APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1983, p. 232-237
0099-2240/83/010232-06$02.00/0

Vol. 45, No. 1

Concentration of Coliphage from Water and Sewage with Charge-Modified Filter Aid

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Received 23 July 1982/Accepted 1 October 1982

Methods of detecting and concentrating animal viruses from large volumes of water and wastewater have experienced rapid development in recent years, but only a few methods are available for the concentration of bacteriophages. The present study describes the use of a charge-modified (Zeta Plus) filter aid (AMF Cuno, Meriden, Conn.) for the concentration of coliphages from large volumes of water and sewage. Coliphages MS-2 and f2 were efficiently adsorbed from water and sewage to the positively charged filter aid. Elution was accomplished with 4% beef extract–0.5 M NaCl adjusted to pH 9.5. The recovery of f2 from 10- to 20-liter volumes of tap water ranged between 11 and 70%, and the recovery of MS-2 ranged between 43 and 70%. The efficiency of recovery of naturally occurring coliphages from secondarily treated sewage ranged between 16 and 44%. This technique appears to be promising because it requires low-cost equipment (47-mm polypropylene filter housing), is easy to handle, and can filter large volumes of water (≥20 liters) with good recoveries. Filtrations can be conducted at the ambient pH of the water, and the unit cost per filtration (i.e., the cost of filter aid) comes to less than three cents per sampling. The technique could be useful in evaluation of viral water quality, study of the ecology and occurrence of phages in natural waters, and isolation of rare phages from natural waters.

It is known that small numbers of enteroviruses may cause overt disease if consumed via contaminated water or food (13). Therefore, methods have been developed to concentrate and detect small numbers of animal viruses from large volumes of water and wastewater. However, the isolation of animal viruses is time consuming and costly. Because of the ease and simplicity of coliphage assay, a number of investigators have advanced coliphages as indicators of water pollution by animal viruses (7, 17, 18). Kott and associates (10) have studied coliphage and enterovirus levels in wastewater treatment plants, surface waters, and tap waters and have shown coliphage levels invariably exceeding animal virus levels but being less than coliform counts. The resistance of a typical coliphage to chlorination was shown to be greater than that of poliovirus type 1, and they concluded that for routine microbiological examination, phage recovered on *Escherichia coli* may provide a measure of the viral quality of waters. This view was further substantiated in their later investigations (8, 9).

The ecology and distribution of bacteriophages in natural waters has not been well elucidated, possibly because of the unavailability of simple and efficient methods to detect small numbers of phages in natural waters. Enrichment methods of phage assay have been proposed which may be used for detecting low levels of phages active against specific host bacteria (6). However, with these methods, usually only one phage type (the one that replicates fastest or the one that predominates in the inoculum) is isolated. Primrose and Day (14) have described a method for concentrating bacteriophages by adsorption and elution to hydroxylapatite, with the recoveries ranging from 33 to 90%, depending on the type of coliphage. In other investigations, membrane filter filtration has been used to concentrate small amounts of phage (11, 19).

Methods of detecting and concentrating animal viruses from water and wastewater have experienced rapid development in recent years (3, 21, 22). The most commonly used method involves adjusting water to pH 3.5 and passing it through microporous filters after the addition of trivalent salts. Under these conditions the viruses have a net positive charge, enabling them to adsorb to the negatively charged membrane filters. The viruses are eluted by small volumes of high-pH buffer (3, 4, 21, 22). Although this method is good for the recovery of animal viruses from large volumes of water, it is not easily applied to bacteriophages, which are very sensitive to inactivation at the low and high pHs used

in these concentration techniques.

