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Using bacteriophages, indicator bacteria, and viral pathogens for assessing the health risk of drinking water obtained by bank filtration.

“bacteria”; Federal Environmental Agency of Germany (UBA)

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1.1 Introduction

Indicator bacteria and coliphages along the transects of Lake Tegel, Lake Wannsee and the artificial recharge pond Tegel

The indicator microorganisms, Escherichia coli, intestinal enterococci, and coliphages were analysed in water samples from both transects for bank filtration at the Lakes Tegel and Wannsee as well as from the artificial recharge pond of Tegel. Surface water samples of both lakes were examined from May 2002 to December 2004. In addition, samples were analysed from four shallow observation wells and three pumping sites for drinking water supply along the transect Wannsee, as well as four deep and seven shallow observation wells and 2-3 pumping sites along the transect of Lake Tegel. Sampling at the artificial recharge pond of Tegel was carried out during four field surveys (August 2002-December 2002) in two shallow, three deep wells and one pumping well for drinking water supply. In addition, the microbial flora was studied in the sand filter of the recharge pond in Tegel.

1.2 Results

1.2.1 Bank filtration field site Lake Tegel

1.2.1.1 Surface water

During the field surveys from May 2002 to December 2004, lake water samples were taken in monthly intervals and investigated for the indicator bacteria E. coli, intestinal enterococci and somatic coliphages. The concentration of indicator bacteria in lake water samples was very low and ranged from below detection limit (<1/100ml) to about 100 cfu/100 ml. Coliphages were detected in concentrations of 1 to 160 pfu/100 ml. No clear seasonal pattern of the concentration of indicator organisms was observed (Figure 1).

Lake Tegel receives faecal contaminated water from two sources: Canal Nordgraben discharging secondary effluent and Creek Tegel draining run off water from agricultural fields. These waters are, however, treated by a phosphate elimination plant (PEA Tegel) before entering the lake. The hygienic quality of the water was, therefore, higher than expected. The effect of this advanced treatment process (flocculation) on the reduction of indicator organisms was investigated during the sampling period from February to August 2004. Concentrations of E. coli, intestinal enterococci, and coliphages were determined in influent and effluent samples of the PEA Tegel (see Table 1).

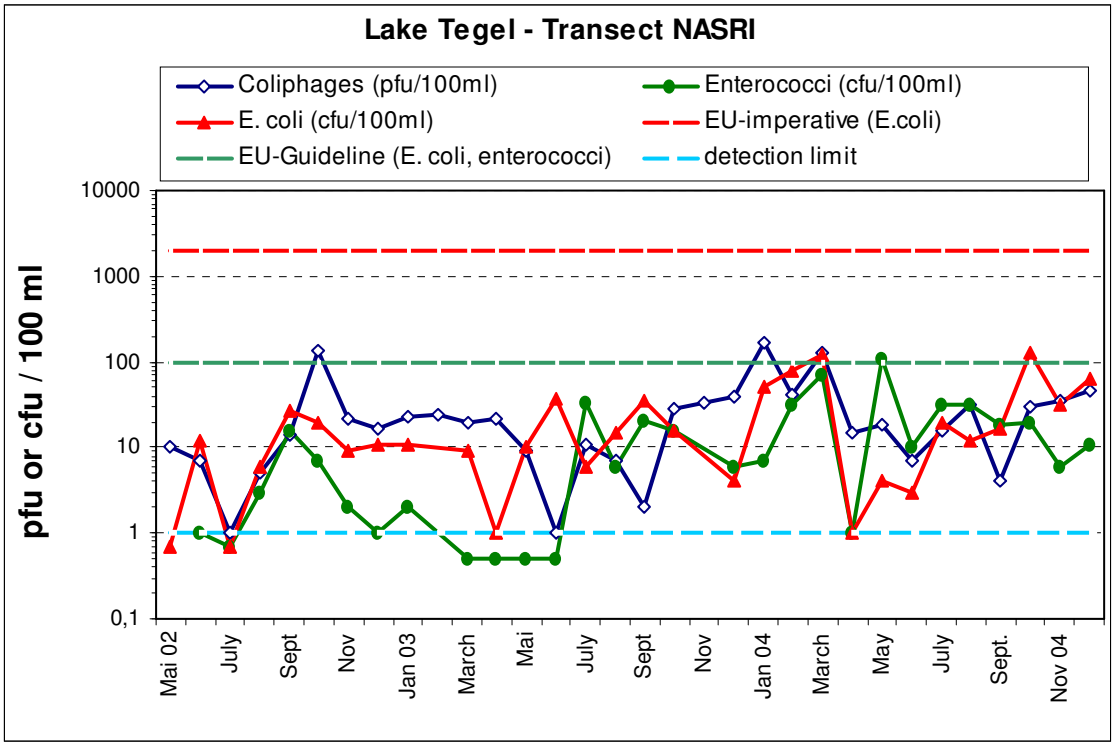

Figure 1 Occurrence of indicator organisms in surface water samples from Lake Tegel

Table 1 Reduction of indicator micro organisms in the PEA Tegel by flocking and rapid sand filtration. (* additional sampling at the same day)

Sampling	Coliphages (pfu/100ml)			E. coli (cfu/100ml)			Enterococci (cfu/100 ml)		
	influent	effluent	Reduction	influent	effluent	Reduction	influent	effluent	Reduction
Jan. 04	1653	232	0,86	1000	150	0,85	175	24	0,86
Jan. 04*	1511	209	0,86	1200	97	0,92	312	10	0,97
Feb. 04	2800	999	0,64	12483	725	0,94	1689	78	0,95
March 04	1032	51	0,95	500	120	0,76	273	72	0,74
April 04	1310	560	0,57	100	50	0,50	49	10	0,80
May 04	3100	100	0,97	3552	122	0,97	42	1	0,98
June 04	1170	172	0,85	438	22	0,95	42	1	0,98
Aug 04	1040	90	0,91	-	-	-	-	-	-
mean	-	-	0,82 + 0,13	-	-	0,84 + 0,15	-	-	0,9 + 0,09

Reduction of all test organisms was on average about one log unit. The reduction rates varied between 57 % and 97 % for somatic coliphages, 50 % and 97 % for E. coli, 74% and 98% for intestinal enterococci. Despite these relatively low elimination rates, the PEA Tegel contributes substantially to the reduction of indicator organisms and probably also of hygienically relevant microorganisms in the Lake Tegel.

1.2.1.2 Transects

1.2.1.2.1 Investigation on the transect of Lake Tegel in the year 2002

Water samples were taken from the deep observation wells (3301, 3302, 3303 and 3304) using a MP1-pumping module and accessories with a flow capacity of 200 L/min and directly from the faucets of 2 or 3 pumping wells for the regular drinking water supply (12, 13 and 14). The MP1-pumping module was not disinfectable and was used for all sampling points. Sampling of the shallow wells along the transect Tegel was started on August 2002 also using non sterile MP1 or Eijkelkamp pumping module. Sterilized Eijkelkamp pumps and equipments were used for each of the seven shallow observation wells during the last four field surveys (September-December 2002).

Apart from one positive sample from the deep observation well 3302, somatic coliphages were never detected in samples from deep wells (Table 2A). Samples from the shallow observation wells were also negative for coliphages (Table 2B). Only one sample from the first well (3311) located on the lake basin contained coliphages at a low concentration of 2 pfu/100 ml.

Table 2 Occurrence of somatic coliphages (pfu/100ml) in water samples from the transect of Lake Tegel (detection limit: 1 pfu/100ml)

A: deep observation wells and pumping wells

Sampling time	Lake Tegel	3301	3302	3303	3304	Well 12	Well 13	Well 14
16.05.02	10	<1	<1	<1	<1	<1	n.a.	<1
11.06.02	7	<1	<1	<1	<1	<1	n.a.	<1
09.07.02	1	<1	<1	<1	<1	<1	n.a.	<1
21.08.02	5	<1	<1	<1	<1	<1	n.a.	<1
23.09.02	14	<1	<1	<1	<1	<1	n.a.	<1
22.10.02	44	<1	1	<1	<1	<1	n.a.	<1
28.11.02	22	<1	<1	<1	<1	<1	<1	<1
19.12.02	15	<1	<1	<1	<1	<1	<1	<1

B: shallow observation wells

Sampling time	Lake Tegel	3311	3310	3309	3308	3307	3306	3305
22.08.02**	5	<1	<1	<1	<1	<1	<1	<1
23.09.02***	2	<1	<1	<1	<1	<1	<1	<1
22.10.02***	44	<1	<1	<1	<1	<1	<1	<1
27.11.02***	18	<1	*	<1	<1	<1	<1	n.a.
17.12.02***	17	<1	*	<1	<1	<1	<1	n.a.
28.01.03***	23	2	<1	<1	<1	<1	<1	<1

* no sample, ** sampling with non sterile instruments, *** sampling with sterilized instruments

n.a. = not analysed

Apart from one sample from well 3301, non of the water samples from deep wells contained intestinal enterococci (Table 3A).

All water samples were free from intestinal enterococci in 100 ml with the exception of the field survey on October 2002. During this survey intestinal enterococci were detected in 6 out of 7 water samples from the shallow wells in concentrations of 3 - 108 cfu/100ml (source of contamination unknown).

Table 3 Occurrence of intestinal enterococci (cfu/100ml) in water samples from the transect of Lake Tegel (detection limit: 1 cfu/100ml)

A: deep observation wells and pumping wells

Sampling time	Lake Tegel	3301	3302	3303	3304	Well 12	Well 13	Well 14
16.05.02	3	1	<1	<1	*	<1	n.a.	<1
11.06.02	1	<1	<1	<1	<1	<1	n.a.	<1
09.07.02	<1	<1	<1	<1	<1	<1	n.a.	<1
21.08.02	2	<1	<1	<1	<1	<1	n.a.	<1
23.09.02	6	<1	<1	<1	<1	<1	n.a.	<1
22.10.02	7	<1	<1	<1	<1	<1	n.a.	<1
28.11.02	2	<1	<1	<1	<1	<1	<1	<1
19.12.02	<1	<1	<1	<1	<1	<1	<1	<1

B: shallow observation wells

Sampling time	Lake Tegel	3311	3310	3309	3308	3307	3306	3305
22.08.02**	3	<1	<1	<1	<1	<1	<1	<1
23.09.02***	16	<1	<1	<1	<1	<1	<1	*
22.10.02***	6	22	3	108	14	<1	26	31
27.11.02***	1	<1	*	<1	<1	<1	<1	*
17.12.02***	1	<1	*	<1	<1	<1	<1	*
28.01.03***	2	<1	<1	<1	<1	<1	<1	*

* no sample, ** sampling with non sterile instruments, *** sampling with sterilized instruments

n.a. = not analysed

E. coli was not detected in water samples from deep wells during the field surveys in May and December. During all other surveys E. coli was detected in one or several samples in concentrations between 2 cfu/100 ml and 6 cfu/10 ml. In total 2 samples from the pumping sites 12 and 14 were also positive for E. coli (

Table 4a).

Table 4 Occurrence of E. coli (cfu/100ml) in water samples from the transect of Lake Tegel (detection limit: 1 cfu/100ml)

A: deep observation wells and pumping wells

Sampling time	Lake Tegel	3301	3302	3303	3304	Well 12	Well 13	Well 14
16.05.02	<1	<1	<1	<1	*	<1	n.a.	<1
11.06.02	12	<1	<1	<1	1	<1	n.a.	<1
09.07.02	<1	2	2	<1	<1	6	n.a.	<1
21.08.02	3	6	<1	<1	2	<1	n.a.	<1
23.09.02	5	9	<1	<1	<1	<1	n.a.	3
22.10.02	8	6	5	1	<1	<1	n.a.	<1
28.11.02	<1	<1	<1	1	<1	<1	<1	<1
19.12.02	5	<1	<1	<1	<1	<1	<1	<1

B: shallow observation wells

Sampling time	Lake Tegel	3311	3310	3309	3308	3307	3306	3305
22.08.02**	6	27	35	<1	<1	<1	<1	30
23.09.02***	27	<1	<1	<1	<1	<1	<1	n.a.
22.10.02***	10	<1	<1	<1	<1	<1	<1	<1
27.11.02***	9	<1	*	<1	<1	<1	<1	n.a.
17.12.02***	11	<1	*	<1	<1	<1	<1	n.a.
28.01.03***	15	<1	<1	<1	<1	<1	<1	<1

* no sample, ** sampling with non sterile instruments, *** sampling with sterilized instruments

n.a. = not analysed, italic = in 10 ml of sample

During the field survey August 2002, samples from the shallow observation wells were taken using a non sterile Eijkelkamp pump and non sterile tubing at all sampling sites. High densities of E. coli were found in samples from the wells 3311 and 3310 located on the lake basin, as wells as in the sample of the last well (3305) of the transect (Table 4B). E. coli was not detected during all other surveys in September 2002 to January 2003 when sterile equipment was used.

Data on the occurrence of heterotrophic plate count bacteria (according to the German Drinking water Directive; colony counts according to DIN EN ISO 6022 were slightly higher) in water samples from deep wells are shown in Table 5. No retention pattern can be deducted from the data received from the samples of the deep observation wells. Colony counts at 20 °C as well as at 37 °C varied from < 10 cfu/ml to > 103 cfu/ml, indicating external contamination by the non disinfected sampling equipment. At the regularly pumping sites of Water Work Tegel, colony counts were usually less than 100 cfu/ml. High concentrations of up to 500 cfu/ml were detected in three samples.

Table 5: Occurrence of heterotrophic plate count bacteria (cfu/ml) in water samples from deep wells and pumping wells along the transect of Lake Tegel (detection limit: 1 cfu/ml) * no sample

A. incubation at 20 °C

Sampling	Lake Tegel	3301	3302	3303	3304	well 12	well 13	well 14
16.05.02	690	160	20	30	n.a.	3	*	1
11.06.02	200	30	50	8	20	<1	*	1
09.07.02	530	360	60	70	160	60	*	30
22.08.02	1370	120	40	30	50	1	*	<1
23.09.02	20	6	90	7	90	1	*	1
22.10.02	2300	650	1300	60	150	210	*	20
27.11.02	820	40	60	6900	10	<1	<1	9
17.12.02	250	170	6	100	40	<1	3	2

B. incubation at 37 °C

Sampling	Lake Tegel	3301	3302	3303	3304	well 12	well 13	well 14
16.05.02	241	9	5	5	n.a.	<1	*	<1
11.06.02	200	1100	590	490	300	1	*	2
09.07.02	300	650	260	360	150	170	*	530
22.08.02	230	310	160	6	240	1	*	<1
23.09.02	21	300	230	10	50	1	*	<1
22.10.02	1800	1060	820	120	200	<1	*	10
27.11.02	130	20	10	2000	10	1	1	1
19.12.02	130	30	1	100	20	2	1	<1

In water samples from the shallow wells, colony counts were lower than in the deep observation wells and ranged from below detection limit to 320 cfu/ml (Table 6). With exception of samples on August 2002, all these samples were taken by disinfected pumps and silicon tubing. These findings further support the possible contamination of the deep wells during sampling.

Table 6 Occurrence of heterotrophic plate count bacteria in water samples from the shallow wells along the transect of Lake Tegel (detection limit: 1 cfu/ml), n.a: not analysed]

Sampling	Lake Tegel	3311	3310	3309	3308	3307	3306	3305
22.08.02 (20°C)	580	60	80	40	4	8	30	80
(37°C)	310	80	100	20	2	3	3	30
23.09.02 (20°C)	150	30	30	9	4	5	2	n.a.
(37°C)	70	40	30	8	<1	<1	20	n.a.
22.10.02 (20°C)	250	20	20	60	8	40	320	50
(37°C)	160	20	20	30	4	20	140	5
27.11.2002 (20°C)	1100	10	n.a.	5	10	10	80	n.a.
(37°C)	130	10	n.a.	5	1	10	30	n.a.
17.12.2002 (20°C)	20	5	n.a.	9	10	20	20	n.a.
(37°C)	30	2	n.a.	9	20	20	20	n.a.

1.2.1.2.2 Investigation on the transect of Lake Tegel in the years 2003-2004

No further investigations were performed at the transect of Lake Tegel because of the low initial concentrations of indicator microorganisms in the lake water. These low concentrations did not allow to deduct retention characteristics of the organisms during subsurface passage along the transect. Investigations were continued at the transect of Lake Wannsee awaiting new shallow wells to be build near the lake shore (see below).

1.2.2 Bank filtration field site Lake Wannsee

1.2.2.1 Surface water

Data on the occurrence of indicator bacteria and coliphages in surface water of Lake Wannsee taken monthly from May 2002 up to December 2004 are shown in the Fig. 2.

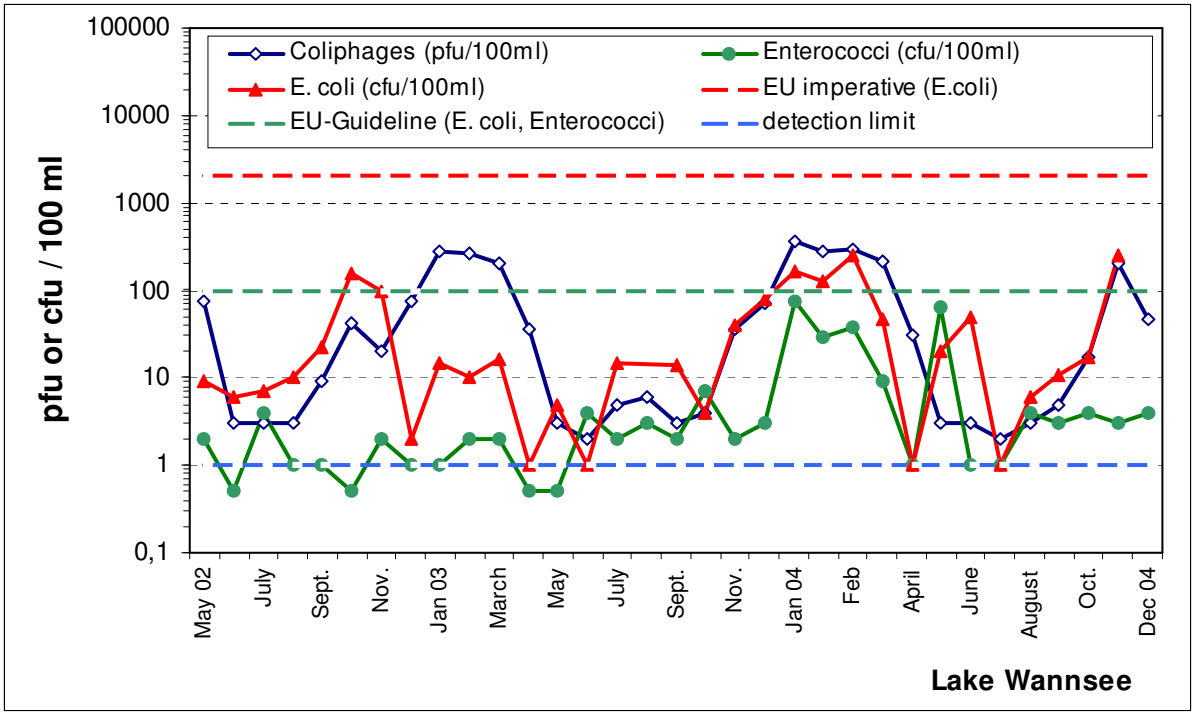

Figure 2 Occurrence of indicator organisms in surface water samples from Lake Wannsee

Concentrations of coliphages varied between 2 and 360 pfu/100 ml. Concentrations of indicator bacteria ranged from detection limit up to 38 cfu/100 ml for intestinal enterococci, and up to 250 cfu/100 ml for E. coli, respectively. A seasonal concentration pattern was observed with low concentrations in summer and higher concentrations in winter. Concentrations of intestinal enterococci were always below the guideline values of the EU bathing water Directive (Figure 2). Concentrations of E. coli were in some samples above the guideline value (100 cfu/100ml) but never reached the imperative value (2000 cfu/100ml).

On 17.3.2004 water samples were collected at 15 different sampling sites closed to the transect area to investigate the spatial variation in the concentration of indicator microorganisms. At one station (FU 9) samples were additionally taken from different depth down to 7 m (0, 2, 4, 6 and 7m).

High concentrations of coliphages (850 pfu/100ml), E. coli (50 mpn/100ml) and intestinal enterococci (32 mpn/100ml) were detected in the sample of station FU5 close to the Griebnitz Sea where water of the Teltow canal - contaminated with secondary effluent - is discharged into Lake Wannsee. Concentrations decreased with increasing distance to sampling site FU5 (see table 7, values above and below FU5) and were lowest at the sampling sites on the transects of Wannsee (**Table 7**).

Concentrations of coliphages at different depths of the lake were in the same order of magnitude ranging from 100 to 180 pfu/100ml. E. coli and intestinal enterococci were only sporadically detected at all sampling depths.

Table 7 Concentration of indicator organisms in Lake Wannsee at different sampling sites close to the transect area.

Sampling sites	Coliphages (pfu/100ml)	Enterococci (cfu/100ml)	E. coli (cfu/100ml)
FU10	70	1	0,1
FU11	80	<1	1
FU9 (0m)	100	<1	<1
FU9 (2m)	140	<1	<1
FU9 (4m)	100	<1	2
FU9 (6m)	150	<1	1
FU9 (7m)	180	1	3
FU8	110	<1	<1
FU7	220	3	2
FU6	240	<1	20
FU5	850	30	50
FU2	280	6	10
FU1	290	4	20
14	350	1	20
15	140	1	2
FU19	110	<1	<1
FU18	120	1	<1
18	70	<1	<1
19	70	<1	<1

1.2.2.2 Investigations on Transect 1 of Lake Wannsee in 2002.

Four shallow observation wells (3335, 3337, 3338 and 3339) and three pumping sites for drinking water supply (wells 3-5) along the transect Wannsee were sampled during the eight field surveys from March to December 2002. During the first four sampling dates, in May, June, July and August, the equipment of the Department of Hydrogeology in the Free University was used for sampling of the shallow observation wells. These samplings were carried out using one or two Eijkelkamp pumps connected in series and plastic tubing for all sampling sites. The flow capacity varied between 3-5 L/min depending on the depth of each sampling site. Sterilised Eijkelkamp pumps and silicon tubing were used for sampling the shallow wells in the last four sampling periods in September, October, November und December. Data on the occurrence of indicator microorganisms in water samples from the wells of transect Wannsee in the year 2002 are summarized in Table 8 - Somatic coliphages were sporadically detected in very low concentrations (1 and 4 pfu/100ml) in the first observation well (3339) located on the lake basin. All other samples located further along the filtration path were negative for coliphages (Table 8).

Table 8 Occurrence of somatic coliphages (pfu/100ml) in water samples from the transect of Lake Wannsee in the year 2002 (detection limit: 1 pfu/100ml)

Sampling time	Lake Wannsee	3339	3338	3337	3335	Well 3	Well 4	Well 5
15.05.2002*	75	4	<1	<1	<1	*	*	*
12.06.2002*	3	1	<1	<1	<1	<1	<1	<1
10.07.2002*	3	<1	<1	<1	<1	<1	<1	<1
21.08.2002*	3	*	<1	<1	<1	<1	<1	<1
25.09.2002**	9	<1	<1	<1	<1	<1	<1	<1
23.10.2002**	43	<1	<1	<1	<1	<1	<1	<1
26.11.2002**	20	*	<1	<1	<1	<1	<1	<1
18.12.2002**	73	<1	<1	<1	<1	<1	<1	<1

* sampling with a non sterilized pump, ** sampling with disinfected pumps

Intestinal enterococci were detected in two out of six samples from the first well 3339. No intestinal enterococci were detected in the other shallow sampling wells and regular pumping sites of the Water Work Beelitzhof (Table 9).

Table 9 Occurrence of intestinal enterococci (cfu/100ml) in water samples from the transect of Lake Wannsee in the year 2002 (detection limit: 1 cfu/100ml)

Sampling time	Lake Wannsee	3339	3338	3337	3335	Well 3	Well 4	Well 5
15.05.2002*	2	<1	<1	<1	<1	*	*	*
12.06.2002*	<1	<1	<1	<1	<1	<1	<1	<1
10.07.2002*	4	<1	<1	<1	<1	<1	<1	<1
21.08.2002*	1	*	<1	<1	<1	<1	<1	<1
25.09.2002**	1	1	<1	<1	<1	<1	<1	<1
23.10.2002**	<1	3	<1	<1	<1	<1	<1	<1
26.11.2002**	2	*	<1	<1	<1	<1	<1	<1
18.12.2002**	1	<1	<1	<1	<1	<1	<1	<1

* sampling with a non sterilized pump, ** sampling with disinfected pumps

In contrast to enterococci and coliphages, E. coli was detected in most samples (4) from wells 3339 and 3337. The detection frequency declined to 3 out of 8 samples in the second well on the sea basin (3338) or on the shore (3335), respectively. During the field survey in October 2002, E. coli was found in all shallow wells (Table 10). Only two out of 21 samples from the pumping sites (wells 3, 4, 5) contained E. coli. Using sterilized pumps and tubing had no effect on the detection frequency of E. coli in the shallow wells. Data on the occurrence of heterotrophic plate count bacteria (according to the German Drinking water Directive; colony counts according to DIN EN ISO 6022 were slightly higher) are summarized in Table 11.

Table 10 Occurrence of E. coli (cfu/100ml) in water samples from the transect of Lake Wannsee in the year 2002 (detection limit: 1 cfu/100ml)

Sampling time	Lake Wannsee	3339	3338	3337	3335	Well 3	Well 4	Well 5
15.05.2002*	9	5	<1	<1	<1	*	*	*
12.06.2002*	6	1	1	10	<1	<1	<1	5
10.07.2002*	7	<1	<1	<1	<1	<1	<1	<1
21.08.2002*	10	*	<1	<1	5	<1	<1	<1
25.09.2002**	22	2	<1	3	7	<1	<1	<1
23.10.2002**	160	2	2	33	11	1	<1	<1
26.11.2002**	100	*	3	<1	<1	<1	<1	<1
18.12.2002**	2	<1	<1	5	<1	<1	<1	<1

* sampling with a non sterilized pump, ** sampling with disinfected pumps

Non sterilized pump and tubing were used for sampling during the first four field surveys and sterilized equipment during the last four surveys. Colony counts in water samples from the shallow wells were usually low (< 100 cfu/ml) but single high concentrations were found especially during the first surveys when non sterilized equipment was used.

Table 11: Occurrence of heterotrophic plate count bacteria (cfu/ml) in water samples from the shallow wells and pumping sites (wells 3,4 and 5) of the Water Work Beelitzhof (detection limit: 1 cfu/ml)

A. incubation temperature 20°C

Sampling sites	Lake Wannsee	3339	3338	3337	3335	well 3	well 4	well 5
15.05.02*	220	50	7	12	6	n.a.	n.a.	n.a.
12.06.02*	80	40	9	500	50	320	60	200
10.07.02*	320	40	50	180	2400	<1	<1	120
21.08.02*	2040	n.a.	10	10	100	1	1	10
25.09.02**	100	50	70	110	110	2	<1	<1
23.10.02**	400	20	10	10	4	1	3	4
26.11.02**	60	n.a.	10	10	10	<1	<1	<1
18.12.02**	20	30	3	40	5	<1	<1	<1

B. Incubation temperature 37°C

Sampling sites	Lake Wannsee	3339	3338	3337	3335	well 3	well 4	well 5
15.05.02*	130	30	6	10	110	n.a.	n.a.	n.a.
12.06.02*	70	20	60	250	220	310	170	300
10.07.02*	200	130	180	270	3800	<1	17	230
21.08.02*	2300	*	10	10	100	1	1	10
25.09.02**	100	40	4	90	20	<1	<1	<1
23.10.02**	140	20	1300	240	20	<1	<1	1
26.11.02**	20	*	30	40	50	<1	<1	<1
18.12.02**	40	20	10	10	20	<1	<1	<1

* sampling with a non sterilized pump, ** sampling with disinfected pump

1.2.2.2.1 Investigations on transect 1 of Lake Wannsee in 2003-2004:

In the year 2003, water samples from transect 1 were taken monthly using sterilised pumps and silicon tubes separately for each station. Sampling concentrated on the shallow and less distant wells because no coliphages had been found after long filtration paths during the previous sampling period. A new observation well -well 201OP- was established additionally.

The observation wells 3337 and the new observation well 201OP were sampled at all sampling surveys. Sampling well 3339 was flooded with lake water and, therefore, not sampled. During some sampling times the shallow observation well 3338 was dry. Sampling at the observation well 3335 was carried out only four times during the intensive sampling period from 15.9.03 to 15.10.03 (data not shown).

Along transect 1 of Lake Wannsee (3338, 3337, 201OP, and well 4), no coliphages were detected in any of the samples (Table 12).

Table 12 Occurrence of somatic coliphages (pfu/100 ml) in water samples at different sampling sites along transect 1 of Lake Wannsee (detection limit: 1/ 100 ml), * no sample, n.a.: not analysed

Sampling time	Lake Wannsee	3338	3337	201OP	Well 4
20.02.2003	267	*	*	<1	n.a.
18.03.2003	290	<1	<1	<1	<1
08.04.2003	40	<1	<1	<1	<1
20.05.2003	3	<1	<1	<1	<1
17.06.2003	1	<1	<1	<1	<1
03.07.2003	5	<1	<1	<1	<1
19.08.2003	6	<1	<1	<1	<1
22.09.2003	2	*	<1	<1	<1
29.09.2003	2	<1	<1	<1	<1
06.10.2003	4	<1	<1	<1	<1
13.10.2003	6	*	<1	<1	<1
21.10.2003	36	*	<1	<1	<1
18.11.2003	n.a.	n.a.	n.a.	n.a.	n.a.
09.12.2003	71	*	<1	<1	<1
20.01.2004	362	<1	<1	<1	<1
17.02.2004	297	<1	<1	<1	<1
16.03.2004	157	<1	<1	<1	<1
20.04.2004	32	<1	<1	<1	<1
positive samples	17 / 17	0 / 12	0 / 16	0 / 17	0 / 16

As in 2002, intestinal enterococci were sporadically detected in water samples of transect 1 (Table 13). Four out of 12 samples contained intestinal enterococci in well 3338 located on the sea basin. The second well 3337 of transect 1 contained enterococci only in one out of 16 samples. No enterococci species were detected in well 201OP and pumping station 4 of the Water Work.

E. coli was again detected more frequently than enterococci and coliphages. Three to four out of 12, 15 or 17 samples from wells 3338, 3337 and 201OP were positive, respectively (Table 14). E. coli was also detected in two samples from pumping well 4 in very low concentrations (1 cfu/100ml).

Table 13: Occurrence of intestinal enterococci (cfu/100 ml) in water samples at different sampling sites along the transect 1 of the Lake Wannsee

(detection limit <1 in 100 ml) * no sample, n.a.: not analysed

Sampling time	Lake Wannsee	3338	3337	201OP	Well 4
20.02.2003	2	*	*	<1	n.a
18.03.2003	1	<1	<1	<1	<1
08.04.2003	<1	<1	1	<1	<1
20.05.2003	<1	<1	<1	<1	<1
17.06.2003	<1	<1	<1	<1	<1
03.07.2003	<1	3	<1	<1	<1
19.08.2003	3	<1	<1	<1	<1
22.09.2003	20	*	<1	<1	<1
29.09.2003	34	<1	<1	<1	<1
06.10.2003	7	6	<1	<1	<1
13.10.2003	<1	*	<1	<1	<1
21.10.2003	1	*	<1	<1	<1
18.11.2003	n.a.	n.a.	n.a.	n.a.	n.a.
09.12.2003	3	*	<1	<1	<1
20.01.2004	29	1	<1	<1	<1
17.02.2004	38	1	<1	<1	<1
16.03.2004	<1	<1	<1	<1	<1
20.04.2004	<1	<1	<1	<1	<1
positive samples	10 / 17	4 / 12	1 / 16	0 / 17	0 / 15

Table 14 Occurrence of E. coli (cfu/100 ml) in water samples at different sampling sites along transect 1 of Lake Wannsee (detection limit: 1 cfu/100ml) *: no sample, n.a.: not analysed, >> invalid results]

Sampling time	Lake Wannsee	3338	3337	201OP	Well 4
20.02.2003	10	*	*	6	n.a.
18.03.2003	16	1	<1	<1	<1
08.04.2003	4	3	1	<1	<1
20.05.2003	5	<1	<1	<1	<1
17.06.2003	<1	<1	>>	<1	1
03.07.2003	15	<1	<1	<1	<1
19.08.2003	>>	1	<1	<1	<1
22.09.2003	19	*	<1	1	<1
29.09.2003	>>	<1	<1	<1	n.a.
06.10.2003	4	<1	8	<1	n.a.
13.10.2003	8	*	<1	<1	1
21.10.2003	8	*	1	<1	<1
18.11.2003	n.a.	n.a.	n.a.	n.a.	n.a.
09.12.2003	150	*	4	5	<1
20.01.2004	129	<1	<1	<1	<1
17.02.2004	250	<3	<3	<1	<1
16.03.2004	<3	<1	<1	<1	<1
20.04.2004	<1	<1	<1	<1	<1
positive samples	13 / 15	3 / 12	4 / 14	3 / 17	2 / 14

1.2.2.2.2 Investigations on transect 2 of Lake Wannsee in 2003-2004:

Four multi level wells were build in 2002 close to the lake shore in order to allow for shorter filtrations paths to be studied: well 207OP (30 cm), well 207MP1 (60 cm), well 207MP2 (120 cm), and well 207UP (180 cm). The new wells were integrated in transect 2 leading to pumping well 3. Unfortunately, three of the new observation wells (207OP, 207MP1, 207MP2) did not work during the sampling period. Well 207MP2 was permanently dry. Wells 207OP and 207MP1 did have water, but the water column in the wells remained at a constant level in spite of continuous pumping for 30 min and more. Obviously, surface water directly penetrated into the wells without infiltration through the filter matrix. The concentrations of indicator microorganisms in these wells were in the same range as in the lake water (Table 15 to Table 17). Sampling of these wells was, therefore, stopped in April 2003. During six field surveys in winter months, the deepest multi level well 207UP (180 cm) had a water column of only 30 cm, which was pumped out in intervals only for microbiological investigations.

Somatic coliphages were detected in five out of six water samples from well 207UP in concentrations of up to 9 pfu/100 ml. Coliphages occurred sporadically in the wells 203, 205 (620 cm depth), 206 and 202OP. No coliphages were detected in samples from pumping well 3.

Table 15 Occurrence of somatic coliphages (pfu/100 ml) in water samples along transect 2 of Lake Wannsee (detection limit 1 pfu/ 100 ml)

* no sample, n.a.: not analysed

Sampling time	Lake Wannsee	205	206	207OP 30 cm	207MP1 60 cm	207UP 180 cm	202OP	203	well 3
23.01.2003	276	1	<1	210	246	9	<1	<1	n.a.
20.02.2003	267	<1	<1	*	*	*	4	1	n.a.
20.03.2003	199	<1	<1	319	260	1	<1	<1	<1
10.04.2003	36	<1	<1	23	4	2	<1	<1	<1
22.05.2003	3	<1	<1	*	*	*	<1	<1	<1
19.06.2003	2	<1	<1	*	*	*	<1	<1	<1
03.07.2003	2	<1	<1	*	*	*	<1	<1	<1
21.08.2003	6	<1	<1	*	*	*	<1	<1	<1
15.09.2003	6	<1	<1	*	*	*	<1	<1	<1
24.09.2003	3	<1	<1	*	*	*	<1	n.a.	<1
29.09.2003	4	<1	<1	*	*	*	<1	n.a.	<1
06.10.2003	4	<1	<1	*	*	*	<1	n.a.	<1
20.10.2003	47	<1	<1	*	*	*	<1	n.a.	<1
11.12.2003	168	<1	<1	*	*	*	<1	n.a.	<1
22.01.2004	276	3	<1	*	*	1	<1	n.a.	<1
19.02.2004	382	1	<1	*	*	2	<1	n.a.	<1
18.03.2004	270	<1	<1	*	*	<1	<1	n.a.	<1
22.04.2004	31	<1	<1	*	*	*	<1	n.a.	<1
positive samples	18 / 18	3 / 18	0 / 18	3 / 3	3 / 3	5 / 6	1 / 18	1 / 9	0 / 16

Concentrations of intestinal enterococci in the lake water were very low and intestinal enterococci were only sporadically detected in water samples from wells 205, 207UP and 202OP (Table 16). No enterococci were detected in samples of wells 203, 206 and pumping well 3.

Table 16: Occurrence of intestinal enterococci (cfu/100 ml) in water samples along transect 2 of Lake Wannsee (detection limit: 1 cfu/100 ml)
*** no sample, n.a.: not analysed,**

Sampling time	Lake Wannsee	205	206	207OP 30 cm	207MP1 60 cm	207UP 180 cm	202OP	203	well 3
23.01.2003	1	<1	<1	<1	<1	<1	<1	<1	n.a.
20.02.2003	2	<1	<1	*	*	*	<1	<1	n.a.
20.03.2003	2	<1	<1	<1	<1	<1	<1	<1	<1
10.04.2003	<1	<1	<1	n.a.	n.a.	n.a.	<1	<1	<1
22.05.2003	<1	<1	<1	*	*	*	<1	<1	<1
19.06.2003	4	<1	<1	*	*	*	<1	<1	<1
03.07.2003	2	<1	<1	*	*	*	1	n.a.	<1
21.08.2003	<1	<1	<1	*	*	*	<1	<1	<1
15.09.2003	<1	<1	<1	*	*	*	<1	<1	<1
24.09.2003	2	<1	n.a.	*	*	*	<1	n.a.	<1
29.09.2003	34	<1	<1	*	*	*	<1	n.a.	n.a.
06.10.2003	7	<1	<1	*	*	*	2	n.a.	n.a.
20.10.2003	2	<1	<1	*	*	*	<1	n.a.	<1
11.12.2003	2	<1	<1	*	*	*	<1	n.a.	<1
22.01.2004	74	<1	<1	*	*	*	1	n.a.	<1
19.02.2004	9	1	<1	*	*	9	<1	n.a.	<1
18.03.2004	<1	<1	<1	*	*	<1	<1	n.a.	<1
22.04.2004	<1	<1	<1	*	*	*	<1	n.a.	<1
positive samples	12 / 18	1 / 18	0 / 18	0 / 2	0 / 2	1 / 4	3 / 18	0 / 8	0 / 14

E. coli was found in higher concentrations in the lake water than intestinal enterococci and more frequently in the shallow observation wells of transect 2 (Table 17). Several samples from wells 205, 202OP and 203 contained E. coli at concentrations ranging from 1 to 50 cfu/100 ml. E. coli was not detected in samples from the lake basin site 206 and from pumping well 3.

Table 17: Occurrence of. E coli (cfu/100 ml) in water samples along transect 2 of Lake Wannsee (detection limit: 1 cfu/100 ml)

*** no sample, n.a.: not analysed, >> invalid result**

Sampling time	Wannsee	205	206	207OP 30 cm	207MP1 60 cm	207UP 180 cm	202OP	203	well 3
23.01.2003	4	<1	<1	<1	<1	<1	2	1	n.a.
20.02.2003	10	<1	<1	*	*	*	<1	<1	n.a.
20.03.2003	16	<1	<1	21	27	<1	<1	<1	<1
10.04.2003	12	<1	<1	n.a.	n.a.	n.a.	<1	<1	<1
22.05.2003	<1	8	<1	*	*	*	<1	<1	<1
19.06.2003	1	1	<1	*	*	*	<1	<1	<1
03.07.2003	15	<1	<1	*	*	*	<1	n.a.	<1
21.08.2003	>>	<1	<1	*	*	*	<1	<1	<1
15.09.2003	8	<1	<1	*	*	*	<1	n.a.	<1
24.09.2003	14	<1	n.a.	*	*	*	52	n.a.	<1
29.09.2003	>>	<1	<1	*	*	*	1	n.a.	n.a.
06.10.2003	4	6	<1	*	*	*	<1	n.a.	n.a.
20.10.2003	40	<1	<1	*	*	*	<1	n.a.	<1
11.12.2003	11	<1	<1	*	*	*	<1	n.a.	<1
22.01.2004	165	<1	<1	*	*	>>	14	n.a.	<1
19.02.2004	46	12	<1	*	*	< 15	<1	n.a.	<1
18.03.2004	<1	1	<1	*	*	*	<1	n.a.	<1
22.04.2004	<1	<1	<1	*	*	*	<1	n.a.	<1
positive samples	13 / 16	5 / 18	0 / 17	1 / 2	1 / 2	0 / 4	4 / 18	1 / 7	0 / 14

2 Artificial recharge pond Tegel

2.1 Investigations on the transect of the recharge pond in 2002

The recharge pond is continuously filled with water from Lake Tegel (influent). Microbiological investigations were carried out in water samples from the pond, from two shallow wells (well 365 and well 366), three deep wells (wells 247, 248, 342) as well as from one regular pumping well of Water Work Tegel (well 20) located at a transect of the groundwater enrichment plant of Tegel. During the first three surveys, samples were taken by one MP1 pump. The same equipment was used at all sampling sites without any pre or post disinfection of instruments. During the field surveys in November and December, sterilized Eijkelkamp modules and silicon tubing were used for sampling of the two shallow wells 365 and 366.

Coliphages were found in pond water samples at concentrations between 2 and 26 pfu/100 ml, but were never detected in water samples from any of the wells (Table 18A). Concentrations of intestinal enterococci in pond water varied between 1 and 5 cfu/100ml. Only two samples from wells 247 and 342 contained enterococci in concentrations of 1 and 3 cfu/100 ml, respectively (Table 18B).

E. coli was detected in pond water samples in concentrations of up to 44 cfu/100 ml. About half of the water samples from the two shallow and three deep wells contained E. coli in concentrations of up to 45 cfu/ 100 ml (Table 16C). Samples of all wells taken on July 2002, as well as samples 4 of 5 samples of the pumping well 20 were free from E. coli in 100 ml volume.

Data on the occurrence of heterotrophic plate count bacteria (according to the German Drinking water Directive; colony counts according to DIN EN ISO 6022 were slightly higher) are shown in Table 18D. No retention pattern can be deducted from the data received. Colony counts at 20 °C as well as at 37°C varied from 1 cfu/ml to > 103 cfu/ml. At the regular pumping well 20, colony counts were usually below 100 cfu/ml. During the November and December surveys very low concentrations were found (1-2 cfu/ml). It is suspected that the well had been disinfected before.

Table 18: Occurrence of somatic coliphages (pfu/100 ml), intestinal enterococci (cfu/100 ml), E. coli (cfu/100 ml), and heterotrophic plate count bacteria (cfu/ml) in water samples from the wells of the groundwater enrichment plant of Tegel

(detection limit: 1 pfu or cfu/100 ml; for colony counts 1 cfu/ml)
* no sample, n.a.: not analysed

A: Somatic coliphages

Sampling time	influent	pond water	365	366	247	248	well 20	342
18.07.02**	5	2	<1	<1	<1	<1	<1	<1
15.08.02**	n.a	4	<1	<1	<1	<1	<1	<1
21.09.02**	6	3	<1	<1	<1	<1	<1	<1
21.11.02***	15	19	<1	<1	<1	<1	<1	<1
12.12.02***	21	26	<1	<1	<1	<1	<1	<1
positive samples	4 / 4	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5

B: Intestinal Enterococci

Sampling time	influent	pond water	366	365	247	248	well 20	342
18.07.02**	4	5	<1	<1	<1	<1	<1	<1
15.08.02**	*	1	<1	<1	<1	<1	<1	<1
21.09.02**	2	2	<1	<1	<1	<1	<1	<1
21.11.02***	1	1	<1	<1	3	<1	<1	<1
12.12.02***	2	2	<1	<1	<1	<1	<1	1
positive samples	4 / 4	5 / 5	0 / 5	0 / 5	1 / 5	0 / 5	0 / 5	1 / 5

C. E. coli

Sampling time	influent	pond water	366	365	247	248	20	342
18.07.02**	4	1	<1	<1	<1	<1	<1	<1
15.08.02**	*	44	<1	2	30	<1	1	45
21.09.02**	8	3	4	2	<1	<1	<1	4
21.11.02***	20	7	3	8	<1	2	<1	<1
12.12.02***	130	8	<1	3	3	2	<1	2
positive samples	4 / 4	5 / 5	2 / 5	4 / 5	2 / 5	2 / 5	1 / 5	3 / 5

D. Heterotrophic bacteria

Sampling	influent	pond water	366	365	247	248	well 20	342
18.07.02 (20 °C)	380	540	150	52	80	200	130	70
18.07.02 (37 °C)	140	340	140	30	30	40	100	40
15.08.02 (20 °C)	n.a.	760	3280	920	290	345	70	250
15.08.02 (37 °C)	n.a	230	3000	500	410	210	70	30
21.09.02 (20 °C)	870	1370	160	270	150	520	170	90
21.09.02 (37°C)	330	230	90	120	30	40	100	50
21.11.02 (20 °C)	70	2420	140	220	420	230	1	280
21.11.02 (37 °C)	10	400	60	120	60	330	1	110
12.12.02 (20 °C)	310	620	540	80	120	1440	2	110
12.12.02 (37 °C)	30	40	50	20	30	150	2	20

* no sample, ** sampling with non sterile instruments, *** sampling with sterilized instruments

italic = in 10 ml of sample

2.2 Investigations in the filter bed of the recharge pond

Core samples were taken from the sand filter of the recharge pond to determine the distribution of bacteria in the filter. Sampling was performed when the filtration site was drained to remove the clogging layer. Sub-samples of the sand core from different depth (2-80 cm) were analysed using cultivation methods as well as cultivation independent methods.

Cultivation was performed using two different colony count agars: i) DEV-agar, according to the German Drinking Water Directive and ii) the agar given in DIN EN ISO 6222 (ISO-agar). For the ISO-agar, the highest concentration of cultivable bacteria was found in the upper 5 cm of the sand core (Figure 3). With DEV-agar no difference in concentration was detected over the whole cell core. No consistent difference in colony counts was observed between DEV and ISO-agar (Figure 3).

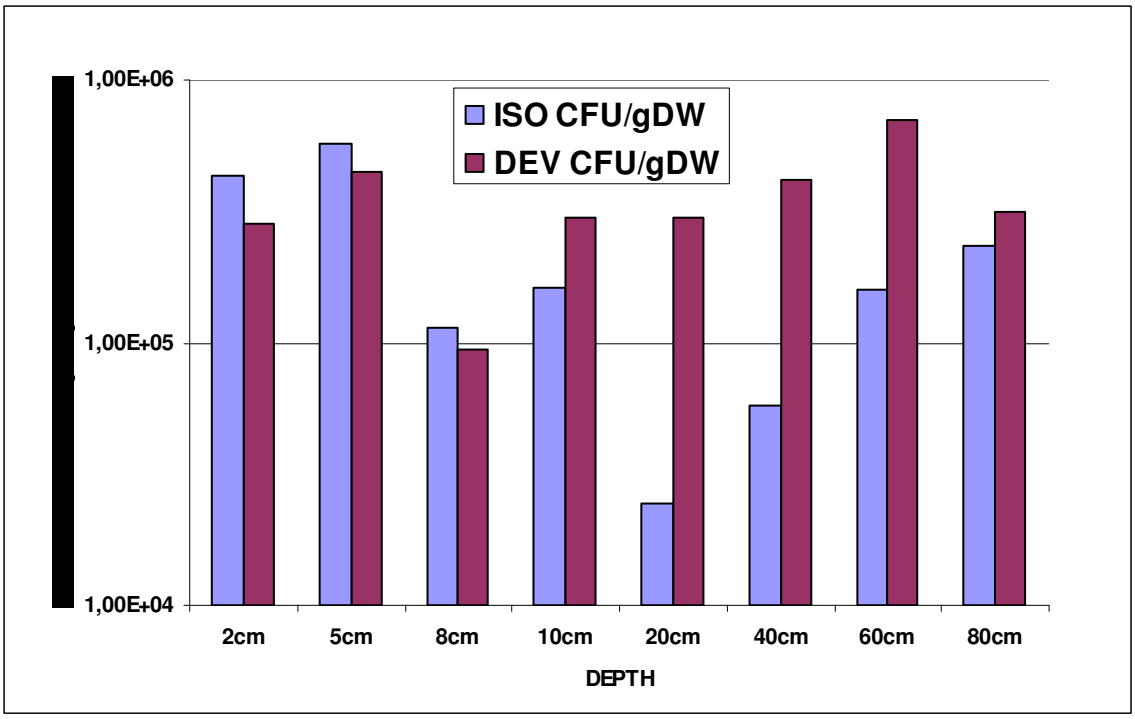

Figure 3 Cultivation of samples from the recharge pond on drinking water media. ISO= agar according to DIN EN ISO 6222, DEV= agar according to the German Drinking Water Directive. Colony forming units (CFU) are based on 1g dry weight (gDW).

Extraction methods with enzymes were used to enhance detachment of the bacteria from the sand. Samples were incubated in a Tris/sodium pyrophosphate solution with the enzymes Alfa-glucosidase, Beta-galactosidase and Lipase. No significant increase in the number of cultivable bacteria was found in the enzyme extracted samples compared to the extraction with buffer only (Figure 4).

