BEHAVIOUR OF VIRUSES IN ACTIVATED SLUDGE TREATMENT

by

SHAFIK ALI BALLUZ

Thesis submitted to the Department of Microbiology
University of Surrey in partial fulfillment
of the requirements for the Degree of Doctor of Philosophy

December 1977

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Acknowledgement

I am deeply indebted to the Lebanese National Council for Scientific Research for a long term scholarship which enabled me to do this work; to the Thames Water Authority for financing the research; to Dr. M. Butler for supervising the work with constructive guidance, valuable advice and encouragement; to Dr. J. H. Darbyshire for guidance and encouragement at the early phase of this work; to all colleagues and members of the Department of Microbiology for their help and assistance; to Mr. J. Dutson, Mr. J. Stacey and Mr. G. McBreaty of the Guildford Sewage Works for the help and assistance they very friendly provided; to Mrs. M. Ellis for typing this thesis; and to Mr. I. Rajkovic for his help with the graphic representations.

My most sincere thanks and gratitude to all of them.

SUMMARY

The behaviour of f2 coliphage and poliovirus I in activated sludge treatment was studied under different operating conditions of flow through time, mixed liquor suspended solids, temperature and virus loading in a bench scale model plant whose performance was similar to that of a full scale treatment plant. The liquid and solids fractions of mixed liquor samples containing virus were assayed separately, with the solids fraction receiving ultrasonic treatment. The recovery of poliovirus from mixed liquor by this method was about 83 per cent, while that of f2 coliphage was in the range of about 54-85 per cent.

The average removal of f2 coliphage across the model plant was about 84 per cent, and was not significantly altered by altering the flow through time, mixed liquor suspended solids and temperature in the plant. The removal was, however, decreased from about 96 per cent to about 70 per cent with higher virus loads. The association of f2 with the mixed liquor solids showed an inverse relation with increased flow, a direct relation with increased mixed liquor solids, and apparently direct relation (with an optimum) with increased temperature and a clear direct relation with increased virus load. The removal of poliovirus across the plant over the range of conditions studied was generally high and reached up to about 99.7 per cent.

The behaviour and removal of both viruses in the model plant correlated with the association of these viruses with the suspended solids. The degree of association, which appeared to depend upon the nature of each virus and was achieved by physical adsorption, was strikingly contrasting with about 18 and 85 per cent of f2 and poliovirus respectively detected on the solids. The striking differences between the behaviour of f2 coliphage and poliovirus I imposed interesting implications on the concept of indicator virus from the public health viewpoint.

Table of Contents

List of Tables

List of figures

List of Abbreviations

BOD_5	5- day biochemical oxygen demand
Cat. #	Catalogue number
CPE	Cytopathogenic Effect
cpm	Counts per minute
DF	Dilution fluid
ML	Mixed liquor
MLL	Mixed liquor liquid fraction
MLS	Mixed liquor solids fraction
MLSS	Mixed liquor suspended solids concentration (in ppm)
$NH_3(N)$	Ammonia nitrogen
nm	nanometer
$NO_3^-(N)$	Nitrates nitrogen
PBS	Phosphate buffered saline
pfu	Plaque forming units
ppm	parts per million
Preinoc.	Pre-inoculation titres
rpm	Revolutions per minute
RSL	Return sludge liquid fraction
RSS	Return sludge solids fraction
s	Standard deviation
s.e.	Standard error
SS	Suspended solids concentration
t	t value for comparison of two means (t test)
TSB	Tryptose soya broth
v/v	Volume per volume
w/v	Weight per volume
$\bar{x}$	Average
$<$	Less than
$>$	More than

I. INTRODUCTION

The cyclic nature of the faecal-oral route for transmission of enteric pathogens presents a constant threat to the health of human populations. The turnover of these pathogens depends upon the contamination of potable water resources with raw or badly (partially) treated sewage. Increased urbanisation and population densities exert ever increasing demands on natural water resources, and at the same time increase, proportionately, the discharge of sewage effluent into these waters. Such pressures, when exerted on the limited water resources of a community, could make the natural self-cleansing mechanisms of water highly inadequate when these could probably be more than adequate for a sparsely populated region. These mechanisms, coupled with conditions in the different zones of this world - whether temperate, arctic, tropical or desert - definitely exert an effect on disease transmission which manifests itself in the various routes for communicable disease transmission (like food, air, water, contact and insect routes).

In urban centres of industrialised states the faecal-oral route for pathogen transmission is probably more important than other routes since the pathogens are in a medium that reaches every household where every individual uses it (Fig. 1) (Schwartzbrod et al., 1973).

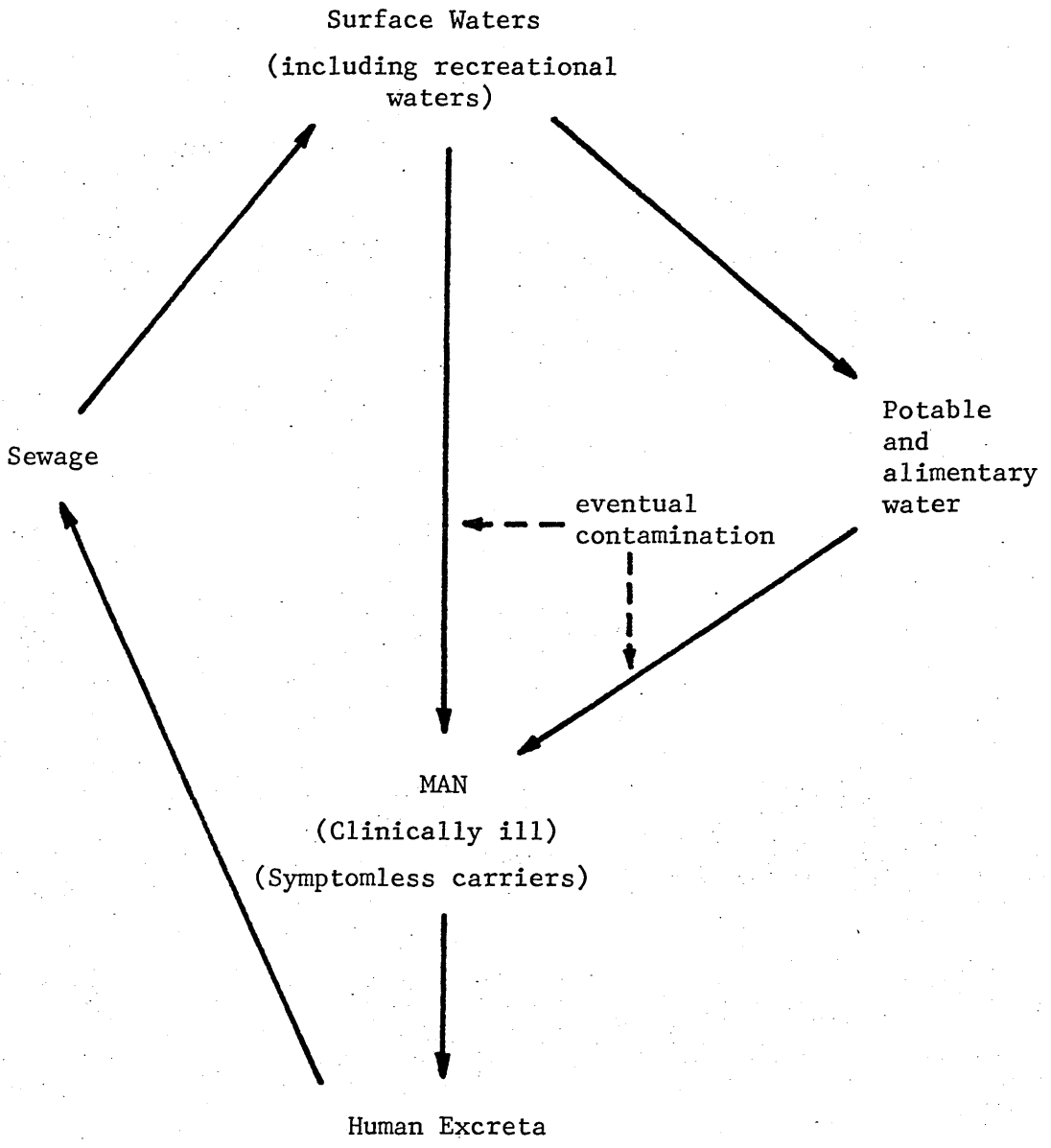


Fig. 1.1 The faecal-oral route of surface and potable water contamination with eventual contamination and infection of man (Modified from Schwartzbrod et al., 1973)

The relationship between the consumption of raw water and the development of an infection depends upon many factors, the principal ones being the number of pathogens present and the susceptibility of the subject. For viruses it is suggested that one 50 per cent tissue culture infective dose ($TCID_{50}$) or one plaque forming unit (pfu) may be infectious to man if the conditions are suitable for it (Katz and Plotkin, 1967; Plotkin and Katz, 1967).

It was not until the 1850's when Dr. John Snow of London established an inferential cause and effect relationship between drinking water and cholera, that the concept of water-borne disease outbreaks was established (Snow, 1936). Decades passed before epidemiological support for Snow's conclusion was obtained, and today records of water-borne outbreaks of disease include those of viral, bacterial and protozoal aetiology apart from chemical poisoning (McCabe and Craun, 1975; McDermott, 1974). Examples of some types of water-borne diseases are given in Table 1 which lists the water-borne outbreaks that occurred in the United States and Canada in 1971 and 1972. Such water-borne outbreaks of disease which were apparently low in 1951-2 (Fig. 1.2) have been on the increase since then. This does not necessarily indicate increased incidence alone because there has also been much better reporting during this time (McCabe and Craun, 1975).

However, all these outbreaks were due to one or more reasons which included (a) use of untreated water for potable purposes

Table 1.1. Water-borne disease outbreaks in the United States and Canada in 1971-72. (Modified from McCabe and Craun, 1975).

	Number of outbreaks	Number of cases
Gastroenteritis	22	5615
Infectious Hepatitis	11	266
Shigellosis	6	614
Giardiasis	3	112
Chemical Poisoning	3	202
Salmonellosis	1*	3
Typhoid	1*	5
Total	47	6817

*Individual and private water supply system.

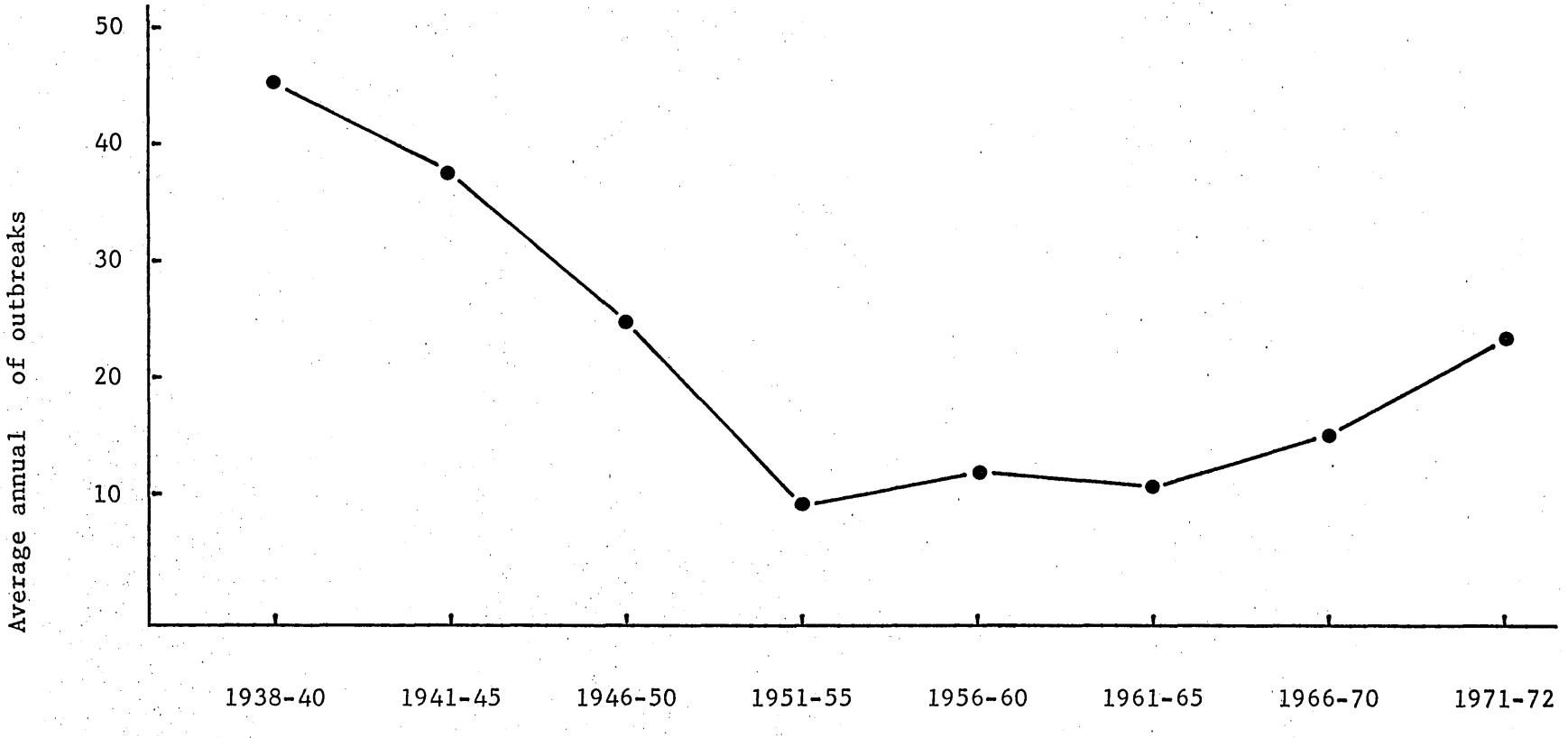


Fig. 1.2. Outbreaks of waterborne disease in the U.S., 1938-1972 (From McCabe and Craun, 1975).

(b) inadequate or interrupted disinfection of the water or (c) contamination through distribution systems. Whatever the reason for the outbreak, the role of the faecal-oral route for pathogen transmission via the water route is emphasised and prevention of such outbreaks rests in disruption of the cyclic nature of this route. This is possible at certain points: (a) at the sewage treatment stage prior to discharge into surface water bodies and (b) at the water treatment stage prior to distribution to the population (see Fig. 1.1) or (c) public health advise on the use of untreated natural sources of water.

1. History of Sewage Collection and Treatment

The evolution of water and waste water treatment processes has a long history that accompanies many disease outbreak problems. Very early Sanskrit writing of 2000 B.C. advised people how to deal with "foul" water by boiling it, exposing it to sunlight and dipping into it a piece of hot copper, then to filter and cool in an earthenware vessel (Baker, 1948). Early Egyptians (1300 B.C.) sedimented drinking water and then filtered it by wick syphons and through earthenware vessels before using it. This method again became widespread in 19th century Europe, to spread inevitably to the United States in the later part of that century (Singley, 1971). It is clear, therefore, that modern water treatment practices have evolved from the early practices with which they closely parallel (sand filtration : filtration through earthenware vessels and by wick syphon, disinfection : boiling; residual disinfection : copper treatment and sunlight).

The proper and hygienic disposal of human excrement, due to its foul and infectious nature, must have concerned man since time immemorial. Other animals, such as cats and dogs, through their basic instinctive behavioural patterns, do attempt to bury or at least cover with earth their excreta. Primitive man was instructed, through religious teachings, of the vile nature of excrement and ordered to dispose of it away from camps and dwellings.

The Old Testament instructs: "Thou shalt have a place also without the camp, whither thou shalt go forth abroad: And thou shalt have a paddle upon thy weapon; and it shall be, when thou wilt ease thyself abroad, thou shalt dig therewith, and shalt turn back and cover that which cometh from thee: For the Lord thy God walketh in the midst of thy Camp, to deliver thee, and to give up thine enemies before thee; therefore shall thy camp be holy: that he see no unclean thing in thee, and turn away from thee." (Deut. XXIV, 12, 13, 14). The Islamic religion regards excreta as defiling; thus the body orifices must be washed after every act of excretion or else man is not clean enough to say his five daily prayers. Domestic dwellings are also considered defiled and unfit as a place for prayer if excreta, or for that matter, water used for body washing, is not properly disposed of but allowed to contaminate the household.

Disposal of excreta and waste water directly into land or water ways is only a satisfactory practice in sparsely populated areas. The urban Greeks and Romans provided effective sewage systems which conducted used water and human excreta to dumping sites or to receiving waters far away from the populated areas (Jones, 1966). Examples of Roman channels constructed to carry waste water can still be seen, for instance, at Colchester. It seems, however, that the Greek and the Roman practice of disposing excreta and waste water was not adopted by later settlers and the knowledge was lost for many centuries (Sidwick and Murray, 1976a).

During mediaeval times, Europe was largely without any public sanitary measures, apart from stone chambers for sewage collection which were used in the houses of the nobility only, and were called privy middens. One of the earliest, and largest, privy midden was built at Guildford Castle in 1296. These privy middens were emptied periodically and the contents used as fertilizer by farmers. It was not until 1594 that Sir John Harrington invented a primitive form of a water closet which again emptied into the privy middens. But, needless to say, the urban poor were without any such "luxuries" as water closets or privy middens (Jones, 1966). The Finns have, for years, had an equivalent system. The privy is built up say, 6 ft. above ground and approached by steps. Faecal material is allowed to accumulate in the space below the privy.

Sanitary reform during the Renaissance (15th and 16th centuries) was most advanced in Italy where health boards were formed as a consequence of the great plague epidemics which swept through the Italian peninsula in the 14th and 15th centuries. The formation of these health boards represented a rejection of the fatalistic attitude - which was prevalent at that time - and soon these boards became a permanent feature of Italian administration. During epidemics, health boards took sweeping powers and they closed markets, prohibited festive gatherings (even religious assemblies), isolated infected households and cleansed or destroyed their possessions. The boards even controlled overcrowding in dwellings, inspected and controlled the quality of food products and initiated regular mortality bills. Proper waste water disposal

was made obligatory, and the Milan Health Board decreed that "every householder who does not have adequate facility for collecting his waters and sewage shall cause to be made one or more tanks in each of his houses according to the instructions and design of an engineer approved by the health board" (Cipolla, 1976). Most of these measures were ultimately copied in Britain by the newly created Health Boards during the cholera epidemic of 1832.

Appalling sanitary conditions had developed in Britain during the Industrial Revolution (1760-1830). The country was largely without any sanitary system or control of environmental hygiene. Increased urbanisation and the build up of large populations of low paid workers coupled with a sharp increase in the cost of living made the expense of cartage of human excreta from middens and cesspits too expensive. Consequently, they started to fill up and overflow and the foul smells spread and with them spread infection and disease. Many people built very large middens which almost never filled because of the percolation of sewage into the water table with subsequent pollution of wells where drinking water was obtained. These conditions were almost certainly responsible for the frequent epidemics of typhoid, cholera and typhus. The relative incidence of typhoid in 1847 in Nottingham was highest (27/1000) in houses with privy middens, followed by houses with pail closet (8.3/1000) and houses with water closet (1.3/1000) (Jones, 1966). These figures demonstrate, to a certain extent, the health hazard posed by the misused domestic methods of disposal of excreta.

Very early in the 19th century, water companies were formed in some urban areas to provide water not only for drinking, and cooking but also for bathing and flushing toilets. One consequence of this practice was that even the extra large privy middens began to overflow. In 1847 it became compulsory to drain houses by sewers often using existing town sewers, the earliest of which were constructed in 1532 primarily to drain subsoil water and rain. The water carriage system polluted rivers to such an extent that, for instance, "river water from the Thames at Blackwell was used as ink to write letters to newspapers." (Sidwick and Murray, 1976a). Much of the pollution of the Thames was due to improper discharge of sewage into the river on the ebb tide, contrary to original design of the sewers and flow balancing reservoirs.

Intensive sanitary reform in England was started by Edwin Chadwick in 1838. In a "Report on the sanitary conditions of the labouring population of Great Britain" he showed that the death rate clearly indicated that their conditions were probably very poor and their susceptibility to epidemic sickness was very high. Cases of diseases unknown in Britain began to appear; cholera which was endemic to India, became pandemic in Europe and reached Britain in 1831. Chadwick succeeded in convincing parliamentarians of the time that environmental health was of prime importance (Jones, 1966).

Chadwick became a member of the General Board of Health which was formed as a result of the Public Health Act of 1848. The responsibilities of this board were related to sanitary, sewarage and disposal arrangements of waste water. Chadwick recommended an arterial system of sewers and proposed the revolutionary idea that the sewers should have an egg-shaped (parabolic) cross section which permits constant flow despite loading and would not block and was self-cleansing. The board decided to manage water supply, sewage disposal and street cleansing by one authority. Chadwick, furthermore, started his researches into sewage treatment as well as of disposal methods like spraying sewage onto land.

A number of commissions were created between 1861 and 1865 and all of them were concerned with the disposal and utilization of sewage from London and other towns. The studies done by those commissions and their committees culminated in the Sewage Utilization Acts of 1866 and 1867. These stipulated that local authorities could purchase land for sewage treatment and cooperate together to protect the waterways from pollution.

The most important public health act was, however, that of 1875 which replaced all previous public health acts concerning disposal of sewage, pollution of rivers, hygiene and sanitation in general. It stated that "nothing in the act shall authorise any local authority to make use of any sewer, drain or outfall, for the purpose of conveying sewage or filthy water into any

natural stream or watercourse, or into any canal, pond or lake, until such sewage or filthy water is free from all excrementitious or other foul or noxious matter, such as would affect or deteriorate the purity of the water in them." This act was re-affirmed by the Rivers Pollution Prevention Acts of 1876 and 1893 which made it an offence for any person to discharge or permit the discharge into any stream, any solid or liquid sewage matter.

The last Sewage Commission of 1882 was appointed to investigate whether there were evil effects resulting from the conditions under which sewage effluent was discharged into the River Thames, and what measures could be applied to prevent them. The Commission's final report was issued in November 1884; it was found that evil effects did exist and demanded a prompt remedy. The report disclosed that chemical precipitation of sewage with lime and iron salts only removed part of the organic matter and the liquid so separated was not sufficiently free from noxious material to justify its discharge from the outfalls as a permanent measure. The report also recommended that, according to the then prevailing state of knowledge, further purification of sewage can only be efficiently done by application on land. This report reflected current thinking at that time and, coupled with the Local Government Act of 1888 which created river boards and authorised county councils to enforce the River Pollution Prevention Act, enhanced the need for better and more efficient sewage treatment methods (Sidwick and Murray, 1976b).

In response to the Commission's report of 1884, Dibdin, the Chemist and Superintending Gas Examiner of the London County Council, recommended sewage treatment by biological principles; he was though to be mad! (Sidwick and Murray, 1976a). Development of sewage precipiting agents continued but District Councils were discouraged to put them into practice because of the 1884 recommendations and because the councils were taking seriously the work of saving streams and rivers from pollution. Dibdin continued his work on biological treatment of sewage and in 1897 he wrote "if any porous material such as coke breeze, or burnt clay, be placed in a vessel and sewage water admitted thereto, a large proportion of the filth contained therein will adhere to the rough sides of the coke or other material, and the organisms will commence their work by feeding and multiplying so that in a short time the whole surface of each particle of coke, or other material which may be employed will be covered by them. Let the water drain off gently after sufficient time has been allowed for the adherence of fine particles of matter to the coke. Air will be admitted as the water is lowered and a fresh impetus will be given to the little workers who will soon be ready for another supply of food to be given to them in the form of a second quantity of foul water." Experiments followed by trials on filter beds made of various media of coke breeze, burnt ballast, pea ballast and sand. Such experimental filter beds were operated for eight hours and left to regenerate for sixteen hours. These experiments resulted with

78 per cent organic matter removal and marked the beginning of the trickling filter or bacteria beds method of sewage treatment. These were soon installed in full scale treatment plants to treat London's sewage, and the trickling filter was thus well on its way as a viable means of sewage treatment. The switch over to percolating filters was met with a lot of opposition especially with the belief that only application to land could purify sewage. However, it was only the huge land requirement (Manchester needed 1000 acres for complete treatment of its sewage with an application rate of 60,000 gallons/acre/day) that affected the change to Dibdin's filtration method that reached the most advanced stage in design as it is known today in 1930 (Sidwick and Murrary, 1976c).

Dibdin, however, forwarded the idea that "the true way of purifying sewage, where suitable land is unavailable, will be first to separate the sludge, and then to turn into the neutral effluent a charge of the proper organism, whatever that may be, specially cultivated for the purpose, retain it for a sufficient period, during which time it should be fully aerated, and finally discharge into the stream in really purified condition." (Dibdin, 1907). This statement surely represents the earliest inception of the activated sludge process.

The history of activated sludge treatment can be traced to very early in the 20th century when the suggestion was put forward that there could be potential in aerating sewage with humus sludge

to treat it. It was later shown that indeed there was such potential, but the production of good quality effluent took several days. Other workers found the same result and were discouraged by this result. Fowler and his colleagues were among these workers who had been experimenting with aeration when Fowler visited the United States in 1912 and saw experiments in aeration where the inside of the bottle was heavy with an algal growth. Upon communicating his observations to his colleagues, work started on aeration of sewage. Their results were positive, high quality effluent and "deposited matter" were produced in a period of several weeks. This activity was generated much more quickly when the floc was conserved and re-fed with fresh sewage and the deposited matter considerably increased. Soon they achieved good quality effluent within 24 hours of aeration (Lockett, 1954a, 1954b; Sidwick and Murray, 1976d). The deposited matter was called activated sludge and soon employed in full scale plants mainly by converting existing settling tanks into aeration tanks and thus activated sludge was put into practice. At present, activated sludge is the most widely spread method of sewage treatment (Genetelli, 1971; Mara, 1976; Southgate, 1969).

2. Composition of Sewage

The foul and infectious nature of sewage is reflected by its chemical, organic and organismal content. The composition of sewage, however, is tremendously variable in strength and flow (volume) since these relate to habits of the population, size of

the inhabited area and length of the sewer. Such variations might be as much as 10 fold (even in dry weather) especially in smaller areas (Hunter and Heukelekian, 1965; Painter, 1971). On average sewage is a dilute solution with at least 99.9 per cent water and solutes and about 0.1 per cent suspended solids comprising about 0.07 per cent organic (Table 1.2a) and 0.03 per cent inorganic matter (Table 1.2b) (Hammer, 1975; Mara, 1976; Painter and Viney, 1959; and Southgate, 1969). The organic constituents are mainly proteins, carbohydrates and fats, whereas the inorganic constituents are soluble and insoluble salts and grit in varying concentrations. In general, sewage is a turbid, grey to brown liquid, but if industrial effluent is pooled with domestic sewage then the colour and turbidity may vary tremendously. Sewage also fluoresces in ultra-violet light due to the presence of "optical brighteners" in detergents. Its temperature is generally about one degree higher than that of the water supply with summer temperatures ranging from 17-20^{o}C and winter temperatures from 8-12^{o}C in the United Kingdom. Temperatures as high as 28-30^{o}C may be observed in hotter climates like certain parts of India. Its pH in soft-water areas is about 6.7-7.5 and in hard-water areas 7.6-8.2. Suspended solids are present in sewage in various particle sizes from the ultra-fine colloidal ($<1\mu$) to the fine (1-100μ) and the coarse ($>100\mu$) (Painter, 1971).

The higher the concentration of waste matter in sewage, the stronger it is said to be. To determine the strength of sewage, however, it is not imperative to determine each of the

Table 1.2a. Organic constituents of domestic sewage. (From Painter, 1971, with modifications)

Constituent	U.K.		U.S.A.	
	In solution mg/l	suspended mg/l	In solution mg/l	suspended mg/l
Carbohydrates	70	34	10	34
non-volatile acids	34		28.5	
Volatile acids	25		0.3	
Amino acids				
Free	5		(	
bound	13		(9.0	
Detergents (ABS)*	17	5.9	4.0	0.3
Uric acid	1		0.33	
Phenols	0.2		0.11	
Cholesterol	present		0.04	
Creatine-Creatinine	6		0.18	
Organic carbon	90		-	
Volatile solids	-		80	
Fats		140		26.0
Proteins		42		27.5
Lignin		present		4.0
Amino sugars		1.7		-
Amides		2.7		-
Soluble Acids		12.5		-
Organic carbon				
by analysis		211		-
by addition		151		-
Volatile matter				
by analysis		-		116
by addition		-		101

* Alkyl benzene sulfonates.

Table 1.2b. Inorganic Constituents of Domestic Sewage. (From Painter, 1971 with modifications)

Constituent	Concentration (mg/litre)	
	Whole Sewage U.S.A. Soft Water Area	Settled Sewage U.K. Hard Water Area
Cl	20.1	68
Si	3.9	-
Fe	0.8	0.8
Al	0.1	-
Ca	9.8	109
Mg	10.3	6.5
K	5.9	20
Na	23.0	100
Mn	0.47	0.05
Cu	1.56	0.2
Zn	0.36	0.65
Pb	0.48	0.08
S	10.3	22
Phosphate (as P)	6.6	22

* Other elements include Sr, Cr, B, Ba, at 0.1-0.5 mg/l.
As less than 0.1 mg/l.
Ag, Cd, Mo, Ni, Co, V, Be less than 0.02 mg/l
O_2 (dissolved in flowing sewage) 1-2 mg/l
N_2, CO_2, H_2S (absent if flow is more than 1.75 ft/sec).

* From Painter, H. A., unpublished data.

organic and inorganic constituents especially when the amount of oxygen needed for this oxidation during sewage treatment presents an adequate measure of their total concentration. There are two types of oxygen demands which can be determined for a sample of polluted water, the chemical and the biochemical oxygen demands (COD and BOD respectively). COD is determined by oxidizing the sample with a boiling acid dichromate solution resulting with oxidation of all organic and inorganic polluting matter. A measure of the oxygen demand thus obtained gives no idea whatsoever about that proportion of organic matter that is oxidizable by microbial action. Thus it is more informative to determine the BOD_5 (5-day BOD) which gives the amount of oxygen consumed by bacteria and microorganisms during the oxidation of the waste for five days. According to its BOD_5, a sewage can be classified as weak, medium, strong or very strong depending whether its BOD_5 value is around 200, 350, 500, or >750 mg O_2 required/liter respectively (Mara, 1976; Southgate, 1969). In addition to BOD_5 and COD values, a knowledge of suspended solids and ammonia content is usually enough to assess the polluting strength of sewage and to determine proper treatment measures for it (Painter, 1971). A summary of these properties for U.K. and U.S.A. domestic sewage is given in Table 1.2c.

The strength of sewage varies from community to community depending on many factors including the standard of living, adequacy of water supply, local habits etc.. Table 1.3 shows differences in sewage between tropical and temperate countries.

Table 1.2c. Properties of domestic sewage. (From Painter, 1971, with modifications).

	Settled sewage from one U.K. town	Unsettled sewage from one U.S. town	
Characteristic		Winter-Spring	Fall-Winter
pH	7.8	7.2	7.2
BOD_5	370	147	136
COD	670	288	282
Total Solids	1309	453	481
Suspended Solids	146	145	146
Ammonia (as N)	46	21	-

Table 1.3. Analyses of tropical and temperate sewage in mg/l. (From Mara, 1976).

