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FATE OF MS2 PHAGES THROUGH MEMBRANE SYSTEMS FOR WASTEWATER AND WATER

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ABSTRACT

Experiments of seeded male-specific (MS2) bacteriophages were devised by the Research Group of the City of LA's Sanitation to characterize the response of microfiltration (MF) and reverse osmosis (RO) systems to varying wastewater quality and operating conditions. Secondary effluent was seeded with commercial bacteriophage, filtered through a trimedia filter, and then processed through the Department of Water and Power (DWP) MF and RO pilot unit at Terminal Island Treatment Plant (TITP). The virus removal by the RO was essentially perfect (100%), but the MF membranes consistently reduced virus concentrations by less than one log. This result agrees with results obtained by other experimenters who used clean water and clean membranes, but it contrasts with the observations of tests carried out in Australia and Maryland over several months, using secondary wastewater effluent. Comparison with other studies adds other possibilities to the Research Group's previous hypothesis that the high concentrations of seeded bacteriophage in the TITP experiments somehow affected MF membrane performance. Another somewhat surprising observation was the modest increase in MF virus removal efficiency with increasing membrane flux, but there was no reliable evidence of a significant affect due to membrane backwashing. As these bacteriophages are far smaller than the pore size of the equipment, virus removal is evidently dependent on the presence of some adsorbing or inactivating material on the filter membranes, and the presently available information suggests that a modest degree of bacterial fouling may contribute to virus removal by microfiltration. Clarification of such results would provide valuable insight for improved membrane technology as a component of large-scale, membrane-based water reclamation systems. Additional findings and cost evaluations will be briefly reviewed during the presentation.

KEYWORDS

efficiency, filtration, MS2 bacteriophage, membrane, osmosis, backwash

INTRODUCTION

The Research Group of Wastewater Engineering Services tested the effectiveness of MF and RO in removing viruses from reclaimed wastewater at the DWP Pilot Facility at TITP in March and April 1996. This was a step towards implementing the City's timetable for reclaiming increasing percentages of its wastewater in coming decades. Seeding with bacteriophages allowed the experiments to be conducted with much higher frequency than many other reported tests of MF and RO, and allowed filter performance to be examined in ways that appear to be new. This paper describes and analyzes the tests, the results, (Iranpour et al., 1998) and comparable work in wastewater and water treatment plants (Dwyer, et al., 1995; Kostelecky, et al., 1995; Olivieri, et al., 1991; Willinghan, et al., 1992; Powelson, et al., 1993, etc.) .

It is preferable to use MS2 bacteriophages (Havelaar, 1993) instead of observing the indigenous enteric viruses because the phages are nonpathogenic to humans and are easy to count by their effect on *E. coli* cultures. Bacteriophages are obligate parasites of bacterial genera that may be found in water, for example salmonella typhi, shigella, escherichia coli, etc. Such virus substitution was only approved by the California Department of Health Services (DHS) for this kind of wastewater pilot test in 1995, so this is an innovative aspect of this study. However, by the time of the approval substantial previous experience had been accumulated by researchers who used MS2 phages as tracers in other types of tests. In one study, Yahya, et al. (1996) compared MS2 and polioviruses for sensitivity to inactivation by UV and found that poliovirus is more sensitive to UV than MS2 is, since 4 logs of inactivation were obtained for poliovirus at a UV dosage of 80 mW-s/cm^2, while 120 mW-s/cm^2 were needed for MS2. On the other hand, Yahya et al. (1991) had previously found that

MS2 was very much more sensitive than poliovirus to ions leached from copper and galvanized pipes. Yahya et al. (1993) and Powelson et al. (1993) used MS2 and PRD1 bacteriophages as tracers in, respectively, tests of virus removal by slow sand filtration and nanofiltration and tests of virus removal in aquifers being recharged with reclaimed wastewater. As PRD-1 phages are larger than MS2 phages (65 nm vs. 28 nm), they were removed to a greater extent in these tests. It seems reasonable that MS2 is more suitable for such mechanical filtration test than for tests of disinfection by chemicals or UV. The results of mechanical filtration are easily extrapolated to other viruses of known sizes without being influenced by factors that affect sensitivity to chemicals or UV.

