Inactivation of f2 coliphage in municipal effluent by the use of various disinfectants

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SUMMARY

Bromine chloride, chlorine and peracetic acid inactivated f2 coliphage in effluent but in order to achieve 99.99% inactivation the three disinfectants were required at about 1, 10 and 100 mg/l respectively. The activity of chlorine was halved by the presence of added organic matter, whereas bromine chloride and peracetic acid were very little affected. When a second successive dose of virus was added to the reaction mixture, the virus was inactivated only by peracetic acid despite the fact that in the chlorine-treated effluent residual chlorine was detected. The addition of a second dose of disinfectant inactivated residual virus in the same way as the first dose.

INTRODUCTION

The inactivation of viruses in waste-water effluents has been studied by several investigators (Lothrop & Sproul, 1969; McKee, Brokaw & McLaughlin, 1960; Olivieri, Donovan & Kawata, 1971; Shuval *et al.* 1966; Warriner, 1967). Where disinfection of effluents is practised chlorine is mainly used because it is the cheapest and easiest disinfectant to handle and its chemistry is very well documented (White, 1972). However, there are disadvantages in its use for effluents as opposed to clean waters, because it reacts with the nitrogenous matter present in the effluent resulting in the formation of large amounts of stable and persistent mono- and dichloramines, as well as other potentially toxic chlorinated compounds (Painter, 1971). Thus in order to achieve a residual of free chlorine, the chlorine demand of the effluent has to be satisfied, a treatment known as 'break-point' chlorination (Palin, 1950).

Alternative disinfectants have been proposed and some of them used. Ozone is one of the more widely tested, although more commonly used for the disinfection of drinking water supplies (Coin, Hannoun & Gomella, 1964; Evison, 1972; Perlman, 1969) than for effluents (Katzenelson & Biederman, 1976; Pavoni *et al.* 1972). The fact that ozone does not introduce a disinfecting residual and is costly to manufacture has restricted its use. Another disadvantage is that there is a large ozone demand by turbid effluents.

The use of halogens other than chlorine has been restricted to small, confined water bodies – for instance, bromine is used for the disinfection of swimming

pools (Brown, McLean & Nixon, 1963; Taylor & Johnson, 1974), and iodine is used for emergency disinfection of drinking water (Chang & Morris, 1953). Bromine has the advantage of forming bromamines in the effluent which are relatively more virucidal than chloramines (Smith, 1978). Iodine has the advantage of not reacting with ammonia at all. However, both halogens have proved to be very expensive for municipal purposes.

Mills (1973) suggested bromine chloride as a potent disinfectant for effluents and it was tested by Keswick *et al.* (1977) who found it to be more virucidal than chlorine. Another halogen compound, chlorine dioxide, is also reported to have considerable activity against viruses in water (Tifft *et al.* 1977) but has not been tried in effluents.

Peracetic acid is known to be virucidal (Kline & Hull, 1960; Sprössig & Mücke, 1969), but there is only one report of its use as a waste-water disinfectant (Meyer, 1975) which was for the decontamination of abattoir effluents.

In this paper the activities of three disinfectants against one virus under standardized conditions were compared.

MATERIAL AND METHODS

f2 coliphage

The method for the propagation and assay of the male-specific coliphage f2 is described elsewhere (Balluz, Butler & Jones, 1978).

Effluent

The collection and storage of the effluent from the Guildford Sewage Treatment Plant and its characteristics have been described previously (Hajenian & Butler, 1980).

Experimental procedure

The apparatus and methods are described in a previous report (Hajenian & Butler, 1980). All experiments were conducted at 15 °C and pH 6. In the chlorine and bromine experiments the pH of the effluent, usually around 7.5, was adjusted to 6.0 before the start of an experiment. With peracetic acid, however, this adjustment was not necessary, because the pH of the effluent was brought down to and stayed at about 5.8 after the addition of the acid.

Disinfectants

(a) Chlorine gas (BDH Air Products) was bubbled into distilled water until a concentration of 5000–7000 mg/ml was obtained (determined iodometrically).

(b) Bromine chloride was prepared by bubbling chlorine gas into liquid bromine (Hopkins & Williams Ltd) in a pressurized vessel until the weight of the contents increased by 44 % (Mills, 1975). BrCl was stored frozen in liquid nitrogen (–180 °C) to prevent evaporation. One ml of BrCl dissolved in 100 ml cold distilled de-ionized water yielded a concentration of 7000 mg/l (determined iodometrically).

Appropriate amounts of both these halogen stock solutions, (a) and (b), were

Fig. 1. Inactivation of f2 coliphage in effluent with bromine chloride, chlorine and peracetic acid, in the presence and absence of 22 mg/l added organic matter, at pH 6 and 15 °C.

dispensed into the experimental vessels and their final concentrations were determined by the DPD method (Palin, 1957).

(c) Peracetic acid (35% aqueous solution; Phase Separation Ltd) was prepared as a 1% working solution by dilution in distilled water at 4 °C and stored at that temperature. Titration of peracetic acid in the experimental vessels was carried out as described by Sulley & Williams (1962).

Analysis of data

The inactivation data obtained were subjected to least-square analysis. The regression lines related the log percentage inactivation of virus to disinfectant residual. The same analysis was also used to calculate the threshold value of disinfectant required to inactivate 99.99% of the virus.

