EVALUATION OF THE SENSITIVITY
OF ENTERIC VIRUSES IN EFFLUENT
TO CHEMICAL DISINFECTION

by

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Thesis submitted to the
Department of Microbiology, University of Surrey
in partial fulfilment of the requirements
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December, 1982
TO MY PARENTS
Acknowledgment

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I would also like to thank my supervisor Dr M Butler and my colleagues, in particular Dr B Lloyd, Mr D Wheeler, Mr A Medlen, Mr J Berg, Mr G Orwa, Mr L Bowler, Mr G Dexter and Mr P Sad for their help and encouragement throughout the work.

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ABSTRACT

The literature indicates a considerable degree of ambiguity and contradiction in the efficiency of various wastewater disinfectants. This largely reflects on 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. The chosen disinfectants were chlorine, chlorine dioxide, ozone and peracetic acid because they represent those most likely to be useful in wastewater disinfection. The selected viruses were bacteriophage \( \phi_2 \) and poliovirus 1 which have been commonly used, also echovirus 1 and Coxsackievirus B5 to extend the range of enteroviruses and finally simian rotavirus (SA11) and human rotovirus, the latter, being one of the most important enteric viral pathogens present in wastewater. The effect of various parameters which might influence the efficiency of the disinfectants such as pH, temperature, suspended solids and peptone concentration as well as the effects of combining disinfectants were investigated.

The results obtained from this study indicated that lower levels of disinfectants were required than most of those levels already reported in the literature which was probably due to the quality of effluent used and emphasized the need to standardize this before disinfection studies.

Chlorine dioxide, although more expensive than chlorine, may be a useful alternative to chlorine because its virucidal efficacy was only slightly affected by changes in pH, temperature and presence of organic matter. Furthermore it does not react with organic matter to form some classes of chlorinated organic compounds considered hazardous to public health and it was easy to obtain reliable assay of its residual concentration.

Ozone may also be a promising alternative to chlorine because its efficacy is not greatly affected over the pH range 6-10 which is normally that encountered in effluent and temperature (2-30°C). Furthermore, it does not leave a toxic residual and it adds dissolved oxygen to water. However, its disadvantages are the high capital cost
for the establishment of the plant, difficulties in its reliable assay in effluent, it requires good quality effluent and it does not maintain a stable residual.

Peracetic acid is another possible alternative disinfectant. It does not form toxic by-products, effluent does not exert a demand for it which leaves it available for disinfection. However, the high concentrations required reduced the pH of the effluent and increased the BOD$_5$ of the treated product.

It was found that the combination of disinfectants did not produce a synergistic effect but only a complementary, additive or combined effect.

It was interesting to note that the different viruses reacted differently to each disinfectant which explains the argument against the use of model or indicator virus studies. In this regard, it was particularly worth noting that the human rotavirus isolate was the most resistant virus to disinfectants although surprisingly simian rotavirus was the least. Therefore, for the proper evaluation of a wastewater disinfectant it would be advisable to test it against as many representative enteric viruses as practicable.

Addendum

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INTRODUCTION

WATERBORNE DISEASES

The increasing demands on water resources due to concurrent increase in world population and industrial demand make recycling of domestic wastewater inevitable. Even in certain parts of the United Kingdom water supplies are already limited, and this has been recognised in a critical official report, "Taken for Granted" (HMSO, 1970), prepared to consider the treatment and disposal of sewage and wastewater for re-use.

Apart from demands on volume of supply there is an urgent need, especially in developing countries, for clean and safe water because it may act as a vehicle for the transmission of a variety of diseases. Indeed, the WHO (Anon, 1979, 1981) estimates that approximately 80% of all sickness and disease in developing countries can be attributed to inadequate water or poor sanitation and water related diseases have been conveniently classified into five general categories by Feachem (1977):

a. Water borne diseases: the situation in which water simply acts as a passive vehicle for the infecting agent, the risk of which could be eliminated simply by improving the water quality.

b. Water washed diseases: in this case pathogens are transmitted by poor personal hygiene associated with access to too little water and the problem may be overcome by access to sufficient water for washing and sanitary use.

c. Water based diseases: typically diseases caused by parasitic worms which depend on aquatic intermediate hosts to complete their life cycle. The elimination of the intermediate hosts by improved quality and management of surface water would prevent the problem.

d. Water related diseases: diseases mediated by insects which either breed in water or bite near water. Preventive measures include destruction of the breeding sites and decreasing the need to visit open water by provision of piped water supply.
e. Faecal disposal diseases: diseases resulting from pathogens multiplying in the products of defective sanitation with subsequent pollution of drinking water sources. The problem is eliminated by proper disposal of human faecal excreta.

The major diseases transmitted by water are listed in Table 1.1 (after Walsh and Warren, 1979).

Table 1.1 Major diseases transmitted by water

<table>
<thead>
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<th>Disease</th>
<th>Cases  (x10³/year)</th>
<th>Deaths (x10³/year)</th>
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<td><strong>WATER-BORNE DISEASES</strong></td>
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<tr>
<td>Amoebiasis</td>
<td>400,000</td>
<td>30</td>
</tr>
<tr>
<td>Diarrhoes</td>
<td>3-5,000,000</td>
<td>5-10,000</td>
</tr>
<tr>
<td>Poliomyelitis</td>
<td>80,000</td>
<td>10-20</td>
</tr>
<tr>
<td>Typhoid fever</td>
<td>1,000</td>
<td>25</td>
</tr>
<tr>
<td><strong>WATER-WASHED DISEASES</strong></td>
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<tr>
<td>Ascariasis (roundworm)</td>
<td>800,000-1,000,000</td>
<td>20</td>
</tr>
<tr>
<td>Leprosy</td>
<td>12,000</td>
<td>very low</td>
</tr>
<tr>
<td>Thricurirosis (whipworm)</td>
<td>500,000</td>
<td>low</td>
</tr>
<tr>
<td><strong>WATER-BASED DISEASES</strong></td>
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<tr>
<td>Schistosomiasis (bilharzia)</td>
<td>200,000</td>
<td>500-1000</td>
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<tr>
<td><strong>DISEASES WITH WATER-RELATED VECTORS</strong></td>
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<td>African trypanosomiasis (sleeping sickness)</td>
<td>1,000</td>
<td>5</td>
</tr>
<tr>
<td>Malaria</td>
<td>800,000</td>
<td>1,200</td>
</tr>
<tr>
<td>Onchocerciasis (river blindness)</td>
<td>30,000</td>
<td>20-50</td>
</tr>
<tr>
<td><strong>FAECAL DISPOSAL DISEASES</strong></td>
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<tr>
<td>Ancylostomiasis (Hookworm)</td>
<td>7-9,000,000</td>
<td>50-60</td>
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The role of water in the transmission of viral diseases has been recognised for some time (Berg, 1966) as soon, in fact, as it was realised that there were many potentially pathogenic enteric viruses. At least 100 of these have now been identified in human faeces (Table 1.2), with levels reaching up to $10^{10}$ infectious units/gm (Banatvala, 1981). Some of them have been found in raw sewage (Sellwood and Dadswell, 1981) and the others are presumably also present but they are difficult to recover and identify (WHO, 1979). Indeed many enteric viruses are difficult to cultivate in the laboratory (Table 1.3) although some of them are morphologically so distinct that electron microscopy of samples may be diagnostic.

It is well established that some viruses survive sewage treatment and may be detected in large numbers in sludges and effluents (Gerba, 1981; Goddard and Bates, 1981; Irving and Smith, 1981; Ward, 1981). Furthermore, rivers contaminated with effluents also carry enteric viruses (Edwards and Wyn-Jones, 1981; Slade, 1978; Slade and Harris, 1982) and certain enteroviruses have even been isolated from drinking water (Berg, 1975; Coin et al., 1966; Melnick et al., 1978; Nestor et al., 1978; Suchkov, 1964). More interesting is the observation that poliovirus (Hoehn et al., 1977) and rotavirus (Walter, 1982) have been detected in treated drinking water.

The potential routes for the transmission of enteric virus via water are illustrated in Fig. 1.1 leading to the contamination of potable and recreational waters, of shellfish and of crops irrigated with effluents. The ability of viruses to be transmitted via these routes depends, of course, on their resistance to environmental factors (Bitton, 1980) and it is known that certain enteroviruses may survive in tap water for up to 168 days, in seawater up to 130 days, in soil for up to 125 days and up to 90 days in oysters (Gerba et al., 1975; Melnick et al., 1978).

The great majority of outbreaks of waterborne viral diseases are associated with diarrhoea although the evidence for the association is largely circumstantial. This is mainly because diarrhoea is not, of course, specific to virus infection and viral diarrhoeas often occur when the levels of infectious material in incriminated samples are
### TABLE 1.2: Enteric viruses present in human faeces

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<td>MENINGITIS; RESPIRATORY DISEASE; DIARRHOEA</td>
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<td>MENINGITIS; RESPIRATORY DISEASE; DIARRHOEA</td>
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<td>COXSACKIEVIRUS B (6)</td>
<td>MYOCARDITIS; RESPIRATORY DISEASE; MENINGITIS</td>
</tr>
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<td>ENTEROVIRUS (4)</td>
<td>RESPIRATORY DISEASE; MENINGITIS; CONJUNCTIVITIS</td>
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<td>HEPATITIS</td>
</tr>
<tr>
<td>NORWALK AGENTS (5 ?)</td>
<td>GASTROENTERITIS</td>
</tr>
<tr>
<td>&quot;SMALL ROUND PARTICLES&quot; (?)</td>
<td>GASTROENTERITIS</td>
</tr>
<tr>
<td>ASTROVIRUS (1 ?)</td>
<td>GASTROENTERITIS</td>
</tr>
<tr>
<td>CALICIVIRUS (1 ?)</td>
<td>GASTROENTERITIS</td>
</tr>
<tr>
<td>REOVIRUS (3)</td>
<td>RESPIRATORY DISEASE; EXANTHEMA; DIARRHOEA (?)</td>
</tr>
<tr>
<td>ROTAVIRUS (2 ?)</td>
<td>GASTROENTERITIS</td>
</tr>
<tr>
<td>ADENOVIRUS (33)**</td>
<td>RESPIRATORY DISEASE; CONJUNCTIVITIS (GASTROENTERITIS?)</td>
</tr>
<tr>
<td>CORONAVIRUS (1 ?)</td>
<td>GASTROENTERITIS</td>
</tr>
</tbody>
</table>

* NUMBER OF SEROTYPES IN PARENTHESES
** PLUS SEROLOGICALLY UNRELATED GASTROENTERIC STRAINS
(After Butler, 1981)

### TABLE 1.3 Methods for cultivation of human enteric viruses

<table>
<thead>
<tr>
<th>VIRUS GROUP</th>
<th>METHOD OF CULTIVATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARVOVIRUS</td>
<td>- ve</td>
</tr>
<tr>
<td>POLIOVIRUS</td>
<td>+ ve (SEVERAL CELL CULTURES)</td>
</tr>
<tr>
<td>ECHOVIRUS</td>
<td>+ ve (SEVERAL CELL CULTURES)</td>
</tr>
<tr>
<td>COXSACKIEVIRUS A</td>
<td>+ ve (NEO-NATAL MICE; SOME CELL CULTURES)</td>
</tr>
<tr>
<td>COXSACKIEVIRUS B</td>
<td>+ ve (SEVERAL CELL CULTURES)</td>
</tr>
<tr>
<td>ENTEROVIRUS</td>
<td>+ ve (SEVERAL CELL CULTURES)</td>
</tr>
<tr>
<td>HEPATITIS A</td>
<td>+ ve (MONKEY LIVER AND EMBRYONIC CELL CULTURES; INCOMPLETE REPLICATION IN SOME OTHER CELL CULTURES)</td>
</tr>
<tr>
<td>NORWALK AGENTS</td>
<td>- ve</td>
</tr>
<tr>
<td>&quot;SMALL ROUND PARTICLES&quot;</td>
<td>- ve</td>
</tr>
<tr>
<td>ASTROVIRUS</td>
<td>+ ve (INCOMPLETE CELL REPLICATION IN SOME CELL CULTURES)</td>
</tr>
<tr>
<td>CALICIVIRUS</td>
<td>- ve</td>
</tr>
<tr>
<td>REOVIRUS</td>
<td>+ ve (SEVERAL CELL CULTURES)</td>
</tr>
<tr>
<td>ROTAVIRUS</td>
<td>+ ve (INCOMPLETE REPLICATION IN SOME CELL CULTURES)</td>
</tr>
<tr>
<td>ADENOVIRUS*</td>
<td>+ ve (INCOMPLETE REPLICATION IN SOME CELL CULTURES)</td>
</tr>
<tr>
<td>CORONAVIRUS</td>
<td>- ve (? HUMAN ORGAN EXPLANT CULTURE)</td>
</tr>
</tbody>
</table>

* NEW GASTROENTERIC SEROTYPES. OTHER SEROTYPES CULTIVATED IN SEVERAL CELL CULTURES.
(After Butler, 1981)
Fig 1.1 Routes for the transmission of viruses via wastewater treatment

(After Butler, 1981)
undetectable. It is also true that inapparent infections are common but may well result in a chain of infection ultimately leading to clinical cases which are remote from the original source case and material. Another difficulty, often experienced, is that the disease outbreaks may not be immediately investigated so that the source material, contaminated water or food, is no longer available. The only viral disease for which there is unequivocal evidence both from reports of transmission via recreational and drinking water or shellfish routes is infectious hepatitis (McCabe, 1978; Verber, 1972). The best documented example of this was during 1955 - 1956 in New Delhi where 30,000 - 50,000 cases were identified (Dennis, 1959; Vismanathan, 1957). The point of particular interest in this classic case history was that despite disinfection which eliminated the faecal bacteria as demonstrated by the standard coliform test, this did not apparently inactivate the hepatitis virus.

There have been other reports of waterborne disease outbreaks attributed to enteroviruses and of some interest was an outbreak of poliomyelitis associated with consumption of drinking water (Mosley, 1967) and Coxsackievirus type A and B outbreaks associated with recreational use (Denis et al., 1974; Hawley et al., 1973). However, in these instances, the waterborne route was questionable. Recently, however, there were two particularly significant reports of 'waterborne' gastroenteritis. The first clearly showed Norwalk Agent as the etiological agent from some 2,000 cases of gastroenteritis associated with consumption of oysters (Murphy et al., 1979) and the second event indicated that the same agent was responsible for some 200 cases of swimming associated gastroenteritis (Centre for Disease Control, 1979). There have also been several outbreaks of gastroenteritis associated with the consumption of shellfish in which a small round virus was consistently demonstrated in the faeces of affected individuals (Appleton, 1981). Cabelli (1981, 1982) has suggested that human rotavirus and to a lesser extent Norwalk agents are the most common agents associated with illness derived from sewage polluted waters.
THE INFLUENCE OF WASTEWATER TREATMENT ON SURVIVAL OF VIRUSES

The amount of enteric virus in raw sewage is highly variable and is, of course, largely related to the prevalence of infection in the community (Sellwood et al., 1981) and may well reflect on their socioeconomic status, the time of the day and the season. In fact, the seasonal appearance of viruses in temperate regions is well established, for instance enteroviruses peak from late summer to early autumn (Bottiger, 1973; Buras, 1974; Palfi et al., 1970) whereas rotavirus usually decline in the Summer (Banatvala, 1981; Davidson et al., 1975). But cases may continue sporadically throughout the year, for instance, Steinman (1981) detected these viruses in a European sewage collected in June and July.

The survival of viruses in raw sewage is governed by a number of physical and chemical factors. Collection from the domestic source involves great dilution of the faecal material but this is unlikely to affect the virus present. Thereafter, conditions in the sewers may be adverse if heavily contaminated with toxic industrial wastes. However, normally the buffering capacity of raw sewage, which is at approximately neutral pH, and the presence of organic and particulate matter is likely to aid the survival of viruses (Slade, 1981). Factors generally considered as likely to cause inactivation of viruses during collection and treatment of sewage include the presence of inhibitory substances, microbial predation and temperature. Ward and Ashley (1977, 1978) have shown that ammonia and certain detergents may promote inactivation of some types of virus but in most situations these are not present at toxic concentrations. Not much is known about microbial predation on viruses. Kelly et al. (1961) suggested that Klebsiella and Enterobacter usually present during the activated sludge treatment, may be possible predators. More recently, Cliver and Herman (1972) reported that Pseudomonas aeruginosa was capable of inactivating Coxsackievirus A9 by proteolysis and Bitton (1980) reported that Rhodophyta (a red alga) displayed some antiviral activity toward Coxsackievirus B5. Protozoa belonging to the ciliata (e.g., Tetrahymena pyriformis) or sarcodina (e.g., Naegleria gruberi) groups are certainly able to ingest enteroviruses and it is believed that subsequent inactivation of the viruses occur within the protozoa (Bitton, 1980). The survival of viruses in sewage is certainly related directly or
indirectly to temperature (Slade, 1978) for example, poliovirus 1 can survive for over 12 weeks in sewage at 4°C (Subrahmanyan, 1977) but at room temperature no virus was recovered from the same sewage after 14 days. Other reports, for example Lefler and Kott (1975), showed that a 99.9% reduction of poliovirus took place within 42 days at 18-25°C and 231 days at 4 - 8°C.

