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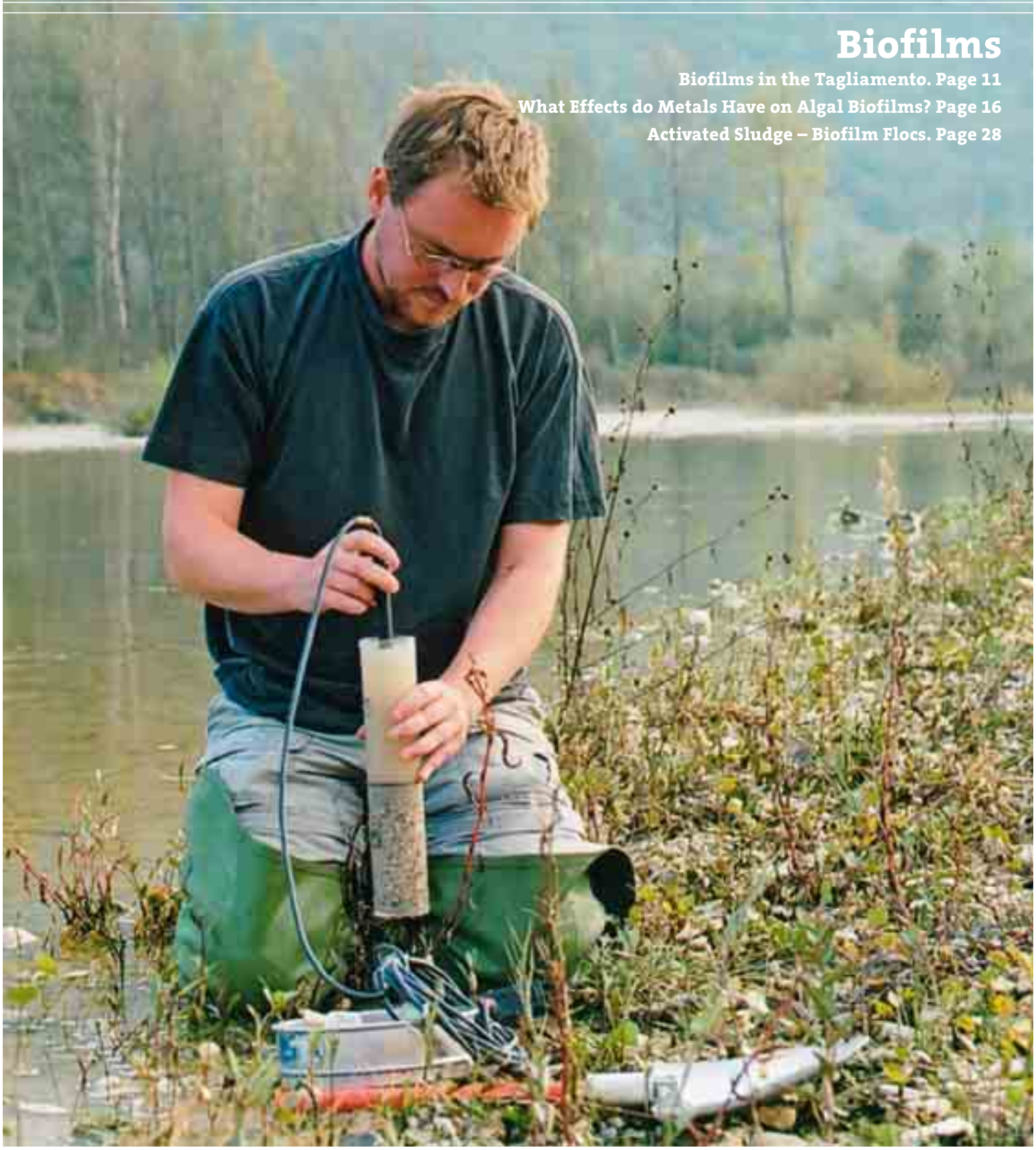
Eawag: Swiss Federal Institute of Aquatic Science and Technology

# Eawag News

## Biofilms





# Editorial

Thomas Egli, microbiologist and executive head of the "Environmental microbiology" department.

# Biofilms: Both a Curse and a Blessing

Who hasn't been annoyed: by teeth coated again so soon after brushing or by the slime that constantly forms in the reservoirs of coffee machines and atmospheric humidifiers or by the slippery rock that can give one an unexpected dip when crossing a stream on a country walk. This is all due to the growth of the smallest of the creatures with which we share our planet, the microorganisms. It is a fact of life that they usually settle in communities – known as biofilms – on any possible, or seemingly impossible surface. Their spectrum extends from microcolonies of only a few cells thick to layers measuring a few millimetres or even centimetres in which the microorganisms are embedded in an aqueous mucus.

Biofilms are fascinating because they consist of physiologically highly diverse groups of organisms, such as bacteria, algae and fungi, which live together in a very small space to their mutual benefit. This is why biofilms are such an advantageous form of life: the organisms often feed each other, are protected from predators and biocides and are less likely to succumb to stress situations such as desiccation because they are embedded in mucus. However, there are some adverse factors too, such as the physical limitation of nutrient transport, or immobility.

Do microorganisms deliberately choose this sedentary lifestyle, or is this process an obligatory rather than an active response to external conditions? This question has split the scientific community into two camps. At any rate, a sometimes vigorous debate on the subject has been ongoing for over three decades now.

Regardless of the higher-level strategy being implemented, here as elsewhere, good and bad aspects are simultaneously at work. The formation of anaerobic niches that can seed corrosion in metal pipes and conduits, hygiene problems in drinking water supply systems, medical issues due to films growing on artificial implants or catheters, impaired performance of heat exchangers, and increased drag on ships' hulls – are just a few areas in which biofilms make life difficult for us. On the other hand, biofilms play a vital part in the metabolism of natural aquatic environments and hence in their capacity for self-purification. In sewage treatment plants, they are deliberately cultivated on special large-area substrates with large surfaces in order to eliminate contaminants from the effluent. Ecotoxicologists would like to use biofilms to help estimate the pollutant load of surface waters. In the cosmetics industry, substances extracted from biofilm mucus are used as water-retaining components: in this way the ingredients can be "stretched" with large quantities of water whilst the cream still retains a voluminous appearance.

While detailed research over the last few decades has taught us a great deal about the behaviour of microorganisms in biofilms, even today, their lifestyle remains a phenomenon that never ceases to astonish and bewilder us. Some of the relevant aspects and advances are featured in this issue of Eawag News.

Thomas Egli

Eawag, the Swiss Federal Institute of Aquatic Science and Technology, adopted a new graphic design for its public face in May 2005. We have now adapted the layout of Eawag News accordingly. We look forward to receiving your suggestions and comments.
*Martina Bauchrowitz, Editor*

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# Content

## Lead Article



## Research Reports



## Various




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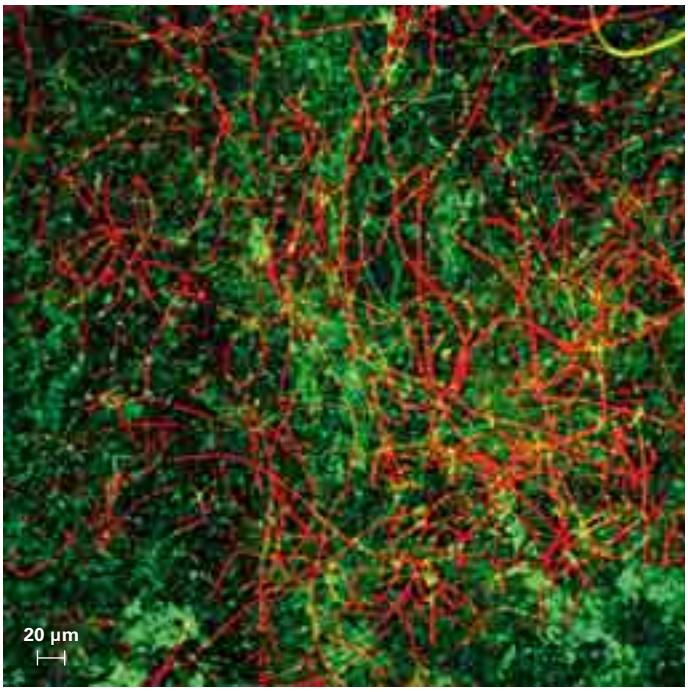
# Biofilms are Ubiquitous

Oskar Wanner, system analyst and scientist in the department of "Urban Water Management", and Martina Bauchrowitz, biologist and editor of Eawag News.

The slime we brush off our teeth in the morning is a bacterial biofilm. If we neglect this daily cleaning, we get mouth odour, our gums become inflamed, and our teeth would in time be damaged by caries. This is well-known, but it is perhaps less well-appreciated that in many fields, biofilms can serve a useful role as well.

Biofilms are accumulations of micro-organisms which form on solid surfaces in aqueous or wet environments [1]. We find them everywhere, in surface and ground waters, in drinking water piping and wastewater treatment plants, and on other technical equipments (e.g. in the medical field). Not only do their locations vary enormously, but also their shape and size: anything is possible from scattered colonies to millimetre-thick layers. Biofilms interact strongly with their environment, so they are greatly affected by, and in return affect, the chemical and physical conditions in which they exist. This is exactly what happens in our mouths, where our teeth provide an ideal substratum for biofilms thanks to the conditions of high humidity and frequent nutritional intake. Biofilms convert carbohydrates to acids and act as a barrier to prevent them from being transported away. This creates the acidic conditions which attack tooth enamel.

Heterotrophic biofilm from the river Saale. Red = filamentous bacteria, green = biofilm matrix.

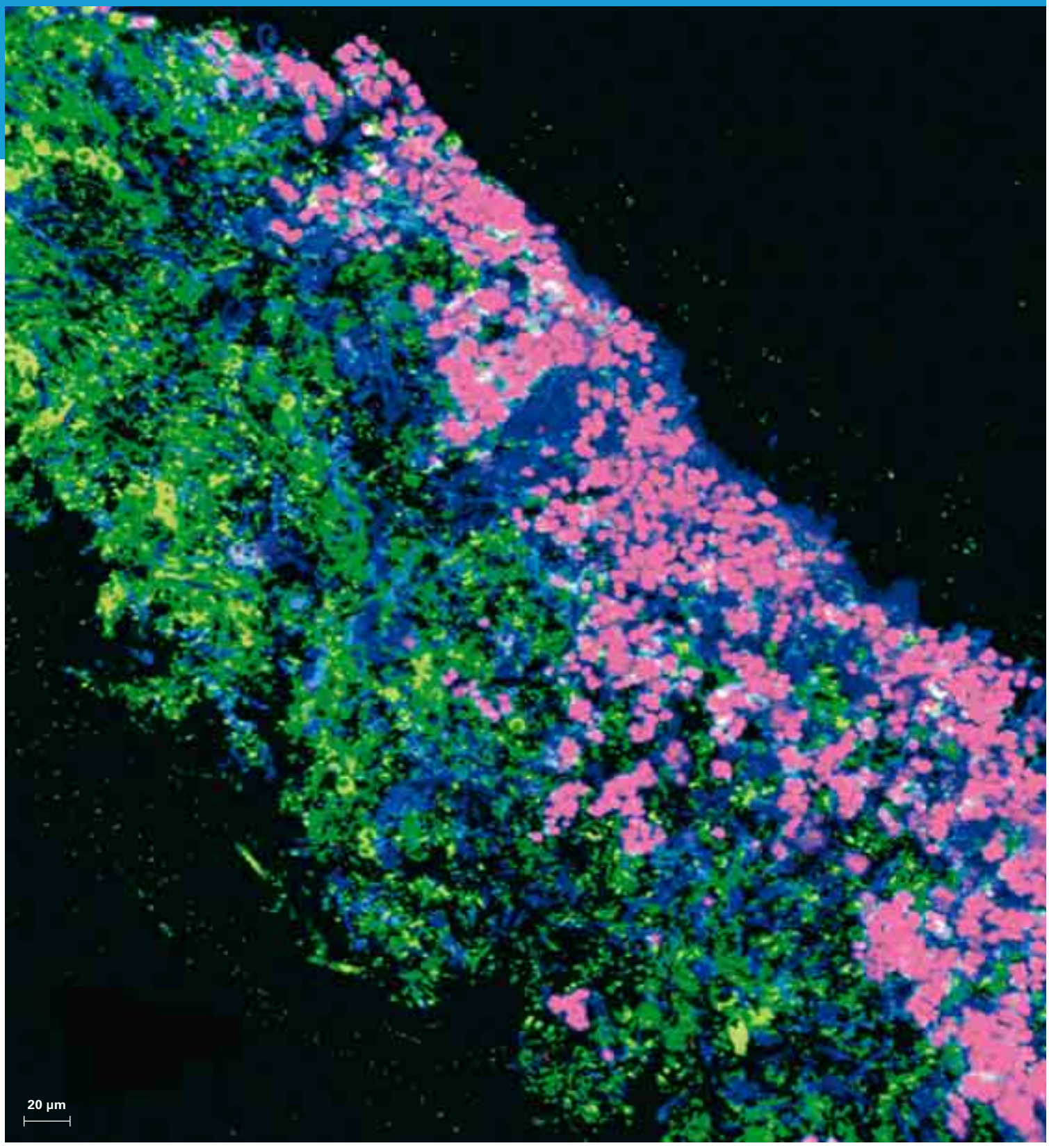



Photos: Thomas Neu, UFZ-Leipzig

**Biofilm Advantages for Microbes.** Micro-organisms organize in a sessile life form where a good nutritional supply is ensured. Freely-suspended organisms, on the other hand, are easily transported away. As a result, the density of micro-organisms in biofilms is higher by orders of magnitude than that of suspended organisms. However, biofilms not only protect from being washed away, but also against biocides and other toxic substances. Apparently, these substances are fixed by the slime layer in which the micro-organisms are embedded and therefore do not reach the cells. Furthermore, biofilms facilitate synergistic cooperation between different microbial species. For example, in wastewater treatment, nitrification is a two-stage process in which *Nitrosomonas* uses ammonium to produce nitrite, which *Nitrospira* converts to nitrate. For *Nitrospira,* the close proximity to its substrate producers is an enormous advantage – a foodstore literally on its doorstep.

**How are Biofilms Structured?** With all of these advantages, it is no wonder that micro-organisms prefer to live in sessile communities [2]. These include bacteria, algae, amoebae, ciliates and fungi in a great variety of compositions. Sunlight favours the growth of photoautotrophic organisms in biofilms, such as algae and cyanobacteria. They conduct photosynthesis and build up their biomass from inorganic substances, and are thus primal species in the food-chain. In the absence of sunlight, on the other hand, biofilms are formed mostly by heterotrophic bacteria, which degrade organic substances. Less frequent are chemoautotrophic bacteria which use inorganic substances.

In biofilms, micro-organisms are embedded in a slimy mass, the biofilm matrix. It consists of extra-cellular polymeric substances, EPS for short, which are excreted by the organisms themselves. EPS contain mainly high-molecular polysaccharides. In addition, there are proteins, other carbohydrates such as uronic acid, and small amounts of lipids and nucleic acids.




Freshwater photoautotrophic biofilm. Pink = cyanobacteria autofluorescence, blue = green algae autofluorescence, green = biofilm matrix.

Biofilms typically show an intricate architecture, with pores, channels and finger- or coral-like protuberances. Sharp spatial gradients are observed within biofilms: this may concern the oxygen or substrate concentration or the pH value. Such gradients may be responsible for the development of physiologically different and clearly distinguished microbial communities. Substrates are transported with the water in the channels and pores of the biofilm via diffusion and advection. Last but not least, biofilms are highly dynamic systems, as the micro-organisms grow, change their location in the biofilm, die, get ingested or are washed away. Due to these processes, biofilms undergo continuous expansion and contraction.

**Biofilms – Complex by Nature.** The diversity of microbial communities, and the large variety of chemical, biological and physical processes which occur in the biofilms, explain why these are such

complex systems. Accordingly difficult is their experimental investigation which relies on an intact biofilm structure. Therefore, mathematical models are useful research tools in helping us to express our ideas in a quantitative form, and to verify them on the basis of experimental observations. Eawag has developed biofilm models and simulation programmes which are widely used today (see contribution by Oskar Wanner on p. 8).

**Biofilms in Natural Water Bodies – Sites of Intense Biological Activity.** In streams, algae-dominant autotrophic biofilms gather on the riverbed and bacteria-dominant heterotrophic biofilms are found in the pore systems under the riverbed [3]. As biomass producers and decomposers, they are important components in the foodchain. On the Tagliamento in north-eastern Italy, one of the last mainly intact wild rivers of Europe, Michael Döring (see contribution p. 11) has investigated whether the exchange of water between the water column and the pore system under the riverbed influences the growth and activity of biofilms.

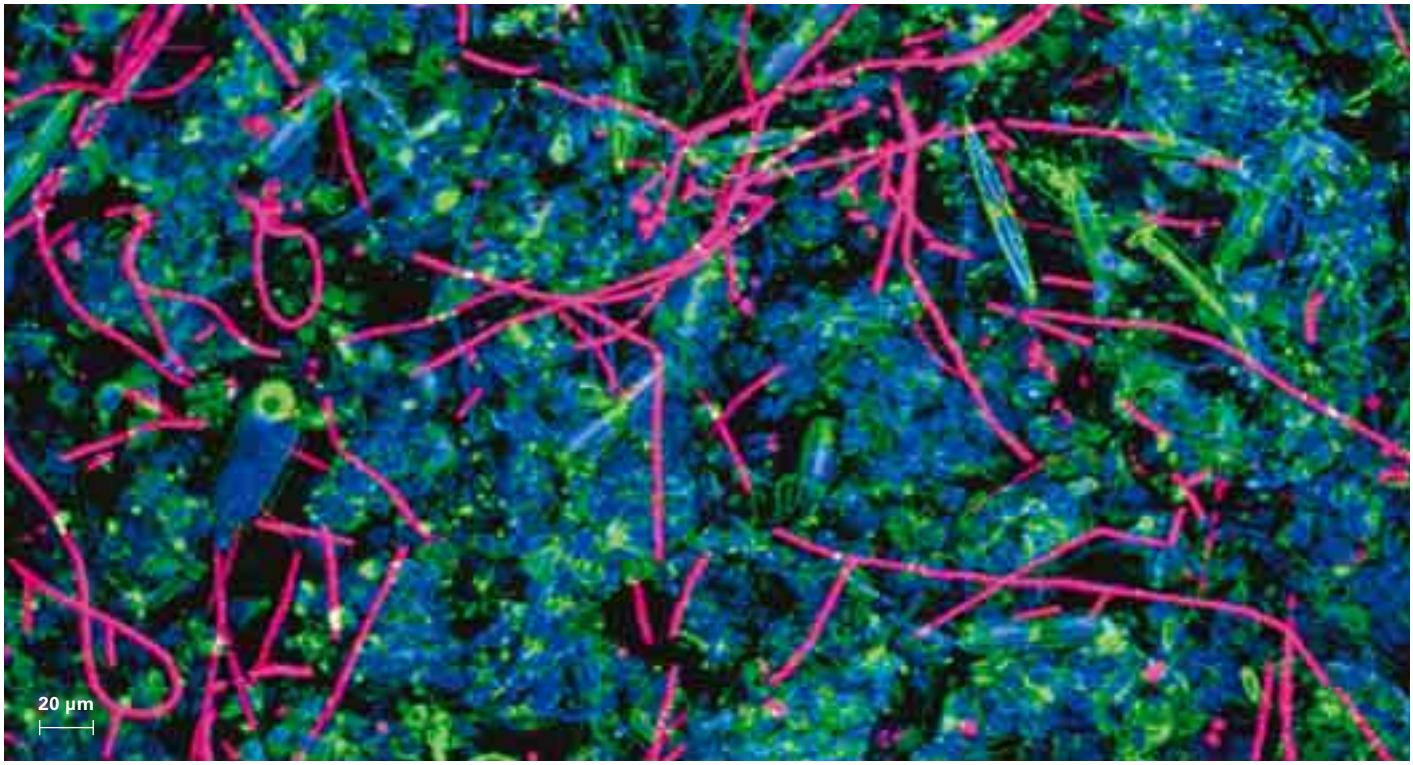
A white sediment of calcium carbonate, known as sea chalk, accumulates on the bed of many lakes. Sabine Sibler and Maria Dittrich (see contribution p. 14) wanted to find out how it is formed. They suspect that the EPS of cyanobacteria is involved in the precipitation. This bacteria group is the main component of the phytoplankton biomass in nutrition-poor lakes and seas. In fact, they found evidence that the bacteria are important for the metal cycle, reduce the metal concentration in water, and thus contribute to the lake's self-cleaning capacities.