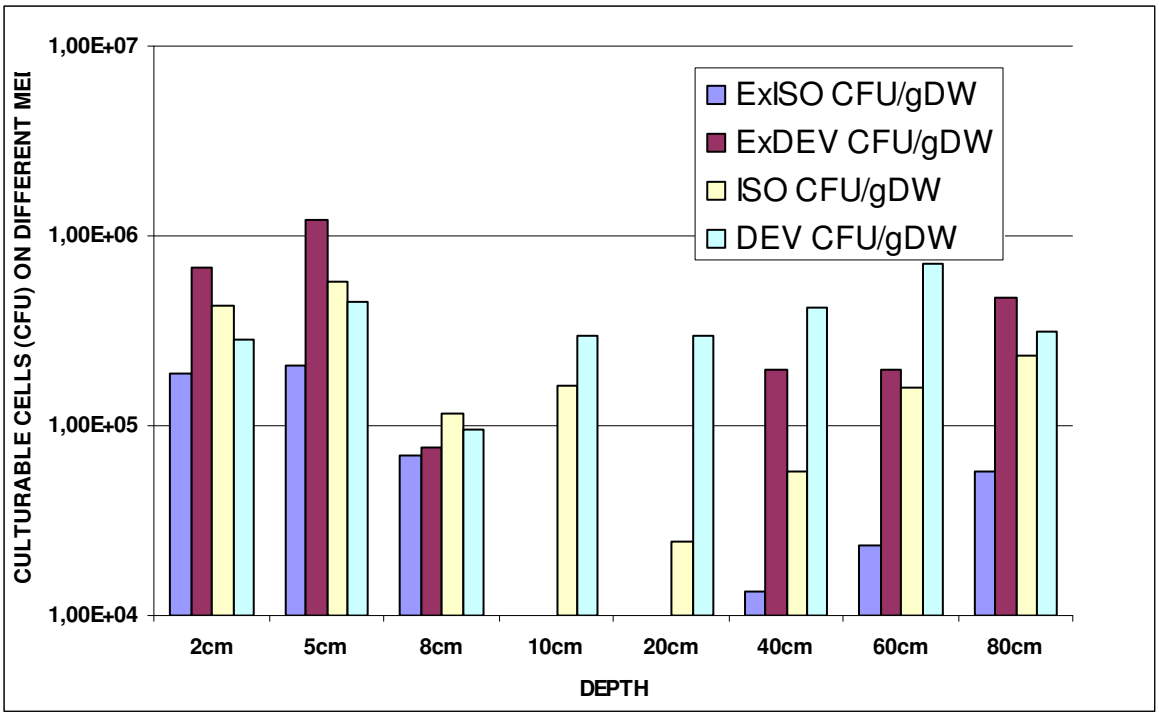

Figure 4 **Comparison of extraction with and without enzymes. ISO= agar according to DIN EN ISO 6222, DEV= agar according to the German Drinking Water Directive. ExISO, ExDEV=enzyme extracted samples; ISO, DEV=samples treated without enzymes. Colony forming units (CFU) are based on 1g dry weight (gDW).**

Total cell counts were determined microscopically after staining with DAPI. The total concentration of bacteria detected was in the range of 108 to 109 cells per gram dry weight in all depth of the filter core (Figure 5).

The fraction of cultivable bacteria was calculated from the comparison between the total cell count and the colony counts (Figure 6). Cultivability was between 0,01 and 0,5 %. No consistent difference was obtained on the two different media.

No further examinations of the sand core were performed since the transect at the recharge pond was not further investigated due to very low concentrations of bacteria and coliphages in the pond water.

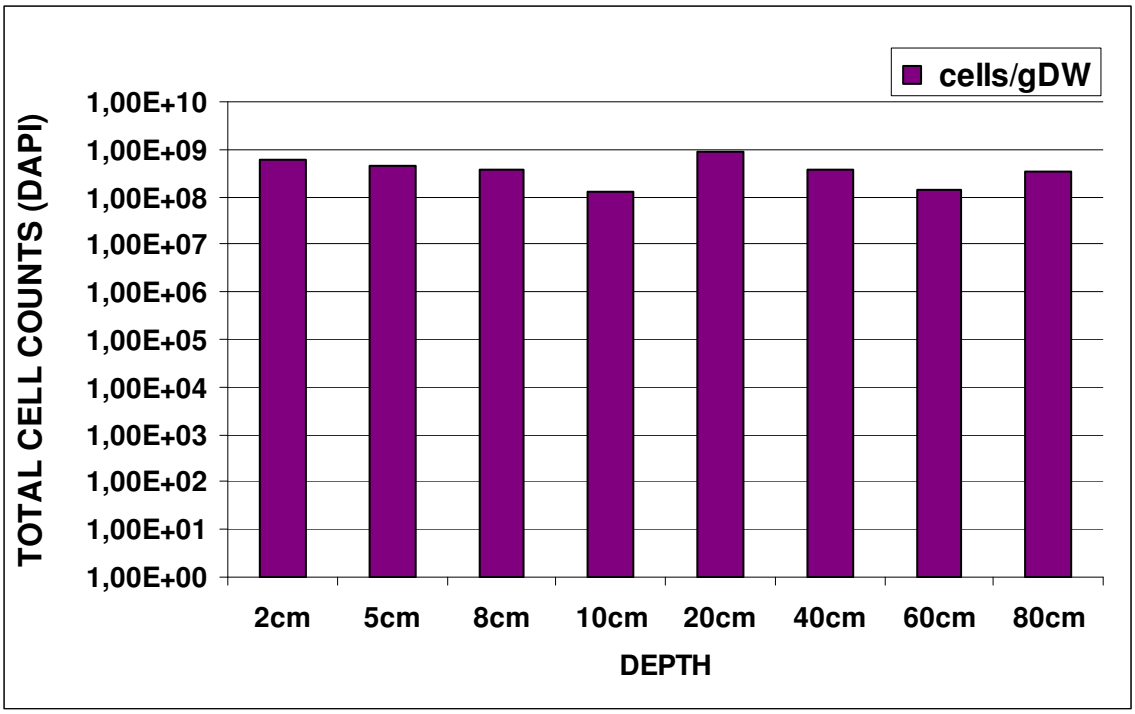

Figure 5 **Total cell counts of core samples from the recharge pond determined by DAPI staining. Cells/gDW=cells per 1g dry weight**

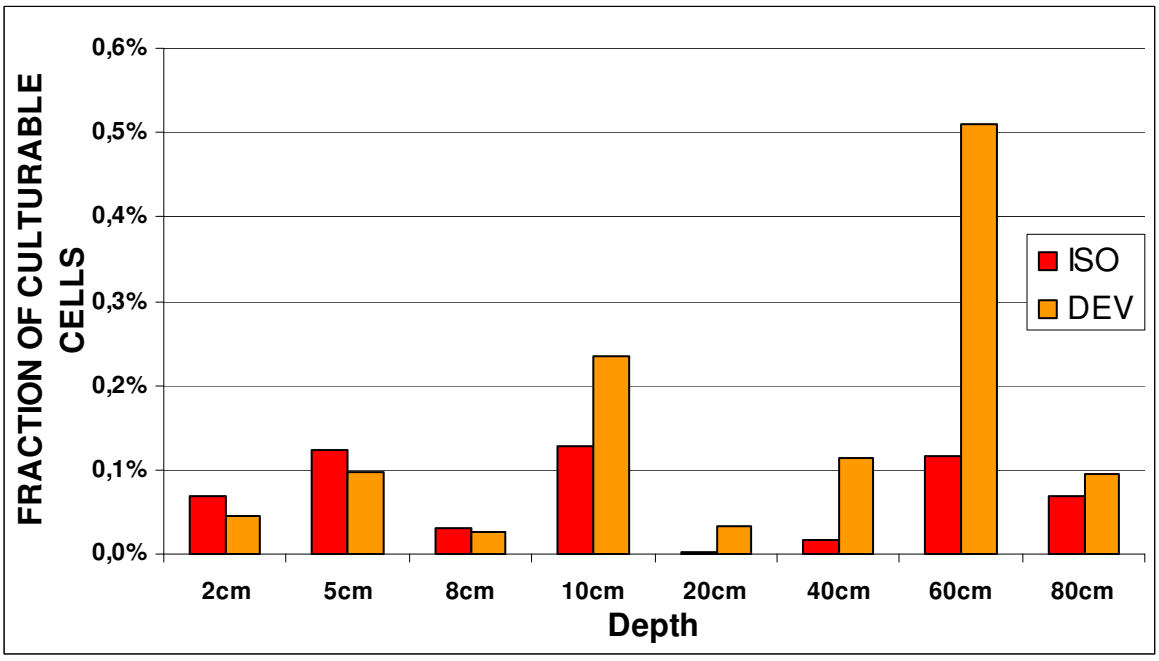

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Figure 6 **Percentage of culturable cells on two different media in core samples from the recharge pond. Fraction determined in relation to DAPI counts. ISO= agar according to DIN EN ISO 6222, DEV= agar according to the German Drinking Water Directive**

2.3 Discussion

2.3.1 Surface water

The results of the microbiological investigations indicated a high hygienic quality of the surface water at both transects and at the artificial recharge pond Tegel. In the surface waters of Lake Tegel and Lake Wannsee, the concentration of intestinal enterococci never exceeded 100 cfu/100 ml (guideline value of the EU Bathing Water Directive), and that of E. coli only occasionally was higher than 100 cfu/100 ml (guideline value of the EU Bathing Water Directive) but never reached 2000 cfu/100 ml (imperative value of the EU Bathing Water Directive). The highest concentration of coliphages was 850 pfu in 100 ml (Fig. 1, 2, Tab.7). Concentrations of coliphages were at some sampling surveys more than 100 times higher than the concentrations of E. coli. This point to a more rapid inactivation of bacteria compared to coliphages under environmental stresses.

Concentrations of the indicator microorganisms examined were lower in Lake Tegel than in Lake Wannsee and showed less seasonal fluctuations during the field surveys between May 2002 and December 2004. An explanation for this is the improvement in hygienic quality of the surface water in Lake Tegel due to treatment of discharges (e.g. Creek Nordgraben) in the flocking and filtration plant of Tegel prior to entering the lake. With flocculation and rapid sand filtration of the inflow to Lake Tegel, an elimination of 84 % for E. coli, 92 % for intestinal enterococci, and 84 % for somatic coliphages was achieved.

Concentrations of indicator microorganisms in Lake Wannsee were higher than in Lake Tegel and clearly showed seasonal fluctuations with highest concentrations in the winter months. High biological activity of aquatic microorganisms and UV irradiation in the summer months are probably responsible for the rapid inactivation of indicator organisms that are not able to proliferate under environmental conditions. Rapid inactivation of coliphages and indicator bacteria by UV irradiation were also observed in a river simulation plant (Dizer et al. 2005).

The Teltow canal discharging a large amount of secondary effluent from the sewage treatment plants in Berlin flows through the Griebnitz Lake into Lake Wannsee. Concentrations of indicator microorganisms were elevated close to the inflow from the Griebnitz Lake. Concentrations decreased, however, with distance from the inflow and reached background close to the transect area.

2.3.2 Pumping wells

No somatic coliphages or enterococci were detected in any of the samples from regular pumping wells of the water works. E. coli was sporadically detected in samples from all but one well in concentrations near the detection limit of 1 cfu/100ml. Sampling was performed from especially installed taps. Although this procedure strongly reduces the risk of contamination, sporadic external contamination cannot be completely ruled out.

2.3.3 Deep observation wells

Deep observation wells were sampled at the transect of Lake Tegel and at the artificial recharge pond Tegel during the surveys in the year 2002. Somatic coliphages and enterococci were detected in one sample each from the four wells at the transect of Lake Tegel. In deep wells from the artificial recharge pond, no coliphages were detected and only 2 out of 15 samples contained enterococci. E. coli was detected in about one third of the samples from deep observation wells in concentrations of between 2 cfu/100 ml and 6 cfu/10 ml (Table 18, 19). Sampling from these wells was only possible using unsterile pumps and equipment which are not optimal for microbiological sampling. Only one MP1 pump and accessories were used during all field surveys for all sampling sites without any treatment before or after operations. The contamination of wells was reduced through pre-pumping groundwater for 20-30 min from each well at a relatively high flow rate of 100 L/min before taking samples. Nevertheless, the problem with possible contamination of the wells remained. Sampling of deep observation wells was not continued in 2003 because of the sterility problems and the very low detection frequency of coliphages not allowing the deduction of retention patterns.

Table 19 Frequency of positive findings of test organisms in deep observation wells sampled by non-sterile MP1 pump and equipment along the transect of Lake Tegel.

	Coliphages	Intestinal enterococci	E. coli
MP1 pumps	1 / 32	1 / 32	10 / 31

2.3.4 Shallow observation wells

Seven shallow observation wells were sampled from the transect of Lake Tegel. Somatic coliphages were only detected in one sample of the well closest to the lake. Intestinal enterococci were not detected in the shallow wells except for the survey in October where all but one wells contained enterococci. E. coli was detected in three wells in concentrations of about 30 cfu/ml during the survey in August when non sterilized equipment was used. In all following surveys - when sterile equipment was used – no E. coli was detected in any of the samples. It is, therefore, assumed, that the high concentrations of E. coli found in August were due to contamination of the wells by sampling.

No retention pattern along the transect flow paths can be obtained from these results. The concentrations of indicator microorganisms in the lake were relatively low and – with a few exceptions –indicator bacteria and coliphages were not or only sporadically detected in the observation wells.

Two shallow wells were sampled at the transect of the artificial recharge pond Tegel. No enterococci or coliphages were detected in samples from these wells. In contrast, E. coli was

detected in most of the samples. Using disinfected equipment did not lower the frequency of positive findings.

Similar results were obtained for the shallow wells at the transects 1 and 2 of Lake Wannsee. Nine shallow wells from transect 1 and transect 2 were sampled. Enterococci and coliphages were only sporadically detected while E. coli was found in 70 out of 135 samples in concentrations of 1-50 cfu/ 100 ml. Again there was no difference in the frequency of positive findings when using non sterile compared to disinfected equipment.

The source of E. coli found in the shallow wells remains unclear. External contamination during sampling cannot be ruled out. Although sampling for microbiological investigations was carried out using disinfected pumps and tubing, other sampling and measurement tasks at the wells were performed using non sterile equipment. Furthermore, the disinfected pumps and tubing could not be introduced in the groundwater without contact with biofilm layers at the inside of the wells which might act as a source of contaminants. Swap samples were taken from the wall of some wells (3337, 3335, 207, and 201OP) as well as from the surface of several non disinfected instruments (tubing, sensors) used by other working groups. No E. coli were detected in biofilm samples from inside the wells. E. coli was, however, detected in one sample from the instruments.

False positive results in the detection method for E. coli might also explain positive findings especially in samples where other indicators were not detectable. To reduce the risk for false positive results, three different detection methods were used - partly in parallel. Aeromonades - identified in one method as giving false positive results - were thereby excluded. Therefore, this explanation for the occurrence of E. coli seems unlikely.

Since external contamination cannot be ruled out, the concentration of E. coli in the wells cannot be used to deduct retention characteristics. The concentration of the other indicators was too low to be able to see log reduction rates. A clear reduction in positive findings with length of filtration path is, however, obvious when compiling all results (see Table 20).

Table 20 Frequency of positive findings in shallow observation wells and pumping wells at the Lake Wannsee

A. Transect 1

Parameters	3339	3338	3337	201OP	3335	Well 4
depth	5	5	7	15	12	25
located	in the lake (40m)	in the lake (9 m)	at the shore 2 m	at the shore (7 m)	at the shore (15 m)	33 m
percolation time	< 1 month	30 d	27 d	?	30 d	90 d
Coliphages	2 / 6 (33%)	0 / 20	0 / 16	0 / 17	0 / 12	0 / 16
Enterococci	2 / 6 (33%)	4 / 20 (20%)	1 / 16 (6%)	0 / 17	0 / 12	0 / 15

B. Transect 2

Parameters	207 UP	205	206	202 OP	203	Well 3
depth (m)	1,8	5	7	12	12	25
located	in the lake (10 m)	in the lake (30 m)	in the lake (20 m)	at the shore (3m)	at the shore (20 m)	42 m
percolation time	a few days	1 month	25 d	72 d	3 month	3 month
Coliphages	5 / 6 (83%)	3 / 18 (2%)	0 / 18	1 / 18 (0.5%)	1 / 9 (1%)	0 / 16
Enterococci	1 / 4 (25%)	1 / 18 (1%)	0 / 17	3 / 18 (2%)	0 / 8	0 / 14

The highest frequency of positive results was found in the observation wells closest to the lake (3338, 3339, 207UP). The number of positive samples declined clearly with increasing distance from the lake along the shore. No positive samples were obtained from the pumping wells even when one liter of sample was analysed.

The data presented indicate a weak migration of indicator microorganisms along the filtration path to the shallow wells of the transects. The concentrations of indicator bacteria and coliphages in both, lake Wannsee and lake Tegel, were, however, never high enough to allow a quantitative approach for the elimination of microorganisms along the transects.

Therefore, further investigations focused on the three model filtration plants of Marienfelde: the sandy soil column of 5 m length, the enclosure and the slow sand filtration pond with a filtration path of 100 or 80 cm, respectively (see NASRI Report 5, chapter 9.2).

2.3.5 Microorganisms in the filter bed

Core samples were taken from the sand filter of the artificial recharge pond Tegel to determine the distribution of bacteria in the filter. The bacteria were evenly distributed over the whole length of the filter bed. The concentration was not higher in the upper zone (clogging layer) than in 80 cm depth. Colony counts as well as total cell counts were in the same order of magnitude at all sampling depth. Culturability ranged from 0.01 to 0.5 %. These results indicate a high potential for biological activity – elimination and degradation – in the whole sand filter.

3 Batch experiments

3.1 Introduction

The elimination of bacteria and viruses by slow sand or bank filtration is to be regarded as a cumulative function of different physically, chemical and biological procedures. The inactivation of micro organism in capillary water of sandy soil and their reversible and irreversible adsorption on to solid particles along the filtration path determine their survival, retention and transport behaviour. The physically chemical characteristics of the filter matrix regulate the interactions between micro organisms and sediment surface. The goal of the batch attempts is therefore the experimental determination of the information, which can be consulted with differential explanation of these interactions and served as basis or factor value for statistical assessment or modelling of the column attempts.

3.2 Survival of test organisms in water and water soil suspension

As groundwater was applied by all experiences on enclosure and slow sand filtration basin, the batch experiments were also accomplished in groundwater taken in the Water Work of Marienfelde. Native sand applied by filling slow sand filtration pond and enclosure, top layer of filtration pond (< 1cm) consisting of clogging layer formed during continuously operation for one year, and upper layer of the sediment (0-5 cm) from bottom of Lake Wannsee were used for batch experiments. The sampling and preparation of the sediment from the Lake Wansee were carried out by the working group hydrogeology of FU Berlin. A part of the sand and sediment samples was sterilised by autoclaving at 120°C for 30 min, in order to eliminate the biological inactivation of test organisms in different sets of soil suspension.

In a large glass flask, 2 L groundwater was inoculated with a dilution of culture suspension (1ml) of test coliphages and indicator bacteria, respectively. After genteelly shaking of samples for 5 min on a horizontally shaker, the culture suspension was allocated in 200 ml volumes in sterile erlenmayer flasks. 50 g sand of each origin were added to an aliquot of culture suspension and incubated on a horizontal shaker (30 rpm) at room temperature for 7-30 d. Incubation of indicator bacteria in water and different soil suspensions was carried out for 7 d. The sampling took place in regular intervals without any settling and centrifugation directly from the liquid phase of sand suspension. Two parallel sets of samples were applied for testing survival of indicator bacteria in water and different soil suspensions.

The amount of viruses present in the water phase was measured as a function of time. Virus density in control container with or without sand was measured to calculate inactivation in liquid phase as function of time (y´= eat).

The concentrations of test organisms in different sets of samples and their exponential regression lines and equations were demonstrated in figures and tables. The decreasing constant of each line corresponds to the inactivation coefficient of test virus which can be calculated through the derivative function y' = eax with x=1d. The reduction of the phage density in 30d of incubation was also calculated and given as factor or log units in each tables.

The adsorption equilibrium of test organisms in virgin sand of the filter pond was also tested by kinetic batch experiments under shaking at 60 rpm for 6h. Five ml samples from liquid phase were taken in interval of 30 min. After centrifugation of sample at 3000 rpm, concentration of test organisms was detected in supernatant. A set of culture suspension without sand was used as control for determination of total amount of test organisms during operation time. Viruses partitioned between solution and sorbent phases was characterised as adsorption rate (dimensionless) and distribution coefficient, Kf, (g-1).

Adsorption rate was calculated through the relation: (Co – Cf) / Co [Co: Total amount of coliphages in water without sand as control (pfu/ml), Cf: amount of free, non adsorbed coliphages in supernatant of centrifuged water sand suspension (pfu/ml)].

Dispersion coefficient (Kf) was calculated: [(Co – Cf) / sand weigth (g)] / Cf. A semi-log plot of virus adsorption to sandy soil as a function of time suggests the adsorption equilibrium.

3.3 Survival of coliphages in water and water soil suspensions

F+ phage 138 and somatic coliphage 241 have shown high persistence in native groundwater from Marienfelde (Table 21). After 30 d incubation at 20°C, survival ratio of both viruses was about 0,65 corresponding with an inactivation rate of 0,2 log units/30d.

In all sterile sandy soil suspensions, very low or no lost of virulent of both coliphages were observed too. Less than one log unit of F+phage 138 disappeared in the native sand or the sandy soil from clogging layer of filter pond. Inactivation rate of somatic coliphage 241 was found only 1,4 log units/30d in native sand while they were not inactivated in sand containing clogging layer. Both coliphages likewise survived fully in the sterile sediment from bottom of the Lake Wannsee (Table 21).

Table 21 **Survival and inactivation ratio of test coliphages in suspensions of different sandy soils at room temperature [SD: Schmutzdecke, *)50 g sandy soil + 200 ml culture suspension in groundwater]**

	F+ coliphage 138				Somatic coliphage 241			
Samples	Derivation of regression line	survival ratio (x=1d)	survival ratio (x=30d)	inactivation rate (log / 30d)	y'=e-0,0x	survival ratio (x=1d)	survival ratio (x=30d)	inactivation rate (log / 30d)
Groundwater Marienfelde (non sterile)	y'=e-0,015x	0,98	0,64	0,2	y'=e-0,015x	0,99	0,65	0,2
Sandy soil* Marienfelde (sterile)	y'= e-0,037x	0,96	0,33	0,5	y'=e-0,106x	0,90	0,04	1,4
Sandy soil* Marienfelde (non sterile)	y'=e-0,09x	0,91	0,07	1,2	y'=e-0,1291x	0,88	0,02	1,7
Sandy soil* + SD Marienfelde (sterile)	y'=e-,0236x	0,98	0,49	0,3	y'=e0,1113x	1,01	1,40	< 0
Sandy soil* + SD Marienfelde (non sterile)	y'=e1,5278x	0,22	<0,00001	>5	y'=e-1,0865x	0,34	<0,00001	>5
Sandy soil* - Wannsee (sterile)	y'=e0,01x	1,01	1,35	< 0	y'=e0,0304x	1,03	2,5	< 0
Sandy soil* - Wannsee (non sterile)	y'=e-,4049x	0,67	0,00001	5	y'=e-0,3194x	0,73	0,001	3

In contrast to the high survival ratios in sterile sand suspensions, all test organisms were rapidly inactivated in non sterile suspension of each sandy soil. Survival rates of F+ phage 138 and somatic phage 241 in non sterile sand of clogging layer were about 0,2 and 0,3.d^{-1}, respectively, resulted in absolutely inactivation of both phages, more than 5 log units within 30 d (

Table 21). Both coliphages have shown a survival rate of about 0,7.d-1 in upper layer of the sediment from Lake Wannsee corresponding an inactivation ratio of 3 or 5 lo units within 30d, respectively. In non sterile native sandy soil of filtration pond, low amount of F+ and somatic phages lost their virulent, 1,2 and 1,7 log units in 30d, respectively (

Table 21).

3.4 Survival of indicator bacteria in water and water soil suspensions

Both indicator bacteria, E. coli A and E. faecalis, have shown high persistence in native groundwater. Survival ratios were calculated 0,74.d^{-1} for E. coli A (Table 22), and 0,61.d^{-1} for E. faecalis (Table 23). Less than one log unit of indicator bacteria was inactivated during an incubation time of 7d. Results of two parallel sets of samples have shown no or low deviation (Table 22 and Table 23).

Concentration of E. coli A significantly enhanced in sterile suspensions of native sandy soil and top layer of filter pond containing clogging layer (Table 22). An increase of the amount of E. faecalis was observed only in sterilized sand with clogging layer (Table 23). These results point to the growth of both indicator bacteria, if there is no biological stress in their aquatic or terrestrial environment.

In the non-sterile sets of both sand suspensions, E. coli A was strongly inactivated within 7d. The survival ratios were found 0,57.d^{-1} in the native sand, and 0,53.d^{-1} in the top layer of filter pond with colmation layer. The inactivation ratio of E. coli A reached approximately 2 log units after 7 d of exposition in both soil suspensions (Table 22).

Table 22 Survival of E. coli A in groundwater and different water-sand suspensions during incubation for 7 d at a temperature of 20 + 2°C

Samples	Parallel experiments	Derivation of regression line	Survival rate (d-1)	Survival rate (in 7 d)	Inactivation (log / 7 d)
Groundwater Marienfelde (non sterile)	a	y'=e-0,2951x	0,74	0,13	0,9
	b	y''=e-0,2972x	0,74	0,12	0,9
	mean		0,74	0,10	0,9
native sandy soil - Marienfelde (sterile)	a	y'=e0,4247x	1,5	> 1	0
	b	y'=e0,2733x	1,3	> 1	0
	mean		1,4	> 1	0
native sandy soil - Marienfelde (non sterile)	a	y'=e-0,6099x	0,54	0,01	1,9
	b	y'=e-0,5268x	0,59	0,03	1,6
	mean		0,57	0,02	1,8
Clogging layer of filtration pond (sterile)	a	y'=e0,842x	2,3	> 1	0
	b	y'=e0,7892x	2,2	> 1	0
	mean		2,3	> 1	0
Clogging layer of filtration pond (non sterile)	a	y'=e-0,6082x	0,54	0,014	1,8
	b	y'=e-0,6563x	0,52	0,01	2,0
	mean		0,53	0,01	1,9

E. faecalis was also strongly inactivated in both non sterile sandy soil suspensions (Table 23). After one week of exposition 2,3 log or 3,2 log units of E. faecalis disappeared in native or sandy soil with clogging layer, respectively. In contrast to E. coli A, there was no clear difference between relative high inactivation ratios of E. faecalis in sterile or non-sterile native sand of filter pond.

These results point to the importance of the biological activity of native microorganism population of sandy soil by elimination of hygienic related bacteria and viruses which are not adapted to the biological stress conditions in capillary water of filtration units.

Table 23 Survival of E. faecalis in groundwater and different water-sand suspensions during incubation for 7 d at a temperature of 20 + 2°C

Samples	Parallel experiments	a (y'=e-ax)	Survival rate (x = 1)	Survival rate (x =7 d)	Inactivation (log / 7 d)
Groundwater Marienfelde (non sterile)	a	-0,588	0,556	0,016	1,8
	b	-0,401	0,670	0,060	1,2
	mean		0,61	0,04	1,5
native sandy soil - Marienfelde (sterile)	a	-1,048	0,351	0,0007	3,2
	b	-0,912	0,402	0,0017	2,8
	mean		0,38	0,0012	3,0
native sandy soil - Marienfelde (non sterile)	a	-1,041	0,353	0,0007	3,2
	b	-1,060	0,346	0,0006	3,2
	mean		0,35	0,0006	3,2
Clogging layer of filtration pond (sterile)	a	2,242	9,410	> 1	0,0
	b	2,009	7,452	> 1	0,0
	mean		8,43	> 1	0
Clogging layer of filtration pond (non sterile)	a	-0,754	0,470	0,0051	2,3
	b	-0,690	0,502	0,0080	2,1
	mean		0,49	0,0065	2,2

3.5 Adsorption of test organisms on sandy soil

Adsorption of F+ phage 138 and somatic phage 241 were tested in soil water suspensions, containing 50 g sterile sand and 200 ml groundwater.

Each of test organisms were separately inoculated in soil samples from there selected origins. After an incubation on a horizontally shaker at 60 rpm for 6h, and room temperature, 5 ml of each samples were centrifuged at 3000 rpm for 10 min. The free, not adsorbed phages in supernatant were quantified. Relation of the free phage density to the amount of phages in control sample without sand served the adsorption rate.

Both coliphages have shown a low adsorption on all sterilized sandy soil samples. Highest adsorption of F+ coliphage 138 was found factor 0,86 on the native sand of filter pond, and factor 0,75 on the sediment of Lake Wannsee (Table 24). Adsorption on the sandy soil of filter pond containing clogging layer was about 0,32. Relatively high kf value (0,12.g^{-1}) was calculated for F+ phage 138 adsorbed per g of native sand of filter pond.

Less adsorption of somatic coliphage 241 was found than those of F+ phage 138 ranging from 0,15 on sandy soil with clogging layer to 0,25 on the native sand of filter pond (Table 24). Correspondingly low kf values varied between 0,004 and 0,007.g^{-1} in all soil samples.

Table 24 Adsorption ratios and dispersion coefficients (kf values) of test coliphages in different water - sand suspensions (SD= clogging layer)

Samples (survival)	F+ phage 138		Somatic phage 241	
	Adsorption	Kf value	Adsorption	Kf value
native sandy soil - Marienfelde (sterile)	0,86	0,12	0,25	0,007
sandy soil + SD Marienfelde (streile)	0,32	0,01	0,15	0,004
sandy soil - Wannsee (sterile)	0,75	0,06	0,17	0,004

Low adsorption of both coliphages on sandy soil from colmation layer of filter pond points to the dispering effect of water soluble organic clogging matter on viruses which obviously reduced sorptive interaction between virus and grain surface.

Due to growth of indicator bacteria in sterile sets of sandy soils and sediments we could not analyse their adsorption on sand by batch experiments. In non sterile sets of soil-ground water suspensions, coliphages and indicator bacteria were differently inactivated during the incubation time. Therefore, the physically chemical adsorption may not be differed from biological inactivation of test organisms in non sterile water-soil suspension.

For testing adsorption of indicator bacteria, sand suspensions containing each bacteria culture was set up for 5 min, after shaking samples for 30 min. The still murky supernatant with colloid particles was used for estimation of the free non adsorbed bacteria. In spite of the short-term sedimentation, 67% or 96% of respective E. coli or E. faecalis remained on the sediment particles of the virgin sand of filtration units (Table 25).

Adsorption ratio of indicator bacteria was found in the same order of magnitude in the soil suspension containing clogging layer of the filter pond (Table 25). In compliance with high adsorption ratio, high dispersion coefficients were found in both soil suspensions of E. faecalis.

Table 25 Adsorption ratios and dispersion coefficients (kf values) of indicator bacteria in different water - sand suspensions (SD= clogging layer)

Samples (pH 7)	E. coli		E. faecalis	
	Adsorption	Kf value	Adsorption	Kf value
native sandy soil - Marienfelde	0,67	0,04	0,96	0,48
sandy soil + SD Marienfelde	0,76	0,06	0,89	0,16

4 Laboratory Experiments: Column studies

Retention and migration behaviour of indicator organisms during sand filtration was investigated in different laboratory columns in addition to the experiments in the semi technical plants of Marienfelde. Laboratory columns of UBA, Technical University of Berlin and Free University of Berlin were used for the investigations.

4.1 Experiments with laboratory columns of UBA

Two experiments were carried out to compare retention of test organisms under aerobic and anaerobic conditions.

4.1.1 Materials and methods

For test organisms, sampling, cultivation and detection see chapters “Enclosure” and “Slow Sand Filtration Pond”. Concentrations of test organisms in each surface water and filtrate fractions were used for calculation of relative breakthrough ratios through the quotient of $C_{filtrate}$ to C_{input}. The mean value of all fractional breakthrough ratios was used for further calculations as described previously.

4.1.1.1.1 Column

An acrylic glass column with a diameter of 14 cm and a length of 110 cm was filled with the same sandy soil which was used for the enclosures of Marienfelde. Native sandy soil - free from organic matter - was not previously used for experiments. Four perforated drain tubes enabled sampling at 20, 40, 60 and 80 cm depths of column. Outlet samples were collected from the end of the column at 100 cm. After upwards saturation of the sandy soil column, tap water was percolated top down at a filtration velocity of 30 cm/d corresponding to a pore velocity of 100 cm/d for one week to stabilise the hydraulic conditions.

At a pore velocity of 100 cm/d, the following percolation or residence times were calculated at five sampling sites: 4,75 h at 20 cm; 9,5 h at 40 cm; 14,25 h at 60 cm; 19 h at 80 cm; and 24 h at 100 cm.

4.1.1.1.2 Anaerobic conditions

Further experiments in the same laboratory column were carried out after filling total volume of column with biologically active sandy soil in a length of 110 cm. Because of the higher filling, sampling sites were displaced to 30, 50, 70, 90 and 110 cm. During continuous percolation of tap water in this prolonged sandy soil column, anaerobic conditions developed in the filter bed. The filter matrix became black and the redox potential dropped to -100 mV.

Inoculation: For peak inoculation (4.1.2.1), suspensions of laboratory cultures of test organisms - F+ phage 138, somatic phage 241, E. coli A and E. faecium - were mixed in a total volume of 500 ml and put onto the surface of the sand filter. After gentle percolation of the suspension into the surface layer of the filter bed, pore water velocity was set on 100 cm/d by means of a regulatory pump. For continuous inoculation (4.1.2.2), culture suspensions were mixed with 50 L water in a reservoir tank from which the filter column was fed.

4.1.2 Results

4.1.2.1 Experiment I - 05.05.2004 (aerobic conditions)

Concentrations of test organisms in the initial suspension were about 7.10^7 pfu/100 ml for F+ phage 138, 5.10^5 pfu/100 ml for somatic phage 241, $8 .10^7$ mpn/100 ml for E. coli A, 2.10^7 mpn/100 ml for Enterococcus faecium.

After inoculation of $3{,}5.10^8$ pfu/500 ml, F+phages 138 were detected in filtrates after 4 h at 20 cm, 9 h at 40 cm, 14 h at 60 cm, 20 h at 80 cm, and 26 h at 100 cm (Figure 7). Following this early breakthrough, filtrate concentrations rapidly increased and reached the peak at 30 cm after 6h, at 60 cm after 17h, and at 100 cm after 30h (Figure 7).

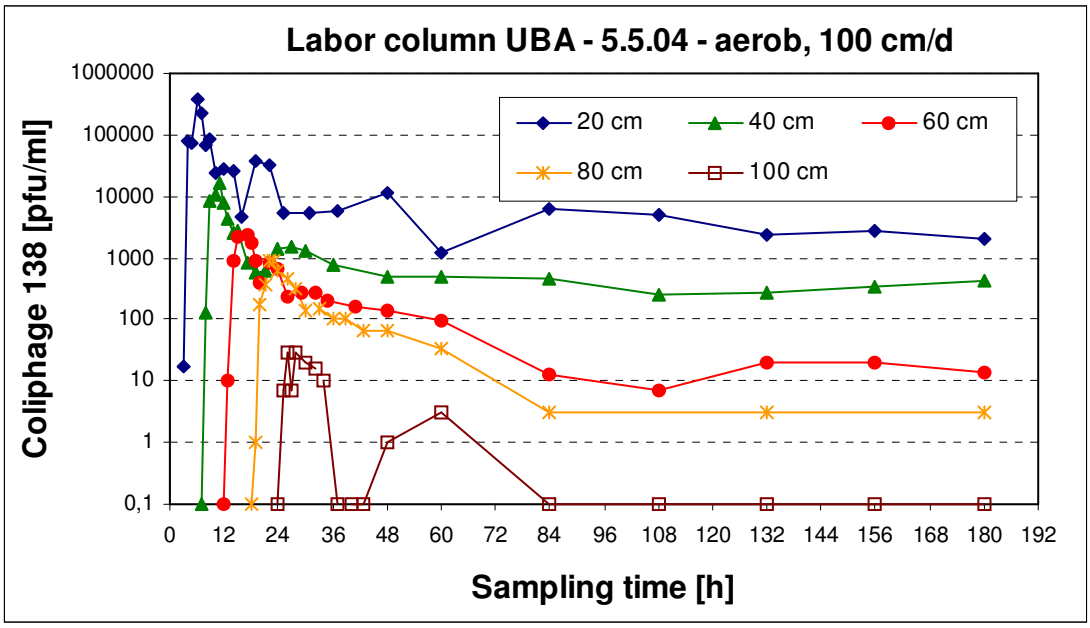


Figure 7: **Concentration of F+phage 138 in the laboratory column of UBA at a pore velocity of 100 cm/d under aerobic conditions (Input of F+phage 138: 3,52.108 pfu/500ml, not demonstrated)**

Breakthrough ratio was 0,0001 for the total effluent, 0,01 at 60 cm, and 0,07 at 40 cm depth. (Table 26). There was no removal of F+phages in the surface layer within 20 cm. Log-

retention increased with increasing depth to 1,2 log units within 40 cm and 4 logs within 100 cm. No homogenous removal of phages was observed along the filtration path between successive sampling sites. Δ log retention varied from 0,4 to 1,6 log units. Elimination rate coefficients (λA) were calculated for the different sampling sites ranging from 0,28 to 0,37 logs.h^{-1} (Table 26). Based on the retention rate of F+phage at 100 cm depth, an elimination of one log unit would occur in a filter path of 26 cm.

Table 26: Retention of F+ phage 138 in the laboratory column of UBA at a pore velocity of 100 cm/d under aerobic conditions

sampling sites (cm)	Cumulative breakthrough ratio*	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm	λA (log.h-1)*
20	1,1	0	-	-	-
40	0,07	1,2	1,2	0,06	0,28
60	0,01	1,9	0,7	0,04	0,30
80	0,005	2,3	0,4	0,02	0,28
100	0,0001	3,9	1,6	0,08	0,37

[*) Calculation by integrative balancing virus concentrations in influent and filtrate samples, +) Retention of phages between two neighbours sites, λA : elimination rate coefficient)]

Initial concentration of somatic coliphage 241 in the inoculum was about (2,6.10^6 pfu/500 ml). The behaviour of somatic coliphage 241 in the column was similar to that of F+phage 138. Highest concentrations of phages were found in filtrate samples from 20 cm after 6 h, 40 cm after 11 h, 60 cm after 16 h, 80 cm after 22h, and in the effluent after 30 h (Figure 8). Retention of phage 241 was distinctly less than observed for F+phage 138. Breakthrough ratios were about 0,03 in the effluent and 0,4 in filtrates from 80 cm. There was no retention in the upper filter paths of 60 cm (Table 27). After a moderate virus retention in the filter path between 60 and 80 cm, highest virus removal of 1,5 logs was observed in the end of the filter path from 80 cm to 100 cm. Correspondingly, elimination rate coefficients of somatic phage 241 were 0,04 and 0,14 logs.h^{-1} at the sampling sites from 80 cm and 100 cm depth. For elimination of one log unit, water would have to be filtered through a filter path of 67 cm.

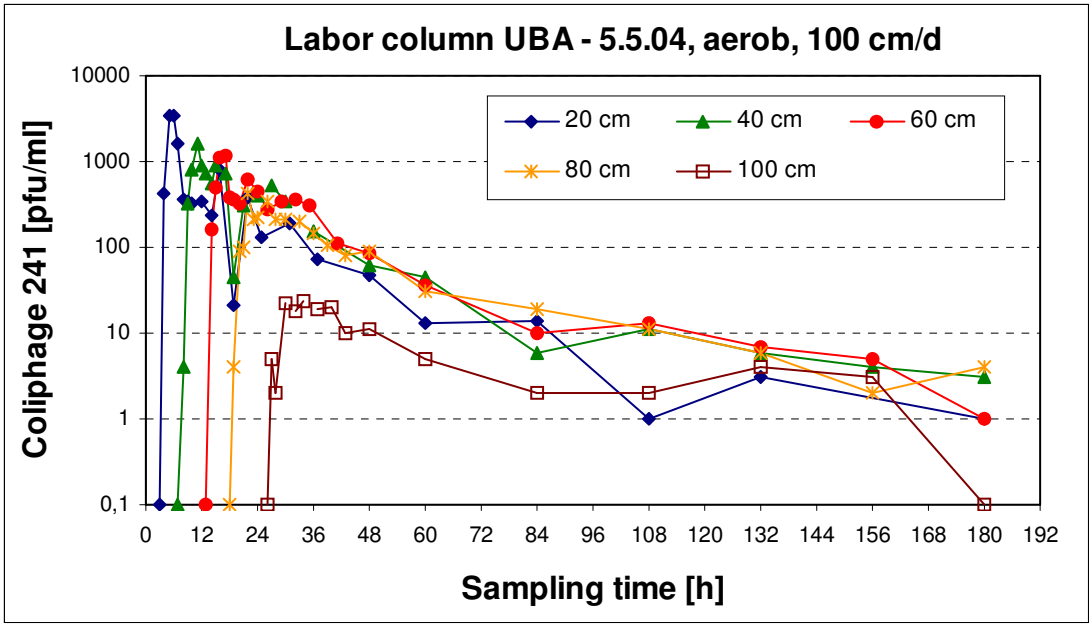


Figure 8: **Concentration of somatic coliphage 241 in the laboratory column of UBA at a pore velocity of 100 cm/d under aerobic conditions (Input of phage 241: 1,6.10^6 pfu/500ml, not demonstrated)**

Initial concentration of E. coli A was 4.108 mpn/500ml. An early breakthrough of E. coli was observed at all sampling sites. It appeared with the front of percolating water after 8 h at 40 cm and after 25 h in the effluent (Figure 9). After this rapid break through, highest concentrations were found in the next filtrate samples. After an operation time of 84 h, cumulative breakthrough ratios of E. coli A were 0,3 at 20 cm, 0,004 at 60 cm and 0,00002 in the effluent (Table 28). Correspondingly, log-retention increased with filtration path from 0,5 to 4,8 log units. A filter path of 21 cm would enable to reduce E. coli A for one log unit.

Table 27: **Retention of somatic phage 241 in the laboratory column of UBA at a pore velocity of 100 cm/d under aerobic conditions.**

sampling sites (cm)	Cumulative breakthrough ratio*	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm	λA (log.h-1)*
20	1,6	0	-	-	-
40	1,5	0	-	-	-
60	1,1	0	-	-	-
80	0,4	0,3	0,4	0,02	0,04
100	0,03	1,5	1,1	0,06	0,14

* Calculation by integrative balancing virus concentrations in influent and filtrate samples,
+ Retention of phages between two neighbouring sites, λA : elimination rate coefficient)]

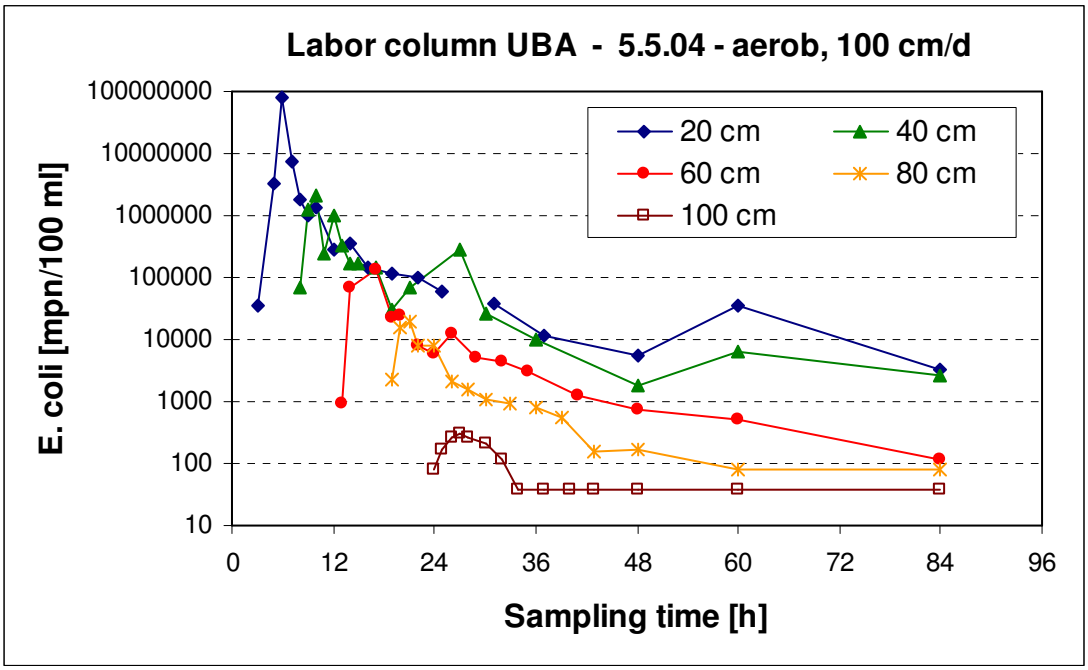


Figure 9: **Concentrations of E. coli A in the laboratory column of UBA at a pore velocity of 100 cm/d under aerobic conditions (Input of E. coli A: 4,76.107 pfu/500ml, not demonstrated)**

After inoculation of 9.10^7 mpn/500ml E. faecium was detected in the first filtrate samples at all sampling sites (Figure 10). Highest concentrations were observed two or four hours after its early breakthrough, e.g. $5{,}6.10^6$ mpn/100 ml after 6 h at 20 cm, $2{,}5.10^6$ mpn/100 ml after 17 h, and $1{,}6.10^5$ mpn/100 ml after 28 h at the end of column (100 cm). During further percolation of the column, concentrations of E faecium slightly decreased to detection limit within 84 h. Cumulative breakthrough ratios were calculated approximately 0,1 up to 80 cm and 0,006 in the effluent, corresponding with a retention ratio of 1 and 1,7 log units, respectively (Table 29).

Table 28: **Retention of E.coli A in the laboratory column of UBA at a pore velocity of 100 cm/d under aerobic conditions**

sampling sites (cm)	Cumulative breakthrough ratio*	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm	λA (log.h-1)*
20	0,3	0,5	0,5	0,03	0,23
40	0,05	1,3	0,8	0,04	0,10
60	0,004	2,4	1,1	0,05	0,39
80	0,0005	3,3	0,9	0,04	0,40
100	0,00002	4,8	1,5	0,07	0,46

* Calculation by integrative balancing virus concentrations in influent and filtrate samples,
+ Retention of phages between two neighbouring sites, λA : elimination rate coefficient)

Retention of intestinal enterococci was low and not homogenous in all part of the filter bed.

Highest retention (0,02-0,04 log/cm) was observed in the surface layer of 20 cm and at the end of the filter path between 80 and 100 cm. Log-retention over the whole filter was 1,7 log units (Table 29). A filter path of 57 cm would be necessary for elimination of one log unit.

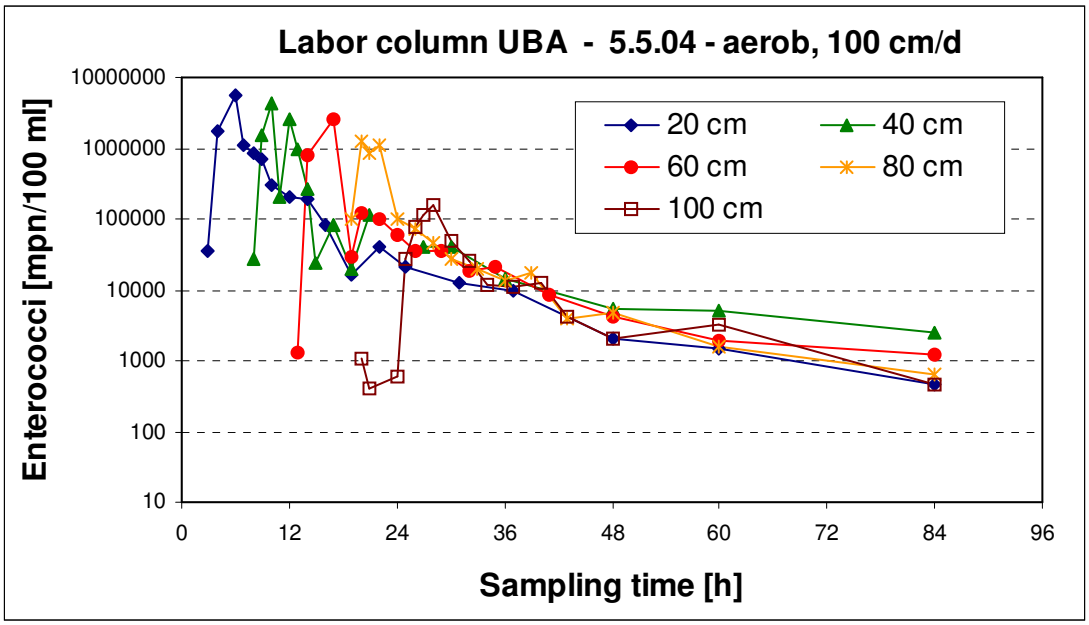


Figure 10: **Concentrations of E. faecium in the laboratory column of UBA at a pore velocity of 100 cm/d under aerobic conditions (Input of E. faecium: 2.108 pfu/500ml, not demonstrated)**

Table 29: **Retention of E. faecium in the laboratory column of UBA at a pore velocity of 100 cm/d under aerobic conditions**

Sampling sites (cm)	Cumulative breakthrough ratio*	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm	λA (log.h-1)*
20	0,4	0,4	0,4	0,02	0,21
40	0,3	0,5	0,1	0,005	0,13
60	0,2	0,7	0,2	0,006	0,10
80	0,1	1,0	0,3	0,016	0,12
100	0,02	1,7	0,7	0,04	0,17

* Calculation by integrative balancing virus concentrations in influent and filtrate samples,
+ Retention between two neighbours sites, λA : elimination rate coefficient)]

4.1.2.2 Experiment II - 12.7.2005 (anaerobic conditions)

In the first part of the experiment F+phage 138 and somatic phage 241 were continuously inoculated into the column. Sampling was carried out in daily intervals from drain tubes at 30, 50, 70, 90 and 110 cm.

Concentrations of F+phage 138 in the water reservoir varied between $2{,}2.10^5$ and $1{,}2.10^6$ pfu/ml during the experiment. Average concentrations of F+phage 138 were $7{,}2.10^5$ pfu/ml in input, $4{,}4.10^5$, 2.10^5, and $3{,}1.10^5$ pfu/ml in filtrate fractions at 30, 70 and 110 cm, respectively (Figure 11). Correspondingly, mean breakthrough ratios of F+phages were in the same order of magnitude at all sampling sites, 0,07 at 20 cm; 0,05 at 70 cm; and 0,06 in the total effluent (Table 30). In comparison to the first experiment under aerobic conditions, relatively low retention of F+ phages was observed under anaerobic conditions (Table 26 and Table 30).

