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The behaviour of f2 coliphage in activated sludge treatment

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SUMMARY

A model activated sludge treatment plant was used which was functionally very similar to a full-scale plant. It was inoculated with f2 coliphage and the titres of virus in the influent, the mixed liquor and the effluent were monitored regularly. The distribution of the virus in the solids and liquid fractions of the mixed liquor was in the ratio of 18:82 and 20·4% of the influent virus was recovered in the effluent. After inoculation was stopped the titre of virus in the solids fraction of the mixed liquor remained high and unaltered for up to 70 h, whereas the value for effluent reverted to the low background titre originally present. These results are discussed in relation to those reported for poliovirus and it is concluded that f2 coliphage is not a suitable model for studies of the behaviour of human enteroviruses.

INTRODUCTION

Carstens, Coetzee, Malherbe & Harwin (1965) suggested that bacteriophage would be a suitable model for indicating the efficiency of sewage treatment for the removal of viruses. This was quickly followed by the more specific recommendation from Buras & Kott (1966) that the f2 coliphage, a picornavirus, might be a highly suitable indicator for the behaviour of human enteroviruses. More recently, Kott, Roze, Sperber & Betzer (1974) reiterated this claim supported by data on the relative frequency of f2 coliphage and human enteric viruses in both percolating filters and oxidation-pond effluents examined at different times of the year. Since then there have been several reports on the distribution and survival of inoculated f2 coliphage in full-scale and pilot-scale plants. For instance, Naparstek, Olivieri, Kawata & Sherman (1976) monitored the survival of the virus in the different treatment stages of activated sludge treatment and final chlorination. As expected the highest reduction of virus (72%) was observed after chlorination but some reduction also occurred after the preliminary comminutor treatment (7·5%), aeration (11·1%), sedimentation (46·8%) and sand filtration (22·2%), giving a cumulative reduction of 80·5% which confirmed a rate of 80·2% reported earlier (Naparstek, 1973) for a larger treatment plant, 2·9 as opposed to 0·75 million gallons/day.

A pilot scale study has been reported by Safferman & Morris (1976) who seeded f2 coliphage into a three-stage activated sludge plant processing 80000 gallons/day. This type of complex treatment plant, which introduces a sequence of nitrification, clarification and denitrification stages, is very efficient and there was a reduction

of phage across the plant of about 99·97%. The greatest reduction rates were observed in the denitrification and high-rate activated sludge stages, each effecting at least 95% reduction of the relevant influent virus titre.

The removal of f2 coliphage by percolating filter sewage treatment plants has also been investigated. Sherman, Kawata, Olivieri & Naparstek (1975) seeded two full-scale plants with virus and monitored it through all the treatment stages (grit chamber, comminutor, primary settling, filtration, secondary settling and chlorination) and showed a reduction across the plant of 70·0 to 80·0% including the chlorination stage. Reduction across the trickling filter alone, however, was low and ranged from 9·0 to 18·9%.

In general, it would appear from these observations that the behaviour of f2 coliphage in sewage treatment was similar to that of poliovirus although Ranganathan, Malina, Sagik & Moore (1974) produced evidence from radioactive tracer studies that f2 coliphage did not behave exactly like poliovirus. However, Balluz, Jones & Butler (1977), pointed out that there are inherent difficulties in interpreting the results of such complex studies in sewage and it was thought desirable to investigate the behaviour of f2 coliphage under conditions similar to those they used for poliovirus, giving special attention to the association of the virus with the solids fractions of the mixed liquor.

MATERIALS AND METHODS

Activated sludge treatment plant model

The model based on that devised by Curds & Fey (1969) and modified by Balluz, Jones & Butler (1977) was used (Plate 1).

Propagation of stock virus and bacterial host

Eschericia coli (K12 Hfr) and the f2 coliphage were obtained from Dr J. Slade, Thames Water Authority.

The bacterial culture was grown at 37 °C in 3% tryptose soya broth and vigorously aerated until a titre of approximately $10^{8·3}$ cells/ml was reached. Sufficient f2 coliphage was added at this stage to achieve a multiplicity of 5 coliphage particles to each bacterium and the culture was re-aerated vigorously at 37 °C for approximately 3 h by which time the bacteria were lysed and the medium had become clear.

Chloroform (10%) was added and mixed vigorously with the culture which was then allowed to stand at room temperature for 10 min. It was then centrifuged (5000*g*, for 10 min at 20 °C) and the aqueous supernatant was removed and stored over 10% chloroform at 4 °C until required.

