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Comparison of Microporous Filters for Concentration of Viruses from Wastewater

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The 1-MDS Virosorb filter and the 50S and 30S Zeta-plus filters, all with a net positive charge, were compared with the negatively charged Filterite filter for concentration of naturally occurring coliphages and animal viruses from sewage effluent. When Filterite filters were used, the effluent was adjusted to pH 3.5 and $AlCl_3$ was added before filtration to facilitate virus adsorption. No adjustment was required with the positively charged filters. Sets of each filter type were eluted with 3% beef extract (pH 9.5) or eluted with 0.05 M glycine (pH 11.5). A maximum volume of 19 liters could be passed through 142-mm diameter Filterite filters before clogging, whereas only 11, 11, and 15 liters could be passed through the 1-MDS, 50S, and 30S filters, respectively. For equal volumes passed through the filters, coliphage recoveries were 14, 15, 18, and 37% in primary effluent and 40, 97, 50, and 46% in secondary effluent for the Filterite, 1-MDS, 50S, and 30S filters, respectively. No statistically significant difference was observed in the recovery of animal viruses among the filters from secondary effluent, whereas in the Filterite and 50S filters, higher numbers of viruses from primary effluent were recovered than in the 1-MDS and 30S filters in two of three collections. Glycine was found to be a less-efficient eluent than beef extract in the recovery of naturally occurring viruses.

Various types of microporous filters have been used to recover viruses from large volumes of water. In the past, electronegative filters have been employed which require the acidification of the water and addition of multivalent cations for optimal virus adsorption (5, 6, 16, 19, 21). Because of the need to condition the water to achieve good recoveries, the method can be cumbersome, and many viruses, especially bacteriophages, may be sensitive to the low pH required (18).

Recently, electropositive filters have been used for virus concentration. These filters eliminate the need to condition the water, thus simplifying the procedure. Sobsey and coworkers (20, 22) first evaluated the positively charged filters for concentrating seeded poliovirus from tap water. Subsequently, the filters were tested for the recovery of bacteriophage, enteroviruses, influenza virus, and bacteria from a variety of media in seeded laboratory studies (3, 8–10, 12, 14). Some of the positively charged filters have also been successfully used for the detection of naturally occurring viruses in swimming pools, drinking water, and wastewater (3, 10, 11, 13).

The purpose of this study was to compare electropositive and electronegative filters in their ability to recover naturally occurring coliphages and animal viruses from primary and secondary sewage.

MATERIALS AND METHODS

Filters. The filters were used as 142-mm flat disks and were housed in stainless steel holders. The electronegative or Filterite filters (Filterite Corp., Timonium, Md.) (22) have nominal pore sizes of 3.0 and 0.45 μm. The electropositive filters were of three types. The first two types were 50S and 30S Zeta-plus depth filters (series S, Zeta-plus; AMF Cuno, Meriden, Conn.) (22) with nominal porosities of 0.75 and 1.0 μm, respectively. The third positive filter, the 1-MDS Virosorb, has a nominal pore size of 0.2 μm and was used as a double layer as previously recommended for maximum virus retention (20). In some instances, the 30S filter was used as a prefilter in combination with one sheet of the 1-MDS filter.

Concentration experiments. Primary (collected after passage through grit chambers) and secondary sewage (collected before chlorination) were collected from an activated sludge treatment plant. The pH and turbidity for the primary sewage were 7.4 and 30 nephelometric turbidity units, respectively, and for the secondary sewage they were 6.8 and 5.0 nephelometric turbidity units, respectively, and remained very constant throughout the study. A volume of up to 100 liters was taken at each collection, and from this single-grab sample, the sewage was processed through the filters at random. The sewage was placed in a 20-liter pressure vessel, and the desired volume was passed through each filter tested with positive pressure. In the case of the Filterite filter, the sewage was first adjusted to pH 3.5 with 1 N HCl, and 5 mM $AlCl_3$ was added before filtration. The adsorbed viruses were eluted with 50 ml of a 3% beef extract solution at pH 9.5. A duplicate filtration was run for each filter and eluted with 50 ml of 0.05 M glycine at pH 11.5. The eluates were immediately adjusted to a neutral pH and assayed for coliphages and animal viruses.

