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Surprise is always relative, which explains why, whenever something unexpected befalls us, there is always someone who “saw it coming”.

Michael Thompson

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I. Abstract

In natural aquatic ecosystems, surface-associated microorganisms vastly outnumber organisms in suspension (Costerton *et al.* 1987; de Carvalho 2007; Marsh and Bowden 2000; Pickett and White 1985; Zobell 1943). Cells aggregate in highly structured matrix-enclosed communities — so called biofilms. Biofilms are now considered as microbial landscapes where local communities become part of the larger microbial network or metacommunity (Battin *et al.* 2007). Exchange of cells through the landscape occurs via dispersal and is thought to shape local community composition. The aim of our study was to investigate colonization patterns of initially suspended microorganisms in established stream biofilms. Biofilms were grown on ceramic coupons in laboratory flumes under laminar and turbulent flow conditions. After 1, 2 and 6 weeks of growth, respectively, cells labeled with a vital stain (5-Carboxy-fluoresceindiacetat-acetoxymethylester) were co-injected with latex microbeads ($\varnothing$ 1 μ m) to the flumes and recirculated for 36 hours. Loss of particle concentration from the bulk water was monitored to estimate removal rates for microorganisms and microbeads. Biofilms were collected after 24 hours to determine the abundance of colonizing cells and beads, and their spatial distribution. Cryosections were made 24 and 36 hours after the injection. Both particles displayed a fast initial decrease, although removal of microbeads was always faster from the water than cells. Microscopic analysis of the cryosections indicated clustering of both colonizers and beads in the biofilms. We present evidence that hydrodynamics and biofilm characteristics collectively drive the spatial distribution of colonizers.

In natürlichen aquatischen Ökosystemen übersteigt die Zahl der an Oberflächen anhaftenden Mikroorganismen die Zahl jener Mikroorganismen, die in der freien Wassersäule suspendiert sind. Mikrobielle Zellen bilden eine hoch strukturierte, in eine Matrix eingeschlossene Gemeinschaft, so genannte Biofilme. Biofilme werden heute als mikrobielle Landschaften begriffen, in denen lokale Gemeinschaften Teil eines großen mikrobiellen Netzwerks sind. Der Austausch von Zellen durch diese Landschaften erfolgt mittels Verbreitung und es wird vermutet, dass diese Vorgänge die lokalen Biozönosen gestalten und formen. Das Ziel dieser Studie ist die Untersuchung von möglichen Kolonisationsmustern der anfangs suspendierten Zellen in einem bestehenden Biofilm. Die Biofilme wuchsen auf Keramikkacheln in Laborrinnen unter laminaren und turbulenten Strömungsbedingungen. Nach 1, 2 und 6 Wochen Biofilmwachstums wurden die, mit einem Vitalfarbstoff (5-Carboxy-fluoresceindiacetat-acetoxymethylester) gefärbten suspendierten Zellen, zusammen mit Latex Mikrokugeln ($\varnothing$ 1 μ m) in die Rinnen zugegeben und für 36 Stunden rezirkuliert. Die Verringerung der Partikelkonzentration in der Wassersäule wurde überwacht, um die Abnahmeraten für Mikroorganismen und Mikrokugeln zu bestimmen. Biofilmproben wurden nach 24 Stunden genommen, um die Abundanz von kolonisierenden Zellen und Mikrokugeln als auch deren räumliche Verteilung zu ermitteln. Für Letzteres wurden Gefrierschnitte der Biofilmproben nach 24 und 36 Stunden angefertigt. Beide Partikel zeigten anfangs eine starke Abnahme; besonders die Abnahme der Mikrokugeln aus der Wassersäule erfolgte schneller als die der Zellen. Die mikroskopische Analyse der Gefrierschnitte zeigte eine Klumpung, sowohl der Mikrokugeln, als auch der mikrobiellen Zellen im Biofilm. Diese Studie gibt einen Hinweis darauf, dass hydrodynamische Prozesse und Biofilmeigenschaften gemeinsam die räumliche Verteilung der kolonisierenden Zellen bestimmen.

II. Introduction

“Most surfaces on this planet teem with microbial life, creating ecosystems of diverse organisms that flourish in slimy beds of their own making” (Kolter and Greenberg 2006). The structure and architecture of biofilms has been subject of recent studies. The coupling of structural properties and their functions have been emphasized. Application of CLSM showed that biofilms are highly hydrated open structures containing a high fraction of EPS and large void spaces between micro-colonies (Lawrence *et al.* 1991; Tolker-Nielsen and Molin 2000). Secondary structures such as mushroom-like caps, filamentous streamers and migratory ripples separated by channels and voids can be seen as an adaptation of biofilm structure for survival in varying environments (Hall-Stoodley *et al.* 2004). In streams, biofilm structural development is the net result of growth and detachment of microbial biomass, hydrodynamics, substrate availability, and grazing (Battin *et al.* 2003b). Microbial biofilms affect particle transport dynamics through additional mechanisms besides changes in bed roughness and pore-water hydrodynamics. The proliferation of benthic biofilms at the stream bed surface often produces algal filaments (streamers) that protrude into the main stream flow and can capture suspended particles (Packman *et al.* 2002).

In order to deal with the relationships between biodiversity, ecosystem function and the effects of scales (composition, structure or function) in biofilm research the view of a microbial landscape has been introduced by (Battin *et al.* 2007). Generally, the interaction between dispersal processes and landscape pattern influences the temporal dynamics of populations (Turner 1989).

(1) Dispersal and Migration

The processes of growth, death, and replacement ensure that biological systems are dynamic, if only on a local scale (Pickett and White 1985). Sessile organisms are dependent on dispersal, which is their premier spatial demographic process. The seed-dispersal pattern not only determines the potential area of recruitment, but also serves as a template for subsequent processes, such as predation, competition and mating (Nathan and Muller-Landau 2000). Many studies (Hall-Stoodley and

Stoodley 2005; Stoodley *et al.* 2002) indicate that dispersal is an integral part of the dynamic nature of life in surface-associated microbial communities. There is a need for individual cells/cellular-consortia within each biofilm to become dispersed throughout the environment in order to maximize the overall genetic diversity of the community and to promote the competitive demands of the gene (Dawkins 1976). The important role of physical transport in regulating the supply of recruits to an area has been emphasized in aquatic ecology (McNair *et al.* 1997; Roughgarden 1987). The continual flux of individuals to and from regional dispersal pools and their residence times in or on the substrate may profoundly impact local assemblage dynamics (Palmer *et al.* 1996). The mechanisms that control immigration can be quite separate from the physical and biological processes that determine local resource availability, however, which complicates efforts to understand how consumer densities are related to spatial variations in habitat suitability. Thus, it is important to examine the potential role of immigration processes when studying the ecology of local populations (Fonseca and Hart 2001). Lawton (1999) has argued that, although the details of individual organisms and ecological systems matter, ecologists would profit most from trying to uncover underlying patterns, rules and laws. Furthermore, he (Lawton 1999) has argued that such generalizations would be most likely to be discovered at very small scales (e.g. populations) and at very large scales (e.g. aggregate patterns in the distribution of biodiversity). Research on microbial biofilms and quorum-sensing systems has helped drive the understanding that most microbes live out their life histories in social contexts that involve complex webs of both cooperative and competitive interactions (Kjelleberg and Molin 2002; Kolenbrander *et al.* 2002; Miller and Bassler 2001). In mixed biofilms, bacteria distribute themselves according to who can survive best in the particular microenvironment and also based on symbiotic relationships between the groups of bacteria (Watnick and Kolter 2000). Different dispersal capabilities and landscape patterns might differentially affect dispersal-assembled communities as well (Battin *et al.* 2007). Biofilm community dynamics at quasi-steady state involve a fine balance between the forces of attachment and those associated with detachment and colonization resistance of the community (McBain *et al.* 2000).

(2) Hydrodynamics

The hydrodynamics of aquatic and aqueous environments have a significant influence on biofilm development and activity and are in turn influenced by biofilms in various industrial and natural systems (Characklis 1990). The hydrodynamic environment in natural streams seems to be dominated by turbulent flow but flow through transient storage zones is controlled by low shear rates and laminar conditions. The development of freshwater multispecies biofilms at solid-liquid interfaces occurs both in quiescent water and under conditions of high shear rates (Rickard *et al.* 2004). Turbulence plays a key role in a variety of ecological processes involving transport through or exchange with the water-column. It is characterized by complex, three dimensional fluid motions in which the paths of individual fluid packets are unpredictable (McNair *et al.* 1997). On the contrary, laminar flow is characterized by low Reynolds numbers and unidirectional flow. In theory, the flow velocity immediately adjacent to the substratum/liquid interface, termed hydrodynamic boundary layer, is negligible (Donlan 2002). Nevertheless, shear rates over surfaces have been reported (Rickard *et al.* 2004) to directly influence the bacterial composition and govern the abilities of individual species to immigrate to biofilms and to colonize surfaces thus controlling the diversity of multispecies biofilms that form on surfaces. Hydrodynamic conditions can also strongly influence biofilm structure (Purevdorj *et al.* 2002) and mechanisms that facilitate bacterial adhesion (e.g. co-aggregation) might be flow dependent. If the biofilm is a highly compliant material the shape will vary not only through the growth cycle of the biofilm but also with variations in fluid shear stress as suggested by Stoodley (1997). Changes in biofilm shape will affect its porosity and density and therefore the transfer of solutes into and through the biofilm (Stoodley *et al.* 1999). Shear-mediated migration of biofilms represents a previously unrecognized mechanism for dissemination in flowing systems (Purevdorj *et al.* 2002). Turbulent transport, rather than passive sinking, may be the primary mode by which particles are delivered directly to streambed surfaces (Denny and Shibata 1989; McNair *et al.* 1997). Influence of two different hydrodynamic settings on biofilm parameters (bacterial abundance, chlorophyll *a* concentration), the settling rate of particles and resulting spatial colonization patterns were analyzed in our study.

(3) Transport

Transport and retention of organic seston are important functions of stream ecosystems because they determine the rate at which particle-associated energy and nutrients are lost from a stream and the degree to which downstream reaches are linked to upstream processes (Hall 1996; Vannote *et al.* 1980). Bacteria represent an important component of the seston, and they are important agents of nutrient cycling (Kondratieff and Simmons 1985) which is important in distributing energy within streams at multiple spatial and temporal scales (Schlesinger 1981). The influence of the seston depends on the ability of its settling or rather uptake at the solid-fluid interface as well as the biological processes active at the site of deposition (Thomas *et al.* 2001). Few of the particles that reach the stream-subsurface interface are retained there. The problem of particle deposition becomes a matter of retention, which in turn suggests that surface properties of both the transported particles and the bed sediment play a critical role in overall particle capture (Packman *et al.* 2002). Biofilms can serve as environmental reservoirs by transferring colloidal particles such as latex beads, bacteria and virus-sized particles from the surrounding bulk fluid to the biomass (Drury *et al.* 1993; Flood 2000; Searcy *et al.* 2006). The heterogeneous structure of biofilms might facilitate the capture and retention of colloid particles. A positive relationship between particle deposition in biofilms and biofilms sinuosity (Battin *et al.* 2003a) as well as biofilm thickness (Drury *et al.* 1993) has been shown. The internal biofilm channel system and the highly hydrated matrix constitute important transient storage zones (Battin *et al.* 2003a) facilitating deposition. The ability of bacteria to adhere to biofilm may influence their travel distance (Hall 1996).

(4) Attachment and Biofilm formation

At maturity, biofilms are often subjected to challenge with planktonic cells (Kadouri and O'Toole 2005). These might constitute individual cells that have grown in suspension or ones that have been derived from biofilms upstream of the community. Immigrant organisms will depend upon their ability to displace, compete or co-operate effectively with the resident biofilm (McBain *et al.* 2000). From an evolutionary standpoint, the selective advantage of bacterial adhesion has been

postulated to favor the localization of surface-bound bacterial populations in nutritionally favorable, non-hostile environments and at the same time provide some level of protection (Dunne 2002). Selection pressures will be exerted disproportionately throughout the biofilm, leading to the establishment of mosaics of sub-communities within the global biofilm architecture (McBain *et al.* 2000).

Biofilm formation can occur by at least three different mechanisms. One is by the redistribution of attached cells by surface motility. A second mechanism is from binary division of attached cells. A third mechanism of aggregation is the recruitment of cells from the bulk fluid to the developing biofilm (Stoodley *et al.* 2002). We will focus here on the recruitment of cells from the bulk fluid. Once microorganisms reach the proximity of a surface, attachment is determined by physical and chemical interactions, which may be attractive or repulsive, depending upon the complex interplay of the chemistries of the bacterial and substratum surfaces, and the aqueous phase (Katsikogianni and Missirlis 2004). The division of the adhesion process in two phases is still the dominating view of bacterial adhesion (Hermansson 1999). An initial attraction of the cells towards a surface due to van der Waals attractions forces, Brownian motion, gravitational forces, electrostatic charges and hydrophobic interactions (Gottenbos *et al.* 2002), and second molecular and cellular interactions by use of microbial surface polymeric structures such as capsules, fimbriae or pili and EPS (Mayer *et al.* 1999; Pratt and Kolter 1998). Both specific and non-specific interactions may play an important role in the ability of the cell to attach to the surface. The relative contributions of specific and non-specific mechanisms are likely to depend on the surface properties as well as the associated flow conditions (Katsikogianni and Missirlis 2004).

The initial attraction of the cells to the surface is followed by adsorption and attachment (Rijnaarts *et al.* 1995). Adsorption is the accumulation of molecules onto a solid by mutual forces, which are a function of the distance surface at a concentration exceeding that in the bulk fluid, and free energy, which is brought about as a result of random Brownian motion. Deposition is normally used to describe the accumulation of particles at a fluid interface that is brought as hydrogen bonding, ionic and dipole interactions, and about by the application of an external force (An and Friedman 1998). Sedimentation theory views particle deposition as a gravitational transfer onto the bed surface. A constant proportion of the suspended particles deposit per unit time, though, it appears that the deposition of particles in

natural streams is little influenced by fall velocity (Packman *et al.* 2002) rather it appears that particle size plays a significant role (Hall 1996; Thomas *et al.* 2001). Several factors and mechanisms other than the fall velocity may be involved in determining the deposition velocity. Shear stress, bed roughness, sorption kinetics of microorganisms and the availability of streambed deposition sites may alter field deposition rates from estimated fall velocities (Leopold *et al.* 1964; Lindqvist *et al.* 1994). The review by (Katsikogianni and Missirlis 2004) on bacterial adhesion highlights the active and passive movement of cells effected by physical forces, such as Brownian motion, van der Waals attraction forces, gravitational forces, the effect of surface electrostatic charge and hydrophobic interactions (Gottenbos *et al.* 2002), while chemotaxis and perhaps haptotaxis contribute to the initial adhesion process (Kirov 2003). In the second phase of adhesion, molecular-specific reactions between bacterial surface structures and substratum surfaces become predominant. This implies a firmer adhesion of bacteria to a surface by the selective bridging function of bacterial surface polymeric structures. Production of bio-polymers 'glue' the cell and its daughter cells onto the surface until detachment takes place (Hermansson 1999; Mack 1999; O'Gara and Humphreys 2001).

