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THE REMOVAL OF VIRUSES FROM WATER BY CONVENTIONAL WATER-TREATMENT PRACTICES

(TITLE)

BY

JANET S. ZIEGLE

B.S., Eastern Illinois University, 1978

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

1980
YEAR

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ABSTRACT

Ziegle, Janet S. M.S., Eastern Illinois University. July 1980. The Removal of Viruses from Water by Conventional Water-Treatment Practices.

The purpose of this investigation was to determine the effectiveness of conventional water-treatment methods in removing viruses from raw water sources. Those processes studied included alum flocculation, rapid sand filtration of flocced and unflocced water samples, and chlorine disinfection.

This study was approached using coliphage f2 as a seeded indicator of treatment efficiency. Coliphage f2 was chosen as an indicator because of its similarities to enteric viral pathogens in both morphology and sensitivity to water-treatment processes. Coliphage f2 was found to occur naturally in surface waters, but not in numbers sufficient to interfere with calculations.

A sand filter for laboratory use was constructed with thin-walled PVC pipe, which was supported in a vertical position. Filter columns of 10-, 20-, and 30-inch depths were constructed either entirely of sand or as dual-media filters with a 1:2 ratio of sand to anthracite coal. Flow rate in the 30in column was adjusted to that reported for rapid sand filtration by adjusting the head of water above the filter bed.

Duplicate one-liter samples of chlorinated final effluent were seeded with 10ml of an f2 stock (ca. 10^6pfu/ml). To one sample was added 0.12g alum and the mixture was stirred and allowed to settle. Phage titer of the supernatant was determined by the agar overlay method.

The samples were then run through duplicate filters and the titers

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of the filtrates were determined. Resulting titers were compared to the original titer levels, and the efficiency of the processes were calculated as percent recovery. Turbidity was also measured on both samples before and after filtration.

Alum flocculation followed by settling resulted in a wide range of reduction of the seeded f2. In most cases at least a one-log (90%) reduction was obtained and in several cases at least a two-log (99%) reduction occurred. The variability in phage reduction was attributed to the quality and quantity of floc produced.

Filtration of unflocced, seeded water through sand and dual-media filters of various lengths resulted in generally low f2 removal. In only a few instances did such treatment result in a one-log (90%) reduction and in most cases the removal by filtration was negligible.

Flocculation without settling, followed by filtration, removed significant numbers of the seeded f2. In several instances a three-log (99.9%) reduction occurred and generally a two-log (99%) reduction was observed.

No correlation of f2 removal vs. column depth was observed. In addition, neither matrix was shown to remove f2 better than the other.

The removal of turbidity by filtration roughly paralleled f2 removal. However, the large variations in turbidity and phage removal levels precludes the use of turbidity reductions as a satisfactory criterion for judging the efficiency of the process in removing viruses.

Chlorine disinfection at 2ppm total residual chlorine resulted in an 80% reduction in f2 after a 2hr contact time; most of this reduction (68-79%) occurred within the first two minutes of chlorine contact, and corresponded with a rapid decrease in the free residual chlorine level. Further disinfection occurred throughout the 2hr contact time, but at a

markedly slower rate.

It was concluded that, connected in series in a water-treatment system, flocculation, filtration, and chlorination could be expected to reduce indigenous viral populations by at least 99.8-99.98%.

ACKNOWLEDGEMENTS

I wish to thank my adviser Dr. William Weiler, for his guidance, direction and patience during the course of this study and throughout the preparation of the manuscript.

I would also like to thank the members of my graduate committee, Dr. Richard Smith and Dr. John Speer, for their constructive criticism in the preparation of this paper and for their help throughout my course of study at Eastern Illinois University.

Thanks are due to Dr. Norton D. Zinder, of the Rockefeller Institute, New York, for the donation of coliphage f2 and host cell cultures used in this study.

Finally, a special thanks to my parents and family, for their support and encouragement.

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INTRODUCTION

Viruses transmitted by water pose a hazard to public health. Most of the rivers that serve as sources of drinking water carry varying amounts of wastewater and viruses enter these water systems with sewage. Over 100 types of viruses are known to be excreted in human feces, and all have been recovered from sewage and polluted waters. These viruses, when ingested can cause diseases such as gastroenteritis, poliomyelitis and infectious hepatitis. Such viruses must be removed or destroyed if the water is to be entirely safe for public consumption.

Extreme dilution of viruses in the environment reduces, but does not eliminate, the probability of infection of individuals in an exposed population. Since many viruses are stable in the environment for long periods of time, the effect of viral pollution of surface waters is a low-grade seeding of the population, resulting in low-level transmission of infection in the community. For many years the accepted criterion of water quality has been the coliform index, which has been an invaluable aid in evaluating the efficiency of treatment processes for public water supplies and has been the basis for bacteriological water quality standards. It has been more recently suggested, however, that the coliform index applies only to intestinal pathogens of bacterial nature and may not be indicative of the presence or absence of viral pathogens.

In June 1974, an international conference sponsored by the American Public Health Association met in Mexico City to discuss detection and control of waterborne viruses. Recommendations proposed at this conference

called for (1) development and standardization of virus detection methods, with special attention to the critical need for quantitative methodology for the recovery of small numbers of viruses from large volumes of water, (2) focusing of resources on projects aimed at reducing or eliminating viruses from sewage, a major source of viruses in drinking water, (3) development of a viral indicator system in order to avoid situations where, despite gross viral contamination, waters are presumed acceptable because bacterial indicators are destroyed, and (4) establishment of monitoring procedures to ensure consistent quality of wastewater and water-treatment operations.

The purpose of this study was to evaluate the effect of conventional water-treatment methods on viruses, using a seeded bacteriophage indicator system. This study was approached with the following questions in mind:

(1) What effect does flocculation and settling have on virus concentrations in wastewater?

(2) What is the effect of rapid sand filtration (with consideration given to column depth and matrix composition) on these viruses?

(3) To what extent does flocculation combined with filtration affect viral populations in wastewater?

(4) What is the virucidal capacity of chlorine disinfection?

(5) And finally, since viruses should occur in low numbers in finished waters, are there suitable means of concentrating small numbers of viruses from large volumes of water?

HISTORICAL REVIEW

Although any human virus excreted in the feces may be present in raw water, the viruses of major concern are those that grow in or near the intestinal tract and are discharged in large numbers in the feces. This group, the enterics, include the enteroviruses (Poliovirus, Coxsackievirus, and Echovirus), infectious hepatitis virus, the Adenoviruses, and the Reoviruses. Enteric viruses are intracellular parasites; therefore they cannot multiply outside a host. Their numbers in aquatic environments decrease because of removal and inactivation. Akin et al. (1971) state that virus survival in water is affected by factors such as (1) the amount of time the virus is in the water, (2) the nature of the water, (3) the rate of flow, (4) the temperature, (5) the chemical content of the water and (6) the organic content of the water.

Enteric virus concentrations in wastewater cannot be determined accurately because of limitations in virus detection methods and because of variability in the amounts and types of viruses that may be present under different conditions (Sobsey, 1975). Several workers have assayed for various enteric and bacterial viruses in raw and treated waters. Their findings are summarized in Table 1.

Although available evidence indicates that enteric virus concentrations in drinking water are likely to be very low, the presence of small numbers is important if small numbers are capable of causing infection. Plotkin and Katz (1967) concluded that as little as one viral unit is capable of causing infection in man.

Table 1. The occurrence of viruses in water.

	VIRUS	CONC. (PFU/l)	REF.
Raw Sewage			
	Coxsackievirus B	4000	Kelly & Sanderson, 1959
	Poliovirus	1600	Kelly & Sanderson, 1959
	enterics	5000	Clarke et al., 1969
	coliphage	$3.6X10^4$-$1.6X10^7$	Dhillion et al., 1970
	virus	7000	Metcalf, 1971
	virus	7000	Pavoni et al., 1972
	coliphage	$3.9X10^7$	Wolf et al., 1974
	coliphage	$5X10^5$-$3X10^6$	Fannin et al., 1976
	coliphage	$1.2X10^4$-$8.5X10^5$	Shaffer, 1977
	virus	8000	Satter, 1978
	virus	1000	Satter, 1978
Partially treated Sewage			
	animal virus	100	Fannin et al., 1977
	coliphage	$8.6X10^5$	Fannin et al., 1977
Primary Effluent			
	virus	109-427	Berg et al., 1978
	virus	1000	Satter, 1978
Secondary Effluent			
	virus	4000	Shaffer, 1977
Chlorinated Secondary Effluent			
	virus	1.5-15	Shaffer, 1977
	virus	27	Satter, 1978
Polluted Surface Waters			
	enteroviruses	30	Chang, 1968
	virus	1.5-15	Metcalf, 1971
	coliphage	$5X10^4$-$8X10^5$	Safferman & Morris, 1976
Ground Water			
	enteric viruses	7.78	Schaub & Sorber, 1977
Finished Water			
	virus	0.01	Shaffer, 1977

Epidemiology

Documented evidence for waterborne transmission of enteric viral disease exixts only for Type A Infectious Hepatitis and Poliovirus. Only a fraction of the reported cases of these diseases can be traced to waterborne sources. Water transmission has been implicated in only 66 of some 50,000 outbreaks of infectious hepatitis since 1946 (Craun et al., 1976). viraraghaven (1973) links only two polio epidemics to sewage-contaminated water.

