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Inactivation of viruses in municipal effluent by chlorine

By H. G. HAJENIAN and M. BUTLER
University of Surrey, Guildford, Surrey

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SUMMARY

The influence of pH and temperature on the efficiency of chlorine inactivation of two unrelated picornaviruses in a typical urban wastewater effluent was examined. Temperature, unlike pH, had relatively little effect on the rate of inactivation. The pH effect was complex and the two viruses differed. The f2 coliphage was more sensitive to chlorine at low pH, but at all values there was a threshold above which additional chlorine resulted in very rapid inactivation. The amount of chlorine required for this was less at low than at high pH, although at pH values above 7 the extent of inactivation was about the same. There was no apparent correlation between pH and rate of inactivation of poliovirus but there was a suggestion that at a pH close to the isoelectric point of the virus it was less sensitive to chlorination.

INTRODUCTION

The increased need for high quality potable water, especially in larger metropolitan areas and arid countries, has imposed more recycling of waste water and this has introduced problems associated with the control of chemical and biological pollutants. Advanced waste water treatment methods, like the activated sludge process, may greatly reduce chemical pollution, but potential microbial pathogens may persist (Berg, 1973; Clarke & Chang, 1959). However, it has been suggested that low-level circulation of pathogens in our water systems may assist in the maintenance of general immunity (Chang, 1968; Mosley, 1967), although it is more generally agreed that pathogen-free supplies are desirable. To achieve this the chemical disinfection of effluents has been introduced, and chlorine is widely used (Chamberlin, 1948; Krusé *et al.* 1973; Shuval *et al.* 1966), but there are problems in this procedure associated with the unpredictable and uncontrollable chemical composition of the effluents which influences not only the amount of residual chlorine available for disinfection but also results in the formation of toxic chlorinated by-products (Ward, 1974; Ward & DeGraeve, 1978). This paper reports on the influence of pH and temperature on the efficiency of chlorine inactivation of two unrelated picornaviruses in an attempt to determine an optimal standardized procedure.

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MATERIALS AND METHODS

Viruses

The male-specific bacteriophage f2 and poliovirus type 1 (Sabin attenuated vaccine strain LSc-2ab) were used. The propagation of stock cultures and titration of infectivity are reported elsewhere (Balluz, Jones & Butler, 1977; Balluz, Butler & Jones, 1978).

Effluent

A large batch of activated sludge effluent was collected from the Guildford Sewage Treatment Plant and stored at −10 °C. All experiments were conducted on thawed portions of this effluent after it had been shown that the pH, suspended solids, BOD_5, COD and ammonia-N were little changed by freezing.

Experimental procedure

The effluent was adjusted to the desired pH before disinfection and was dispensed into six clean chlorine-demand-free pyrex beakers. These were placed in a water bath for an hour in order to reach the desired temperature. One beaker contained 300 ml of effluent to which chlorine was added at the highest dose required for that experimental run. After 1 and 30 min 100 ml portions were removed and analysed for chlorine by the DPD method (Palin, 1957). A second beaker contained 100 ml of effluent and 1 ml of virus (normally in standard diluent which introduced 22 mg/l organic matter in the final mixture) to give a final concentration of approximately 1×10^5 p.f.u./ml. No chlorine was added to this vessel and samples were removed to determine viral infectivity. The remaining four beakers each had 100 ml of effluent, 1 ml virus and varying concentrations of chlorine. The beakers were covered with foil and fitted with glass stirrers driven at 100 rev./min. Samples (5 ml) were withdrawn after 5 and 30 min and were mixed immediately with sodium thiosulphate (30 mg/ml) to neutralize the chlorine. These samples were kept at 4 °C until titrated for viral infectivity.

Chlorine stock solution

Chlorine gas (BDH Air Products) was bubbled into distilled water until a concentration of 5000–7000 mg/l was obtained (determined iodometrically). Appropriate volumes of this were added to the experimental vessels to provide the desired concentration in the effluent.

Analysis of data

The inactivation data obtained were subjected to least-square analysis. The regression lines related the log per cent inactivation of virus to chlorine residual. The same analysis was also used to calculate the threshold value of chlorine residual required to inactivate 99·99 % of the virus.

RESULTS

Selected chemical and physical characteristics of the activated sludge effluent, both fresh and thawed, are presented in Table 1. By the *t*-test analysis only the pH had significantly altered after storage, but this was not thought to be important as the pH was anyway adjusted before conducting each experiment. The effluent exerted a slight chlorine demand throughout the experimental period which increased with rising pH (Table 2).

