A series of studies conducted on laboratory-scale sand columns confirmed that *Escherichia coli (E. coli)* removals in slow-rate biological filters occur primarily at the interface (schmutzdecke) and are statistically related to operational (schmutzdecke ripening state, biological activity, temperature, and protistan abundance) and design (empty bed contact time and media grain size) parameters. Biological activity, as measured by carbon dioxide respiration in the top 2.5 cm, as well as protistan abundance in the top 0.5 cm of the schmutzdecke, correlated positively to *E. coli* removal. The role of predation deserves further investigation. The ability of a slow-rate biofilter to reestablish an effective schmutzdecke-removal layer after a scouring or scraping event was not strongly related to biomass concentrations or biological ripening conditions of the supporting media prior to scraping or scouring. Slow-rate biological filters should be operated in such a way as to enhance biological activity (in addition to biomass accumulation alone) and increase protistan abundance in the schmutzdecke.

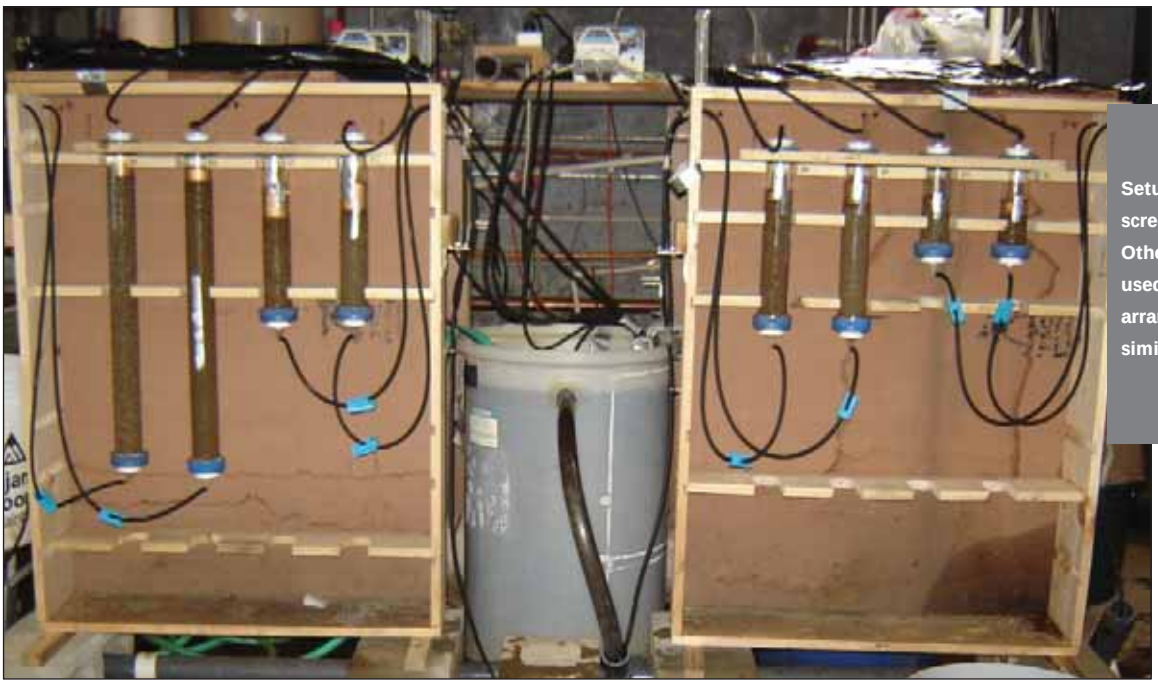
Assessing *Escherichia coli* removal in the schmutzdecke of slow-rate biofilters

BY MICHAEL UNGER

AND M. ROBIN COLLINS

The microbial removal role of the interface between the overlying water and the media bed in biological filters such as a slow sand filter (SSF) or a riverbank filtration (RBF) system has been a subject of much debate over the years. The term schmutzdecke comes from the German words "schmutz," meaning dirt, and "decke," meaning covering, and is typically applied to slow sand filtration. *The Manual of Design for Slow Sand Filtration* (the manual; Barrett et al, 1991) defines it as, "a layer of material, both deposited and synthesized, on the top of the filter bed that causes headloss disproportionate to its thickness." The nature of the schmutzdecke varies, however, based on source water characteristics. In New England, it is often gelatinous, highly biologically active, and somewhat bound to the top of the sand bed (Collins et al, 1989) Conversely, in Empire, Colorado, the schmutzdecke has been described as an "inert carbonaceous deposit" (Barrett et al, 1991). In all cases, the schmutzdecke houses a highly biologically active conglomerate of bacteria, algae, protists, and macroinvertebrates (McNair et al, 1987).

The literature is unclear about the actual definition of what constitutes a schmutzdecke and how to distinguish between it and the sand bed of an SSF. Weber-Shirk and Dick (1997) provide a detailed review of the history of the term and the discrepancies in its usage. Briefly, the schmutzdecke can be divided into two regions, a filter cake or slime layer above the sand, and the biologically active



Setup for the screening experiment. Other experiments used varying arrangements of similar filter columns.

region in the sand bed. Weber-Shirk and Dick prefer to define the schmutzdecke narrowly as the filter cake only, while the manual's definition includes the biologically active sand region because the headloss through that region is much greater than the headloss through the rest of the filter (Barrett et al, 1991).

For the purpose of this article, the manual's definition has been used. A difficulty arises when using this definition, however, because no clear delineation exists between the biologically active sand and the rest of the filter, where biological activity does extend but to a lesser degree. For the purposes of uniform comparison, when characterizing the schmutzdecke, sand was typically sampled to a depth equivalent to 7.5 min of empty bed contact time (EBCT). This EBCT was determined using operational values common to SSF. A typical hydraulic loading rate (HLR) for an SSF is 0.12 $m^3/m^2/h$, and operators will usually remove 1–2 cm of media in order to reduce headloss enough to put a filter back on line. EBCT is related to the depth of a filter, L, and the HLR by the relationship EBCT = L/HLR. For the typical SSF described previously, EBCT = (1.5 cm)/(0.12 $m^3/m^2/h$) = 7.5 min. During the research described in this article, various HLRs ranging from 0.2 m/h to 0.6 m/h were tested in different experiments, so the depth of sand sampled varied correspondingly.

Researchers have witnessed riverbeds in RBF systems behaving very similarly to a traditional SSF schmutzdecke. First, biological activity is present in both an SSF schmutzdecke and a riverbed. Dissolved organic carbon concentrations were reduced up to 50% in the "first few centimeters" during a Torgau, Germany, RBF study, and in Düsseldorf, Germany, water from the "first few decimeters" of riverbed sediment showed evidence of biodegradation (Ray et al, 2002). Second, bacterial removal occurs primarily at the water/media interface in both SSF and RBF systems. For example, during riverbank filtration in Louisville, Ky., concentrations of aerobic spores decreased by more than 2 log units in the first meter of subsurface passage and only slightly more than 1 log unit in the remaining 14 m (Wang et al, 2002).

Unfortunately, the exact role this interface layer, or schmutzdecke, exerts in microbial removals in both SSF and RBF systems has not been well-defined. The development of a schmutzdecke has been shown to alter conditions within a filter such that the applicability of conventional filtration theory (e.g., Yao et al, 1971) is questionable. Most important, the adsorption coefficient or sticking factor, α, is not uniform with depth in biological filters. Researchers of RBF have reported that attachment of viruses (Schijven et al, 1999) and bacteria (Hendry et al, 1997; Zhang et al, 2001) decrease with increasing travel distance. Schijven et al (1999) hypothesize that improved removal in early stages of transport is the result of a high concentration of "favorable" attachment sites, possibly iron oxyhydroxides. Findings by other researchers also support the theory that adsorption to favorable aluminum sites in a filter is responsible for much of the bacterial removal in biological filters treating a synthetic raw water (Weber-Shirk & Chan, 2002). Other research has shown, however, that the influence of these metal-based adsorption sites will be significantly reduced in the presence of humic acid or dissolved organic matter (Foppen et al, 2006) and "biogrowth" on the sand media (Chen et al, 1998).

