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Research Article

Influence of hydraulic conductivity on communities of microorganisms and invertebrates in porous media: a case study in drinking water slow sand filters

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Abstract. The impact of reduced hydraulic conductivity on the abundance and diversity of microorganisms and invertebrates was examined in an artificial ecosystem consisting of a slow sand-filter. Sand-filters processed pre-treated lake water under high flow rates and acted as small ecosystems inhabited by a complex community. The first trophic level consisting of microorganisms serves as a food source for a dense community of protists, micro- and macro-invertebrates. The reduction of hy-

draulic conductivity due to the development of larger bacterial and fungal biomass induced a shift of the microbial community towards anaerobiosis that may increase clogging by carbonate precipitation. The presence of more bacterial prey seems to favour the development of higher trophic levels. Predation and bioturbation by eukaryotes were not able to counteract the reduction of hydraulic conductivity due to prokaryotic clogging.

Key words. Biofilm; carbonate; predation; clogging; fatty acid; bioturbation.

Introduction

Little is known about how complex biological communities of interstitial habitats affect the development of their physical environment. According to the general theory of ecological succession (Engstrom et al., 2000), this system should become more productive with age and the biomass in the sediment should increase with time, leading to bioclogging. By taking advantage of an anthropic system, we observed that the evolution of an infiltrating sedimentary system toward clogging is unpredictable

based on physical and chemical parameters. Surprisingly, “identical” systems have a completely different clogging behavior due to different development rates in the bacterial exopolymeric substances (Mauclaire et al., 2004).

In the present study, we determined how eukaryotic and prokaryotic communities responded to the clogging process, and attempt to identify factors driving the system progression towards clogging. Sand filters represent specific habitats for micro-, meio- and macro-organisms (Adam et al., 1998; Aepli 1990; Moll et al., 1999) and we expected that the biological compartment of the artificial infiltrating porous media is highly adapted to its environmental conditions. As microbial development may be controlled by both bottom-up (nutrient resources) and top-down (bioturbation, predation) factors, the aim of this study was to elucidate the relationships between the physical functioning of the system, the microbial

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compartment, and the meio- and macrofauna. Considering that the clogging process would control the nutrient resources of the system (bottom-up control), we hypothesized that an increase in clogging of porous media would lead to a general development of system productivity by increasing abundance, activity, and diversity of the prokaryotic and eukaryotic community.

Materials and methods

Slow sand filters

At the water plant Zurich-Lengg, raw water is collected from Lake Zurich (Switzerland) at a depth of 30 m (more than 20 m below the thermocline). Primary ozonation is used for disinfection prior to a coarse cleaning by rapid filters. Secondary ozonation and active carbon filtration are conducted before the mechanical and biological superfine cleaning with slow sand filters. These filters (1120 m² each) are isolated systems with their floors constructed on special bricks for drainage. The sediment tanks consist of four layers of sediment with increasing grain size from the top to the bottom: 85 to 50 cm of fine sand (diameter 0.2–2 mm), 5 cm of coarse sand (diameter 4–8 mm), 5 cm of fine gravel (diameter 8–15 mm), and 5 cm gravel (diameter 15–30 mm). The thickness of the fine sand layers varies according to number of cleaning cycles (removal of the top 5 cm). The sand originated from Lake Obersee and was extracted by the sand washing plant Kibag. All filters are operated indoors in the dark at 4 to 8 °C.

Newly constructed filters (clean sand) exhibit an initial hydraulic conductivity of approximately 4–7 m h⁻¹. During the following years, the hydraulic conductivity decreases gradually to 0.5 m h⁻¹, indicating the increasing clogging of the system. At this point, the filters must be cleaned by removing the top 5 cm of sand to raise the hydraulic conductivity by 1–2 m h⁻¹.

Sampling procedure

To study the influence of reduced hydraulic conductivity on the biotic system, filters were grouped by the degree of clogging (high and low, Fig. 1). When sampled, highly clogged (C+) and less clogged (C-) filters had an average hydraulic conductivity of 0.5 m h⁻¹ and 1.2 m h⁻¹, respectively. For each filter type (C+ and C-), three filters were selected. The investigated filters were not subject to maintenance for at least 19 mo, a sufficient time to allow the equilibration of the system.