MS2 phages have a nomimal size of 0.01-0.02 um roughly five times smaller than the nominal pore size of MF. They are typically present in wastewater in concentrations comparable to those of human enteric viruses, but both types are removed by standard wastewater treatment processes at approximately the same rate (Havelaar, 1993). Thus, the background concentration of MS2 phages in the secondary effluent, which is used as the feed to MF pilot units (e.g., Willingham, et al., 1991) is relatively low, often in the range 10 to 100 viruses per mL. Moreover, a high concentration of coliform bacteria and temperatures above 30°C seem to be necessary for significant multiplication of MS2 phages under natural conditions (Havelaar 1993). Thus, tracer tests that seed a wastewater stream with a higher concentration of viruses provide confidence that the observed viruses after filtration are indicators of filtration effectiveness.

Other tests of MF units (e.g., Water Board, 1992; Willingham, et al., 1992; and Olivieri et al., 1991) have observed the filtration efficiency for indigenous MS2 phages. However, they have taken samples no more frequently than once a day, except for the Australians' intensive sampling runs, each of which took samples every five minutes for an hour. The need to use a higher concentration in a seeded test and the limited supplies of concentrated virus culture dictated the use of much briefer laboratory tests, with sampling conducted every few minutes. This also allowed observation of the effect of the backwash cycle on MF performance.

Jacangelo (1995) notes that from a practical stand point higher loading tests are negligible since natural waters typically contain low concentrations of viruses. He also studies filtration efficiencies of MF based on physical sieving or adsorption of MS2, cake layer formation, and fouling state of the membrane and other conditions. When the MF is first started physical sieving or adsorption is the primary removal mechanism of MS2. The MS2 removal efficiency increases as solids in the water forms a cake layer on the membrane surface and decreases when the cake layer is removed by backwashing.

The references quotes here and many other papers including DWP (1996) have tested others water parameters with MF and RO units, e.g., turbidity, oil and grease, total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), silt density index (SDI), total organic carbon (TOC), total phosphorus, total kjeldahl nitrogen (TKN), total coliform, fecal coliform, fecal streptococci, coliphage, etc.

Most of the effort in this study focused on viral parameters of the MF unit, for two reasons. First, the virus removal by RO was essentially complete in the first two days of testing, so that additional testing of the RO effluent was considered a poor use of limited laboratory resources. Second, MF is also planned for use as a pretreatment for other forms of disinfection, such as UV (e.g., Jolis and Hirano, 1993, Iranpour et al. 1998) so that understanding MF performance is more critical for future planning than RO performance.

METHODS AND MATERIAL

Figure 1 is a simplified schematic of the experimental facility, showing only the equipment that was used in the virus testing. MMF consists of three trimedia pressure filters, each with anthracite on the top, sand in the middle, and garnet supporting these on the bottom. The Memcor 3M10C MF consisted of three parallel, hollow fiber, polypropylene membrane cartridges(Memtec,1995). The FS RO used thin film composite (TFC) (polyamide) membranes (model TFCL 4820 HR), which cannot tolerate free chlorine. The FS RO system consisted of a 4-vessel first stage and a 2-vessel second stage. The Dow RO used TFC membranes (Filmtec Model BW30-4040), which are coated with a polymer for biofouling resistance and are intolerant to free chlorine. The Dow RO system also consisted of a 4-2 two stage system. Each vassel contained three cartridges.

For the experiments the virus culture concentrate was mixed with the wastewater in the 750 gallon tank. The phage concentrate was supplied by BioVir Laboratories Inc. These experiments were done on March 27, April 3, April 23, and April 29, 1996.

On March 27, approximately 750 gallons of TITP secondary effluent from MMF were seeded with approximately 10^{12} viruses processed through the MF/RO system. Multiple samples were taken at the tank where the virus was mixed with the MMF effluent, at the MF inlet, MF outlet and at the RO outlet. On April 3, approximately 700 gallons of MMF effluent were seeded with approximately 10^{11} viruses.

On April 23, after evaluation of the results from the previous experiments, a larger test observed virus removal by MF units. Instead of processing one tankful, the mixing tank was refilled three times, so that 2800 to 2900 gallons of MMF effluent were processed over about 4 hours to simulate more closely the condition of full scale operation. Each tankful was seeded with approximately 10^{12} viruses. As the previous tests had shown that the virus levels in the mixing tanks and MF inlet were not significantly different, and that virus removal by reverse osmosis was perfect within available measurement capabilities, samples were taken only at MF inlet and MF outlet. To reduce the total number of samples that the lab had to process and to provide a more comprehensive monitoring of virus levels than would be provided by single samples taken at widely spaced times, each analyzed sample was composited from two or three samples taken over an interval of 3 to 6 minutes at one of the two sampling points. Corresponding samples were taken at each time at each sampling point. Another innovation compared to the two previous experiments was to separate the measurements taken during periods of normal MF operation from those taken immediately after a backwash.