Increased adsorption in the schmutzdecke may have a biological cause in addition to the physical/chemical mechanisms described here. Bellamy et al (1985) operated 30-cm-diameter pilot SSFs in parallel using the same source water and varied operational or design parameters. Cold (2–5°C) filters removed fewer coliforms than warm (17°C) filters. A filter receiving chlorinated water during ripening removed only 60% of challenge bacteria versus a control filter removing 97% and a nutrient-amended filter removing 99.9%. The authors conclude that the biological community is responsible for the improved removal. Hirschi and Sims (1994) calculated an increase in α from 0.12 to 0.51 during 54 days of ripening.

The scientific community is not entirely convinced that development of a biofilm improves bacterial removal in granular filters. For example, Weber-Shirk and Dick (1997) used azide to inhibit biological growth after ripening filters for five days. Introduction of the azide induced an immediate reduction in *Escherichia coli (E. coli)* removal. They argued that the structure of the biofilm remained, so any increase in adsorption attributable to the biofilm should have continued to contribute to removal. The azide-induced reduction in removal efficiency was immediately reversible when the azide feed was discontinued, suggesting that the only plausible biological removal mechanism was predation or nonspecific degradation resulting from antagonistic bacteria—not adsorption to a biofilm.

The development of a schmutzdecke has been shown to alter conditions within a filter such that the applicability of conventional filtration theory is questionable.

Another bacterial removal mechanism of interest in the schmutzdecke is straining. Hijnen et al (2004) found that SSFs with a schmutzdecke demonstrated a removal of bacteria 1–2 logs greater than filters without a schmutzdecke, but virus removal was unchanged by the presence of a schmutzdecke. This finding indicates that straining is important in bacterial removal at the sand–water interface as a slime (cake) layer develops, but viruses are small enough to pass without being strained. Mauclaire et al (2006) observed a decrease in hydraulic conductivity during filter runs and compared the state of the biological communities in clogged (hydraulic conductivity < 0.5 m/h) and unclogged (average hydraulic conductivity of 1.2 m/h) SSFs in Switzerland. They observed a higher abundance of bacteria and microfauna in clogged filters and attributed much of the observed decrease in hydraulic conductivity to bioclogging.

PURPOSE OF THIS RESEARCH

Establishing or reestablishing the primary bacterial removal mechanism(s) as soon as possible after SSF cleaning or riverbed scouring events is required for more efficient and reliable treatment from slow-rate biofilters. In light of these varying and sometimes conflicting removal mechanisms that have been hypothesized for slow-rate biofilters, an effort was made in the current research to evaluate the biological-based mechanisms in the uppermost regions of a biological filter (i.e., the schmutzdecke) where most of the removals are expected to take place. The specific objectives and corresponding tasks of this research included:

Objective 1: Ranking the relative importance of various media characteristics and operational conditions on biofiltration removals of *E. coli* bacteria to assist design engineers and operators enhance performance.

Objective 2: Assessing the effect of a sudden removal of the schmutzdecke on pathogen removal in the event of scouring or cleaning and a filter's subsequent ability to recover from such an event as a function of temperature.

Objective 3: Assessing *E. coli* removal mechanisms associated with the schmutzdecke biofilm. Specifically, determining first whether the extracellular polymeric substances (EPS) excreted by the schmutzdecke biofilm enhance the "stickiness" of filter media, a phenomenon referred to here as "biologically mediated adsorption," and second whether toxic substances in the schmutzdecke enhance death or inactivation of *E. coli.*

Objective 4: Estimating the potential influence of protistan predation as a pathogen removal mechanism.

GENERAL METHODOLOGY

The task of researching a deeper understanding of pathogen removal mechanisms in the schmutzdecke was approached systematically. Separate experiments were performed using 4.8-cm-diameter laboratory-scale test filter columns with spiked concentrations of *E. coli* to meet the research objectives listed previously. The results of early experiments influenced the design of later experiments. The experimental setup and laboratory methods are described in this section, and the specific experimental design and research goals of each individual experiment are provided in a later section in this article.

Experimental setup. Each sand filter column was constructed in a 4.8-cm-inner-diameter glass chromatography column[1] with tetrafluoroethylene adapters. Sand depth depended on the particular experimental design of

each study. Space (3–5 cm) was left at the top of each filter for a water reservoir. Stainless-steel screens supported the sand in the columns. Columns were incrementally packed under saturated conditions by adding approximately 50 g of sand and tapping the side of the column lightly three times between increments. The sand[2] for each column came from the same source and had similar physical properties as sand used in the construction of numerous filtration plants in New England. Sand was washed with tap water in a bucket using a forceful hose nozzle in order to remove fines. Dirty water was decanted, and sand was washed repeatedly until the wash water was no longer visibly dirty. Washed sand had an effective size of 0.39 mm and a uniformity coefficient of 2.2. Inert rubber tubing connected a constant-flow raw water tank to the top of each column, with energy provided by peristaltic pumps.[3] Raw water from the Durham, N.H., water treatment plant, which is drawn from the Oyster River, was used to ripen the filters and was spiked with *E. coli* for microbial challenges. The sand filter columns were ripened and/or challenged individually, in series, or in parallel, depending on the individual study's experimental design.

Operational parameters and raw water quality. Raw water quality was monitored daily by the staff of the University of New Hampshire (UNH)/Durham Water Treatment Plant for conductivity, alkalinity, dissolved oxygen (DO), ultraviolet absorbance at 254 nm (UV_{254}), temperature, apparent and true color, pH, and turbidity. The Oyster River reservoir, the raw water source for the plant, is a small impoundment subject to spikes in turbidity and organic content. During the two-year period of this research, turbidity typically ranged from 2 to 8 ntu with single-day spikes as high as 41 ntu. Apparent color typically ranged from 75 to 150 units with spikes above 200, and as high as 490 on one occasion. Alkalinity was low (20–50 mg/L as calcium carbonate). Additional details are available in Unger (2006).

Periodically the authors performed basic water quality analyses including DO, temperature, and turbidity on the effluent of experimental filter columns in addition to enumeration of influent and effluent challenge microorganisms. Flow rates were monitored with a stopwatch and 25-mL graduated cylinders. Flow rates varied between 6 and 18 mL/min corresponding to HLRs between 0.2 $m^3/m^2 \times h$ and 0.6 $m^3/m^2 \times h$ depending on