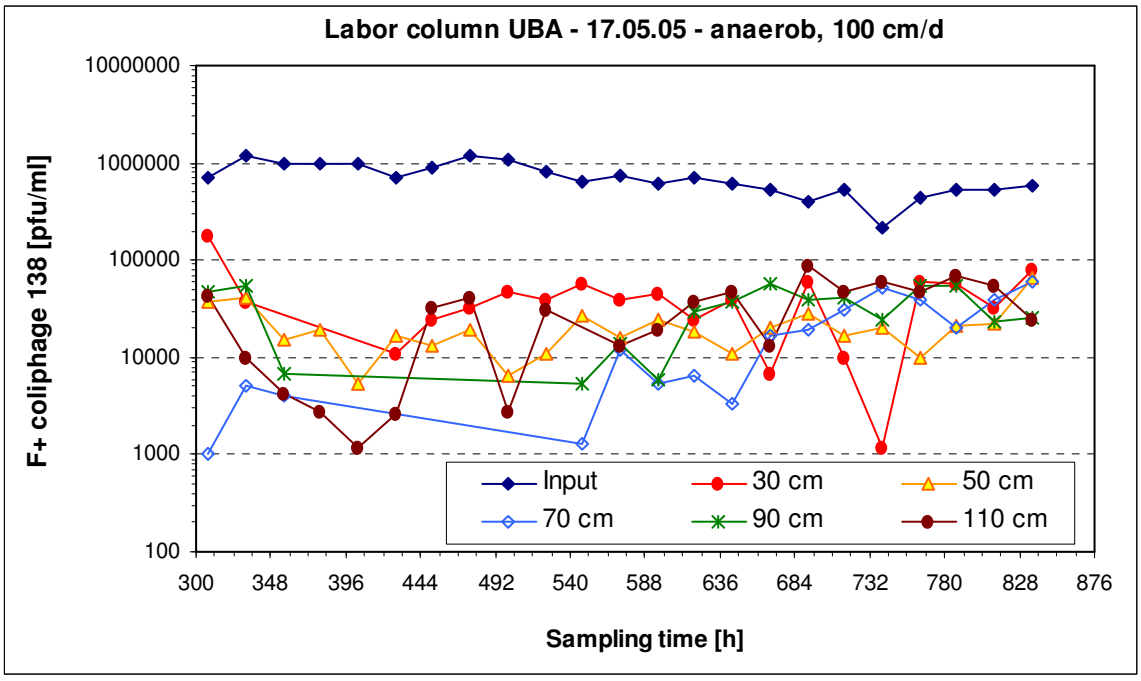


Figure 11: Concentrations of F+phage 138 in the laboratory column of UBA at a pore velocity of 100 cm/d under anaerobic conditions

Highest virus retention was about 1,2 log units in the top filter layer of 30 cm which resulted in a specific retention factor of 0,04 log/cm. Retention of F+phage declined in the deeper filtration path. Elimination rate coefficients decreased significantly from 0,37 log.h-1 at the surface layer to 0,11 log.h-1 in total effluent (Table 30).

Table 30: Retention of F+phage 138 in the laboratory column of UBA at a pore velocity of 100 cm/d under anaerobic conditions,

sampling sites (cm)	Breakthrough ratio*	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm	λA (log.h-1)*
0,3	0,07	1,2	1,2	0,04	0,37
0,5	0,03	1,4	0,2	0,02	0,28
0,7	0,05	1,3	0	-	0,18
0,9	0,06	1,2	0	-	0,13
1,1	0,06	1,2	0	-	0,11

* Calculation by integrative balancing virus concentrations in influent and filtrate samples,
+ Retention of phages between two neighbours sites, λA : elimination rate coefficient

Concentrations of somatic phage 241 in the influent ranged from $1{,}4.10^5$ to $5{,}1.10^5$ pfu/ml, (average 3.10^5 pfu/ml). Mean concentrations in filtrate samples were $5{,}3.10^4$ pfu/ml at 20 cm, $2{,}2.10^4$ pfu/ml at 70 cm, and $1{,}2.10^4$ pfu/ml at 110 cm (Fig. 6). Correspondingly, breakthrough ratios of phage 241 were about 0,2, 0,1, and 0,05 at the sampling sites of 30, 70 and 110 cm depth, respectively (Table 6). The highest elimination rate coefficient of 0,22 $log.h^{-1}$ was determined in the top layer of 30 cm which decreased with increasing filter depth to 0,12 $log.h^{-1}$ at 100 cm (Table 31).

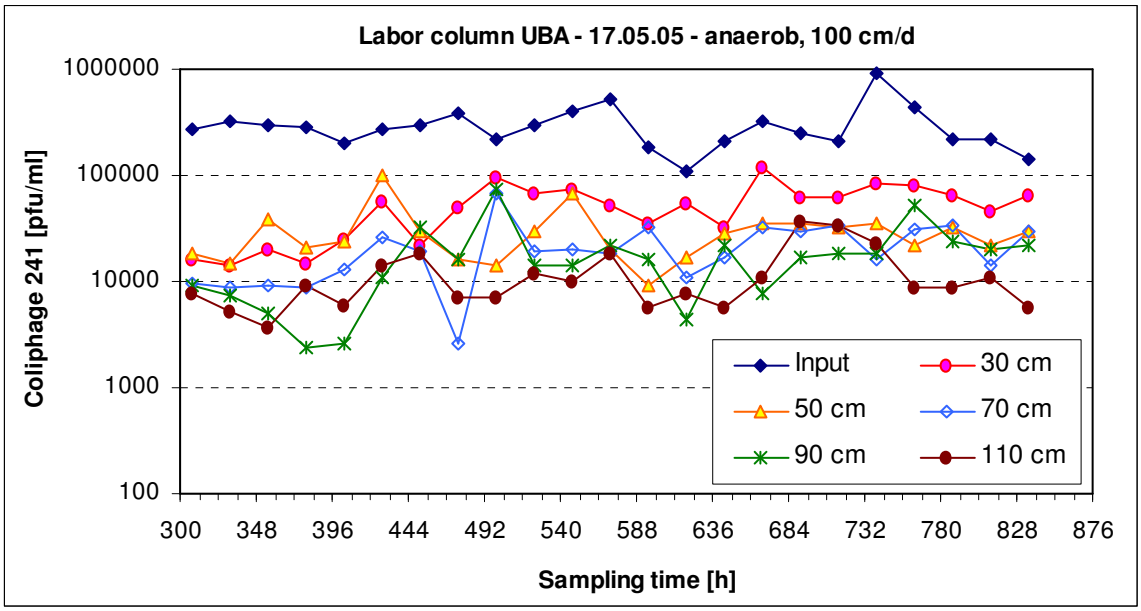


Figure 12 Concentrations of somatic coliphage 241 in the laboratory column of UBA at a pore velocity of 100 cm/d under anaerobic conditions

In the second part of the experiment, suspensions of E. coli A and E. faecium were inoculated as peak in the top layer of the filter bed. Percolation was continued at a pore

velocity of 100 cm/d. Samples were taken in regularly intervals according to movement of frontal filtrate fraction. Redox potential was –190 mV in the effluent of the column at 110 cm.

Table 31 **Retention of somatic coliphage 241 in the laboratory column of UBA at a pore velocity of 100 cm/d under anaerobic conditions**

sampling sites (cm)	Cumulative breakthrough ratio*	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm	λA (log.h-1)*
0,3	0,2	0,7	0,7	0,02	0,22
0,5	0,1	1	0,3	0,01	0,18
0,7	0,1	1	0	0,005	0,14
0,9	0,1	1	0	0,005	0,12
1,1	0,05	1,4	0,4	0,01	0,12

* Calculation by integrative balancing virus concentrations in influent and filtrate samples,
+ Retention of phages between two neighbours sites, λA : elimination rate coefficient

Initial concentration of inoculated E. coli A was about 8.10^9 pfu/100 ml. Relatively high concentrations were detectable in all frontal fractions of percolating water at all sampling sites. Following this early breakthrough, concentrations of E. coli A were highest in the next two or three filtrate fractions at all sampling sites. In further filtrate samples, E. coli A concentrations decreased slightly to detection limit (Figure 13).

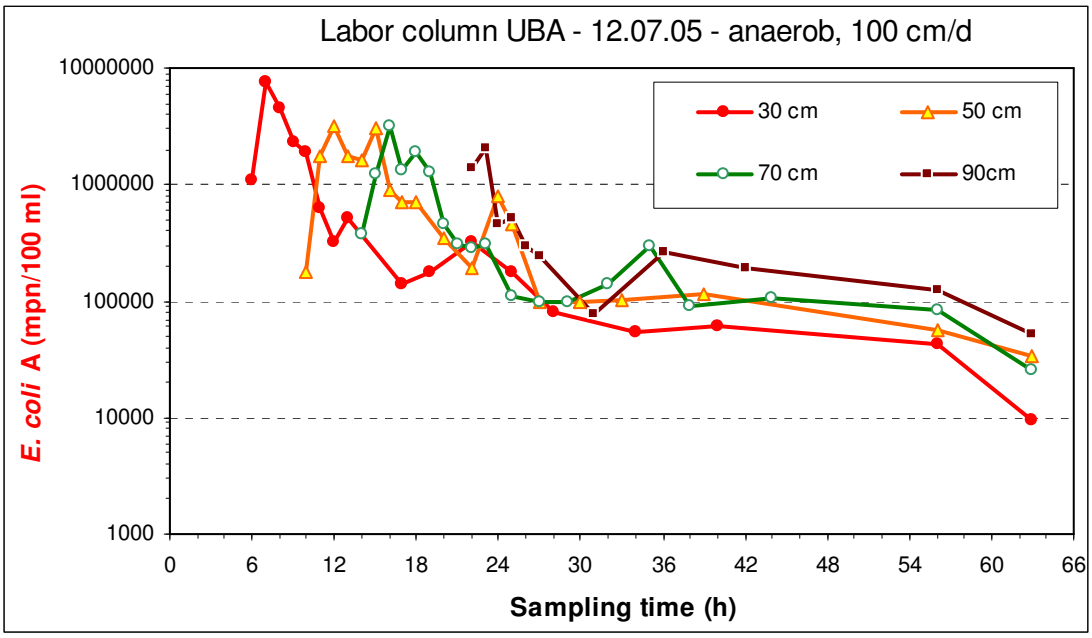


Figure 13: **Concentrations of E. coli A in the laboratory column of UBA at a pore velocity of 100 cm/d under anaerobic conditions (Input of E. coli A: 8,3.109 pfu/100ml, not demonstrated)**

Relatively high breakthrough ratios were calculated for all sampling sites compared to aerobic conditions. Cumulative breakthrough ratios were 0,5 in the upper filter path of 50 cm; 0,4 at 70 cm; and 0,2 at 90 cm depth (Table 32). Elimination rate coefficients were similar (0,07 $log.h^{-1}$) at all filter paths of this anaerobic filter column.

Table 32: Retention of E. coli A in the laboratory column of UBA at a pore velocity of 100 cm/d under anaerobic conditions

sampling sites (cm)	Cumulative breakthrough ratio*	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm	λA (log.h-1)*
0,3	0,5	0,3	0,3	0,009	0,08
0,5	0,5	0,3	0	-	0,06
0,7	0,4	0,5	0,2	0,006	0,06
0,9	0,2	0,6	0,1	0,009	0,07

* Calculation by integrative balancing virus concentrations in influent and filtrate samples,
+ Retention of phages between two neighbours sites, λA : elimination rate coefficient

Concentration of E. faecium was about 6.109 mpn/100 ml in the inoculated culture suspension. Synchronic with the frontal fraction of percolating water, E. faecium was detected in all filtrate samples at all sampling sites (Figure 14). Highest concentrations were found in the following filtrate fractions within one hour.

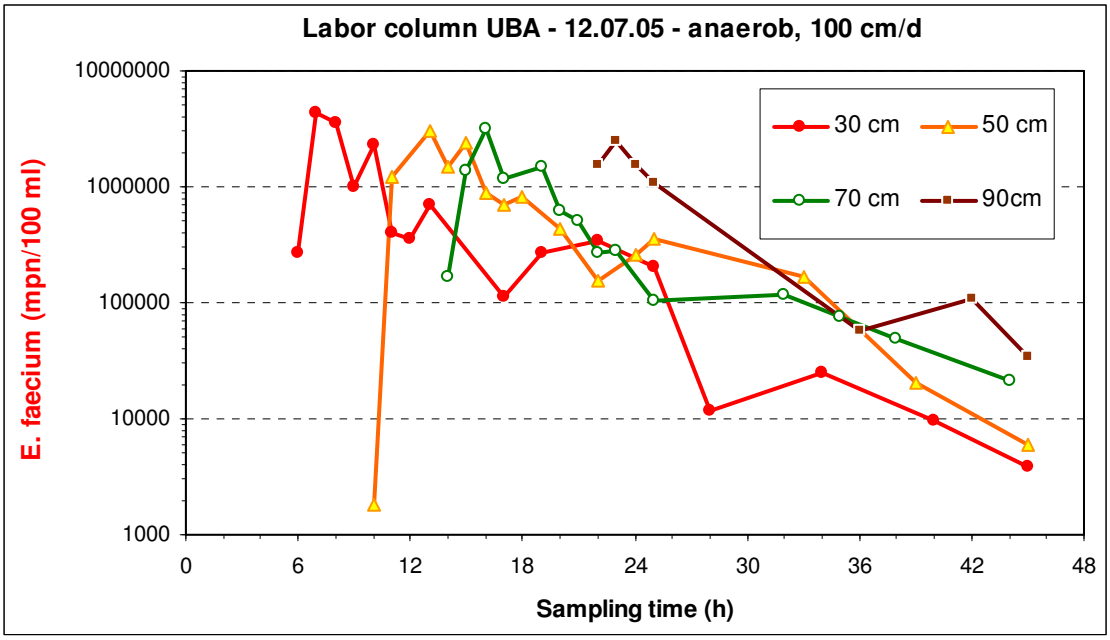


Figure 14: Concentrations of E. faecium in the laboratory column of UBA at a pore velocity of 100 cm/d under anaerobic conditions (Input of E.faecium: 6,7.109 pfu/100ml, not demonstrated)

Retention of E. faecium was even lower under anaerobic conditions compared to aerobic conditions. High relative breakthrough ratios of intestinal enterococci were determined at all sampling sites: 0,5 at 30 cm; 0,6 at 50 cm; 0,4 at 70 cm; 0,3 at 90 cm; and 0,2 at 110 cm (Table 33). Log-retention over the whole column was only 0,7 log units. Specific retention was 0,01 log/cm over the whole length of the filtration path. An elimination rate coefficient of 0,09 $log.h^{-1}$ was calculated for the surface layer while it was about 0,06 $log. h^{-1}$ in the further filtration paths of the column.

Table 33: Retention of E. faecium in the laboratory column of UBA at a pore velocity of 100 cm/d under anaerobic conditions

sampling sites (cm)	Cumulative breakthrough*	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm	λA (log.h-1)*
0,3	0,5	0,3	0,3	0,01	0,09
0,5	0,6	0,3	0	-	0,05
0,7	0,4	0,4	0,1	0,01	0,06
0,9	0,3	0,6	0,2	0,01	0,06
1,1	0,2	0,7	0,1	0,01	0,06

* Calculation by integrative balancing virus concentrations in influent and filtrate samples,
+ Retention of phages between two neighbours sites, λA : elimination rate coefficient

4.2 Experiments with the clogging column (FU-Berlin)

The aim of this experiment was to test adsorption and transport behaviour of test coliphages in a native and non destroyed sandy soil column from a lake basin consisting of natural layers of filter matrix.

Addition of bacteria was not possible in this experiment due to concerns about possible interactions between the bacteria and the trace chemicals added by the “chemical group”.

4.2.1 Materials and methods

A filtration column was prepared with undisturbed sandy soil taken directly in an acryl glass cylinder from the bottom of Lake Wannsee.

After placing horizontal drain tubes in this plexi glass column at different depths, it was percolated with surface water from Lake Wannsee at a pore velocity of 100 cm/d. The length of the column was 100 cm. Seven drain tubes enabled continuous sampling at different sampling sites of the filter matrix using a multi canal peristaltic pump. Consecutive filtrate samples (à 3 ml) were continuously pumped out from each drain tube within a percolation

time of 12 h and separately collected by means of an auto sampler over 6 weeks. Samples were taken from the reservoir and the drain tubes at 4, 12, 25, 50, 60 80 and 90 cm as well as from the total outlet at 100 cm (figure 62, NASRI Report 1, chapter 1.5.5). Samples were daily analysed.

Culture suspensions of the test coliphages F+ phage 138, and somatic phage 241 were added to the reservoir filled with 10 L of lake water. The reservoir was weakly refilled with lake water and inoculated with both phage cultures at the same concentrations.

4.2.2 Results

Initial concentrations of F+ phage 138 in the input varied between $1{,}1.10^4$ and $5{,}7.10^4$ pfu/ml during the operation time of two weeks. F+ phages were detected in all filtrate samples at 4 cm depth. Phages were not detected in filtrate samples at 25 cm and only very sporadically in filtrate samples at 12 cm. Phage concentrations increased to 480 pfu/ml in the filtrate sample at 4 cm after 12 h. Concentration varied synchronic with the concentrations in the water reservoir (Figure 15). Retention of F+ phage was very high. A log-retention of 2,7 log units was calculated within the top 4 cm of the filter bed. Retention of F+ phage was at least 4 log units within the upper 12 cm. Correspondingly, the highest elimination rate coefficient was found in the surface layer of 4 cm (6,4 log. h^{-1}) which decreased to 3,1 $log.h^{-1}$ within a filter path of 12 cm (Table 34).

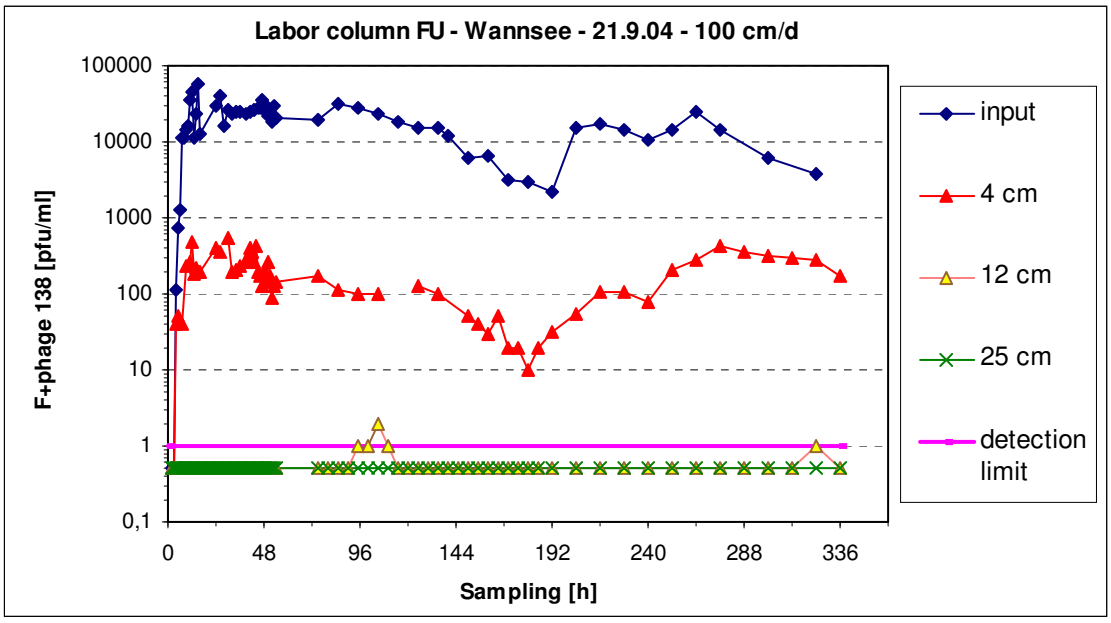


Figure 15 **Concentrations of F+phage 138 in the laboratory column of FU Berlin at a pore velocity of 100 cm/d under aerobic conditions**

Table 34 **Retention of test coliphages in the laboratory column of the FU-Berlin (Pore velocity; 100 cm/d; filter path 100 cm, 21.9 – 1.11.2004)**

Test organisms	sampling sites (cm)	Break-through ratio*	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm	λA (log.h-1)*
F+ phage	4	0,002	2,7	2,7	0,67	6,4
138	12	< 0,0001	> 3,9	> 1,2	> 0,15	3,1
somatic	4	0,08	1,1	1,1	0,28	2,7
phage 241	12	< 0,00006	> 4,2	> 3,1	> 0,39	3,4

* Calculation by integrative balancing virus concentrations in influent and filtrate samples,
+ Retention between two neighbours sites, λA : elimination rate coefficient

Initial concentrations of somatic phage 241 ranged from $1{,}9.10^4$ to $4{,}6.10^4$ pfu/ml during the continuous inoculation of the filter column for two weeks (Figure 16). Coliphages were detected in all filtrate samples from the first drain tube at 4 cm in concentrations around 1000 pfu/ml. Phages were only very sporadically detected at the second sampling site at 12 cm. No phages were detected in the filtrate samples at 25 cm. Log-retention was relatively high and increased from 1,1 log units in the upper 4 cm to more than 4 log units within the upper 12 cm. (Table 34). Correspondingly, high elimination rate coefficients were found in both filter layers (2,7 and 3,4 $\log.h^{-1}$).

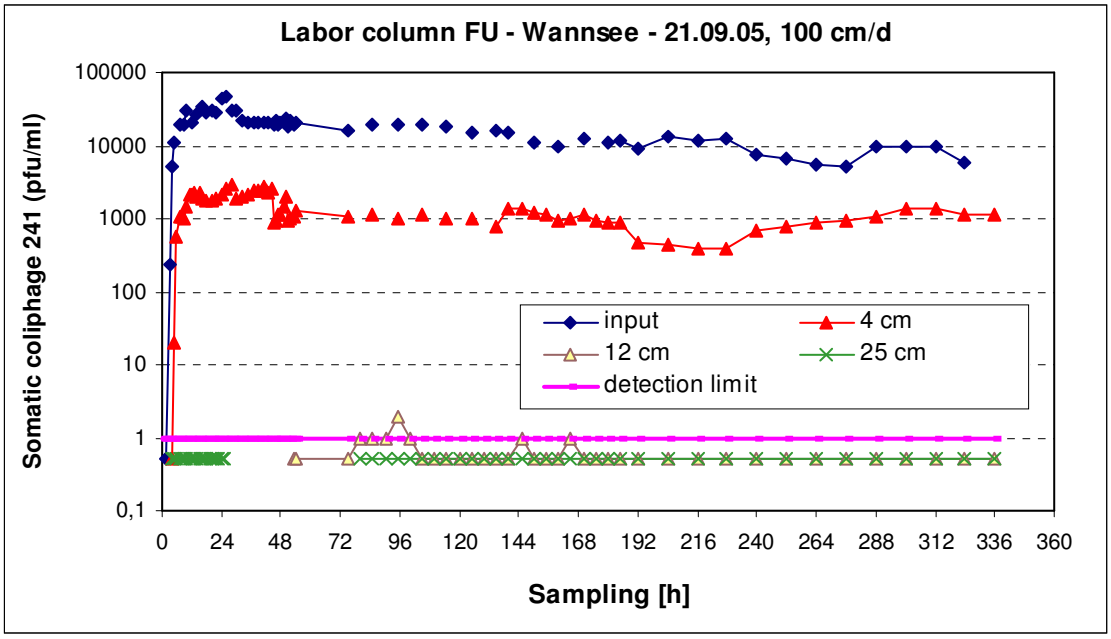


Figure 16: **Concentrations of somatic coliphage 241 in the laboratory column of FU Berlin at a pore velocity of 100 cm/d under aerobic conditions**

4.3 Experiments with the column system (TU-Berlin)

Columns operated at different temperatures were used to study the effect of temperature on phage retention.

Addition of bacteria was not possible in these experiments due to concerns about possible interactions between the bacteria and the trace chemicals added by the “chemical group”.

4.3.1 Materials and methods

Three acryl glass columns, each of them with a length of 50 cm and a diameter of 10 cm were filled with sandy soil and saturated with water from Lake Tegel. Each column was placed in an incubator at a temperature of 5°C, 15°C and 25°C, respectively. Retention of test coliphages was tested at a pore water velocity of 30 cm/d at three different temperatures. Phages were added to the water reservoir of the columns. This enabled a continuous inoculation of the columns. Samples were taken daily from the input and the effluent of the columns.

4.3.2 Results

Input concentrations of F+ phage 138 varied between 1.10^4 and 8.10^4 pfu/ml during the total operation time of 6 weeks. At a temperature of 5 °C, F+ phages appeared in filtrate fraction after three days. The first breakthrough at 15 °C and 25 °C was observed in filtrate fractions after 4-5 days (Figure 17).

Relatively high amount of F+ phages broke through the filter column at 5°C. Breakthrough ratio reached the highest level of 0,15 in the filtrate fraction at day 7 (Figure 17). During further operation time, the retention of F+ phages moderately increased resulting in a lower breakthrough ratio of 0,02 which remained stabile at this order of magnitude up to end of the experiment. Mean breakthrough ratio of samples from 12th to 42th day was calculated 0,02 (Table 35).

At 15° and 25°C, F+ phage was detected in low concentrations in the filtrate during the first 12 days. This resulted in a high log-retention of more than 4 log units. After two weeks, breakthrough of phages increased, and the log-retention declined to about 3 log units. In the further run of the experiment, retention of phages was very variable resulting in log-retentions of 3-5 log units.

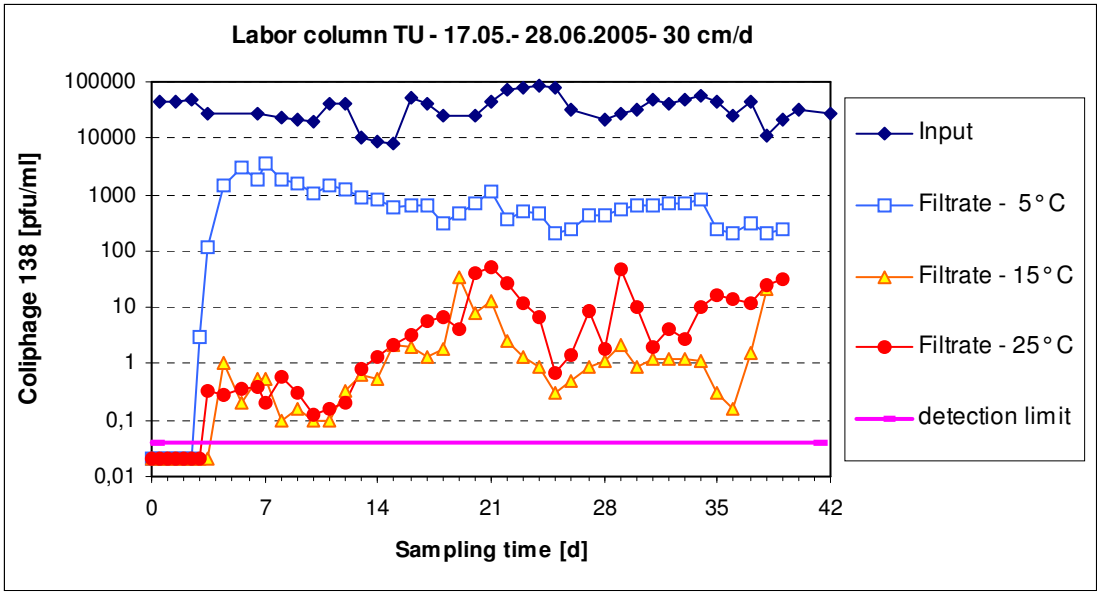


Figure 17: **Effect of temperature on retention of F+phage 138 in the laboratory column of TU Berlin at a pore velocity of 100 cm/d under aerobic conditions**

Taking into account all results of experiment during last 4 weeks all results of the experiment during 4 weeks the mean retention ratio of F+phage at 5°C was 1,7 log units corresponding to an elimination rate coefficient of 0,6 log.h-1. Retention ratios averaged 3,4 log units at 25°C and 3,8 log units at 15°C (Table 35). Both specific retention and elimination rate coefficient were slightly higher at 15°C than at 25°C.

Table 35: **Retention of coliphages by slow sand filtration in dependence on temperature in laboratory column of Technical University Berlin (Pore velocity; 30 cm/d; filter path 50 cm; 29.5 – 28.6.2005)**

Phages	Temperatur (°C)	Breakthrough ratio*	Retention (log)	specific retention (log/cm)	λ* (log . h-1)
F+ coliphage 138	5	0,02	1,7	0,03	0,57
	15	0,0002	3,8	0,08	1,30
	25	0,0004	3,4	0,07	1,16
somatic coliphage 241	5	0,2	0,8	0,02	0,26
	15	0,01	2,0	0,04	0,70
	25	0,003	2,5	0,05	0,86

* Calculation by integrative balancing virus concentrations in influent and filtrate samples,
+ Retention of phages between two neighbours sites, λA: elimination rate coefficient

The concentration of somatic coliphage 241 in the water reservoir was relatively constant ranging from $1{,}6.10^4$ to $5{,}6.10^4$ pfu/ml. Phage 241 occurred at first in the filtrate fraction which was collected at day 3 at 5°C and at day 4 at 15°C and 25°C. The breakthrough ratio of phage 241 increased rapidly, and remained at a mean ratio of 0,2 up to the end of the experiment (Figure 18, Table 35).

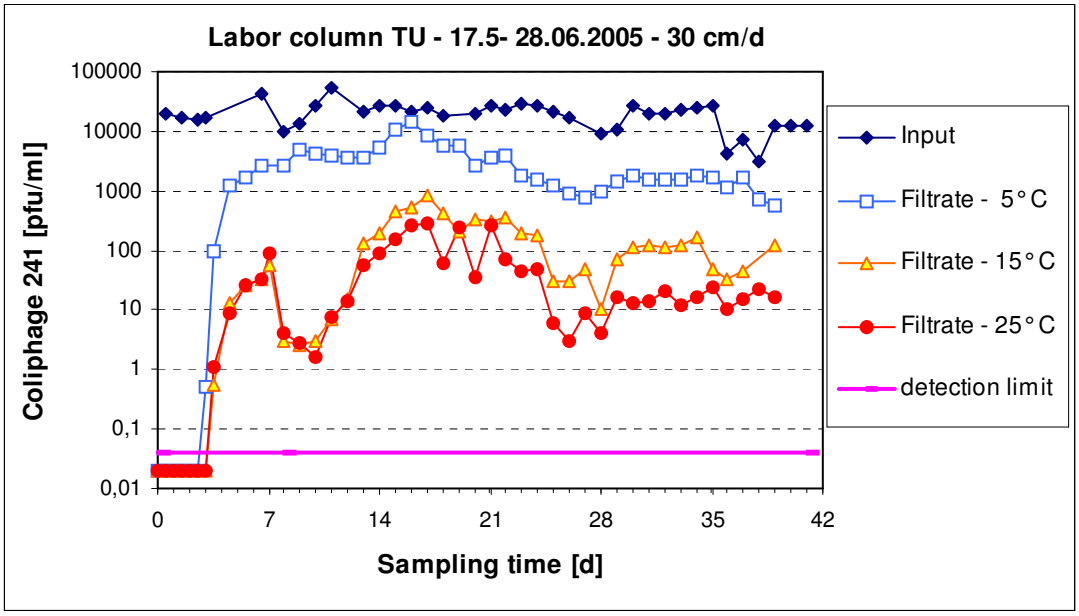


Figure 18: Effect of temperature on retention of somatic coliphage 241 in the laboratory column of TU Berlin at a pore velocity of 100 cm/d under aerobic conditions

At 15°C and 25°C, breakthrough of phage 241 was significantly less than at 5°C. Based on the results from 12 up to 42 d, mean retention ratios of phage 241 were 2,2 logs at 15°C and 2,6 logs at 25°C, respectively (Table 35).

4.4 Long filtration column (UBA)

Simultaneous addition of coliphages and bacteria was not possible in the experiments with the long column due to concerns about possible interactions between the bacteria and the trace chemicals added by the “chemical group”.

4.4.1 Materials and Methods

For test organisms, sampling, cultivation and detection see chapters “Enclosure” and “Slow Sand Filtration Pond”. For assessment of retention, relative breakthrough ratio was

calculated through the quotient of phage concentrations in each fraction of filtrate and input samples. The mean value of all fractional breakthrough ratios was used for further calculation as described previously.

In the first experiment under aerobic conditions somatic coliphage 241 was directly injected in the input tube as sluggish pick. The amount of phage 241 in this inoculum was used as basis for calculation of phages broke through the column at different sampling sites. In comparison to this approach, densities of phage 241 in the filtrate samples from 20 cm depth were taken as basis for calculation breakthrough ratio in deeper sampling sites.

The long sandy soil column - for simulation of ground water stream in an aquifer for 50 d - was inoculated with both coliphages and percolated continuously with water from Lake Tegel.

Percolation rate of lake water was adjusted to 0,5 L/h corresponding to a filtration velocity of 33 cm/d or a pore water velocity of 100 cm/d for about 4 weeks (NASRI Report 4). After four weeks the flow rate was increased to 4 L/h (pore water velocity 800 cm/d) for two weeks and to about 12 L/h for 3 weeks (pore water velocity 24 m/d). Subsequently, percolation of the column was continued at a flow rate of 100 cm/day for further 8 months (see 1.4).

In the year 2005 the long filter columns were operated under “anoxic” conditions (see 1.4). Experiments were carried out with coliphages at a pore velocity of 100 cm/d for 3 months.

4.4.2 Results

4.4.2.1 Short term experiment during 35 days

Somatic coliphage 241 was added as a peak whereas F+phage 138 was continuously added to the column. To achieve this, a suspension (500 ml) of coliphage 241 was directly inoculated into the inlet of the column within 30 minutes. A suspension of coliphage 138 was added into the lake water reservoir of 500 L. Sampling was carried out daily from the water reservoir and all drain tubes of the first column (20 cm, 40 cm, 80 cm, 160 cm, 340 cm and 500 cm).

At a pore velocity of 100 cm/d, the following percolation times are calculated for each filtration path: 16 h for 20 cm, 32 h for 40 cm, 64 h for 80 cm, 5 days for 160 cm, 11,3 days for 340 cm and 16,6 days for 5 m.

Inoculation of the lake water reservoir with a suspension of F+ phage 138 resulted in an initial concentration of 2 x105 pfu/ml which decreased to 550 pfu/ml after 35 d percolation time (Figure 19). At 20 cm and 40 cm, F+ phage 138 was found in relatively high concentrations of about 300 pfu/ml already at the second day. The first breakthrough of phage 138 at 80 cm and 160 cm, was observed at the days 3 and 7, respectively.

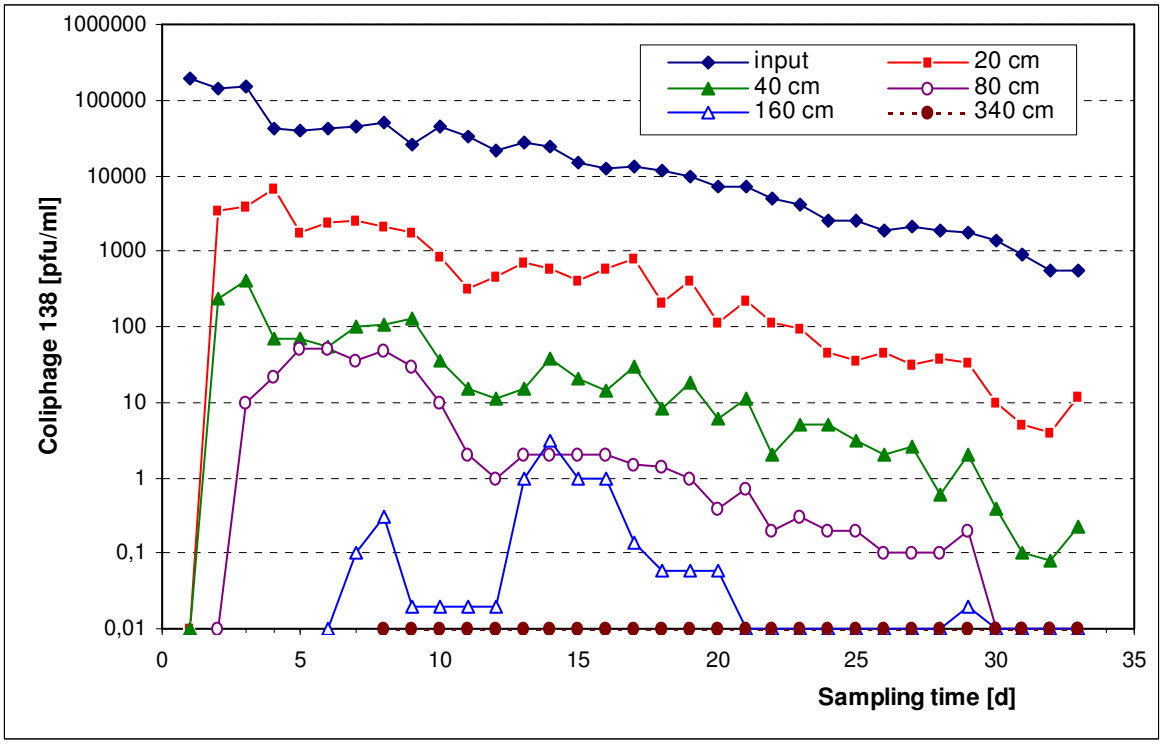


Figure 19: **Concentration of coliphage 138 in different levels of the sandy soil column at a pore velocity of 100 cm/d; detection limit = 0.01 pfu/ml**

After 35 d, the concentration of coliphage 138 was about 10 pfu/ml at 20 cm and less than 1 pfu/ml at 40 cm. At 80 cm and 160 cm coliphage 138 was no longer detected in 100 ml of samples collected after 30 d (Figure 19). No coliphage 138 was found at 340 cm during the entire experiment.

Breakthrough of phages in each fraction was calculated through integrative balancing virus concentrations in influent and filtrate samples (Table 36). During an operation time of 35 days, breakthrough ratios were 0,03 at 20 cm and 0,0003 at 80 cm depth (Figure 20, Table 36). Adequately, removal of F+ phage was found 1,5 log units within the upper 20 cm, 2,8 log units within 40 cm and 5,1 log units within 160 cm. Results of specific retention (log/cm) suggested that the removal of F+phage was not homogenous in all section of the filter matrix. Highest specific retention factor of 0,08 log.cm^{-1} was detected in the surface layer of 20 cm which clearly diminished with increasing filter path and amounted to 0,02 log.cm^{-1} within 40 and 160 cm.

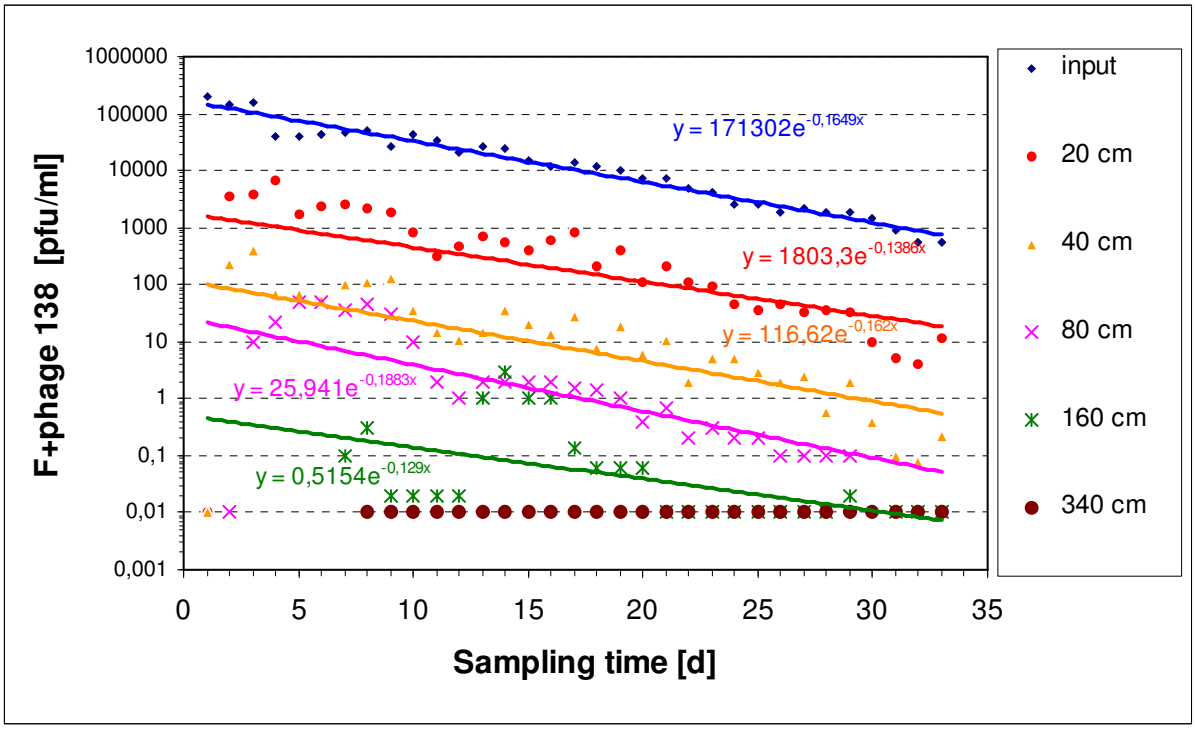


Figure 20 : Regression lines of F+phage 138 in different levels of the sandy soil column at a pore velocity of 100 cm/d

Correspondingly, the highest elimination rate coefficient (λ) was calculated as 0,72 log.h^{-1} in the upper 20 cm. In deeper parts of the filter λ-values decreased clearly to 0,3 log.h $^{-1}$ at 160 cm (Table 36).

Table 36: Retention of F+ coliphage 138 in the long sandy soil column at a pore velocity of 100 cm/d.

Sampling sites (cm)	Breakthrough ratio*	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm	λA (h-1)*
20	0,03	1,5	1,5	0,08	0,72
40	0,001	2,8	1,3	0,07	0,68
80	0,0003	3,6	0,7	0,02	0,42
160	0,00001	5,2	1,6	0,02	0,31
340	-	-	-	-	-

* Calculation by integrative balancing virus concentrations in influent and filtrate samples,
+ Retention of phages between two neighbours sites, λA : elimination rate coefficient

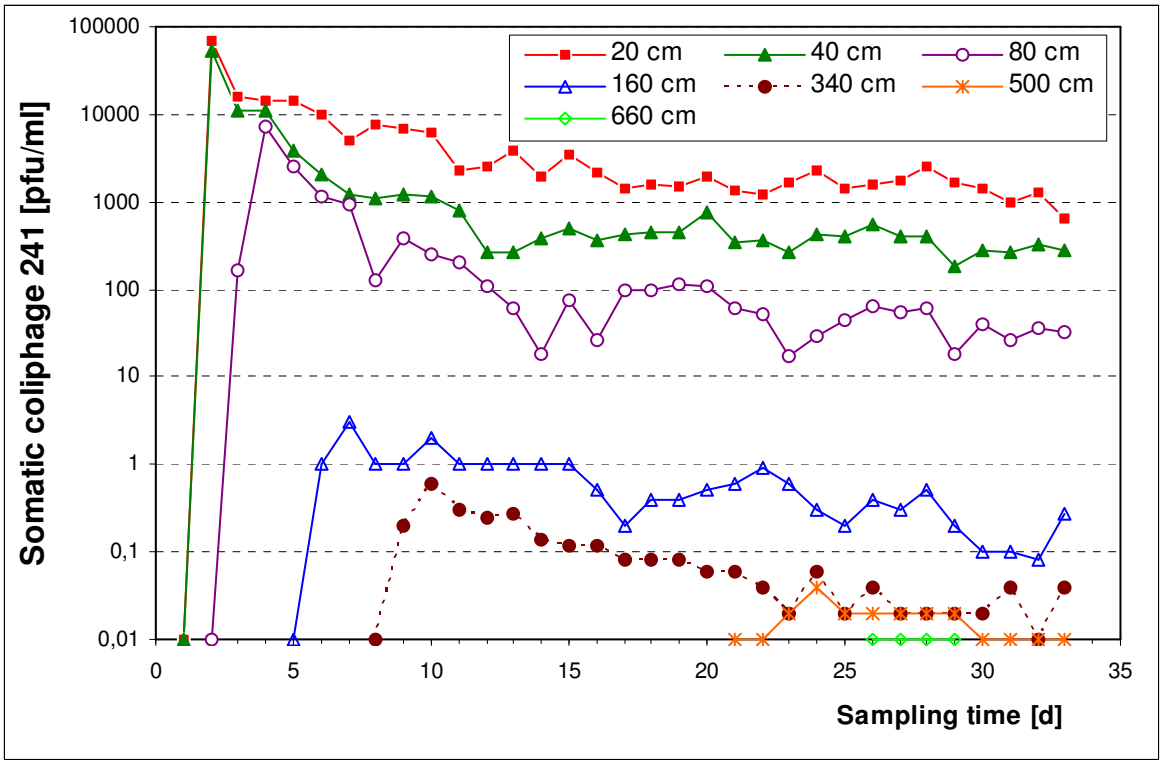
Additionally, regression lines were fitted for phage concentrations measured in samples from each sampling site (Figure 20). Interception point of these regression lines (a values) were also used for determination of breakthrough and retention of phages at each filter path tested (Table 37). Similar breakthrough ratios were estimated as detected by integrative balancing of virus densities (Table 36 and Table 37).

Table 37: Calculation of breakthrough and retention ratios of F+phage 138 using regression lines (Fig. 2) from each sampling sites

Sampling sites (cm)	a (pfu/ml)	log a	Breakthrough ratio*	Retention (log)	Δ Retention+ (Δ log a)	Specific retention (Δ log) / cm	λB (log.h-1)**
input	171302	5,2	-	-	-	-	-
20	1803	3,4	0,01	2	2	0,10	0,95
40	117	2,1	0,0007	3,2	1,2	0,06	0,76
80	26	1,4	0,0002	3,8	0,6	0,02	0,46
160	0,5	-0,3	0,000003	5,5	1,7	0,02	0,33
340	-	-	-	-	-	-	-

a: interception point of each regression line; *) Calculation through the relation of a values
+) Retention of phages between two neighbours sites; λB: elimination rate coefficient

The concentration of somatic coliphage 241 in the stock suspension was 5,8 x 10^7 pfu/ml corresponding to a total peak inoculum of 2,9 x 109 pfu (500 ml). Somatic phages broke through the upper filter paths of 20 and 40 cm already in the first day of operation, and detected at deeper filter sections time delayed e.g. after 6 d at 160 cm, and after 9 d at 340 cm. Less concentrations of phage 241 were found in effluent of the first column (500 cm) after 23 d. No phage was detectable in the second column at a filter path of 660 cm (Figure 21).



F

Figure 21: **Concentration of somatic coliphage 241 in different levels of the sandy soil column at a pore water velocity of 100 cm/d (detection limit = 0,01 pfu/ml)**

In a first attempt, the total amount of phages in the inoculum was used as basis for estimation of breakthrough and retention ratio of phages in filter matrix (Table 38). In comparison with F+phage 138, migration of somatic phage 241 in the column was relatively high. Breakthrough ratios were 0,08 within 20 cm and factor 0,006 within 80 cm (Table 38, Figure 21). Nevertheless, specific retention factor of phages in the surface filter path of 20 cm was clearly higher (0,06 log/cm) than in the filter path between 20 and 80 cm (0,02 log/cm). Log-retention of somatic phage 241 was 5, 6 or 7 log units in the deeper filter paths within 160, 340 or 500 cm, respectively. A relatively high specific retention of 0,04 log/cm was found only in the filter path between 80 and 160 cm. Significantly less specific retention was calculated for the deeper filter passages from 160 cm to 500 cm.

Correspondingly, the highest elimination rate coefficient λ (0,53 $\log.h^{-1}$) was detected in the surface filter layer of 20 cm. Virus elimination remained at about 0,3 $\log.h^{-1}$ between 20 and 160 cm, and clearly declined in deeper filter paths to 0,13 $\log.h^{-1}$ between 340 and 500 cm (Table 38).

Table 38: Retention of somatic coliphage 241 in the long sand filtration column at a pore velocity of 100 cm (Inoculum concentration of phages was taken as 1)

Sampling sites (cm)	Breakthrough ratio*	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm	λA (h-1)*
20	0,08	1,1	1,1	0,06	0,53
40	0,04	1,4	0,3	0,02	0,34
80	0,006	2,2	0,8	0,02	0,27
160	0,00001	5,1	2,9	0,04	0,30
340	0,000001	6	0,9	0,005	0,17
500	0,0000001	7,0	1,1	0,007	0,13

*) Calculation by integrative balancing virus concentrations in influent and filtrate samples,
+) Retention of phages between two neighbours sites, λA : elimination rate coefficient)]

As a second way for calculating virus retention in the filter column, we used the virus concentrations in filtrate samples from the first drain tube at 20 cm as basic input values which were continuously measured during the operation time of 35 days. In comparison to the first calculation model (Table 38), we found a relatively high breakthrough ratio of 0,28 in the upper filter path between 20 and 40 cm (Table 39). Removal of phages was relatively homogenous in a filter path between 20 and 160 cm with relatively stable specific log-retentions varying only between 0,02 and 0,03 $log.h^{-1}$. In contrast, log-retention was significant less (0,005 log/cm) in deeper filter paths up to 500 cm.

Adequately, relative high elimination rate coefficient was found in upper filter matrix between 40 and 160 cm ranging from 0,19 to 0,29 $log.h^{-1}$. Virus elimination rate declined to 0,13 or 0,11 $log.h^{-1}$ in a filter path of 320 or 480 cm, respectively (Table 39).

Table 39: Integrative balancing of the migration and retention of somatic phage 241 by slow sand filtration at a pore velocity of 100 cm (concentrations of phages in filtrates from 20 cm were taken as 1)

Sampling sites (cm)	Breakthrough ratio*	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm	λA (h-1)*
20	-	-	-	-	-
40	0,28	0,6	0,6	0,03	0,27
80	0,06	1,2	0,6	0,02	0,19
160	0,0002	3,6	2,4	0,03	0,25
340	0,00003	4,5	0,9	0,005	0,13
500	0,000004	5,3	0,9	0,005	0,11

* Calculation by integrative balancing virus concentrations in influent and filtrate samples;
+ Retention of phages between two neighbours sites, λA : elimination rate coefficient

Breakthrough and retention ratios were also calculated by means of regression lines as demonstrated in Figure 22. Results of both calculations approaches, integrative balancing and comparison of regression lines, resulted in similar breakthrough and retention ratios (Table 39 and Table 40.

