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The behavior of viruses on disinfection by chlorine dioxide and other disinfectants in effluent

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1. SUMMARY

A comparative study on the efficacy of chlorine dioxide, chlorine, ozone and peracetic acid in inactivating viruses was carried out against 6 viruses in a municipal sewage effluent. The viruses selected were bacteriophage f_2 and poliovirus 1, which have been commonly used; also echovirus 1 and coxsackievirus B5 to extend the range of enteroviruses; and simian rotavirus (SA11) and human rotavirus, the latter being one of the most important enteric viral pathogens present in waste water. The results indicated a wide range in the response of these viruses to chlorine dioxide. Of the 3 enteroviruses tested, coxsackievirus B5 was the most resistant, with a dose of 17.25 ppm required for 99.99% inactivation in 5 min. In the case of the 2 rotaviruses tested, human rotavirus was distinctly more resistant than SA11. On the other hand, the other viruses tested responded differently to the other disinfectants. The most resistant virus on nearly all occasions under selected conditions was the human rotavirus; the least resistant virus was SA11. The enteroviruses, with phage, were somewhat similar in their response, although coxsackievirus B5 was usually the most resistant.

2. INTRODUCTION

There is a considerable degree of ambiguity and contradiction in the efficacy of various wastewater disinfectants. This largely reflects the absence of standardization in these studies and the use of only a single disinfectant and virus rather than multiple studies. It thus seemed appropriate to carry out a comparative study on several commonly used wastewater disinfectants against representative enteric viruses and of a bacteriophage. The disinfectants chosen were chlorine dioxide, chlorine, ozone and peracetic acid, because they represent those most likely to be useful in wastewater disinfection.

The use of chlorine for the disinfection of water and wastewater has been an accepted practice in many countries. However, chlorination, as is practised in water and wastewater, results in the formation of certain potentially toxic by-products. An alternative disinfectant to chlorine should be cheap, easy to produce, transport and store. It

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should also be potent at low dosage and readily decompose, either spontaneously or by the application of a neutralizing agent, into harmless by-products. Furthermore, it should be simply and reliably assayed and unreactive with other chemical and physical constituents of the effluent. Chlorine dioxide, although more expensive than chlorine, has several advantages over chlorine and may be a possible alternative to the latter. Chlorine dioxide does not react with ammonia nitrogen while it reacts with oxidizable material [6] and has the important advantage of not forming potentially toxic by-products like trihalomethanes [16,27], but it can produce halogenated organic compounds in some cases [2]. It is also a powerful oxidant over a wide range of pH and its efficacy is relatively unaffected at pH levels between 6 and 10, but is higher in the alkaline region, making it attractive for use with high pH, lime-softened water [9].

The main purpose of this study was to examine the response of viruses to disinfection by chlorine dioxide, and 3 commonly used disinfectants chlorine, ozone and peracetic acid in a municipal sewage effluent.

3. MATERIALS AND METHODS

3.1. Viruses

Stock cultures of poliovirus type 1 (LSC 2ab), coxsackievirus B5 and echovirus 1 were prepared in BGM cell cultures, and viral infectivity was assayed by the microtiter method in the same cells. Human rotavirus and SA11 were cultivated in MA-104 cells and assayed by the plaque test in the same cells [3,26]. The bacteriophage f_2 was cultivated in *Escherichia coli* K12 Hfr and assayed by the soft agar overlay technique [1].

3.2. Disinfectants

Chlorine solution was obtained by bubbling the gas through chilled distilled water. Chlorine dioxide was prepared by heating to 80 °C a mixture of powdered potassium chlorate and oxalic acid in water [18]; the gas evolved was dissolved in chilled, distilled water. Peracetic acid was obtained as a 35% aqueous solution. Ozone was produced by an ozonator (Wallace and Tiernan). The liquid disinfectants, present in water or effluent, were assayed by the DPD method [20] and ozone by the colorimetric version of the DPD method [19].