Around 70 % of our drinking water comes from ground water. During the passage through the subsoil, water is cleaned naturally by biofilms, before it reaches a drinking water catchment. To some degree, biofilms can even be employed to clean contaminated ground water, such as in the wake of a chemical accident. If the contaminants are biodegradable, specialised micro-organisms can be pumped into the aquifer, where they settle, and, in the absence of alternative substrates, use the contaminants as food source.

**Biofilms as Indicators of Environmental Impacts.** Micro-organisms are often used in ecotoxicology to estimate the pollutant loading of natural water bodies and the hazard potential of toxic substances. In this field, natural stream biofilms are particularly interesting model systems: on the one hand, because they play a central role in ecosystem metabolism and interact with the toxic substances, and on the other hand because, as immobile biological elements, they accumulate pollutants over a long period and may thus reveal chronic impacts. Eawag is currently testing whether biofilms can be used as indicators for metal stress.

The contribution by Sébastien Meylan (see p. 19) reports on an Eawag field study. The sediments of the Furtbach in Canton Zurich are contaminated by copper and zinc. Due to increased discharge after heavy rainfall, the sediments are entrained with the flow and the metals are released. The biofilms react quickly to such changed environmental conditions: shortly after the rain starts, the metal concentration in biofilms increases significantly. Other experiments (see contribution by Séverine Le Faucheur on p. 22) show that the

Freshwater photoautotrophic biofilm. Pink = cyanobacteria autofluorescence, blue = green algae autofluorescence, green = biofilm matrix.



algae in the biofilms respond to metal stress with the formation of phytochelatines – polypeptides which bind metals and render them inert.

Thus, metals trigger reactions on the cellular level. But does this also mean that the organisms are damaged? A comprehensive investigation at Eawag deals with this question (see contribution of Renata Behra on p. 16). In addition to cellular reactions, the project aims at identifying consequences of metals on the community level, such as changes of species diversity and inhibition of photosynthesis. Thus, it should reveal the causal relationship between metal stress and ecological effects.

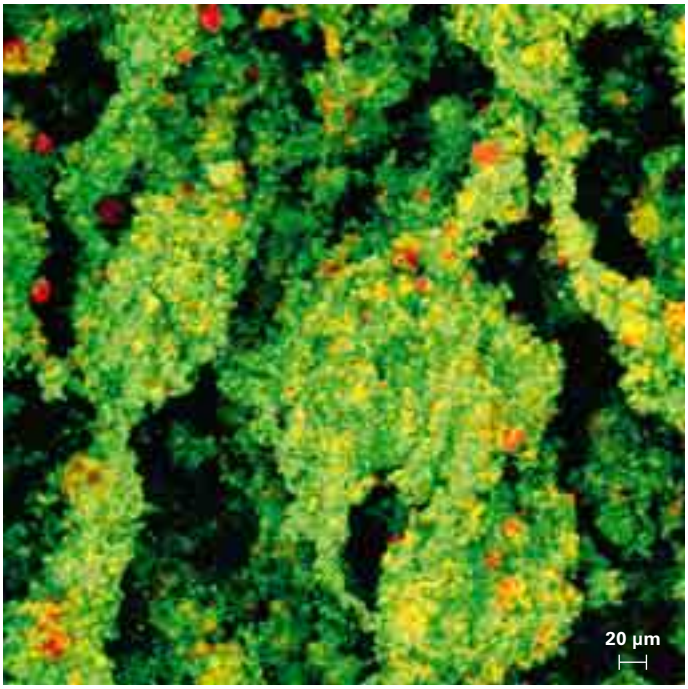
**Biofilms in Wastewater Treatment.** Micro-organisms play an important role in wastewater treatment. They degrade the pollutants present in the waste water. In Switzerland, activated sludge reactors are commonly used. Therein, suspended bacteria grow and aggregate in flocs of up to 2 millimetres in diameter. These activated sludge flocs contain millions of bacteria, and since they demonstrate structures and processes which are typical for biofilms, they are often referred to as "suspended biofilms". In his project, Reto Manser investigated whether floc size has an effect on the microbial biocoenosis forming in the flocs, and consequently on the effectiveness in pollutant elimination (see contribution p. 28).

In addition, real biofilm reactors are also adopted in Swiss wastewater treatment plants. In such reactors, the bacteria settle on the support material provided. Adriano Joss (see contribution p. 24) characterised the decomposition of micro-pollutants in the Altenrhein sewage treatment plant. At this site, half of the waste water is treated in a conventional active sludge reactor, while the other half is purified in a biofilm reactor, the dimensions of which are considerably smaller. The question was whether the two plants would achieve the same elimination rate.

**Undesired Biofilms.** Along with their useful functions, biofilms can have very undesirable effects. Biofilms in drinking water distribution systems and air-conditioning equipment can contain legionelles, which cause the dangerous legionnaire's disease. In the medical field, biofilms grow on instruments, tubes and on implants such as pacemakers, and can cause infections and even rejections by the body. The bacteria can be prevented from settling by the selection of adequate surface properties, such as smoothness, or coating catheters with antibiotics.

Larger facilities are also not immune. Algae and fungi lead to costly cleaning of building facades and can damage monuments. Biofilms slow ships by increasing the drag on hulls, which leads to excessive fuel use. Sulphate-reducing bacteria can lead to the dreaded pitting corrosion of metallic surfaces and cause great damage. These processes, designated as biofouling and biocorrosion, cost around 200 billion dollars annually in the USA alone [4].

Biofilm growth can seriously reduce the performance of heat exchangers. This problem occurs especially with heat exchangers which gain their heat from waste water in sewage pipes, as the untreated water provides an abundance of nutrient supply. Therefore, Eawag investigated how biofilm growth can be controlled on such heat exchangers (see contribution by O. Wanner on p. 31).




Activated sludge chemoautotrophic biofilm. Red = bacteria, green = biofilm matrix.

By increasing the speed of water flow, biofilms can be partly detached from the heat exchanger surface by the resulting increase in mechanical shear forces. Mechanical cleaning is therefore a tried and tested means of controlling unwanted biofilms – just like on our teeth. ○ ○ ○

The photos were taken with a confocal laser scanning microscope, and were kindly provided by Thomas Neu (UFZ Leipzig) [5].


[1] Costerton J.W., Lewandowski Z., De Beer D., Caldwell D., Korber D., James G. (1994): Minireview: biofilms, the customized microniche. Journal of Bacteriology *176,* 2137–2142.

[2] Flemming H.C., Wingender J. (2001): Biofilme – die bevorzugte Lebensform der Bakterien. Biologie in unserer Zeit *31,* 169–180.

[3] Lock M.A. (1993): Attached microbial Communities in rivers. In: Aquatic Microbiology (ed. T.E. Ford). Blackwell Scientific Publications, Oxford, pp. 113–138.

[4] Okabe S., Jones W.L., Lee W., Characklis W.G. (1994): Anaerobic SRB biofilms in industrial water systems: a process analysis. In: Biofouling and Biocorrosion in industrial water systems (ed. G.G. Geesey et al.). Lewis Publishers, Boca Raton, pp. 189–204.

[5] Neu T.R., Lawrence J.R. (2002): Laser scanning microscopy in combination with fluorescence techniques for biofilm study. In: The encyclopedia of environmental microbiology (ed. G. Bitton) Volume 4. John Wiley & Sons, New York, pp. 1772–1788.

Oskar Wanner, system analyst and scientist in the department of "Urban Water Management".

# Biofilm Models: Tools for Research

Scientific investigation is a process of rigorously testing hypotheses by observation and experiment. Mathematical models provide valuable aids to science, expressing data in a quantitative form, which allows experimental evidence to support or refuse the model. Through the modelling of complex systems, such as wastewater treatment plants, engineers are able to reproduce, control and optimise the behaviour of the plants.

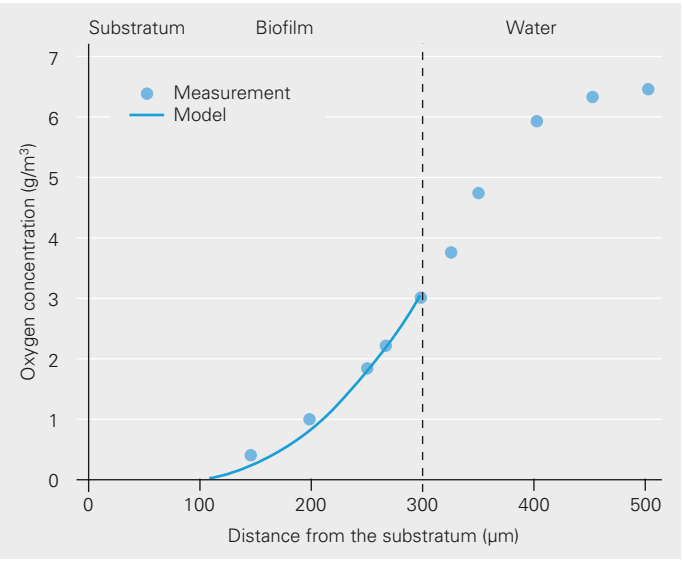
A single cubic millimetre of biofilm is home to millions of micro-organisms. They take up oxygen, carbon and nitrogen compounds from the surrounding water, and use these substances (which we refer to as "substrates" in this article) for their growth. Depending on the type and concentration of the available substrates, the cell density of some microbial species in the biofilm quickly increases while other species are less successful. Thus, the interactions between the substrates and the micro-organisms determine the relative abundance, spatial distribution and changes over time of the different microbial species, and may result in dramatic variations in the chemico-physical conditions over just a few tens of millimetres. For example, by using all of the oxygen diffusing into the biofilm, aerobic micro-organisms living near the surface create an environmental niche in the biofilm depth for anaerobic micro-organisms. Biofilms are therefore particularly interesting and complex habitats, whose behaviour is determined by a multitude of concurrent biological, chemical and physical processes.

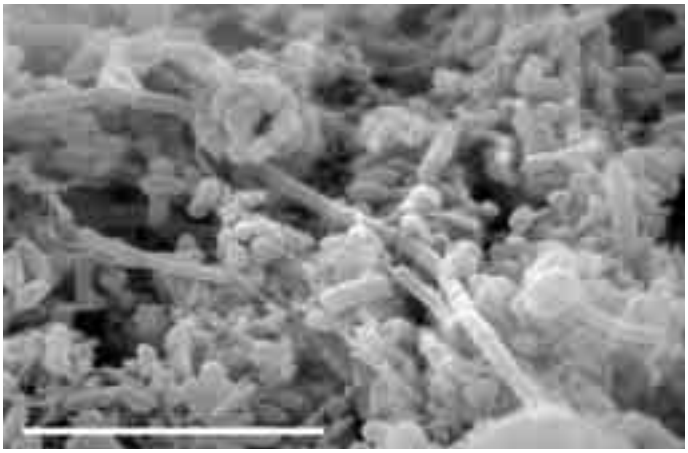
Experimental methods for the characterisation of biofilms have seen continuous development and refinement over recent decades, and today furnish us with very detailed information about biofilm structures. Concomitantly, mathematical models have been designed and enable us to analyse and simulate the processes occurring in biofilms. This co-development of experimental and mathematical methods has greatly advanced our knowledge on biofilms.

**The Original Simple Trickling Filter Model.** The first trickling filter plant for wastewater treatment was installed in St. Gallen in 1912. Waste water was fed through a container filled with stones, on which micro-organisms quickly settled. By using the pollutants in the waste water as substrates, micro-organisms effectively purified the water. As more and more trickling filter plants were installed, the engineers wished to have a tool to help them design new plants. Therefore, simple mathematical equations were formulated with which the capacities of trickling filters for various pollutant loads, wastewater flows and filter volumes could be calculated. These equations were known as empirical or black-box biofilm models. Since they relied entirely on measurements of pollutant loads at the inputs and outputs of the plants, they failed to take into account the processes occurring within the filter itself.

**Model Refinement**. By the advent of microelectrodes in the 1970s, it was possible to measure substrate concentrations within the biofilm directly. It was discovered that these concentrations could vary greatly over tiny distances within the biofilm (Fig. 1). This finding led to the development of mechanistic models which described the processes taking place in biofilms on the basis of physical laws. They explained the substrate concentrations in biofilms as a result of the interactions between "substrate transport" and "substrate

Fig. 1: Oxygen profile in the biofilm measured by a microelectrode and calculated with a model.





Hansruedi Siegrist, Eawag

Fig. 2: Scanning electron microscope image of a biofilm. Length of the white bar = 10 µm.

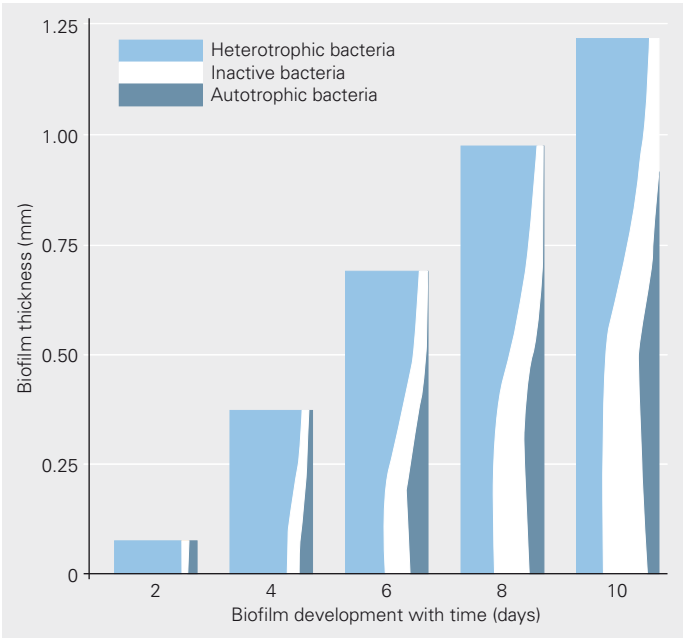



Fig. 3: Biofilm growth and change over time of the relative proportions of different microbial species from the substratum (bottom) to the biofilm surface (top).

consumption by micro-organisms". However, they were based on the simplifying assumption that the various micro-organism types are distributed evenly throughout the biofilm. Models permitting the calculation of the microbial composition in biofilms could only be worked out after microscopic imaging had revealed that microbial species distribution can vary enormously, as a function of substrate distribution (Fig. 2). The first of such models was developed at Eawag in 1984. It enabled us to calculate the spatial distribution and temporal changes of the various micro-organism types throughout the biofilm depth (Fig. 3). Over the following years, new experimental insights were gradually integrated into the model.

**Today's Models Reflect the Complexity of Biofilms.** Today, all of the transformation and transport processes, which are known to be relevant for the behaviour of biofilms, are integrated in the Eawag model [1]. The transformation processes include:

- substrate consumption and production,
- growth, inactivation and decomposition of micro-organisms.

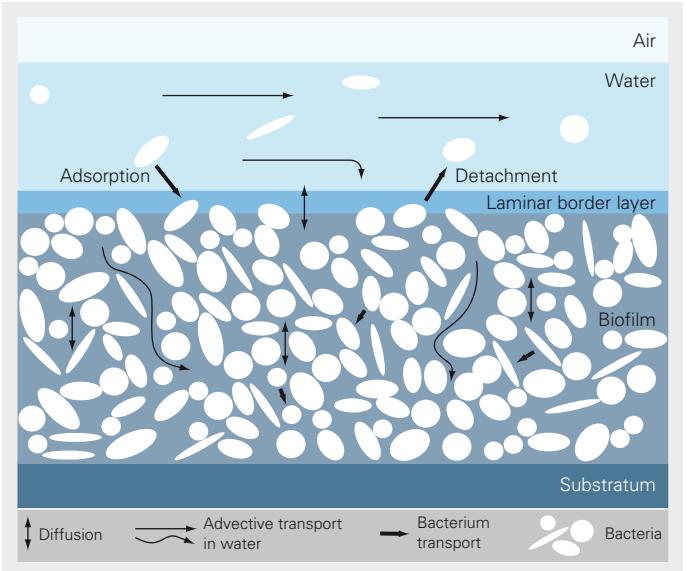
The transport processes describe (Fig. 4):

- transport of the substrates through advection and diffusion from the overlying water to the biofilm surface, and further into the large water-filled pores and tight cell intermediary spaces of the biofilm,
- adsorption and detachment of micro-organisms at the biofilm surface,
- active and passive displacement of micro-organisms in the biofilm,
- volume changes of the biofilm matrix as a consequence of growth and death of micro-organisms within biofilms.

The model is part of the Aquasim simulation programme (see box) developed at Eawag, and can be relatively simply applied to solve practical problems. It has, though, one serious limitation: it assumes that biofilms consist of homogeneous, dense layers of micro-organisms, and that any spatial gradients of micro-organisms and substrates are relevant only in the direction perpendicular to the substratum, and are insignificantly small in other dimensions.

Since the 1990s, however, we have known that biofilms exhibit a great range of spatial structures: they can, for example, be pervaded by large pores or possess mushroom-shaped outgrowths (Fig. 5, top right). Consequently, a new generation of multi-dimensional models [2] attempts to simulate the full range of possible

Fig. 4: Transport processes for micro-organisms and substrates accounted for in the Eawag model.



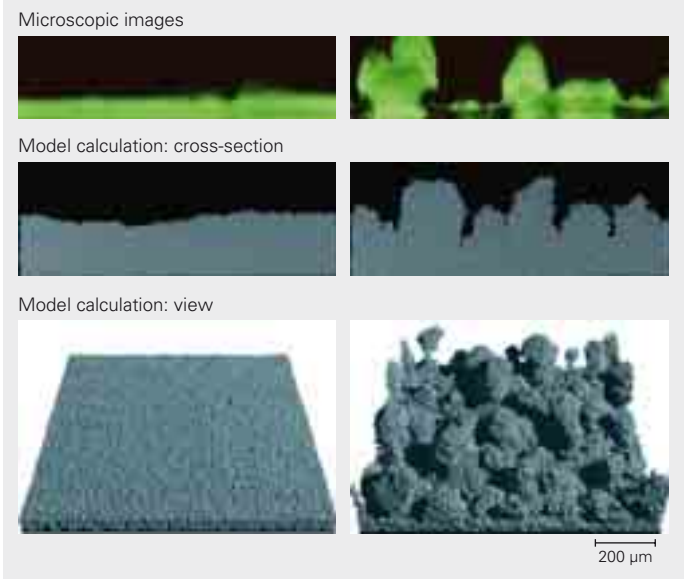
**The Aquasim Simulation Programme**

The computer programme Aquasim was developed by Eawag and is used worldwide for the identification and simulation of aquatic systems [4]. The programme also contains built-in mathematical functions for statistical data analysis. With the "parameter estimate" function, it attempts to determine unknown values of model parameters by iteratively best-fit matching time-series of calculated and measured values. With the "sensitivity analysis" function, it investigates whether the time series of the calculated values are affected noticeably by a change in the value of a model parameter. Only in this case can the parameter value be determined with the measured time series. Aquasim contains various models, in part developed at Eawag, for environmental systems such as rivers and lakes, one of which is a model which enables the simulation of biofilm systems with several microbial species and substrates [1]. After input of the necessary biological, chemical and physical data, Aquasim calculates the performance of a biofilm reactor or the substrate uptake of a biofilm in an aquatic ecosystem. Furthermore, it models the growth of the biofilm and the spatial distribution of the microbial species and substrates over the biofilm depth, the temporal change of these values in the water phase outside and inside the biofilm, as well as the exchange of substrates, micro-organisms and particles between the biofilm and the water flowing over it.

structures (Fig. 5, centre and bottom). However, they are limited in practice since they are exceptionally calculation intensive.

