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Reovirus Removal and Inactivation by Slow-Rate Sand Filtration

LINDA K. McCONNELL,[1] RONALD C. SIMS,[1]* AND BILL B. BARNETT[2]

Utah Water Research Laboratory[1] and Department of Biology,[2] Utah State University, Logan, Utah 84322

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Laboratory column studies were conducted at the Utah Water Research Laboratory, Logan, Utah, to evaluate reovirus removal from drinking water supplies by slow-rate sand filtration (SSF). Columns, constructed to simulate a full-scale SSF field operation, were inoculated with reovirus at ca. 1,000-times-greater concentrations than those typically found in domestic sewage. Reovirus removal and inactivation were investigated as functions of filter maturity and other filter sand characteristics. Reovirus removal studies demonstrated that the SSF process is capable of reducing reovirus in influent water by a minimum of 4 log concentration units under certain conditions of water quality, flow rate, and sand bed construction. Infectious reovirus was not detected in effluent samples from any of the sand beds studied, after inoculation of the SSF columns; therefore, removal efficiencies were not affected significantly by characteristics, including age, of the two filter sands evaluated. Studies conducted with radioactively labeled reovirus demonstrated that reovirus removed from influent water was distributed throughout the entire length of the filter beds. Concentrations of reovirus in the filter sands decreased with increasing bed depth. The greatest removal occurred in the top few centimeters of all sand beds. No infectious reovirus could be detected in clean or mature sand bed media, indicating that reoviruses were inactivated in the filter.

Slow-rate sand filtration (SSF) has been used as a method for purification of drinking water supplies for over a century, although use of this method has declined over the last several decades with the introduction of more advanced treatment technologies. However, for small and rural communities with limited financial and technical resources, SSF may be the most appropriate technology. Simplicity of design, low operation and maintenance costs, ease of operation, and production of bacteriologically improved water supplies are some of the potential benefits of SSF. Although the SSF process can potentially be used to treat waters of any quality, its use is generally limited to treating surface waters with low turbidities (less than 50 nephelometric turbidity units) for providing optimum treatment efficiency and filter cycle length (19).

A primary concern of any water treatment system is the removal and inactivation of pathogenic microorganisms. Domestic sewage can contain a variety of pathogenic viruses at concentrations ranging from 10^1 to 10^5 infectious units per liter (5, 22). Reovirus is readily isolated from raw sewage at concentrations of 10^2 to 10^5 viruses per liter (1). Irving and Smith (21) assayed the influent and effluent of an activated-sludge sewage treatment plant for enteroviruses, adenoviruses, and reoviruses. Over a one-year period, the mean influent levels of all of these viruses were approximately equal. However, at each step in the treatment process, reovirus was the most stable, and after final chlorination, the overall reduction in enterovirus titers was 93%, the adenovirus titers were reduced by 85%, and the reovirus titers were reduced by 28%. Reovirus is consistently present in sewage and is relatively stable through sewage treatment processes. For these and other reasons, reovirus has been proposed as a viral indicator (1, 30). Although reoviruses are not known to cause disease in humans, they are closely related to rotaviruses, and both are members of the reovirus subgroup in the family Reoviridae (27). Rotavirus is a major human pathogen which is the leading cause of nonbacterial gastroenteritis in children (3). Surface waters receiving sewage inputs constitute a potential health hazard since many receiving streams are also used for drinking-water supplies. Because one virus particle can potentially induce infection (5, 20), the need for virus-free drinking water supplies is extremely important.

SSF is recognized as a biological- as well as a physical-treatment process. High-treatment efficiency is associated with the maturation of the schmutzdecke, or filtering mat, on the surface of the filter sand. The schmutzdecke is a biologically active layer composed of particulates and micro- and macroorganisms including algae, bacteria, protozoa, and rotifers.

Viruses are present in surface waters as aggregated, solids-associated, and monodispersed particles. Removal of solids-associated and aggregated virions in SSF may occur primarily by straining. Free viruses are believed to be removed largely by adsorptive processes since their small size (20 to 300 nm) precludes removal by straining (4, 7, 12, 16, 31, 38). Sand is generally regarded as a relatively poor adsorbent, compared with clay minerals and soils, for nonaggregated virions. However, studies have been conducted indicating that even coarse materials like sand have a relatively high adsorption capacity for viruses (6, 28).