Table 40: Calculation of breakthrough and retention ratios of somatic coliphage 241 using regression lines from each sampling sites (Fig. 5),

Sampling sites (cm)	a* (pfu/ml)	log a	Break-through ratio*	Retention (log)	Δ log Retention+ (log a)	Specific retention (Δ log) / cm	λB (h-1)*
20	14632	4,2	-	-	-	-	-
40	4300	3,6	0,29	0,5	0,5	0,03	0,25
80	850	2,9	0,06	1,2	0,7	0,02	0,30
160	3,0	0,5	0,0002	3,7	2,5	0,03	0,44
340	0,50	-0,3	0,00003	4,5	0,8	0,004	0,27
500	0,1	-1	0,000007	5,2	0,7	0,004	0,62

a: interception point of each regression line; *) Calculation through the relation of a values + Retention of phages between two neighbours sites; λ: elimination rate coefficient

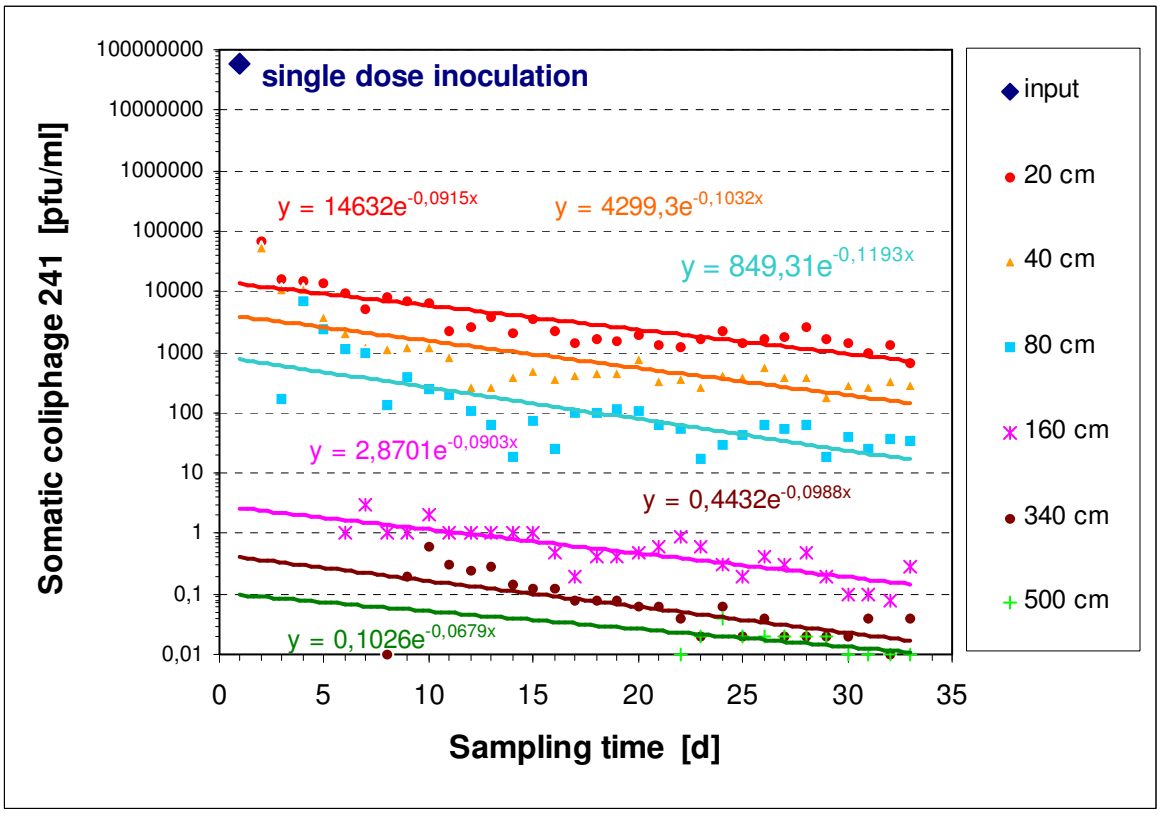


Figure 22: Regression lines of somatic phage 241 in different levels of the sandy soil column at a pore velocity of 100 cm/d

Modelling approach for determination of retention factor and deactivation rate coefficient (λC) of both test organisms will be separately reported by modelling group.

4.4.2.2 Effect of long term percolation on the migration of coliphage 241

Percolation of the sandy soil column with lake water was continued at a flow rate of 100 cm/d for 8 months without further inoculation of test coliphages in the column. Sampling was carried out weekly from 7.4.03 up to 7.12.03 from all drain tubes of the first column (20 cm, 40 cm, 80 cm, 160 cm, 340 cm and 500 cm).

F+coliphage 138 was only sporadically detected in the water samples from different sampling sites of the column during this long term experiment.

Somatic phage 241 was detected in all water samples during the 8 months of investigation. Concentrations of coliphage 241 were in the range of 103 to 104 pfu/ml in water samples from 20 and 40 cm within the first months, and declined about one log unit during the experiment (Figure 23). Similar behaviour at lower concentration levels was observed for the other sampling sites of 80, 160, and 340 cm of the column. At 500 cm coliphage 241 was only sporadically detected in low concentrations up to week 27.

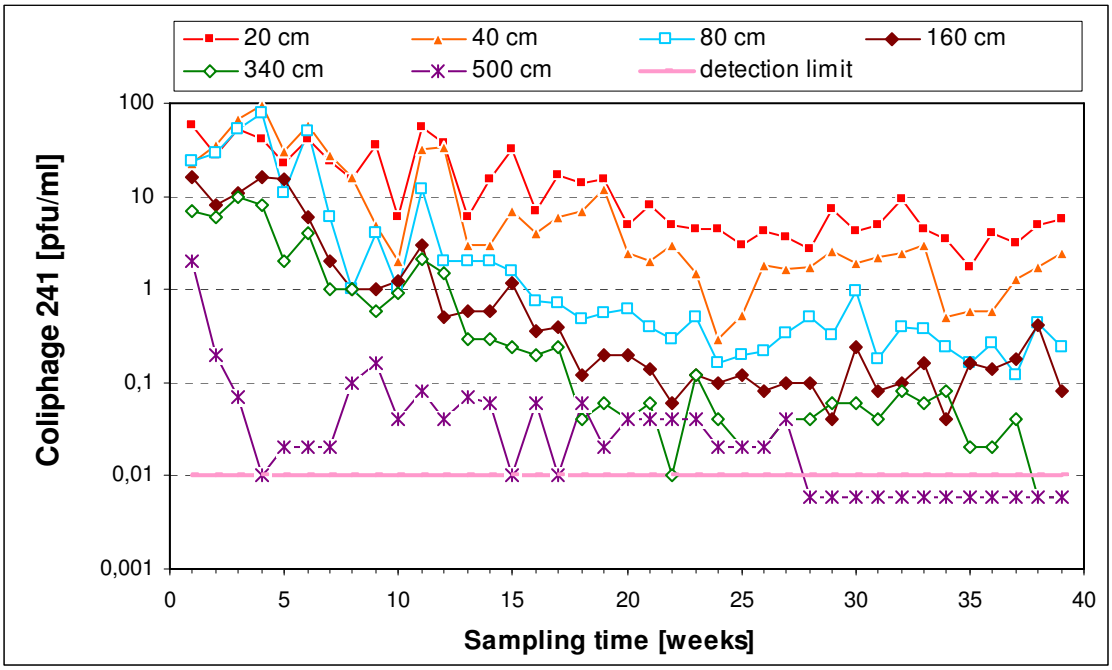


Figure 23: **Concentration of coliphage 241 in the long sandy soil column during 8 months at a flow rate of 100 cm/d; detection limit: 0.01 pfu/ml**

For the estimation of virus retention, the concentrations of phage 241 in samples from the first drain tube at 20 cm were taken as input values and compared with concentrations of phages at other sampling sites.

During this operation time of about 42 weeks, breakthrough ratios were 0,6 0,2 or 0,1 at the filter depths of 20, 60 or 140 cm, respectively. In deeper filtration paths at 340 cm 500 cm, breakthrough ratios were lower (0,04 and 0,01). In the upper filtration paths between 20 and 80 cm, retention of phages was relatively high with a specific retention of 0,01 log/cm. In deeper filter paths between 60 and 320 cm, relatively high virus migration was observed resulting in a low specific retention of 0,002 or 0,003 log/cm between 140 and 480 cm (Table 41) Elimination rate coefficients were 0,1 $log.h^{-1}$ in the upper filter path up to 80 cm and slightly decreased to 0,07 $log.h^{-1}$ within 160 cm and to 0,04 $log.h^{-1}$ at the end of the first column (500 cm).

Table 41: Retention of somatic coliphage 241 (inoculated in the first experiment) during a further operation time of 8 months at a pore velocity of 100 cm/d

Sites (filtepaths) (cm)	Breakthrough ratio*	Retention (log)*	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm	λA (h-1)*
20 (-)	1	0	-	-	0,11
40 (20)	0,6	0,2	0,2	0,012	0,11
80 (60)	0,2	0,6	0,4	0,010	0,10
160 (140)	0,1	1,1	0,5	0,005	0,07
340 (320)	0,04	1,4	0,3	0,002	0,04
500 (480)	0,01	1,9	0,5	0,003	0,04

* Calculation of breakthrough and retention ratios by integrative balancing of virus concentrations in filtrate samples; virus concentrations in samples from 20 cm depth was taken as 1;
+ Retention of phages between two neighbours sites, λA : elimination rate coefficient

4.4.2.3 Effect of flow rate on migration of coliphages in the sandy soil column

After 35 d of percolation, the flow rate through the column was increased from 0,5 L/h (1 m/d) to 4 L/h (8 m/d) for the next three weeks without any additionally inoculation of the column with coliphages. Subsequently, the flow rate was further increased to 12 L/h (24 m/d) for a further 3 weeks.

The concentrations of coliphage 138 were very low after the first 35 h of operation. Nevertheless, an increase in concentration was detected at all sampling points after increase in flow rate (Figure 24). After two weeks, concentrations of coliphages 138 further decreased to values close to the detection limit. Therefore, the possible effects of a further increase in flow rate could not be detected.

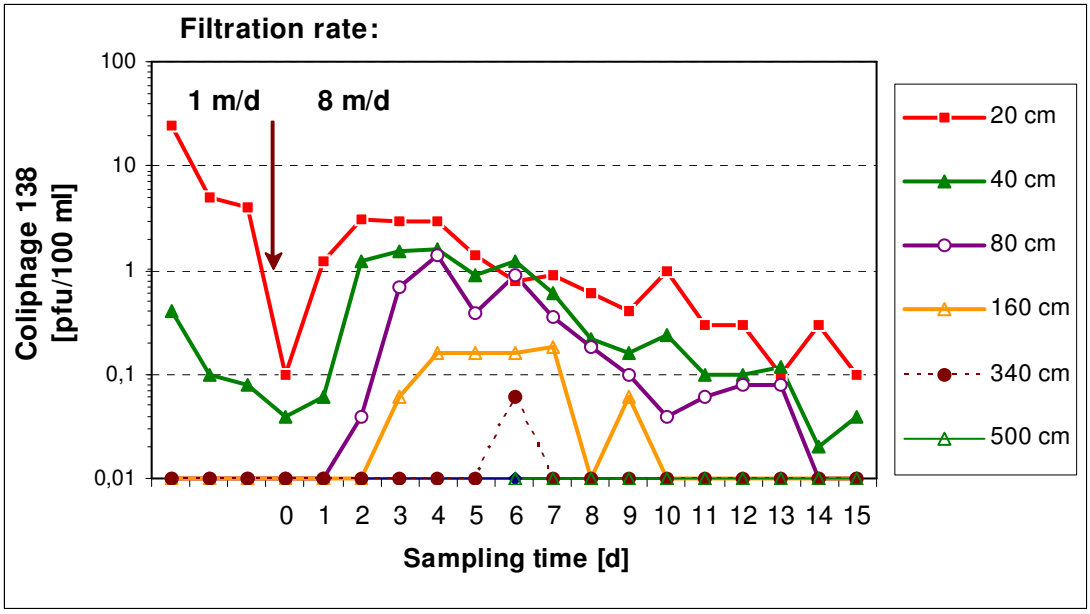


Figure 24: **Concentration of F+ coliphage 138 in the sandy soil column after an increase of pore water velocity to 8 m/d.**

Concentrations of coliphages 241 within the column high of 20 cm did not change by increasing the flow rate to 8 m/d (Figure 25). But migration of coliphages significantly increased in all other filtrates. At the sampling sites of 40 cm and 80 cm, coliphage 241 concentrations reached highest concentrations following a percolation of 2 and 3 days. At all other sampling sites, concentrations continuously increased up to 7 days and remained at this level until the end of experience.

A further increase in flow rate to 24 m/d did not alter the concentrations of coliphages 241 in the water samples from the drain tubes of 20, 40 and 80 cm high, but clearly induced coliphage mobilisation at a column high of 340 and 500 cm (Figure 25).

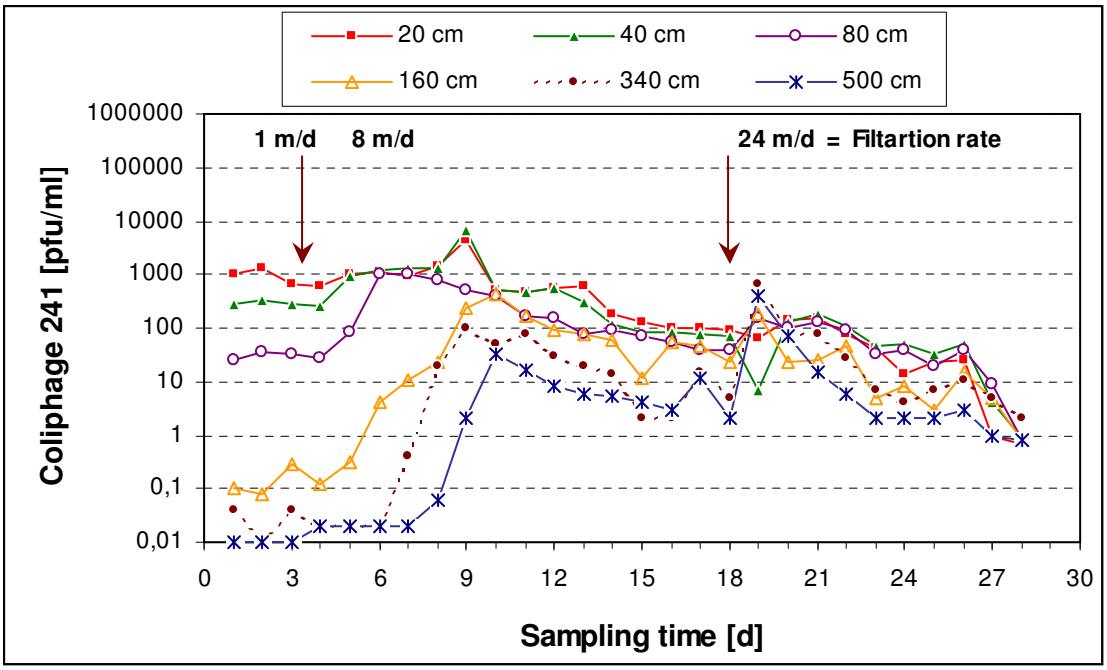


Figure 25: **Concentration of coliphage 241 in the sandy soil column after increasing pore water velocity to 8 m/d and 24 m/d.**

4.4.2.4 Effect of "anoxic" conditions on the retention of test coliphages

In the year 2005 reduced oxygen conditions (O2 < 0,1 mg/L) were introduced at the long sandy soil column. This was achieved by gassing the influent with nitrogen in the reservoir tank. Pore water velocity was regulated to 100 cm/d.

Both test coliphages were continuously added to the column. The initial concentration of coliphages was adjusted at different levels during the operation time of 90 days.

During the initial 7 weeks of the experiment, relatively low concentrations of F+ phage 138 were inoculated in the influent. Concentrations of phages in the influent varied between 10 and 100 pfu/ml (Figure 26). Phages were detected in all filtrate samples from 20 cm and 40 cm depth. They were sporadically detected at 80 cm depth but not in deeper sampling sites (Figure 26). Mean breakthrough ratio was 0,03 or 0,006 at 20 and 40 cm depths, corresponding to a log-retention of 1,5 or 2,2 log units, respectively (Table 42A).

Concentrations of F+ coliphage 138 were increased weakly up to 5x106 pfu/ml during the operation time between weeks 7 and 13 weeks. Phages were found in all filtrate samples up to 160 cm. In filtrates from 340 or 500 cm they did not appear before week 47 or 63, respectively (Figure 26).

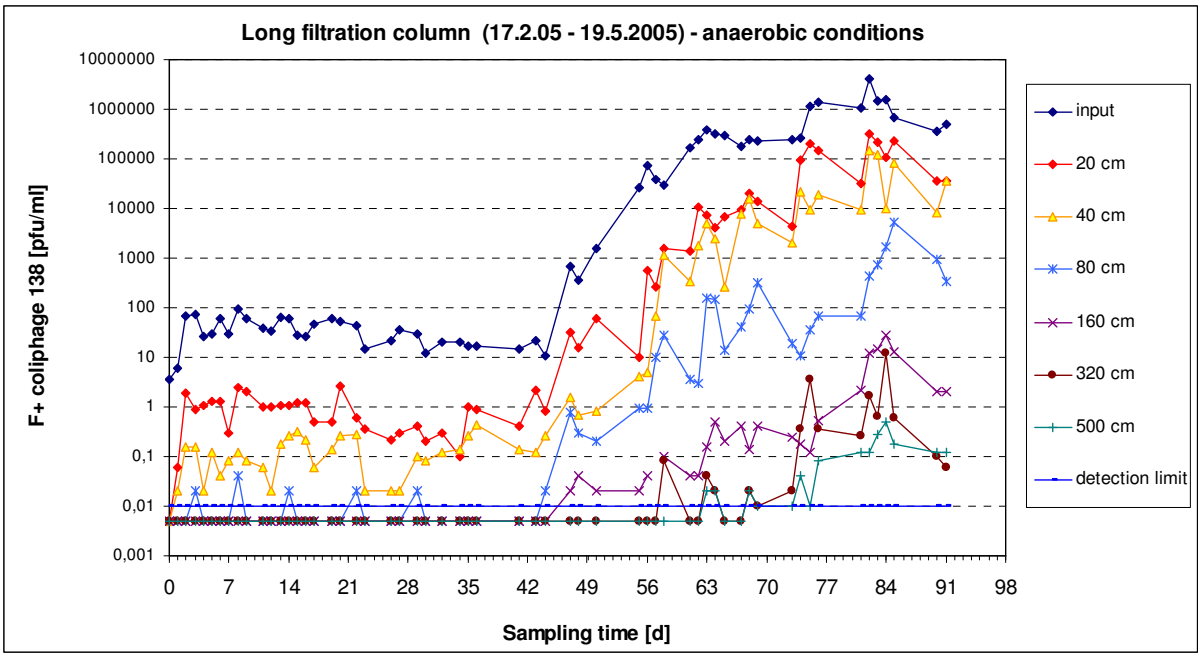


Figure 26: **Concentrations of F+ coliphage 138 in influent and filtrate samples from different depths in the long column under "anoxic" conditions at a pore water velocity of 100 cm/d.**

Table 42 : **Retention of F+ coliphage 138 in long sandy soil column by anaoxic conditions: A. operation at low concentrations of phages in input (0-42 d)**

Sampling sites (cm)	Break-through ratio	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm)	λA (log.h-1)
20	0,03	1,5	1,5	0,08	0,72
40	0,006	2,2	0,7	0,035	0,53

+ Retention of phages between two neighbours sites, λA : elimination rate coefficient]

B. operation at high concentrations of phages in input (45-91 d)

Sampling sites (cm)	Break-through ratio	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm))	λA (log.h-1)
0,2	0,08	1,1	1,1	0,06	0,52
0,4	0,03	1,5	0,4	0,02	0,37
0,8	0,0008	3,1	1,6	0,04	0,37
1,6	0,000004	5,5	2,4	0,03	0,33
3,4	0,000001	6	0,5	0,003	0,17
5	0,0000001	7	1	0,006	0,13

+ Retention of phages between two neighbours sites, λA : elimination rate coefficient]

Log- retention of F+phage138 was one to two log units within the upper 20 or 40 cm. Log-retention increased to 3 log units after 80 cm, 6 log units after 340 cm, and 7 log units after 5 m filtration path (Table 42B). Compared to the results of obtained in experiments under aerobic conditions, retention ratios of F+ phage from the first and the second filter paths (20 and 40 cm) were relatively low (Table 1 and 7B). Retention of phages in deeper filter paths up to 340 cm was found at a similar order of magnitude under aerobic and "anoxic" conditions.

Removal of F+phages was not homogenous in all sections of the filter column. Highest specific retention rate (0,06 log/cm) was found within the first 20 cm of filter (Table 42B). It decreased to 0,03 log/cm within 160 cm, and to 0,003 log/cm between 160 and 340 cm.

Correspondingly, the highest elimination rate coefficient (0,52 $log.h^{-1}$) was found in the first 20 cm which decreased with increasing filter paths to 0,13 $log.h^{-1}$ at 5m filtration path.

Concentrations of somatic coliphage 241 in the influent of the filter column varied between 80 and 800 pfu/ml during the initial seven weeks of the experiment. Somatic phages were detected in all filtrate samples from 20, 40 cm, and 80 cm. After week 10 they appeared in filtrates from 160 and – sporadically - 160 cm (Figure 27).

Log-retention was especially low at 20 and 40 cm depths, with 0,7 log units and 1,2 log units, respectively (Table 43A). Log-retention increased to 2,8 log units at 80 cm, and 3,5 log units at 160 cm depth.

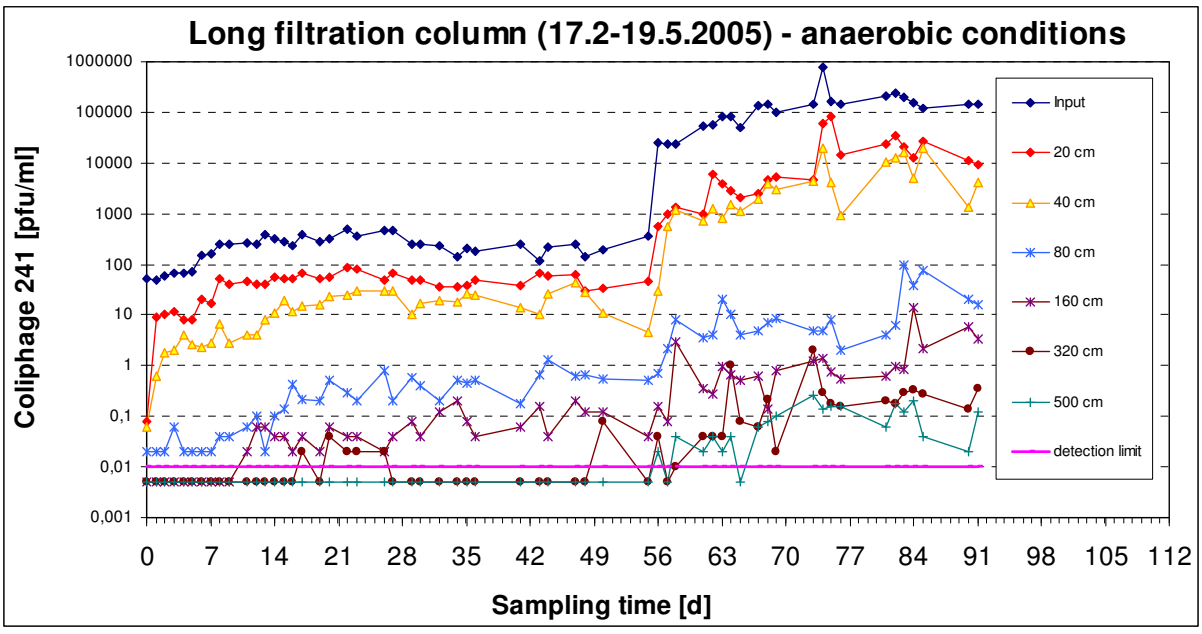


Figure 27: **Concentrations of somatic coliphage 241 in influent and filtrate samples from different sampling sites by slow sand filtration under "anoxic" conditions at a pore water velocity of 100 cm/d.**

Concentrations of coliphage 241 in the influent were increased up to $1x10^6$ pf/ml during further operation between weeks 7 and 13. Coliphage 241 was subsequently detected in all filtrate samples up to 500 cm filtration path (Figure 27).

Table 43 : Retention of somatic phage 241 in long sandy soil column under "anoxic" conditions

A. operation at low concentrations of phages in input (0-45 d)

Sampling sites (cm)	Break-through ratio	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm)	λA* ($log.h^{-1}$)
20	0,2	0,7	0,7	0,04	0,35
40	0,06	1,2	0,5	0,03	0,30
80	0,002	2,8	1,6	0,04	0,34
160	0,0003	3,5	0,7	0,01	0,21

+ Retention of phages between two neighbours sites, λA : elimination rate coefficient]

B. operation at a high concentration of phages in input (55-91 d)

Sampling sites (cm))	Break-through ratio	Retention (log)	Δ log Retention+ (heterogeneity)	Specific retention (Δ log) / cm)	λA* ($log.h^{-1}$)
20	0,1	1,1	1,1	0,05	0,50
40	0,03	1,5	0,4	0,02	0,35
80	0,0001	3,9	2,4	0,06	0,46
160	0,00002	4,7	0,9	0,01	0,28
340	0,000002	5,6	0,9	0,005	0,16
500	0,0000007	6,2	0,5	0,003	0,12

+ Retention of phages between two neighbours sites, λA : elimination rate coefficient]

Log-retention of somatic phages was 1,1 log units at 20 cm, 4 log units at 80 cm 4,7 log units at 160 cm and 6,2 log units at 500 cm. There was no clear difference between removal of coliphage 241 under aerobic and "anoxic" conditions (Table 38 and Table 43B).

Highest log-retention of phages (2,4 log units) was observed in the filter section between 40 and 80 cm with a specific retention rate of 0,06 log/cm comparable to the rate of 0,05 log/cm at 20 cm (Table 43B). In deeper filter sections specific retention rate decreased clearly to 0,003 log/cm.

Elimination rate coefficient was 0,5 $log.h^{-1}$ in the upper 20 cm und decreased clearly with increasing filter path to 0,12 $log.h^{-}1$ at 500 cm (Table 43B).

5 Slow sand filtration experiments

5.1 Material and Methods

5.1.1 Test organisms.

See Part enclosure (Chapter 6).

5.1.2 Filtration pond

The slow sand filtration pond in is a semi technical plant with a quadratic filtration surface of 60,4 m2, filled with sandy soil of a depth of 80 cm. Effective (d_{10}) and median grain sizes of sand were 0,28 mm and 0,7 mm, respectively. As the d_{60}/d_{10} ratio was about 3,28 which is more than 2, the grain size is not considered as uniform (Clement, 2002). The nominal porosity of the sand was measured as 0,335. The experimental set up is shown in Part 6. Some physical and chemical characteristics of the sandy soil are listed in Table 44

Table 44: Selected characteristics of the native sandy soil in the filtration pond

Characteristics of sandy soil in filtration pond	
effective grain size	0,15 - 0,30 mm
uniformity coefficient	3
water charging particles	< 1%
Porosity	31,90%
Dispersion	0,04
dispersion coefficient	0,036 m2/d
average residual time	2,25 - 9 h

Ground water from the water work Marienfelde was used for filling and continuous percolation of the sand filter. During filling of the filter pond, ground water flowed upwards. At a water level of 45 cm corresponding 40 m^3 surface water volume, filtration process was started and stabilised four weeks at each flow adjusted. The flow velocity was regulated to 1,5, 3 and 6 m^3/h corresponding to a filtration velocity of 60, 120 and 240 cm water columns per day or a pore water velocity of 180, 360 and 720 cm/d. Theoretical detention time of water for the total length of the filtration path was measured as 2,25 h, 4,5 h or 9 h at the three filtration velocities, respectively. Table 45 shows some characteristics of the surface

water percolated in the filtration pond. Selected geochemical properties of the sediments are listed in Table 46.

Table 45: Selected characteristics of surface water in the filtration pond

Characteristics of percolated water	
Natrium	46,4 mg/L
Kalium	4,3 mg/l
Calcium	125 mg/L
Magnesium	17,7 mg/L
Sulphate	236 mg/L
Nitrate	0,3 mg/L
Phosphate	<0,1mg/L
DOC	5,5 mg/L
pH	7,8
Conductivity	963 µS/cm

Table 46: Selected geochemical properties of the sediments

Parameters	Native	Clogging layer
Fe-ox [mg/kg]	275	605
Fe-red [mg/kg]	850	1700
Fe-total [mg/kg]	1125	2305
Mn-ox [mg/kg]	11.0	68.8
Mn-red [mg/kg]	17.5	100.0
Mn-total [mg/kg]	28.5	168.8
C-org [weight %]	0.022	0.343
C-inorg [weight %]	0.118	1.395
S-total [weight %]	0.010	0.048
CECeff [mmol(eq)/100g	0.127	1.127

5.1.3 Inoculation

Experiments with chemicals and coliphages were performed simultaneously. Reference compounds of all working groups were mixed in a stock solution of 100 L volume and added as a sluggish peak to the surface water of the filter pond (see 5.2.1.1, 5.2.2.1). Bacteria were not added simultaneously with chemicals because of possible interactions.

Inoculation of coliphages 138 and 241 and indicator bacteria in the reservoir was started in separate experiments synchronic with the tracer salt NaCl (5%) after completion of the experiments with chemical compounds (see 5.2.1.2, 5.2.2.3, 5.2.3). Approximate concentrations of test organisms in the stock solution were $1x10^{11}$ pfu/ml for F+ coliphage 138, $1x10^{10}$ pfu/ml for somatic coliphage 241, and $1x10^{9}$ mpn/ml for indicator bacteria.

After mixing, the solution of all reference compounds was evenly sprayed to the surface water of the filter pond within 5 min. The chloride concentration of surface water increased from 150 mg/L up to 500 mg/l, whereby an increase of conductivity from 895 to about 1400 µS/cm was measured. A water pump operated continuously to homogenize the reference contaminants in the surface water.

5.1.4 Sampling

Sampling was only possible from the water reservoir above the filter and from the outlet of the filter. Samples from reservoir water were collected 20 cm below the water surface in sterile flasks at regular intervals. Sampling from the effluent of filter pond was carried out in short intervals (30 – 60 min.) during the first 12 h after inoculation and were prolonged to several hours and days during the further course of the experiment. An auto sampler was used for sampling the outlet over night.

Core samples from the filter bed were taken before and after one of the experiments to investigate the survival of bacteria and coliphages in the filter.

The first experiment in the filtration pond was carried out on the native sandy soil without any apparent formation of biomass or clogging layer on the surface. During continuous operation, biomass rapidly developed in the filter pond in particular through algae growth which was apparent on the surface of the sand filter (“clogging layer”). After formation of a clogging layer, the filtration experiment was repeated with the same flow rate. At the end of all experiments - approximately after an operation time over 10 months – the filter bed was drained dry and samples from the clogging layer up to 1 cm depth was collected for batch experiments and chemical analysis. Some chemical properties of the clogging layer are given in Table 46.

5.1.5 Assessment of the retention of test organisms

5.1.5.1 Cumulative breakthrough ratio A - CBTA

Concentrations of test organisms in surface water and filtrate samples were used for calculation of relative breakthrough ratios. The quotient of the concentration in each filtrate sample to the total concentration of test organisms in pond water corresponds to the relative

breakthrough of each filtrate fraction. The addition of these single relative breakthrough ratios as a function of time over the whole experiment resulted in a cumulative breakthrough ratio (CBT) of test organisms at a given time according to equation (1):

$$CBTA = \sum \frac{C\ filtrate}{Cinput} \quad (1)$$

Multiplication of the CBT values by 100 gives the breakthrough as percentage of the input concentration.

Log-Retention. The negative decade logarithm of the CBT gives the retention or removal of test organisms in the filter pond as log units.

5.1.5.2 Specific retention rate (filter factor F).

The specific retention rate demonstrates retention of test organisms per cm of filter path.

Retention as log unit per cm filter path has also been defined as filter factor F (Pang et al., 2005).

$$F = \frac{(-)\log CBT}{Filter\ path\ (cm)} \quad (2)$$

5.1.5.3 Elimination rate coefficient (λA)

On the basis of the filtration theory of Yao et al. (1971), transport of colloid particles through saturated porous media can be represented by a connective dispersion equation, augmented by adsorption and desorption terms to account for bacterial interaction with the collector surface. In a column with characteristic length L (m), operated by a constant flow rate V(m/h), assuming steady state conditions, and neglecting dispersion (<0,001), virus inactivation and detachment or virus transport is described by (Schijven and Hassanizadeh, 2001):

$$(-)\log (CBT) = [\lambda \cdot L] / [2{,}3 \cdot V]$$

The elimination rate coefficient λ can be derived from this equation:

$$\lambda = \frac{(-)\log (CBT) \cdot V \cdot 2{,}3}{} \quad (3)$$

L

5.1.5.4 Modelling parameter: λc(h-1), Retardation factor (R)

The adsorption and transport behaviour of test organisms was simulated using a one-dimensional, one-site kinetic model under following assumptions (see NASRI Report 2, chapter 2.4.1). Deactivation or elimination rate coefficient λC (h^{-1}), and retardation factor (R) were taken from the calculation model of Dr. Holzbecher. The λ obtained by modelling is listed as λC in the tables. λC was in the same order of magnitude as λA in all experiments.

5.1.5.5 One log removal distance (D) and time (T)

The elimination rate coefficient (λ) and filter factor (F) are the mean parameter to use to determine setback time or distances for removal of microorganisims in the aquifer. From equation 4, it can be deduced that virus removal in a saturated sand filter under steady state conditions with a constant attachment, detachment and inactivation coefficient, should decline in a linear fashion with travel distance. One log removal distance (D) may be calculated from the log retention of test organisms in the total length of filtration unit (L):

D (m) = log removal / L (m) (4)

or

D = F / 0,01

The reciprocal of the filter factor gives also the distance (D) for one log unit reduction of the test organisms:

D = 1 / F D= 1 log removal per m (5)

One log retention time (T) of test organisms in each filter unit is the reciprocal of the elimination rate coefficient (λ)

T (h) = 1 / λ (6)

Distance or filtration time required for e.g. 8 log removal of test organisms are 8 time higher product of the D and T values.

5.1.5.6 Biofilm investigations

Biofilm investigations were made in the slow sand filtration pond at three different sites. Plastic pipes were installed in three different depth (10 cm, 30 cm and 60 cm). In each pipe a holder with four glass slides was introduced and left there for a period of several weeks. Sampling of glass slides with biofilms was done by pulling the glass slides out of the pipes and immersing them in 50 ml Falcon tubes either with 3.7 % formaldehyde or water from the reservoir.

5.1.5.6.1 Determination of total cell counts (DAPI staining) and fluorescent in situ hybridization

For the determination of total cell counts different areas (at least 6 points) on the glass slide with biofilm were covered with 10 µl DAPI solution (4`6-diamidino-2-phenylindole, Sigma, Deisenhofen; Germany, 10 µg/ml) for 5 min, in the dark. After rinsing with water and air-drying DAPI cell signals were counted. At least 10 microscopic fields (100 x 100 µm) were chosen randomly and a minimum of 1000 cells were enumerated microscopically.

For in situ hybridization, the glass slides with the biofilms were fixed in 50 ml formaldehyde solution (3.7%) in Falcon tubes (Kisker, Germany), for 4 h at 10°C. Subsequent removal of the formaldehyde solution was performed by washing the slides in 50 ml 1 x PBS solution 3 times for 3 min each. After this, samples were air-dried and could be stored at room temperature in the dark at this step of the procedure for several weeks. To prepare wells for the hybridization five to eight grommets were sticked on each slide as shown in Figure 28.

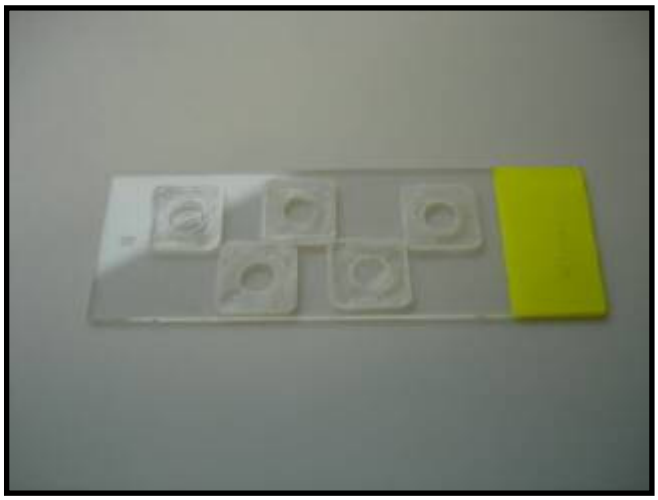


Figure 28: Glass slide with a two week old biofilm with grommets used as hybridization chambers

All custom synthesized oligonucleotide probes used in this study are listed in Table 47. They were 5'-labelled with the indocarbocyanine dye Cy3 (Metabion, Planegg, Germany) and stored in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) at –20°C prior to usage. Hybridization solutions were adjusted to a final concentration of 5 ng probe/µl. The pre-warmed (46°C) hybridization solution (0.9 M NaCl, 20 mM TRIS/HCl, pH 7.2, 0.01% SDS, 35% v/v formamide) was mixed in a ratio of 9:1 with the fluorescently labelled oligonucleotide. To

each well of the slides 10 µl hybridization solution was added and hybridized for at least 2 h at 46°C in the dark. To prevent evaporation the slides were put into Falkon tubes with a piece of wet paper. After the hybridization unbound oligonucleotides were carefully removed by a washing steps with 50 ml pre-warmed washing buffer (20 mM TRIS/HCl, 0.01% SDS, 88 mM NaCl) for 20 min.

After air-drying, the slides were counterstained with 10 µl DAPI solution (10 µg/ml) for 5 min, in the dark for the detection of total cell counts.

Table 47: Oligonucleotide probes used for biofilm analysis

Probe	Target organisms	Reference
EUB338	Eubacteria	Amann et al. 1990
EUB338 II	Eubacteria	Daims et al. 1999
EUB338 III	Eubacteria	Daims et al. 1999
ALPHA1b	alpha-Proteobacteria	Manz et al. 1992
BETA42	beta-Proteobacteria	Manz et al. 1992
GAM42	gamma-Proteobacteria	Manz et al. 1992
CF319	Cytophaga-Flavobacteria	Manz et al. 1996
PLA46	Planctomycetales	Neef et al. 1998
HYPHO1241	Hyphomicrobiaceae	Layton et al. 2000
AQUA841	Aquabacteriaceae	Kalmbach et. al 1999
AERO1244	Aeromonadaceae	Böckelmann et al. 2000
FLAVO1004	Elbe river snow isolate 8	Böckelmann et al. 2000
BETA21	Elbe river snow isolate 21	Böckelmann et al. 2000
Probe D	Enterobacteriaceae	Gutell et al. 1994

5.1.5.6.2 Microscopy and documentation

FISH signals were detected by epifluorescence microscopy using a Zeiss Axioskop (Oberkochen, Germany) equipped with Zeiss light fliter set no. 01 for DAPI (excitation 365 nm, dichroic mirror 395 nm, suppression 397 nm), and HQ light filter 41007 (AF Analysentechnik, Tübingen, Germany) for CY3-labelled probes (excitation 535-550 nm, dichroic mirror 3565 nm, suppression 610-675 nm). Pictures were taken with the digital camera ColorView12 (SIS, Münster, Germany).

5.1.5.6.3 CTC and LIVE/DEAD staining

The 5-cyano-2,3-ditolyl tetrazolium chloride (CTC, Polysciences, Eppelheim, Germany) is a monotetrazolium redox dye which produces a fluorescent formazan (CTF) when it is chemically or biologically reduced. Four week old glass slides were sampled and washed in 1 x PBS to remove non-adherent or loosely-attached bacteria, and then stained by dipping the slides alternatively in 15 ml of a 4 mM solution of CTC or CTC solution plus R2A medium

in a petridish for 2 h. After incubation the slides were rinsed in 1 x PBS and air-dried before microscopical examination.

For the determination of live and dead bacterial cells kit no. L7007 from Molecular Probes, Heidelberg, Germany was used. The kit contains the green-fluorescent nucleic acid stain SYTO9 and the red-fluorescent nucleic acid stain propidium iodide. SYTO9 stains generally all bacteria in a population those with intact membranes and those with damaged membranes. In contrast, propidium iodide penetrates only bacteria with damaged membranes, causing a reduction in SYTO9 stain fluorescence when both dyes are present. Therefore live cells fluoresce green and dead cells red. Glass slides with biofilms were divided into six squares by pencil drawing. 10 µl of staining solution A (SYTO9, 1:1000 diluted) and staining solution B (propidium iodide, 1:1000 diluted) were pipetted on each square and incubated at room temperature in the dark for 15 min. After that slides were rinsed gently with water, air-dried, and covered with mounting oil and coverslips before microscopical examination.

5.2 Results

Experiments with coliphages and chemicals were performed simultaneously (see 2.1.1 and 2.2.1). Bacteria were not added in these experiments because of possible interactions. Inoculation of coliphages and indicator bacteria was started in separate experiments after completion of the experiments with chemical compounds (see 5.2.1.2, 5.2.2.3, 5.2.3).

The following three filtration velocities were tested:

- 240 cm/d corresponding to a pore water velocity of 720 cm/d and a flow rate of 6000 L/h (see 5.2.1)
- 120 cm/d corresponding to a pore water velocity of 360 cm/d and a flow rate of 3000 L/h (see 5.2.2)
- 60 cm/d corresponding to a pore water velocity of 180 cm/d and a flow rate of 1500 L/h (see 5.2.3)

Biofilm investigations were performed during experiment SSF8 (see 2.4).

5.2.1 Experiments with a filtration velocity of 240 cm/d

The heterogeneity of the filtration in the filter was tested in a first experiment using one coliphage (see 5.2.2.1). Two further experiments were conducted with both coliphages and chemicals (5.2.1.1) or bacteria (5.2.1.2) added simultaneously.

5.2.1.1 Experiment SSF1 – 19.3.03 (240 cm/d, without clogging layer)

In the first experiment with the slow sand filtration pond - during a continuous operation time for one week - the function of the six drainage tubes and pumps was tested. After inoculation of F+ phage 138 and tracer salt NaCl into the reservoir water, concentration of phages was

about 2 x 105 pfu/ml. Concentrations decreased to 1,6x104 pfu/ml after 5 h and about1 x 103 pfu/ml after 22 h (Figure 29 and Figure 30).

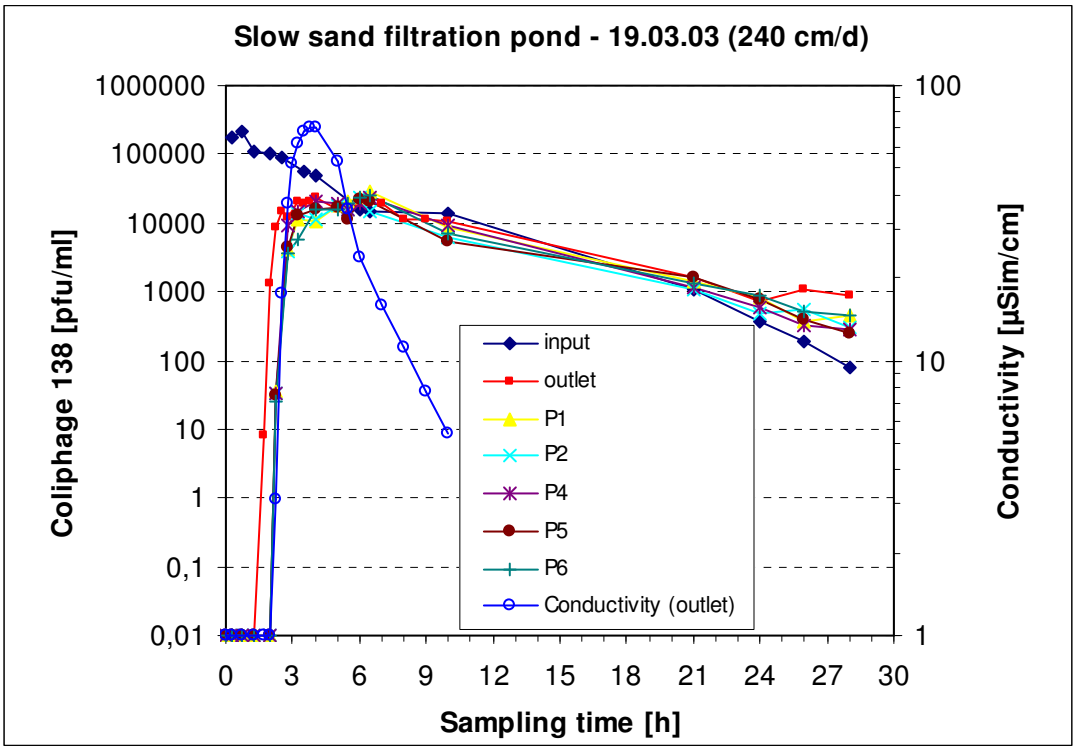


Figure 29: **Concentration of F+ phage 138 in influent and filtrate samples from different drain tubes (P1-P6) and total effluent of the filtration pond, electro conductivity (µSim.cm-1) of total effluent samples (operation time: 11 – 28 h, pore water velocity: 720 cm/d, without clogging layer**

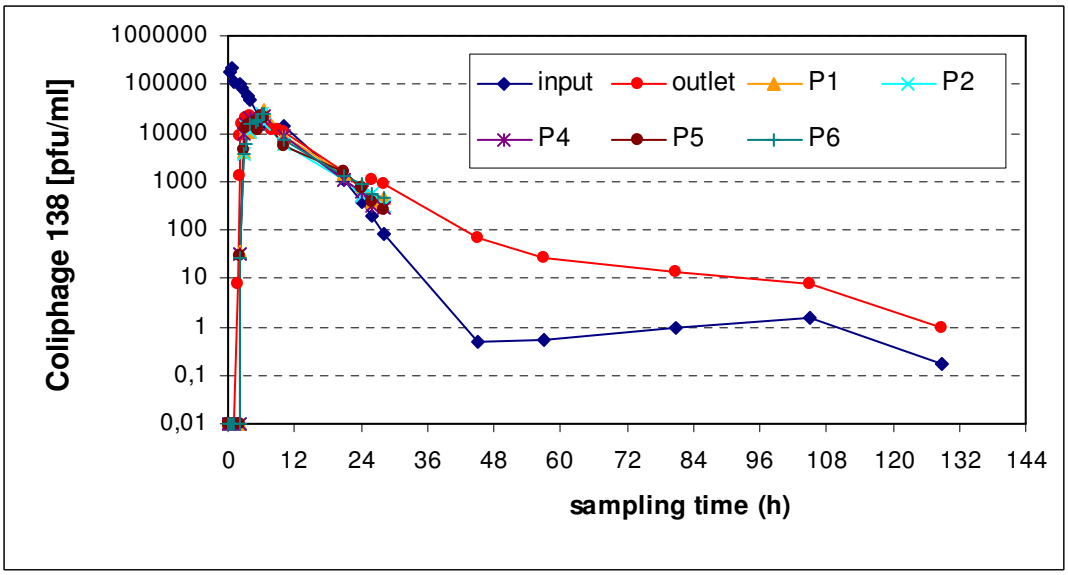


Figure 30: **Concentration of F+ phage 138 in influent and filtrate samples from different drain tubes (P1-P6) and total effluent of the filtration pond (operation time: 128 h)**

In filtrate samples, F+ phage 138 was already detectable synchronic with tracer salt NaCl in the initial filtrate fractions sampled after 2 h (Figure 29). Breakthrough of phages was observed in the total effluent approximately 30 min earlier than in the single drainage tubes P1, P2, P4, P5 and P6 (tube P3 was blocked). Concentrations in samples from the six drainage tubes were comparable over the whole run of the experiment.

Concentrations increased to the highest concentration (about 2 x 104 pfu/ml) after 4 h in all filtrates samples and moderately decreased to to 5 x 102 pfu/ml after 24 h.

Breakthrough ratio and log retention of F+ phage 138 were calculated (Table 48). A breakthrough ratio of 0,69 was found for the total effluent. For the 5 drain tubes, relative breakthrough ratios varied between 0,43 and 0,52. Due to this relative low variation in migration of coliphages, samples in further experiments were not taken from single drain tubes but only from the total outlet tube of the filtration pond.

Table 48: Cumulative breakthrough of F+ coliphage 138 in filtrate samples from different drain tubes and total effluent during an operation time of 28 h

(P1-P6: different drain tubes at 100 cm depth)

Parameters	total effluent	P1	P2	P4	P5	P6	mean
Breakthrough ratio	0,7	0,5	0,4	0,5	0,5	0,5	0,5
Retention (log)	0,16	0,29	0,37	0,29	0,33	0,31	0,29

Sampling from the total effluent was continued in regular intervals during further filtration up to 7 d. Concentrations of coliphages were 100 pfu/ml after 2 days and 10 pfu/ml after 5 days (Figure 30). At the end of operation a cumulative breakthrough ratio of 0,41 was calculated corresponding to a log-retention of 0,39 logs and a filter factor of 0,005 logs/cm (Table 49). Elimination rate coefficient was determined as 0,33 $log.h^{-1}$. The results were not analysed by modelling approaches.

Table 49: Retention of F+ coliphage 138 in the sand filtration pond of Marienfelde by a pore water velocity of 720 cm/d (filter velocity 240 cm/d, SSF1 – 19.3.03)

Test organisms	Breakthrough ratio (CBTA)	Retention (log)	Specific retention (log)/cm	λA* (log.h-1)	Retardation (R)**	λC** (h-1)
F+Phage 138	0,4	0,4	0,005	0,33	-	-

* Calculation according to integrative balancing of total virus concentrations in surface water and filtrate samples,
** calculation according to modelling approaches

5.2.1.2 Experiment SSF7 - 14.6.04 (240 cm/d, without clogging layer)

After completion of the investigations of other working groups in the slow sand filter, retention of coliphages and indicator bacteria could be analysed. The first experiment was conducted with a filtration velocity of 240 cm/d corresponding to 720 cm pore water column per day.

Culture suspensions of test organisms (F+ phage 138, somatic coliphage 241, E. coli and intestinal enterococci) were simultaneously added into the reservoir water and mixed with a surface water pump.

Concentration of F+ phage 138 in the reservoir water was $1{,}1x10^5$ pfu/ml in the beginning of the experiment. Concentrations decreased to $2{,}1\ x10^4$ pfu/ml after 3 h, 2,6.103 pfu/ml after 5h, and $2x10^2$ pfu/ml after 8 h. Phages were no longer detected after 24h (Figure 29). In filtrate samples, coliphage 138 was already detected in the effluent fraction collected after 2h, synchronic with the tracer salt NaCl. Concentrations increased to the highest concentration of $1{,}7x10^3$ pfu/ml after 4 h and moderately declined to $1x10^2$ pfu/ml up to 24 h (Figure 31).

Cumulative breakthrough ratio was 0,3 after an operation time of 192 h. The breakthrough of phages, already in the initial phase of filtration was relatively high. Breakthrough ratios were 0,2 after 6 h and 0,3 after 24h (Figure 32). The retention equilibrium obviously occurred within 12-24 of filtration process.

