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Changes in the algal composition, bacterial metabolic activity and element content of biofilms developed on artificial substrata in the early phase of colonization

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Changes in the algal composition and metabolic profiles of bacterial communities as well as the inorganic components were studied on artificial substrata during the early phase of biofilm formation under laboratory conditions in September 2002 and 2003. Sterile Perspex and polished quartz glass discs with a diameter of 3 cm were placed into a Perspex rack, which was immersed vertically in an aquarium containing water from a shallow soda lake. The temperature was kept constant and sufficient oxygen supply was provided. The samples were illuminated for 12 hours a day. Periphyton communities were sampled from 2 to 126 hours of exposure. In both experiments, the alteration of the number of algal species and cells as well as the carbon source utilization of microbial communities was logarithmic. In the two years, considerable differences were revealed in the magnitude of algal cell numbers. The proportion of benthic and planktonic algae showed an undulating pattern in the second experiment. One of the dominant benthic species was the diatom *Achnanthidium minutissimum* Kütz., while that of the planktonic, the cyanobacterium *Microcystis aeruginosa* Kütz. During the experiments an increase in the bacterial activities could be observed; the higher the microbial diversity and abundance that was detected, the more BIOLOG carbon sources were utilized. The examined element contents indicated interactions among algae and bacteria in the biofilms from the beginning of the colonization processes.

Key words: biofilm, bacteria, activity, algae, composition, periphyton, colonization, artificial substratum

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Introduction

The complex microbial communities of biofilms – including algae and bacteria –play a key role in many processes of their environment: algae contribute to the primary production of the lakes – sometimes exceeding that of planktonic species –, bacteria are of great importance in the production as well as the degradation of organic matters (MANZ et al. 1993).

Several algal-bacterial interactions have been investigated and described previously: bacteria involved in the community metabolism of biofilms can utilize not only dissolved organic materials and debris drifting from the water body but the metabolic products released by certain algal species (MAKK et al. 2003). Thus, microbial communities may utilize allochthonous and/or autochthonous organic matter, the latter including algal detritus and exudates as well (ROMANÍ et al. 2004). On the other hand, microscopic studies have proved that algae play a key role in biofilm formation; their surfaces provide potential habitats for several bacteria. A study on bacterial interactions revealed that in the case of submerged plant surfaces, bacterial abundance was significantly higher in areas of diatom colonization (DONNELLY and HERBERT 1999).

Numerous studies have been carried out on the spatial structure and morphology of periphyton, which depends upon many environmental factors. However, the different adherence strategies of algae are also of high importance in the actual formation of biofilms.

The ability to produce gelatinous stalks or tubes by certain diatom species attached to surfaces was described a long time ago (CHOLNOKY 1927). Later many authors classified the adhering types (e.g. ROUND 1981, ROSOWSKI at al. 1986, CATTANEO 1990). HOAGLAND et al. (1993) give an overall summary of diatom adhesion mechanisms, including several forms not detailed in the studies described above, such as adhesion with extracellular polymeric substances in the form of cell coatings, special adhering films or fibrils.

Apart from the physical structures that enable different species to attach to the substratum surface, there are certain »colonization strategies« that can be distinguished among algae. These strategies (ruderal, competitive and stress tolerant) are explained and reviewed by BIGGS et al. (1998). Revealing the type of organisms appearing and the course of events taking place in the initial phase of colonization of submerged surfaces in aquatic systems may lead to further understanding the process of biofilm formation and the possible microbial interactions within the biofilm.

The aim of this study was to examine the early phase of biofilm formation under controlled (laboratory) conditions and to gain an overall view of the changes of metabolic profiles of bacterial communities, the appearance of algae with different adherence strategies and the changes in the inorganic components of the biofilms.

Materials and methods

During the experiments, altogether 30 pieces of sterile Perspex discs (in September 2002) and 48 pieces of polished quartz glass (in September 2003) with a diameter of 3–3 cm were placed vertically in a Perspex rack (serving as sample holder), which was then immersed in an aquarium (100 cm x 60 cm x 30 cm) containing water from a Hungarian shallow soda lake. Water samples collected from 20–30 cm below the surface of the open

water region of Lake Velencei (47°13'N, 18°39'E; average depth of 1.4 m; and characterized by sodium-magnesium-hydrocarbonate-sulfate dominance with 1000–3000 mg L^{-1} total dissolved salt and pH 7.8–10.0) were transported into the laboratory and processing was done within 2–3 h. The algal cell count of the lake water was 103,000 ind. mL^{-1} in 2002 and 55,000 ind. mL^{-1} in 2003.