Component	Kenya (Nairobi)	Kenya (Nakuru)	India (Kodun-gaiyur)	Peru (Lima)	Israel (Herzliya)	U.S.A. (Allentown)	U.K. (Yeovil)
- BOD_5	448	940	282	175	285	213	324
- suspended solids	550	660	402	196	427	186	321
- total dissolved solids	503	661	1060	1187	1094	502	-
- Ammonia - N.	67	72	30	-	76	12	29

The strength of sewage (or BOD_5) depends on the dilution factor - i.e. water consumption per capita - and the BOD_5 contribution per capita. These vary from country to country over a wide range. Mara (1976) reported the daily per capita BOD_5 contributions as follows:-

Zambia	36 gm
Kenya	23 gm
S.E. Asia	43 gm
India	30-45 gm
Rural France	24-34 gm
U.K.	50-59 gm
U.S.A.	45-78 gm

Sewage also contains most types of microorganisms and some larger parasites or parasite eggs. The source of these organisms is human excreta, but some others grow in sewage channels or reservoirs and they include bacteria, protozoa and fungi.

Bacterial populations in sewage could reach viable counts of 6.3×10^6 (while total counts may reach 5.6×10^8) bacteria per millilitre of sewage (Pike and Carrington, 1972). Some of the bacterial genera and species identified include Escherichia, Clostridium, Nitrobacter, Nitrosomonas, Streptococcus, Vibrio cholerae, Salmonella typhi, S. paratyphi, Shigella, Brucella, Mycobacterium tuberculosis, and Leptospira (Painter, 1971; Mara, 1976). These examples of bacteria contained in sewage, of course, include

pathogenic and non-pathogenic species with the pathogenic species particularly abundant at epidemic times. At least 112 species of fungi have been identified in sewage. Sewage also contains intestinal worms like Taenia spp. (a platyhelminth), Ascaris spp. (a nematode) and Schistosoma spp. (a trematode) (Mara, 1976).

Of the protozoan organisms identified in sewage there are Entamoeba histolytica, Trichomonas spp. and Giardia intestinalis as examples of pathogenic species (Jawetz, Melnick and Adelberg, 1974). Many other non-pathogenic protozoans are seen in sewage like the free swimming ciliate Paramecium caudatum; the crawling ciliate Aspidisca spp.; the stalked ciliates Vorticella spp., Opercularia spp., Epistylis spp. and Carchesium spp.; the flagellates like Bodo spp. and Euglena spp.; and the rhizopods like Amoeba spp. (Curds, 1969; Hawkes, 1963; Painter, 1971). These non-pathogenic protozoa, especially the stalked ciliates, act as pollution indicators whereby their abundance or rarity shows the saprobic conditions of the sewage in question (Curds, 1969).

The occurrence of viruses in sewage will be dealt with further in the review of the Literature.

3. Treatment of Sewage

There are two main purposes for treating sewage, namely:

1. To prevent pollution of natural receiving waters with organic and inorganic pollutants and,

2. To prevent spread of enteric pathogenic microorganisms which potentially contaminate sewage.

The first purpose is achieved by oxidation of the pollutants by microorganisms (mainly bacteria) which utilize the pollutants as food. This process could be broadly represented in an over simplified way by the following equation:

$$\text{Wastes} + \text{Oxygen} \xrightarrow{\text{Microorganisms}} \text{Treated wastes} + \text{New microorganisms.}$$

It is evident that the new microorganisms - and their breakdown products - could exert their own oxygen demand; but these are easily removed by gravity sedimentation before discharge of the treated effluent into receiving waters. The process of autolysis or autooxidation - which is observed in pure or batch cultures after nutrient exhaustion - also plays a major role in removal of excess or dead microorganisms in the treatment process. Since treatment is mainly by aerobic oxidation the final hydrogen acceptor is oxygen. This process liberates energy for bacterial cell metabolism (Genetelli, 1971, Mara, 1976).

Activated sludge treatment is the most commonly used process for municipal waste water treatment. In this respect it is followed by biofiltration (bacteria or trickling filter beds) and treatment in lagoons and ditches. Septic tank treatment is most commonly used privately by small communities of less than 300 persons or for individual households (Mara, 1976; Pipes, 1966).

A. Septic tanks

Sewage treatment in septic tanks proceeds anaerobically in rectangular, water-tight and usually underground chambers in which sewage from dwellings is retained for 1-3 days. During this time, solids settle to bottom forming a thick sludge, whereas a thick scum forms on the surface. About halfway down the depth of the chamber a layer of relatively clear liquid forms. It is from this clear layer that the septic tank effluent is drawn off for disposal or further treatment (Fig. 1.3) (Mara, 1976).

Septic tank effluent, from a public health point of view, is regarded as hazardous as sewage itself. This is because the primary function of the tank is to remove unsightly scum and sludge, and because anaerobic reactions take place only at the very bottom of the tank thus effecting very little oxidation of pollutants in the clear layer. To remedy this incomplete treatment, septic tanks are often built with coarse, upflow filters which effect further anaerobic oxidation of its effluent. Alternative methods include the incorporation of a small scale percolating filter and allowing the effluent to trickle over it, or by using the effluent for subsurface irrigation, or by allowing the effluent to percolate into soil with sufficient percolative capacity (Mara, 1976). These facts dictate a minimum distance for locating a septic tank and its drainfield from the nearest building, well, stream, embankment, pool, water pipe, paths and large trees. These minimum distances could be as long as 60

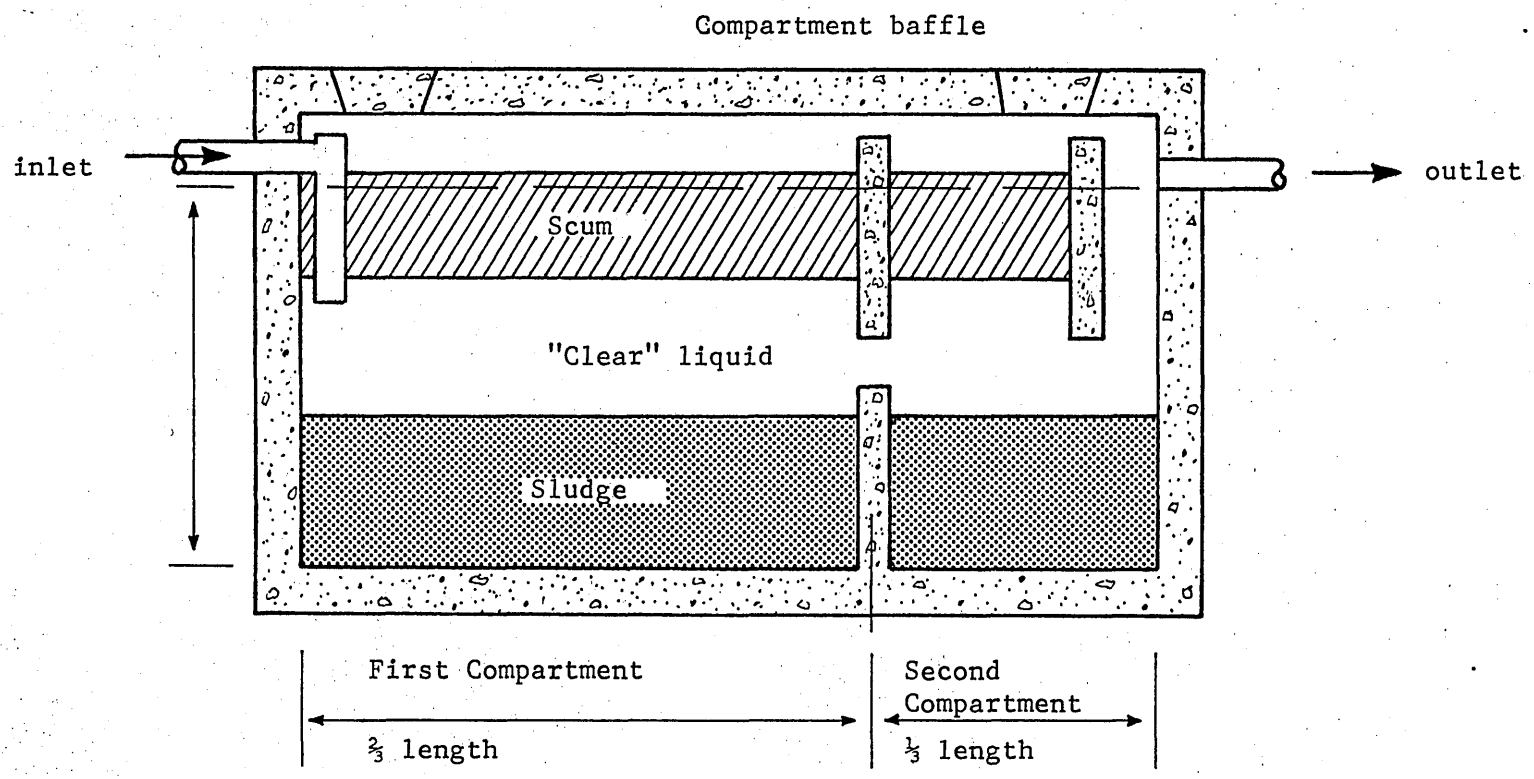


Fig. 1.3. Generalized design plan for septic tank. (from Mara, 1976).

meters from a water body if this is used as a domestic water supply, but in any case the distance cannot be shorter than 1.5 meters for all of the above locations (Cotteral and Norris, 1969).

Since the desired desludging frequency of a septic tank is between 1-5 years, and since this should be done when sludge volume reaches a maximum of $\frac{1}{3}$ total tank volume, then the size of the tank could easily be calculated knowing the rate of sludge accumulation per person per year according to the following formula:

$$F = \frac{\frac{1}{3} V}{S \times P}$$

or $\frac{1}{3}V = F \times S \times P$

where V = Tank volume in cubic metres.

F = Frequency of desludging.

S = Sludge accumulation per person per year.

P = Population served.

Vincent (1963) estimated sludge accumulation rate in Zambia to be 0.03-0.04 m^3/person per year, and Malan (1964) estimated the same rate in South Africa to be 0.032 m^3/person per year. A value of 0.04 m^3/person per year is probably a reasonable value for design.

One modification of the septic tank process is an emergency, anaerobic sanitation unit for 500 persons in disaster stricken areas (Howard, Lloyd and Webber, 1975). The unit is easily installed in one day from the contents of a wooden crate that weighs 500 kilograms and has an estimated working life of 5-10 years. It consists of a number of squatting plates connected in series to two 21,000-litre flexible, reinforced rubber sedimentation tanks which provide a retention time of 8-10 days. An optional trickling filter may be connected to the outlet of the second tank for better effluent quality (Fig. 1.4). The effluent from this sanitation unit was of acceptable chemical quality, and its removal efficiency for Salmonellae, Vibrios, and other bacterial pathogens was at least 83 per cent.

B. Waste Stabilization Ponds

This method of sewage treatment simply takes place by leaving sewage in ponds where bacterial and algal populations gorw and oxidize the sewage. The ponds are usually shallow basins enclosed by earthen embankments thus they have a considerable economic advantage in the cost of their construction and maintenance. They are also the most important and frequently used sewage treatment processes in hot climates where land is usally available and the climate suitable for their proper functioning. However, their use is not restricted to hot coutnries as they are used in all latitudes, even as far north as Alaska.

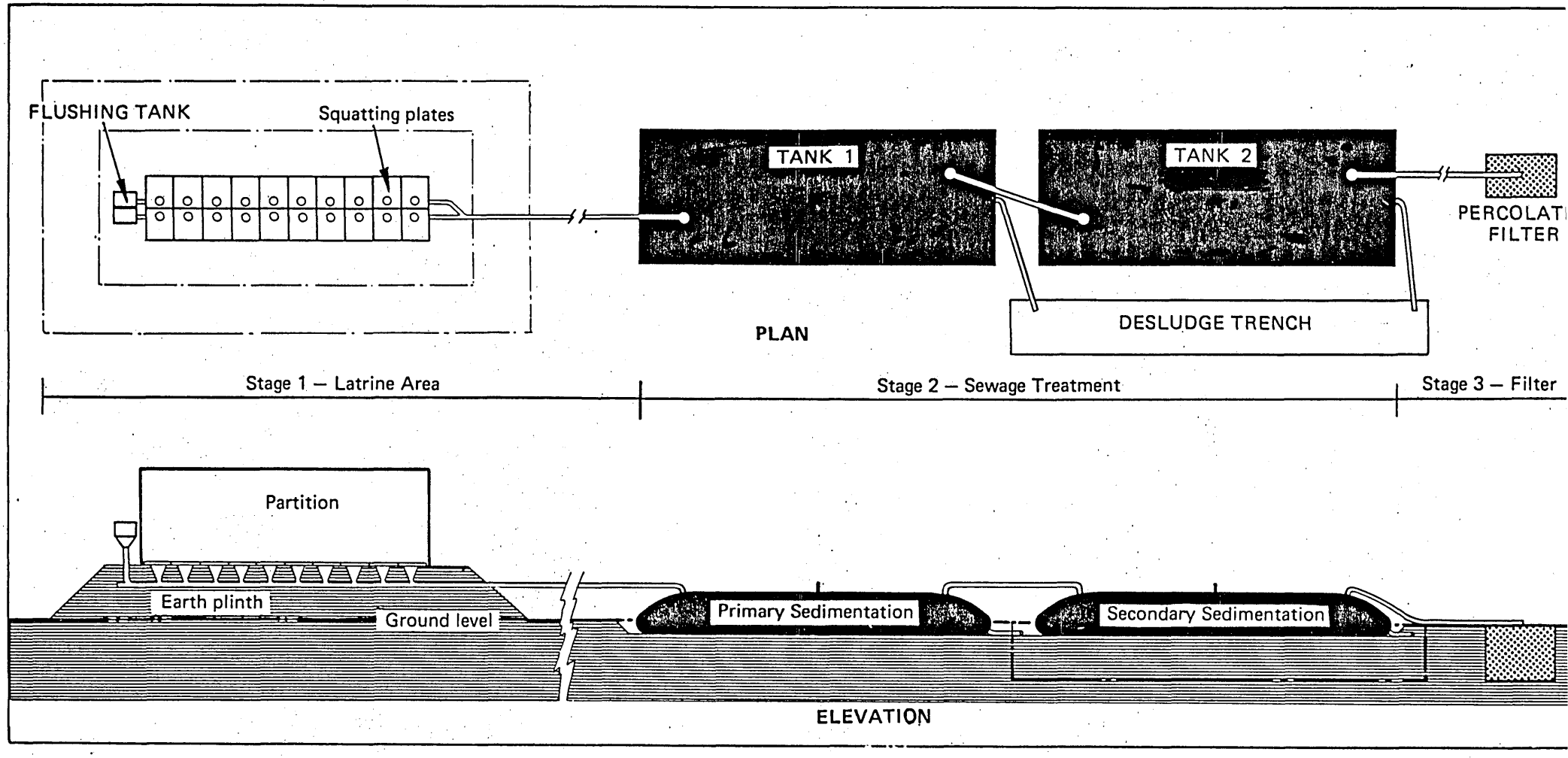


Fig. 1.4. General layout of emergency sanitation unit (from Howard, Lloyd and Webber, 1975).

The most common waste stabilization ponds are the facultative ponds. These affect treatment by a mixture of aerobic and anaerobic processes with the aerobic processes prevailing near the top layers of the pond. Two processes keep the top layers in aerobic conditions namely: reaeration and production of photosynthetic oxygen by the algal populations. Thus a symbiotic relationship exists between the algae which supply the bacteria with O_2 and receive metabolic CO_2 from them (Fig. 1.5).

Proper performance of the pond resides in proper mixing which depends solely on heat and wind. These create currents in the pond and bring up to the photic layers of the pond those non-motile algae which would otherwise sink to the bottom and exert an extra oxygen demand. Maximum mixing by wind action occurs when an unobstructed contact distance of 100 metres is allowed on the surface of the pond (Mara, 1976).

Intense anaerobic sludge digestion occurs at the bottom of the pond at a temperature of at least $15^{o}C$. If temperatures rise to above 20 or $22^{o}C$, intense methane production could float sludge and heavy sludge mats form on the surface of the pond. These of course prevent light penetration into the photic zone and must be removed manually. Pond depth is usually set between 1 and 1.5 metres; the lower limit is the minimum depth for prevention of emergence of vegetation and the upper limit prevents

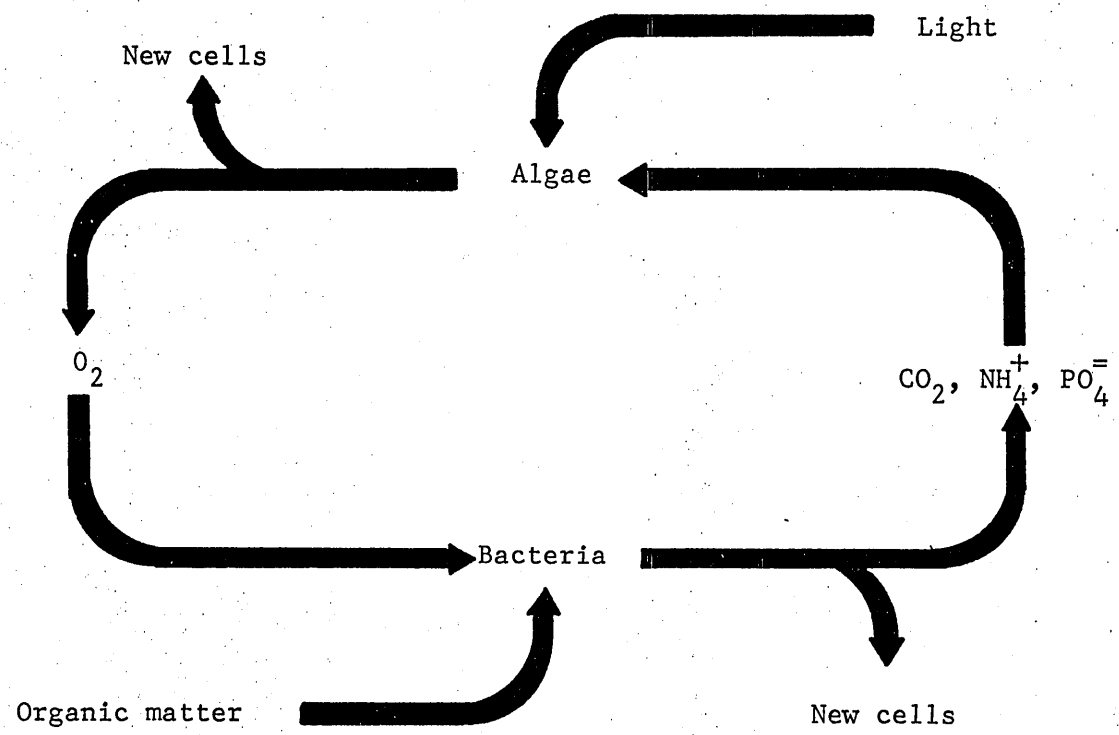


Fig. 1.5. Symbiosis between bacteria and algae in stabilization ponds.

(From Feachem, McGarry and Mara, 1977)

anaerobic conditions from becoming too prevalent in the pond. Depths of at least two metres are employed in extreme climates; this preserves heat in cold climates and minimizes evaporation in hot climates.

Stabilization ponds have a number of advantages that establish their value in sewage treatment:

1. Any degree of sewage treatment can be achieved at the lowest cost and with minimum maintenance.
2. The environment of a stabilization pond is hostile enough to affect high degrees of pathogen inactivation. Snails do not survive in such a habitat, and thus the spread of Schistosoma is checked. Christie in 1966 reported that stabilization pond contents were capable of reducing poliovirus titres by 100,000 fold.
3. Stabilization ponds tolerate high organic and shock loadings.
4. They can effectively treat a wide variety of industrial, domestic and agricultural wastes.
5. Easy alteration of design for altered degree of treatment.
6. Their construction offers easy land reclamation should the land be required.
7. The algae produced in the pond is a potential high protein food source which could easily be coupled with fish farming (Mara, 1976).

C. Trickling filters

As Genetelli (1971) puts it, "the name is a misnomer since the biological unit does not filter nor does it trickle." Other names for this process, in addition to trickling filters, are percolating, biological filters, bacteria beds. The process is also called biofiltration (Mara, 1976).

Trickling filters are circular or rectangular beds of coarse, porous, impervious, hard and rough material usually stone such as clinker or coke breeze of 30 to 60 mm. grading. The beds are usually 1.8 metres deep but could have any depth between 0.9 to 3.6 metres. Settled sewage is sprayed or otherwise distributed over the bed (via a distributor) through the air to flow on the surface of the bed packing material in a thin film in contact with air. Distributors are in the form of rotating perforated pipes for circular beds, and in the form of a single perforated pipe usually travelling (back and forth) parallel to the width and along the length of a rectangular bed. A trickling filter bed is also provided with underdrains which lead to collecting channels. The system of underdrains also allows ventilation of the filter bed.

Oxidation of sewage is accomplished by the film of micro-organisms on the surface of the bed packing material. It is evident that a new bed would be lacking this microbial film which soon develops on the surface of packing material and

eventually reaches a population equilibrium. The seed for these microorganisms, mainly bacteria and protozoa (Genetelli, 1971), comes with the sewage and attaches itself to the filter. These microorganisms or biota oxidize the organic matter in sewage as it diffuses into the microbial film. Suspended, colloidal as well as dissolved organic matter is utilized as nutrients for these microorganisms and are removed and oxidized in this way. Treated sewage goes from the filter to settling tanks to remove any suspended matter - also called humus solids - that might be in it. The origin of such suspended matter is often due to the sloughing off of the microorganisms which die and leave the filter with the treated sewage (Fish, 1973; Genetelli, 1971; Mara, 1976).

Full scale trickling filter plants are operated in single or multiple filter beds, in series or in parellel, with various proportions of their effluents recycled through the filter (Genetelli, 1971). Supernatent liquor from humus settling tanks is also returned and pooled with the filter beds influent. When trickling filters are operated and maintained properly without overloading they produce a good quality effluent within 20-30 mg/l BOD_5. It is evident that a filter continues to function properly if conditions do not allow blockage of the filter and if the influent does not contain substances toxic to filter organisms such as cyanide, copper, chromium and other heavy metals (Genetelli, 1971).

D. Activated Sludge Treatment

Activated sludge process is the alternative to bio-filtration and is the most common waste water treatment method in the U.K. and other regions of the world. In this process settled sewage is led to the aeration tank where it is mixed with the sludge that is composed of bacterial flocs on which protozoa and some metazoa graze. After mixing and aerating the sewage and activated sludge for 6-8 hours, the mixed liquor - as it is now called, is led to the final settling tanks where the activated sludge is settled and returned to the aeration tanks to treat further incoming sewage. Activated sludge solids concentration is usually maintained at 2,000 to 8,000 mg/l and the plant operates at a BOD_5 loading of 500-600 mg per litre of mixed liquor. Excess sludge is usually disposed of on sludge drying beds, used as fertilizer or for land fills or incinerated. The supernatant liquid from settling tanks forms the final effluent from the plant which is discharged into receiving waters (Fish, 1973; Genetelli, 1971; Mara, 1976; Pipes, 1966; Southgate, 1969).

When a new activated sludge plant starts operation, it is either seeded with activated sludge from another plant, or, if the sewage is aerated, sludge will form and it will have the desired suspended solids within 4-6 weeks provided it is properly settled and returned to aeration. These solids are mainly composed of bacteria in flocculated masses of slimy matter

on which protozoa attach and graze. The majority of these bacteria are gram-negative bacteria which may be of the nitrifying or non-nitrifying varieties mainly belonging to the genera Pseudomonas, Achromobacter, and Flavobacterium-Cytophaga (Pike and Carrington, 1972). Lighthart and Oglesby (1969) classified these principal genera into three clusters, I faecal forms, II Pseudomonas aeruginosa and III undefinable types. Of these types, the main nitrifying species of bacteria, Nitrosomonas and Nitrobacter are present in activated sludge and they are responsible for oxidizing ammonia (Hawkes, 1963; Pike and Carrington, 1972; Pipes, 1966). Many other bacterial genera have been isolated from activated sludge, but due to the complex nature of activated sludge, no single list of bacteria or other organisms can be taken as complete or exclusive. Difficulties relating to the isolation procedure especially with the mass of floc in which the organisms are present, coupled with specificity and sensitivity of the media or procedures chosen, make any such study far from being complete (Hughes and Stafford, 1976).

Floc formation in activated sludge was first thought to be due to the slime production by Zooglea, into which bacteria and inert debris get entangled. Butterfield, (1935) concluded that Zooglea was the main species responsible for floc formation, but this was disputed when many bacteria of activated sludge were induced to flocculate in aerated pure cultures (McKinney and Harwood, 1952).

The mechanisms of floc formation in activated sludge largely depend on the nature of the gelatinous, slimy substance secreted by the bacteria (Hughes and Stafford, 1976). The bulk of the slimy substances on activated sludge flocs was found to be composed of polysaccharides, heteropolysaccharides and a polyester (β-hydroxybutyric acid), (Forster, 1971; Steiner, McLaren and Forster, 1976; Wallen and Davis, 1972). There are four theories for mechanisms by which flocculation of bacteria and colloidal particles takes place in activated sludge. All these depend, to a lesser or greater extent, on a combination of van der Waal's forces, ionic and electrical charges and the ability of polymers to neutralize them, complexing and binding capacities of polyvalent cations and an affinity of activated sludge flocs to air-water interfaces (Hughes and Stafford, 1976).

Activated sludge also contains a multitude of organisms ranging over the whole spectrum of microorganisms including protozoa with a number of metazoa. The sources of activated sludge organisms are from natural freshwater, human microfauna and microflora. Organisms from animal origin, for example cattle, sheep or swine almost certainly exist in sewage and sewage treatment plants especially where farm effluent is pooled with domestic sewage.

Of these groups of organisms, protozoa are abundantly represented in activated sludge, making up 5 per cent of the mixed liquor solids and numbering at least 228 species of which 160 are ciliates (Pike and Curds, 1971) which were considered the

most important species by these authors. When a new sludge forms, there is an ecological succession in the type of protozoa present that corresponds to the conditions of the sludge. The first protozoa to populate activated sludge would be rhizopods (e.g. Amoeba), followed by flagellates (e.g. Euglena), free swimming ciliates (e.g. Paramecium), crawling ciliates (e.g. Aspidisca) and finally stalked ciliates (e.g. Vorticella, Opercularia) (Pipes, 1966). Whether protozoa are responsible for breakdown of organic matter or for clarification of wastewater is a debated issue. No matter what, these organisms live in the environment of activated sludge and obviously get their food from it and ciliates are known to belong to one of three groups as far as their feeding habit is concerned;

(1) bacteria feeding,

(2) omnivorous, and

(3) carnivorous (Curds, 1969). Curds, Cockburn and Vandyke, 1968 , however, reported that withdrawal of ciliates from the treatment process produced turbid effluent while their re-introduction clarified it. Similar experiments with industrial effluent did not produce the same result (Hughes and Stafford, 1976)

Fungi are also present in activated sludge but they are not as abundant as bacteria or protozoa and this may well be due to lack of reports in the literature. However, some of the genera that were isolated include Aspergillus, Rhizopus, Penicillium and others. Some other genera were reported in very large numbers in activated sludge (Pipes, 1966).

It is not unlikely that fungi play an important role especially in floc formation of activated sludge, but research in this field is still lacking. Some fungi, however, were isolated from bulking activated sludge - a condition characterized with poor settling and effluent - and these were mainly filamentous fungi like Sphaerotilus, Geotrichum, and the rotifer trapping fungus Arthrobotrys (Pipes, 1966).

Invertebrates like rotifers and nematodes are frequently present in activated sludge as well as insects and insect larvae. No definite roles have been attributed to these in activated sludge (Pipes, 1966).

In addition to the basic activated sludge process and the operational modifications like tapered aeration, stepped aeration and contact stabilization, there are other methods like the aerated lagoons, oxidation ditches (Mara, 1976) and the newest ICI deep shaft aeration process. All these processes utilize the same medium of activated sludge but their process design is different. Aerated lagoons are activated sludge units without sludge return. They evolved from waste stabilization ponds with simple addition of aerators to supplement algal oxygen, but as soon as aerators were put into operation the algae disappeared and the microbial flora resembled that of activated sludge. Aerated lagoons could achieve a BOD_5 removal of at least 90 per cent with relatively long retention time of 2-6 days (Mara, 1976).

Another modification of activated sludge is the oxidation ditch (Pasveer ditch) which is a long or oval channel and about 1-1.5 metres deep. The oxidation ditch liquor is aerated by one or more cage rotors (brush aerators) placed across the channel. These rotors propel the liquor along the ditch at a speed of 0.3-0.4 metres per second. A sludge concentration of 3000-5000 mg/l is usually maintained in the ditch by recirculation of the settled sludge and the excess disposed of usually on sludge drying beds (Mara, 1976; Southgate, 1969).

A novel sewage treatment process devised by Imperial Chemical Industries Ltd. is called the ICI deep shaft aeration process for effluent treatment. The process was originally designed for large scale production of protein employing pure cultures of microorganisms, but was later utilized as a sewage treatment process. The process is mainly aimed at land saving in populated areas, and employs a deep, partitioned shaft in which the liquor circulates, mixed with air in a downward flow section called the downcomer and in the upward flow section called the riser. The process is aimed at meeting the 20:30 effluent standard in about two hours retention time, the standard achieved by the conventional methods in a retention time of 6-8 hours. The increased efficiency of this process is imparted by increased solubility of air at pressures of 100-300 metres of water, this being the depth of the shaft. Other processes, both pre- and post-aeration remain the same as the conventional methods (Hines et al., 1975)

E. Marine Disposal of Sewage

Another method of sewage disposal is that of the disposal of raw, whole sewage into the sea or ocean. This practice is still the main method of sewage disposal in many countries. This method is properly done by discharging sewage at least ten metres deep under the low water level and in such a location as to allow currents to carry it away from the seashore. Dilution and other natural oxidation processes coupled with settling usually inactivate the sewage, but this is a slow process and results in coastal pollution if not done properly.

F. Treatment with Water Hyacinth

A new method for sewage treatment was reported recently, it simply involved leading sewage into a lagoon where a water hyacinth (Eichhornia crasspipes) was planted. The hyacinth was highly prolific in sewage with as high BOD_5 removal as 80 per cent. This simple method involves no high cost and has advantages in that the hyacinth itself is very good for production of fertilizer, biogas (60-80 per cent methane) and animal feed. All these features make it quite suitable totreat sewage on large and small scales equally (Wolverton and McDonald, 1976).

The survival and persistence of pathogens in sewage and sewage treatment processes will be dealth with in the review of the literature.

II. OCCURRENCE AND TRANSMISSION OF VIRUSES BY WATER AND WASTE WATER

(Review of Literature)

Feachem (1977) and Bradley (1977) classified pathogens which may be transmitted by water according to their relationship to the water cycle as water-borne (faecal-oral transmitted agents e.g. cholera and infectious hepatitis), water-washed (skin and eye infections, e.g. trachoma), water-based (Schistosoma in snails) and water-related insect vectors (which transmit diseases such as sleeping sickness and malaria). The control of the incidence of diseases caused by these pathogens and parasites entails (a) proper treatment and disposal of sewage and excreta, (b) improved hygiene, (c) improved management of surface water quality, and (d) elimination of host (e.g. snails) and vector (e.g. insects) organisms.

The removal of viruses from water and waste water is far from satisfactory (Berg 1973a, Chang, 1968). However, the larger organisms may be removed by filtration and sedimentation and the coliform test, which is considered an adequate and reliable indicator of faecal pollution and water quality (Wolf, 1972), provides an index of the success of these measures. Although it is worth noting that Dutka (1973) and Scarpino (1971, 1975) have expressed doubts about the validity of the coliform test on the

basis that it did not strictly conform to the criteria which should be met by an indicator organism. These criteria, include (1) presence (in greater numbers than pathogens) whenever pathogens are present, (2) higher resistance to treatment or disinfection than pathogens, (3) inability to proliferate in the water medium, (4) have simple characteristic and unambiguous identification procedure, and (5) the indicator must be harmless to man.

A more satisfactory bacterial group which could meet these criteria are the faecal streptococci since, unlike coliforms, they rarely (if ever) multiply in natural waters (Geldriech and Kenner, 1969).

