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The influence of operating conditions of activated-sludge treatment on the behaviour of f2 coliphage

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SUMMARY

The behaviour of f2 coliphage during activated-sludge treatment was influenced by the temperature, flow-through-time, concentration of mixed liquor suspended solids and the virus load.

The most sensitive way to detect behavioural changes was to examine the regression coefficients for the rate of uptake or loss of virus by the mixed liquor solids. This type of analysis revealed, for instance, high values when the solids concentration was high and even greater values occured when high inocula were used. At high temperature the rate of loss of virus titre after inoculation had stopped was greater than the rate of uptake of virus during inoculation although in all other conditions uptake occurred at a greater rate than the loss of virus. The coefficients were relatively low when the flow rate was increased, when the temperature was low or when the inoculum was small.

The distribution of virus between the solids and liquid fractions of the mixed liquor varied somewhat for all conditions but was notably different when (*a*) the plant was incubated at 5 °C when there was much less virus in the solids fraction than usual, and (*b*) when the inoculum was low and a much higher proportion of virus was found in the solids.

The efficiency with which virus was removed across the plant was the least-sensitive determinant of viral behaviour and the value was about the same for most treatment conditions. However, low or high inocula did result in some increased or decreased removal of virus, respectively.

INTRODUCTION

Malina *et al.* (1975) have shown that the removal of poliovirus was apparently not dependent on the operating conditions of the activated sludge treatment plant. For example, alterations in the organic loading of the plant, the retention time (or rate of flow), the concentration of the suspended solids in the aeration tank or the amount of oxygen present in the aerating gas did not affect the amount of virus recovered from the effluent. However, Balluz, Butler & Jones (1978) showed that there was a distinct difference between the behaviour of poliovirus and f2 coliphage in a model plant which was directly related to the differences in the adsorption of virus to the solids in the aeration tank. It was thought, therefore, that alterations in the concentration of the suspended solids, the retention

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time or virus load would inevitably lead to alterations in the behaviour of the virus and in the efficiency of the plant in removing virus. The report below shows that this was the case.

MATERIALS AND METHODS

The model plant

Experiments were done using a model plant based on that devised by Curds & Fey (1969), modified as described by Balluz, Jones & Butler (1977) and Balluz *et al.* (1978).

The virus

The propagation of f2 coliphage, the cultivation of *Escherichia coli* (K_{12}Hfr), the procedure for inoculating the plant, for the collection and treatment of samples and their assay for virus, was described by Balluz *et al.* (1978). The only variant of the inoculation procedures previously described occured when the high load inoculum was tested. In this case the plant was permitted to equilibrate with a normal inoculum for several days before the higher load of virus was introduced into the influent reservoir.

Numerical analysis

The regression coefficients (slopes) were calculated using the following formula:

$$\text{regression coefficient} = \frac{n\Sigma xy - \Sigma x \Sigma y}{n\Sigma x^2 - (\Sigma x)^2},$$

Where x and y were the coordinates of each observation (point) and n was the number of observations (Loveday, 1975). This analysis gave a numerical value which directly reflected the rate of uptake or release of virus in total infectious units, thus a high positive or negative value meant, respectively, a large accumulation or loss of virus.

RESULTS

Under all operating conditions of the plant the pattern of behaviour of the virus was essentially the same (Fig. 1*a–f*). After inoculation of the influent reservoir the titre of virus in the mixed liquor liquid (MLL) and effluent reached their plateaux in 3–4 h while the plateau in the mixed liquor solids (MLS) was reached a little later. The same pattern of behaviour and distribution of virus was observed after the introduction of a high load of virus to the plant already carrying a normal load (Fig. 2). In all cases, as soon as inoculation was stopped there was a sharp drop in the titre of the virus in the effluent, a moderate drop in that of the MLL, but little change in the titre in the MLS.

A detailed comparison of the behaviour of the virus under various operating conditions revealed widely different regression coefficients, some differences in the distribution of the virus between the MLL and the MLS, and slight differences in the total removal of the virus across the plant. For these comparisons the plant conditions represented in Table 1, row 1, were taken as the standard.