The importance of extracellular polymeric substances (EPS) on biofilm structure and function is undeniable. It seems likely that the earliest living organisms produced exopolysaccharides and their secretion is a feature of the biology of many contemporary bacteria, cyanobacteria and unicellular algae (Wotton 2004). EPS synthesized by microbial cells vary greatly in their composition and hence in their chemical and physical properties (Sutherland 2001). Exopolymers are important in the attachment of bacteria to substrata and the development of the biofilms (Costerton *et al.* 1987). Matrix polymers not only glue the biofilm to the surface but also enable spatial organization to be imposed on the community (McBain *et al.* 2000). Their ability to adsorb and retain nutrients is especially significant in biofilms (Lock 1994) and the gel matrix also allows the conservation of exoenzymes that might otherwise be lost to the surrounding water. In addition to providing a physical barrier against predation it is also likely that exudates have chemical and microbiological defensive properties (Wotton 2004).

(5) Spatial organization

The spatial patterns of organisms primarily result from abiotic factors but organisms can also physically alter their environment and create spatial heterogeneity (Turner 2005). It is evident that many different environmental factors influence the settling and adherence of particles. The questions we posed are: “Is there a pattern that microbial cells display as they settle onto the biofilm?” and if so “Does this pattern change in time?”. Highly organized patterns with relatively regular cell-cell spacing have been observed in single species biofilms (Stoodley *et al.* 2002). Spatial structure is necessary to facilitate the evolution of cooperation in biofilms (Kreft 2004). Theories on the spatial patterns formed in microbial communities have been proposed (Battin *et al.* 2007; Green *et al.* 2004; Sloan *et al.* 2006). Since we looked at the colonization of established biofilms so-called secondary colonizers set the focus of this study. Secondary colonizers will interact with either vacant sites on the surface or the primary colonizers. There are three possible outcomes to the encounter between a potential immigrant and a newly colonized surface. (i) The surface may be hostile to the potential colonizer due to lack of available/unoccupied binding sites and the immigrant will therefore fail to bind. (ii) The immigrant cells may displace physically, from the surface, one of the early colonizers by virtue of a possibly higher binding affinity for a common binding site. This is most likely to occur during the initial attachment phase of film formation and before the deposition of polymer cements. The duration of this phase will therefore be indirectly related to the metabolic potential at each colonized site. (iii) Both the immigrant species and the primary colonizer are retained at the surface, either at separate sites or attached to each other or to matrix polymers. Where a surface is co-colonized, then the degree of interaction between the colonizers will be minimal in the first instance but will increase as the community grows and adjacent microcolonies come into closer proximity. Such interactions might be mediated through the production of cell-cell signaling compounds, specific, and nonspecific inhibitors or they might involve competition for available nutrients (McBain *et al.* 2000). Indeed, these interactions appear to be essential for the attachment, growth and survival of species at a site (Rickard *et al.* 2002). In addition to possible patterns during adhesion, movement of sessile cells within the biofilm matrix was predicted by (Stoodley *et al.* 2002). Motility of bacterial cells over surfaces such as gliding, twitching and swarming has been

reported (Fenchel 2002; Harshey 2003; Kaiser 2007). Movement within biofilms and underlying mechanisms has not been investigated to our knowledge.

(6) Spatial analysis

The importance of spatial processes and spatial heterogeneity is widely recognized and especially dealt with in landscape ecology. Intense interest in issues of spatial scale (Wiens 1989), metapopulation dynamics (Hanski 1998) and spatio-temporal dynamics (Hassell *et al.* 1991) evolved from this awareness. Landscapes can be defined as the configuration of patterns at any scale relative to the ecological processes or organisms under investigation, and the concept of landscape can therefore be applied to any scale or system (Turner 2005). We integrated analytical methods already used in landscape ecology in our work on microbial landscapes. We stress the point that microbiological research will be improved by such interdisciplinary approach. Patterns may result from different processes and forces such as seed dispersal, intraspecific competition, interspecific competition, disturbance, or environmental heterogeneity (Jackson *et al.* 2001; Kaplan and Fine 2002; Leff 2000; Xu *et al.* 1998), which may operate at different spatial scales (Wiegand and Moloney 2004). Information given by the location of the labeled particles within our study region (biofilm) was defined by point patterns. The density of these points is referred to as the “intensity” of the pattern. If a point process is stationary, there is a close mathematical relationship between the second-order intensity and an alternative characterization of second-order properties known as the K function (Ripley 1981). Essentially, the K function describes the extent to which there is spatial dependence in the arrangement of events (Bailey and Gatrell 1995; Wiegand and Moloney 2004). The pair-correlation function $g_{12}(r)$ is the analogue of Ripley’s $K_{12}(r)$ when replacing the circles of radius r by rings with radius r . The pair-correlation function $g_{12}(r)$ is related to Ripley’s K-function (Ripley 1981; Stoyan and Stoyan 1994):

$$g_{12}(r) = \frac{dK_{12}(r)}{dr} \bigg/ (2\pi r)$$

Values of $g_{12}(r) > \lambda_2$ indicate that there are on average more points of pattern 2 at distance r of points of pattern 1 as one would expect under independence, thus

indicating attraction between the two patterns up to distance r . Similarly, values of $g_{12}(r) < \lambda_2$ indicate repulsion between the two patterns at distance r . The estimated $g_{12}(r)$ function is calculated for a sequence of distances r and the results of $g_{12}(r)$ are then plotted against distance (Wiegand and Moloney 2004). We attribute differences between the pattern distribution of microbes and microbeads to biological interactions and biotic processes influencing the transport, attachment and movement during colonization.

III. Methods

(1) Flume Design and Settings

The advantage of laboratory flumes to study the coupling between hydrodynamics, chemistry, and microbial biofilm structure and function has been emphasized in previous studies (Besemer *et al.* 2007; Singer *et al.* 2006). In this study, microcosms consisted of flumes made of Plexiglas (length 1.3 m, width 0.02 m, height 0.02 m) with inlet baffles made from 5-cm-long tubes (diameter 5 mm) to streamline water flow. At the flume outlet tailgates ensured uniform flow. To avoid bias due to inflow and backflow sampling several coupons at the beginning and end of the flume served as buffers to stabilize the hydrodynamic environment and were not used for sampling. Each flume was paved with low porosity, unglazed ceramic coupons that served as substratum for biofilm growth. Coupons were made of natural clay (Westerwald, Germany) and baked at 1060°C to yield a porosity of approximately 10%. Coupons were acid-washed (1 N HCl) to remove clay-bound inorganic nutrient ions, rinsed in sterilized de-ionized water, and finally combusted (450°C 4 h) to remove organic coatings.

From a water tank filled with 120 liter lake water submersed pumps (Aquamedic Ocean Runner 3500 and 2500) distributed the water to four header tanks. Each header tank supplied five flumes with water. Overflow outlets ensured constant head within the tanks. Water would run through the flumes into a gutter which drained into the main water tank to close the cycle.

Water samples were taken for analysis from the water tank before and after water exchange with lake water (Lunzer Untersee, Austria). Water chemistry data such as pH, dissolved oxygen (DO), conductivity, phosphate, ammonium, nitrite, and nitrate were analyzed with a multi-parameter digital meter (HACH LANGE HQ40d, Germany). Table 1 lists these parameters.

Table 1: Chemical parameters, nutrient conditions in the water tank

Parameters	Values
pH	8.4
Dissolved Oxygen	$9.1 \pm 0.5 \text{ mg L}^{-1}$
Conductivity	$238.0 \pm 1.4 \text{ }\mu\text{S cm}^{-1}$
PO ₄ -P	$2.5 \pm 1.2 \text{ }\mu\text{g L}^{-1}$
NH ₄ -N	$26.7 \pm 30.0 \text{ }\mu\text{g L}^{-1}$
NO ₂ -N	$7.8 \pm 5.6 \text{ }\mu\text{g L}^{-1}$
NO ₃ -N	$303.6 \pm 164.6 \text{ }\mu\text{g L}^{-1}$

Microcosms were designed to yield laminar and turbulent flow regimes with initial parameters as described in Table 2. This was achieved by regulating flow rate and the slope of the flumes. Flow rate, the prime determinant of the hydrodynamic environment was regularly checked throughout the experimental period and adjusted if necessary. Since turbulent flow is typically associated with Reynolds number >2000, turbulent flow was confirmed injecting rhodamine for visualizing flow patterns in a test run.

Table 2: Hydrodynamic setting and flow conditions

Treatment	Flow rate. mL s^{-1}	Depth. cm	v. cm s^{-1}	Re
laminar	10.1 ± 0.8	1.0	5	277
turbulent	63.0 ± 2.3	0.9	35	1894

Water in the water tank was exchanged with new lake water every third day, exchanging approximately 80 liters. The water was filtered through a 100- μm mesh to remove particles and insect larvae.

Fluorescent tubes (58 W, TLD33 Philips) with reflectors yielded a mean photosynthetic active radiation of $50 \text{ }\mu\text{mol m}^{-2} \text{ s}^{-1}$ above the flumes. Dark:light period was 12:12 hours. Lateral white diffusers minimized spatial heterogeneity of irradiance.

(2) Inoculum

Colonizer bacteria were taken from 3 μm filtered lake water amended with nitrate, ammonium, phosphate (250 mg L^{-1} , respectively), and glucose (1000 mg L^{-1}) and incubated overnight at 28 $^{\circ}\text{C}$ at dark in a shaker. One and a half liters of the culture were centrifuged at 4000 G for one hour using a Beckman Coulter centrifuge (Avanti J-26XP, USA). The supernatant was discarded and the pellet diluted with 30 ml PBS puffer solution and transferred to two sterile Greiner tubes (15 ml). Cells were stained with 0.25 μl 25 mM CFDA/SE (5-(and-6-)-carboxyfluorescein diacetate, succinimidyl ester) per milliliter suspension and incubated for at least 2 hours in the shaker at 28 $^{\circ}\text{C}$. Cells were then centrifuged (Eppendorf Centrifuge 5810R, Germany) at 3220 G (1 h) to reduce background fluorescence of CFDA/SE residuals. Stained cells were dissolved in 20 ml tap water which was used to minimize nutrient and substrate supply. The culture was diluted to yield 2×10^7 stained cells per milliliter. Most cells were rod shaped with average length of $2.13 \pm 0.36 \mu\text{m}$ and width $0.97 \pm 0.20 \mu\text{m}$.

Fluorescent blue carboxyl polystyrene latex beads (Postnova Analytics, Germany) were used as conservative tracers. Characteristics of the microbeads can be found in the appendix. The polystyrene microspheres dispersion (in distilled de-ionized water) was vortexed and 600 μl were added to 2 liters of the cell suspension which converts to 10^7 beads mL^{-1} .

Each flume was fed with an inoculum (800 ml) tap water containing $10.8 \pm 1.2 \times 10^6$ CFDA/SE-cells mL^{-1} and 10×10^6 microbeads mL^{-1} .

In order to re-circulate microbes and microbeads during the experiments, duplicate flumes were fed with the inoculum. A small pump was placed in a sink at the flume outlet from there the inoculum was transferred in 2-m-long PVC tubes to the header tank.

During the recirculation optical density was measured with a photometer at 600nm (HACH LANGE DR2800 spectrophotometer, Germany) taking 2 ml water samples, which were returned to the water cycle after measurement. In addition, 2 ml water samples were collected in a time series and contained cells preserved with 0.1 mL 37% formaldehyde and stored in the dark at 4 $^{\circ}\text{C}$ for subsequent microscopic analysis. After 24 hours of recirculation, coupons were randomly sampled from each flume using sterile forceps. Coupons were collected from downstream to upstream to avoid changing the hydrodynamic environment in the flume. Sampled coupons were

replaced immediately with sterile coupons to minimize wake interference and skimming flow (Singer *et al.* 2006). Furthermore, 3 coupons per flume were removed for chlorophyll *a* analysis, 3 coupons were transferred into 2.5 ml 2.5% formaldehyde for the determination of CFDA/SE-cell and bead settlement, and 3 coupons per flume were sampled for the spatial analysis of CFDA/SE-cells and beads.

(3) Chlorophyll *a* and bacterial abundance

Coupons with biofilm were kept frozen (-4°C). 4 ml acetone (p.a.) was added and extracted over night at 6°C (4° C). Tubes were vortexed and the solution was filtered through a GF/F Filter. Absorbance was measured at 665 nm and 750 nm with a spectrophotometer (Shimadzu, UV-1700 Pharma Spec). Length and width of each tile was measured to refer chlorophyll *a* to the surface area.

Biofilm samples which were taken for settlement analysis were sonicated for 30 seconds (20% energy output) to disintegrate possible aggregates. The abundance of microbeads and CFDA/SE cells in the inoculum was determined. Samples were kept dark for most of the processing steps to avoid fluorescence fading (Fuller *et al.* 2000).

(4) Cryosectioning

For spatial analysis, biofilms were embedded in O.C.T.[™] compound (Sakura Finetek Europe B.V., Netherlands) and frozen (-80°C) pending cryosectioning and further analysis. XZ-cryosections were transferred onto poly-l-lysine coated microscope slides, air-dried and mounted in Citifluor. Images were obtained using epifluorescence microscopy (Zeiss Imager M1, Zeiss AxioCam MRc5) and digital image processing to record the spatial distribution of CFDA/SE-cells and fluorescent microbeads. A 40x/0.80W EC Plan Neofluar objective was used for magnification. Images were randomly selected within the biofilm and recorded with three different filters.

(5) Data analysis

Statistics were calculated using STATISTICA (StatSoft) software, whereas plots were designed with SigmaPlot (SYSTAT Inc.) graphing software. A two-factorial ANOVA model, where biofilm age and flow treatment were fixed factors and microbial abundance respectively chlorophyll *a* values were the dependent factors, was used to compare biomass changes for each treatment. Values of the microbial abundance respectively chlorophyll *a* concentration were square-root transformed to achieve homogeneity of variances. The Tukey honest significant difference (HSD) test was employed for pairwise post-hoc comparisons between treatments. The same routine was calculated for the deposition of microbeads and CFDA/SE stained cells. Therefore we used the ratio of CFDA/SE stained cells to microbeads found in the biofilms as dependent factors.

Three general hypotheses were tested: The two main effects respectively biofilm age and flow treatment, and the third was the test of the interaction of both effects.

(6) Image analysis

Digitized cryosections (32 bits RGB TIFF files, 1,024 by 1,024 pixels) were imported into ImageJ 1.41k (Abramoff *et al.* 2004) for image processing. Each imported image layer (red-green-blue [RGB]) was split into channels and set to scale. We applied a fixed threshold to each image set using the multithreshold plugin. This plugin performs automatic thresholding based on the entropy of the histogram. Thresholding results in a matrix with “zero values” in positions where the numbers of RGB pixels are below the threshold values and “one values” where the numbers of RGB pixels are between the threshold values and 255. One value represents positions containing microbial cells, or microbeads. Using the command “analyze particles”, coordinates and area size of all selected particles were listed. Cell aggregates that could not be differentiated into single cells were weighted in respect to their area size. Identification of the study region was performed by merging images of 3 different wavelengths and by defining the outer boundaries using multiple point selection. Coordinates of the study regions boundaries were saved as shape files. Point-pattern analysis was done with Programita (Wiegand and Moloney 2004).