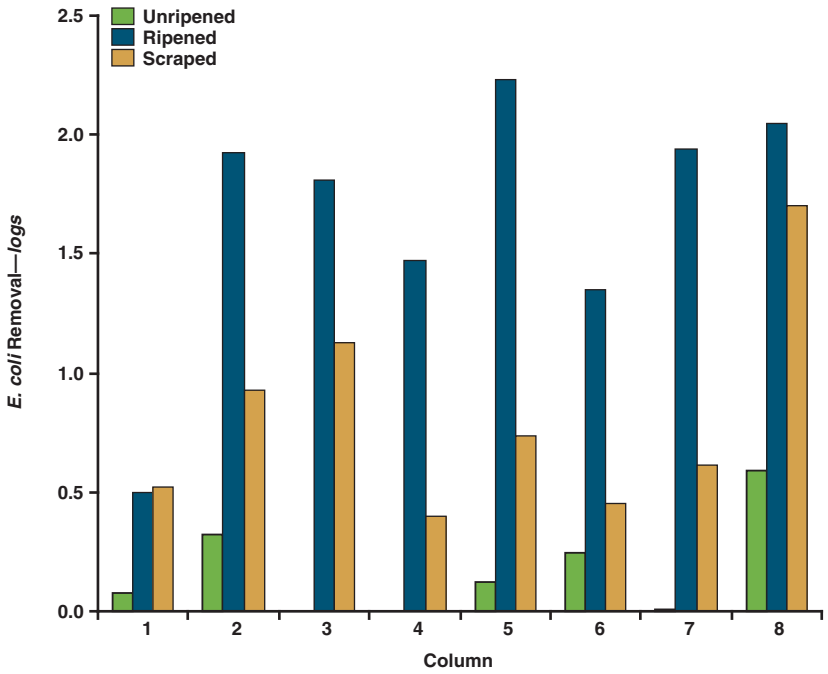
TABLE 1 Assignment of factor levels to sand filter columns for the screening experiment

Column	Size*	HLR—*m/h*	Biomass	EBCT—*min*
1	Large	0.2	Virgin	15
2	Large	0.2	Acclimated	60
3	Large	0.6	Virgin	60
4	Large	0.6	Acclimated	15
5	Small	0.2	Virgin	60
6	Small	0.2	Acclimated	15
7	Small	0.6	Virgin	15
8	Small	0.6	Acclimated	60

EBCT—empty bed contact time, HLR—hydraulic loading rate

*The entire stock was sieved through a number 16 sieve (1.18 mm). All sand retained was considered "large," and all sand passing was considered "small."

FIGURE 1 Log removal of *E coli* in designed sand columns under varying conditions



E. coli—Escherichia coli, *MPN—most probable number*

Spiking concentrations: unripened = 8.8 x 10^5 MPN/100 mL, ripened = 7.7 x 10^3 MPN/100 mL, scraped = 1.0 x 10^4 MPN/100 mL

the specific experimental design. EBCTs were in the range of 15–60 min.

Experimental challenge microorganism. A strain of *E. coli* (F-amp) resistant to ampicillin and streptomycin was cultured in tryptic soy broth in the Water Treatment Technology Assistance Center laboratory at UNH. During an experimental challenge, 20 L of raw water would be spiked with stock *E. coli* to a goal concentration of 10^4 most probable number (MPN)/100 mL. *E. coli* were enumerated using a US Environmental Protection Agency (USEPA)–approved MPN method.[4]

E. coli exhibiting the green fluorescent protein (GFP) were used as challenge organisms in some studies because they could be differentiated from naturally occurring bacteria. In a water matrix, samples were fixed with a 10% glutaraldehyde/10% cacodylic acid fixative and filtered through 0.22-µm filters. To extract *E. coli* from sand samples, 3 g were placed in a 50 mL Erlenmeyer flask. Forty millilitres of 0.1% sodium pyrophosphate solution was added, and the mixture was shaken twice on a shaker table at 320 rpm for 30 s with a 30-s pause between shakes. The flask was allowed to sit 30 s to allow the heaviest particulate matter to settle out. An appropriate volume of supernatant was then extracted, fixed, and filtered in the same manner as aqueous samples. Filters were mounted on slides for counting *E. coli*. The microscope used was equipped with a 100-W mercury lamp and a filter cube with settings of 510 nm dichroic mirror, 450–490 nm excitation, and 515 nm barrier filter.[5]

The task of researching a deeper understanding of pathogen removal mechanisms in the schmutzdecke was approached systematically.

Biofilm characteristics. Four analyses were used to characterize the biofilm colonizing filter media: a general phospholipids measurement for biomass; total carbohydrate and total protein measurements to estimate the mass of EPS excreted; and carbon dioxide (CO_2) respiration to monitor biological activity. Details of these procedures can be found elsewhere (Unger, 2006).

Toxic effects of schmutzdecke extract. An experiment with microbe-free extracts from the schmutzdecke (equivalent to 7.5-min EBCT, the top 2.5 cm in this experiment) of filter columns from the "Effect of ripening time on protistan abundance in the schmutzdecke" study (described later) tested the hypothesis that the release of toxic substances into the schmutzdecke increases *E. coli* removal. Duplicate aliquots of the extract were transferred to test tubes, spiked with *E. coli* and shaken gently (approximately 50 rpm) on a shaker table for 1.5 h, the same time as most other challenges in this research, and surviving *E. coli* were enumerated.

Enumeration of protists. For live protist counts, samples were taken from random locations within the schmutzdecke of a sand filter column using a 3-mm-diameter plastic straw to a depth of 5 mm. The mass of the samples was determined before they were diluted with water. Forty microlitres of diluted sample were placed on a slide under a 25-mm^2 coverslip. Duplicate slides were made from each sample. Slides were observed at 400× magnification. Protists were grouped into four categories: flagellates, amoebae, attached ciliates, and unattached ciliates. Protists were counted inside a whipple disk field, and fields were randomly selected until at least 300 protists were counted. The concentration of protists in the original sample was calculated using the average count per field, the ratio of the field size to slide size, and the dilution of the sample.

In other experiments, samples were quantified by fixing protists and staining with primuline, a fluorescent yellow dye that causes eukaryotes to fluoresce under a mercury lamp. Samples were shaken three times in phosphate buffer to dislodge protists. Supernatant buffer was fixed with a 10% glutaraldehyde/10% cacodylic acid fixative and then filtered through 0.8-µm filters, which were stained for 15 min with primuline according to the method in Hines (1998). The same fluorescent lamp–equipped microscope and settings were used for protist enumerations as were used for GFP *E. coli*.

DESIGN OF INDIVIDUAL EXPERIMENTAL TASKS, RESULTS, AND DISCUSSION

Objective 1: Ranking operational and filter design parameters. *Screening Experiment.* The first study was a screening experiment, in which an orthogonal array of sand filter columns was used to evaluate the effect of selected media characteristics (sand grain size and amount of attached biomass) and operational parameters (HLR and EBCT) on *E. coli* removal. Factors were assigned as shown in Table 1.

Log-removal results and spiking concentrations for three stages of filter ripening are presented in Figure 1. Filters referred to as "unripened" were challenged with raw water spiked with *E. coli* 30 min after packing the columns with sand and placing them on line. Filters then operated for three weeks filtering Oyster River water before being challenged again ("ripened"). An amount of sand equivalent to 7.5-min EBCT was scraped from the top of each column, and the filters were operated for an additional 24 h before being challenged a third time ("scraped"). Analysis of variance (ANOVA) of the four factors mentioned (media size, attached biomass, HLR,

and EBCT) was conducted on log-removal data from the challenges of ripened and scraped filters. Table 2 ranks the relative importance of each experimental factor and two-factor interactions for the ripened and scraped filters.

EBCT and grain size were found to be the most significant factors for explaining the variation in *E. coli* removals from the ripened columns. Bellamy et al (1985) reached the same conclusion using 30-cm-diameter pilot-scale filters, which suggests that the laboratory-scale filters used for the current research perform similarly to larger filters. However, the 4.8-cm-diameter laboratory-scale columns used in the current research give a conservative estimate of removal because their larger circumference-to-surface-area ratio allows more opportunity for short-circuiting on the laboratory-scale column walls; roughening the glass walls would have helped minimize short-circuiting. The relatively low error terms in Table 2, however, imply that the experimental factors studied adequately explained the variation in *E. coli* removals. Typically, experimental errors < 15% imply that no major factors were excluded, the factors studied were adequately controlled, and the analytical errors were minimal (Ross, 1996). For the scraped filters, error was < 15%, and for the ripened filters, error was only slightly > 15%.