The presence of bacteria as indicators of pathogenic viruses is unsatisfactory. Forinstance, Gilcrease and Kelly (1954) compared the survival of E. coli, the E. coli B bacteriophage and Coxsackievirus A in samples of spring water, stored, autoclaved salt water, river water, brackish, swamp and hard water. On the basis of their results they concluded that the absence of coliform bacteria indicated the absence of intestinal viruses; but this conclusion is rather puzzling since their results show that the viruses tested survived better than E. coli (Table 2.1).

Further evidence for a lack of correlation between the extent of bacterial and viral pollution of water was provided in an analysis of the hepatitis A epidemic in New Delhi in 1955-56, in which the number of hepatitis cases was in stark contrast to the absence of concomitant increase in typhoid or dysentery cases which led Dennis (1959) to conclude that the control of bacteria and

protozoa at the Chandrawal water treatment works remained adequate while hepatitis A escaped these measures.

Table 2.1. Survival of some viruses compared to that of *E. coli*. (data from Gilcrease and Kelly, 1954).

	Reservoir water	Salt water (8-10°C)	Spring Water		Chlorination (0.1 ppm, 10 minutes)
			8-10°C	59-60°C	
E. coli.	12 weeks	5 weeks	3-4 weeks (1%)	15 minutes	50%
E. coli. B phage	36 weeks	25 weeks	3-4 weeks (58%)	4 hours	40%
Mouse encephalomyelitis			3-4 weeks (100%)	1 hour	100%
Coxsackievirus A			3-4 weeks (100%)		

1. Occurrence of viruses in Sewage

Much of the direct evidence for the presence of enteric viruses in sewage is from reports of surveillance and monitoring of water and waste water. Indirect evidence, however, contributes significantly especially in accounts of previous epidemics of viral disease where the water source had been incriminated and reports of experimental infection of human volunteers by administration of stools filtrates from clinically ill patients with suspected viral disease. The viruses include polio, Echo, Coxsackie, adeno, reo and rotaviruses and the agents of infectious hepatitis and winter vomiting disease, all of which may be excreted in large numbers in the faeces (Berg, 1973a; Kollins, 1966; Appleton and Pereira, 1977).

In theory, of course, all enteric viruses may be present in sewage; and, depending upon the type of sewage treatment, these viruses may reach and infect humans. Some viruses which are excreted by man in the urine may also be present in sewage. For example, these include the viruses of herpes simplex, rubella, Coxsackie, Echo, vaccinia, measles, mumps, lassa and rabies (Utz, 1974). But the role of water in the ecology and transmission of disease caused by these viruses is probably neglibible. Whatever the case, the transmission of enteric viral infections depends upon the social factors and the way of life people lead. Hygiene and the quality of municipal sewage works provided for the community are of prime importance in the control of enteric disease transmission (Krejs, Gassner and Blum, 1974).

The viruses of hepatitis A and infectious gastroenteritis (rotaviruses), in addition to other viruses, are probably the most important examples of viruses which may be present in water and, of course, may be responsible for outbreaks of disease (Fattal and Nishmi, 1977; Flewett, 1977; Kollins, 1966; Krejs, Gassner and Blum, 1974; Nupen, 1970; Thornton and Zuckerman, 1975). Evidence for the transmission of rotavirus by the oral route is essentially experimental and in a formative stage of investigation.

There were fifty documented outbreaks of water-borne infectious hepatitis from around the world (Berger, 1970; Mosley, 1967). Later, McCabe and Craun (1975) listed eleven water-borne outbreaks in the United States and Canada in 1971-72 alone. The worst of all these reported epidemics, by far, was that which occurred in New Delhi in 1955-56 where about 29,000 cases were reported (Dennis, 1959; Viswanathan, 1957). The cause of that epidemic was attributed to a heavy contamination with sewage of the raw water supply of the Chandrawal treatment plants. Attempts to prevent contamination of the Jumnah river at the point of raw water intake by the heavily contaminated sewage channel of Najafgarh Nallah that drained the sewage of an industrial community of 40,000 failed, and the explosive outbreak occurred. Dennis (1959) concluded that the capacity of the water treatment to cope with bacterial contaminants was unimpaired because there was no untoward increase in typhoid or dysentery despite an enormous hepatitis epidemic, already noted.

However, agents responsible for gastrointestinal disorders may be bacterial, protozoal or viral. Many such outbreaks are documented; and viral aetiology is usually incriminated when no other agent is isolated (Mosley, 1967). Weibel et al. 1964 listed 126 epidemics of gastroenteritis and 16 epidemics of diarrhea in the United States between 1946 and 1960. Causative agents for the majority of these outbreaks were not identified but viral aetiology was suspected. Dolin et al. (1971) experimented with the transmission of gastroenteritis to healthy volunteers by oral administration of stools filtrates from one gastroenteritis epidemic on a U.S. Navy ship and three others on continental United States. These workers confirmed the transmission of clinical gastrointestinal illness through the administration of a filterable - thus probably viral - agent. Earlier studies (Gordon, Ingraham and Korns, 1947; Jordan, Gordon and Dorrance, 1953) also confirmed probable viral aetiology of gastroenteritis using the same technique.

Poliovirus may also be transmitted via faecally contaminated water (Mosley, 1967). Kling as the director of the State Bacteriological Institute in Sweden - the first country in which paralytic poliomyelitis became apparant in epidemic proportions - went so far as to advocate the idea that all poliomyelitis epidemics were spread only by water:"sans eau aucune formation de foyers poliomyelitiques" (Fenner et al., 1974; Mosley, 1967). Mosley (1967), however, did not rule out other routes for the epidemics which occurred in Sweden (during 1930's and 1940's) and attributed them to

an over enthusiastic search (sparked by Kling's conclusions) for instances of water-borne poliomyelitis. Mosley lists other epidemics of poliomyelitis but only two examples are reasonably well documented. The first occurred in Huskerville, Nebraska in 1952 (Bancroft, Englehard and Evans, 1957) and another in Edmonton, Alberta in Canada in 1953 (Little, 1954). The Huskerville epidemic took place in a university community of families living in temporary houses and claimed seventeen cases of paralytic and twenty eight cases of non-paralytic illnesses. The cases were distributed in two and a half rows of the houses, with only one non-paralytic case in the remaining one and a half rows. The distribution of cases followed a pattern which closely correlated with the locations of those water closets whose flush valves were not provided with vacuum breakers which prevent aerosol spread during flushing, and with the location of water mains in which wide fluctuations in pressure (down to negative) occurred before and during the outbreak.

The 1953 Edmonton epidemic was marked with an upsurge in the number of incidences and 75 cases were reported in the first two weeks of November. The coincidence between these cases and the failure of sewage effluent chlorination facilities in Devon - the town 20 miles upstream - where there were 5 confirmed and 30 suspect poliomyelitis cases. These facts, coupled with the previous knowledge that poliomyelitis patients continue to excrete the virus for weeks, led to an incrimination of the water supply to Edmonton (Little, 1954).

An important question which arises from the evidence for transmission of viral disease via faecally contaminated water is would disease have been prevented had a test indicator virus been available? Bacteriological disease is certainly controlled on the basis of the coliform test. But, unfortunately, no equivalent indicator virus or test has yet been formulated or accepted as adequate for viral pollution.

There are only a few reports on the efforts to find an indicator of viral pollution, for instance, Gilcrease and Kelly (1954), recommended the use of E. coli based on comparative studies with Bacteriophage, Coxsackie viruses A and mouse encephalomyelitis virus. Cohen and Shuval (1973) re-examined the suitability of coliforms, faecal coliforms and faecal streptococci as indicators of viral pollution. They monitored two biological filtration sewage treatment plants, an open sewer, a polluted river, a lake, polluted springs and wells for coliforms, faecal coliforms and streptococci, and related their incidence with that of selected enteric viruses. All three bacteria had an apparently high ratio to virus, $1:10^6$ for coliforms, $5:10^5$ for faecal coliforms and $2.5:10^4$ for faecal streptococci in polluted waters. They concluded that it would be best to take the faecal streptococci as indicators of viral pollution but it should be noted that there was no comment about the sensitivity of their methods for isolation of the viruses.

It is, of course, possible to use the enteric viruses themselves as indicators of viral and faecal pollution. Coin et al. (1964) after an extensive survey on monitoring bacteria and viruses in the Paris water supply and waste water systems found that poliovirus was most frequently isolated from their samples.

Based on this observation, they recommended the use of poliovirus as a suitable indicator of faecally polluted waters. Whether poliovirus would be as an indicator for hepatitis A is less than clear as for other faecally transmitted viruses, for there is little information on the relative numbers of different viruses with respect to each other. Metcalf (1971), in a review article warned that the use of poliovirus as an indicator was unsatisfactory. He further argued that the presence or absence of any of the enteroviruses does not provide any information on another unrelated enterovirus.

The presence of bacteriophages as natural inhabitants of the human intestines, and their consequent presence in sewage of domestic origin led many investigators to consider them as potential indicators of faecal and viral pollution (Scarpino, 1975). Their frequency in sewage and surface waters, coupled with the economic, simple, rapid and reliable estimation of their numbers gave them added advantages. Ware and Mellon (1956) reported the presence of $10^{2.57}$ pfu/ml and Pretorius (1962) reported the presence of $10^{3.42}$ pfu/ml in sewage. Much wider fluctuations were reported by other authors varying from $10^{1.26}$ - $10^{3.98}$ pfu/ml (Dias and Bhat, 1965), and from $10^{1.56}$ - $10^{4.20}$ pfu/ml for sewage from various rural and urban localities in Hong Kong (Dhillon et al., 1970). The ratio of coliphages to coliform bacteria, however, showed wide variations from 1:52 (Pretorius, 1962) to 1:358 (Ware and Mellon, 1956).

Buras and Kott (1966) put forward a recommendation that coliphages be used as indicators of viral pollution of water. Later, Shah and McCamish (1972) recommended the use of f2 as an indicator virus in water and waste water based on its resistance to chlorination. Kott et al. (1974) reaffirmed this recommendation based on their findings on the relative survival of f2 coliphage and poliovirus I in an experimental oxidation pond in which the survival of both viruses was unaltered. Their results also yielded coliphage to enterovirus ratios from 1:1 to 10^5:1 in flood waters, waste water in various seasons, trickling filter and oxidation pond effluents with the highest ratios for waste water and the lowest ratios for flood waters. Seasonal variations were observed in waste water and effluents with high to moderate ratio values for srping and fall. Attempts to isolate coliphages from well water during 3 outbreaks of enteric disease in small communities yielded no coliphages, and only two samples were positive for enterovirus. All the above results were obtained by filtering samples through 0.45μ filter, followed by further concentration by alginate ultrafiltration and recovery of virus therefrom - a somewhat complex procedure.

Experiments on the relative resistance of viruses to disinfectants have shown that f2 was most resistant to chlorination followed by poliovirus and T2 (Shah and McCamish, 1972). Similar results were obtained by Scarpino (1975) with chloramines, whereas hypochlorous acid showed a reversal of that observation with poliovirus I being the most resistant followed by Coxsackie virus A9, MS2 and

f2 coliphages, E. coli and T5 coliphage. Preliminary results in this laboratory using chloramine-T as a disinfectant at levels of 1-18 ppm (determined as active chlorine) in effluent (pH 7.6-7.8) did not seem to effect any inactivation of the f2 coliphage tested (Hajenian, 1977, personal communication).

There have been other recommendations for the use of bacteriophages as indicators. Carstens et al. (1965) recommended that bacteriophages of Serratia marcescens could be used as indicators for pathogenic bacteria and animal viruses in water. This was based on the observations that Serratia marcescens bacteriophages were inactivated at rates similar to that of E. coli. and slower than that of Mahoney strain of poliovirus I. Smedberg and Cannon (1976) have made a provocative recommendation that the cyanophage LPP-1, a parasite of the filamentous, non-blooming, blue-green algae Lyngbya, Phormidium and Plectonema, be used as a replacement of the coliform index for bacterial and viral contaminants of water. This recommendation was based on the authors' conclusion that the virus, which is easily and rapidly titrated, is always present in polluted water in large numbers and is more resistant to chlorination than E. coli. The authors' conclusions and recommendations were in striking contradication to their data which show LPP-1 to be much more sensitive to chlorination. These facts make their paper entirely unsatisfactory, and the authors did recognize the drawbacks of their paper but claimed that later unpublished results confirmed and supported their recommendation (Cannon, 1977, personal communication).

The search for a bacteriophage (or other phage) as an indicator for viral pollution has intensified in recent years. The justification for using these organisms as indicators for enteroviruses is the alleged similarity in behaviour although this behaviour is not yet adequately understood (Metcalf, 1971).

2. Removal of virus during waste water treatment

The removal or inactivation of virus during the various methods of waste water treatment has received increasing attention for at least thirty years. But it was not until 1952, when Dulbecco introduced the plaque assay method to animal virology, that (accurate and meaningful) quantitative studies on the efficiency of virus removal during waste water treatment were possible. Since then, many studies were reported employing different viruses, laboratory models or full scale treatment plants.

A. Laboratory model studies:

The first reported study of viruses in waste water treatment was that of Carlson, Ridenour and McKhann (1943). They inoculated a batch type aeration unit filled with mixed liquor from a full scale activated sludge treatment plant and noted that great reductions in the infectivity of the virus were achieved. However they did not feel justified in expressing their results quantitatively since the virus was assayed in mice and the animals died at different times with or without the development of paralysis.

Kelly, Sanderson and Neidl (1961) also used a "fill-and-draw" laboratory model for their studies of the removal of viruses, including poliovirus I, Coxsackie virus B type 5 and T2 coliphage. However their results were presented in a confusing way which makes an assessment difficult. Forinstance, they mention that they used Coxsackie virus B5 in some experiments but do not give the results. In the studies on poliovirus, 78 per cent was removed while for T2 coliphage only about 40 per cent was removed. The authors also reported no loss of virus when they bubbled nitrogen gas instead of air through the tank. Furthermore the incorporation of glucose at final concentrations of 0.001-0.01 molar had no effect on the behaviour of the viruses. But the addition of the metabolic inhibitor, malonic acid (which inhibits succinic acid dehydrogenase) greatly reduced the removal of virus, whereas a respiratory stimulant had no detectable effect. This information may indicate a certain relationship between the metabolism of activated sludge and virus removal; and it is regretted that the authors did not follow it up to make its interpretation possible. Their low recovery of virus from the inoculated sewage, mixed liquor liquid and "sludge", led them to conclude that inactivation was taking place. Although the authors report virus titres for "sludge", it is not clear whether this refers to the solids fraction of the mixed liquor or whole sludge.

Clarke et al. (1961) were the first to use a laboratory model of a continuous process of activated sludge for the study of removal of virus. The model had been devised by Ludzak (1960) for other types of studies and consisted of an aeration tank, subdivided to provide a settling compartment. They studied the removal of Coxsackie virus A9 and poliovirus I during the continuous process while continuously inoculating the plant with virus and observed that 79-94 per cent of poliovirus I was removed while the values for Coxsackie virus A9 ranged from 96.1 to 99.4 per cent with rates as high as 99.99 per cent removal in six hours also reported. These authors also studied the fate of poliovirus I introduced into batches of sewage which were then allowed to settle out. In the uppermost layers of the sewage, only 29-62 per cent of the virus was recovered after 24 hours. While in control experiments in buffered aqueous suspensions, poliovirus was not significantly reduced by aeration or storage. None of their experiments, were unfortunately aimed at an examination of the deposited solids in the process.

Laak and McLean (1967) investigated the survival of poliovirus III in an activated sludge treatment plant model by conducting single inoculation experiments and monitoring the virus in the centrifuged effluent and mixed liquor (liquid). Poliovirus was found to persist in the mixed liquor (liquid) for up to six days, and in the effluent for at least 3 days. Sand column effluent, through which the model plant effluent was allowed to percolate,

were positive for virus for about as long as the model plant effluent was positive. But sand core samples from these columns were positive for virus for about 2-5 days after the model plant effluent ceased to be positive.

Sobsey and Cooper (1973) investigated the survival of poliovirus I in a laboratory model of a stabilization pond treatment process and deduced from measurement of virus in the liquid fraction that 25-50 per cent of the virus was adsorbed to the solids. They compared this with virus survival in bacterial, algal, algal-bacterial batch cultures and in pond bacteria. They observed highest virus "inactivation" in the pond bacteria cultures reaching 99.999 per cent over 280 hours. Again the study, like that of Clarke et al. (1961), was incomplete in that there was no attempt to recover viruses directly from the solids.

Other authors (Malina et al., 1974; Malina et al., 1975) studied poliovirus inactivation in a laboratory model of the activated sludge process. Their plant consisted of a mixed liquor aeration tank subdivided to accommodate a settling tank. Poliovirus was assayed by both infectivity assays and by tritium radioactivity counts. The virus particles associated with solids were eluted by shaking solids, after centrifugation, in distilled deionized water for fifteen minutes at high speed using a mechanical shaker and the virus suspension obtained used for infectivity assay. Their data on batch studies indicated immediate adsorption of the virus onto activated sludge solids. The distribution of radioactivity counts at six minutes after inoculation was 179 cpm/ml of

the liquid fraction and 6000 cpm/gm (presumably grammes wet weight) of solids at a suspended solids concentration of 2200 ppm.

Malina and his co-workers considered that poliovirus which did not appear in the effluent was inactivated by activated sludge solids which have constantly changing surfaces and are constantly oxidizing substrates. They stated in the results that the activated sludge solids exhibited a capacity permanently to "inactivate" adsorbed virus with "consequent release of degraded viral components." Their results showed average reduction through their model plant of 98 per cent with no differences between experiments aerated with oxygen or air. They obtained similar results in experiments in a model of the contact stabilization process. Their attempts to recover and assay virus adsorbed to the solids by eluting the virus in a 10 per cent calf serum in borate saline buffer at pH 9 yielded only about 1 per cent of the influent virus on the mixed liquor solids. They also reported no accumulation of virus on the solids during prolonged inoculation of their model plant up to 8 days.

Malina et al. (1975) concluded that the capacity of activated sludge to inactivate poliovirus was independent of organic loading of the model plant, retention time, suspended solids concentration or amount of oxygen in the aerating gas. Furthermore, they concluded that their model of the contact stabilization process (with contact time of 16 minutes) was no less efficient in removing poliovirus than their model of the conventional activated sludge process (with contact times of 5 to 15 hours). None of their data indicated any

loss or decrease in the capacity of sludge to inactivate virus upon continuous and prolonged inoculation.

Clarke and Chang (1975) have been the only group to report on the removal of enteroviruses by a bench scale rotary-tube trickling filter. The model they used was composed of three lucite tubes whose inside smooth surfaces were roughened to provide a suitable surface for slime growth. The tubes were fed with domestic sewage and rotated at 16 revolutions per minute. Eighty five, 83 and 94 per cent of poliovirus I, echovirus 12 and Coxsackie virus respectively were removed at medium filtration rates (corresponding to a 10 million gallon per day (mgd)/acre of filter area). Lower rates of 59, 63 and 81 per cent were reported for poliovirus I, echovirus 12 and Coxsackievirus A9 (respectively) at a high filtration rate (of 23 mgd/acre).

B. Full scale plant studies:

Kelly and Sanderson (1959) surveyed sewage treatment plants in the United States and found that sewage treatment may not destroy virus. The frequency of isolation from activated sludge influent was greater than from the effluent, but the frequency of isolation from trickling filter influent and effluent was about the same. The frequency of virus isolations followed a consistent pattern with a minimum (10 per cent of samples) around February and March and a maximum (80 per cent of samples) around July and August. This pattern of distribution agrees with the pattern reported by Mack et al.

(1962) who monitored an activated sludge plant by collecting dip (grab), pad and sludge samples. All their samples (including sludge samples) were centrifuged and only the supernatant fluid was assayed for virus. The frequencies of isolation of virus, which those authors observed, were 0.3 per cent of the dip samples, 16.4 per cent of the gauze pad samples and 10.9 per cent of the sludge samples. Gradual decrease in virus content of sewage was observed as it was processed and the highest reduction (92-95 per cent) was observed after chlorination.

Seasonal fluctuation in the virus content of sewage was also observed by Bagdasaryan and Kazantseva (1965). These authors employed the gauze swab method to collect their samples, and their findings confirmed that treatment reduces the virus content of sewage; no complete de-contamination, however, was obtained.

Isherwood (1965) and Bush and Isherwood (1966) studied the fate of Coxsackie virus A13 in a combined activated sludge and trickling filter sewage treatment plant by inoculating the influent and monitoring the virus through the plant. Fluid from the gauze pads which were used to monitor the incidence of virus was centrifuged and assayed by intramuscular inoculation of mice. Although no quantitative results for the efficiency of the plant were reported, they concluded, in general, that Coxsackie virus A13 could be detected in the effluent.

Virus reduction in waste stabilization ponds was studied by Christie (1966), who used tube cell cultures for the assays of the virus. He deduced that the bulk of the poliovirus was "inactivated"

on the basis of low titres of virus in the liquid fraction. He also reported that no virus was recovered from the sludge at the bottom of the pilot stabilization pond (5525-litre capacity) but gave no details of the method for examining the sludge nor did he define what he meant by "sludge".

Malherbe and Strickland-Cholmley (1967) were apparently the first to assay virus in "whole sewage" by the direct inoculation of cell cultures. By whole sewage they meant influent sewage without further settling, filtration or centrifugation, they did however treat it with chloroform to reduce bacterial contaminants. They were examining trickling filters and found that these effected insignificant reductions in the numbers of enteroviruses and reoviruses between influent and secondary humus tanks, but the overall reduction across the plants tested resulted in recovery of only low titres of virus which they did not consider to present a public health risk. These authors also reported that no poliovirus I survived in Ringer's solution which was incubated at 37°C for 18 days. They concluded, somewhat surprisingly, that this would be representative of the behaviour of poliovirus during sludge digestion at 37°C.

England et al. (1967) assessed the sewage treatment facilities at Santee, California after about 60 per cent of the population of 12,000 served by this treatment facility had been vaccinated against the three poliovirus types. The treatment facility included in series, activated sludge tanks, oxidation pond, sand and gravel

filtration and finally chlorination. The activated sludge facility effected 76-90 per cent reduction in the number of samples positive for virus between influent and effluent, whereas virus became non-detectable in later stages and, not surprisingly, especially after chlorination. Further studies with known titres of poliovirus III seeded into the sand and gravel filtration stages led them to conclude that activated sludge removed virus more efficiently than filtration and settling. In studies on other viruses, these authors recovered adenoviruses, reoviruses, polioviruses, Coxsackieviruses B and echoviruses in raw sewage but in lower numbers after each stage of treatment specially activated sludge.

In a review of the virology of waste water treatment, Grabow (1968) outlined the methods used for monitoring and isolating viruses from waste water. He pointed out that viruses may survive waste water treatment processes and alluded to the suitability of standards set for bacterial indicators and the health risks involved. His survey showed that virus removal by the trickling filters was poor and probably inadequate; whereas activated sludge removed viruses much more efficiently. However, further work on the quantitative recovery of virus from all stages of the process still required careful study.

The efficiency of the activated sludge process alone in removal of enteric virus was studied by Lund, Hedstrom and Jantzen (1969) who collected weekly dip samples of raw sewage and effluent from a plant serving 40,000 inhabitants. Their method employed direct inoculation onto HeLa cells and they observed about 97 per cent drop in titre across the plant.

Naparstek (1973) assessed the removal of virus in two full scale activated sludge plants by seeding the influent with f2 coliphage as a model of enterovirus behaviour. His results indicated that removal ranged between 80-90 per cent. Later, the same group of researchers (Sherman et al., 1975) studied the removal of f2 coliphage in full scale trickling filters by seeding the influent sewage with about 10^6 pfu/ml of f2 coliphage. Their observations showed reasonably consistent removal by primary sedimentation, 32-37 per cent, while the trickling filter beds effected removals between 1.3-40.4 per cent with averages between 9-18.9 per cent for both plants. Removal in the secondary clarifiers agreed with results of removal in the primary clarifiers and averaged 28.4-30.1 per cent. Chlorination, however, gave the highest and most consistent removal (inactivation) rates reaching 85.2-88.6 per cent which led them to conclude that if a goal of one virus particle in each ten gallonswas to be achieved for recreational waters, greater efficiency of the terminal disinfection of effluent must be achieved.

The same group (Naparstek et al., 1976) investigated f2 removal in a 750,000 gallon per day stepped aeration activated sludge plant. The background counts of phage capable of forming plaques on the host bacterium used (E. coli K-13) fluctuated between 10^2 and 10^4 pfu/ml. The average removal of virus in the successive units in the plant ranged from 0-16.7 per cent, average 7.5 per cent, for the preliminary grit units, 20.0-68.4 per cent, average 46.8 per cent, for primary sedimentation,

0-33.3 (their figures were 2nil removal values - which were really negative removals - and a third value of 33.3 per cent ; average 11.1 per cent) for the aeration stage, 11.8-31.0 per cent for sand filtration (average 22.2 per cent) and 66.0-79.2 per cent for chlorine contact basin (average 72.0 per cent). The average cumulative removal across the plant was reported as 80.5 per cent (but when calculated from these authors figures by the present author the value should have been 90.5 per cent). Naparstek et al. again stress the importance of terminal disinfection for improved viral quality of effluents.

Safferman and Morris (1976) investigated the fate of f2 coliphage in a multi-stage activated sludge plant including a denitrification stage. Coliphage background titres in the primary effluent (settled sewage) ranged from 5×10^1 to 8×10^2 pfu/ml with peak values in the afternoon (16.00 hrs). The plant was seeded at 500 times these indigenous levels and the observed reduction in the activated sludge module ranged between 89 to 98 per cent with an average of more than 95 per cent. The authors thus concluded that modifying the basic activated sludge process by increasing the loading did affect the capcity of the plant to remove virus. Similar values were achieved after denitrification (96.9 per cent), while nitrification effected removals between 58-78.6 per cent and filtration, removals on the average were 18.2 per cent (ranging from 0-48 per cent). The overall removal across the whole plant was at least 99.98 per cent.

3. Methodology and Interpretation

From the above account it is clear that the removal of virus by the activated sludge process is superior to most other treatment systems. A better understanding of the mechanism of removal, though, is warranted especially as it relates to the role of suspended solids and microorganisms found in these solids. Such deficiencies in the state of knowledge of the removal (inactivation?) mechanisms are also stressed by other authors (Grabow, 1968; Schwartzbrod, 1973).

The methodology of virus recovery from waste water and waste water sludges seems to be of paramount importance if an understanding of the mechanism of removal and inactivation (if any) is to be achieved. Cliver (1971) pointed out the apparant inability of many researchers in the field to distinguish between the phenomena of non-detection, inactivation, adsorption or aggregation of virus. This is a very difficult distinction to be able to make clearly and without mixing up one with the other. Kelly, Sanderson and Neidle (1961) considered non-detection to mean inactivation without attempting to assay the virus on the solids. Clarke et al. (1961) deduced, probably rightly, by monitoring titres of the mixed liquor liquid only that activated sludge removed viruses by physical adsorption according to a Freundlich isotherm. The establishment of such a relationship requires a measurement of the proportion adsorbed, especially in the case of viruses, when it is probable that they have more than one fate (Treybal, 1968).

It was only recently that more promising methods were devised for the recovery of viruses from sewage sludges and these include direct inoculation, ultrasonic treatment and radioactive nuclide labelling of viruses. Direct inoculation of whole sewage was used by Malherbe and Strickland-Cholmeley (1967), Lund, Hestrom and Jantzen (1969) and Buras (1974) and indeed these authors reported better recovery of virus from sewage. The excellent tool of radioactive labelling of virus was used by Cliver (1971), Malina et al. (1974) and Malina et al. (1975), but this method offers no clue as to whether the radiation detected is from a viable, inactivated, adsorbed, monodisperse, aggregated, virus or other material. Homogenization of the sedimented solids offersthe advantage of better accuracy in the serial dilution of the suspension with finer particles. To this end, workers in the field used homogenization (Cliver, 1975) and ultrasonic disintegration (Subrahananyan, 1977; and Wellings, Lewis and Mountain, 1976), the technique which improved greatly (222 per cent) the recovery of viable bacteria from activated sludge (Williams et al., 1970; Williams, Forster and Hughes, 1971). Combinations of the above methods may well prove advantageous in the recovery of virus from solids especially when agents facilitate elution or which prevent readsorption (Bitton, 1975) are coupled with ultrasonic disintegration and direct inoculation (Sattar and Westwood, 1976; Subrahmanyan, 1977). These methods could still be greatly improved although recovery rates as high as 95 per cent are reported (Sattar and Westwood, 1976).

Review papers by Berg (1971, 1973a, 1973b) also stressed the need for better methods for recovery of virus from water and waste water. He evaluated the activated sludge treatment process as the most efficient with reported removal rates ranging from 70 per cent to at least 90 per cent.

Trickling filters were much more erratic in their capacity to remove virus with values ranging from 16-100 per cent. Berg also stressed that such wide fluctuations in the recovery of viruses from water and waste water are the result of inherent characteristics of the purification system and the virus in question. No practical recommendations, however, were offered to improve the detectability of virus in these systems. Metcalf (1971) also stressed the need to understand the biological parameters involved in removal, inactivation and transmission of viruses in the water environment. In particular he referred to the problem of detection of virus when it finds shelter in organism (e.g. clams) or other constituents (solids) in relation to the knowledge of their persistence and the possible hazard to public health.

Thus, the present study was planned to reappriase the "removal" or "inactivation" of f2 coliphage and poliovirus I in a model activated sludge treatment plant under defined conditions. The model plant, first devised by Curds and Fey (1969), has the advantages of allowing sampling from all stages of activated sludge treatment in order to focus attention on the solids fraction of

activated sludge. The model plant also allowed control of such parameters as suspended solids concentration, temperatures and flow times through the aeration tanks.

The study also included batch experiments of inoculated sewage, mixed liquor and effluent as controls on the isolated effects of each of the fluids mentioned on the inoculated virus.

Two methods of inoculation of the model plant were used, namely: continuous and single inoculation. These methods would offer opportunity to study behaviour of the viruses used under different loadings of the activated sludge with virus.

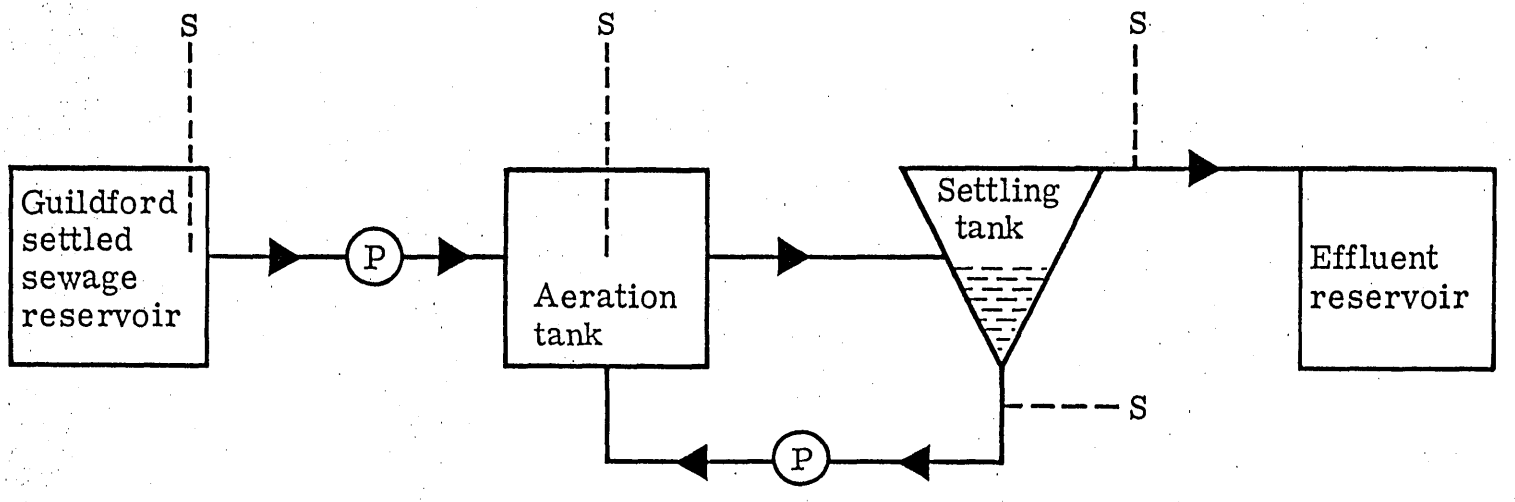
1. Activated sludge treatment plant model

A. Construction:

A laboratory scale model of the activated sludge treatment process was constructed based upon that devised by Curds and Fey (1969). The model plant conformed with the basic stages of aeration and final settling in the activated sludge process (Fig. 3.1).