That documented waterborne viral disease is almost exclusively limited to Hepatitis Type A outbreaks does not necessarily mean that waterborne transmission of other enteric viruses does not occur. Lack of evidence of waterborne transmission of other viruses has been attributed to several causes. Berg (1967) has suggested that low-level transmission of viruses is likely to produce asymptomatic carriers rather than overt disease. Infected individuals serve as sources of further infection in the community and secondary person-to-person transmission obscures the true source. In addition, a substantial proportion of the population is likely to possess prior immunity to the virus and would have subclinical infections (Chang, 1968). Clinical illness is observed in only a small fraction of those who become infected. The ratio of clinical causes to subclinical cases has been estimated to range from 1/10 to 1/1000 (Long and Bell, 1972), depending upon the species of virus involved. Finally, most enteric viruses cause such a broad spectrum of enteric and respritory disease syndromes (Table 2) that the scattered cases of illness are probably too varied in symptomatology to be attributed to a single viral agent (Sobsey, 1975).

Thus, the nature of enteric virus transmission by water makes it difficult, if not impossible, to recognize unless there is an explosive

Table 2. Enteric viruses and their associated diseases (modified from Sobsey, 1975).

Virus Group	# of types	nucleic acid content	common disease syndromes
ENTEROVIRUSES			
Poliovirus	3	RNA	poliomyelitis, aseptic meningitis (AM)
Coxsackievirus A	23	RNA	herpangia, AM, exanthem
Coxsackievirus B	6	RNA	AM, epidemic myalgia, myocarditis, pericarditis
Echoviruses	3	RNA	AM, exanthem, gastroenteritis
ADENOVIRUS	31	DNA	upper respiratory illness, pharyngitis, conjunctivitis
REOVIRUSES	3	RNA	upper respiratory illness, diarrhea, exanthem
HEPATITIS A VIRUS	1?	?	viral hepatitis
GASTROENTERITIS VIRUS(ES)	?	?	acute infectious, non-bacterial gastroenteritis

outbreak caused by extensive sewage contamination.

Water Renovation

Water renovation is the application of water treatment procedures to wastewaters. All sewage and water treatment procedures remove or destroy viruses to some extent. Some treatment methods are better than others, and none is likely to remove all viruses present in sewage or raw water (Berg, 1973a).

A 1955 outbreak of infectious hepatitis in Delhi, India challenged the adequacy of conventional water treatment practices. Infectious hepatitis was shown to have been spread by a water supply which had been treated by flocculation, rapid sand filtration and chlorination (Dennis, 1959).

Flocculation

Chemical flocculation is an important process in advanced wastewater treatment and renovation systems. Flocculation has proven to be effective in removing turbidity and organics from water. Much of the information concerning virus removal by flocculation is contradictory, and quantitative information regarding flocculation is difficult to compare because of the wide range of viruses and coagulants that has been used in these investigations.

According to Clarke and Chang (1959), the removal of viruses from water by chemical flocculation is a result of a metal-cation-protein reaction in which a metal-virus complex is formed and aggregates to form a precipitate. This reaction, they conclude, is non-specific and the results obtained with one virus should be applicable to other viruses.

From a study in 1973, Berg stated that good removal of viruses by

chemical flocculation was dependent on coagulant concentration. These results are in agreement with results from a study by Chang and coworkers (1958a) which showed increased removal of seeded viruses with an increase in coagulant (alum) dosage. However, later studies by Clarke and Chang (1959) demonstrated high removal with low dosages of alum, which indicates that coagulant dosage alone is not a measure of the efficiency of the process.

Chang et al. (1958a) also reported that satisfactory floc formation occurs with common coagulants under a wide range of natural conditions. Good floc formation, and ultimately good virus removal, is affected by various physical and chemical factors such as pH, turbidity, flow rate and the organic content of the water.

Generally the optimum pH for a flocculation system is considered to be one that facilitates the earliest appearence of floc. However, in a study by Chang et al. (1958a), earliest floc formation occurred at a low pH (5.2), but optimum removal (99%) of seeded Coxsackie and bacterial viruses occurred at pH values of 7.7 and 6.2, respectively. It appeared that if the floc formed too rapidly, virus removal was reduced, possibly due to reduced contact of virus and metal ion.

Several workers have reported decreased virus removal with increases in turbidity and organic content of the water: higher concentrations of coagulant effected increased removal in these instances. Chang (1968) concluded that flocculation is effective in reducing the virus concentration as well as the total amount of organic matter in the water, thus rendering chlorination (or some other chemical disinfection system) more effective in destroying viruses and other pathogens. Flocculation should not be considered a disinfection process by itself, since viruses are not

destroyed by flocculation but merely concentrated in the floc.

Filtration

Current theory regarding filtration emphasizes at least two separate and distinct steps (Kawamura, 1975). First, the transport step, which involves the transport of the suspended particles to the surface of the filter media, and second, the attachment step, which involves the attachment of the particle to the filter medium surfaces or to other particles previously retained in the filter bed. The particle may be transported to the filter medium by one or more physical processes including diffusion, interception, gravity settling, internal impingement, and hydrodynamic transport. In none of these is the chemical nature of the particle of much significance. The attachment step, however, is affected by chemical and physio-chemical variables such as temperature, flow rate, pH, ionic composition of the water, nature and dosage of coagulant and the composition and surface condition of the medium grains.

There are two principle mechanisms involved with filtration of water through porous materials (Rice, 1974). The first, a straining mechanism, involves the trapping of large particles (with diameters greater than the pore size of the medium). Straining leads to clogging of the upper surface of the bed, and should be minimized for efficient operation.

Most removal, then, is by chance adhesion of the relatively small suspended particles to the relatively large surface of the filter medium or onto previously deposited material. Adhesion is, in effect, flocculation upon the filter medium grains.

The effectiveness of sand filtration alone has been questioned by several authors. Clarke and Chang (1959) stated that filtration is an adsorption phenomenon and that sand, being a relatively poor adsorbent, has

low removal efficiency. In an American Water Works Association committee report (1972) it was concluded that rapid sand filters, including dual-media filters, are not capable of removing colloidal-form suspended matter to an acceptable degree and that pretreatment ahead of filtration is generally essential. The most common pretreatment is alum flocculation.

Flow Rate. The flow rate through the filter is a critical variable. Water tends to drag particles of floc past the filter grains. As more particles accumulate, the flow cross-section is reduced and the velocity of the water increases (Rice, 1974). Eventually, equilibrium is reached so that the dragging action of the water balances the adhesive force and no further deposition occurs.

For a given suspension and a given filter, there will be a flow rate that will result in most of the suspended matter appearing in the effluent, i.e., there will be gross filtration failure at some flow rate. For well-designed filters, the point of gross filtration failure is above the flow rate obtainable with the available head pressure.

Water Temperature. Water temperature is a major variable in filtration. The effects are complex and quantitative estimates of these effects are uncertain. However, cold water is always more difficult to filter than warm water (Rice, 1974), since floc penetration into and through the filter is greater in cold water than in warm water. Rice (1974) attributes the cause of filtration problems to the relatively high viscosity of cold water, since the viscosity of water decreases 44% from 0 to 20C.

Filter Depth. A thin layer of granular filter medium will remove a certain percentage of the applied solids. The depth of the filter required is a function of the applied load and the desired quality.

As a filter run continues, floc accumulates in the medium voids and

and the percentage in each layer will change. The top layer voids are filled more quickly than the bottom layer ones and there is a gradual shift of suspended solids deeper into the filter. The top part of the filter becomes less useful with time and the bottom part becomes more useful. Therefore, the deeper the filter bed, the longer it can be run before floc penetrates through it.

Medium Grain Size. Small sand grains remove a higher percentage of the applied suspended matter than do large sand grains (Rice, 1974) for two reasons. First, the surface area-to-volume ratio of the smaller grains is larger and greater surface area offers more opportunity for floc particles to accumulate. Second, the opportunity for bridging between grains is greater for the smaller grains because distances are shorter.

The rate of head-loss increase is greater for small medium particles than for large medium particles. It is generally agreed by workers in the field of filtration that length of run and depth of floc penetration into to filter increase as effective grain size increases.

Medium Composition. After the filter has been in operation for a period of time, the filter must be cleaned to remove the sediments that have accumulated in the upper layer of the medium. This is accomplished by backwashing the filter. After backwashing, the finer sand grains will settle on top and the larger toward the bottom, resulting in a significant difference in filtration performance from top to bottom. The head-loss effect is magnified because the smaller grains at the top of the filter are exposed to the maximum concentration of suspended solids. Head-loss would be less if the larger grains were associated with the higher concentration of suspended solids, a situation which is not possible with a filter composed of a material of uniform specific gravity.

From a quality standpoint, sand grains should be small to trap more particles, but from a head-loss standpoint the grains should be large to permit longer filter runs. One alternative is to use the coarser medium but to increase the depth of the bed. A more practical approach is the use of materials of different specific gravity in the same filter.

After settling, following backwash of a dual-media filter, filter medium grains are positioned in the filter according to specific gravity and size. Anthracite coal with a specific gravity of 1.6 and a grain size of 1.0mm will be above silica sand with a specific gravity of 2.6 and a grain size of 0.5mm. By correct relative sizing of coal and sand, one can produce a filter that is, in effect, a coarse filter on top of a finer filter. A typical design is 20in (50cm) of 0.8-2.0mm anthracite coal over 10in (25cm) of 0.4-1.0mm silica sand.