TABLE 2 ANOVA and ranking of experimental factors and interactions for ripened and scraped columns

Ripened Filters

Source	DF	Sum of Squares	F Ratio	Probability > F	% Contribution
EBCT	1	1.623	18.940	0.0024*	39.4
Size	1	1.474	17.195	0.0032*	35.8
HLR	1	0.171	1.991	0.1959	4.1
HLR × EBCT	1	0.144	1.677	0.2314	3.5
Error	8	0.683			16.6

Scraped Filters

Source	DF	Sum of Squares	F Ratio	Probability > F	% Contribution
EBCT	1	2.505	40.306	0.0004*	63.5
HLR	1	0.315	5.073	0.059†	8.0
Size	1	0.264	4.255	0.078†	6.7
Size × EBCT	1	0.138	2.214	0.1804	3.5
Block‡	1	0.178	2.867	0.1343	4.5
Size × HLR	1	0.082	1.316	0.2890	2.1
Error	7	0.435			11.0

ANOVA—analysis of variance, DF—degrees of freedom, *F* ratio—a test statistic for comparing multiple independent variables, EBCT—empty bed contact time, HLR—hydraulic loading rate

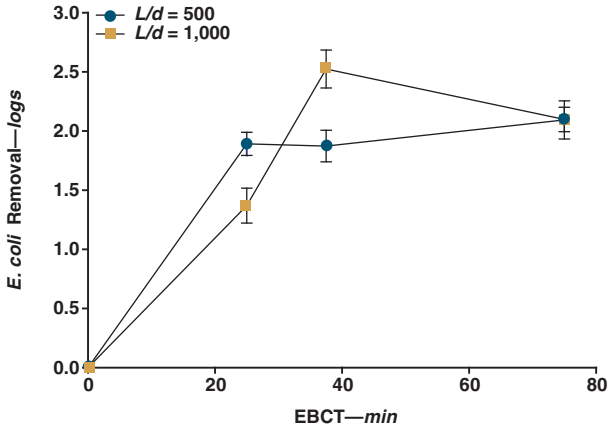
*Significant at 95% confidence level
†Significant at 90% confidence level
‡Block term accounts for differing conditions (raw water, influent *Eschirichia coli* concentration) between two separate challenges.

For recently scraped filters, the optimal settings were smaller sand and longer EBCT, as for the ripened case, but also the faster hydraulic loading rate of 0.6 m/h. The improved removal at higher loading rates is an unexpected result because it would be assumed that slower flow rates should theoretically allow more time for *E. coli* to attach to the sand or be consumed by predators. However, a long EBCT and fast HLR correspond to a deeper filter as shown by the relationship L = HLR × EBCT, in which L is the filter depth. So, in fact, the result that removal in scraped filters is greatest for long EBCTs and high HLRs further demonstrates the need for a schmutzdecke because a deeper bed is needed to obtain equivalent removals when the schmutzdecke is not fully developed. In other words, a conservative approach to maximize removals is to increase bed depth at higher HLRs. However, similar removals can be achieved for shallower beds if the HLR is reduced to maintain an equivalent EBCT.

Empty bed contact time and grain size were found to be the most significant factors for explaining the variation in Escherichia coli removals from the ripened columns.

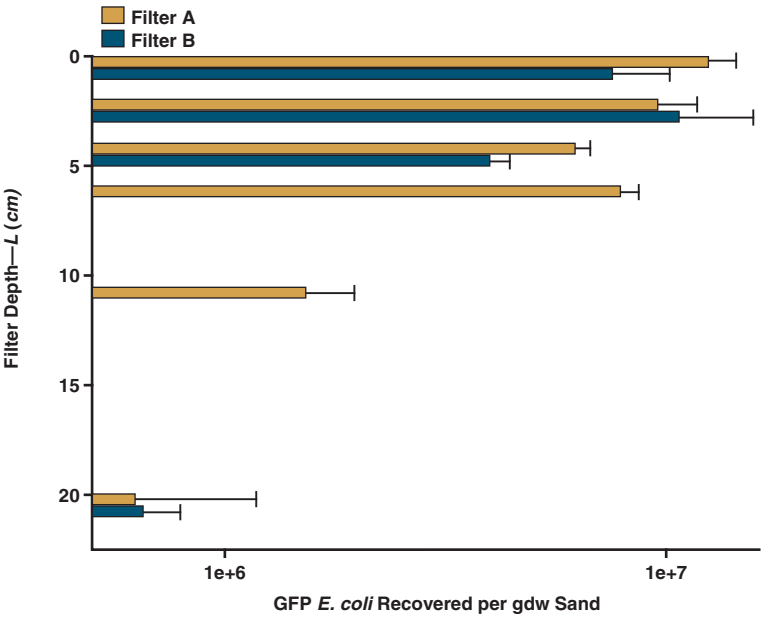
In addition, the scraped filters in this experiment were allowed to ripen for 24 h before being challenged. As such, the ANOVA of scraped filters assessed ripening rate, not steady-state filtration. The conclusion that filters operating at higher HLRs for 24 h after scraping removed more *E. coli* implies that operators may be able to run scraped filters at faster loading rates to ripen filters more quickly without compromising removal efficiency, so long as the filters' EBCT is adequate. A study by Thames Water (United Kingdom; Renton et al, 1994) supported this finding. In that study, mean loading rates of 0.36 m/h were achieved for full-scale filters, with individual filters operating at peak HLRs as high as 0.5 m/h. The authors

FIGURE 2 *E. coli* removal with varied filter length-to-sand media effective size ratios (*L/d*) and EBCT



d—*sand media effective size, EBCT—empty bed contact time,* E. coli—Escherichia coli, L—*filter length,*

FIGURE 3 Distribution of GFP challenge *E. coli* recovered from two duplicate filters operated at 0.6 m/h HLR



E. coli—Escherichia coli, *EBCT—empty bed contact time, gdw—gram dry weight, GFP—green fluorescent protein, HLR—hydraulic loading rate,* L—*filter length*

The operationally defined schmutzdecke (7.5 min EBCT) at 0.6 m/h HLR is 7.5 cm. Error bars indicate standard deviations determined from triplicate extractions at each depth from each filter. Bars are plotted at the midpoint of the range of sampling depth.

state that filter clogging and not water quality degradation limited the maximum loading rate. They hypothesize that faster loading rates accelerate ripening by increasing the flux of organic substrate, oxygen, and nutrients required for biofilm development.

Influence of filter-depth-to-media-diameter (L/d) ratio and EBCT. A follow-up experiment sought to narrow the scope of the screening experiment by focusing on the effect of EBCT and *L/d*—the ratio of the depth of the filter bed (*L*) to the effective diameter of the media (*d*)—on *E. coli* removal by SSF. *L/d* was chosen as an experimental factor because it incorporates both EBCT and grain size and because it has been used as a measure to predict filter performance in applications other than SSF. For example, *L/d* is used as a general rule for designing rapid-rate filters. Filter media surface area is not linearly dependent on grain size, but *L/d* is a rough measure of filter surface area applicable to comparing filters of the same media. (MWH, 2005)

E. coli log removal is plotted for each combination of design factors on Figure 2. These results indicate that the *L/d* ratio did not significantly influence *E. coli* removal compared with EBCT. In addition, above a certain EBCT (20 min for this study), *E. coli* removal appears to plateau. The occurrence of such a plateau in *E. coli* removal with increasing EBCT reemphasizes the importance of the schmutzdecke. Once below the schmutzdecke, adding filter depth at a constant hydraulic loading rate (i.e., increasing EBCT) does not proportionately improve removal. Thus removal in the schmutzdecke is not dependent on depth-filtration variables.