Samples were collected on three dates: 2 November 2000, 20 March 2001 and 17 May 2001. The last two sampling dates were before and after lake stratification. Saturated hydraulic conductivity of the entire filter was estimated by measuring the pressure head as the difference in water level between the inlet and outlet of each

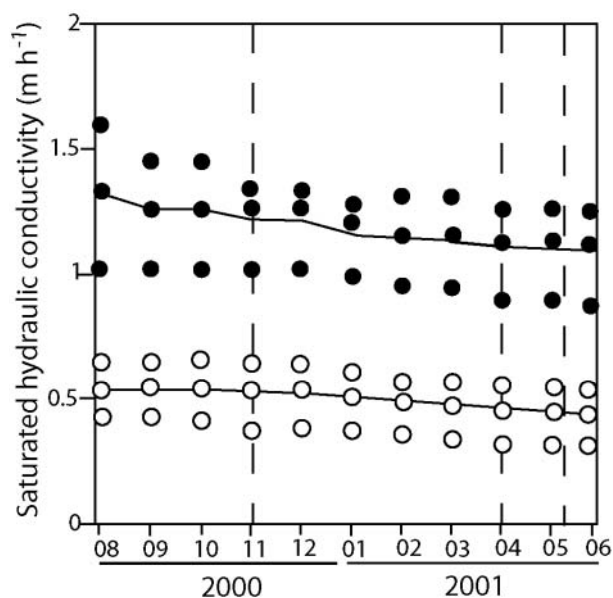


Figure 1. Average saturated hydraulic conductivity of the two sand filter types with sampling dates indicated by the dashed lines. The dots indicate the measurements obtained for each individual filters (triplicates). The black lines and dots correspond to the C+ filters, the grey line and white dots to the C- filters.

filter at a constant flux rate of 500 m³ h⁻¹. Samples of sand for microbiological analysis were taken from each filter at 3 points spaced approximately 1 m apart, forming an equilateral triangle. The triplicate samples of each filter were pooled for subsequent analyses. Sand was collected by coring with a Plexiglas tube (diameter 50 mm). The cores were sectioned, and two parts (0–5 cm and 15–20 cm) were preserved in an icebox for later analysis. The top layer (0–5 cm) corresponded to the sand removed when slow sand filters are cleaned. Samples of fauna were taken at 100 cm and 20 cm depth at one point from each filter using a Bou-Rouch pump (Bou and Rouch, 1967).

SEM observations

Electron-microscope studies were conducted on a Zeiss Leo 1530 scanning electron microscope (SEM). The sand samples were fixed with 4% glutaraldehyde for 4°C. The fixed samples were washed twice in distilled water and dehydrated in ethanol (50%, 80%, 98%, 3 min × 3 times each; absolute ethanol 10 min × 3 times) and critical-point dried in liquid CO₂. The samples were coated with platinum prior to SEM imaging.

Biological analysis

Lipids were extracted as described previously (Mauclair et al., 2003). The fatty acids were methylated and the fatty acid methyl esters separated by gas chromatography (Hewlett Packard HP 5890 series II equipped with a HP

Ultra 2 capillary column and a Flame Ionization Detector). Fatty acids with chain lengths of C3 to C20 were identified by the MIDI microbial identification system (MIDI, Inc. version 4.0) using TBSA40 and Eukary peak libraries. The fatty acids were grouped as indicated on Table 1. The total amount of PLFAs was converted to cell estimates assuming that, on average, bacteria contain 1 pmol of PLFA within 2×10^4 cells (Green and Scow, 2000).

Meio- and macrofauna were collected from 2L of pumped interstitial water filtered through a 180- μ m mesh net. The fauna was preserved with formaldehyde (4 % final concentration) and extracted under a stereomicroscope.

Data analysis and statistical methods

The impact of clogging intensity was tested using 3 way-analysis of variance (ANOVA) with clogging status, date and depths as main effects. When necessary, data were ln-transformed to homogenize variances. The 3-way ANOVAs were performed using Statistica 5 TM (Statsoft, Tulsa, Oklahoma).