Sobsey et al. (36) compared sands and clays as virus adsorbents. When tested as suspensions in water, sands adsorbed less than 10% of the added poliovirus and less than 40% of the added reovirus, compared with over 99% adsorption of either virus by clays. However, the sands did adsorb over 95% of either virus when used in a 10-cm column configuration. The degree of virus adsorption that occurs in sand filter beds is a function of the virus type, sand characteristics, and physicochemical characteristics of the suspending medium (2). Virus types and even viral strains vary in their affinity for a particular filtering medium. Sand characteristics that have been shown to influence virus adsorption include percentage of clay (8, 10, 15), cation exchange capacity (10, 12), specific surface area (10, 12), and organic-matter content (13, 17, 32). Physicochemical properties of the suspending medium that have been found to affect virus adsorption include pH, ionic strength, and organic-carbon content (5).

Most investigators agree that adsorptive processes play a

* Corresponding author.

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major role in virus removal and possible inactivation in soils (28). However, it has been proposed that biological inactivation of viruses may be an equally important process (29, 34). Several strains of bacteria isolated from marine water (14, 26, 33), activated sludge (11, 23), and oxidation pond water (18, 35) have been found to have antiviral activity. Although no similar investigations have been conducted to identify antiviral bacteria in the SSF process, the presence of large numbers of bacteria and grazing organisms has been noted in slow-sand filters (25). Other laboratory column studies have demonstrated that clean, sterile filter sand does not remove viruses, indicating that biological activity resulting from maturation of the sand bed may affect the removal and inactivation of viruses in the SSF process (29, 31).

Even though SSF has been used as a water treatment technology for many decades, relatively few studies have been conducted to investigate processes of virus removal. SSF process variables that have been previously shown to affect virus removal include water temperature, filtration rate, sand bed depth, filter maturity, and filter cleaning (9, 19, 29, 31, 34, 37). This study was undertaken to evaluate the effectiveness of the SSF process in removing an enteric virus under specified conditions of water quality, flow rate, and sand bed construction. Virus removal and inactivation were investigated as functions of sand characteristics and filter maturity. Experimental results might be used to evaluate the adequacy of standard design criteria for SSF bed depth in preventing a breakthrough of infectious viruses into the treated water.

MATERIALS AND METHODS

SSF filter sands. Recommended design criteria for SSF media particle size generally require preparation by sieving. Use of locally available unsieved construction sand may be more cost effective and require less labor for preparation. Two locally available construction sands were selected for use as experimental filter media. One construction sand was sieved to meet State of Utah specifications for SSF filter media (sand A [Table 1]). The other construction sand was used unsieved (sand B [Table 1]). Characteristics of filter sands A and B are presented in Table 1. Sieve analyses were conducted on both sands to determine the percentages of fines passing a no. 300 U.S. Standard sieve (0.05-mm diameter).

Influent water source. Logan River water was piped directly to SSF columns via facilities at the Utah Water Research Laboratory. Logan River has been designated as a Class A water source. Class A sources are specified for the protection of cold-water game fish and waterfowl and for agricultural use, and these sources have water quality restrictions requiring a biological oxygen demand of less than 5 mg/liter, a nitrate (NO_3 N) concentration of less than 4 mg/liter, and a P_i (PO_4 P) concentration of less than 0.25 mg/liter. The raw-water quality of the Logan River during the experimental period is shown in Table 2. The temperature of the Logan River water during the experimental period ranged from 14.5 to 22°C.

TABLE 1. Characteristics of SSF filter sands[a]

Parameter (units)	Sand A	Sand B
Uniformity coefficient	2.1	4.4
Effective size (mm)	0.28	0.18
Sand (%)	99.9	98.4
Silt/clay (%)	0.1	1.6
Bulk density (g/cm^3)	1.55	1.61
Porosity (%)	35	31
CEC (meq/100 g of sand)	0.9	1.3

[a] Sand A, sieved; sand B, unsieved; CEC, cation-exchange capacity.

TABLE 2. Quality of raw water in the Logan River during the experimental period[a]

Parameter	Avg value
Turbidity	1.0 NTU[b]
pH	8.3
Electrical conductivity	280 μS/cm
Alkalinity	184 mg/liter as $CaCO_3$
Total dissolved solids	170 mg/liter
Calcium	48 mg/liter
Ammonia (NH_3 N)	10.7 μg/liter
Sulfate (SO_4 S)	5.2 mg/liter
P_i (PO_4 P)	36.2 μg/liter
Temperature range	15–22°C

[a] October to September 1982.
[b] NTU, Nephelometric turbidity unit.

Virus and virus assay. A large pool of reovirus type 1 Lang was grown in roller bottle cultures of Madin-Darby bovine kidney cells (1). The reovirus pool contained 1×10^7 fluorescent cell-forming units (FCFU) of reovirus per ml as determined by an immunofluorescent cell count assay (30). The reovirus pool was dialyzed against distilled water to reduce the total salt concentration to 10^{-4} and then diluted 1:10 in Logan River water just before being placed on the sand filters. The water samples were assayed for reovirus by an immunofluorescent cell count procedure (30) after dilution in Eagle minimal essential medium (GIBCO Laboratories).