Recently, Sobsey and Jones (20) have observed that poliovirus adsorbs efficiently to positively charged Zeta Plus filters in the pH range of 5.5 to 7.5 without the need for added polyvalent cations. Goyal et al. (5) have found that Zeta Plus depth filters adsorb coliphages from tap water, sewage, and lake water at neutral pH and that the coliphages could be eluted with 4% beef extract–0.5 M NaCl. Using this method, these workers could concentrate coliphages from tap water with recoveries ranging from 34 to 100% and from secondarily treated sewage with an average efficiency of 55%. Logan et al. (12) also have observed that Zeta Plus filters give good recoveries (50 to 60%) of coliphages from 65-liter volumes of river water. The present study describes the use of charge-modified filter aids to concentrate and detect coliphages from water and wastewater.

MATERIALS AND METHODS

Phages and phage assays. The bacteriophages MS-2 and f2 used in this study were obtained from American Type Culture Collection and were grown and assayed with the host bacterium, *E. coli* B (ATCC 15595). These phages are small (28 nm), single-stranded RNA viruses. The coliphages were assayed by the PFU method. Culture and assay procedures were similar to those described by Adams (1), Davis and Sinsheimer (2), and Rovozzo and Burke (16).

Filter and filter aids. AP-20 prefilters (Millipore Corp., Bedford, Mass.) were used as bases for overlaying the slurry of charge-modified filter aids in 47-mm-diameter polypropylene Millipore filter housings. The filter aids used in this study were supplied by AMF Cuno (Meriden, Conn.). The charge-modified filter aids are prepared from perlite and have large surface areas per unit weight. The filter aids are mainly used to facilitate filtration and retard clogging during filtration processes. The electrokinetic adsorption by the filter aids is achieved by modifying the surface of the filter aid with resin to create a positive cationic charge on its surface. Since most suspended contaminants exhibit a net negative charge in the solution, the positive charge of the filter assists in the electrokinetic capture of contaminates.

Presently, AMF Cuno offers a positively charged surface-modified perlite which is available in three grades: coarse, medium, and fine. In this study, the coarse and fine grades were studied for the concentration of coliphages from water.

Preparation of filters. The desired quantity of filter aid was suspended in 30 to 50 ml of sterile distilled water, and the resultant slurry was passed through a 47-mm polypropylene filter holder containing an AP-20 prefilter with the aid of a syringe.

Virus concentration. Coliphages were seeded in dechlorinated tap water and then filtered through appropriate filters. Dechlorination of tap water was done by the addition of 1 to 3 mg of sodium thiosulfate per liter. Samples were collected before and after filtration, and the difference between the two was taken to be the amount of virus adsorbed to the filter. The adsorbed virus was eluted by passage of different types of eluents slowly through the filters with positive pressure. The eluents used were 3% beef extract (pH 9.5) (Scott Laboratories, Inc., Fiskeville, R.I.) and 4% beef extract containing 0.5 M sodium chloride (pH 9.5) (Mallinckrodt Chemical Works, St. Louis, Mo.).

RESULTS

Effect of pH and filter aid grade on f2 adsorption. To determine whether the pH and the type of filter aid play a role in f2 phage adsorption, tap water was adjusted to various pHs and seeded with virus, and various quantities of either coarse or fine filter aid were added. The mixture was stirred and centrifuged, and the supernatant was assayed for phage. The fine filter aid had more adsorption capacity than the coarse filter aid (Table 1). The adsorption of the viruses was maximal at pH 6.0 and decreased as the pH of the water was increased. The fine filter aid adsorbed more than 99% of the viruses at the ambient pH (7.4 to 7.6) of the tap water. Virus adsorption to negatively charged diatomaceous

TABLE 1. Effect of pH and filter aid on phage f2 adsorption[a]

Filter aid type	Amt of filter aid (g)	% Adsorption of f2 at the following pH:			
		6.0	7.6	8.5	9.5
Coarse	0.001	ND[b]	0	ND	ND
	0.01	ND	4	ND	ND
	0.1	99	98	98	93
Fine	0.001	ND	35	ND	ND
	0.01	ND	65	ND	ND
	0.1	99.9	99.7	99.1	99
Diatomaceous earth	0.1	ND	6	ND	ND

[a] A 100-ml sample of dechlorinated tap water was adjusted to the desired pH, and virus was added. This was followed by centrifugation at 3,000 rpm for 15 min and assay of the supernatant. The difference between the viral PFU in seeded water and that in the supernatant was taken as the amount of virus adsorbed to the filter aid. The input virus concentration was approximately 5.0×10^6 PFU/ml.