Viral assays. Coliphages were assayed in the orginial water sample as well as the eluate so that percent recoveries could be determined. The soft-agar overlay technique was employed with *Escherichia coli* ATCC 15597 as the host (1).

Animal viruses were enumerated on buffalo green monkey cells by an agar-overlay technique for determination of PFU (15). With cytopathogenic effect production as a positive test, the five-tube most probable number (MPN) method with 2, 0.2, and 0.02-ml volumes was also used in enumeration of the viruses.

Statistical analysis. MPN values were calculated by a program written by one of the authors (L.M.K.). Analysis of variance was performed by the FACTAN program described by Sokal and Rohlf (24). Transformation of the data was required to satisfy the necessary assumptions for valid analysis of variance. Plaque counts were transformed to

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TABLE 1. Comparative capacity of 142-mm disk filters

Filter	Nominal pore size (μm)	Vol (liter) passed through filter before clogging[a]
Filterite	3.0 + 0.45	19
1-MDS	0.2	11
50S	0.75	11
30S + 1-MDS	1.0 + 0.2	15

[a] Secondary sewage with a turbidity of 5.5 nephelometric turbidity units.

$\log_{10}$, or MPN values and low plaque counts that had a Poisson distribution were transformed to $\sqrt{N + 0.5}$ (24). The computations were performed with the Cyber 175 and Dec-10 computers at the University of Arizona Computer Center.

RESULTS

The four filter systems tested in this study showed some differences in the volumes of sewage which could be passed through before clogging (Table 1). The Filterite filter was able to pass a maximum volume of 19 liters of secondary effluent at a turbidity of 5.5 nephelometric turidity units. The 1-MDS and 50S filters passed 11 liters, whereas the capacity of the 1-MDS filter was increased by 4 liters when the 30S filter was used as a prefilter.

For evaluation of the ability of the filters to concentrate coliphages, 1 liter of primary effluent and 5 and 10 liters of secondary effluent were passed through each filter system. Coliphages were enumerated in the grab samples from both primary and secondary sewage before concentration by filtration and in each eluate so that the percent recovery could be determined. The Filterite filter had the lowest efficiencies for secondary effluent of all the filters tested (Table 2). The filters performed equally in their ability to recover phage from primary effluent, and the percent recovery was found to be consistently lower than that in the secondary effluent. Overall, glycine was a poor eluent compared with beef extract for coliphage recovery.

A total of six samples of secondary effluent was processed and assayed for animal viruses by either the PFU or MPN method. A statistical analysis showed no significant difference in the number of viruses isolated between the negatively or positively charged filters. In this case, the 30S and the 1-MDS filters were evaluated separately. The results indicate that glycine is a less-efficient eluent (Table 3). Three samples of primary effluent were processed and assayed by both the PFU and MPN methods. In two of the samples, the Filterite and the 50S filters recovered higher numbers of viruses than did the 1-MDS or 30S filters. Again, the results indicate that glycine is a less-efficient eluent (Table 4).

TABLE 2. Recovery of coliphages from sewage

Filter	% Recovery of coliphages with the following effluents at the indicated volumes					
	Secondary				Primary	
	5 liter[a]		10 liter[a]		(1 liter)	
	BE[b]	G[c]	BE	G	BE	G
Filterite	22	12	40	23	17	12
1-MSD	61	36	92	44	14	15
50S	100	62	50	52	14	5
30S + 1-MDS	58	38	46	35	31	6

[a] Average of two experiments.
[b] Beef extract (pH 9.5).
[c] Glycine (pH 11.5).