Table 1. *Physical and chemical characteristics of fresh and thawed effluent*

Characteristic	Fresh effluent	Frozen and thawed effluent
pH	7·48 ± 0·23	8·03 ± 0·24
Suspended solids (mg/l)	14·28 ± 3·85	18·50 ± 8·56
Ammonia-N (mg/l)	0·27 ± 0·09	0·19 ± 0·08
BOD_5 (mg/l)	6·68 ± 2·97	5·92 ± 1·77
COD (mg/l)	30·50 ± 8·16	36·66 ± 11·54

Table 2. *Chlorine dose/residual relationships at pH* 6, 7 *and* 8·8, *at* 15 °C

Contact time	pH	Chlorine dose (mg/l)	Residual chlorine (mg/l)		
			Free Cl_2	Combined Cl_2	Total Cl_2
1 min	6	10	6·7	0·9	7·6
	7	10	5·9	0·8	6·7
	8·8	10	4·6	1·1	5·7
30 min	6	10	3·6	1·1	4·7
	7	10	3·0	1·2	4·2
	8·8	10	2·2	1·0	3·2

The amount of residual chlorine required to inactivate f2 coliphage was least at the lowest pH values (Fig. 1). Furthermore, at that pH, increasing amounts of chlorine were relatively more active than the same amount of chlorine at higher pH values, and this is illustrated by the solid lines in Fig. 1, where the slope gets less steep as the pH rises. However, at each pH value a threshold was reached above which the addition of further chlorine achieved 99·99 % or greater inactivation of virus within 5 min or less (Fig. 1, dotted lines). Above pH 7 the maximum required was about 10 mg/l residual chlorine but at low pH as little as one fifth of this chlorine was active.

In contrast to f2 coliphage, the amount of residual chlorine required to inactivate poliovirus did not have an inverse relationship with pH, in fact at pH 6·8 the virus was more resistant than at 4 and 7·7 (Fig. 2, solid lines). Furthermore, the threshold value for 99·99 % inactivation was highest at pH 6·8 (Fig. 2, dotted lines).

Temperature had relatively little effect on the linear part of the regression curves of f2 coliphage. At pH 9 there was some indication of less sensitivity at 5 °C than at 25 °C but at pH 6 the small observed differences did not obviously correlate with temperature (Fig. 3).

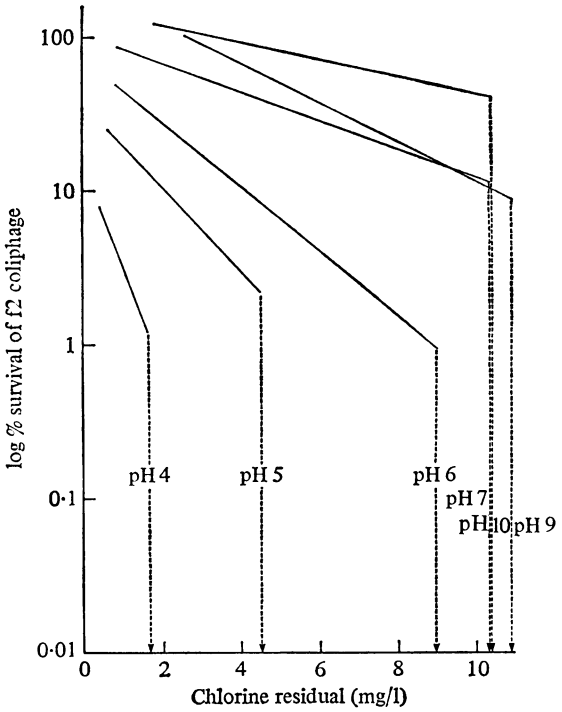

Fig. 1. The influence of pH on the inactivation of £2 coliphage by different concentrations of chlorine, incubated at 15 °C for 30 min.

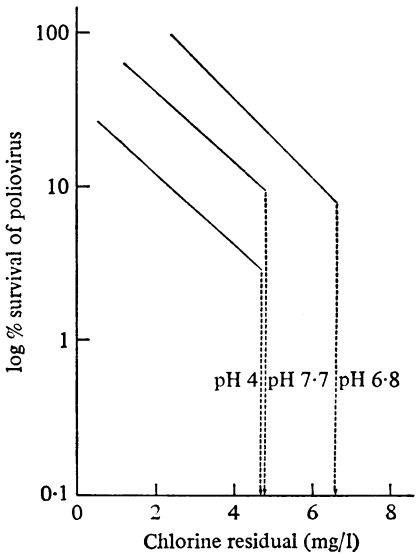

Fig. 2. The influence of pH on the inactivation of poliovirus by different concentrations of chlorine, incubated at 15 °C for 30 min.