[b] ND, Not done.

TABLE 2. Effect of filter aid concentration on removal of phage f2 from tap water[a]

Vol of water filtered (ml)	Amt of filter aid (g)	Overall % adsorption
100	0.1	69
100	0.5	95
100	1.0	99.9
1,000	1.0	91
1,000	1.75	99.9
10,000	1.75	99
20,000	1.75	85

[a] Different volumes of dechlorinated seeded tap water were passed through 47-mm-diameter filters with AP-20 prefilters overlaid with the amount of Zeta Plus fine filter aid indicated. The flow rate of filtration was between 95 and 115 ml/min. The results are the average of three to four experiments. The input virus concentration was approximately 5.0×10^6 PFU/ml.

earth (Grade II, medium particle size; Sigma Chemical Co., St. Louis, Mo.) was also tested. The negatively charged filter aid retained few viruses. This was an indication that the higher efficiency of virus removal of charge-modified filter aids was probably a function of the positive charge present on the surface.

The effect of filter aid concentration when retained on an AP-20 prefilter is shown in Table 2. These results indicated that 1.0 g of fine filter aid was required to adsorb 99.9% of the virus from 100 ml of water under these conditions. To achieve higher adsorption from large volumes (10 to 20 liters) of water, the quantity of filter aid required was increased to 1.75 g. This was also the maximum quantity of filter aid which could be loaded in a 47-mm filter housing.

Comparison of eluents. A number of different eluents are effective in the elution of human enteric viruses adsorbed to microporous filters. For coliphages, either 3% beef extract (pH 9.0) or 0.5 M NaCl containing 4% beef extract adjusted to pH 9.0 is a good eluent (5). Therefore, these two eluents at pH 9.5 were compared for elution efficiency of adsorbed f2 phage under different conditions (Table 3). It was observed that 4% beef extract–0.5 M NaCl gave consistently better recovery than 3% beef extract alone. Therefore, 4% beef extract–0.5 M NaCl was selected as the eluent for further experiments. To determine the optimum volume of eluent, four separate 20-ml volumes of eluent (4% beef extract–0.5 M NaCl, pH 9.5) were passed through the filter aid serially, and each of them was assayed separately. Most of the viruses were eluted in the first two 20-ml volumes (Table 4). Therefore, 40 ml was selected as the optimum eluent volume.

TABLE 3. Effect of eluent on recovery of phage f2[a]

Vol of eluent (ml)	Eluent	% Recovery
10	BE[b]	21
10	BE + NaCl[c]	26
20	BE	25
20	BE + NaCl	33
40	BE	35
40	BE + NaCl	48

[a] Ten liters of dechlorinated seeded tap water at ambient pH (7.4 to 7.6) was filtered through 47-mm-diameter polypropylene filters with AP-20 prefilters overlaid with 1.75 g of Zeta Plus charged fine-grade filter aid. The adsorbed viruses were eluted by twice back passing different amounts of eluent. The input virus concentration was approximately 5.0×10^6 PFU/ml.

[b] BE, 3% beef extract (pH 9.5).

[c] BE + NaCl, 4% beef extract–0.5 M NaCl (pH 9.5).

TABLE 4. Influence of eluent volume on recovery of phage f2[a]

Expt	% Recovery with the following 20-ml vol of eluent:				Total % recovery
	1	2	3	4	
1	29	9	2	0.46	40
2	26	3	2	1	32
3	28	12	3	2	45

[a] Ten liters of dechlorinated seeded tap water was passed through 47-mm filters with AP-20 prefilters overlaid with 1.75 g of fine Zeta Plus filter aid. Thereafter, elution of adsorbed viruses was carried out by passing four separate 20-ml volumes of eluent (4% beef extract–0.5 M NaCl, pH 9.5) serially. The input virus concentration was approximately 5.0×10^6 PFU/ml.