Objective 2: Assessing *E. coli* removal with depth, filter scouring and recovery, and temperature influence. ***Distribution of captured* E. coli.** Previous research summarized in the manual (Barrett et al, 1991) has demonstrated that 1–3 orders of magnitude more bacteria are removed in the schmutzdecke than in the rest of the filter. The distribution of captured *E. coli* with depth should follow the observed removal trend with

depth. As such, it was expected that extracting captured *E. coli* from various depths in a filter with a surfactant would yield much higher numbers in the schmutzdecke than in deeper sections. *E. coli* expressing the gene for the GFP were used as challenge organisms, so they could be enumerated independently of naturally occurring bacteria.

The distribution of recovered GFP *E. coli* confirmed the hypothesis that a significantly greater number of challenge bacteria were entrapped in the schmutzdecke (equivalent to 7.5 min EBCT, the top 7.5 cm at 0.6 m/h HLR) than at other depths as shown in Figure 3. Filters A and B were duplicate filters 22.5 cm deep operated identically at an HLR of 0.6 m/h, ripened for 15 days from May 23 to June 6, 2006, and challenged with a spiking concentration of 3.5×10^7 GFP *E. coli*/mL.

In addition to the overarching conclusion that the schmutzdecke is responsible for most *E. coli* removal, two major trends can be observed from these results:

- Despite an order of magnitude greater removal in the schmutzdecke, a large number of bacteria reached and attached to the lower region of the 22.5-cm test filter, and a large number also escaped the filter (1.3×10^6/mL for filter A and 1.6×10^6/mL for filter B, respectively). Such incomplete removal reinforces the recommendation of slow sand filter standards such as the IRC (1989) to maintain a minimum bed depth during normal operation (typically 45 cm).
- In both filters, the concentration of challenge bacteria attached to the slime or cake layer (L = 0–1 cm) was not significantly different from the concentration in the biologically active sand region (L = 2–3 cm) below the slime layer. This result contradicts the hypothesis put forth (e.g., Foppen et al 2005) that straining in the slime layer is primarily responsible for *E. coli* removal. It also reinforces the need to define the schmutzdecke to include the biologically active sand region below the surface slime or cake layer.

Scouring simulation. The screening experiment (as previously discussed) had indicated that scraping can significantly reduce *E. coli* removal. To examine this result in more detail, a scouring simulation was conducted with the goals of

- first, further quantifying pathogen removal with depth in a biological filter;
- second, reevaluating the ability of a filter to recover from a sudden loss of the schmutzdecke, for example riverbed scouring or SSF scraping; and

FIGURE 4 Schematic of filter arrangement for parallel (A) and series (B) operation during the scouring simulation experiment

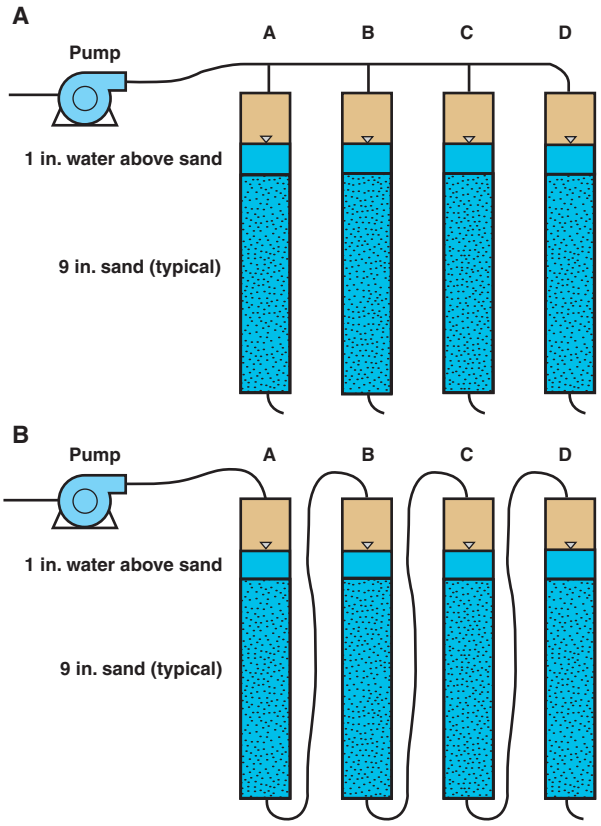
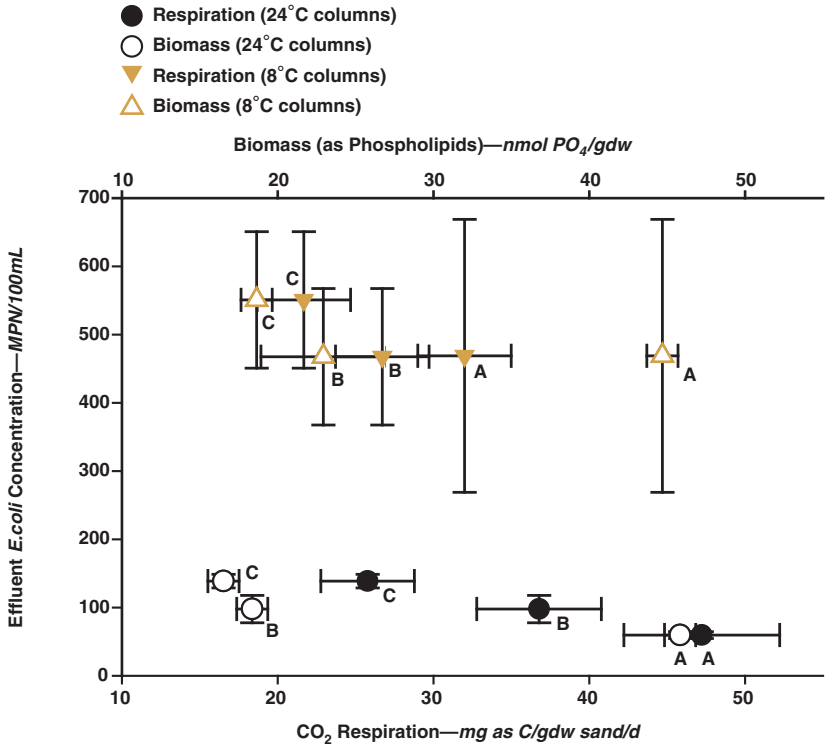


TABLE 3 Relationship between total coliform removal and biomass (as phospholipid) for various filter-ripening conditions during scouring simulation

Column Parameters	Challenge in Parallel After 2 Weeks' Ripening in Series		Challenge in Parallel After 2 Weeks' Ripening in Parallel	
	Log Removal of Total Coliforms	Biomass, Top 2.5 cm ***nmol PO_4/gdw***	Log Removal of Total Coliforms	Biomass, Top 2.5 cm ***nmol PO_4/gdw***
Column A—*0.0–22.5 cm*	1.3	36 ± 2	1.6	42 ± 4
Column B—*22.5–45.0 cm*	0.8	14 ± 7	1.6	29 ± 2
Column C—*45.0–67.5 cm*	0.2	9 ± 1	1.4	31 ± 2
Column D—*67.5–90.0 cm*	0.3	11 ± 4	1.6	39 ± 2
Full train of four challenged in series—*90 cm total*	2.1			

gdw—grams dry weight, PO_4—phosphorus (phosphate)

FIGURE 5 Correlation between schmutzdecke biofilm and *E. coli* removal in sand columns at different temperatures



C—carbon, CO_2—carbon dioxide, E. coli*—Escherichia coli, gdw—grams dry weight, MPN—most probable number, PO_4—phosphorus (phosphate)*

Influent concentration was 21,000 ± 6,000 MPN/100 mL for cold columns (8°C) and 19,000 ± 3,000 MPN/100 m/L for warm columns (24°C). The vertical axis shows the effluent concentration; lower values indicate better removal. Letters correspond to column identification in Table 4.