In Fig. 1, the three collections of primary effluent are compared for the numbers of PFU obtained in each individual assay for the four filters and two eluents by the square root transformation (24). In the first collection, the Filterite and 50S filters did equally well, whereas the 1-MDS and 30S filters recovered lower numbers of viruses. In the second collection, the Filterite filter barely outperformed the 50S filter, which in turn again did better than the 1-MDS and 30S filters. In the third collection, all filters recovered equally low numbers of viruses. In 6 of 12 cases, glycine recovered significantly lower numbers of viruses than did the beef extract, whereas in only two cases did the beef extract recover fewer viruses.

There have been legitimate concerns when enumerating viruses by either the MPN or PFU method. The MPN method, a statistical estimation with a wide range, has been universally accepted in the determination of coliforms (2). The PFU method gives an exact count but may inhibit some environmental viruses from plaquing due to the stress of the overlay or could show false plaques, depending on the nature of the water (17). The number of viruses obtained by assay of primary effluent by both techniques were almost identical (Table 4).

DISCUSSION

Previously published comparative studies with negatively and positively charged filters involved the use of laboratory strains of animal viruses and were concerned with virus

TABLE 3. Recovery of animal viruses from 10 liters of secondary effluent

Sample no.[a]	No. of viruses recovered with the following effluents and filters:							
	BE[b]				G[c]			
	Filterite	1-MDS	50S	30S	Filterite	1-MDS	50S	30S
1[d]	32	18	11	20	ND[e]	ND	ND	ND
2[d]	30	109	24	ND	ND	ND	ND	ND
3	325	5	<2	<2	13	13	58	ND
4	57	82	57	13	85	13	<2	43
5	<2	600	ND	5	5	<5	<2	<2
6	28	<2	<2	23	<2	<2	<2	<2

[a] 100-liter samples.
[b] Beef extract (pH 9.5).
[c] Glycine (pH 11.5).
[d] Enumerated by PFU; the other samples (3 through 6) were enumerated by MPN.
[e] ND, Not determined.

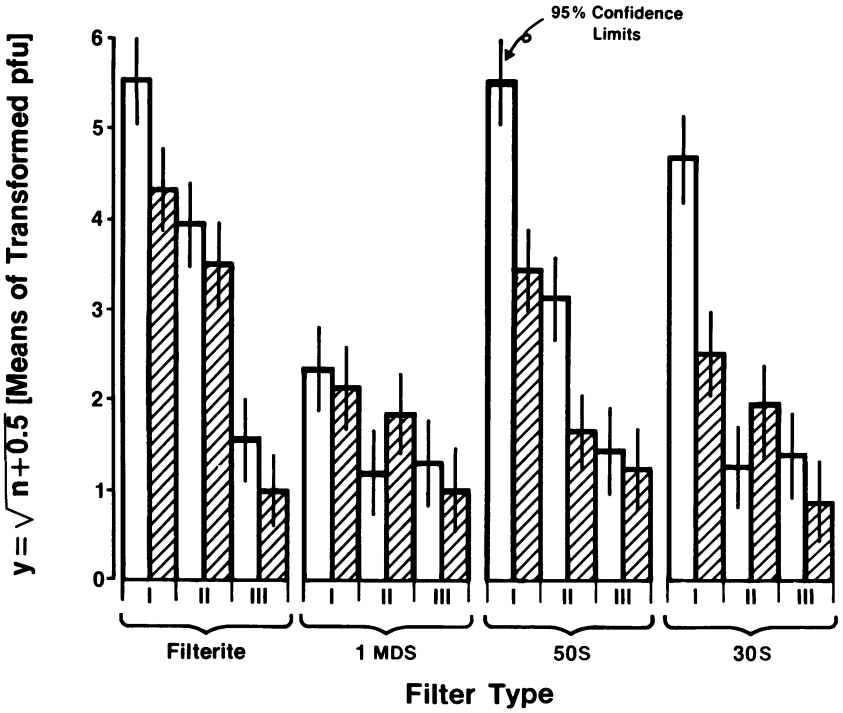

FIG. 1. Evaluation of beef extract (□) and glycine (▨) for elution of animal viruses from filters from three separate collections (I, II, and III) of primary effluent.

concentration from tap water (20, 23). The goal of the present study was to compare these filters and two of the most commonly used filter eluents for recovery of indigenous virus from sewage.