The water temperature of the aquarium was kept constant (at 20±1 C) in a thermostat room and sufficient oxygen supply was provided by an aquarium filter. The samples were illuminated for 12 hours each day.

Periphyton communities were sampled by taking the discs out of the aquarium after 2, 4, 8, 16, 32 and 64 hours of exposure in 2002, and after 2, 6, 14, 30, 62 and 126 hours of exposure in 2003. The biofilms developed on the discs were washed in sterile tap water of known volume. For the bacteriological and algological analyses the same composite samples, originating from five replicates, were used.

For the mapping of the potential biochemical activities and changes of the bacterial communities on the disc surfaces, an analysis of functional diversity using BIOLOG GN2 MT plates (BIOLOG Inc., Hayward, California, USA) was applied. This community-level sole-carbon-source utilization assay is a rapid and cultivation-independent method to distinguish between microbial communities based on temporal, spatial and physicochemical parameters (GARLAND 1997, PRESTON-MAFHAM et al. 2002).

The turbidity of the samples was set according to the GN NENT standard provided by the manufacturer, and 150–150 µL were inoculated into the BIOLOG GN2 microplates, providing 95 different carbon sources and a redox indicator (tetrazolium violet). The detection of the substrate utilization was based on the reduction of the tetrazolium dye, therefore the optical density values were measured at 590 nm following an incubation at 21°C after 24, 48, 72 and 96 hours. To reduce the number of variables, the 96 hour absorbance data of the utilized carbon sources was applied for principal component analysis (PCA) by using the SYN-TAX 2000 statistical software package (PODANI 2001). The types and the numbers of the carbon sources utilized by the bacterial communities were evaluated according to GARLAND and MILLIS (1991), so the carbon source utilization was regarded positive if absorbance values were higher than the averages of absorbance values on a given microplate.

The algae were counted from the samples treated with Lugol's iodine solution, according to UTERMÖHL (1958). The species were also identified and the ratio of originally planktonic and benthic algae was determined in each sample.

The element content was measured only in the second experiment. In this experiment, the outer area of the disc surface was covered to avoid colonization. Therefore, only the central spot of disc surfaces with a diameter of about 1 cm was investigated in three replicates.

For elemental analysis, the discs were dried at 80 °C on a ceramic hot plate placed in a clean box. After siliconising the surface, gallium as internal standard was dropped onto the discs and dried again. At every sampling time 3 parallel samples were measured. The analytical measurements were carried out with EXTRA IIA total reflection X-ray fluorescence spectrometer (Atomika Instruments GmbH, Oberschleissheim, Germany) equipped with Mo- and W-tubes, cut-off filter and Si(Li) detector. The integration time was 1000 s.

Results

Bacteriological activities

Principal component analysis of the community-level bacteriological sole carbon source utilization data originating from the biofilm samples of both (2002 and 2003) experiments resulted in a distinct separation of the samples along PC1 (Fig. 1). The samples from the very early colonization (obtained after 2, 4, 6, 8 and 16 hours of exposure) grouped together (K2-$_{2002}$, K2-$_{2003}$, K4-$_{2002}$, K6-$_{2003}$, K8-$_{2002}$ and K16-$_{2002}$) and clearly separated from the ones originated after more than 30 hours of exposure. These latter samples (K32-$_{2002}$, K64-$_{2002}$ and K30-$_{2003}$, K92-$_{2003}$, K126-$_{2003}$) showed a second separation along PC2 according to the year of the experiment. The increase in the number of carbon sources utilized by the bacterial communities showed a logarithmic tendency in both experiments (Fig. 2). At the first period of the experiment (from 2 to 16 hours) the number of the utilized carbon sources was much smaller in 2002 than in 2003, which indicates the presence of a longer initial lag period, during which the inoculum density increased. By the second half of the experiment (62 and 64 hours) no differences between the numbers of utilized carbon sources were detected. Among the carbon sources mostly sugars or sugar derivatives (D-fructose, D-galactose, maltose, β-methyl-D-glucoside and glucose-1-phosphate) were preferred at the very beginning of the experiment, in 2003 together with amino acids (such as L-alanine, L-aspartic acid, L-glutamic acid and L-proline). After 30 hours of incubation additional carbohydrates (e.g. L-fucose, gentiobiose, D-glucose, m-inositol, D-lactose, lactulose, D-mannitol, D-mannose, D-melibiose, β-methyl-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose), polymers (cyclodextrin, dextrin, glycogen, tween 40, tween 80) and amino acids (e.g. L-alanyl-glycine, L-asparagine, glycyl-L-aspartic acid, glycyl-L-glutamic

