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Virus Removal by Disinfection of Effluents

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ABSTRACT

The safe disposal of effluents can present a major problem to large urban communities because of their inevitable content of potentially pathogenic enteric viruses. At least one hundred types of virus may be present although many of these are difficult or even impossible to characterise under these conditions. Wastewater treatment does not greatly effect the survival of many enteric viruses and some survive well even after effluent disposal. The use of disinfectants for the inactivation of virus in effluent is practicable but requires careful manipulation in order to avoid the disemination of byproducts toxic to man or capable of interferring with the ecology of the receiving waters or soils. No one system is likely to be either universally acceptable because of the variable quality of effluents and much research remains to be done before guidelines can be recommended or established.

KEYWORDS

Enteric-viruses, effluent, disinfection, halogens, chlorine dioxide, ozone, peracetic acid

INTRODUCTION

Is disinfection of effluents necessary? Seven years ago Berg (1973) made the point that although it was not easy to remove all viruses from sewage, the technology to do so existed and we should be prepared to pay the cost. It is the object of this brief review to re-examine the practicability of achieving virus-free effluents, a subject recently reviewed by several others in particular, Grabow (1979), White (1979) and Bitton (1980).

The safe disposal of effluents and the associated sludges can present a major problem to urban communities where the volumes involved may exceed millions of litres each day. Although these products of wastewater treatment are potentially valuable for irrigation and fertilisation of land, they are usually discarded because it is simpler and cheaper . Sludges are frequently dumped in the sea, but may be spread on land where the treatment works are too far from the coast for transportation to be economical. Effluents typically pour into rivers, estuaries or the sea depending on the location of the treatment works, but, where water is scarce,they may be used as an irrigant or even recycled for

potable supply (Grabow, 1979). However, apart from the technical and engineering problems involved in such productive distribution of effluent, serious consequences may result from their use because of their load of chemical contamination and the presence of pathogenic microorganisms including viruses. The degree of chemical and biological contamination reflects on the origin of the wastewater which may range from purely domestic sewage to principally industrial effluents. Furthermore, the quality of products of wastewater treatment depends on the type of process used which range very widely indeed. The final processing or finishing of effluents and sludges is necessarily different because of their different constitution and this is particularly obvious so far as the removal of viruses is concerned. The removal of viruses from sludges has only relatively recently attracted serious attention (Cliver, 1975; Berg, 1978; Osborn and Hattingh, 1978) but for effluents, various procedures have been adopted for some time, particularly disinfection with chlorine, a treatment now under critical review.

The problems associated with the removal of viruses from effluents are complex and it is essential to pay close attention to the following interdependant questions if a proper evaluation of suitable methods of treatment is to be reached:

1. How many types of viruses are present in effluent, how numerous are they and what is their pathogenic potential?

2. How reproducible and sensitive are the methods for the isolation and characterisation of viruses in effluent and is there a representative virus.

3. How does wastewater treatment affect the distribution and fate of viruses and how well do viruses survive after the disposal of untreated effulent?

4. What methods are available and practicable for the removal of viruses from effluents, how do these work and is there a recommended procedure?

1. Viruses get into wastewater with the faecal solids within which they may be present at levels of up to 10^{10}/g as judged by electron microscopy (Flewett, 1977). However, levels of infectious virus in faeces may be much lower (Madely, 1979) and since faecal solids represent only a minute fraction of wastewater, the predicted input of infectious virus may be no more than 10^{4}/litre (Melnick, Gerba and Wallis, 1978).

It is well known that at least a hundred different types of enteric viruses(Table 1) may be found in human faeces (Melnick, Gerba and Wallis, 1978), of which at least some could be expected to be in final effluents. It is also possible that certain viruses excreted with the urine could also be present (Utz, 1974) but in general, it is likely that the frequency and pathogenic potential of enteric viruses will be influenced by geographic, seasonal,as well as socioeconomic factors. Most enteric virus infections in developed countries are sporadic and episodic but in developing regions they may well be seriously epidemic. The range of symptoms they may cause include not only gastroenteritis but such divergent clinical features as meningitis, exanthema and even respiratory symptoms (Andrews, Periera and Wildy, 1978). Some enteric viruses, for instance poliovirus vaccine strains, may for obvious reasons be regularly isolated from effluent or contaminated waters although apparently not necessarily so even during a vaccination campaign (Katzenelson & Kedmi, 1979). Of the other common enteric viruses, the echoviruses, the coxsackieviruses, the reoviruses and the adenoviruses many may be isolated throughout the year, but