According to Kawamura (1975) a dual-medium filter can be operated at a high rate with low head-loss and can produce a high quality effluent. The upper flow limit depends upon water temperature, depth of the filter bed, and medium grain size.

Virus Removal. From studies by Clarke and Chang (1959) it was concluded that rapid sand filtration is relatively ineffective in removing viruses from water. This low efficiency was attributed to the inherently poor adsorption properties of the sand. These results are in accord with those of other workers who found that rapid sand filtration alone is not an effective means of removing viruses.

Berg (1973a) reported that filtration through clean sand does not remove viruses, but that filtration of coagulated effluents does. This removal is due to the fact that the layering floc itself adsorbs viruses. Further studies by Guy et al. (1977) showed that sand and carbon columns

were 50% as effective in virus removal after backwashing, which suggests that adsorption (of viruses to sand grains) is not the sole, nor even the principle, mechanism of virus removal.

Chang (1968) reported that the virus removal efficiency of both flocculation and rapid sand filtration combined appear to be slightly better than the combined efficiency of the two processes conducted independently. He attributes this improved performance to the greater efficiency of the sand filter in removing virus particles in the unsettled floc, than those freely suspended in the unflocced water.

Disinfection

Since treatment procedures do not remove all viruses from either sewage effluents or water, the safety of such waters largely depends upon terminal disinfection. The term disinfection is used to describe a process which removes or destroys all organisms capable of causing disease. These organisms include bacterial spores and vegetative cells, protozoan cysts, and viruses.

Studies have shown that current treatment procedures adequate to remove bacterial pathogens may not be as effective in removing the more resistant spores, cysts, and viruses. In a study by Berg et al. (1978) chlorine levels which removed 99.999% of fecal coliforms in sewage, removed only 85-99% of the viruses present. Studies by Hoehn (1976) showed enteric bacteria to be less resistant to all forms of chlorine than enteric viruses and amoebic cysts.

Disinfectants must be selected according to need. Effluents and waters containing solids can probably be disinfected only by heat or by penetrating radiation (Berg, 1973a). Effluents discharged into streams should not be disinfected with anything which would react with the effluent and produce compounds which would kill or injure aquatic life, yet drinking waters

should carry a disinfecting residual. The most commonly used disinfectants in sewage and water treatment are the halogens.

Chlorine. Chlorine is the most widely used disinfectant for sewage effluents and drinking water. Chlorine hydrolizes in water to form the strongly virucidal hypochlorous acid (HOCl). Chlorine also occurs as the hypochlorite ion (OCl^-) in alkaline solutions, as chloramines when ammonia is present, and as organic chloramines when organic nitrogen compounds are available. Since only HOCl is a rapid virucide, the use of chlorine as an effective disinfectant depends upon the removal of ammonia and organic compounds from water which is near-neutral to acidic.

Pretreatment processes such as flocculation and filtration are needed to purify the raw water to a degree suitable for chlorination. In sewage, a 99.99% reduction must be obtained to reach the suggested standard of 1pfu/100gal. Chang (1968) reports that 99% reduction of indigenous viruses can be expected with flocculation and filtration. The remaining reduction is dependent upon the disinfection process.

Culp (1974) maintains that four conditions must be met for virus inactivation by chlorination:

(1) The turbidity of the water should be less than 1.0 Jackson turbidity units (Jtu), and preferably less than 0.1Jtu.

(2) The pH of the water should be close to 7.5 for waters containing ammonia or less than 7.0 for ammonia-free waters.

(3) Rapid, uniform mixing of water and chlorine must be provided.

(4) A concentration of 0.5-1.0mg/l of undissociated hypochlorous acid (HOCl) must be maintained in the water being treated for a contact period of 30min.

In studies by Kelly and Sanderson (1960), Poliovirus and Coxsackie-

virus in water were inactivated by combined residual chlorine. It was concluded that the effective concentration depended upon pH and contact time. At 25C and a pH of 7, 9mg/l of combined chlorine residual inactivated Poliovirus in 30min. A one-hour contact time was required to inactivate Poliovirus at a chlorine concentration of 6mg/l and over 7 hours at a concentration of 0.5mg/l. Decreasing the pH decreased the rate of inactivation at all concentrations.

The efficiency of the disinfectant varies with the characteristics of the water and the resistance of the infectious agent concerned (Neefe et al., 1947). Great variations in chlorine resistance among viruses has been observed. Lothrop and Sproul (1969) reported that a combined chlorine residual of 28mg/l was required to produce a 99.99% inactivation of the T2 bacteriophage in settled raw water after a 30min contact time, while a combined chlorine residual of 40mg/l was required to produce a similar reduction of Type I Poliovirus (30min contact time).

There is also evidence to suggest variations in chlorine resistance among different strains of the same type virus. In studies by Kelly and Sanderson (1960) Poliovirus Type I, Mahoney strain, proved to be less resistant to chlorine than the MK 500 strain of the same virus. They found Coxsackievirus B5 to be more sensitive to chlorine than both strains of the Polio Type I virus, while Poliovirus Type III proved to be the most resistant of those studied.

Ozone. Culp (1974) feels ozone is more effective against viruses than is chlorine because its potency is not effected by pH or ammonia content. With proper mixing and recommended dosage of not less than 1mg/l ozone, a contact time of 5min is adequate in clear water (1.0Jtu). The disadvantage of ozone is that it must be generated locally as it is needed and it can-

not be stored, making it difficult to adjust ozone-application rates to meet changes in water quality or variations in flow.

In studies by Pavoni et al. (1972) seeded coliphage f2 was totally destroyed in 5min in secondary effluent by 15mg of applied ozone per liter. It appeared that the kill mechanism for bacterial cells and viral particles was probably that of oxidation. They theorized that ozone acts as a general cytoplasmic oxidant which causes cell lysis.

Iodine. Molecular iodine (I_2) is a convenient and practical disinfectant for emergency treatment of small quantities of drinking water (Clarke and Chang, 1959), but its use as a routine disinfectant for sewage and water treatment has been questioned. According to Hsu (1964) inactivation of a virus by iodine merely means modification of its protein coat. The genetic properties, particularily the reproducability, of the virus may be preserved.

The effects of elemental iodine on viruses are not consistent. Viruses show varying degrees of sensitivity to disinfection by iodine. In studies by Clarke and Chang (1959) Coxsackieviruses A2 and B1 were shown to have the same resistance to molecular iodine, but twice the resistance (in terms of contact time) of Poliovirus I and Echovirus 7. In addition, Coxsackievirus A9 appeared to be more resistant than Coxsackies A2 and B1. In order to achieve 99.6% destruction of Coxsackieviruses A2 and B1, contact times of 6, 3.5, and 2.5min at 25C were required with 5, 10, and 15mg/l residual iodine, respectively.

When the data on Coxsackie B1 is compared with those of Clarke and Kabler (1954) on Coxsackie A2 virus, it is seen that molecular iodine is much less virucidal than is free chlorine, even when comparison is made on a molar basis.

Detection of Viruses

When a body of water receives sewage or sewage effluent after conventional treatment, the presence of enteric viruses in the polluted water cannot be excluded. Viral density in the polluted water depends upon (1) their original density in the polluting material, (2) the dilution factor, (3) the time elapsing after discharge, and (4) the physical and chemical properties the water (Chang, 1968).

Berg (1973b) advocated the development of quantitative methods to measure accurately the efficiency of virus removal by treatment processes. Grinstein et al. (1970) theorized that the concentration of viruses in water must be at least 4000pfu/gal (ca. 1pfu/ml) to be detected without concentration. Concentration of virus particles in water and sewage samples before they are inoculated into cell cultures permits the recovery of viruses that might otherwise be undetected.

Chang (1968) maintains that any method suitable for the detection of enteroviruses in water would have to fulfill the following conditions: (1) It should concentrate small numbers of viral units from large volumes of water, (2) the virus material thus concentrated should be suitable for inoculation into tissue culture for determination of viral density, and (3) ideally it should separate the viruses from the bacteria, protozoa and other microbes, as well as from toxic material to avoid the detrimental effects of the latter on tissue cells. Several techniques have been developed along these lines, and the most promising appears to be a membrane adsorption technique.

Membrane Filtration

The theory of the membrane-adsorption technique is related to the specific surface properties of the virion. That is, under certain specific

conditions viruses frequently adsorb to a variety of substances, including microporous membranes. The concentration of viruses by the membrane-adsorption technique depends upon adsorption of the viruses to the membrane and their subsequent removal by elution. The membranes, which are composed of cellulose derivatives, are commonly used to partially purify or clarify crude virus-cell harvest material by filtration. Cliver (1965) attributed an observed loss in virus titer in the filtrate to the adsorption of virions to the matrix of the membrane.

Virus adsorption phenomena were shown to be influenced by chemical composition of the membrane, the ratio of pore diameter to the diameter of the virion, and the absence of substances, such as those occuring in serum, which interfere with virus adsorption (Cliver, 1968). Cellulose triacetate membrane filters were shown to adsorb viruses poorly when the porosity exceeded the virion diameter by as much as three times. Conversely, cellulose nitrate membrane filters were shown to adsorb viruses efficiently even when the porosity exceeded the virion diameter by as much as 285 times.