3.3. Inactivation experiment

Tests on the 3 liquid disinfectants (chlorine dioxide, chlorine and peracetic acid) were done in 500-ml Pyrex beakers mounted in a water bath and each provided with a stirrer with an overhead drive. To each beaker 100 ml of effluent was added followed by the virus to provide approx. 10^7–10^8 infectious units/ml. Finally, the disinfectant (chlorine dioxide or chlorine) was added at a dose selected to provide a predetermined free and combined residual to take account of effluent demand. There was negligible demand for peracetic acid and demand of ozone was not determined, because it was pumped through Drechsel bottles containing 200 ml of effluent (seeded with virus) to provide a steady-state residual. After the samples had been collected, the residual disinfectant was immediately neutralized with sodium thiosulphate and aliquots were stored at −20 °C until required for viral assay.

3.4. Effluent

All experiments were done using a representative activated sludge effluent from large batches stored at −12 °C. All experiments were conducted on thawed portions of this effluent. The pH, suspended solids, biological oxygen demand, chemical oxygen demand and ammonia concentration were determined before freezing and after thawing.

3.5. Data presentation

Each data point represents the average of triplicate experiments conducted on the same day under exactly the same conditions. The *t*-test analysis of the data indicated that variations among triplicate trials were not significant at the 0.05 level. Resistance has been quantified in terms of % survival after t minutes of contact with the disinfectant.

4. RESULTS

When a halogen is added to water or effluent, some of it reacts with ions and/or organic matter present in the system. Some of these reactions are instantaneous, while others are slow. Part of the disinfectant is taken up by this 'demand' and the remainder acts as free and combined residual disinfectant.

To establish the chlorine dose: residual relation, different doses of chlorine were added to the effluent at pH 7.2 and 15 ° C. The free residual was titrated at 1 min. The same procedure was also used for chlorine dioxide and the results are reported elsewhere [8].

In the disinfection experiments with ozone, the gas was provided continuously. Ozonized air was bubbled through the reaction flask and samples were taken to determine when the concentration of the absorbed ozone in the reaction flask reached equilibrium. This equilibrium was dependent on the concentration of ozone in the air passing through the flask.

In the case of peracetic acid, the effluent did not exhibit a measurable demand and the concentration initially administered did not change after 30 min of contact time.

Once the behavior of disinfectants in effluent was established, disinfection of the 6 viruses was attempted. Chlorine dioxide inactivated all the 6 test viruses but the dose response relationship varied widely. Of the three enteroviruses tested at pH 7.2 and 15 ° C, coxsackievirus B5 was the most resistant (data not presented) with a dose of 17.25 ppm (5 ppm, free residual chlorine dioxide) required for complete inactivation in 5 min and 99.9% inactivation being achieved by 15.25 ppm (4 ppm) in the time interval. At 13.25 ppm (3 ppm), all the enteroviruses tested behaved similarly. The behavior of bacteriophage f_2 appeared to be more sensitive than the enteroviruses with total inactivation being at a dose of 13.25 ppm. In the case of the two rotaviruses tested, human rotavirus was distinctly more resistant than SA11. For instance, a higher level of disinfectant was required to achieve the same level of inactivation of the human virus. The comparative behavior of all these viruses (Fig. 1) shows that human rotavirus was