During treatment varying quantities of viruses are removed from the aqueous phase of sewage largely by sedimentation, but no conventional process effects total removal (Table 1.4). The bulk of residual virus is of course in the sludges (Balluz et al., 1977; Goddard et al., 1982, 1983) and these may indirectly result in later contamination of surface and underground water when disposed of to land (Slade and Edworthy, 1981).

Table 1.4 Estimated enterovirus reduction by various sewage treatment methods

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Virus removal</th>
<th>Virus remaining in effluent (pfu/litre)</th>
<th>expected (pfu/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw sewage</td>
<td>-</td>
<td>7,000</td>
<td>500,000</td>
</tr>
<tr>
<td>Primary treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedimentation</td>
<td>50</td>
<td>3,500</td>
<td>250,000</td>
</tr>
<tr>
<td>Secondary treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trickling filters</td>
<td>50</td>
<td>3,500</td>
<td>250,000</td>
</tr>
<tr>
<td>Activated sludge</td>
<td>90</td>
<td>700</td>
<td>50,000</td>
</tr>
<tr>
<td>Stabilization ponds</td>
<td>90</td>
<td>700</td>
<td>50,000</td>
</tr>
<tr>
<td>Tertiary treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(after sedimentation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excess lime precipitation</td>
<td>90-99.99</td>
<td>700 - 0.7</td>
<td>50,000-50</td>
</tr>
<tr>
<td>Alum precipitation (after lime)</td>
<td>90</td>
<td>7 - 0.07</td>
<td>5,000-5</td>
</tr>
<tr>
<td>Activated carbon adsorption</td>
<td>0 - 50</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
The processes involved during sewage treatment are described as primary, secondary and tertiary. Primary treatment is physical and involves the removal of large objects by screening and/or sedimentation. Secondary treatment refers to biological processes carried out by microorganisms and tertiary treatment is a final, sometimes referred to as an advanced stage where undesirable components are removed from sewage typically by physico-chemical processes such as coagulation, filtration, adsorption and sedimentation (Fig. 1.2). The products are essentially of two contrasting types - clear fluids or effluent on the one hand and sludges which contain 2 - 6% solids consisting essentially of material brought into the works as well as solids consisting of spent microbial population which were generated during fermentation and oxidation of organic matter during treatment.

**Primary sedimentation**

Primary sedimentation which usually comprises storage of sewage for up to 3 hours may result in the removal of viruses particularly that portion which is adsorbed to solid particles. The remaining primary effluent or settled sewage is free from large solids but still contains a high percentage of organic matter in the form of colloidal or soluble substances and detailed examination of individual stages has revealed wide differences in efficiency. Berg (1971) reported only a 0-3% removal of added poliovirus during the 3 hours sedimentation period even though more than 50% of the solids had settled but he also noted that extending the retention time to 24 hours yielded additional, 40 - 70%, virus removal. These observations confirmed those made earlier by Clarke et al. (1961) who noted in an experimental system that about 50% of suspended solids precipitated during the first 3 hours of retention in the primary sedimentation tanks without appreciable alteration in the detectable levels of virus in the settled sewage but after 24 hours 25 - 55% reduction of poliovirus from the aqueous phase was observed. It is worth noting here that according to Sproul (1973) it was not uncommon to detect increases rather than decreases in the virus levels after primary sedimentation because some of the faecal solids, which include viral aggregates, were broken up. Indeed, Feachem et al. (1981) in their review reported that the sludge removed from sedimentation tanks will normally contain a concentration of enteroviruses 10-100 times higher than the
concentration of enteroviruses found in the raw sewage. Others, particularly Rao and colleagues (1977) working in different climatic conditions have drawn somewhat different conclusions, for instance during the wet season in Bombay they demonstrated that with a 2 hour retention time there was a 24-33% removal of enteroviruses during sedimentation. At other seasons of the year the removal ranged from 41-83%. The lower virus removal during the wet season was almost certainly attributable to the continuous disturbance of the process by heavy rains (Gerba, 1981). In full scale treatment plants Kelly and Sanderson (1959) reported no significant removal of Coxsackievirus B and echovirus and later, England et al. (1967) also reported little or no (0-12%) removal of the poliovirus strains. This was confirmed by Shuval (1970) who noted that removal of poliovirus 1 was insignificant. However, Balluz et al. (1978) showed that there was a distinct difference between the behaviour of poliovirus and f2 coliphage in a laboratory scale model activated sludge sewage treatment plant which was directly related to differences in the adsorption of virus to solids in the aeration tank.

Secondary treatment

These mainly comprise

a. Trickling filters
b. Activated sludge
c. Waste stabilization ponds
d. Septic tank treatment

a. Trickling filters

In this system, the settled sewage is passed downward through a 'percolating filter' sometimes called a 'bacterial bed' which, in its usual form, consists of carefully graded stones of either coke or clinker, although more recently plastic materials have been used. The bed may be circular or rectangular in plan and is commonly 6 feet or so deep. Settled sewage is distributed over the surface from rotating arms or, in rectangular filters, by a distributor moving backwards and forwards. The bed rests on a concrete floor provided with underdrains and with ventilating tiles to provide aeration by natural draught.
The biological reaction leading to the purification of sewage is achieved by adsorption and agglomeration of wastewater nutrients onto the biological film. The oxygen is then supplied by the diffusion of oxygen into the moving stream of wastewater percolating through the filter, and this enables microbial oxidation of the nutrients to take place.

Virus removal by trickling filters or percolating filtration takes place by adsorption onto the biological slime which metabolises the organic matter but most studies suggest that they are inefficient in the removal of enteric viruses (Grabow, 1968; Malherbe and Strickland-Chomley, 1967). Kollins (1966) isolated viruses as frequently from trickling filter effluents as from the influent sewage which confirmed the earlier observation of Kelly and Sanderson (1959) who recovered viruses as frequently from filter effluents as from influent sewage, although smaller numbers of viruses were detected in the effluent. Later, Kelly et al. (1961) examined a number of sewage treatment plants and demonstrated virus in 34 of 49 effluents. However, with bench scale, rotary tube trickling filters, at medium filtration rates (equivalent to 10mgd) through the plants, poliovirus 1, echovirus 12 and Coxsackievirus A9 were all markedly reduced (85, 83 and 94% respectively; Clarke and Chang, 1975). A higher flow rate (23mgd) proved less efficient and on average reduced virus removals by 19%. Not all viruses may be removed with similar efficiencies, for instance on the one hand Gilerease and Kelly (1954) reported a 60% reduction of Coxsackievirus A after filtration whereas Sherman et al. (1975) reported as little as 9-12% of seeded coliphage f2 was removed. Sellwood and Dadswell (1981), in a qualitative study, found that total virus content of the effluent was apparently 50% less than at the inlet, but polioviruses were reduced by a further 25%. One of the reasons for the inefficiency of filtration may simply be that it does not permit sufficiently good contact between the viruses and the organic/biological layers for adsorption and consequent inactivation to occur. Even when viruses are adsorbed they may be replaced by other substances and eventually leach out.

b. **Activated sludge**

In this process, a large mass of bacteria is uniformly dispersed
in suspension in settled sewage, the mixture being maintained under aerobic conditions for a period of several hours - often 4 to 8 in Great Britain. The process by which wastewater nutrients are removed is similar to that of trickling filtration with the only difference being that the biological active unit is in the form of a flocculent suspension kept in an aerated and agitated system. The flocs are biologically active because they consist of different bacterial and protozoal populations which are held together within a matrix of inert and inorganic material. Kelly and collaborators (1961) demonstrated that the removal of viruses by this procedure consists of at least 2 steps: (1) aeration in the presence of sludge floc and nutrients and (2) settling floc.

Activated sludge treatment has been studied extensively and appears to be a relatively efficient way of removing enteric viruses from the liquid phase. In fact quite high levels of removal have been reported, for instance, at least 90% removal of enteroviruses has been noted in both laboratory and field conditions (Balluz et al., 1977; Clarke, et al., 1961; Heikal et al., 1981; Kelly et al., 1961; Roa et al., 1977. Gerba (1981), furthermore, showed that each enterovirus had a characteristic removal rate in the activated sludge. There is also some evidence for inactivation of virus but the mechanism was not clear (Balluz, 1977; Grabow, 1982).

A recent modification of the activated sludge process is one in which the aeration basin is replaced by a deep shaft. The effluent fed to the deep shaft circulates through the shaft and rapidly mixes with the entire mass of activated sludge in the system. The circulation of the liquid in the shaft is brought about by injection of air mainly or wholly into the downcomer. This air also serves to supply the microorganisms with the oxygen they need. This has shown to be a more economical way of treating wastewater and has been found to possess advantages over normal treatment in that it does not require primary treatment (Hines et al., 1975). The behaviour of viruses within this system has yet to be reported but there is no reason to believe that it will differ greatly from traditional activated sludge treatment.
c. **Waste stabilization ponds**

The treatment process here depends on the action of numerous microscopic and macroscopic forms of plant and animal life. This varies in number and kind with season and with the nature of fluid entering the pond. Hence, one would expect from this that there would be a wide variation in the efficiency of the removal of virus from ponds in different geographic areas serving different communities. Malherbe and Coetzee (1965) investigated the survival of attenuated poliovirus seeded every 20 days into a model system of four stabilization ponds with a theoretical retention time of 38 days. They concluded that biological processes which resulted in effluent quality which was improved chemically and biologically, did not necessarily have a significant effect on the virus content. Malherbe and Strickland-Cholmley (1967) showed some reduction in enterovirus and reovirus titres from a series of oxidation ponds although removals varied widely partly due to poor pond design, poor experimental procedures and short-circuiting of sewage flow across the ponds. Shuval (1970) reported virus reductions ranging from 0-96% in ponds with a 20-day retention time and Nupen et al. (1974) showed greater removal of virus in the summer, when algal growth was abundant and the temperatures were higher. Although reductions as high as 99.9% were achieved, viruses were still routinely detected in all the effluents. Rao et al. (1978) reported that virus removal ranged from 68.1% in the winter to 98.7% during the summer in waste stabilization ponds in India. This could be caused at least in part by blooms of algae leading to an increase in alkalinity to pH9 or above. The same authors (1981) indicated that experimental pilot stabilization ponds operated at depths of 3-5ft at different loading rates were efficient in removing viruses in the range of 86-95% while full scale ponds with retention times ranging from 2.7 to 17.2 day showed virus removal efficiencies ranging from 88-98% so it is apparent that well designed ponds can remove a very high proportion of viruses.

The overall conclusion must be that no single secondary treatment process or combination of procedures is totally effective in removing viruses, thus, the application of tertiary or advanced physico-chemical process is of great importance.
d. **Septic tank treatment**

A septic tank is simply a settling chamber, or chambers, with a mean retention time of about 3 days or less. These tanks serve small populations (5 - 200 people) and the virus concentrations may change dramatically but very little is known about them. Feacham et al. (1981) reported that viruses in such tanks are thought to be removed both by inactivation in the anaerobic liquor and by adsorption to solids which settle to the sludge layer. In one report (EPA, 1978) a 99% reduction of poliovirus was observed in septic tank effluent in 14 day at 20°C or 43 day at 7°C. One might suspect that the sludge which results from those septic tanks would be very rich in enteroviruses and would require treatment by digestion, drying or composting. In those developing countries, where septic tanks are common, warm temperatures may enhance the inactivation of enterovirus, both in septic tanks and in the drainfields.

**Conventional tertiary treatment**

Tertiary treatment is an advanced stage which is designed to polish effluents thereby rendering them safe and aesthetically acceptable. It usually involves some type of chemical or physical or chemical and physical treatment such as the addition of a coagulant or polyelectrolyte precipitation, filtration or the passage through activated carbon or resins to remove residual contaminants (Gerba, 1981). An analysis of the various different treatment processes for removing of virus from biological effluents is shown in Table (1.5).
Table 1.5 Enterovirus removal during tertiary treatment of municipal effluent

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>% virus removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical coagulation</td>
<td></td>
</tr>
<tr>
<td>Alum and ferric chloride</td>
<td>40-99</td>
</tr>
<tr>
<td>Polyelectrolytes</td>
<td>2-99.99</td>
</tr>
<tr>
<td>Lime (calcium hydroxide)</td>
<td>90-99.999</td>
</tr>
<tr>
<td>Sand filtration</td>
<td>10-90</td>
</tr>
<tr>
<td>Carbon adsorption</td>
<td>0-50</td>
</tr>
</tbody>
</table>

After Gerba, 1981

a. Coagulation

Coagulation is generally very effective for the removal of viruses and in a laboratory study by Bruner and Sproul (1970) removals of up to 98% of poliovirus type 1 could be obtained by precipitation with alum or calcium hydroxide. Berg (1971) reported that coagulating with lime can remove 99-99.9% of poliovirus and Sattar et al (1976), in laboratory studies, showed that 99.9% or more of added poliovirus could be removed from sewage by lime treatment at pH 11.5 which was doubtless due primarily to denaturing by high alkalinity rather than the act of precipitation. The removal of viruses can be enhanced by low or high pH and by the presence of cations (Jenkins et al., 1980) and very much improved by coagulation before filtration (Berg, 1971). Wolf et al. (1974) reported at least 99.6% removal of seeded coliphage f2 and poliovirus in a laboratory model coagulation-sedimentation system employing alum. Feachem et al. (1981) recommends that for maximum efficiency coagulation should be followed by sand filtration but in general, it must be assumed that virus is removed with the precipitate and not inactivated.

b. Sand filtration

A high proportion of viruses can be removed from secondary effluents by sand filtration alone provided appropriate conditions are
used, such as the grade of sand, the rate of flow and the type of coagulant previously applied (Feachem et al., 1981). It is important to note that clean sand alone does not adsorb viruses efficiently and it must be enriched with a complex microbial population depending on the organic and other substances present in effluent. Sproul (1973) reported that filtration of treated effluents through sand filters will remove all viruses from the effluent provided that the bed is sufficiently deep. Safferman and Morris (1976) achieved only 0-48% removal of coliphage by dual and multimedia filters without precoagulation and in contrast Berg (1971) reported 82-99.8% removal in a filter operating at 2.25gpm/sq ft on lime-flocced effluent. Slade (1978) found that at filtration rates between 1.12 and 4.15 m/day and temperatures of 6-11°C virus removal varied from 97 to more than 99.8%. Partial removal of poliovirus from water by 10-50ppm activated carbon has been reported by Grabow (1968).

**DISINFECTION**

For water and wastewater disinfection, the available methods fall into 2 major groups:

a) Physical agents such as ionizing irradiation, non-ionizing irradiation and photodynamic oxidation and

b) Chemical agents in particular the oxidizing agents. Chemicals falling into this category are a. halogen, b. halogen compounds, c. ozone and d. peracetic acid.

**Physical agents**

a. **Ionizing irradiation** A good source is either cobalt 60 or caesium 137 which produce gamma irradiation. Alternatively, high energy electrons may be produced by electron accelerators as a source of irradiation (Grabow, 1979). When gamma irradiation interacts with materials, high energy electrons are produced at particular sites which disrupt essential electronic structures, they are most efficiently deployed when living cells are packed together, as in the tissue of higher forms of life, for instance for destruction of cancer cells (White, 1978). In the case of small scattered organisms, for instance viruses in sewage, some of the radiation energy will be wasted in the...
support medium. However, the ionizing rays may have an indirect effect, via production of active radicals in the support medium.

Gamma-rays are the most commonly used and a good microbial kill was observed when a secondary effluent had a total irradiation dosage of 50,000 rads (Environment Canada (EC), 1978) but Vajdic (1975) reported that at this dosage some bacteriophage was still detectable in the reactor effluent. Lessel and Suess (1978) used gamma-irradiation for the inactivation of poliovirus and with 300 Krads produced 90% inactivation but for very high reduction of various enteric viruses (poliovirus, Coxsackievirus B3, echovirus 6, reovirus 1 and adeno virus 5) no less than 1000 Krads were required (White, 1978). An important limiting factor in this treatment is restricted penetration in turbid effluents, also the safety aspects associated with the handling of radioactive material.

b. Non-ionizing irradiation

The most usual source is ultra-violet and its germicidal properties were recognized for many years before the first attempt was made to use it as a disinfectant. It was as early as 1878 that Down and Blunt observed the effect of sunlight on a mixture of microorganisms and concluded that light of short wave length was responsible for the destruction of these organisms (Jepson, 1968, 1973). However, the use of ultraviolet light for the inactivation of viruses in wastewater has not been studied extensively and according to White (1978) the virucidal effect of ultraviolet light is thought to be associated with adsorption by the nucleic acid and a progressive lethal biochemical change occurs by excitation, disruption of unsaturated bonds, particularly of the purine and pyrimidine components of the nucleo-proteins.

Hughes et al. (1979) clearly demonstrated in their study on the kinetics of inactivation of rhinoviruses by ultraviolet inactivation, that the nucleic acid was the major site affected. Vajdic (1969) used a bacteriophage of E. coli throughout a series of investigations and found that levels up to $10^4$ per ml could be inactivated quite satisfactorily in a test unit utilizing a 36 watt lamp and a 25.4mm (1in) radiation distance provided retention times were of the order of
Ultraviolet radiation has a limited penetration efficiency which means that effective treatment is restricted to waters with low turbidities and present as a thin film (Grabow, 1979; Oliver and Carey, 1976) but under carefully controlled conditions has great promise (Johnson et al., 1982).

Another disadvantage, in certain circumstances, is the absence of disinfecting residuals of any kind remaining in the treated water to protect supplies from any subsequent contamination (Vajdic, 1970). Also it has no value for eliminating taste or colour problems but once set up the equipment needs little attention other than cleaning.