Wallis and Melnick (1967a) observed that enteroviruses can be made to adsorb to or pass through membrane filters simply by manipulating the virus suspending medium. The addition of salts to the medium, particularly those containing divalent cations, such as $MgCl_2$, was shown to enhance viral adsorption. The adjustment of the medium to pH 5 (close to the isoelectric point of the virus in question) was shown to enhance significantly the adsorption of viruses. Conversely, the presence of organic or proteinaceous substances was observed to interfere significantly with virus adsorption to the membrane matrix, presumably by the competition of these substances for membrane adsorption sites. The writers referred to these interfering substances as membrane-coating-components (MCC). Nevertheless, they achieved

an 80-100 fold concentration of viruses. Several authors have reported some success in removing the interfering MCC by various prefiltration and flocculation techniques.

Elution of the adsorbed virus usually occurs at a high pH (8-9). Substances used to elute viruses from the membrane surface are usually proteinaceous, including whole serum, albumin, beef extract, and nutrient broth. The use of proteinaceous substances as elutants is based on an observation by Cliver (1965), who noted that viruses adsorbed to the membrane unless the membranes were pretreated with serum. After the adsorption process, the membranes are soaked/ground up in the elutant to release the adsorbed viruses. The resulting small volume of elutant is then assayed for virus concentration (density).

The membrane filter procedure is tentatively recommended for the quantitative recovery of viruses from large volumes of clean water (Standard Methods for the Examination of Water and Wastewater, 14th ed.). The major advantages of the membrane adsorption technique, as reported by Hill et al. (1971), are its simplicity, speed and sensitivity. Its major disadvantage is related principally to the types of water to be examined for viruses. For example, the presence of MCC in natural waters, especially sewage and sewage effluents, adversely affects virus adsorption. In addition, waters that are highly turbid tend to clog the filter, unless this turbidity is removed by pretreatment.

Another aspect of detection methodology of enteric viruses in water concerns the availability of a suitable assay system based upon virus infectivity for a laboratory host, since no single type cell culture or laboratory animal is capable of detecting all enteric viruses and there is often considerable variability in host sensitivity to different virus types.

Indicators

To resolve the inadequacies and expenses associated with current virus testing and to provide an accurate measure of virus concentrations in the environment, it has been proposed (DeMichele et al., 1974) that a test procedure for an indicator be developed in a manner paralleling that using E. coli as an indicator organism.

Any organism that occurs naturally in the feces and not elsewhere is an indicator of feces. Viruses and other pathogens are not a normal part of fecal flora: they occur in domestic sewage in varying numbers that reflect the combined infection and carrier status of the population. Since pathogens are not always in the feces, the presence of fecal organisms does not always indicate the presence of pathogens. However, since domestic sewage is usually derived from the feces of many people, some pathogens are always likely to occur there, and the greater the number of people contributing to the sewage, the more uniform the numbers of pathogens. But extensive fluctuations in numbers, especially of individual pathogens, is bound to be the rule. Since the fraction of the population infected with or carrying any given pathogen changes continuously over an extended period of time, and since normal fecal flora remains relatively constant, it is only the individual pathogen which can actually indicate its own presence. Thus, the fecal indicator of domestic sewage can only indicate that pathogens may be present, and this indicator must almost always be non-quantitative. Many different pathogens occur in sewage, and it is because the task of detecting all of them is so complex and costly that indicators of their presence is sought.

In drinking water and other treated waters the validity of indicator

usage is further complicated by the relative rates of removal and destruction of the indicator and pathogen by treatment methods.

Therefore, for any organism to be a good indicator of pathogens in water it must (1) occur in large numbers in the source and (2) be removed by treatment methods to a similar extent as are the pathogens.

Coliforms

Coliform bacteria are the traditional bacteriological tool for measuring the effectiveness of water treatment against fecal contamination. The total coliform group includes a broad spectrum of aerobic and facultatively anaerobic, gram negative, nonspore-forming bacilli which ferment lactose and produce gas within 48 hours at 35C.

During the past 75 years, the mass of data accumulated permits the conclusion that an absence of coliforms in water is evidence of a bacteriologically safe supply. Much of the criticism concerning the adequacy of the coliform index to measure fecal pollution is based upon data which suggest that viruses respond differently to treatment methods than do coliform bacteria.

Gilcreas and Kelly (1955) studied the effect of storage, flocculation, filtration, and chlorination on the densities of Escherichia coli, Coxsackievirus A5 and Thieler's virus. They found that E. coli was removed to a significantly greater degree in all of the treatment processes than was either virus.

These results are in accord with studies by Berg et al. (1978), who found that quantities of combined chlorine that destroyed more than 99.999% of the indigenous fecal coliforms, total coliforms, and fecal streprococci in primary effluent destroyed, in contrast, only 85-99% of the indigenous viruses present. Viruses were recovered from 5 of 8 chlorinated primary effluent samples from which fecal coliforms were not recovered with MPN

procedures.

These results indicate that in chlorinated effluents, fecal coliforms and other indicator bacteria are destroyed so much more quickly than viruses that it is possible for all or most of the indicator bacteria to be destroyed long before all of the viruses are destroyed; this situation gives a false indication of properly processed water.

Berg et al. (1978) concluded that although fecal coliforms and fecal streptococci are useful indicators of viruses in effluents from which they are recovered, the absence of these bacteria, and even total coliforms, from disinfected effluents does not assure that viruses are also absent.

Coliphages

In studies by Scarpino (1978) coliforms were found to be more sensitive to chlorine than the indigenous coliphages, which therefore survived longer and were present in the final effluents in higher concentrations than were the coliforms. Thus it was suggested that the indigenous coliphages might be useful for evaluating the performance of sewage treatment plants in removing animal viruses.

The use of coliphages as indicators of virus in water has also been advocated by Kott et al. (1974). Vaughn and Metcalf (1975) pointed out, however, that it may be difficult to establish the validity of coliphages as viral indicators, since they may be present in high numbers even when enteroviruses are in low concentrations or absent. Although the need for in-plant studies has been recognized, such studies have been limited because the native virus populations in wastewater are extremely variable. It was suggested by Cramer et al. (1976) that perhaps the best assessment of virus survival patterns may be obtained by seeding the treatment plant with a high titer of one virus type.

The time and expense required to obtain sufficient amounts of Poliovirus or any other animal virus makes frequent seeding of a large treatment facility impractical. But, since some coliphages behave similarily to the enteroviruses and are more resistant to environmental stress than the coliforms, it has been argued that they may be used, as a seeded indicator, to evaluate the effectiveness of wastewater treatment more accurately than the traditional indicator organisms (Kott et al., 1974).

Much of the effort to determine the stability of coliphages and to study their suitability as animal virus models has been directed toward the T-coliphages. However, the more recently isolated, small RNA phages (Loeb, 1960) may be better models for enteroviruses than the T-phages since they share a greater number of similar characteristics (Table 3). Moore et al. (1975), for example, advocated the use of f2 phage as an enterovirus model because it more closely resembles the enterics in size and morphology (Table 4). In fact, f2, a single-stranded RNA phage, so closely resembles the members of the enterovirus group that, in the Lwoff, Horne and Tourier classification system, it is included in the same family as the enteroviruses, the Naporivirdae (Cramer et al., 1976). On the basis of physical and chemical similarity, ease of procurement and saftey of handling, f2 seems to be a good candidate for an enteric virus model.

Studies by Wolf et al. (1974) showed that bacteriophage f2 and Poliovirus were similarily removed by alum flocculation. This same trend of removal was also reported by Graeser in 1974. Kott et al. (1974) showed f2 to be more resistant than MS2 which was more resistant than Poliovirus Type I to chlorination in oxidation ponds. Further studies by Tifft et al. (1977) showed f2 to survive longer in chlorinated effluents than Poliovirus Type I, which survived longer than the DNA phage ØX174. Cramer et al. (1976)

Table 3. Characteristics of coliphages in water and wastewater (Scarpino, 1978. Table 8.7).

Coliphage	Host	Nucleic Acid	Characteristic of head	Characteristic of tail	Receptor Site
T1	*Escherichia coli*	2-DNA	icosahedral 500A	100 X 500A	cell wall
T2,4,6	*E. coli*	2-DNA	prolated icosahedral 650 X 950A	250 X 1100A	cell wall
T3,7	*E. coli*	2-DNA	icosahedral 470A	100 X 150A	cell wall
T5	*E. coli*	2-DNA	icosahedral	100 X 1700A	cell wall
f2, MS2	*E. coli* male (f^+)	1-RNA	icosahedral 250A	none	f^+ pili

2-DNA = double stranded DNA

1-RNA = single stranded RNA

Table 4. Physical and chemical characteristics of f2 bacteriophage and Poliovirus (Cramer et al., 1976. Table 1).

Characteristic	Poliovirus	f2
VIRION		
nucleic acid	RNA	RNA
capsid symmetry	cubic	cubic
envelope	none	none
capsid diameter	270-300A	200-250A
molecular weight (daltons)	$5.5\text{-}6.8 \times 10^6$	3.0×10^6
pH stability	3-10	3-10
stability at 50C, 30min	cationic stabalized	stable
NUCLEIC ACID		
number of strands	one	one
shape	linear	linear
molecular weight (daltons)	$2.5\text{-}2.7 \times 10^6$	$0.7\text{-}1.2 \times 10^6$
nucleotides	6000	3300
PROTEIN		
number of different polypeptides	4	2
amino acids not found	none	Histidine

advocate the use of f2 as an indicator because f2 was equally or more resistant than Poliovirus Type III to inactivation by both chlorine and iodine, and also because it can be grown in large quantities and assayed with relative ease.