The aeration tank was made of a one-litre Quickfit fermenter vessel (Cat.# FV1L) covered with a Quickfit flange lid (Cat.# MAF 2/2) having five ground glass socket fittings. Both fermenter and lid were fitted together with a Quickfit flange clip (Cat.# JC100F). The aeration tank was different from the original design in that it had a water jacket fitted on it for temperature regulation (Fig. 3.2).

The settling tank was a 500 ml capacity inverted conical flask sealed at the neck and fitted with three Quickfit screw fittings size SQ 18 along a diameter of its top surface with one of these screw fittings at the centre. A curved side arm with Quickfit ground glass cone fitting (Cat.# SRB size 19/26) was fitted at the midpoint of the side of the tank and pointing towards the top, (Fig. 3.2, 14). The ground glass cone fitting on the side arm was used in conjunction with a Quickfit T-tube with cone and 2 threads (Cat.# MQ1, cone size 19/26, screwthread size 18).



P = Pump
S = Sampling point

Fig. 3.1. Flow Diagram of laboratory model for activated sludge sewage treatment.

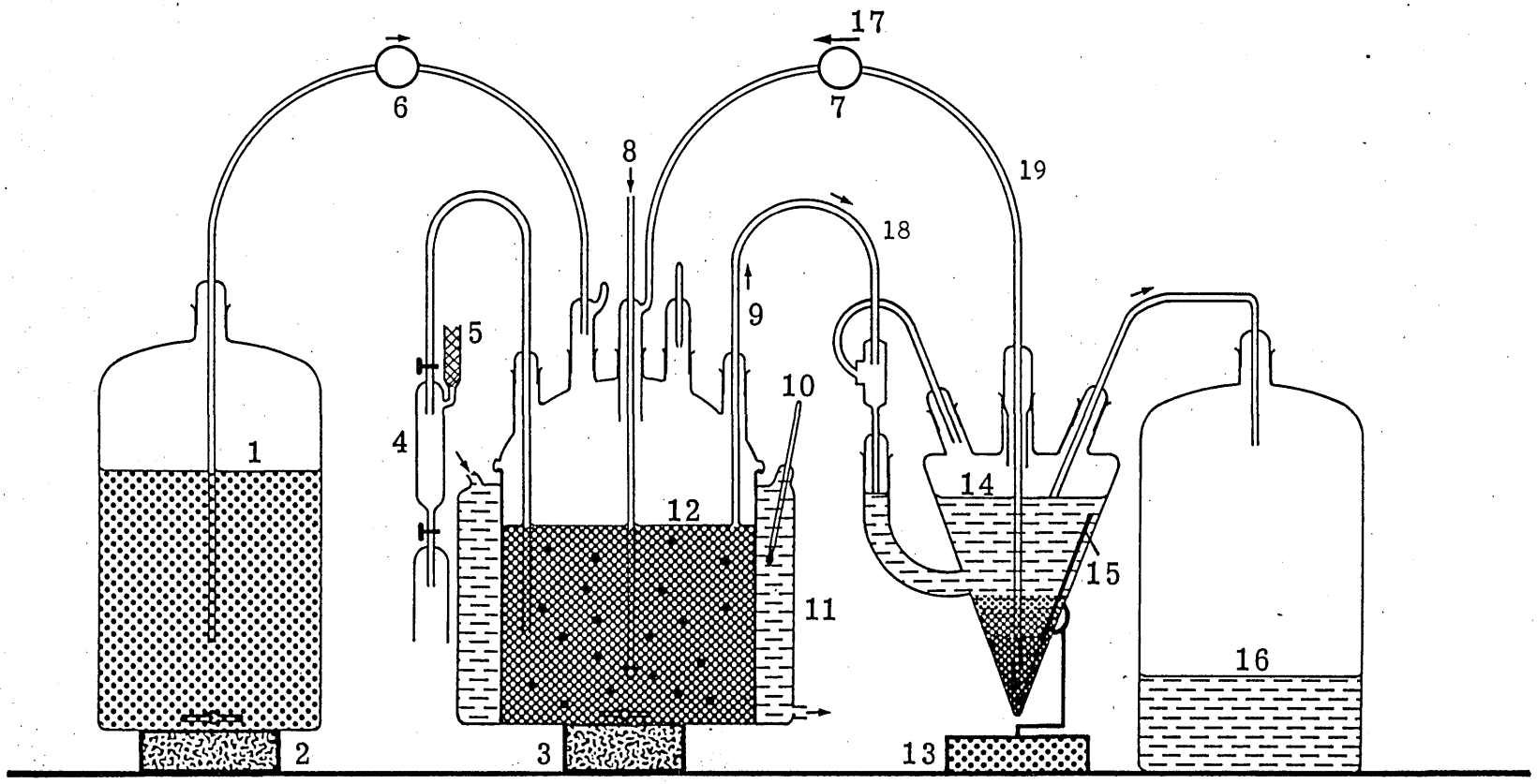


Fig. 3.2. Diagram of laboratory model activated sludge plant.

1.	Guildford settled sewage	7.	Pump	13.	Motor
2.	Magnetic stirrer	8.	Air	14.	Settling tank
3.	Magnetic stirrer	9.	Mixed liquor overflow	15.	Scraper
4.	Sampling device	10.	Thermometer	16.	Effluent
5.	Air filter	11.	Water jacket	17.	Return sludge
6.	Pump	12.	Aeration tank		

Another part of the plant was a sampling device that consisted a piece of 2.5 cm internal diameter glass tubing (10 cm in length) fitted with a ground glass stopcock (Quickfit Cat.#TH 1/3) at either end with a 5 cm length of 8 mm internal diameter glass tubing as inlet and outlet. A side arm tube (8 mm internal diameter glass tubing) was added to vent the sampling device chamber near the inlet, and a hood made of a 7-cm length of 2.5 cm internal diameter glass tubing was also added over the outlet tube (fig. 3.2 (4)).

The model plant was provided with two 2.5-litre bottles for influent sewage and effluent. The influent reservoir was connected to the aeration tank via a peristaltic pump into one of the ground glass fittings through a Quickfit cone-screwthread adapter (Cat.#ST52/18). Air from an air pump (3 l/min.) was provided through another of the ground glass cone fittings on the flange lid via a Quickfit T-tube with ground glass cone and 2 screwthreads (Cat.#MQ1). The air tube was provided with an aquarium air diffuser that was lowered as low as possible in the aeration tank without hindering movements of the stirrer magnet. An outlet tube from the aeration tank was provided via a third of the ground glass cone fittings to the top inlet on the T-fitting on the side arm of the settling tank. The remaining free opening of the T-fitting on the side-arm of the settling tank was connected with a tube to one of the side screw fittings on the top of the conical settling flask. A straight piece of 10 mm

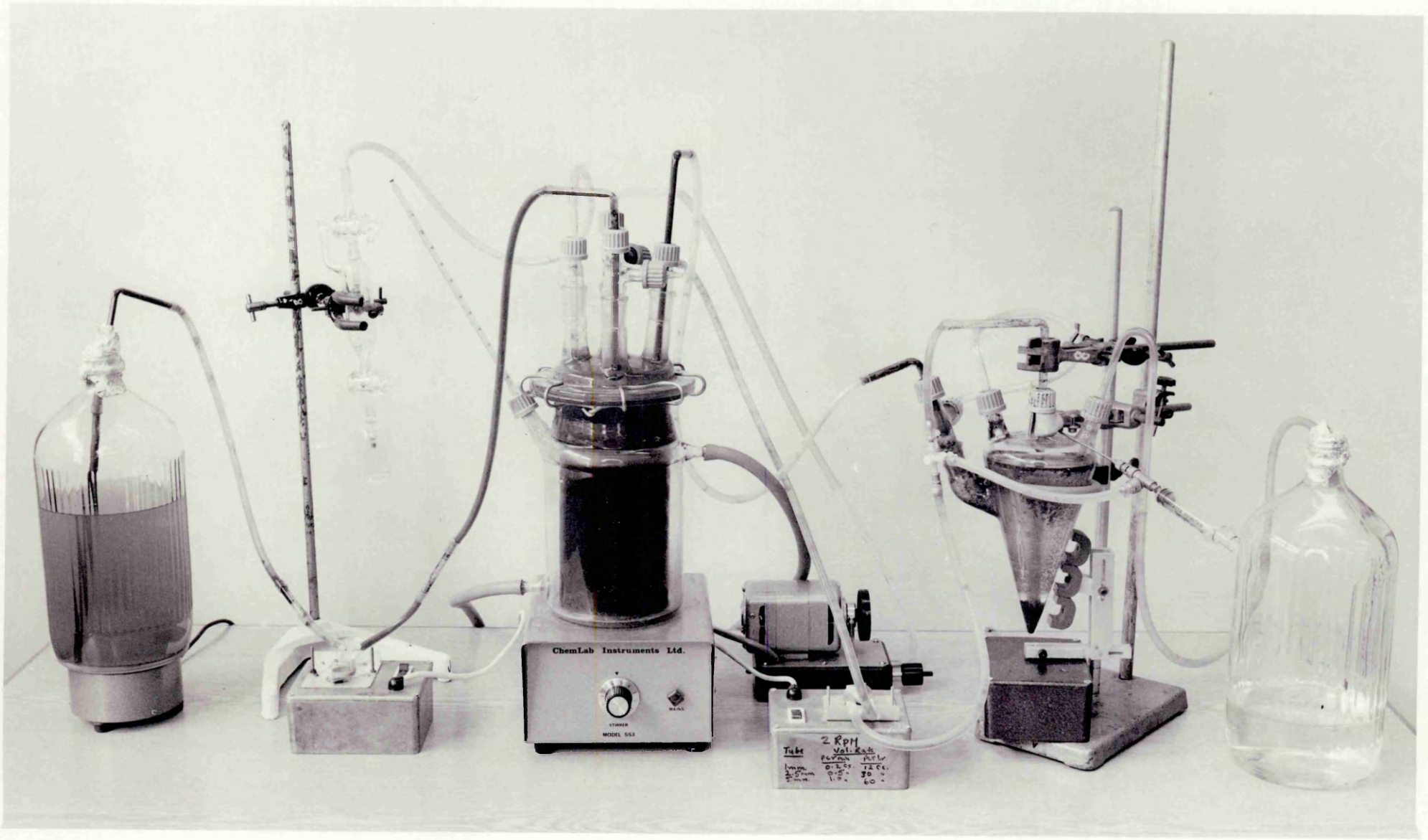
diameter glass tubing was inserted into the settling flask and its tip kept at a distance of 1.5-2 cm away from the bottom of the flask. This tube was connected, via a peristaltic pump, to the aeration tank using the same ground glass fitting through which air was introduced. A three-way valve, used to sample return sludge, was included in this tube at point 19 (Fig. 3.2). The sampling device was connected to a fourth ground glass fitting via a length of silicone rubber tubing (10 mm diameter) and a straight length of glass tubing that was pushed to about midway down the aeration tank.

An iron wire (0.5 mm thick, 12 cm long) encased in glass was driven around the inner surface of the settling tank by a motor driven, externally located magnet at a speed of one revolution per minute (r.p.m.). Stirrer magnets were placed in the aeration tank and in the influent sewage reservoir; and these were driven by magnetic stirrers at 200 r.p.m.

B. Operation:

The plant was operated as an air-tight system which receives influent sewage and air into the aeration tank and discharges effluent and air into the effluent reservoir. The aeration tank was initially filled with mixed liquor from the Guildford sewage works and any adjustment of the mixed liquor suspended solids (MLSS) concentration was done by withdrawing (through the sampling device) or adding (through the extra port in the aeration tank flange cover) an appropriate volume of mixed liquor. Figure 3.2a

Fig. 3.2a. The Model activated sludge plant.


ChemLab Instruments Ltd.
STIRRER
MODEL 553
MAINS
2 RPM
Vol. Rods
Tube
1 mm.
2.5 mm.
5 mm.
0.5 cc.
0.5 "
1.0 "
12 cc.
30 "
60 "

shows the plant in operation. The influent and effluent reservoirs were covered loosely with a piece of aluminium foil each.

The movement of liquids through the plant and from one compartment to another in the plant was either propelled by the movement of air through it or by the aid of peristaltic pumps. Rate of sludge return was kept constant at 60 ml/hr. throughout, whereas influent sewage rate of flow was regulated to give different flow-through times of 12.5, 10.0 and 5.4 hours as required. Flow-through times across the plant were taken as the times required for one litre of influent sewage to be collected as effluent. The level of the mixed liquor outlet tube in the aeration tank was adjusted to maintain the volume of mixed liquor at exactly one litre. Sampler stopcocks were normally kept closed.

The temperature of the aeration tank was regulated by connecting the water jacket to a Grant Thermocirculator unit with rubber tubing and filling the jacket and the thermocirculator unit with distilled water (with 10% v/v industrial methylated spirit (IMS) as anti-freeze).

The plant was provided with raw settled sewage and mixed liquor collected from the Guildford sewage treatment plant. Raw settled sewage was used in all experiments on the model plant unless otherwise indicated.

2. Collection of settled sewage, mixed liquor and effluent

Field samples of settled sewage (henceforth referred to as sewage), mixed liquor for use in running the model plant and to perform other batch experiments; together with activated sludge effluent for use in batch experiments were collected from the Guildford sewage treatment plant (fig. 3.3).

The Guildford sewage treatment plant at present employs both activated sludge and percolating filter processes for sewage treatment. It is designed to function for a population of 89,000 and the present population is about 77,500 (1973 estimate). The dry weather flow into the plant is 3 million gallons per day (mgd), and the retention time in the activated sludge aeration tank is 9.6 hours under these conditions. The plant was designed to meet the Thames River Authority effluent standard of 20 mg/l suspended solids and 15 mg/l BOD_5.

Sewage was collected in 2.5-litre bottles as grab samples after the primary settling tanks (fig. 3.3, point A) and immediately taken to these laboratories where it was either used immediately or was stored at $4^{o}C$ to be used within 48 hours. Filtered sewage samples were prepared by passing them through Whatman GF/C glass fibre filters.

Mixed liquor samples were also collected from the Guildford works (fig. 3.3, point B) as grab samples, usually in 1-litre bottles. The samples were immediately used in the laboratory

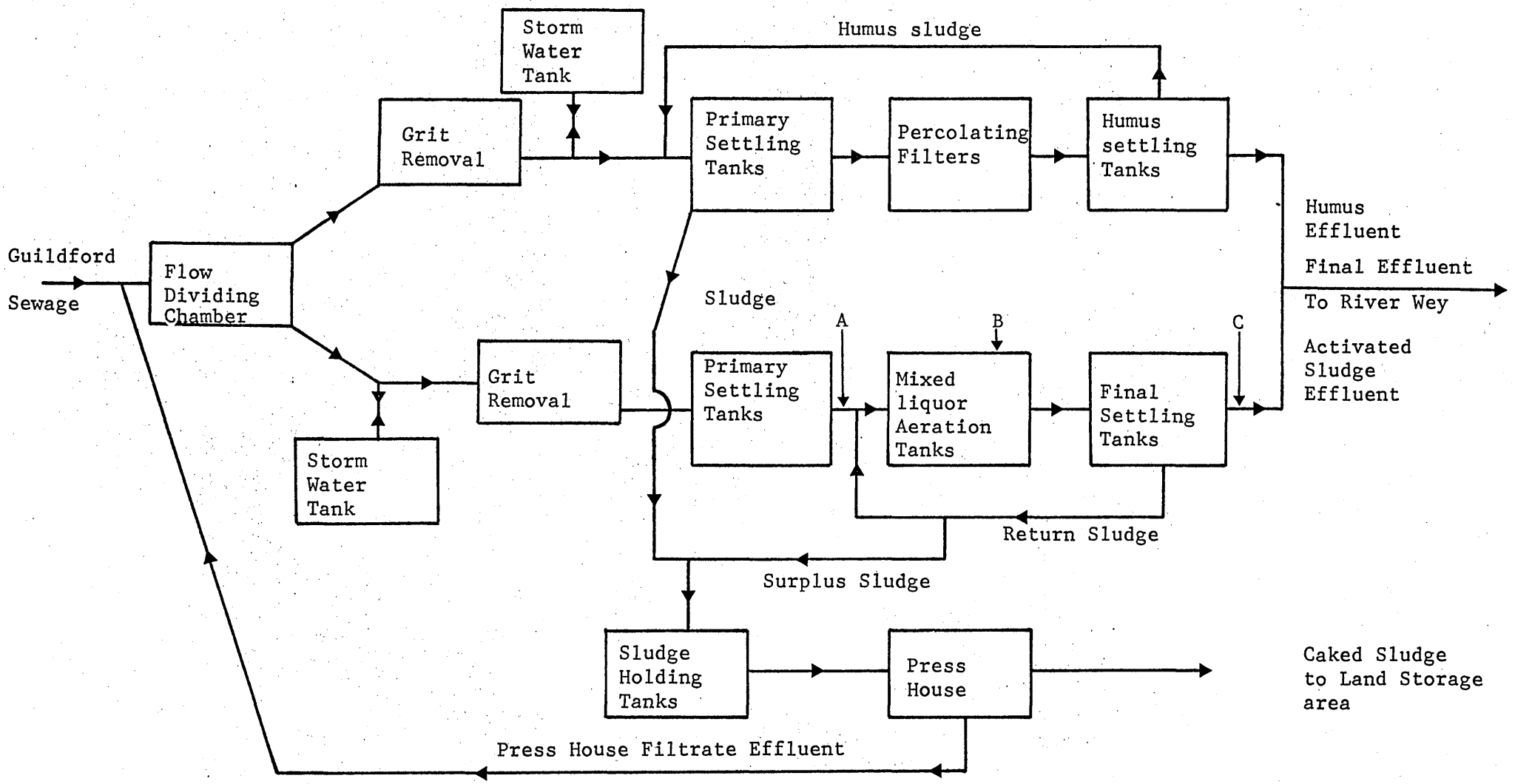


Fig. 3.3, Guildford Sewage Treatment Plant (Schematic)

either in the model plant aeration tank or as material for batch experiments.

When required, effluent samples were also collected as grab samples (fig. 3.3, point C), usually in 1-litre bottles. These samples were used immediately.

3. Performance of the Model Plant

The performance of the model plant was monitored twice a week by characterising both the effluent and influent with respect to its BOD_5, ammonia, nitrate and suspended solids.

A. Determination of biochemical oxygen demand :

Assay of the 5-day biochemical oxygen demand of sewage and effluent samples required the following reagents (Department of the Environment, 1972):

I. Dilution water nutrients:

a) Ferric chloride solution (Analar, 0.0005 M)

b) Calcium chloride solution (Analar, 0.25 M)

c) Magnesium sulphate solution (Analar, 0.1 M)

d) Phosphate buffer stock solution pH 7.2: KH_2PO_4 (Analar) 0.45 M, NaOH (Fisons) 0.22 M, dissolved in 0.7 x the final volume and the pH was adjusted to 7.2. Enough ammonium sulphate (Analar) was added to make the final solution 0.015 M in $(NH_4)_2SO_4$.

II. Manganous sulphate solution (2.1 M $MnSO_4$ (BDH); dissolved in water and filtered if necessary).

III. Alkaline iodide solution: enough NaOH (Fisons) to make 10 N solution was dissolved in ½ the final volume to which was added enough NaI (BDH) to make the final solution 6 M in NaI. The mixture was kept hot (at least 70°C) until all NaI was dissolved, which was then made up to the final volume after cooling to room temperature.

IV. Sulphuric acid: H_2SO_4 (Analar) 50% v/v in distilled water was prepared.

V. Standard sodium thiosulphate: N/80 $Na_2S_2O_3.5H_2O$ (HBL) solution was prepared.

Sewage samples usually required dilution up to 50 times with dilution water prepared from one litre of aerated distilled water with 1 ml of each of the dilution water nutrients mentioned above (effluent samples did not normally need dilution). Two 250-ml glass stoppered bottles were filled to overflowing with the sample of effluent (or diluted sewage) and left to settle down for at least two minutes and then were tapped with the glass stopper to allow any trapped small air bubbles to escape. The bottles were numbered 1 and 2, and bottle 1 was stoppered firmly to avoid further aeration. Bottle 2 was stoppered and then unstoppered to make space for 1 ml of alkaline iodide and 1 ml of manganous sulphate, and both bottles were incubated at 20°C in the dark for five days.

A series of reactions would take place (in bottle 2) involving the production of a brownish precipitate of manganic hydroxide equivalent to the amount of oxygen present in solution as follows:

$$MnSO_4 + 2NaOH \longrightarrow \underset{\text{white ppt.}}{Mn(OH)_2} + Na_2SO_4$$

$$2Mn(OH)_2 + \underset{\text{Dissolved in sample}}{O_2} \longrightarrow \underset{\text{brown ppt.}}{2Mn\,O(OH)_2}$$

At the end of 5 days, both bottles were acidified with 2 ml of 50% H_2SO_4; this dissolves the brown precipitate and liberates the iodine from KI in an amount equivalent to the oxygen present, and the difference between the two bottles gives the amount of oxygen consumed by microorganisms. The reactions are as follows:

$$MnO(OH)_2 + 2H_2SO_4 \longrightarrow Mn(SO_4)_2 + 3H_2O$$

$$Mn(SO_4)_2 + 2KI \longrightarrow MnSO_4 + K_2SO_4 + I_2$$

A suitable aliquot (25-200 ml) from each bottle was separately titrated against N/80 (0.0125 N) thiosulphate until the colour changes from honey to straw colour. At this point 2 drops of starch indicator were added and titration continued to a colourless endpoint.

Oxygen content of each of the two bottles at day 5 was calculated using the formula (Golterman, 1971):

$$O_2 \text{ (mg/l)} = \frac{\text{vol. of thiosulphate (ml) x N x 8 x 1000}}{\text{Volume titrated (ml)}}$$

(where N is the normality of thiosulphate). The difference between the O_2 concentration of the two bottles was taken as the BOD_5 of the sample after accounting for dilution factors if any.

B. Determination of Ammonia:

Ammonia was determined by a direct Nesslerisation procedure (The British Drug Houses, undated (a)). An appropriate (1-5 ml) sample of sewage or effluent was filtered through a Whatman number 1 fluted filter paper and placed in a 50 ml Nessler's cylinder and the volume made up to 50 ml with distilled deionized water. Two millilitres of Nessler's reagent (BDH) were added and the contents thoroughly mixed. A blank cylinder was prepared similarly with distilled deionized water and Nessler's reagent. Both cylinders were read by comparing colours at 5 minutes using BDH Nessleriser and appropriate colour discs, (BDH's NAA, NAB, NAC discs). The amount of ammonia was read directly on the matching colour disc as parts per million for each millilitre used in the sample with proper consideration for initial dilution (i.e. NH_3 ppm = γ reading x No. of ml used x dilution).

C. Determination of nitrates Nitrogen:

Nitrates nitrogen in influent settled sewage and in effluent samples were assayed by direct Nesslerisation employing the phenol disulphonic acid method (The British Drug Houses Ltd., undated (b)).

The method required the following reagents:

1. Phenoldisulphonic acid reagent (BDH).
2. Ammonia solution (BDH) 10% NH_3 w/w.
3. Glacial acetic acid (BDH).
4. Silver sulphate (Analar).

The sample (10 ml) to be analysed was treated with 1 ml glacial acetic acid and 0.1 gm silver sulphate, mixed and left to stand for 10 minutes after which it was filtered through a Whatman No. 32 filter paper. After chlorides were removed by that preliminary treatment, a 1-ml aliquot was dried in a porcelain evaporating dish by placing it in a shallow boiling water bath. The residue was then cooled and dissolved in 1-ml phenoldisulphonic acid and the liquid transferred with 30-ml distilled water into a 50 ml Nessleriser cylinder. Ten millilitres of ammonia solution were then added into the cylinder, the contents were mixed and cooled and their volume made up to the 50-ml mark. A blank was prepared similarly using distilled water as the initial sample. Nitrate nitrogen (in parts per million) was read directly on the matching colour disc (BDH, NHP disc) in a Nessleriser. Dilution and amount of sample used were accounted for as follows:

Nitrate (N) ppm = γ reading x No. of ml used x dilution factor).

D. Determination of suspended solids:

Suspended solids were determined by filtering a suitable sample (50 ml for mixed liquor, 100-200 ml for settled sewage and

effluent) through a dry, pre-weighed, 4.7 cm diameter Whatman GF/C filter paper. The filter paper, with the solids, was then dried at 105°C in a drying oven for one hour and cooled in a desiccator and weighed. The difference between the final and the initial weights was taken as the weight of the suspended solids in the sample and the quantity contained in one litre of the sample in milligrams was expressed as parts per million (ppm), (Department of the Environment, 1972).

4. Inoculation of the model plant

Two types of experiments, single dose and continuous inoculation, were performed on the model plant. The former method of inoculation was done by the introduction of enough suspension into the aeration tank to give an approximate titre of 10^5 plaque forming units (pfu/ml) in the mixed liquor. The other method of inoculation involved inoculating the influent sewage with enough virus suspension to give a titre of about 10^5 pfu/ml, and by repeating this operation daily when the influent reservoir was changed in order to maintain this influent titre for a number of days. Inoculation was stopped by supplying the plant with uninoculated sewage.

5. Sampling from the model plant

Samples of influent sewage, mixed liquor, return sludge and effluent were taken from the model plant for virus assay in the following ways:

I. Influent sewage samples were taken by pipetting 5 ml from the influent reservoir directly.

II. Mixed liquor samples were obtained from the aeration tank by opening the top sampling device stop-cock and constricting the mixed liquor outlet from the aeration tank (at point 18 fig. 3.2). The increased air pressure in the aeration tank filled the sampling device chamber and when about 20 ml was collected, the constriction (at point 18) was released and the sampling device top stopcock closed. The mixed liquor volume was then emptied into a 20-ml bottle by opening the bottom stopcock. Five millilitres were pipetted from that bottle into a numbered bijou bottle and the remaining contents of the universal bottle returned to the aeration tank via a spare port in the flange lid. The numbered bijou bottle contents were kept for virus assay.

Samples of mixed liquor to be used for determination of suspended solids were also taken via the sampler as described above.

III. Samples of return sludge were obtained from the 3-way valve at point 19 (fig. 3.2) by the following method which ensured selection of a representative specimen. A five millitre capacity syringe was filled once with the return sludge and emptied into a 20 ml bottle to be returned to the aeration tank later. The syringe was filled again

to the five millilitre mark and this sample was retained in a bijou bottle for testing.

IV. Effluent samples (5 ml) were collected directly from the effluent tube as it emptied into the effluent reservoir (fig. 3.2).

6. Inoculation and sampling of volumes of water, sewage and mixed liquor

Known volumes of distilled de-ionized water, sewage, mixed liquor and 10% mixed liquor (prepared as 9 + 1 volumes of MLL and ML respectively) were inoculated with known volumes of virus preparation. After thorough mixing, the liquid was sampled at intervals usually over a 24 hour period. The volumes were thoroughly mixed by swirling occasionally between times of sampling and especially prior to sampling. Samples were stored and assayed for the appropriate virus using the appropriate method for the fluid concerned.

7. Treatment of the samples

Samples from experiments with f_2 coliphage were treated in a different way to those containing poliovirus I.

A. Samples from experiments with f_2 coliphage:

All samples containing f_2 coliphage were shaken with 0.5 ml of chloroform (BDH) for thirty seconds and were stored in the

refrigerator at 4°C. Samples of influent settled sewage and effluent did not receive any further treatment until assayed.

Mixed liquor samples were further treated by bubbling nitrogen gas through them to evaporate all chloroform. Later they were centrifuged at 1500 x g for 10 minutes at 20°C and the supernatent liquid fraction decanted into a bijou bottle and labelled as a mixed liquor liquid (MLL) sample. The solids fraction was resuspended up to a final volume of 5 ml with dilution fluid into which 10% v/v newborne calf serum (Flow) was incorporated. The resuspended solids were subjected to ultrasonic disintegration for one minute using an exponential probe (pt. No. 34041) in an MSE 150 watt ultrasonic disintegrator for one minute at 2°C at a peak to peak amplitude of 13μ (giving an effective amplitude of 91μ since the probe used has a transformation ratio of 7:1). All ultrasonic treatment was similar to this except where otherwise indicated.

After the ultrasonic treatment, samples were again stored at 4°C until assayed. All samples with f_2 coliphage were assayed within 48 hours of sampling.

B. Samples from experiments with Poliovirus I:

All samples containing poliovirus I were stored at -28°C until assayed. Mixed liquor and return sludge samples, however, were centrifuged at 1500 x g and the supernatant liquid fraction decanted into bijoux bottles, labelled and then stored at -28°C

until assayed. The pelleted solids were resuspended up to 5 ml in 10% newborne calf serum (Flow Labs.) in diluent, and subjected to ultrasonic disintegration under exactly the same conditions described for f_2 coliphage. The solids fractions, after this treatment, were stored at -28°C until assayed. No time limit was set before assay.

8. Tissue culture media

A. Growth medium:

Tissue culture growth medium had the following constituents in sterile distilled, de-ionized water:

Medium 199 (10 x) (Wellcome Labs. Ltd.)	10.0%	v/v
Foetal calf Serum (Flow Labs.)	5.0%	"
Bicarbonate buffer (Wellcome Labs. Ltd.)	2.5%	"
Penicillin (Crystapen, Glaxo)	200	units/ml
Streptomycin (Glaxo)	100	µg/ml

B. Maintenance medium:

Tissue culture maintenance medium contained the following constituents in sterile distilled, de-ionized water:

Medium 199 (10 x) (Wellcome Labs. Ltd.)	10.0%	v/v
Foetal calf Serum (Flow Labs.)	2.5%	"
Bicarbonate buffer (Wellcome Labs. Ltd.)	5.0%	"
Penicillin (Crystapen, Glaxo)	200	units/ml
Streptomycin (Glaxo)	100	µg/ml

C. Diluent: The diluent had the same constituents as maintenance medium without the serum.

D. Agar overlay medium:

Two solutions, A and B were prepared for the agar overlay medium and both solutions were used in equal volumes. Solution A was a double strength maintenance medium containing 0.0004% neutral red (GTG stains and reagents) and Amphotericin B(Flow Labs.) at a final concentration of 2.5μg/ml, and solution B was a 1.6% solution of Difco purified agar. Solution B was autoclaved (15lb./in^2 for 15 minutes), cooled to about 50°C and held at 43°C in a water bath. Solution A was warmed to 43°C and then added to solution B and the mixture was held at 43°C until used.