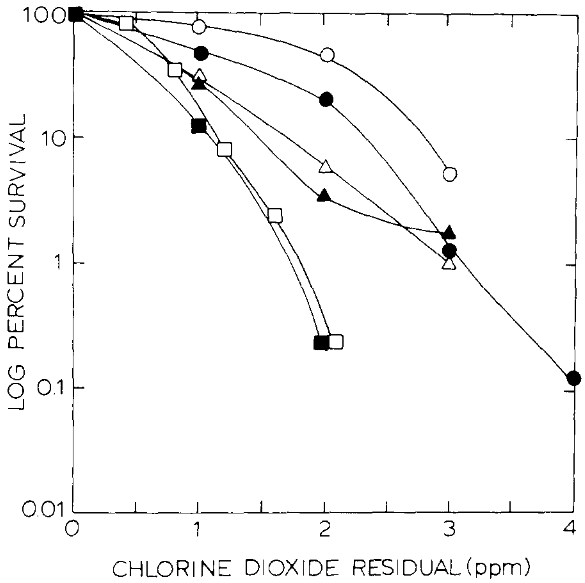

Fig. 1. Inactivation of viruses by various concentrations of chlorine dioxide (ppm) in a municipal sewage effluent (pH 7.2, 15 ° C). ○, Human rotavirus; ●, coxsackievirus B5; △, echovirus 1; ▲, poliovirus 1; □, bacteriophage f_2; ■, simian rotavirus.

the most resistant followed by coxsackievirus B5 and that these two viruses, unlike the other four, showed more resistance to chlorine dioxide at low concentration. In contrast, the curves for bacteriophage f_2, echovirus 1, poliovirus 1 and SA11 all showed a more even decline in infectivity with increasing chlorine dioxide.

The inactivation of viruses by chlorine at 15 ° C and pH 7.2 is represented in Fig. 2. Among the 6 viruses tested, coxsackievirus B5 was the most resistant with 99.99% inactivation achieved at a residual of 11.0 ppm, while simian rotavirus was most sensitive with only a residual of 1.1 ppm needed to achieve 99.99% inactivation. A striking difference was noted between the two rotaviruses. The human strain was 7 times more resistant to chlorine than simian rotavirus.

Ozone (Fig. 3) was a more efficient disinfectant than the others and concentrations of 0.5 ppm were required for complete inactivation of the viruses tested. Again, the response of the viruses varied widely. Bacteriophage f_2 was the least resistant. The 3 enteroviruses reacted similarly, but, in contrast to the 2 rotaviruses, responded quite

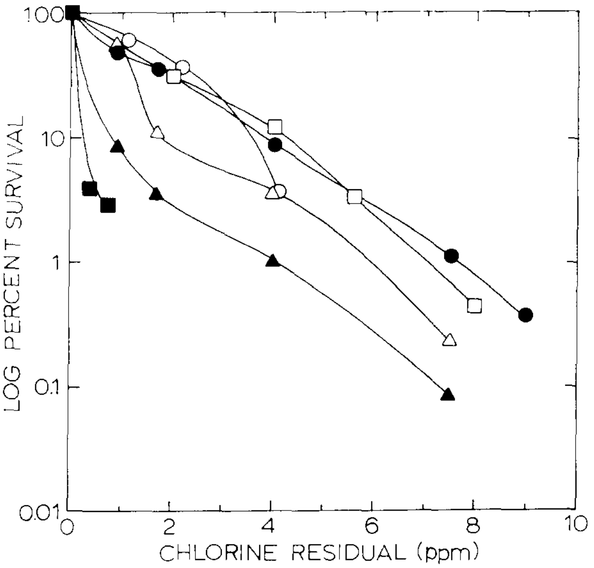

Fig. 2. Inactivation of viruses by chlorine. Conditions and symbols as in Fig. 1.

differently, with the human rotavirus proving to be the most resistant of all the viruses tested.

The inactivation of the various viruses by peracetic acid is illustrated in Fig. 4 and the results indicate that relatively high concentrations of acid

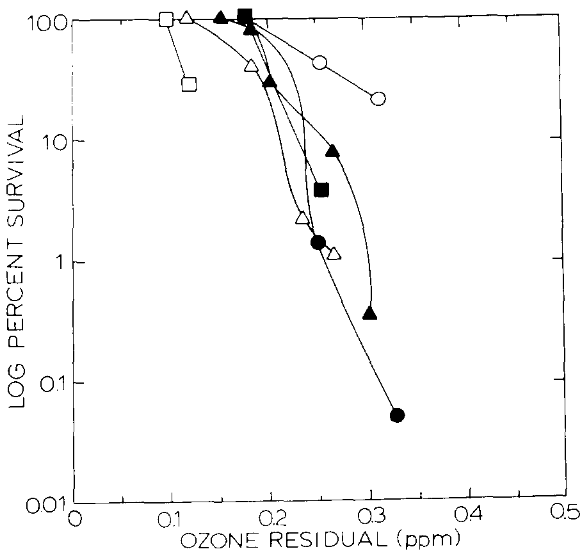

Fig. 3. Inactivation of viruses by ozone. Conditions and symbols as in Fig. 1.