The rate of reovirus inactivation in Logan River water was evaluated. The diluted (1:10) reovirus pool was assayed immediately, after 7 h at 20°C, and after 14 days at 4°C.

Reovirus was eluted from sands by mixing 10 g of sand with 10 ml of sterile beef extract broth (Oxoid Ltd.) adjusted to pH 9.5. The slurry was gently mixed for 30 min at room temperature. The resulting eluants were assayed for reovirus after neutralization and dilution with Eagle minimal essential medium.

Radioactive-labeled reovirus. Reovirus was purified by differential centrifugation, followed by equilibrium density gradient banding in CsCl solution. The purified reovirus was then covalently labeled with ^{125}I by a lactoperoxidase iodination procedure with the New England Nuclear Corp. NEZ-151 radioiodination system.

The ^{125}I-labeled reovirus was then separated from other forms of the ^{125}I by pelleting the virus from suspension at 200,000 × *g* for 90 min. Of the ^{125}I in the labeled reovirus suspension, 90% could be precipitated by the addition of reovirus-specific antiserum. The ^{125}I was assayed with an Abbot Laboratories model 221 gamma counter.

SSF laboratory columns. SSF columns were constructed to simulate a full-scale SSF field system. A typical SSF column is shown in Fig. 1. Columns used for the unlabeled-reovirus removal studies were constructed of glass pipe (inner diameter, 15 cm). Each column consisted to two 1.5-m pipe sections to facilitate removal and addition of filter sand and support media. Filter sand (ca. 1.2 m) was placed on top of 30 cm of graded support gravel. Water column height was maintained at 1.2 m above the filter bed by an adjustable float valve. Effluent flow rates were controlled by a Teflon stopcock metering valve attached to the bottom support plate. Effluent was discharged through a glass tube (inner

diameter, 6 mm) connected to the outlet of the metering valve. The glass tubing was ca. 1.6 m in length, extending from the bottom of a column to a sufficient height above the filter bed to prevent negative pressures from occurring in the sand bed (Fig. 1).

Three replicate columns of each sand type were operated continuously at a filtration rate of 0.2 m/h. Column tops were left uncovered to permit gas exchange between the sand bed, column supernatant, and atmosphere. To prevent algae growth in the sand beds due to incidental light, the bottom 1.2 m of each column was covered with black plastic. Daily measurements were taken to determine headloss development through the filter bed, supernatant temperature, and influent and effluent turbidity.

^{125}I-reovirus studies were conducted in glass columns (inner diameter, 5.1 cm) to minimize the quantity of radioactive reovirus required. Sand bed construction and filter operation were similar to that for the columns previously described. Two replicate columns of each sand type were utilized.

Dye tracer studies. Before SSF columns were challenged with reovirus, fluorescein dye studies were conducted to ensure that the flow pattern approximated plug flow characteristics and to identify any significant hydraulic short-circuiting. Dye tracer curves were also used to establish the flow pattern and average detention time within the columns for nonadsorbing constituents. It is recognized that viruses are colloids and do not travel at the same rate as solutes through porous media. Therefore, the dye tracer cures were used as a basis for selecting a range of effluent-sampling times for viruses. Effluent-sampling times spanned a period four times as long as the average hydraulic-detention time to ensure that viruses traveling more slowly through the sand media would be sampled.