**So, Which Model is Best to Solve a Specific Problem?** To answer this question an in-depth study was undertaken by researchers from six different countries. A series of typical problems were solved by the models available today and the results of the calculations compared across the board [3]. It was revealed that the suitable model depends primarily on the problem to be solved: for example, to describe the development of a small cluster of micro-organisms of a certain species embedded in a biofilm and the substrate concentration around the cluster, a two or three-dimensional model was best. To calculate the effluent concentrations for a biofilm reactor with heterotrophic and autotrophic micro-organisms, the application of the one-dimensional Eawag model is still recommended. To represent a biofilm in which only one microbial species and one substrate are significant, simple models could still be used. Thus the study came to the conclusion that in practice the old, simplistic models give almost as good results for many problems as the new, more complex models. ○ ○ ○

Fig. 5: Different spatial structures of *Pseudomonas aeruginosa* biofilms imaged by a confocal laser scanning microscope by Soren Molin, TU Denmark in Lyngby (top) and simulated by a model (centre and bottom) [5].




[1] Wanner O., Reichert P. (1996): Mathematical modeling of mixed-culture biofilms. Biotechnology and Bioengineering *49,* 172–184.
[2] Wanner O. (2002): Modeling of biofilms. In: Encyclopedia of Environmental Microbiology (ed. G. Bitton). John Wiley & Sons, New York, pp. 2083–2094.
[3] Wanner O., Eberl H.J., Morgenroth E., Noguera D.R., Picioreanu C., Rittmann B.E., van Loosdrecht, M.C.M. (2006): Mathematical modeling of biofilms. Scientific and Technical Report *18,* IWA Publishing, London, 179 p.
[4] Reichert P. (1998): Aquasim 2.0 – User Manual. Eawag, Dübendorf.
[5] Picioreanu C., van Loosdrecht M.C.M., Heijnen J.J. (1998): Mathematical modeling of biofilm structure with a hybrid differential-discrete cellular automaton approach. Biotechnology and Bioengineering *58,* 101–116.

# Biofilms in the Tagliamento

Michael Doering, geographer and PhD student and Urs Uehlinger, biologist and scientist in the department of "Aquatic Ecology".

Biofilms play an important role in the metabolism of streams and rivers. In the Tagliamento River, their growth and activity depend essentially on the water exchange between surface waters and the sediments in the river bed.

In running waters, biofilms grow on the surface of stones in the riverbed as well as on water saturated subsurface sediments, the hyporheic zone. Their development is influenced by a number of factors such as shading, feeding of insects, the nutrient content of the water, the morphology of the river bed and the discharge regime. Further, the exchange between the surface water and the interstitial water of the hyporheic zone plays an important role.

Generally the strength of this exchange increases with a higher variability of the river morphology, i. e when fast and slow-flowing stretches alternate (riffle-pool sequence) and when the riverbed is subject to sediment movement during periods of high water flow. What happens, however, if the entire surface water runs exclusively through the hyporheic zone for kilometers and reappears at the surface again much further downstream? This situation can be found in the Tagliamento River. What influence does this have on biofilm development?

**The Tagliamento River, One of the Last Free Flowing Rivers in Europe.** This last large natural Alpine river originates in the Venetian Alps and drains, after about 170 km, into the Adriatic sea between Venice and Triest [1, 2]. Its average discharge in the mid section is about 110 $m^3$ per second (mid section), but can exceed more than 4000 $m^3$ per second during local storm events and heavy rainfall especially in autumn. Between river kilometer 92 and 114, the river exclusively runs in the hyporheic zone during dry seasons.

For one and a half years, we measured the discharge between kilometer 85 and 125 (Fig. 1). Up to kilometer 114, in the so-called losing zone, the Tagliamento lost between 1.6 and 4.5 $m^3$ of water per second and kilometer to the hyporheic zone. The end of this zone is defined by a layer of impermeable marine sediments of the former coastline, the "linea delle risorgive" acting as an aquiclude. In the adjacent gaining zone, surface discharge increases by about 0.2 to 0.4 $m^3$ per second and kilometer. The amount of surface water lost to the hyporheic zone and the length of the dry section depend on the flow conditions in the Tagliamento River. During long dry periods, as recorded in July and August 2003, surface flow lacks completely over a length of more than 20 km. In contrast, during periods of high water level as for example in October 2004 the river is characterized by continuous surface flow (Fig. 1).

**Characterization of the Losing and Gaining Zone.** The vertical hydrological gradient (VHG) emphasizes the results of the discharge

Fig. 1: Discharge of the Tagliamento River in the losing and gaining zone.

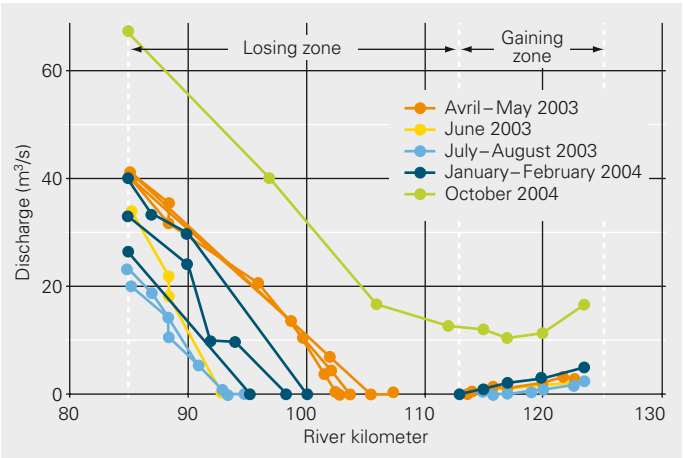
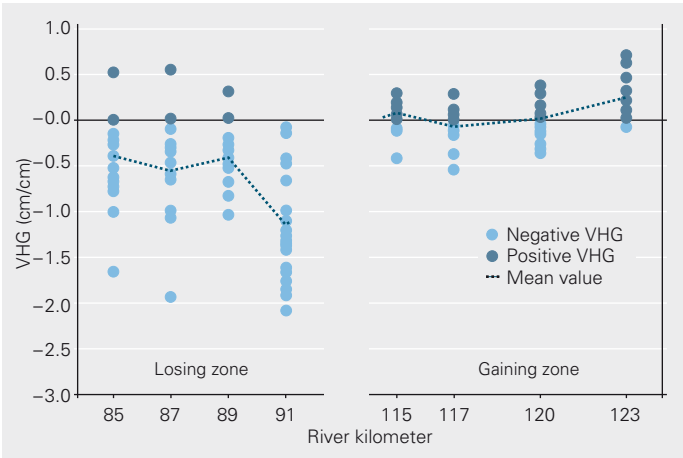



Fig. 2: Vertical hydrological gradient (VHG) in the losing and gaining zone.



Dimitry van der Nat, Eawag

The losing zone of the Tagliamento River. Seepage of the Tagliamento and the dry river bed.

The beginning of the gaining zone of the Tagliamento River.

Michael Döring, Eawag

measurements (Fig. 2). For the measurement of the VHG, PVC pipes driven about 50 cm deep into the sediments are used. The lower 10 cm of these pipes are perforated, to ensure an unlimited water exchange. After some time, the water levels inside and outside of the pipes are compared: if the level of the water in the tube is lower than that outside, the river loses water to the hyporheic zone (negative VHG); contrarily, i. e. when the level of the water in the pipe is higher than outside, water enters the river from the hyporheic zone (positive VHG).

Depending on the direction of this water exchange, habitat conditions change. The daily and seasonal temperature fluctuations are lower in the gaining zone than in the losing zone. Differences in nutrient concentrations are also detectable: e. g. average nitrate concentration in the losing zone (0.75 mg per liter) was lower than in the gaining zone (1.1 mg per liter). This can be partly explained by nitrification i. e. the conversion of ammonium to nitrate by bacteria in the hyporheic biofilm.

**More Biofilm in the Gaining Zone than in the Losing Zone.** For the quantification of the biofilm abundance, 5 stones were collected at each of 4 sites in the losing and gaining zone. The biofilm on the stones was scraped off with a wire brush, weighed and ashed. The ash-free dry weight (AFDW) per $m^2$ of stone surface was then calculated and used as a measure of biofilm abundance (Fig. 3A). On average biofilm abundance was twice as high in the gaining zone (25.3 g per $m^2$) than in the losing zone (12.2 g per $m^2$). The reason for this is presumably the higher nutrient concentration in the gaining zone. Changing environmental conditions are responsible for seasonal variations of biofilm abundance. The higher abundance in July and August was probably the result of favorable light and temperature conditions during the summer months. High discharge in conjunction with scouring of the sediment in August and October (Fig. 3C) reduced the biofilm abundance. In the following winter, the amount of biofilm recovered.

**Active Biofilm in the Losing Zone.** The activity of the hyporheic biofilm was determined by its respiration activity i. e. the amount of oxygen micro-organisms consume while decomposing organic matter. We used PVC cylinders of a defined size, half filled with hyporheic sediment and half filled with river water. Initial oxygen content was measured with an oximeter. Subsequently, the sealed cylinder was buried into the sediment. After four hours, the cylinder was removed from the sediments and measured for oxygen content again (see cover picture). Oxygen consumption was calculated by the difference between initial and final oxygen concentration. Average respiration activity was about twice as high in the losing zone (0.4 mg of oxygen per kg sediment and hour) than in the gaining zone (Fig. 3B). High-quality organic substances (e. g. algae) accumulating in the hyporheic zone by downwelling may account for these differences.

Large-scale exchange processes and their effects on habitat conditions exhibited a considerable influence on biofilm development. These findings help to upscale results from small-scale investigations (riffle-pool sequence) to large natural rivers. Overall the study has demonstrated the importance of an extensive hyporheic zone for natural rivers. However, the function of such hyporheic zones is limited in today's mostly regulated rivers. ○ ○ ○

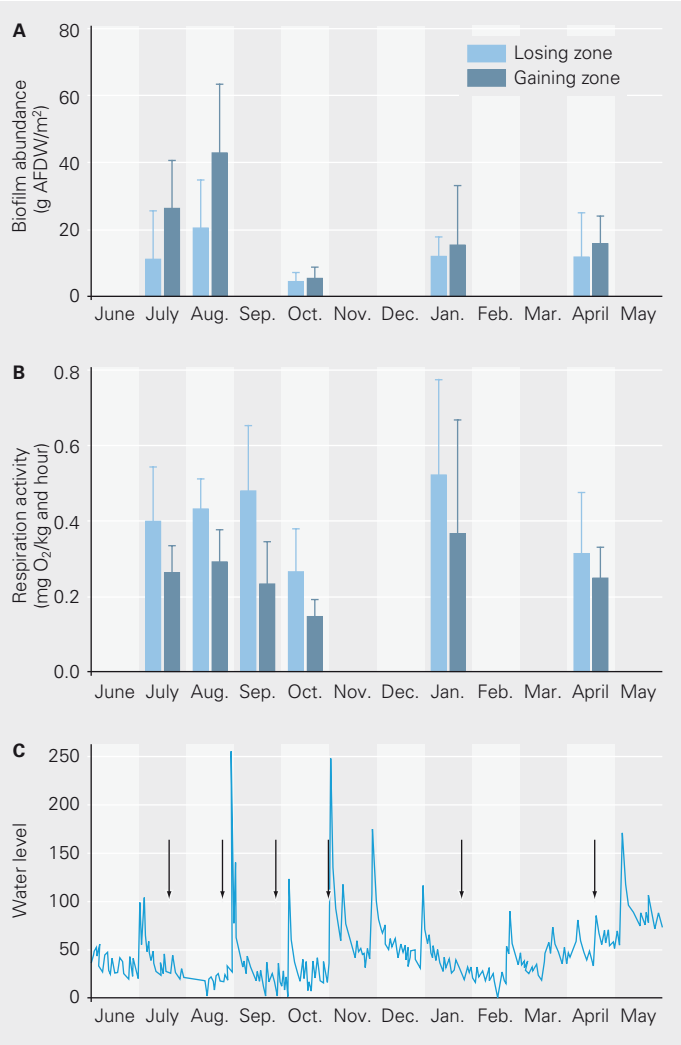



Fig. 3: Biofilm abundance at the river surface (A), respiration activity of the hyporheic biofilm (oxygen consumption per kg sediment <8 mm and hour at 20°) (B), and water level of the Tagliamento River (C) from June 2003 to April 2004. Error bar = standard deviation, AFDW = ash-free dry weight.


[1] Tockner K., Ward J.V., Arscott D.B., Edwards P.J., Kollmann J., Gurnell A.M., Petts G.E., Maiolini B. (2003): The Tagliamento River: a model ecosystem of European importance. Aquatic Sciences *65,* 239–253.

[2] Tockner K., Ward J.V., Edwards P.J., Kollmann J., Gurnell A.M., Petts G.E. (2001): Der Tagliamento (Norditalien): Eine Wildflussaue als Modellökosystem für den Alpenraum. In: Laufener Seminarbeitrag, Laufen/Salzach: Bayerische Akadademie für Naturschutz und Landschaftspflege, S. 25–34.

Sabine Sibler, engineer in environmental protection and scientific employee and Maria Dittrich, physicist and head of the "Biomineralisation" group in the "Surface water" department.

# Calcite Precipitation on Cyanobacteria

Calcite, or calcium carbonate $CaCO_3$, is found as a natural product in calcium-rich lakes all over the world. It precipitates in the water column and is then deposited at the bottom of the lakes. What actually causes the formation of calcite?

Large amounts of calcite are formed in the course of a year in Swiss lakes too. The precipitation rate in Lake Sempach and Lake Lugano is estimated at about 2500 and 8000 t per annum respectively [1]. Two observations indicate that cyanobacteria (see box) are involved in the precipitation of calcite: In Lake Lucerne, we found particles, very probably cyanobacteria, whose surface was completely covered with calcite crystals (Fig. 1). In particular, a large amount of calcite is precipitated during the period when these photosynthetically active organisms are "in full bloom" [2]. This led to an initial link being made between calcite precipitation and the photosynthesis during which $HCO_3^-$ is taken up by the cells. Our experiments with both photosynthetically active and photosynthetically inhibited cyanobacteria, however, spoke against this idea as in both cases calcite crystals were precipitated on the cells. It is now assumed that the calcite precipitation is encouraged by substances on the surface of the cells.

Cyanobacteria exist either like plankton, i. e. freely floating in water, or form a biofilm on solid substrates such as floating particles or aquatic plants [2]. Like many other micro-organisms, cyanobacteria form so-called extra-cellular polymeric substances on their cell surface (EPS, see leading article). Especially large quantities of EPS are deposited when cyanobacteria or other micro-organisms form a biofilm. They serve as "glue" between the cells [3]. In our research project, we pursued the particular question of whether the EPS on the cyanobacteria caused the precipitation of calcite. Additionally, we wanted to characterise the surface and EPS qualities of the cyanobacteria more precisely.

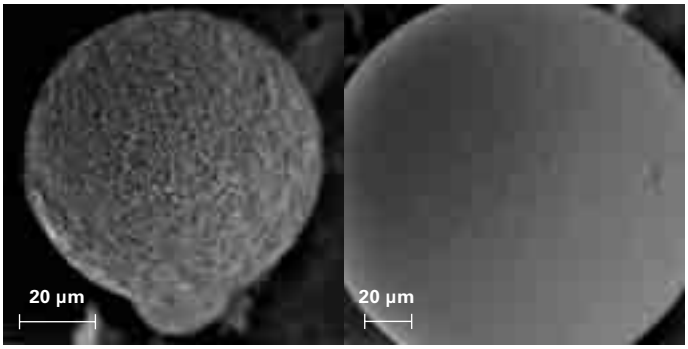
**EPS from Two Different Strains.** For our experiments, we employed two differently pigmented strains of the cyanobacterium *Synechococcus elongatus:* one was coloured by phycoerythrine red (syn-red) and one by phycocyanine green (syn-green). The syn-red strain was isolated from Lago Maggiore and syn-green from the Plönersee in northern Germany. The EPS was separated from the cyanobacteria using phenol extraction, freeze-dried and resuspended in water before being further used.

For characterisation, we separated the EPS samples by means of agarose gel electrophoresis and were able to observe band patterns that were clearly divergent. From this we concluded that the EPS from the two *Synechococcus* strains are distinguished by the sizes of their molecules.

Fig. 1: Rod-shaped particles from Lake Lucerne, completely covered with calcite. These are, in all probability, cyanobacteria.

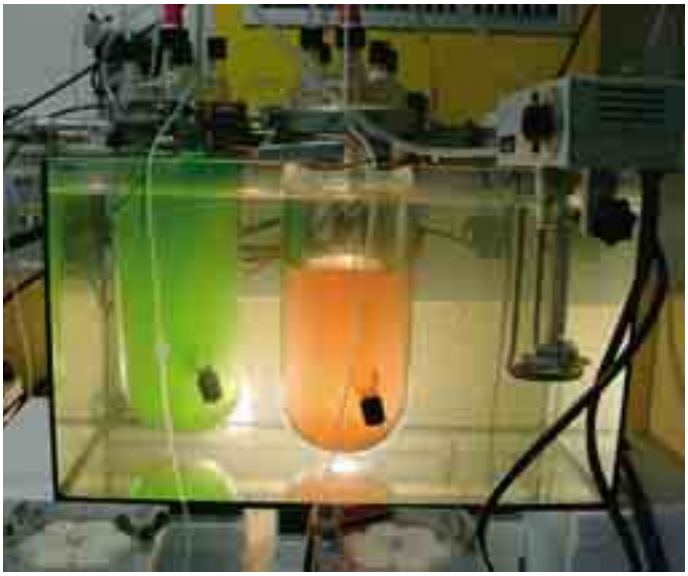
**Cyanobacteria and Calcite Precipitation**

Cyanobacteria belong to the group of the so-called pico-plankton [2]. Although this group usually dominates the entire phytoplankton biomass of oligotrophic lakes and oceans, they were only discovered about 20 years ago [4]. This was because of the extremely small size of the organisms which lies between just 0.5 and 3 µm. As opposed to classical phytoplankton with sizes of up to 100 µm, however, the cyanobacteria are not algae but bacteria. Like algae, the cyanobacteria also act as primary producers and are an important starting point in the food chain; i. e. they perform photosynthesis and convert carbon dioxide, water and inorganic salts with the aid of solar energy into sugar and organic substances, thus in the long run supplying all aquatic organisms with food.




Fotos: Maria Dittrich, Eawag

Fig. 2: Calcite crystals form on the surface of EPS-coated agarose globules, (left); on the other hand, no calcite is deposited on uncoated control-group globules (right).



Culture reactors with green and red-pigmented cyanobacteria.

**Characterisation of the Functional Groups on the Bacteria Surfaces.** A functional group is defined as a terminal reactive group (Tab. 1). These groups are responsible for the chemical behaviour of a substance in the environment and determine how rapidly and with which other groups the substance prefers to react and which kind of bond is created. With the aid of acid-base titration and subsequent data evaluation using the FiteQL computer model for bacteria surfaces, we succeeded in determining the percentages of the different functional groups on the surfaces of the syn-red and syn-green strains (Tab. 1). For both strains, the percentage of carboxyl groups is highest and is followed of the amino and phosphate groups [5].

However, with this method we did not only measure the functional groups of the EPS but also those of the bacterial cell wall. In order to get an idea of which particular functional groups are available in the EPS, we carried out the titration experiments in an analogous way with EPS isolated from syn-green. It turned out that in the EPS, no other functional groups other than carboxyl groups are found, and that 90 % of the entire carboxyl groups on the cell surface proved to be in the EPS with only 10 % occuring on the cell wall.