Therefore, coordinates and area size of the observed patterns as well as the study region shape file had to be imported. The data file must be a space (or tab) delimited ASCII file with the *.dat extension. Information on the grid size, the number of cells with data, and the coordinates of cells that contain points has to be provided.

Information given by the location of the labeled particles within our study region (biofilm) was defined by point patterns. The density of these points is referred to as the “intensity” of the pattern. If a point process is stationary, there is a close mathematical relationship between the second-order intensity and an alternative characterization of second-order properties known as the K function (Ripley 1981). The bivariate K-function $K_{12}(r)$ is defined as the expected number of points of pattern 2 within a given distance r of an arbitrary point of pattern 1, divided by the intensity λ_2 of points of pattern 2:

$$\lambda_2 K_{12}(r) = \mathbf{E}[\#(\text{points of pattern 2} \leq r \text{ from an arbitrary point of pattern 1})] \quad (1),$$

where $\mathbf{E}(\)$ denotes expectation, $\#$ means ‘the number of’ and λ is the intensity or mean number of events per unit area. Essentially, the K function describes the extent to which there is spatial dependence in the arrangement of events (Gatrell *et al.* 1996; Wiegand and Moloney 2004). The pair-correlation function $g_{12}(r)$ is the analogue of Ripley’s $K_{12}(r)$ when replacing the circles of radius r by rings with radius r , and the O-ring statistic $O_{12}(r) = \lambda_2 g_{12}(r)$ gives the expected number of points of pattern 2 at distance r from an arbitrary point of pattern 1:

$$O_{12}(r) = \mathbf{E}[\#(\text{points of pattern 2 at distance } r \text{ from an arbitrary point of pattern 1})] \quad (2).$$

Using rings instead of circles has the advantage that one can isolate specific distance classes and can therefore detect mixed patterns. The pair-correlation function $g_{12}(r)$ is related to Ripley’s K-function:

$$g_{12}(r) = \frac{dK_{12}(r)}{dr} \bigg/ (2\pi r) \quad (3).$$

Values of $g_{12}(r) > \lambda_2$ indicate that there are on average more points of pattern 2 at distance r of points of pattern 1 as one would expect under independence, thus indicating attraction between the two patterns up to distance r . Similarly, values of $g_{12}(r) < \lambda_2$ indicate repulsion between the two patterns at distance r . The estimated $g_{12}(r)$ function is calculated for a sequence of distances r and the results of $g_{12}(r)$ are

then plotted against distance (Wiegand and Moloney 2004). Edge effects were corrected utilizing only points lying inside the main study area as centres in calculating the point-pattern statistics (Haase 1995).

Running point pattern analysis with data input as list with coordinates (no grid) and irregularly shaped study region setting the ring width to 'one', Programita creates a temporary file (tempshape) which gives data as coordinates in grid. This file is needed to weight points in terms of their area size. Point pattern analysis was run again this time with ring width of 'four' since strong fluorescence signals of clustered particles reduced resolution. The use of rings that are too narrow will produce jagged plots as not enough points will fall into the different distance classes. On the other hand, the O-ring statistics will lose the advantage that it can isolate specific distance classes if the rings are too wide (Wiegand 2004).

Ten different images of biofilm sections of the same treatments (biofilm age, flow velocity, time after injection of inoculum) were combined as replicates. Testing the null model against real data is necessary to take uncertainty due to the stochastic character of the point process and the limited sample size, into account. Random labeling involves repeated simulations using the fixed $n_1 + n_2$ locations of pattern 1 and 2, but randomly assigning "case" labels to n_1 of these locations. Each simulation generates a $g_{12}(r)$ function, and approximate $n/(n + 1) \times 100\%$ confidence envelopes are calculated from the highest and lowest values of $g_{12}(r)$ taken from n simulations of the null model (Wiegand 2004). Due to relatively low particle densities in the images we set the number of simulations to 499 resulting in 99.8% confidence envelopes.

Two different variants of random labeling were applied. First, we compared the aggregation (or regularity) of the univariate component patterns to reveal whether one pattern is more clustered (or less regular) than the other, conditional on the structure of the joined pattern. Second, analysis revealed information about the symmetry of the correlation of type 1 and type 2 points. A positive difference indicates that type 2 points have at distance r more neighbors (= type 1 and type 2 points) than type 1 points. Thus, type 2 points are mainly located in areas with higher intensity of the joined pattern whereas type 1 points are mainly located in areas of lower intensity. Thus, departure from random labeling depicted in this analysis

indicates that the process that assigns the labels to the points interacts with the heterogeneity of the joined pattern (Wiegand and Moloney 2004).

IV. Results

(1) Biofilm characteristics

Biofilm age had a significant effect on microbial cell abundance ($F(2.12)=111.6$, $p=.000$) and chlorophyll *a* ($F(2.12)=2548$, $p=.000$). Significant differences in cell abundance were also shown for flow treatments ($F(1.12)=210$, $p=.000$) for chlorophyll *a*, but not microbial cell abundance. Chlorophyll *a* showed a moderate interaction effect ($F(2.12)=54$, $p=.000$), which, was not found for microbial abundance.

The mean microbial abundance increased 27-fold within 5 weeks in the laminar flow and 13-fold in the turbulent treatment. Chlorophyll *a* in the 6- week old biofilms similarly increased 28-fold in the laminar treatment, and was 37-fold higher than in the 1-week old biofilms. Mean chlorophyll *a* concentration was twice as high for turbulent flow as for laminar flow (Figure 1).

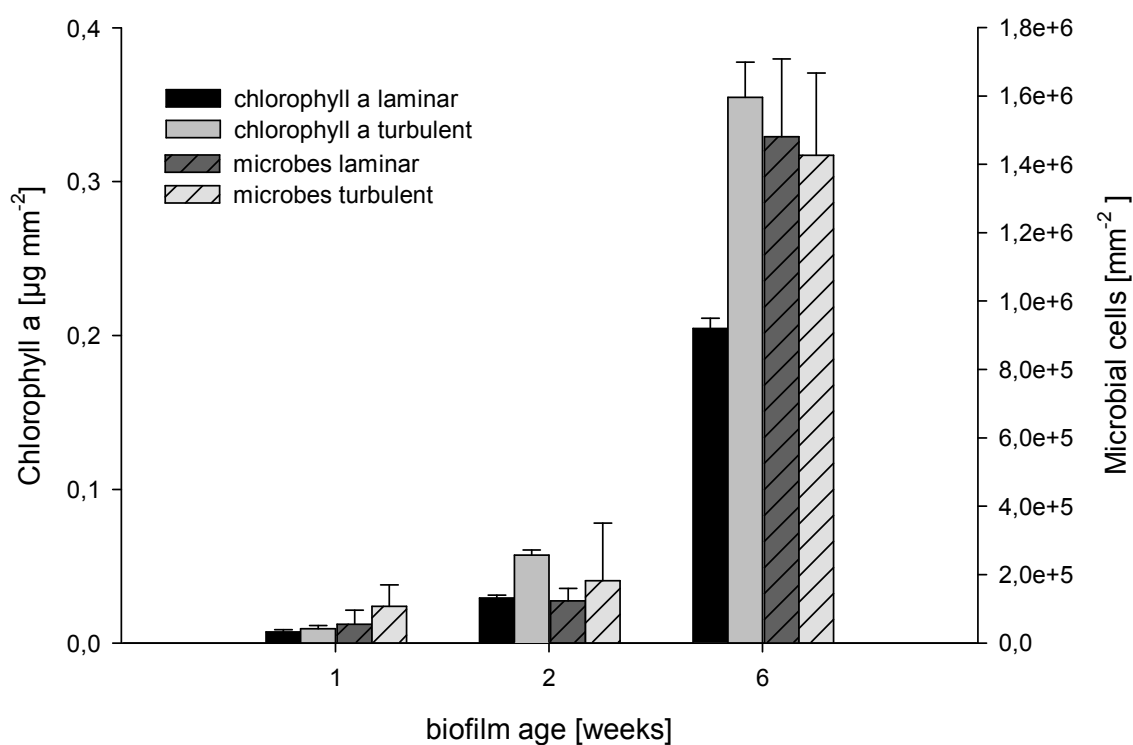


Figure 1: Biofilm biomass parameters chlorophyll *a* content and microbial cell densities

(2) Deposition of particles and CFDA/SE stained cells

Depending on biofilm age and flow regime, turbidity decreased within the first hour after the injection. Older biofilms and turbulent flow caused the fastest removal rates. Measurements and graphs of the optical density can be found in the appendix.

Despite similar densities of CFDA/SE stained cells and microbeads, significant differences in particle removal were observed. The number of microbeads decreased faster than CFDA/SE-stained cells, which indicates faster deposition. Removal was especially high in the beginning of the experiments and was slowing down after 2 to 3 hours of recirculation.

First, I will look at the abundance of microbeads and CFDA/SE-stained cells, in the water column and then compare them to the values predicted by the perfect sink model.

Flow velocity did not affect the deposition of CFDA/SE-stained cells in the 1-week old biofilms. The microbead concentration in the water column changed within 12 hours after the injection, as a consequence microbead concentration was three times higher in laminar flow than in the turbulent flow treatment (Figure. 2.1 a).

2-week old biofilms remarkably influenced the deposition of CFDA/SE-stained cells and microbeads. Twice as many CFDA/SE-stained cells were removed after 12 hours in laminar flow compared to turbulent flow. In the laminar flow, microbeads removal was less than in the turbulent flow resulting in a 10-fold higher microbead concentration in the water column in laminar flow after 12 hours (Figure. 2.1 b).

In the 6-week old biofilm removal of CFDA/SE-stained cells and microbeads in turbulent flow decelerated. The ratio of CFDA/SE-stained cells in laminar flow compared to turbulent flow was similar. Removal of microbeads was different, regarding microbeads would settle faster in laminar flow so that microbead concentration was 4-fold higher in the laminar flow regime, 12 hours after the injection (Figure. 2.1 c).

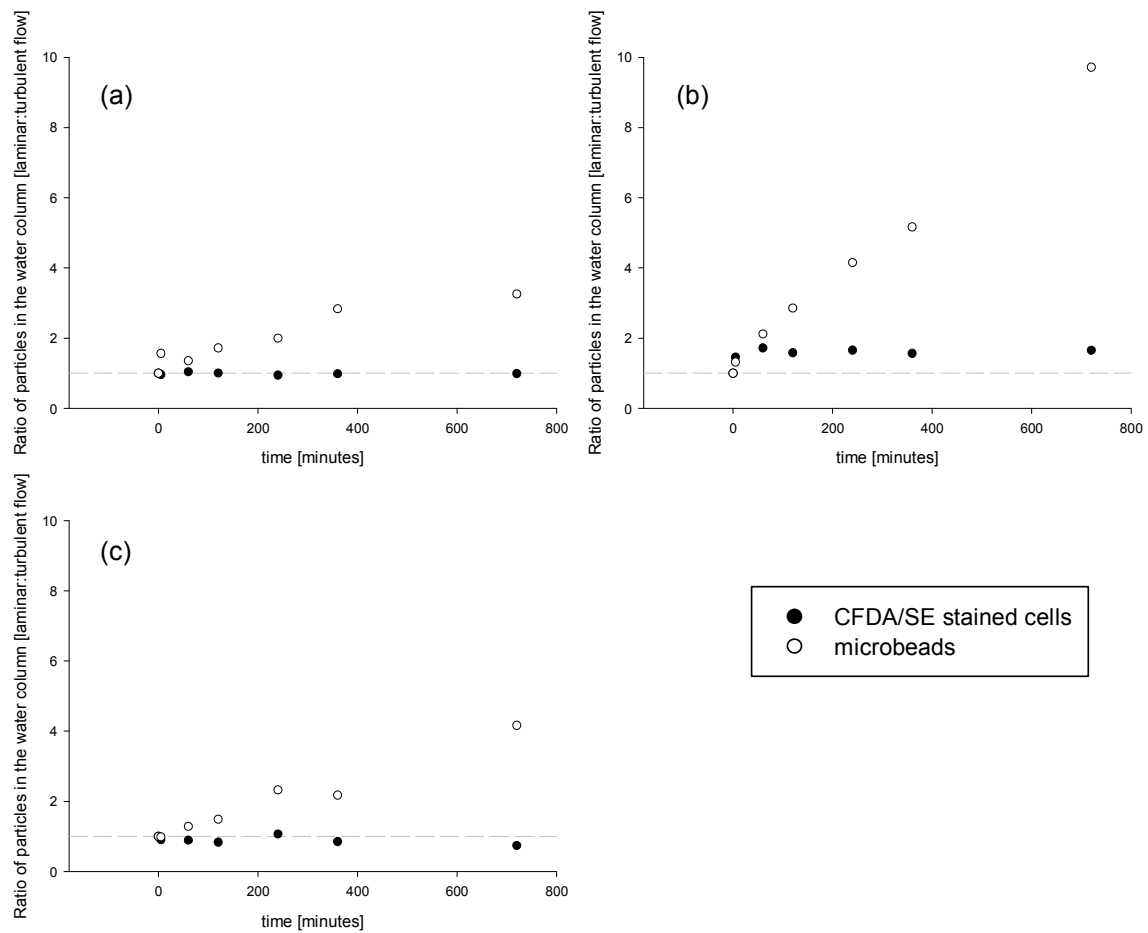


Figure 2.1: Comparing the change of concentration at different flow treatments for (a) 1-week, (b) 2-week and (c) 6-week old biofilm.

I will now compare these observed values with the deposition behavior of microbeads in the control flumes without biofilms — referring to the perfect sink model (Figure 2.2).