Titration of virus

The procedure for plaque assay was according to d'Herelle's soft agar technique as described by Adams (1959). The volume of the inoculum was 0·5 ml/plate, taken from a tenfold serial dilution prepared in dilution fluid containing peptone (0·1%), sodium chloride (0·03%) with $MgSO_4$ (0·0005 M) and tris (hydroxymethyl

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methylamine) buffer (0·01 M at pH 7·8). The lawn agar was prepared by a modification of the method of Loeb & Zinder (1961). The medium contained Bacto tryptone (1%), yeast extract (0·5%), sodium chloride (0·5%) and glucose (0·1%) and was adjusted to pH 7·0 with N-sodium hydroxide, after which calcium chloride and magnesium sulphate were each incorporated at a final molarity of 0·0025. For the soft agar technique the medium contained nutrient broth (0·8%) and sodium chloride (0·5%) in 0·65% agar.

The results of the titrations were expressed as plaque forming units/ml (p.f.u./ml).

Inoculation of the plant and sampling

The reservoir of influent settled sewage was inoculated with sufficient virus to give an average initial titre of $10^{5\cdot9}$ p.f.u./ml. The retention time of the plant was kept at 5·4 h and the temperature and suspended solids at 15 °C and 4000 parts/10^6 respectively.

Samples (5 ml) of the influent, mixed liquor and effluent were taken at intervals and to each was added 0·5 ml chloroform which was thoroughly mixed with the sample in order to destroy viable bacteria. After this treatment the samples of influent and effluent were stored at 4 °C to await later assay. The mixed liquor was fractionated by centrifugation (1500*g*, for 10 min at 20 °C) after the chloroform had been evaporated by bubbling nitrogen gas through it. The supernatant (liquid fraction) was decanted and stored at 4 °C. The deposit (solids fraction) was resuspended to a final volume of 5 ml in the dilution fluid containing 10% calf serum. It was then subjected to ultrasonic treatment with an MSE 150 watt 20 kHz ultrasonic disintegrator with a logarithmic probe No. 34041 at 13 μm for 1 min at 2 °C. After treatment the sample was stored with the other samples at 4 °C. All samples were assayed within 48 h of sampling.

RESULTS

In the period before the introduction of f2 coliphage some unspecified bacteriophage activity was detected in the influent, mixed liquor and effluent (Fig. 1). The greatest background titre was detected in the influent and the effluent titre was 19% of this (Table 1). In the mixed liquor the solids fraction carried the greater proportion of the detectable phage with the ratio between solids and liquid fraction being 68·6:31·4. No attempt was made to characterize the phage beyond the observation that it produced plaques in the bacterial host which were more or less similar to those produced by f2 coliphage.

After inoculation of the influent reservoir with f2 coliphage the titres of virus rapidly increased in both the mixed liquor and effluent and reached a plateau by about 20 h later (Fig. 1). Thereafter the phage titres remained constant and in the mixed liquor the phage was distributed between the liquid and solids fractions in the ratio 84:16 respectively (Table 1). The titre of phage in the effluent was about 20·4% of that in the influent which indicated that the total reduction across the plant was 79·6%.