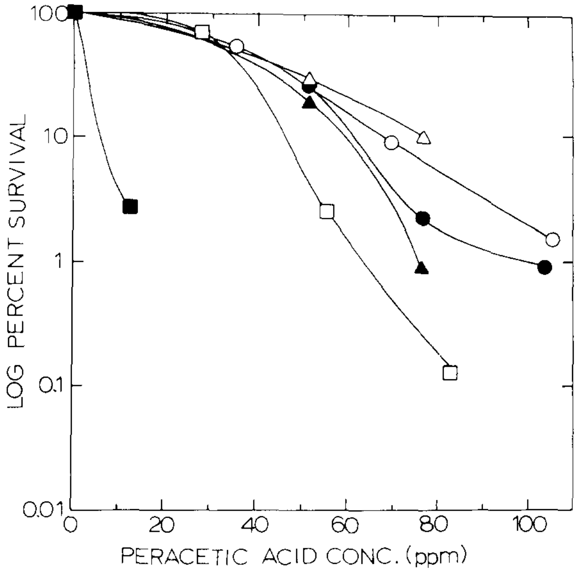

Fig. 4. Inactivation of viruses by peracetic acid. Conditions and symbols as in Fig. 1.

were required to achieve significant effects. For instance, up to about 140 ppm were necessary to give 99.99% inactivation of human rotavirus, which was the most resistant. On the other hand, simian rotavirus, which was the least resistant, required only as little as 20 ppm to give 99.99% inactivation. The three enteroviruses reacted similarly and the bacteriophage was slightly less resistant than the enteroviruses, especially at high concentrations of peracetic acid.

The order of sensitivity of the enteroviruses and bacteriophage f_2 was different to each disinfectant. For instance, bacteriophage f_2 was the

Table 1

Relative sensitivity of enteric viruses to disinfection Highest sensitivity at top of list. Cl_2, Chlorine; ClO_2, chlorine dioxide; PAA, peracetic acid; O_3, ozone.

Cl_2	ClO_2	PAA	O_3
Human rota	Human rota	Echo	Human rota
f_2	Coxsackie	Human rota	Polio
Coxsackie	Echo	Polio	SA11
Echo	Polio	Coxsackie	Echo
Polio	f_2	f_2	Coxsackie
SA11	SA11	SA11	f_2

least sensitive to the other disinfectants (Table 1). The 3 enteroviruses also differed from each other with regard to resistance to the 4 disinfectants, with poliovirus being the most sensitive to chlorine and chlorine dioxide but least sensitive to ozone.

5. DISCUSSION

The efficacy of effluent disinfection depends to a great extent on the quality of the effluent [29]. For this reason, the characterization of the effluent undergoing disinfection experiments is important since there are often variations in the physical and chemical quality [2]. To minimize such variations, it was decided in the present work to use large batches of effluent which were stored at $-12°$C, a procedure which resulted in minimal variation in the effluent quality. The chemical analysis of the effluent after thawing was as follows: pH 7.8, suspended solids 12.5 mg/l, NH_3 as nitrogen 1.55 mg/l, BOD 10.56 mg/l and COD 37.22 mg/l. The most obvious change was a shift towards higher pH, but this was not thought important because the pH of the system was always adjusted before experiments. There was a characteristic biphasic mode of inactivation with a sharp loss of viral infectivity within the first 5 min, followed by an extended phase where little, if any, further inactivation occurred after 30 min.