TABLE I - HUMAN VIRUSES FOUND IN FAECES

VIRUS GROUP	COMMON CLINICAL SYMPTOMS
PARVOVIRUS (3) *	Respiratory disease (?)
POLIOVIRUS (3)	Paralysis, meningitis.
ECHOVIRUS (34)	Meningitis, respiratory disease, diarrhoea.
COXSACKIEVIRUS A (24)	Meningitis, respiratory disease.
COXSACKIEVIRUS B (6)	Myocarditis, respiratory disease, meningitis.
ENTEROVIRUS (4)	Respiratory disease, meningitis, conjunctivitis.
HEPATITIS A (1)	Hepatitis.
NORWALK AGENTS (5)	Gastroenteritis.
ASTROVIRUS (1)	Gastroenteritis.
CALICIVIRUS (1)	Gastroenteritis.
REOVIRUS (3)	(None ?).
ROTAVIRUS (4)	Gastroenteritis
ADENOVIRUS (3)	Respiratory disease, conjunctivitis,gastroenteritis
CORONAVIRUS (1)	Gastroenteritis.

* (number of serotypes)

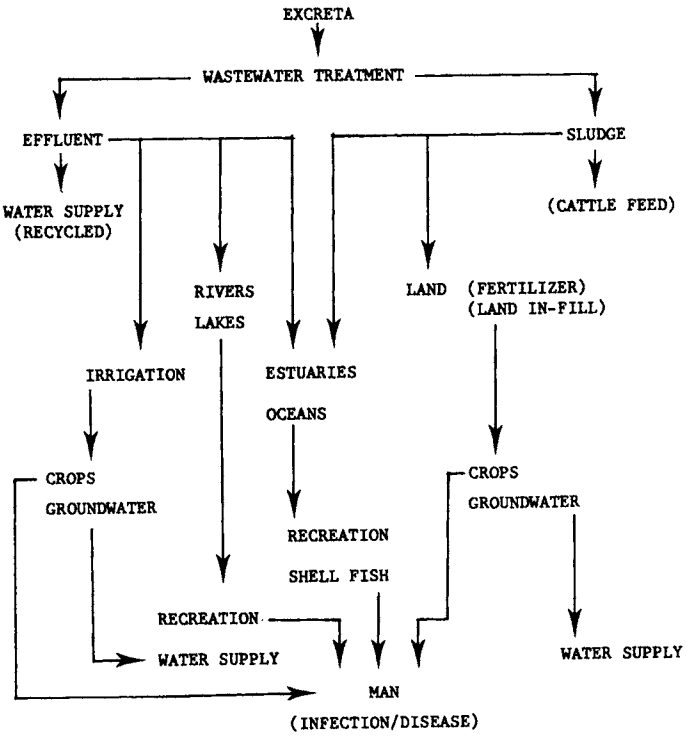
TABLE 2 - HUMAN VIRUSES FOUND IN FAECES

VIRUS GROUP:	CULTIVATION IN LABORATORY:
PARVOVIRUS	- ve
POLIOVIRUS	+ ve (several cell cultures)
ECHOVIRUS	+ ve (several cell cultures)
COXSACKIEVIRUS A	+ ve (neo-natal mice)
COXSACKIEVIRUS B	+ ve (several cell cultures)
ENTEROVIRUS	+ ve (several cell cultures)
HEPATITIS	+ ve (monkey only)
NORWALK AGENTS	- ve
ASTRO VIRUS	- ve
CALICIVIRUS	- ve
REOVIRUS	+ ve (several cell cultures)
ROTAVIRUS	+ ve (incomplete replication in cell culture)
ADENOVIRUS	? +ve (HEK cells only)
CORONAVIRUS	- ve

the serotypes may vary from year to year and valuable corroborative data for this has been provided in the U.K., by the Public Health Authority through its weekly and quarterly reports (CDSC, 1980). Unfortunately, several important enteric viruses such as Hepatitis A, the rotaviruses and several so-called 'small round viruses' cannot be readily cultivated in the laboratory (Table 2) and evidence for them depends wholly on clinical or immunological data, or on their visualisation in the electron microscope.

Although many different enteric viruses are likely to be present in wastewater, the risks of transmission of infection via contaminated water in developed countries by various routes (Fig. 1) is thought to be slight although probably increasing, but elsewhere the risks may be very great indeed (WHO, 1976). Undoubtedly, outbreaks of gastroenteritis have been associated with effluent-polluted lake water (CDC, 1979; Denis et al., 1974) and bathing beaches (Cabelli et al., 1979) and many cases of Hepatitis A as well as other enteric virus infections have resulted from the consumption of shell fish harvested from contaminated waters (Gerba and Goyal, 1978).

Figure 1. Routes for the transmission of viruses via wastewater treatment.



In general, where epidemiological evidence for gastroenteritis and diarrhoeal diseases has been assembled, it accounts for about half the recorded outbreaks of waterborne diseases (Craun, McCabe and Hughes, 1976), although in relatively few cases is the aetiological agent identified either by isolation or by serological means. It is however believed that a substantial proportion of these infections could be of viral origin because of the development of symptoms between one and two days after infection and the recovery of the subject within two to three days without significant sequelae.

