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Regeneration of Pleated Filters Used to Concentrate Enteroviruses from Large Volumes of Tap Water

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Pleated cartridge filters are capable of concentrating enteroviruses from large volumes (well over 2,000 liters) of tap water. These epoxy-fiberglass filters can be regenerated if they are treated with 0.1 N NaOH or autoclaved to inactivate any contaminating virus. The regenerated filters regained their ability to concentrate viruses from water at high flow rates.

In a recent paper (1), we described the use of pleated filters as virus adsorbents in a modified version of the virus concentrator that was developed by Wallis et al. (4) and is now available as the Aquella virus concentrator (Carborundum Co., Niagara Falls, N.Y.). These filters, coupled with fiberglass prefilters, are capable of processing large volumes of tap water. Since neither the flow rate (26 liters/min) nor the ability of the filters to adsorb virus decreased after processing 19,000 liters of treated tap water (1), reuse of the filters to process additional test samples of water was examined.

It was determined that adsorbed virus not removed by the standard elution with basic buffer could be inactivated by treating pleated filters with 0.1 N NaOH or by autoclaving. These treatments did not reduce the capacity of the filters to concentrate virus anew from tap water at high flow rates.

MATERIALS AND METHODS

Virus and viral assays. Plaque-purified poliovirus type 1 (strain LSc) was used in this study. The BGM cell line was used for viral assays. Plaque assays were performed as described in detail elsewhere (3). Samples were assayed after being made isotonic and after fetal calf serum was added to make a final concentration of 2% or after dilution in tris(hydroxymethyl)aminomethane-buffered saline containing 2% fetal calf serum.

Filters. Epoxy-fiberglass filters (Filterite Corp., Timonium, Md.) were used as virus adsorbents. They were used as flat, circular sheets in 13- and 25-mm diameter holders or as pleated 25.4-cm cartridge filters. Honeycomb, fiberglass depth filters (model K-27, Commercial Filters Division, Carborundum Co., Lebanon, Ind.) were used as prefilters.

Treatment of filters. Tap water at pH 3.5 containing 0.0005 M aluminum chloride was passed through flat filters in 25-mm holders at 30 lb/in^2 until the flow rate decreased by approximately 75%. To lessen the volume of water required to produce this decrease, prefilters were not used to protect the filters. Regeneration of clogged filters was accomplished with 50 ml of 0.05 M, pH 10.5 glycine and 50 ml of one of the following solutions: 0.1 N NaOH, 0.1 N H_2SO_4, 0.1 N HNO_3, 0.1 N HCl, 0.1% Tween-80 (wt/vol), 0.1% Triton X-100 (wt/vol) in 0.1 N NaOH, or 1% Rovar (wt/vol). The filters treated with Tween-80 or Triton X-100 were washed with 100 ml of 95% ethanol and 100 ml of water. Filters treated with the other regenerants were washed with 100 ml of water only.

Viral studies. Virus (10^7 to 10^8 plaque-forming units [PFU]) was added to 2,000 ml of 0.05 M glycine at pH 3.5 with 0.0005 M $AlCl_3$. The sample was then passed through a cartridge, 0.25-μm porosity filter at 1,000 ml/min. Under these conditions, greater than 99% of the virus adsorbed to the filter. Two thousand milliliters of 0.05 M glycine at pH 10.5 was passed through the filter five times to elute adsorbed virus as previously described (1). Next, either 2,000 ml of 0.1 N NaOH was passed through the filter or the filter was autoclaved (15 lb/in^2, 15 min). Residual base was removed by passing 20 liters of dechlorinated tap water through the filter. To measure the extent of viral inactivation, surviving virus was eluted from the filter using pH 10.5 glycine as described above and concentrated by a multiple adsorption-elution scheme using membrane filters as described below. The eluate was neutralized with pH 2, 0.05 M glycine and assayed for virus. The neutralized eluate (approximately 3,000 ml) was adjusted to pH 3.5 using pH 2 glycine, mixed with aluminum chloride to produce a final concentration of 0.0005 M, and passed through a series of flat 3.0-, 0.45-, and 0.25-μm porosity epoxy-fiberglass filters in 25-mm holders. Virus adsorbed to the flat filters was removed by passing 20 ml of pH 10.5 glycine through them one time. This eluate was neutralized with pH 2 glycine, assayed for virus, adjusted to pH 3.5 and 0.0005 M $AlCl_3$, and passed through the filter series described above contained in 13-mm holders. Adsorbed virus was removed by passing 2 ml of pH 10.5 glycine through the filters one time. The eluate was neutralized as above and assayed for virus. Using this procedure, virus in 3,000 ml of eluate could be concentrated in a final volume of 5 ml with an efficiency of 75 to 90%. It should be noted that this reconcentration procedure can be used for eluates from filters used to process a clean sample such as the glycine solution, but cannot be used for eluates from filters used to process large volumes of tap water (1) or sewage effluent (2).