• third, assessing the effect of initial biomass levels in the filter on ripening.

The arrangement of columns for the scouring simulation experiment is shown schematically in Figure 4. Filter columns ripened in series (Figure 4, part B) to simulate a single, deeper filter for two weeks. Each section was then challenged individually (in parallel) with *E. coli* to assess removal with depth. The columns were then ripened in parallel (Figure 4, part A) for two weeks before a second challenge. Selected results for the series- and parallel-ripened columns are shown in Table 3. For the filter sand columns ripened in series, the majority of total coliform removals and biomass content (as quantified by phospholipids) was again associated with the first column (A), which contained the schmutzdecke.

The original amount of biomass on the sand of a filter prior to ripening did not appear to have any influence on the filter's ability to remove coliforms after two weeks of additional ripening in parallel. Total coliform removal in column A, which in the first two weeks had ripened significantly compared with the following three columns, was equivalent to removal in the later three columns. This result suggests that both an SSF can recover from scraping and an RBF can recover from scouring in two weeks or less under conditions similar to those used in this study, and this recovery is independent of initial filter biomass levels as quantified by phospholipids. It is noted that this simulation did not reproduce the disturbance to the filter bed that would occur during a full-scale scouring or scraping event.

Influence of temperature on schmutzdecke redevelopment. In light of the first scouring simulation result indicating that two weeks of ripening was sufficient to ripen a filter regardless of its initial biological state, a second scouring simulation was conducted. The initial ripening time of two weeks with columns in series was repeated, but the ripening period with columns in parallel was shortened to four days. In addition, to evaluate the influence of temperature on filter ripening, the four days of parallel ripening were conducted at two temperatures: 8 and 24°C. Results are shown in Figure 5 and Table 4 and can be summarized as follows:

• *E. coli* removals in the first column (A)—meant to simulate the top of a full-scale filter—and the remaining columns (B and C) were not significantly different even though the first column received two additional weeks of ripening time at the top of the filter. This result is in agreement with that of the first scouring simulation, but in this second simulation, columns B and C were allowed to ripen only four days in parallel as opposed to 14 days for the first simulation.

Operators need to be aware that scraping invariably reduces a filter's ability to remove *Escherichia coli*.

• Warm (24°C) biological columns outperformed colder (8°C) biological columns with respect to *E. coli* removal. This result is in agreement with previous research that had shown increasing removal of viruses (Poynter & Slade, 1977) and total coliforms (Bellamy et al, 1985) in slow sand filters as raw water temperature increased. Pyper (1985) observed the worst removal of *Giardia* cysts when the temperature was below 1°C.

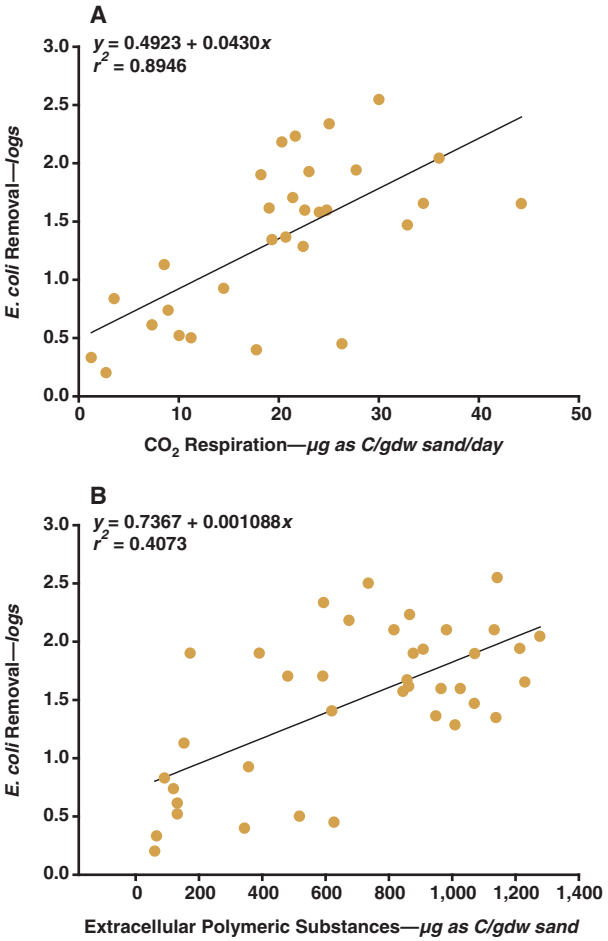
• Biomass levels, as quantified by phospholipids, and EPS, as quantified by carbohydrates and proteins, were not significantly different among respective cold and warm biological columns under the conditions of this study. The activity of the biological columns, however, as quantified by CO_2 respiration, was higher in the warmer columns.

Objective 3: Assessing various bacterial removal mechanisms associated with the schmutzdecke. As previously explained, the diverse environment of the schmutzdecke provides many possible mechanisms for enhanced bacteria removal. In light of the previous results that most removal occurs in the schmutzdecke and that temperature affects *E. coli* removals, it was decided that the extent of schmutzdecke development needed to be assessed and individual components of the schmutzdecke be compared with removal. First, measures of biomass, biological activity, and EPS mass were compared with *E. coli* removal in order to investigate whether the presence of EPS and/or an active biofilm enhanced removal beyond the presence of biomass alone. Correlations would provide evidence of biologically mediated adsorption. Next, the possibility of exotoxin release by antagonistic organisms in the schmutzdecke biofilm was investigated. Finally, it was hypothesized that the biofilm supports a large population of protists, some of which either intercept bacteria in the pore water or graze on the attached biofilm, clearing adsorption sites for incoming bacteria. Each of these three removal mechanisms was evaluated in turn.

Correlating schmutzdecke biological characteristics to **E. coli** *removal.* The first step in assessing the role of the biofilm in bacterial removal in the schmutzdecke was to correlate various indicators of the biofilm's development to *E. coli* removal. Biological activity in the schmutzdecke, as measured by CO_2 production, correlated well to *E. coli* log removal (Figure 6, part A). The mass of EPS in the schmutzdecke as measured by carbohydrates and proteins did not show a significant correlation to *E. coli* removals (Figure 6, Part B). Although a linear correlation did not exist between phospholipids and *E. coli* log removal, an "exponential rise to max" nonlinear regression fit the phospholipid data better (Figure 7), suggesting that not all of the biomass (as quantified by phospholipids) contributes to *E. coli* removal.