Although clinically characteristic infections such as Hepatitis A may well occur, it must be borne in mind that only a small proportion of infected subjects, especially children, develop symptoms (Evans, 1978) and this is also true of virtually all enteric virus infections even those which may be very serious, such as poliomyelitis. The significance of this observation is that infected symptomless individuals may well represent foci for further infection in the community, and should this happen, then disease of epidemic proportion could subsequently develop. Of course, in these circumstances, it would be very difficult to relate such an epidemic to an original waterborne infection. Incidentally, it is suggested by some that low levels of infection through the water route may be advantageous to a community by providing it with a relatively harmless mechanism for the circulation of viruses which could otherwise become dangerously epidemic in a largely susceptible population (Mosley, 1967). It has also been argued (Gamble, 1979) that even if the elimination of such low-level transmission of viruses were possible it would be unlikely to have much effect on endemic infection which is commonly by the direct person to person method. Neverhteless, it is thought that viruses in water represent an underestimated problem (Mahdy, 1979).

2. An assessment of the extent of the hazards of viral contamination of effluents and the effectiveness of any treatment for its removal depends critically on the sensitivity and reliability of the procedures for the recovery and characterisation of the viruses. Successful characterisation of viruses depends essentially on the infectivity test because not only is this the paramount property under scrutiny but the levels likely to be present are very small indeed. Certainly they would not be sufficient, without impractical levels of concentration, for detection by electron microscopy or serological techniques, both of which require at least 10^5 particles/ml.

Although infectivity assays may be quite sensitive, it must be borne in mind it is possible that only one infectious particle is sufficient to infect a ,pa susceptible subject (Westwood and Sattar, 1976) and in this regard, it is important to note the difficulty which exists in defining an infectious particle (Floyd and Sharp, 1979) which may be an aggregate of particles, or even particles embedded in organic floc. Another impoortant limitation on the interpretation of infectivity assays relates to the sensitivity of the selected cell culture. The choice of this is often dictated by such pragmatic considerations as cost and practicability, for instance, it is claimed that one of the most sensitive cell systems for a wide range of enteric viruses is the primary rhesus kidney cell culture but this is now virtually unobtainable and one or more monkey kidney cell lines like vero and BGM have become popular alternatives (Schmidt et al., 1978). None of these cell cultures is equally sensitive to all the enteric viruses, furthermore, some viruses replicate in them much more slowly than others so that overgrowth of the culture by one virus may occur, a not unlikely event, from an effluent sample which could well be expected to be contaminated with several different viruses. This problem would be less troublesome where the assay for infectivity was the plaque test, but it would be intractable where a quantal assay was employed based on degeneration of

the whole culture. Even greater problems occur when the virus is non-cytopathogenic, and this could be compounded if such a virus interfered with the growth of a cytopathogenic virus. Another limitation which must be applied to the interpretation of infectivity assays is that cell sensitivity to laboratory-adapted virus and to fresh viral isolates is known to differ, especially to isolates from faecal samples (Madeley, 1979) with which virus in effluents are comparable. These various points highlight the difficulties in developing viral standards for effluent quality where the assay of infectivity is only effective for some, uncertain for others and totally unavailable for those in which we are particularly interested

Another constraint on the sensitivity of the infectivity assay relates to the small number of infectious particles which may be present, such that concentration of the sample before isolation may well be essential. Many methods for concentration of viruses from water have been recommended, such as filtration, flocculation or two phase liquid separation, but their value for effluent treatment will be greatly influenced by the effluent quality especially its content of suspended solids. (Seeley and Primrose, 1979). One particular problem is the concentration of cytotoxic substances which may be difficult to characterise and remove (Glass, Sluis and Yanko, 1978; Schmidt et al., 1978).

Having isolated and cultured a virus from effluent, there remains its characterisation and identificationwhich is a major task. This, to a large extent, depends on the development of a characteristic cytopathology and the application of serological tests, particularly the neutralisation test. Other serological tests like immunoflourescence for non-cytopathogenic viruses may have to be used and characterisation by electron microscopy may also be necessary.