One advantage of ultraviolet irradiation is that it may enhance subsequent chemical disinfection (Akkad, 1980) and it is worth noting that its own activity may be enhanced by interaction with other factors such as ultrasonication (Oliver and Carey, 1976) or interaction with H$_2$O$_2$ (Bayliss and Waites, 1980). Mystrad (1979) and Severin (1980) recommended that ultraviolet disinfection of secondary effluent should be considered as an alternative to chlorination.

c. Photodynamic oxidation

This method of disinfection involves the addition of a suitable photosensitizing chemical such as methylene blue, neutral red, to water or effluent (Bitton, 1980). This is followed by irradiating the sensitized virus by monochromatic light or ordinary sunlight (Grabow, 1979; Marzouk, 1982). It is believed that the dye penetrates the virus and attaches itself to nucleic acids and when activated by light induces an oxidation reaction which disrupts the substrate (Acher and Juven, 1977; Gerba et al., 1977; Hobbs et al., 1977). The photodynamic action on microorganisms is dependent on dye concentration, pH, temperature, sensitization time in the dark, and the turbidity of the sewage (Grabow, 1979). Bitton (1980) reported that nearly $6 \log_{10}$ reduction in poliovirus titre is achieved at 25°C in sand filtered sewage, pH 10, treated with 5 mg/l methylene blue for a period of 16 hour in the dark. But its practicability is strictly limited (Marzouk, 1982) due to difficulties in the design of the plant.

To provide adequate mixing and exposure to light...
Chemical agents

a. Halogens

The choice of chemical disinfectant is limited by the requirement for a high quality final effluent free from toxic by-products and the disinfectant must be cheap, easy to produce, transport and store. It must be potent at low dosage and readily decompose, either spontaneously or by the application of a neutralising agent into harmless by-products. Furthermore, it must be simply and reliably assayed and unreactive with other chemical and physical constituents of the effluent. In this regard, the characterisation of effluent is an important consideration because wide variations in physical and chemical quality are known to occur and are likely to influence the effectiveness of disinfection (Tonelli, 1976). To achieve efficient disinfection the following criteria must apply:

1. All organisms must be equally exposed.
2. Systems must be plug-flow or batch operated for the entire contact period.
3. The water treated must not contain any material likely to neutralize the disinfectant or its effect on the pathogens.
4. No cross contamination between treated and untreated water.

It may also be necessary to pre-treat effluents by such methods as sand filtration (Berg, 1973a) or chemical flocculation (Gerba, 1981) before the application of disinfectants.

The most commonly used oxidizing agents are the halogens, particularly chlorine, bromine and iodine, of which the most important is chlorine. Indeed, for many years the word "disinfection" has been associated with chlorine which has been very widely used for the disinfection of potable water. It has also been commonly used for the treatment of swimming pools as well as for the disinfection of wastewater effluents.

Chlorine was discovered by Scheele, a Swedish chemist, in 1774 and it was first used in the 19th century as a disinfectant by Moreveau to provide adequate mixing and exposure to light.
In order to understand how chlorine and other halogens act as disinfectants, their chemical properties must be examined. There are in fact exhaustive reports. (Fair et al., 1947; Palin, 1950a; White 1972, 1978) and what is important to note is that when a halogen is added to pure water two reactions result that is the formation of the hypohalous acid which ionizes to the hypohalite ion. The following formulae summarise the essential features (X denotes the halogen):

\[ \text{X}_2 + \text{H}_2\text{O} \rightarrow \text{HOX} + \text{H}^+ + \text{X}^- \]

\[ \text{HOX} \rightarrow \text{H}^+ + \text{OX}^- \]

A well defined equilibrium is established depending on the pH, but problems arise in the presence of organic components the commonest of which is ammonia and the reactions are represented thus:

\[ \text{HOX} + \text{NH}_3 \rightarrow \text{NH}_2\text{X} + \text{H}_2\text{O} \text{ (monohaloamine)} \]
\[ \text{HOX} + \text{NH}_2\text{X} \rightarrow \text{NHX}_2 + \text{H}_2\text{O} \text{ (dihaloamine)} \]
\[ \text{HOX} + \text{NHX}_2 \rightarrow \text{NX}_3 + \text{H}_2\text{O} \text{ (trihaloamine)} \]

Increased acidity and increased Cl:NH3 ratio favour the highly substituted derivatives of ammonia (Palin, 1950b). The mono and dihalomines decompose to release nitrogen and those formed with chlorine are the most stable:

\[ 2\text{NH}_2\text{X} + \text{HOX} \rightarrow \text{N}_2 + 3\text{HX} + \text{H}_2\text{O} \]
\[ 2\text{NHX}_2 \rightarrow \text{N}_2 + 2\text{HX} + \text{X}_2 \]

In the case of chlorine, the hypochlorous acid is a "weak" acid which means that it tends to undergo partial dissociation to produce a hydrogen ion and a hypochlorite ion. The relative amounts of HOC1 and OCl- depend on the activity, ionization constant and temperature and the extent of this reaction can be calculated from the equation:

\[ \text{K}_{\text{ion}} = \frac{(\text{H}^+)(\text{OCl}^-)}{(\text{HOC1})} \]
For instance, the $K_{\text{ion}}$ for chlorine in water is $2.7 \times 10^8$ moles/l at 25°C. At pH 5.0 chlorine is almost entirely present as hypochlorous acid and at pH 10 as hypochlorite, and hypochlorous acid starts to ionize into hypochlorite at pH 6 and above 9 only hypochlorite ion is present (Fig 1.3).

Available chlorine will also react with such compounds in sewage as sulphides, manganous and ferrous salts, ammonia, cyanides, phenols and other aromatic compounds and humic acids so reducing or neutralizing the disinfection action. Chlorine demand is the discrepancy between the concentrations of added chlorine and the measurable residual chlorine. The chlorine demand varies with respect to the quality of water and contact time. As it was pointed out in the reactions of hypohalous acid with ammonia, chloramines are produced and these have reduced powers of disinfection. The point at which the chlorine demand is satisfied and when the concentration of free available chlorine increases proportionally with the dose of chlorine is termed the "break point" and Fig. 1.4 illustrates the reactions that occur during breakpoint chlorination. Bromine also displays a breakpoint curve similar to that of chlorine, and it is the decomposition of the dibromamine which is the basis for this reaction. Tribromamine is the major species of combined residual bromine present beyond the breakpoint. White (1978) has shown that the breakpoint curves occur when the bromine to ammonia ratio is 1.5. For wastewater disinfection, the predominant species of bromine compounds is dibromamine over a pH range of 7-8.5. Dibromamine has a germicidal efficiency almost equal to free chlorine. For this to be formed there must be an excess ammonia nitrogen present for this to occur which is usually the case in all highly nitrified effluents. Free bromine residuals (HOBr), which would occur in highly nitrified effluents do not decompose nearly as rapidly as bromamines. Their persistence increases with decrease in halogen demand in the environment. According to White (1978), the literature reveals a considerable lack of agreement on the germicidal efficiency of bromine compounds, which is due to the difficulty in sorting out which species of bromine is being investigated. It is worth noting that dibromamine has a comparable germicidal efficiency to hypobromious acid (free bromine) but not as germicidal as molecular bromine ($\text{Br}_2$) which only exists
Fig 1.3 Relative amounts of HOC1 and OCl⁻ present in water at various pH values (after Fair et al., 1947)
below pH7. Taylor and Johnson (1974) reported that at 0.1μM concentration, the effectiveness of bromine compared with chlorine ranked Br₂>HOCl>HOBr>HBr and they demonstrated that five log₁₀ of inactivation of the DNA phage φX174 was achieved at pH4.5 and 0°C using 0.4mg/l bromine. In the presence of ammonia, bromine appears to be a better virucide than chlorine. Sollo et al., (1975) noted that bromine was more effective at high pH than chlorine. Olivieri et al., (1975) found that bromine inactivated the naked RNA but how bromine penetrated the protein coat was not understood.

The virucidal effects of iodine (I₂) were reported by White (1978) who found that 10.2 mg/l (at pH 5.0 and 5°C) were needed to inactivate 99% of f₂. Unlike chlorine, the rate of inactivation was enhanced at pH9 but the higher resistance of enteroviruses than enterobacteria to iodine has been effectively demonstrated in by Berg et al., (1964) who found that 1 mg/l of iodine destroyed 99% of E. coli in about 1 min but similar inactivation of Coxsackievirus A9 required over 3 hour. They also found that hypoiodous acid (HOI) was much more virucidal form than I₂.

b. Chlorine dioxide

Chlorine dioxide in aqueous solution produces chlorous and chloric acids

\[ 2\text{ClO}_2 + \text{H}_2\text{O} \rightarrow \text{HClO}_2 + \text{HClO}_3 \]

The germicidal activities of chlorine dioxide (ClO₂) were first recognized by Ridenour and Ingols (1947). However, early work on chlorine dioxide lacked adequate analytical techniques to differentiate between ClO₂ and the various chlorine residuals available. For this reason, the early reported results are not easy to interpret. Ridenour and Ingols (1947) found that ClO₂ was at least as effective against bacteria as chlorine, and its efficiency was not affected by pH values between 6 and 10. The same authors (1946) also found that chlorine, chlorine dioxide or a mixture of the two, were efficient against poliovirus provided that free chlorine or chlorine dioxide was present. Ridenour and Armbruster (1949) reported that if chlorine dioxide was applied in an amount to give a residual of not less than 0.1ppm, it
destroyed pathogens at temperatures between 5°C and 20°C, and at pH values above 7, within a five min contact period.

Tift et al. (1977) in their study on the disinfection potential of chlorine dioxide relative to chlorine for combined wastewater flow found that four and five \( \log_{10} \) reductions of exogenous poliovirus 1 were observed at chlorine dioxide doses of 12 and 16 mg/l respectively, over a 120 sec contact time. Chlorine dioxide at 12 mg/l resulted in approximately the same bacterial and viral kills as chlorine at 25 mg/l in 120 sec contact time in the system. The inactivation efficiency of chlorine dioxide was improved by the sequential addition of both disinfectants. Walters (1976) also found that chlorine dioxide had bactericidal and virucidal properties superior to those of chlorine. Bacterial and viral inactivation of four \( \log_{10} \) reduction units at approximately 30 sec contact time were achieved when chlorine dioxide was introduced at concentrations of 5.0 and 7.5 mg/l into a secondary wastewater effluent seeded with \( E. \ coli \) and bacteriophage f\(_2\). Longley et al. (1980) reported that a high degree of inactivation of f\(_2\) coliphage was obtained using chlorine dioxide doses of 5.0 mg/l. In contrast, chlorine used at the same concentration, produced a much lower level of inactivation of this virus.

c. Ozone

In water and wastewater treatment ozone came into use basically to remove colour and to overcome odours by oxidizing the materials which caused these. However some, although relatively little, work has been reported on the inactivation of viruses in effluents and most of these papers have unequivocally shown that ozone is an efficient and powerful disinfectant. In the earliest studies Hettche and Schulz-Ehlbeck (1953) indicated that ozone was slightly more virucidal than free chlorine, for instance 0.05 to 0.45 mg/l of ozone destroyed the same quantity of poliovirus in 2 min as did 0.5 to 1.0 mg/l of residual gaseous chlorine in 1.5 to 3 hour.

Coin et al. (1964) observed that inactivation of poliovirus 1 in potable water was incomplete unless a residual greater than 0.3 mg/l was obtained and it was noted by Diaper (1968) that an ozone residual
of 0.1 - 0.2ppm was adequate for inactivation of all enteric viruses in 5 min in drinking water supply. Later, Perlman (1969) reported that a residual ozone concentration of 0.7 mg/l resulted in a virus reduction to a level of 0.01% in 4 min. Furthermore, 0.7 mg/l of free ozone was found to be a threshold value below which inactivation was not satisfactory and above which more than 99.9% virus inactivation was achieved. Evison (1972 & 1978) reported that the presence of peptone affected ozonation and that 5 log₁₀ inactivation of the bacteriophage required 4 times more ozone (0.2 mg/l) in domestic sewage than in sterile river water (0.052). Majumdar et al. (1973, 1974) and Sproul & Majumdar (1975) studied the ozonation of poliovirus 1 in triple distilled water, secondary effluents and primary effluents and demonstrated that the threshold value for inactivation of poliovirus was 1mg/l. They derived from their work equations to describe two different reaction rates relating to ozone dosages of >1mg/l and <1mg/l., respectively which possibly reflected on the existence of clumping of the organisms. Katzenelson et al. (1974) reported a two-stage inactivation curve for poliovirus 1 and other organisms by ozone and the study was extended (1976) to disinfection of poliovirus in sewage using batch and continuous experimental procedures. In the batch studies, when filtered sewage seeded with poliovirus 1 was added to a buffered ozone mixture, complete disappearance of ozone occurred with concurrent decrease in the virus titre. However, in continuous experiments, it was found that there was virus inactivation even before an ozone residual appeared in the treated effluent and virus inactivation could be detected 30 sec after bubbling had started.

In pilot plant studies on sewage effluent Pavoni et al. (1972) found that f₂ virus was completely inactivated after a contact time of 5 min at a total ozone dosage of approximately 15ppm and a residual of 0.015ppm. Bollyky and Seigel (1977) reported that holding secondary effluent for up to 10min subsequent to ozone contact improved disinfection, the product responsible for this was thought to be an ozonide or peroxide reaction rather than ozone residual.

Farooq et al. in a series of publications (1977a,b; 1978) noted that application of bubbling ozone enhanced inactivation, and that an increase in temperature led to an increase in the inactivation efficiency and that ozone was more stable and more efficient at a lower
pH because higher ozone residuals resulted. Burleson et al. (1975) and Dahi and colleagues (1976, 1977, 1979, 1980) also studied the effect of sonication on ozonation and they concluded that the increase in the inactivation efficiency was due to the fact that ultrasound increased the gas-liquid ozone transfer, and decomposed the dissolved ozone. It also disaggregates the viable microbial units.

Ozone is known to be an unstable gas that decomposes slowly in the gaseous phase to ordinary oxygen; however, the mechanism and kinetics of its dissociation in aqueous solution is complex (Peleg, 1976).

\[
\begin{align*}
O_3 + H_2O &\rightarrow O_2 + 2OH^- \\
O_3 + OH &\rightarrow O_2 + HO_2 \\
O_3 + HO_2 &\rightarrow 2O_2 + OH \\
OH + OH &\rightarrow \frac{O_2 + H_2O}{2} \\
OH + HO_2 &\rightarrow H_2O + O_2 \\
OH + OH^- &\rightarrow O^- + H_2O \\
O^- + O_2 &\rightarrow O_3^- \\
HO_2 + HO_2 &\rightarrow H_2O_2 + O_2
\end{align*}
\]

Recently, Hoigne and Bader (1976, 1979) noted the formation of the hydroxyl radical upon ozonization of organic matter at high pH and that this radical reacted primarily with any organic solute when these are present in clean water systems. The hydroxyl radical may well be available to inactivate viruses, once organic substances are present they will preferentially react with any slightly reactive species (such as OH radical) produced by the ozone on decomposition in water. Further studies need to be initiated on the direct effect of the dissociation species of ozone and disinfection such as OH, HO_2, O_3, O_2 and O^-.

d. **Peracetic acid (PAA)**

Peracetic acid (PAA) in water hydrolyzes to give acetic acid and nascent oxygen.
Kline and Hull (1960) were the first to study the virucidal efficiency of PAA and they found that a substantial inactivation of poliovirus was achieved at 0.04% aqueous solution of PAA in 5 min time. Sprossig and Mucke (1969) noted that solutions of PAA in alcohols appeared to be more effective against poliovirus 1 and Coxsackievirus B2 than aqueous solutions. More recently, Hajenian and Butler (1980), found that coliphage and poliovirus reacted differently to PAA. Levels up to 0.032% PAA were needed to achieve 99.43% inactivation of poliovirus 1 and only 0.013% PAA for the same degree of inactivation of f² coliphage.

Combination of various disinfectants

The combination of drugs in order to obtain enhanced effects is a well established practice (Dahi, 1976) but the combination of various disinfectants has not generally been tried or even recognized as potentially valuable. However, there are some reports on the synergistic effects of various treatment process. For instance, the synergistic effect of sonication and ozonation has been reported (Burleson et al., 1975; Dahi, 1976; Dahi and Lund, 1980; Kazenelson et al., 1974) and Oliver and Carey (1976) found that ultraviolet disinfection was improved by sonication.

Another example of synergism is the use of two chemical disinfectants together, for instance, it was as early as 1946 that Ridenour and Ingols reported on the synergistic effects between chlorine and chlorine dioxide. They found that chlorine dioxide was not as efficient a bactericide as a mixture of ClO₂ and Cl₂, in ratio of 3:1. More recently, Tift et al. (1977) demonstrated that the sequential addition of chlorine and chlorine dioxide improved disinfection and Sprossig and Mucke (1965) showed that the virucidal efficiency of peracetic acid was enhanced in the presence of alcohol.
The advantages of such treatment or equivalent procedures with chlorine and ozone (Ross et al., 1976; Wyatt and Wilson, 1979) is mainly in the production of a better quality water free from irritating or toxic residuals and Kott et al. (1980) found that the simultaneous application of ozone and chlorine did not produce a true synergistic effect.