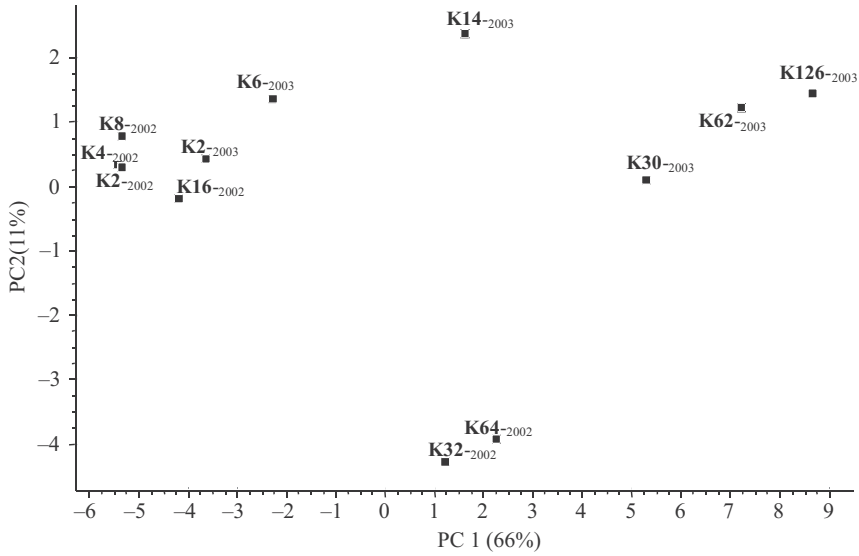

Fig. 1. Scores plot of the principal component analysis based on the 96 -hours' absorbance data of BIOLOG GN2 microplates inoculated with periphyton biofilm samples from artificial substrata. (Characters indicate the sampling times and numbers of years studied.)

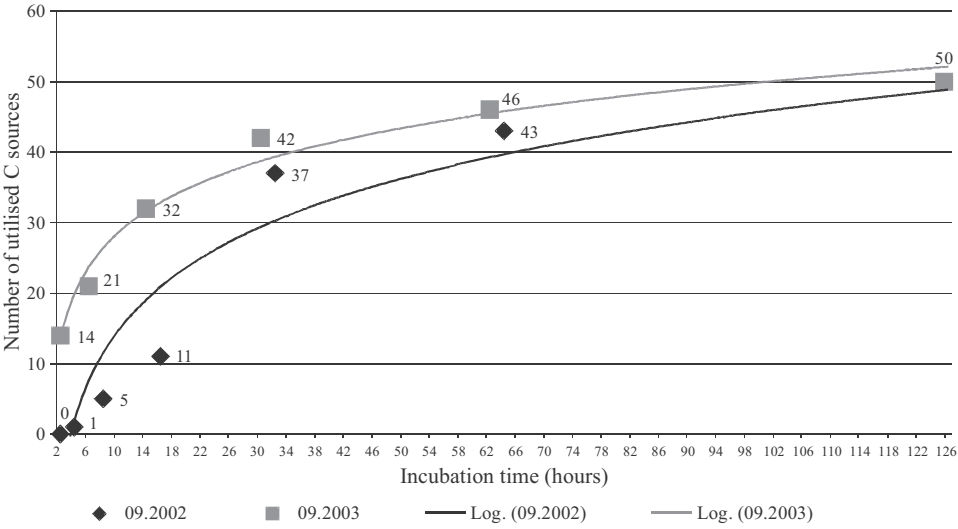

Fig. 2. Changes in the number of utilized BIOLOG carbon sources of the bacterial communities from the biofilm samples in September 2002 and 2003.

acid, L-histidine, L-leucine, L-ornithine, L-phenylalanine) were utilized by both samples. Therefore, neither the number nor the type of the utilized substrates resulted in the separation of the 2002 and 2003 samples after 30 hours of incubation (Fig. 1), but did result in the differences of the average well color development (AWCD) and the sum of activities. After 32 hours 0.683 and 64,918, after 64 hours 0.731 and 69,421 were the AWCD and the sum of absorbance values on the BIOLOG plates in 2002, while after 32 hours 0.983 and 93,396, after 62 hours 1,140 and 108,262 in 2003, respectively.