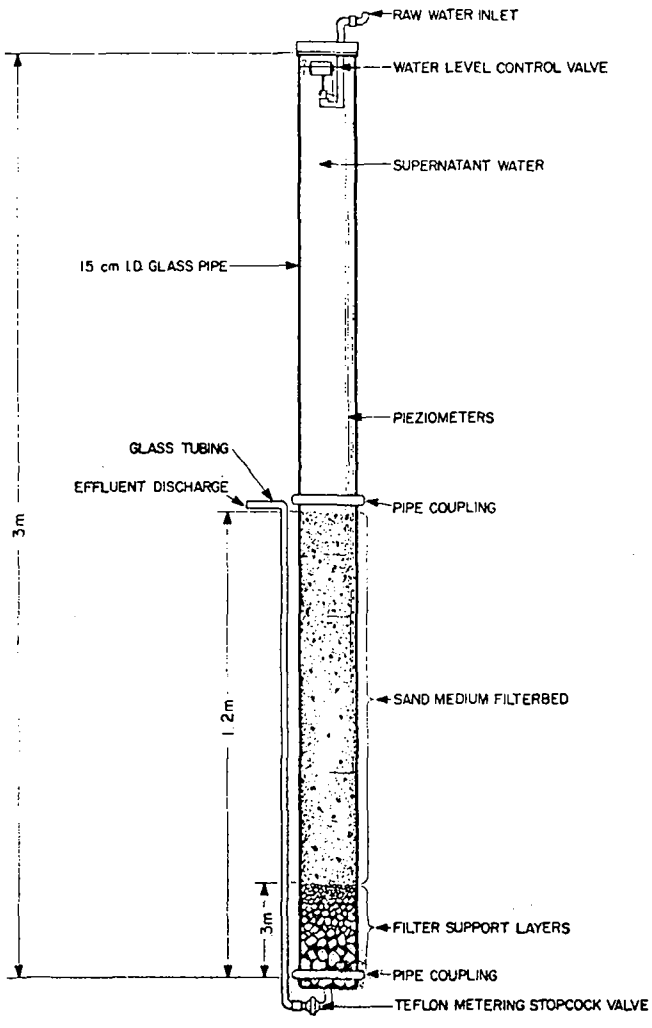

FIG. 1. Typical SSF laboratory column model.

Reovirus removal studies with unlabeled virus. Sieved- and unsieved-sand columns (inner diameter, 15 cm) were challenged with reovirus at 1, 13, and 21 weeks after initiation of filter operation at an average loading of 3.0×10^8 FCFU per column. Reovirus inoculum was applied to the sand surface through Tygon tubing. Reovirus solutions were chilled ca. 4°C below the supernatant temperature, providing a density differential to minimize dispersion of inoculum in the column supernatant. Beginning immediately after inoculation, effluent samples were collected as composites over varying lengths of time for an 8-h period, representing approximately four theoretical-detention times. Sampling intervals were based on the dye tracer curves for hydraulic-detention time and on characteristics of each column as discussed above.

Immediately after the week-1 experiment, a sieved-sand column was dismantled, and a core sample was taken through the entire bed depth. The sand core was divided into 7.6-cm sections and assayed for infectious reovirus. The same procedure was repeated after the week-13 experiment with an unsieved-sand column and after the week-21 experiment with one column of each sand type.

^{125}I-reovirus experiments with clean filter sands. SSF columns (inner diameter, 5 cm) containing clean sieved and unsieved construction sands were inoculated with ^{125}I-reovirus and unlabeled (infectious) reovirus to evaluate virus removal, inactivation, and distribution in SSF filter beds. Columns were each challenged with a 20-ml volume of reovirus inoculum containing 40,000 cpm of ^{125}I-reovirus and 10^6 FCFU of infectious reovirus per ml. The inoculum was applied to the sand surface, after the supernatant was temporarily removed, and was allowed to penetrate the surface of the filter bed. Supernatant was subsequently replaced, and the filters were operated at a filtration rate of 0.2 m/h. Effluent samples were collected as composites over an 8-h period immediately after inoculation and were assayed for ^{125}I-reovirus and infectious reovirus concentrations. Sampling times were based on dye tracer characteristics of each column as previously described. After each experiment, all columns were dismantled, and core samples were taken through the entire bed depth. Sand core samples were assayed for ^{125}I-reovirus concentrations before and after elution. The resulting eluants were assayed for infectious reovirus and ^{125}I-reovirus concentrations.

^{125}I-Reovirus experiments with aged, unsieved sand. Experiments were conducted to evaluate the effect of filter maturity (biological growth) on reovirus removal, inactivation, and distribution in filter beds. The effect of scraping the schmutzdecke of a mature filter to simulate filter cleaning was also investigated. Filter sand was obtained from a field scale SSF system at the Utah Water Research Laboratory that had been in continuous operation for 8 months. The field scale slow-sand filter had been used to treat raw Logan River water with the same quality as that described in Table 2. The sand used in the field scale facility was identical to the unsieved construction sand used in laboratory column studies. A diagram of the SSF field scale facility is presented in Fig. 2. A complete description of the design, operation, and