The absence of a correlation between EPS and *E. coli* removal supports the claims of Weber-Shirk and Dick (1997) that the development of a schmutzdecke biofilm does not improve *E. coli* removal. Vandevivere and Baveye (1992) also found that contaminant removal and hydraulic conductivity did not decrease when a biofilm developed because much of the pore space was not filled. However, they investigated dissolved—not particulate—contaminants. Therefore, while it is true that the schmutzdecke biofilm does not constrict all pore spaces, it is also premature to assert that the biofilm plays no role in increasing adsorption. In the present study, total biomass and the total mass of EPS were measured in composite samples taken from the full column diameter and a depth

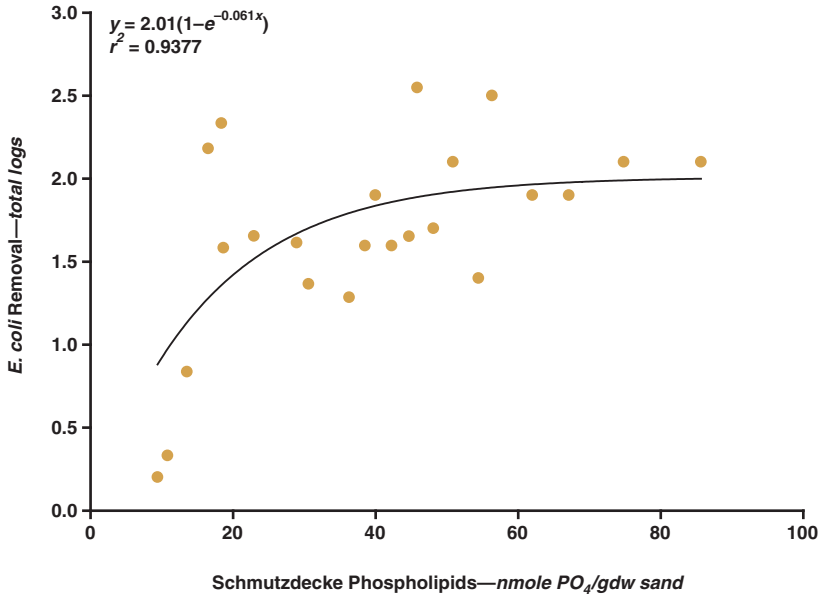
FIGURE 6 Linear correlation between log *E. coli* removal in a filter and either respiration (A) or extracellular polymeric substances (B) measured as total carbohydrate and total protein* in the schmutzdecke of sand columns†



C—carbon, CO_2—carbon dioxide, EBCT—empty bed contact time, E. coli—Escherichia coli, gdw—grams dry weight, HLR—hydraulic loading rate

**Normalized to units as C*
†Equivalent of 7.5 min. EBCT (the top 2.5 cm at 0.2 m/h HLR)

FIGURE 7 Exponential rise to maximum fit for biomass (as phospholipids)*



EBCT—empty bed contact time, E. coli*—*Escherichia coli*, gdw—grams dry weight, HLR—hydraulic loading rate, PO_4—phosphorus (phosphate)*

**Measured in the top 7.5 min. EBCT (2.5 cm at 0.2 m/h HLR)*

equal to 7.5 min EBCT (2.5–7.5 cm). More precise tests and a better spatial distribution of sampling points would help to better answer whether the biofilm contributes to increased adsorption. Thus, a biological removal mechanism appeared to contribute to *E. coli* removal, but the nature of the action exerted by the biomass was not apparent, so experiments were designed to examine the possibility of toxic excretions and protistan predation.

Toxic effects of schmutzdecke extract. Microbe-free extracts were prepared from the schmutzdecke of filter columns as described in the "General Methodology" section. The number of *E. coli* spiked into each extract did not decrease significantly compared with the control ($p = 0.6538$), implying the schmutzdecke extract was not responsible for *E. coli* death by toxic exposure. However, the exact conditions (e.g., temperature) inside the filters were not reproduced in this laboratory experiment; it is possible that death caused by conditions in a filter other than the presence of toxic materials contributes to *E. coli* removal in biological filters. Additional details on this experiment may be found elsewhere (Unger, 2006).

Objective 4: Estimate potential influence of protistan predation. Finally, the hypothesis that the schmutzdecke provided an environment for a large number of protists—and especially phagocytic heterotrophs—responsible for consuming *E. coli* was tested.

Protist enumeration. Flagellates, amoebae, and ciliates were counted in live schmutzdecke samples from a filter column that had been filtering raw water for 61 days from Oct. 7 to Dec. 7, 2005, as described previously and in Unger and Collins (2006). Flagellates were at least one order of magnitude more abundant than amoebae and ciliates. The population of flagellates in the top 5 mm of the schmutzdecke was calculated to be 4×10^6 to $8 \times 10^6/cm^2$ of filter cross sectional area. A first estimate of consumption rate was calculated using the size-specific uptake rate reported

TABLE 4 Temperature influence on log removal of *E. coli* and biomass distribution in sections of a sand filter during scouring simulation

Column Parameters	*E. coli* Log Removal	Biomass *nmol PO_4/gdw*	Carbohydrate *µg as glucose/gdw*	Protein *µg as BSA*/gdw*	Activity *µg C respired/gdw/day*
Cold (8°C)					
Column A—*0.0–22.5 cm*	1.7	45 ± 1	1,600 ± 200	12,400 ± 800	32 ± 3
Column B—*22.5–45.0 cm*	1.7	23 ± 4	900 ± 40	8,800 ± 500	27 ± 3
Column C—*45.0–67.5 cm*	1.6	19 ± 1	620 ± 40	8,900 ± 600	22 ± 3
Warm (24°C)					
Column A—*0.0–22.5 cm*	2.5	46 ± 1	1,700 ± 100	11,300 ± 500	48 ± 5
Column B—*22.5–45.0 cm*	2.3	18 ± 1	1,100 ± 400	5,700 ± 400	37 ± 4
Column C—*45.0–67.5 cm*	2.2	17 ± 1	620 ± 90	7,000 ± 300	26 ± 3

BSA—bovine serum albumin, *E. coli—Escherichia coli*, gdw—grams dry weight, PO_4—phosphorus (phosphate)

*Protein standard contained in the Modified Lowry Total Protein test kit (Pierce Protein Research Products, Rockford, Ill.) used for this research.

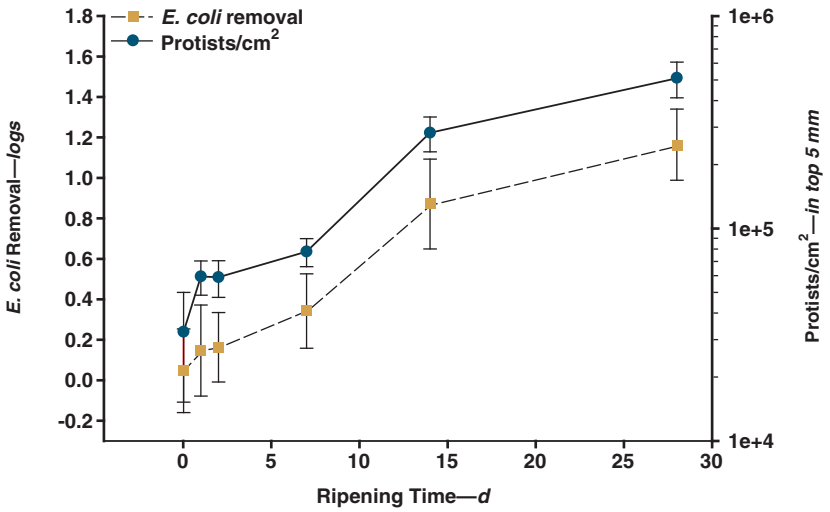
by Kinner and co-workers (1998) of 0.77 bacteria/flagellate/hour, based on laboratory grazing experiments in flasks using protists extracted from a wastewater-contaminated sand and gravel aquifer. This uptake rate is considered conservative because it is lower than other uptake rates reported in the literature and specific to a size range of bacteria (0.8–1.5 µm), which includes *E. coli*. Multiplying the concentration by the uptake rate results in a potential bacterial consumption rate of 3×10^6 to 7×10^6/cm^2 filter cross section/hour. An actual *E. coli* removal rate of 1.3×10^2 bacteria/cm^2/hour was observed, which is four orders of magnitude less than the calculated potential consumption rate.