Testing treated filters. The ability of treated cartridge filters to concentrate virus from tap water was determined by passing virus in 1,900 liters of dechlorinated tap water at pH 3.5 and 0.0005 M aluminum chloride through the filters at approximately 25 liters/min. Adsorbed virus was eluted using pH 10.5 glycine as described above (1).

RESULTS

Filterite filters are capable of adsorbing greater than 90% of virus added to glycine at pH 3.5 and 0.0005 M aluminum chloride. The mean recovery upon elution with basic buffer was 69% of the adsorbed virus. Routine elution does not remove all adsorbed virus but does reduce the number of viruses adsorbed to the filter by about 70% (Table 1). Treating filters with 0.1 N NaOH produces a reduction of 4 to 5 logs. Treating filters for 5 min was as effective in inactivating viruses as longer treatments. Since treating virus suspended in tap water with 0.1 N NaOH results in a 5.7-log reduction in 10 s and >7.5-log reduction in 1 min, the inability of 0.1 N NaOH to inactivate all adsorbed virus seems to be due to failure of the base to contact all adsorptive surfaces or to the formation of protective organic or inorganic flocs around the virus. After autoclaving, no virus could be detected in the concentrated filter eluate. These treatments do not reduce the ability of the filter to concentrate virus, even after repeated treatments (Table 2).

Since the pleated cartridge filters are capable of processing several thousand liters of tap water without clogging (1), a model system using flat filters in 25-mm holders without prefilters was used in studies on regeneration of clogged filters. Under these conditions, the filters clogged after processing 5 to 10 liters of tap water at pH 3.5 with 0.0005 M aluminum chloride. The surface area of a cartridge filter (2,800 cm^2) is 700 times the area of the 25-mm flat filter. Furthermore, a filter protected by a prefilter is capable of processing approximately 20 times more water than an unprotected filter. Therefore, 10 liters being processed by the 25-mm flat filter indicates that 140,000 liters could be processed by the cartridge filter.

Treating clogged filters with 0.1 N NaOH solubilized organic compounds still adsorbed to the membrane after elution of the filters with glycine buffer and increased the flow rates as compared with similar filters that had not been regenerated. Regenerating filters with 0.1 N NaOH restored the flow rate to approximately 75% of that obtained with a fresh filter. Continued clogging and regenerating of the filters produced similar results. The flow rate of water through clogged filters could be increased by treatment with base. However, the initial flow

TABLE 1. *Inactivation of virus adsorbed to Filterite filters*[a]

Virus influent (PFU)	Virus adsorbed to the filter[b]	Virus eluted[c] PFU	Virus eluted[c] %	Treatment	Post-treatment eluted virus	Virus reduction in logs $\left[\frac{\text{column 2 - column 3}}{\text{column 5} \times 1.44}\right]$
6.4×10^6	6.4×10^6	4.6×10^6	72	None	4.0×10^5	0.49
3.5×10^7	3.4×10^7	2.9×10^7	85	0.1 N NaOH, 5 min	1.7×10^2	4.3
1.2×10^8	1.2×10^8	6.4×10^7	53	0.1 N NaOH, 15 min	3.4×10^2	5.1
2.1×10^7	2.1×10^7	6.6×10^6	31	0.1 N NaOH, 15 min	2.4×10^2	4.6
7.6×10^7	7.6×10^7	5.4×10^7	71	0.1 N NaOH, 60 min	6.5×10^2	4.4
1.5×10^7	1.5×10^7	1.1×10^7	73	0.1 N NaOH, 18 h	9.9×10^1	4.4
4.1×10^6	3.9×10^6	3.8×10^6	97	Autoclaving	<1	>5.0
7.4×10^6	7.2×10^6	4.9×10^6	68	Autoclaving	<1	>6.4

[a] Virus in glycine buffer at pH 3.5 containing 0.0005 M aluminum chloride was passed through 10-inch (25.4 cm), 0.25-μm Filterite filters. Adsorbed virus was eluted using buffer at pH 10.5, and the filters were then subjected to the indicated treatment. Virus surviving the treatment was eluted from the filters using pH 10.5 buffer and concentrated in a small volume for assay using membrane filters. The details of the procedure are given in the text.