Algal composition

In both experiments, the species number as well as the number of cells showed detectable logarithmic growths. In spite of this fact, considerable differences could be observed in the cell number per cm^2. In samples from 2003 the abundance was much higher: after 2

Tab. 1. Cell and species number in the biofilms at each sampling hour and experiment.

September 2002			September 2003		
Cell number (cells cm^{-2})	*Species number*	Time (hours)	Cell number (cells cm^{-2})	*Species number*	Time (hours)
115	**18**	2	**1769**	**4**	2
407	**33**	4	**3538**	**6**	6
317	**35**	8	**13471**	**6**	14
353	**34**	16	**14288**	**7**	30
1149	**33**	32	**30209**	**26**	62
853	**46**	64	**932123**	**21**	126

hours 1769 cells cm^{-2} were present on the disc surfaces already, while this value was only 115 cells cm^{-2} in September 2002. We counted 14,288 cells in the sample washed from the disc after 30 hours in September 2003; while 1149 cells cm^{-2} were found in the 32-hour biofilm in 2002. By the end of the experiment, the number of cells in the biofilm reached a value 500 times higher than that of the initial (932,000 cells cm^{-2}, Fig. 3). The tendency of changes in the species number, however, differed from that of the cell numbers (Fig. 4). In 2002 the number of species in the samples was higher than in 2003, during the experiment.

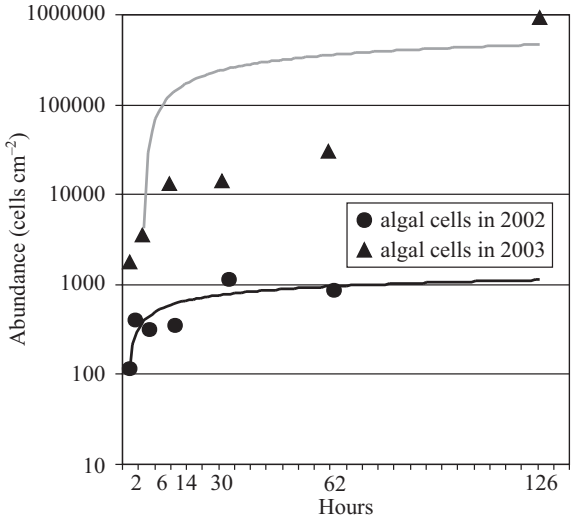

Fig. 3. The temporal changes of algal cell abundance on the disc surfaces in September 2002 and 2003.

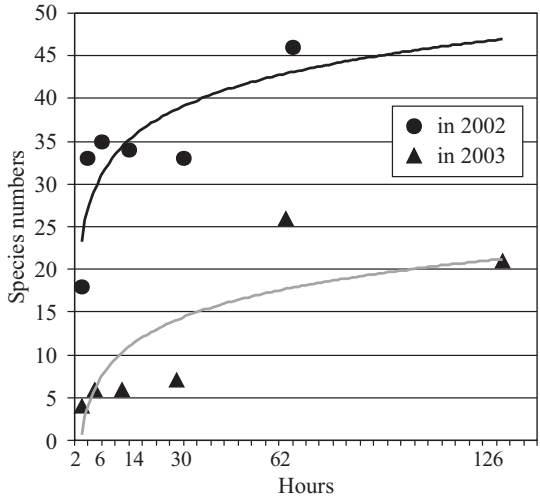

Fig. 4. The temporal changes of algal species numbers on the disc surfaces in September 2002 and 2003.

On the first sampling (after 2 hours of incubation), 18 species were identified and this value reached 46 by the end of the studied period. In 2003 in the first half of the investigation, the species numbers lagged behind those of the samples from 2002.

When the proportion of planktonic and benthic algae is taken into account, the dominance of the benthic algae at the beginning (in the second hour) of both experiments was detected (Figs. 5a, b). With time, the originally planktonic organisms (especially *Microcystis aeruginosa* Kütz.) represented themselves in increasing numbers on the disc surfaces. Compared to the initial ratio, the relative abundance of benthic algae increased after 14 hours in 2003, while at the end of the experiment the number of planktonic species (especially *M. aeruginosa*) increased drastically (they covered over 90 per cent of the algae in the biofilm). One of the dominant benthic species was the diatom *Achnanthidium minutissimum* Kütz. The relative abundance of this species and that of *M. aeruginosa* is also indicated (Fig. 5).