An increase in the rate of flow (Table 1, row 2) resulted in a decreased positive

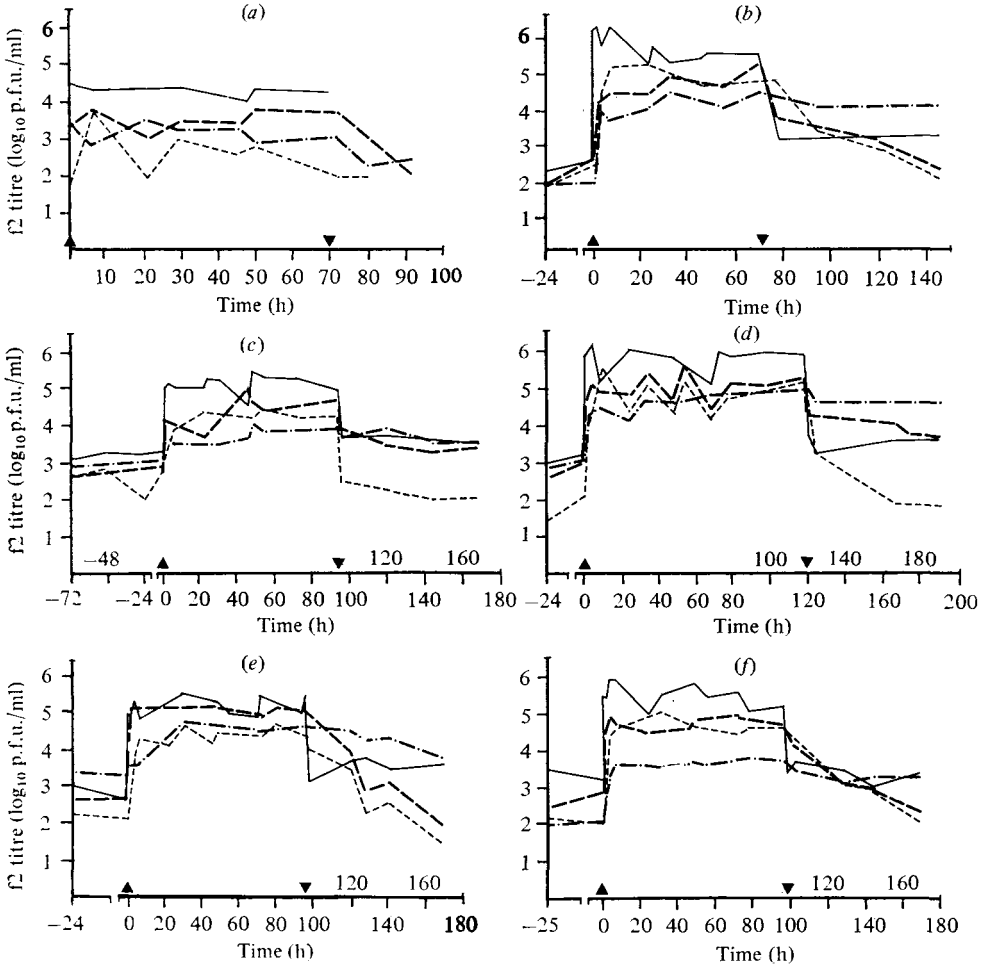

Fig. 1. Distribution of f2 coliphage during continuous inoculation of the model plant under different operating conditions. (▲, Inoculation started; ▼, inoculation stopped; ——, influent; —·—, MLS; ————MLL; ------, effluent). (*a*) MLSS (mixed liquor suspended solids) 2000 parts/10^6, 10 h retention time and 15 °C (Low virus loading). (*b*) MLSS 2000 parts/10^6, 10 h retention time and 15 °C (Normal virus loading). (*c*) MLSS 2000 parts/10^6, 5·4 h retention time and 15 °C (Normal virus loading). (*d*) MLSS 6000 parts/10^6, 10 h retention time and 15 °C (Normal virus loading). (*e*) MLSS 4000 parts/10^6, 10 h retention time and 25 °C (Normal virus loading). (*f*) MLSS 4000 parts/10^6, 10 h retention time and 5 °C (Normal virus loading).

regression coefficient and a consequent slight decrease in the proportion of virus in the MLS. The proportion of virus recovered from the effluent was slightly lower than under standard conditions.