**Are the EPS Actually Involved in Calcite Precipitation?** In order to answer this question, the isolated EPS were fixed onto agarose globules with a diameter 50–150 µm. These coated globules were then incubated for 5 days in a $NaCO_3/CaCl_2$ solution. We did the same with uncoated agarose globules. If the EPS are responsible for the formation of calcite, we should have been able to find calcite crystals on the coated globules but not on the uncoated globules of the control group. This was in actual fact the case: under the scanning-electron microscope (SEM) (Fig. 2), we saw that the agarose globules, independent of whether they were coated with EPS from syn-red or syn-green, were completely covered with calcite crystals. On the other hand, no calcite had been formed on the uncoated control-group globules.

As a result of our experiments, we were able to verify that the EPS on the surface of the cyanobacteria actually act as crystallisation points for calcite precipitation [5]. Further, our results allow us to assume that cyanobacteria not only play an important role in the calcium cycle of aquatic systems but also that, thanks to their large surface area, the calcite crystals may co-precipitate other metals, like poisonous heavy metals, for example. This would mean that cyanobacteria could also be of great importance in the self-cleaning of lakes and rivers. ○ ○ ○

Tab. 1: Proportion of functional groups on the cell surfaces of the two *Synechococcus* strains examined, syn-red and syn-green.

| Functional groups | Formula | Syn-red | Syn-green |
|---|---|---|---|
| Carboxyl group | –COOH | 44 % | 37 % |
| Amino group | –NH | 26 % | 36 % |
| Phosphate group | $-PO_4^{2-}$ | 26 % | 27 % |

[1] Ramisch F., Dittrich M., Mattenberger C., Wehrli B., Wüest A. (1999): Calcite dissolution in two deep eutrophic lakes. Geochimica et Cosmochimica Acta *63,* 3349–3356.
[2] Dittrich M., Kurz P., Wehrli B. (2004): The role of autotrophic picocyanobacteria in calcite precipitation in an oligotrophic lake. Geomicrobiology Journal *21,* 45–53.
[3] Sutherland I.W. (2001): Biofilm exopolysaccharides: a strong and sticky framework. Microbiology *147,* 3–9.
[4] Weisse T. (1993): Dynamics of autotrophic picoplancton in marine and freshwater ecosystems. In: J.G. Jones (Ed.), Advances in microbial ecology 13. Plenum Press, p. 328–370.
[5] Dittrich M., Sibler S. (2005): Cell surface groups of two picocyanobacteria strains studied by zeta potential investigations, potentiometric titration, and infrared spectoroscopy. Journal of Colloid and Interface Science *286,* 487–495.

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Co-authors: W. Ruperez, B. Wagner, D. Kistler, L. Sigg, E. Navarro, C. Robinson

# What Effects do Metals Have on Algal Biofilms?

Much smaller quantities of metals are currently discharged into water bodies than in the past. However, even low metal concentrations can have negative impacts on water organisms and thus on the whole ecosystem. This is the challenge for ecotoxicologists. It is important to draw up concepts and methods for assessing the ecological consequences.

Algal biofilms react very rapidly to elevated metal concentrations in waters. We know that biofilms take up metals from the water and accumulate them and that biofilms activate mechanisms for metal detoxification (see also articles by S. Meylan on p. 19 and S. Le Faucheur on p. 22). However, it cannot be established on the basis of these two processes whether the metals actually have a negative effect on the algae and whether consequently this leads to changes at the level of the community.

The aim is therefore to develop a multi-level investigation method which allows to identify both the secondary reactions and the causal relationships [1]. Thus, the observed ecological effects may be traced back to the metal stress.

For our investigations, we grew algal biofilms in so-called microcosms (cf. box) and exposed them to elevated concentrations of the two heavy metals copper (Cu) and cadmium (Cd).

**Cellular Effects: Metal Accumulation and Phytochelatin Formation.** Unlike in the past, we now have to deal with lower, often continuous, pollution levels from metals in water. We therefore set out firstly to investigate whether long-term exposure to low metal concentrations has the same effect at the cellular level as short-term exposure to greatly elevated metal levels: the algal biofilms grown in the microcosms were therefore exposed for 6 weeks to 2 different concentrations of both copper and cadmium (Cu-1 = 0.1 µM, Cu-2 = 0.5 µM, Cd-1 = 5 nM und Cd-2 = 50 nM). The tested copper and cadmium concentrations are respectively 3 and 15 times and 5 and 50 times higher than the quality targets laid down in the Water Protection Ordinance of 2 µg Cu (~30 nM) and 0.05 µg Cd per litre of water (~0.5 nM). The control biofilms, on the other hand, were incubated in water from the river Glatt whose level of Cu and Cd at 25 nM and 0.2 nM was just under the legal quality objevtives.

The algal biofilms will only be damaged, if the metals are taken up by the algae. As expected, the Cu and Cd concentrations rose in

### Microcosms for Experimental Investigation of Biofilms

Algal biofilms are grown and investigated under relatively natural conditions in so-called microcosms. These are containers or channels (Fig. 1) in which biofilms grow on carrier materials, in our case on small glass slides. Microcosms are operated with water from rivers either statically (water is not changed), semi-statically (water is changed after a time) or as flow-through system (continuous inflow of fresh water). The microorganisms contained in the water colonise the surfaces provided in the microcosm. Mixed biofilms are formed in the microcosms and their species composition is comparable to biofilms occurring in the water body. This allows a comparison of the effects identified in experiments with single species with the reactions of a mixed community and an evaluation of their ecological relevance. In our study, the microcosms were operated semi-statically with water from the river Glatt.

Fig. 1: Microcosm installation, in which natural algal biofilms are grown and exposed to elevated metal concentrations.

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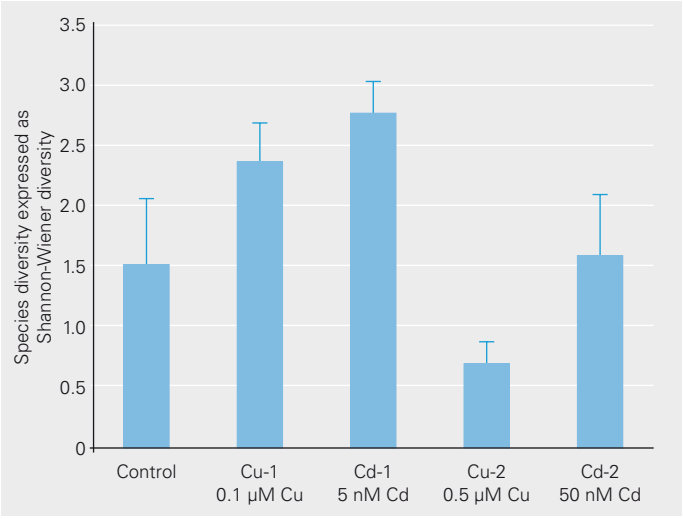
the biofilms after metal exposure and remained unchanged in the control biofilms (Tab. 1).

We also determined the content of phytochelatins; these peptides are formed in the presence of elevated metal concentrations and play a part in detoxification of metals. It was shown that more phytochelatins were formed in the biofilms exposed to the metals. However, we also detected phytochelatins in the control biofilms (Tab. 1). Thus, it is not possible to infer definitive conclusions regarding the metal contamination.

**Effects at Community Level: Population Size of Individual Algae Species and Species Diversity.** Which and how many species of algae occur in a biofilm is determined both by the environmental conditions and the physiological characteristics of the individual species. Accordingly, another important question is how chronic, low-level metal pollution affects the population size of individual species and the species diversity in the biofilms. We therefore examined the biofilms under the microscope after a 6-week exposure to the metals and noted which species occurred and how often they were found.

The biofilm communities reacted very sensitively to small changes in the metal concentrations. It appeared that on the one hand, metal stress affected the population size of individual species in the biofilm: some species decreased or disappeared completely, others became more abundant. On the other hand, species diversity changed, expressed here by the so-called Shannon-Wiener diversity index (Fig. 2). The higher this index, the greater the species diversity. There was a loss of diversity with the Cu-2 concentration. Probably in this case the most sensitive species are excluded from the biofilm community. Diversity after exposure to Cd-2, however, was similar to the control, even though the two biofilm communities were composed of different species. With exposure to Cd-1 or Cu-1, there was even an increase in species diversity. This is probably due to the decrease of species that are dominant in the absence of metal stress, whilst previously less abundant species do prevail in presence of metal stress.

Fig. 2: Species diversity in the algal biofilms studied, expressed as Shannon-Wiener diversity index, after 6-week exposure to different metal concentrations.




| Parameter | Copper biofilms | Cadmium biofilms |
|---|---|---|
| Copper accumulation | ↑ | not exposed to Cu |
| Cadmium accumulation | not exposed to Cd | ↑ |
| Phytochelatin content | ↑<br>Problem: ↑ also in control biofilms | ≠↑ |
| Population sizes of individual species | depending on species: ↑, — or ↓ | depending on species: ↑, — or ↓ |
| Species diversity | ↑ or ↓ | ↑ or ↓ |
| Metal tolerance | ↑ towards Cu + Cd | ↑ towards Cd |

Tab. 1: Reactions of the studied biofilms to metal stress.
↑ = increasing, — = remaining constant, ↓ = decreasing.

In order to determine whether the changed species distribution in the biofilms can actually be attributed to the metal stress, the data were analyzed statistically. It was shown that some types of algae correlated negatively and others positively with the copper content of the water and with copper accumulation in the biofilm. The correlation analysis for cadmium was less informative.

**Physiological Effects: Metal Tolerance.** On the one hand, algae have different strategies for coping with excess metals [2] and on the other, react with a varying degree of sensitivity to metals. Metals have a correspondingly variable effect on biofilm communities in which several species occur: sensitive species are damaged in their physiological performance or are even eliminated, whilst tolerant species are favoured. As a result, metal stress-induced succession takes place in the biofilm. It is assumed that the newly formed algal community becomes overall more tolerant to the metal which has restructured the community [3]. We wanted to test this statement.

Algal biofilms which had been exposed for 1–6 weeks to Cu-2 or Cd-2 concentrations were, in a second step, exposed for three hours to much higher metal concentrations (20 µM Cu, 25 µM Cd). We subsequently measured the photosynthesis activity and determined the extent to which photosynthesis was inhibited by comparison with the control biofilms. Although, as in the control biofilms, inhibition after 1–3 weeks was still at around 60 % for Cd and 80 % for Cu, we observed a clear increase in tolerance after 6 weeks (Fig. 3). Both communities actually became more tolerant towards the metal which caused the succession. In addition, the biofilms exposed initially to Cu-2 were also better protected against Cd stress. We concluded that different defence mechanisms were triggered in the Cu and Cd biofilms. The processes which are effective in the Cu communities probably also help to cope with Cd stress.

**Metal Stress Clearly Diagnosable.** We were able to show by our experiments that chronic exposure of biofilms to slightly elevated copper and cadmium concentrations has impacts at all levels of the biological organisation (Tab. 1). Species diversity proved to be the most sensitive parameter. In the case of the Cu biofilms, the

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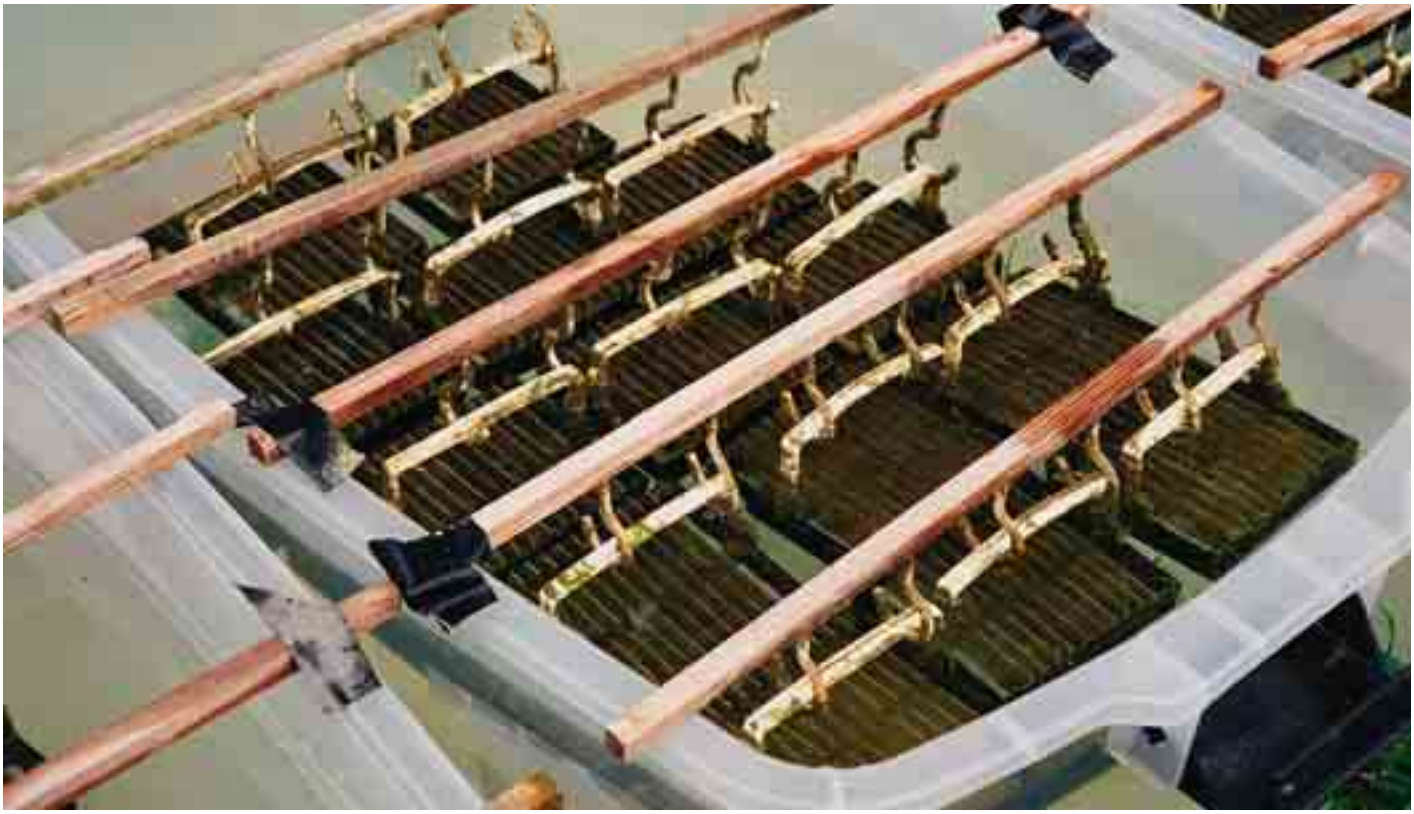
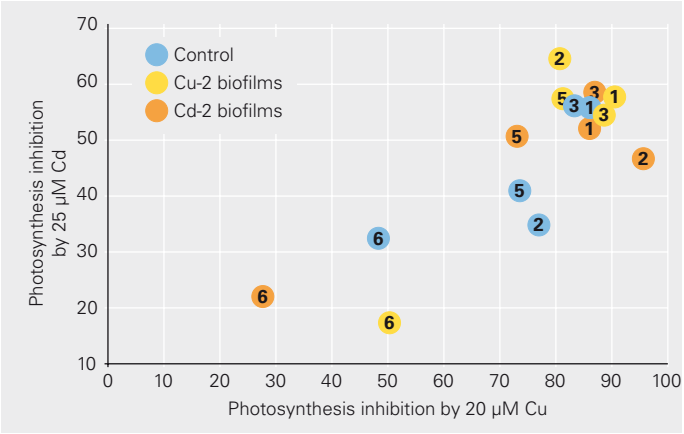


Algal biofilms in microcosms.

tolerance experiments point clearly to a causal relationship between changed diversity and metal exposure. If accumulation data are also included in the assessment, then the structural changes in the Cu communities can be ultimately traced back to the Cu stress. In the case of the Cd biofilms, the raised Cd tolerance is clearly due to the Cd stress, which is again confirmed by the accumulation data. Tolerance analyses are therefore an important tool for detecting effects which are due to metal-induced changes to the biofilm community. ○ ○ ○

Fig. 3: Temporal development of metal tolerance in control biofilms and Cu-2 and Cd-2 biofilms. The figures indicate how many weeks the biofilms were exposed to low Cu-2 or Cd-2 concentrations, before being exposed in a 3-hour short-time test to higher concentrations of Cu or Cd. The less photosynthesis is inhibited, the greater the metal tolerance.




[1] Eggen R.I.L., Behra R., Burkhardt-Holm P., Escher B.I., Schweigert N. (2004): Challenges in ecotoxicology. Environmental Science & Technology *38,* 58A–64A.

[2] Soldo D., Hari R., Sigg L., Behra R. (2005): Tolerance of *Oocystis nephrocytioides* to copper: intracellular distribution and extracellular complexation of copper. Aquatic Toxicology *71,* 307–317.

[3] Soldo D., Behra R. (2000): Long-term effects of copper on the structure of freshwater periphyton communities and their tolerance to copper, zinc, nickel and silver. Aquatic Toxicology *47,* 181–189.

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# Metal Accumulation in Algal Biofilms

When it rains, the metal content of rivers can increase dramatically. Algal biofilms react particularly sensitively to such changes. They have a tendency to accumulate metals. Uptake by the algae depends on whether the metals are present in free or bound form.

Metals such as copper and zinc are important trace nutrients for water organisms, but they can also be toxic when concentrations are too high. However, whether metals have a harmful effect depends essentially on the availability of the metals for the organisms. This so-called bioavailability is influenced in turn by the speciation of the metals. Speciation describes the chemical form in which the metals are present. Metals are readily available when they occur freely in the water, i. e. when ions such as copper $Cu^{2+}$ or zinc $Zn^{2+}$ are bound only to water molecules. The metals are less readily available when they form complexes with weak organic or inorganic ligands, and they are not available at all when they are bound to strong organic ligands.

During sampling in the Furtbach.

Eawag

In line with this, laboratory studies showed that algae accumulate metals when the concentration of free metal ions increases [1, 2]. As yet, these relationships have scarcely been studied in natural waters because these systems are much more complex. We wanted to close this gap and investigated in a field study whether algal biofilms in a stream accumulate more metals when the metal content of the water temporarily increases during the course of a rain event.

**Study in a Small Stream.** We conducted our experiments in the Furtbach in the canton of Zurich. The Furtbach flows through a predominantly agricultural area and the industrial zone at Regensdorf to the Limmat. It takes up the treated waste water from the Regensdorf sewage plant. Because this sewage treatment plant had insufficient capacity to treat the waste water adequately in the eighties, the stream sediments below the discharge point are heavily contaminated with metals. Since construction of the new sewage plant in the year 2000, the level of pollution in the stream has fallen. Nevertheless, the metal-contaminated sediments continue to pass metals into the stream water. This happens to an even greater extent during rain when the sediments are stirred up by the increased flow.

We investigated whether the copper and zinc concentrations in the Furtbach changed when it rained and analysed in what speciation the metals occurred in the water. At the same time, we determined the metal accumulation in natural algal biofilms [3]. These biofilms formed on glass surfaces which we installed on the stream bed. As soon as Meteo-Swiss (the Swiss Meteorological Office) forecasted heavy rain, we went out into the field to take samples.