Shah and McCamish (1972), in agreement with previous reports, found that phage T2 was more sensitive to the action of chlorine than was Poliovirus or f2. A comparison of the inactivation curves of f2 and Poliovirus revealed the following information: (1) At any point on the curves, f2 was more resistant to chlorine than was Poliovirus. (2) Detectable numbers of f2 persisted long after poliovirus was no longer detectable. (3) Both curves were consistent and relative numbers of Poliovirus could be derived from known numbers of f2 at any point on the curve. They concluded that this property of greater chlorine resistance makes f2 an eminent candidate for the role of indicator of proper treatment methods.

Scarpino (1978) feels that when a model is proposed solely to study (by phage addition) patterns of enterovirus reduction in water and wastewater treatment processes, the phage need not be present in any quantity relative to enteric virus numbers; it has only to be established that the indicator phage is as resistant as are the enteric viruses.

MATERIALS AND METHODS

Propagation and Maintenance of Stock Cultures. Bacteriophage f2 and host Escherichia coli K37 used in this study were kindly donated by Dr. N. D. Zinder of the Rockefeller Institute, New York.

Stock cultures of E. coli K37 were initially grown at 35C and subsequently maintained on Difco Plate Count Agar slants at 3C. Nutrient broth cultures in 750ml spectrophotometric flasks were grown for 3-4hr at 35C in a New Brunswick Model G76 Gyratory Waterbath at 140cpm. When turbidity was visible in the flasks, 5ml of a purified f2 stock (ca. 10^6 pfu/ml) was added to each flask. The cultures were then incubated for another hour.

A purified f2 suspension was obtained by filtering the cultures through a 47mm diameter Gelman GN-6 membrane filter (pore diameter of 0.45μm). Phage stock cultures were held at 3C or frozen in 50mm screw-capped test tubes.

Phage Titers. Phage titers were determined by the agar overlay method as described by Adams (1959). When necessary, dilutions were made using 0.5% Tryptone (Difco) blanks (99ml). Phage titers are means of five replicate 1ml portions.

Media. All media were prepared using water which had been processed by a Miil-Q (Millipore) Reagent-Grade Water System (laboratory-pure water). Bacterial broth cultures were grown in flasks of nutrient broth containing 8g Nutrient Broth (Difco) and 5g NaCl per liter of laboratory-pure water. For agar overlay plates, base layer agar was prepared by

adding 23g Nutrient Agar (Difco) and 5g NaCl to a liter of laboratory-pure water. Soft agar pours were prepared by adding 12g Agar (Difco), 12g Nutrient Broth (Difco) and 7.5g NaCl to one liter of laboratory-pure water and dispensing in 11ml portions to 25mm diameter screwcapped test tubes. Dilution blanks (99ml) were prepared using 5g tryptone (Difco) per liter of laboratory-pure water. Sterilization of all media was at 121C and 15psi for 20min in a Castle Horizontal Autoclave.

Natural Populations of E. coli K37 Infective Phage. The incidence of natural populations of phage infective for E. coli K37 was determined by filtering 10ml samples of surface waters, finished waters, and sewage effluents through Gelman GN-6 microporous membranes, followed by 10ml of concentrated Nutrient Broth (10X NB) to elute any phage that might have adsorbed to the membrane. The resulting 20ml volume was assayed for phage without further dilution.

Filtration. A sand filter for laboratory use was constructed with thin-walled polyvinylchloride pipe, 24mm in diameter, which was supported in a vertical position. Filter columns of 10-, 20-, and 30-inch depths were constructed either entirely of sand or as dual-media filters with a 1:2 ratio of sand to anthracite coal. Sand filters consisted of silica sand with a specific gravity of 2.58 (Standard Methods for the Examination of Water and Wastewater, 14th ed.) and a uniformity coefficient of 1.41 (Curry,1974). Dual-media filters consisted of silica sand topped with anthracite coal having a specific gravity of 1.49 and a uniformity coefficient of 1.71. The media components used in this study were obtained from a fresh shipment recieved by the Mattoon, Illinois Water Treatment Plant. A support above the filter held a one-liter side-arm flask containing the seeded sample. The water head ranged from 24 to 19 inches above

the matrix surface during each run.

The column void volume was determined by placing 5ml of a concentrated NaCl solution on top of a water-saturated column. Laboratory-pure water was then filtered through the column until the NaCl was evident in the filtrate; the NaCl concentration of the filtrate was monitored, *in situ*, using a Markson Model 10 Conductivity Meter.

Duplicate one-liter samples of chlorinated final effluent, collected from the Charleston, Illinois sewage treatment plant, were seeded with 10ml of an f2 stock solution (ca. 10^6pfu/ml). Sodium thiosulfate was added (1ml of a 10% solution/liter of sample) at the time of collection to neutralize residual chlorine.

To one of the samples was added 0.12g alum (technical grade $Al_2(SO_4)_3$) and the mixture was stirred magnetically for 30min at room temperature. The resulting floc was allowed to settle for 30min and the titer of the supernatant (FOT) was determined. The titer of the unflocced control sample (NFOT) was also determined at that time.

The two samples were then run through duplicate filters by gravity filtration. The samples were run through columns which had been previously saturated with laboratory-pure water. The column void volume was discarded and one liter was quantitatively collected. Passing the unflocced sample through represented filtration alone, while passing the flocced sample through represented combining flocculation and filtration into one system. The titers of the flocced (F) and unflocced (NF) filtrates were determined, and calculations were as follow:

% reduction by flocculation and settling: $\frac{NFOT - FOT}{NFOT} \times 100$

% reduction by filtration: $\frac{NFOT - NF}{NFOT} \times 100$

% reduction by flocculation (without settling) followed by filtration:

$$\frac{NFOT - F}{NFOT} \times 100$$

Turbidity was measured before and after filtration on both flocced and unflocced samples, using a Model DRT-15 Turbidimeter, standardized to 0.14 NTU.

Chlorine Resistance. To determine the effect of chlorine on phage f2, one-liter samples of finished water were seeded with the phage. To 990ml of finished water, obtained from the Charleston, Illinois Water Treatment plant, was added 10ml of f2 stock (ca. 10^6pfu/ml). The mixture was stirred magnetically throughout the experiment. Phage titers were determined at the time of phage addition and after 2, 5, 10, 15, 30, 60, and 120 minutes. Chlorine levels (free and total residual chlorine) were determined at the outset of the experiment and at half-hour intervals during the course of the experiment, using a DPD Chlorine test kit (LaMotte Chemicals).

Phage Concentration. The membrane filteration method, similar to that of Wallis and Melnick (1967b), was used to concentrate seeded f2 coliphage from an original one-liter sample volume into a 10ml elutant volume.

Several variables were manipulated to obtain the optimum conditions for virus adsorption to, and subsequent elution from, a Gelman GN-6 membrane filter. Those variables controlled included pH and divalent cation concentration of the virus-suspending medium and the pH and concentration of the elutant.

Divalent cation concentration in the one-liter sample was controlled using a 5M $MgCl_2$ stock solution. The pH of both the sample and elutant was adjusted using 1M solutions of NaOH and HCl.

The concentration method was first attempted by seeding laboratory-pure water, then later with effluent samples obtained from Charleston and

Mattoon, Illinois sewage treatment plants. Residual chlorine was neutralized in chlorinated effluents by the addition of 1ml of sodium thiosulfate (10% solution) per liter of sample.

One-liter samples of water were seeded with 2ml of an f2 stock to a final concentration of approximately 1pfu/ml. The original titer was determined by assaying serial dilutions of the stock culture. The pH and $MgCl_2$ levels were adjusted to the desired levels and the sample was stirred magnetically. The seeded sample was then passed through a Gelman GN-6 membrane filter by gravity filtration. Elution of viruses from the membrane involved soaking the membrane <u>in situ</u> for 30min, with 2 successive 5ml portions of nutrient broth, for a total elutant volume of 10ml.

The efficiency of the procedure was expressed as % recovery, calculated as follows: $$\frac{\text{elutant titer/ml X 10ml}}{\text{original titer/ml X 1000ml}} \times 100$$

RESULTS AND DISCUSSION

f2 as an Indicator

To be an acceptable model for use in in-plant studies, f2 must not multiply or significantly die off in wastewater. Cramer et al. (1976) offered some evidence that it will not. RNA phages, like f2, do not possess a tail structure and attach only to male pili on the bacterial host cell. The attachment does not require energy, but penetration by the virus does. Consequently, multiplication is temperature-dependent. The optimum temperature for most enteric viruses is around 37C, and it is unlikely that wastewater in the natural environment would ever reach this temperature. A lack of suitable numbers of male hosts also makes it doubtful that f2 would multiply to a significant degree in wastewater.

Data obtained, using f2 as an indicator, are accurate provided that the wastewater is seeded to a titer sufficiently high that the presence of other E. coli K37 bacterial viruses is rendered negligible. As can be seen in Table 5, the background count of E. coli K37-infective phage in raw and finished waters was always less than 10pfu/ml. By seeding the wastewater so that the f2 titer is around 10^4pfu/ml, the ratio of seeded f2 to background virus is at least 1000:1. The probability of a plaque not originating from a seeded f2 is very low ($p \leq 0.001$).