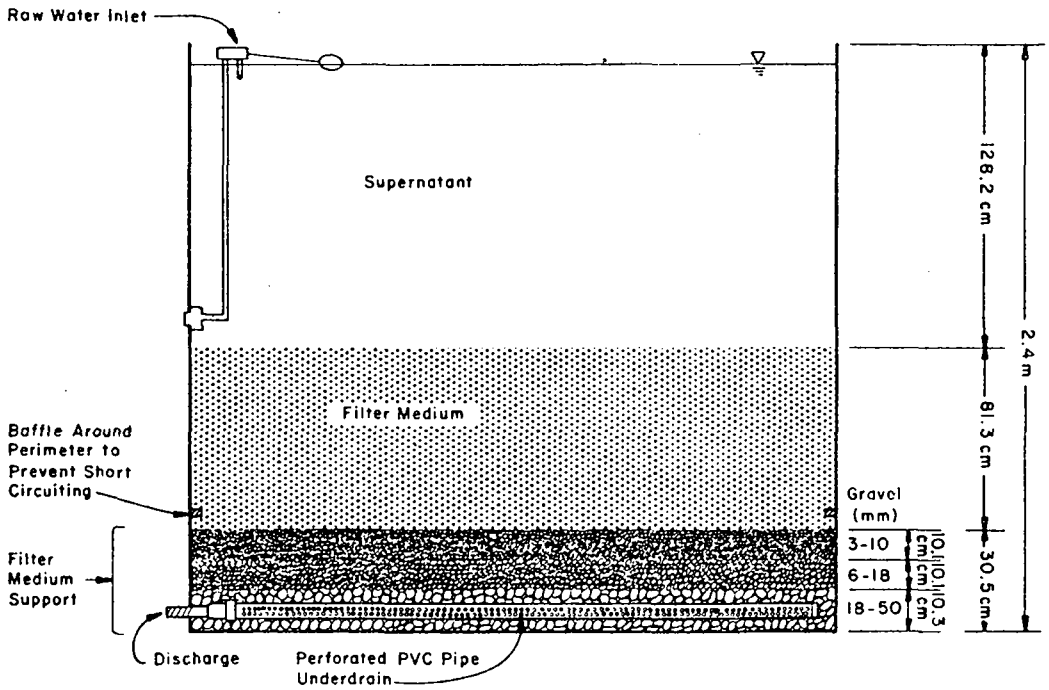

FIG. 2. Cross-sectional view of the SSF field scale facility.

cost effectiveness of the SSF field facility is given by Slezak (M.S. thesis, Utah State University, Logan, 1983). Core samples were taken of the entire sand bed depth (53 cm). Because the sand cores could not be transferred directly to the laboratory columns, each core was divided into 7.6-cm sections, mixed, and added to the columns. To simulate the sand bed depth used in previous experiments (1.2 m), we added additional unsieved sand to the columns. Two columns were used with the schmutzdecke present and, to simulate scraping of a slow-sand filter, two columns were used with the schmutzdecke removed. The procedure used for sampling the sand and effluent of each column was that previously described for ^{125}I-reovirus experiments.

One of the core samples taken from the field facility was analyzed for bacteria counts. Analyses were conducted in triplicate with standard plate count media by the procedure of the American Public Health Association (2). Standard plate counts were also conducted on clean sands A (sieved) and B (unsieved).

RESULTS

Unlabeled-reovirus removal studies. Analyses of effluent water samples collected from SSF columns containing sieved and unsieved sand inoculated at 1, 13, and 21 weeks of filter operation showed no detectable infectious reovirus. Based on a detection limit of 80 FCFU per ml, the reovirus concentration was reduced more than 4 log concentration units by SSF treatment of the reovirus-laden waters. Tests conducted to evaluate the rate of reovirus inactivation in Logan River water demonstrated that there was no significant difference in the virus titer after 0 or 7 h (at 20°C) of holding time. The reovirus titer dropped 25 to 50% after 14 days at 4°C in Logan River water.

Analyses of sand samples taken from a sieved-sand column after the week-1 experiment demonstrated that infectious reoviruses were present and were distributed through the top 107 cm of filter sand. The majority of the adsorbed reovirus was found in the first 42 cm (Fig. 3). A mass balance conducted on the reovirus added to the column indicated that the total quantity of infectious reovirus that could be eluted from the sand with the high pH proteinaceous eluter represented ca. 2% of the added reovirus. This low level of infectious reovirus was very near the lower detection limit of the assay. No infectious reovirus could be detected in unsieved-sand samples assayed after the week-13 experiment or in sieved- and unsieved-sand samples assayed after the week-21 experiment.