**Mechanisms of action of disinfectants**

Most enteric viruses consist essentially of a protein coat around a nucleic acid core and a disinfectant may affect either of the essential features (Bitton, 1980). Generally very little is known about the mechanism of inactivation even with such commonly used disinfectants as chlorine and other halogens. It is known that chlorine causes physical, chemical and biochemical changes in the cell membrane of bacteria (Venkobachar et al., 1975; 1977) and Kruse et al. (1971) suggested that the -SH groups on the protein coat of virus were oxidized by halogens, thus denaturing the protein and preventing viral infectivity. O'Brien and Newman (1979) reported that chlorine inactivation of poliovirus resulted in the loss of RNA converting the virus from 156S particles to 80S particles. However, it was found that virus inactivation occurred before RNA was released from the virions. Alvarez and O'Brien (1982) also reported that chlorination of poliovirus led to the release of RNA from the capsids of chlorinated virus and Dennis et al. (1979) found that chlorine inactivates f₂ phage by acting on its RNA. However, Tenno et al. (1980) concluded that HOCL inactivates poliovirus by acting on the protein component of the virus and that the inactivating reaction does not result in any detectable change in the structure of the virus nor does it affect the infectivity of the viral RNA. Degradation of the protein structure of the virus occurs only after prolonged contact and presumably multiple reactions of HOCL with the protein coat.

The mechanism of inactivation of poliovirus 1 and f₂ coliphage by iodine has been investigated by Hsu (1964) who found that the RNA of both viruses was unaffected by iodine concentrations as high as 200 mg/l. In addition, f₂ was found to be more resistant to the sulphydryl blocking agent, p-chloromercuri-benzoate (PCMB) than were poliovirus 1 and f₂. Furthermore, Li et al. (1942, 1944) showed that
tyrosine and histidine were iodinated and the rate of iodination of tyrosine was 100 times faster than histidine and could be virtually instantaneous under alkaline conditions and Hsu et al. (1966) concluded that the most probable mechanism of $f_2$ inactivation was by iodination of tyrosine.

Ingols and Ridenour (1948) provided an explanation of the mechanism of inactivation of virus by chlorine dioxide. They found that the interaction between chlorine dioxide and peptone, chosen to represent a large colloidal molecule, had a quantitative relationship and they suggested the disinfectant adsorbed to the peptone. If this was the case then its action against viruses could be directly on the protein coat. The adsorption of the disinfectant would cause higher local concentrations on the surface of the virus than would be expected from the measured residual and could account for the claimed effectiveness of the virucidal effect of chlorine dioxide.

As far as the mechanism of inactivation of viruses by bromine chloride (BrCl) is concerned, Keswick et al. (1981) concluded that BrCl as HOBr or bromines inactivate poliovirus by reacting with the protein coat of the virus. Moreover, this inactivating reaction did not result in the degradation of the structure of the virion, nor the biological activity of the RNA.

Kim et al. (1980) investigated the mechanism of inactivation of bacteriophage $f_2$ by ozone and found that the protein capsid of $f_2$ phage coat was disrupted into many subunits. Furthermore, the specific adsorption of the phage to host pili was inversely related to the extent of phage breakage liberating RNA and disrupting adsorption to the host pili and that the RNA may be secondarily sheared by a reduction with or without the coat protein molecules, which have been modified by ozonation. Roy et al. (1981) observed that ozone altered two of the four polypeptide chains present in the viral protein coat of poliovirus 1. However, this did not significantly impair virus adsorption on the host cells or alter the integrity of the virus particle. Damage to the viral RNA after exposure to ozone was demonstrated by velocity sedimentation analysis. It was concluded that the damage to the viral nucleic acid was the major cause of poliovirus inactivation by ozone.
Toxic by-products that result from the use of various disinfectants

Chlorine has been accepted as one of the most efficient and economic disinfectants of water supplies. However, the discoveries of Rook (1974) and Bellar et al. (1974) that chlorination of water supplies containing organic matter produced chloroform and other volatile organohalogens has challenged the concept that chlorine is the ideal disinfectant and much concern has recently been devoted to the toxic by-products that result from chlorination. Bellar et al. (1974) detected chloroform and other trihalogenated methanes in several municipal water supplies and Symons and Henderson (1977) noted that chloroform and other trihalomethanes (THM) were known to be widespread in drinking water. Environment Canada (1978) reported that chlorinated aromatic hydrocarbons were readily formed during aqueous chlorination at room temperature and the extent of reaction was shown to depend on the pH of the solution and on the concentrations of both aromatic hydrocarbon and the active chlorine.

As far as ozone is concerned, it was reported in Environment Canada (1978) that ozone reacts with a variety of organic compounds present in water. For instance, glyoxal and methylglyoxal are formed as ozonolysis products of 2-butanol, benzene and methylated derivatives and Elia et al. (1978) indicated that ozonation yielded acetone, acetaldehyde and acetic acid as volatile oxidation products. Lawrence et al. (1980) showed that ozonation of aqueous solutions of fulvic acid, under simulated water treatment conditions, resulted in the formation of various oxidation products which included numerous alkyl phalates, mono and dicarbolic aliphatic acid and a few cyclic keto-compounds all of which are toxic. Leitzke et al. (1980), however, did not detect any chloroform after ozone treatment of chlorinated hydrocarbons in the main river water. Leinhard and Sontheimer (1979) reported that the intermittent addition of ozone is significantly more effective in the removal of humic acid, and that this will produce more polar and small molecule oxidation products with lower trihalomethane formation potential and therefore less toxicity.

Chlorine dioxide is usually reduced during water treatment to the chlorite ion (Dowling, 1974) which may be toxic. Stevens et al.
(1976) reviewed the reactions of chlorine dioxide with organic matter and concluded that the predominant products formed were aldehydes, carboxylic acid, ketones and quinones. Trihalomethane was not formed and Hubbs et al. (1981) found, interestingly, that chlorine dioxide was effective against trihalomethane formation.

**Aims of the Project**

The relevant literature indicates a considerable degree of ambiguity and contradiction in the efficiency of various disinfectants which is largely the result of individuals not standardising their studies with those of others and working largely on single disinfectants and viruses. It thus seemed appropriate to attempt to resolve some aspects of this ambiguity by doing a comparative study of several commonly used disinfectants against representative enteric viruses. The chosen disinfectants were chlorine, chlorine dioxide, ozone and peracetic acid because they represented those most likely to be useful in wastewater. The selected viruses were bacteriophage f₂, echovirus 1, poliovirus 1, Coxsackievirus B5, simian rotavirus (SA 11) and human rotavirus and the choice was determined by several factors.

Bacteriophages have frequently been used as indicators of enteric and other virus behaviour but doubts about the validity of this have often been expressed. It is certainly notable that some phages meet many of the requirements of an indicator system as they are frequently present in higher numbers than animal viruses in wastewater (Kott et al., 1974) and indeed may be present when there are no detectable enteroviruses (Safferman and Morris, 1976; Vaughn and Metcalf, 1975) and some have been shown to be more resistant to chlorination processes (Berg et al., 1978; Hajenian and Butler, 1980).

Because of the potential of enteroviruses to survive in the aquatic environment, Cliver (1971) suggested that enteroviruses may be themselves the most valid model agents for indication of viral pollution and several investigators have examined the possibility of using polioviruses as indicators for enteric viral pollution, but conflicting findings were reported. Payment et al. (1979) concluded that vaccine strains of polioviruses may be a good virological indicator of faecal pollution in an area where very few other
Enteroviruses are circulating in the population and regular vaccination is carried out. However, the unsuitability of poliovirus as indicator of viral pollution was noted by Katzenelson and Kedim (1979) who did not often detect poliovirus in samples shown to contain high numbers of other enteric viruses. These observations focused attention on the need in this project to investigate the behaviour of at least two other enterovirus representatives and the easily cultivable echovirus 1 and Coxsackievirus B5 were selected.

It was also thought highly desirable to select as many relevant representative enteric viruses as practicable which meant the inclusion of two members of the rotovirus group. In this instance the simian strain SA 11 was used because it has been taken as representative of human rotovirus (Walter, 1982) and a naturally occurring or indigenous strain of human rotovirus was introduced because it had become feasible to assay its infectivity by indirect immunofluorescence (Banatvala et al., 1975).
**MATERIALS AND METHODS**

**Propagation and assay of viruses**

a. **Enteroviruses**

Poliovirus type 1 LSc 2ab (Sabin attenuated vaccine virus), Coxsackievirus B5 and echovirus 1 (provided by Dr. Slade, Thames Water Authority) were grown by inoculating 3 day old drained and washed monolayer cultures of buffalo green monkey cell line (BGM) in 8oz bottles with 0.5ml virus sample. Maintenance medium (Appendix Ia) was then added and the cultures were incubated at 37°C until the development of extensive cytopathic effect. Virus was harvested after successive freeze thawing (-20°C + 20°C, 3 times) of the culture and the product was clarified by centrifugation (4000 g, 10 min, 20°C). The clear supernatent was distributed aseptically into sterile bijoux bottles and stored at -20°C.

Viral infectivity was assayed by the microtitre method (Grist et al., 1978). A three day old monolayer of BGM cells was washed with phosphate buffer saline (PBS; Appendix IIa), cells were removed by trypsinization (Appendix IIb) and were resuspended in growth medium (Appendix Ia) containing twice the normal concentration of serum. Aliquots (0.75 µl) of cells were distributed into the wells of microtitre plates with a micropipette. Serial tenfold dilutions of virus sample were prepared in diluent (Appendix Ia) and 0.75 µl of the appropriate dilutions were added to the wells containing the cells. A minimum of 5 wells were inoculated for each dilution, the plates were then sealed with sellotape and incubated at 37°C. They were examined for cytopathic effect (cpe) at 48hour and thereafter until no more cpe developed. The infectivity was determined by the Karber equation to provide the 50% tissue culture infective dose (TCID 50/ml).

b. **Rotaviruses**

(i) Simian rotavirus SA-11 obtained from Dr. M. Estes, (Baylor College, Houston, U.S.A.) was cultivated in MA-104 cells provided by Dr. Macrea, (Warwick University, U.K.). For preparation of stocks of virus, the cells were grown in 8oz bottles and were changed to...
free growth medium (Appendix Ib) 24 hour prior to inoculation with virus. The serum-free growth medium was discarded and the cell monolayers were washed with PBS. The culture s were then inoculated with 0.2 ml of virus suspension and the bottle incubated at 37°C for 1 hour, rocking every 15 min to ensure uniform distribution of the inoculum. After allowing the virus to adsorb, the inoculum was poured off, and replaced with 40 ml of serum-free growth medium containing 7.5 μg/ml trypsin (Appendix Ib). The cell monolayer was then reincubated until cytopathic effect were observed. When marked degeneration of the cell monolayer was visible the culture was subjected to two cycles of free-thawing (-20°C to +20°C respectively) and the resulting suspension was centrifuged (4000 g, 10 min, 20°C) to deposit the cell debris. the supernatant containing the virus was distributed aseptically in approximately 1.5 ml volumes into sterile universals and stored at -20°C until required.

Virus infectivity was assayed by the plaque test in MA104 cells (Smith et al., 1979). The cells were grown in 25cm³ flasks and were inoculated with 0.1 ml of test virus. Equal volumes of double strength MA104 overlayer medium and agar (Appendix Ib), with the addition of DEAE dextran and pancreatin were mixed together and kept at 45°C. An agar overlayer was applied and flasks were incubated in a 5% CO₂ incubator and titres were expressed as pfu/ml.

(ii) Human rotavirus (HR) was obtained from Dr. M. Goddard (Public Health Laboratory, Reading) in the form of stool specimens suspended in Hanks BSS. They were stored in 0.1 ml volumes at -196°C and infectivity was assayed by indirect immunofluorescence of infected MA104 cells (Banatvala et al., 1975). Two ml of cells in growth medium (Appendix Ib) were seeded into each well of an 8 well plate (Multiwell, T.A. Stones, U.K.). When confluent the medium was replaced by serum-free medium, cultures were reincubated for 24 hour and drained before inoculation with 0.1 ml suspension of virus. After adsorption for 10 min at 37°C, 2 ml serum-free medium containing 7.5 μg/ml trypsin was added to each well. Plates were then subjected to centrifugation (300 x g, 90 min, 25°C) and the medium replaced with fresh medium. Cultures were reincubated for a further 24 hour at 37°C, the medium was removed and cultures were fixed with cold industrial methylated spirits for 10 min and finally air dried. Before the addition of 1:40 dilution of
antiserum (calf rotavirus antiserum provided by Dr. J. Bridger, ARC, Compton, U.K.) cells were moistened with PBS for 10 min. The plates were incubated for 1 hour at 37°C, then washed 3 times with PBS (Appendix IIa) before flooding with 1:40 dilution of fluorescein isothiocynate antibovine IgG conjugate. Cultures were incubated for 1 hour, washed 3 times with PBS, air dried and fluorescent foci counted using incident light fluorescence with Ploem Pak (Leitz Orthoplan).

Propagation of cell cultures

a. Buffalo green monkey cell (BGM)

Ampoules containing 1.5ml of BGM cells were removed from the liquid nitrogen refrigerator, were rapidly thawed at 37°C, and the contents were then transferred to 8oz bottles containing 35ml growth medium (Appendix Ia). The cultures were incubated at 37°C until a confluent cell monolayer was formed at 3 days. The growth medium was discarded and the monolayer was washed with PBS before the addition of maintenance medium (Appendix Ia). After a further period of incubation the cells were passaged at a 1:4 ratio. The maintenance medium was discarded, the culture was washed with PBS and then exposed to versene-trypsin (Appendix IIb). To the resultant cell suspension was added sufficient growth medium for the preparation of 4 new cultures. The storage of cells is described in Appendix II.

b. MA104 cells

These cells were cultivated in 80cm³ or 25cm³ plastic tissue culture flasks (Nunc, Gibco-Biocult). They were passaged at a 1:4 ratio twice a week. Old medium was discarded and confluent monolayers were washed with PBS (Appendix IIa) and then exposed to versene-trypsin (Appendix IIb) at 37°C until the cell sheath was detached from the surface. The detached cells were resuspended in growth medium (Appendix Ib) and between $10^4$-$10^5$ cell/ml were seeded into culture flasks (35ml per 80cm³ flask; 10ml per 25cm³ flask). The new cultures were incubated for 3 days at 37°C or until the cultures were confluent. At this stage the cells were again passaged. Cells were preserved as described in Appendix II. For experiments, cultures were required in
tissue culture flasks and multi-plates (Lux, Scientific Corporation) and incubated at 37°C in 5% CO₂ in air. After 2 to 3 days confluent cells were washed and incubated for 24 hour in serum-free medium.

Propagation and assay of bacteriophage f₂

a. **Propagation of Escherichia coli K12 Hfr (E.coli)**

A culture of the host bacterium E.coli K12 Hfr provided by Dr. J. Slade, (Thames Water Authority) was used to inoculate 3% tryptone soya broth (TSB) (Appendix IIIa). The resultant 24h culture was stored at 4°C for up to 14 days and used as the stock culture for the preparation of daily working culture. These were obtained by the inoculation of 0.5ml of the stock culture into 15 ml of 3% TSB (Appendix IIIa) and incubated overnight. The daily working culture was used for one working day.

b. **Propagation of bacteriophage f₂**

Stocks of bacteriophage f₂ were prepared by inoculating a 50 ml portion of Tryptone yeast extract medium (TYE) medium (Appendix IIIb) with 2 ml of an overnight E.coli culture. The culture was placed in a shaking water bath at 37°C and aerated. Two hours later, when the bacterial concentration had reached about 2 x 10^8 cells/ml, the culture was infected with bacteriophage f₂ (provided by Dr. J. Slade, T.W.A.). The flask was aerated for another 3 hours by which time lysis was complete. Five ml of chloroform were added and the flask was left at room temperature for 10 min, thus any remaining bacteria were lysed. The cell debris was removed by centrifugation (4000 g, 10 min, 20°C) and the resultant clear supernatant fluid, containing the bacteriophage f₂, was stored at 4°C in bijoux bottles.

c. **Assay of infectivity of bacteriophage f₂**

Soft agar (Appendix IIIId) in 4 ml volumes in bijoux bottles, was kept molten in a water bath at 43°C. Ten-fold dilutions of virus were prepared in diluent (Appendix IIIc) and 0.1ml volumes of virus with an
equal volume of *E.coli* daily working culture were dispensed into the soft agar. The contents of each bijoux was mixed well on a rotamix (Hook and Tucker Ltd.) poured immediately onto a lawn plate (Appendix IIIe) and allowed to set. The plates were then incubated at 37°C and the number of plaques were counted after 18 hour.

**Effluent**

Activated sludge effluent was collected from Mogdon or Guildford sewage treatment plant in plastic containers, and 4 l aliquots were distributed into 5 l plastic bottles which were then stored at -12°C. All experiments were done on thawed samples. The pH, suspended solids, biological oxygen demand (BOD), chemical oxygen demand (COD) and ammonia concentration of which was then determined by standard methods (Appendix IV).