[b] Influent virus − effluent virus.

[c] Mean = 69%. The reciprocal of the mean is 1.44, which is used to calculate the virus reduction in the last column.

TABLE 2. *Concentration of virus by treated Filterite filters,*[a] *in 1900-liter sample size*

Treatment	No. of times treatment was applied	Virus in filter		
		Influent PFU	Eluate	
			PFU	%
None		7.5×10^6	4.7×10^6	63
0.1 N NaOH, 5 min	6	5.8×10^6	3.3×10^6	57
Autoclaving	2	1.8×10^7	1.2×10^7	67
Autoclaving	5	1.1×10^7	6.4×10^6	58

[a] Dechlorinated tap water at pH 3.5 with 0.0005 M aluminum chloride was seeded with virus and passed through a K-27 prefilter and a 0.25-μm Filterite filter at 26 liters/min. Virus adsorbed to the filters was recovered by passing 2,000 ml of pH 10.5 glycine through the filters five times.

rate with regenerated filters was always less than the initial flow rate through the original filter. After three clogging and regenerating cycles, the initial flow rate decreased to 25% of that obtained with a fresh filter. On the basis of these studies using 25-mm flat filters without prefilters as a model, fresh pleated cartridge filters with K-27 prefilters should be capable of processing over 100,000 liters of tap water before clogging. Regenerating these filters with base should permit their use for processing additional large volumes of water (Table 3). As shown in Table 2, both fresh and regenerated filters were capable of concentrating virus from large volumes (1,900 liters) of water. Treating the clogged filters with the other regenerants listed in Materials and Methods was no more effective in increasing the flow rate than was treating with 0.1 N NaOH. Stronger solutions of acid and base were not used because of the hazards associated with passing these solutions through filters under pressure.

TABLE 3. *Characteristics of fresh and base-regenerated filters*

Times filter was regenerated with 0.1 N NaOH	Initial flow rate (liters/min)	Capacity[a] (liters)
0 (Fresh filter)	38[b]	100,000[b]
1	27	60,000
2	16	40,000
3	10	20,000

[a] Volume of tap water at pH 3.5 with 0.0005 M aluminum chloride that can be processed before a 75% reduction in the initial flow rate occurs.

[b] Values are estimates for 0.25-μm porosity, 25.4-cm pleated filters with K-27 prefilters and were obtained using 0.25-μm flat filters in 25-mm holders as a model system, as described in the text.

DISCUSSION

Organic compounds present in low amounts in tap water may be concentrated along with viruses on membrane filters using procedures designed for virus concentration (1, 4). Buildup of these organics reduces the flow rate and eventually clogs the filters. Individual filters vary in the amount of water they can process before clogging. Filterite epoxy-fiberglass filters are capable of processing larger volumes of treated tap water than are Cox, Acropor, or Millipore filters of similar size (1). In addition, the Filterite filters can be pleated, and the 25.4-cm cartridges can be housed in conventional holders. This permits a filter with a large surface area to be contained in a relatively small holder. The combination of large surface area and resistance to clogging permits reuse of the epoxy-fiberglass filters to process several large volume samples of water. However, routine elution does not remove all adsorbed virus and organics. Treating used filters with 0.1 N NaOH reduces by 4 to 5 logs the residual virus not removed during routine elution with basic buffer. Also, treatment with base removes adsorbed organic compounds and permits reuse of clogged filters. As an alternative, autoclaving may be used; it reduces adsorbed virus to levels below detectability (>6.5-log reduction) and allows reuse of filters that have not been clogged. Since the pleated filters protected by a K-27 prefilter can likely process between 19,000 and 100,000 liters of tap water before clogging, autoclaving alone should permit reuse of the filters several times before regeneration with base is required.

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LITERATURE CITED

1. **Farrah, S. R., C. P. Gerba, C. Wallis, and J. L. Melnick.** 1976. Concentration of viruses from large volumes of tap water using pleated membrane filters. Appl. Environ. Microbiol. 31:221-226.
2. **Farrah, S. R., C. Wallis, P. T. B. Shaffer, and J. L.**

Melnick. 1976. Reconcentration of poliovirus from sewage. Appl. Environ. Microbiol. 32:653-658.

3. **Melnick, J. L., and H. A. Wenner.** 1969. Enteroviruses, p. 529-602. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral and rickettsial infections, 4th ed. American Public Health Association, New York.
4. **Wallis, C., A. Homma, and J. L. Melnick.** 1972. Apparatus for concentrating viruses from large volumes. J. Am. Water Works Assoc. 64:189-196.