^{125}I-reovirus experiments. No infectious reovirus could be detected in sand samples obtained from columns containing clean sieved or unsieved sands or aged, unsieved sand. However, analyses for ^{125}I-reovirus did demonstrate that reovirus was distributed through the entire bed depth for all filter beds and was present at very low levels in effluent samples. The percentages of total ^{125}I-reovirus detected in

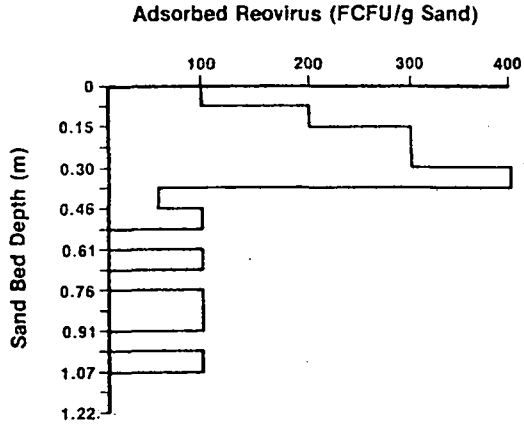

FIG. 3. Distribution of infectious reovirus detected in an SSF laboratory column containing sand A (sieved).

TABLE 3. Percentage of added ^{125}I-reovirus detected in effluent and sand samples collected from SSF columns containing clean sieved and unsieved sands and aged, unsieved sand

Sand type	% Recovered in effluent	% Recovered in sand
Sieved (clean)	3.3	96.7
Unsieved		
Clean	5.2	94.8
Aged with schmutzdecke	5.9	94.1
Aged without schmutzdecke	4.9	95.1

sand and effluent samples for each experiment are reported in Table 3. The values shown are averages of duplicate-test results.

The distributions of ^{125}I-reovirus with respect to bed depth in clean sieved- and unsieved-sand beds, in aged-, unsieved-sand beds with the schmutzdecke removed, and in aged-, unsieved-sand beds with the schmutzdecke present are presented in Fig. 4 through 7, respectively.

The results of standard plate counts conducted on sand core samples collected from the SSF field facility, in addition to those on clean filter sands A and B, are reported in Table 4.

DISCUSSION

Results from these experiments demonstrated that the SSF process is capable of reducing influent reovirus loadings by greater than 4 log concentration units under certain conditions of water quality, flow rate, and sand bed construction. In other SSF studies with similar flow rates, poliovirus levels have been reduced 3 to 5 log concentration units (29). Earlier studies had indicated that a 61-cm column of saturated sand could remove influent poliovirus at application rates of 50% at 0.6 m/h, 58% at 0.3 m/h, and 69% at 0.15 m/h (31). In this experiment no infectious reovirus could be detected in any effluent samples. There was no difference observed in removal efficiencies for sieved and unsieved sands. These results indicate that locally available, unsieved construction sands may be as effective as presieved SSF filter sands for providing virologically safe drinking water.

Reovirus removal efficiency in sieved- and unsieved-sand columns did not appear to change with aging of the filter sands. These results are in contrast to results obtained from other SSF studies. Taylor (37) and Poynter and Slade (29) observed that clean filter sand was less effective for poliovirus removal than ripened filter sand. Both studies were conducted with SSF columns operated at the same filtration rate as in this study (0.2 m/h). However, the absence of detectable reovirus in any of the effluent samples in our study makes it difficult to comment on the merits of aging the filter sands. Perhaps the use of shorter bed depths would have allowed reovirus to break through in the effluent and thus would have facilitated comparisons of the varied parameters.

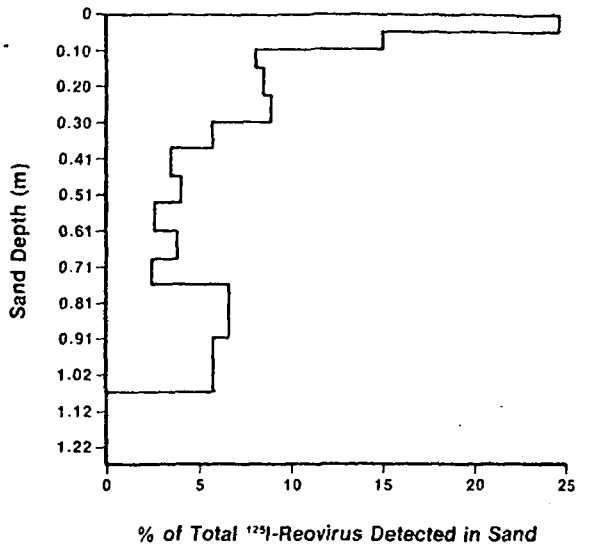

FIG. 5. Distribution of ^{125}I-reovirus in an SSF column containing clean sand B (unsieved).