E. Phosphate buffer saline:

To prepare a litre of phosphate buffer saline at pH 7.3, the following solutions were required:

Solution A

Sodium chloride (Analar)	8.0 grams	
Potassium chloride (Analar)	0.2 "	
Disodium hydrogen Phosphate	(Na_2HPO_4, BDH)	1.15 gm
Potassium dihydrogen phosphate	(KH_2PO_4, BDH)	0.20 gm
Distilled de-ionized water	800 ml	

Solution B

Calcium chloride (BDH)		0.1 gm
Magnesium chloride (BDH)	($MgCl_2.6H_2O$)	0.1 gm
Distilled de-ionized water		200 ml

After dissolving the salts, the solutions were autoclaved (A at 15 lb./in^2 for 15 minutes and B at 10 lb/in^2 for 20 minutes). When cool, the two solutions were mixed and dispensed aseptically into 100 ml bottles and stored at 4°C until used.

F. Versene - trypsin solution:

An ampoule of freeze dried trypsin (Wellcome Reagents Ltd.) was reconstituted with 10 ml sterile de-ionized water to give a 5% w/v solution which was stored frozen (-28°C) in 1 ml amounts.

The versene - trypsin solution was prepared by adding 1 ml of 5% trypsin to a bottle containing 100 ml of 1:5000 Versene (Wellcome Reagents Ltd.)

9. Bacterial culture media

The following media were used to grow the bacterial host Escherichia coli. K_{12} Hfr and to assay f2 coliphage (Poynter and Slade, 1977).

A. Tryptose soya broth (TSB, Oxoid): A 3% w/v solution of TSB was prepared and autoclaved at 15 lb/in^2 for 15 minutes. It was then stored at room temperature (22°C) until used.

B. Lawn Agar: The following constituents were used in the preparation of the lawn agar:

Bacto tryptone (Difco)	1.0%	w/v
Yeast Extract (Difco)	0.5%	"
Sodium Chloride (Analar)	0.5%	"
Glucose (BDH)	0.1%	"

All were dissolved in distilled de-ionized water and the pH adjusted to 7.2 with 1 N NaOH. Agar (Davis) was later added at a concentration of 1.0% w/v and the mixture autoclaved at 15lb/in^2 for 15 minutes. When the medium was cooled down to 45^{o}C, $CaCl_2$ and $MgSO_4$ were each incorporated at a final concentration of 0.0025 M (actually done by adding aseptically 5 ml of a 0.5 M sterile stock solution of the $CaCl_2$ and $MgSO_4$). The medium was then distributed in 15 ml volumes into 90 mm petri dishes which were stored at 4^{o}C until used.

C. Soft agar: The soft agar used in the overlay method was prepared according to Douglas (1975) with the following constituents in distilled de-ionized water:

Agar (Davis)	0.65%	w/v
Nutrient Broth (Difco)	0.80%	"
Sodium chloride (Analar)	0.50%	"

The agar was autoclaved at 15 lb/in^2 for 15 minutes and distributed aseptically into 100 ml bottles which were stored at 4^{o}C until used.

D. Dilution fluid: The coliphage dilution fluid was prepared according to Poynter and Slade (1977) with the following constituents in distilled de-ionized water:

Sodium Chloride (Analar)	0.03%	w/v
Peptone (Difco)	0.10%	"
Magnesium Sulphate (GPR)	0.0005	M
Tris (Hydroxy methyl) methylamine (pH 7.8) BDH	0.01	M

The solution was autoclaved and stored in 100 ml volumes at 4°C until used.

10. Tissue Culture

Stock cultures of Vero cells (African green monkey, Cercopithecus aethiops, kidney cells - MacFarlane and Sommerville, 1969) were grown in 20-oz glass medical flat bottles, in 40 ml of growth medium. At 3 days, when the cell layer was confluent, each bottle was drained of the growth medium and washed with ten millilitres of PBS (pre-warmed to 37°C) which was drained off and replaced with 40 ml of maintenance medium. At seven days, the cultures were drained of the maintenance medium and the cell layer was washed with 10ml of warm PBS. The PBS was drained off and 10ml of versene-trypsin solution was added. The bottle was rocked gently to ensure complete contact of the versene-trypsin with the cell layer and about half of it was then poured off and discarded. After further incubation at 37°C for about 5

minutes the cell layer was stripped. The cell suspension from all bottles was removed and added to growth medium and the number of cells enumerated in a haemocytometer. The final volume of the cell suspension was adjusted to give at least 5×10^4 cells/ml as a seeding level (this was usually 4 x the original volume). This cell suspension was used to grow more stock cultures in 20-oz bottles and to prepare cultures in 2-oz bottles for the plaque tests. The 2-oz bottles were seeded with 7.5 millilitres of cell suspension and became confluent at 3-4 days, when they were ready for use.

The cell culture was maintained in this manner for about six months when new deep-frozen, cell suspension was taken from liquid nitrogen, thawed and used as a younger seed to propagate the cell line. This ensured that the cell line was always within 6 months old from the starting stocks which were obtained from Dr. J. S. Slade of the Metropolitan Water Board Scientific Services Laboratories.

The deep frozen cell suspensions were prepared by centrifuging the versene-trypsin cell suspension for 5 minutes at 300 xg and decanting the versene-trypsin. The cells were resuspended in growth medium containing 7.5% v/v dimethyl sulfoxide (BDH, Analar) at a cell concentration of at least 4×10^5 cells/ml. This cell suspension was distributed in 0.5ml volumes into glass ampoules that were sealed, and placed in a polystyrene container at $4^{\circ}C$

for about 4 hours. Afterwards, the polystyrene container with the ampoules was placed at the top of the liquid nitrogen container to ensure gradual cooling down before storing in the liquid nitrogen at -196°C.

When the deep frozen ampoules were used to propagate the cell line, they were removed from liquid nitrogen and immersed in a water bath at 37°C for rapid thawing. The ampoule was then broken and the cell suspension added to 15ml growth medium that was placed in two 2-oz bottles and incubated for 3 hours when the cells were observed under the microscope to have attached to the glass surface. The medium was then poured off and replaced with fresh growth medium. These cells were propagated in the same way as described above when they became confluent.

11. Bacterial Cultures

Escherichia coli (K_{12}Hfr) obtained from Dr. J. S. Slade of the Metropolitan Water Board, Scientific Services Laboratories, was grown in 3% tryptose soya broth (TSB) at 37°C to a multiplicity of about 10^8 cells/ml. Stock slant cultures were grown on nutrient agar (2.8% w/v oxoid nutrient agar autoclaved at 15 lb/in^2 for 15 minutes) at 37°C until visible growth was observed. Replicate slants were stored at 4°C until needed.

Working cultures of E. coli K_{12}Hfr were grown overnight by inoculating a 15-20 ml volume of 3% TSB with a loop of E. coli K_{12}Hfr from a nutrient agar slant. The working culture was stored at 4°C.

Daily working cultures of E.coli K_{12}Hfr were grown overnight by inoculating 15-20 ml of 3% TSB with 0.5 ml of the working culture and incubating at 37°C. These cultures were discarded after use and within 24 hours.

12. Viruses

A. Poliovirus I:

Stocks of Poliovirus type I, LS-c, 2ab obtained from Dr. J. S. Slade of the Metropolitan Water Board Scientific Services Laboratories were prepared in vero cells. Confluent (3-day) vero cell monolayers in 20-oz medical flat bottles were drained and washed with warm (37°C) PBS and were inoculated with 1 ml of a virus suspension at about 10^6 plaque forming units (pfu/ml). The bottles were returned to 37°C for virus adsorption. After 30 minutes the bottles were drained of the inoculum and 40 mls maintenance medium was added to each and the bottles returned to 37°C. Control, uninoculated, similarly treated, bottles were included.

The cell monolayers were observed daily for evidence of cytopathogenic effects (CPE), and when all cells showed CPE the virus was harvested by three cycles of freezing and thawing (-28°C to +20°C) and pooling the infective fluid. The virus

preparation was further clarified by dividing the pooled fluid into 8 ml volumes in sterile universal bottles and shaking each with an equal volume of 1, 1, 2-trichlorotrifluorethane (arcton). The mixture was subjected to ultrasonic disintegration using an MSE 150 watt ultrasonic disintegrator at 13μ probe tip amplitude at 2°C using an exponential probe (Pt. No. 34041, end diameter 1/8th of an inch, having a transformation ratio of 7:1 resulting with an effective amplitude of 91μ). The mixture was centrifuged at 3000 xg for ten minutes and the upper aqueous layer containing the virus preparation was decanted into another sterile universal bottle. The arcton extraction and ultrasonic treatment were repeated twice again. The clarified virus suspension from each universal bottle was pooled, mixed thoroughly, and distributed aseptically into sterile bijoux bottles in 2ml volumes and stored at -28°C. For all experiments with poliovirus the fluid from a thawed bijou bottle was used as the inoculum. Once a bijou bottle was opened to take an inoculum, it was kept for assay after which it was discarded.

B. f2 Coliphage:

The f2 coliphage was obtained from Dr. J. S. Slade of the Metropolitan Water Board Scientific Services and stock culture were prepared in the following way. Fifty millilitres of 3% TSB were placed in a sterile, cotton plugged 125 ml capacity erlenmeyer flask and inoculated with 1ml of a working culture of *E. coli* K_{12}Hfr.

The inoculated medium was vigorously aerated in a shaking water bath at 37°C until a cell count of 2×10^8 cells/ml was obtained. Enough f2 coliphage suspension was added to achieve an inoculation ratio of 5 coliphage particles to the bacterial cell. Incubation was resumed with aeration for about 3 hours when the medium cleared indicating a good degree of lysis of bacterial cells. Incubation was then stopped and 5 ml chloroform was added and mixed by swirling the flask. The mixture was then distributed equally into 4 universal bottles and centrifuged at 400 xg for ten minutes. The pooled supernatant aqueous fraction that contained the coliphage was stored at 4°C over 5 ml chloroform. Inocula were taken aseptically from this pool.

13. Virus Assay

A. Poliovirus microtitre assay method:

Vero cell suspension, at a cell count of 10^5 cells/ml, were prepared in maintenance medium containing Amphotericin B (Flow Laboratories) at a final concentration of 2.5µg/ml and double the normal concentration of newborne calf serum. Using a standard dropper (40 drops/ml, Flow Laboratories), 0.05 ml of the cell suspension was added to each well of a Linbro microtitre plate (Flow Laboratories). Ten-fold serial dilution of virus were prepared by carrying over 0.3 ml in a series of bijou bottles containing 2.7 ml diluent) of either the experimental sample or control poliovirus I suspension. Using a standard dropper

(40 drops/ml, Flow Laboratories) 0.1 ml of each dilution was added to the cell-seeded wells. Eight replicas were prepared from each virus dilution. Virus controls and blanks were prepared in the same way, and the plates were covered with sellotape and incubated at 37°C.

The microtitre wells were observed under the microscope for CPE which was taken as evidence for presence of virus in the sample. Titres were calculated using the Reed and Muench equation and expressed as $TCID_{50}$/ml.

B. Poliovirus plaque assay method:

Confluent vero cell monolayers in 2-oz bottles were drained of their medium, washed with PBS (37°C) and inoculated with 0.5ml of a ten-fold serial dilution of poliovirus I (or experimental sample). The virus sample was allowed to adsorb to the cells for 30 minutes at 22°C after which 7.5ml agar overlay medium at 43°C was added into the bottle by pouring it onto the side with no cell layer and then allowing the agar to flow ;and set on the cells as it cooled.

The bottles were incubated inverted at 37°C in the dark and observed for plaque formation daily, and the total plaques counted and recorded at 3 days of incubation. Control virus and blank bottles were similarly treated and included in all assays performed.

Titres were calculated from plaque counts by averaging duplicate counts and accounting for dilution factors and inoculum sizes.

C. f2 coliphage plaque assay:

Coliphage inocula were taken from ten-fold serial dilutions prepared by carrying over 0.5ml into successive tubes containing 4.5ml dilution fluid. To 0.5ml of each dilution, 0.1ml E. coli culture at 10^8 cells/ml was added and the mixture added to a tube containing 2.5ml of molten soft agar at 43°C. The contents of the tube were thoroughly mixed for 10 seconds using a vortex mixer and immediately poured over an agar plate that had been pre-warmed to 37°C. The plates were left at room temperature to set, and were incubated overnight in an inverted position at 37°C.

Plaque counts were made after incubation for at least six hours (overnight) and titres calculated by averaging duplicate counts, accounting for dilution factors and inoculum size, and the titres expressed as Pfu/ml.

14. Statistical methods:

The statistical methods used were as follows (Loveday, 1975):

- Mean ($\bar{x}$) and Standard deviation(s) of a set of observations,

$$\bar{x} = \frac{\Sigma x}{n} , \quad s = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n-1}}$$

where ΣX is the sum of the values, X is each individual value, n is number of values.

- Significance of the difference between two means (t-test),

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S_{1,2}\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \quad , \text{ where } s_{1,2} = \sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1 + n_2 - 2}}$$

where $\bar{X}_1$ and $\bar{X}_2$ are the means of the first and second measurements, s_1 and s_2 their standard deviations and n_1 and n_2 the number of observations in each.

$s_{1,2}$ is the combined standard deviation for the two means.

t is the computed test value.

- Confidence limits of the mean,

$$\bar{x} \pm \frac{ts}{\sqrt{n}} \quad ,$$

(symbols are as above).

- Coefficient of regression (slope).

$$C.R. = \frac{n\Sigma xy - \Sigma x \Sigma y}{n\Sigma x^2 - (\Sigma x)^2}$$

X and Y are the coordinates of each observation.

Other symbols as above.

- Standard error of the mean

$$s.e. = \frac{s}{\sqrt{n}}$$

- Coefficient of variation

$$C.V. = \frac{s}{\bar{x}} \%$$

IV. RESULTS

1. Performance of the model plant:

The performance of the model plant with respect to its capacity to remove suspended solids, BOD_5 and ammonia is summarized in table 4.1 (a-h) for each set of operating conditions. On the average, the plant was capable of removing 93.6 per cent of the influent suspended solids, 94.4 per cent of the influent BOD_5 and at least 88.7 per cent of the influent ammonia (table 4.2).

The pH of sewage, mixed liquor and effluent from the Guildford plant, as well as those from the model plant was consistently between 6.8-7.5.

The performance of the model plant parelleled the performance of the Guildford sewage treatment plant as summarized in table 4.3, and was well within the Thames River Authority treatment standards of 20 mg/l suspended solids and 15 mg/l BOD_5 in the effluent.

2. Recovery of f2 Coliphage from inoculated water, sewage and mixed liquor

The behaviour (or fate) of f2 coliphage in inoculated sewage and mixed liquor was studied by measuring recovery of the inoculated f2 usually over 24 hours. The use of samples of

Table 4.1. Chemical and physical monitoring of influent and effluent from the model plant

a. Average results of monitoring model plant operated at 2000 ppm MLSS, 15°C and 10 hrs. flow through time for nineteen weeks (mg/l).

	Influent*			Effluent			
	SS	BOD_5	$NH_3(N)$	SS	BOD_5	$NO_3^-(N)$	$NH_3(N)$
$\bar{x}$ **	247.4	262.9	37.1	12.6	10.1	28.9	<1
s **	66.3	74.8	6.2	2.3	3.0	5.3	-

b. Average results of monitoring model plant operated at 4000 ppm MLSS, 15°C and 10 hrs. flow through time for fourteen weeks (mg/l).

	Influent*			Effluent			
	SS	BOD_5	$NH_3(N)$	SS	BOD_5	$NO_3^-(N)$	$NH_3(N)$
$\bar{x}$	209.9	189.4	37.9	13.1	10.6	32.7	<1
s	49.3	70.8	5.2	1.5	1.6	3.5	-

c. Average results of monitoring model plant operated at 6000 ppm MLSS, 15°C and 10 hrs. flow through time for ten weeks (mg/l).

	Influent*			Effluent			
	SS	BOD_5	$NH_3(N)$	SS	BOD_5	$NO_3^-(N)$	$NH_3(N)$
$\bar{x}$	237.3	201.7	33.8	13.2	10.3	29.6	<1
s	58.9	57.2	6.0	1.8	1.3	6.0	-

Table 4.1. (continued)

d. Average results of monitoring model plant operated at 4000 ppm MLSS, 5°C and 10 hrs. flow through time for eleven weeks (mg/l).

	Influent*			Effluent			
	SS	BOD_5	$NH_3(N)$	SS	BOD_5	$NO_3^-(N)$	$NH_3(N)$
$\bar{x}$	180.4	185.9	34.4	20.0	17.6	22.5	<1-2
s	51.6	53.9	5.3	4.8	1.3	3.0	-

e. Average results of monitoring model plant operated at 4000 ppm MLSS, 25°C and 10 hrs. flow through time for fourteen weeks (mg/l).

	Influent*			Effluent			
	SS	BOD_5	$NH_3(N)$	SS	BOD_5	$NO_3^-(N)$	$NH_3(N)$
$\bar{x}$	225.6	207.4	34.3	12.9	10.5	33.0	<1
s	41.2	62.3	4.0	2.6	1.2	3.6	-

f. Average results of monitoring model plant operated at 6000 ppm MLSS, 25°C and 12.5 hrs. flow through time for five weeks (mg/l).

	Influent*			Effluent			
	SS	BOD_5	$NH_3(N)$	SS	BOD_5	$NO_3^-(N)$	$NH_3(N)$
$\bar{x}$	210.6	161.8	33.6	12.4	8.7	37.6	<1
s	44.8	39.6	4.6	1.1	0.8	4.6	-

Table 4.1. (continued)

g. Average results of monitoring model plant operated at 4000 ppm MLSS, 15°C and 5.4 hrs. flow through time for six weeks (mg/l).

	Influent*			Effluent			
	SS	BOD_5	$NH_3(N)$	SS	BOD_5	$NO_3^-(N)$	$NH_3(N)$
$\bar{x}$	195.2	227.2	34.7	14.0	13.4	32.3	<1
s	66.2	49.7	4.1	2.4	2.1	5.1	-

h. Average results of monitoring model plant operated at 2000 ppm MLSS, 15°C and 5.4 hrs. flow through time for seven weeks (mg/l).

	Influent*			Effluent			
	SS	BOD_5	$NH_3(N)$	SS	BOD_5	$NO_3^-(N)$	$NH_3(N)$
$\bar{x}$	183.9	139.9	35.4	12.9	12.0	33.1	1-2
s	40.7	28.3	2.8	1.6	1.5	4.5	-

* Influent sewage $NO_3^-(N)$ values were consistently less than 2.5 mg/l.

** Mean and standard deviation.

Table 4.2. Cumulative averages for chemical and physical analyses on model plant influent and effluent (mg/l).

	SS	BOD_5	$NO_3(N)$	$NH_3(N)$
Influent	217.0	206.6	-	35.5
Effluent	13.9	11.5	30.5	2.0*
Removal (per cent)	93.6	94.4	-	94.4**

* Maximum value ** minimum removal.

Table 4.3. Chemical and physical analyses, of influent and effluent from the Guildford activated sludge sewage treatment plant from September 1975 to August 1976 (mg/l)*.

	SS	BOD_5	$NH_3(N)$	$NO_3(N)$
Influent				
$\bar{x}$	123.9	188.9	26.3	-
s	11.5	26.3	1.6	-
Effluent				
$\bar{x}$	13.4	9.1	0.8	21.8
s	4.3	3.64	0.5	4.1
Removal (per cent)	89.2	95.2	97.0	-

* Data provided by the Area Chemist, Guildford Sewage Treatment plant. During this period, MLSS averaged 4863 ppm (s = 480).

serially diluted whole sewage, mixed liquor and effluent as inocula for virus titration had no adverse effects on the formation, size or shape of f2 plaques.

A. Recovery of f2 coliphage from water:

The recovery of f2 coliphage, and its survival in distilled, deionized water at pH 7.0 at 23°C was measured over 24 hours. The inoculated water was mixed well prior to each sampling and also between sampling times. The total recovery was as high as 43.65 per cent at time zero (Fig. 4.1 and table 4.4) and declined down to 18.62 per cent after 24 hours. It must be stressed at this point, and for all experiments, that zero-time sampling was done immediately after inoculation after complete mixing was insured.

To avoid ambiguity, figures are reported as calculated to two decimal places and not rounded off to the nearest whole unit, although their absolute significance is doubtful.

Another experiment represented in Figures 4.2 and table 4.5 was done to recover f2 coliphage from water (distilled and deionized) at pH 4, 7 and 9. The recovery at zero time from water at pH 7 was 54.95 per cent , at pH 9 was lower at 16.59 per cent , whereas at pH 4 it was much less at 0.44 per cent. Later recoveries declined moderately at pH 7, sharply at pH 9 and was increased to a peak of 25.12 per cent and finally to 14.13 per cent at pH 4 as shown by the regression coefficients of +0.12 for pH 4, -1.78 for pH 7 and -3.68 for pH 9. The pH variation observed at 24 hours in the three liquids were from 4.0 to 5.8, from 7.0 to 7.4 and from 9.0 to 7.5.

Table 4.4. Titres and per cent recoveries of inoculated f2 from deionized water pH7, 23°C. (calculated titre $10^{5.83}$ pfu/ml).

Time	Titre *	Recovery %
0	5.47	43.65
3	5.29	28.84
18	5.24	25.70
24	5.10	18.62
Regression coefficient		-0.78

* Log_{10} pfu/ml

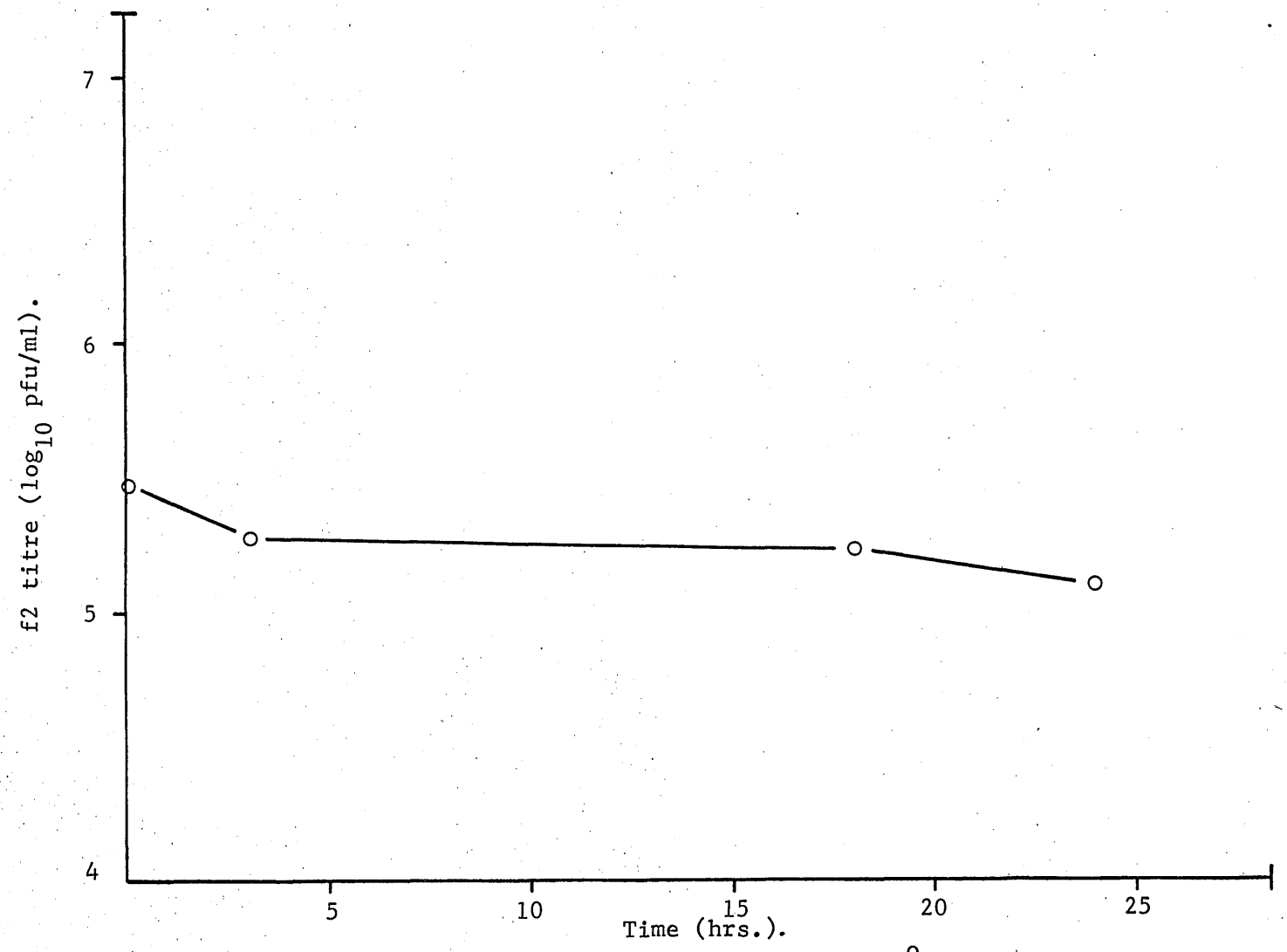


Fig. 4.1. Recovery of f2 from deionized water, pH 7.0, 23°C.

Table 4.5. Titres and per cent recoveries of inoculated f2 from deionized water at pH 4, 7 and 9, 23°C. Calculated titre $10^{6.20}$ pfu/ml.

Time	pH 4	Per cent Recovery	pH 7	Per cent Recovery	pH 9	Per cent Recovery
0	3.85 *	0.44	5.94 *	54.95	5.42 *	16.59
4.5	5.60	25.12	5.05	7.08	3.00	0.06
19	5.18	9.55	3.40	0.16	-	0.00
24	5.35	14.13	3.40	0.16	-	0.00
Regression coefficient		+0.12		-1.78		-3.67
Final pH	5.8		7.4		7.5	

* f2 titres, $\log_{10}$ pfu/ml.

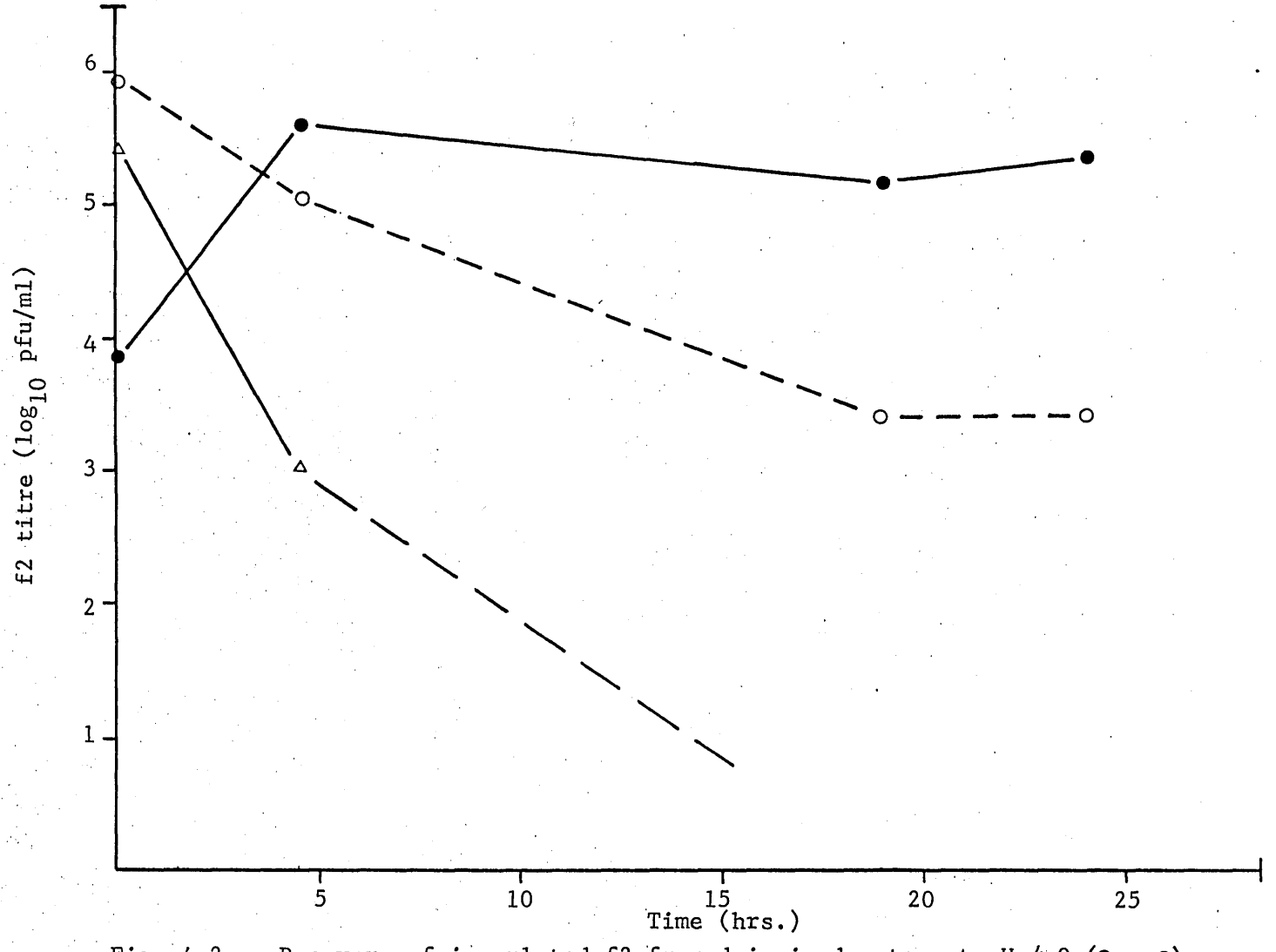


Fig. 4.2. Recovery of inoculated f2 from deionized water at pH 4.0 (●—●), 7.0 (○--○) and 4.0 (△—△).

B. Recovery of f2 coliphage from sewage:

The recovery of inoculated f2 from sewage over 24 hours is represented graphically in figure 4.3 and in table 4.6. Of the calculated initial titre of 6.38 $\log_{10}$ pfu/ml, only 73.51 per cent was recovered at zero time. The titres peaked at around 5 hours after inoculation giving a recovery of 87.06 per cent that of the calculated inoculum. At 24 hours the recoverywas as low as 18.62 per cent thus giving an overall regression coefficient of -2.55 thus indicating almost the same decline rate in comparison to the decline of the background phage in the uninoculated control sample (regression coefficient -2.88).

A sample of uninoculated sewage was monitored for virus capable of producing plaques on E. coli K_{12}Hfr to estimate the background titres of naturally existing phage particles (which replicate on E. coli K_{12}Hfr) in sewage. The initial titre was 3.63 $\log_{10}$pfu/ml which declined steadily to 3.05 $\log_{10}$pfu/ml(26 per cent of initial titre) at 24 hours (Fig. 43. and Table 4.6).

The background count of naturally existing phage was, in this case, 0.002 per cent of the inoculum and no evidence of substantially increased titre was detected in any experiment.

In another experiment aimed at measuring inoculated f2 recovery from natural and pasteurized sewage (30 minutes at 60°C) the pattern of recovery of f2 in pasteurized sewage was very similar to that in natural (Fig. 4.4, Table 4.7) with an

Table 4.6. Titres and per cent recoveries of inoculated f2 and background phages in sewage ($23^{o}C$), calculated titre at zero time $10^{6.38}$ pfu/ml.

Time	Recovery of background phage		Titre	Per cent of zero time titres
	Titre	Per cent of calculated titre		
0 hr.	6.24*	73.51%	3.63*	100.00
1	6.21	66.02%	-	-
5	6.32	87.06%	3.41	60.32
17	5.85	29.64%	3.13	31.64
24	5.65	18.62%	3.05	26.31
Regression coefficients		-2.52		-2.88

* Log_{10} pfu/ml.