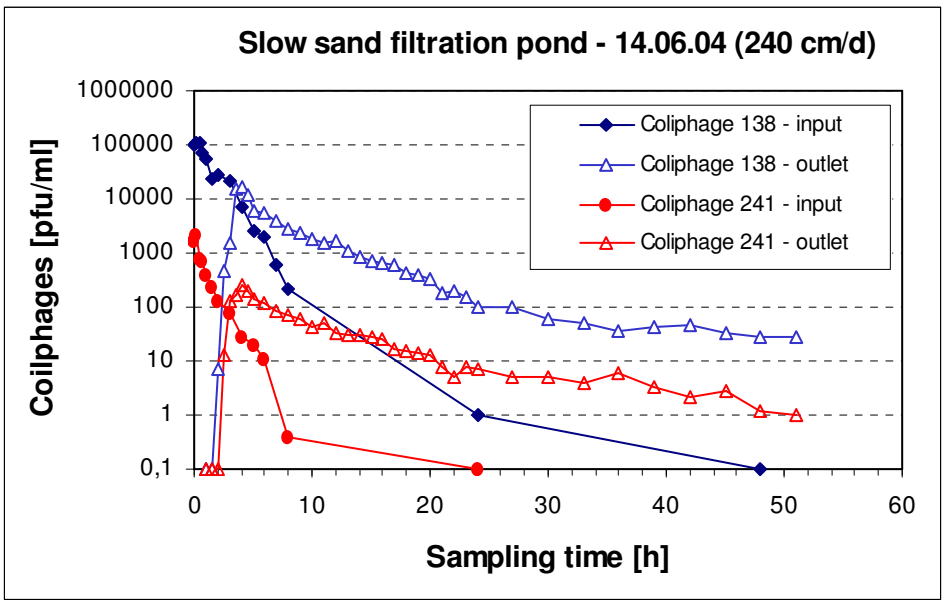


Figure 31: **Concentration of F+ phage 138 and somatic coliphage 241 in influent and effluent of the filtration pond without clogging layer at a pore velocity of 720 cm/d.**

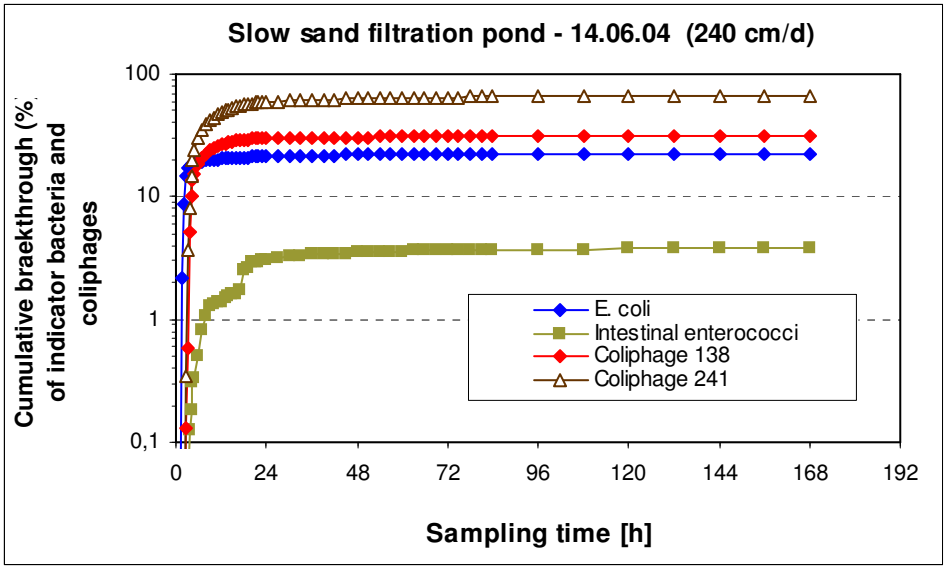


Figure 32: **Cumulative breakthrough of test organisms by slow sand filtration without clogging layer at a pore velocity of 720 cm/d.**

Corresponding to this relative high breakthrough ratios, a low retention of 0,5 log units was found at the end of the experiment, resulting in a specific retention ratio of 0,006 log/cm filter path (Table 50). The elimination rate coefficient (λA) of F+phage 138 at this high filtration velocity calculated by equation (3) was 0,44 log units.h-1. Statistical analysis of input and filtrate concentrations by modelling resulted in a retardation factor of 1,25 and a λc value of 0,4 h^{-1}.

Table 50: **Retention of test organisms in the sand filtration pond of Marienfelde with a pore water velocity of 720 cm/d (filter velocity 240 cm/d, SSF7 – 14.6.04) *) Calculation according to integrative balancing total virus concentrations in surface water and filtrate samples, ** calculation according modelling approaches**

Test organisms	Breakthrough ratio (CBTA)	Retention (log)	Specific retention (log)/cm	λA* (log.h-1)	Retardation (R)**	λC** (h-1)
F+Phage 138	0,3	0,5	0,006	0,44	1,25	0,40
somatic Phage 241	0, 7	0,2	0,002	0,15	1,36	0,17
E. coli	0,2	0,7	0,008	0,56	-	-

A relatively low concentration of somatic coliphage 241 (2.103 pfu/ml) was found in the reservoir water after inoculation. Concentrations decreased to 100 pfu/ml after 3h, and 10 pfu/ml after 6h (Figure 31). Somatic coliphages appeared in the effluent at a concentration of 13 pfu/ml after a percolation time of 2,5 h simultaneous with the tracer salt. The highest

concentration of coliphage 241 was about 250 pfu/ml in the filtrate fraction taken after 4 h. Concentrations decreased moderately up to less than 1 pfu/ml after an operation time of 100 hour.

Cumulative breakthrough ratios were relatively high with 0,3 at 6 h, 0,5 at 12h and 0,6 at 24h (Figure 32). Cumulative breakthrough ratio was 0,7 after a filtration time of 150 h. Correspondingly, retention of F+ phage was low (0,18 log), with a specific retention rate of 0,002 log/cm filter path (Table 50).

The elimination rate coefficient λA of phage 241 was 0,15 log units.h-1 which was clearly less compared to phage 138. The modelling approach revealed a retardation factor of 1,36 and also a low elimination rate coefficient λc of 0,17.h-1.

The concentration of E. coli reached $1{,}5 \times 10^4$ mpn/100 ml in the reservoir water which gradually decreased to 100 mpn/100ml after 24 h and 1 mpn/100ml after 48h (Figure 33). In the filtrate samples, E. coli was detected for the first time (6 mpn/100ml) after 1 h. The concentration increased rapidly in the next filtrate fractions, and reached the highest level of 4×10^4 mpn/100 ml already in the sample taken after 2,5 h (Figure 32). Concentrations of E. coli decreased in further filtrate fractions, to 98 mpn/100 ml after 5 h, 10 mpn/100 ml after 24h and 1 mpn/100 ml after 48h.

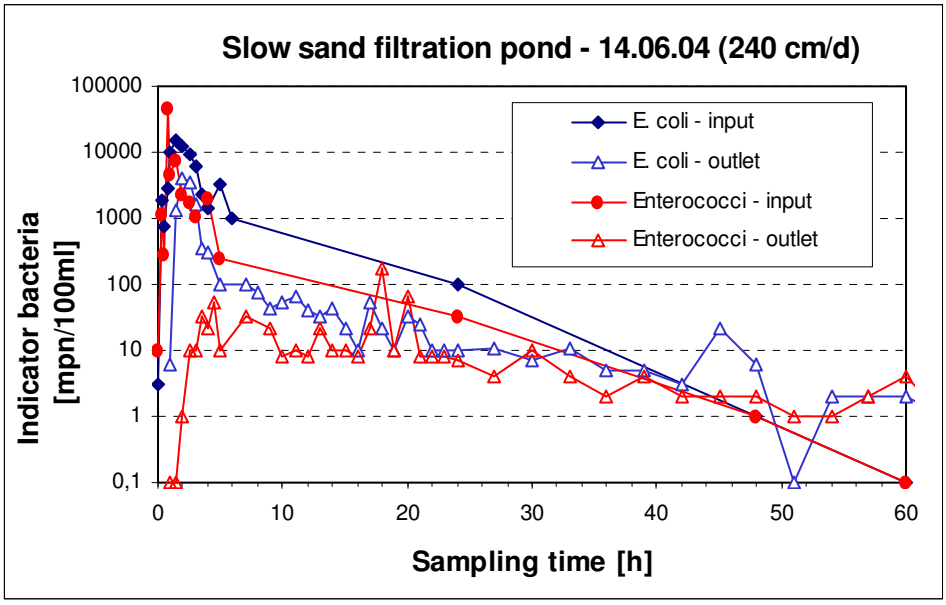


Figure 33: Concentrations of E. coli and Enterococcus sp. in influent and effluent of the filtration pond without clogging layer at a pore velocity of 720 cm/d.

Due to the rapid breakthrough of E. coli in high concentrations in the initial filtrate fractions, the cumulative breakthrough ratio was 0,2 % already after 3h. Similar breakthrough ratios were calculated after 12 h and at the end of operation after 150 h (Figure 33). Consequently, retention of E. coli was high (about 0,65 logs) with a specific retention rate of 0,008 log/cm (Table 50).

The elimination rate coefficient (λA) was 0,56 log units.h-1. No modelling was carried out on these results. The highest concentrations of Enterococcus faecalis in reservoir water was

4,6.105 mpn/100ml declining to 250 mpn /100 ml after 5 h, and 32 mpn/100 ml after 24h (Figure 33). In the filtrate samples, they were mostly detected in concentrations less than 100 mpn/100ml. Two high densities were observed with 54 mpn/100 ml after 5 h and 170 mpn/100 ml at 18 h.

Cumulative breakthrough ratio of enterococci was 0,005 after 6h, 0,01 after 12h, 0,03 after 24 h and 0,04 at the end of experiment after 150h (Figure 32). The retention of enterococci was, therefore, relatively high (1,4 log units) and resulted in a specific retention rate of 0,02 logs/cm filter path.

Correspondingly, the highest elimination rate coefficient (1,2 log units. h-1) was found for intestinal enterococci (Table 50). No modelling was carried out on these results.

5.2.2 Experiments with a filtration velocity of 120 cm/d

5.2.2.1 Experiment SSF4 – 26.05.03 (120 cm/d, without clogging layer)

In this experiment only phages were tested and the filtrate flow velocity was reduced to 120 cm/d water column or 360 cm/d pore water velocity. Immediately after inoculation of the reservoir water, the concentration of F+ coliphage 138 reached 6,1.105 pfu/ml and then gradually decreased to 5,2.104 pfu/ml after 6 h (Figure 34). After 48 h of operation 100 pfu/ml were detected in the reservoir water. After a rapid breakthrough, the concentration of coliphage 138 in the effluent reached 258 pfu/ml after 6 h and remained at a relatively constant level between 200 and 300 pfu/ml up to 24 h. Concentration subsequently declined to a level of 1 pfu/ml during further operation up to 90 h. Detection of coliphage 138 in the filtrate samples was synchronic with the increase in conductivity.

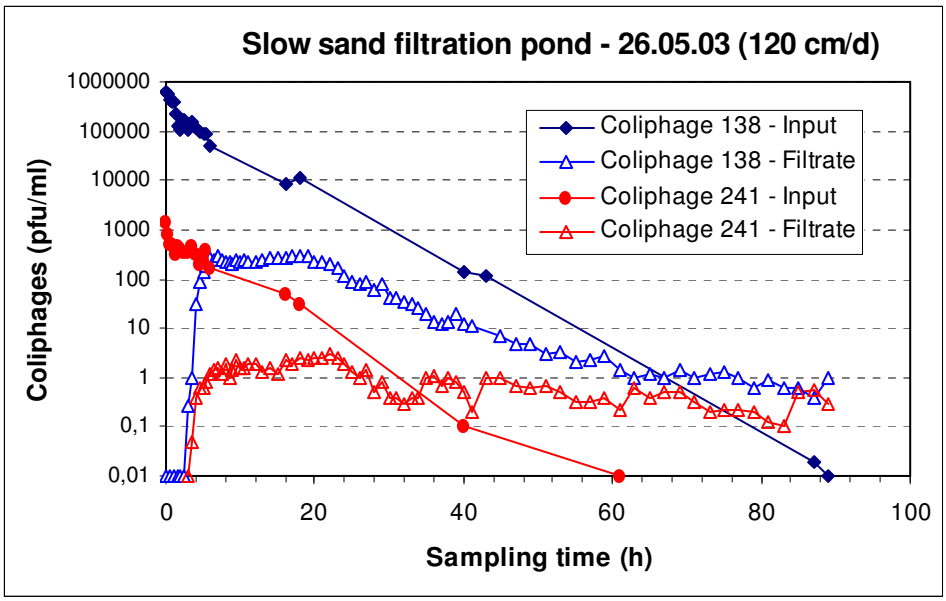


Figure 34: **Concentration of F+ phage 138 and somatic phage 241 in influent and effluent of the filtration pond without clogging layer at a pore water velocity of 360 cm/d.**

Cumulative breakthrough ratio of phage 138 was calculated as 0,001 after a filtration time of 12 h which increased marginally to 0,004 in the further run of the experiment (Figure 35, Table 51). Based on this total virus retention of 2,4 log units, a specific retention rate of 0,03 log units per cm filter length was calculated.

With a filtration velocity of 0,05 m.h-1 and a cumulative breakthrough of 0,004, the elimination rate coefficient was calculated (equation 3) as 1,02 log units.h-1 (Table 51). No modelling was carried out on these results.

After inoculation of the reservoir water, concentrations of coliphage 241 were much lower (103 pfu/ml) than that of coliphage 138. Concentrations decreased to 170 pfu/ml during an operation time of 6 h (Figure 31). Despite these relatively low concentrations in the reservoir water, coliphage 241 appeared synchronic with the tracer salt in the initial filtrate samples (Figure 32). Coliphage 241 was detected in all filtrate samples at low concentrations (10 - 200 pfu/100 ml) during the further run of the experiment.

Retention of coliphage 241 in filter pond was not as good as for coliphage 138. Cumulative breakthrough ratios reached 0,004 and 0,01 after a percolation time of 12 or 24 h, respectively. At the end of experiment the ratio increased up to 0,024 (Figure 32, Table 51). With a relative retention of 1,6 log units, the specific retention rate amounted to 0,02 log units per cm filter path. Correspondingly, an elimination rate coefficient of 0,7logh^{-1} was determined at this filter velocity of 0,05 mh^{-1} which was clearly less than that of phage 138 (Table 52). The results were not analysed by modelling approaches.

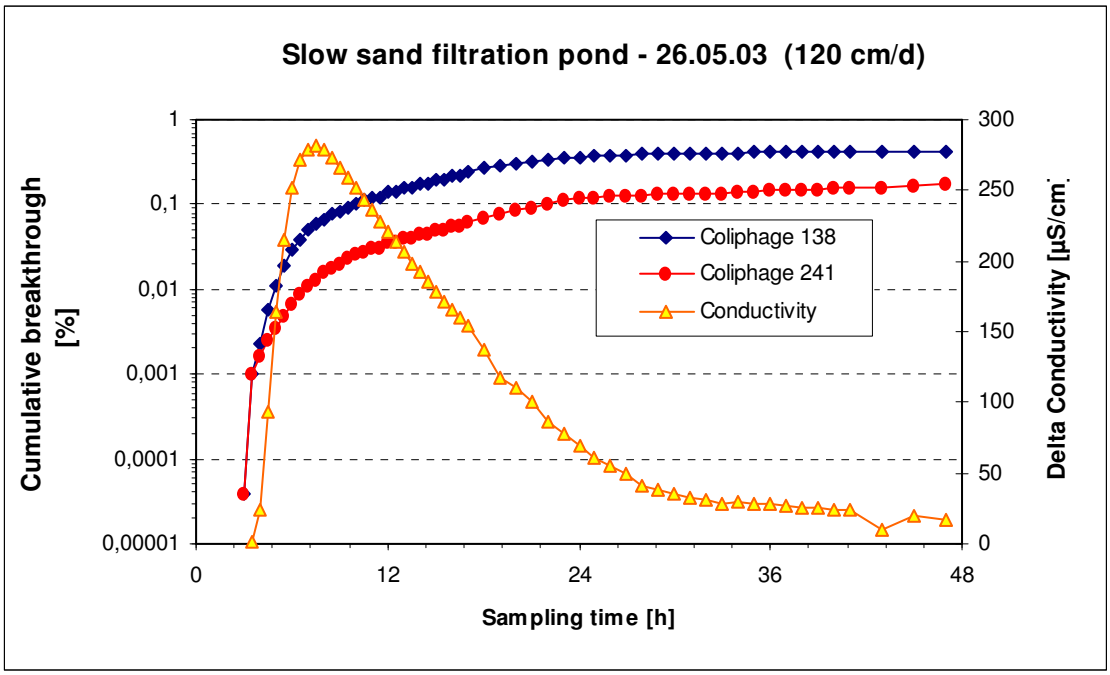


Figure 35: **Cumulative breakthrough of test phages and mobility of tracer salt NaCl in slow sand filtration pond without clogging layer at a pore water velocity of 360 cm/d (3000 L/h).**

Table 51: Breakthrough ratio of F+ phage 138, somatic coliphage 241 and E. coli in slow sand filtration pond at a filtration rate of 120 cm/d (360 cm pore water/d) before and after formation of a clogging layer

time (h)	26.05.03 (see 2.2.1) without clogging layer		17.06.03 (see 2.2.2) with clogging layer		22.11.04 (see 2.2.3) with clogging layer		
	f+ phage 138	Phage 241*	f+ phage 138	Phage 241*	f+ phage 138	Phage 241	E.coli
6	0,0003	0,001	0,0008	0,003	0,0001	0,0001	0,0003
12	0,0014	0,004	0,0013	0,012	0,0005	0,0004	0,0010
24	0,0036	0,012	0,0015	0,017	0,0008	0,0009	0,0014
48	0,0042	0,018	0,0016	0,024	0,0012	0,0018	0,0017
72	0,0042	0,021	0,0016	0,024	0,0014	0,0031	0,0018
96	0,0043	0,023	0,0016	0,024	0,0016	0,0041	0,0019
120	0,0043	0,024	0,0016	0,024	0,0017	0,0055	0,0019
144	0,0043	0,024	0,0016	0,024	0,0018	0,0064	0,0020
168	0,0043	0,024	0,0016	0,024	0,0019	0,0074	0,0020

5.2.2.2 Experiment SSF5 - 17.06.03 (120 cm/d, with clogging layer)

The experiment was repeated with both coliphages after an apparent biomass or clogging layer had formed on the filter surface. The clogging layer was not uniform but patchy with different thickness and structure.

F+ coliphage 138 reached a concentration of about 105 pfu/ml in the reservoir water after inoculation which rapidly declined to 1000 pfu/ml within 12 h (Figure 36). In the initial filtrate samples, concentration of F+phage increased synchronic with conductivity and reached the highest level of 100 pfu/ml after 6 h of operation. Concentration of phages declined gradually to 1 pfu/ml or 10 pfu/100ml after 36 h or 100 h, respectively.

Cumulative breakthrough ratios were calculated as 0,0013 after 12 h and 0,0016 after 60 h which did not change up to the end of the experiment (Figure 37, Table 52). Retention of phage 138 was about 2,79 log units and therewith in the same order of magnitude as in the experiment without clogging layer. A specific retention ratio of 0,035 logs per cm filter length was calculated. Consequently, a similar elimination rate coefficient of 1,2 $log.h^{-1}$ was calculated like in the experiment before formation of an apparent biomass. Modelling of results showed no retardation (R=1) but a moderate elimination rate coefficient of 1,36.h^{-1} (Table 52).

The initial concentration of somatic coliphage 241 in the reservoir water was very low (about 100 pfu/ml). Nevertheless, phages were rapidly observed in the initial filtrate samples. Only low concentrations were detected in the effluent with a maximum of 24 pfu/100 ml in the effluent sample after 7 h of operation. Coliphage 241 was sporadically detected in 100 ml of other filtrate samples up to the end of operation at 180h (Figure 36).

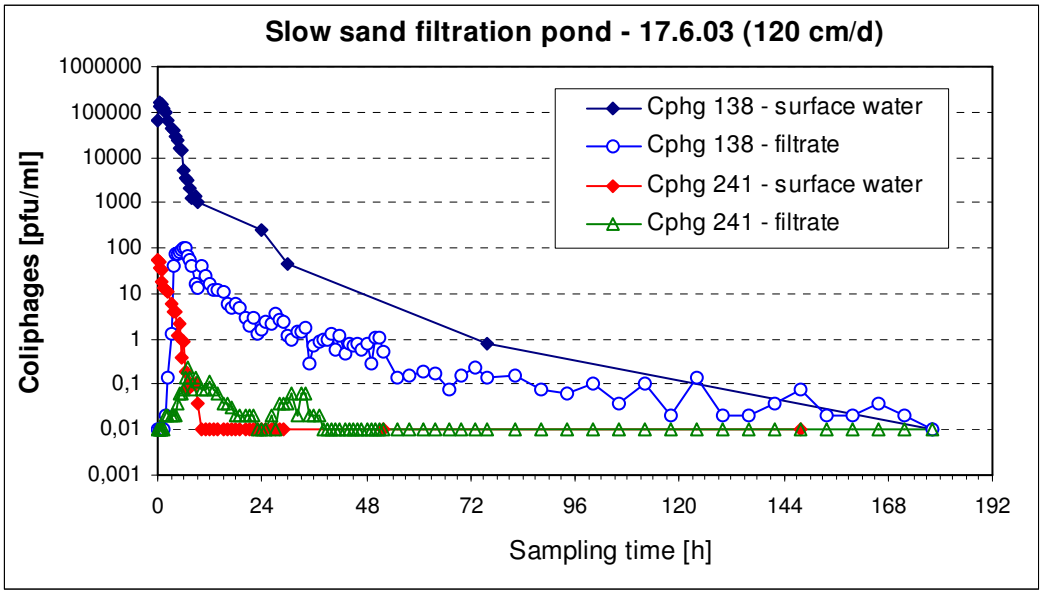


Figure 36: **Concentration of coliphages 138 and 241 in influent and effluent of the filtration pond with clogging layer at a pore water velocity of 360 cm/d.**

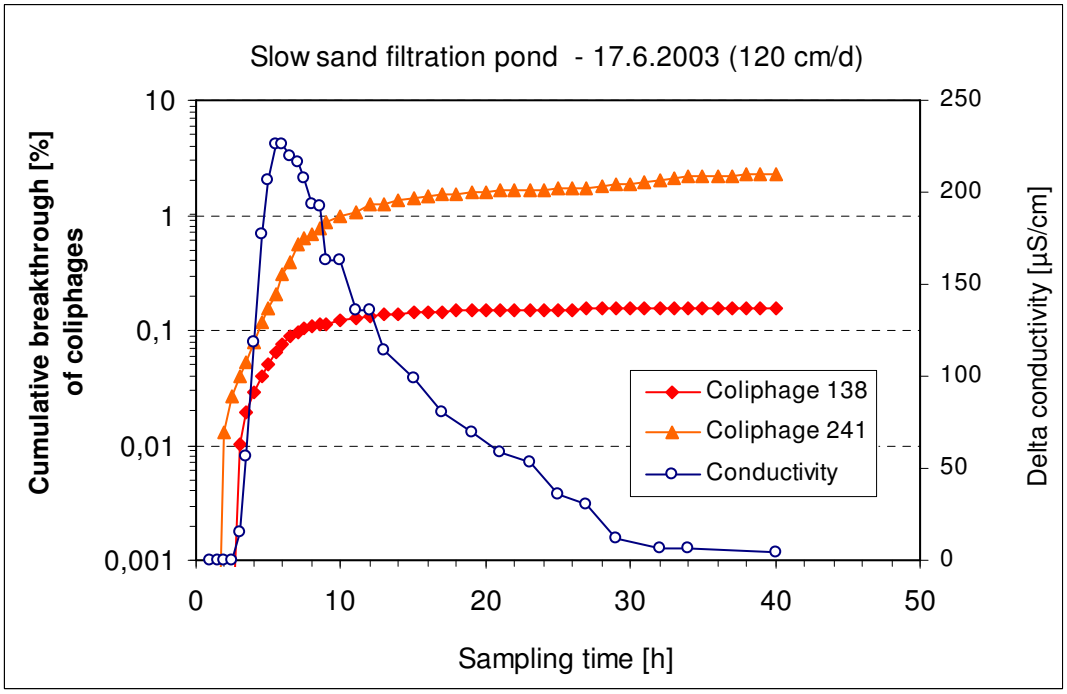


Figure 37: **Cumulative breakthrough of coliphage 138, 241 and mobility of tracer in slow sand filtration pond with clogging layer at a pore water velocity of 360 cm/d.**

Breakthrough ratios of somatic phage 241 were calculated as 0,012 after 12 h and as 0,024 at the end of the experiment after 150 h (Figure 35, Table 51). The same breakthrough ratio of 0,024 was also found in the previous experiment without clogging layer. The specific

retention ratio of 0,02 log/cm and the elimination rate coefficient of 0,70 were also similar to those of the previous experiment (Table 52). Adaptation of results by modelling approaches showed an elimination rate coefficient of 0,81 $log.h^{-1}$ and a retardation factor of 1,3.

Table 52: Retention of test organisms in the sand filtration pond of Marienfelde before (-) and after (+) formation of an apparent biomass at a filtration velocity of 120 cm/d (360 cm pore water/d),)

Test organisms	Date	Clogging layer	Breakthrough ratio (CBTA)	Retention (log)*	Specific retention (log)/cm	λA* (log.h-1)	Retar-dation**	λC** (h-1)
F + phage 138	26.05.03	(-)	0,004	2,4	0,03	1,02		
	17.06.03	(+)	0,002	2,8	0,03	1,20	1,0	1,36
	22.11.04	(+)	0,002	2,7	0,03	1,16		
somatic coliphage 241	26.05.03	(-)	0,024	1,6	0,02	0,70		
	17.06.03	(+)	0,024	1,6	0,02	0,70	1,3	0,81
	22.11.04	(+)	0,012	1,9	0,02	0,83		
E. coli	22.11.04	(+)	0,002	3,3	0,04	1,44		

* Calculation according to integrative balancing total virus concentrations in surface water and filtrate samples (equation 3),
** calculation according modelling approaches, λ: elimination rate coefficient

5.2.2.3 Experiment SSF8 – 22.11.2004 (120 cm/d, with clogging layer)

Coliphages and bacteria were applied simultaneously in this experiment with a filtration velocity of 120 cm/d or 360 cm pore water per day and the results were compare to the results of previous experiments at the same flow rate (see 5.2.1.1, 5.2.2.2). An apparent, thick, and uniform biomass had developed on the sandy soil surface.

F+ coliphage 138 reached an initial concentration of $1,27x10^6$ pfu/ml and decreased slowly to $4,5x10^4$ pfu/ml after 24 h, $1,8x10^3$ pfu/ml after 72 h, and 1 pfu/ml after 192 h (Figure 38). In the effluent, F+ phages appeared in filtrate sample after 3 h at a concentration of 14 pfu/100 ml before the tracer salt NaCl was detectable. Concentrations of phages in filtrate samples increased to a maximum value of 1250 pfu/ml after 6 h of operation and remained relatively long time at a high level similar to steady state conditions. After 72 of operation, concentration of F+ phage 138 declined to 100 pfu/ml. Phages were still detectable at the end of operation after 384 h in concentrations of approximately 10 pfu/ml.

Despite relatively high initial concentrations of F+ phage 138 in the reservoir water, cumulative breakthrough was low increasing from 0,0005 after 12 h, to 0,0009 after 24 h, and to 0,0016 after 10 days (Table 51, Figure 39). Similar breakthrough ratios were found in the

experiment in June 2003 (see 5.2.2.2, Table 51). Retention of F+ phage 138 was about 2,7 log units, corresponding to a specific retention rate of 0,034 log/cm filter path (Table 52).

Elimination rate coefficient (λ) was calculated as 1,16 log unit.h^{-1} which was also similar to that of the experiment in June 2003 (Table 52).

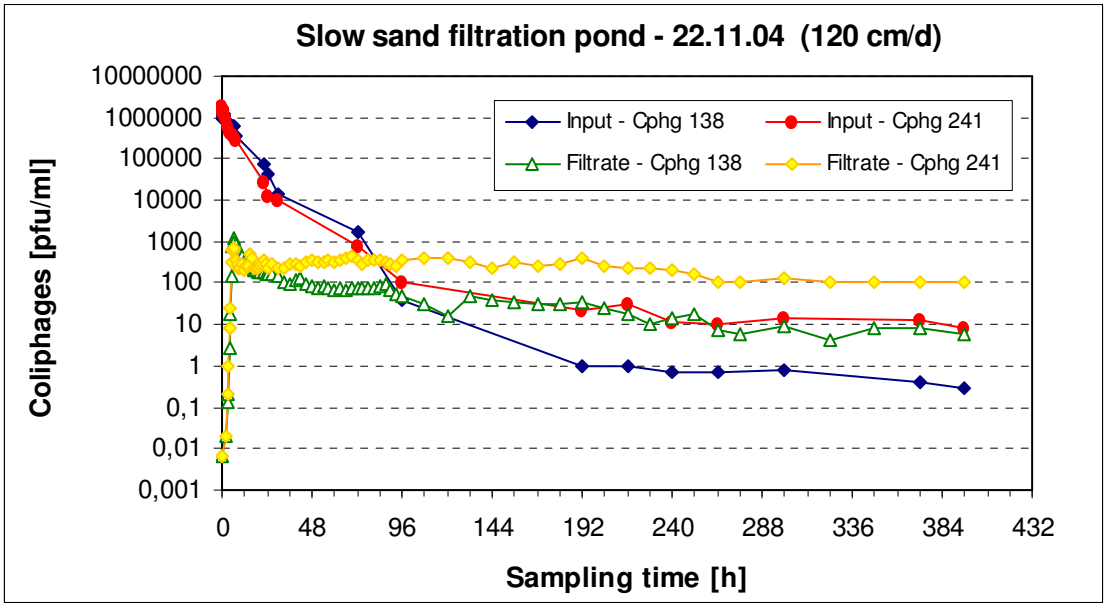


Figure 38: Concentration of coliphages 138 and 241 in influent and effluent of the filtration pond with clogging layer at a pore water velocity of 360 cm/d

Concentration of somatic phage 241 in the reservoir water was 1,9x10^6 pfu/ml directly after inoculation declining to 3,4x10^5 pfu/ml after 6 h, 1,3x10^4 pfu/ml after 24h and 10^2 pfu/ml after 96h of operation (Figure 38). Phage 241 was still detectable in pond water after 10 -18 days at concentrations of about 10 pfu/ml.

Effluent samples were already positive after 3 h of filtration. Highest concentration (700 pfu/ml) in the filtrate was found after an operation time of 3,5 h. Concentrations of phage 241 in all other filtrate samples varied between 210 and 360 pfu/ml within the next 10 days and marginally declined to 105 pfu/ml at the end of experiment after 396 h.

Cumulative breakthrough ratio increased slightly from 0,0004 within 6 h to 0,0008 after 12 h, and 0,005 after 120 h. After 15 d of operation the cumulative breakthrough ratio was 0,012 (Figure 39).

Retention of somatic phage 241 was 2 log units resulting in a specific retention rate of 0,024 log/cm filter path. Elimination rate coefficient was calculated as 0,83 log.h-1 which was slightly higher than those of other experiments at the same filtration velocity (Table 52).

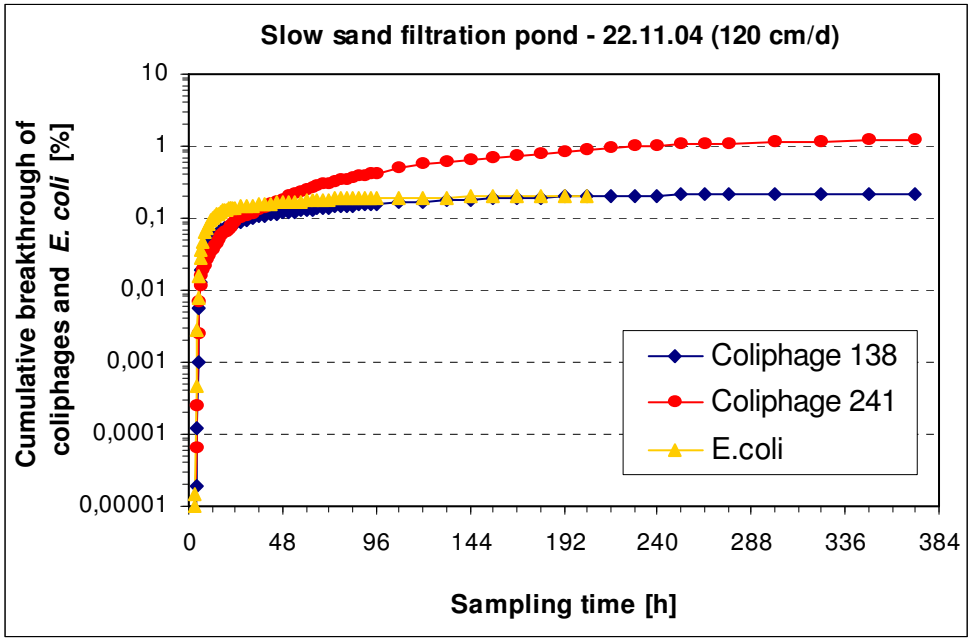


Figure 39: **Cumulative breakthrough of the test coliphages and E. coli in slow sand filtration pond with clogging layer at a pore water velocity of 360 cm/d (3000 L/h)**

Initial concentration of E. coli was 3,5.106 mpn/100 ml in the reservoir water which rapidly decreased to 3,8.105 mpn/100 ml after 6 h, and 575 mpn/100 ml after 24. E. coli was sporadically detected in reservoir water after 96h (Figure 37). Synchronic with other test organisms, E. coli was found in the effluent sample collected after 3 h of operation. Concentrations of E. coli were highest (2446 mpn/100 ml) already after 6h and decreased to 122 mpn/100 ml within 24 h. Concentrations remained at a stable level between 10 – 100 mpn/100ml during the further run of the experiment (Figure 40).

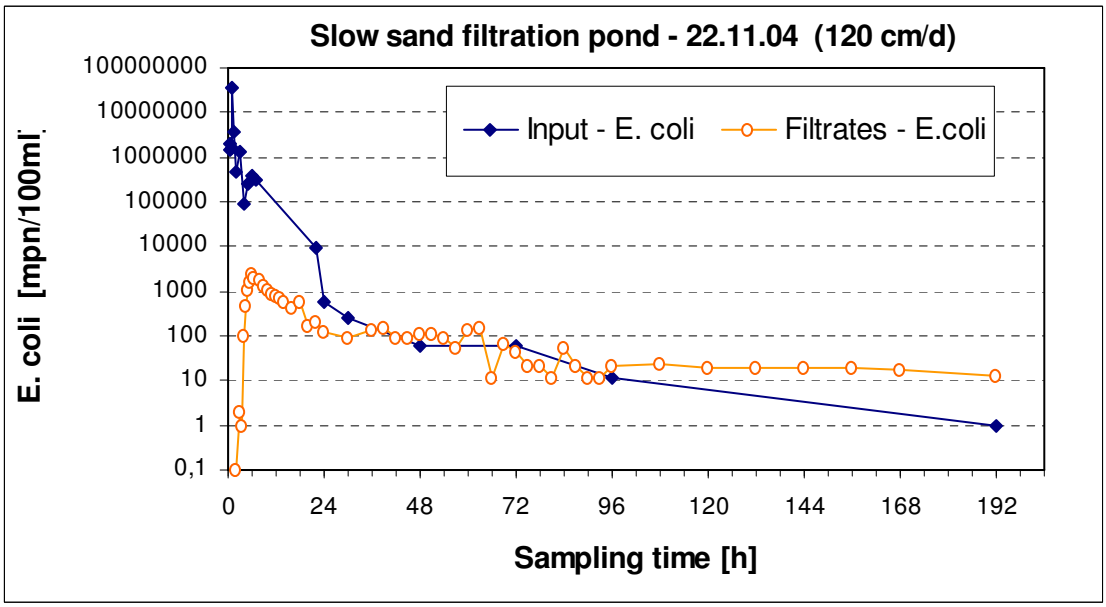


Figure 40: **Concentration of E. coli in influent and effluent of the filtration pond with clogging layer at a flow rate of 360 cm pore water column per day**

Despite early and continuous breakthrough, retention of E. coli in the slow sand filter was relatively high. Cumulative breakthrough ratios were about 0,001 at 12 and 24 h. Cumulative breakthrough ratio increased to 0,002 after 240 h of operation (Figure 39). Based on the relative high retention of 3,3 logs, specific retention capacity of filter pond was calculated as 0,042 log/cm corresponding to a relative high elimination rate coefficient of 1,44 log.h-1 (Table 52). The initial concentration of enterococci in the reservoir water was too low to deduct any retention behaviour.

5.2.3 Experiment SSF6 – 19.11.2003 (60 cm/d, with clogging layer)

Indicator bacteria and coliphages could be applied after completion of the experiments of other working groups at the end of the year 2003. The surface of the sandy soil was covered with a thick and homogenous biomass which reduced the filtrate volume significantly. The pore water velocity was set to 60 cm/d corresponding to a pore velocity of 180 cm/d.

F+phage 138 reached an initial concentration of 2.105 pfu/ml which declined one log unit after 5 h, and remained at a relatively stable level during the next two days. Concentration in reservoir water was 6,2.104 pfu/ml after 48 h, and 2 pfu/ml after 72 h Figure 42). Relatively low concentrations of F+ phage 138 were found in filtrate samples. However, they appeared in the initial filtrate samples. Concentrations reached a maximum of 6 pfu/ml after 18 h and slightly decreased to 1 pfu/ml after 48 h (Figure 41).

Retention of phage 138 was high. Cumulative breakthrough ratio was about 0,0001 after 24 h and at the end of the experiment after 150 h (Figure 42). Based on the total retention of 3,8 log units, a specific retention ratio resulted in a value of 0,05 logs/cm filter length (Table 53) Elimination rate coefficient was calculated as 0,82 log units.h-1 which was clearly less than at a flow rate of 120 cm/d. Modelling approach also showed a similar elimination rate of 0,89.h-1 and a retardation factor of 1,3 (Table 53).

Table 53: Retention of test organisms in the sand filtration pond with a filtration velocity of 60 cm/d (180 cm pore water/d),

Test organisms	Breakthrough ratio (CBTA)	Retention (log)	Specific retention (log)/cm	λA * (log.h-1)	Retardation (R) **	λC** (h-1)
F+ phage 138	0,0001	3,8	0,05	0,82	1,3	0,89
somatic phage 241	0,004	2,4	0,03	0,53	1,1	0,68
E. coli	0,0001	4	0,05	0,83		

[λ : elimination rate coefficient, *) calculation according to integrative balancing total virus concentrations in surface water and filtrate samples (equation 3),
** calculation according modelling approaches]

Initial concentrations of somatic phage 241 were low (about 6 x 10^2 pfu/ml) after inoculation into the reservoir and further decreased to 14 pfu/ml after 48 h (Figure 41). Phages rapidly appeared in the initial filtrate samples and reached a maximum concentration of 18 pfu/100ml in the effluent after 12 h of operation. Concentrations ranged from 1 to 10 pfu/100ml during the further run of the experiment.

A low cumulative breakthrough of phage 241 was calculated with 0,0002 after 12 h that slightly increased to 0,004 at the end of experiment after 150 h (Figure 42). High retention of phage 241 (2,4 logs) resulted in a specific retention ratio of 0,03 logs/cm.

In contrast to the low breakthrough ratio a moderate elimination rate coefficient of 0,53 log units.h^{-1} was determined. Modelling approaches also showed an elimination rate coefficient of 0,68.h^{-1} and retardation factor of 1,1.

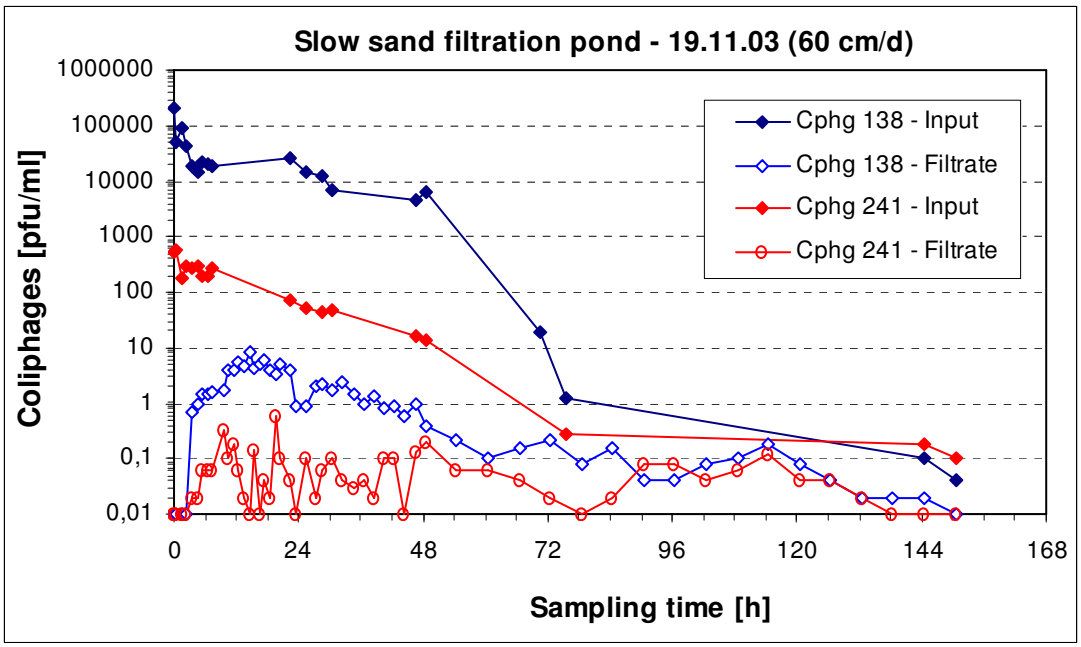


Figure 41: **Concentration of F+ phage 138 and somatic coliphage 241 in influent and effluent of the filtration pond with clogging layer at a pore water velocity of 180 cm/d (1500 L/h)**

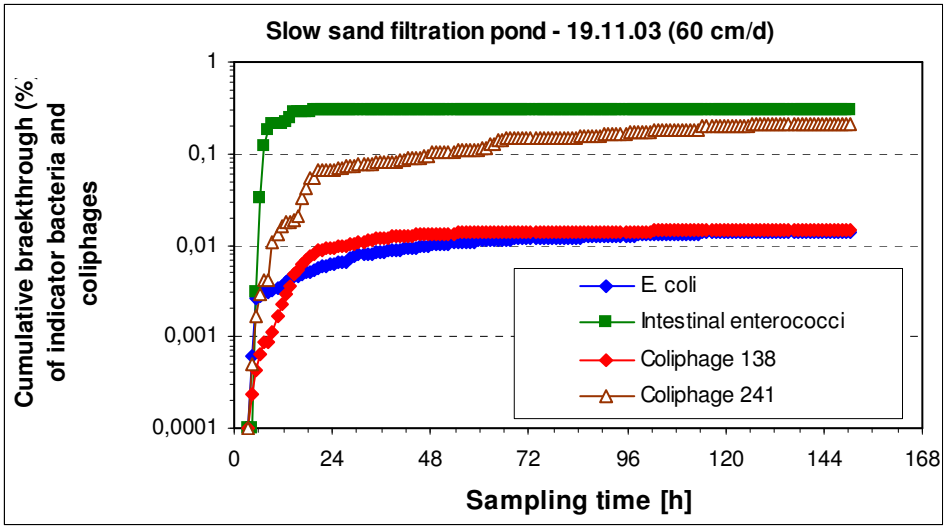


Figure 42: **Cumulative breakthrough of test organisms by slow sand filtration with clogging layer at a pore water velocity of 180 cm/d (1500 L/h).**

The initial concentration of E. coli in pond water was about $4{,}4 \times 10^7$ mpn/100 ml and decreased to $5{,}2 \times 10^5$ mpn/100 ml within 8 h of percolation. E. coli concentrations further decreased to 382 mpn/100ml or 42 mpn/100ml after 12 h or 24h, respectively. E. coli was already detected in the filtrate sample taken after 4 h despite a calculated percolation time of 9 h for water at this low flow rate to reach the effluent. Following this rapid breakthrough, the highest concentration of E. coli (240 mpn/100 ml) was detected after 5 h. Concentrations of E. coli varied between 15 and 75 mpn/100 ml in other effluent fractions collected within a filtration time of 48 h. After 60 h it was detected in all filtrate samples in concentrations below 10 mpn/100 ml (Figure 43).

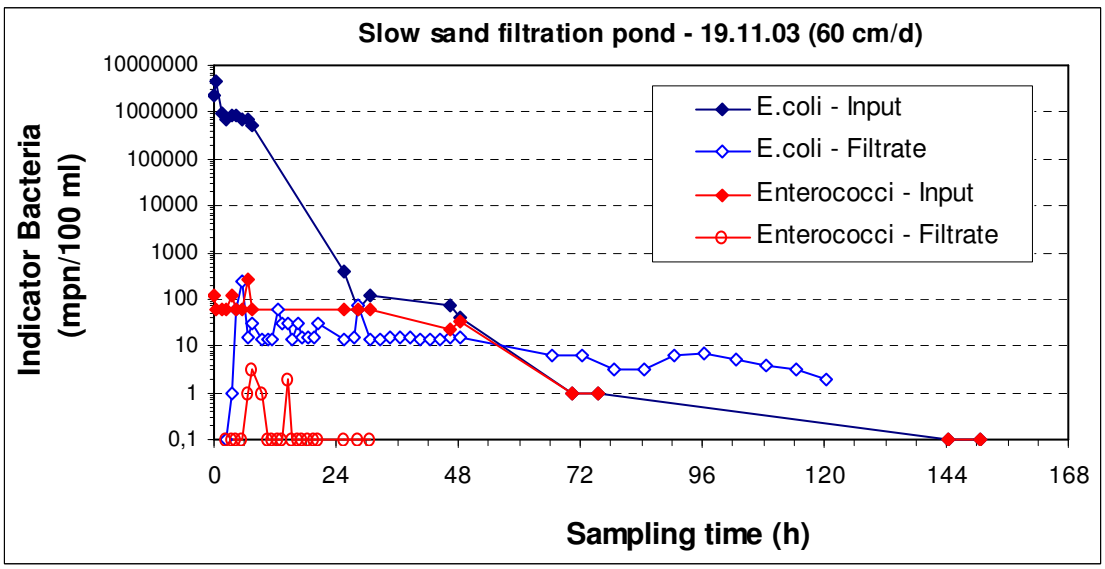


Figure 43: **Concentration of E. coli and Enterococcus faecium. in influent and effluent of the filtration pond with clogging layer at a pore water velocity of 180 cm/d (1500 L/h)**

Cumulative breakthrough ratio was 0,00004 within a filtration time of 12 h and increased to 0,0001 at the end of experiment after 150 h (Figure 42). The high retention of E.coli at this flow rate (4 log units) resulted in a high specific retention ratio of 0,05 log/cm filter length. Elimination rate coefficient reached 0,83 log unit.h^{-1} (Table 53).

After inoculation of filter pond, the concentration of Enterococcus faecium was extremely low (about 100 mpn/100 ml). After 6 h of operation it was no longer detectable in the reservoir water (Figure 43). As enterococci were only sporadically detected in filtrate samples, a reliable estimation of their removal or retention could not be carried out.

5.2.4 Biofilm Investigations before and after Experiment SSF8

Total bacterial cell counts were determined by DAPI staining (see 1). Counts in the biofilms varied between 1,8 x 10^5 and 6,3 x ^{106} cells/cm2 slide. A variety of different cell morphologies was detected in the biofilm; a selection is shown in Figure 44 (A-F).

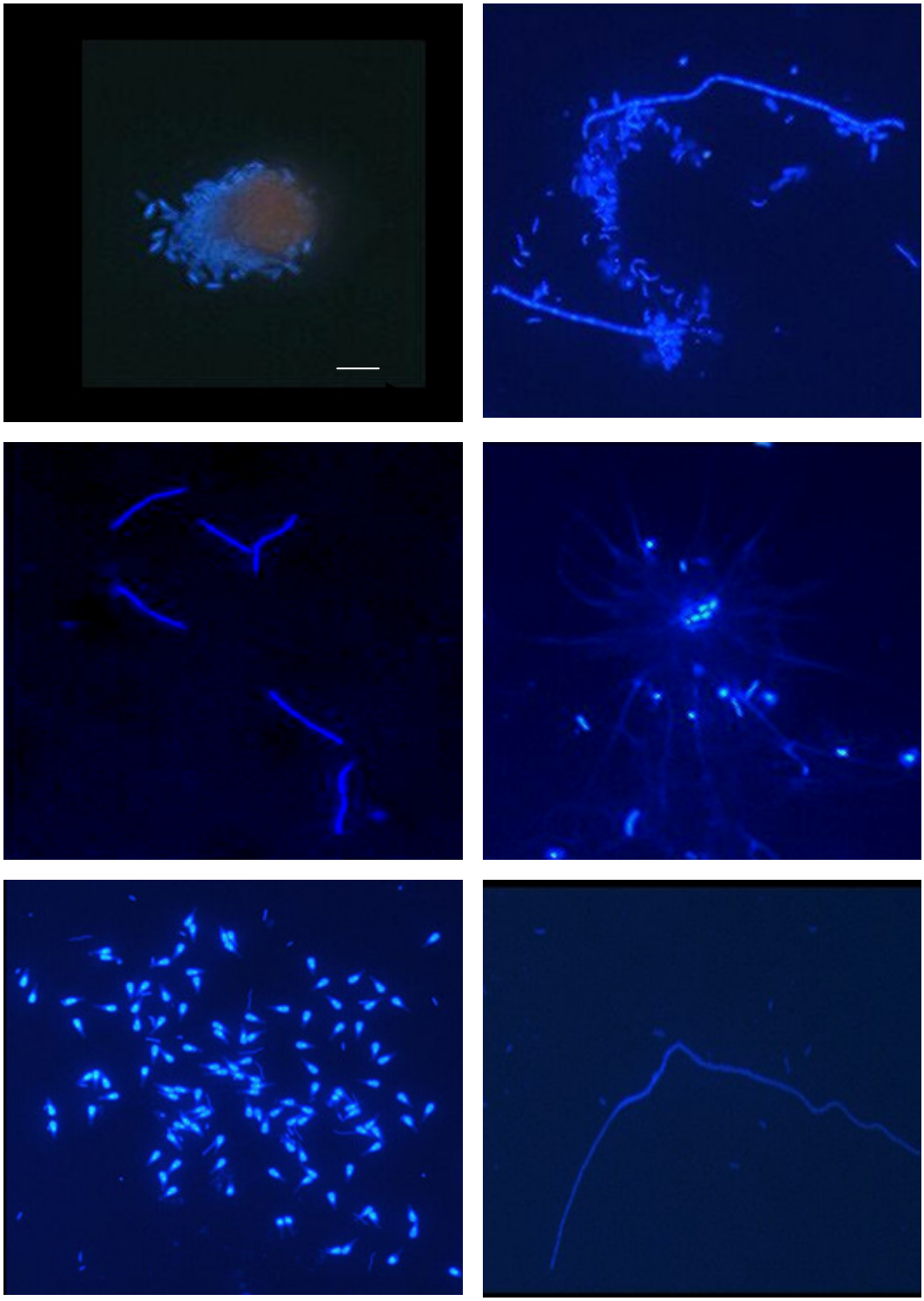


Figure 44: **Various cell morphologies in a four week old biofilm at site one as detected by DAPI staining. Scale bar in (A) equals 5µm and belongs to all images. (A) microcolony of small rod shaped bacteria attached to an alga body. (B) single rod shaped bacteria and long chains of rod shaped bacteria. (C) slightly curved bacteria, the dominant type in the two, four and five week old biofilms in 10 cm and 30 cm depth. (D) a remarkable microcolony with a center of encapsulated bacteria with radial branches of a material around it. (E) bacterial cells composed of a "head" and a "tail". (F) thin and long filamentous bacterium.**

Cell counts on the slides increased with increasing age of the biofilm and decreased with sampling depth. Figure 45 shows the results of one sampling site. Cell counts in the young, two week old biofilm were 2 x 10^6 cells/cm2 at 10 cm depth decreasing to 2 x 10^5 cells/cm2 at 30 cm and 60 cm depth. Cell counts of the four week old biofilm were higher than in the younger biofilm at 10 cm and 30 cm (about 3 x 10^6 cells/cm2). In 60 cm depth low concentrations were detected even after four weeks.