**Effect of Rain on Metal Speciation.** Our results show that total concentrations of the dissolved metals copper and zinc in the Furtbach increased during rainfall (Fig. 1). Figures 2A + B give a breakdown of the proportions of bioavailable metal species in the total

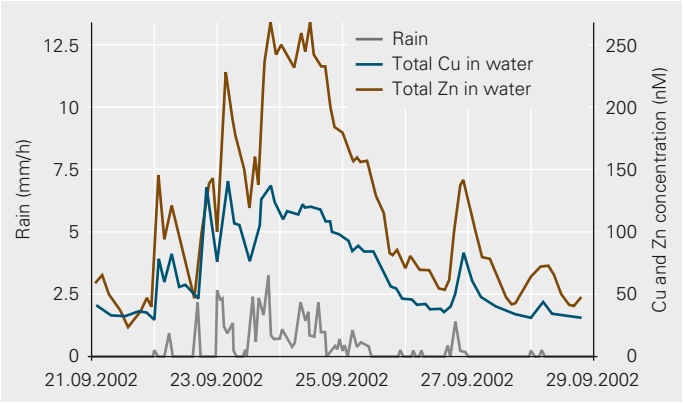



Fig. 1: Copper and zinc concentrations in the Furtbach vary with the amount of rain.

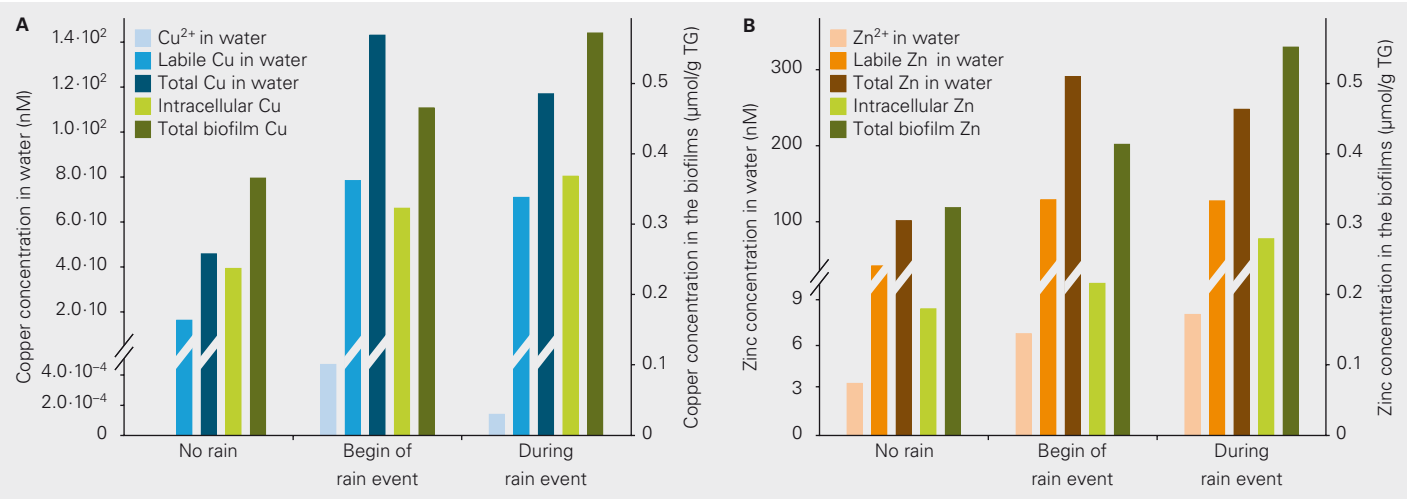
concentration: they show the dissolved free $Cu^{2+}$ and $Zn^{2+}$ ions and the labile metal species (= free ions + weakly complexed metals). Concentrations of most copper and zinc species continually increase during rainfall. This is due to desorption of the metals from the sediment. The only exception are the free $Cu^{2+}$ ions. After an initial increase, their concentration drops again over the later course of the rain event. Unlike the $Zn^{2+}$ ions, probably a larger proportion of the desorbed $Cu^{2+}$ ions combine with the ligands present in the water. It is likely that the $Cu^{2+}$ ions have a greater affinity with the ligands than the $Zn^{2+}$ ions or there are more ligands which can only form complexes with $Cu^{2+}$ ions.

**Accumulation of Copper and Zinc in the Biofilm.** The algal biofilms react extremely rapidly to the changing metal concentrations during rainfall. Soon after the start of the rain event and during the subsequent rainfall, the total levels of both metals copper and zinc continually increase in the biofilm (Fig. 2A + B). Once the rain has stopped, they begin to fall quite slowly, finally reaching their original values after about 2 days. The total content includes both intracellular metals and those adsorbed on the biofilm surface. Relatively large proportions of both metals are adsorbed on the biofilms when it rains. On the other hand, fewer metals are taken up by the algae into the cell interior (Fig. 2A + B). This is mainly due to the fact that the algae actively regulate the uptake of metals.

**Different Metal Species Determine Bioavailability.** Which metal species in the water are decisive for the bioavailability of metals and thus for metal accumulation in the algal biofilm? In order to answer this question, we studied the relationships between the intracellular metal contents and the different metal species in the water (Fig. 3). It was shown that there was a positive correlation between the intracellular zinc content and the concentration of the free $Zn^{2+}$ ions in the water. However, in the case of copper we observed a positive correlation between the intracellular copper content and the labile copper species. This finding is new. The reason probably lies in the extremely low concentration of free $Cu^{2+}$ ions in the water: in order for the algae to be able to cover their copper requirement at all, they must also have access to copper in its weakly complexed species. The bioavailability and thus the risk potential of copper and zinc are therefore determined by different metal species. We were able to confirm these results later in an experimental microcosm study [4].

**Relevance of the Results for Water Assessment.** In the short term – e.g. during heavy rainfall – increased copper and zinc concentrations in rivers have a measurable effect on natural algal biofilms. Copper and zinc are quickly taken up and only slowly eliminated again. We were able to prove under field conditions for the first time that speciation of the metals plays an important part in bioaccumulation. This should be taken into account in future assessments of water quality. At present, however, it is mainly the

Fig. 2: Concentrations of different copper (A) and zinc species (B) in the water of the Furtbach and in the algal biofilm. Before rain, the concentration of free $Cu^{2+}$ ions in the water is so low that it cannot be shown.

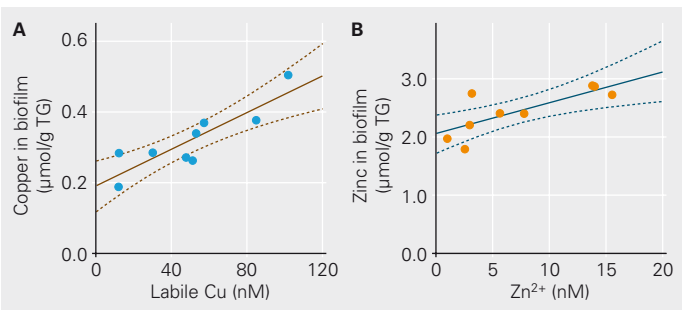


Eawag

The sampling system in the Furtbach. The algal biofilms grow on glass plates.

total concentration of a metal which is used as a parameter to assess the pollution load. In fact bioavailability of the metals and thus the risk potential for micro-organisms depends on the metal species present. Moreover, the complexing strength of the water also plays a part: type and quantity of the ligands present determine the proportion of complexed metal species. If the complexing strength is low, the majority of the metals are present as free ions i. e. in readily available form. In this case, negative effects on water organisms can occur even with a relatively low total concentration. Therefore in order to be able to assess pollution more accurately in future, it is sensible to develop simple field methods for detecting different metal species. ○ ○ ○

Fig. 3: The intracellular copper (A) or zinc content (B) is regulated by the concentration of labile copper species or free $Zn^{2+}$ ions.





[1] Knauer K., Behra R., Sigg L. (1997): Effects of free $Cu^{2+}$ and $Zn^{2+}$ ions on growth and metal accumulation in freshwater algae. Environmental Toxicology and Chemistry *16,* 220–229.

[2] Campbell P.G.C., Errécalde O., Fortin C., Hiriart-Baer V.P., Vigneault B. (2002): Metal bioavailability to phytoplankton-applicability of the biotic ligand model. Comparative Biochemistry and Physiology, C-Toxicology & Pharmacology *133,* 189–206.

[3] Meylan S., Behra R., Sigg L. (2003): Accumulation of copper and zinc in periphyton in response to dynamic variations of metal speciation in freshwater. Environmental Science & Technology *37,* 5204–5212.

[4] Meylan S., Behra R., Sigg L. (2004): Influence of metal speciation in natural freshwater on bioaccumulation of copper and zinc in periphyton: a microcosm study. Environmental Science & Technology *38,* 3104–3111.

Séverine Le Faucheur, chemist, did her PhD on this topic. Laura Sigg, chemist and head of the group "Biogeochemistry of metals".
Co-author: R. Behra

# Phytochelatins as Bioindicators of Metal Exposure?

Algae protect themselves against toxic metals with phytochelatins. They synthesize these short polypeptides when metal concentrations in the water are elevated. Is it possible to use phytochelatins as bioindicators for metal stress? We discovered some surprising answers.

Metals are naturally present in the environment and enter aquatic ecosystems through various processes such as soil erosion, precipitation, emissions from volcanoes etc. Metals are also found in industrial products, building materials and agrochemicals such as fertilizers which may then enter the environment and waters.

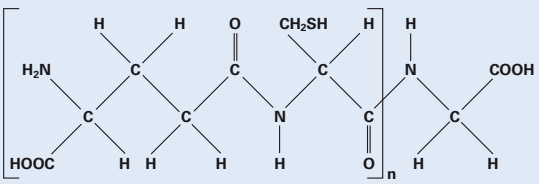
On the one hand, all organisms need metals to live, but on the other, metals can also have harmful effects depending on their concentration [1]. In order to protect themselves against this toxicity, algae have developed a special detoxification mechanism. They produce intracellular molecules, so-called phytochelatins ($PC_n$, see box). Their role is to bind the accumulated excess metals and thus render them harmless. Phytochelatin formation in algae is triggered by an elevated metal content in the water. We wanted to know whether we could evaluate metal pollution of water from the opposite direction, based solely on the phytochelatin concentration in algae and therefore, whether phytochelatin concentration is a suitable bioindicator.

**Are Phytochelatins Produced under Metal Stress?** It was important for us not to work as in the past with pure algal cultures under artificial conditions in the laboratory, but to study how algae behave in a natural system. For this reason, we tested the effects of the metals copper, zinc and cadmium in natural algal biofilms [2] which were grown in microcosms in the field (see also article by R. Behra p. 16). For five weeks, water was pumped through the microcosms from the river Glatt, which itself has only low concentrations of the studied metals: 25 nM copper, 34 nM zinc and 0.2 nM cadmium.

Algal biofilms were colonized on the glass slides mounted inside the microcosms. For our tests, we took the glass slides together with the biofilms and exposed them to metals for 24 hours. Water from the river Glatt, to which known quantities of copper, zinc or cadmium had been added, was used as the basis, so that the following metal concentrations were reached: 500 and 1500 nM copper, 250 and 1000 nM zinc and 1000 nM cadmium. The control biofilms were exposed to water from the Glatt without added metals. After exposure the biofilms were scraped from the glass slides and examined for their phytochelatin content. The phytochelatins were stepwise extracted from the biofilms by freeze drying, acidifying and centrifuging. After derivatization of the thiol

**What are Phytochelatins?**

Phytochelatins are small intracellular polypeptides with the amino acid sequence $(\gamma\text{-Glu-Cys})_n\text{-Gly}$, where n = 2–11. Due to their thiol (SH) and carboxyl groups (COOH), they have a very strong affinity to metals. They are formed enzymatically by algae and also by plants and fungi from glutathione, the predominant thiol. Phytochelatin production is induced when the metal content of the environment is increased. In an earlier laboratory investigation, we were able to detect this induction in the single-cell green alga *Scenedesmus vacuolatus*, after the cadmium content of the culture media had been raised experimentally [3].The algae produced the phytochelatins in different polymerisation grades depending on the cadmium concentration. In the literature, cadmium is often described as the strongest inducer, yet the production of phytochelatins is also induced by other metals such as copper, zinc or lead, although to a lesser extent. The production of phytochelatins also depends on the species of algae in question.



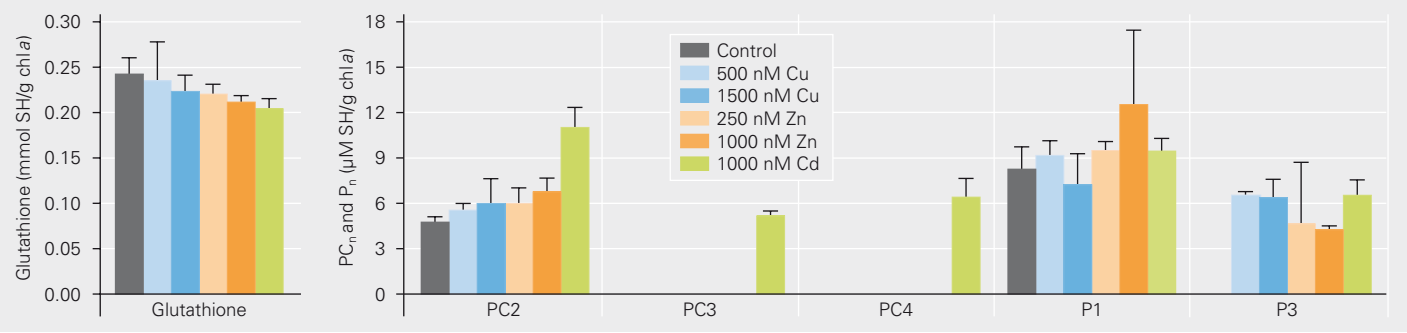



Fig. 1: Concentration of glutathione, phytochelatins (PC2-4) and other unidentified thiols (P1–3) in algal biofilms under metal stress.

groups (addition of a fluorescent tag to thiol groups), analyzes were conducted using high pressure liquid chromatography (HPLC).

**Unexpected Results.** Surprisingly, in addition to glutathione, the precursor for phytochelatins, we also detected the phytochelatin PC2 in the control biofilms (Fig. 1, grey bars). Biofilms which had been exposed to elevated copper concentrations (blue bars) showed neither a reduction in the glutathione content nor an increase in the phytochelatin content. Zinc and cadmium (orange and green bars) on the other hand caused a slight reduction in glutathione and a significant increase in PC2. With cadmium stress, moreover, the phytochelatins PC3 and PC4 were also synthesized. In addition, we observed two unidentified thiols, P1 und P3 in all biofilms. As only exception, the control biofilms did not synthesize P3.

**Other Influential Factors.** Unexpectedly, phytochelatins are already present in the algal biofilms even with low metal concentrations. Phytochelatin production seems therefore to depend also on other factors. Nutrients, light and temperature are known to affect the content of intracellular thiols. In addition, phytochelatins are synthesized from glutathione, and are therefore dependent on its concentration. Glutathione in turn is not only a precursor for phytochelatins but has also other tasks in the cell. For example, it participates in the detoxification of other pollutants and in defence mechanisms against oxidative stress. The glutathione content of the cell and thus the relative content of glutathione and phytochelatins are indirectly influenced in this way.

Moreover, the species composition of the algal biofilm can have an effect on the phytochelatin content. Thus, algal biofilms producing phytochelatins at low metal concentrations will contain more phytochelatins than biofilms which are less sensitive (in induction) to metals. In our natural biofilms, scarcely any more phytochelatins are formed with higher amounts of copper in the water. By contrast, phytochelatin production increases with elevated zinc and cadmium concentrations. The concept of using phytochelatins as bioindicators for zinc and cadmium stress should therefore be pursued further.

Thiol P3 seems to be another promising candidate. It is not found in the control biofilms, but is detectable after exposure to elevated copper, zinc and cadmium concentrations (Fig. 1). P3 may possibly be more suitable as a bioindicator for metal stress than the phytochelatins studied here. Further studies are therefore needed to clarify the role of the thiol P3 and to determine its structure.

○ ○ ○

**Metals –**
**Necessary for Life, but Toxic in High Doses**

All living organisms are dependent on certain so-called essential metals (copper, zinc, iron, nickel etc.). They are needed in very small quantities as co-factors for enzymes or proteins [1]. The non-essential metals include cadmium, mercury or lead. Depending on their concentration, both essential and non-essential metals act as cell toxins. The toxic action is based on the unspecific binding of the metals to important biomolecules and results in:

- functional groups being blocked,
- essential metals being displaced,
- or the active form (conformation) of the biomolecules being changed.


[1] Mason A.Z., Jenkins K.D. (1995): Metal detoxification in aquatic organisms. In: Tessier A., Turner D.R. (Eds.) Metal speciation and bioavailability in aquatic systems. John Wiley & Sons: Chichester, p. 479–608.

[2] Le Faucheur S., Behra R., Sigg L. (2005): Thiol and metal content in periphyton exposed to elevated copper and zinc concentrations: a field and microcosm study. Environmental Science and Technology *39,* 8099–8107.

[3] Le Faucheur S., Behra R., Sigg L. (2005): Phytochelatin induction, cadmium accumulation and algal sensitivity to free cadmium ion in *Scenedesmus vacuolatus.* Environmental Toxicology and Chemistry *24,* 1731–1737.

# Biofilters on the Test Bed

Adriano Joss, micro-biologist, and Max Maurer, chemical and process engineer, are both researcher in the "Environmental engineering" department.
Co-author: H. Siegrist

Biofilters are currently experiencing a revival in the area of wastewater treatment. In such systems, biofilms develop on solid surfaces such as polystyrene globules. The great advantage of the biofilters is their low space requirement due to the short retention time of waste water in the reactor. But how is the elimination efficiency? Are the pollutants effectively removed from the waste water?

Biofilters take up much less space than conventional activated-sludge reactors (see photo on p. 26). This is because the retention time of waste water in the biofilter is considerably shorter. We wanted to know if the elimination efficiency of the biofilters comes nevertheless near to that of the activated sludge process. Experience shows that for nutrients this is actually the case. But what about so-called micropollutants (see box) that are usually relatively hard to remove? This is where the emphasis of our project was placed.

**Comparison of Biofilters and Conventional Activated Sludge Processes.** We found ideal conditions for our investigations at the wastewater treatment plant in Altenrhein (see photo on p. 26). Here, half of the sewage is processed in a conventional plant using the activated sludge process while the other half is processed in a biofilter installation. The hydraulic properties of the two installations are quite different: While the sewage in the biological stage of the conventional plant (9000 $m^3$) is in contact with the activated sludge for between 6 and 20 hours, the retention time in the biofilter (approx. 450 $m^3$ = throughput volume minus displacement of the substrate material) is only 0.5 to 1.4 hours. In total, the sewage treatment plant Altenrhein is laid out for a population equivalent of 90 000 (sum of waste water from the number of natural persons and of industrial origin correspondingly converted) and processes an average dry-weather flow of 400 l of waste water per second.

**Micropollutants**

Pollutants that occur in lakes and rivers in very low concentrations (micro- and nanograms per litre) are designated as micropollutants. Even in such low concentrations, they can have a toxic effect on water organisms. Micropollutants come from industry (e. g. de-greasing agents, additives in plastics), agriculture (e. g. pesticides) as well as from hospital and household waste water (e. g. medicines, biocides, natural and synthetically produced hormones as well as toiletries). Many micropollutants are removed only in part in sewage treatment plants or, like pesticides, pass directly into lakes and rivers without passing through sewage treatment plant.

**Degradation of 18 Different Micropollutants.** The aim of our project was to quantify the degradation of 18 different micropollutants (see table in Fig. 1). We therefore took samples from the biofilter and the activated sludge reactor in the sewage treatment plant Altenrhein at three sampling campaigns of one week each. We fitted a device for the automatic sampling of influent and effluent waste water on both plants. As the wastewater quantities as well as the degree of loading vary strongly during the day, we employed a flow-weighted sampling program, i. e. in the course of a week, samples were always taken as soon as a specific amount of water had passed the sampling device. In this way, it was possible to realistically estimate the weekly input and output pollutant quantities and, thus, to calculate the amounts of pollutants actually removed in the biofilter and the activated sludge plant.