Removal of Viruses by Water Treatment

All water-treatment processes remove or destroy viruses to some extent. Some treatment methods are more effective than others and none is likely to remove all the viruses present in raw water. A maximum removal by each

Table 5. Naturally-occuring Escherichia coli K37-infective bacteriophage populations.

Sample Site	Sample Type	pfu/ml[A]
Charleston Water Department	Raw	2.0
	Finished	*
	Distribution	*
Charleston Sanitary District	2 Effluent	1.6
	Final Effluent	6.0
Riley Creek Station 1	Surface	*
6	Surface	0.4
7	Surface	*
8	Surface	*
Cassel Creek	Surface	1.2

[A] mean of 5 replicate 1ml portions

* less than one plaque per 5ml

process in a treatment system should be achieved in order that no viruses, or a minimum number, remain in the final effluent.

Flocculation

In nine separate trials, the seeding of surface water with about 10^4 f2 particles per ml followed by alum flocculation and subsequent settling resulted in a wide range of population reductions (Table 6). In most cases at least a 1-log (90%) reduction was obtained and in several cases at least a 2-log (99%) reduction occurred. These results are in agreement with those of Chang et al. (1958b), who stated that more than a 95% removal of added virus should be obtained by alum flocculation of moderately turbid waters. Their experiments also showed that virus removal roughly paralleled the removal of coliforms, total bacteria, and turbidity. Chaudhuri and Engelbrecht (1970) reported a 98-99.9% reduction of seeded T4 and MS2 bacteriophage in wastewater with alum flocculation.

Success of the process in practice with various sources of water (Chang et al., 1958a) shows that satisfactory floc formation occurs with common coagulants under a wide range of natural conditions. The variability in phage reduction, in our experiments, was attributed to the quality an d quantity of floc produced. However, no attempt was made to control or assess these parameters.

Filtration

Physical Parameters. The physical characteristics of the filtration columns used in these experiments are summarized in Table 7. Filtration columns of 10, 20, and 30 inches were constructed and the flow rate of the 30-inch column was adjusted to that reported for rapid sand filtration (Curry, 1974) by adjusting the head of water above the bed. The flow rates of the 10- and 20-inch columns were significantly higher than that of the 30-inch column with the same amount of head pressure. Flow rates in the

Table 6. Coliphage f2 removal by flocculation and settling.

Trial	Unflocced control[A]	Flocced/Settled[A]	Reduction[B]
1	4.43	1.90	2.53
2	4.23	2.00	2.23
3	4.18	2.48	1.70
4	4.34	3.52	0.82
5	4.53	1.78	2.75
6	4.52	2.89	1.63
7	4.46	3.20	1.26
8	4.34	3.15	1.19
9	3.71	1.43	2.28

[A]Log of coliphage f2 titer (pfu/ml)

[B]Control minus flocced/settled

Table 7. Physical parameters of filter columns.

Medium	Column Depth (in)	Flow Rate* mean	Flow Rate* range	Void Volume**
Sand	10	9.0	7.7-10.3	34
	20	4.4	3.5-5.2	28
	30	3.5	3.4-3.6	25
Coal/Sand	10	1.8	1.1-2.5	27
	20	1.3	1.1-1.5	29
	30	1.5	1.2-1.8	29

*gal/min/sq. foot of surface area

**as percent of total volume

dual-media filters were significantly slower than those obtained with the sand columns. This is contrary to the report of Curry (1974), who stated that the use of dual-media filters is conducive to higher rates of filtration.

Void volume was measured to determine the homogeneity of packing. Percent void was essentially the same in both sand and dual-media columns, as well as in all three depths of both.

Phage Removal. Filtration without flocculation, of seeded water through these columns resulted in generally low f2 removal (Table 8). In only a few instances did such treatment result in a 1-log (90%) reduction and in most cases the removal was negligible. These results are in accord with those of other who found that rapid sand filtration is not an effective means of removing viruses. Clarke and Chang (1959) attributed this low efficiency to the inherently poor adsorptive properties of the sand.

No correlation of f2 removal vs. column depth was observed. In addition, neither matrix was shown to remove f2 better. In general, a layer of anthracite over the top of sand is used to achieve higher rates of filtration and longer filter runs (Curry, 1974) rather than to reduce turbidity or remove viruses.

Turbidity Removal. As shown in figure 1, the removal of turbidity by filtration roughly parallels removal of seeded phage. However, the large variations in turbidity and phage removals (Table 9) leads the writer to agree with Chang and coworkers (1958b), who maintain that turbidity in itself is not a satisfactory criterion for evaluating the efficiency of the process in removing viruses and bacteria.

Flocculation and Filtration

Flocculation without clarification, followed by filtration removed

Table 8. Coliphage f2 reduction by filtration alone and by flocculation and filtration combined.

Medium	Column Depth (in)	Reduction* by Filtration	Reduction* by Floc/Filt.
SAND	10	0.36	1.69
		0.32	0.62
	20	1.02	3.63
		0.53	2.49
	30	1.15	3.02
		0.23	1.34
COAL/SAND	10	0.07	2.58
		0.06	1.79
	20	0.28	2.34
		1.19	0.74
	30	0.35	3.18
		0.66	1.10

*log reduction of coliphage f2 titer

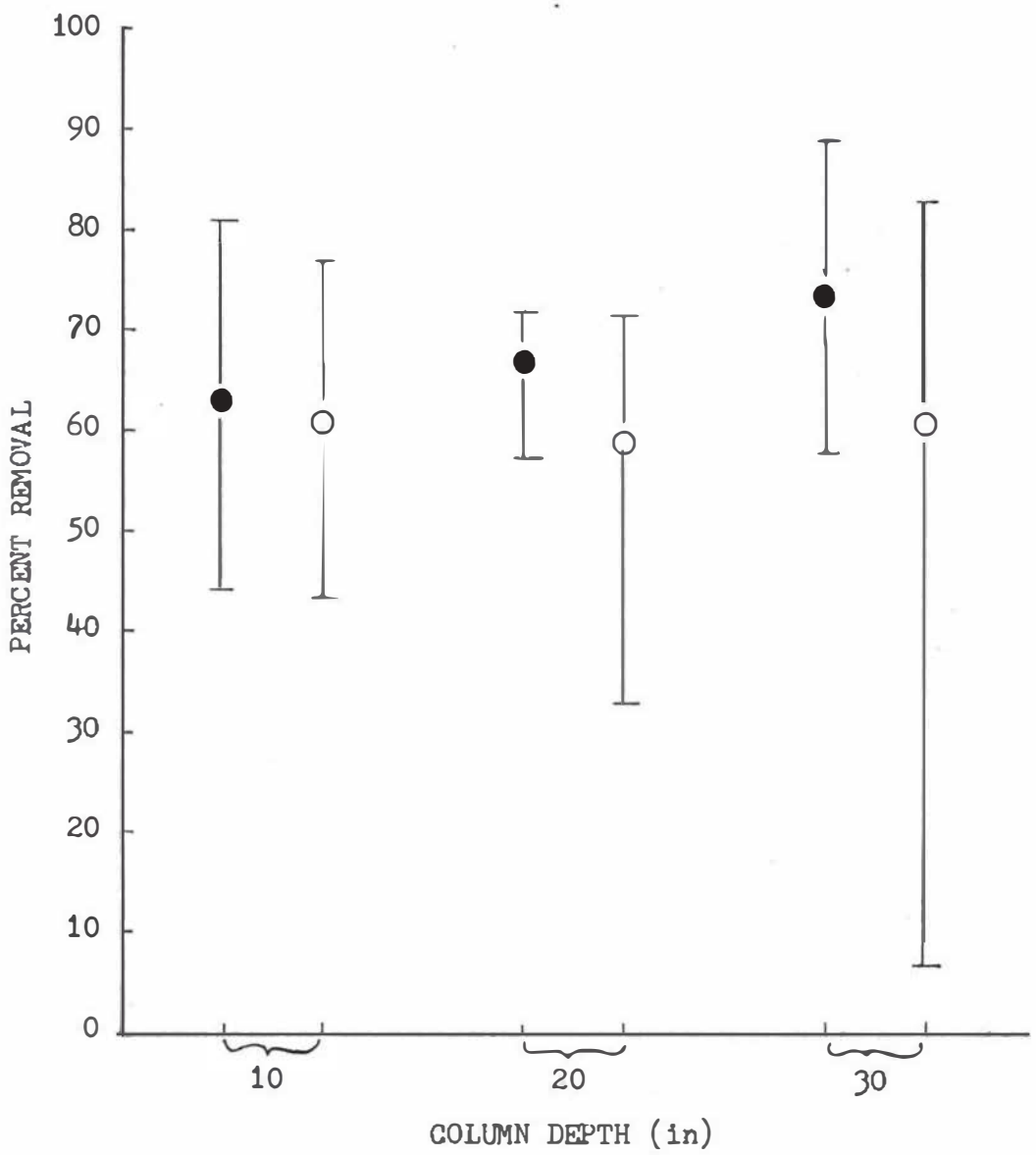

Figure 1. Range and average reduction of turbidity (dark circle) f2 (light circle) by rapid sand filtration.