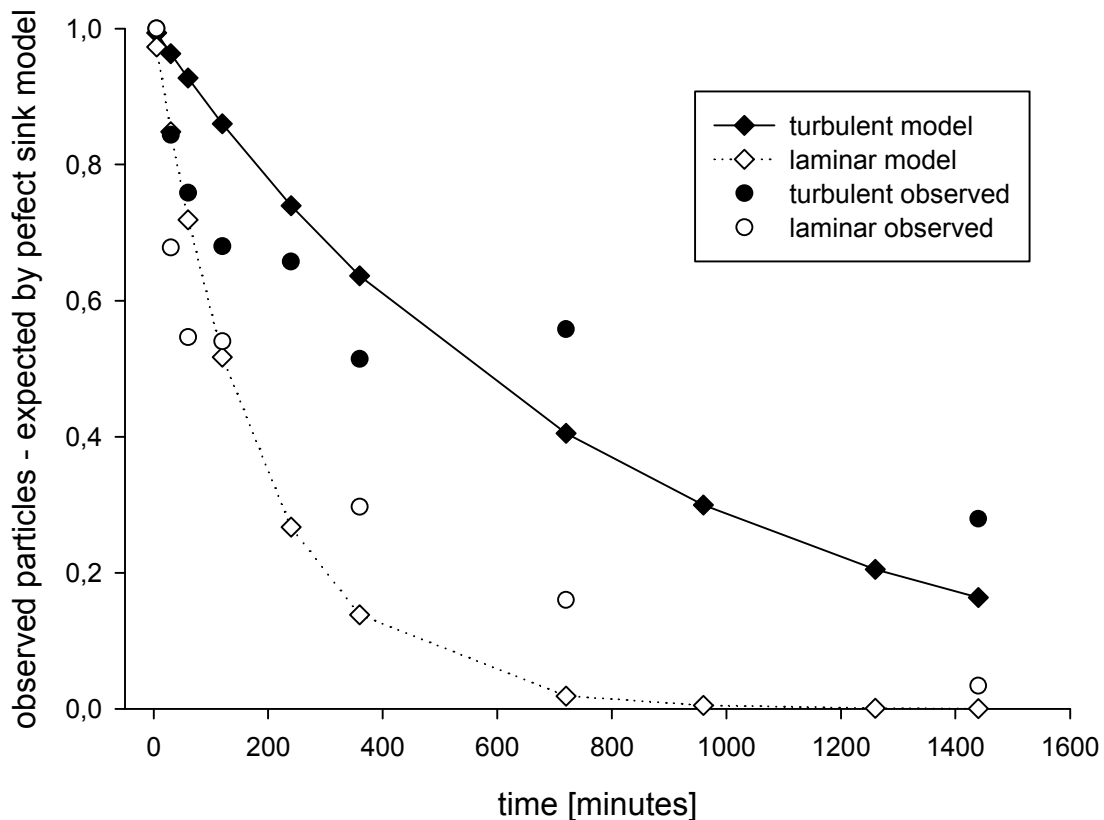


Figure 2.2: Perfect sink model versus observed microbead concentration in the water column

The model expects more microbeads to settle at higher flow velocities. Deposition of microbeads exceeded the perfect sink model independent of flow velocity and biofilm age (Figure. 2.3).

In the 1-week old biofilms (Figure. 2.3 a), the fast initial deposition of microbeads and CFDA/SE-stained cells is shown except for CFDA/SE-stained cells at turbulent flow, which were not “captured” by the biofilm. Fast flow decreased the deposition rate of CFDA/SE stained cells for all biofilm ages, removing fewer cells from the water column than expected by the perfect sink model. The initial deposition velocity of CFDA/SE stained cells decreased in older biofilms in laminar flow. Differences between the 2- and 6- week old biofilms are small and appear symmetric. However, fewer microbeads and CFDA/SE stained cells were removed in the 6-week old biofilm except for microbeads in laminar flow (Figure. 2.3 c). Microbeads settled on the biofilms rapidly and only a slight difference in removal between all 3 biofilm stages was detected in turbulent flow. In laminar flow, microbeads were immobilized pre-eminently at each biofilm age.

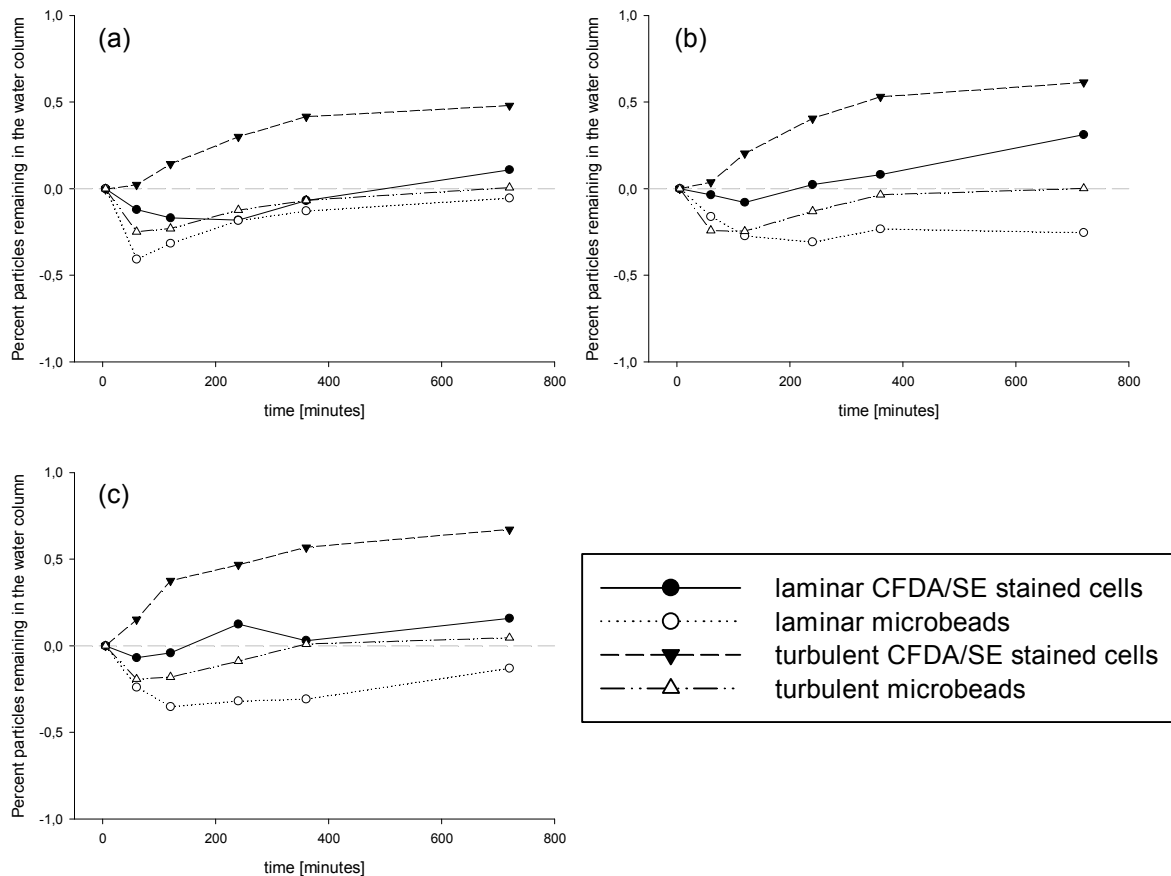


Figure 2.3: Differences between the observed and expected microbeads and CFDA/SE-stained cells concentration in the water column for (a) 1-week, (b) 2- week and (c) 6- week old biofilm.

(3) Number of particles found in biofilms after 36 hours of recirculation

Observations from microbead and CFDA/SE-stained cells removal could not be confirmed by actual cell and microbead counts in biofilm samples. CFDA/SE-stained cells were more abundant in the biofilms from turbulent flow. Laminar flow had little effect on settling abilities on 1-week old biofilms as no major differences in abundance between CFDA/SE stained cells and microbeads were found.

Microbead and CFDA/SE stained cell deposition increased little over time in both flow treatments. High variability was found in all turbulent flow treatments, notably for CFDA/SE-stained cells (Figure. 3.1).

CFDA/SE-stained cells were 4-fold higher in the turbulent than in the laminar treatment in the 1-week old biofilm. This difference became slightly reduced in the 2-week old biofilm but still CFDA/SE stained cells were transferred predominantly to the biofilm subjected to turbulent flow.

Increased deposition of both microbeads and CFDA/SE-stained cells was also observed in turbulent flow in 6-week old biofilms although fewer CFDA/SE-stained cells were found than in the 2-week old biofilms. In contrast, CFDA/SE-stained cells settled less in laminar flow.

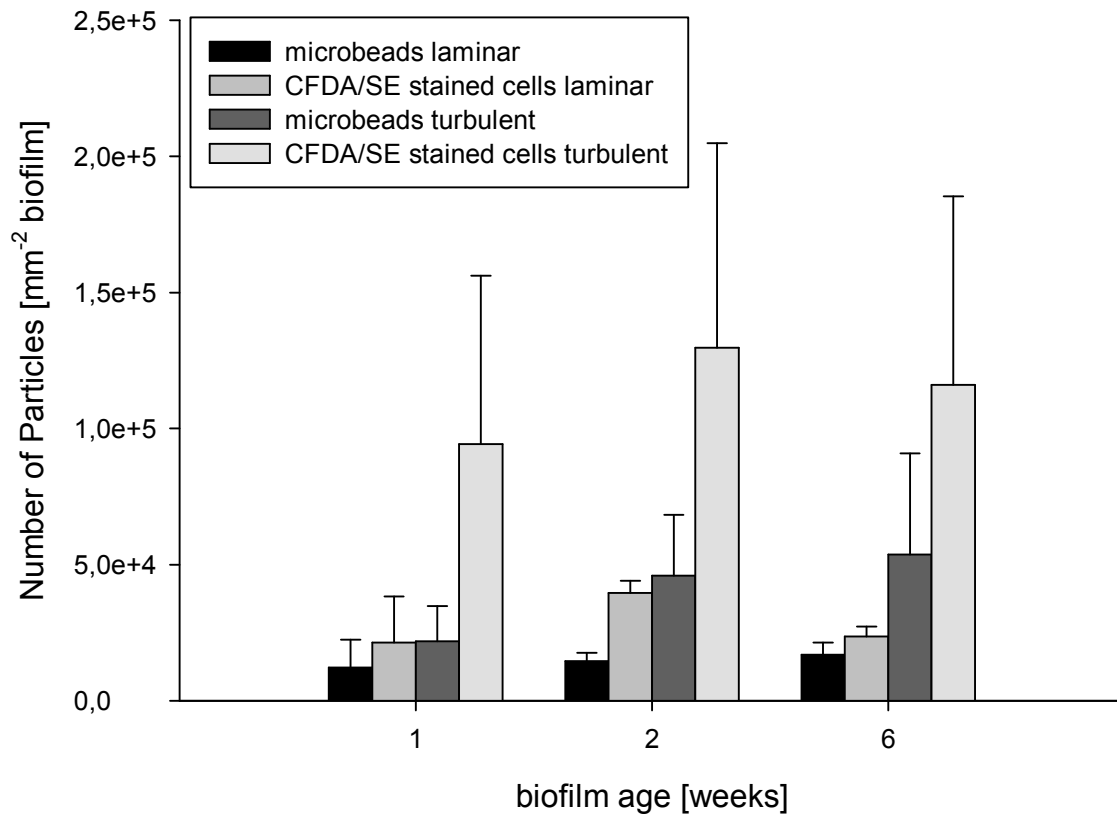


Figure 3.1: Abundance of microbeads and CFDA/SE-stained cells in biofilms differing in age and flow regime.

For statistics we used the ratio of CFDA/SE stained cells to microbeads found in the biofilms to facilitate direct comparison of treatments. Two-factorial ANOVA analysis revealed significant effects comparing biofilm age ($F(2,12)=12.11$, $p=.001$), flow treatments ($F(1,12)=26.89$, $p=.000$) and for the interaction of both ($F(2,12)=13.7$, $p=.001$).

The CFDA/SE-stained cells to microbeads ratio decreased over time, which is due to higher microbead abundance in older biofilms. Turbulent flow enhanced microbial deposition significantly in comparison to the laminar flow (Figure. 3.2). The ratio of deposited CFDA/SE stained cells to microbeads was not significantly different between 1- and 2- week old biofilms, but was significantly smaller in the 6-week old

biofilms (Tukey HSD $p=.001$). Table 1 shows results of the Tukey HSD test for interaction of the main effects

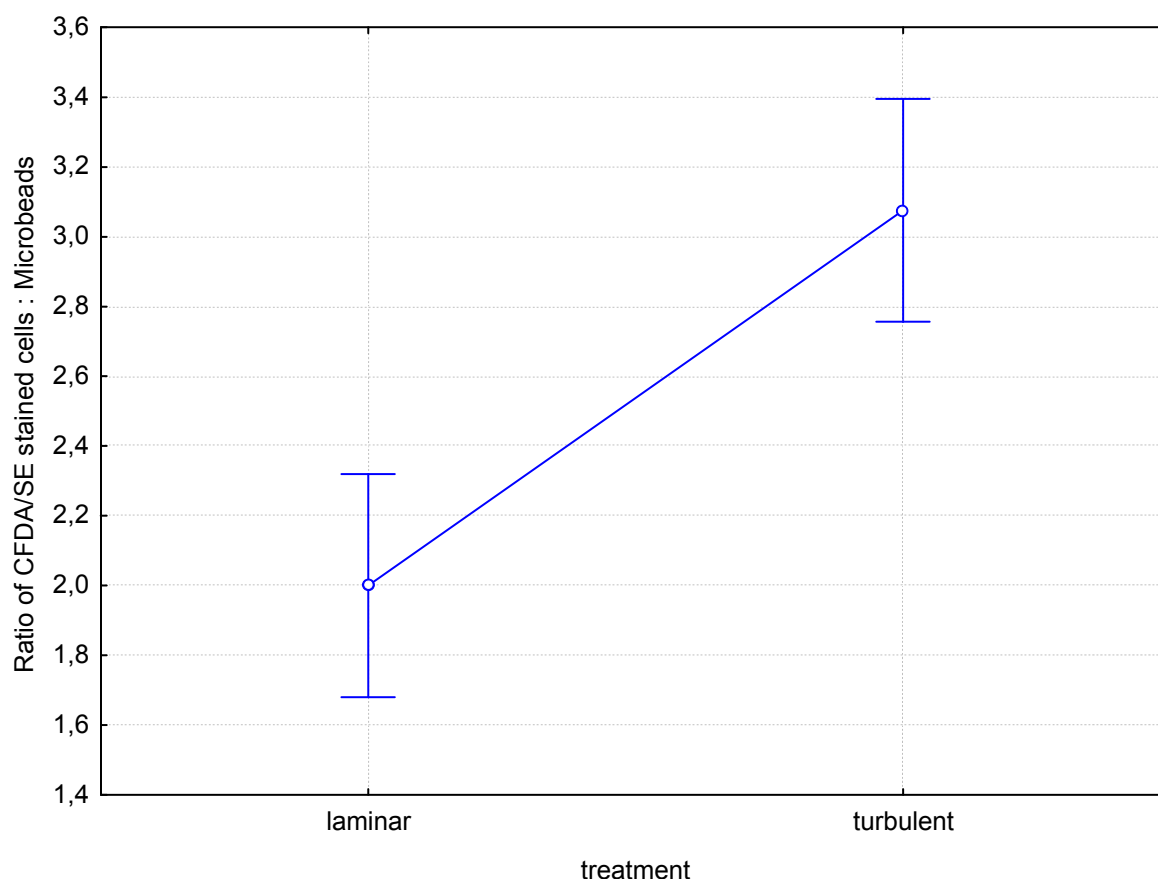


Figure 3.2 Two factorial ANOVA shows a significant difference ($F(1,12)=26,88$, $p=.000$) between flow regimes for the ratio of CFDA/SE stained cells to microbead abundances

Flow Treatment	Biofilm age	1.807	2.765	1.436	4.322	2.648	2.257
laminar	W1		0.160	0.899	0.000	0.251	0.804
laminar	W2	0.160		0.030	0.009	1.000	0.733
laminar	W6	0.899	0.030		0.000	0.049	0.272
turbulent	W1	0.000	0.009	0.000		0.006	0.001
turbulent	W2	0.251	1.000	0.049	0.006		0.877
turbulent	W6	0.804	0.733	0.272	0.001	0.877	

Table 1: Tukey HSD post-hoc test for two factorial ANOVA showing the interaction of treatment and biofilm age. Significant results are marked red.