On April 29 a final experiment was done to observe virus removal at flow rates of 14, 22, and 27 gpm. This was done because previous tests had all been done with flow rates of 18-20 gpm, but the MF units are capable of operating with a wider range of membrane fluxes. Two tanks were processed at each flow rate, during an experiment period totaling about four and a half hours. Each tank was seeded with approximately 10^{13} viruses, and as on April 23 samples were taken only at the inlet and outlet of the MF unit. The procedure also followed the April 23 pattern in the other respects: composited samples to suit available laboratory resources, and separation of periods of normal and post-backwash operation. The period between the backwashes was adjusted to match the flow rate to maintain the same schedule of backwashes after filtering every 350 to 400 gallons as in the previous tests.

RESULTS

Figure 2a shows the bacteriophage counts and their times for March 27. The "MFin" line is perfectly horizontal because the only available MF inlet value is an estimate based on an erroneous dilution, and the time of the sample was lost in the laboratory. All but two of the 1 values for the RO output are upper bounds, since no viruses were detected in these samples.

Figures 2b shows the results from April 3 in the same way. All of the RO values are upper bounds. Figure 2c shows the results from April 23 for the times of normal MF operation. Figures 2d shows the data from Figure 2c with additional values recorded during the periods immediately after backwashes to show a full set of measurements. Figure 2e shows the corresponding post-backwash values. Figure 2f is like Figure 2c, showing the data from April 29th for the times of normal MF operation. The first four points were recorded at approximately 14 gpm, the next four at 22 gpm and the last four at 27 gpm. Figure 2g is like Figure 2d, combining normal and post-backwash data and Figure 2h shows the corresponding post-backwash values.

The quickest and easiest inference from these data is that the RO unit provides essentially perfect virus removal, as would be expected from the physical nature of reverse osmosis.

The most prominent feature of the April 3 data is an upward trend in the MF outlet counts. However, the April 23 and 29 data do not show such a trend but show a relatively stable level with modest fluctuations. The logarithmic plots also show that the percentage fluctuations of the inlet and outlet concentrations are similar in magnitude, although not well correlated in time.

During normal operation of the MF about 0.8 log virus reduction occurred, but immediately after backwashing the virus reduction is typically in the range of 0.4 to 0.5 logs. However, in the results from April 29 this difference between normal and post-backwash operation did not persist.

The results in April 29 show a definite trend toward increased virus removal efficiency with increased flow rate during normal operation, but the post-backwash measurements do not show this trend. At 14 gpm about 0.4 logs of virus removal are observed in normal operation; at 22 gpm about 0.6 logs are observed; and at 27 gpm the factor of reduction is nearly 0.8 logs, with one pair of samples showing a full log.

Comparing the results from all four days suggests substantial variability from one day to another, and it is not clear now whether this is the result of some sort of intrinsic variability in filtration efficiency of such a microfiltration unit or is a result of the large variation in the input concentration of viruses. However since the result for each day appear to be relatively stable, the rise in efficiency with flow rate appears to be trustworthy.

Some perspective on the many fluctuations in the data could be provided by a more careful assessment of their uncertainty. One estimate of the uncertainty of a given set of points that are supposed to be samples from the same population is the familiar procedure of evaluating their mean and standard deviation. This is what was done above to show that the observed counts for the mixing tank on March 27 were significantly different from the count predicted by the dilution calculation. However, another approach to the uncertainty would be provided by considering that Poisson statistics (Iranpour, et al. 1988) apply to the counts of plaques on culture plates from which the PFU per ml values are derived. Thus, each plaque count n has a standard deviation of square root of n, and the relative uncertainty that this provides carries over to the estimate of PFU/ml. In this way each of the count values in the figures and tables could be given an error bar from the raw laboratory data. This would greatly assist judging the significance of these results, and could be useful in related future work.

COMPARISONS

Table 1 summarizes the specification of the MFs used in similar studies. Table 2 gives the test results for the tests in Table 1.