RESULTS

A comparison of the disinfecting abilities of chlorine, bromine chloride (BrCl) and peracetic acid (PAA) in effluent (Fig. 1) revealed that for 99.99% inactivation of the f2 coliphage BrCl and PAA, unlike chlorine, were little affected by the presence of additional organic matter. It was also shown that BrCl was active at one-tenth the concentration of chlorine whereas PAA was required at ten times that of chlorine on a weight per volume basis. There was a linear negative correlation between the amount of disinfectant and extent of inactivation (Fig. 1,

Fig. 2. Chlorination of two successive doses of f2 coliphage in effluent at pH 6 and 15 °C. Arrow indicates time when second dose of f2 was added. *Chlorine residual (mg/l).

solid lines) up to a certain threshold value of disinfectant, above which 99.99 % inactivation was achieved (Fig. 1, dotted lines).

The protection afforded to the effluent by residual disinfectant was studied by adding a second dose of f2 coliphage. Chlorine, despite the presence of a residual of 'free chlorine' (Fig. 2) or 'combined chlorine' (Fig. 3) was unable to inactivate added virus. However, in contrast, PAA fully retained its capacity to inactivate a second dose of virus (Fig. 4). Furthermore, unlike chlorine, there was very little loss of measurable PAA throughout the experiment.

The addition of a second dose of any one of the three disinfectants achieved the same percentage of inactivation of virus as obtained with the original dose (Figs. 5, 6 and 7). In the case of two-step chlorination and bromination the final residual obtained at 65 minutes was about the same as at 30 minutes; in other words, there was again a demand for the halogen, whereas with peracetic acid there was an increased residual observed after the addition of the second dose (Fig. 7) which confirmed that there was no demand on the acid.

Fig. 3. Chlorination of two successive doses of f2 coliphage in effluent at pH 6 and 15 °C (22 mg/l organic matter in the medium). Arrow indicates time when second dose of f2 was added. *Chlorine residual (mg/l).

DISCUSSION

The disinfection of effluents with chlorine has usually been conducted by the 'break-point' procedure in order to take account of the chlorine demand of the system. However, the concentration of the nitrogenous compounds in an effluent may be so highly variable that a clearcut break-point concentration could not be predicted. Furthermore, requirement for chlorine would also be influenced by the undefined and variable organic matter present in the effluent (Painter, 1971). In this respect it was interesting to observe that neither bromine chloride nor peracetic acid was much affected by the presence of added organic matter, and this was not the only advantage in the use of these two disinfectants in contrast to chlorine. For instance, bromine chloride hydrolyses to produce 90% HOBr, the active form (Mills, 1973), at pH values around neutrality, which is the normal pH of the effluent. Chlorine, on the other hand, produces only 19% HOCl at around neutrality, so that acidification of effluent to about pH 6.0 would be desirable in order to achieve at least 90% hypochlorous acid, the active form. The addition of peracetic acid resulted in a drop of the pH to just below 6.0, and it would have been interesting to examine the disinfecting activity of this acid in conjunction with chlorine. In certain cases, like abattoir effluents, hospital

Fig. 4. Disinfection of two successive doses of f2 coliphage in effluent with peracetic acid at pH 7.6 and 15 °C (22 mg/l organic matter in the medium). Arrow indicates time when second dose of f2 was added. *Peracetic acid residual (mg/l).

Fig. 5. Two-step chlorination of f2 coliphage in effluent at pH 6 and 15 °C (22 mg/l organic matter in the medium). Arrow indicates time when second dose of chlorine was added. *Chlorine residual (mg/l).

Fig. 6. Two-step bromination of f2 coliphage in effluent at pH 6 and 15 °C (22 mg/l organic matter in the medium). Arrow indicates time when second dose of BrCl was added. *Bromine residual (mg/l).

Fig. 7. Two-step disinfection of f2 coliphage in effluent with peracetic acid at pH 7.6 and 15 °C (22 mg/l organic matter in the medium). Arrow indicates time when second dose of peracetic acid was added. *Peracetic acid residual (mg/l).

discharges and swimming pools, where the load of micro-organisms is expected to be high and extensive disinfection desirable, the use of two disinfectants may prove advantageous. Recently Wyatt & Wilson (1979) reported that the use of ozone in conjunction with chlorine for swimming pool disinfection rendered the water bacteriologically safe.

Peracetic acid belongs to a different group of chemicals to the halogens but it exhibits a similar biphasic pattern of disinfection. It was interesting to note that effluent did not seem to have a demand for it; that is to say, unlike halogens, the concentration of peracetic acid did not decrease within 30 or 60 min contact time. Neither did its virucidal activity decrease after 30 min. This was readily demonstrated by the addition of more coliphage, which was inactivated to the same degree as the original dose of coliphage. This was not the case with chlorine, where the second dose of virus was not inactivated, whether the residual was in the 'free' or 'combined' form of chlorine. The implications are that chlorine residual did not protect the effluent, as noted by Smith (1978).

It was also interesting to note that the two small doses of disinfectant added successively did not present any advantage over one equivalent large dose. This observation was in agreement with those of Krusé, Olivieri & Kawata (1971) who achieved 1.5 log reduction of f2 coliphage with a single dose of 30 mg/l chlorine or 3 doses of 10 mg/l each. The linear nature of the relation between concentration and degree of inactivation is thus apparent. The relative reduction in titres after each point of addition of disinfectant was the same whether, for instance, the initial titre was 1×10^5 p.f.u./ml or 8×10^3 p.f.u./ml; that is to say, the percentage law applied.

Evidently, therefore, bromine chloride and peracetic acid had some distinct advantages over chlorine, but a cost-benefit estimation of their use might well prove that they would be too expensive to adopt as substitutes for chlorine in those instances where disinfection of effluent was required.

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