Among the first diatoms settling on the surface were *Gomphonema* species. These initially adhered directly to the substratum through their apical pad without any stalks. Towards the end of the experiment, however, these species attached to the disc with the help of short stalks. Apart from *Microcystis aeruginosa*, other planktonic species that were dominant in one or more samples included the diatom *Chaetoceros muelleri* Lemm. and the dinophyte *Peridiniopsis borgei* Lemm.

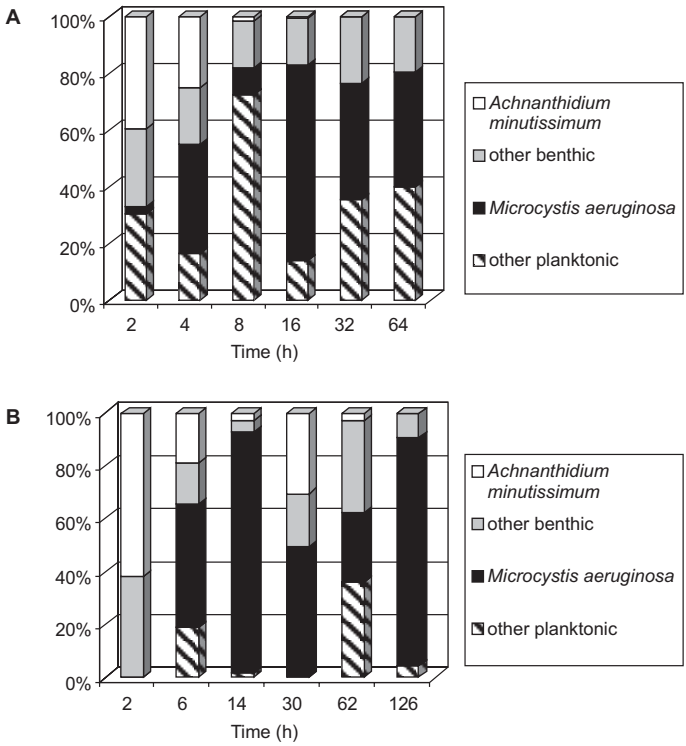

Fig. 5. The proportion of benthic and planktonic algae in the biofilms in September 2002 (A) and 2003 (B).

In 2002, from the 16th hour practically only some *Nitzschia* species were present as benthic algae on the substrate (e.g. *N. gracilis* was the dominant), while in 2003 a relatively diverse benthic species composition was found (among them *Achnanthidium minutissimum*, *Cymbella lacustris*, some *Gomphonema* and *Fragilaria* and *Nitzschia* species, as well).

According to the cluster analysis (UPGMA method, Bray-Curtis index), the samples from 2002 and 2003 clearly separate. In these groups the samples from early and late phases of colonization are also well distinguished (Fig. 6).

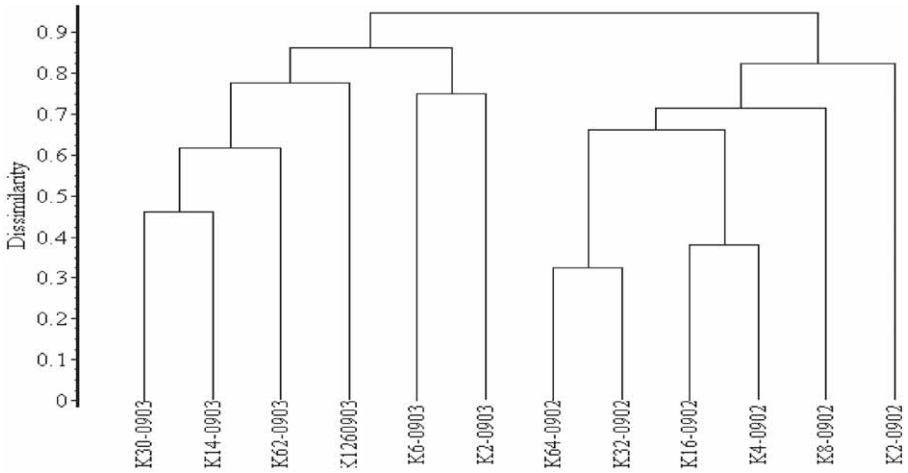

Fig. 6. Dendrogram of the cluster analysis (UPGMA, Bray-Curtis) based on the algal abundance on the substrate in September 2002 and 2003.