From comparing these results to, for example, the Blackheath, Australia study (Water Board, 1992), the Baltimore study (Willingham, et al., 1991), and Southern California Metropolitan Water District (SCMWD) study (Kostelecky, et al., 1995), it is clear that the virus removal in the April 3, 23, and 29 experiments was much less than the approximately two to three logs that other experimenters have usually observed. However, the Baltimore study observed one period of four weeks when only 1 log reduction occurred, and this also occurred on several other locations when it lasted only one week.

The most consistent success in removing viruses from screened secondary wastewater effluent by microfiltration has been reported from Australia by the Sydney-Illawarra-Blue Mountains Water Board. Their test of the Memcor microfiltration unit lasted five months, with samples recorded once or twice a day, and estimated the mean reduction of native MS2 coliphage during this period to be 3.2 logs. This, however, is almost certain to be an underestimate of the actual mean reduction of the virus concentration achieved by their equipment, since many of the filtrate samples contained no detectable viruses.

The Baltimore, Maryland study of screened secondary wastewater effluent also observed large reductions of native bacteriophages by similar Memcor microfiltration equipment, but the results were not as consistent as in the Australian study. Most of the Maryland measurements also showed filtrate concentrations below the detectable limit. Their preliminary data (Olivieri, el at. 1991) reported reduction factors of 1.3 to 4.2 logs, with a mean of 3.1.

Substantial variability was also observed by Jolis and Hirano, who obtained reductions of 1.5 to 4 logs when they performed seeded virus tests on secondary effluent in three days. Since they used seeding, their results were obtained under slightly different experimental conditions from those in the Australian and Maryland studies. Another difference is that the seeded tests were performed at higher feed concentrations.

Seeded tests were also performed on Memcor microfiltration units as part of the pilot testing for a study of using microfiltration to purify Colorado River water for the personnel at the five pumping stations on the Colorado River Aqueduct. In these tests, removal factors of 1.7 to 2.9 logs were observed. However, in seeded tests of Memcor units carried out on fresh water by Jacangelo et al. (1995), covering a feed ranging 10^3 to 10^9 pfu/mL, the reduction factors varied only modestly. Jacangelo found 0.2-1.2 logs of removal of MS2 from bench-scale batch experiments. The bench-scale experiments were set-up to model conditions where viruses cold easily penetrate the membrane. These conditions included using membranes with minimal cake layers, distilled buffered water, and maximum membrane operating pressure. He then ran pilot-scale experiments running the membranes in a continuous mode. The overall removal of MS2 ranging from < 0.5 to 3 logs were obtained.

These results from other studies provide perspective on the observations of variable virus removal in the Research Group's seeded virus studies at TITP. The variation of the reduction factor in our results from around 0.2 logs to 3.5 logs is consistent with observations at several other places that fall short of the performance observed in Australia. The correspondence with the other seeded tests is particularly close.

CONCLUSIONS

The chief conclusions from TITP and other experiments are: a) virus removal by RO is essentially perfect as expected; b) virus removal efficiency of the MFunit at TITP for April 3, 23 and 29 is relatively stable and removal efficiency on these days is less than one log (order of magnitude); c) the efficiency values are low compared to other MF studies; d) variability is also observed in other studies, and some differences are noted in virus reduction factors in wastewater compared treatment to water.

Additional studies are needed to further clarify the variations in removal performance of MFs. We hope to discuss the additional findings in the presentation.

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Fig. 1. Schematic of DWP filtration-reverse osmosis (MF/RO) pilot unit at TITP