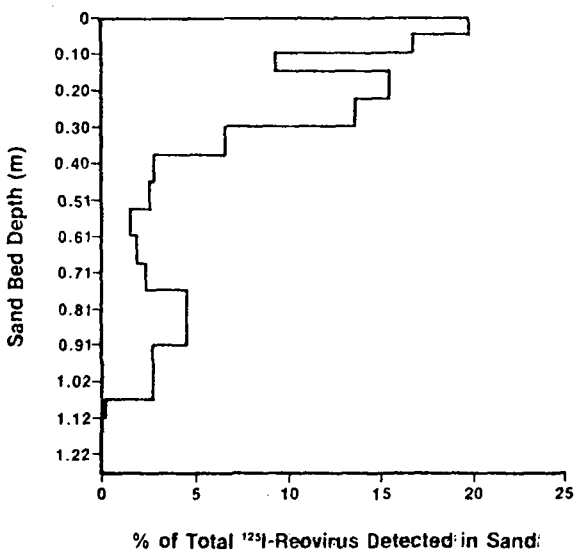

FIG. 4. Distribution of ^{125}I-reovirus in an SSF column containing clean sand A (sieved).

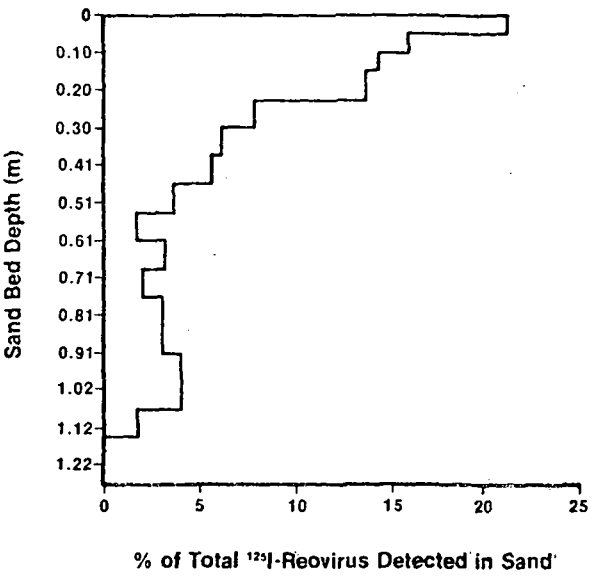

FIG. 6. Distribution of ^{125}I-reovirus in an SSF column containing aged sand B (unsieved) with the schmutzdecke removed.

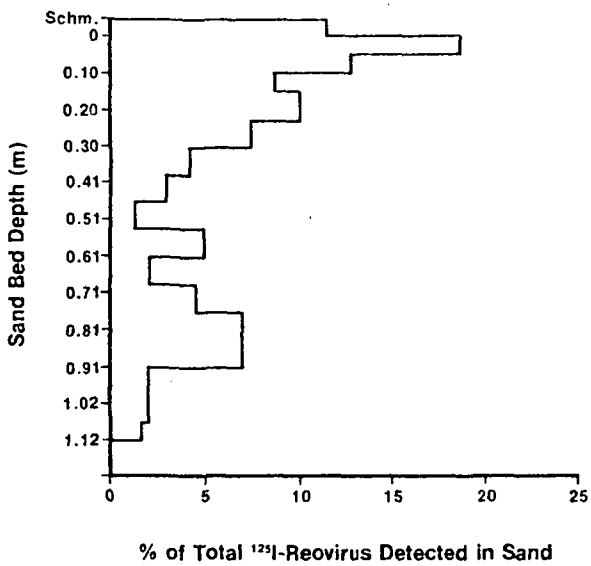

FIG. 7. Distribution of ^{125}I-reovirus in an SSF column containing aged sand B (unsieved) with the schmutzdecke (Schm.) present.

Infectious reoviruses were detected at very low levels in sieved-sand samples collected after the week-1 experiment (Fig. 3). The majority of the sand-associated infectious reovirus (>70%) was detected in the first 42 cm of the filter bed. These results support the observations of other investigators that microorganism removal in SSF is greatest in the upper portion of the filters (9, 25). Analyses of sieved- and unsieved-sand samples collected after the experiments at weeks 13 and 21 showed that no infectious reovirus could be detected. It was believed that these results could be due to one of several phenomena: (i) the reovirus might have been inactivated in the sand beds; (ii) reoviruses might have been tightly adsorbed to the sand and could not be eluted; or (iii) some constituent in ripened filter sand might have interfered with the elution procedure. Labelle and Gerba (24) reported similar problems in trying to elute echovirus and coxsackievirus from marine sediments.

TABLE 4. Standard plate counts conducted on clean filter sands A (sieved) and B (unsieved) and on schmutzdecke and sand bed samples collected from the SSF field facility

Sample	Avg no. ($\times 10^{-3}$) of colonies per g of sand
Clean sand A	29
Clean sand B	63
SSF field facility[a]	
Schmutzdecke	5,410
1.3	3,050
5.1	640
11.4	1,280
19.0	1,290
26.7	1,390
34.3	480
41.9	330
49.5	730

[a] Sand bed samples are designated by the average depth in centimeters from the filter surface.