Element analysis

In the early phase of colonization K, Ca, Fe, Zn, Cu, Sr, Ti and Mn were detectable with TXRF-spectrometry. At the first sampling (after 2 hours) calcium, potassium, iron and zinc were found on the surfaces of discs. Copper was detected after 6 hours and strontium after 14 hours, while manganese and titanium were present only in the 126-hour biofilm samples. The amount of the studied elements increased during the investigated colonization time (Fig. 7). The relative standard deviation amounted to 10–24%. RSD is higher in case of direct analysis of biofilm samples, while the primer photons scatter on the surface of the microorganisms.

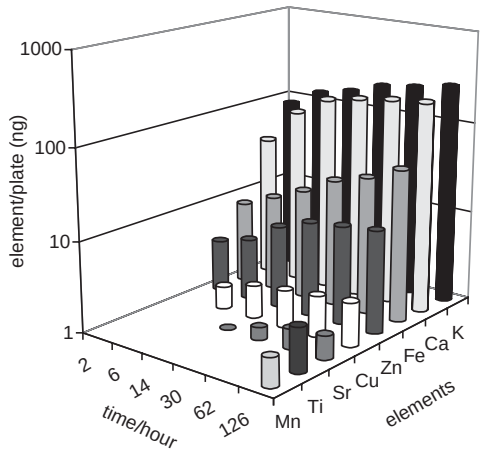

Fig. 7. Changes in the element content of the biofilms in September 2003 (logarithmic scale)

Discussion

Studying the colonization processes of artificial substrata is a hard task due to their complexity. Several events take place on the substratum after its immersion in the water: the number of algae settling on the substratum is increased by immigration and reproduction and decreased by emigration, mortality and grazing. During sampling, neither the number of individuals immigrating onto the substratum nor those emerging due to reproduction can be separated and thus determined. However, it has been observed that the level of immigration is the determining factor at the beginning of colonization. After that, the reproduction of the already settled algae gradually takes over and plays the main role (BIGGS 1996). On a longer scale, a special undulation can be observed in biofilm formation (ÁCS and KISS 1993).

The approximately logarithmic increase in the species and cell numbers of the biofilm washed off the disc surfaces is in good correlation with the natural succession processes occurring under undisturbed, non-laboratory conditions. The biofilm formation, as a phenomenon aiming at the colonization of a given available »niche« or habitat, can be considered as succession (ÁCS and BUCZKÓ 1994, STEVENSON 1996). Similar results were obtained in experiments carried out on a longer scale (HILLEBRAND and SOMMER 2000).

From the lower individual numbers and relatively high proportion of planktonic organisms in 2002, it can be concluded that no real biofilm formation took place during the experiment, as can be seen well on the dendrogram of cluster analysis (the samples from 2002 and 2003 are separated). Instead, the intense settlement of primarily planktonic individuals could be observed in the first year. The reason might be the shorter time for the colonization, comparing the two years, as well as the difference in the type of substratum. In the initial hours, more than 70% of the algae were benthic in the samples in 2002 and 100% in 2003. This ratio slightly decreased until the 16^{th} hour in 2002, then stayed around 20% till the end of experiment. After an analysis of the species composition in more detail, it can be seen that the formation of biofilm did not really get started. From the 16^{th} hour practically only some *Nitzschia* species began to grow on the substratum, while in 2003 a relatively diverse benthic community was found. In 2003 the proportion of benthic algae drastically decreased until the 14^{th} hour, then markedly increased and again decreased. This »undulating« feature in the proportion of benthic and planktonic algae coincides well with the physico-chemical conditions of biofilm formation and the adherence strategies of algae of different life forms.