An increase in the mixed liquid suspended solids to 6000 parts/10^6 (Table 1, row 4) increased the positive regression coefficient quite sharply without greatly affecting the MLS:MLL ratio. The amount of virus recovered from the effluent was slightly greater than under standard conditions and was related to the

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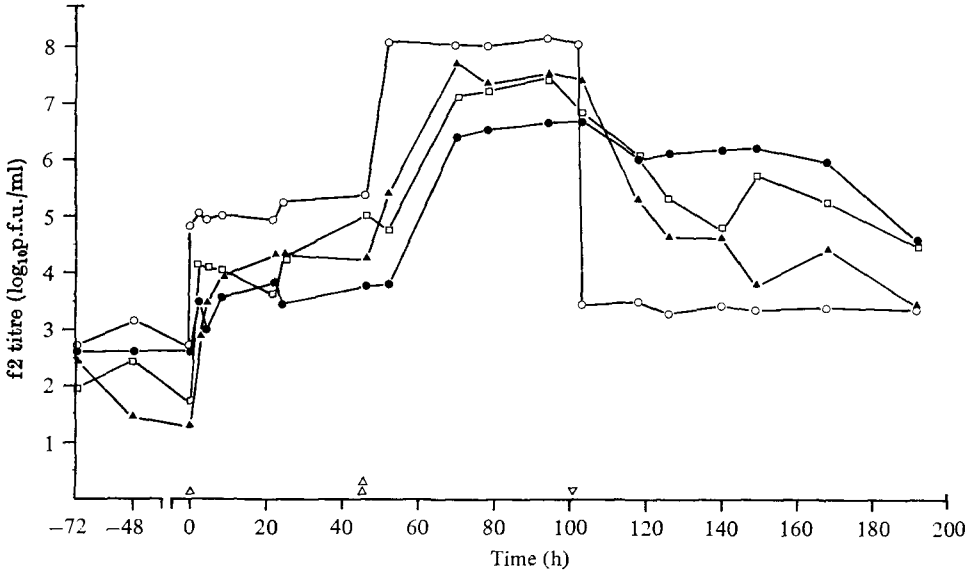

Fig. 2. Distribution of f2 coliphage during continuous (normal and high) inoculation of the model plant at 2000 parts/10^6 MLSS, 15 °C and 10 h retention time. △, Inoculation started, normal virus loading; ▲, inoculation increased, high virus loading; ▽, inoculation stopped; ○—○, influent □—□, MLL; ●—●, MLS; ▲—▲, effluent.

inevitable increase in the suspended solids found in effluents under these conditions.

At low temperature (5 °C; Table 1, row 5) the regression coefficients were low and there was a shift in the distribution between MLS and MLL in favour of the liquid fraction with a consequent increase in the proportion recovered from the effluent.

At high temperature (25 °C; Table 1, row 6), although the MLS:MLL ratio was similar to the standard, a somewhat higher proportion of virus was recovered from the effluent. This correlated with a large negative regression coefficient after inoculation had stopped, which was numerically much greater than the positive coefficent recorded during inoculation. In all other tests the negative coefficient was less than the positive coefficient.

Changes in the behaviour of f2 coliphage also occurred when low or high inocula were tested. During low-level inoculation the regression coefficients were low, but there was a shift in the MLS : MLL ratio towards MLS with a consequent low recovery of virus from the effluent (Table 1, row 7). High-level inoculation (Table 1, row 8; Fig. 2) resulted in high regression coefficients, so that although the distribution of f2 coliphage between MLS and MLL was normal, the rate of release of virus from the solids after inoculation was stopped was high, which contributed to the high value for virus recovered from the effluent.