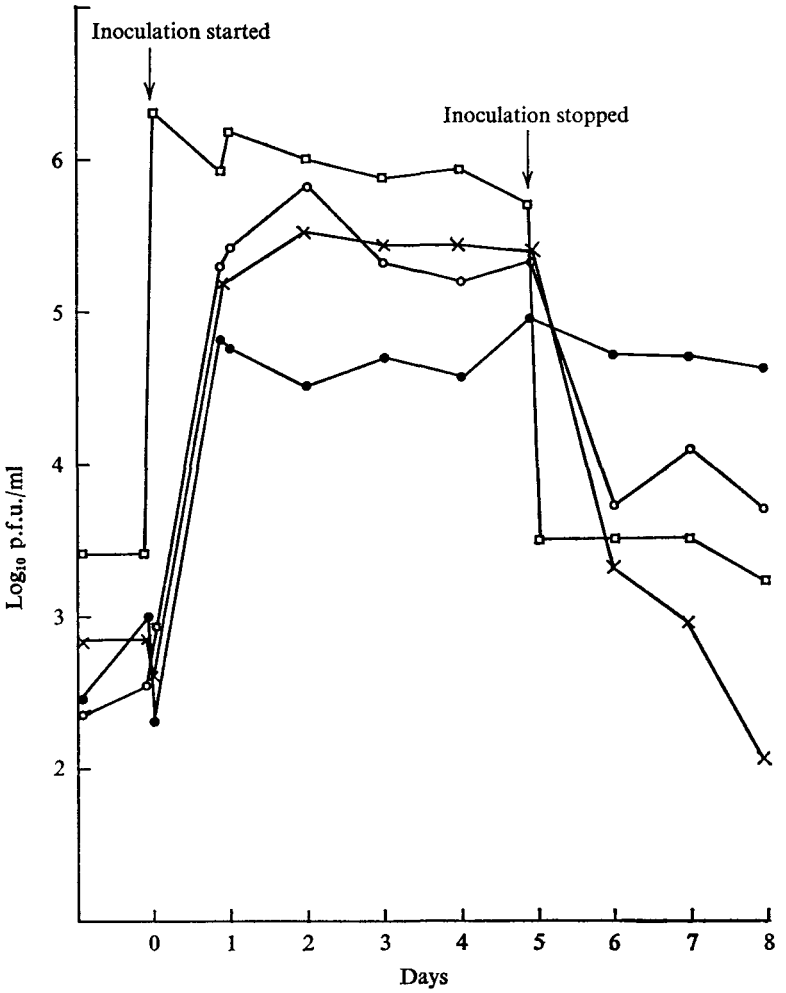

Fig. 1. Distribution of virus in the model plant before, during and after cessation of inoculation with virus. Virus in raw settled sewage (□—□), effluent (×—×), mixed liquor liquid fraction (○—○) and mixed liquor solids fraction (●—●).

When the influent reservoir was replaced with normal influent sewage, the titres of f2 coliphage in the effluent dropped quite sharply to a value about the same as that recorded before inoculation (Table 1). However, in the mixed liquor, even 70 h after inoculation had ceased the phage titre in the solids fraction remained almost as high as during inoculation, while that in the liquid fraction, although also declining quickly, maintained a value which was greater than that recorded before inoculation. The reduction across the plant at this time appeared less efficient (26·3%) than during the period of inoculation (Table 1) but the titre of phage in the effluent was obviously influenced, in particular, by residual virus associated with the mixed liquor solids.

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Table 1. *Distribution of f2 coliphage on different parts of the model activated sludge plant before, during and after inoculation*

	Preinoculation*	Inoculation	Post inoculation
Influent	3·24	6·01	3·44
Mixed liquor			
(*a*) Liquid fraction	2·54 (31·4)†	5·38 (84)†	3·93 (36)†
(*b*) Solids fraction	2·88 (68·6)†	4·66 (16)†	4·17 (64)†
(*c*) Total	3·04	5·45	4·37
Effluent	2·52 (19)‡	5·32 (20·4)‡	2·86 (26·3)‡

* Preinoculation figures: the mean of 25 samples collected from time to time; inoculation figures: the mean of samples collected between days 1 and 5; post inoculation figures: the mean of samples collected between days 6 and 8 ($\log_{10}$ p.f.u./ml).

† % of the total titre of mixed liquor.

‡ % of appropriate influent titre.

DISCUSSION

Bacteriophages are invariably present in raw settled sewage of domestic origin but it was thought unlikely that the background titres which were detected would affect the interpretation of the subsequent experimental values since they represented only 0·3% of the titre achieved after the addition of f2 coliphage. Furthermore, it was assumed that neither the background nor added bacteriophage replicated in host cells at 15 °C which was the temperature of the plant.

After the addition of f2 coliphage it was not surprising that the titre in the mixed liquor and effluent soon rose to plateaux and continued at those levels, with minor variations until inoculation was discontinued. It was noted that the ratio of virus in the liquid and solids fractions before and during inoculation differed (31:68 and 84:16) possibly indicating that the solids had become saturated with adsorbed virus at the higher dose rate although this did not appear to affect the general efficiency of the plant. However, what was of particular interest was that the behaviour of the phage was different from that previously reported for poliovirus in the same model activated sludge plant (Balluz, Jones & Butler, 1977). The most striking contrast was in the distribution of the two viruses between the liquid and solids fractions of the mixed liquor. Thus, the values for f2 coliphage were 84:16 and for poliovirus were virtually the opposite at 15:85. Another dissimilarity was the percentage removal of virus across the plant, which for the phage was 79·6% and for poliovirus 99·96%, which implied that the efficiency of the plant for the removal of poliovirus was very much greater than for f2 coliphage. The fact that the value for the reduction of phage was somewhat greater than that reported by Naparstek *et al.* (1976) which was 66% (calculated from their figures excluding the final chlorination stage) presumably reflects the differences in the function of the two plants, model and full-scale.