The lack of »real« biofilm formation (with diverse attached algae) in 2002 is clearly visible from the individual number of algae, too, which was approximately 40 times lower after 60 hours than in 2003. The bacterial investigations also showed a less mature biofilm in 2002, because the number of utilized carbon sources was much lower in every investigated hour in 2002 than in 2003. However, the logarithmic distribution of the number of utilized carbon sources in both experiments indicated biofilm formations. Therefore, the biofilm formation in 2002 might have been the result mainly of bacterial activities. The adherence of living organisms on surfaces is always preceded by the formation of a so-called »conditioning film«, which provides the appropriate charge and nutrients on the substratum. Among the algae, those without stalks, adhering tight with their whole valve surface are capable of attaching to the substratum surface first (STEVENSON 1996). They are followed by further individuals developing other adhering strategies. This phenomenon is in

accordance with the fact that the species *Achnanthidium minutissimum* started to grow rapidly at the very beginning of the colonization. This diatom is considered as a pioneer colonist species, which is supported by many of its features, such as size and adherence abilities (ROEMER et al. 1984, IVORRA et al. 1999). For the attachment of planktonic algae, the biofilm structure has to be at a certain level, for these species do not possess any special mechanism or structure necessary for adherence. Instead, they are able to »settle«, to attach to the filaments and fibrils of the already developed biofilms. With time, however, the real benthic algae start to grow inside the biofilm (this is the above-mentioned reproduction phase). This layer becomes more and more thick and dense; hence the relative quantity of planktonic algae decreases gradually. This is when the emigration phase starts but our experiments were not continued beyond this point.

Concerning the species attaching to the substratum, the results are in good accordance with the findings of ÁCS (1998) and ÁCS et al. (2000). In both studies, short-term changes during biofilm colonization on an artificial substratum (glass slides) were examined in the Danube River. ÁCS et al. (2000) also found the first algae at a very early phase of colonization, after 6 hours. As in our experiment in September 2003 (when signs of real biofilm formation were observed), planktonic species were among the first algae to appear on the substratum surface and had a considerable proportion. During the first week of their investigation, the periphyton was dominated by diatoms adhering with their apical pads. The differences between the two results lie not in the adherence characteristics but in the actual species that colonize the surface. In our case, the first colonizers attached to the discs were dominant species found in the natural periphyton communities of Lake Velencei.

In the same reference, *Gomphonema* and other diatom species capable of stalk formation appeared in the biofilm but first attached to the surface directly, as well. The occurrence of diatoms adhering through short, non-branching stalks followed later on in both experiments.

During our investigations of these colonization processes, a consequent increase in the types of bacterial activities could be observed. The higher the microbial (including bacteria) diversity and abundance became, the more carbon sources were utilized in the biofilms. The number of carbon sources utilized by the microbes was higher and higher with time; however, the tendency was decreasing. This logarithmic increase agrees well with the changes in the species and cell numbers of algae and the examined element contents. The more algae attached to the substratum, the more additional surface was provided for other microbes, including bacteria. Electron microscopic observations demonstrated large numbers of biofilm bacteria attached to the outer surfaces of algae, especially diatoms (MAKK et al. 2003). Moreover, diatoms served not only as substratum for bacteria but metabolic interactions took place among them, as well (COOKSEY (1992). The increasing bacterial cell densities in the experiment resulted in a significantly increasing amount of attached algae. The increasing adsorption of suspended algae in the bacterial biofilm is an important factor in algal immigration during the initial development of periphyton communities (HODOKI 2005).

Combining the results of the bacteriological and algological investigations with the changes in the element contents of the studied biofilms, it can be assumed that the measured increase derived from the living fraction of the biofilms.

In conclusion, the findings demonstrate strong interactions between algae and bacteria in biofilm communities from the very early phase of colonization processes. TXRF proved

to be suitable for direct analysis of the element content of biofilms, although this analysis had higher relative standard deviations because of the photons scattered from the surface of microorganisms.

References

ÁCS, É., 1998: Short-term fluctuation in the benthic algal compositions on artificial substratum in a large river (River Danube, near Budapest). Verh. Int. Ver. Limnol. 26, 1653–1656.

ÁCS, É., BUCZKÓ, K., 1994: Daily changes of reed periphyton composition in a shallow Hungarian Lake (Lake Velencei). Proc. 13 Int. Diatom Symp., Bristol, 1–10.

ÁCS, É., KISS, K. T., 1993: Colonization process of diatoms on artificial substrate in the River Danube near Budapest (Hungary). Hydrobiologia 269/270, 307–315.

ÁCS É., KISS, K. T., SZABÓ, K., MAKK, J., 2000: Short-term colonization sequence of periphyton on glass slides in a large river (River Danube, near Budapest). Algol. Stud. 100, 135–156.