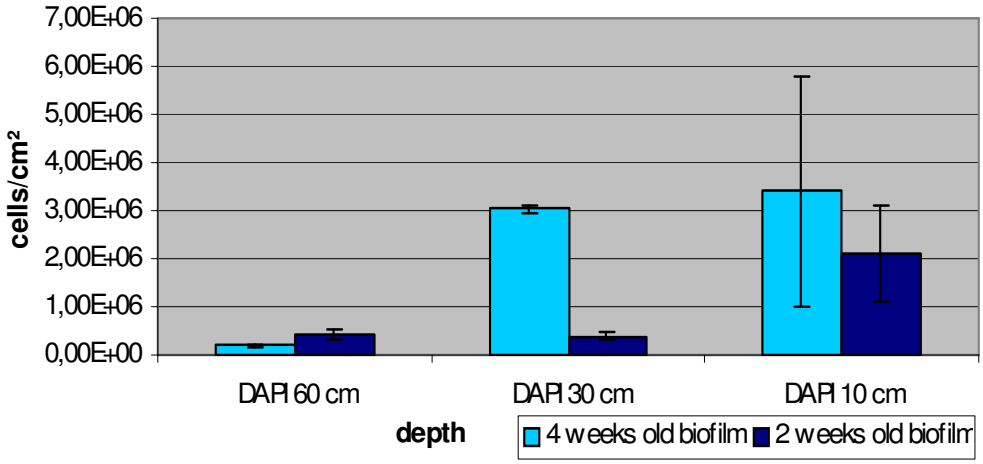


Figure 45: DAPI counts of biofilm bacteria at site one

No big differences in cell counts were found between the three sampling sites. Differences were highest at 10 cm depth were cell counts varied between 5 x 10^5 cells/cm2x and 2,5 x 10^6 cells/cm2 (Figure 46). At all three sampling sites cell counts decreased with depth to 3,5 x 10^5 cells/cm2 in 30 cm and even lower counts in 60 cm. Slides at 60 cm depth at site three were lost during the experiment because of a technical defect and, therefore, no values could be achieved for this point.

Phylogenetic characterization of the bacterial biofilm population (Figure 51) was performed in addition to DAPI total cell counts. The bacterial biofilm population was characterized with regard to (i) depth profile within 10 cm, 30 cm and 60 cm depth; (ii) biofilm age with two, four and seven week old biofilms and (iii) three different sites within the slow sand filter pond.

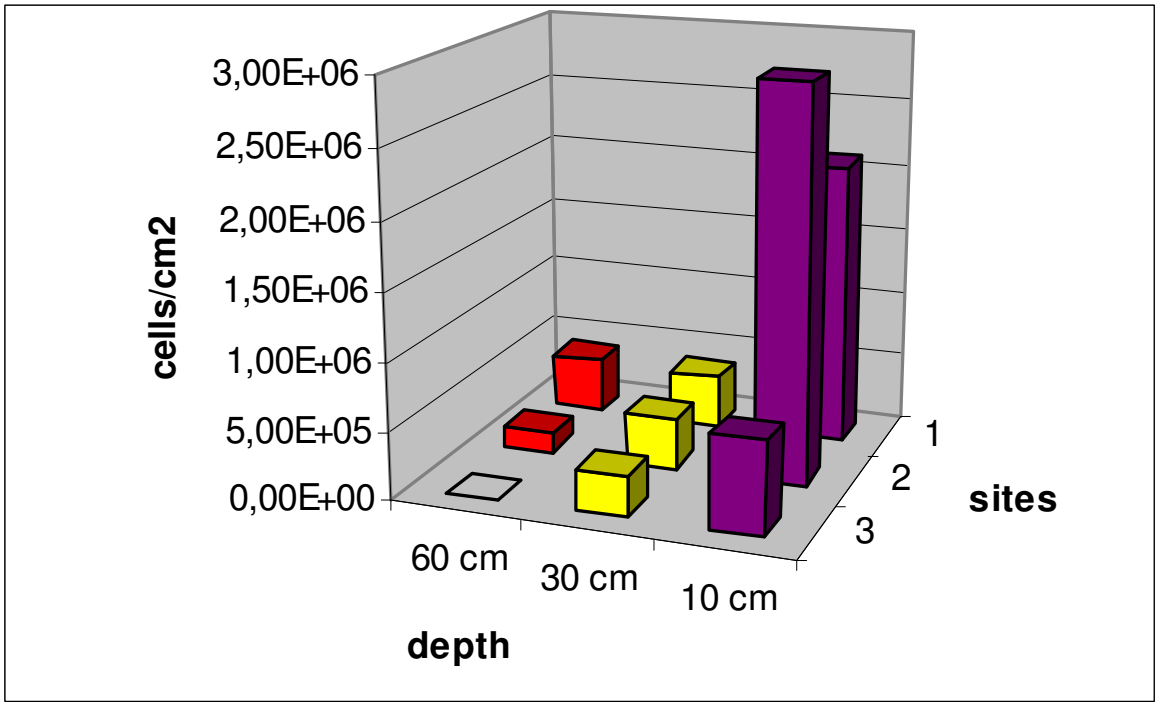


Figure 46 **DAPI counts of biofilm bacteria at different sites**

A high percentage of total cell counts was detectable with the Eubacteria probe. As exemplarily shown in a four week old biofilm (Figure 47) about 60 % of total cell counts in 10 cm and 30 cm depth and 40 % in 60 cm depth were detected. Within the Proteobacteria the alpha-group was dominant in the two upper layers with remarkable 22 % of total cell counts in 30 cm depth. In contrast to this, the alpha-Proteobacteria nearly disappeared in the deepest layer. Beta-Proteobacteria were only rarely detected in the top layer in contrast to 13 % in 30 cm depth and 8 % in 60 cm depth. Gamma-Proteobacteria were nearly equally distributed within the depth profile with values ranging between 3 % and 9 % of total cell counts. The group of Cytophaga-Flavobacteria was detected in the same range as the gamma-Proteobacteria with their highest amount of 9 % in the 30 cm depth. Planctomycetales were only detected in the top layer in low amounts.

The phylogenetic composition of the two week old biofilms was similar to the four week old biofilms (Figure 48). Alpa- and beta-Proteobacteria were, however, dominant in the 10 cm layer in the young biofilm and in the 30 cm layer in the older biofilm.

The phylogenetic characterization of two week old biofilms at the three different sites in the slow sand filter pond revealed a great diversity of the detected bacterial groups (Figure 49).

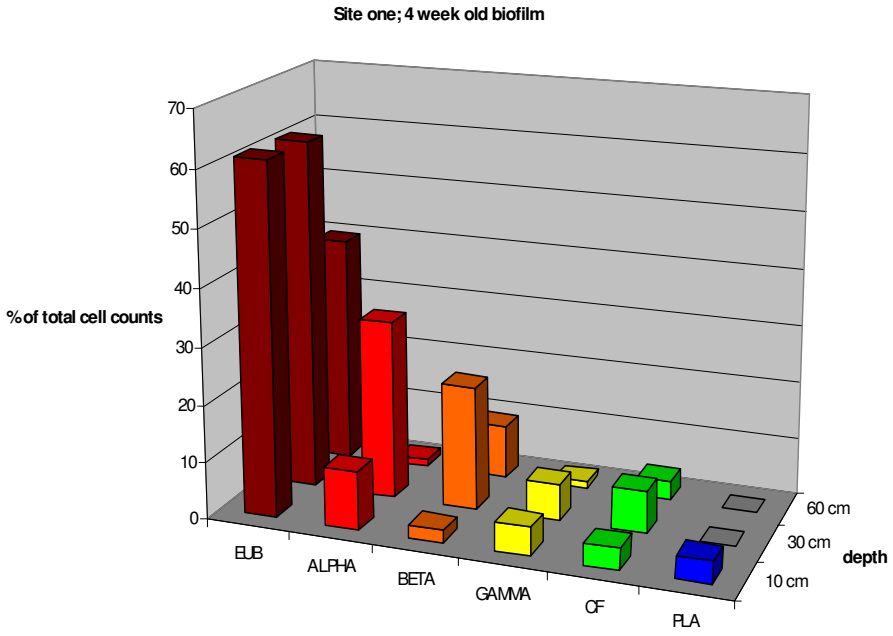


Figure 47: **Phylogenetic characterization of four week old biofilms at site one at different depths**

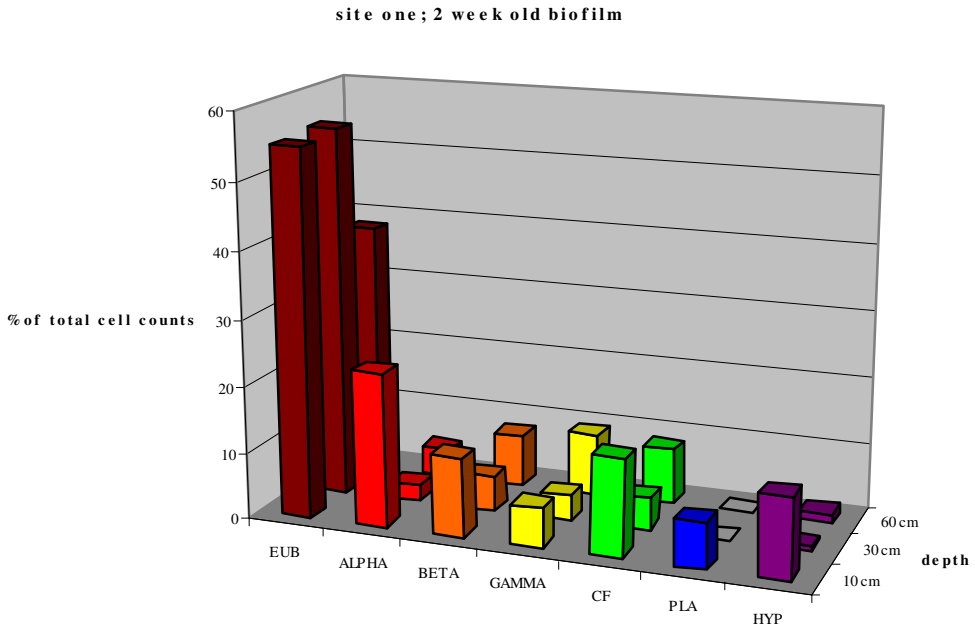


Figure 48: **Phylogenetic characterization of two week old biofilms at site one at different depth**

Eubacteria were nearly equally distributed at all three sites and depths ranging from 30 % to 60% of total cell counts. Within the Eubacteria the alpha-Proteobacteria were the dominant group at all three sites in 10 cm depth but not in deeper layers. In contrast to this the amount of beta-Proteobacteria remained constant over depth. Gamma-Proteobacteria were only rarely present at all three sites with up to 5 % of total cell counts. Cytophaga-Flavobacteria showed their highest amount at site one in all depths. Planctomycetales were only detected in the 10 cm layer at site one and two.

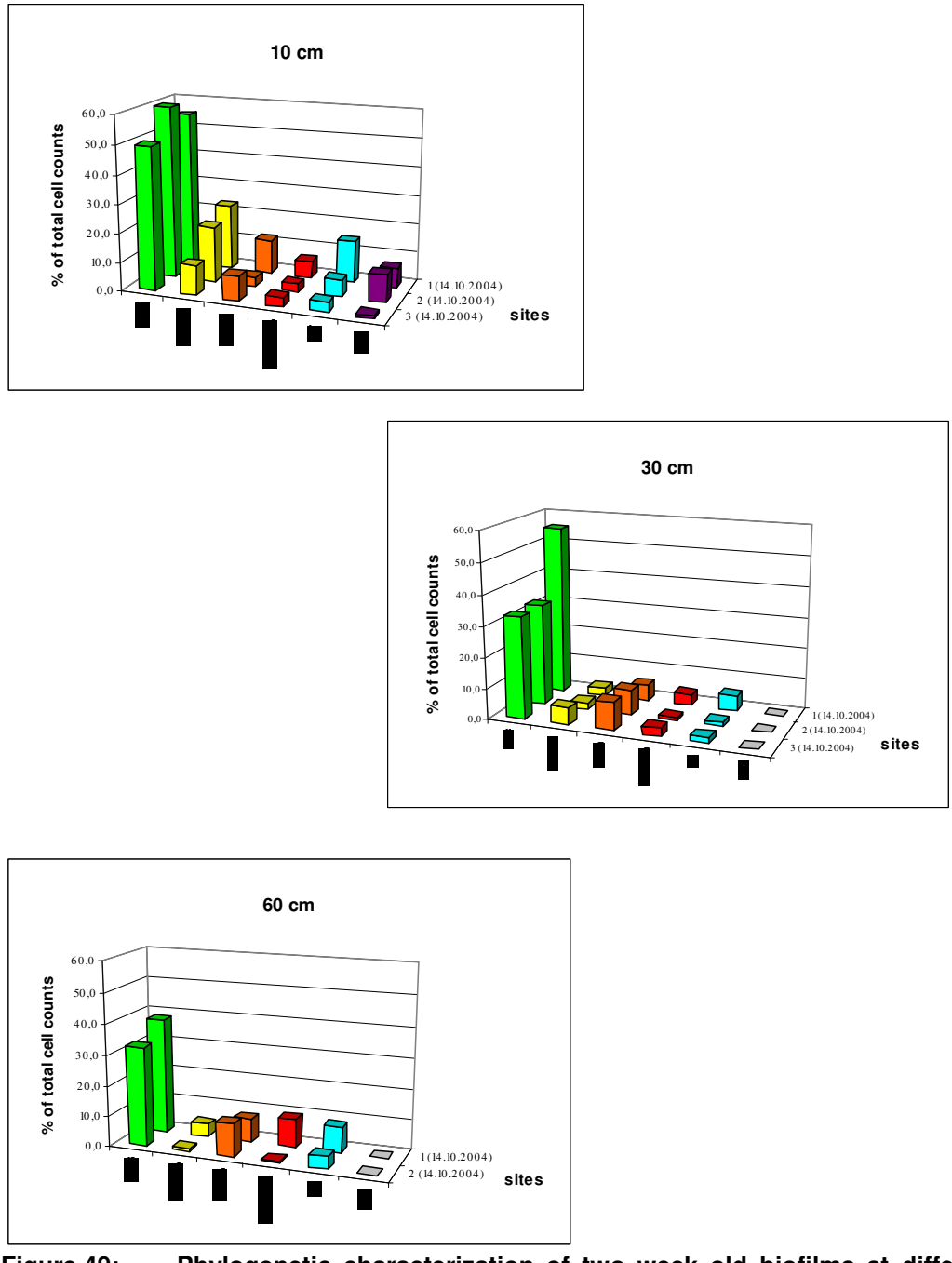


Figure 49: **Phylogenetic characterization of two week old biofilms at different sites and different depths**

A two and a five week old biofilm were hybridized with various probes targeting specific organisms within the Proteobacteria. Interestingly, bacteria hybridizing with probe HYPHO1241 in the dominant alpha-group accounted for 12 % of total cell counts in the two weeks old biofilm (see Figure 48). These bacteria had not the typical cell morphology of Hyphomicrobium but were slightly curved bacteria as shown in Figure 44.

Within the beta-Proteobacteria the amounts of Aquabacterium and those bacteria which hybridized with probe ISO21, a dominant bacterial member in the river Elbe, were nearly the same with 2 % -3 % of total cell counts. Within the gamma-Proteobacteria the specific probe for Aeromonadaceae detected an amount 2 % of total cell counts, whereas probe FLAVO1004 showed no signal in any depth of the biofilm (data not shown). After experiment SSF8, the presence of Enterobacteriaceae in the biofilms – the slides had been introduced in the filter 4 weeks prior to the experiment - was tested. Two weeks after the beginning of the experiment glass slides with biofilms were sampled and hybridized with probe D, specific for members of the Enterobacteriaceae (Figure 50). No signals were detected at site one at any depth. At site two 1 % of total cell counts gave positive signals with this sample in 10 cm depth and even remarkable 3 % of all DAPI stained cells hybridized with sample D at site three in 10 cm depth. No signals were detected in the deeper layers of the filter.

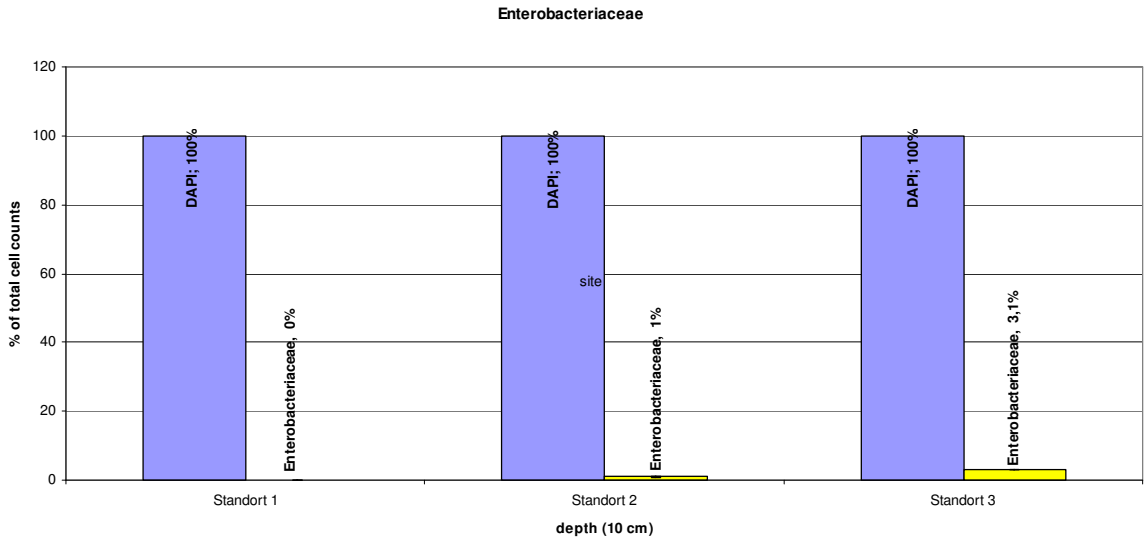


Figure 50: Detection of Enterobacteriaceae at the three different sites in the slow sand filter pond after deploying strain E.coli A3 and a retention period of two weeks

The amount of actively respiring bacteria in the biofilm (Figure 52A) was relatively low and decreased with increasing depth from 20 % of total cell counts in 10 cm depth to 9 % in 60 cm depth. No significant differences could be detected for the use of pure CTC or CTC with carbon supplementation due to high standard deviations. LIVE/DEAD staining of biofilm bacteria (Figure 53) revealed that the ratio of live and dead bacteria were similar in all biofilms examined. In 10 cm depth about 55 % of all bacteria were detected as living and 45 % were detected as dead cells. In 30 cm depth 65 % were detected as living cells and 35 % as dead. In the deepest layer of 60 cm live and dead cells were nearly equally distributed.

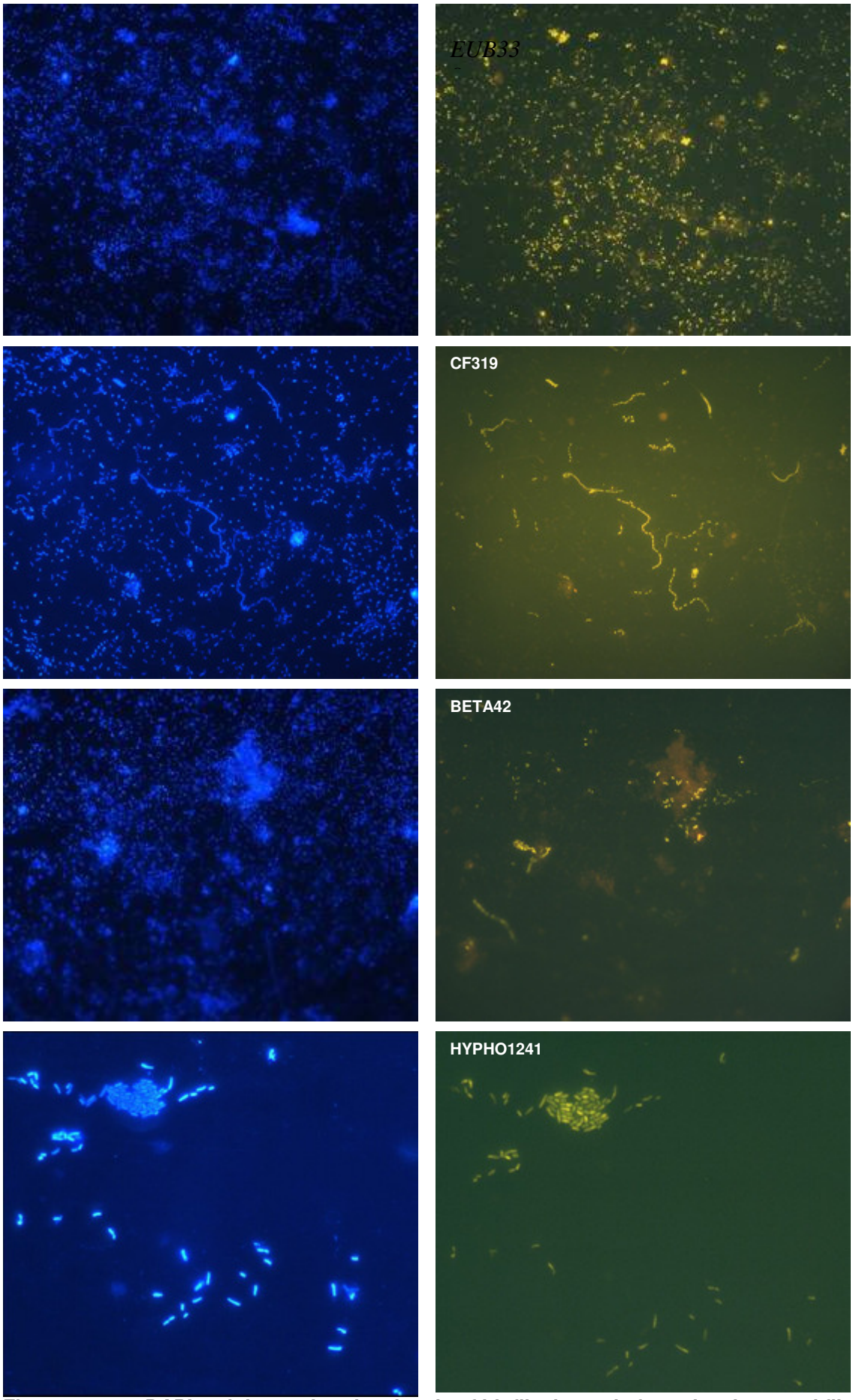
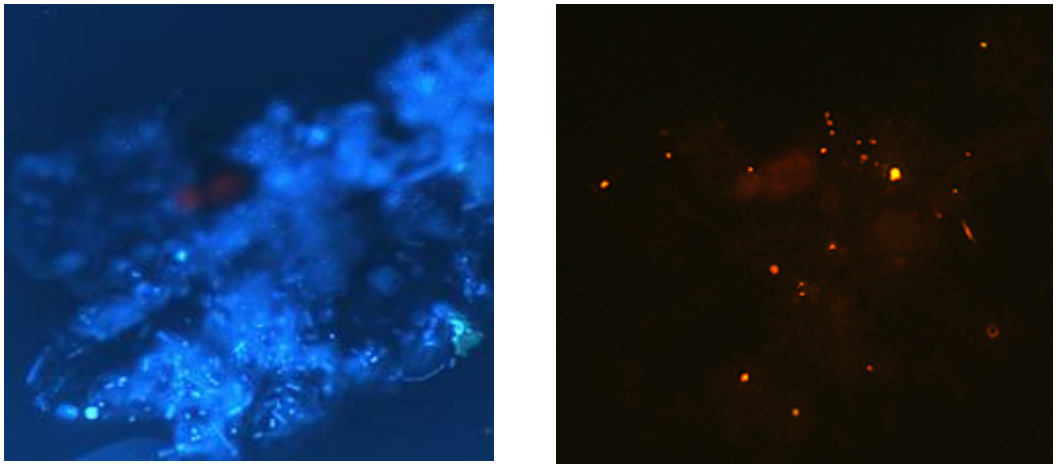


Figure 51: DAPI staining and probe signals of biofilm bacteria from the slow sand filter



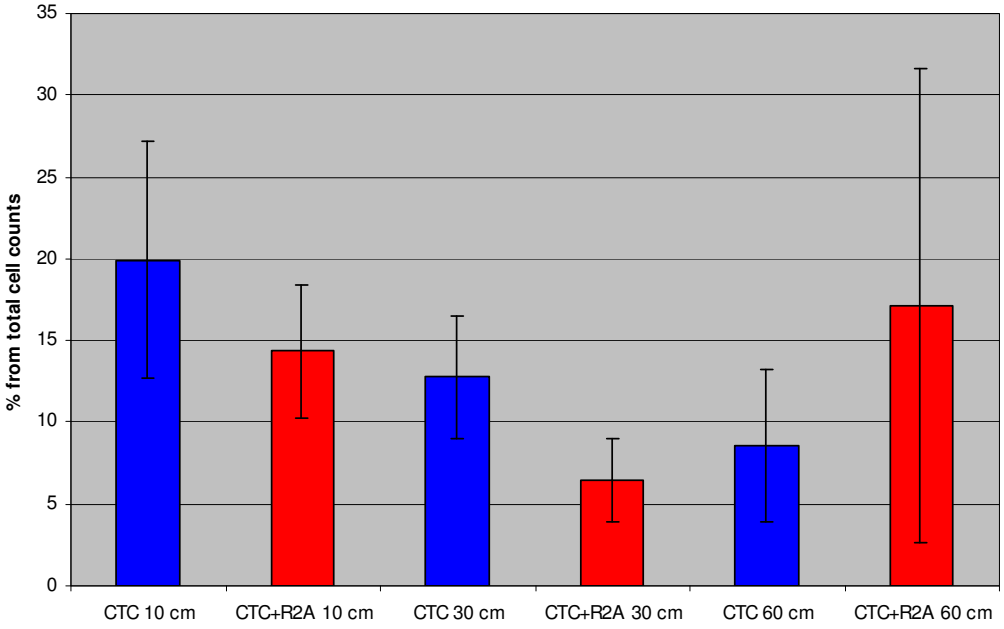


Figure 52: A: Epifluorescence photomicrographs of biofilm bacteria on glass slides in the slow sand filter pond (1) bacterial cells stained with DAPI (2) corresponding micrograph with CTC signals of active cells B. Percentage of actively respiring cells after incubation with 4mM CTC (blue) and 4mM CTC plus R2A medium (red)

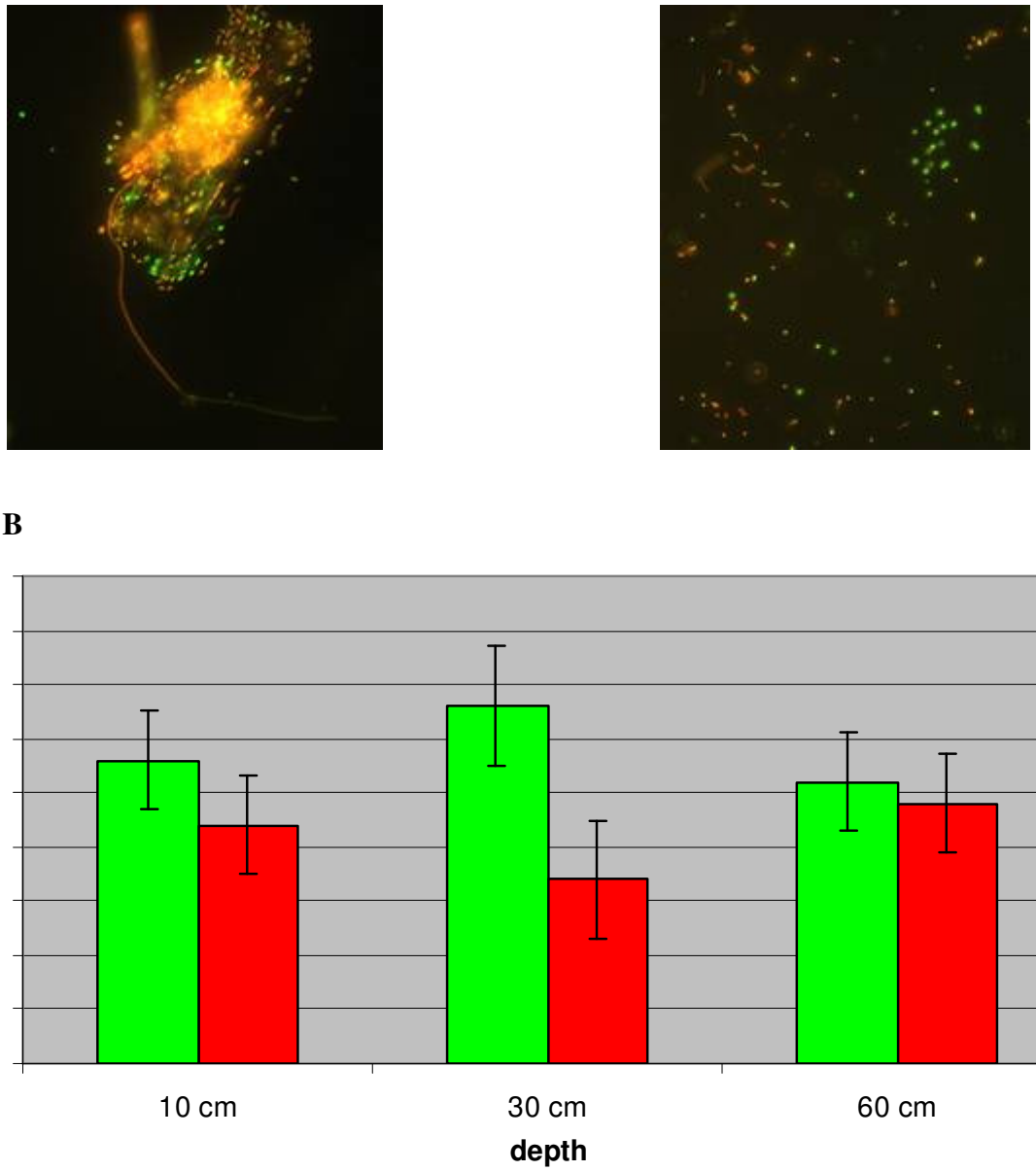


Figure 53 **A: Epifluorescence photomicrographs of biofilm bacteria on glass slides in the slow sand filter pond after LIVE/DEAD staining (1) accumulation of different bacterial cells (2) single cells within the biofilm: green: live cells; red: dead cells. B: Percentage of live and dead cells in the biofilm after Live/Dead staining: green: live cells; red: dead cells**

6 Enclosure experiments

Retention of coliphages and indicator bacteria by slow sand filtration in the enclosure

6.1 Material and Methods

6.1.1 Test organisms.

Both test coliphages were isolated from the Teltow canal discharging secondary effluent and run off water in Berlin. For isolation, cultivation and detection of somatic coliphage 241, the host bacterium E. coli WG5 was used as described in ISO 10705-2 (2000b). E. coli K13 was used as host for the phage 138 as described in EPA-methods 1601 (2001).

Detection limit was 1 plaque forming unit (pfu)/ 100 ml for both test coliphages.

The environmental E. coli strain A3 was obtained from the Department of Environmental Microbiology of the Technical University Berlin (Prof. Dr. Szewzyk). Enterococcus faecalis strain Teltow 11 was isolated from the Teltow canal. Detection and enumeration of E. coli and E. faecalis in water samples were carried out according to DIN EN ISO 9308-3, DIN EN ISO 7899-1 and DIN EN ISO 7899-2. Detection limit was 1-15 colony forming units (cfu)/100 ml or most probable number (mpn)/100 ml depending on the method used. All test organisms were selected for high survival potential in aquatic environments.

6.1.2 Enclosures.

Enclosures were cylindrical sand filtration units with a length of 200 cm and a diameter of 120 cm. Three enclosures were installed in an open infiltration pond and were filled from bottom to top with 0,3 m of gravel and 1 m of filter sand leaving about 0.8 m depth of water reservoir at the top. The effective (d_{10}) and median grain size of the sand was 0.28 mm and 0.7 mm, respectively. The d_{60}/d_{10} ratio was 3.28. The sand was, therefore, not considered uniform (ratio > 2, Clement, 2002). The porosity of the sand was measured to be 0.335. In Enclosure I, sampling sites were located at horizontal drain tubes at 20, 40, 60, and 80 cm depth as well as at the outlet at 100 cm depth (Figure 54 in NASRI Report 5, chapter 9.2). Enclosure II had three sampling sites at 40 cm, at 80 cm depth, and at the outlet tube at 100 cm depth. Sampling in Enclosure III was carried out only from the outlet tube at 100 cm depth. The water work Marienfelde regularly delivers ground water to all filtration ponds of the research area that was also used for filling and continuous percolation of the enclosures. By means of a hydraulic gradient, surface water in the reservoir of enclosure was maintained at a level of 70 cm corresponding to a volume of 500 L. The outlet of the enclosure had to be connected to a suction pump to regulate the filtration velocity to 50 L/h corresponding to 360 cm pore water per day (Vp) or 120 cm water column per day (Vf). Theoretical elapsed time of water during enclosure passage was calculated 6,6 h. Table Table 54 ,Table 55 and Table 56 show some characteristics of the surface water and sand used for the experiments in the enclosure.

Table 54: Selected characteristics of surface water in the filtration pond

Characteristics of percolated water	
Natrium	46,4 mg/L
Kalium	4,3 mg/l
Calcium	125 mg/L
Magnesium	17,7 mg/L
Sulphate	236 mg/L
Nitrate	0,3 mg/L
Phosphate	<0,1mg/L
DOC	5,5 mg/L
pH	7,8
Conductivity	963 µS/cm

Table 55: Selected characteristics of the native sandy soil in the filtration pond

Characteristics of sandy soil in filtration pond	
effective grain size	0,15 - 0,30 mm
uniformity coefficient	3
water charging particles	< 1%
Porosity	31,90%
Dispersion	0,04
dispersion coefficient	0,036 m2/d
average residual time	2,25 - 9 h

Table 56: Selected geochemical properties of the sediments

Parameters	Native	Colmation layer
Fe-ox [mg/kg]	275	605
Fe-red [mg/kg]	850	1700
Fe-total [mg/kg]	1125	2305
Mn-ox [mg/kg]	11.0	68.8
Mn-red [mg/kg]	17.5	100.0
Mn-total [mg/kg]	28.5	168.8
C-org [weight %]	0.022	0.343
C-inorg [weight %]	0.118	1.395
S-total [weight %]	0.010	0.048
CECeff [mmol(eq)/100g	0.127	1.127

6.1.3 Inoculation

Experiments with chemicals and coliphages were performed simultaneously. Reference compounds of all working groups were mixed in a stock solution of 10 L volume and added as a sluggish peak to the surface water reservoir of the enclosure containing 500 L of ground water. Bacteria were not added simultaneously with chemicals because of possible interactions. Inoculation of coliphages 138 and 241 and indicator bacteria in the reservoir was started in separate experiments synchronic with the tracer salt NaCl. Approximate densities of test organisms in the stock solutions were $1x10^{11}$ pfu/ml for f+ coliphage 138, $1x10^{10}$ pfu/ml for somatic coliphage 241, and $1x10^{9}$ mpn/ml for indicator bacteria, respectively. The stock solutions of the test organisms were diluted in 10 L groundwater percolated and added into the water reservoir simultaneously with tracer salt solution of NaCl.

In the experiment Enclosure I-10 (see 6.2.5) both test coliphages were continuously injected into the reservoir water over a time period of 6 weeks.

During the experiment in the Enclosure III-13 (see 6.2.6), primary effluent from the sewage treatment plant of Berlin was daily added into the water reservoir in concentrations ranging from 2 % to 10 %.

6.1.4 Sampling

Samples from reservoir water were collected 20 cm below the water surface in sterile flasks at regular intervals. Sampling from drain tubes was carried out in sterile flasks separately from the other groups. Sampling intervals were short (30 – 60 min) during the first 12 h after inoculation and were prolonged to several hours and days during the further course of the experiment. An auto sampler was used for sampling the outlet over night.

Core samples from the filter bed were taken before and after one of the experiments to investigate the survival of bacteria and coliphages in the filter (see 6. 2.7).

After an operation time of over one year, filter bed was drained, and samples from the clogging layer up to 1 cm depth were collected for chemical analysis.

6.1.5 Assessment of the retention of test organisms

6.1.5.1 Cumulative breakthrough ratio A - CBTA

Concentrations of test organisms in reservoir water and filtrate samples were used for calculation of relative breakthrough ratios. The quotient of the concentration in each filtrate sample to the total concentration of test organisms in the reservoir water corresponds to the relative breakthrough of each filtrate fraction. The addition of these single relative breakthrough ratios as a function of time over the whole experiment resulted in a cumulative breakthrough ratio (CBT) of test organisms at a given time according to equation (1):

$$CBTA = \sum \frac{Cfiltrate}{CInput} \quad (1)$$

An example for the calculation of the cumulative breakthrough using phage 138 in experiment 1 of Enclosure III is given in Figure 67.

6.1.5.2 Cumulative breakthrough ratio B - CBTB

The breakthrough ratio was also calculated using the actual concentrations of microorganisims reached at each sampling point during the first 12 h of the experiment. Best fit straight lines were used to obtain the intersection points “a” for each sampling site (see Figure 66). These were used to determine the retention and the breakthrough of microorganisims after different filtration paths according to equation 2.

$$CBTB = \sum \frac{afiltrate}{ainput} \quad (2)$$

Examples for the use of best fit straight lines of the equilibrium stage at five sampling sites are demonstrated in figure 1b for F+phage 138 and in Figure 69 for coliphage 241.

The values obtained for CBTB using this calculation method (see Table 57) were comparable to the CBTA-values (not shown in all tables) calculated as described above.

Multiplication of the CBT values by 100 gives the breakthrough as percentage of the input concentration.

6.1.5.3 Δ-Log-Retention (Δ-log a values, heterogeneity)

The decade logarithms of the concentrations of test organisms resulting from the interception points “a” of each regression line at each sampling site are demonstrated in tables 1-6 as log a values. The differences between two log a values from two neighbour sampling sites can be used to assess the heterogeneity of removal of test organisms in different path of the filter matrix.

6.1.5.4 Specific retention rate (filter factor F)

Retention of test organisms was also calculated through the (-)log10 of CBT values. The specific retention rate demonstrates the retention of test organisms per cm filter path.

Retention as log units per cm filter path has also been defined as filter factor F (Pang et al., 2005).

$$F = \frac{(-)LogCBT}{\text{Length of filter path (cm)}} \quad (3)$$

The specific retention rate can also be calculated for the different parts of the filter by dividing the heterogeneity (Δ log a) through 20 cm.

6.1.5.5 Elimination rate coefficient (λ)

On the basis of the filtration theory of Yao et al. (1971), transport of colloid particles through saturated porous media can be represented by a connective dispersion equation, augmented by adsorption and desorption terms to account for bacterial interaction with the collector surface. In a column with characteristic length L (m), operated by a constant flow rate V(m/h), assuming steady state conditions, and neglecting dispersion (<0,001), virus inactivation and detachment or virus transport is described by (Schijven and Hassanizadeh, 2001):

$$(-)Log(CBT) = \frac{\lambda L}{2{,}3V} \quad (4)$$

The elimination rate coefficient λ can be derived from this equation:

$$L = \frac{(-)Log(CBT) * V * 2{,}3}{L} \quad (5)$$

λ was calculated using both cumulative breakthrough ratios CBTA and CBTB (see λA and λB in the tables).

6.1.5.6 Modelling parameter: λC (h-1), Retardation factor (R)

The adsorption and transport behaviour of test organisms was simulated using a one-dimensional, one-site kinetic model (see NASRI Report 2, chapter 2.4.a). The λ obtained by modelling is listed as λC in the tables. λC was in the same order of magnitude as λA and λB in all experiments.

6.1.5.7 One log removal distance (D) and time (T)

Elimination rate coefficient (λ) and filter factor (F) are the main parameters to characterise removal of microorganisms in the aquifer. From equation 6, it can be deduced that virus removal in a saturated sand filter under steady state conditions with a constant attachment, detachment and inactivation coefficient, should decline in a linear fashion with travel distance.

One log removal distance (D) may be calculated from the log retention of test organisms in the total length of the filtration unit (L):

$$D\ (m) = L\ (m) / \log \text{removal} \qquad (6)$$

Or as reciprocate of filter factor (F) $D = 1 / F$

One log retention time (T) of test organisms in each filter unit is the reciprocal of the elimination rate coefficient (λ)

$$T\ (h) = 1 / \lambda \qquad (7)$$

Distance or filtration time required for e.g. 8 log removal of test organisms are 8 time higher than the D and T values.

6.2 Results

Four experiments (experiments 1, 3, 3 cont. and 13) were conducted in Enclosure III (6.2.1-6.2.3 and 6.2.6) and one experiment each in Enclosure II (experiment 9, 6.2.4) and Enclosure I (experiment 13, 6.2.5). Core samples were taken before and after experiment 3 in Enclosure III and analysed for coliphages and bacteria (6.2.7).

6.2.1 Experiment in enclosure III without apparent biomass on the filter surface (Enc.III-1 - 05.08.03)

The first experiment in Enclosure III - with five sampling sites at 20, 40, 60, 80 and 100 cm depth - was carried out directly after saturation of the native sandy soil with groundwater that was used for percolation of the enclosure. Filter velocity was adjusted to 120 cm/d or 50 L/h corresponding to a pore velocity of 360 cm/d. The surface layer of the enclosure was free from visible biomass.

After inoculation of the reservoir, the concentration of F+phage 138 was 8×10^5 pfu/ml decreasing to 2×10^5 or 4.7×10^4 pfu/ml after a percolation time of 5 or 12 h, respectively (Figure 65, Figure 66). An early breakthrough of F+phage 138 was observed synchronic with the tracer salt NaCl at all sampling sites. Concentrations in the filtrate samples increased rapidly, achieving highest levels after 4 h. Concentrations remained stable up to 24 h before gradually decreasing (Figure 66). Despite the rapid breakthrough in the initial filtrate fractions, retention of phages in the column was relatively high. Retention ratios were found 0,96 0,99 or 0,999 in filtrate samples from 20 cm, 60 cm or in the effluent, respectively, after 12 h of percolation time (Figure 67). After an operation time of 278 h, cumulative breakthrough of coliphage 138 at each sampling site remained at the same level as observed after 12 h.

Log-Retention was highest in the upper and lower part of the sand filter (1,3 and 1,1) and lower (0,5-0,8) in the middle part of the filtration path (Table 57).

The elimination rate coefficients (λ) calculated in three different ways - balancing total virus concentrations in reservoir and filtrate samples, using regression lines of each sampling site at their equilibrium stage as well as by two site kinetic adsorption modelling - were found to be in the same order of magnitude (Table 57). The highest elimination rate coefficients (about 5 log units.h^{-1}) were calculated for the filtration path in the upper 20 cm of the sand filter. With increasing filter length, elimination rates declined to about 2 log units.h-1 after 40 cm and to about 1,5 log units.h^{-1} in 60 cm or 80 cm filter path.

Table 57: Retention of F+phage 138 in Enclosure III without apparent biomass on the filter surface (pore velocity: 360 cm/d, 5.8.2003)

sampling sites	a (pfu/ml)	log a	log-Retention (total) +	Δ log-Retention (Heterogeneity) (Δ log a)++	λB* (log.h-1)	Break-through ratio (CBTA)**	λA** (log.h-1)	Retardation (R)***	λC*** (h-1)
surface water	514471	5,7	-	0	-	-	-	-	-
P1 (20 cm)	25486	4,4	1,3	1,3	4,8	0,04	5,4	2,1	5,5
P2 (40 cm)	8371	3,9	1,8	0,5	2,1	0,01	2,3	1,1	2,3
P3 (60 cm)	3210	3,5	2,2	0,4	1,5	0,004	1,7	1,1	1,5
P4 (80 cm)	550	2,7	3,0	0,8	1,5	0,001	1,6	1,1	1,6
effluent (100 cm)	43	1,6	4,1	1,1	1,3	0,0001	1,3	1,1	1,2

+) Retention of phages between surface and each sampling site
++) Retention of phages between two neighbour sites
*) Calculation according to the best fit straight line of the equilibrium phase,
**) Calculation according to balancing total virus concentrations in surface water and filtrate samples,
***) Calculation by modelling

The concentration of somatic coliphage 241 was much lower in the reservoir after inoculation compared to coliphage 138. Only 2000 pfu/ml were detected and concentration decreased to 40 pfu/ml during a percolation time of 24 h (Figure 68).

The detection of coliphage 241 in filtrate samples was also synchronic with the tracer NaCl at all sampling sites. After a rapid increase the densities of phages achieved maximum levels of 64 pfu/ml or 3 pfu/ml at the sampling sites 20 cm or 80 cm after a percolation time of 2 h or 6 h, respectively (Figure 68, Figure 69). The stationary phase of phage density in filtrate samples at all sampling sites occurred within a filtration time of 12 h. These characteristic events, early breakthrough and filtration equilibrium within 12-24 h, were observed in all further experiments in the enclosures.

Table 58: Retention of somatic phage 241 in Enclosure III without apparent biomass on the filter surface (pore velocity: 360 cm/d, 09.03.03)

sampling sites	a (pfu/ml)	log a	log-Retention (total) +	Δ log-Retention (Heterogeneity) (Δ log a)++	Break-through ratio (CBTA)*	λA* (log.h-1)	Retardation (R)***	λC** (h-1)
surface water	532	2,7	-	-	-	-	-	-
P1 (20 cm)	74	1,9	0,9	0,9	0,12	3,3	1,5	2,4
P2 (40 cm)	16	1,2	1,5	0,7	0,03	1,7	1,2	1,6
P3 (60 cm)	5	0,7	2,0	0,5	0,01	1,1	1,1	1,1
P4 (80 cm)	-	-	-	-	0,004	1,2	1,1	1,2
effluent (100 cm)	-	-	-	-	-	-	n.a.	n.a.

+) Retention of phages between surface and each sampling site
++) Retention of phages between two neighbour sites
*) Calculation according to balancing total virus concentrations in surface water and filtrate samples,
**) Calculation by modelling

Despite the low initial concentration of coliphage 241 in the influent, retention was lower than for F+phage 138. After a percolation time of 12 h, retention ratios of 0,92 and 0,99 were observed after 20 cm or in the effluent, respectively, (Figure 69). Coliphage 241 was detected in all filtrate samples during continued operation of the filter up to 278 h, but at relatively low concentrations (Figure 68).

As with F+phage 138, the highest log-retention (0.9) was found in the upper 20 cm of the sand filter (Table 58). Retention in the middle part of the filter was reduced (Log-Retention: 0,5-0.7). Due to very low concentrations of coliphage 241 in filtrate samples at 80 cm and 100 cm it was not possible to calculate the retention in the lower part of the filter.

High elimination rate coefficients (about 2-3 log units.h^{-1}) were calculated for the first 20 cm depth. Elimination rate coefficients clearly decreased to about 1 log unit.h^{-1} with increasing filtration path.

6.2.2 Experiment in enclosure III after visible biomass had formed on the filter (Enc.III-3 –10.09.2003)

The second experiment with coliphages in the enclosure was started after formation of apparent biomass on the filter surface. Some characteristics of sediment from the surface layer of enclosure (up to 1 cm depth) is summarised in table B. The accumulation of biomass on the surface reduced the flow rate at the same hydraulic conditions to 42 L/h corresponding to 100 cm water column or 300 cm pore velocity per day.

In the first part of the experiment, all reference chemicals and F+phage 138 were inoculated into the water reservoir of enclosure. The initial concentration of F+phage in the reservoir water was 6.8 x 10^4 pfu/ml and decreased by about 4 log units during the 140 h of operation. Similar curves were obtained at the different filtration levels at lower concentrations (Figure 71).

The retention of F+ phage 138 was slightly better than in the previous experiment without apparent biomass on the filter surface. After an operation time of 12 h, the retention of coliphage138 amounted 97 % or 99 % at a filtration path of 20 or 40 cm, respectively (Figure 72).

The elimination rates of coliphages attained 3 or 4 log units at the sampling sites of 80 cm and 100 cm depths (Table 59). Similar relative retentions were obtained at the end of operation after 140 h. The highest log-retentions were detected in the upper (1,5 log units) and lower (1,0 log units) filtration path of the enclosure. In the middle part lower log-retentions of 0,5-0,8 log units were calculated.

Elimination rate coefficients (λ) were highest (about 2-3 log units.h^{-1}) in the first 20 cm and decreased to 1.2-1.8 in the further filtration path. Due to the early breakthrough of phages, low and relatively constant retardation factors were found at all sampling sites of the enclosure, ranging between 1,1 and 1,2.