**Disinfection procedures**

a. **For tests on chlorine, chlorine dioxide and peracetic acid**

The pH of the effluent was adjusted to the required value by the addition of either N/NaOH or or N/HCl. It was then distributed into 500 ml pyrex beakers which were placed in a water bath and incubated to reach the required temperature. Before every experimental run, chemical titrations of known volumes of the disinfectant were carried out and the required concentrations were added to each experimental beaker. To each beaker 100 ml of effluent was added plus 1 ml suspension of virus to give a concentration of approximately 1 x 10^5 pfu/ml bacteriophage f₂ and simian rotavirus, 1 x 10^5 TCID₅₀/ml poliovirus 1, echovirus 1 and Coxsackievirus B5 or 1 x 10^5 ff*/ml human rotavirus. Disinfectant was then added to provide the required concentration range. A virus control beaker (no disinfectant) was incorporated in each experimental run. All beakers were covered with plastic covers and fitted with glass stirrers which were driven by an overhead variable speed stirrer (Citenco type KQRs) and a series of pulleys and a drive belt (Figures 2.1 and 2.2). The speed of the motor was adjusted to about 100 rpm and stirring was maintained throughout.

*ff: fluorescent foci*
the test. Samples (5ml) were collected at 5 and 30 min and pipetted into bijoux bottles containing sufficient Na$_2$S$_2$O$_3$ (15 mg/l) to neutralize the disinfectant.

b. **For test on ozone**

A laboratory ozonator (Wallace and Tiernan, BA-023) was used. Air was pumped through a flask of silica gel crystals into the ozonator, at a constant rate of 100 l/hour. The amount of ozone produced by the generator was varied by voltage control and the flasks were 250 ml capacity Drechsel bottles fitted with sintered glass diffusers, porosity 2, on the distribution tubes. Each reaction vessel was also fitted with a side arm and tap to facilitate removal of samples from the vessel. Gas passed from these flasks to a Drechsel bottle charged with 7% potassium iodide for adsorption of excess ozone, then through a gas meter and finally vented to the atmosphere (Figures 2.3 and 2.4). The disinfection experiments were all operated on a batch basis, with continuous ozonation throughout the experimental period. Effluent was seeded with 2 ml of virus to give a concentration of about $1 \times 10^5$ pfu for f$_2$, and simian rotavirus, $1 \times 10^5$ TCID$_{50}$ for enteroviruses, and $1 \times 10^5$ ff/ml for human rotavirus. Samples were withdrawn at the start and after 2.5, 5 and 10 min via the side arm tap. The first 3 ml were discarded and a second 3ml portion was immediately neutralized with sodium thiosulphate. A 10 ml portion was removed to determine the ozone concentration using the colourimetric version of Palin's DPD method (Palin 1967, 1974). Sonication experiments were carried out using a sonic water bath at a frequency of 50 KHZ (Kerry Pulsatron 55; Fig. 2.5). The bicarbonation of the effluent was done by treating the effluent with sodium bicarbonate at a concentration of 0.5m before ozonation.
Fig 2.1  Liquid disinfectants: experimental set up

Fig 2.2  Schematic diagram of the experimental apparatus for liquid disinfectants
Fig 2.3 Ozone: experimental apparatus

Fig 2.5 The ozonation experimental apparatus with reaction flasks placed in a sonication water bath
Fig. 2.4 Schematic diagram of the experimental apparatus for ozone.
Preparation of disinfectants

a. Chlorine

was obtained by bubbling chlorine gas into chilled distilled water for about 5 min. The concentration of the stock solution was determined by the iodometric method (Standard Methods, 1971) and calculated amounts of the concentrated solution were then added in the experimental vessels to obtain the required levels which were determined by the DPD method (Palin, 1974).

b. Chlorine dioxide

was prepared by heating to 80°C a mixture of powdered potassium chlorate, oxalic acid, plus a little water, and dissolving the evolved gas in chilled distilled water (Palin, 1948) the concentration of stock solution was determined by the iodometric method (Standard Methods, 1975) and calculated amounts of the concentrated solution were then added to the effluent and it was assayed by using the DPD method (Palin, 1974, 1979).

c. Peracetic acid

was obtained from Phase Separation Ltd., as a 35% aqueous solution. It was stored at 4°C and 1% working stock solution was prepared by dilution in cold distilled water. It was assayed by the method of Sully and Williams (1962).

d. Ozone

preparation has been described in section disinfection procedure b.

Preparation of glassware

In all experiments glassware was carefully washed with Homesol.
(Mertz and Dade) and then thoroughly rinsed with distilled water.

**Statistical and computer analysis**

The statistical analysis of the data was by method described by Parker, (1979) these included the determination of the mean and standard deviation, confidence limits of the mean, t-test and least square analysis.

The results were presented using a curve fitting program written in FORTRAN IV by Mr. Peter Sad (Dept. of Chemical Engineering) The program was graphic routine from the GINO-F Library and was run on the University of Surrey prime computer system. Graphs were plotted on a CALCOMP Plotter.

| All experiments were the mean of at least three replicate tests. |
Chemical analysis of effluent samples

The results of five chemical tests conducted on fresh and thawed portions of effluent are presented in tables 3.1 and 3.2. A rise in pH upon freezing and thawing was always noted, however, only very slight changes were observed in the other parameters tested. The slight alkaline shift was not thought likely to be important because the pH of the system was always adjusted before each experiment.

The monthly averages for the chemical tests on daily composite samples of the effluent were provided by the area chemists of Mogden sewage treatment plant and Guildford sewage treatment plant. The chemical analyses on the samples collected for experimental work were compared to the daily and the monthly averages and no substantial differences were observed. Tables 3.3 and 3.4.
Table 3.1 Chemical tests conducted on fresh effluents used in this study

<table>
<thead>
<tr>
<th>Date effluent collected</th>
<th>pH</th>
<th>Suspended solids (mg/l)</th>
<th>NH$_3$(N) (mg/l)</th>
<th>BOD$_5$ (mg/l)</th>
<th>COD (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.4.80</td>
<td>7.50</td>
<td>8.0</td>
<td>1.0</td>
<td>6.0</td>
<td>37.5</td>
</tr>
<tr>
<td>15.10.80</td>
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<td>6.0</td>
<td>0.6</td>
<td>7.11</td>
<td>37.0</td>
</tr>
<tr>
<td>28.1.81*</td>
<td>7.20</td>
<td>22.0</td>
<td>3.1</td>
<td>19.0</td>
<td>18.0</td>
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<td>17.2.81</td>
<td>7.20</td>
<td>9.6</td>
<td>3.95</td>
<td>15.2</td>
<td>44.0</td>
</tr>
<tr>
<td>24.2.81*</td>
<td>7.70</td>
<td>2.0</td>
<td>---</td>
<td>2.3</td>
<td>---</td>
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<tr>
<td>26.5.81*</td>
<td>7.45</td>
<td>9.0</td>
<td>0.2</td>
<td>5.1</td>
<td>---</td>
</tr>
<tr>
<td>27.5.81*</td>
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<td>---</td>
</tr>
<tr>
<td>16.6.81</td>
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<td>9.2</td>
<td>0.15</td>
<td>10.7</td>
<td>44.0</td>
</tr>
<tr>
<td>4.10.81</td>
<td>7.30</td>
<td>8.8</td>
<td>1.81</td>
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<td>9.3.81</td>
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<td>20.0</td>
<td>1.18</td>
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</tbody>
</table>

$\bar{x} = 7.35$, $s = 0.19$

* Effluent batches were not frozen

Table 3.2 Chemical tests conducted on thawed effluents used in this study

<table>
<thead>
<tr>
<th>Date effluent collected</th>
<th>pH</th>
<th>Suspended solids (mg/l)</th>
<th>NH$_3$(N) (mg/l)</th>
<th>BOD$_5$ (mg/l)</th>
<th>COD (mg/l)</th>
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</thead>
<tbody>
<tr>
<td>20.4.80</td>
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<td>10</td>
<td>0.85</td>
<td>6.11</td>
<td>38.10</td>
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<td>15.10.80</td>
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<td>40</td>
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<td>17.2.81</td>
<td>7.7</td>
<td>12</td>
<td>4</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>16.6.81</td>
<td>7.7</td>
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<td>0.12</td>
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<td>40</td>
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<tr>
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<td>7.9</td>
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<td>1.85</td>
<td>16</td>
<td>44</td>
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<tr>
<td>9.3.82</td>
<td>7.7</td>
<td>22</td>
<td>1.7</td>
<td>3.7</td>
<td>24</td>
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</table>

$\bar{x} = 7.83$, $s = 0.16$

55
Table 3.3 Monthly averages for some analyses conducted on daily composite samples of the activated sludge effluent, provided by the area chemist of Sewage Treatment Plant (Mogden)

<table>
<thead>
<tr>
<th>Month</th>
<th>pH</th>
<th>Suspended solids (mg/l)</th>
<th>NH$_3$(N) (mgm/l)</th>
<th>BOD$_5$ (mgm/l)</th>
<th>COD (mgm/l)</th>
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</thead>
<tbody>
<tr>
<td>August 1979</td>
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<td>7.9</td>
<td>1.2</td>
<td>7.2</td>
<td>39</td>
</tr>
<tr>
<td>September</td>
<td>7.2</td>
<td>7.0</td>
<td>1.0</td>
<td>6.6</td>
<td>37</td>
</tr>
<tr>
<td>October</td>
<td>7.2</td>
<td>5.0</td>
<td>0.6</td>
<td>3.0</td>
<td>37</td>
</tr>
<tr>
<td>November</td>
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<td>6.5</td>
<td>1.1</td>
<td>7.0</td>
<td>39</td>
</tr>
<tr>
<td>December</td>
<td>7.2</td>
<td>6.1</td>
<td>2.0</td>
<td>7.2</td>
<td>39</td>
</tr>
<tr>
<td>January 1980</td>
<td>7.2</td>
<td>7.2</td>
<td>2.9</td>
<td>7.0</td>
<td>41</td>
</tr>
<tr>
<td>February</td>
<td>7.2</td>
<td>12.6</td>
<td>4.4</td>
<td>12.8</td>
<td>52</td>
</tr>
<tr>
<td>March</td>
<td>7.2</td>
<td>16.3</td>
<td>2.3</td>
<td>11.8</td>
<td>51</td>
</tr>
<tr>
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<td>5.7</td>
<td>1.2</td>
<td>4.2</td>
<td>35</td>
</tr>
<tr>
<td>May</td>
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<td>6.4</td>
<td>0.5</td>
<td>5.9</td>
<td>40</td>
</tr>
<tr>
<td>June</td>
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<td>6.6</td>
<td>1.8</td>
<td>5.7</td>
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</tr>
<tr>
<td>July</td>
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<td>8.6</td>
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<td>5.4</td>
<td>38</td>
</tr>
<tr>
<td>August</td>
<td>7.2</td>
<td>7.7</td>
<td>0.4</td>
<td>6.1</td>
<td>36</td>
</tr>
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</table>

$x = 7.2$  $s = 7.97$  $1.51$  $6.91$  $40.46$

$s = 0.00$  $3.12$  $1.17$  $2.69$  $5.27$
Table 3.4 Monthly averages for some chemical analyses conducted on daily composite samples of the activated sludge effluent, provided by the area chemist of the Sewage Treatment Plant (Guildford)

<table>
<thead>
<tr>
<th>Month</th>
<th>pH</th>
<th>Suspended solids (mg/l)</th>
<th>NH$_3$(N) (mg/l)</th>
<th>BOD$_5$ (mg/l)</th>
<th>COD (mg/l)</th>
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<td>0.73</td>
<td>6.4</td>
<td>---</td>
</tr>
<tr>
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<td>---</td>
<td>9.5</td>
<td>1.2</td>
<td>6.0</td>
<td>---</td>
</tr>
<tr>
<td>October</td>
<td>---</td>
<td>14.8</td>
<td>1.1</td>
<td>10.6</td>
<td>---</td>
</tr>
<tr>
<td>November</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>December</td>
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<td>35.0</td>
<td>0.58</td>
<td>14.3</td>
<td>---</td>
</tr>
<tr>
<td>January 1977</td>
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<td>10.8</td>
<td>1.85</td>
<td>13.4</td>
<td>---</td>
</tr>
<tr>
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<td>---</td>
<td>18.6</td>
<td>2.04</td>
<td>12.4</td>
<td>49.3</td>
</tr>
<tr>
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<td>19.3</td>
<td>0.68</td>
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<td>---</td>
</tr>
<tr>
<td>April</td>
<td>---</td>
<td>15.3</td>
<td>1.25</td>
<td>5.4</td>
<td>---</td>
</tr>
<tr>
<td>May</td>
<td>---</td>
<td>14.0</td>
<td>0.65</td>
<td>5.8</td>
<td>---</td>
</tr>
<tr>
<td>June</td>
<td>---</td>
<td>12.8</td>
<td>0.93</td>
<td>6.5</td>
<td>---</td>
</tr>
<tr>
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<td>---</td>
</tr>
<tr>
<td>August</td>
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<td>7.9</td>
<td>0.85</td>
<td>3.9</td>
<td>---</td>
</tr>
<tr>
<td>September</td>
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<td>6.8</td>
<td>0.78</td>
<td>5.9</td>
<td>---</td>
</tr>
<tr>
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<td>11.9</td>
<td>0.48</td>
<td>4.7</td>
<td>---</td>
</tr>
<tr>
<td>November</td>
<td>---</td>
<td>8.5</td>
<td>1.15</td>
<td>7.6</td>
<td>37.0</td>
</tr>
<tr>
<td>December</td>
<td>---</td>
<td>24.5</td>
<td>2.10</td>
<td>16.4</td>
<td>---</td>
</tr>
<tr>
<td>January 1978</td>
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<td>12.4</td>
<td>0.41</td>
<td>5.8</td>
<td>53.4</td>
</tr>
<tr>
<td>February</td>
<td>7.35</td>
<td>11.0</td>
<td>0.78</td>
<td>10.6</td>
<td>43.2</td>
</tr>
<tr>
<td>March</td>
<td>7.2</td>
<td>10.5</td>
<td>0.70</td>
<td>11.2</td>
<td>44.9</td>
</tr>
</tbody>
</table>

$x = 7.28$  $14.02$  $1.003$  $8.65$  $45.56$

$s = 0.07$  $6.66$  $0.500$  $3.63$  $6.21$
Disinfectant dose

When 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. In the case of chlorine, part of the dose is taken up by this 'demand' and the rest acts as free and combined residual disinfectant.

To establish the chlorine dose: residual relation different doses of chlorine were added to the effluent at pH7.2 and 15°C. The free residual was titrated at 1 and 30 min. The same procedure was also used for chlorine dioxide and the results are presented in Tables 3.5 and 3.6.

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 in the reaction flask reached equilibrium. This equilibrium was dependent, of course, on the concentration of ozone in the air passing through the flask.

In 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.

Table 3.5 The relation between chlorine dose and chlorine residual in effluent (pH7.2, 15°C)

<table>
<thead>
<tr>
<th>Dose (ppm)</th>
<th>1 min (ppm)</th>
<th>30 min (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Residual</td>
<td>Total</td>
</tr>
<tr>
<td>5.0</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>7.5</td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td>10.0</td>
<td>6.5</td>
<td>7.2</td>
</tr>
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<td>15.0</td>
<td>8.7</td>
<td>11.0</td>
</tr>
<tr>
<td>20.0</td>
<td>12.6</td>
<td>14.5</td>
</tr>
</tbody>
</table>
Table 3.6  The relation between chlorine dioxide dose and chlorine dioxide residual (as ppm available chlorine) in effluent (pH 7.2, 15°C)

<table>
<thead>
<tr>
<th>Dose ppm</th>
<th>1 min (ppm)</th>
<th>30 min (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5</td>
<td>1.0</td>
<td>0.75</td>
</tr>
<tr>
<td>8.25</td>
<td>2.0</td>
<td>1.60</td>
</tr>
<tr>
<td>11.00</td>
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<td>2.50</td>
</tr>
<tr>
<td>13.75</td>
<td>4.0</td>
<td>3.00</td>
</tr>
</tbody>
</table>
Disinfection experiments

Once the behaviour of disinfectants in effluent was established, disinfection of the six viruses was attempted under selected conditions likely to influence the behaviour of both virus and disinfectants.

a. Chlorine dioxide

Chlorine dioxide inactivated all the six test viruses but the dose response relationship varied widely. Of the three enteroviruses tested at pH7.2 and 15°C, Coxsackievirus B5 almost was the most resistant (Fig 3.1b - 3c), with 5ppm required for complete inactivation in 5 min and 99.9% inactivation being achieved by 4ppm in the same time. However, thereafter little or no further inactivation was detectable to give a characteristic biphasic response. At 3ppm all these viruses behaved similarly but Coxsackievirus B5 showed more resistance than the other two enteroviruses. The behaviour of bacteriophage f₂ appeared to be more sensitive than the enteroviruses with total inactivation being achieved at concentrations as low as 3ppm (Fig 3.1a). In the case of the two rotaviruses tested, human rotavirus was distinctly more resistant than simian rotavirus (SA11) (Fig 3.1e-1f), for instance a higher level of disinfectant was required to achieve the same level of inactivation of the human virus. The comparative behaviour of all these viruses is best represented by the regression lines (Fig 3.1g). These show that human rotavirus was 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 indicated by the shoulder-like curve. In contrast the curves for bacteriophage f₂, echovirus 1, poliovirus 1 and simian rotavirus (SA 11) all showed a more even decline in infectivity with increasing chlorine dioxide concentration.

The influence of pH and temperature on the disinfecting capacity of chlorine dioxide was tested with poliovirus 1 and bacteriophage f₂ only. The pH range tested was 4 to 9, at 5°C, 15°C and 25°C.