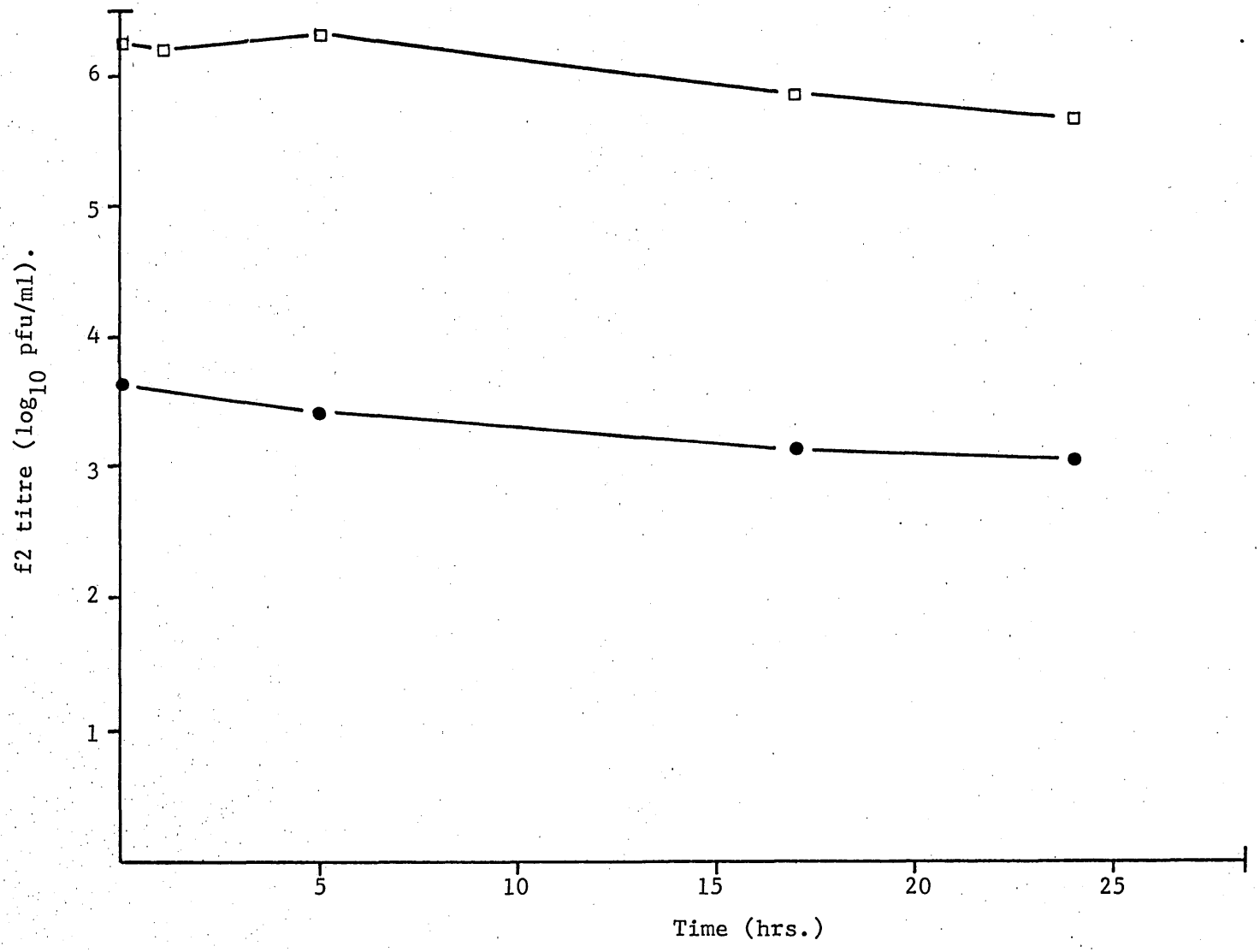


Fig. 4.3. Recovery of inoculated (□——□) f2 coliphage and background (●——●) phage from sewage

Table 4.7. Titres and per cent recoveries of inoculated f2 from natural and pasteurized sewage. ($23^{\circ}C$), calculated titre at zero time $10^{6.14}$ pfu/ml.

Time	Pasteurized		Natural	
	Titre	Per cent recovery	Titre	Per cent recovery
0 hr.	6.11*	93.31	6.21*	117.00
1	6.20	115.00	6.20	115.00
3	6.24	129.00	6.21	117.00
18	5.60	28.81	5.60	28.81
24	5.50	22.92	5.53	24.55
Regression coefficients		-4.04		-4.34

* Log_{10} pfu/ml.

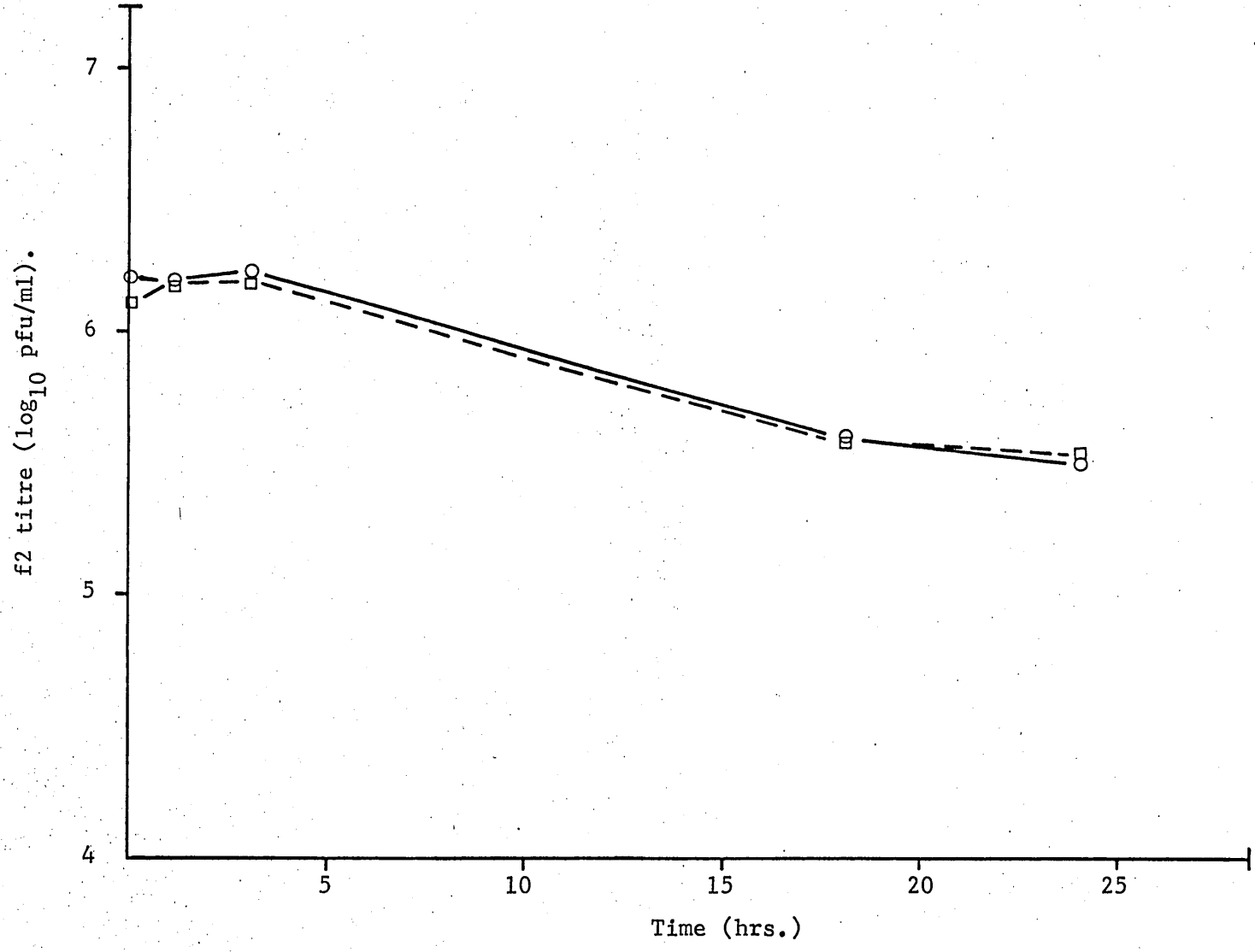


Fig. 4.4. Recovery of f2 from inoculated natural (□— — —□) and pasteurized (○——○) sewage.

initial recovery at zero time of 93.31 per cent and 117 per cent for pasteurized and natural sewage respectively. A peak titre was also observed here at 5 hours with recoveries of 129.00 per cent and 117.00 per cent from pasteurized and natural sewage respectively. The recovered titre at 24 hours was 22.92 per cent and 24.50 per cent of the inoculum for pasteurized and natural sewage respectively. The decline slopes were very close being -4.04 for pasteurized and -4.34 for natural sewage.

C. Recovery of f2 coliphage from sewage - statistical analysis:

The reproducibility of f2 recovery from sewage was tested in an experiment summarized in table 4.8. Twenty replicate samples of inoculated sewage taken at zero time were assayed and gave an average recovery of 41.06 per cent with 95 per cent confidence limits from 28.62 per cent to 53.50 per cent. This would result with the rejection of the values indicated (70 per cent of the determinations), had rejection been employed.

To evaluate f2 recovery from sewage in relation to f2 recovery from the virus stock itself, ten replicate samples of a 10^{-4} dilution of f2 stock (approximate titre 10^{8} pfu/ml) were assayed (Table 4.9). The average recovery was 99.11 per cent with confidence limits (at 95 per cent level) of 86.03 - 112.19 per cent. Only four values fell outside this range and would be rejected had rejection been employed.

The recovery of f2 from inoculated sewage and filtered sewage from ten replicate samples taken at zero time and 4 hours is summarized in table 4.10. The f2 recoveries at zero time in both sewage and filtered sewage were similar (47.30 per cent and 45.77 per cent respectively) and with no significant difference at the 95 per cent significance level of the t-test. The average recovery from sewage at 4 hours was greater (67.48 per cent) than that at zero time (47.30 per cent), but with no significant difference at the 95 per cent. confidence level, whereas the same difference was significant at the 90 per cent significance level. The difference between recoveries from filtered sewage at zero and 4 hours was not significant, whereas the difference between recoveries from sewage and filtered sewage at 4 hours were again significant at the 90 per cent significance level.

The effect of ultrasonic treatment alone and ultrasonic treatment coupled with the incorporation of serum was also studied. Table 4.11 summarizes results from an experiment where fifteen replicate samples were taken from an inoculated sample of sewage at zero time and five of them assayed without treatment, the next five were subjected to ultrasonic treatment and then assayed, and to the third group of five enough calf serum was added to make them 10 per cent in calf serum and then they were treated ultrasonically and assayed. Exactly the same method was followed for another group of fifteen samples that were taken

Table 4.8. Recovery of f2 from sewage, results of 20 replicate samples.

Sewage titre $\log_{10}$pfu/ml.	Per cent recovery (% of expected titre)*
5.20	16.98**
5.47	31.62
5.73	57.54**
5.28	20.42**
5.83	72.44**
5.37	25.12**
5.45	30.20
5.41	27.54**
5.44	29.51
5.82	70.79**
5.77	63.10**
5.26	19.50**
5.42	28.18**
5.23	18.20**
5.44	29.51
5.18	16.22**
5.98	102.33**
5.94	93.33**
5.43	28.84
5.57	39.81
$\bar{x}$ 5.58	41.06 %
s	26.11 %
C.V.	63.59 %
s.e.	5.84 %

95% confidence limits $\bar{x} \pm \frac{(ts)}{\sqrt{N}} = 41.06 \pm \frac{(2.13 \times 26.11)}{\sqrt{20}}$

$= 41.06 \pm 12.44$

* Calculated expected titre 6.00 $\log_{10}$ pfu/ml.

** These values would be rejected since they are outside than confidence limit.

Table 4.9. Recovery of f2 from stock virus.

Titre	Recovery (% of x)
7.94	93.33
7.90	85.11*
7.85	75.86*
7.94	93.33
8.10	134.90*
8.01	109.65
7.94	93.33
7.95	95.50
8.06	123.03*
7.91	87.10
$\bar{x}$ 7.97	99.11%
s	18.13%
C.V.	18.29%
s.e.	5.73%

Confidence limits $\bar{x} \pm \frac{(ts)}{(\sqrt{N})} = 99.11 \pm \frac{(2.26 \times 18.13)}{\sqrt{10}}$

$= 99.11 \pm 13.08$

* These values would be rejected (outside confidence limits).

Table 4.10. Recovery of f2 from inoculated sewage and filtered sewage (calculated titres $10^{5.86}$ pfu/ml).

Sewage titres ($\log_{10}$ pfu/ml)							
	0-time			4 hrs.			
			Per cent recovery			Per cent recovery	
	5.62		57.54	5.65		61.66	
	5.67		64.57	5.87		102.33	
	5.42		36.31	5.70		69.18	
	5.35		30.90	5.50		43.65	
	5.34		30.20	5.85		97.72	
	5.70		69.18	5.46		39.81	
	5.70		69.18	5.58		52.48	
	5.77		81.28	5.74		75.86	
	5.79		85.11	5.61		56.23	
	5.20		21.88	5.74		75.86	
$\bar{x}$	5.60	A*	47.30%	5.69	B*	67.48%	$t = 1.95 < t_{95} = 2.13$
s			25.06%			21.06%	
C.V.			52.98%			31.21%	not significant at t_{95}
s.e.			7.92%			6.66%	

(significant at $t_{90} = 1.$

*(means A and B compared)

Table 4.10. (continued)

Filtered Sewage titres ($\log_{10}$ pfu/ml).

	0-time		Per cent recovery	4 hrs.		Per cent recovery	
	5.75		77.62	5.42		36.31	
	5.50		43.65	5.50		43.65	
	5.62		57.54	5.66		63.10	
	5.00		13.80	5.84		95.50	
	5.20		21.88	5.18		20.89	
	5.40		34.67	5.36		31.62	
	5.63		58.88	5.55		48.98	
	5.67		64.57	5.58		52.48	
	5.61		56.23	5.72		72.44	
	5.32		28.84	5.32		28.84	
$\bar{x}$	5.52	C*	45.77%	5.61	D*	49.38%	$t = 0.37 < t_{95} = 2.1$
s			20.53			22.63%	
C.V.			44.85			45.82%	not significant
s.e.			6.49			7.16%	*(means C and D compared

$t = 0.16 < t_{95} = 2.13$

not significant

*(means A and C compared)

$t = 1.85 < t_{95} = 2.13$

not significant

(significant at $t_{90} = 1.75$)

*(means B and D compared)

Table 4.11. Recovery of f2 from sewage, effect of ultrasonic treatment and addition of serum. (Calculated titre $10^{5.62}$ pfu/ml).

	Sewage		Sonicated Sewage		Sonicated sewage (with 10% serum)*	
	Titres ($\log_{10}$ pfu/ml)	Per cent Recovery	Titres $\log_{10}$ pfu/ml	Per cent Recovery	Titres $\log_{10}$ pfu/ml	Per cent Recovery
0-time	(5.72	125.89	5.72	125.89	5.51	77.62
	(5.41	61.66	5.67	112.20	5.61	97.72
	(5.44	66.07	5.51	77.62	5.63	102.33
	(5.67	112.20	5.50	75.86	5.35	53.70
	(5.70	120.23	5.73	128.82	5.61	97.72
	x 5.61	97.21%	5.64	104.08%	5.55	85.82%
	s	30.87%		25.74%		20.34%
	C.V.	31.75%		24.73%		23.70%
	s.e.	13.80%		11.51%		10.60%
24-hrs.	(5.42	63.10	5.37	56.23	5.53	81.28
	(5.51	77.62	5.51	77.62	5.55	85.11
	(5.48	72.44	5.32	50.12	5.55	85.11
	(5.52	79.43	5.45	67.61	5.53	81.28
	(5.41	61.66	5.36	54.95	5.47	70.79
	$\bar{x}$ 5.47	70.85%	5.41	61.31%		80.71%
	s	8.16%		11.15%		5.87%
	C.V.	11.52%		18.19%		7.27%
	s.e.	3.64%		4.99%		3.25%
	Decline in recovery over 24 hrs.	27.12%		41.09%		5.95%

* 0.5 ml serum added to those 5 ml samples, accounted for in the calculation of the titres.

Table 4.11a. t-test values for comparing means of Table 4.11 (t_{95} = 2.31).

	means compared	
Time	Sewage-Sonicated Sewage	Sewage-Sonicated sewage (10% serum)
Zero	0.38	0.69
24 hrs.	1.54	2.19

24 hours after inoculation. Recoveries at zero time were 97.21 per cent , 104.08 per cent and 85.82 per cent from sewage, sonicated sewage and sonicated in 10 per cent serum in sewage. The decrease in recoveries at 24 hours was varied, being least in the sonicated 10 per cent serum in sewage (5.95 %) followed by the sewage samples (27.12 %) and finally by sonicated sewage (41.09 %). Table 4.11a shows t-test values for comparing the means of each of the sonicated samples against the untreated samples of the same time. No significant differences were shown by this test.

D. Recovery of f2 coliphage from mixed liquor:

The recovery of f2 from mixed liquor was done on both liquid and solids fractions of the mixed liquor. Solids were assayed for f2 after resuspending in dilution fluid. Background phage counts were assayed in all experiments and it was generally observed that their titres constituted only a very small proportion of the count due to the inoculated f2.

Results of f2 recovery over 24 hours from mixed liquor solids and liquid phases are presented in figure 4.5 and table 4.12. The overall decline in titre of virus was 87.41 per cent over the 24-hour period with the majority of this decline occurring in the MLL (regression coefficient -0.93 as opposed to regression coefficient of MLS titres of -0.03). In fact, the ratio of solids to liquid titres assumed an ascending mode with a regression coefficient (slope) of +0.57. This indicated persistence of the f2 coliphage on the solids fraction.

Table 4.12. Titres, per cent recoveries and regression coefficients of inoculated f2 from MLL and MLS fractions resuspended in dilution fluid (calculated titre at zero time $10^{5.83}$ pfu/ml; 23

Time	Titres		Total Recovered Titre	Total Recovered (%)	Per cent of initial calculated titre		MLS/MLL (%)
	MLS	MLL			MLS	MLL	
0	3.11*	5.14 *	5.14 *	20.42	0.19	20.42	0.93
3	4.63	5.57	5.62	61.66	6.31	54.95	11.48
18	4.14	5.25	5.28	28.18	2.04	26.30	7.76
24	4.19	4.84	4.93	12.59	2.29	10.23	22.39
		Regression coefficients		-0.96	-0.03	-0.93	+0.57

* Log_{10} pfu/ml.

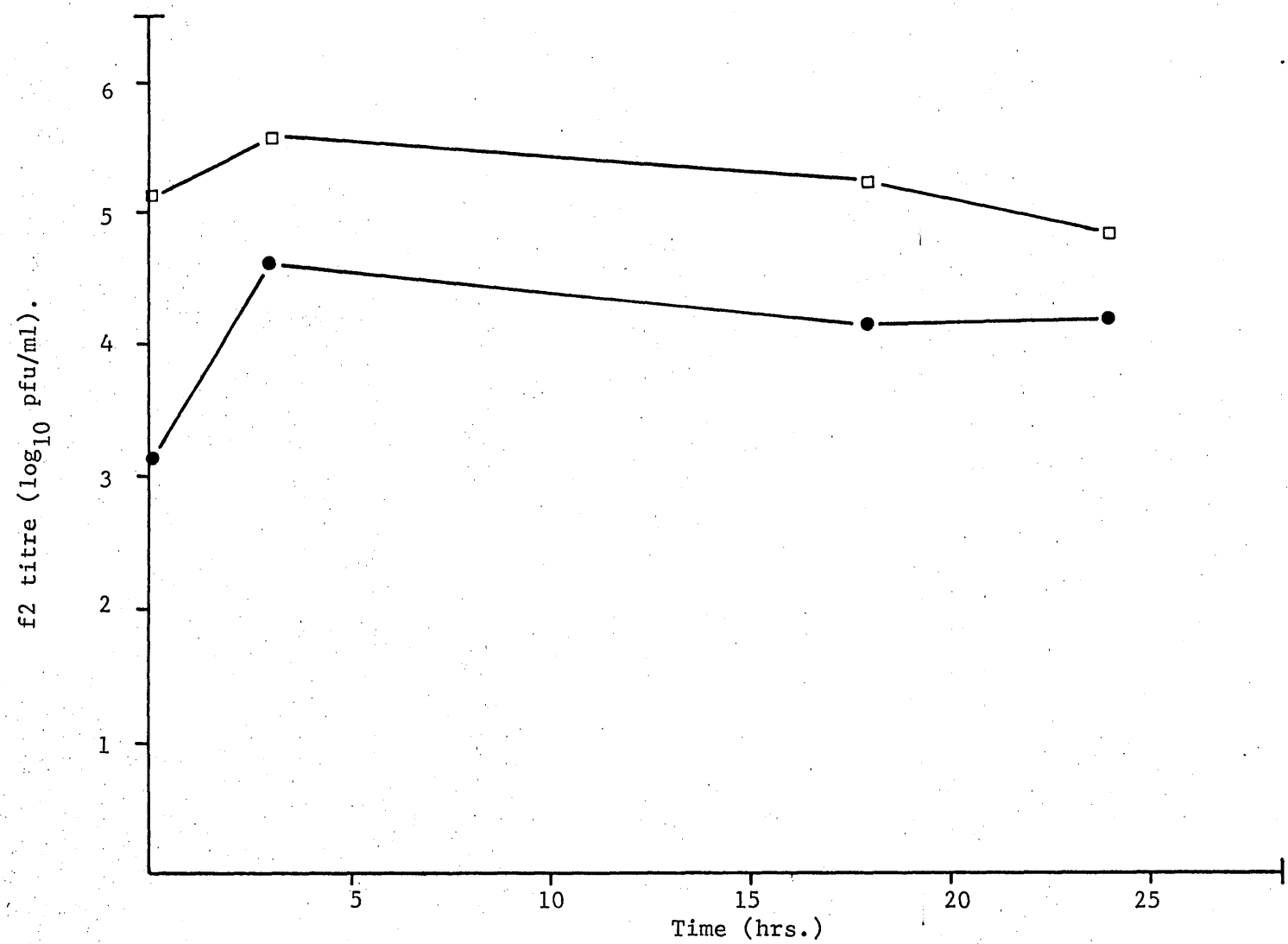


Fig. 4.5. Recovery of f2 from ML(MLSS = 3600 ppm). (MLL titres □——□, MLS titres ■——■).

Table 4.13. Titres, per cent recoveries and regression coefficients of inoculated f2 into 10 per cent mixed liquor (MLSS = 360 ppm, 23°C, calculated titre $10^{5.83}$ pfu/ml).

Time (h)	Titres MLS	Titres MLL	Total Recovered Titre	Total Recovered (%)	Per cent of initial calculated titre MLS	Per cent of initial calculated titre MLL	MLS/MLL (%)
0	- *	5.39*	5.39*	36.31	-	36.31	-
3	3.96	5.75	5.77	87.10	1.35	83.20	1.62
18	3.60	5.26	5.27	27.54	0.59	26.91	2.19
24	3.00	5.12	5.12	19.50	0.15	19.50	0.77
		Regression coefficients		-1.72	-0.06	-1.65	-0.02

* Log_{10} pfu/ml.

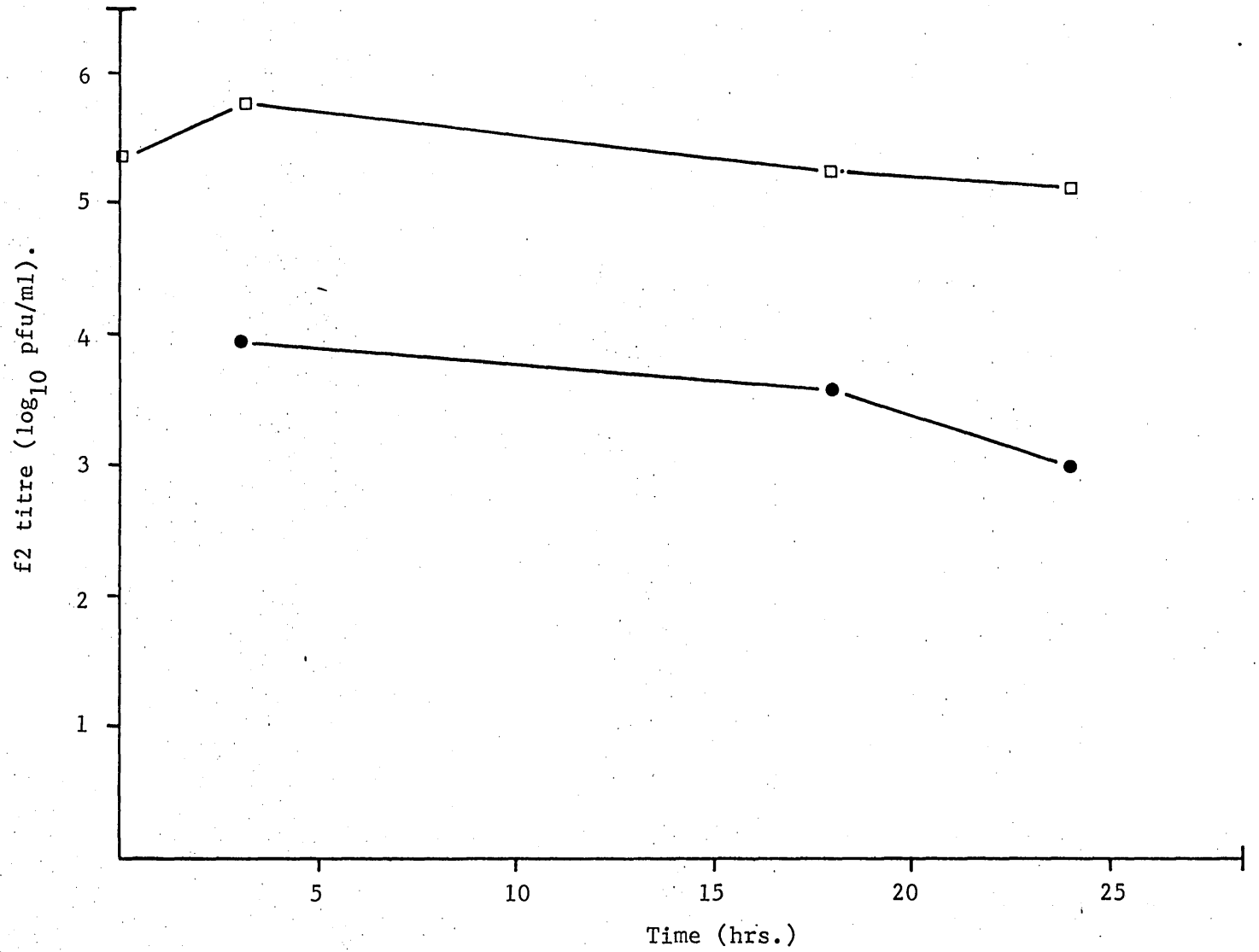


Fig. 4.6. Recovery of f2 from 10% ML (MLS = 360 ppm) (MLL titres □—□, MLS titres ●—●).

The following experiment was designed to study the effect of reduced MLSS in the mixed liquor on the recovery of inoculated f2 (Fig. 4.6 and Table 4.13). Ten per cent ML was prepared from the same sample of ML used for the previous experiment. In this experiment, solids were assayed after resuspending in dilution fluid. Similar to the results in fig. 4.5, decline in the titre of f2 was observed both in MLL and MLS fractions, with regression coefficients of -1.65 and -0.06 respectively. The maximum total recovery was 87.10 per cent of the input titre, with a regression coefficient of -1.72. Greater rate of decline and total maximum recovery were observed in this experiment in comparison to that of figure 4.5. The ratio of titres of solids to liquids assumed a slight decline with a slope of - 0.02, thus implying also a certain degree of persistence of f2 on the solids.

Further data in support of the persistence of f2 on MLS is presented in figure 4.7 and table 4.14. The same features of recovery of f2 coliphage from mixed liquor wereobvious as in figures 4.5 and 4.6 with a maximum total recovery of f2 of 76.85 per cent with a regression coefficient of -2.26 over the 24-hour period. The ratio of solids to liquid titres again assumed an ascending mode with a regression coefficient of +6.00, indicating continued uptake and persistence of f2 on the solids fraction.

Table 4.14. Titres, per cent recoveries and regression coefficients of inoculated f2 from MLL and MLS fractions (MLSS = 2690 ppm, 22°C, calculated titre $10^{6.13}$ pfu/ml).

Time	Inoculated Titres ($\log_{10}$ pfu/ml) MLL	MLS	Per cent of calculated titre MLL	MLS	Total(%) Recovery	MLS/MLL (%)
Zero	6.00	4.44	74.81	2.04	76.85	2.75
3 hrs.	5.51	4.79	23.98	4.57	28.55	19.05
18	4.80	4.77	4.68	4.36	9.04	93.33
24	4.72	4.91	3.89	6.03	9.92	154.88
	Regression coefficients		-2.38	+0.11	-2.26	+6.00

Time	Control titres ($\log_{10}$ pfu/ml) MLL	MLS	Total titres ($\log_{10}$ pfu/ml)	Total	Per cent of total at zero time MLL	MLS
Zero	1.70	2.48	2.55	100.00	14.13	85.11
3	2.10	2.51	2.65	125.89	35.48	91.20
18	2.00	2.60	2.70	141.25	28.18	112.20
24	1.85	2.23	2.38	67.60	19.95	47.86
	Regression coefficients			-0.85	-0.02	-0.85

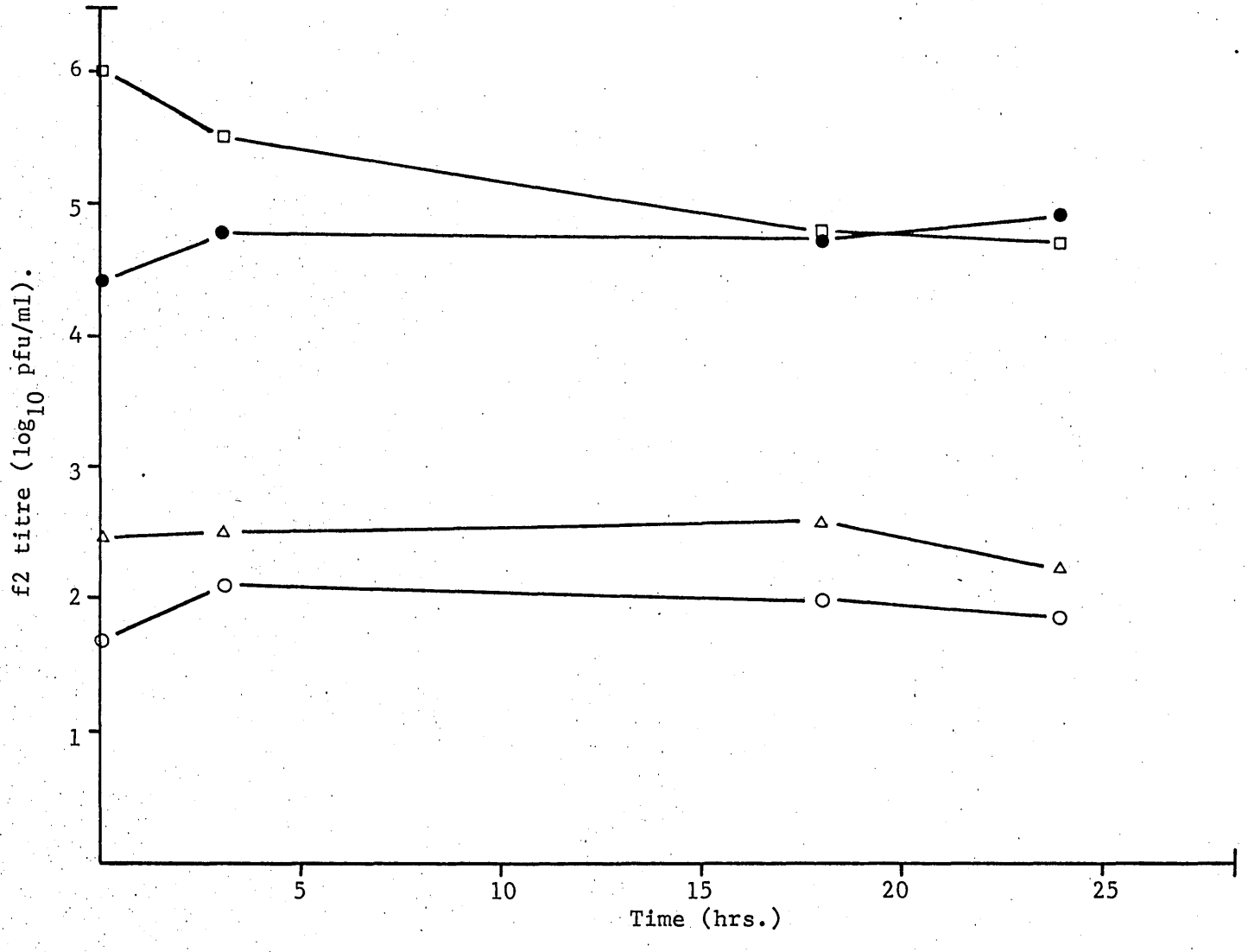


Fig. 4.7. Recovery of f2 from ML. (MLSS = 2690 ppm, inoculated MLS ●——●, MLL □——□, control MLS △——△, and MLL □——□ titres).