BIGGS, B. J. F., 1996: Patterns in benthic algae of streams. In: STEVENSON, R. J., BOTHWELL, M. L. and LOWE, R. L. (eds.), Algal ecology in freshwater benthic ecosystems, 31–56. Academic Press, San Diego, London.

BIGGS, B. J. F., STEVENSON, R. J., LOWE, R. L., 1998: A habitat matrix conceptual model for stream periphyton. Arch. Hydrobiol. 143, 21–56.

CATTANEO, A., 1990: The effect of fetch on periphyton spatial variation. Hydrobiologia 206, 1–10.

CHOLNOKY, B., 1927: Beiträge zur Kenntnis der Bacillariaceen-Kolonien. Nova Hedwigia 67, 223–236.

COOKSEY, K. E., 1992: Bacterial and algal interactions in biofilms. In: MELO et al. (eds.): Biofilms- science and technology, 163–173. Kluwer Academic Publishers, Dordrecht.

DONNELLY, A. P., HERBERT, R. A., 1999: Bacterial interactions in the rhizosphere of seagrass communities in shallow coastal lagoons. J. Appl. Microbiol. Symp., Suppl. 85, 151–160.

GARLAND, J. L., 1997: Analysis and interpretation of community-level physiological profiles in microbial ecology. FEMS Microbiol. Ecol. 24, 289–300.

GARLAND, J. L., MILLIS, A. L., 1991: Classification and characterization of heterotrophic microbial communities on the basis of pattern of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. 57, 2351–2359.

HILLEBRAND, H., SOMMER, U., 2000: Diversity of benthic microalgae in response to colonization time and eutrophication. Aquat. Bot. 67, 221–236.

HOAGLAND, K. D., ROSOWSKI, J. R., GRETZ, M. R., ROEMER, S. C., 1993: Diatom extracellular polymeric substances: function, fine structure, chemistry, and physiology. J. Phycol. 29, 537–566.

HODOKI, Y., 2005: Bacterial biofilms encourage algal immigration onto substrata in lotic systems. Hydrobiologia 539, 27–34.

IVORRA, N., HETTELAAR, J., TUBBING, G. M. J., KRAAK, M. H. S., SABATER, S., ADMIRAAL, W., 1999: Translocation of microbenthic algal assemblages used for *in situ* analysis of metal pollution in rivers. Arch. Environ. Cont. Toxicol. 37, 19–28.

MAKK, J., BESZTERI, B., ÁCS, É., MÁRIALIGETI, K., SZABÓ, K., 2003: Investigations on diatom-associated bacterial communities colonizing an artificial substratum in the River Danube. Arch. Hydrobiol. Suppl. 147, Large Rivers 14, 249–265.

MANZ, W., SZEWZYK, U., ERICSSON, P., AMANN, R., SCHLEIFER, K. H., STENSTRÖM, T. A., 1993: In situ identification of bacteria in drinking water and adjoining biofilms by hybridization with 16S and 23 S rRNA-directed fluorescent oligonucleotide probes. Appl. Environ. Microbiol. 59, 2293–2298.

PRESTON-MAFHAM, J., BODDY, L., RANDERSON, P. F., 2002. Analysis of microbial community functional diversity using sole-carbon-source utilisation profiles – a critique. FEMS Microbiol. Ecol. 42, 1–14.

PODANI, J., 2001: SYN-TAX 2000 Computer programs for data analysis in ecology and systematics (User's Manual).

ROEMER, S. C., HOAGLAND, K. D., ROSOWSKI, J. R., 1984: Development of a freshwater periphyton community as influenced by diatom mucilages. Can. J. Bot. 62, 1799–1813.

ROMANÍ, A. M., GIORGI, A., ACUÑA, V., SABATER, S., 2004: The influence of substratum type and nutrient supply on biofilm organic matter utilization in streams. Limnol. Oceanogr. 49–50, 1713–1721.

ROSOWSKI, J. R., HOAGLAND, K. D., ALOI, J. E., 1986: Structural morphology of diatom-dominated stream biofilm communities under the impact of soil erosion. In: EVANS, L. V., HOAGLAND, K. D. (eds.), Algal biofouling – Studies in environmental sciences 28, 247–298. Elsevier, Amsterdam.

ROUND, F. E., 1981: The ecology of Algae. Cambridge Univ. Press, Cambridge.

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 in freshwater benthic ecosystems, 3–30. Academic Press, San Diego.

UTERMÖHL, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt. Int. Ver. Limnol. 9, 1–38.