Inactivation of bacteriophage f₂ at 5°C is illustrated in Fig
3.2a - 2e with regression line analysis in Fig 3.2f. The striking feature is the largely similar response over the whole range tested, with most resistance at pH7.5 and least at pH4. At 15°C the response was very similar to that at 5°C (Fig 3.3a-3e). It was noted (Fig 3.3f) that bacteriophage f2 also responded similarly to inactivation up to 1ppm but that at 2ppm it was distinctly more sensitive at pH 4. At 25°C bacteriophage was only slightly affected by pH changes (Fig 3.4a - 4f). Again, as with the experiments conducted at 15°C, the phage was least resistant at pH4.

Poliovirus 1 responded quite differently to bacteriophage f2 under the same experimental condition. For instance, at 5°C (Fig 3.5a-5e) it was apparent that poliovirus 1, unlike bacteriophage under same test conditions, was only slightly affected by pH changes (Fig 3.5f). A similar result was obtained at temperatures of 15°C (Fig 3.6a-6f) and 25°C (Fig 3.7a-7f).

Organic matter in the form of peptone had only a marginal effect on the efficiency of the disinfectant against bacteriophage f2 (Table 3.5, Fig 3.8). The effect of various amounts of exogenous suspended solids (obtained from activated sludge) on the efficacy of chlorine dioxide was also investigated and it was observed that the extent of inactivation, as expected, decreased with increasing concentration of suspended solids (Table 3.6, Fig 3.9). So it is evident that suspended solids have a detrimental effect on chlorine dioxide disinfection.
Fig 3.1a  Bacteriophage f2

Fig 3.1b  Coxsackievirus B5

Fig 3.1c  Echovirus 1

Fig 3.1d  Poliovirus 1

The inactivation of virus by chlorine dioxide in effluent (pH 7.2, 15°C)
Fig 3.1g: Regression lines for inactivation of virus by chlorine dioxide in effluent after 30 min prepared from data given in fig 3.1a-1f.

- Poliovirus 1
- Echovirus 1
- Coxsackievirus B5
- Bacteriophage F2
- Simian rotavirus (SA11)
- Human rotavirus
The inactivation of bacteriophage $f_2$ by chlorine dioxide in effluent at various pH values and $5^\circ$C
Fig 3.2f Regression lines for inactivation of bacteriophage φ2 by chlorine dioxide in effluent after 30 min prepared from data given in Fig 3.2a-2e.
Fig 3.3a Bacteriophage $f_2$ (pH4)  

Fig 3.3b Bacteriophage $f_2$ (pH5)  

Fig 3.3c Bacteriophage $f_2$ (pH5)  

Fig 3.3d Bacteriophage $f_2$ (pH7.5)  

Fig 3.3e The inactivation of bacteriophage $f_2$ by chlorine dioxide in effluent at various pH values and 15°C
Regression lines for inactivation of bacteriophage f2 by chlorine dioxide in effluent after 30 min prepared from data given in Fig 3.3a-3e.
Fig 3.4a Bacteriophage f2 (pH4)  

Fig 3.4b Bacteriophage f2 (pH5)  

Fig 3.4c Bacteriophage f2 (pH6)  

Fig 3.4d Bacteriophage f2 (pH7.5)  

Fig 3.4a-4e The inactivation of bacteriophage f2 by chlorine dioxide in effluent at various pH values and 25°C
Fig 3.4f Regression lines for inactivation of bacteriophage $f_2$ by chlorine dioxide in effluent after 30 min prepared from data given in Fig 3.4a-4e.
Fig 3.5a Poliovirus 1 (pH 4)

Fig 3.5b Poliovirus 1 (pH 5)

Fig 3.5c Poliovirus 1 (pH 6)

Fig 3.5d Poliovirus 1 (pH 7.5)

Fig 3.5e The inactivation of poliovirus 1 by chlorine dioxide in effluent at various pH values and 5°C
Fig 3.5f  Regression lines for inactivation of poliovirus 1 by chlorine dioxide in effluent after 30 min prepared from data given in Fig 3.5a-5e.
Fig 3.6a Poliovirus 1 (pH 4)

Fig 3.6b Poliovirus 1 (pH 5)

Fig 3.6c Poliovirus 1 (pH 6)

Fig 3.6d Poliovirus 1 (pH 7.5)

Fig 3.6a-6e The inactivation of poliovirus 1 by chlorine dioxide in effluent at various pH values and 15°C
Fig. 3.6f Regression lines for inactivation of poliovirus by chlorine dioxide in effluent after 30 min prepared from data given in Fig 3.6a-6e.
The inactivation of poliovirus 1 by chlorine dioxide in effluent at various pH values and 25°C
Fig 3.7f Regression lines for inactivation of poliovirus 1 by chlorine dioxide in effluent after 30 min prepared from data given in Fig 3.7a-7e.
Fig 3.8 Regression analysis for inactivation of bacteriophage f<sub>2</sub> by 1.60ppm chlorine dioxide in effluent in the presence of various concentrations of peptone (pH 4, 25°C)

Table 3.7 The inactivation of bacteriophage f<sub>2</sub> by 1.6ppm chlorine dioxide in effluent in the presence of various concentrations of peptone (pH 4, 25°C)

<table>
<thead>
<tr>
<th>Disinfectant Level (ppm at 0 time)</th>
<th>Virus input conc. mg/1</th>
<th>Peptone</th>
<th>Virus recovered*</th>
<th>%Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.65</td>
<td>22</td>
<td>4.01</td>
<td>22.67+</td>
</tr>
<tr>
<td></td>
<td>4.65</td>
<td>22</td>
<td>3.25</td>
<td>3.96</td>
</tr>
<tr>
<td></td>
<td>4.65</td>
<td>44</td>
<td>3.19</td>
<td>3.47</td>
</tr>
<tr>
<td></td>
<td>4.65</td>
<td>66</td>
<td>3.39</td>
<td>5.42</td>
</tr>
<tr>
<td></td>
<td>4.65</td>
<td>110</td>
<td>3.25</td>
<td>3.96</td>
</tr>
</tbody>
</table>

Regression Coefficient
- Slope: a = 0.004, b = 4.02
- Intercept: r = -0.15, a = 0.004

*Log<sub>10</sub> PFU/ml. The Log<sub>10</sub> value is presented as 0 in cases where no virus is recovered.
+ values were not considered in estimating the slope.
Fig 3.9 Regression analysis for inactivation of bacteriophage \( f_2 \) by 2ppm chlorine dioxide in effluent in the presence of various concentrations of suspended solids (pH 4, 5°C).

Table 3.8 The inactivation of bacteriophage \( f_2 \) by 2ppm chlorine dioxide in effluent in the presence of various concentrations of suspended solids (pH 4, 5°C).

<table>
<thead>
<tr>
<th>Disinfectant Level (ppm at 0 time)</th>
<th>Virus Input Suspended solids conc. mg/l</th>
<th>Virus recovered*</th>
<th>%Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5 min</td>
<td>30 min</td>
</tr>
<tr>
<td>0.00</td>
<td>4.65</td>
<td>10.0</td>
<td>4.02</td>
</tr>
<tr>
<td>2.00</td>
<td>4.65</td>
<td>10.0</td>
<td>3.41</td>
</tr>
<tr>
<td>2.00</td>
<td>4.65</td>
<td>23</td>
<td>3.77</td>
</tr>
<tr>
<td>2.00</td>
<td>4.65</td>
<td>76</td>
<td>3.92</td>
</tr>
<tr>
<td>2.00</td>
<td>4.65</td>
<td>92</td>
<td>3.98</td>
</tr>
</tbody>
</table>

Regression Coefficient
- Slope: \( r = 0.94 \)
- Intercept: \( a = 0.16 \), \( b = 6.61 \)

*\( \log_{10} \) PFU/ml. The \( \log_{10} \) value is presented as 0 in cases where no virus is recovered.
+ values were not considered in estimating the slope.
b. Ozone

The results (Fig 3.10a-10f) show that the response of the viruses varied widely. Bacteriophage f2 was the least resistant and the three enteroviruses reacted in a similar fashion but in contrast the two rotaviruses responded quite differently with the human rotavirus proving to be the most resistant of all the viruses tested.

The effect of pH was studied to determine whether the rate of inactivation was affected by the rate of decomposition of ozone at various pH values. The ozonation of poliovirus 1 was tested at pH values 4, 7.2 and 9.0 and the regression analysis (Fig 3.11) suggests that ozone was most effective at pH4.

Temperature had relatively little effect on the rate and amount of inactivation by ozone (Fig 3.12) with the least activity as expected at the lowest temperature tested.

The addition of various amounts of organic matter in the form of peptone (Fig 3.13) showed that there was some reduction in the efficacy of ozone with increasing concentration of peptone.

When varying amounts of suspended solids (obtained from activated sludge) were added to the effluent at pH7.2 and room temperature, the extent of inactivation decreased, as expected, with increased amounts of suspended solids (Fig 3.14).

As described in Chapter 2, a sonication waterbath at a frequency of 50KHZ was used to determine the effect of sonication on ozone disinfection. The treatment was applied for (a) 10 min before ozonation, and (b) 10 min before ozonation and for another 10 min while ozonating. The results are illustrated in Fig 3.15. This shows that sonication enhanced disinfection when applied before disinfection, but an even better effect was obtained when sonication was applied before and during ozonation.

In an attempt to investigate whether the O3 molecule or the OH radical is the disinfecting species in ozonation, the effluent was carbonated before ozonating. This was done as described in the methods
by adding sodium bicarbonate at a concentration of 0.5M before ozonation. Also, the treated effluent was ozonated for 10 min only and non-ozoniated air was bubbled through for another 5 min to find out whether there was any further inactivation by ozone. The regression line analyses are depicted in Fig 3.16 which show that bicarbonating the effluent before ozonating it enhanced inactivation. Again, the results indicated that ozone was most effective at acidic pH.
Fig 3.10a Bacteriophage f₂

Fig 3.10b Coxsackievirus B5

Fig 3.10c Echovirus 1

Fig 3.10d Poliovirus 1

The inactivation of virus by ozone in effluent (pH 7.2, 20°C).
Fig 3.10g Regression lines for inactivation of virus by ozone in effluent after 10 min prepared from data given in Fig 3.10a-10f.

- Poliovirus 1
- Echovirus 1
- Coxsackievirus B5
- Bacteriophage F2
- Simian rotavirus (SA11)
- Human rotavirus
Fig 3.11 Regression lines for inactivation of poliovirus 1 by 0.2ppm ozone in effluent at various pH values and 20°C.
Fig 3.12 Regression lines for inactivation of poliovirus 1 by 0.2ppm ozone in effluent at pH7.2 and various temperatures.
Fig 3.13 Regression lines for inactivation of poliovirus 1 by 0.2 ppm ozone in effluent in the presence of various concentrations of peptone (pH 7.2, 20°C)
Fig 3.14 Regression lines for inactivation of poliovirus 1 by 0.2 ppm ozone in effluent in the presence of various concentrations of suspended solids (pH 7.2, 20°C)
Fig 3.15 Regression lines showing the effect of sonication on the inactivation of poliovirus 1 by 0.25ppm ozone in effluent (pH7.2, 20°C)
Fig 3.16 Regression lines for inactivation of poliovirus 1 in carbonated effluent by 0.2ppm ozone at various pH values and 20°C
c. Chlorine

The inactivation of viruses by chlorine at 15°C and pH7.2 is represented in Fig 3.17a-17f. The reaction was characteristically biphasic with optimal chlorine residuals for any one treatment occurring by 5 min. Among the six viruses tested (Fig 3.17g) Coxsackie virus B5 was the most resistant with 99.99% inactivation achieved at 11.0ppm (5 min contact time), while simian rotavirus was most sensitive with only 1.1ppm needed to achieve 99.99% inactivation. A striking difference was noted between the two rotaviruses with the human strain seven times more resistant to chlorine than simian rotavirus.

The results of tests in which pH was varied at room temperature (Fig 3.18a-18c) show clearly (Fig 3.18d) that the inactivation of poliovirus 1 by chlorine was greatly enhanced, as expected, by decreasing pH.

The effect of temperature (Fig 3.19a-19c) clearly showed that resistance was highest at the lowest temperature tested.

The predictable effect of organic matter (in the form of peptone) on the efficacy of chlorination was only tested at room temperature and pH7.2 and the results (Fig 3.20a-20b) demonstrated loss of activity.

The addition of suspended solids (obtained from activated sludge effluent) also resulted in reducing the chlorine efficacy (Fig 3.21a-21b) and this is best illustrated in Fig 3.21c.
Fig 3.17a  Bacteriophage f2  

Fig 3.17b  Coxsackievirus B5  

Fig 3.17c  Echovirus 1  

Fig 3.17d  Poliovirus 1  

Fig 3.17a-17f The inactivation of virus by chlorine (pH 7.2, 15°C)
Fig 3.17g Regression lines for inactivation of virus by chlorine in effluent after 30 min prepared from data given in Fig 3.17a-17f.
Fig 3.18a - 18c  The inactivation of poliovirus 1 by chlorine in effluent at various pH values and 20°C
Fig 3.18d Regression lines for inactivation of poliovirus 1 by chlorine in effluent after 30 min prepared from data given in Fig 3.18a - 3.18c
Fig 3.19a-19b The inactivation of poliovirus 1 by chlorine in effluent at various temperatures and pH 7.2

Fig 3.19c Regression lines for inactivation of poliovirus 1 by chlorine after 30 min prepared from data given in Fig 3.19a-19b
The inactivation of poliovirus 1 by chlorine in effluent in the presence of various concentrations of peptone (pH 7.2, 20°C).

Regression lines for inactivation of poliovirus 1 by chlorine in effluent after 30 min prepared from data given in Fig 3.20a-20b.
Fig 3.21a-21b The inactivation of poliovirus 1 by chlorine in effluent in the presence of various concentrations of suspended solids (pH7.2, 20°C).

Fig 3.21c Regression lines for inactivation of poliovirus 1 by chlorine in effluent after 30 min prepared from data given in Fig 3.21a-21b.
d. Peracetic acid

The inactivation of the various viruses by peracetic acid is illustrated in Fig 3.22a-22g and the results indicated that relatively high concentrations of acid were required to achieve significant effects. For instance, up to about 140ppm 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 20ppm 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.

Temperature (4°C and room temperature (20°C); Fig 3.23a-23c) had little effect on the inactivation efficiency. Furthermore the presence of organic matter (50ppm exogenous peptone) had negligible effect (3.24a-24c) as was also the case with suspended solids (Fig. 3.25a-25c)
Fig 3.22a-22f  The inactivation of virus by peracetic acid in effluent (pH7.2, 15°C)
Fig 3.22g Regression lines for inactivation of virus by peracetic acid in effluent after 30 min prepared from data given in Fig 3.22a-22f.
Fig 3.23a-23b The inactivation of poliovirus 1 by peracetic acid in effluent at various temperatures and pH 7.2

Fig 3.23c Regression lines for inactivation of poliovirus 1 by peracetic acid in effluent after 30 min prepared from data given in Fig 3.23a-23b

Peracetic acid cane (ppm)
Fig 3.24a-24b The inactivation of poliovirus 1 by peracetic acid in effluent in the presence of various concentrations of peptone (pH 7.2, 20°C).

Fig 3.24c Regression lines for inactivation of poliovirus 1 by peracetic acid in effluent after 30 min prepared from data given in Fig 3.25a-25b.
Fig 3.25a-25b The inactivation of poliovirus 1 by peracetic acid in effluent in the presence of suspended solids (pH 7.2, 20°C)

Fig 3.25c Regression lines for inactivation of poliovirus 1 in effluent prepared from data given in Fig 3.26a-3.26b

- △ - 50 ppm suspended solids
- ▼ - 0 ppm suspended solids
The effect of combinations of disinfectants

These experiments were on the effects of simultaneous and sequential application of liquid disinfectants. In the case of simultaneous application of two liquid disinfectants, samples were taken after 5 min and 15 min. The experiments on sequential addition of disinfectant the first disinfectant, followed after 5 minutes by addition of the second disinfectant, and samples were taken at 5 min after the application of the first disinfectant and 10 min after the application of the second disinfectant.

Fig 3.26 illustrates the effects of the simultaneous application of chlorine and chlorine dioxide whereby chlorination was enhanced in the presence of chlorine dioxide and that a similar degree of inactivation was obtained upon the sequential or simultaneous application of both disinfectants, but a slightly better effect was obtained when chlorine was added before chlorine dioxide.

The results of the application of chlorine and peracetic acid are demonstrated in Fig 3.27. This figure shows a noticeable enhancement whenever peracetic acid and chlorine were present together. The greatest effect was noted when peracetic acid was added five minutes before chlorination. However, similar results were obtained when HCl was used instead of peracetic acid which implies that peracetic acid provided an acidic medium for chlorine as did HCl.

The effects of the application of both peracetic acid and chlorine dioxide are shown in Fig 3.28. This figure indicates that maximum efficacy was noted upon the application of peracetic acid five minutes before chlorine dioxide. A slight enhancement was achieved when chlorine dioxide was applied 5 min before the application of peracetic acid.