Table 59: Retention of F+ coliphage 138 in Enclosure III with apparent biomass on the filter surface (pore velocity: 300 cm/d, 10.09.03)

sampling sites	a* (pfu/ml)	log a*	log-Retention (total) +	Δ log-Retention (Heterogeneity) (Δ log a)++	λ B * (log.h-1)	Break-through ratio (CBTA)**	λA** (log.h-1)	Retardation (R)***	λC*** (h-1)
surface water	68211	4,8	-	-	-	-	-	-	-
P1 (20 cm)	2443	3,4	1,5	1,5	2,1	0,03	2,1	1,1	3,2
P2 (40 cm)	741	2,9	2,0	0,5	1,4	0,01	1,6	1,1	1,8
P3 (60 cm)	131	2,1	2,7	0,8	1,3	0,001	1,4	1,2	1,8
P4 (80 cm)	12	1,1	3,8	1,0	1,3	0,0002	1,3	1,1	1,5
effluent (100 cm)	-	-	-	-	-	0,00001	1,4	1,1	1,1

+) Retention of phages between surface and each sampling site
++) Retention of phages between two neighbour sites
*) Calculation according to the best fit straight line of the equilibrium phase,
**) Calculation according to balancing total virus concentrations in surface water and filtrate samples,
***) Calculation by modelling (R: retardation factor, λ: elimination rate coefficient)

6.2.3 Experiment in enclosure III with indicator bacteria cultures (Encl.III-3 continued - 17.09.2003)

In the second part of experiment Enclosure III-3, coliphage 241 and indicator bacteria (E. coli and enterococci) were inoculated together into the water reservoir of Enclosure III. Core samples of the sand filter were taken before and after the experiment and analysed for bacteria and coliphages (see 6.2.7). The initial concentration of coliphage 241 in the water reservoir was 2,1 x 10^3 pfu/ml (Figure **73**). Concentration decreased about 3 log units during the first 100 h of operation. Similar curves were obtained at the different filtration levels at lower concentrations. Coliphage 241 was detected in low numbers from all filtrate samples up to an operation time of 800 h. The retention of coliphages 241 at all sampling sites was lower than in the previous experiment without apparent biomass on the filter surface (see 6.2.1). This may be due to preferential flow phenomena due to the core samples taken at the beginning of the experiment. Breakthrough ratios (51 h of operation) were 0,6 within 20 cm, 0,2 within 40 cm, and 0,004 in total effluent of the enclosure at a depth of 100 cm (Figure 74, Table 60). Highest retention was measured with 0,84 log units in the surface filtration path. It decreased to 0,42 log units between 20 and 40 cm. In the further filtration paths up to 80 cm depth, retention rates were measured as 0,65 and 0,8 log units. Relatively low elimination rate coefficients of 0,4-0,8 were calculated for all parts of the filtration path. Modelling

approaches showed a high retardation factor of 2,80 but very low λ value of 0,4.h^{-1} within the surface layer of 20 cm.

Table 60: Retention of the somatic coliphage 241 Enclosure III with apparent biomass on the filter surface (pore velocity: 300 cm/d, 17.09.03)

sampling sites	a* (pfu/ml)	log a*	log-Retention (total) +	Δ log-Retention (Heterogeneity) (Δ log a)++	Break-through ratio (CBTA)**	λA** (log.h-1)	Retardation (R)***	λC*** (h-1)
surface water	1655	3,2	-	-	-	-	-	-
P1 (20 cm)	241	2,4	0,8	0,8	0,6	0,5	2,8	0,4
P2 (40 cm)	92	2,0	1,3	0,4	0,2	0,6	1,6	0,5
P3 (60 cm)	21	1,3	1,9	0,6	0,07	0,8	2,1	0,5
P4 (80 cm)	3	0,5	2,7	0,8	0,02	0,8	1,5	0,5
effluent (100 cm)	-	-	-	-	0,004	0,6	n.a.	n.a.

+) Retention of phages between surface and each sampling site, ++) Retention of phages between two neighbour sites *) Calculation according to the best fit straight line of the equilibrium phase, **) Calculation according to balancing total virus concentrations in surface water and filtrate samples, ***) Calculation by modelling (R: retardation factor, λ: elimination rate coefficient)

The indicator bacteria E. coli and Enterococcus faecalis were added to the enclosure at the same time as coliphage 241. The addition of bacteria was possible for this experiment since the investigations with the trace chemicals had already been completed. Simultaneous addition of trace chemicals and bacteria was previously not allowed due to expected interactions.

Initial concentration of E. coli in the reservoir water was 108 cfu/100 ml (Figure 75, Table 61). It decreased about one log unit during the first 14 h of the experiment. The indicator bacteria also occurred synchronic with the tracer NaCl in all filtrate samples. Concentration in the effluent stabilized at about 1000 cfu/ 100 ml during the stationary phase of the filtration process within 24 h.

Retention of E. coli in the enclosure was higher than that of coliphages. About 99 % of the inoculated E. coli was eliminated after 20 cm of filtration after 14 h of operation. Elimination increased to 4 log units after 80 cm and 5 log units in the effluent at a depth of 100 cm (Figure 76).

Retention of E. coli was highest (2,1 log) in the upper part of the sand filter and lowest (0,6 log) between 60 cm and 80 cm (Table 5). Correspondingly, the elimination rate

coefficient was 2-3 log units.h^{-1} in the upper part and lower (1,3 - 1,7 log units.h^{-1}) in the further filtration path.

Table 61: Retention of E. coli in Enclosure III with apparent biomass on the filter surface (pore velocity : 300 cm/d, 17.09.03)

sampling sites	a* (pfu/100ml)	log a*	log-Retention (total) +	Δ log-Retention (Heterogeneity) (Δ log a)++	λB* (log.h-1)*	Break-through ratio (CBTA) **	λA** (log.h-1)	Retardation (R)***	λC*** (h-1)
surface water	236000000	8,4	-	-	-	-	-	#	#
P1 (20 cm)	1963800	6,3	2,1	2,1	3,0	0,01	2,8	#	#
P2 (40 cm)	-	-	-	-	3,0	0,0002	2,6	#	#
P3 (60 cm)	58823	4,8	3,6	1,5	1,7	0,0002	1,8	#	#
P4 (80 cm)	14441	4,2	4,2	0,6	1,5	0,0001	1,4	#	#
effluent (100 cm)	1748	3,2	5,1	0,9	1,5	0,00002	1,4	#	#

+) Retention of phages between surface and each sampling site , ++) Retention of phages between two neighbour sites, *) Calculation according to the best fit straight line of the equilibrium phase,, **) Calculation according to balancing total virus concentrations in surface water and filtrate samples, ***) Calculation by modelling (R: retardation factor, λ: elimination rate coefficient), #) in preparation.

The concentration of enterococci in the reservoir water was about 106 cfu/100 ml after inoculation (Figure 77). Survival potential of enterococci was much lower than that of the E. coli strain. Concentration in the reservoir water decreased rapidly within a few hours and only 1000 cfu/100 ml were detected after 3 h. As for E. coli, the retention of enterococci was higher than that for coliphage 241. Only 0,9 % or 0,1 % of inoculated enterococci were found in filtrates at 20 or 80 cm depth, respectively. No enterococci were detected in the effluent (Figure 78).

Table 62: Retention of intestinal enterococci in Enclosure III with apparent biomass on the filter surface (pore velocity: 300 cm/d, 17.09.03)

sampling sites	a* (pfu/100 ml)	log a*	log-Retention (total) +	Δ log-Retention, Heterogeneity (Δ log a)++	λB* (log.h-1)*	Break-through ratio (CBTA)**	λA** (log.h-1)	Retardation (R)***	λC*** (h-1)
surface water	6095830	6,8	-	-	-	-	-	#	#
P1 (20 cm)	4082	3,6	3,2	3,2	4,6	0,01	4,0	#	#
P2 (40 cm)	378	2,6	4,2	1,0	3,0	0,002	2,3	#	#
P3 (60 cm)	125	2,1	4,7	0,5	2,5	0,001	2,0	#	#
P4 (80 cm)	10	1,0	5,8	1,1	2,1	0,0003	1,5	#	#
effluent (100 cm)	-	-	-	-	-	0,00001	1,3	#	#

+) Retention of phages between surface and each sampling site
++) Retention of phages between two neighbour sites
*) Calculation according to the best fit straight line of the equilibrium phase,
**) Calculation according to balancing total virus concentrations in surface water and filtrate samples,
***) Calculation by modelling (R: retardation factor, λ: elimination rate coefficient), #) in preparation.

Retention was more than 6 log units over the total filter path of 100 cm. Retention was highest (3,2 log) in the upper part of the sand filter and lowest (0,5 log) between 40 cm and 60 cm (**Table 62**).

Elimination rate coefficients were highest (about 4 log units.h^{-1}) within the top layer of 20 cm. It decreased to 2-3 log units.h^{-1} for a filtration paths of 40 and 60 cm and to 1,3 log units.h^{-1} in the total length of filter path.

6.2.4 Experiment in Enclosure II under microaerophilic conditions (Enc.II-9 – 30.08. 04)

Enclosure II - with two drain canals at 40 and 80 cm as well as the outlet at 100 cm depth - was continuously percolated with 0.1 M acetic acid for three weeks to reduce the oxygen content of the percolation water and to obtain micro aerophilic or anoxic conditions. At a relatively constant level of < 0,1 mg/L oxygen in effluent samples we inoculated the water reservoir with culture suspensions of coliphages and E. coli. Enclosure II was operated with a filtration velocity of 72 cm/d corresponding to a pore velocity of 210 cm/d.

Concentration of F+ phage 138 was $2x10^6$ pfu/ml in the reservoir water directly after inoculation. Due to a relatively low filtration velocity, dilution of phages in the water reservoir was low and the concentration of phages did not decreased rapidly. After 10 h or 26 h, 1.106 or $1x10^5$ pfu/ml phages were found, respectively (Figure 79). Retention of phages under micro- aerophilic filtration conditions in Enclosure II was very low. All filtrate samples contained more than 10^4 pfu/ml within the first 48 h. The highest concentration ($8,6x10^5$ pfu/ml) was detected in the filtrate sample at 40 cm after 7 h of operation. In correspondence with the pore velocity, the highest phage concentrations were observed after 9 h at 80 cm and after 24 h at 100 cm. Concentrations in filtrate samples decreased slightly in the further course of the experiment. F+ phages were still detectable in all filtrate samples after 3 weeks.

Breakthrough of F+ phage 138 under micro aerophilic conditions was much higher than under aerobic conditions. Cumulative breakthrough ratios were 0,5 in the upper part of the enclosure and decreased to 0.2 in the lower part compared to 0,0002 under aerobic conditions (Table 59, Table 63). Correspondingly, low elimination rate coefficients (λ) were calculated at all sampling sites. Highest λ values of 0,4-0,6 log units.h^{-1} were calculated for the upper part of the filtration path decreasing to 0.6-1,0 in the lower part (Table 63). Statistical analysis of the results indicated no retardation of the coliphages (R = 1).

Table 63: Retention of F+ phage 138 in Enclosure II under micro-aerophilic conditions at a pore velocity of 210 cm/d (30.08.04).

sampling sites	a* (pfu/ml)	(log a)*	log-Retention (total) +	Δ log-Retention, Heterogeneity (Δ log a)++	Break-through ratio (CBTA)**	λA** (log.h-1)	(R)***	λC (h-1)***
surface water	1968000	6,3	-	-	-	-	-	-
P2 (40 cm)	784048	5,9	0,4	0,4	0,47	0,42	1,1	0,67
P4 (80 cm)	548000	5,7	0,6	0,2	0,32	0,13	1,0	0,16
Effluent (100 cm)	201446	5,3	1	0,4	0,17	0,13	1,0	0,06

+) Retention of phages between surface and each sampling site , ++) Retention of phages between two neighbour sites, *) Calculation according to the best fit straight line of the equilibrium phase,, **) Calculation according to balancing total virus concentrations in surface water and filtrate samples, , ***) Calculation by modelling (R: retardation factor, λ: elimination rate coefficient)

The initial concentration of somatic phage 241 was about $5,4x10^4$ pfu/ml in the water reservoir of the enclosure. Concentration decreased to 104, 103, and 10^2 pfu/ml after a percolation time of 10, 24 and 48 h (Figure 80). After a rapid breakthrough of phages in the filtrate fractions at 40 cm the highest concentration of $8,5x10^4$ pfu/ml was found after a percolation time of 4 h. Highest concentrations at other sampling sites were observed after 10 h at 80 cm and 28 h at 100 cm depth.

Retention of somatic phage 241 was much lower in the enclosure under micro-aerophilic conditions compared to aerobic conditions and even lower than for F+ phage 138. Highest retention was determined in the upper part with 0,6 log units which significantly decreased to

0.05-0.08 log units in the lower part of the enclosure. Accordingly, the highest elimination rate coefficient (0,3-0,4 log units.h^{-1}) were calculated for the upper part. In the lower part, the elimination rate coefficient decreased to 0,07-0,1 log units.h-1 in the lower part (Table 64). Modelling results showed a moderate retardation in the surface filter layer (R=1,5) but no retardation (R = 1) in the lower part.

Table 64: Retention of somatic coliphage 241 in the Enclosure II under micro aerophilic conditions at a pore velocity of 210 cm/d (30.08.04).

sampling sites	a* (pfu/ml)	(log a)*	log-Retention (total) +	Δ log-Retention, Heterogeneity (Δ log a)++	Break-through ratio (CBTA)**	λA** (log.h-1)	(R)***	λC (h-1)***
surface water	38170	4,6	-	-	-	-	-	-
P2 (40 cm)	9019	4,0	0,6	0,6	0,55	0,33	1,50	0,43
P4 (80 cm)	7531	3,9	0,7	0,08	0,40	0,11	1,04	0,14
Filtrate (100 cm)	6784	3,8	0,8	0,05	0,30	0,09	1,00	0,07

+) Retention of phages between surface and each sampling site
++) Retention of phages between two neighbour sites
*) Calculation according to the best fit straight line of the equilibrium phase,
**) Calculation according to balancing total virus concentrations in surface water and filtrate samples,
***) Calculation by modelling (R: retardation factor, λ: elimination rate coefficient)

After inoculation of E. coli, a high initial concentration of $7x10^9$ mpn/100 ml was measured in the reservoir of the enclosure. Concentration of E. coli decreased to $1x10^7$ mpn/100 ml after 12 h and $1,4x10^6$ mpn/100 ml after 24 h (Figure 81). Highest concentrations of E. coli were found in filtrate samples already after 3 h at 40 cm, after 8 h at 40 cm, and after 24 h in the effluent of the enclosure. Concentrations of E. coli in filtrate samples reduced slightly during the further run of the experiment. E. coli was detectable in all filtrate samples over 3 weeks of operation.

Breakthrough of E. coli was also much higher under micro aerophilic conditions of this experiment compared to aerobic conditions. Cumulative breakthrough ratios were 0,84 in the upper part of the enclosure and decreased to 0,01 in the lower part compared to 0,00002 under aerobic conditions (Table 61, Table 65).

Breakthrough was again lower for E. coli than for coliphages.

Table 65: Retention of E. coli in Enclosure II under micro aerophilic conditions at a pore velocity of 210 m/d (30.08.04).

sampling sites	a* (mpn/100 ml)	(log a)*	log-Retention (total) +	Δ log-Retention, Heterogeneity (Δ log a)++	Break-through ratio (CBTA)**	λA** (log.h-1)	(R)***	λC (h-1)***
surface water	70341000	7,8	-	-	-	-	-	-
P2 (40 cm)	949126	6,0	1,9	1,87	0,84	0,19	#	#
P4 (80 cm)	263826	5,4	2,4	0,56	0,08	0,3	#	#
Filtrate (100 cm)	94105	5,0	2,8	0,45	0,01	0,33	#	#

+) Retention of E. coli between surface and each sampling site
++) Retention of E. coli between two neighbour sites
*) Calculation according to the best fit straight line of the equilibrium phase,
**) Calculation according to balancing total virus concentrations in surface water and filtrate samples,
***) Calculation by modelling (R: retardation factor, λ: elimination rate coefficient) #) in preparation

6.2.5 Experiment in Enclosure I with continuous inoculation (Enc.I-10 - 12.10.04)

A suspension of test coliphages was continuously injected into the surface water reservoir of Enclosure I - simultaneously with the reference suspension of microcystin - by means of a two canal pump. Filter velocity was regulated to 70 cm water column or 210 cm pore water column per day. Sampling was only possible in the water reservoir and at the outlet after a filtration path of 100 cm.

Mean concentration of F+ phage 138 in the water reservoir was $1,3x10^5$ pfu/ml during the first part of the experiment for 15 days (Figure 54, Table 66). The cumulative breakthrough ratio (0.0001 log units) was similar to the ratios obtained for pulse experiments under aerobic conditions (see 6.2.1, 6.2.2). Based on a retention of 3,9 log units, an elimination rate coefficient (λ) of 0,67 log unit.h^{-1} was calculated for a filtration path of 100 cm.

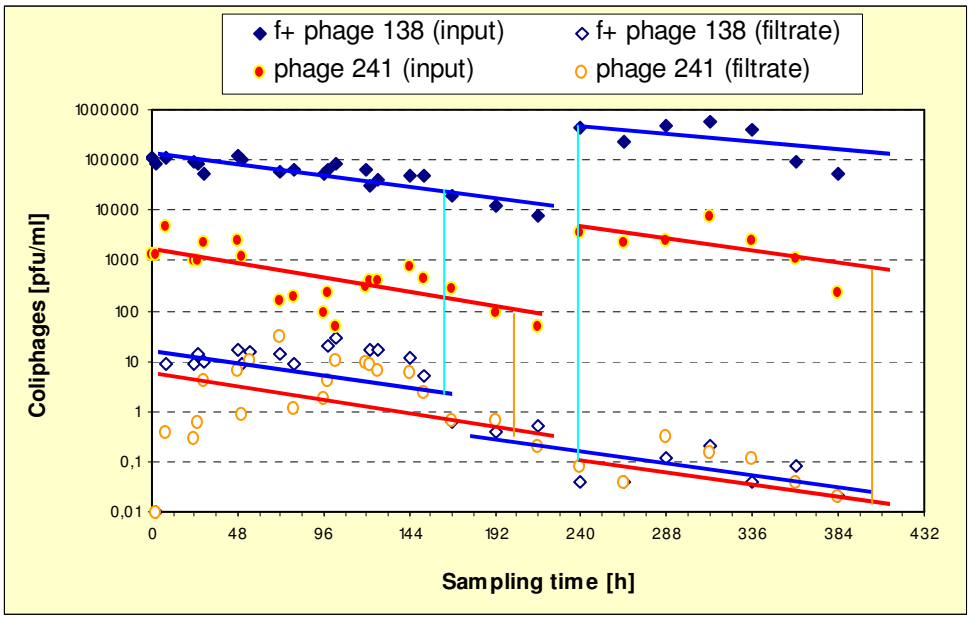


Figure 54: **Retention of coliphages in Enclosure I (with clogging layer) during continuous inoculation at a pore water velocity of 210 cm/d**

After 15 days of operation, inoculation dose of phages was increased to $1{,}2 \times 10^7$ pfu/ml . Nevertheless, F+ phage 138 was still only sporadically detected in the filtrate samples. Retention was, therefore, 7 log units and the elimination rate coefficient increased to 1,28 log units per h (Table 66). In the modelling approach, all results of this experiment were analysed as a block. No or only weak retardation (R=1,1) and also a relatively low elimination rate coefficient (λC) of 0,72 h^{-1} were found.

The concentration of somatic coliphage 241 averaged $1{,}7 \times 10^3$ pfu/ml in the reservoir water during the initial phase of experiment. Mean concentration of phages in filtrate samples was 14 pfu/ml corresponding to a retention of 2,1 log units. The elimination rate coefficient of phage 241 was calculated as 0,36 logs. h^{-1}.

After 15 days the concentration of somatic coliphage 241 was increased more than 2 log in the water reservoir to $1{,}7 \times 10^5$ pfu/ml. Concentrations in the filtrate samples were even lower than with lower initial concentrations in the beginning of the experiment. Retention, thereby, increased significantly to 5,2 log units resulting in a specific elimination rate of 0,92 logs. h -1. Modelling approach of results showed a relatively high retardation factor of 3,5, but extremely low elimination rate coefficient (λC) of 0,13 logs. h^{-1}.

Table 66: Retention of test coliphages in Enclosure I with continuous inoculation at a pore velocity of 210 cm/d (12.10.04)

F+ phage 138	a (pfu/ml)*	log a *	Δ log-Retention, Heterogeneity (Δ log a) +	Break-through ratio (CBTA)**	λA** (log.h-1)	(R)***	λC (h-1)***
surface water	128986	5,11	-	-	-	-	-
Filtrate (100 cm)	15	1,18	3,9	0,00012	0,67	1,1	0,72
surface water	11983696	7,08	-	-	-	-	-
Filtrate (100 cm)	1	0,00	7,1	0,00000003	1,28	-	-
somatic coliphage 241	a (pfu/ml)*	log a *	Δ log-Retention, Heterogeneity (Δ log a) +	Break-through ratio (CBTA)**	λA** (log.h-1)	(R)***	λC (h-1)***
surface water	1691	3,23	-	-	-	-	-
Filtrate (100 cm)	14	1,14	2,1	0,008	0,36	3,5	0,13
surface water	171509	5,23	-	-	-	-	-
Filtrate (100 cm)	1	5	5,2	0,000004	0,92	-	-

+) Retention of phages between two neighbour sites
*) Calculation according to the best fit straight line of the equilibrium phase,
**) Calculation according to balancing total virus concentrations in surface water and filtrate samples,
***) Calculation by modelling (R: retardation factor, λ: elimination rate coefficient)

6.2.6 Experiment in Enclosure III with primary effluent – (Enc.III-13 - 26.10 – 18.12.04, pore velocity: 210 cm/d)

In the last experiment with Enclosure III, surface water was continuously infected with domestic primary effluent from the sewage treatment plants of southern Berlin. The primary effluent was treated by sieving through 0,1 mm mesh size before usage. The concentration of primary effluent was regulated between 2 and 10 % to obtain a constant level of coliphages and indicator bacteria.

The concentration of F+ phages in the reservoir water varied between 2.105 – 2.106 pfu/100 ml in the first four days when 2 % of primary effluent was added to the water reservoir (Figure 55). After one week, the density of F+ phages declined to about 7.103 pfu/100ml in spite of permanent addition of 2 % waste water. Therefore, the amount of primary effluent was increased to 4 % which caused a moderate rise in the concentration of concentration F+ phages up to $9x10^4$ pfu/100 ml, but concentrations decreased again to $1x10^4$ pfu/100 ml during the third week of operation. After four weeks, 10 % of primary effluent was added. Concentrations of F+ phages did not increase but remained at a similar level as measured previously (Figure 55).

Despite increasing volume of primary effluent, concentrations of coliphages remained at a relatively stable concentration. One explanation might be a high daily fluctuation of contaminants in the primary effluent added to the enclosure. The chemical and physical characteristics of the primary effluent were, however, similar over the whole run of the experiment. Some chemical parameters of primary effluent are demonstrated in Fig. 11A. Mean concentrations of these selected parameters averaged 638+217 mg/L for chemical oxygen demand (COD), 5,04 + 1,19 mg/L for total organic carbon (TOC), 64,6 + 12,4 mg/L total nitrogen (total N), and 0,95 + 0,23 g/L for dry matter. Constant concentrations of phages in the reservoir water despite increasing amount of primary effluent might be explained by changing microbial activity or increasing deactivation of phages in water reservoir of Enclosure III.

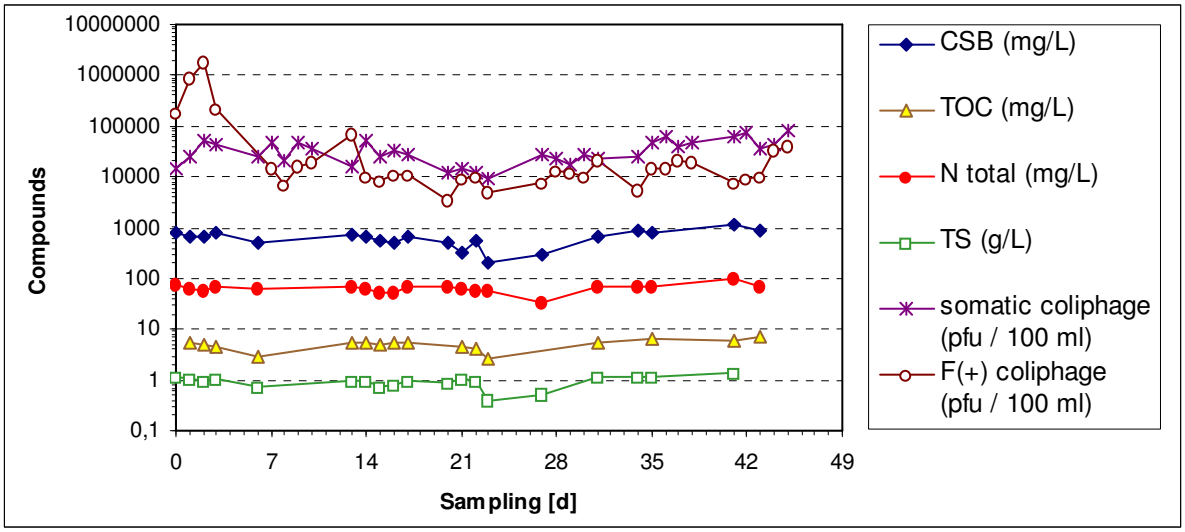


Figure 55: Concentrations of selected chemical parameters (mg/L) and coliphages (pfu/100 ml) in primary effluent spiked to the reservoir of Enclosure III

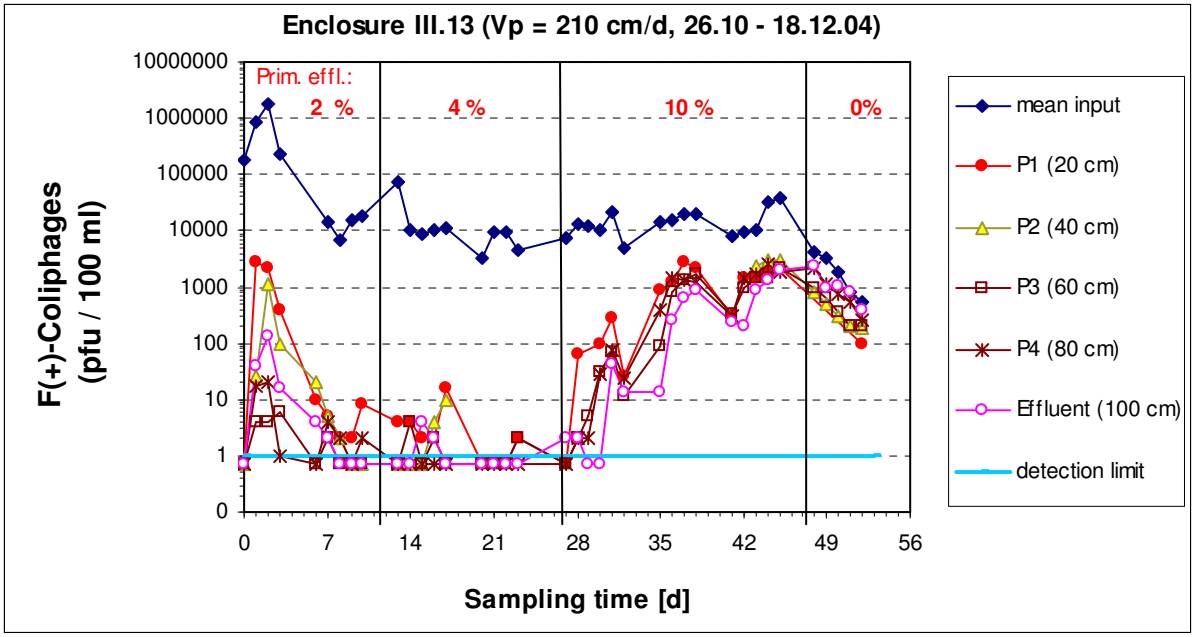


Figure 56: **Retention of F+phages in Enclosure III with continuous percolation of primary effluent at different concentrations (pore velocity = 210 cm/d)**

Relatively high concentrations of F+ phages were found in filtrate samples taken after 2 or 3 days (Figure 56). Consequently, breakthrough ratios of F+ phages during the initial phase of the experiment were relatively high, e.g. 0,002 or 0,0004 (corresponding to a log-retention of 2,7 and 3,4 log units) after a filtration path of 20 or 40 cm, respectively (Figure 56, Table 67). Retention increased to 3,5 or 3,7 log units in the further run of the experiment, during an operation time of 10 days. After rising of wastewater portion in the reservoir to 4 %, removal of F+ phages persisted at a high level of 4 log units during the further percolation time of two weeks. As the amount of waste water in the reservoir increased to 10 %, concentrations of F+ phages increased about three log units in filtrate samples at all sampling sites. Correspondingly, retention of F+ phages decreased to about one log unit at all sampling sites.

Table 67: **Retention (log10) of F+ coliphages in Enclosure III during continuous filtration of primary effluent at different concentrations (pore velocity 210 cm/d, 26.10. – 18.12.04)**

Wastewater (%)	P1 (20 cm)	P2 (40 cm)	P3 (60 cm)	P4 (80 cm)	Effluent (100 cm)
2 (initial)	2,7	3,4	3,6	4,9	4,2
2 (after 10 d)	3,5	3,7	3,6	3,5	3,0
4	3,5	3,5	3,8	4,1	3,8
10	1,2	1,2	1,4	1,3	1,7
0	0,9	0,6	0,6	0,3	0,2

The concentration of somatic phages in reservoir water varied between 1.104 and 8.104 pfu/ml during continuously contamination with primary effluent in increasing portions up to 10 % (Figure 57). Less retention of somatic coliphages has been observed than that of F+ phages. During the first three days, a relatively high amount of phages were detected in filtrate samples at all sampling sites that resulted in a breakthrough ratio of 0,04 at the upper part of the enclosure (20 cm), and 0,0006 in the total effluent at 100 cm depth. These breakthrough ratios were comparable to the ratios found when a sluggish peak inoculum of coliphages was applied (see 6.2.1-6.2.3).

During further percolation of the enclosure with 2 % primary effluent, retention of somatic phages increased clearly to 3,1 log units within 20 cm, and more than 4 log units in the effluent samples. A relatively high and stable retention of somatic phages in the enclosure was still observed when the amount of primary effluent was increased to 4 %. Log-retentions were 2,7 log at 20 cm and about 4 log units in the effluent (Table 68).

When the amount of primary effluent was increased to 10%, concentration of somatic phages was increasing by three log units in all filtrate samples (Figure 57). Correspondingly, retention decreased to about one log unit at all sampling sites (Table 68). Breakthrough ratios were 0,14 at the surface filter path, and 0,08 in the total effluent of the enclosure.

Table 68: Retention (log10) of somatic coliphages in Enclosure III during continuous filtration of primary effluent at different concentrations (pore velocity 210 cm/d, 26.10. – 18.12.04)

Wastewater (%)	P1 (20 cm)	P2 (40 cm)	P3 (60 cm)	P4 (80 cm)	Effluent (100 cm)
2 (initial)	1,4	1,8	3,7	2,5	3,6
2 (after 10 d)	3,1	3,2	4,1	3,5	4,2
4	2,7	3,0	3,6	3,3	4,0
10	0,9	0,8	0,9	0,9	1,1
0	0,6	0,6	0,4	0,2	0,04

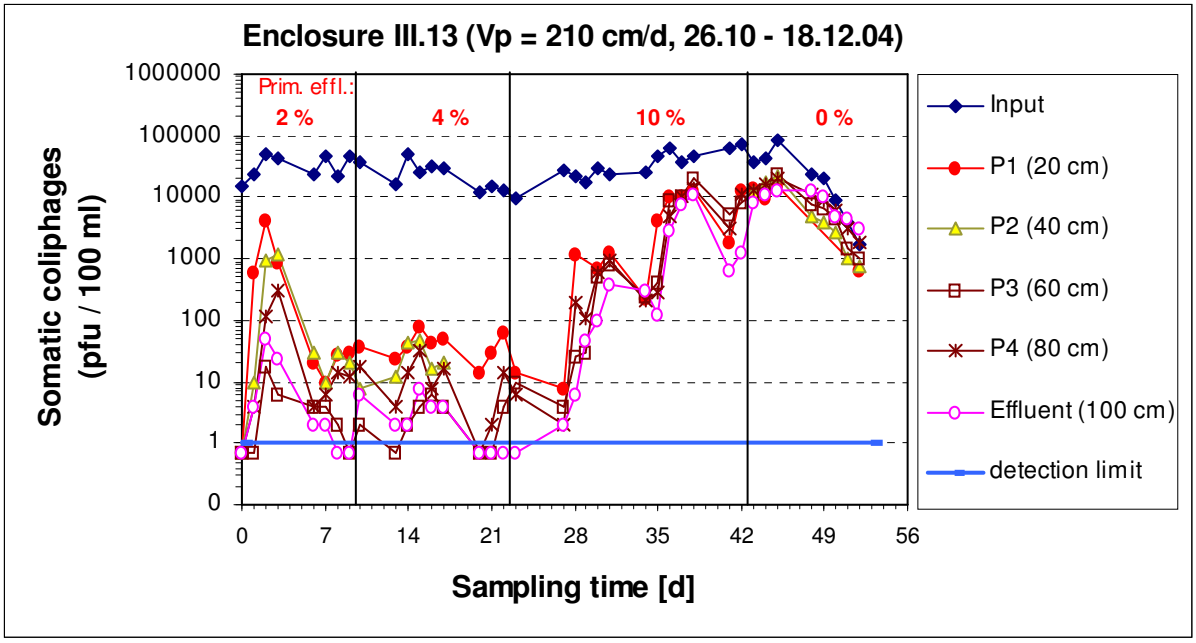


Figure 57: **Retention of somatic coliphages in the Enclosure III with continuous percolation of primary effluent at different concentrations (pore velocity = 210 cm/d)**

Following continuously spiking of primary effluent, concentration of E. coli in the reservoir water varied between 2.104 and 2.105 mpn/100 ml at the initial phase of the experiment with 2 % of primary effluent and slightly enhanced up to 1.106 mpn/100 ml as the amount of primary effluent was increased to 4 % and 10 % (Figure 58). Retention of E. coli was high during the first four weeks of operation with 2 % to 4 % of primary effluent. Most of the filtrate samples were free from E. coli in 100 ml volume. E. coli was only sporadically detected in filtrate samples from different sampling sites at concentrations of 3-100 mpn/100 ml. During this part of the experiment retention of E. coli varied between 3 and 5 log units (Table 69).

Increase of primary effluent concentration to 10 % led to a breakthrough of E. coli in filtrates at all sampling sites. Concentrations of E. coli in filtrate samples increased with percolation time up to concentration levels in the reservoir water. Correspondingly, log-retention decreased to 0,3 to 0,7 log units (Table 69). Breakthrough ratios averaged to 0,35 at 20 cm, 0,31 at 60 cm, and 0,19 in effluent from 100 cm.

Table 69: **Retention (log10) of E. coli in the Enclosure III during continuous filtration of primary effluent at different concentrations (pore velocity 210 cm/d, 26.10. – 18.12.04)**

Wastewater (%)	P1 (20 cm)	P2 (40 cm)	P3 (60 cm)	P4 (80 cm)	Effluent (100 cm)
2	3,9	5,0	3,0	3,6	3,7
4	4,9	5,7	5,3	4,2	4,3
10	0,5	0,3	0,5	0,8	0,7

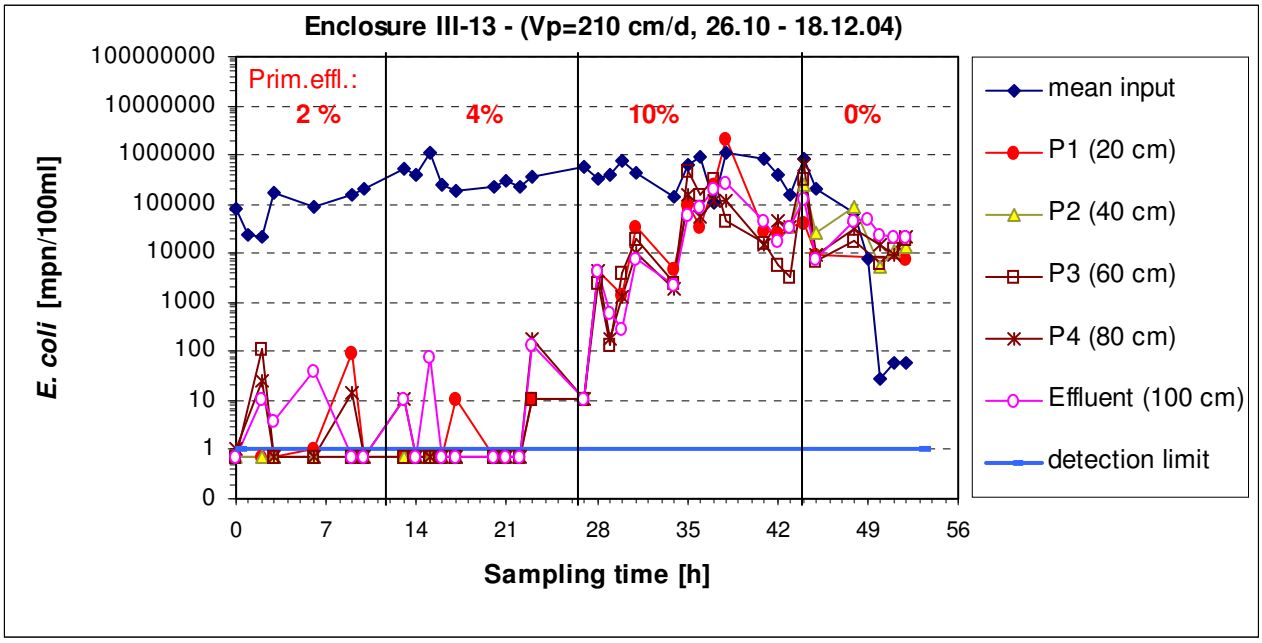


Figure 58: **Retention of E. coli in the Enclosure III with continuous percolation of primary effluent at different concentrations (pore velocity = 210 cm/d)**

Concentrations of intestinal enterococci in the water reservoir of the enclosure were in most cases between 104 and 105 mpn/100 ml. Filtrate samples from different sampling sites were sporadically positive for enterococci during the infiltration of 2 % and 4 % primary effluent (Figure 59). About 4 log units of intestinal enterococci were removed in the enclosure during this part of the experiment (Table 14). After increasing the amount of primary effluent to 10 %, enterococci were detected in filtrates from all sampling sites. The removal of enterococci diminished to about one log unit at most sampling sites (Table 70).

Table 70: **Removal of intestinal enterococci (log10) in Enclosure III during continuous filtration of primary effluent at different concentrations (pore velocity 210 cm/d, 26.10. – 18.12.04)**

Wastewater (%)	P1 (20 cm)	P2 (40 cm)	P3 (60 cm)	P4 (80 cm)	Effluent (100 cm)
2	3,0	4,3	3,6	4,4	4,4
4	4,1	4,9	3,5	3,4	3,6
10	0,2	1,1	1,1	1,2	1,0

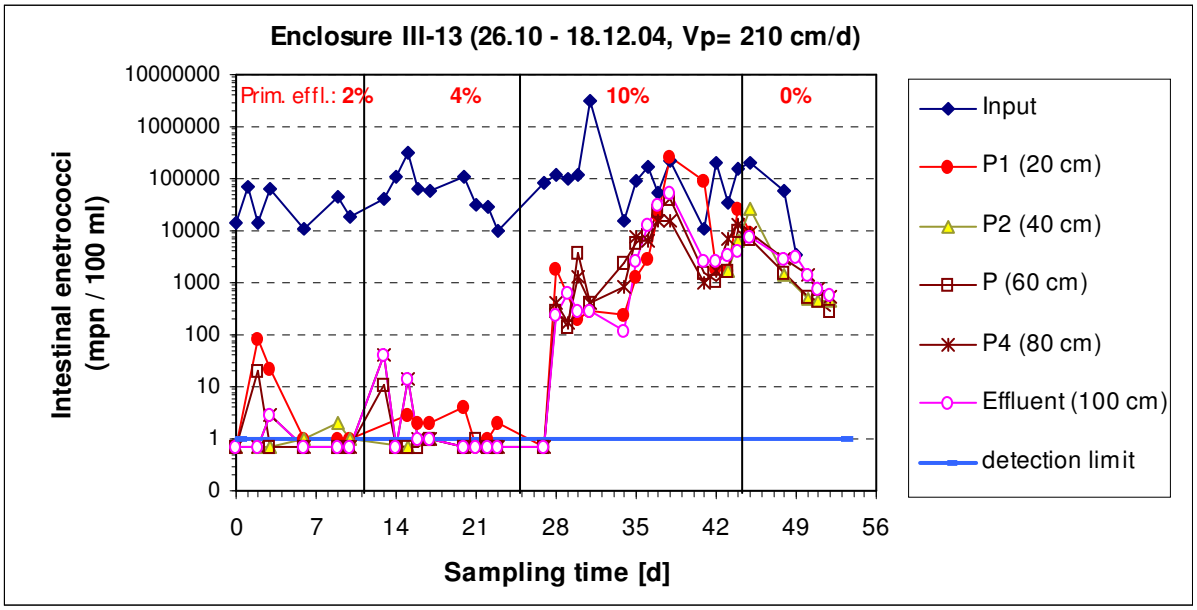


Figure 59: **Retention of intestinal enterococci in Enclosure III with continuous percolation of primary effluent at different concentrations (pore velocity = 210 cm/d)**

6.2.7 Core samples

Seven cores of the filter material in the enclosure were taken during and after experiment 3 in Enclosure III (days 1, 3, 4, 11, 23, 53, and 71. The cores were divided into 5-10 cm sections which were analysed for total bacteria (DAPI), E. coli, enterococci as well as the two coliphages. Additionally, the clogging layer and the core samples were examined microscopically.

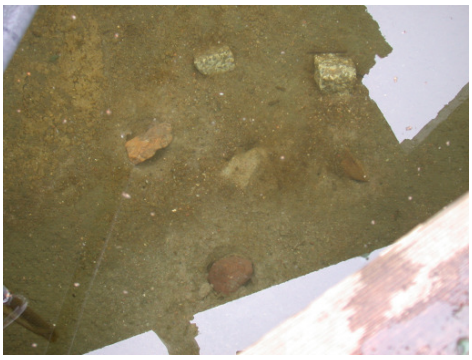
Both, bacteria and coliphages survived in the filter sand for extended periods of time. Concentrations of 1-100 cfu/g E. coli were, for example, detected in core samples from all depths 71 days after inoculation. This long term survival potential in the filter bed may explain the sporadicac appearance of indicator bacteria and coliphages in the effluent of the filters several weeks after inoculation.

6.2.7.1 Characterisation of the clogging layer

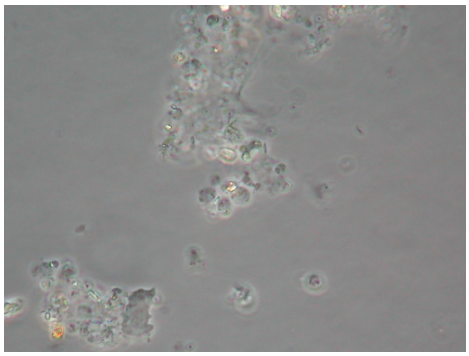
The dominating organisms found in the clogging layer were algae of different groups (diatoms, gold and green algae) together with amoebae. Occasionally higher organisms (e.g. gastrotrichs) were found (Table 71, Figure 60). Cyanobacterium microcystis, which had been applied to the filter together with the microcystin was found in the clogging layer at all sampling times.

Table 71: Organisms identified in the clogging layer

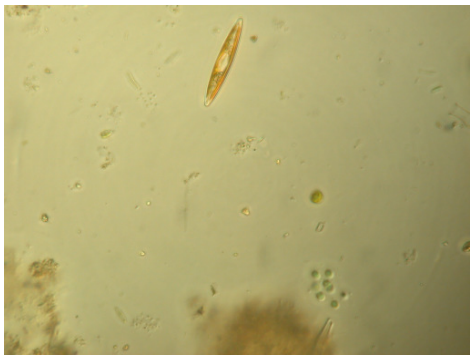
Cyanobacteria :	Microcystis spec.
Diatoms :	Navicula spec. Synedra spec. Anomoeoneis spec. Nitzschia spec.
Gold algae :	Dinobryon spec.
Green algae :	Chlorococcum spec. Euastrum spec.
Amoebae :	Actinosphaerium spec. Testacea , ambiguous
Gastrotichs :	Chaetonotus spec.



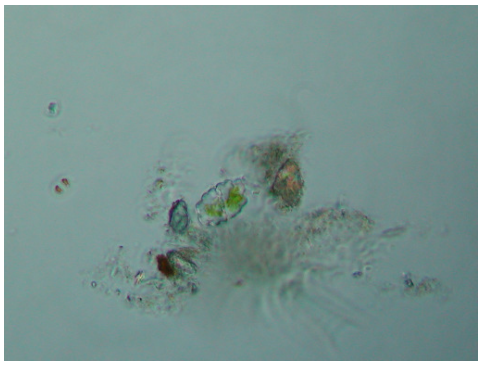
„Schmutzdecke“ enclosure



Amoeba, sediment et al.
magnification1000



Diatoms and single cell greenalgae
magnification 400



„Jochalge“ Euastrum spec.
magnifigation 1000

Figure 60: Typical organisms in the upper sand layers of the enclosure

6.2.7.2 Examination of the filter cores

Filter cores were analysed for total bacteria using DAPI staining and fluorescence microscopy. Concentrations between 10^8 and 10^9 cfu/g dry weight were found in all samples (Figure 61). No correlation between the concentration of the bacteria and the depth of the filter was detected. Similar results were obtained from the GWA Tegel (results not shown).

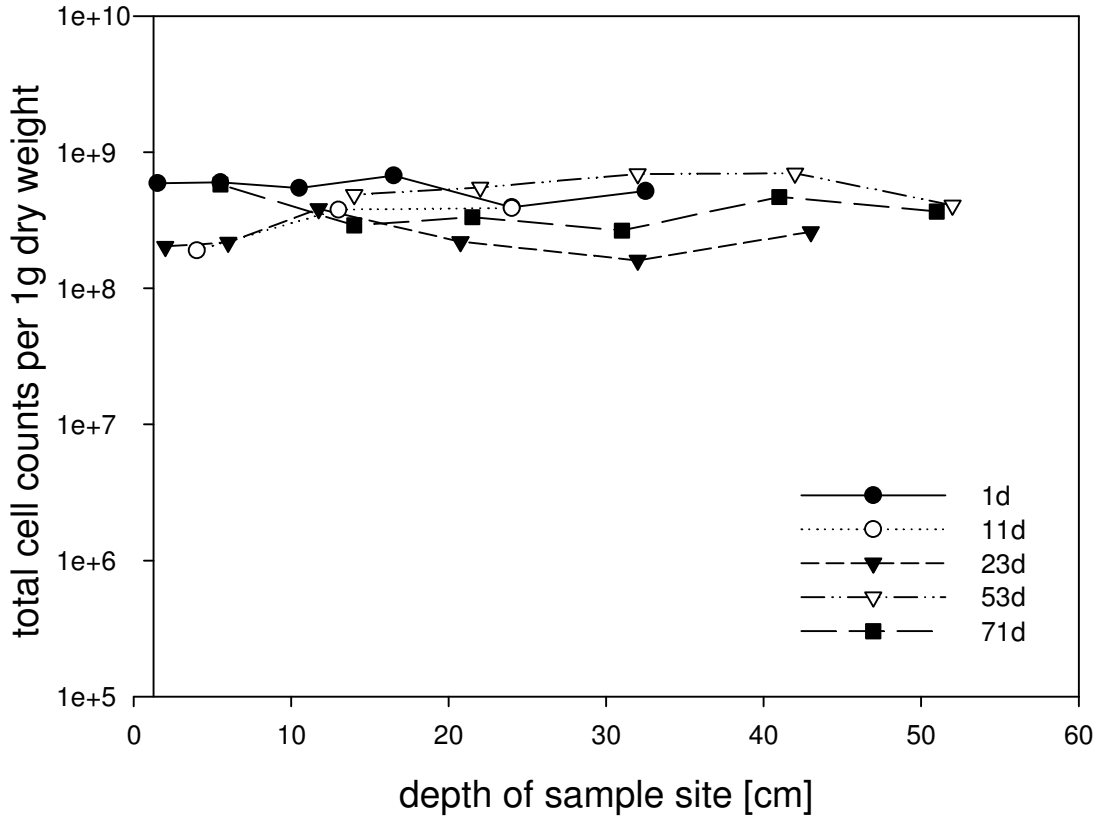


Figure 61: Total cell counts per 1g dry weight determined by DAPI staining in sediment samples taken from the enclosure during the filtration experiment.

The filter cores were analysed for both bacteriophages since the previous experiment had been carried out with coliphage 138. Coliphage 138 was still detected in concentrations of 10-70 pfu/g wet weight in the beginning of the experiment. After 23 days, these coliphages were only detected sporadically in very low numbers (< 1-3 pfu/ g, data not shown).

Coliphage 241 was detected in very low concentrations (1-5 pfu/g wet weight) before the start of the experiment in all sampling depths. High concentrations were found at day 3 and 4 after inoculation. Concentrations decreased with depths (Figure 62). In the upper layers

concentrations ranged from 3×10^3 – 1.5×10^4 pfu/g wet weight. In the lower layers, concentrations increased from day 2 to day 3 from 10 to several hundred pfu/g. Coliphage 241 was still present after 23 days in all sampling depth at concentrations of 100-300 pfu/g.