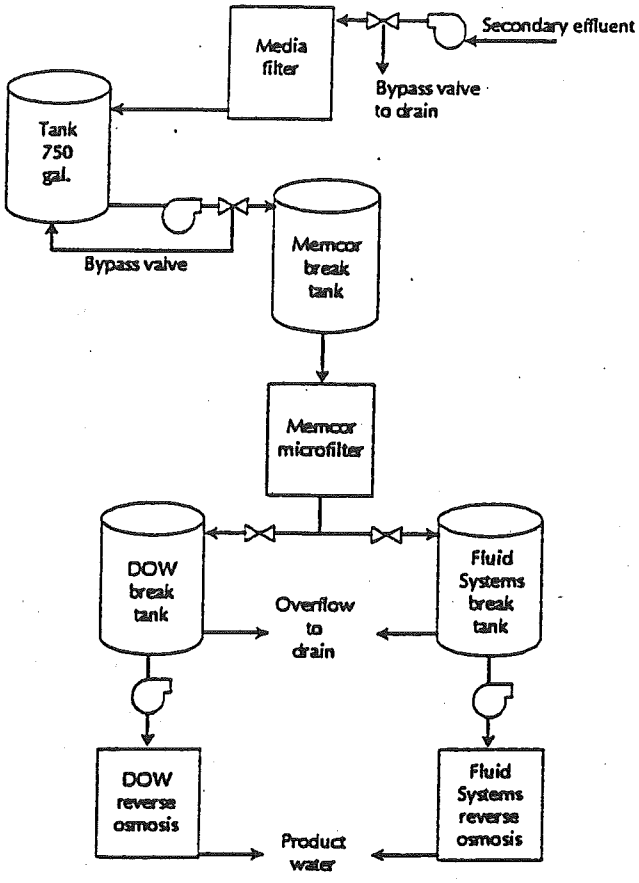
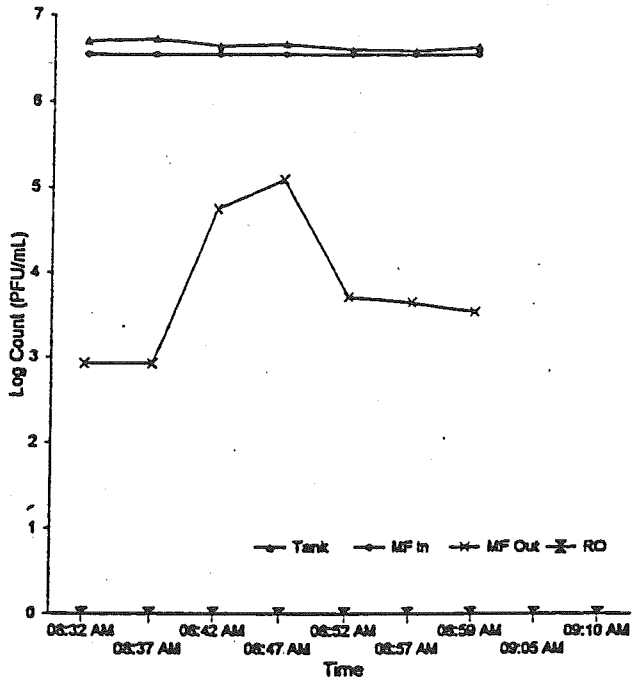


Figure 2. MS2 bacteriophage counts



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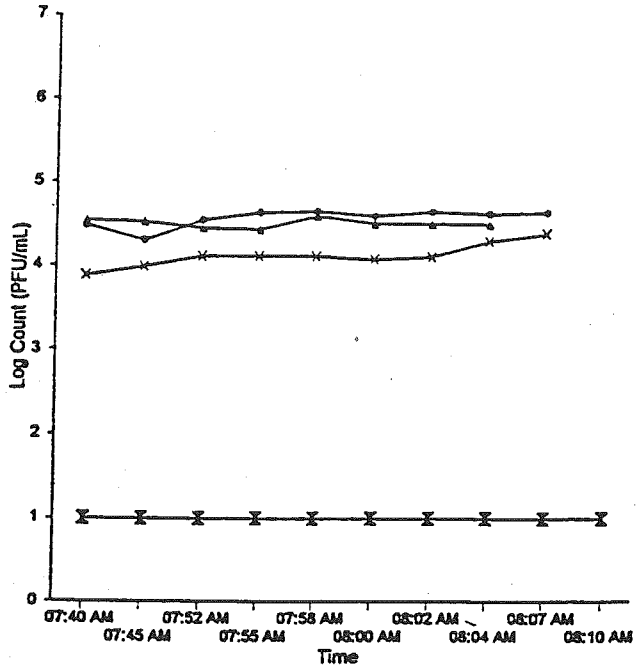