The effects of the application of ozone with chlorine, chlorine dioxide and peracetic acid were also investigated. The simultaneous addition of ozone with the other disinfectants was achieved by first applying the liquid disinfectant and then ozonating the mixture. The sequential application was performed either by applying the liquid disinfectant for 5 min and when ozonating the effluent or by ozonating
the effluent for 5 min and then applying the liquid disinfectant to the treated effluent for another 5 min. Fig 3.29-31 present the effects of the application of ozone with the liquid disinfectants. Fig 3.29 shows that the simultaneous application of both ozone and chlorine yielded better results than separate application. Only slight enhancement was noted upon the addition of Cl2 first followed by ozone. However, chlorine was less efficient when applied after ozonation. Fig 3.30 shows that only slightly better results were achieved when chlorine dioxide and ozone were applied simultaneously. Maximum enhancement was noted when ozone was applied for 5 min and then followed by chlorine dioxide. Only slightly increased efficiency was noted when chlorine dioxide was applied 5 min before ozone. Because the disinfecting action of ozone enhanced at acidic pH, the combined effects of ozone and peracetic were examined first and a control was run using hydrochloric acid (HCl) instead of peracetic acid. Fig 3.31 shows that very good enhancement was achieved when ozone and peracetic acid were applied together and when peracetic acid was applied 5 min before ozonation. Comparable results were obtained when HCl was used to lower the pH of the effluent. Ozonation was slightly more efficient when peracetic acid was applied 5 min after ozonation.
Fig 3.26 The effect of the application of chlorine and chlorine dioxide on poliovirus 1 in effluent (pH 7.2, 20°C).  

A: Nil  
B: Cl₂ for 5 min (1ppm)  
C: ClO₂ for 5 min (1ppm)  
D: B and C simultaneously  
E: B followed by C (extended to 15 min)  
F: C followed by B (extended to 15 min)  

Fig 3.27 The effect of the application of chlorine and peracetic acid on poliovirus 1 in effluent at 20°C.  

A: Nil  
B: Cl₂ for 5 min (1ppm)  
C: PAA for 5 min (22.98 ppm)  
D: B and C simultaneously  
E: Same as D but acidify with H instead of PAA  
F: B followed by C (extended to 15 min)  
G: C followed by B (extended to 15 min)  
H: Same as G but acidify with H of PAA
Fig 3.28 The effect of the application of chlorine dioxide and peracetic acid on poliovirus 1 in effluent at 20°C.

- **A:** Nil
- **B:** ClO₂ for 5 min (1 ppm)
- **C:** PAA for 5 min (22.98 ppm)
- **D:** B and C simultaneously
- **E:** B followed by C (extended to 15 min)
- **F:** C followed by B (extended to 15 min)

Fig 3.29 The effect of the application of ozone and chlorine on poliovirus 1 in effluent (pH 7.2, 20°C).

- **A:** Nil
- **B:** Cl₂ for 5 min (1 ppm)
- **C:** O₃ for 5 min (0.2 ppm)
- **D:** B and C simultaneously
- **E:** B followed by C (extended to
- **F:** C followed by B (extended to
**Fig 3.30** The effect of the application of ozone and chlorine dioxide on poliovirus 1 in effluent (pH 7.2, 20°C).

A: Nil
B: ClO₂ for 5 min (0.8ppm)
C: O₃ for 5 min (0.2ppm)
D: B and C simultaneously
E: B followed by C (extended to 10 min)
F: C followed by B (extended to 10 min)

**Fig 3.31** The effect of the application of ozone and peracetic acid on poliovirus 1 in effluent at 20°C.

A: Nil
B: PAA for 5 min (25.96ppm)
C: O₃ for 5 min (0.2ppm)
D: B and C simultaneously
E: Acidify with HCl and then ozonate for 10 min
F: B followed by C (extended to 10 min)
G: C followed by B (extended to 10 min)
DISCUSSION

The efficacy of effluent disinfection depends to a great extent on the quality of the effluent (Tonelli, 1976) and for this reason, the characterisation of the effluent undergoing disinfection experiments is important since there are often variations in the physical and chemical quality (Aieta et al., 1980; Painter et al., 1971). To minimize such variations it was decided in the present work to limit experiments to as few different batches of effluent as possible and as described, these were collected and stored at -12°C, a procedure which resulted in minimal alteration of the effluent quality. The most obvious change was a slight shift towards alkalinity, also noted by others (Hajenian and Butler, 1980; Painter et al., 1971) but this was not thought important because the pH of the system was always adjusted before experiments.

In all the liquid disinfectants experiments, there was a characteristic biphasic mode of inactivation with a sharp loss of viral infectivity within the first five minutes, followed by an extended phase where little, if any, further inactivation occurred. This phenomenon has been reported and discussed by many others the most recent of which include Aieta et al. (1980); Hajenian and Butler, (1980); Longley et al. (1980) and Olivieri et al. (1971) but Chick (1908) was the first to attempt to explain the laws of disinfection which she predicted should obey first order kinetics. However, in her experiments, first order kinetics were only obeyed when anthrax spores were inactivated by phenol, whereas with all other bacteria tested other patterns of inactivation were followed. The divergences from the first order kinetics mainly fell into two categories; those which showed an initial lag before maximum or optimum rates of inactivation developed, and those where after an initial period of rapid inactivation, a plateau developed representing a persistent infectious fraction. Sometimes both features were observed in the same system and various explanations have been sought for these different phenomena all of which are thought to apply equally to the inactivation of viruses. For instance, an initial shoulder would appear when the multihit response occurred which would be expected with most suspensions of infectious virions which, of course, would almost
certainly include viral aggregates of various size (Floyd and Sharp, 1977). There would be, in such circumstances, a delay in the measured loss even though much virus was actually inactivated. Aggregation could equally explain residual infectivity where infectious particles remained inaccessible to the disinfectant (Boardman and Sproul, 1977). It is also possible that viral populations are genetically heterogenous with respect to sensitivity to disinfection, indeed some studies have resulted in the selection of populations with increased resistance (Bates et al., 1977) but it is also possible that some residual infectivity is the result of multiplicity reactivation (Young and Sharp, 1979), that is, the restoration of the complete replication mechanism due to the multiple infection of cell with virions with differently but only slightly damaged genomes. Such doubts re-emphasize the need for reliable and reproducible infectivity assays and in most situations each virus particle must be assumed to be infectious and its infectivity determinable.

It was noted in the present work that for any parameter tested, there was a linear negative correlation between disinfectant residual and survival of virus up to a certain threshold value of disinfectant after which there was a sharp decline in survival. The linear portions of these reactions were presented as the regression lines. The main points to emerge from the present work relate to the peculiar advantages and disadvantages of the different disinfectants and combinations thereof.

With regard to chlorine dioxide this has long been suggested as a possible alternative to chlorine particularly in water with high chlorine demand (Holden, 1970). It has had extensive use as a water disinfectant in Europe, although early on its main value was for the removal of taste and odour in water treatment (Longley, 1980). The first reports on its germicidal efficacy lacked the adequate techniques to differentiate between chlorine dioxide and the various chlorine residuals present simultaneously (White, 1978), indeed the aqueous chemistry of chlorine dioxide is not even now completely understood (White, 1978). It has been thought of as a more powerful oxidant than chlorine (Rosenblatt, 1975) but this is not so in practice when it is applied under the narrow pH ranges encountered in water or wastewater. Its main advantages over chlorine are that it does not react with
nitrogenous compounds, including simple aminoacids, and its virucidal capacity is not influenced by ammonia (Malpas, 1973).

It was observed in the present work that the rate of inactivation by chlorine dioxide was, as expected, a function of disinfectant dosage, however, the results obtained in this study seemed to indicate a greater potency than reported elsewhere. For instance, Walters (1976) required it at concentrations as high as 7.5 mg/l, rather than the 3 mg/l used in the present work, to achieve 4log10 reduction of f2 bacteriophage in secondary wastewater effluent. An even higher concentration, 12 mg/l, was required by Tift et al. (1977) and the reason for these differences were almost certainly a reflection of the quality of the effluent used.

In the present study the efficiency of chlorine dioxide was, as expected, only slightly affected by changes in pH because the chemistry of the hydrolysis of the molecule is independent of pH. Furthermore, chlorine dioxide unlike chlorine probably retains its molecular configuration in water (Ridenour et al. 1947; Ridenour et al. 1949). However, it is worth noting that the resistance of bacteriophage to chlorine dioxide varied slightly with temperature at different pH values. For instance at 50°C the phage was most resistant at pH5 and 6, while at 25°C, it was most resistant at pH7.5. However, whatever the temperature bacteriophage appeared to be most sensitive at pH4. This sensitivity could have been due to the presence of minute amounts of chlorine known to be present but which were not detected by the DPD method (Aieta, 1982). The reaction of poliovirus to chlorine dioxide appeared to be even less affected than phage by changes in pH and temperature.

Another not unexpected finding was that the virucidal efficiency of chlorine dioxide was not affected by the presence of organic matter in the form of peptones shown earlier by Ingols and Ridenour (1948). Furthermore, its efficiency was inversely proportional to the concentration of suspended solids, which was, presumably, due to the demand exerted by the floc which may offer the virus some physical protection. It has also been suggested that the disinfectant may actually adsorb to the suspended solids and become unavailable. In relation to this Ingols and Ridenour (1948) noted as mentioned in
Chapter one that it reacted with peptone in a way that appeared to follow the laws of surface adsorption and this supposed adsorption phenomenon was used to interpret the mechanism by which chlorine dioxide inactivated viruses as a result of direct adsorption to the protein coat. Under these conditions it was suggested that local high concentrations of disinfectant could occur on the surface of the virus leading to inactivation rates greater than would be expected from the measured residual. Bernarde et al. (1967) reported that chlorine dioxide abruptly inhibits protein synthesis which is probably the mechanism by which it destroys microorganisms but this would not apply to viruses. Olivieri et al. (1982) suggested that the primary target of chlorine dioxide inactivation of viruses was the viral capsid and not nucleic acid.

As far as ozone is concerned, the lack of exact knowledge about the chemistry and kinetics of it in solution and the uncertainty of analytical techniques are serious obstacles to the interpretation of the results of its disinfection activity. Hoigne and Bader (1976, 1979) postulated that when ozone hydrolizes in water, about half of it will be converted to OH radicals, the most reactive oxidant known, furthermore the hydrolysis equilibrium is pushed further to the right by the presence of the OH radical which dominates at high pH values. In clear water systems, the hydroxyl radical will probably be available to inactivate microorganisms such as viruses, but if organic substances are present, these will preferentially react with the radical. Molecular O$_3$ itself is thought to be germicidal and both this and the OH radical have similar oxidation potentials and similar reactions with organic matter such as phenols and aminoacids containing sulphur.

The rate of the hydrolysis of ozone in water depends on the concentration of the ozone, the pH, and the concentration of carbonate and bicarbonate ions (Hoigne and Bader, 1979). Hydrolysis is accelerated by raising the pH since the formation of OH radical is favoured at alkaline pH values and is inhibited by carbonate/bicarbonate because these ions scavenge the OH radical in some way which is yet to be fully elucidated. It was noted in the present study that the inactivation by ozone of all the viruses tested was essentially a function of time which was almost certainly due to the use of the continuous bubbling injection system which was in
contrast to the single dose system used with the liquid disinfectants. Farooq et al. (1977, 1978) reported similar observations and showed that the continuous bubbling of ozone was the most efficient because ozonation is governed by gas transfer and this is best achieved, of course, in the presence of small ozone bubbles. In the present work, like that of Farooq et al. (1977), the virucidal efficiency of ozone was best at the lowest pH tested, pH4, and it is worth noting that the hydrolysis of ozone in water decreases with decreasing pH thus leaving the active O$_3$ molecule predominant. It is also interesting that Kinman (1974), unlike Farooq et al. (1977), reported that at very low temperatures, between 1 and 5°C, the efficiency of ozone was independent of pH, and Katzenelson et al. (1974) suggested that there is a minimum temperature below which decomposition is not further affected by pH, that is to say, it seems likely that O$_3$ exists as molecular ozone at low temperature or low pH.

In the present work on the inactivation of poliovirus 1, the disinfectant as shown previously by Farooq et al. (1977), was not significantly affected by temperature and from this it can be concluded that ozone may inactivate viruses via either the molecular O$_3$ or the hydrolysis product, the OH radical. Further support for the suggestion that the O$_3$ molecule was active was from studies on the influence of carbonation when there was enhancement of the disinfecting ability. This was presumably because, as described earlier, carbonate interferes with hydrolysis. An interesting question is, is it possible to determine whether the OH radical or molecular ozone is the most potent disinfecting species. From the present study it would appear that molecular O$_3$ is the most efficient because the best disinfection was achieved at low pH or in the presence of carbonate both of which favour the presence of molecular O$_3$.

The presence of peptone interfered with ozonation as was shown earlier by Evison (1978). Furthermore, the presence of suspended solids, not surprisingly, also interfered with disinfection. Bollyky and Siegel (1977) believed this was mainly because suspended solids provided the virus with physical protection against disinfectant as well as creating an ozone demand.

Sonication has been shown to influence the rate and
effectiveness of ozone disinfection (Dahi and Lund, 1980) provided that it was administered during ozonation and not before application of the disinfectant. One probable explanation for this is that sonication results in the disaggregation of virus aggregates to smaller and more vulnerable infectious units. Katzenelson et al. (1974) reported that pretreatment of poliovirus with ultrasound increased subsequent ozone disinfection dramatically, an observation confirmed by the present study. In both cases the result was almost certainly due to breakup of aggregates to smaller and more vulnerable infectious units and, indeed, it was shown in the present work that there was an increase in infectivity after sonication alone. It is also thought likely that ultrasonic treatment enhances the solubility of ozone in some way, for instance by bringing the bubbles of ozone into intimate contact with the water and the microorganisms, or even resulting in the rapid dispersion of ozone molecules. Dahi (1976) attributed this enhancement to two physicochemical mechanisms: (1) ultrasound decomposes ozone causing augmentation in the activity of free radicals and (2) simultaneous ultrasonic treatment enhances the aeration constant. Both mechanisms increase the gas-liquid ozone transfer and utilization of the applied ozonated gas. Burleson et al. (1975) also reported the synergistic effect of simultaneous ultrasonics and ozone treatment.

Any discussion of ozonation of viruses in effluent is limited by the fact that there are relatively few reports especially of work on this medium. Also, difficulties arise in comparing the results of different investigators because so many different experimental conditions have been utilized, and some investigators have expressed their results simply in terms of ozone dosage, without making it clear whether the dosage was that which was applied or the residual which was recorded. The levels of dissolved ozone residual should always be quoted after a given contact time. But in general the accurate determination of ozone concentrations is quite difficult especially in sewage effluents, and many different methods have been used (Rice et al., 1981). Bearing these factors in mind, the present results indicate that quite low levels of ozone were required for useful inactivation, certainly lower than even those reported by Katzenelson and Biederman (1976) who achieved good inactivation of poliovirus 1 only upon applying 0.6-1.0 mg/l of ozone. Much higher concentrations still were reported by Kott (1978) who found that dosages of 15 to 20
mg/l of ozone added to secondary effluents were required to achieve reductions of 3 orders of magnitude for enteroviruses and Pavoni et al. (1972) reported complete of \( f_2 \) coliphage with an ozone dose of 15 mg/l. In the present study, findings similar on poliovirus 1, echovirus 1, and Coxsackievirus B5 were similar to those of Evison (1978). However, she also reported that poliovirus 3 and Coxsackievirus B3 were the most resistant to ozonation and that 1.1 mg/l and 3.0 mg/l respectively were required for 99.9% kill in 10 min contact time. These viruses were not included in the present study.

The mechanism of inactivation by ozone is little understood. As mentioned in Chapter 1, Kim et al. (1980) reported that ozone interfered with the specific adsorption mechanism of the phage. Also the phage capsid was broken into protein subunits, liberating RNA which may itself be sheared by the disinfectant. Roy et al. (1981) observed that ozone altered two of the four polypeptide chains present in the viral protein coat of poliovirus 1. However, this did not significantly impair virus adsorption on the host cells or alter the integrity of the virus particle, however, damage to the viral RNA after exposure to ozone was demonstrated by velocity sedimentation analysis and it was concluded that the damage to the viral nucleic acid was the major cause of poliovirus inactivation.

Turning now to chlorine it is well established that for adequate disinfection of effluents it is essential to use sufficient amounts to satisfy nitrogenous demand, the so called "break point" phenomenon (White, 1972, 1978). The virucidal efficacy of the products of hydrolysis of chlorine and its combined forms has been widely studied and the free species HOCl and OCl\(^-\) are superior to such combined forms as the chloramines (Engelebrecht et al. 1978, 1980). Of the combined forms, Kelly and Sanderson (1960) reported that the dichloramines were superior to the monochloramines. In general, the HOCl\(^-\) ion which is predominant at low pH is most active species (White, 1978) but it is worth noting that Scarpino et al. (1972, 1974) in a comparative study on chlorination of poliovirus 1 and E. coli concluded that OCl\(^-\) was a better virucide than HOCl which is contradictory to most of the published data and this draws attention to the fact that certain other factors may also play a role in the efficacy of chlorine disinfection. For instance, Hajenian and Butler (1980) reported that poliovirus 1
required less chlorine for 99.99% inactivation at pH 4 and 7.7 than at pH 6.8, and they suggested that the virus may be more stable at a pH close to one of its suggested isoelectric points pH 7.0 (Mandel, 1971), Taylor (1980) confirmed this.

The influence of temperature although measurable requires less complex explanation and is probably simply due to slower reaction kinetics of chlorine to HOCl and OCl⁻ (White, 1978). Earlier, White (1972) had reported that for adequate chlorination in the winter season as much as five fold increase in contact time was required to equal that achieved by the standard dose and in the summer.