Concentration of coliphages from water and wastewater. To determine the efficiency for coliphage concentration from tap water, 10- to 20-liter volumes of coliphage-seeded tap water were passed through the filter aid contained in 47-mm-diameter filter housings. The recovery of f2 phage averaged 48% when 10 liters was processed, whereas the efficiency decreased to 20% for 20-liter volumes (Table 5). The recovery of MS-2 phage from 10- to 20-liter volumes of tap water ranged between 48 and 67% (Table 5).

Under natural conditions, low numbers of phages may be present in water. Therefore, tap water was seeded with low numbers of MS-2 phage to determine the efficiency of this method for detecting and concentrating small numbers of phages from water. The method detected small numbers of phages from water, with recoveries similar to that found when large numbers were present (Table 6).

The method was next evaluated for the concentration of naturally occurring coliphages from secondarily treated sewage. Since the den-

TABLE 5. Concentration of coliphages from tap water[a]

Coliphage	Mean initial vol (liters)	Mean concn factor	Mean % adsorption[b]	Mean % recovery[b]
f2	10	250	92 (7.40)	48 (12.12)
	19.5	487	86 (7.93)	20 (6.45)
MS-2	10	250	99.6 (0.46)	67 (3.06)
	20	500	99 (0.79)	28 (8.85)

[a] Tap water pH, 7.4 to 7.6. Adsorbed viruses were eluted by passage of two 20-ml volumes of 4% beef extract–0.5 M NaCl (pH 9.5). The total amount of input virus in the influents ranged between 4.4×10^8 and 3.7×10^9 PFU. The results are averages of three to seven experiments.

[b] Numbers within parentheses are standard deviations of the means.

sity of coliphages in sewage is high enough to be easily detected before and after concentration, the efficiency of concentration could easily be determined. The recovery of coliphages ranged between 16 and 44% from secondarily treated sewage (Table 7).

DISCUSSION

Presently a number of methods are available for concentration of animal viruses from water and wastewater, but only a few methods are available for use with bacteriophages, and most of them require costly filtration equipment and filters. The most commonly used method for concentrating enteroviruses is the use of negatively charged microporous filters (21, 22), which are unsuitable for concentrating bacteriophages from natural waters (19). This method requires the adjustment of the water sample to pH 5.0 or below, which inactivates many bacteriophages. In addition, the use of high-pH eluents (pH 10.0 to 11.5) may also inactivate additional phages (15).

The microporous filters such as Zeta Plus and 1MDS (Virosorb) which are positively charged in the pH range (2 to 8) of most natural and tap waters have a definite advantage over negatively charged filters as virus adsorbents since they eliminate the need for acid or salt addition. Zeta Plus series filters efficiently concentrate coliphages at neutral pH from tap water, lake water, sewage, and river water (5, 12). Our observations (Table 2) with the coarse- and fine-grade positively charged filter aids also revealed that these could efficiently adsorb coliphages from tap water at ambient pH values (7.4 to 7.6). When comparing the coliphage adsorption efficiency of the coarse- and fine-grade filter aids, we found the fine-grade filter aid to be more efficient. This could possibly be attributed to the larger surface area per unit weight of the fine filter aid as compared with the coarse filter aid. That virus adsorption was due to electropositive charges present on the filter aid was substantiated by the low viral adsorption to the negatively charged diatomaceous earth, which is structurally similar to the perlite which is used in the preparation of the Zeta Plus filter aids (Table 1).