Recovery of coliphages from secondary effluent was significantly better with the positively charged filters than with the Filterite filter, but there was little difference in overall recovery from primary sewage. Differences in recovery from the secondary effluent are probably due to the sensitivity caused by the low pH conditions used for virus concentration with Filterite filters (10, 18). The higher turbidities and organic concentration present in primary sewage probably account for the generally poor recoveries of coliphages.

In waters such as primary effluent with high turbidities and organic matter, high adsorption and recovery efficiencies may be lost due to viruses associated with solids (7) and competition for adsorption sites (19). Sobsey et al. (23) have found that water quality influenced virus adsorption and recovery from both the Filterite and 1-MDS filters. The Filterite filter had better recoveries than did the 1-MDS filter with the addition of 5 mM $MgCl_2$, but it also showed a greater variability. In this study, the coliphage recoveries in primary effluent were lower for all filters investigated, and the numbers of animal viruses recovered varied greatly between different collections, with the Filterite filter showing the greatest variability and the 1-MDS filter the least.

Overall, the positively charged filters can be used to efficiently concentrate viruses from sewage effluents. Although the 1-MDS and 30S filters performed as well as the 50S filter in secondary effluent, they did not do as well with primary effluent. The 1-MDS filter, however, has an advantage as it is available in cartridge form, thus with the increased surface area, larger volumes of water could be processed (20). All the positively charged filters offer an advantage over the negatively charged filters, as preconditioning of the water sample is unnecessary, although Chang et al. (3) have found improved recovery from seeded sewage samples with the 30S filter when the effluent was adjusted to a pH below 6.0.

Positively charged filters thus far have shown great versatility for concentrating bacteria and endotoxins (12) as well as viruses. It is still uncertain as to what conditions are optimal when using these filters for various types of water or

TABLE 4. Comparison of MPN and PFU methods for enumerating animal viruses from 4 liters of primary effluent

Filter	Sample no.[a]	No. of viruses recovered with the following eluents by the following methods:			
		Beef extract		Glycine	
		MPN	PFU	MPN	PFU
Filterite	1	677	830	98	387
	2	58	60	58	19
	3	198	59	58	35
1-MDS	1	123	130	123	110
	2	32	40	20	15
	3	54	35	32	30
50S	1	83	165	123	170
	2	58	40	19	30
	3	54	70	58	60
30S	1	69	170	ND[b]	ND
	2	58	45	11	10
	3	32	25	58	35

[a] 50-liter samples.
[b] ND, Not determined.

whether all virus types are efficiently concentrated and further investigation is necessary; however, electropositive filters are indeed part of the answer to a simpler, more-reliable means for detecting viruses in the water environment. Percent recoveries of coliphages from secondary effluent concur closely with those determined in a study by Goyal et al. (10), in which the Zeta-plus filters were evaluated. It was not surprising to find that glycine at pH 11.5 was a poorer eluent than the beef extract for both coliphages and animal viruses. This has been reported by other investigators, possibly due to the inactivation of some viruses at the high pH (21) and superior performance of a protein solution for eluting viruses from filters (4, 10).

No statistically significant difference was observed in the recovery of animal viruses among the filters from secondary effluent (Table 3), whereas the Filterite and 50S filters recovered greater numbers of viruses from primary effluent than did the 1-MDS and 30S filters in two of three collections (Table 4). These data demonstrate that no major differences exist between the abilities of negatively and positively charged filters for recovery of indigenous animal viruses from effluents. The lower efficiencies observed with two of the positively charged filters used with primary sewage is probably due to the increased concentration of organic matter which competes with virus for adsorption onto the filter. The larger surface area of the 50S filter compared with the other positively charged filters may account for its better performance.

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