Clearly, the complexity of this situation makes the testing for the presence of all possible enteric virus out of the question, yet it is equally true that the monitoring for the effectiveness of effluent treatment is essential, so the selection of an indicator virus would be useful. For bacteria it is generally agreed that certain coliform bacteria may be regarded as representative, but with viruses it is not at all obvious which, if any one type, could be selected. The idea that enteric bacteria could be used as models for enteric viral contamination has been rejected, not only because there is little correlation between the levels found of the two groups, but because many viruses are more resistant to disinfection, which is the usual object of the study (Berg, et al., 1978). Furthermore, the range of types of enteric viruses which may be present vary widely because, unlike many enteric bacteria, they do not appear to form part of the natural gut flora but occur sporadically. However, it has been frequently suggested that a coliphage,which would be expected to be part of the natural flora, would be suitable indicator of enteric virus pollution (Scarpino, 1975; Kott et al 1974). The coliphages certainly fulfil a number of recommended prerequisites (Haas, 1977) that they are present whenever their pathogenic hosts are present, they are incapable of regrowth in the effluent, but are at least equally resistant to environmental stress, including disinfection. Furthermore, they are present in large numbers and are readily enumerated. However the selection of a particular coliphage poses a problem, for instance, an obvious contender like the f2 coliphage recommended by Shah and McCabe (1972) is not the only phage to replicate in its specific host Escherichia coli (K_{12}Hfr). The use of the less selective E. coli B strain would isolate even more types of coliphages (Kott, et al., 1974). The deliberate introduction of a phage type unlikely to occur naturally, for example, a phage of Serratia marcesens (Castens and Coetzee, 1965) would be impractical for routine monitoring of effluent treatment, even supposing it had the right properties, like high resistance to disinfectant. To take a phage dependent on a host thought to be commonly present

in wastewater treatment like a cyanophage (Smedberg and Cannon, 1976) would only be useful if it occurred reliably and was also very resistant to effluent treatment. Nearly all these studies, as well as those in which a model enterovirus was propsed, suffer from the disadvantages peculiar to such laboratory studies, in that it is not certain how far results can be extrapolated to field conditions.

3. So how worried need we be about viruses in effluent? The answer depends essentially on the nature of the wastewater treatment providing the effluent and, of course, on the fate of the effluent and the survival of the viruses in the receiving waters or soil. The nature of the wastewater treatment will inevitably influence the behaviour and fate of viruses, for instance, it is well known that much more virus is found in effluents after percolating filtration than those from the activated sludge treatment (Berg, 1973).

The degritting and settling procedures carried out during the early stages of sewage treatment lead to an unpredictable dispersion of the virus originally present in the faecal solids. For instance, although a substantial proportion of the solids settle out into the primary sludges there is little apparent loss of infectivity in the settled sewage effluent (Kollins, 1966) presumably due to a break up of vvviral aggregates and flocs to release individual infectious virions and the fact that viruses have a strong predeliction to adsorp to solids (Bitton, 1976) accounts for their effective removal during the activated sludge aeration. Indeed, good quality effluents from such treatment should have a very low suspended solids content and a good correlation between low solids content and low viral infectivity of effluents has been observed (Balluz and Butler, 1979). The absence of solids from effluent is also important because they provide a mechanism for the protection of adsorbed virus against disinfection (Boardman and Sproul, 1977; Hajkal, et al, 1979) Virus in untreated final effluents is ultimately subject to various forms of environmental stress in the receiving waters or soil (Bitton, 1980), but the fact that virus is readily isolated from effluent polluted river water obviously implies reasonable survival and experimental studies in different types of water provide additional evidence of good survival (Gerba, Wallis and Melnick, 1975) especially in clean (Mehnel et al., 1977) or heavily polluted waters but surprisingly and inexplicably, less so in moderately dirty water (Clarke, et al 1969). Virus apparently survives less well in sea water (Gerba and Schaiberger, 1975b) indeed there is some evidence that it is specifically inactivated (Fujioka, Loh and Lau, 1980). Disposal of effluent to land may result in percolation of virus through the soil to contaminate ground water supplies (Schaub and Sorber, 1977) but normally viruses eventually disappear, due to adsorption and ultimately inactivation. .pa
The distribution of viruses in soil is known to be strongly influenced by the degree of hydration, the pH, the ionic strength and the organic content of the medium (Akin et al., 1971). It is clear from all this that the ecology of viruses during wastewater treatment and after the disposal of the products is evidently very complex. Furthermore, it is also worth noting that virions enter the sewage plant discontinuously and in various states of aggregation so that no one sample at any one stage in the process can be taken as representative of anything other than what is present at that time in that sample.

4. The methods available for the inactivation of viruses in effluent differ little in principle from those applied to potable water, but are distinct from the disinfection of viruses contaminating, laboratory or medical equipment, where highly toxic chemicals like detergents, phenols, formaldehyde or permanganate may be used (Spalding et al 1977). For effluents, the choice of treatment is limited by the requirement for a high quality final effluent free from harmful by-products. The treatment 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 or 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).