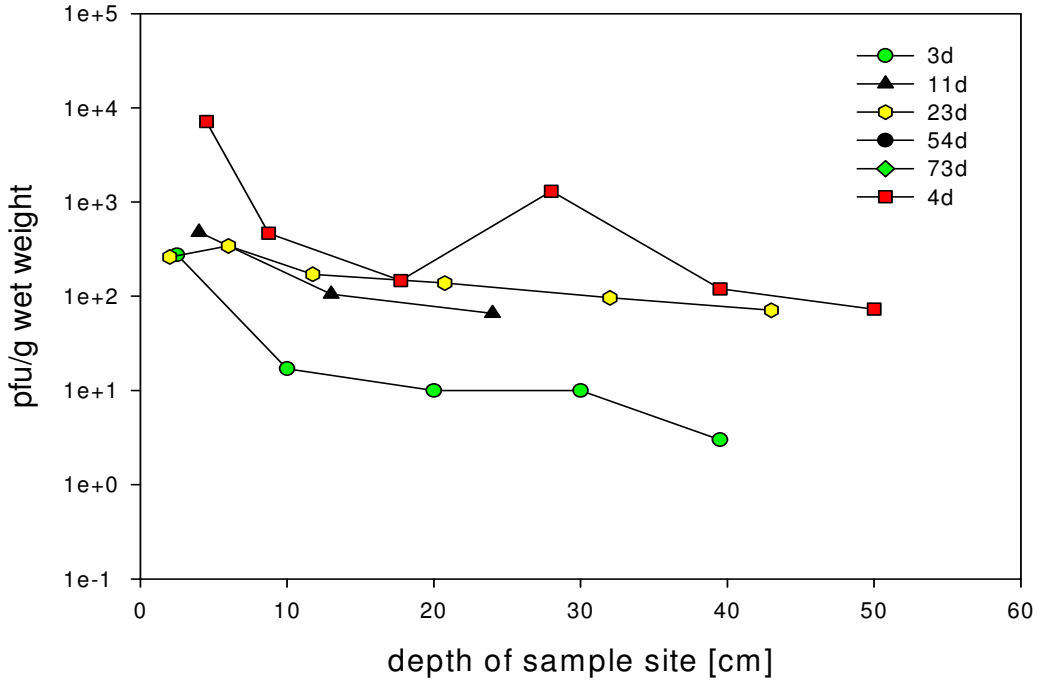


Figure 62: **Concentration of coliphage 241 in sediment samples taken from the enclosure during the experiment (no data at day 54 and 73).**

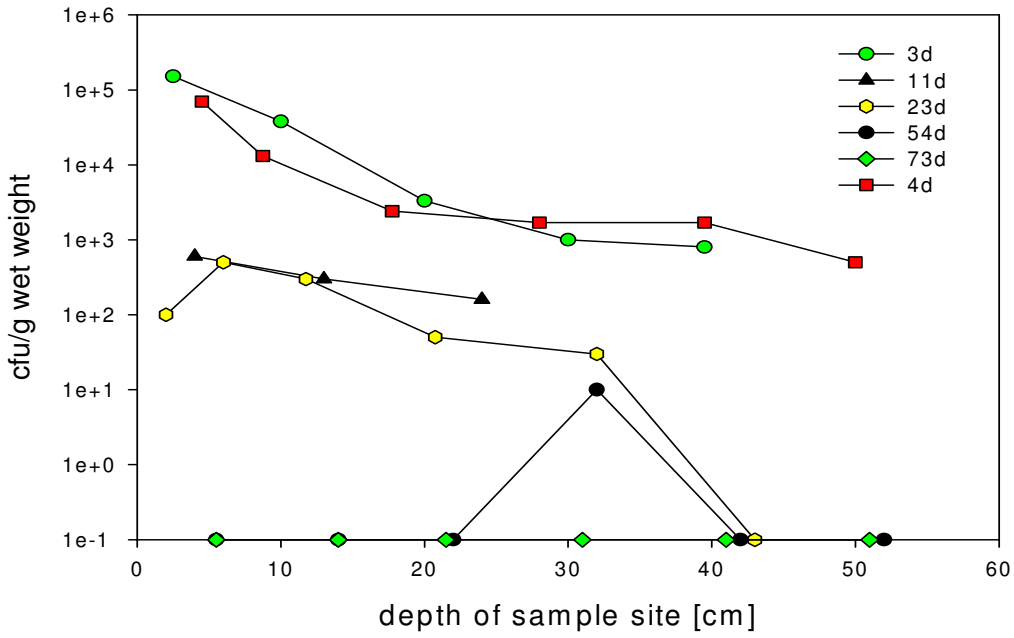


Figure 63: **Concentration of enterococci in sediment samples taken from the enclosure during the filtration experiment (detection limit 1 cfu/g).**

Enterococci were not detected in core samples before inoculation. After inoculation, concentrations were in the range of 1-8 x 10^4 cfu/g wet weight in the upper layers and decreased to about 1000 cfu/g in a depth of 40-50 cm (Figure 63). Concentrations decreased after 2-3 weeks to 100-600 pfu/g in the upper layers and to below detection limit in 40-50 cm depth. Enterococci were detected in only one core sample at day 53 and in none of the core samples at day 71.

E. coli was not detected in core samples before inoculation. Concentrations were above the detection limit at day 3 after inoculation (data not shown). At day 4 very high concentrations of 10^5 cfu/g wet weight were detected in the upper layers of the filter (Figure 64). Concentrations decreased with depths and were in the range of 10^3-10^4 cfu/g in 20-50 cm. E. coli survived better in the filter than the enterococci. Concentrations of 1-100 cfu/g were detected in all core samples even after 71 days of percolation.

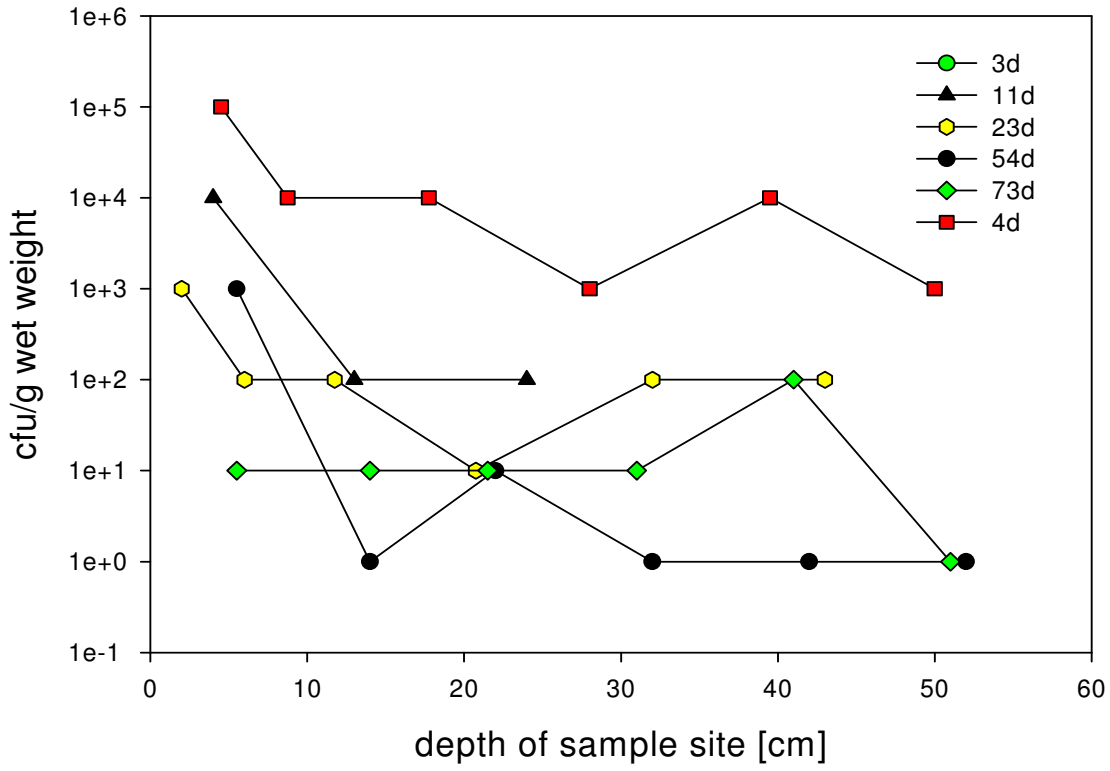


Figure 64: **Concentration of E. coli in sediment samples taken from the enclosure during the experiment (no data at day 3, detection limit 1 cfu/g).**

6.3 Summary

After a bloc injection, a part of test organisms break through the filter column synchronic with tracer salt NaCl. The filtration (retention) equilibrium occurred (was observed) within a percolation time of 12 h. The cumulative breakthrough of test organisms has marginally increased during the further operation time up to two weeks.

Despite to this early breakthrough, high retention of test organisms was determined in the enclosure by aerobic conditions which averaged to 3 log units for both coliphages and 2 log units for the indicator bacteria.

Developing a clogging layer had no clear effect on removal or mobility of test organisms.

The elimination of test organisms in the filter column was not homogenous, the highest level of retention was detected in the top layer of filtration column which declined significantly in the deeper layers tested.

On continuously contamination of enclosure with test organisms and by micro aerophile conditions corresponding to a dissolved oxygen content less than 0,2 mg/L, the removal of all test organisms decreased significantly.

Continuously inoculation of primary effluent at a concentration ranged from 2 - 10 % of filtrate volume has shown a moderate elimination rate of coliphages, approximately 2 log units during the initial phase of process. The retention of phages increased significantly, up to four log units during an operation time of 4 weeks. Removal of indicator bacteria was measured not less than four log units within this phase of operation. An increase of primary effluent rate to 10 % abruptly led to a breakthrough of all test organisms. Less than one log unit viral and bacterial contaminants were removed by further operation in enclosure.

Annex – ENCLOSURE

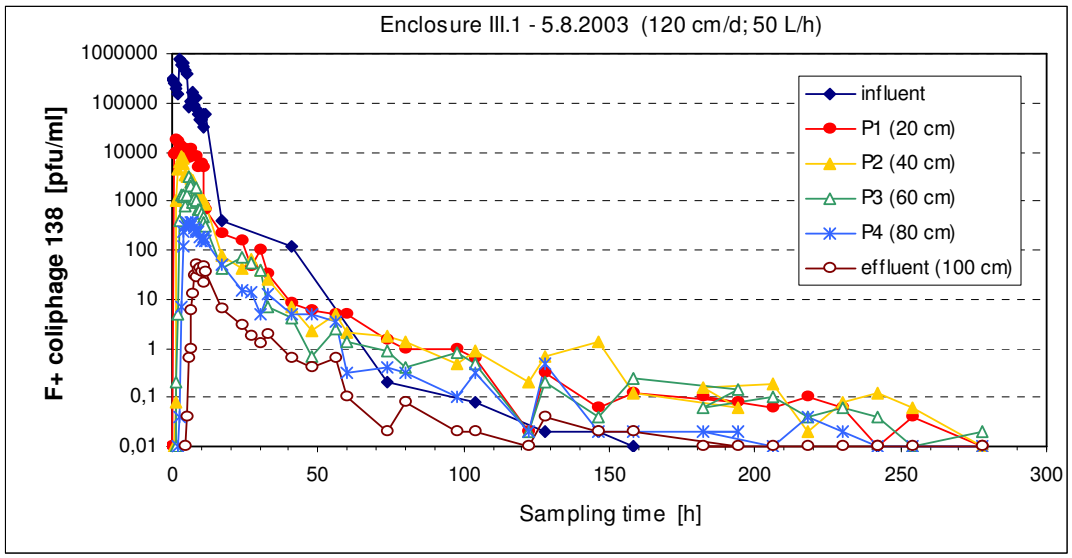


Figure 65 **Concentration of F+phage 138 at different sampling levels of the enclosure by absence of an apparent biomass on the filter surface and at a pore velocity of 360 cm/d.**

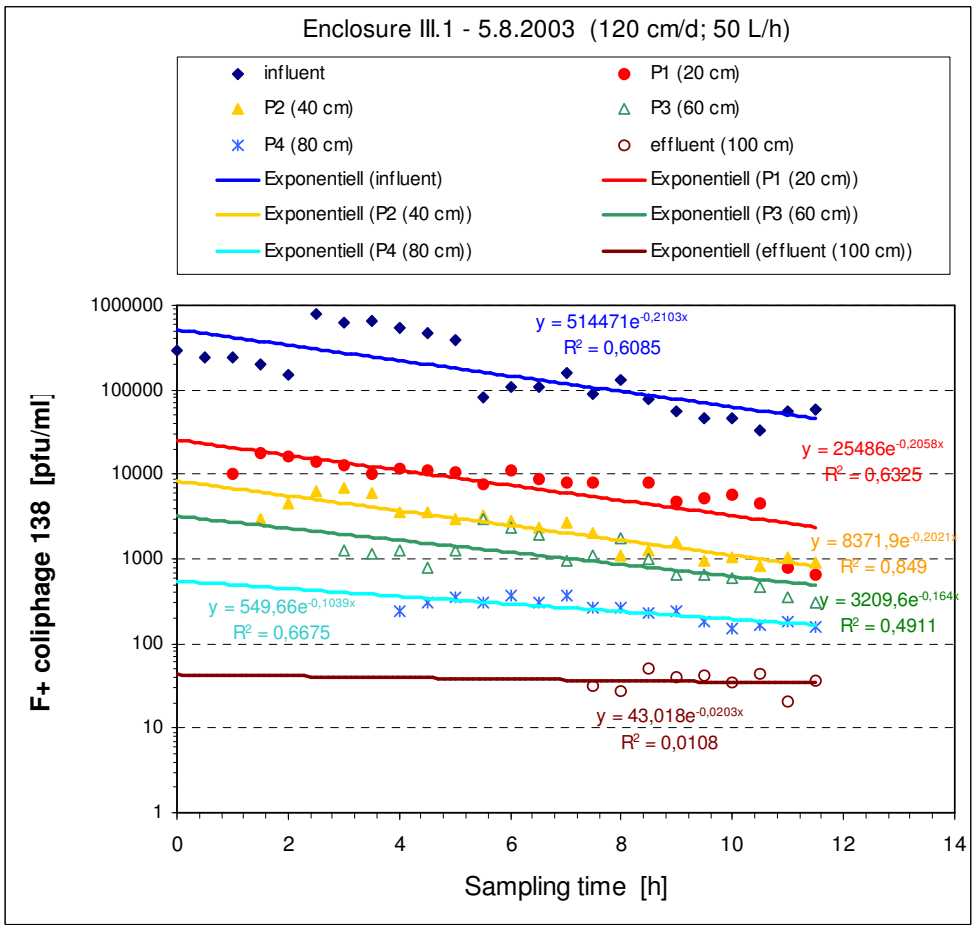


Figure 66 **Regression lines of the F+ coliphage 138 concentrations at different sampling levels of the enclosure before forming any apparent biomass and at a pore velocity of 360 cm/d.**

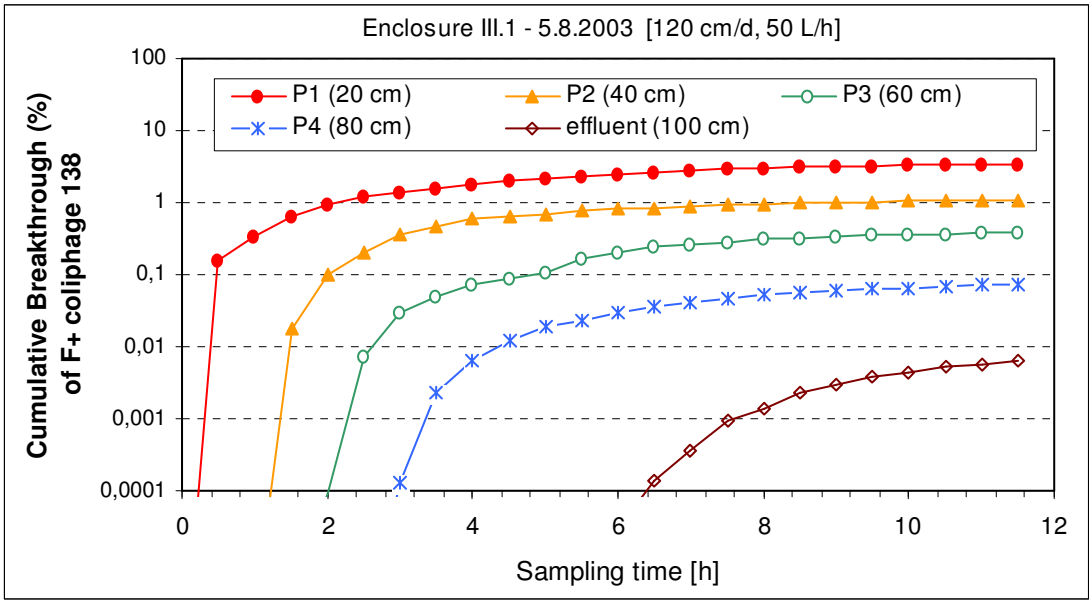


Figure 67: **Cumulative breakthrough of F+ coliphage 138 at different sampling sites of the enclosure before forming any apparent biomass and at a pore velocity of 360 cm/d.**

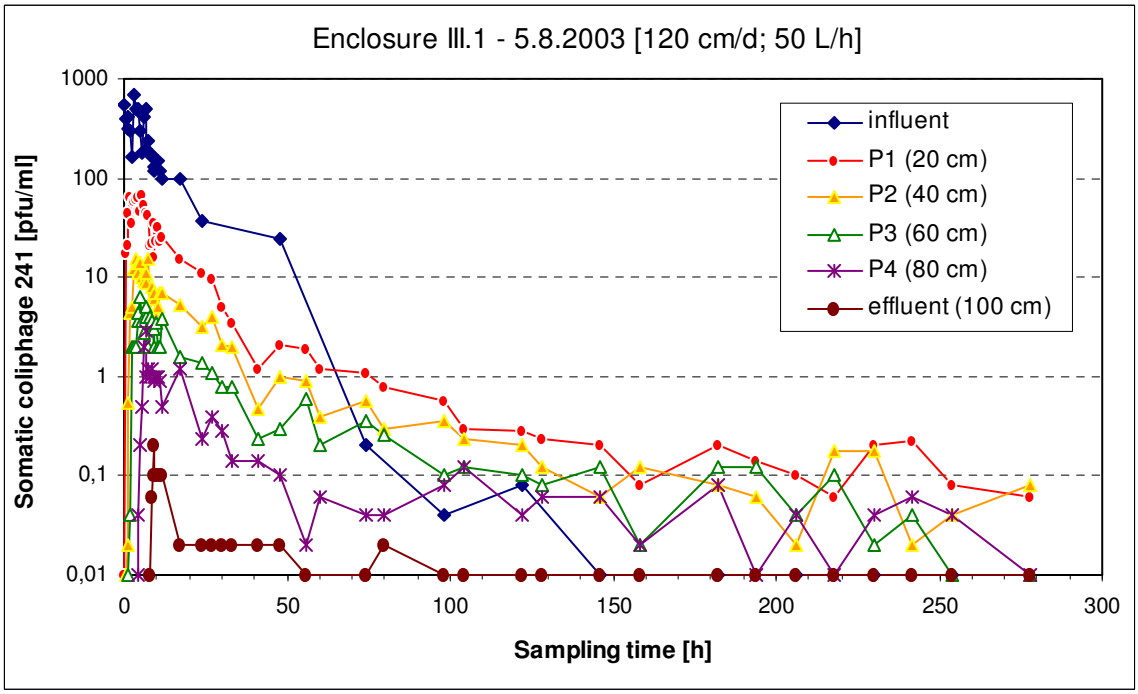


Figure 68: **Concentration of somatic coliphage 241 in different sampling levels of the enclosure before forming any apparent biomass and at a pore velocity of 360 cm/d.**

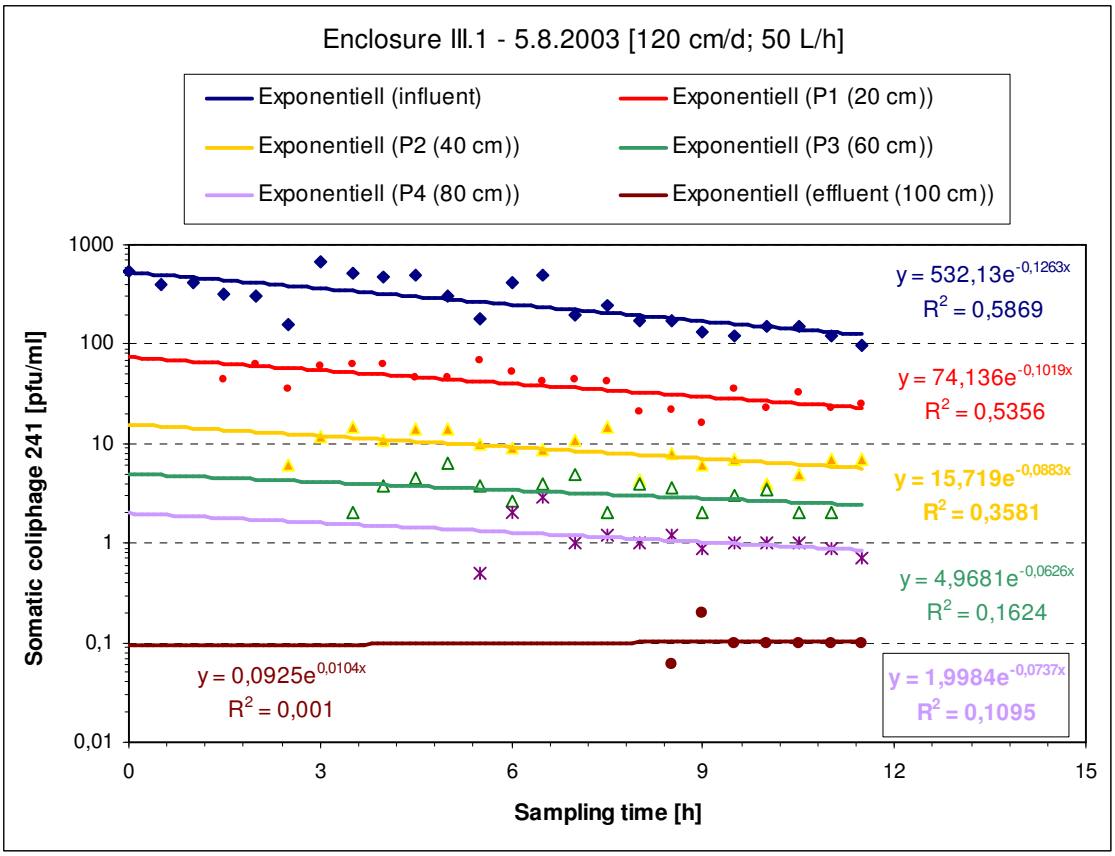


Figure 69 **Regression lines of somatic coliphage 241 concentrations at different sampling levels of the enclosure before forming any apparent biomass and at a pore velocity of 360 cm/d.**

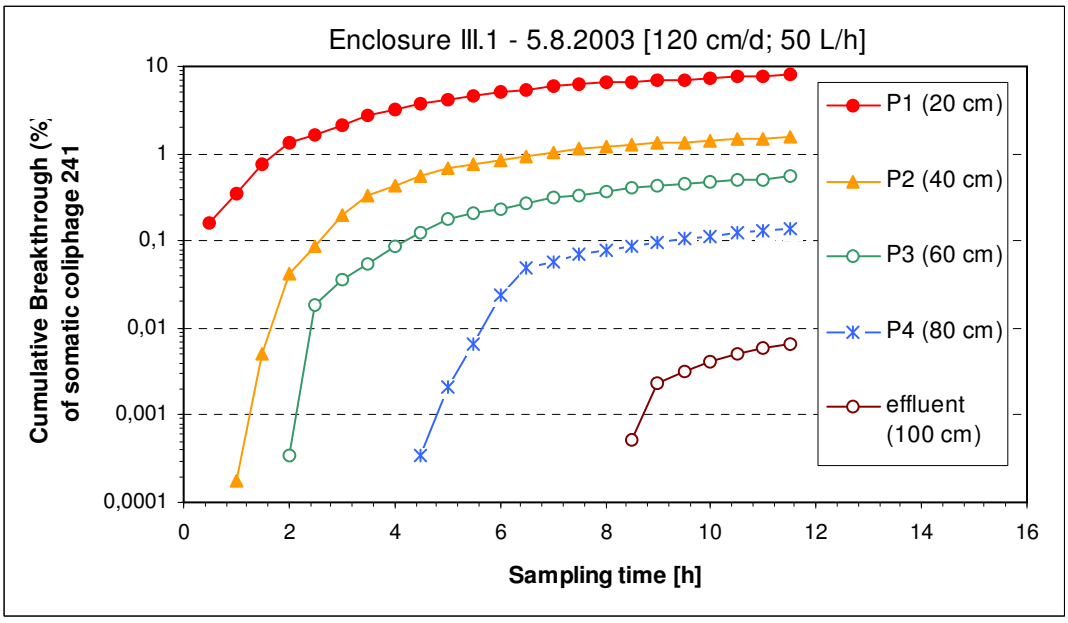


Figure 70: **Cumulative breakthrough of somatic coliphage 241 at different sampling sites of the enclosure before forming any apparent biomass and at a pore velocity of 360 cm/d.**

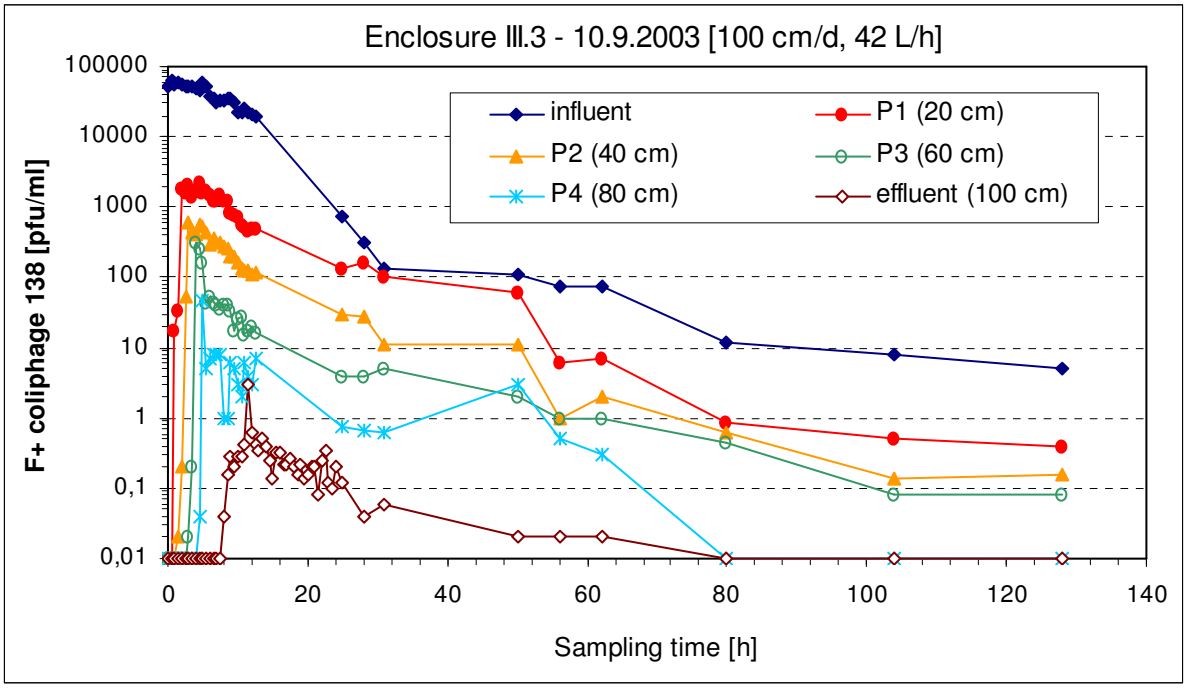


Figure 71 **Concentration of F+ coliphage 138 in different sampling levels of the enclosure after forming an apparent biomass and at a pore velocity of 300 cm/d.**

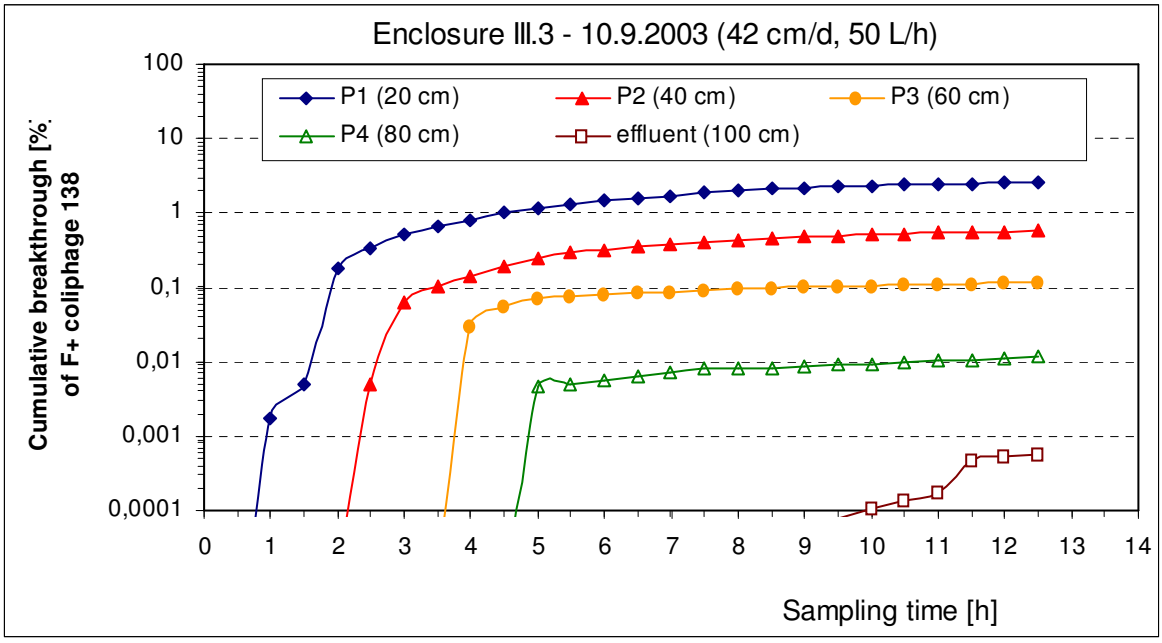


Figure 72 **Cumulative breakthrough of F+ coliphage 138 at different sampling sites of the enclosure after forming an apparent biomass and at a pore velocity of 300 cm/d.**

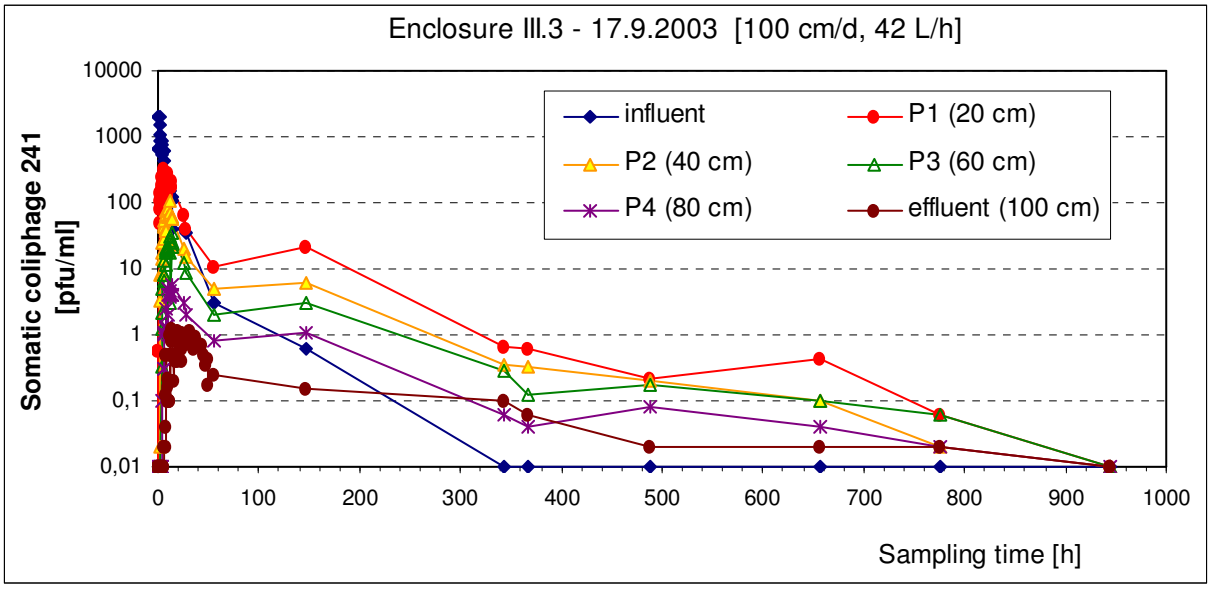


Figure 73 **Concentration of somatic coliphage 241 in different sampling levels of the enclosure after forming an apparent biomass and at a pore velocity of 300 cm/d.**

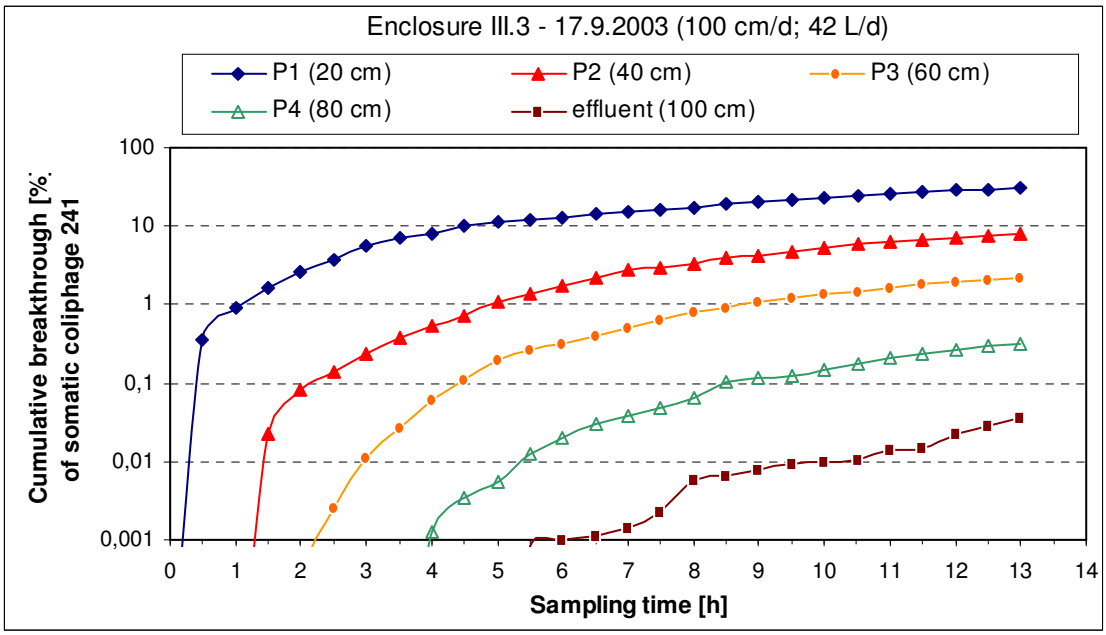


Figure 74 **Cumulative breakthrough of somatic coliphage 241 at different sampling sites of the enclosure after forming an apparent biomass and at a pore velocity of 300 cm/d.**

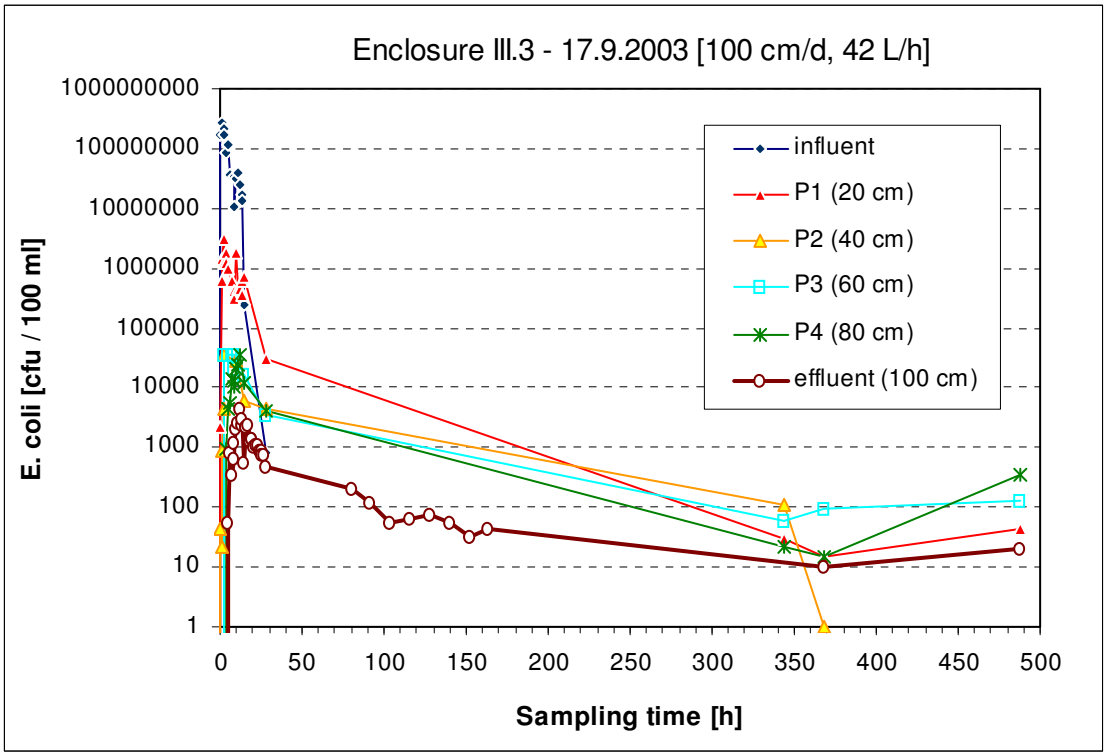


Figure 75 **Concentration of E. coli in different sampling levels of the enclosure after forming an apparent biomass and at a pore velocity of 300 cm (detection limit 10-15 cfu/100 ml)**

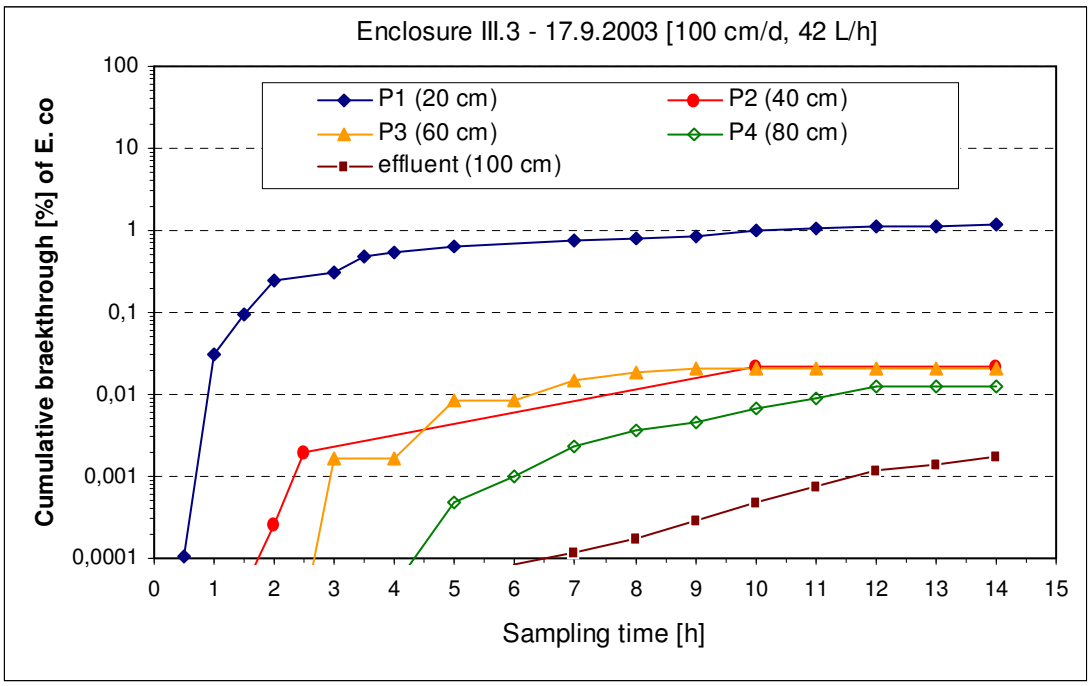


Figure 76 **Cumulative breakthrough of E. coli at different sampling sites of the enclosure after forming an apparent biomass and at a pore velocity of 300 cm/d**

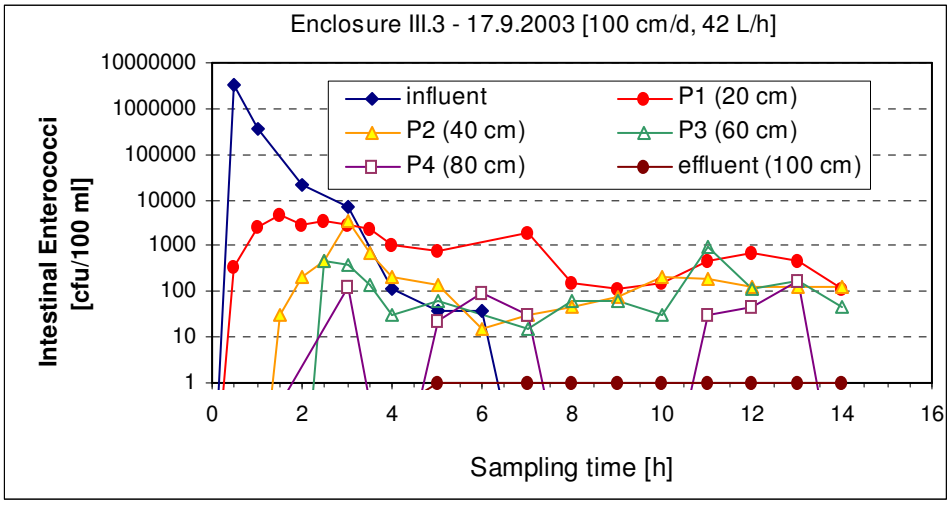


Figure 77 **Concentration of E. faecium in different sampling levels of the enclosure after forming an apparent biomass and at a pore velocity of 300 cm (detection limit 10-15 cfu/100 ml)**

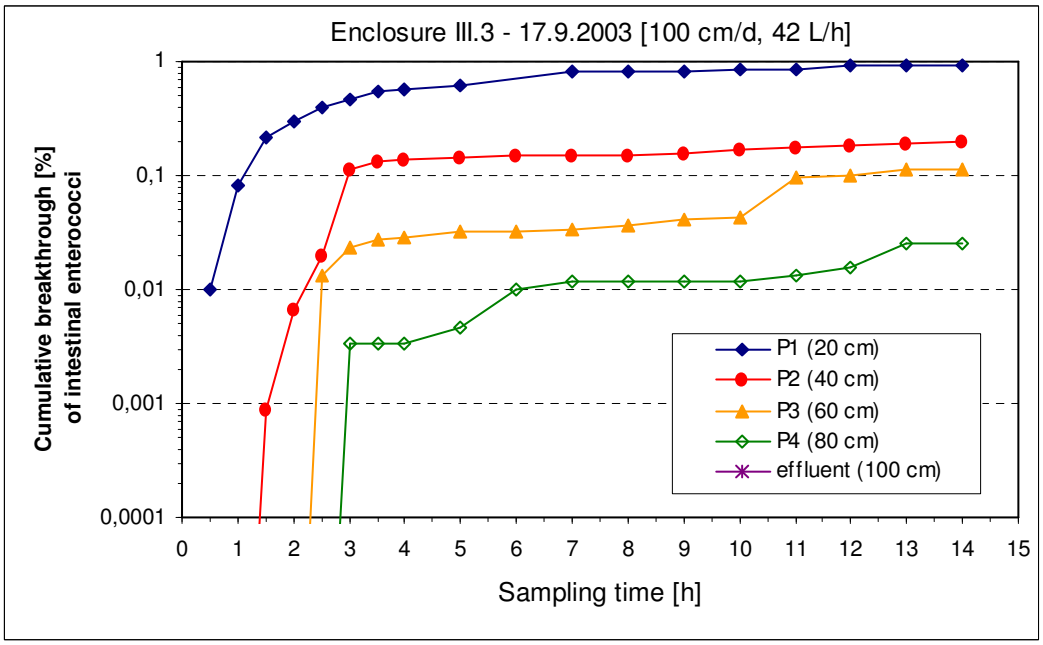


Figure 78 **Cumulative breakthrough of E. faecium at different sampling sites of the enclosure after forming an apparent biomass and at a pore velocity of 300 cm/d**

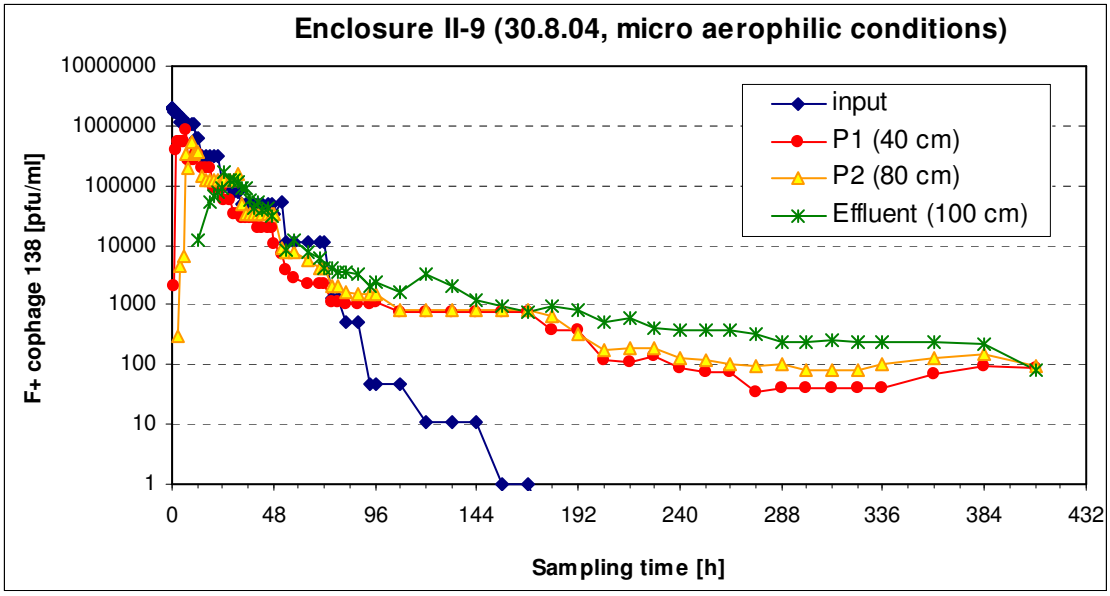


Figure 79 **Retention and transport behaviour of F+ coliphages in the enclosure II under micro aerophilic conditions at a pore velocity of 210 cm/d.**

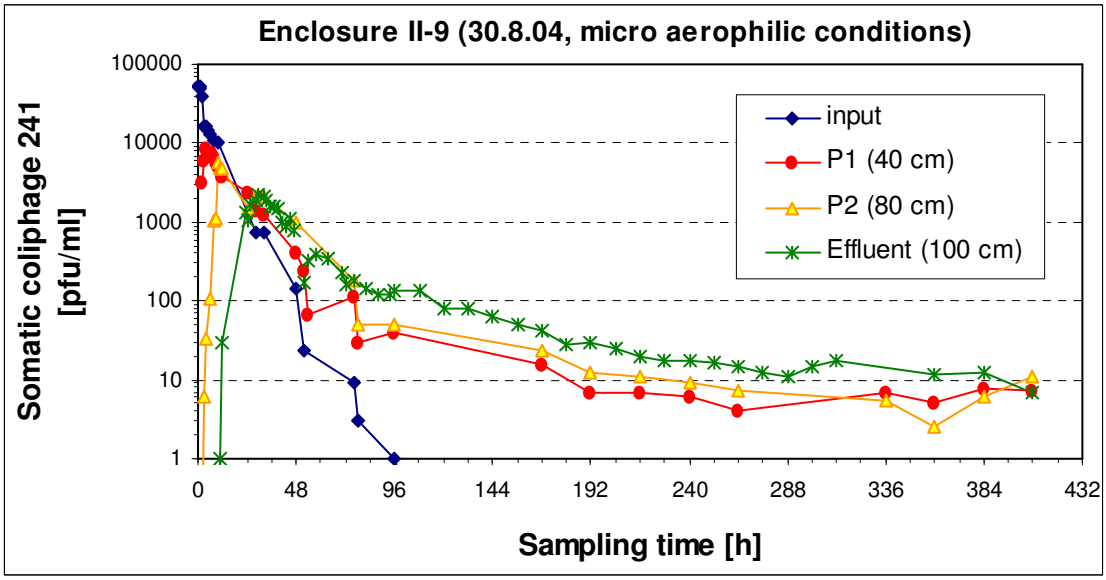


Figure 80 . **Retention and transport behaviour of somatic coliphages in the enclosure II under micro aerophilic conditions at a pore velocity of 210 cm/d.**

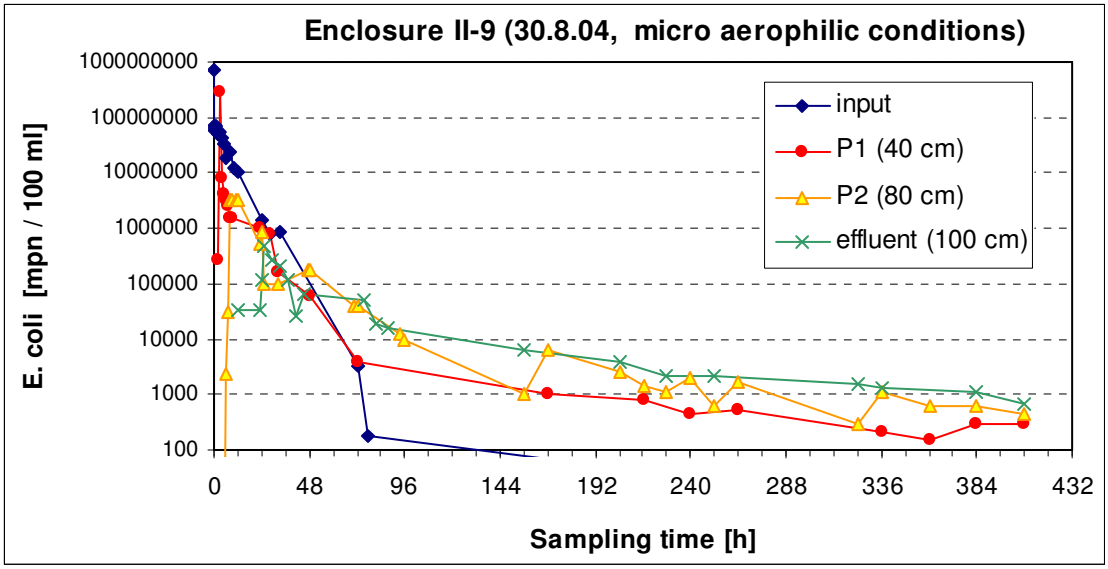


Figure 81 **Retention and transport behaviour of E. coli in the enclosure II under micro aerophilic conditions at a pore velocity of 210 cm/d.**