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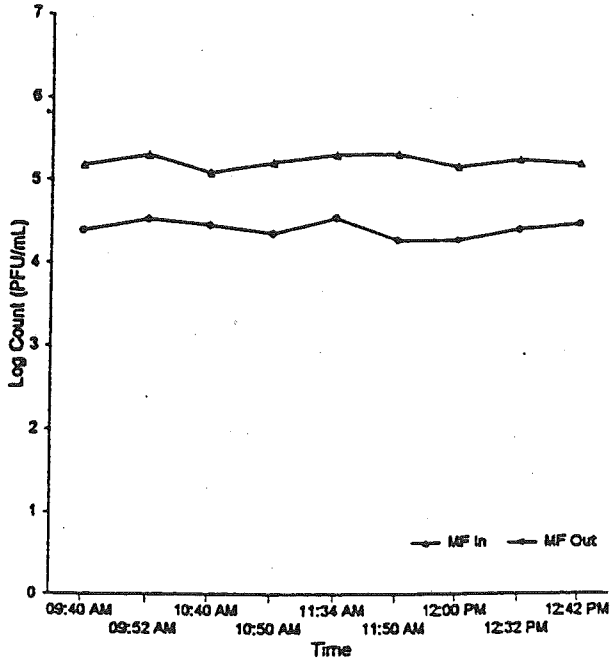
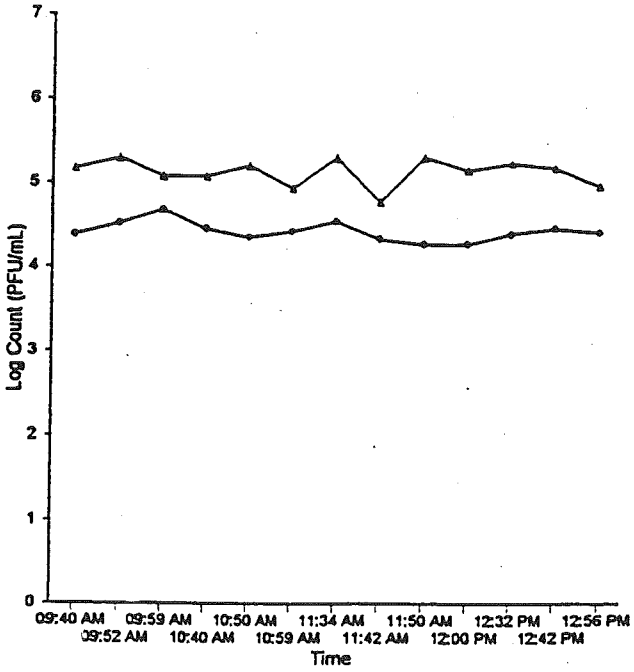
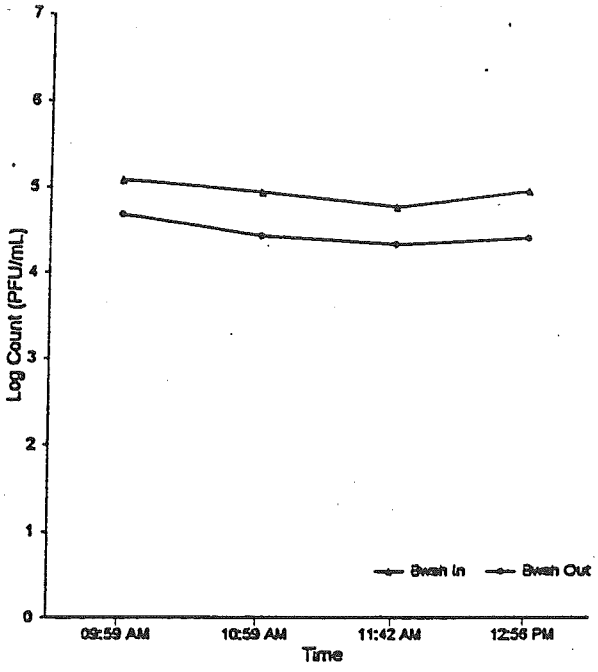


(c) 04/23/96 (normal operation samples)

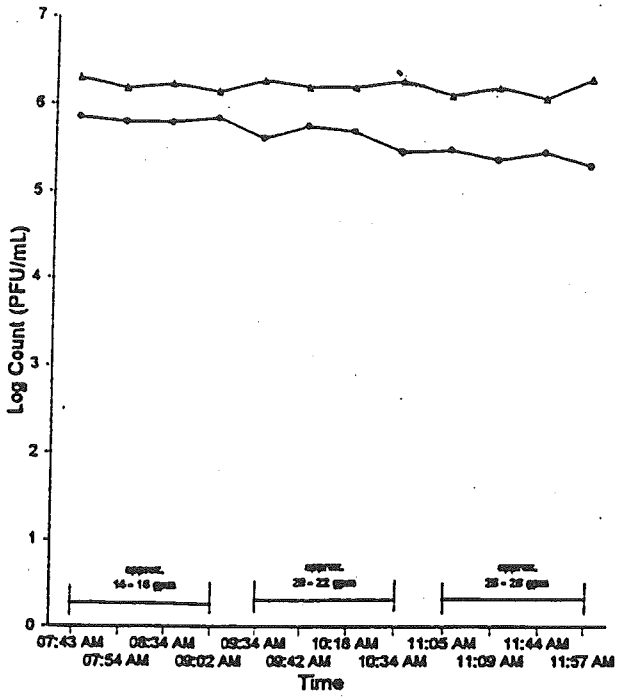


(d) 04/23/96 (all samples)