(4) Aggregation of particles analyzed by comparing the mean size of cell clusters

Treatment	Particle type	Mean Cluster Size [μm]	Standard dev. [μm]	N
1WL_24h	microbeads	3.25	7.07	165
1WL_24h	CFDA/SE stained cells	1.38	1.73	384
1WL_36h	microbeads	3.98	10.34	131
1WL_36h	CFDA/SE stained cells	2.35	3.10	117
1WT_24h	microbeads	4.19	8.54	193
1WT_24h	CFDA/SE stained cells	2.17	3.20	266
1WT_36h	microbeads	4.7	11.51	401
1WT_36h	CFDA/SE stained cells	3.34	9.78	723
2WL_24h	microbeads	3.42	5.24	147
2WL_24h	CFDA/SE stained cells	2.12	4.49	512
2WL_36h	microbeads	2.5	2.47	195
2WL_36h	CFDA/SE stained cells	1.7	3.31	681
2WT_24h	microbeads	3.72	9.73	344
2WT_24h	CFDA/SE stained cells	3.37	10.84	637
2WT_36h	microbeads	5.58	16.80	545
2WT_36h	CFDA/SE stained cells	2.66	6.61	541

Table 2: Cluster sizes of particles found in the biofilms 24 hours and 36 hours after inoculum was injected to the flumes

Cluster size increased within 12 hours in the 1-week old biofilms independent of flow treatment. This trend reversed in the 2-week old biofilms under laminar conditions. In turbulent flow, CFDA/SE stained cell cluster size decreased while aggregation of microbeads did not or even increased. Turbulent flow increased cluster size of both microbeads and CFDA/SE-stained cells in all flow treatments. In general, CFDA/SE-stained cells were less aggregated than microbeads.

(5) Spatial analysis

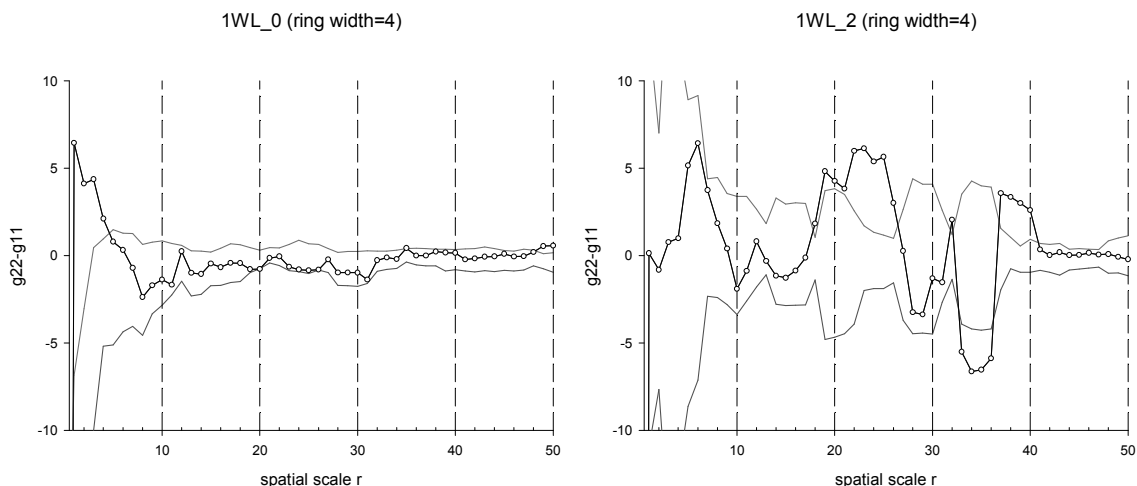
a. Comparison of the aggregation (or regularity) of the univariate component patterns

In the 1-week old biofilm, under laminar flow conditions, CFDA/SE-stained cells were more aggregated than microbeads within the first 10 μm after 24 hours of recirculation. Twelve hours later, CFDA/SE-stained cells showed an increased aggregation from 10 to 40 μm compared to microbeads.

Aggregation of microbial cells was observed from 0 to 10 μm and from 30 to 40 μm in the turbulent flow. Twelve hours later, CFDA/SE-stained cells were more clumped than microbeads over the entire range except for 10 to 20 μm where no significant difference between living and non-living particles could be observed.

In 2- week old biofilm from the laminar flow, microbeads clumped from 0 to 10 μm while CFDA/SE stained cells showed repulsion within the same range. More distant (20 to 40 μm) the opposite was found. No significant difference between the two population patterns (CFDA/SE-stained cells and microbeads) could be observed after 36 hours of recirculation.

CFDA/SE-stained cells showed repulsion in the 2- week old biofilms in turbulent flow as indicated by the univariate analysis. Compared to microbeads, CFDA/SE-stained cells were more aggregated within the first ten micrometers and from 30 to 50 μm . Samples collected 12 hours later showed aggregation of CFDA/SE stained cells from 0 to 20 μm , repulsion from 20 to 30 μm , and aggregation from 30 to 40 μm in reference to microbeads.



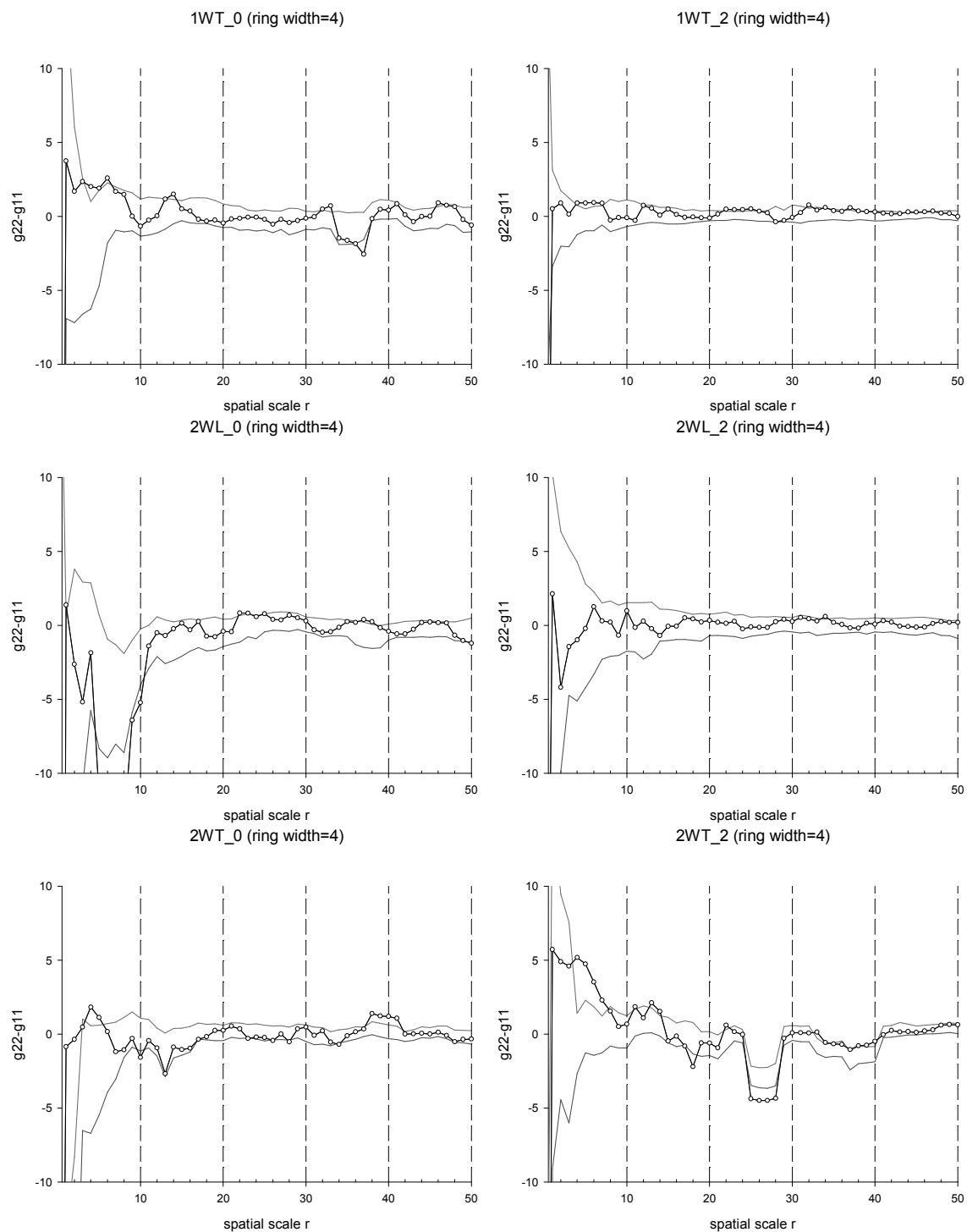
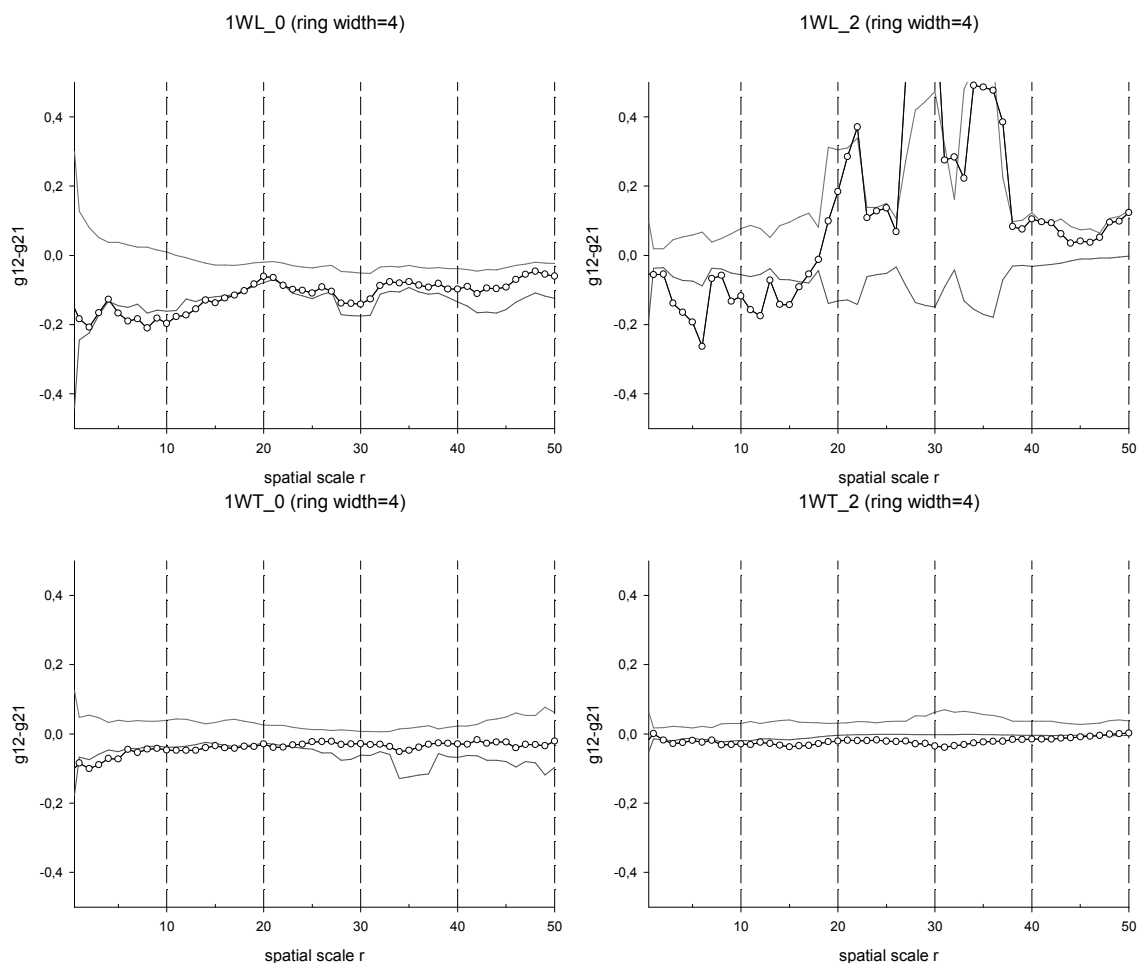


Figure 4.1 Comparisons of the univariate patterns reveal whether one pattern is more clustered (or less regular) than the other, conditional on the structure of the joined pattern. Outliers above the confidence interval indicate aggregation of CFDA/SE stained cells, below aggregation of microbeads.

b. Symmetry of the correlation of CFDA/SE stained cells and microbeads

Analysis of 1-week old biofilms revealed similar results for both treatments after 24 hours of recirculation. Microbeads were located in zones of high mutual particle abundance within the first 20 μm in comparison to CFDA/SE stained cells. Twelve hours later, microbeads in the turbulent flow displayed this effect over the entire range (0-50 μm), whereas the opposite was true for laminar conditions. Elevated abundances of CFDA/SE-stained cells were found from 20 to 50 μm though no changes within the first 20 μm were revealed.

2-week old biofilms from the laminar flow showed microbeads in areas of high joined pattern densities over the entire scale except for 10 to 20 μm where no significant differences were found. After 36 hours, CFDA/SE-stained cells were dominant in regions of high particle concentrations reaching from 10 to 30 μm . Pattern analysis of the turbulent flow treatment revealed microbeads within the joined pattern at 20 to 30 μm at 24 hours. Later (36 hours) microbeads ranging from 0 to 30 μm were located at the high particle density area.