TABLE 4

CHEMICAL DISINFECTANTS

CHLORINE	BROMINE
CHLORINE DIOXIDE	BROMINE CHLORIDE
IODINE	OZONE
COMBINATIONS OF THESE CHEMICALS	
OTHERS, e.g. PERACETIC ACID	

TABLE 3

PHYSICAL DISINFECTANTS

IONISING IRRADIATION:
e.g. GAMMA RAYS OR ELECTRONS
NON-IONISING
e.g. ULTRAVIOLET LIGHT
PHOTODYNAMIC OXIDATION
HEAT

The disinfection methods available range from the purely physical (Table 3), to purely chemical (Table 4) like the application of the halogen disinfectants. In addition, there is the more complex and interesting application of a combination of one or more of these basic methods. Indeed, the distinction between the categories is by no means clear cut, especially in effluents where, because of the impurities, a number of mechanisms may operate antagonistically or synergistically. For example, ionising irradiation although able to act directly on the virus, may also function chemically in so far as it may induce the production of toxic free radicals. In contrast, a chemical disinfectant like chlorine, may react preferentially with organic and inorganic contaminants so that its full potential is lost. Thus, the effectiveness of any one of these treatment systems may be much influenced by the presence of chemical and physical impurities and the pretreatment of effluents to remove these may be essential for the effective, economic and safe disinfection of viruses (Guy and McIver, 1977). Culp (1971) questioned the need for disinfection after effective pertreatment but this very much depends on the fate of the effluent and a number of procedures have been adopted for the improvement of effluent quality before disinfection. Of the most commonly applied methods, flocculation with a variety of salts or synthetic polyelectrolytes is probably the most useful, but not all viruses behave in the same way, for instance rotavirus is less efficiently adsorbed to aluminium hydroxide than poliovirus, (Farah et al., 1978), but in practice such differences might not be important.

Filtration of effluents by slow sand filteration (Poynter and Slade, 1977) results in the removal of virus by adsorbtion to the complex microbial population which grows in the upper layers,but rapid sand filtration although removing solids from effluent and therefore effecting some useful clarification does not remove suspended virus well (Guy and McIver 1977) furthermore its function in this regard is greatly influenced by pH, ionic concentration and organic contamination. Adsorption of virus to such substances as activated charcoal is efficient from clean water (Oza and Chang, 1975) but such substances are rapidly blocked by organic matter (Sproul, 1968). Incidently all these

treatments which result in the removal of virus merely defer the problem of its inactivation, to the treatment of the resultant sludge.

Excess lime treatment has the greatest promise for the treatment of effluent with resultant inactivation of virus (Grabow, Middendorf and Basson, 1978) It is thought that the single-stranded RNA viruses are particularily susceptible to the high pH values obtained with the genome as the prime target (Ward et al 1978) although Sproul (1972) thought that inactivation was the result of denaturation of the capsid. In general, enteric viruses have a wide pH tolerance which is, presumably, a reflection on their natural history as gut parasites transmitted by the faecal oral route and, therefore, exposed to the acidity of the stomach and compensating alkaline secretions of the small intestine.

Inactivation of virus present in good quality effluents by physical of physico-chemical methods has attracted increasing attention because disinfection by chlorine has come increasingly under attack. The most promising developments use ionising (Sinsky, 1977) and non-ionising irradiation (Vajdic, 1970) not only alone but in conjunction with chemical methods. The main disadvantages in these methods is that ionising irradiation by gamma rays or high energy electrons, like that with ultraviolet, has poor penetrating power and its effect is greatly diminished by turbidity. However, although expensive in comparison to the application of conventional disinfectants they may, as a result of improved, technology become competitive and acceptable (Singer and Nash ,1979) Heat also would work well but remains impractical for effluents, although applicable to sludges (Ward and Ashley, 1978) where anaerobic digestion results in the development of elevated temperature. However, studies of heat inactivation of viruses in water have, incidently, provided a useful basis for understanding viral inactivation in general. In particular, of the characteristic biphasic inactivation where after treatment there remains a residual of apparently unaffected virus. The influence of pH, divalent cations and redox potential on heat inactivation may also provide some insight into the influence of these factors on chemical disinfection, indeed the action of pH, cations or redox potential alone on viruses should not be ignored (Poynter, Slade & Jones 1973) because both redox potential and pH are both believed to act on viral capsid proteins (Mandel, 1971) which may either alter their sensitivity to disinfectants or their ability to adsorb to sensitive cells.

The most practical and successful disinfectants of wastewater effluents are the oxidising agents like the halogens, ozone and peroxides, although it is likely that the action of these chemicals is not exclusively through oxidation. The general principles of disinfection were established by Chick (1908) who formulated a law which stated that the ratio of a given population decreased exponentially with time, that is that the reaction obeyed 'first order' kinetics. However this only applies if the disinfectant is in excess (Hiatt, 1964), if the system is homogeneous and if the interaction between virus and the disinfectant is direct. Such conditions do not, of course, apply in sewage effluent which has a complex and variable constitution.