The comparison of recoveries of f2 coliphage from mixed liquor solids after ultrasonic treatment of the resuspended solids in dilution fluid or in 10 per cent calf serum in dilution fluid is represented in table 4.15. The table also includes data from the MLL samples because values for total recoveries from the inoculated samples were required.

It is evident that there was no significant difference between mean virus recovery in the MLL samples, whereas, a significance up to the 99.9 per cent confidence level occurred between average titres obtained from the two methods of recovery of virus from MLS. No significant difference was observed in the total recovery by the two methods, although the absolute recovery by the serum method averaged more ← 54.37 per cent VS 46.29 per cent. The differences between the means of percentage ratios of MLS/MLL titres by both methods was significantly different.

The adopted method of recovery of virus from MLS was sonic treatment in 10 per cent serum and the relative efficiency of the method after one or more successive treatments was tested. For this purpose, an experiment was performed whereby an inoculated 100-ml volume of mixed liquor was stirred for 16 hours, and six 5-ml samples were taken from it. The samples were treated in a series of fractionations followed by resuspension as illustrated in figure 4.8. The recovery of virus (Table 4.16) showed a slight increase amounting to an additional 9.65 per cent after the second

Table 4.15. Recovery of f2 from ML. Comparison of solids resuspension in DF and 10 per cent serum in DF. (23°C, SS = 3150 ppm, calculated titre 6.02 $\log_{10}$pfu/ml)

	Solids resuspended in 10 per cent serum in DF					Solids resuspended in DF				
	Recovered Titres MLL	MLS	Total Titre MLL & MLS	Total Recovery (%)	$\frac{MLS}{MLL} \times 100$	Recovered Titres MLL	MLS	Total Titre MLL & MLS	Total Recovery (%)	$\frac{MLS}{MLL} \times 10$
	5.37*	4.52*	5.43*	25.52	14.13	5.74*	4.37*	5.76*	54.56	4.27
	5.76	4.63	5.79	58.46	7.41	5.39	4.26	5.42	29.94	7.41
	5.68	4.62	5.72	49.76	8.71	5.88	4.54	5.90	75.32	4.57
	5.86	4.54	5.88	71.93	4.79	5.61	4.45	5.64	41.39	6.92
	5.87	4.55	5.89	73.60	4.79	5.83	4.37	5.84	65.60	3.46
	5.66	4.83	5.72	49.76	14.79	5.89	4.41	5.90	75.32	3.31
	5.87	4.76	5.90	75.32	7.76	5.46	4.41	5.50	29.98	8.91
	5.48	4.46	5.52	31.40	9.55	5.62	4.30	5.64	41.39	4.79
	5.53	4.60	5.58	36.05	11.75	5.18	4.11	5.22	15.74	8.51
	5.86	4.60	5.88	71.93	5.50	5.58	4.41	5.61	38.63	6.76
$\bar{x}$	5.73	4.63		54.37	8.92	5.67	4.38		46.29	5.89
s	5.30	4.07		18.80	3.64	5.34	3.77		20.79	2.06

Comparing the means

t value for MLL titres means = 0.67, not significant at 95 per cent level of confidence.

" " " MLS " " = 4.49 > 2.13, significant at 95 per cent (also at 99.9) confidence levels

" " " Total recovery = 0.91, not significant.

" " " MLS/MLL per cent means = 2.29 > 2.13, significant at 95 per cent confidence level.

* Log pfu/ml

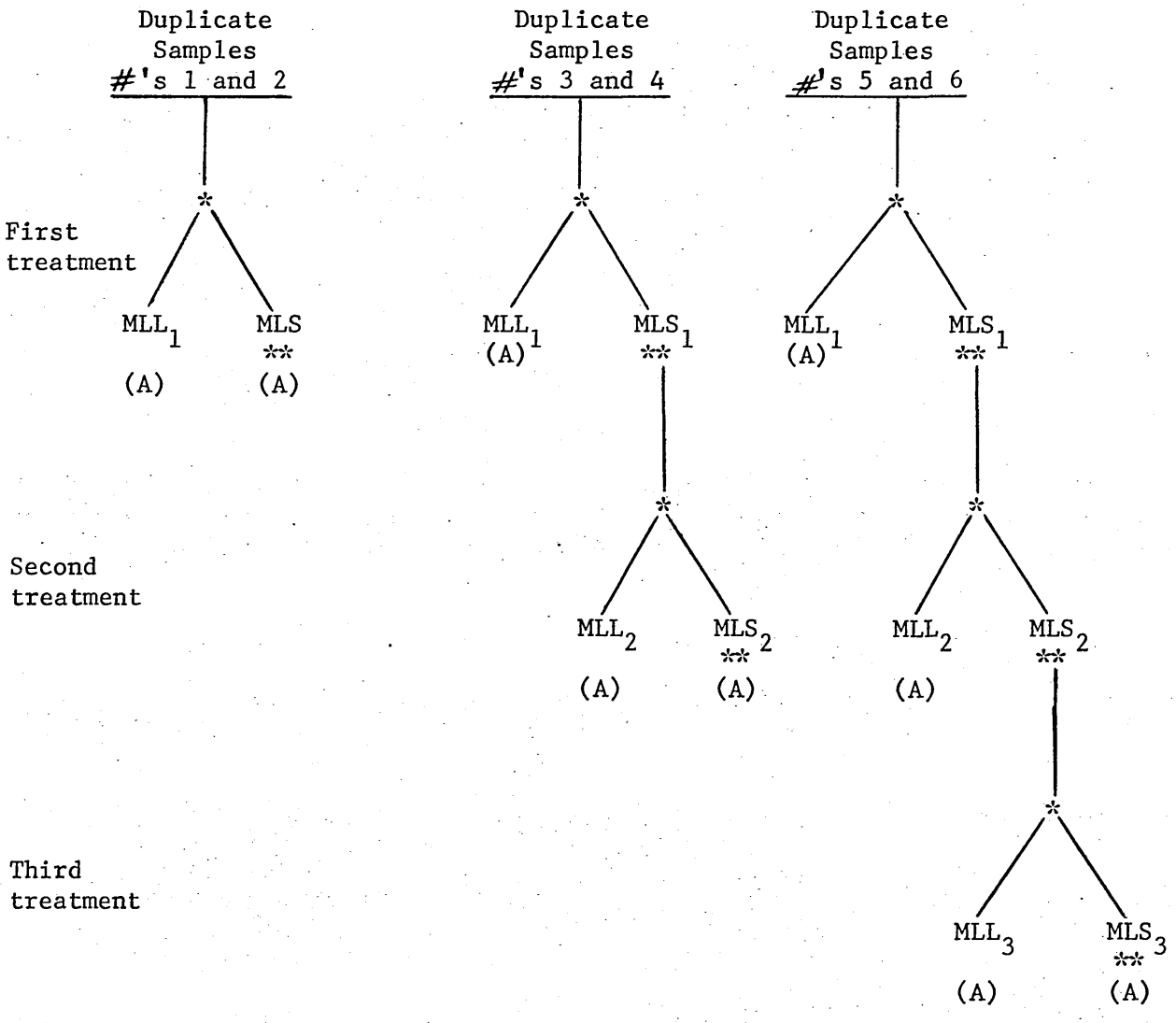


Fig. 4.8. Sample treatment protocol for experimental results on Table 4.16.

* Samples centrifuged, MLL and MLS separated.

** MLS resuspended in 10 per cent serum in dilution fluid, and treated ultrasonically.

(A) Fractions assayed.

Table 4.16. Recovery of f2 coliphage from mixed liquor after after repeated resuspension and ultrasonic treatment of solids (titres $\log_{10}$pfu/ml, calculated titre 8.50 $\log_{10}$pfu/ml)

Sample	MLS 1st treatment	MLS 2nd treatment	MLS 3rd treatment	MLL 1st treatment	MLL 2nd treatment	MLL 3rd treatment	Total treatment Titre	Average of duplicate total	Solids / Total recovered per cent	Per cent recovery
1	7.20			7.28			7.54	7.58	45.33%	12.05
2	7.27			7.36			7.62			
3		6.89		7.45	6.77		7.62	7.62 (9.65%*)	12.93%	13.21
4		6.48		7.34	7.26		7.63			
5			6.39	7.52	7.09	6.23	7.70	7.65(17.49%*)	6.37%	14.16
6			6.51	7.37	6.97	6.48	7.59			

* Increase in recovery of f2 after the second and third successive treatments 7.58 $\log_{10}$ pfu/ml taken as 100 per cer

treatment and 17.49 per cent after the third treatment (as compared to the first amount recovered). The amount of virus on the solids, however, was reduced from 45.33 per cent to 12.93 per cent to 6.37 per cent (as percentage of total ML titre) after each successive ultrasonic treatment, thus implying increased elution with every successive treatment.

3. Behaviour of f2 coliphage in the model plant:

The behaviour of f2 coliphage in the laboratory model plant of the activated sludge treatment process was investigated by inoculating the influent sewage with f2, and monitoring it through the model plant. Samples of influent, effluent and mixed liquor were monitored with special attention given to the solids contained in the mixed liquor.

The results from a preliminary experiment done at 15°C, 2000 ppm MLSS, and 10 hours flow through time are presented in figure 4.9 and table 4.17. The average recovery of f2 coliphage from the inoculated influent was 60.26 per cent. Titres in the mixed liquor fractions and effluent rapidly reached plateaux after six hours, and the comparison of average plateaux titres showed an overall f2 coliphage reduction across the plant of 95.53 per cent of the total recovered input. The recovered mixed liquor total titres averaged 18.62 per cent that of the influent, and the distribution between solids and liquid phases was in the order of 36.31:63.69 respectively.

Table 4.17. Titres ($\log_{10}$pfu/ml) during continuous inoculation experiment with f2 coliphage (also represented in figure 4.9)

	Titres			
Time	INF	MLL	MLS	EFF
preinoc.	3.50	2.48	2.74	-
zero	4.66			
1 hr.	4.52	3.40	3.48	2.00
6	4.40	3.80	2.90	3.74
21		3.00	3.56	2.00
(preinoc. 3.14)	3.14			
24 hrs.	4.47			
30	4.18	3.48	3.30	3.07
46		3.38	3.30	2.66
(preinoc. 3.19)	3.19			
48	4.45			
50	4.42	3.80	2.95	2.84
70	4.38			
INOCULATION	STOPPED			
72	3.20	3.74	3.05	2.00
80	-	2.96	2.30	2.00
92	-	2.00	2.48	-
from 0 time to inoc. stop $\bar{x}$ =	4.48	3.56	3.31	3.13
s =	3.84	3.34	3.05	3.31
		ML Total	$\bar{x}$=3.75	

Inoculum $10^{8.10}$ Expected titre in inf. $\frac{10^{8.10}}{2500} = 10^{4.70}$ pfu/ml Average recovery up to inoc. stop $= \frac{10^{4.48}}{10^{4.70}} \times 100 = 60.26\%$

$\frac{EFF}{TNF}$ % = 4.47%, $\frac{ML}{TNF}$% = 18.62%, $\frac{MLS}{MT}$% = 36.31% MLS regression coefficients from 1-70 hours = - .45 from 72 - 92 hours = - .37

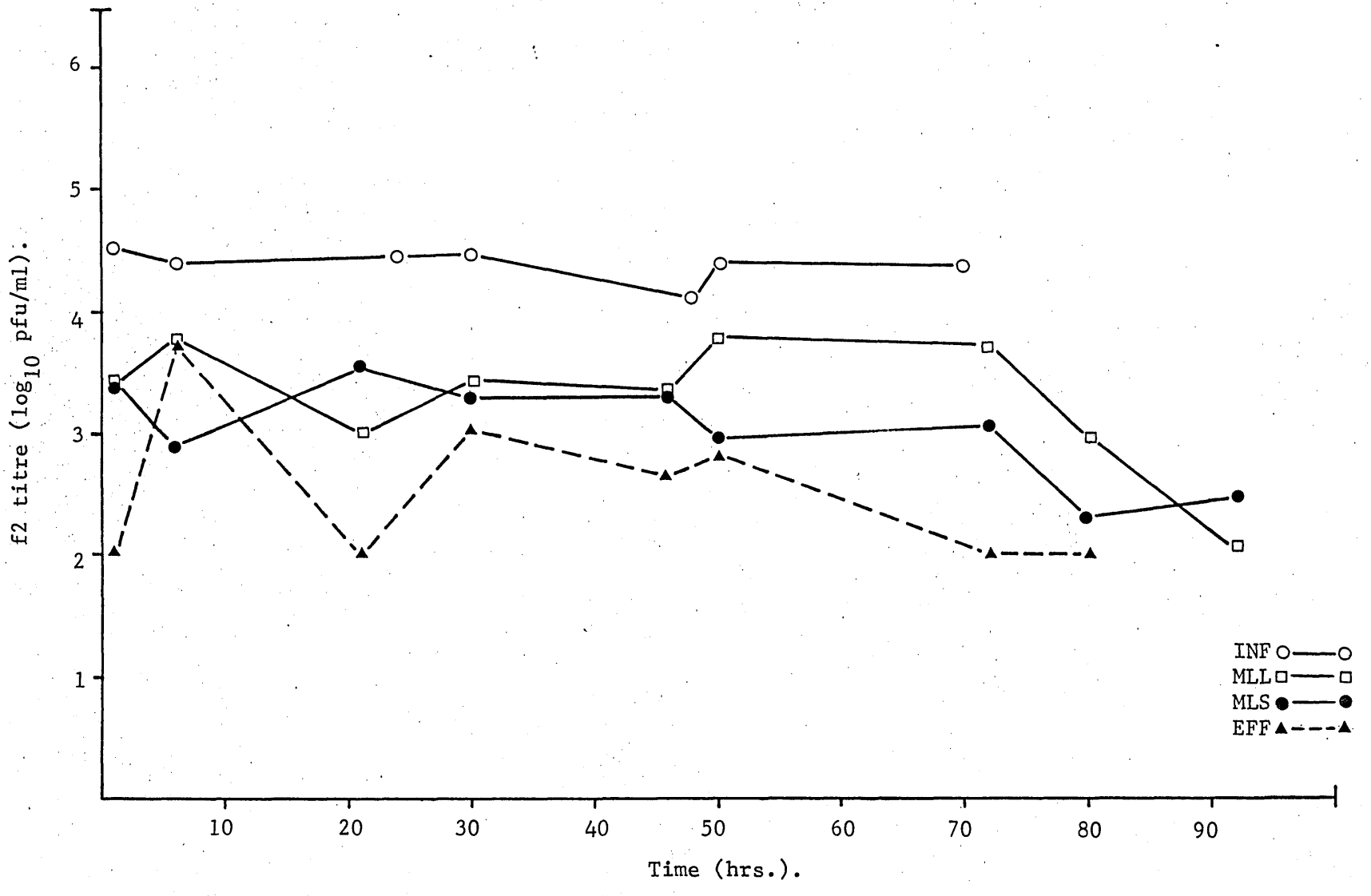


Fig. 4.9. Distribution of f2 coliphage during continuous inoculation experiment in model plant at 2000 ppm MLSS, 10 hours flow through time and 15°C(inoculated from 0 to 72 hours).

Titres of mixed liquor fractions and effluent reverted to pre-inoculation titres within 24 hours of inoculation stop.

Another experiment under the same conditions (15°C, 2000 ppm MLSS, and 10 hours flow through time) showed a similar pattern of behaviour and distribution of f2 in the model plant (table 4.18 and figure 4.10). The recovery of f2 coliphage from the influent averaged 56.23 per cent of the calculated titre, and the mixed liquor fractions and the effluent titres reached their plateaux values at about seven hours from the start of inoculation. The efficiency of the model plant in removing f2 was 88.88 per cent (11.22 % in the effluent). and the total mixed liquor average amounted to 10.23 per cent that of the influent. The distribution of f2 between the solids and liquid fractions of the mixed liquor was 21.88:78.12 respectively. Another feature of this experiment was the rapid decline in effluent and MLL titres to near background values within 60-70 hours after inoculation stoppage; whereas the MLS fraction appeared to sustain its titre during the same period. In fact, the regression coefficient of the MLS titres between 7-70 hours was +29 9, and -121 after inoculation stoppage indicating slower decline in titres after inoculation stoppage whereas the buildup of titre was more accelerated.

Information on the effect of mixed liquor suspended solids on the behaviour of f2 in the model plant was sought by doubling the MLSS to 4000 ppm while leaving the temperature at 15°C and

Table 4.18. Titres ($\log_{10}$pfu/ml) during continuous inoculation experiment with f2 coliphage (also represented in figure

Time		Titres INF	MLS	MLL	EFF
- 24		2.30	1.95	1.95	1.90
0	preinoc. 2.60)	6.15	2.00	2.60	2.48
1		6.35	2.08	4.15	3.13
3		5.86	3.95	4.26	4.48
7		6.42	3.72	4.48	5.20
24	(preinoc. 2.50)	5.40	4.11	4.46	5.29
25	(" " 3.05)	5.80			
32		5.40	4.55	4.93	5.08
46		5.52	4.34	4.79	4.61
48	(preinoc. 3.30)	5.66			
55		5.60	4.11	4.62	4.68
70		5.66	4.51	5.24	4.67
INOCULATION STOP					
78		3.20	4.35	3.74	4.77
94		3.32	4.10	3.53	3.37
120		3.23	4.09	3.11	2.81
144		3.26	4.10	2.32	1.95
	$\bar{x}$	5.95*	4.30**	4.85**	5.00**
	s	5.92	4.08	4.74	4.82
			Total ML $\bar{x}$ =	4.96	

* 0-time to inoculation stop ** 7 hrs. to inoculation stop

Calculated INF $=10^{6.20}$, per cent recovery $\frac{\text{INF}}{10^{6.20}}\%$ = 56.23 %.

$\frac{\text{EFF}}{\text{INF}}\%$ = 11.22% $\frac{\text{ML}}{\text{INF}}\%$ = 10.23 % $\frac{\text{MLS}}{\text{ML}}\%$ = 21.88 %

MLS regression coefficients from 3-70 hours = +299, from 78-144 hours = - 121

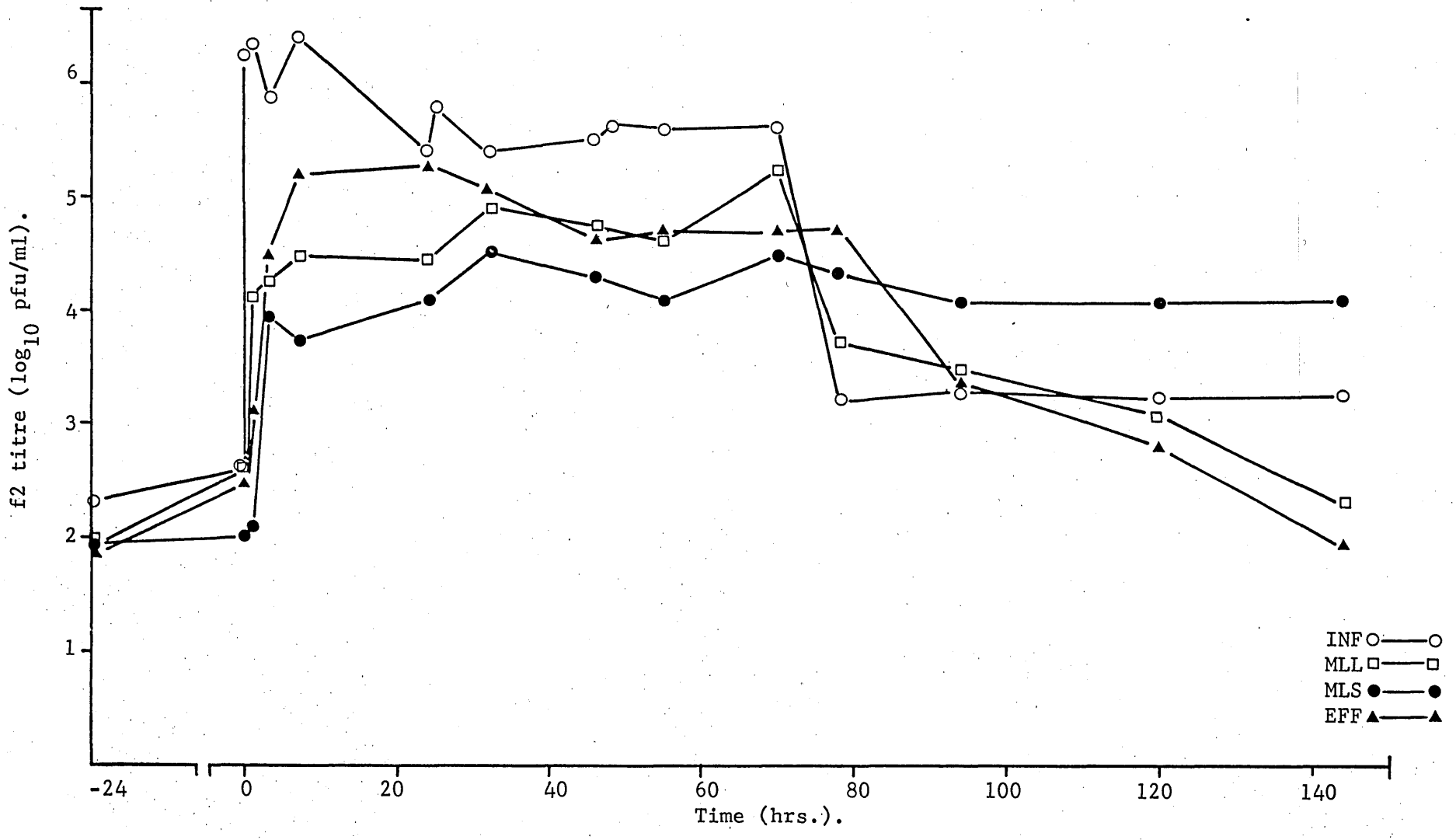


Fig. 4.10. Distribution of f2 coliphage during continuous inoculation experiment at 2000 ppm MLSS, 15°C and 10 hours flow through time (inoculation 0 to 72 hours).

the flow through time at 10 hours. The results of a continuous inoculation experiment are presented in table 4.19 and figure 4.11. In this experiment, the average recovery of f2 coliphage from influent titres was 89.31 per cent and the average titre of the total mixed liquor amounted to 29.51 per cent that of the influent. The average titres of MLS only represented 4.79 per cent those of the ML, and the MLS had regression coefficient values of +485 from 5 hours until inoculation stop, and -328 from inoculation stop until 221 hours thus indicating a slower rate of decline.

Further information on the effect of MLSS was sought by stepping up the MLSS to 6000 ppm while leaving the temperature at 15^{o}C and the flow through time at 10 hours. The results of this experiment are represented in figure 4.12 and table 4.20. The efficiency of recovering f2 coliphage titres from the influent sewage was 51.29 per cent and the titre of the average total mixed liquor amounted to 25.70 per cent. The efficiency of the plant in removing f2 coliphage during the experiment averaged 86.20 per cent and the MLS titre represented 26.92 per cent that of the total mixed liquor.

Again it was observed that titres of effluent and mixed liquor reached their plateaux values rather rapidly in about four hours, and were maintained at that level throughout inoculation.

Table 4.19. Titres ($\log_{10}$pfu/ml) during continuous inoculation experiment with f2 coliphage (also respresented in figure 4.11)

	TITRES			
Time	INF	MLL	MLS	EFF
preinoc.	3.41	2.20	2.16	3.04
zero	6.20			
1.5 hrs.	6.28	5.28	3.60	4.75
5	6.56	5.77	3.80	5.42
20	6.33	6.07	4.26	5.84
24 (preinoc. 3.45)				
25	6.37			
30	6.39	6.10	4.26	6.10
46	6.34	6.00	4.34	6.03
48 (preinoc. 3.30)				
	6.82			
54	6.38	6.17	4.68	6.07
69	6.38	5.76	4.59	5.79
72 (preinoc. 3.34)				
	6.52			
78	6.56	5.65	4.75	5.96
95		5.78	4.69	6.06
96 (preinoc. 3.30)				
	6.44			
117	5.93	5.58	4.56	5.75
120 (preinoc. 3.95)				
	6.64			
125	6.62	5.73	4.86	6.14
141	6.38	5.93	4.94	6.36
INOCULATION STOPPED				
155	3.55	5.27	4.66	4.62
166	3.50	4.03	4.33	4.62
171	3.50	4.15	4.30	4.31
190		3.26	4.03	3.37
192	3.76	3.76	4.30	3.67
212		3.21	4.32	3.08
216	3.46			
221		3.20	4.04	3.05
$\bar{x}$	6.46*	5.91	4.61	6.01
s	6.12	5.57	4.39	5.73

Total ML $\bar{x}$ 5.93

inoculum 9.90 $\log_{10}$pfu/ml, Calculated titre $\frac{10^{9.90}}{2500}$ = 6.51

average recovery from INF $\frac{10^{6.46}}{10^{6.51}} \times 100 = 89.31\%$

$\frac{EFF}{INF}\%$ = 35.48% $\frac{ML}{INF}\%$ = 29.51% $\frac{MLS}{ML}\%$ = 4.79

MLS regression coefficient
from 5-141 hrs + 485 and from 155-221 hrs. - 328

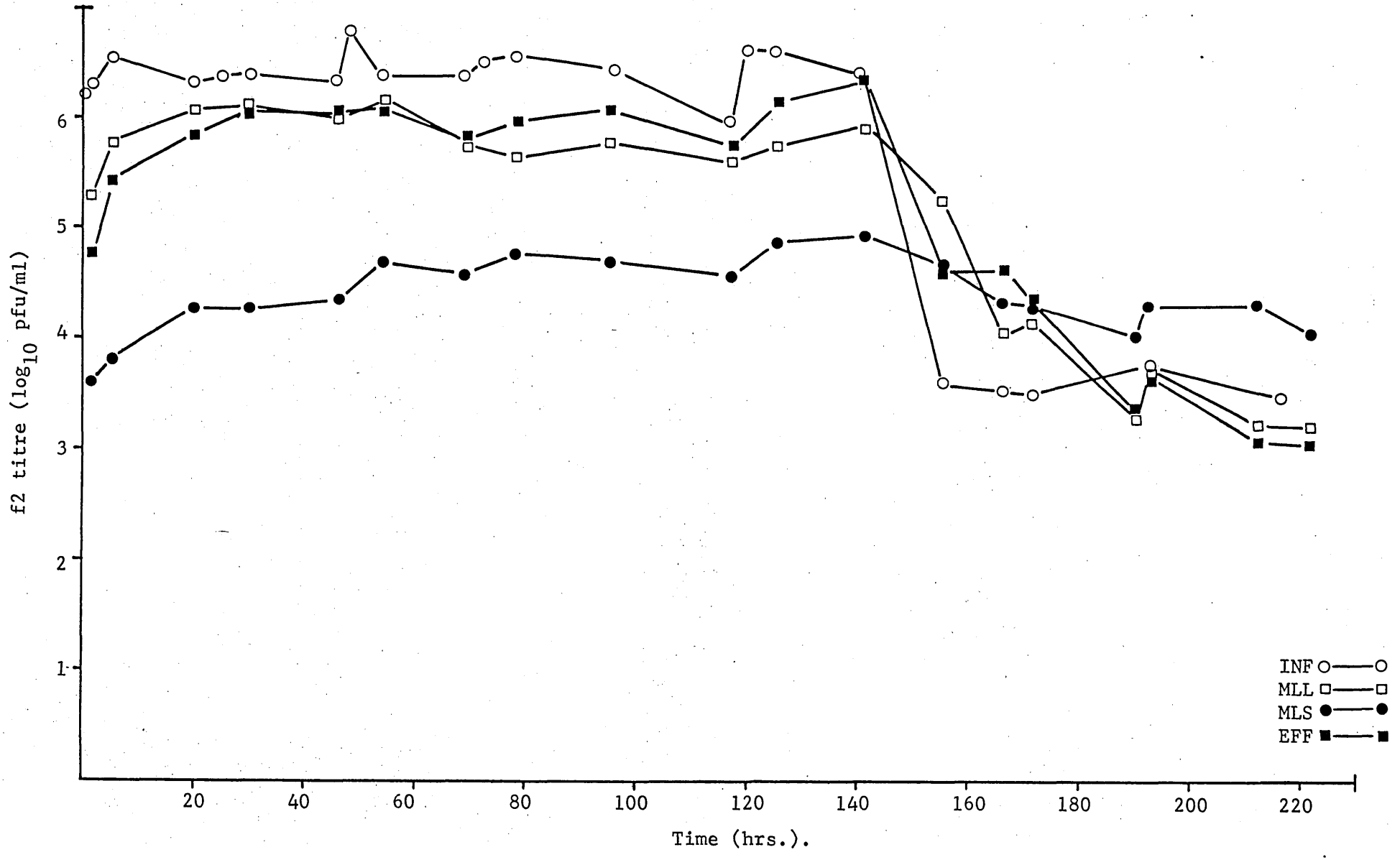


Fig. 4.11. Distribution of f2 coliphage during continuous inoculation of the model plant at 4000 ppm MLSS, 15°C and 10 hours flow through time (inoculation 0 - 141 hrs).

Table 4.20. Titres ($\log_{10}$pfu/ml) during continuous inoculation experiment with f2 coliphage at 6000 ppm, 15°C and 10 hrs. flow through time. (also represented in figure 4.12).