Results

Results from fatty acid concentrations of the different biological groups (Fig. 2), fatty acids of anaerobes (Fig. 3), invertebrate species richness (Fig. 4), and SEM observations (Fig. 5) showed that filters were inhabited by a complex community of micro- and macro-organisms. The microbial community was composed mainly of bacteria (Fig. 2), as indicated by a relatively high abundance of branched fatty acids (used as bacterial markers, Table 1). The presence of dissolved oxygen in interstitial water was indicated by chemical measurements, and both aerobic and anaerobic bacterial markers were detected in all the filters. This result indicates the presence of anaerobic micro-niches within the pore spaces. The prokaryotic community was dominated by Gram-negative bacteria as shown by the high relative abundance of short chain monounsaturated PLFA (Fig. 2). Vertical gradients of PLFA concentrations varied significantly according to the clogging stage of the filter (Table 2). Higher PLFA contents were recorded in the surface of the C+ filters (Fig. 2). Based on published conversion factors, we calculated that the top layer contained 8.7×10^7 bacterial cells g sed^{-1} and 6.4×10^8 bacterial cells g sed^{-1} for C- and C+, respectively. The SEM pictures (Fig. 5) confirmed the presence of an abundant microbial community. In C+ filters (Figs. 5, 4a,e, 6a, 8b), most of the bacteria were embedded in a thick EPS matrix, whereas the EPS matrix was less dense in C- filters (Figs. 5, 1b, 3b). Furthermore, while small carbonate precipitates were detected in the C-filters (Figs. 5, 3c, 5c), carbonate cementation between the sand grains

Table 1. Biomarker groups of PLFA. PLFA nomenclature is in the form A:B ω C, where A designates the total number of carbons, B the number of double bonds and C the distance of the closest unsaturation from the aliphatic end of the molecule. The prefixes "i" and "a" refer to iso- and anteiso-methyl branching, and mid-chain methyl branches are designated by "Me" preceded by the position of the branch from the acid end. The cyclopropyl ring is indicated as "cy".

Gram-positive bacteria	Terminally branched saturated (mainly i14:0, i15:0, a15:0, i16:0, i17:0, i18:0, i19:0, i21:0)
Gram-negative bacteria	Short monounsaturated (mainly 15:1 ω 6, 15:1 ω 7, 16:1 ω 11, 16:1 ω 7, 16:1 ω 5, 17:1 ω 8, 17:1 ω 6)
Anaerobes	10Me16:0, 17:1 ω 6, cy17:0, cy19:0
Actinomycetes	i14:1, i15:1, i16:1, i17:1, a17:1, i18:1, i19:1
Microeukaryota	18:3 ω 6, 18:2 ω 6, 18:1 ω 9, 18:1 ω 5, 10Me18:0, 20:4 ω 6, 20:3 ω 6
Fungi	18:3 ω 6, 18:2 ω 6, 18:1 ω 9, 18:1 ω 5, 10Me18:0
Protists	20:4 ω 6, 20:3 ω 6

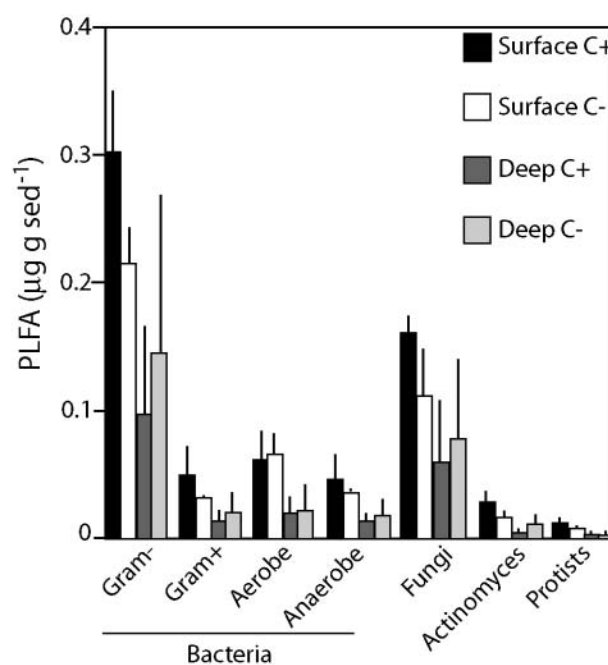


Figure 2. Spatial variation of the biomarker groups in the top (0–5 cm depth) and deep layers (15–20 cm depth) of the C+ and C- filters as averages with standard deviations for the three sampling dates (n = 9).

(Figs. 5, 2a) and calcification of micro-organisms (Figs. 5, 4c) were observed in the C+ filters.