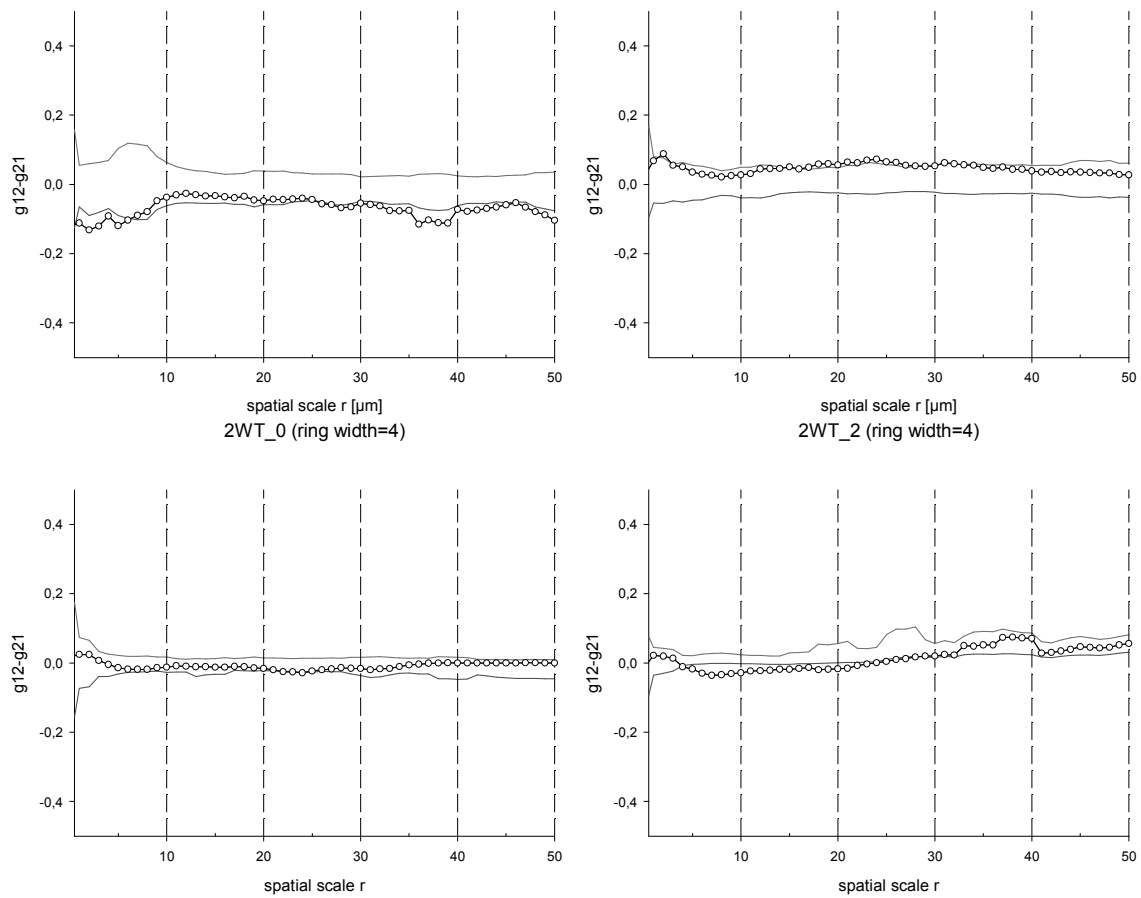


Figure 4.2.: A positive difference indicates that CFDA/SE stained cells have at distance r more neighbors (= stained cells and microbeads) than microbeads. Thus, CFDA/SE stained cells are mainly located in areas with higher intensity of the joined pattern whereas microbeads are mainly located in areas of lower intensity. Departure from random labeling depicted in this analysis indicates that the process that assigns the labels to the points interacts with the heterogeneity of the joined pattern.

V. Discussion

The immigration of suspended microbial cells into resident biofilms was investigated. The study examined the “filtration” abilities of stream biofilms, the spatial distribution of abiotic particles and microbes settled onto the biofilm, and displacement of microbes within the biofilm after “landing”. This study indicates that the dispersal of microbial cells and the colonization of stream biofilms are spatially heterogeneous and collectively controlled by hydrodynamics, biofilm structure and possible biotic interactions between dispersers and resident biofilms. The transport of dispersers to the biofilms seems to be primarily affected by the interplay between hydrodynamics and biofilm topography which changes with biofilm age.

(1) Removal, Adhesion or Filtration?

Microbeads used in this study act as conservative tracer particles; their transportation behavior is driven by physical processes alone. If physical processes govern landing sites for cells and beads in a similar way, but the rate at which cells and beads reach these areas differs, then this suggests that another, non-physical process is involved. Minshall *et al.* (2000) pointed out that adhesion or other forms of filtration, rather than gravitation, may be the primary mode of immobilization once seston particles are delivered to the benthic zone. We were able to show that microbial and abiotic particle transport display different settling rates. Flow velocity is a major factor contributing to settling rates since turbulent flow enhances settling probability (Packman *et al.* 2002). (McNair *et al.* 1997) suggested that turbulence could be viewed as assisting small particles of near-neutral buoyancy in reaching the bottom, but hindering particles with substantially greater fall velocity. This effect was reproduced in the study for microbeads especially for the two week old biofilms but suspended microbial cells wouldn't follow this trend (Figure 2.1). Significant fractions (35 to 60% of the initial concentration for laminar and turbulent flow) of the CFDA/SE-cells remained suspended after 24 hours, which are indicative of their dispersal kernels under different flow conditions. Almost all microbeads were removed from the water during that same travel length. It can not be excluded that differences in deposition between microbes and non-living particles in the experiment are due to motility and chemotaxis capabilities, reduced sinking rates of small planktonic

organisms owing to EPS or simply different drag forces. Bacteria are small enough that Brownian motion plays an important role in dispersal. Shape influences the rate of sinking, and some bacteria may adopt shapes that minimize sinking rate, whereas spherical shapes (microbeads) have the largest diffusion coefficient for any given volume (Dusenbery 1998). Images of the inoculum revealed rod shaped microorganisms, certainly emphasizing the contribution of different diffusion coefficients. Tumbling, a behavior frequently found in microorganisms, reduces the efficiency of encountering resource patches (Kiorboe *et al.* 2002). We cannot exclude biotic interactions mediated by cell-to-cell signaling. It was, in fact, hypothesized that cell-to-cell signaling could help invading cells to differentiate between “self” and “non-self” (i.e., resident cells) and therefore influence the invasibility of resident biofilms (Battin *et al.* 2007). Indeed there seems to be interactions between the CFDA/SE-stained cells in the bulk water and the microbial community in the biofilm. Bacterial growth in streams can increase particle deposition velocity through enhanced filtration and adhesion (Battin *et al.* 2001). The contribution of biofilm structure on settling rate relies on: modifying the hydrodynamic transport processes (Packman *et al.* 2002) the inherent ‘stickiness’ and capacity for absorption of particles of EPS (Wotton 2004) as well as offering preferred landing sites on canopy structures such as mushroom caps or streamers that are exposed to the bulk liquid flow (Battin *et al.* 2007). This study shows that biofilm age, which clearly altered biofilm characteristics (Figure 1), affect the deposition of particles.

The perfect sink model has been shown to be useful for comparison with actual decrease rates. Deviations from the null model, which applies to flat and homogenous surfaces, will be due to the surface characteristics of the biofilms and the activity of the suspended microorganisms.

The fast initial decrease of microbeads exceeds the model predictions, especially in the one week old biofilms (Figure 2.3 a). This indicates high uptake capabilities of nascent biofilms. The perfect sink model underestimates the initial decrease rates of microbeads, particularly in the laminar flow. The suspended microbial cells in turbulent flow displayed decrease rates differing from the microbeads. The difference for CFDA/SE-stained cells at different flow velocities may be influenced by biological interactions of the disperser cells and the biofilm.

(2) Settling

Once microbes settle on the biofilm matrix attachment processes take place. A regulated developmental sequence of stages has been identified for some species (Kaiser 2007; Stoodley *et al.* 2002). EPS production glues the cell to the surface. Every new organism that binds to the linking film presents a new surface and therefore forms a basis for the accretion of defined organism groupings (Jenkinson and Lappin-Scott 2001). The capacity of biofilms to take up microbial cells in comparison to microbeads decreased with biofilm age in turbulent flow. Nascent biofilms still building up biomass might offer more favorable spots and are less resistant to invaders. Invading species select favorable habitats; on the other hand it has been shown that microbial communities can rebuff invaders (Upper *et al.* 2003; Woody *et al.* 2007). Antibiotic activity seems widespread among freshwater microalgae (Kellam *et al.* 1988) and bacteria (Be'er *et al.* 2009; Narisawa *et al.* 2008). My results suggest that the “landing” sites for disperser cells on the resident biofilms are influenced by the interplay of hydrodynamics and surface topography. For instance, roughness elements can induce small turbulences even under laminar flow, and such wake-induced turbulence could enhance settling of near-buoyant immobile particles (McNair *et al.* 1997) in these areas. Microbeads become trapped in older biofilms with higher biomass in greater extend, which could be due to increased surface roughness.

(3) Spatial distribution

Spatial distribution patterns not only determine the potential area of recruitment, but also serve as a template for subsequent processes, such as competition and mating (Nathan and Muller-Landau 2000). Different dispersal patterns have been described (Legendre and Legendre 1998; May 1984) using the environmental control model, where environmental variables are responsible for the observed variations in the presence or abundance of the organisms (Bray and Curtis 1957; Whittaker 1956) and the biotic control model, where the links among organisms, are considered to be the primary factors structuring communities (Southwood 1987). A different approach to the understanding of biological phenomena by viewing alternative causes in the non-mutually-exclusive sense, that is, in terms of the relative contribution of each alternative has been proposed (Borcard *et al.* 1992).

Differences in the distribution of both patterns could be described by the behavior of suspended cells. Clumped patterns within the first 10 μm of scale might be due to previous aggregation. Aggregation between aquatic bacteria has been reported (Rickard *et al.* 2002). Only a few adhering, sessile microorganisms can stimulate the adhesion of other, still suspended planktonic microorganisms. This may occur by sessile microorganisms slowing down an approaching, planktonic microorganism, thus increasing its chance of adhering to the substratum surface, as is frequently observed under flow (Busscher *et al.* 1991; Dabros 1989) or through strong attractive interactions between sessile and planktonic microorganisms, a phenomenon known as 'co-adhesion' (Bos *et al.* 1999; Bos *et al.* 1995; Liljemark *et al.* 1988).

Microbeads and microbial cells were distributed non-randomly. Disperser cells seemed to move in a non-random fashion in the biofilm, as they generally aggregated more strongly than microbeads. This pattern may be attributable to the similar environmental demands of the dispersers. Flow and topography determine the "landing" sites of the dispersers irrespective of whether these are favorable or not to the dispersers. Therefore, the ability to actively leave these sites towards favorable spots is an advantage during this stage of biofilm colonization. The comparison of spatial patterns found 24 hours and 36 hours after injection indicate cell movement after hitting the bottom. Microbes would move away from areas of high particle densities, which possibly representing boundary regions. We were able to show that the invading species were moving closer together.

Previous studies have shown that examining microscale spatial structure and transport in natural environments may be essential to understand how communities of microbes interact (Price-Whelan *et al.* 2006) and perform community-level functions in natural ecosystems (Garland and Mills 1991) and how species diversity of microbial communities is maintained on the microscale (Kim *et al.* 2008).

VI. Synopsis

In nature, microbial dispersal takes place over highly heterogeneous streambeds. Three distinct biofilm dispersal strategies have been identified: 'swarming/seeding dispersal', in which individual cells are released from a microcolony into the bulk fluid or the surrounding substratum; 'clumping dispersal', in which aggregates of cells are shed as clumps; and 'surface dispersal', in which biofilm structures move across surfaces (Hall-Stoodley *et al.* 2004). Local and temporal variations in flow can induce detachment of biofilms. These in-stream processes continuously contribute to the pool of microorganisms transported in the stream water, which is further supplemented by soil (Hullar *et al.* 2006) and wastewater (Byappanahalli *et al.* 2003) microbial communities. The implication of the spatial analysis suggests that once settled microbial cells start to create a pattern by selecting a favorable environment. Despite its model character, this study on micro-scale controls on dispersal and colonization may provide mechanistic clues to the propagation and fate of suspended microorganisms in streams.

VII. Literature

Abramoff, M. D., P. J. Magelhaes, and S. J. Ram. 2004. Image Processing with Imagej. *Biophotonics International* **11**: 36-42.

An, Y. H., and R. J. Friedman. 1998. Concise Review of Mechanisms of Bacterial Adhesion to Biomaterial Surfaces. *Journal of Biomedical Materials Research* **43**: 338-348.

Bailey, T. C., and A. C. Gatrell. 1995. *Interactive Spatial Data Analysis*. Longman.

Battin, T. J., L. A. Kaplan, D. J. Newbold, and C. M. Hansen. 2003a. Contributions of Microbial Biofilms to Ecosystem Processes in Stream Mesocosms. *Nature* **426**: 439-442.

Battin, T. J., L. A. Kaplan, J. D. Newbold, X. Cheng, and C. Hansen. 2003b. Effects of Current Velocity on the Nascent Architecture of Stream Microbial Biofilms. *Appl. Environ. Microbiol.* **69**: 5443-5452.

Battin, T. J., W. T. Sloan, S. Kjelleberg, H. Daims, I. M. Head, T. P. Curtis, and L. Eberl. 2007. Microbial Landscapes: New Paths to Biofilm Research. *Nat Rev Micro* **5**: 76.

Battin, T. J., A. Wille, B. Sattler, and R. Psenner. 2001. Phylogenetic and Functional Heterogeneity of Sediment Biofilms Along Environmental Gradients in a Glacial Stream. *Appl. Environ. Microbiol.* **67**: 799-807.

Be'er, A., H. P. Zhang, E. L. Florin, S. M. Payne, E. Ben-Jacob, and H. L. Swinney. 2009. Deadly Competition between Sibling Bacterial Colonies. *Proceedings of the National Academy of Sciences* **106**: 428-433.

Besemer, K., G. Singer, R. Limberger, A.-K. Chlup, G. Hochedlinger, I. Hödl, C. Baranyi, and T. J. Battin. 2007. Biophysical Controls on Community Succession in Stream Biofilms. *Appl. Environ. Microbiol.* **73**: 4966-4974.

- Borcard, D., P. Legendre, and P. Drapeau. 1992. Partialling out the Spatial Component of Ecological Variation. *Ecology* **73**: 1045-1055.
- Bos, R., H. C. van der Mei, and H. J. Busscher. 1995. A Quantitative Method to Study Co-Adhesion of Microorganisms in a Parallel Plate Flow Chamber. II: Analysis of the Kinetics of Co-Adhesion. *Journal of Microbiological Methods* **23**: 169-182.
- Bos, R., H. C. van der Mei, and H. J. Busscher. 1999. Physico-Chemistry of Initial Microbial Adhesive Interactions--Its Mechanisms and Methods for Study. *FEMS Microbiol Rev* **23**: 179-230.
- Bray, J. R., and J. T. Curtis. 1957. An Ordination of the Upland Forest Communities of Southern Wisconsin. *Ecological Monographs* **27**: 325-349.
- Busscher, H. J., J. Noordmans, J. Meinders, and H. C. van der Mei. 1991. Analysis of the Spatial Arrangement of Microorganisms Adhering to Solid Surfaces - Methods of Presenting Results. *Biofouling* **4**: 71 - 79.
- Byappanahalli, M., M. Fowler, D. Shively, and R. Whitman. 2003. Ubiquity and Persistence of *Escherichia coli* in a Midwestern Coastal Stream. *Appl. Environ. Microbiol.* **69**: 4549-4555.
- Characklis, W. G. 1990. *Biofilms*. Wiley.
- Costerton, J. W., K. J. Cheng, G. G. Geesey, T. I. Ladd, J. C. Nickel, M. Dasgupta, and T. J. Marrie. 1987. Bacterial Biofilms in Nature and Disease. *Annual Review of Microbiology* **41**: 435-464.
- Dabros, T. 1989. Interparticle Hydrodynamic Interactions in Deposition Processes. *Colloids and Surfaces* **39**: 127-141.
- Dawkins, R. 1976. *The Selfish Gene*. Oxford University Press.
- de Carvalho, C. C. C. R. 2007. Biofilms: Recent Developments on an Old Battle. *Recent Patents on Biotechnology* **1**: 49-57.