For eluting coliphages adsorbed to Zeta Plus filters, the eluents of choice have been either 3% beef extract (pH 9.0) or 4% beef extract–0.5 M NaCl (pH 9.0) (5). The 4% beef extract–0.5 M NaCl (pH 9.5) gave better recoveries than 3% beef extract alone (Table 3). The optimum volume of eluent for recovery of viruses was 40 ml (Table 4), but it was necessary to pass the eluent

TABLE 6. Concentration of low numbers of phage MS-2 from seeded tap water[a]

Initial vol (liters)	Final vol (ml)	Concn factor	Total PFU in influent	PFU recovered	% Recovery[b]
10	40	250	1,660	1,740	100
10	40	250	1,660	640	39
10	40	250	1,660	744	45
10	40	250	450	190	42
10	40	250	375	104	28
10	40	250	325	208	64

[a] Dechlorinated tap water (125 ml) was seeded with a low number of phage MS-2, and a sample from this was assayed to determine the viral PFU. Of this, 100 ml was mixed with 10,000 ml of dechlorinated tap water, and the mixture was filtered through a 47-mm-diameter AP-20 prefilter overlaid with 1.75 g of Zeta Plus fine filter aid.

[b] The adsorbed viruses were eluted by back passing two 20-ml volumes of 4% beef extract–0.5 M NaCl (pH 9.5).

TABLE 7. Concentration of naturally occurring coliphages from secondarily treated sewage[a]

Initial vol (liters)	Final vol (ml)	Concn factor	Viral PFU in influent	Viral PFU in filtrate	% Adsorbed	Viral PFU recovered	% Recovery
1	40	25	6.7×10^4	2.1×10^4	69	2.3×10^4	34
1	40	25	6.7×10^4	2.3×10^4	66	2.9×10^4	44
2	40	50	1.3×10^5	5.8×10^4	57	4.1×10^4	31
2	40	50	3.8×10^5	2.7×10^5	29	9.2×10^4	24
2	40	50	3.8×10^5	2.7×10^5	29	1.1×10^5	28
3	40	75	4.3×10^5	2.9×10^5	33	1.6×10^5	38
3	40	75	2.0×10^5	9.1×10^4	55	5.8×10^4	29
3	40	75	2.0×10^5	1.0×10^5	49	6.1×10^4	31
4	40	100	2.7×10^5	8.9×10^4	66	4.2×10^4	16
4	40	100	5.8×10^5	3.9×10^5	28	1.9×10^5	33
4.5	40	112	3.0×10^5	1.7×10^5	44	6.4×10^4	21
5	40	125	2.4×10^5	1.2×10^5	53	7.0×10^4	28

[a] The filtration was carried out at ambient pH (6.6 to 6.8) through a 47-mm AP-20 prefilter overlaid with 1.75 g of Zeta Plus fine filter aid. Adsorbed viruses were eluted by passage of two 20-ml volumes of 4% beef extract—0.5 M NaCl (pH 9.5).

twice through the filter aid to obtain the maximum virus recovery.

The present method efficiently concentrated coliphages from tap water as well as naturally occurring coliphages from secondarily treated sewage (Table 7), with good recoveries. As expected, lower recoveries of phage were observed when concentration from sewage was attempted. This is probably due to competition for adsorption sites with organic matter.

Although concentration efficiencies are somewhat lower than with other methods using filters, the use of charged filter aids has several major advantages. High concentration factors are possible because of the use of small filter housings. It was possible to easily process 10- to 20-liter volumes of water with a lightweight plastic, 47-mm-diameter housing, whereas previously used systems required the use of 147-mm or larger metal housings to process similar volumes. Average flow rates were close to 0.8 liter per min in the present method, and the unit cost per filtration (i.e., the cost of the filter aid) was less than three cents.

An added advantage is that filtrations can be conducted at the ambient or natural pH values of water and wastewater, thereby avoiding any loss or inactivation of bacteriophages due to extreme pH conditions. This method could be useful in the isolation of rare phages from natural waters or in the evaluation of viral water quality. The technique could also be useful for the study of the ecology and occurrence of viruses in natural waters.

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