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Positively Charged Filters for Virus Recovery from Wastewater Treatment Plant Effluents

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Positively charged Zeta Plus filters were used to concentrate enteroviruses from 19 liters of effluent from activated sludge units. Neither the addition of salts nor the acidification of the effluent was required for adsorption of viruses to the filters. Viruses adsorbed to the filters were eluted by treating the filters with a solution of 4 M urea buffered at pH 9 with 0.05 M lysine. Eluted viruses were concentrated into final volumes of 1 to 2 ml by using a two-step concentration procedure that employed inorganic and organic flocculation. Approximately 50% of the viruses added to effluents could be recovered in the final sample. The procedure was used to monitor effluents from activated sludge units at two wastewater treatment plants for the presence of enteroviruses.

Membrane filtration with microporous filters as adsorbents is considered to be one of the best procedures for recovering viruses from relatively large volumes of water and wastewater (1). Previously, the filters used for concentrating viruses from water had a net negative charge at the pH of most tap waters (pH 7 to 8) and did not adsorb viruses efficiently unless the water was acidified or treated with certain salts (1). Recently described filters retain a positive charge at pH values near neutrality and have been used to concentrate viruses from tap water at ambient pH values without the addition of salts (20). Positively charged filters have been used for virus concentration from tap water in several studies (6, 18, 20). Small amounts of poliovirus in a large volume (380 liters) of drinking water were concentrated to a final volume of 25 ml by using positively charged microporous filters, and the overall virus recovery was 22.5% (20). Goyal et al. (13) evaluated the positively charged membrane filters for coliphage concentration from tap water and sewage effluents and obtained recoveries ranging from 34 to 100%. Coliphage occurring naturally in raw sewage and secondarily treated effluent were recovered with average efficiencies of 56.5 and 55.0%, respectively. Similar results were obtained by Bitton and his co-workers (2) with the same method. No work was done, however, on animal virus concentration from wastewater effluents with positively charged microporous filters.

Farrah and Bitton (6) have evaluated a series of eluents for virus recovery from various filters, including positively charged filters. They found that 4 M urea-0.05 M lysine, pH 9, efficiently eluted poliovirus from positively charged filters. It was found later that urea-lysine also performed well in virus recovery from sludge (8) and marine sediments (3).

The purpose of this study was to test the performance of positively charged filters in virus concentration from wastewater effluents.

The activated sludge effluent was sampled at the outlet, before chlorination, at the University of Florida sewage treatment plant, Gainesville, and at the Gainesville sewage treatment plant.

Poliovirus type 1 (strain LSc) and coxsackievirus B3 were used in the seeded experiments and assayed on the MA-104 cell line and buffalo green monkey kidney cells (BGM), respectively, using a methylcellulose overlay as previously described (4). Samples were assayed after dilution in phosphate-buffered saline with 2% fetal calf serum.

Indigenous viruses were detected by using the BGM cell line overlaid with Eagle minimum essential medium with 2% fetal calf serum as previously described (7).

Positively charged Zeta Plus (30S series) filters (AMF, Cuno Div., Meriden, Conn.) were used. The filters were 273 mm in diameter and were housed in a flat Plexiglas holder. An MDS cartridge filter, kindly supplied by AMF, was also used for recovery of idigenous viruses from 114 liters of activated sludge effluent.

Five gallons (19 liters) of activated sludge effluent was seeded with poliovirus type 1 or coxsackievirus B3 and passed through Zeta Plus filters housed in a 273-mm-diameter Plexiglas holder at a rate of 5 liters/min. Viruses adsorbed to the filter surface were eluted by

passing 400 ml of 4 M urea–0.05 M lysine, pH 9, through the filters. The eluent was allowed to cover the entire filter surface. The contact time with the filter was approximately 30 s. The eluate was assayed for viruses to determine the efficiency of the elution step and was then adjusted to 0.05 M $AlCl_3$, neutralized with 1 M sodium carbonate, and mixed for 5 min. The resultant flocs were collected by centrifugation at 4,000 × *g* for 5 min, and the supernatant was discarded. The flocs were mixed with 5 volumes of 3% beef extract–0.1 M ethylenediaminetetraacetic acid at pH 9. Most of the floc could be dissolved in this solution. After mixing, the sample was centrifuged at 4,000 × *g* for 5 min.

The supernatant from this centrifugation step was neutralized and dialyzed against phosphate-buffered saline at pH 7.2 for 8 h at 4°C. After dialysis, the sample was further concentrated via organic flocculation by the method of Katzenelson et al. (14). This concentration procedure resulted in a final volume which did not exceed 2 ml.

This concentration procedure was also used for the recovery of indigenous viruses from 5 gallons (19 liters) of activated sludge effluent. In one experiment, an MDS positively charged cartridge filter was used to recover indigenous viruses from 30 gallons (114 liters) of activated sludge effluent to compare its efficiency with the 19-liter sample collected on the same day.

In preliminary experiments (data not shown) it was found that Zeta Plus series 30S filters gave a higher recovery of poliovirus than did Zeta Plus series 30C filters. Furthermore, to prevent clogging of the filters, larger-pore-size Zeta Plus filters (30S) were used instead of the series 50S and 60S filters previously used by other workers (2, 13). Series 30S filters have a 1-μm porosity, whereas series 50S and 60S filters have porosities of 0.65 and 0.45 μm, respectively. Thus, 30S Zeta Plus filters were subsequently used in all experiments.