Another feature of great interest was that, in complete contrast to poliovirus, the titre of f2 coliphage in the solids fraction of the mixed liquor was relatively low yet remained stable after the inoculation stopped which implied that the phage was

more strongly bound to the solids than poliovirus; this may not be surprising if the phage had an affinity for the bacteria present in the sludge.

The main implications of these dissimilarities between the behaviour of f2 coliphage and poliovirus are that the phage is an unsuitable indicator for the behaviour of human picornaviruses and that in studies of viruses in sewage it may well be necessary to examine the behaviour of each virus individually.

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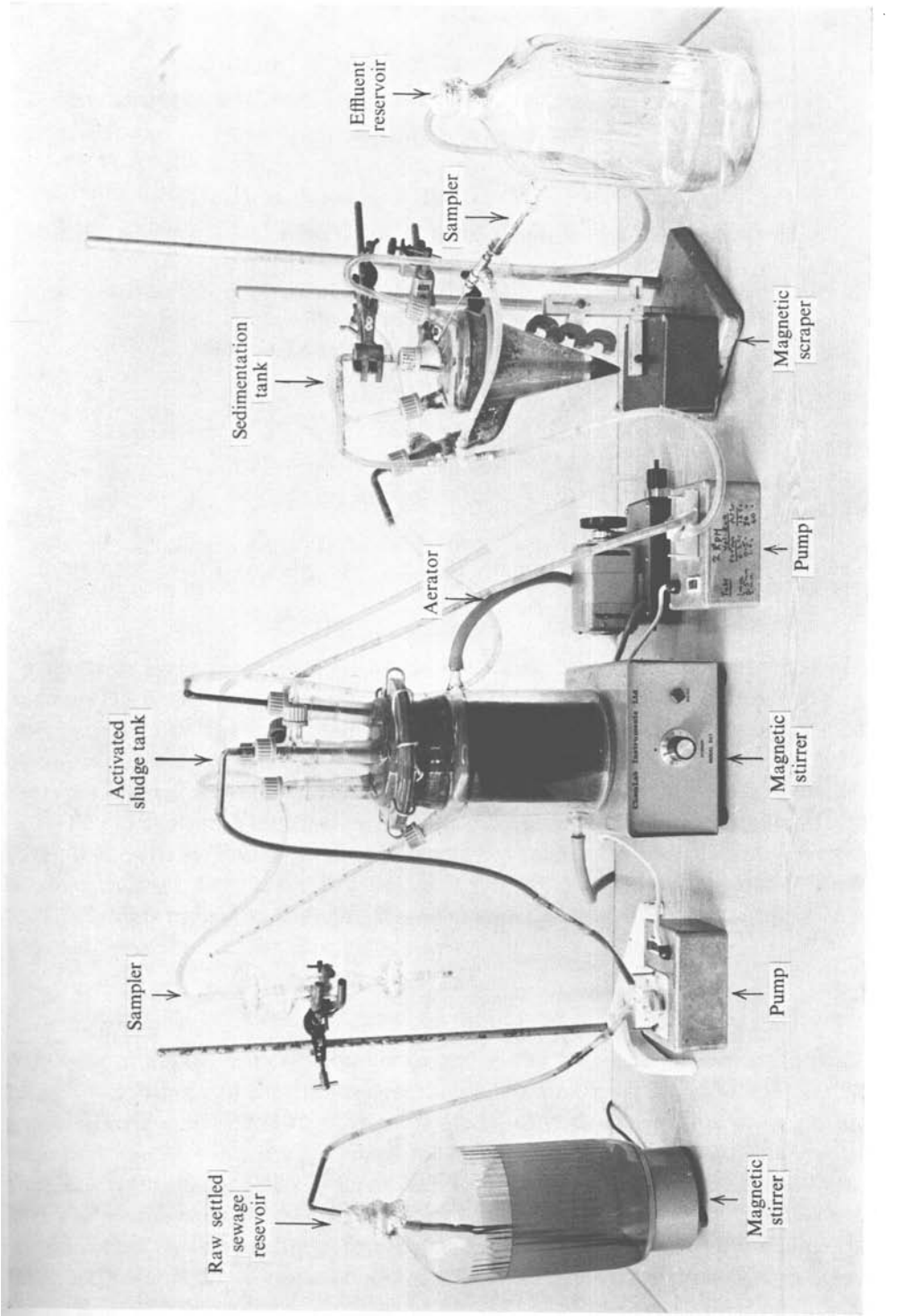
EXPLANATION OF PLATE

Plate 1

Laboratory model activated sludge plant.



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