- Denny, M. W., and M. F. Shibata. 1989. Consequences of Surf-Zone Turbulence for Settlement and External Fertilization. *The American Naturalist* **134**: 859.
- Donlan, R. M. 2002. Biofilms: Microbial Life on Surfaces. *Emerg Infect Dis* **8**: 881-890.
- Drury, W. J., P. S. Stewart, and W. G. Characklis. 1993. Transport of 1-Mum Latex Particles in *Pseudomonas aeruginosa* Biofilms. *Biotechnology and Bioengineering* **42**: 111-117.
- Dunne, W. M., Jr. 2002. Bacterial Adhesion: Seen Any Good Biofilms Lately? *Clin. Microbiol. Rev.* **15**: 155-166.
- Dusenbery, D. B. 1998. Fitness Landscapes for Effects of Shape on Chemotaxis and Other Behaviors of Bacteria. *J. Bacteriol.* **180**: 5978-5983.
- Fenchel, T. 2002. Microbial Behavior in a Heterogeneous World. *Science* **296**: 1068-1071.
- Flood, J. A., Ashbolt, N.J. 2000. Virus-Sized Particles Can Be Entrapped and Concentrated One Hundred Fold within Wetland Biofilms. *Adv. Environ. Res.* **3**: 403-411.
- Fonseca, D. M., and D. D. Hart. 2001. Colonization History Masks Habitat Preferences in Local Distributions of Stream Insects. *Ecology* **82**: 2897-2910.
- Fuller, M. E., S. H. Streger, R. K. Rothmel, B. J. Mailloux, J. A. Hall, T. C. Onstott, J. K. Fredrickson, D. L. Balkwill, and M. F. DeFlaun. 2000. Development of a Vital Fluorescent Staining Method for Monitoring Bacterial Transport in Subsurface Environments. *Appl. Environ. Microbiol.* **66**: 4486-4496.
- Garland, J. L., and A. L. Mills. 1991. Classification and Characterization of Heterotrophic Microbial Communities on the Basis of Patterns of Community-Level Sole-Carbon-Source Utilization. *Appl. Environ. Microbiol.* **57**: 2351-2359.

- Gatrell, A. C., T. C. Bailey, P. J. Diggle, and B. S. Rowlingson. 1996. Spatial Point Pattern Analysis and Its Application in Geographical Epidemiology. *Transactions of the Institute of British Geographers* **21**: 256-274.
- Gottenbos, B., H. J. Busscher, H. C. van der Mei, and P. Nieuwenhuis. 2002. Pathogenesis and Prevention of Biomaterial Centered Infections. *Journal of Materials Science: Materials in Medicine* **13**: 717-722.
- Green, J. L., A. J. Holmes, M. Westoby, I. Oliver, D. Briscoe, M. Dangerfield, M. Gillings, and A. J. Beattie. 2004. Spatial Scaling of Microbial Eukaryote Diversity. *Nature* **432**: 747-750.
- Haase, P. 1995. Spatial Pattern Analysis in Ecology Based on Ripley's K-Function: Introduction and Methods of Edge Correction. *Journal of Vegetation Science* **6**: 575-582.
- Hall-Stoodley, L., J. W. Costerton, and P. Stoodley. 2004. Bacterial Biofilms: From the Natural Environment to Infectious Diseases. *Nat Rev Microbiol* **2**: 95-108.
- Hall-Stoodley, L., and P. Stoodley. 2005. Biofilm Formation and Dispersal and the Transmission of Human Pathogens. *Trends in Microbiology* **13**: 7-10.
- Hall, R. O. J., Peredney C. L., and Meyer J. L. 1996. The Effect of Invertebrate Consumption on Bacterial Transport in a Mountain Stream. *Limnology and Oceanography* **41**: 1180-1187.
- Hanski, I. 1998. Metapopulation Dynamics. *Nature* **396**: 41-49.
- Harshey, R. M. 2003. Bacterial Motility on a Surface: Many Ways to a Common Goal. *Annual Review of Microbiology* **57**: 249-273.
- Hassell, M. P., H. N. Comins, and R. M. Mayt. 1991. Spatial Structure and Chaos in Insect Population Dynamics. *Nature* **353**: 255-258.
- Hermansson, M. 1999. The DLVO Theory in Microbial Adhesion. *Colloids and Surfaces B: Biointerfaces* **14**: 105-119.

- Hullar, M. A. J., L. A. Kaplan, and D. A. Stahl. 2006. Recurring Seasonal Dynamics of Microbial Communities in Stream Habitats. *Appl. Environ. Microbiol.* **72**: 713-722.
- Jackson, C. R., P. F. Churchill, and E. E. Roden. 2001. Successional Changes in Bacterial Assemblage Structure During Epilithic Biofilm Development. *Ecology* **82**: 555-566.
- Jenkinson, H. F., and H. M. Lappin-Scott. 2001. Biofilms Adhere to Stay. *Trends in Microbiology* **9**: 9-10.
- Kadouri, D., and G. A. O'Toole. 2005. Susceptibility of Biofilms to *Bdellovibrio Bacteriovorus* Attack. *Appl. Environ. Microbiol.* **71**: 4044-4051.
- Kaiser, D. 2007. Bacterial Swarming: A Re-Examination of Cell-Movement Patterns. *Current Biology* **17**: R561-R570.
- Kaplan, J. B., and D. H. Fine. 2002. Biofilm Dispersal of *Neisseria subflava* and Other Phylogenetically Diverse Oral Bacteria. *Appl. Environ. Microbiol.* **68**: 4943-4950.
- Katsikogianni, M., and Y. F. Missirlis. 2004. Concise Review of Mechanisms of Bacterial Adhesion to Biomaterials and of Techniques Used in Estimating Bacteria-Material Interactions. *Eur Cell Mater* **8**: 37-57.
- Kellam, S. J., R. J. P. Cannell, A. M. Owsianka, and J. M. Walker. 1988. Results of a Large-Scale Screening Programme to Detect Antifungal Activity from Marine and Freshwater Microalgae in Laboratory Culture. *European Journal of Phycology* **23**: 45 - 47.
- Kim, H. J., J. Q. Boedicker, J. W. Choi, and R. F. Ismagilov. 2008. Defined Spatial Structure Stabilizes a Synthetic Multispecies Bacterial Community. *Proceedings of the National Academy of Sciences* **105**: 18188-18193.

- Kiorboe, T., H.-P. Grossart, H. Ploug, and K. Tang. 2002. Mechanisms and Rates of Bacterial Colonization of Sinking Aggregates. *Appl. Environ. Microbiol.* **68**: 3996-4006.
- Kirov, S. M. 2003. Bacteria That Express Lateral Flagella Enable Dissection of the Multifunctional Roles of Flagella in Pathogenesis. *FEMS Microbiology Letters* **224**: 151-159.
- Kjelleberg, S., and S. Molin. 2002. Is There a Role for Quorum Sensing Signals in Bacterial Biofilms? *Current Opinion in Microbiology* **5**: 254-258.
- Kolenbrander, P. E., R. N. Andersen, D. S. Blehert, P. G. Egland, J. S. Foster, and R. J. Palmer, Jr. 2002. Communication among Oral Bacteria. *Microbiol. Mol. Biol. Rev.* **66**: 486-505.
- Kolter, R., and E. P. Greenberg. 2006. Microbial Sciences: The Superficial Life of Microbes. *Nature* **441**: 300-302.
- Kondratieff, P., and G. Simmons. 1985. Microbial Colonization of Seston and Free Bacteria in an Impounded River. *Hydrobiologia* **128**: 127-133.
- Kreft, J.-U. 2004. Biofilms Promote Altruism. *Microbiology* **150**: 2751-2760.
- Lawrence, J. R., D. R. Korber, B. D. Hoyle, J. W. Costerton, and D. E. Caldwell. 1991. Optical Sectioning of Microbial Biofilms. *J. Bacteriol.* **173**: 6558-6567.
- Lawton, J. H. 1999. Are There General Laws in Ecology? *Oikos* **84**: 177-192.
- Leff, L. G. 2000. Longitudinal Changes in Microbial Assemblages of the Ogeechee River. *Freshwater Biology* **43**: 605-615.
- Legendre, P., and L. Legendre. 1998. *Numerical Ecology*. Elsevier.
- Leopold, L. B., M. G. Wolman, and J. P. Miller. 1964. *Fluvial Processes in Geomorphology*. Freeman.

- Liljemark, W. F., C. G. Bloomquist, M. C. Coulter, L. J. Fenner, R. J. Skopek, and C. F. Schachtele. 1988. Utilization of a Continuous Streptococcal Surface to Measure Interbacterial Adherence in Vitro and in Vivo. *J Dent Res* **67**: 1455-1460.
- Lindqvist, R., J. S. Cho, and C. G. Enfield. 1994. A Kinetic Model for Cell Density Dependent Bacterial Transport in Porous Media. *Water Resour. Res.* **30**: 3291–3299.
- Lock, M. A. 1994. Dynamics of Particulate and Dissolved Organic Matter over the Substratum of Water Bodies. Lewis Publishers.
- Mack, D. 1999. Molecular Mechanisms of *Staphylococcus epidermidis* Biofilm Formation. *Journal of Hospital Infection* **43**: S113-S125.
- Marsh, P. D., and G. H. W. Bowden. 2000. Microbial Community Interactions in Biofilms, Society for General Microbiology: Symposia of the Society for General Microbiology. 1949 - ed. Cambridge Univ. Press.
- May, R. M. 1984. An Overview: Real and Apparent Patterns in Community Structure. Princeton University Press.
- Mayer, C., R. Moritz, C. Kirschner, W. Borchard, R. Maibaum, J. Wingender, and H.-C. Flemming. 1999. The Role of Intermolecular Interactions: Studies on Model Systems for Bacterial Biofilms. *International Journal of Biological Macromolecules* **26**: 3-16.
- McBain, A. J., D. G. Allison, and P. Gilbert. 2000. Population Dynamics in Microbial Biofilms. Cambridge Univ. Press.
- McNair, J. N., J. D. Newbold, and D. D. Hart. 1997. Turbulent Transport of Suspended Particles and Dispersing Benthic Organisms: How Long to Hit Bottom? *Journal of Theoretical Biology* **188**: 29.
- Miller, M. B., and B. L. Bassler. 2001. Quorum Sensing in Bacteria. *Annual Review of Microbiology* **55**: 165.

- Minshall, G. W., S. A. Thomas, J. D. Newbold, M. T. Monaghan, and C. E. Cushing. 2000. Physical Factors Influencing Fine Organic Particle Transport and Deposition in Streams. *Journal of the North American Benthological Society* **19**: 1-16.
- Narisawa, N., S. Haruta, H. Arai, M. Ishii, and Y. Igarashi. 2008. Coexistence of Antibiotic-Producing and Antibiotic-Sensitive Bacteria in Biofilms Is Mediated by Resistant Bacteria. *Appl. Environ. Microbiol.* **74**: 3887-3894.
- Nathan, R., and H. Muller-Landau. 2000. Spatial Patterns of Seed Dispersal, Their Determinants and Consequences for Recruitment. *Trends in Ecology & Evolution* **15**: 278-285.
- O'Gara, J. P., and H. Humphreys. 2001. *Staphylococcus epidermidis* Biofilms: Importance and Implications. *J Med Microbiol* **50**: 582-587.
- Packman, A. I., T. J. Battin, and J. D. Newbold. 2002. Challenges in Ecohydraulics: Biophysicochemical Processes at the Stream-Subsurface Interface. *Proceedings of the 5th International Conference on Hydroscience and Engineering*.
- Palmer, M. A., J. D. Allan, and C. A. Butman. 1996. Dispersal as a Regional Process Affecting the Local Dynamics of Marine and Stream Benthic Invertebrates. *Trends in Ecology & Evolution* **11**: 322-326.
- Pickett, S. T. A., and P. S. White. 1985. *The Ecology of Natural Disturbance and Patch Dynamics*. Academic Press.
- Pratt, L. A., and R. Kolter. 1998. Genetic Analysis of *Escherichia coli* Biofilm Formation: Roles of Flagella, Motility, Chemotaxis and Type I Pili. *Molecular Microbiology* **30**: 285-293.
- Price-Whelan, A., L. E. P. Dietrich, and D. K. Newman. 2006. Rethinking 'Secondary' Metabolism: Physiological Roles for Phenazine Antibiotics. *Nat Chem Biol* **2**: 71-78.

- Purevdorj, B., J. W. Costerton, and P. Stoodley. 2002. Influence of Hydrodynamics and Cell Signaling on the Structure and Behavior of *Pseudomonas aeruginosa* Biofilms. *Appl. Environ. Microbiol.* **68**: 4457-4464.
- Rickard, A. H., S. A. Leach, L. S. Hall, C. M. Buswell, N. J. High, and P. S. Handley. 2002. Phylogenetic Relationships and Coaggregation Ability of Freshwater Biofilm Bacteria. *Appl. Environ. Microbiol.* **68**: 3644-3650.
- Rickard, A. H., A. J. McBain, A. T. Stead, and P. Gilbert. 2004. Shear Rate Moderates Community Diversity in Freshwater Biofilms. *Appl. Environ. Microbiol.* **70**: 7426-7435.
- Rijnaarts, H. H. M., W. Norde, E. J. Bouwer, J. Lyklema, and A. J. B. Zehnder. 1995. Reversibility and Mechanism of Bacterial Adhesion. *Colloids and Surfaces B: Biointerfaces* **4**: 5-22.
- Ripley, B. D. 1981. *Spatial Statistics*. Wiley.
- Roughgarden, J., S. D. Gaines, and S. W. Pacala 1987. *Supply Side Ecology: The Role of Physical Transport Processes*. Blackwell.
- Schlesinger, W. H. M., John M. 1981. Transport of Organic Carbon in the World's Rivers. *Tellus* **33**: 172-181.
- Searcy, K. E., A. I. Packman, E. R. Atwill, and T. Harter. 2006. Capture and Retention of *Cryptosporidium parvum* Oocysts by *Pseudomonas aeruginosa* Biofilms. *Appl. Environ. Microbiol.* **72**: 6242-6247.
- Singer, G., K. Besemer, I. Hödl, A. K. Chlup, G. Hochedlinger, P. Stadler, and T. J. Battin. 2006. Microcosm Design and Evaluation to Study Stream Microbial Biofilms. *Limnol. Oceanogr. Methods* **4**: 436-447.
- Sloan, W. T., M. Lunn, S. Woodcock, I. M. Head, S. Nee, and T. P. Curtis. 2006. Quantifying the Roles of Immigration and Chance in Shaping Prokaryote Community Structure. *Environmental Microbiology* **8**: 732-740.