**Identical Elimination Efficiencies.** Figure 1 shows the results of our investigation [1]. Each of the data points represents a micropollutant and indicates which percentage of the particular substance was biologically degraded in the activated sludge (X-axis) and the biofilter (Y-axis). Most pollutants are removed in the biofilter installation just as well as in the conventional plant with activated sludge. Their data points are either on the diagonal straight line or in its proximity within the 10 % stray zone shown in blue (Fig. 2). In the two plants, the removal efficiency is only different for three micro-

# Biofilms: Successful Revival in Sewage Treatment

The first processes for biological wastewater treatment resemble a vertical river bed. Waste water runs over stones piled on top of each other, where the pollutants are converted and decomposed. The purification of the water occurs in biofilms which form on the surface of the stones. These biofilm systems have been continually developed and, in recent years, have experienced an previously undreamt upswing in the sewage treatment business.

**Biofilms Feel Good in Both Fixed and Moving Beds.** This is mainly due to the fact that the stones mentioned above were replaced with modern substrates that offer the micro-organisms a considerably larger surface per unit volume. Today, two biofilm methods are mainly applied: the fixed-bed and the moving-bed methods [1, 2]. In both cases, surfaces are available in the reactors on which 0.02–1 mm thick biofilms can develop.

In the fixed-bed process, which is also known as the biofilter, polystyrene globules have proven to be a suitable substrate. They are put into a cage and immersed in the waste water. The polystyrene globules are pushed upwards by hydrostatic pressure. Because the globules can only move against each other, to a limited extent, this method is designated as fixed-bed. Expanded clay granulate materials or structured plastic surfaces can also be used as an alternative.

In the moving-bed system, plastic particles in various forms are used. They have a specific weight similar to that of water and are kept suspended by aeration or by using agitators. As a result, they require no specific reactor shape and can therefore also be used for the simple upgrading of conventional activated sludge plant.

**Hybrid Installations: Biofilms and Activated Sludge at the Same Time.** The latest development is the so-called hybrid procedure. Here, moving-bed technology is combined with the activated sludge process. In this case, the slow-growing bacterial groups such as the nitrifyers settle mainly in the biofilms on the plastic particles as opposed to the fast-growing generalists, which favourably grow in the sludge suspension. Thus, the two bacterial populations do not interfere with each other. The hope is to combine the advantages of both systems in order to build smaller plants with higher treatment efficiencies. In the last few decades, more and more urban areas have grown around the sewage treatment plants and there is no longer enough space available for expansions. Compact design is therefore becoming increasingly important. Practice will show whether the hybrid system will meet the expectations placed on it.


[1] Tschui M., Boller M. (1997): Abwasserreinigung mit submersen Festbettreaktoren. GWA Gas Wasser Abwasser *77*, 796–781.

[2] Maurer M., Siegrist H. (1999): Nitrifikation und Denitrifikation im Wirbelbett. Mitteilungen zum Gewässerschutz Nr. 36, Buwal, Bern


Photos: Eawag

Polystyrene globules and plastic particles as substrates for the fixed and moving-bed systems.

pollutants. Estron is decomposed better in the biofilter (85 %) than in the activated sludge stage (50 %) whereas for azithromycin and sulfapyridin, it is the other way round: 50 % and 70 % are eliminated in the activated sludge process and only 20 % and 35 % are removed in the biofilter. In a second measurement campaign, however, sulfapyridin was equally well degraded in both plants. Additionally, we found out that only partial biological elimination occurs for most micropollutants, independent of the sewage treatment method (Fig. 1). Merely 3 of the 18 substances examined are eliminated to more than 80 %. On the other hand, 10 of the 18 pollutants examined are degraded by less than 50 %.

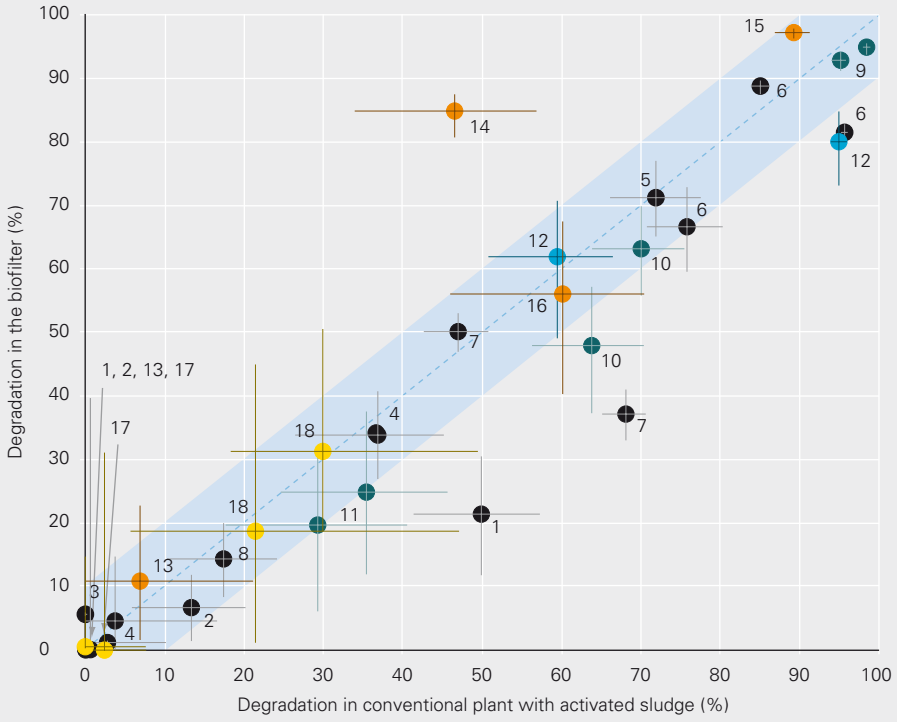
**Biofilters – Small and Efficient.** In spite of their 20 times smaller size and the between 10 and 20 times shorter contact-times, the biofilter in the sewage plant Altenrhein provides an elimination efficiency comparable with that of the conventional plant. What are the reasons?

► Presumably sequential elimination occurs within the biofilter along the direction of flow: the easily-degradable organic substances are removed in the first layers so that the pollutant load is much lower in the direction of the outlet. Accordingly, one can assume that different decomposition specialists settle in the different layers of the biofilter, which leads to the presence of an increased variety of micro-organisms.

► Moreover, the daily flushing of the system guarantees that fast-growing generalists are eliminated from the biofilter. These bacteria, which feed on easily-degradable substrate, would otherwise overgrow the biofilm. Consequently, an extremely efficient biofilm of slow-growing specialists develops in the biofilter: This category includes nitrification specialists that convert the urea from urine into nitrate as well as the slow-growing bacteria that metabolise recalcitrant organic substances. Due to the thin biofilm structure, these specialists are in direct contact with the waste water and can take up and degrade pollutants more efficiently than in a particle of

Bird's eye view of the sewage plant Altenrhein: the biofilter facility (ringed) requires 8 times less space than the conventional plant with its 3 rectangular activated-sludge and 3 round settling tanks; the plants each handle half of the incoming waste water.

ARA Altenrhein




| No. | Substance | Substance classe |
|---|---|---|
| 1 | Azithromycin | ● Antibiotics |
| 2 | Clarithromycin | |
| 3 | Erythromycin | |
| 4 | Roxithromycin | |
| 5<br>6 | Sulfamethoxazol and its waste product $N^4$-Acetyl-sulfamethoxazol | |
| 7 | Sulfapyridin | |
| 8 | Trimethoprim | |
| 9 | Ibuprofen | ● Pain Killer |
| 10 | Naproxen | |
| 11 | Diclofenac | |
| 12 | Iopromid | ● X-ray contrast medium |
| 13 | Carbamazepin | ● Anticonvulsant |
| 14 | Estron | ● Hormones |
| 15 | Estradiol | |
| 16 | Ethinylestradiol | |
| 17 | Tonalid | ● Perfumes |
| 18 | Galaxolid | |

Fig. 1: Comparison of degradation efficiencies for 18 different micropollutants in a biofilter and in a conventional plant with activated sludge. Since some substances were measured several times, more than 18 data points are represented in total. Horizontal and vertical lines at each data point indicate the estimated areas of uncertainty for the degradation computed [1]. The 18 micropollutants examined are shown in the table.

activated sludge, whose surface is dominated by rapidly growing bacteria.

**Disadvantages of the Biofilter.** In addition to the advantages already described, the biofilter process also exhibits some disadvantages when compared to conventional activated sludge plants:

► Due to the short hydraulic retention time in the biofilter reactor maximum loads cannot be coped with so easily.

► The biofilter reactor must be rinsed daily. 30 % of treated waste water is used for such flushing purposes. The rinsing must be accompanied by a certain amount of turbulence, which is achieved by a raised flow and the simultaneous injection of air.

► Energy demands in biofilter installations of 0.4–0.6 kWh per $m^3$ waste water [2, 3] are higher than in conventional sewage plants, which use between 0.2 and 0.5 kWh per $m^3$ of waste water [4].

► Due to high particle loads in their effluent, biofilter installations in many locations have to be provided with an additional sand filter. This is the case in Switzerland, when the limiting value prescribed by law of 15 mg particulate matter per litre of purified waste water is exceeded for a fifth of the random samples taken within 24 hours. Thus, the biofilter in the sewage treatment plant Altenrhein is equipped with a sand filter.

The lower space requirement of biofilter reactors is, however, of greater importance than their disadvantages, in particular at sites where wastewater treatment installations have to be enlarged.

○ ○ ○


[1] Joss A., Keller E., Alder A.C., Göbel A., McArdell C.S., Ternes T., Siegrist H. (2005): Removal of pharmaceuticals and fragrances in biological wastewater treatment. Water Research *39,* 3139–3152.

[2] Keller U. (2005): Personal communication, Abwasserverband Altenrhein.

[3] Kunz H. (2005): Personal communication, ARA Region Bern AG.

[4] Müller E.A., Thommen R., Stähli P. (1994): Energie in ARA. BUWAL, Bern.

Reto Manser, an environmental engineer, recently completed his doctoral dissertation in the department of "Environmental Engineering", which is headed by Hansruedi Siegrist.

# Activated Sludge – Biofilm Flocs

Activated sludge flocs in wastewater treatment plants are biofilms of a special kind, lacking a carrier material. Depending on the type of system used, the size of flocs formed in treatment plants varies: flocs in membrane bioreactors are smaller than those in conventional systems. Does this affect purification performance?

Aggregations of bacteria play a key role in biological wastewater treatment. In biofilm systems, they are attached to solid surfaces as growths (see article by A. Joss, p. 24). In activated sludge systems, by contrast, they take the form of flocs with a diameter of 0.1–2 mm, held in suspension by stirring or aeration. Structurally, these so-called activated sludge flocs are very similar to conventional biofilms – they merely lack the carrier material.

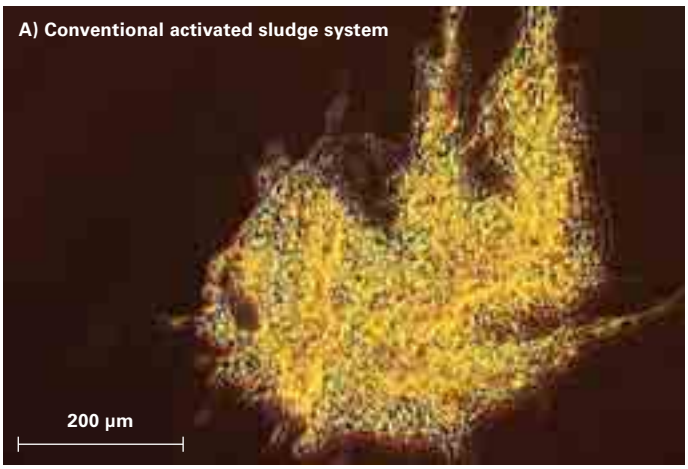
**Secondary Clarification: Sedimentation Versus Submerged Membranes.** In the activated sludge system, the flocs are usually separated from the treated waste water by sedimentation in the secondary clarifier. Recently, the use of membranes submerged in activated sludge for separation purposes has increasingly been discussed. Submerged membrane bioreactors appear to represent a highly promising alternative to the conventional system (e. g. [1]).

The benefits offered by the new system are the complete retention of biomass, the resultant high quality of the treated waste water and a markedly reduced footprint.

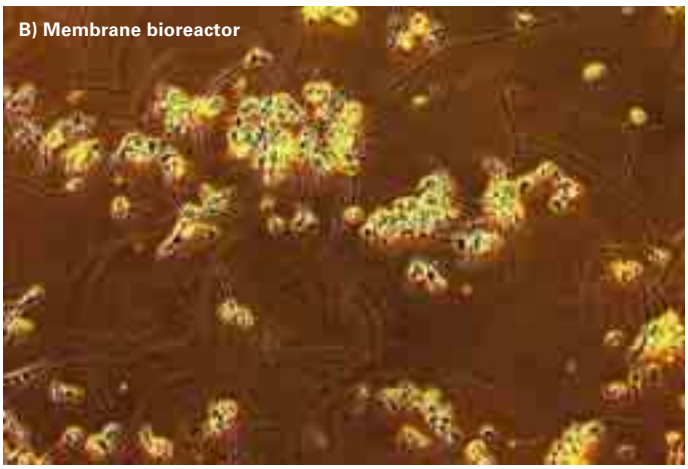
However, it is known from the literature that flocs in membrane bioreactors are substantially smaller than in plants with conventional secondary clarifiers. Floc size has a direct influence on supplies of oxygen and nutrients to the bacteria in the interior of the floc and hence on their activity. We therefore wished to establish whether the smaller floc size adversely affects the purification performance of a treatment plant with a membrane bioreactor.

**Comparison of Two Pilot Treatment Plants.** For this reason, we compared two pilot treatment plants installed at Eawag: a conventional plant with a secondary clarifier and a membrane bioreactor plant. Both plants are connected to the municipal sewage system and fed with waste water from the Dübendorf area. The treated

Fig. 1: Phase-contrast micrographs of activated sludge flocs from the conventional activated sludge plant with a secondary clarifier (A) and from the membrane bioreactor (B).




Fotos: Reto Manser, Eawag



water is returned to the sewage system. The basic settings are the same for both plants. Samples were taken every week for two and a half years. In addition to effluent volume and quality, we analysed the bacterial composition of the activated sludge, floc size and nitrification rates.

**Activated Sludge Floc Size.** As described in the literature, floc size in our membrane bioreactor was always smaller than in the conventional system (Figs. 1A and B). Flocs from the conventional plant had a mean diameter of 200–500 µm, although the size showed considerable seasonal variation. In the membrane bioreactor, the sludge received flocs with a diameter of approx. 100 µm at the beginning of the project and after almost 2 years of operation the floc size had declined to no greater than 40 µm.

But why are the flocs in conventional plants always larger? One main reason is presumably that, in a conventional secondary clarifier, small flocs do not settle adequately and are washed out with the treated water. Thus, selection occurs for bacteria that preferentially become established in medium-sized to large flocs (>100 µm). By contrast, the membrane in the alternative system poses an insuperable barrier for all bacteria, and there is no natural selection for large flocs. In addition, it is possible that the shear forces arising from coarse bubble aeration of the membrane modules prevent the growth of larger flocs. Finally, for the bacteria, small flocs may represent an optimum balance between oxygen and nutrient supplies and protection against predation.

**Nitrifiers in Activated Sludge Flocs.** In municipal wastewater treatment, nitrifiers are an important family of bacteria, even though they account for less than 5% of the total bacterial population. Within the nitrifier family, two groups are distinguished: ammonia-oxidizing bacteria, which transform ammonia derived from urine into nitrite, and nitrite-oxidizing bacteria, which subsequently convert nitrite to nitrate. Both groups are autotrophic (generating their own organic matter from inorganic substances) and also obligately aerobic (dependent on oxygen).

In order to investigate whether the nitrifier communities in flocs differ between the two pilot plants, we used a molecular biological analytical method – fluorescence *in situ* hybridization (FISH). This method allows bacteria to be identified directly in their habitat [2].

The composition of the ammonia-oxidizing communities in flocs was found to be similar in the two plants. The dominant bacterial species in the flocs was *Nitrosomonas oligotropha.* The pilot plants predominantly receive water with low ammonia concentrations, which is preferred by *N. oligotropha.* In addition, members of the *Nitrosomonas communis* and *Nitrosomonas eutropha* lineages were found in the activated sludge flocs in both plants, although these bacterial species generally prefer nutrient-rich habitats. The occurrence of these two species may have been due to marked variations in the influent load, with distinct peaks in nutrient concentrations, and possible inoculation with bacteria from the sewage system. We assume that a heterogeneous community enhances the stability of the nitrification process.

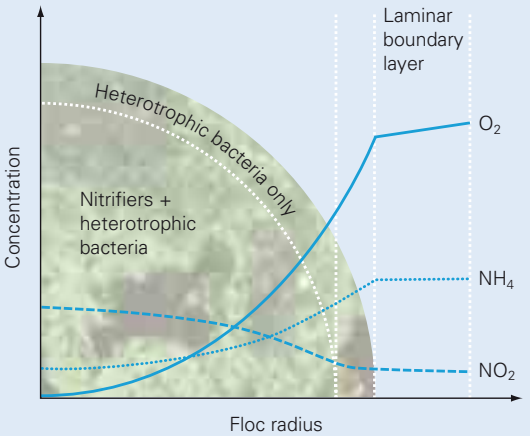
In both plants, the nitrite-oxidizing bacteria belonged to the *Nitrospira* genus. It has now been shown in various publications (e. g. [3]) that in most treatment plants, nitrite is converted to nitrate by *Nitrospira* rather than – as previously claimed – *Nitrobacter. Nitrospira* is significantly better adapted to low substrate concentrations than Nitrobacter.

Nitrifiers generally form dense clusters of 10–10 000 cells, growing only in the floc interior (Fig. 2, yellow stain). They are presumably overgrown by more rapidly replicating heterotrophic bacteria (i.e. bacteria dependent on organic substances).

**Influence of Floc Size on Nitrification Rates.** Another question of interest was whether floc size affects the activity of nitrifiers. To investigate this, the nitrification rate was both measured and calculated using the mass transfer model developed by the authors (see Box and Figs. 2 and 3). The results showed a good match between the measured and calculated values, suggesting that our mass transfer model provides a realistic reflection of the actual distribution of nitrifiers in the flocs. At the same time, clear differences

### Mass Transfer Model for Activated Sludge Flocs

In our mass transfer model, activated sludge flocs are represented as spheres [4, 5]. It is assumed that the available substrates and the oxygen in the boundary layer between floc and aqueous phase and in the floc interior are only transferred by diffusion. This gives rise to pronounced concentration gradients in the activated sludge flocs. Oxygen ($O_2$) and ammonium ($NH_4$) decrease towards the floc interior, as consumption exceeds transfer into the floc. In contrast, nitrite ($NO_2$) is only produced inside the floc and is therefore scarcely influenced by diffusion. Although the results are associated with a number of uncertainties, the model is a useful tool for understanding the processes involved.




Distribution of bacterial groups and of oxygen and nutrients in the idealized floc.