In comparing the results obtained in this study with those reported by others, one should keep in mind the effects that the quality of effluent has on disinfection. In this study, a dose of 17.25 ppm with a residual of 5 ppm after 1 min was required to achieve inactivation of coxsackievirus B5 which was the most resistant. All other viruses needed a dose of 15.25 ppm (residual 4 ppm after 1 min) or less for their inactivation. A dose of 12 mg/l of chlorine dioxide was reported by others [28] to inactivate total coliforms, fecal coliforms, fecal streptococci and seeded poliovirus 1 and coliphage f_2 and 174 within 2 min in partially purified wastewater. Others (30) found that bacterial and viral inactivation of four log reduction units at approximately 30 sec contact time was achieved at chlorine dioxide concentrations of 5.0 and 7.5 mg/l in a secondary wastewater effluent seeded with *E. coli* and bacteriophage f_2. Results of laboratory and field studies [22] on the disinfection of wastewater effluents indicated that chlorine dioxide inactivates poliovirus 1 and natural coliphage populations more efficiently than chlorine. Similar findings were reported by others [10,15].

An important feature of the results of all the disinfectants tested was the considerable variation in the resistance of various viruses to these disinfectants which emphasized that the choice of any one virus as a model was inappropriate. Furthermore, in making any comparison of the behavior of different viruses, it is worth bearing in mind the essentially different assay systems used for each virus studied. In the present work, these ranged from a monolayer cell culture for the enteroviruses, a plaque assay for SA11, an indirect immunofluorescence technique for detecting human rotavirus and a bacterial pour-plate for bacteriophage f_2. Although these tests were valid and reproducible, there are no satisfactory grounds for assessing whether each virus behaved in exactly the same way after disinfection with regard to its infectivity test. In addition, the infectivity ratios of different viruses are believed to be different. For instance, 1 pfu of bacteriophage f_2 is thought to represent one phage particle [1], whereas 1 pfu of poliovirus may be anything up to 1000 particles [5]. The implication of these facts is that what is experimentally shown as a more resistant virus may not be actually more resistant, but its assay method may not be sensitive enough to detect every infectious particle.

The wish to standardize conditions for disinfection of effluents has led to the suggestion that an indicator should be selected and there has been much debate on the value of viral indicators, a subject well reviewed [13,17,24,25]. There have, for instance, been proposals to use naturally occurring bacteria and viruses to monitor wastewater treatment and disinfection [22]. However, most workers have tried to defend the use of either bacteriophages or poliovirus 1, both of which are readily assayed in the laboratory and are commonly present [13,21], although not always so [11,23]. There have also been suggestions that laboratory grown viruses can be satisfactory models for the test behavior of viruses. For example, simian rotaviruses are thought to be representative of human rotaviruses but it was apparent from the present study that human rotavirus isolated from faeces was much more resistant to all disinfectants tested than the laboratory-grown SA11. For a virus to survive relatively harsh environmental conditions, it must be innately resistant or be protected by such physical means as association with particulate matter or occulsion with a biological film on a surface. There are several suggestions for innate resistance. For instance, it was shown [7] that the male specific bacteriophage isolated from wastewater varied widely in their sensitivities to chlorination. Furthermore, it was reported [14] that the resistance of wildtype strains of bacteriophages and certain vertebrate viruses varied widely. Kelly and Sanderson [12] reported that poliovirus (strain MK500), which was isolated from sewage, was much more resistant to inactivation by chlorine than the laboratory-grown strain of poliovirus 1 and it was even demonstrated [4] that resistant strains of poliovirus could be developed by selection from disinfected samples.

It is worth drawing attention again to the interesting observation that the test viruses behaved differently to all 4 disinfectants. The most resistant virus on nearly all occasions under selected conditions was the human rotavirus and the least resistant was the simian rotavirus. The enteroviruses with phage were somewhat similar in their response although coxsackievirus B5 was usually the most resistant. It must be emphasized again that over a narrow threshold value, all viruses could be inactivated. It is somewhat arbitrary, perhaps, to say that one virus is more resistant than another and care must be exercised in defining optimum conditions for disinfection. Therefore, when a proper evaluation of a disinfectant is required it would be advisable to test it against as many representative enteric viruses as practicable.

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