Deviations from first order reactions have been commonly observed and they mainly fall into two categories, those which show an initial lag before maximum or optimum rates of inactivation develop and those where, after an initial period of rapid inactivation, a plateau develops representing a persistant infectious fraction. Sometimes both features are observed in the same system (Fujoka and Ackerman, 1975). Various explanations have been sought for these phenomena. For instance, an initial shoulder would appear when the multihit response occurred as would be expected with most suspensions of infectious virions which, of course, include viral aggregates of various size (Floyd &

Sharp 1977). There would be,in such examples, a delay in the measured loss even though much virus was actually inactivated. Aggregation could equally explain residual infectivity where infectious particals remained inaccessible to the disinfectant (Broardman 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 population with increased resistance (Bates, Shaffer and Sutherland, 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 a cell with virions with differntly but only slightly damaged genomes. Such doubts re-emphasise the neeed for reliable and reproducable infectivity assays and in such situations each virus particle must be assumed to be infectious and its infectivity determinable.

There are infact few examples where the total number of virions present corresponds to the number of infectious units and usually there is a considerable difference. This also emphasises that a disinfection activity may have to be determined empirically for each virus in question if precise data is required (Hajenion and Butler, 1980). Certainly, assay of its efficacy depends not only on effective assay of infectivity but also on that of the disinfectant.

The mechanism by which the oxidative disinfectants work is probably complex and a basic understanding is required. So far as the halogens are concerned they are known to react with viral proteins and nucleic acids(Olivieri et al., 1975; O'Brien and Newan, 1979) and in this debate it is worth noting the essential structural features of the enteric viruses which have a proteinaceous capsid enclosing the single or double stranded nucleic acid genome. The capsid proteins may be organised as specific receptors, the integrity of which is vital to the infectious process but damage to the receptors may not result in denaturation of the genome which, of course, leaves the possibility that viral replication could ultimately occur. Furthermore, as noted above, partial denaturation of the genome may not inevitably result in failure to replicate. A disinfectant may dissociate or denature viral proteins or react with the genome or both and one which reacts specifically with the genome must be able to penetrate the capsid.

Of the halogen disinfectants, chlorine has a long history as a successful disinfectant (White, 1972). It is most commonly used for finished potable waters and swimming pools but is widely used for treatment of effluents, particularly storm waters and even for oxidation ponds (Kott, 1973). Its chemial properties have been exhaustively examined and described. Basically, when a halogen is dissolved in water it hydrolizes to form the hypohalous acid.

$$X_2 + H_2O \rightarrow HOX + X^- + H^+$$

and the acid ionises to the hypohalite ion.

$$HOX \rightarrow OX^- + H^+$$

Hydrolysis and ionisation are pH, temperature and concentration dependant and the three halogens chlorine, bromine and iodine behave differently. It is important to know this because it is the hypohalous acid which is the most active molecule. For instance at low pH the chlorine molecule is predominant whereas above pH 9 the hypochlorite ion is present. Chlorine functions best against viruses at about pH 6 when optimum levels of the acid are formed (Kott, Nupen and Ross, 1975) and it is worth noting, here, that the pH of many effluents is about pH 8.

Another interesting and important phenomonon is that hypohalous acids react with ammonia to form mono, di and tri halomines:

$$HOX + NH_3 \rightarrow NH_2X + H_2O.$$

$$HOX + NH_2 \rightarrow NHX_2 + H_2O$$

$$HOX + NHX_2 \rightarrow NX_3 + H_2O$$

These reactions are concentration dependant such that, for instance, when the ratio of chlorine to ammonia is greater than 20:1, free chlorine is again available for hydrolysis, a phenomenon well known as break-point chlorination (Palin, 1950). The reaction is also pH dependant with the highly substituted derivatives being found in acid conditions. The mono and dihalomines decompose to release nitrogen and those formed with chlorine are the most stable:

$$2NH_2X + HOX \rightarrow N_2 + 3HX + H_2O$$

$$2NHX_2 \rightarrow N_2 + 2HX + X_2$$

Halogens react with other nitrogenous matter to form similar derivatives but the chemistry of such compounds is complex. Halogens also react with inorganic matter to produce stable but non-disinfecting compounds and the loss of halogen this way is referred to as the halogen demand of the system, which is usually high in effluents.

In the case of chlorine it is clear that it has some remarkably useful characteristics especially in water with slight nitrogenous contamination when it forms stable, persistent and disinfecting chloramines. These are, however, less active against viruses than against bacteria (Shah and McCamish 1972; Hart, 1974). In heavily polluted effluents the loss of chlorine, its conversion into chloramines and many even less acceptable byproducts may be so great as to render its highly unsuitable (Ward and DeGraeve, 1978; Smith, McCall and Chen, 1977) mainly because of their toxicity to the natural flora and fauna of the receiving waters and their carcinogenic potential. Furthermore, it may be necessary to acidify effluent to obtain optimum conditions for chlorine disinfection (Mills, 1973).