Farrah and Bitton (6) have found that 4 M urea with 0.05 M lysine was a good eluent for Zeta Plus filters as well as other filters (Acropor, Filterite, and Millipore). The urea likely interferes with hydrophobic interactions between the virus and the filter (9), whereas the lysine is used to buffer the solution at a pH value at which both the filter and virus have net negative charges (16). Therefore, both electrostatic and hydrophobic interactions between the virus and the filter are reduced, and elution occurs. We have used this eluent for virus concentration from activated sludge effluent throughout the entire study. The results of five trials are shown in Table 1. Virus recovery from the elution step was highly efficient, and mean recoveries of 98 and 97% were achieved for poliovirus and coxsackievirus, respectively. The mean overall recoveries after elution and two concentration steps were 49 and 44% for poliovirus and coxsackievirus, respectively.

Another merit of this method is that 19 liters of activated sludge effluent could be concentrated to volumes as low as 0.8 ml. Such a small volume can be entirely and economically assayed on tissue cultures. This gives an advantage in the detection of low virus concentrations in wastewater effluents. The concentration factor ranged from 15,200 to 23,750 (Table 1).

Other workers have used membrane filters to concentrate viruses from large volumes of secondarily treated effluents. Farrah et al. (10) were able to concentrate viruses from 390 liters of clarified sewage effluent to a final volume of approximately 40 ml, with a mean recovery of 40%. However, the samples were passed through a series of clarifiers to remove suspended solids before adding the viruses, and the eluate was passed through a carbon column and through ion-exchange resins to remove organic compounds from the eluate. Gerba et al. (12), using pleated membrane filters, concentrated 19 to 190 liters of secondarily treated sewage with an average efficiency of 50%. However, the eluent they used, glycine buffer, had a pH of 10.5 to 11.5, and this may lead to inactivation of certain viruses (5, 11, 19). Their method (12) also included salt and acid addition to wastewater samples before membrane filtration, and this complicated the concentration procedure.

We have also evaluated our method with regard to the recovery of indigenous viruses from activated sludge effluent. Results of seven trials are shown in Table 2. Poliovirus type 1, type 2,

TABLE 1. *Concentration of poliovirus type 1 (LSc) and coxsackievirus B3 from 19 liters of activated sludge effluent by using positively charged membrane filters*[a]

Virus type	Total virus input (PFU)[b]	Elution step (% recovery)	% Overall recovery	Final concentrate vol (ml)	Concn factor
Polio 1	8.91×10^7	91.5	42.2	0.8	23,750
	1.56×10^8	100.2	47.4	1.0	18,095
	9.96×10^7	102.0	55.8	1.2	15,200
Coxsackie B3	1.88×10^7	94.1	46.8	1.1	17,270
	1.41×10^7	100.0	40.1	1.1	17,270

[a] Seeded activated sludge effluent was passed through a 273-mm-diameter disk Zeta Plus 30S filter. Viruses adsorbed on the filter were eluted with 4 M urea–0.05 M lysine at pH 9. The eluate was flocculated with 0.005 M $AlCl_3$, and the aluminum hydroxide flocs were dissolved in a 3% beef extract–0.1 M ethylenediaminetetraacetic acid solution at pH 9. This concentrate was further concentrated by organic flocculation after dialysis against phosphate-buffered saline to eliminate ethylenediaminetetraacetic acid. The final floc was dissolved in 0.15 M Na_2HPO_4 at pH 9.

[b] PFU, Plaque-forming units.

TABLE 2. *Detection of indigenous enteroviruses in activated sludge effluent by using positively charged membrane filters*[a]

Date (1980)	Sample source (sewage treatment plant)	Sample vol (liters)	Concn factor	Final concentrate vol (ml)	$TCID_{50}$/liter[b]	Virus types[c]
28 November	Gainesville	114	34,545	3.3	0.4	Polio 2
28 November	Gainesville	19	9,500	2.0	1.4	Polio 1 and 3
31 October	Gainesville	19	9,500	2.0	1.4	Polio 1
21 November	Gainesville	19	12,667	1.5	10.0	Polio 1 and 2
3 October	U.F.[d] campus	19	10,555	1.8	0.1	Coxsackie B3
2 August	U.F. campus	19	9,048	2.1	0.1	Polio 2
10 October	U.F. campus	19	9,048	2.1	ND[e]	ND

[a] Technique described in text.
[b] The 50% tissue culture infective dose ($TCID_{50}$) was determined by the procedure of Reed and Muench (17).
[c] Enterovirus types were determined by serum neutralization tests or the Lim and Benyesh-Melnick pool method (15).
[d] U.F., University of Florida, Gainesville.
[e] ND, Not detected.

and type 3 and coxsackievirus B3 were found. Virus concentrations ranged between 0.1 and 10.0 50% tissue culture infective doses per liter of activated sludge effluent. The concentration of indigenous viruses with the MDS cartridge filter to recover virus from 114 liters of effluent was 0.4 50% tissue culture infective dose per liter, which was lower than the concentration of viruses from the 19-liter sample collected on the same date. Since the samples were nonhomogeneous, the low 50% tissue culture infective dose did not necessarily mean that the MDS cartridge filter was less efficient.

The method that we have developed for virus concentration from activated sludge effluent is economical, efficient, and easy to perform. There is no need to add salt or acid to the effluents used in these studies to enhance virus adsorption onto the positively charged membrane filters. However, pH adjustment of some effluents may be required to obtain efficient adsorption of viruses. We have found that recovery of seeded or indigenous viruses in effluents from other treatment plants is improved by adjusting the pH of the samples to below 6 (unpublished data). The urea-lysine eluent used works well at pH 9, and this helps avoid the potential harmful effect of high pH on virus infectivity (5, 11, 19). Finally, the most important feature of this method is that the final volume of the concentrate is small, which means lower costs with respect to viral assay.

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