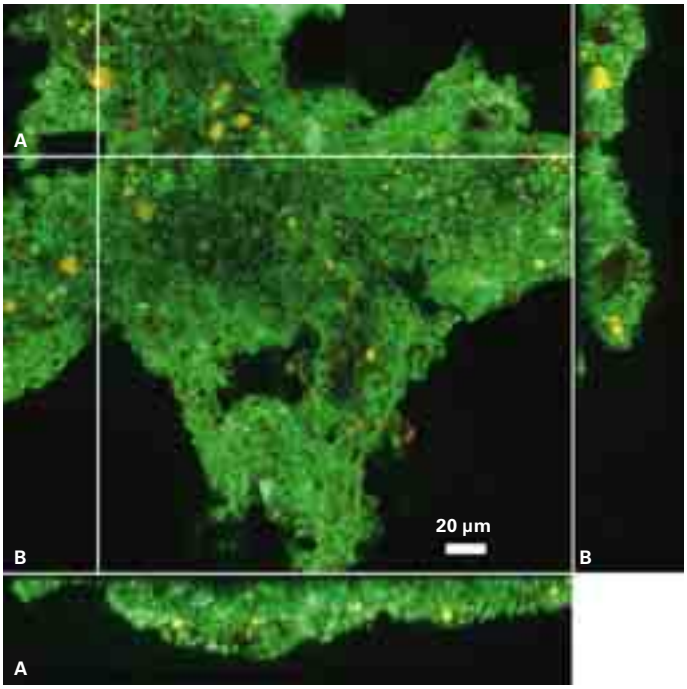



Fig. 2: Confocal laser scanning microscopic sections through an activated sludge floc, stained with gene probes. Green: general probe for all bacteria; yellow: specific probe for a group of ammonia-oxidizing bacteria. The floc was slightly deformed by compression during preparation.

emerged between the two treatment systems. At an oxygen concentration of 1 g/m$^3$, when the nitrification rate for the smaller flocs in the membrane bioreactor has already almost attained its maximum level, the larger flocs from the conventional system are not yet fully supplied with oxygen and the nitrifiers are only partly active. These flocs only become fully aerobic at oxygen concentrations of more than 3 g/m$^3$.

In contrast, both small and larger flocs are well supplied with ammonium, and no transfer limitation appears to be present. This may also be due to the fact that in the nitrification process one molecule of ammonium is converted per unit reaction, but two molecules of oxygen are consumed.

**Consequences for Operations.** Floc size is decisively influenced by the system used, i. e. whether activated sludge flocs are separated out by sedimentation or membrane filtration. In addition, floc size affects oxygen supplies to the bacteria in the floc interior and hence also nitrification performance: in a membrane bioreactor, 90 % of the maximum nitrification rate is already attained at an oxygen concentration of 1 g/m$^3$.

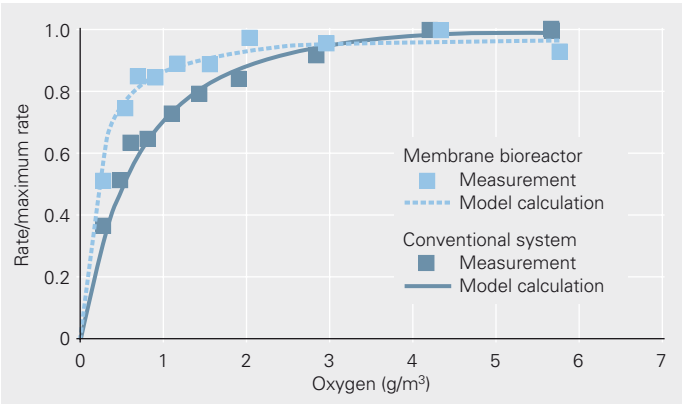
It therefore makes sense to operate an aerobic tank in a membrane bioreactor at an oxygen concentration of 1 g/m$^3$ for two reasons:

► The aeration rate can be reduced, as less oxygen needs to be introduced than in the conventional system. Aeration energy requirements are thus decreased by 10–20 %.

► As a result of the lower oxygen concentration, denitrification performance is improved. This is because overall less oxygen passes from the aerobic into the anoxic denitrification zone. In the denitrification process, nitrate is converted to molecular nitrogen.

Conclusion: In our study, floc size was shown to have substantial effects on purification performance and the efficiency of wastewater treatment plant operations. ○ ○ ○

Fig. 3: Measured and calculated nitrification rates as a function of oxygen concentration under excess substrate conditions.




[1] Stephenson T., Judd S., Jefferson B., Brindle K. (2000): Membrane bioreactors for wastewater treatment. IWA Publishing, London.

[2] Amann R., Fuchs B.M., Behrens S. (2001): The identification of microorganisms by fluorescence *in situ* hybridisation. Current Opinions in Biotechnoogy *12,* 231–236.

[3] Daims H., Nielsen J.L., Nielsen P.H., Schleifer K.H., Wagner M. (2001): *In situ* characterization of *Nitrospira*-like nitrite oxidizing bacteria active in wastewater treatment plants. Applied and Environmental Microbiology *67,* 5273–5284.

[4] Manser R., Gujer W., Siegrist H. (2005): Consequences of mass transfer on the kinetics of nitrifiers. Water Research *39,* 4633–4642.

[5] Schwarzenbach R., Gschwend P., Imboden D. (2003): Environmental organic chemistry. John Wiley & Sons, Inc., New Jersey.

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# Biofilms Hamper Heat Recovery

With our ever-increasing need for energy, its efficient use becomes more and more important. An attractive possibility is to recover heat energy from the relatively warm waste water. Biofilms, however, developing rapidly on heat exchangers in sewage pipes, can hamper heat recovery from waste water in the sewer. Is this avoidable?

Waste water contains a lot of heat energy because it is released into the drainage system at quite high temperatures. A simple calculation shows that the recovery of this heat is worthwhile: An amount of 8000 kW of energy, enough to power 80 000 100-watt lightbulbs, could theoretically be recovered from Zurich's sewage system, reducing the temperature of the waste water by a mere 1 °C. Waste water is thus an interesting and also continuously available energy source. Instead of emitting the heat to the environment, it could be used to heat buildings and to generate hot water. In Switzerland this is already being done at more than 50 locations, where heat pumps and heat exchangers integrated in the wastewater stream provide heat energy. In Zurich-Wipkingen, for example, a heat exchanger built into the sewage system (Fig. 1) supplies the heating for more than 900 residences.

A serious problem, however, of the heat recovery technology are biofilms. Favoured by the high levels of nutrients available in the waste water, they rapidly grow on the surfaces of heat exchangers, reducing the efficiency of the exchangers considerably. Our task, therefore, was to determine how the formation of these biofilms can be controlled.

**Heat Exchanger Test Rig.** For this reason, we developed a system for testing possible measures to control the formation of biofilms (Fig. 2). It consists of a Plexiglas trough channel, in the base of which a small 1 m-long heat exchanger with a cold-rolled stainless steel surface is placed. Four extractable coupons, made of the same steel as the heat exchanger, are also placed on the floor of the channel. These coupons have differing surface qualities, providing a measure of the effect of surface characteristics on the biofilm formation. Pre-treated municipal waste water is fed to the test channel. The heat exchanger itself works with de-ionised water. The temperature at the inlet and outlet of the heat exchanger and of the waste water is measured continuously and recorded on a computer. A cooling device simulates a consumer for the heat extracted by the heat exchanger.

Fig. 1: A 200 m-long heat exchanger integrated in a sewage pipe.

EWZ

**Heat Recovery from Waste Water and Sewage Plant Operation**

In a research project financed by the Federal Office of Energy, we investigated how much heat can be recovered from waste water, without affecting the operation of a downstream sewage treatment plant [1]. It was revealed that the natural cooling of the waste water in the sewage system lay at around 1 °C, and that the weather-dependent variations of the wastewater temperature were considerably larger than that. If the decrease in wastewater temperature through a heat exchanger in the sewage pipes is kept within certain limits, and the treatment plant has a reserve capacity (i. e. is not fully loaded), then the effect of heat recovery on the operation of a sewage treatment plant is low [2, 3]. Furthermore, heat recovery offers the advantage of a reduced heat load to our surface waterbodies.

Photos: Eawag

Fig. 2. The laboratory test rig, on which the effect of biofilm formation on heat exchanger efficiency was tested.

**Biofilm Formation can be Kept Within Limits.** When waste water flows over the heat exchanger in the Plexiglas channel, the first bacteria begin to settle after only a couple of hours, and after a few days a biofilm has formed, which can be hundreds of micrometers thick (Fig. 3). This biofilm creates a resistance to the transfer of heat from the waste water into the heat exchanger, and therefore reduces the efficiency of the latter. The efficiency of the heat exchanger can be calculated from the temperature difference between the inlet and outlet water, and the volumetric flow through the heat exchanger. Figure 4 shows how the performance decreases over time with the formation of the biofilm. After 18 days, it is down to 50 % of the original efficiency of the clean heat exchanger.

Now the speed of the wastewater flow is raised from 0.4 m/s to 1.0 m/s for 20 min. This augments the friction that the waste water asserts on the biofilm, part of the biofilm is washed away, and the heat exchanger efficiency rises again. This type of rinsing never failed to recover at least some of the performance [4]. When the wastewater speed remained constant at the higher value of 1.0 m/s, the efficiency did not fall below 80 % even after 2 months.

Fig. 4: Effect of biofilm formation on the development over time of the heat exchanger efficiency, relative to the efficiency of the clean heat exchanger. The arrows indicate the short-time increase in wastewater speed.

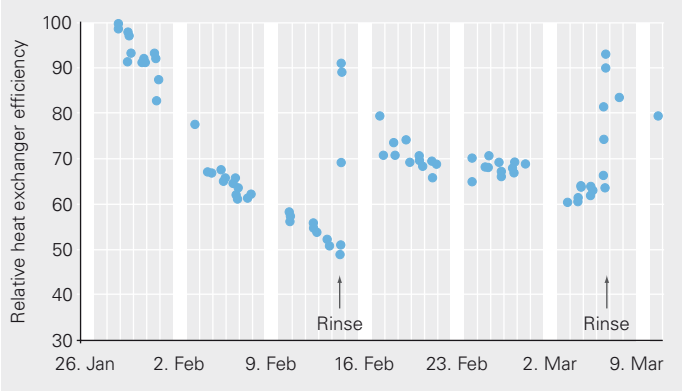


Fig. 3: Biofilm formation on steel coupons with differing surface characteristics. After 0 (left), 2 (middle) and 7 days (right) exposure in the wastewater channel.

With the surface tests, on the other hand, the results were less promising. Irrespective of the type of surface preparation of the steel coupons – different degrees of smoothness were obtained through grinding and polishing (sandpaper, cloth, electrical and diamond) – no significant reduction in biofilm formation could be established. Less biofilm was observed only on Teflon-coated surfaces. The best results were obtained with the combination of Teflon coating and higher flow speeds. Although, the coatings can not withstand the sand and gravel in the waste water in sewage pipes, in other systems their use could be promising.

**Conclusions.** The test rig investigations showed that it is not possible to prevent biofilm formation on heat exchangers in sewage systems entirely. It can, however, be limited through a temporary or continuous increase of the wastewater speed by means of structural (installation of rinsing) or operational (mechanical cleaning) measures by which a heat exchanger efficiency of about 80 % can be maintained. ○ ○ ○


[1] Wanner O. (2004): Wärmerückgewinnung aus Abwassersystemen. Schlussbericht BFE-Projekt Nr. 44 177. www.waermepumpe.ch: Forschung/Entwicklung, Berichte, Wärmequellen.

[2] Wanner O., Clavadetscher P., Siegrist H. (2005): Auswirkungen der Abwasserabkühlung auf den Kläranlagenbetrieb. Gas Wasser Abwasser *2*, 111–118.

[3] Wanner O., Panagiotidis V., Clavadetscher, P., Siegrist H. (2005): Effect of heat recovery from raw waste water on nitrification and nitrogen removal in activated sludge plants. Water Research *39*, 4725–4734.

[4] Wanner O., Delavy P., Hany R., Panagiotidis V., Zinn M.: Control of heat exchanger biofilms. In preparation.

# Publications

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[4115] **Markard J., Truffer B.** (2004): Innovation processes in large technical systems: market liberalization as a driver for radical changes? In: "Innovation, Sustainability and Policy", (Eds.). Kloster Seeon, Germany, 23.

[4117] **Yoshimura C., Omura T., Furumai H., Tockner K.** (2005): Present state of rivers and streams in Japan. River Res. Appl. *21,* (2–3), 93–112.

[4118] **Göbel A.** (2004): Occurence and fate of sulfonamide and macrolide antimicrobials in wastewater treantment. Diss., Naturwissenschaften ETH Zürich, Nr. 15703.

[4119] **Karaus U.** (2004): The ecology of lateral aquatic habitats along river corridors. Diss., Naturwissenschaften ETH Zürich, Nr. 15 841.

[4120] **Kaech A., Vallotton N., Egli T.** (2005): Isolation and characterization of heterotrophic bacteria able to grow aerobically with quaternary ammonium alcohols as sole source of carbon and nitrogen. Syst. Appl. Microbiol. 28, (3), 230–241.

[4121] **Karaus U., Alder L., Tockner K.** (2005): "Concave islands": Habitat heterogeneity of parafluvial ponds in a gravel-bed river. Wetlands *25,* (1), 26–37.

[4122] **Strassmann K.M., Brennwald M.S., Peeters F., Kipfer R.** (2005): Dissolved noble gases in the porewater of lacustrine sediments as palaeolimnological proxies. Geochim. Cosmochim. Acta *69,* (7), 1665–1674.

[4123] **Müller B., Maerki M., Schmid M., Vologina E.G., Wehrli B., Wüest A., Sturm M.** (2005): Internal carbon and nutrient cycling in Lake Baikal: sedimentation, upwelling, and early diagenesis. Global and Planetary Change *46,* (1–4), 101–124.

[4124] **Kaech A., Hofer M., Rentsch D., Schnider C., Egli T.** (2005): Metabolites and dead-end products from the microbial oxidation of quaternary ammonium alcohols. Biodegradation *16,* (5), 461–473.

[4125] **Ternes T.A., Bonerz M., Herrmann N., Loffler D., Keller E., Lacida B.B., Alder A.C.** (2005): Determination of pharmaceuticals, iodinated contrast media and musk fragrances in sludge by LC/tandem MS and GC/MS. J. Chromatogr. A *1067,* (1–2), 213–223.

[4126] **Leuz A.-K., Johnson C.A.** (2005): Oxidation of Sb(III) to Sb(V) by $O_2$ and $H_2O_2$ in aqueous solutions. Geochim. Cosmochim. Acta *69,* (5), 1165–1172.

[4127] **Escher B.I., Bramaz N., Eggen R.I.L., Richter M.** (2005): In vitro assessment of modes of toxic action of pharmaceuticals in aquatic life. Environ. Sci. Technol. *39,* (9), 3090–3100.

[4128] **Zwank L., Berg M., Elsner M., Schmidt T.C., Schwarzenbach R.P., Haderlein S.B.** (2005): New evaluation scheme for two-dimensional isotope analysis to decipher biodegradation processes: Application to groundwater contamination by MTBE. Environ. Sci. Technol. *39,* (4), 1018–1029.

[4129] **Tillman D.E., Larsen T.A., Pahl-Wostl C., Gujer W.** (2005): Simulating development strategies for water supply systems. J. Hydroinform. *7,* (1), 41–51.

[4131] **Diemer M., Billeter R., Hooftman D.A., Oetiker K., Lienert J.** (2005): Die langfristigen Auswirkungen von Nutzungsänderungen auf häufige Pflanzenarten montaner Kalkflachmoore in der Schweiz. Natur und Landschaft *80,* (2), 63–68.

[4132] **Rosakis A., Koster W.** (2004): Transition metal transport in the green microalga *Chlamydomonas reinhardtii* – genomic sequence analysis. Res. Microbiol. *155,* (3), 201–210.

[4133] **Boller M.** (2005): Bedeutung von Schwermetalleinträgen durch Niederschlagswasser. In: "38. Essener Tagung für Wasser- und Abfallwirtschaft", (Eds.). GWA Gewässerschutz Wasser Abwasser, Aachen, 37/31–37/15.

[4134] **Boller M.** (2005): Eawag – Eidg. Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz: Forschung im Dienste des Wassers. GWA Gas, Wasser, Abwasser *3,* 191–202.

[4135] **Soldo D., Hari R., Sigg L., Behra R.** (2005): Tolerance of *Oocystis nephrocytioides* to copper: intracellular distribution and extracellular complexation of copper. Aquat. Toxicol. *71,* (4), 307–317.

[4136] **Lorke A., Wüest A.** (2005): Turbulence and mixing regimes specific to lakes. In: "Marine turbulence: theories, observations, and models. Results of the CARTUM Project." H.Z. Baumert, J. Simpson J. Südermann (Eds.). Cambridge University Press, 346–354.

[4137] **Wüest A., Lorke A.** (2005): Validation of microstructure-based diffusivity estimates using tracers in lakes and oceans. In: "Marine turbulence: theories, observations, and models. Results of the CARTUM Project." H.Z. Baumert, J. Simpson, J. Südermann (Eds.). Cambridge University Press, 139–152.

[4138] **Bloesch J.** (2005): IAD International Workshop "Hydrologie und Limnologie – eine andere Grenze im Donau Einzugsgebiet" in Petronell bei Wien, Oktober 14–16, 2004 – IAD International Workshop "Hydrology and Limnology – another boundary in the Danube River Basin" in Petronell near Vienna, October 14–16, 2004. Donau Aktuell/ Danube News *11,* 10–11.

[4139] **Zobrist J., Hoehn E.** (2005): Umgang mit Indikatorwerten im Grundwasser. GWA Gas, Wasser, Abwasser *5,* 359–364.

[4140] **Mavrocordatos D., Pronk W., Boller M.** (2004): Analysis of environmental particles by atomic force microscopy, scanning and transmission electron microscopy. Water Sci. Technol. *50,* (12), 9–18.

[4141] **Krejci V., Rossi L., Kreikenbaum S., Fankhauser R.** (2004): Projekt "STORM": Abwassereinleitungen aus Kanalisationen bei Regenwetter – Einführung in das Projekt. GWA Gas, Wasser, Abwasser *6,* 419–422.

[4142] **Krejci V., Kreikenbaum S.** (2004): Projekt "STORM": Abwassereinleitungen aus Kanalisationen bei Regenwetter – Konzepte des Gewässerschutzes. GWA Gas, Wasser, Abwasser *6,* 423–430.

[4143] **Rossi L., Krejci V., Kreikenbaum S.** (2004): Projekt "STORM": Abwassereinleitungen aus Kanalisationen bei Regenwetter – Anforderungen an die Abwassereinleitungen. GWA Gas, Wasser, Abwasser *6,* 431–438.

[4144] **Kreikenbaum S., Krejci V., Fankhauser R., Rauch W.** (2004): Projekt "STORM": Abwassereinleitungen aus Kanalisationen bei Regenwetter – Berücksichtigung von Unsicherheiten in der Planung. GWA Gas, Wasser, Abwasser *8,* 587–594.

[4145] **Krejci V., Kreikenbaum S., Fankhauser R.** (2004): Projekt "STORM": Abwassereinleitungen aus Kanalisationen bei Regenwetter – Akute Ammonikak- und hydraulische Beeinträchtigungen. GWA Gas, Wasser, Abwasser *9,* 671–679.

[4147] **Brennwald M.S., Kipfer R., Imboden D.M.** (2005): Release of gas bubbles from lake sediment traced by noble gas isotopes in the sediment pore water. Earth Planet. Sci. Lett. *235,* 31–44.

[4148] **Wüest A., Zeh M.** (2005): Dem Felchenfangrückgang im Brienzersee auf der Spur. Bulletin SEV/AES *10,* 25–28.

[4149] **Dittrich M., Sibler S.** (2005): Cell surface groups of two picocyanobacteria strains studied by zeta potential investigations, potentiometric titration, and infrared spectroscopy. J. Colloid Interface Sci. *286,* (2), 487–495.

[4150] **Dittrich M., Obst M.** (2004): Are picoplankton responsible for calcite precipitation in lakes? Ambio *33,* (8), 559–564.

[4151] **Huber M.M., Gobel A., Joss A., Hermann N., Loffler D., McArdell C.S., Ried A., Siegrist H., Ternes T.A., von Gunten U.** (2005): Oxidation of pharmaceuticals during ozonation of municipal

wastewater effluents: A pilot study. Environ. Sci. Technol. *39,* (11), 4290–4299.

[4152] **Treude T., Niggemann J., Kallmeyer J., Wintersteller P., Schubert C.J., Boetius A., Jorgensen B.B.** (2005): Anaerobic oxidation of methane and sulfate reduction along the Chilean continental margin. Geochim. Cosmochim. Acta *69,* (11), 2767–2779.