To determine whether reoviruses were inactivated in the sand beds as well as to determine reovirus distribution patterns in the filters, tracer studies were conducted with ^{125}I-reovirus. The concentrations of infectious reovirus per gram of sand applied to tracer columns were approximately equal to the concentrations used in the unlabeled-virus experiments. Influent reovirus loadings were reduced by greater than 5 log concentration units.

No infectious reovirus could be detected in clean sieved and unsieved sands or in aged, unsieved sand. The same sands assayed for ^{125}I-reovirus showed that radiolabeled reovirus was present throughout the entire length of all filter beds and that the elution procedure efficiently eluted the adsorbed reovirus. From these results it was concluded that the infectious reovirus added to SSF columns was inactivated in the sand beds. The processes of viral inactivation operating in the sand beds could not be determined. Microbial action may not necessarily be responsible for the observed inactivation in the sand, but some type of soil surface virus interaction may cause breakdown of the virus. Results from this study indicate that biological activity in the sand beds was not responsible for inactivation of viruses. Clean, unsieved sand, which had bacteria counts 10 to 90 times lower than those for ripened, unsieved sand (Table 4), showed the same removal efficiencies as did mature filters.

Although radiolabeled reovirus was distributed through the entire sand bed depth of all filters, the greatest removal (>65%) occurred in the top 35 cm of the sand beds. Distribution diagrams (Fig. 4 through 7) of ^{125}I-reovirus with respect to bed depth show that the patterns of reovirus removal were similar for sieved and unsieved sands and aged and clean sands. The results indicate that sand characteristics and filter maturity did not significantly affect virus distribution in slow-sand filters. However, the pattern of virus distribution through the sand emphasizes the importance of sand bed depth as an SSF design criterion for protecting drinking water supplies from viral contamination. Other SSF studies have demonstrated that the depth of filter sand will influence microorganism removal efficiencies (29, 31).

Radiolabeled iodine was detected at very low levels in the effluent of all SSF columns. However, the absence of infectious virus in these effluents suggests that this small fraction of the radiolabel may have been associated with nonviral proteinaceous materials known to be present in the purified reovirus pool that was iodinated or that the iodine was associated with reovirus particles that were degraded or otherwise inactivated on the columns. Another possibility is that iodination changed the adsorption characteristics of the reovirus. In subsequent studies we will evaluate the use of tritium-labeled reovirus in place of the iodinated reovirus.

The effect of cleaning a mature filter bed on reovirus removal was evaluated. Typically, when slow-sand filters are cleaned, the filters are temporarily drained, and the top 2 cm of the sand surface (schmutzdecke) is removed. In this study, columns containing ripened, unsieved filter sand were challenged with ^{125}I-reovirus before and after the schmutzdecke was removed. Scraping off the schmutzdecke did not adversely affect reovirus removal efficiencies. Other investigators have observed similar results after filter cleaning (29, 34). However, Poynter and Slade (29) reported that filter cleaning in full-scale SSF facilities resulted in lower virus removal efficiencies. They speculated that the effect of filter cleaning on virus removal is influenced more by the length of time that the filter is drained, and therefore exposed to the air, than by removal of the sand surface.

Exposing the sand surface to air during filter cleaning may adversely affect the microbial community in the filter bed (34).

Virus removal efficiencies observed in these experiments were much higher than those reported in some other SSF studies (31, 34) and were of the same magnitudes as those seen in yet other studies (29) conducted by using the same filtration rates. The higher removal efficiencies obtained in this study could be attributed to several factors. First, the sand bed depths used were twice as deep as those used in the other investigations. The greater bed depth provides a longer residence time for microorganisms within the sand bed and, therefore, a greater chance to be inactivated. Second, removal efficiencies of different virus types will vary for a given filtering medium. Most of the previous SSF studies have investigated removal of poliovirus. Poynter and Slade (29) observed that removal of poliovirus and a bacteriophage were quite different when applied to the same system. It is recommended for future research that removal of other virus types in SSF be evaluated to determine the variability of removal efficiencies as a function of virus type. Finally, the quality of the influent water source used in this study was probably much better than that used in the other studies. Two of the other studies were conducted with reservoir water from the Thames River in England, from which pathogenic viruses have been frequently isolated (29).

Recommendations for future research based on the results of this study include the evaluation of the effect of water quality in terms of turbidity, organic-carbon content, and ionic strength on filter performance with respect to virus removal. Also, the presence and extent of antiviral activity of microorganisms present in the SSF filter beds could be investigated.

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