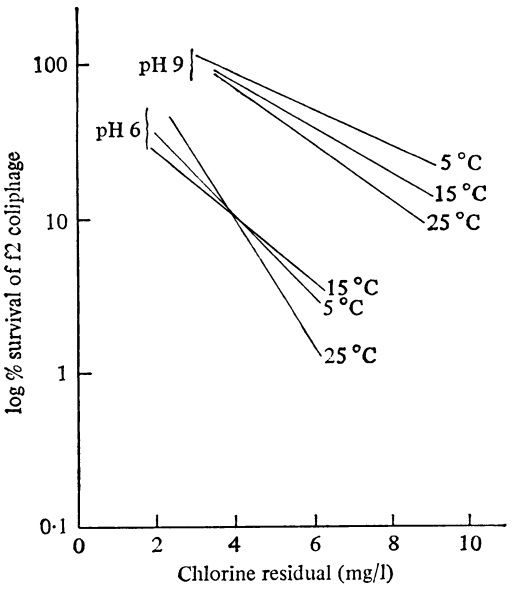

Fig. 3. The influence of pH and temperature on the inactivation of f2 coliphage by different concentrations of chlorine applied for 30 min.

DISCUSSION

In disinfection of clean water with chlorine the inactivation of micro-organisms follows Chick's laws of unimolecular reactions (Chick, 1908; Weidenkopf, 1958), but not so with disinfection in effluents. This divergence is due to the transformation of the pure halogen into a complex of halogenated compounds each with different disinfectant abilities and varying rates of disinfection. Thus in effluent it is desirable to add the minimum amount of chlorine to effect maximum inactivation in order to avoid the production of chlorinated by-products which would be released with the effluent into the waterways and may be toxic, This is in contrast to chlorination of clean water where it is important to maintain a chlorine residual, perhaps by break-point treatment (Palin, 1950), in order to inactivate secondary contamination with potential microbial pathogens, and where no chlorinated by-products are likely to be formed.

The characterization of effluent in which disinfection experiments are to be conducted is very important because wide variations in physical and chemical quality are known to occur (Painter, Viney & Bywaters, 1961) which are likely to influence the effectiveness of the disinfectants (Tonelli, 1976). In order to limit this a large batch of effluent was taken and held at -10 °C, a procedure which apparently resulted in minimal alteration of the effluent quality, also noted by Painter (1971).

In the present study it was interesting to note that inactivation of f2 coliphage or poliovirus was achieved at lower levels of chlorine dose or residual than those reported by others. For instance Krusé, Olivieri & Kawata (1971) reported 1·5 log reduction in titre of f2 in activated sludge effluent held at 4 °C and pH 7 with a chlorine dose as high as 30 mg/l. However, the residual chlorine, whether free or combined, was not reported, so it is possible that very high chlorine demand had

occurred. In the present studies with f2 under similar conditions (5 °C and pH 6) only 9 mg/l chlorine was required to achieve as much as 4 log inactivation. The lower pH could have accounted for the greater inactivation, since we found an inverse correlation with pH and inactivation. Cramer, Kawata & Krusé (1976), who tested as much as 30 mg/l of chlorine, failed to achieve the degree of inactivation found in the present study. However, they used autoclaved effluent which may perhaps have altered the chlorine demand in some way. Similarly, Shuval (1970) could not achieve more than 90% reduction in poliovirus in effluent at pH 7·7–7·8 with 11 mg/l chlorine and 30 min contact time, whereas in the present study half that dose removed 99·99% of the infectivity at 15 °C. Shuval (1970) did not report the temperature, which is an important factor in disinfection studies.

It was interesting that poliovirus required less chlorine for 99·99% inactivation at pH 4 and 7·7 than at pH 6·8, which suggests that the virus was more stable at a pH close to one of its suggested isoelectric points, pH 7·0 (Mandel, 1971). This may imply that the effect of pH on inactivation kinetics is not only on the chlorine species predominant in the system but also on the ionic state of the virus. It would be interesting to test the influence of isoelectric points of other viruses in relation to their susceptibility to disinfection.

In comparative studies with different viruses Scarpino *et al.* (1974) have demonstrated that poliovirus required more disinfectant than f2 coliphage for the same degree of inactivation, but contrary evidence had been presented by Shah & McCamish (1972). This apparent conflict almost certainly represents differences in effluent quality, the influence of pH, buffers and experimental procedures. These and similar reported inconsistencies in disinfection efficiency almost certainly reflect simply on the type of effluent and methodology and powerfully illustrate the need for standardized experimental technique. It is also worth bearing in mind the innately different assay systems used for the two viruses, a monolayer cell culture for poliovirus and a bacterial pour-plate for f2 coliphage. Although both are plaque tests there are no satisfactory grounds for assessing whether each virus behaves in exactly the same way after disinfection with regard to its infectivity test. In addition, the infectivity ratios of the two viruses are believed to be different, thus one p.f.u. of f2 coliphage is thought to represent one phage particle (Adams, 1959), whereas one p.f.u. of poliovirus may be anything up to 100 particles (Floyd & Sharp, 1977). The implication of these facts is that what is experimentally shown as a more resistant virus may not be actually resistant, but that its assay method may not be sensitive enough to detect every infectious particle.

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