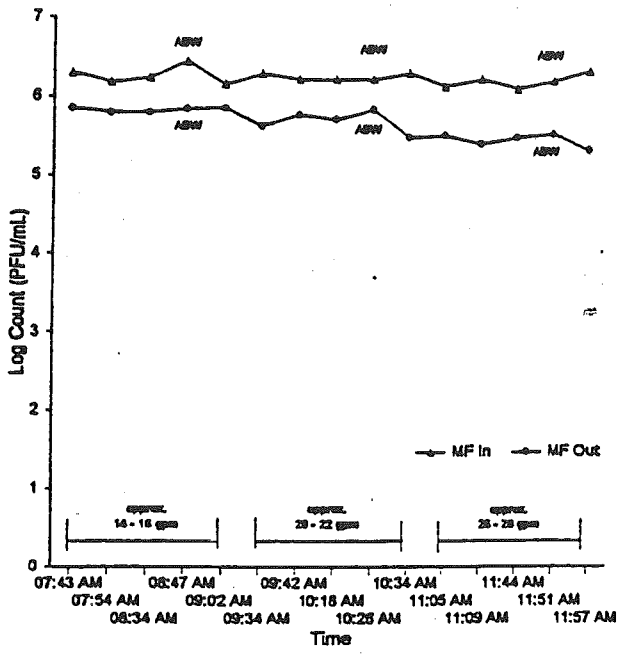
Figure 2. Continued.



(e) 04/23/96 (post-backwash samples)



(f) 04/29/96 (normal operation samples, varying flow rates)



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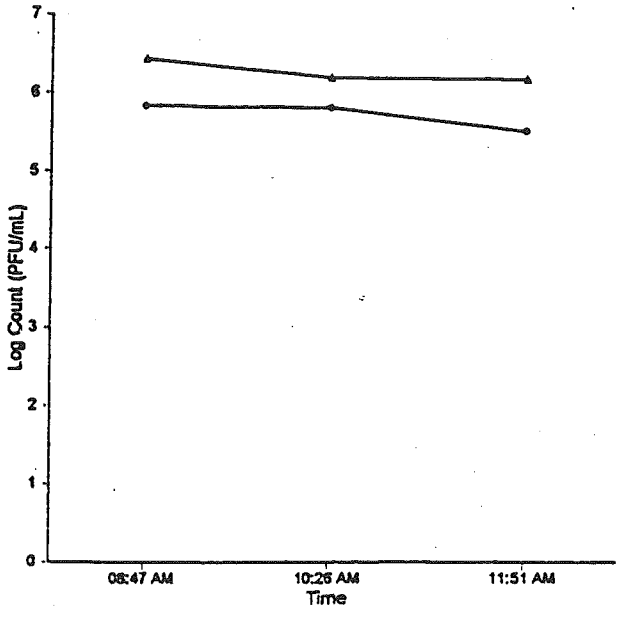

(h) 04/29/96 (post-backwash samples)

Table 1 - MF specifications for other studies.