Faunal abundance showed similar spatial distribution to microbial abundance. In C+ filters, faunal abundance significantly decreased with depth, whereas in C- filters no significant vertical gradient was observed (Fig. 4 and Table 3). Although the bottom part of both filters contained similar densities of organisms, invertebrate abun-

dance was significantly higher in C+ filters (331 ind l^{-1}) compared to C- filters (133 ind l^{-1}). Nematodes and oligochaetes always represented more than 50% of the overall faunal abundance. The majority of the biomass

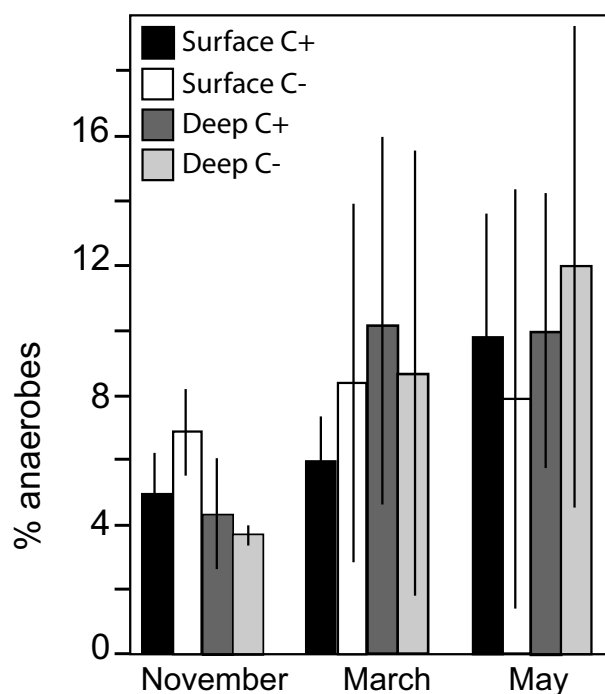


Figure 3. Spatial and temporal variation of the anaerobic biomarkers relative to the total fatty acid concentrations in the top (0–5 cm depth) and deep (15–20 cm) layers of the C+ and C- filters as average ($n = 3$).

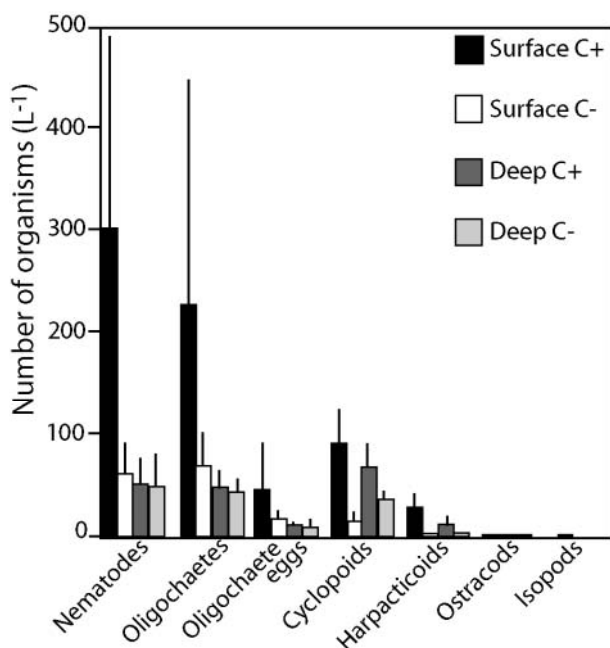


Figure 4. Spatial variation of the fauna in the surface (20 cm) and deep (100 cm) layers of the C+ and C- filters as averages with standard deviations for the three sampling dates ($n = 9$).

within the system consisted of oligochaetes, which, like other taxa were more abundant in the top part of the C+ filters (Fig. 4). Micro-crustacea (cyclopoids, harpacticoids and ostracods) represented 1–45% of the fauna. The only macro-crustacean found, *Asellus aquaticus*, was rare and only present in the top layer of C- filters.