Table 9. Phage f2 and turbidity reductions by filtration alone and by flocculation followed by filtration.

Column Depth (in)	Reduction (%) by Filtration		Reduction (%) by Floc/Filt	
	Turbidity	Phage	Turbidity	Phage
10	81.0	56.7	82.0	98.0
	44.2	67.3	28.6	83.7
	60.0	43.4	96.0	99.8
	66.1	76.7	97.3	86.4
	$\bar{x}_{10}$= 62.8	61.0	$\bar{x}_{10}$= 76.0	92.0
20	72.0	65.4	97.0	99.9
	66.0	71.4	91.5	99.7
	71.0	67.0	92.0	99.7
	57.7	33.3	94.5	73.3
	$\bar{x}_{20}$= 66.7	59.3	$\bar{x}_{20}$= 93.8	93.2
30	89.0	69.9	98.0	99.6
	58.2	7.1	92.0	92.9
	74.0	82.6	98.0	99.97
	73.2	83.4	98.3	94.0
	$\bar{x}_{30}$= 73.6	60.8	$\bar{x}_{30}$= 96.6	96.6

significant numbers of the seeded f2 (Table 8). In several instances, a 3-log (99.9%) occurred and generally a 2-log (99%) reduction was observed. Variations in phage reduction was, again, attributed to the quality and quantity of floc prodiced.

Because layering of floc on the surface of the filter bed tends to increase the efficiency of removal by filtration, it would be incorrect to compare the percent removal by flocculation and filtration combined with the sum of the two processes conducted independently. While filtration is relatively inefficient in removing viruses from unflocced waters, Chang (1968) reported a greater efficiency of the sand filter in removing virus particles in the unsettled floc. Nevertheless, whether conducted independently or combined into one system, flocculation and filtration can be expected to reduce virus concentrations by 90-99%.

Generally, the 20- and 30-inch columns removed more f2 than the 10-inch columns. Low removal in the 10in column was attributed to floc breakthrough, which usually resulted in higher turbidities in the effluent. Turbidity removal paralleled phage removal (Figure 2), but again the large variations would preclude the use of turbidity removal as an indicator of treatment efficiency.

Disinfection

Standard Methods for the Examination of Water and Wastewater (14th ed.) defines the chlorine requirement for disinfection as "the amount of chlorine that must be added per unit volume of effluent to produce the desired residual chlorine concentration after a definate (sic) contact time." Residual chlorine concentration and contact time are chosen to meet the U. S. EPA drinking water standard of less than four coliform organisms per 100ml of finished water.

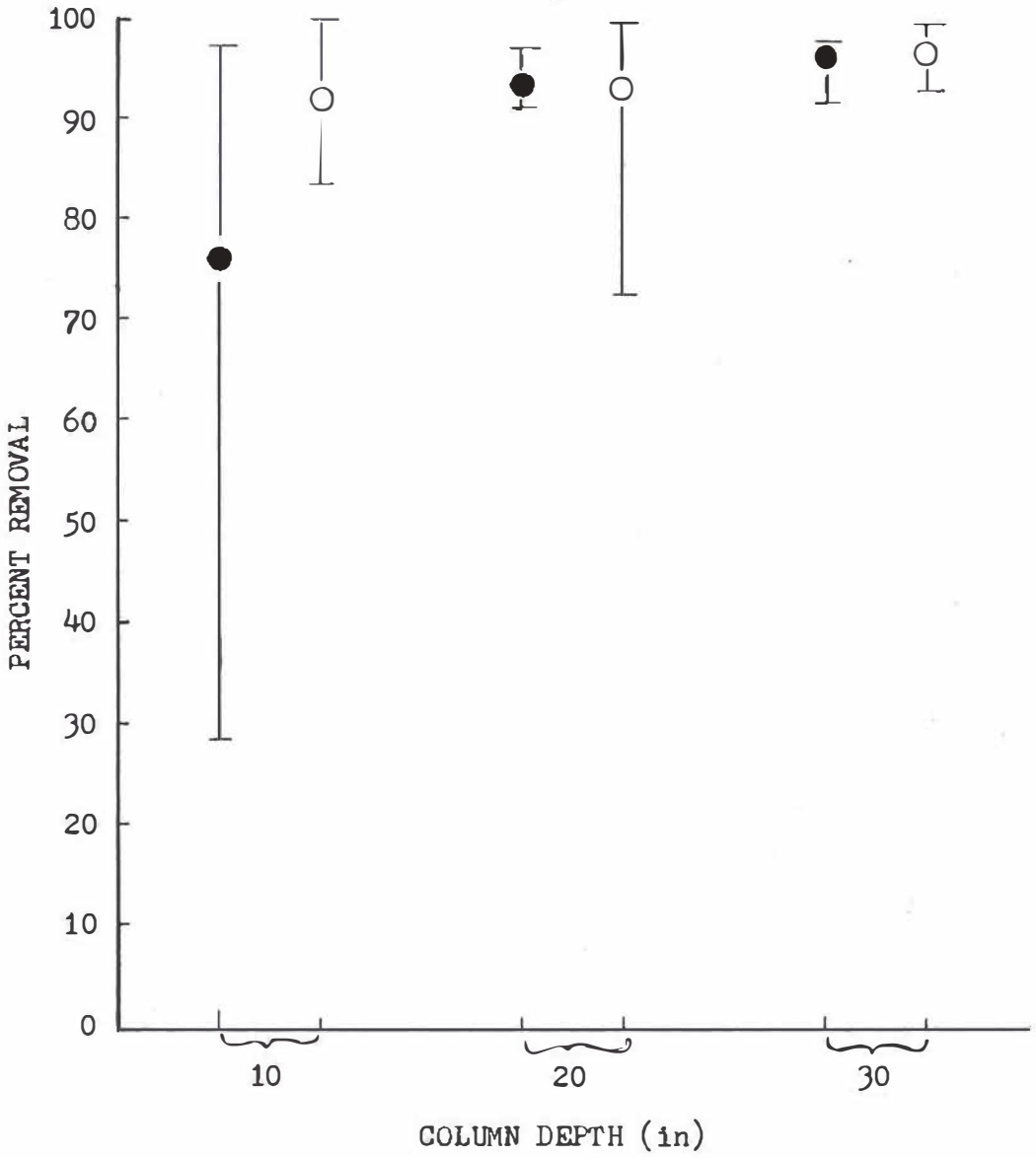

Figure 2. Range and average reduction of turbidity (dark circle) and f2 (light circle) by flocculation followed by filtration.

Common water-treatment practice is to chlorinate to 2.0ppm total residual chlorine after a 2hr contact time. This practice has proven to be effective in removing indicator bacteria. However, in this study, only a 70-80% reduction of coliphage f2 occurred at this chlorine level after a 2hr contact time (Table 10). These results are in accord with those of Berg et al. (1978) which showed that chlorine levels adequate to remove 99.999% of fecal coliforms in sewage removed only85-99% of the viruses present.

In three separate trials, 68-79% of the f2 kill occurred within the first two minutes of chlorine contact (Table 10). Further disinfection occurred throughout the 2hr contact time, but at a markedly slower rate. This phenomenon can be explained by examining the residual chlorine levels throughout the contact time (Figure 3). The free chlorine residual, a rapid virucide, was completely dissipated by 30min. This loss of free chlorine corresponded with the rapid drop in f2 titer. The remaining chlorine residual was in combined forms, which are much less virucidal and require longer contact times for adequate disinfection (White, 1975). Culp (1974) stated that a free chlorine residual of at least 0.5mg/l must be maintained for 30min for adequate disinfection of drinking water.

The results of this chlorine sensitivity experiment may be somewhat misleading. Because it is necessary to add organic material with the phage during seeding, much of the free chlorine residual was rapidly converted to the less virucidal combined forms (mono- and di-chloramines). This drastic reduction in free chlorine would not generally be the case in a continuous water-treatment system where a free chlorine residual is maintained at a constant level. These data are assumed only to approximate minimal virus destruction by chlorination, as they are most likely an

Table 10. Destruction of coliphage f2 throughout a 2hr contact time, with an initial chlorine concentration of 2.0ppm total residual chlorine.