Investigator	Location	No. of samples	Source	Filter				Flow		Backwash
				Type	Material	Pores um	Area sqm	Rate flux	Mode	Interval min
INDIGENOUS										
wastewater										
(1) Water Board	Blackheath TF Plant Australia	96	200 um screened sec. effluent	Memtec CMF	hollow fiber, polypropylene	0.2	960	35 gsfd (avg) 74 gsfd (peak)	direct	18
(2) Olivieri, V.P., et al. and Willingham, et al.	Back River AS Plant Baltimore, MD	160	100-200 um screened sec. effluent	Memtec CMF	hollow fiber, polypropylene	0.2	30	70 gsfd	direct	10-15 (auto)
(3) Iranpour, et. al.	Terminal Island TP San Pedro, CA	6	trimedia sec. effluent	Memcor 3M10CMF	hollow fiber, polypropylene	0.2	45	14-27 gpm	direct	13-24
water										
				——— No references ———						
SEEDED										
wastewater										
(4) Jolis, et. al.	Southeast Plant San Francisco, CA	17	3175 um screened sec. effluent	Memtec 60M10CMF	hollow fiber, polypropylene	0.2	10	0.4-0.5 gsm/sqm	direct	18 (auto)
(5) Iranpour, et. al.	Termial Island TP San Pedro, CA	46	trimedia sec. effluent	Memcor 3M10CMF	hollow fiber, polypropylene	0.2	45	14-27 gpm	direct	13-24
water										
(6) Jacangelo, et. al.	Bull Run Reservoir; Portland, OR Lake Elsman; San Jose, CA Seine River; Vigneux, France				hollow fiber, polypropylene	0.2			direct	30
(7) Kostelecky, et. al.	Colorado River Aqueduct	9	150 um screened	Memtec 30M10CMF	hollow fiber, polypropylene	0.2		0.33-0.50 gpm/sqm	direct	

Table 2 - MS2 bacteriophage counts for other studies.

Investigator	Date	MF (pfu/mL) inlet	MF (pfu/mL) outlet	Log Removal	Comments
Indigenous wastewater					
(1) Blackheath Water Board	09/91			3.01 ± 0.61	16 days
	10/91			3.48 ± 0.40	22
	11/91			3.15 ± 0.46	21
	12/91			2.97 ± 0.37	16
	01/92			3.05 ± 0.38	21
				3.17 ± 0.48	
(2) Olivieri, V.P., et al. and Willingham, et al.		2.00E-01	1.00E-02	1.30	
		5.00E+00	1.00E-02	2.70	
		4.00E+00	1.00E-02	2.60	
		5.00E+00	1.00E-02	2.70	
		5.00E+01	1.00E-02	3.70	
		1.00E+02	1.00E-02	4.00	
		3.20E+01	1.00E-02	3.50	
		6.30E+01	1.00E-02	3.80	
		1.60E+02	1.00E-02	4.20	
		1.00E+01	1.00E-02	3.00	
		4.00E+00	1.00E-02	2.60	
				3.10 ± 0.8	
(3) Iranpour, et. al.	03/12/96	1.60E+01	3.00E+00	0.7	
	03/13/96	4.00E+00	1.00E+00	0.6	
	03/14/96	5.00E+00	1.00E+00	0.7	
	03/19/96	1.30E+01	3.00E+00	0.6	
	03/20/96	1.00E+00	1.00E+00	0	
	03/21/96	1.00E+00	1.00E+00	0	
Seeded wastewater					
(4) Jolis, et. al.	11/03/93	2.80E+03	9.10E-01	3.5	
		2.80E+03	3.80E-01	3.9	
		2.80E+03	3.80E-01	3.9	
		3.00E+03	3.80E-01	3.9	
		3.40E+03	3.80E-01	4.0	
	11/15/93	3.10E+03	1.10E+02	1.4	
		3.60E+03	1.10E+02	1.5	
		3.70E+03	4.90E+01	1.9	
	12/08/93	1.80E+03	1.00E+01	2.3	
		3.50E+03	1.00E+01	2.5	
		3.60E+03	1.00E+01	2.6	
		4.10E+03	1.00E+01	2.6	
		5.80E+03	1.00E+01	2.8	
		4.90E+03	4.50E+00	3.0	
		3.60E+03	2.90E+00	3.1	
		4.60E+03	2.50E+00	3.3	
		3.40E+03	9.00E-01	3.6	
				2.9 ± 0.8	
(5) Iranpour, et. al.	Figs. 2a to 2h				
water					
(6) Jacangelo, et. al.				0.2 ± 0.2	bench
				2.0 ± 0.8	continous, 23 samples
				0.4 ± 0.4	continous, 33 samples
				0.8 ± 0.8	continous, 12 samples
		9.00E+01		1.3	
		1.70E+04		1.5	
		1.60E+05		1.9	
		1.10E+06		1.6	
		1.70E+08		0.3	
		1.50E+09		0.2	
(7) Kostelecky, et. al.		1.30E+06	2.80E+04	1.7	
		3.00E+07	3.40E+05	2	
		1.60E+07	2.20E+04	2.9	
				2.2 ± 0.6	