The 3-way ANOVAs indicated a significant increase in microbial and faunal abundance and diversity with time, especially at the surface of the C+ filters (effect “date”, Table 2). The relative importance of anaerobes compared to the total amount of PLFA increased from about 4% in November to 10% in May, indicating the development of anaerobic microniches in sediments (Fig. 3). Furthermore, anaerobes changed over time according to the clogging intensity (effect of “date × clogging”, Table 2). In the C- filters, the percentage of anaerobes remained stable at the surface of the filters, whereas it regularly increased deeper. In C+ filters, similar development was observed between November and March, although on the last sampling date (May), the prevalence of anaerobes increased at the surface of sediment (Fig. 3). Similarly, the vertical gradients of Gram-positive and Gram-negative bacteria, actinomycetes, microeukaryotes, fungi, and protists were significantly different according to the clogging intensity (effect of “depth × clogging”, Table 2). PLFA concentrations were higher in the top layers of C+ than in C- filters, whereas no significant differences in PLFA contents were measured between C+ and C- filters in the deep layer. For the invertebrates, both nematode and micro-crustacean abundances were significantly higher in clogged filters (effect “clogging”, Table 2).

Discussion

Biodiversity of the artificial infiltrating porous media

The community structure of micro-organisms inhabiting the slow sand filters supports the results of other studies showing that Gram-negative bacteria are the most abundant microbial group in drinking water systems (Keinänen et al., 2003; Lahti, 1993; Payment et al., 1988; Smith et al., 2000). Eukaryotic markers did not represent the major part of the microbial community in terms of biomass, although they were detected in all the filters. Like Moll et al. (1999), we observed an increasing prevalence of Gram-negative bacteria and microeukaryotes in our filters operating at low temperatures (between 4 to 8°C). The identification of 22:6 ω 3, a biomarker of psychrophilic bacteria (Bowman et al., 2003), in nearly all the samples indicated that the microbial population living in the filters is well adapted to this particular environment. Low temperature also affects the development of the invertebrates (Adam et al., 1998). Furthermore, the filters (indoor tanks) are completely isolated from natural

Table 2. ANOVA results table with date, clogging status and depth as tested effects. * data log transformed, df degrees of freedom.

		date	clog. status	depth	date x clog.	date x depth	clog. x depth	date x depth x clog.
df		2	1	1	2	2	1	2
PLFA								
Abundance *	<i>F</i>	12.17	2	71.66	2.71	8.06	10.59	0.05
	<i>p</i>	<u>0.000</u>	.17	<u>0.000</u>	0.09	<u>0.002</u>	<u>0.003</u>	0.96
Number of peak	<i>F</i>	2.99	0.83	13.05	2.15	3.7	0.19	2.96
	<i>p</i>	0.07	0.37	<u>0.001</u>	0.14	<u>0.04</u>	0.67	0.07
Biomarker group of PLFA								
Gram-positive *	<i>F</i>	4.62	1.85	34.3	2.97	4.73	6.42	0.7
	<i>p</i>	<u>0.02</u>	0.19	<u>0.000</u>	0.07	<u>0.02</u>	<u>0.018</u>	0.51
Gram-negative	<i>F</i>	15.1	0.99	71.5	2.07	6.86	7.37	0.12
	<i>p</i>	<u>0.000</u>	0.33	<u>0.000</u>	0.15	<u>0.004</u>	<u>0.012</u>	0.88
Anaerobes	<i>F</i>	0.05	0.7	30.1	3.94	8.13	3.29	1.4
	<i>p</i>	0.95	0.41	<u>0.000</u>	<u>0.03</u>	<u>0.002</u>	0.08	0.27
Actinomycetes *	<i>F</i>	0.01	0.26	19.2	2.53	5.82	9.28	0.02
	<i>p</i>	0.99	0.61	<u>0.000</u>	0.10	<u>0.009</u>	<u>0.006</u>	0.978
Microeukaryota	<i>F</i>	9.24	1.51	25.1	0.48	2.24	5.4	0.54
	<i>p</i>	0.001	0.23	<u>0.000</u>	0.62	0.13	<u>0.03</u>	0.59
Fungi	<i>F</i>	8.96	1.1	21.2	0.48	2.18	4.98	0.45
	<i>p</i>	<u>0.001</u>	0.30	<u>0.000</u>	0.63	0.13	<u>0.03</u>	0.64
Protists	<i>F</i>	1.79	3.47	69.03	2.61	4.9	5.45	1.13
	<i>p</i>	0.19	0.07	<u>0.000</u>	0.09	<u>0.02</u>	<u>0.03</u>	0.34
Invertebrates								
Number of taxa *	<i>F</i>	102.8	2.2	1.5	2.4	0.6	0.7	0.3
	<i>p</i>	<u>0.000</u>	0.15	0.24	0.11	0.56	0.41	0.71
Oligochaetes *	<i>F</i>	1.37	2.04	5.07	0.14	0.93	0.15	0.42
	<i>p</i>	0.27	0.17	<u>0.03</u>	0.87	0.41	0.71	0.66
Nematodes *	<i>F</i>	1.63	4.46	4.84	0.26	0.38	1.14	0.34
	<i>p</i>	0.22	<u>0.04</u>	<u>0.04</u>	0.77	0.69	0.29	0.72
Microcrustaceans*	<i>F</i>	3.15	12.51	0.030	0.32	0.30	0.78	0.45
	<i>p</i>	0.06	<u>0.002</u>	0.87	0.73	0.74	0.39	0.64