Viewed from the perspective of clearance rate, however, the potential of the protists in the schmutzdecke to remove bacteria appears more limited. Kinner et al (1998) also report a clearance rate of 1.4 nL/flagellate/hour. At this rate, the number of flagellates counted in the top 0.5 cm of the schmutzdecke could clear between 5.6×10^6 and 1.1×10^7 nL/hour. The feed rate of 0.2 m^3/m^2/hour is equal to 2×10^7nL/hour. Thus, the flagellates counted could be expected to clear roughly one quarter to one half of the influent challenge solution of bacteria.

Effect of ripening time on protistan abundance in the schmutzdecke. In light of the large disparity between potential and observed uptake, a follow-up study was undertaken to explore the connection between *E. coli* removal and protistan abundance after various filter run times. Seven filters were each ripened for different lengths of time ranging from 0 to 30 days. Immediately after the *E. coli* challenge, a pipette tip was used to remove two samples of known cross sectional area (0.50 cm^2) and depth (0.5 cm) from the schmutzdecke of each filter. The protist counting results are summarized in Table 5.

E. coli removal and the associated protistan abundance showed a strong correlation to one another (Figure 8) with both increasing with increasing ripening time. *E. coli* removal was slightly lower in these columns than for 22.5-cm-deep columns with the same ripening times in the scouring simulation experiments, but those studies had been conducted in the summer (20–25°C), whereas this

FIGURE 8 Protistan abundance and *E. coli* removal in sand columns after various ripening times



EBCT—empty bed contact time,* E. coli—Escherichia coli*, HLR—hydraulic loading rate, MPN—most probable number

For all filter columns displayed: HLR = 0.3 m/h, EBCT = 45 min. Column 3 data were excluded to maintain uniform loading conditions (equal HLRs) for all points. Raw water ranged from 2.3 to 5.8°C during ripening. Influent* E. coli *concentration was 1.6 x 10^5 MPN/100 mL.

TABLE 5 Ripening times, *E. coli* log removal, and protist abundance for seven filter columns with varying run times

Column	Ripening Time	***E. coli*—log removal**	Protists/gdw	Protists/cm^2 Filter Plan Area—***top 5 mm***
1	4 weeks	1.2 ± 0.2	$4.0 \pm 2.0 \times 10^5$	$5.0 \pm 1.0 \times 10^5$
2	2 weeks	0.9 ± 0.2	$2.3 \pm 0.5 \times 10^5$	$2.8 \pm 0.5 \times 10^5$
3	2 weeks*	0.7 ± 0.2	$3.1 \pm 0.4 \times 10^5$	$2.4 \pm 1.4 \times 10^5$
4	1 week	0.3 ± 0.2	$5.0 \pm 2.0 \times 10^4$	$7.8 \pm 1.2 \times 10^4$
5	48 h	0.2 ± 0.2	$3.8 \pm 0.4 \times 10^4$	$5.9 \pm 1.1 \times 10^4$
6	24 h	0.1 ± 0.2	$3.0 \pm 1.0 \times 10^4$	$5.9 \pm 1.1 \times 10^4$
7	1 h†	0.0 ± 0.2	$2.0 \pm 1.0 \times 10^4$	$3.0 \pm 2.0 \times 10^4$

E. Coli—Escherichia coli, EBCT—empty bed contact time, gdw—grams dry weight, HLR—hydraulic loading rate

For all filter columns: HLR = 0.3 m/h, EBCT = 45 min, except for column 3*

*Column 3 had an HLR of 0.6 m/h, twice as fast as the other columns in order to produce a total throughput equal to that of column 1.
†Column 7 was allowed to run for 1 h after packing to allow for settling and flushing of fines.

study occurred from February 25 to March 28 (1.9–5.2°C).

Other researchers (Mauclaire et al, 2006) have observed a similar connection between longer ripening times and a higher number of protists at the surface of a filter. In the case of their research as well as in the present study, biofilm growth in the schmutzdecke continued to increase even as the number of protists increased. They and others (Kinner et al, 2002) have hypothesized that selective predation strengthens the surviving bacterial community by selecting for better adapted organisms and increasing available substrate. Therefore, in the schmutzdecke, protists may be indirectly improving removal of *E. coli* by accelerating the development of a biofilm, which itself is responsible for straining and improved adsorption.

CONCLUSIONS AND RECOMMENDATIONS

Specific conclusions are summarized here as they relate to each of the four objectives of this article. These conclusions are based on research conducted on laboratory-scale filters and, as such, are intended to inform researchers, operators, and designers of the observed trends. Readers should consider these conclusions and recommendations in conjunction with the existing literature on biological filtration and on-site experience.

Objective 1: Rank operational and design parameters.

• The results of the screening experiment and *L/d* experiment can guide engineers and operators of slow sand filters. Engineers should consider designing filters with smaller sand size and longer EBCTs to facilitate bacterial removal. Operators may be able to increase HLR, which will allow the filter cross-sectional area to be reduced, provided the filter depth is increased to provide the desired EBCT. (Caution: The conclusion that faster flow rates may not negatively impact *E. coli* removal in a significant way should be pilot tested before a full-scale implementation is attempted.)

Objective 2: Assess effect of schmutzdecke removal and recovery.

• Operators need to be aware that scraping invariably reduces a filter's ability to remove *E. coli*. Pathogen removal should be monitored after scraping until removal reaches an acceptable level before filtered water is used for consumption. Under the conditions of this research, filters recovered from a removal of the schmutzdecke and upper layer of sand in fewer than four days, regardless of the depth of sand removed below the schmutzdecke.

• Temperature affected the extent to which *E. coli* removal efficiency recovered after scraping/scouring. Cold (8°C) filters achieved a significantly lower ultimate removal efficiency than warm (24°C) filters after the same ripening period. Longer ripening times would be required for filters scraped during cold temperatures.

Objective 3: Assess removal mechanisms associated with the schmutzdecke biofilm.

• Bacterial removal correlated strongly with biological activity, but assessment of EPS by total carbohydrate and total protein did not provide the strong correlation that would be indicative of biologically mediated adsorption. Use of other assays besides gross phospholipids, carbohydrates, and proteins and more localized sampling may provide more insight into this phenomenon.

• No evidence was observed of substances present in the pore water of the schmutzdecke significantly enhancing *E. coli* removal by death or inactivation.

Objective 4: Estimate potential influence of protistan predation.

• Protistan predation may play an important role in *E. coli* removal in SSF and RBF either by grazing of surface-associated bacteria to limit detachment and free adsorption sites or by intercepting bacteria in pore water, but neither mechanism was confirmed.

• More protists were counted in the schmutzdecke of ripened filters than in unripened filters, correlating with higher *E. coli* removal. In addition, protists were observed grazing surface-associated bacteria from sand grains in live samples under the microscope.

ACKNOWLEDGMENT

The authors acknowledge the USEPA for funding this research through the New England Water Treatment Technology Assistance Center (WTTAC) at UNH. The views presented in this article have not been subjected to USEPA review and therefore do not necessarily reflect the views of the agency. No official endorsement should be inferred. The authors also thank WTTAC staff, especially Melanie Martin-Doole for formalizing biofilm assay standard operating procedures; Wesley East, superintendent, and the staff of the UNH/Durham water treatment plant; and Nancy Kinner, Aaron Margolin, and Bob Mooney, of UNH, for assistance advising the protist predation and schmutzdecke toxicity studies.

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FOOTNOTES

[1]Chromaflex®, Kontes article 420830, Kontes, Vineyard, N.J.
[2]"O" sand, Holliston Sand Co., Slatersville, R.I.
[3]L/S Series, Masterflex, Cole-Parmer, Vernon Hills, Ill.
[4]Quanti-Tray 2000®, IDEXX Laboratories Inc., Westbrook, Maine
[5]Nikon Optiphot microscope and Nikon B-2H filter cube, Melville, N.Y.

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