The presence of suspended solids of both organic and inorganic nature also characteristically present in colloidal suspensions is well known to adversely affect chlorination. It is usually present in complex ill-defined forms and proteins make up a large percentage which will, of course, react actively with chlorine, therefore the removal of such suspended matter from the effluent is essential for optimal disinfection. The suspended solids not only exert an increased demand on chlorine but may also protect the virus (Hejkal et al., 1979; Stagg et al., 1977) however, Boardman and Sproul (1977) concluded that viral adsorption to some particulate surfaces provided negligible if any protection from disinfectant.

Examination of the reports relating to the chlorination of sewage effluent reveals that much higher levels were usually required than have been used in the present work. For example, Kruse et al. (1971) reported that at neutral pH, 30 mg/l chlorine resulted in less than 80 percent of f₂ coliphage inactivation, whereas in the present work as little as 5 mg/l could effect the same degree of inactivation. However, the residual chlorine whether free or combined, was not reported by Kruse et al. (1971) so it is possible that they were reporting total chlorine and that the effluent demand was high. Another example of inconsistency is the work of Cramer et al. (1976) who used autoclaved effluent and applied chlorine at 30 mg/l and failed to achieve much inactivation and this result was probably because heating resulted in the formation of new products capable of creating chlorine demand.
The mechanism by which chlorine inactivates viruses is still not clear. Kruse et al. (1971) postulated that halogenation causes the oxidation of the sulfhydryl (-SH) groups on the protein coat of the virus, thus denaturing the protein and preventing the virus adsorption to the host. However, the halogenation of -SH groups is, apparently, not necessarily the prime inactivation process in all viruses. O'Brien and Newman (1979) reported that chlorination of poliovirus resulted in the loss of viral ribonucleic acid, converting the viruses from 156s 80s particles although it was, in fact, observed that virus inactivation occurred before the nucleic acid was released. In a more recent study Alvarez and O'Brien (1982) reported that chlorination of poliovirus did lead to the release of the RNA suggesting that loss of nucleic acid was a critical event during disinfection. Tenno et al. (1980) concluded that HOCl inactivated poliovirus by acting on the protein component of the virus and that the inactivation reaction did not result in any detectable change in the structure of the virus nor affect the infectivity of the viral RNA.

Regarding the experiments with peracetic acid, it was observed that unlike the other disinfectants, it was only slightly affected by the presence of peptone and suspended solids. However, on the basis of concentration it was the least efficient virucide tested. Indeed such high concentrations were required that they would not be applicable in practice as they would lower the pH of the medium drastically and increase BOD₅ of the product by the formation of acetic acid and its salts (Meyer, 1976; Poffe et al., 1978). However, the results did indicate that lower concentrations were necessary than those reported elsewhere. For instance, Mucke (1970) reported that 2000 ppm of the peracetic acid were required for the inactivation of poliovirus within 5 min. Kline and Hull (1960), on the other hand, showed that poliovirus was inactivated by 400 ppm of peracetic acid in 5 min. In a comparative study on the efficacy of peracetic acid against laboratory grown poliovirus 1 and indigenous faecal bacteria in effluent, Harakeh et al. (1981) showed that peracetic acid at up to 7 ppm was a good bactericide but not an efficient virucide.

An interesting observation reported by Hajenian and Butler (1980) was that not only did effluent not exert a demand but that its disinfecting activity apparently persisted for at least 30 min.
However, despite this the disinfection was characteristically biphasic which was probably because of the presence of viral aggregates.

The main purpose of the experiments in which combinations of various disinfectants were tested, was to investigate whether the simultaneous or sequential application of two disinfectants will yield real synergism or merely produce an additive or complement an effect.

The results for chlorine and chlorine dioxide indicated that the disinfecting efficacy of former was enhanced in the presence of chlorine dioxide. This was probably because whenever either one of these disinfectants is applied first it reacts with various constituents of the medium therefore permitting the other disinfectant to be more potent. However, this enhancement was not high enough to be considered synergistic. Similar effects to those reported here were noted by Ridenour and Ingols (1946) and more recently by Tift et al. (1977) who found that the inactivation efficiency of chlorine dioxide was improved by the sequential addition of chlorine followed by chlorine dioxide.

The complementary effect of peracetic acid and chlorine was clear simply on the basis that the application of peracetic acid provided chlorine with an more acid medium in which to disinfect in which condition it was a better virucide due to the presence of the HOCl ion.

An additive effect was also apparently achieved when peracetic acid was applied 5 min before chlorine dioxide but not vice versa. But when this was simultaneously applied there did not seem to be as much enhancement. It is possible that the two chemicals react with each other in someway but it is difficult to explain this phenomenon because the mechanism of neither is fully elucidated.

The good enhancement that was noted upon the simultaneous addition of chlorine and ozone was almost certainly because one of the disinfectants reacted with various constituents of the medium, thus providing the other with a less inhibitory medium in which to function. However, the decrease in the disinfecting efficacy of chlorine when it was applied after ozone could probably be due to the reaction of ozone.
the effluent and the production of certain compounds that might influence the activity of chlorine. Kott et al. (1980) reported that the application of ozone and chlorine in wastewater did not produce a synergistic effect, but a combined or purely additive effect. Again, a complementary effect was achieved when ozone and peracetic acid were applied together. The presence of the latter provided the former with an acidic medium in which it functions more efficiently. This confirms the previous findings that ozone is more efficient in an acid medium. It may be concluded in general from these data that the application of two disinfectants were rarely synergistic but merely complementary or additive.

An important feature of the results of all the disinfectants tested was the considerable variation in the resistance of various viruses to the various disinfectants which emphasised, that the choice of any one virus as a model was inappropriate. Furthermore, in making any comparison of the behaviour of different viruses, it is worth bearing in mind the innately 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 simian rotavirus (SA 11), an indirect immunofluorescence technique for detecting human rotavirus and a bacterial pour-plate for bacteriophage f₂. 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, one pfu of bacteriophage f₂ is thought to represent one phage particle (Adams, 1959), whereas one pfu of poliovirus may be anything up to 100 particles (Floyd and Sharp, 1977). The implication of these facts is that what is experimentally shown as 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 (Kott et al., 1974; Morris and Waite, 1981; Scarpino, 1975, 1978). There have, for instance, been proposals to use naturally occurring bacteria and viruses to monitor wastewater treatment (Roberts
et al., 1980) and disinfection. 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 (Kott et
al., 1974; Payment et al., 1979; Scarpino, 1975, 1978) although not
always so (Katzenelson and Kedmi, 1979; Safferman and Morris, 1976).
There have also been suggestions that laboratory grown viruses can be
satisfactory models for tests behaviour of viruses for example, simian
rotoviruses 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 simian rotavirus (SA 11). 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 occlusion with a biological film on a surface.
There are several suggestion for innate resistance, for instance,
Hajenian (1979) showed hat the male specific bacteriophages isolated
from wastewater varied widely in their sensitivities to chlorination.
Furthermore, Liu et al., 1971 found that the resistance of wildtype
strains of bacteriophages and certain vertebrate viruses varied widely.
Kelly and Sanderson (1958) reported that poliovirus (strain MK 500),
which was isolated from sewage, was much more resistant to inactivation
by chlorine than the laboratory grown strain of poliovirus 1 and Bates
et al., (1977) even demonstrated that resistant strains of poliovirus
could be developed by selection from disinfected samples.

Probably the most useful way to evaluate a disinfectant in
effluent is to determine its effectiveness against the natural
occurring populations of microorganisms under field operating
conditions, but such work as has been reported is only on bacteria, for
instance, Aieta et al., (1980) reported that a faecal isolate of E.coli
grown in batch culture using nutrient broth was orders of magnitude
more sensitive to chlorine and chlorine dioxide than was its in situ
wastewater grown counterpart. Other studies by Berg et al., (1982)
also endorse the view that antecedent growth conditions greatly
influenced sensitivity to disinfection agents. They found that
populations of E.coli grown under conditions that were more closely
approximate to natural environments were more resistant than those grown
under commonly used laboratory conditions and in the present study as
described above it was found that a faecal isolate of human rotavirus
was substantially more resistant to all the disinfectants tested than a laboratory grown simian rotavirus (SA 11). This high resistance may be attributed to the resistance and ability of naturally present viruses to survive under harsh conditions. If this is true, then the view that antecedent growth conditions alters the resistance of bacteria to disinfectants and that bacterial populations grown under conditions that are more resistant than those grown under optimum laboratory growth conditions would be valid (Berg et al., 1982).

It is worth drawing attention again to the interesting observation that the test viruses behaved differently to all four 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, was somewhat similar in their response although Coxsackievirus B5 was usually the most resistant, but it must be emphasized again that over a narrow threshold value all viruses could be inactivated so 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.

Although the mechanism of the various tested disinfectants is not fully understood they nevertheless work in practice. Chlorine has long been the disinfectant of choice for both water and wastewater treatment by virtue of its virucidal and bacteriocidal efficiency, low cost, convenience and relatively long lived residual. However, the high chlorine dosages needed for the disinfection of some wastewaters came under criticism due to the toxic by products, trihalomethanes and other chlorinated organics, that may be produced. Furthermore, the chlorine residual in effluents does not apparently provide much protection from secondary contamination. Chlorine dioxide, although more expensive than chlorine, may be a good alternative and its advantages over chlorine are: it does not react with ammonia to form chloramines nor is its efficacy influenced by the presence of ammonia, it does not react with organic matter to form some classes of chlorinated organic compounds considered hazardous to the public health (e.g. trihalomethanes), it does not react to form chlorinated phenols...
to the extent that chlorine does and it is a powerful oxidant over a wide pH range. Furthermore it provides a residual that is easily measurable. This feature is also a major advantage that chlorine dioxide has over ozone, but which is quite difficult to determine accurately in sewage effluents, although it is also a promising alternative to chlorine. This is because ozone, like chlorine dioxide, is not affected by ammonia and not greatly dependent on pH(6-10) and temperature (20°-30°C), it does not leave a toxic residual and it adds dissolved oxygen to water. However, disadvantages of ozone are the high capital cost for the establishment of the ozonation plant, it does not maintain a stable residual and requires effluent which has been subject to some form of pretreatment to minimize ozone dosage. Peracetic acid is another possible alternative disinfectant. It has non-toxic by-products is not subject to organic demand leaving it available with a long term potential. However, the present study showed that it was the least efficient virucide and such high concentrations were required tha it would not be applicable in practice. It should be emphasized that further studies need to be done to learn more about the mechanism of action of these and other disinfectants and such information could well lead to the selection of other more effective disinfectants.

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I-Cell culture media

a. **BGM cell culture** The growth medium consisted of 10% (v/v) Eagles MEM with Earles salts (Flow), 10% (v/v) foetal calf serum (Sera Lab), 1% (v/v) 200 mM L-glutamine, (Flow), 1% (v/v) non essential amino acid (NEAA) (Flow), 1.1% (v/v) sodium bicarbonate (Flow), 100 units/ml penicillin (Glaxo) and 100 μg/ml streptomycin (Glaxo) made up to 100 ml with sterile distilled deionised water. Maintenance medium was prepared as for growth medium but with 2% foetal calf serum instead of 10% and the sodium bicarbonate content increased to 2.2% v/v. Diluent was growth medium without serum.

b. **MA-104 cell culture.** The growth medium consisted of 10% (v/v) Eagles MEM with Earles salts (Flow), 10% (v/v) foetal calf serum (Sera labs), 1% (v/v) 200 mM L-glutamine, 1% (v/v) non essential amino acid, 0.13% (w/v) sodium bicarbonate, 100 g/ml penicillin and 100 μg/ml streptomycin made up to 100ml. Serum free growth medium was like the growth medium but without serum and with the sodium bicarbonate content increased to 0.26% (w/v) and 7.5 μg/ml trypsin (Wellcome Reagents). Agar overlayer medium consisted of 10% (v/v) Eagles MEM with Earles salts, 1% (v/v) L-glutamine, 1% (v/v) non essential amino acid, 0.13% (w/v) sodium bicarbonate, 7.5 μg/ml trypsin (tissue culture grade), (Wellcome Reagents) or 20 μg/ml porcine pancreatin (Sigma), 0.003% (w/v) neutral red, (Oxoid), 100 μg/ml penicillin; 100 μg/ml streptomycin, 1.2% (w/v) purified agar (Oxoid) and 100 μg/ml DEAE-dextran (Sigma) made up to 100ml.
II-Storage of cells

Three day old cell monolayers were washed with PBS ([a] below) and then trypsinized ([b] below). The detached cells were dispersed by pipetting vigorously in a small volume of cell freezing medium ([c] below). The concentration of viable cells was determined and cell counts adjusted to $1.5 \times 10^6$ viable cells/ml and were suspended in freezing medium (2.1e) and then distributed in 0.6ml volumes into plastic ampoules. The ampoules were placed at 4°C for 1 hour and then frozen carefully down to -196°C at which temperature they were stored.

a. **Phosphate buffer saline (PBS)** was prepared with tablets of PBS (Oxoid) dissolved in distilled deionized waters autoclaved at 15 psi for 15 min.

b. **Versene trypsin solution** Freeze dried trypsin 1:300; (Wellcome Reagents Ltd) was reconstituted in 10 ml sterile deionized water and stored at -20°C in 1 ml quantities. When needed 1 ml trypsin was added to 100 ml versene solution (1:5000; Wellcome Reagents Ltd). All working solutions were stored at 4°C.

c. **Freezing medium** was growth medium with 20% (v/v) foetal calf serum and 7.5% (v/v) dimethyl sulphoxide (BDH Chemicals).

III-Media for cultivation of Escherichia coli and bacteriophage f2

a. Tryptone soya broth (TSB) was prepared with 3 g tryptone soya broth (Oxoid) in 100 ml distilled water and autoclaved for 15 min at 15 psi.

b. **Tryptone - yeast extract medium (TYE)** consisted of 10g tryptone (Oxoid), 1g yeast extract (Difco) and 8g NaCl (BDH Chemicals) dissolved in 11 distilled water and sterilized by autoclaving for 30 min at 15 psi. To this base medium was added aseptically: 5 ml 20% glucose (BDH Chemicals), 4 ml 0.5 M CaCl$_2$ (BDH Chemicals), 1 ml 10 μg/ml thiamine HCl (BDH Chemicals).
c. Dilution fluid was prepared with 0.3 g NaCl, 1.0 g peptone, 1.0 ml 0.5 M MgSO$_4$.6H$_2$O, (BDH Chemicals), 10.0 ml 1 M Tris HCl pH 7.8 (BDH Chemicals) made up to 1 l with distilled water and autoclaved for 15 min at 15 psi in 100 ml volumes.

d. Soft overlay agar consisted of 8.0 g bacto nutrient broth (Oxoid), 6.5 g standard agar (Davis) and 5.0 g NaCl, made up to 1 l with distilled water, distributed as 4 ml volumes into bijoux bottles and then autoclaved for 10 min at 10 psi.

e. Lawn agar consisted of 10.0 g tryptone (Oxoid), 5.0 g yeast extract (Oxoid), 5.0 g NaCl, 1.0 g glucose (BDH Chemicals) and was made up to 1 l with distilled water, and the pH adjusted to 6.8 - 7.2 with 1 N NaOH. 10 gm Agar (Davis) was added and the medium autoclaved in 500 ml portions for 15 min at 15 psi. After cooling to about 50°C in water bath the following sterile solutions were added to each 250 ml portions. 5.0 ml 0.5 M CaCl$_2$.2H$_2$O (BDH Chemicals) and 5.0 ml 0.5 M MgSO$_4$.6H$_2$O (BDH Chemicals). The media was mixed well and about 15 ml portions of it poured into sterile 9 cm diameter, single vent petri dishes (Sterilin) and allowed to set.

IV-Chemical analysis of effluent

a. pH The pH of the effluent was read using a pH meter 7020 (Electronic Instruments)

b. Determination of ammonia - Nessleriser method

One Nessleriser glass was filled to the 50 ml mark with distilled water and placed in the left-hand compartment of the Nessleriser (BDH Lovibond). Another glass was filled with filtered effluent and placed in the right-hand compartment. Two ml of Nessler's reagent was added to each glass, mixed and the colours produced were compared with the colours on the standard discs exactly 5 min later, with the Nessleriser standing against a uniform source of light. If the colour produced was too intense to read, an appropriate dilution of the sample was prepared and the test repeated. The effluent samples usually did not need any dilution.
The readings on the comparator discs (NAA, NAB and NAC) correspond to the quantity of ammonia (NH$_3$) in µg in the sample used. To convert a reading to ppm, the conventional way of reporting quantities in water systems, the readings were multiplied by a factor of 0.02 thus a reading of 10 µg in a 50 ml sample corresponded to 0.20 ppm (mg/l).

c. **Suspended solids**

The contents of the vessel containing the effluent were mixed well in order to resuspend any solids that has settled. Two hundred ml of effluent were filtered through a 4.7 cm diameter 0.1 µ GF/C filter (Whatman), which was weighed beforehand. The filter was then dried for one hour in an oven at 105°C, cooled in a dessicator and weighed again. The difference in weights was multiplied by 5 to get the weight of suspended solids per litre (ppm) (Dept of the Environment, 1972).

d. **Biochemical oxygen demand**

was determined using the procedure outlined by Golterman (1971).

e. **Chemical oxygen demand**

was determined using the procedure outlined in standard methods (1971).