sediment ecosystems and flying adult insects. This particular filter design may explain the relatively low abundance of invertebrates and the absence of insect larvae in these filters compared to the fauna communities in warmer outdoor slow sand filters (Jellison et al., 2000, Wotton and Hirabayashi, 1999).

The studied filters are artificial systems characterized by a peculiar fauna that differed from that observed in the raw water, which contained mainly micro-crustaceans (Aeppli, 1990; Smart and Harper, 1999). Because of the ozone treatment, direct colonization is unlikely and the animals probably descended from individuals introduced during the sand filling. However, the ozone pretreatments do not impair development of organisms within the filters (Urfer and Huck, 1999; Foncesca et al., 2001; Moll et al.,

1998). Although SEM revealed the presence of numerous diatom skeletons (Figs. 4, 7a) and a structure looking like an extremely small *Spirulina* (Figs. 4, 4d), there was no evidence that the living microbial community established in the filters was derived from the lake. The cleaning procedure of the raw water (ozonation and rapid carbon filtration) appears to be highly efficient since biomarkers of diatoms, microalgae and green algae, which are abundant in the lake of Zurich, were not detected. The absence of long-chain polyunsaturated fatty acids also confirms the absence of phytoplankton in the filters. This result is consistent since the filters are maintained in the dark, preventing phototrophic activity. However, it is interesting to note that there is no direct relation between the phytoplankton bloom observed in the lake between the