[4153] **Lee Y., Yoon J., von Gunten U.** (2005): Spectrophotometric determination of ferrate (Fe(VI)) in water by ABTS. Water Res. *39,* (10), 1946–1953.

[4154] **Sigg L., Behra R.** (2005): Speciation and bioavailability of trace metals in freshwater environments. In: "Metal ions in biological systems", A. Sigel, H. Sigel R.K.O. Sigel (Eds.). Taylor & Francis Group, Boca Raton, 47–73.

[4164] **Stips A., Burchard H., Bolding K., Prandke H., Simon A., Wüest A.** (2005): Measurement and simulation of viscous dissipation in the wave affected surface layer. Deep Sea Research Part II: Topical Studies in Oceanography *52,* (9–10), 1133–1155.

[4165] **Wüest A., Zeh M**. (2005): Dem Felchenrückgang im Brienzersee auf der Spur. natur+mensch *2,* 5–9.

[4171] **Maurer M.** (2005): Vom Transportsystem zum Gewässerschutzelement. Die Schweizer Gemeinde *6,* 25–26.

[4172] **Udert K.M., Larsen T.A., Gujer W.** (2005): Chemical nitrite oxidation in acid solutions as a consequence of microbial ammonium oxidation. Environ. Sci. Technol. *39,* (11), 4066–4075.

[4174] **Larsen T.A., Lienert J., Maurer M., Gujer W.** (2005): Ökologische Infrastrukturinnovationen in der Siedlungswasserwirtschaft – Ansätze und Perspektiven. In: "Die Zukunft der Infrastrukturen – Intelligente Netzwerke für eine nachhaltige Entwicklung", L. Reinhard R. Schaeffer (Eds.). Metropolis-Verlag, Marburg,

[4175] **Fietz S., Sturm M., Nicklisch A.** (2005): Flux of lipophilic photosynthetic pigments to the surface sediments of Lake Baikal. Global and Planetary Change *46,* (1–4), 29–44.

[4176] **Chevre N., Brazzale A.R., Becker-van Slooten K., Behra R., Tarradellas J., Guettinger H.** (2005): Modeling the concentration-response function of the herbicide dinoseb on *Daphnia magna* (survival time, reproduction) and *Pseudokirchneriella subcapitata* (growth rate). Ecotox. Environ. Safe. *62,* (1), 17–25.

[4177] **Hammes F.A., Egli T.** (2005): New method for assimilable organic carbon determination using flow-cytometric enumeration and a natural microbial consortium as inoculum. Environ. Sci. Technol. *39,* (9), 3289–3294.

[4186] **Gianella S., Wohlwend L.** (2005): Langfristige Bewirtschaftung von GEP-Daten und Kanalisationskataster – Problematik, Methodik und Erfahrungen mit den Gemeinden des Abwasserverbandes Altenrhein. In: "61. VSA-Hauptmitgliederversammlung", (Eds.). Verband Schweizer Abwasser- und Gewässerschutzfachleute, 35–43.

[4187] **Kracht O., Gujer W.** (2005): Neue Wege der Fremdwasserbestimmung – Verbesserte Quantifizierung mit Tracermethoden? In: "61. VSA-Hauptmitgliederversammlung", (Eds.). Verband Schweizer Abwasser- und Gewässerschutzfachleute, 17–23.

[4188] **Neumann M., Daebel H., Dominiguez D., Gujer W.** (2005): Unsicherheiten bei der Modellierung siedlungswasserwirtschaftlicher Anlagen – Explizite Berücksichtigung in Planung und Dimensionierung. In: "61. VSA-Hauptmitgliederversammlung", (Eds.). Verband Schweizer Abwasser- und Gewässerschutzfachleute, 1–10.

[4189] **Rieckermann J., Gujer W.** (2005): Abwasserverluste aufspüren. In: "61. VSA-Hauptmitgliederversammlung", (Eds.). Verband Schweizer Abwasser- und Gewässerschutzfachleute, 11–16.

[4190] **Göbel A., Thomsen A., McArdell C.S., Joss A., Giger W.** (2005): Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. Environ. Sci. Technol. *39,* (11), 3981–3989.

[4191] **Blüm W., McArdell C.S., Hoehn E., Schaubert R., Labhart W., Bertschi S.** (2005): Organische Spurenstoffe im Grundwasser des Limmattales – Ergebnisse der Untersuchungskampagne 2004.

[4193] **Muscheler R., Beer J., Kubik P.W., Synal H.-A.** (2005): Geomagnetic field intensity during the last 60,000 years based on $^{10}$Be and $^{36}$Cl from the Summit ice cores and $^{14}$C. Quat. Sci. Rev. *24,* (16–17), 1849–1860.

[4205] **Hendrickx B., Dejonghe W., Boenne W., Brennerova M., Cernik M., Lederer T., Bucheli-Witschel M., Bastiaens L., Verstraete W., Top E.M., Diels L., Springael D.** (2005): Dynamics of an oligotrophic bacterial aquifer community during contact with a groundwater plume contaminated with benzene toluene, ethylbenzene, and xylenes: an in situ mesocosm study. Appl. Environ. Microbiol. *71,* (7), 3815–3825.

[4207] **Burkhardt M., Stamm C., Waul C., Singer H., Müller S.** (2005): Surface runoff and transport of sulfonamide antibiotics and tracers on manured grassland. J. Environ. Qual. *34,* 1363–1371.

[4208] **Lienert J., Larsen T.A.** (2005): Making the first step towards a more sustainable urban water management system – with the NoMix toilet. In: "European Water Day, 84$^{th}$ ASTEE congress", (Eds.). Paris, France, 15.

[4209] **Li W., Nowak W., Cirpka O.A.** (2005): Geostatistical inverse modeling of transient pumping tests using temporal moments of drawdown. Water Resour. Res. *41,* (8),

[4210] **Hug T., Gujer W., Siegrist H.** (2005): Rapid quantification of bacteria in activated sludge using fluorescence in situ hybridization and epifluorescence microscopy. Water Res. *39,* (16), 3837–3848.

[4211] **Hug T., Ziranke M., Siegrist H.** (2005): Dynamics of population and scumming on a full-scale wastewater treatment plant in Switzerland. Acta Hydrochim. Hydrobiol. *33,* (3), 216–222.

[4212] **Ammann A.A.** (2005): Speciation of Aminopolycarboxylate and Aminophosphonate metal complexes by AEX ICP-MS in environmentla water symples. In: "Biochemistry of Chelating Agents", B. Nowack J.M. VanBriesen (Eds.). American Chemical Society, Washington DC, 108–120.

[4213] **Ihssen J.** (2005): Adaptation of *Escherichia coli* to growth with low concentrations of carbon and energy substrates. Diss., Naturwissenschaften ETH Zürich, Nr. 16 019.

[4214] **Gächter R., Steingruber S.M., Reinhardt M., Wehrli B.** (2004): Nutrient transfer from soil to surface waters: Differences between nitrate and phosphate. Aquat. Sci. *66,* (1), 117–122.

[4215] **Göbel A., Thomsen A., McArdell C.S., Alder A.C., Giger W., Theiss N., Löffler D., Ternes T.A.** (2005): Extraction and determination of sulfonamides, macrolides, and trimethoprim in sewage sludge. J. Chromatogr. A *1085,* (2), 179–189.

[4216] **Jansson R., Backx H., Boulton A.J., Dixon M., Dudgeon D., Hughes F.M.R., Nakamura K., Stanley E.H., Tockner K.** (2005): Stating mechanisms and refining criteria for ecologically successful river restoration: a comment on Palmer et al. (2005). J. Appl. Ecol. *42,* (2), 218–222.

[4217] **Gurnell A., Tockner K., Edwards E., Petts G.** (2005): Effects of deposited wood on biocomplexity of river corrridors. Frontiers in Ecology and the Environment *3,* (7), 377–382.

[4218] **Paetzold A., Tockner K.** (2005): Effects of riparian arthropod predation on the biomass and abundance of aquatic insect emergence. J. N. Am. Benthol. Soc. *24,* (2), 395–402.

[4219] **Dodd M.C., Shah A.D., von Gunten U., Huang C.-H.** (2005): Interactions of Fluoroquinolone Antibacterial Agents with Aqueous Chlorine: Reaction kinetics, mechanisms, and transformation pathways. Environ. Sci. Technol. *39,* (18), 7065–7076.

[4220] **Teutsch N., von Gunten U., Porcelli D., Cirpka O.A., Halliday A.N.** (2005): Adsorption as a cause for iron isotope fractionation in reduced groundwater. Geochim. Cosmochim. Acta *69,* (17), 4175–4185.

[4221] **Johnson A.C., Aerni H.R., Gerritsen A., Gibert M., Giger W., Hylland K., Jurgens M., Nakari T., Pickering A., Suter M.J.F., Svenson A., Wettstein F.E.** (2005): Comparing steroid estrogen, and nonylphenol content across a range of European sewage plants with different treatment and management practices. Water Res. *39,* (1), 47–58.

[4222] **Gabriel F.L.P., Heidlberger A., Rentsch D., Giger W., Guenther K., Kohler H.P.E.** (2005): A novel metabolic pathway for degradation of 4-nonylphenol environmental contaminants by Sphingomonas xenophaga Bayram – ipso-hydroxylation and intramolecular rearrangement. J. Biol. Chem. *280,* (16), 15526–15533.

[4223] **Huber M.M., Korhonen S., Ternes T.A., von Gunten U.** (2005): Oxidation of pharmaceuticals during water treatment with chlorine dioxide. Water Res. *39,* (15), 3607–3617.

[4224] **Geueke B., Namoto K., Seebach D., Kohler H.P.E.** (2005): A novel beta-peptidyl aminopeptidase (BapA) from strain 3-2W4 cleaves peptide bonds of synthetic beta-tri- and beta-dipeptides. J. Bacteriol. *187,* (17), 5910–5917.

[4225] **Yildirim S., Franko T.T., Wohlgemuth R., Kohler H.P.E., Witholt B., Schmid A.** (2005): Recombinant chlorobenzene dioxygenase from *Pseudomonas* sp. P51: A biocatalyst for regioselective oxidation of aromatic nitriles. Adv. Synth. Catal. *347,* (7–8), 1060–1072.

[4226] **Geueke B., Namoto K., Agarkova I., Perriard J.C., Kohler H.P.E., Seebach D.** (2005): Bacterial cell penetration by beta(3)-oligohomoarginines: Indications for passive transfer through the lipid bilayer. Chembiochem *6,* (6), 982–985.

[4227] **Wedekind C., Muller R.** (2005): Risk-induced early hatching in salmonids. Ecology *86,* (9), 2525–2529.

[4228] **Le Faucheur S.** (2005): Phytochelatin induction by metals in freshwater algae. Diss., Naturwissenschaften ETH Zürich, Nr. 15 985.

[4229] **Monaghan M.T., Robinson C.T., Spaak P., Ward J.V.** (2005): Macroinvertebrate diversity in fragmented Alpine streams: implications for freshwater conservation. Aquat. Sci. *online first,* 1–11.

[4230] **Vermeirssen E.L.M., Burki R., Joris C., Peter A., Segner H., Suter M.J.F., Burkhardt-Holm P.** (2005): Characterization of the estrogenicity of Swiss midland rivers using a recombinant yeast bioassay and plasma vitellogenin concentrations in feral male brown trout. Environ. Toxicol. Chem. *24,* (9), 2226–2233.

[4232] **Robinson C.T., Kawecka B.** (2005): Benthic diatoms of an alpine stream/lake network in Switzerland. Aquat. Sci. *online first,* 1–15.

[4234] **Jankowski T., Straile D.** (2004): Allochronic differentiation among *Daphnia* species, hybrids and backcrosses: the importance of sexual reproduction for population dynamics and genetic architecture. J. Evol. Biol. *17,* (2), 312–321.

[4236] **Weyhenmeyer G.A., Meili M., Livingstone D.M.** (2004): Nonlinear temperature response of lake ice breakup. Geophys. Res. Lett. *31,* (7),

[4237] **Peeters F., Beyerle U., Aeschbach-Hertig W., Brennwald M.S., Kipfer R.** (2004): Response to the comment by G. Favreau, A. Guero, and J. Seidel on "Improving noble gas based paleoclimate reconstruction and groundwater dating using $^{20}Ne/^{22}Ne$ ratios" (2003) Geochim. Cosmochim. Acta, *67,* 587–600. Geochim. Cosmochim. Acta *68,* (6), 1437–1438.

[4238] **Fette M., Kipfer R., Schubert C.J., Hoehn E., Wehrli B.** (2005): Assessing river-groundwater exchange in the regulated Rhone River (Switzerland) using stable isotopes and geochemical tracers. Appl. Geochem. *20,* (4), 701–712.

[4239] **Jankowski T.** (2004): Predation of freshwater jellyfish on *Bosmina:* the consequences for population dynamics, body size, and morphology. Hydrobiologia *530–31,* 521–528.

[4241] **Joss A., Keller E., Alder A.C., Göbel A., McArdell C.S., Ternes T., Siegrist H.** (2005): Removal of pharmaceuticals and fragrances in biological wastewater treatment. Water Res. *39,* (14), 3139–3152.

[4242] **Le Faucheur S., Behra R., Sigg L.** (2005): Phytochelatin induction, cadmium accumulation, and algal sensitivity to free cadmium ion in *Scenedesmus vacuolatus*. Environ. Toxicol. Chem. *24,* (7), 1731–1737.

[4243] **Sanchez-Polo M., von Gunten U., Rivera-Utrilla J.** (2005): Efficiency of activated carbon to transform ozone into OH radicals: Influence of operational parameters. Water Res. *39,* (14), 3189–3198.

[4244] **Schmid M., Halbwachs M., Wehrli B., Wuest A.** (2005): Weak mixing in Lake Kivu: New insights indicate increasing risk of uncontrolled gas eruption. Geochem. Geophys. Geosyst. *6.*

[4245] **Buschmann J., Canonica S., Sigg L.** (2005): Photoinduced oxidation of antimony(III) in the presence of humic acid. Environ. Sci. Technol. *39,* (14), 5335–5341.

[4248] **Schwarz U., Bloesch J.** (2004): GIS-supported mitigation of the impact of hydropower dams on the flood plains of the Drava-Mura Rivers in Croatia/Hungary. In: "GIS and Remote Sensing in Hydrology, Water Resources and Environment", (Eds.). Procedings of ICGRHWE, Three Gorges Dam, China, 178–187.

[4249] **Bloesch J.** (2004): Water quality monitoring and the morphological paradigm in the Danube River basin – a review. In: "GIS and Remote Sensing in Hydrology, Water Resources and Environment", (Eds.). Procedings of ICGRHWE, Three Gorges Dam, China, 285–292.

[4269] **Blass A., Bühler R., Grosjean M., Margreth S., Sturm M.** (2005): The sedimentation of the last few centuries in three proglacial lakes, Upper Engadine, Switzerland. In: "Sediment 2005", H. Haas, K. Ramseyer F. Schlunegger (Eds.). Schriftenreihe der Deutschen Gesellschaft für Geowissenschaften, Gwatt, Lake Thun, Switzerland, 35.

[4273] **Monecke K., Sturm M.** (2005): Late Glacial to Holocene climate variability and anthropogenic impact as reflected in a high resolution sedimentary record from Baldegger See, Central Switzerland. In: "Sediment 2005", H. Haas, K. Ramseyer F. Schlunegger (Eds.). Schriftenreihe der Deutschen Gesellschaft für Geowissenschaften, Gwatt, Lake Thun, Switzerland, 112.

[4275] **Truffer B., Lienert J., Monstadt J.** (2005): Zukünfte der Siedlungswasserwirtschaft – Eine Szenarioanalyse für die Schweiz. GWA Gas, Wasser, Abwasser *9,* 695–702.

[4277] **Lorke A., Peeters F., Wuest A.** (2005): Shear-induced convective mixing in bottom boundary layers on slopes. Limnol. Oceanogr. *50,* (5), 1612–1619.

[4278] **Tandy S., Schulin R., Suter M.J.F., Nowack B.** (2005): Determination of [S,S]' -ethylenediamine disuccinic acid (EDDS) by high performance liquid chromatography after derivatization with FMOC. J. Chromatogr. A *1077,* (1), 37–43.

[4279] **Nesatyy V.J., Rutishauser B.V., Eggen R.I.L., Suter M.J.F.** (2005): Identification of the estrogen receptor Cd-binding sites by chemical modification. Analyst *130,* (7), 1087–1097.

[4280] **Nesatyy V.J., Suter M.J.F.** (2004): On the conformation-dependent neutralization theory and charging of individual proteins and their non-covalent complexes in the gas phase. J. Mass Spectrom. *39,* (1), 93–97.

[4281] **Ledford H.K., Baroli I., Shin J.W., Fischer B.B., Eggen R.I.L., Niyogi K.K.** (2004): Comparative profiling of lipid-soluble antioxidants and transcripts reveals two phases of photo-oxidative stress in a xanthophyll-deficient mutant of *Chlamydomonas reinhardtii*. Mol. Genet. Genomics *272,* (4), 470–479.

[4282] **Fischer B.B., Krieger-Liszkay A., Eggen R.I.L.** (2004): Photosensitizers neutral red (Type I) and rose bengal (Type II) cause light-dependent toxicity in *Chlamydomonas reinhardtii* and induce the Gpxh gene via increased singlet oxygen formation. Environ. Sci. Technol. *38,* (23), 6307–6313.

# In Brief

## Janet Hering appointed as new Director of Eawag

On 26 June, the Federal Council appointed **Janet Hering** (the candidate proposed by the ETH Board) as the new Director of Eawag. The 48-year-old US citizen is currently Professor of Environmental Science and Engineering at the California Institute of Technology (Caltech). Professor Hering is an expert on water treatment processes (contaminant removal) and the biogeochemical behaviour of trace metals. On 1 January 2007, she will be taking over as Director of the research institute with a staff of 400 from Ulrich Bundi, who has held the post on an interim basis since 1 July 2004. ○ ○ ○

## Global composting network – the "decomp database"

In many developing countries, the local languages lack a word for "compost". Particularly in urban areas, where waste disposal is a major problem, there is little awareness of the possibility of composting organic wastes – despite the fact that pioneering efforts to promote community-level composting are already under way in a number of countries. These activities, however, are rarely networked and frequently face the same difficulties. For this reason, an information platform known as the "decomp database" is being made available by Sandec (the Department of Water and Sanitation in Developing Countries at Eawag). This online resource collects data on decentralized composting schemes worldwide. The aim is to document – and share with interested parties – the experiences of individual countries or regions. ○ ○ ○

Biodegradable waste is distributed on a compost pile (Mumbai, India).

S. Drescher, Eawag

## Relocation to Forum Chriesbach

Eawag has moved into its new headquarters at Dübendorf. The administrative and research centre, which also houses the joint Eawag-Empa library, training facilities and a staff canteen, sets new standards in terms of sustainability. Structurally and technically, the building comes close to the limits of what is currently feasible. Calculations indicate that energy consumption at this centre, designed for some 120 staff, is four times lower than in a conventional building. Conceived as a "zero energy building", the Forum Chriesbach lacks traditional heating and cooling systems. It will be officially inaugurated on 1 and 2 September. ○ ○ ○

F. Rohrer, Eawag

## Award for application of arsenic biosensor in the field

A biosensor used to detect arsenic in water has been successfully applied in the field for the first time by a team of researchers from Eawag and the universities of Hanoi (Vietnam) and Lausanne. The newly developed test permits rapid analysis of large numbers of samples, is inexpensive and can be used directly in the regions concerned. The article describing this research in the journal *Environmental Science and Technology* was selected as the ES&T top technology paper for 2005. This award marks the success not only of a good publication but also of a method in the development of which Eawag played a key role. ○ ○ ○

Workshop in Hanoi: How does the new test for arsenic work?

M. Berg, Eawag