The disinfection potential of chlorine against viruses has been demonstrated by many people with experimental model systems as well as in the field situation. It is important to note that viruses range in their sensitivity (Lund 1964) for instance a laboratory strain of coxsackievirus was the most resistant enterovirus tested and some other enteritis viruses like reovirus and adenovirus were more sensitive than than any of the tested enteroviruses. More interesting is the observation that fresh isolates of enterovirus appeared to be more resistant than laboratory adapted strains(Kelly and Sanderson, 1958, Lui et al 1977). Furthermore, certain viruses may have resistance selectively induced by cultivation in the presence of chlorine (Bates, Shaffer and Sutherland, 1977). This observation raised objections to inadequate chlorination (Nupen and Morgan, 1978) because of the possibility that such resistant viruses may, when released in effluent, ultimately replicate in susceptible people.

The need for thorough mixing of disinfectant to ensure optimal activity is stressed (Longley, 1978) and this is especially important where virus is

adsorbed to particulate matter by which it is protected (Boardman and Sproul, 1977; Hijkal et al, 1979) so the design of efficient chlorinating systems is .pa important (Tickhe, 1976). Furthermore, its meaningful assay in effluent has been closely questioned (Morrow and Martin, 1977). Of these methods chemical assay, especially by the DPD method (Palin, 1974), is the most accurate and reproducible but for automatic monitoring the known relationship between redox potential and disinfection potential (Victorian, Hellstrom and Rylander, 1972) has led to the development of assay based on the electrical charge however, the value of this has been questioned (Rosenblalt, 1975) especially its application to water heavily contaminated with nitrogenous compounds (Johnson, Edwards and Keeslan, 1978) when an electrode responding directly to hypochlorous acid was used. No one method is wholly satisfactory because the chemical property of a disinfectant may not correspond with its disinfecting potential.

The problems resulting from the excessive use of chlorine for effluent disinfection (Comp. General, 1977) has led to quite extensive searches for alternative. Furthermore, the disontinuance of the production and distribution of liquid chlorine, which some critise (Humphrey, 1978),has precitated even more active interest in alternative disinfectants.

Of the other halogens the one least likely to be useful alone is iodine. It is both poorly soluble in aqueous solution and not very reactive. However, it is easily stored and transported and is therefore useful for emergency sterilisation of water. In aqueous solution it forms hypoiodous acid and hydrogen iodide, the acid being the most active molecule:

$$I_2 + H_2O \rightarrow HOI + HI$$

It experiences considerable demand but it persists for longer in effluent than chlorine (Cramer et al., 1976) and is a more effective virucide than chlorine both at neutral pH and, especially, at higher pH values.

Bromine, although known to be a powerful virocide (Taylor and Johnson, 1974, Floyd, Johnson and Sharp, 1976; Hajenian and Butler, 1980) is poorly soluable in water and is a highly corrosive liquid. Although, as such, it is not suitable for effluent treatment its chlorine derivative, bromine chloride has great promise. Indeed this has been found to be more effective against viruses than chlorine on a weight for weight basis (Keswick et al., 1978; Kawata et al., 1979) and experienced less interferance from added ammonia or glycine in sewage effluent. However, its potential against a wide spectrum of enteric viruses has yet to be evaluated as has the effect of its residual on the ecology of the receiving waters (Mills, 1977). It is readily soluable in water and in aqueous solution hypobromous acid is formed which is readily ionised:

$$BrCl + H_2O \rightarrow HOBr + HCl$$

If some hypochlorous acid is also formed it is unstable in the presence of the bromide ion forming further hypobromous acid, the most active molecule

$$HOCl + Br \rightarrow HOBr + Cl^-$$

These reactions are pH dependant and the greatest disinfecting potential is between pH 7 and 8 which would mean that for use in effluent no acidification would be required. The disinfectant reacts, of course, with nitrogenous compounds to form bromamines which are claimed to be highly virucidal (Mills, 1975). However, they are short lived which although a disadvantage for clean water treatment would therefore be a valuable property for effluents treatment.

The excellent potential for disinfection possessed by chlorine dioxide was recognised over thirty years ago (Ridenour and Ingols, 1947) and it has been .pa shown to be very useful against viruses (Dowling, 1974; Kawata et al., 1979).In aqueous solution it produces chlorous and chloric acids

$$2ClO_2 + H_2O \rightarrow HClO_2 + HClO_3$$

The normal method of production of chlorine dioxide which is from chlorine and sodium chlorite means that a residual of chlorine remains which in the form of hypochlorous acid reacts with chlorine dioxide to produce the highly reactive chloride ion (Tifft et al., 1977):

$$2ClO_2 + HOCl + H^+ \rightarrow 2ClO_2 + H_2O + Cl^+$$

Some of the early reports on the disinfecting activity of chlorine dioxide failed to take into account that chlorine was present but in more recent studies, especially where the disinfectant is made by heating potassiun chlorate with oxalic acid (Palin, 1948) its activity is better understood. Fortuitously, chlorine dioxide does not readily react with nitrogenous compounds although it does experience a demand from phenolic chemicals. It is this property, resulting in the elimination of colour, taste and odour which has attracted much attention (Ingols, 1975). Its assay in the presence of chlorine is complex but recently a specific spectrophotometric assay has been reported (Knechtel, Janzen and Davis, 1978) which is useful.