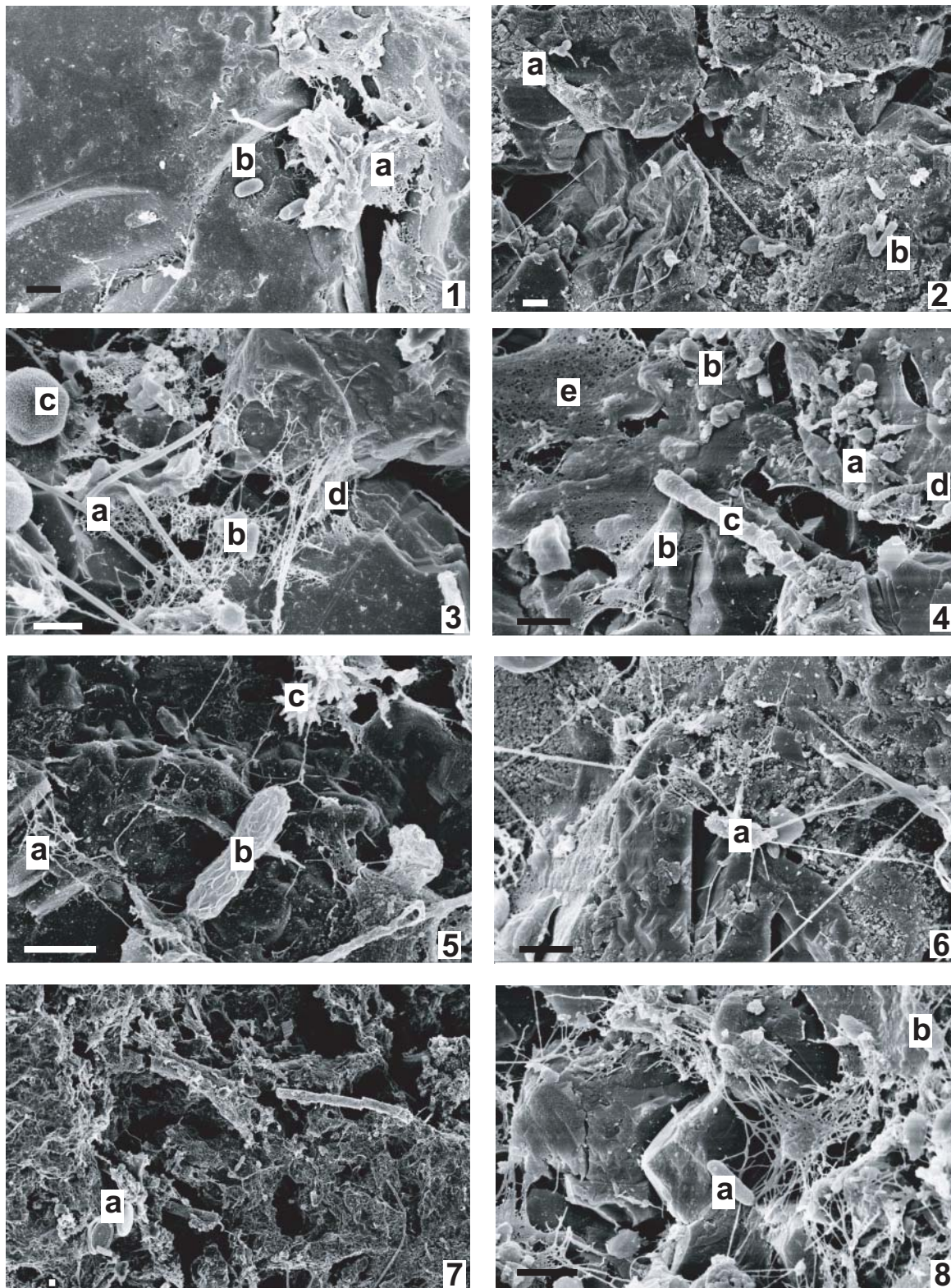


Figure 5. Scanning electron micrographs of sediment from the slow sand filters (top layer): C- filters (odd numbers) and C+ filters (even numbers). Picture 1a- EPS filling the space between sand grains, 1b- bacteria, 2a- carbonate precipitation cementing the space between sand grain, 2b- bacteria, 3a- fungal hyphae, 3b- bacteria, 3c- calcite precipitation, 3d- fine EPS deposit, 4a- small bacterial cocci embedded in EPS, 4b- bacteria rod embedded in EPS, 4c- calcified microorganisms, 4d- "nano spirulina", 5a- fine EPS film, 5b- bacteria covered by fine EPS, 5c- carbonate precipitate, 6a- bacteria covered by a dense EPS accumulation, 7a- diatom skeleton, 8a- bacteria free of EPS, 8b- bacteria embedded in EPS.

March and May sampling and the microbial community established in the filters.

Clogging processes and biological responses

As hypothesised, the increase of clogging provoked significant changes in the abundance and diversity of prokaryotes and eukaryotes, and appeared to stimulate microbial activity. Several studies demonstrated that microbial growth is related to hydrodynamics in porous media (Molz et al., 1986; Murphy et al., 1997) and predicted that a reduction of the hydraulic conductivity prolonged the contact between flowing water and biofilms, stimulating the incorporation of the nutrients transported by water in the top layer of clogged systems. Such nutrient incorporation thus produced a positive feedback to the clogging process by stimulating the bacterial development in the surface layer of sediments. Furthermore, the bacterial stimulation may also increase the clogging process by facilitating the carbonate precipitation. As there was more biofilm in the clogged filter, it is expected that anaerobes would develop better in the C+ filters than in C- filters. Although the smell of H₂S was never detected during the sampling, the presence of 10Me16:0, a biomarker of *Desulfovibrio* species (Edlund et al., 1985), indicated that part of the microbial community reduced sulfate. The possible shift of the microbial community towards sulphate-reducing activity has implications for carbonate precipitation. Although aerobic oxidation favours the carbonate dissolution by releasing carbon dioxide, anaerobic oxidation by sulphate-reducing bacteria at pH 8 favours carbonate precipitation by production of bicarbonate. This hypothesis is confirmed by SEM observation of the sand samples (Fig. 5). Previous observations demonstrated that carbonate precipitate occupied a similar pore volume to the exopolymeric substances in our filters (Mauclaire et al., 2004). Furthermore, the presence of hydrogen sulfide may provoke the precipitation of iron and manganese. This phenomenon, reported in similar filtration systems (Vandenabeele et al., 1992), was not observed in the Zurich-Lengg filters.