Time (min)	% Kill			
	1	2	3	$\bar{X}$
2	79.0	68.2	69.2	72.1
5	78.0	81.4	58.8	72.7
10	89.2	75.2	75.0	79.8
15	84.6	78.8	69.2	77.5
30	--	73.8	76.8	75.3
60	--	--	71.0	71.0
120	--	76.6	78.3	77.5

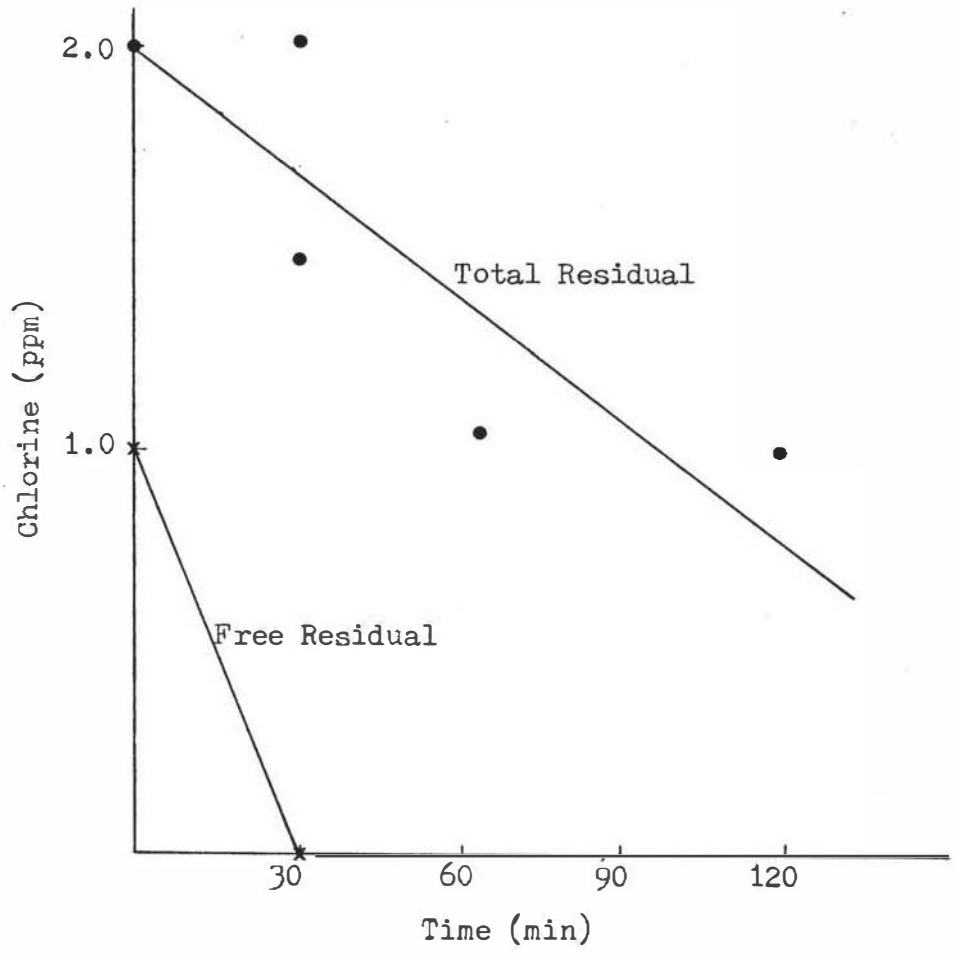

Figure 3. Free and total residual chlorine levels throughout a 2hr contact period.

underestimation of the reduction that would occur in an actual treatment system.

Total Removal by the System

Assuming that f2 is an adequate indicator of virus removal by water-treatment processes, flocculation and filtration should reduce the virus populations by 99-99.9%, and chlorination with 2ppm total residual chlorine for a contact time of 2hr will result in an additional 80% reduction. These processes, conducted in series, should produce approximately a 3- to 4-log (99.8-99.98%) reduction of indigenous virus populations. If a surface water supply contains 1.5-15 enteric viruses per liter, as reported by Metcalf (1971), then enteric virus numbers should be reduced to about one virus particle per 100 to 1000 gallons of finished water by conventional water-treatment practices.

Concentration of Viruses

Assuming little or no inactivation, failure to recover 100% of the seeded phage can be attributed to either (1) failure of the phage to adsorb to the membrane or (2) failure of adsorbed phage to be eluted from the membrane. Insufficient recovery of seeded phage is, most likely, due to a combination of both of these factors. Experimental variables were manipulated in an attempt to obtain maximum levels of adsorption and elution of seeded f2 bacteriophage. Variables, investigated in the adsorption step, include the pH and $MgCl_2$ concentration, as well as the presence/absence of membrane-coating-components (MCC). Those variables adjusted in the elution step were the elutant concentration and pH.

The membrane filter (MF) method of virus concentration was first attempted by seeding laboratory-pure water to negate any possible interference by MCC. These experiments are summarized in Table 11.

Table 11. Concentration of f2 in seeded laboratory-pure-water by MF techniques.

Trial	Adsorption		Elution		Recovery (%)
	$MgCl_2$	pH	elutant	pH	
1	0.05M	3.1	1 X NB	6.8	49.0
2	0.05M	4.0	1 X NB	6.8	4.5
3	0.05M	4.3	1 X NB	6.8	53.0
4	0.05M	4.5	1 X NB	6.8	20.7
5	0.05M	5.0	1 X NB	6.8	4.1
6	0.05M	5.5	1 X NB	6.8	39.0
7	0.05M	4.4	1 X NB	8.0	48.0
8	0.05M	4.5	1 X NB	9.0	32.0
9	0.05M	4.5	10 X NB	6.8	66.3
10	0.05M	4.5	10 X NB	6.8	91.3

1 X NB is single-strength nutrient broth

10 X NB is ten times-single strength nutrient broth

Effect of solution pH. The literature suggests that optimum adsorption occurs within the pH range of 3-5, depending on the virus in question. In experiments 1-6 (Table 11), levels of pH ranging from 3.1 to 5.5 were employed to determine the effect of hydrogen-ion concentration on phage adsorption to microporous membrane. Percent recovery ranged from 4.1-53.0% in these experiments.

Effect of elutant pH. In experiments 7 and 8 (Table 11) the effects of elutant pH on the recovery of viruses by the MF method was tested. In the previous experiments viruses were eluted from the membrane with single-strength nutrient broth at a pH of 6.8. Elutants adjusted to a pH of 8.0 and 9.0 resulted in recoveries of 48% and 32%, respectively. These values are within the range of values resulting from elution at a pH of 6.8. It would appear, then, that elutant pH has no significant effect on the recovery of f2 by MF techniques.

Effect of elutant concentration. Increasing the elutant concentration by ten times had a marked effect on virus recovery. Recoveries of 66 and 91% were obtained using 10X NB (pH 6.8) as the elutant. This marked increase in recovery suggests that previous lack of recovery was due, mainly, to failure of adsorbed viruses to be eluted, rather than a failure of the viruses to adsorb to the membrane. The fact that over 90% of the seeded viruses were recovered suggests that inactivation was minimal.

Effect of $MgCl_2$ concentration. An increase in the $MgCl_2$ concentration in the virus-suspending medium from 0.05M to 0.10M resulted in decreased recovery of seeded phage (Table 12). The addition of divalent cations is known to enhance viral adsorption by forming a salt bridge between the virion and the membrane; however, high concentrations of salts can cause a reversal of the virus' charge and can result in repulsion of the viruses

Table 12. Concentration of f2 in seeded natural waters by MF techniques.

Sample Site	Sample type	Adsorption		Elution		Recovery (%)
		$MgCl_2$	pH	elutant	pH	
Charleston Sanitary District	2 Effluent	0.05M	4.5	10 X NB	6.8	58.8
		0.05M	4.5	10 X NB	6.8	32.0
		0.05M	4.5	10 X NB	6.8	33.6
		0.10M	4.5	10 X NB	6.8	13.8
	Final Effluent	0.05M	4.5	10 X NB	6.8	2.6
		0.05M	4.5	10 X NB	6.8	0.0
		0.05M	4.5	10 X NB	6.8	0.0
		0.10M	4.5	10 X NB	6.8	0.0
Mattoon Sanitary District	2 Effluent	0.05M	4.5	10 X NB	6.8	18.6
	Final Effluent	0.05M	4.5	10 X NB	6.8	16.8

10 X NB is ten times single strength nutrient broth

from each other and from the membrane surface (Mix, 1976).

Effect of MCC. The effect of MCC on virus adsorption was investigated by seeding natural water samples. Secondary and final effluent samples from the Mattoon and Charleston sewage treatment plants were seeded to approximately 10^2-10^3pfu/l with f2. Percent recovery in natural waters ranged from 0-58.8% (Table 12). This decrease in recovery (from the 90% obtained with laboratory-pure-water) is probably due to decreased adsorption of the viruses to the membrane. The presence of MCC interferes with virus adsorption by competing with the viruses for adsorption sites on the membrane (Mix, 1976).

The difference in recovery efficiency between the secondary and final Charleston sewage plant effluent samples is due to differing concentrations of MCC in the two waters. The secondary effluent sample was collected after the water had been processed by degritting, aerobic digestion, and clarification. This water was clear but the presence of some organics was probable. The final effluent, on the other hand, was taken from a holding lagoon which contained high concentrations of organics. The decrease in percent recovery in the final effluent sample is due to the higher level of organics in this water.

The Mattoon plant, conversely, discharges their final effluent directly into the recieving stream. The final effluent sample was taken before discharge, so the secondary and final effluents contain comparable amounts of MCC, which resulted in comparable recovery levels.

CONCLUSIONS

1. Alum flocculation and clarification will reduce virus populations by at least 90%.

2. The removal of viruses in unflocced water by rapid sand filtration is negligible.

3. Flocculation without settling, followed by filtration, will result in a 90-99% reduction of virus populations in wastewater.

4. Chlorination to 2.0ppm total residual chlorine can be expected to reduce viral populations by at least 80% after a 2hr contact time.

5. A water-treatment system including alum flocculation, sand filtration and chlorination will result in at least a 99.8-99.98% reduction of indigenous virus populations in wastewater.

6. In clean waters, containing salt, viruses are adsorbed efficiently by cellulose nitrate membranes and these adsorbed viruses can be efficiently eluted and quantitatively assayed. However, application of the membrane filter method of virus concentration to natural waters is complicated by the presence of membrane-coating-components which interfere with virus adsorption.

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