Table 1. *The influence of functional conditions and virus load on the behaviour of f2 coliphage during treatment*

Conditions	Row No.	MLSS* (parts/10^6)	Retention time (h)	Temperature (°C)	Inoculum level†	MLS: MLL‡ Ratio	Influent virus recovered in effluent %	Regression coefficient in MLS during inoc.	Regression coefficient in MLS after inoc. stopped	Figure ref. No.
Different flow rates	(1)	2000	10	15	Normal	22:78	11·2	+299	−121	1b
	(2)	2000	5·4	15	Normal	16:84	10·5	+ 65	− 45	1c
Different MLSS	(3)	2000	10	15	Normal	22:78	11·2	+299	−121	1b
	(4)	6000	10	15	Normal	27:73	13·8	+665	−512	1d
Different temperatures	(5)	4000	10	5	Normal	8:92	12·6	+ 21	− 14	1f
	(6)	4000	10	25	Normal	24:76	15·9	+253	−622	1e
Different inocula	(7)	2000	10	15	Low	36:64	4·5	− 45	− 37	1a
	(8)	2000	10	15	High	16:84	30·2	+73417	−37369	2

* Mixed liquor suspended solids (parts/10^6).
† Normal, approximately $10^{5\cdot5}$ p.f.u./ml; low, approximately $10^{4\cdot5}$ p.f.u./ml; high, approximately $10^{6\cdot2}$ p.f.u./ml.
‡ Mixed liquor solids titre: mixed liquor liquid titre.

DISCUSSION

The major difficulty regarding the analysis of the behaviour of the virus under different operating conditions of the model plant was that, although it was possible to stabilize the concentration of the solids in the aeration tank, the flow rate and the temperature, there was no satisfactory way to standardize the chemical and biological quality of the influent settled sewage, which was obtained from the local sewage works. Changes in these qualities might very well have influenced the biological population of the floc in the aeration tank as well as the physico-chemical features of the floc, both of which would influence the adsorption and possible inactivation of virus. Nevertheless, some weight can probably be attached to the observed behavioural changes which were related to various alterations in the operating conditions of the plant.

Regression analysis was clearly the most sensitive way of showing up differences in the behaviour of virus under different operating conditions of the plant because each value was distinctive whether relating to the uptake of virus by the solids during inoculation or its release after inoculation was stopped. The distribution of virus between the solids and liquid fractions of the mixed liquor also revealed special features of the behaviour of the virus particularly in the low temperature and low inoculation experiments. The removal of virus across the plant was the least-sensitive determinant of viral behaviour and in only those experiments which tested low or high inocula were there striking differences from the mean. Since this was the method of evaluation chosen by Malina *et al.* (1975) it was not surprising that they did not find noticeable effects when they varied the operating conditions of the plant.

It was interesting to observe that heavy loading of the plant with virus resulted in a high rate of uptake of virus by the floc as well as high total uptake which implied that the floc had a high capacity for the adsorption of virus. The mechanism of the removal of virus was probably simply by physical adsorption, an inherent characteristic of which is that it is directly influenced by the concentration of the reactants and the temperature (Marshall, 1976; Treybal, 1968). In our experiments an increase in the virus load, the suspended solids concentration or the temperature all resulted in changes in the behaviour of the virus.

It is much more difficult to establish from these experiments whether or not inactivation of virus occurred, using the word in its exact sense. The only numerical values which have been used to imply that inactivation of a virus may occur were provided by Balluz *et al.* (1977), who compared the expected losses in poliovirus titres due to dilution with the greater actual losses recorded after inoculation had stopped. In the experiments reported here and elsewhere (Balluz, 1977; Balluz *et al.* 1978) the persistence of f2 coliphage on the MLS implied that negligible inactivation occurred in the MLS. The fact that the titre in the MLL during this phase remained somewhat higher than could be accounted for by dilution was consistent with the likelihood that it was constantly recharged with virus eluting from the MLS.

It has already been noted (Balluz *et al.* 1978) that different viruses behave

differently under the same conditions, thus it would not be surprising if behavioural differences also occurred under different operating conditions of the plant. If this were the case, as is likely, it would support the contention of Metcalf (1971) that there is little justification for the selection of one virus as a general indicator of viral pollution and would dispute the claims made by several others that bacteriophages (e.g. Kott *et al.* 1974) or cyanophages (Smedberg & Cannon, 1977) could be convenient indicators of enteroviral pollution in water and sewage treatment.

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