- Southwood, T. R. E. 1987. The Concept and Nature of the Community. Blackwell Scientific.
- Stoodley, P., J. D. Boyle, I. Doods, and H. M. Lappin-Scott. 1997. Consensus Model of Biofilm Structure.
- Stoodley, P., Z. Lewandowski, J. D. Boyle, and H. M. Lappin-Scott. 1999. The Formation of Migratory Ripples in a Mixed Species Bacterial Biofilm Growing in Turbulent Flow. *Environ Microbiol* **1**: 447-455.
- Stoodley, P., K. Sauer, D. G. Davies, and J. W. Costerton. 2002. Biofilms as Complex Differentiated Communities. *Annu Rev Microbiol* **56**: 187-209.
- Stoyan, D., and H. Stoyan. 1994. Fractals, Random Shapes and Point Fields Methods of Geometrical Statistics. Wiley.
- Sutherland, I. W. 2001. Biofilm Exopolysaccharides: A Strong and Sticky Framework. *Microbiology* **147**: 3-9.
- Thomas, S. A., J. D. Newbold, M. T. Monaghan, G. W. Minshall, T. Georgian, and C. E. Cushing. 2001. The Influence of Particle Size on Seston Deposition in Streams. *Limnology and Oceanography* **46**: 1415–1424.
- Tolker-Nielsen, T., and S. Molin. 2000. Spatial Organization of Microbial Biofilm Communities. *Microb Ecol* **40**: 75-84.
- Turner, M. G. 1989. Landscape Ecology: The Effect of Pattern on Process. *Annual Review of Ecology and Systematics* **20**: 171-197.
- Turner, M. G. 2005. Landscape Ecology: What Is the State of the Science? *Annual Review of Ecology, Evolution, and Systematics* **36**: 319-344.
- Upper, C. D., S. S. Hirano, K. K. Dodd, and M. K. Clayton. 2003. Factors That Affect Spread of *Pseudomonas syringae* in the Phyllosphere. *Phytopathology* **93**: 1082-1092.

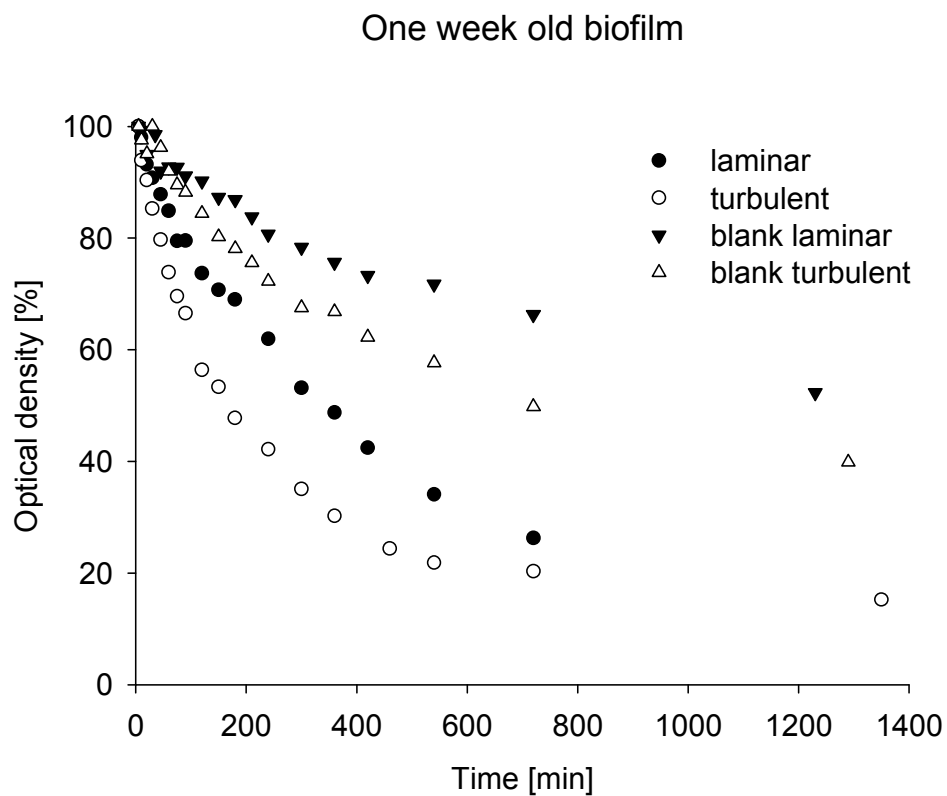
- Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**: 130-137.
- Watnick, P., and R. Kolter. 2000. Biofilm, City of Microbes. *J. Bacteriol.* **182**: 2675-2679.
- Whittaker, R. H. 1956. Vegetation of the Great Smoky Mountains. *Ecological Monographs* **26**: 1-80.
- Wiegand, T. 2004. Introduction to Point Pattern Analysis with Ripley's L and the O-Ring Statistic Using the Programita Software. Department of Ecological Modelling, UFZ-Centre for Environmental Research.
- Wiegand, T., and K. A. Moloney. 2004. Rings, Circles, and Null-Models for Point Pattern Analysis in Ecology. *Oikos* **104**: 209-229.
- Wiens, J. A. 1989. Spatial Scaling in Ecology. *Functional Ecology* **3**: 385-397.
- Woody, S. T., A. R. Ives, E. V. Nordheim, and J. H. Andrews. 2007. Dispersal, Density Dependence, and Population Dynamics of a Fungal Microbe on Leaf Surfaces. *Ecology* **88**: 1513-1524.
- Wotton, R. S. 2004. The Ubiquity and Many Roles of Exopolymers (EPS) in Aquatic Systems. *Scientia Marina* **68**: 13-21.
- Xu, K. D., P. S. Stewart, F. Xia, C.-T. Huang, and G. A. McFeters. 1998. Spatial Physiological Heterogeneity in *Pseudomonas aeruginosa* Biofilm Is Determined by Oxygen Availability. *Appl. Environ. Microbiol.* **64**: 4035-4039.
- Zobell, C. E. 1943. The Effect of Solid Surfaces Upon Bacterial Activity. *J. Bacteriol.* **46**: 39-56.

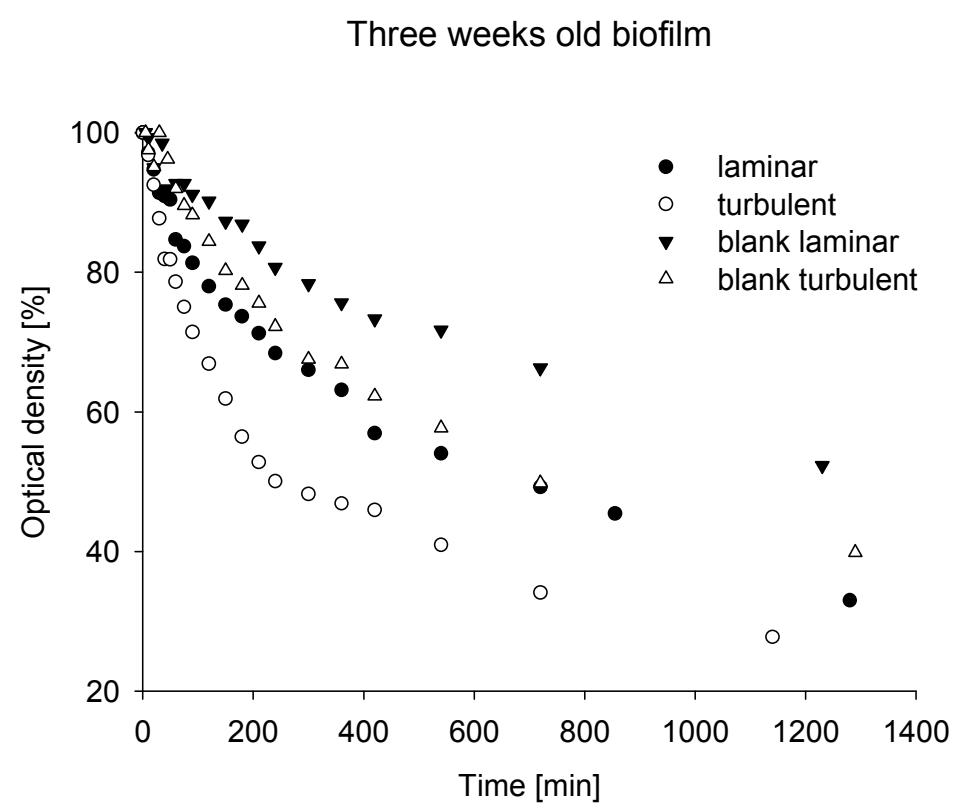
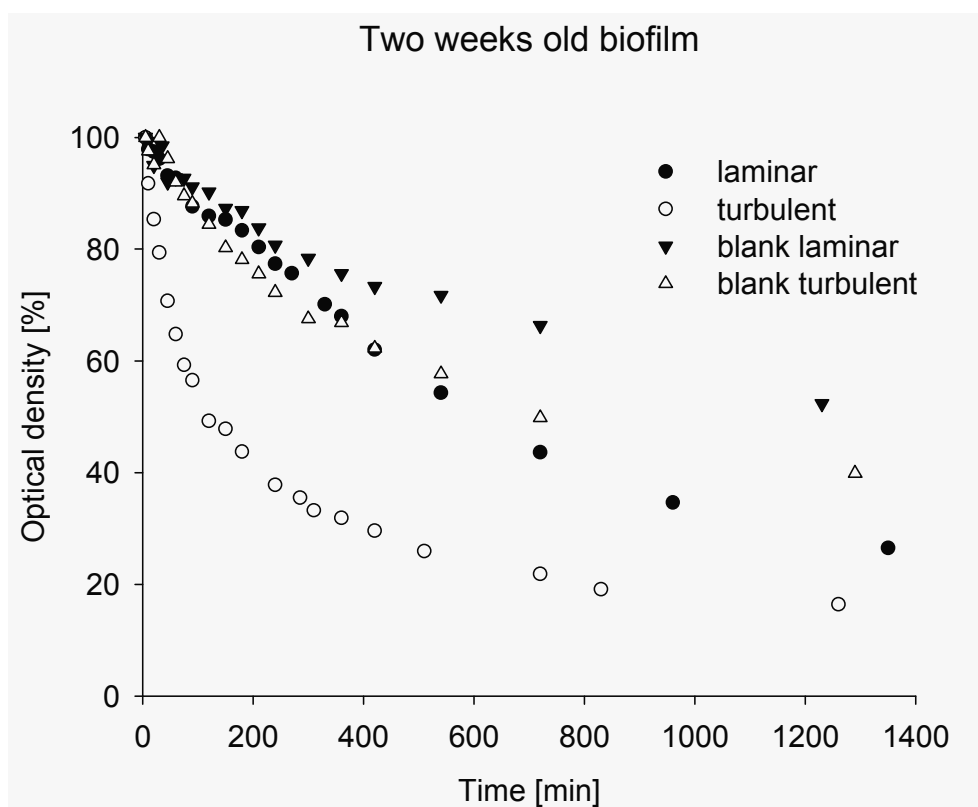
VIII. APPENDIX

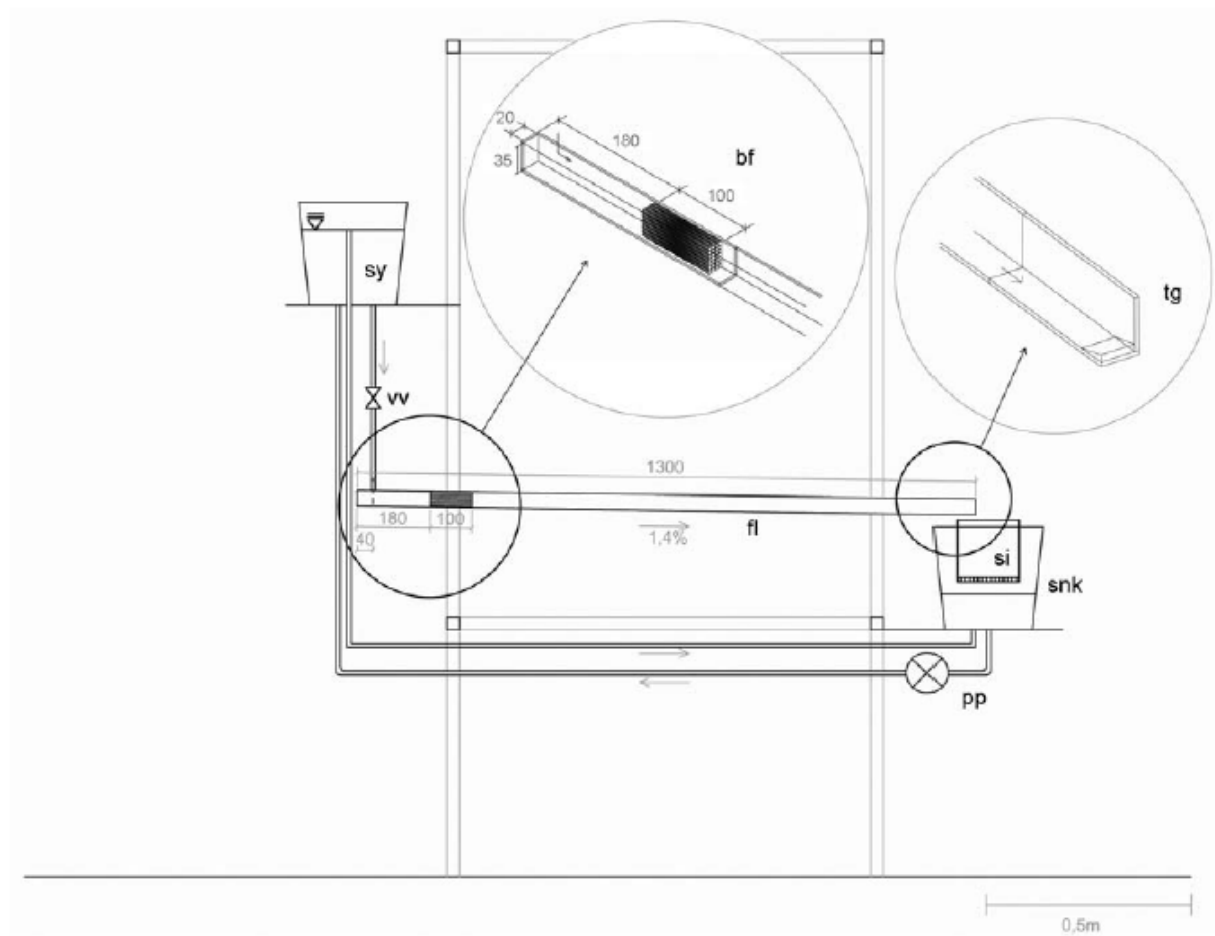
Microsphere characteristics

Fluorescent Blue Carboxyl Polystyrene Latex	
Mean diameter	1 μm
Standard deviation	0.03 μm
Density of polysterene at 20°C	1.055 g cm^{-1}
Surface charge density	24.4 $\mu\text{C cm}^{-2}$
Excitation/Emission wavelength of fluorescent dye	360 / 415 nm

Decrease of opticial density in the bulk water during recirculation







The flume setup (lateral view) (sy: siphon, snk: sink, vv: valve, si: sieve, fl: flume, tg: tailgate, bf: baffle, pp: pump) used during recirculation mode (Singer *et al.* 2006).

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