From the earliest times ozone has attracted a lot of attention as a water disinfectant. Like chlorine dioxide, it has to be produced in situ but the equipment is simple depending on the convertion of oxygen in an electric arc. However, for accurate and reliable delivery considerable care has to be exercised, and its assay is frought with difficulty because its chemistry in aqueous solution is complex (Peleg, 1976).

$$O_3 + H_2O \rightarrow O_2 + 2OH^-$$

$$O_3 + OH \rightarrow O_2 + HO_2$$

$$OH + OH^- \rightarrow O^- + H_2O$$

$$O^- + O_2 \rightarrow O_3^-$$

$$O_3 \rightarrow O_2 + O\cdot$$

Its disinfectant activity probably resides in the hydroxy and oxide radicals (Hoigne and Baden, 1976; Kim, Gentile and Sproul, 1980) but its value also lies in its capacity to react with many organic carbon compounds incidently resulting in the removal of colour, odour and taste, a subject which has been critically reviewed (Kinman, 1975). It has been shown to be active against a range of viruses (Evison, 1978) and its potential is greatly enhanced by good mixing particularly the application of ultrasound (Dahi, 1975, Burleson, Murray and Pollard, 1975) which probably affects not only the bubble size (Farouq, Chian and Engelbrecht, 1977) but may be useful in the break-up of viral aggregates and the release of virus from floc. Its effectiveness has been compared to that of chlorine (Bollyky and Siegel,1077; Wyatt and Wilson, 1979), but its main disadvantage for effluent is the great demand which has to be met before the formation of the active residual, however there is a complete absence of harmful biproducts.

There remains a small miscellany of chemicals which are known to have a disinfecting potential but which have not been adequately tested. Prime amongst these is peracetic acid. Its virucidal activity was first reported by Klein and Hull (1960) and later by Sprossig and Mucke (1969) and there is a recent laboratory study on viruses in effluent (Hajenian and Butler, 1980). This is complemented by a similar, although more extensive study of its bacteriocidal activity in effluent (Poffe et al., 1978) In aqueous solution nascent oxygen is produced which presumably imparts the disinfecting property:-

$$CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{-}O\text{-}OH \rightarrow CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{-}OH + O\cdot$$

The byproduct, acetic acid, is unlikely to be toxic although it is undoubtedly a microbial metabilite. It appears to suffer negligable demand in effluent since its activity is fully retained against added virus up to thirty minutes after its original application (Hajenian and Butler, 1980). Another common peroxide,the peroxide of hydrogen is a very weak disinfectant (Bayliss and Waites, 1980) and is unlikely to be useful for wastewater treatment except for the control of hydrogen sulphide and bulking.

One of the most interesting developments in the disinfection of effluents could be the application of two or more treatments sequentially or simultaneously to achieve a real or apparent synergistic effect. Apparent synergism would occur, of course, when the action of one treamtent simply removed a substance capable of blocking or inactivating the other reactant. Examples of this have already been noted, for instance the pretreatment of effluent to make it more suitable for chemical disinfection or the more special case of the value of ultra sound during ozonisation (Dahi, 1976).A good example of real synergism is demonstrated by the sequential addition of chlorine and chlorine dioxide where the nett disinfection is improved (Tifft et al., 1977). Usually the advantages of such treatment or equivalent procedures with chlorine and ozone (Ross, van Leeuven and Grabow, 1976; Wyatt and Wilson, 1979) is mainly in the production of a better quality water free from irritating or toxic residuals. True synergism has also been thought to be the result of the combination of monochloramine and iodine (Kerman and Layton, 1976) and iodine with ozone (Buddle, 1973).

A number of other combinations of disinfectants could be usefully explored, for instance chlorine with peracetic acid where the application of the latter causes a fall in pH to acid values (Hajenian and Butler, 1980b) at which chlorine is at its greatest efficiency. A particular problem with such studies especially where the chemicals are applied simultaneously could be in the determination of specific disinfectant residual.

In conclusion it should be stressed that few of the viruses present in effluent can be properly characterised. Furthermore, the health hazard that any potentially pathogenic enteric virus represents in effluent can only be guessed at. However, although the disinfection of effluents, will improve the situation the variable quality of effluents will make it difficult to standardise any procedure. It should also be stressed that overdosing with disinfectant, especially chlorine, should be discouraged and critical studies of alternatives continued, especially, perhaps the development of the combination of disinfectants.

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