The source of bioclogging in porous media is usually biofilm that grows on the surface of grains and is smaller than a millimeter. The biofilm volume estimates (Mauclaire et al., 2004) were confirmed by SEM observations (Fig. 5): the EPS were more abundant and thicker in the C+ than in the C- filters. Furthermore, fungi and actinomycetes, which grow as filaments, can occupy many pores with dimensions approaching the centimetre scale (Dupin and McCarty, 1999). Presence of fungal hyphae was confirmed by SEM observations (Figs. 5, 3a). In the C+ filters, fungi and actinomycetes biomarkers were more abundant than in C- filters, further favouring bioclogging.

Protists in porous media have been used to account both for hydraulic conductivity increases and decreases.

In column experiments, the addition of heterotrophic nanoflagellates or amoebae merely delayed the occurrence of clogging (DeLeo and Baveye, 1997; Weber-Shirk and Dick, 1999; Mattison et al., 2002), probably by grazing on attached bacteria. However, in our study, predator abundances were significantly higher at the surface of C+ filters. This finding demonstrates that predation was not sufficient to reduce bacterial growth. In complex environments such as slow sand filters, the increase of predation stimulated microbial growth, which in turn reduced hydraulic conductivity. Furthermore, grazing might weaken adhesion of bacterial colonies (Honda and Matsumoto, 1983; Cullimore and Mansuy, 1987). The resulting biofilm detachment and subsequent filtration of clumps at strategic points such as pore constriction could explain the observed accelerated clogging (Taylor and Jaffé, 1990).

As with predation, invertebrate activities have been related to increases and decreases in hydraulic conductivity (Eder, 1982; Torreiter et al., 1994). Macroinvertebrates, such as nematodes or oligochaetes, generate biogenic structures, bioirrigation and various types of sediment reworking. Collectively, these processes, termed bioturbation, take place at the inhabited part of the benthic habitat (Gerino et al., 1999) and can favour water infiltration by producing tubes and galleries in the clogged layers of sand filters. Although in our study, the sampling point of microorganisms and invertebrates only overlapped at 20 cm depth and densities of invertebrates were probably too low to significantly affect hydraulic conductivity, bioturbator abundance was positively correlated with reduction of hydraulic conductivity. This correlation was probably the result of a trophic interaction between micro- and macro-fauna; as invertebrates living in sediments mostly feed on biofilm associated with particles (see review in Boulton, 2000), the stimulating effect of clogging on the density of micro-organisms may have also favoured the development of macroinvertebrates. Furthermore, invertebrates may have stimulated the microbial growth by modifying the chemical environment of the interstitial habitat, keeping the microbial communities in a growing state, and excreting nutrients into the system (Yingst and Rhoads, 1980; Aller, 1994; Griebler, 1996; Traunsperger et al., 1997; Mermillod-Blondin et al., 2004).

Conclusion

Biological communities inhabiting sand filters comprise organisms from bacteria to invertebrates, that belong to different trophic levels. The study of filters with different clogging intensities demonstrated that the community structure of both prokaryotes and eukaryotes depends on the rate of filtration. The reduction of hydraulic conduc-

tivity could be interpreted as an early stage of sand diagenesis with accumulation of biomass within the pore space and the development of a complex community of micro- and macro-organisms. Furthermore, the shift of microbial community from aerobic to anaerobic conditions might favor carbonate precipitation and thus the clogging process.

This study suggests approaches to prevent or minimize the clogging of the sand filter and obtain more sustainable management. The microbial activity within the filter was limited by the intense pre-treatment of the infiltrated lake water and the design of the filter. As these physical and chemical limitations did not completely prevent clogging, we speculate that the presence of organisms grazing on biofilm such as protists (Mattison et al., 2002) and invertebrates might help prevent clogging. In the present study, invertebrate density was low and essentially linked to the occurrence of microorganisms. Such low densities were probably not sufficient to modify the clogging status of the filters. We can speculate that the introduction of higher densities (e.g. 10.000 individuals per m² or more than 1000 individuals per liter) of invertebrates such as tubificid worms could minimize the clogging process due to grazing and bioturbation. Introduction of invertebrates which produce galleries in the sediments (Mermillod-Blondin et al., 2005; Rogaar, 1980) could maintain the hydraulic conductivity of the system. Such biological treatment has, however, never been tested and future research on clogging of sand filter should consider that metazoans could help to maintain the hydrological functioning of filtration systems.

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