	TITRES			
Time (hrs)	INF	MLS	MLL	EFF
- 24	3.08	2.85	2.65	1.50
0 (preinoc 3.20)	5.60	3.08	3.00	2.10
1	5.91	3.89	4.67	3.57
4	6.21	4.39	5.09	4.69
8	5.20	4.51	4.93	5.60
(preinoc. 3.10)				
24	6.10	4.19	4.83	4.39
33	5.96	4.69	5.43	5.09
48 (preinoc. 3.4)		4.61	4.66	4.32
	5.91			
54	5.66	4.70	5.57	5.16
68	5.17	4.84	4.53	4.17
72	6.00			
(preinoc. 3.65				
78	5.91	4.86	5.15	4.79
96	6.00	4.89	5.07	4.92
118	5.91	5.00	5.26	5.17
INOCULATION STOPPED				
120	3.71	4.96	4.20	4.55
124	3.28	4.65	4.26	3.18
144	3.42	4.66	4.15	2.62
165	3.59	4.63	4.03	1.86
174	3.58	4.60	3.72	1.78
189	3.61	4.55	3.61	1.78
$\bar{x}$	5.89*	4.73	5.16	5.03
s	5.62	4.42	5.02	5.06
		Total ML$\bar{x}$ = 5.30		

inoculum = $10^{9.58}$pfu/ml Calculated INF Titre $\frac{10^{9.58}}{2500}$ = 6.18

INF Recovery = 51.29 %

$\frac{EFF}{INF}$ % = 13.80% $\frac{ML}{INF}$ % = 25.70% $\frac{MLS}{ML}$ % = 26.92%

MLS regression coefficients	
from 4-118 hours	+ 665
from 120-189 hours	- 512

* from zero time to 118 hours ** from 4-118 hours.

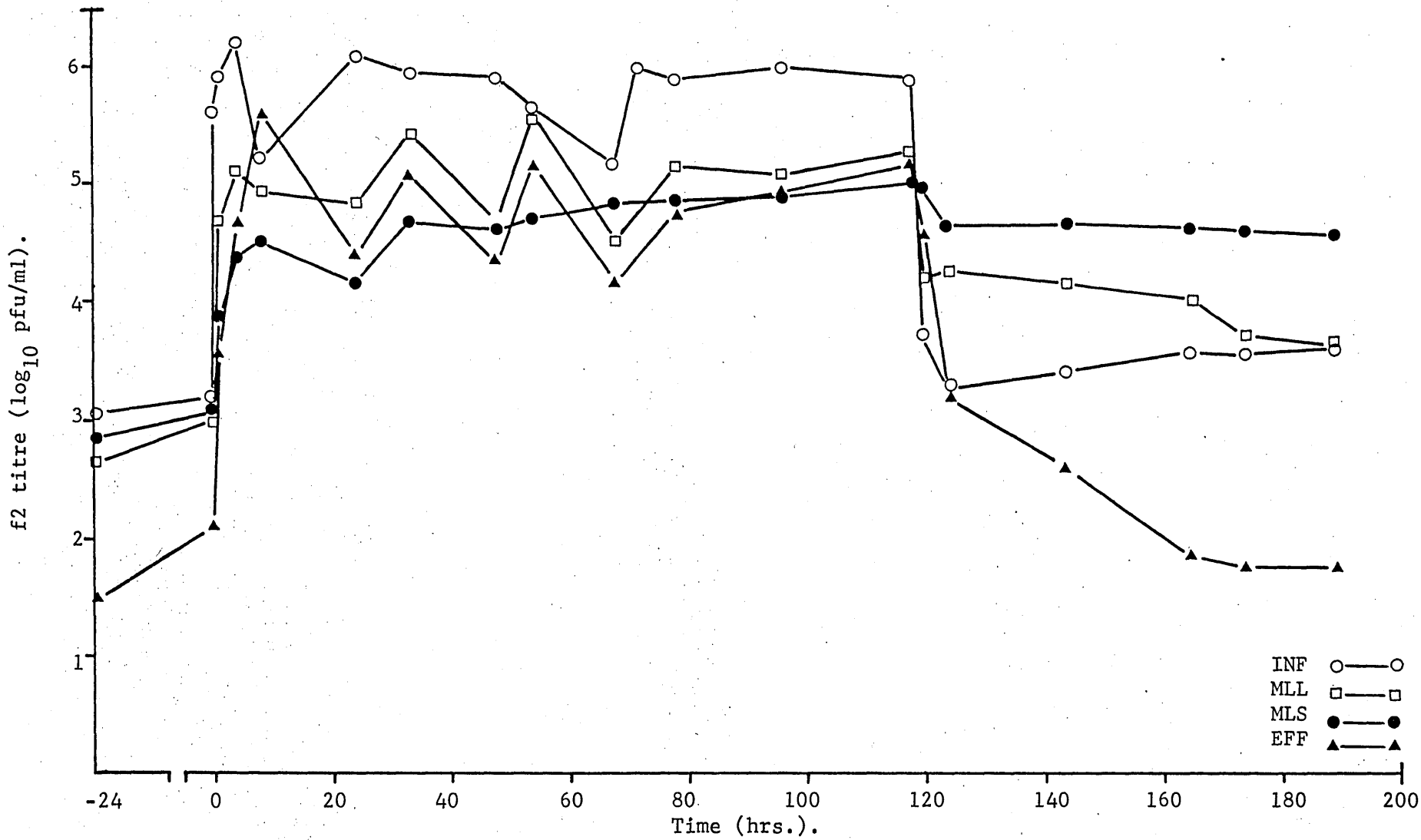


Fig. 4.12. Distribution of f2 coliphage during continuous inoculation of the model plant at 6000 ppm MLSS, 15°C and 10 hours flow through time (inoculation 0 - 118 hrs).

These titres declined rapidly after inoculation stoppage except in the mixed liquor solids. The regression coefficient of the MLS titres from 4 hours to inoculation stop was +665, and that of MLS from inoculation stop to 189 hours was -512.

The following experiments were designed to investigate the effect of reducing the flow through time to 5.4 hours. The first such experiment was done at 2000 ppm MLSS, 15°C and 5.4 hour flow through time (figure 4.13 and table 4.21). The efficiency of recovering f2 coliphage from the influent was 32.36 per cent and the average titre for the total mixed liquor amounted to 20.42 per cent that of the influent. The efficiency of the model plant, removing f2 coliphage was 89.53 per cent and the MLS titre represented 15.85 per cent that of the mixed liquor. The same trend was observed in the rapid attainment of plateaux titres by the effluent and the mixed liquor fractions by about six hours after inoculation also the rapid decline after inoculation stoppage especially in effluent and MLL titres. The regression coefficient for the MLS between 6 and 94 hours was calculated as +65, and -45 between 96 and 168 hours.

The previous experiment was extended by another continuous inoculation experiment done at 5.4 hours flow through time, 4000 ppm MLSS and 15°C and its results are represented in figure 4.14 and table 4.22. In this experiment, the average influent titre was 6.01 $\log_{10}$pfu/ml representing an average recovery of 69.18 per cent

Table 4.21. Titres ($\log_{10}$pfu/ml) during continuous inoculation experiment with f2 coliphage 15°C, 2000 ppm SS and 5.4 hours flow through time (also represented in Figure 4.13)

	TITRES			
Time	INF	MLS	MLL	EFF
- 72	3.18	3.00	2.70	2.70
- 48	3.36	3.00	2.84	2.90
- 24	3.30	3.04	2.90	2.07
0 (preinoc.3.34)		3.15	2.85	2.80
1	5.14	3.60	4.19	2.88
3	5.19	3.70	4.13	3.51
6	5.08	3.58	4.10	3.98
22	5.10	3.55	3.70	4.41
24 (preinoc.3.60)	5.34			
31	5.30	3.55	4.30	4.39
46	4.6	3.75	5.04	4.28
48 (preinoc.3.58)	5.50	4.07	4.59	4.24
54	5.39	3.88	4.42	4.49
72 (preinoc.3.49)	5.31	3.91	4.50	4.26
94	5.00	3.90	4.52	4.31
INOCULATION STOPPED				
96	3.80	3.78	3.90	2.50
120	3.78	3.91	3.45	2.30
144	3.61	3.59	3.28	2.00
168	3.54	3.58	3.40	2.08
$\bar{x}$	5.30*	3.81**	4.54**	4.32**
s	4.93	3.46	4.51	3.81

TOTAL ML $\bar{x}$ = 4.61

inoculum $10^{9.49}$, Calculated influent = $\frac{10^{9.49}}{5000}$ = $10^{5.79}$ pfu/ml

recovery from influent = 32.36%

$\frac{EFF}{INF}$ % = 10.47%, $\frac{ML}{INF}$ % = 20.42%, $\frac{MLS}{ML}$ % = 15.85%

MLS regression coefficients

from 6- 94 hours	+ 65
from 96-168 hours	- 45

* From zero time to 94 hours, ** from 6 hours to 94 hours.

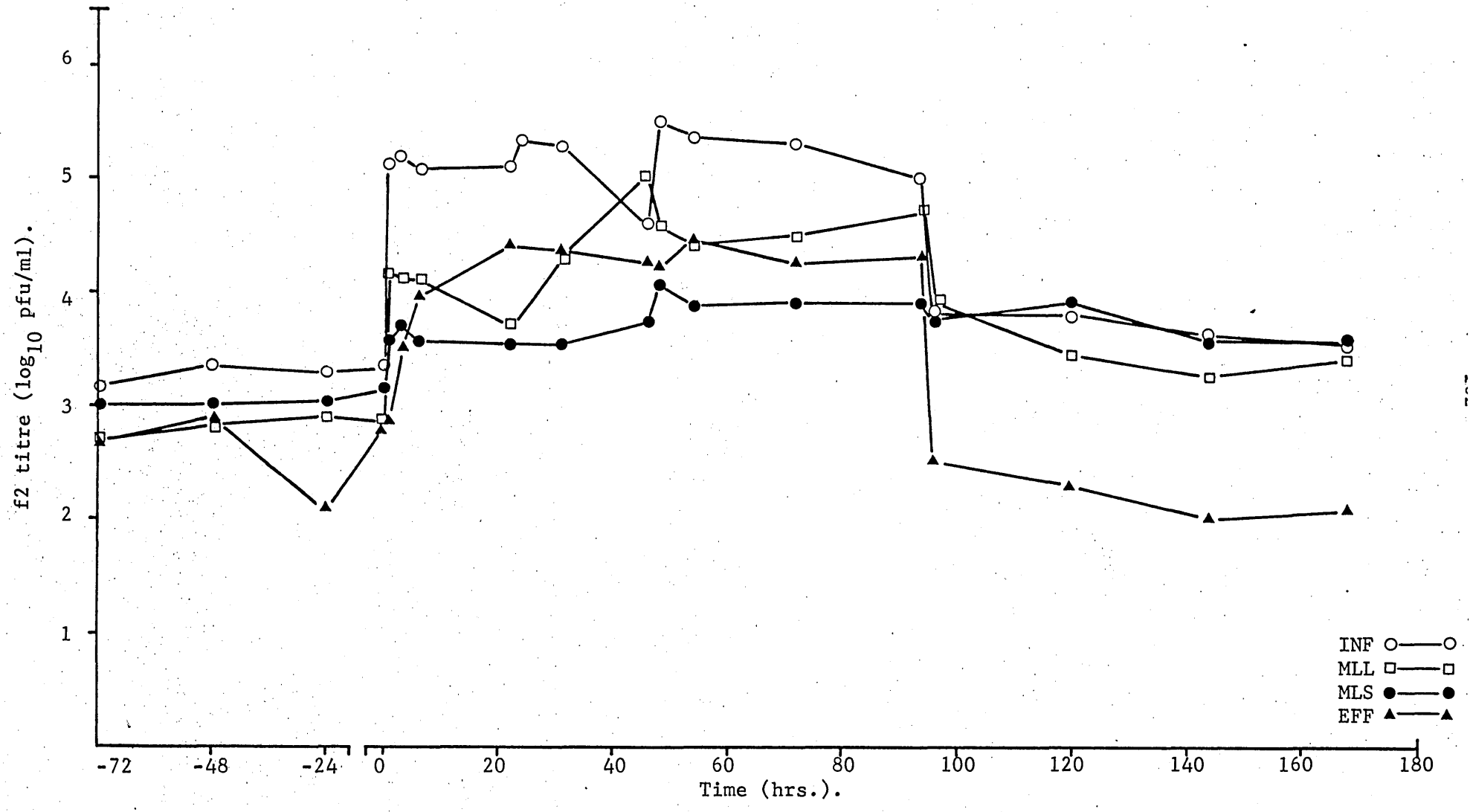


Fig. 4.13. Distribution of f2 coliphage during continuous inoculation of the model plant at 2000 ppm MLSS, 15°C and 5.4 hours flow through time (inoculation 0 - 95 hrs).

Table 4.22. Titres ($\log_{10}$pfu/ml) during continuous inoculation experiment with f2 coliphage at 4000 ppm MLSS, 15°C and 5.4 hrs. flow through time (also represented in Figure 4.13).

Time	TITRES INF	MLL	MLS	EFF
- 24	3.44	2.45	2.32	2.80
- 1	3.41	3.02	2.68	2.83
0	6.28	2.90	2.36	2.65
2	6.24	4.24	3.70	4.32
7	6.07	5.00	4.50	4.80
20	5.90	5.30	4.80	5.13
24 (preinoc. 3.72)				
24	6.21,	5.41	4.71	5.19
29	6.04	5.65	4.90	5.41
46	6.01	5.78	4.76	5.48
48 (preinoc. 3.38)				
48	5.95	5.27	4.50	5.77
53	5.74	5.61	4.72	5.55
72	5.80	5.26	4.70	5.41
(preinoc. 3.27)				
73	5.84			
77	5.86	5.54	4.70	5.07
94	5.90	5.21	4.63	5.36
98 (preinoc. 3.52)				
	5.85			
118	5.74	5.28	4.90	5.32
INOCULATION STOPPED				
140	3.50	3.70	4.69	3.34
149	3.56	4.25	4.53	2.60
166	3.50	4.08	4.71	2.91
173	3.34	3.41	4.52	2.00
188	3.24	3.71	4.59	2.00
$\bar{x}$	6.01*	5.45**	4.73**	5.38**
s	5.65	5.18	4.20	5.16

Total ML $\bar{x}$ = 5.53

inoculum $10^{9.87}$, expected titre = $\frac{10^{9.87}}{5000}$ = 6.17 $\log_{10}$pfu/ml

f2 recovery from Inf. % $\frac{10^{6.01}}{10^{6.17}}$ = 69.18%.

$\frac{EFF}{INF}$ % = 23.44%, $\frac{ML}{INF}$ % = 32.79%, $\frac{MLS}{ML}$ % = 15.85%

MLS regression coefficients: from 7-118 hours, + 113
from 140-188 hours, - 126

* from zero time to 118 hours. ** from 7-118 hours.

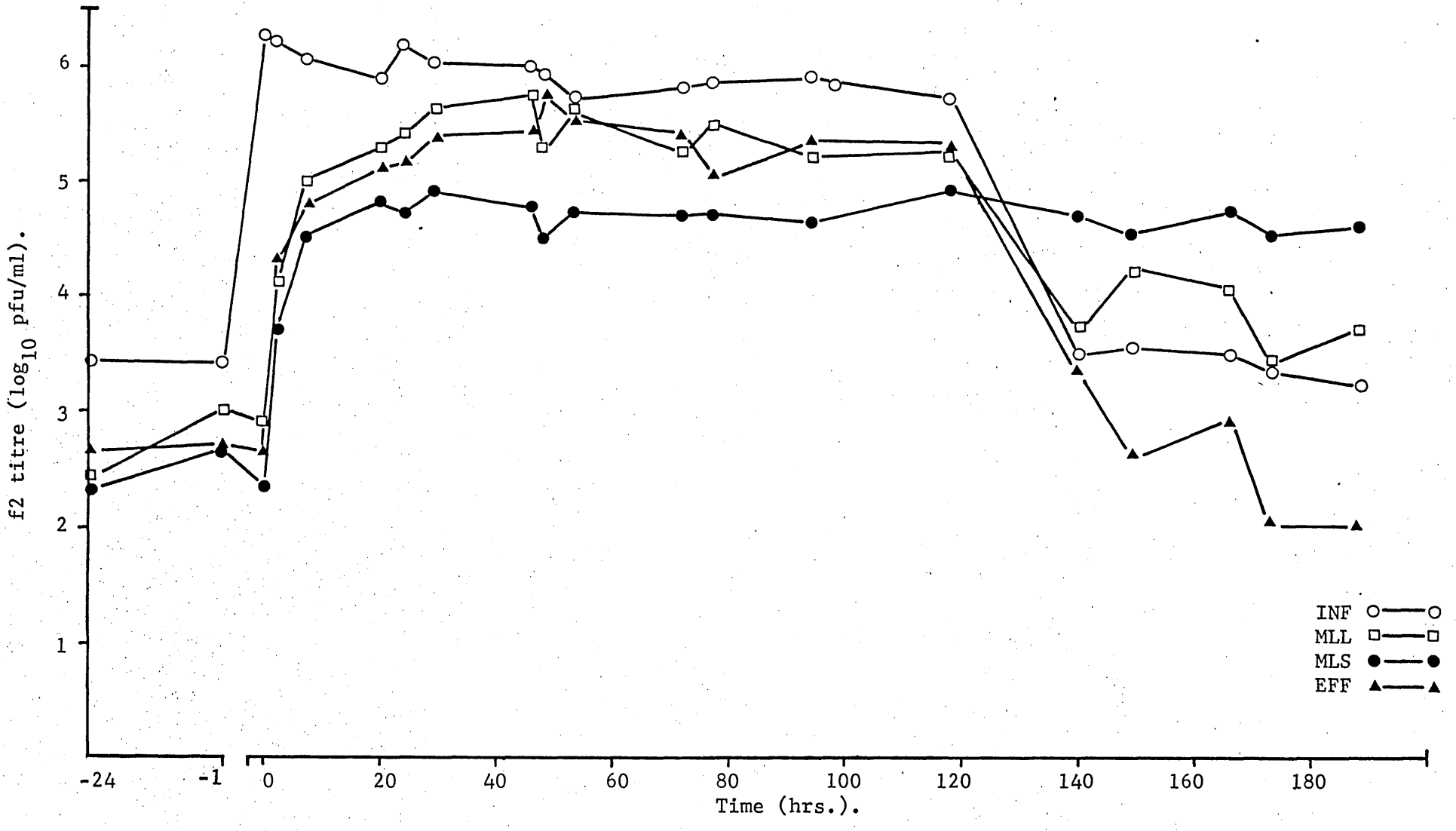


Fig. 4.14. Distribution of f2 coliphage during continuous inoculation of the model plant at 4000 ppm MLSS, 15°C and 5.4 hours flow through time (inoculation 0 - 118 hrs).

and the total efficiency of the model plant in removing the virus was 76.56 per cent. The mixed liquor average titre represented 32.79 per cent of the average influent titre, and the MLS represented 15.85 per cent of the total ML average.

Further observations were made on the rapid attainment of plateaux titres by the mixed liquor solid and liquid fractions and effluent; as well as the rapid decline of the same titres after inoculation stoppage except the MLS titres which remained nearly constant. Regression coefficients of MLS titres from 7-118 hours was +113, and from 140-188 was -126.

In further experiments, presented in figure 4.15 and table 4.23, the effect of increased temperature of the aeration tank to 25°C while leaving MLSS at 4000 ppm and the flow through time at 10 hours was investigated. In this experiment the influent averaged 5.35 $\log_{10}$pfu/ml which represented an overall recovery of 54.95 per cent from the influent to the calculated influent titres. The overall capacity of the plant to remove f2 coliphage under the conditions mentioned was 84.15 per cent, while the total titres of the mixed liquor represented 87.10 per cent of the influent. The MLS titres, at plateaux levels, were 23.99 per cent that of the total ML titres. Contrary to most other experiments, the average effluent titres were less than the MLS titres. The decline in effluent titres after inoculation stoppage (as seen in figure 4.13), was less pronounced

than its decline in other experiments. The regression coefficient of plateaux values of MLS was +253, and after inoculation stoppage to the end of the experiment it was -622.

The study of the effect of temperature on the performance of the model plant was further extended by an experiment performed at 5°C, 4000 ppm MLSS and 10 hours flow through time as represented in figure 4.16 and table 4.24. In this experiment, f2 coliphage recovery from the influent was 45.71 per cent of the capacity of the plant to remove f2 coliphage was 87.41 per cent. The total ML titres, compared to the effluent, averaged 12.77 per cent ; and the MLS titres when compared to total ML titres were as low as 7.94 per cent - being among the lowest recorded MLS/ML per cent ratio in all experiments. The regression coefficient for MLS titres from 6-96 hours was +21, and that from 102-168 hours was -14. These values for regression coefficients mark the lowest absolute values for all experiments with f2.

Further information on the model plant's capacity to remove f2 was sought in a dual-level inoculation experiment. The plant was continuously inoculated at a moderate level for two days after which inoculation was continued at a much higher level (about 170 times) for two additional days. The distribution of f2 coliphage throughout this dual-level inoculation experiment is represented in figure 4.17, and the titres observed are listed in table 4.25.

Table 4.23. Titres (log_{10}pfu/ml) during continuous inoculation experiment with f2 coliphage at 4000 ppm MLSS, 10 hrs. flow through time and 25°C (also represented in Figure 4.15).

Time	TITRES INF	MLS	MLL	EFF
- 24	3.11	3.51	2.79	2.38
0 (preinoc.2.80)	5.03	3.40	2.77	2.30
1	5.00	3.67	4.73	3.00
3	5.49	3.94	5.21	3.84
6	4.99	3.74	5.14	4.41
22		4.56	5.21	4.25
25 (preinoc.3.34)				
30	5.65	4.88	5.17	4.84
46 (preinoc.2.90)				4.30
48	5.40	4.81	5.22	4.59
55	5.10	4.73	5.12	4.50
70	5.01			
72 (preinoc.3.20)	5.59	4.60	5.02	4.49
80	5.40	4.70	5.30	4.72
94	5.13			
96 (preinoc.3.40)	5.60	4.71	5.10	4.49
INOCULATION STOPPED				
98	3.30	4.71	5.01	4.11
120	3.81	4.60	4.04	3.56
128	3.90	4.39	3.00	2.40
140	3.59	4.40	3.20	2.69
168	3.70	3.89	2.11	1.60
$\bar{x}$	5.35*	4.67**	5.17**	4.55**
s	5.12	4.32	4.46	4.21
		Total ML $\bar{x}$ = 5.29		

inoculum $10^{9.01}$, calculated inf titre 5.61 log_{10}pfu/ml.

Recovery from influent 54.95%

$\frac{EFF}{INF}$ % = 15.85%. $\frac{ML}{INF}$ % = 87.10%, $\frac{MLS}{ML}$ % = 23.99%

MLS regression coefficients: from 6- 96 hours + 253
from 98-168 hours - 622

* from zero time to 96 hours ** from 6-96 hours.

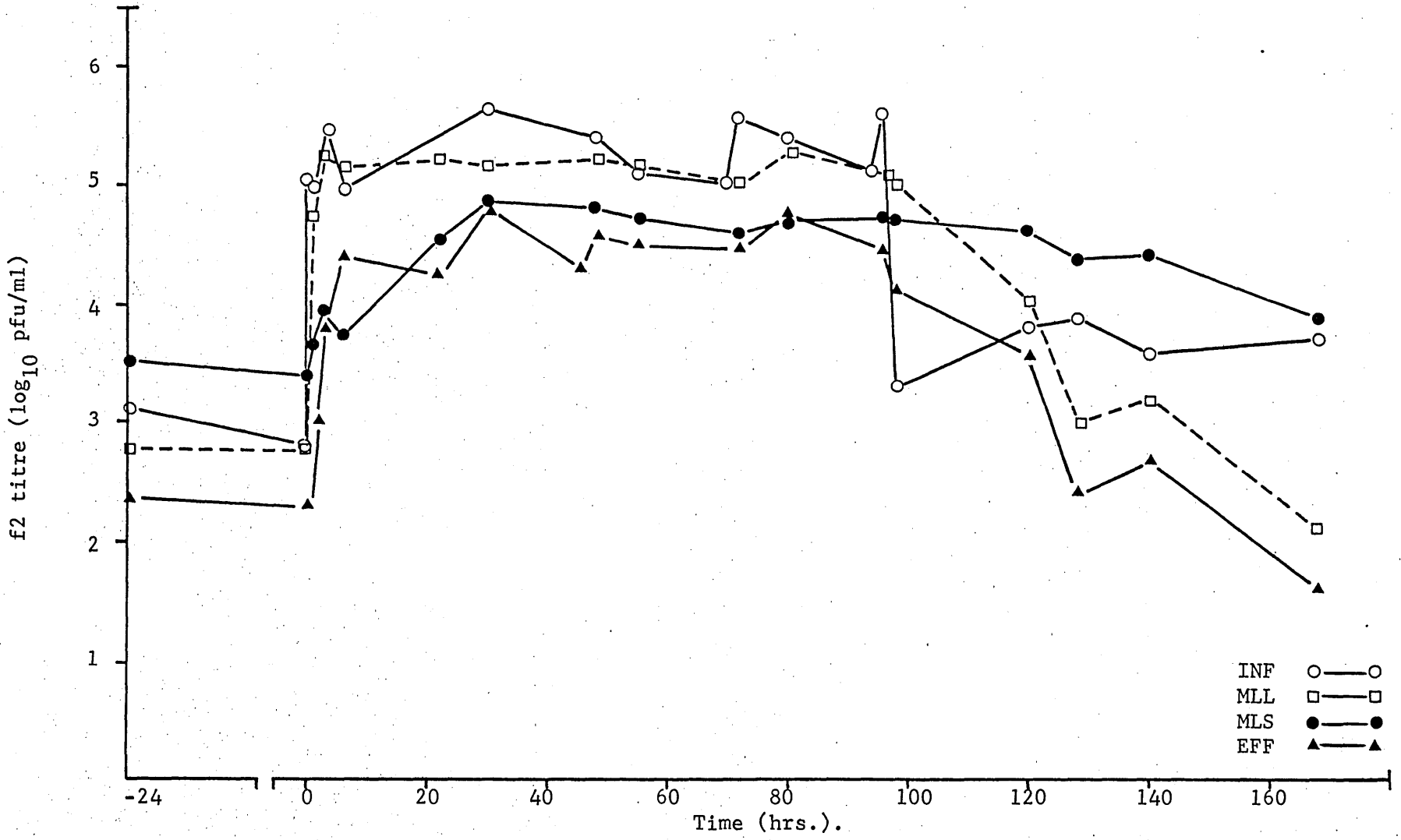


Fig. 4.15. Distribution of f2 coliphage during continuous inoculation of the model plant at 4000 ppm MLSS, 10 hours flow through time and 25°C. (inoculation 0 - 96 hrs).

Table 4.24. Titres ($\log_{10}$pfu/ml) during continuous inoculation experiment with f2 coliphage at 4000 ppm MLSS, 10 hours flow through time and 5°C (also represented in Figure 4.16).

Time	TITRES INF	MLS	MLL	EFF
- 24	3.52	2.08	2.51	2.20
0 (preinoc.3.20)	5.46	2.11	2.86	2.08
1	5.46	2.92	4.61	3.16
3	5.92	3.33	4.94	4.49
6	5.92	3.63	4.72	4.69
22		3.63	4.50	4.89
24 (preinoc.2.78)	4.94			
31	5.52	3.57	4.63	5.10
46		3.69	4.60	4.76
48 (preinoc.3.36)	5.80	3.70	4.81	4.71
54	5.50	3.61	4.80	4.60
72 (preinoc.3.66)	5.62	3.76	4.91	4.49
78	5.10	3.81	4.80	4.61
95 (preinoc.3.53)				
96	5.20	3.71	4.71	4.61
INOCULATION STOPPED				
98	3.40			
102	3.69	3.48	4.10	4.29
128	3.49	3.11	3.11	3.00
144	3.00	3.28	2.95	2.95
168	3.40	3.29	2.28	2.10
$\bar{x}$	5.67*	3.68**	4.74**	4.77**
s	5.44	2.94	4.18	4.46

Total ML$\bar{x}$ = 4.78

inoculum $10^{9.41}$, calculated influent titre = $\frac{10^{9.41}}{2500}$ = 6.01 Log_{10} pfu/ml.

virus recovery from influent = 45.7%

$\frac{EFF}{INF}$ % = 12.59%, $\frac{ML}{INF}$ % = 12.77%, $\frac{MLS}{ML}$ % = 7.94%

MLS regression coefficients from 6- 96 hours + 21
from 102-168 " - 14

* from zero time to 96 hours, ** from 6-96 hours.

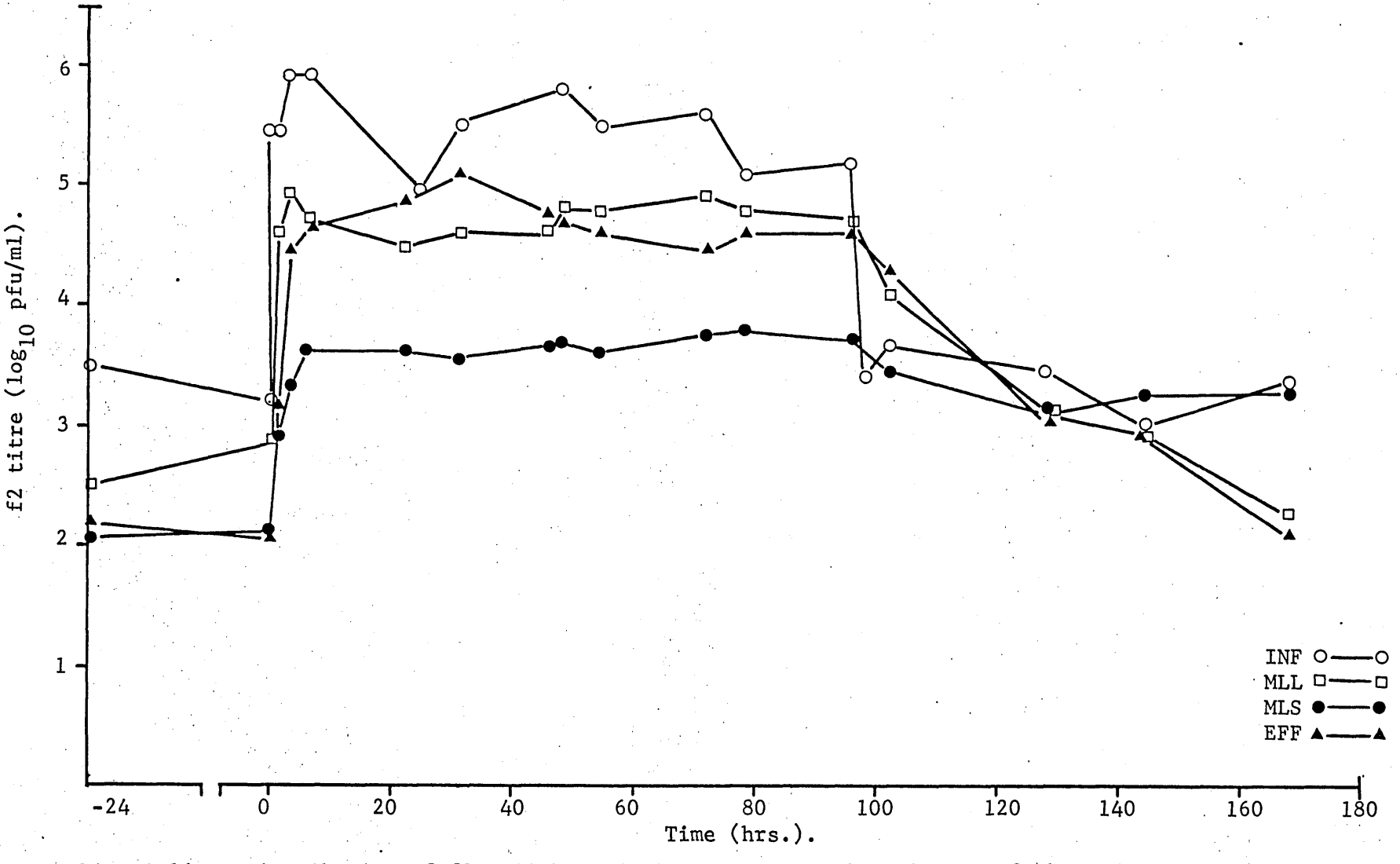


Fig. 4.16. Distribution of f2 coliphage during continuous inoculation of the model plant at 4000 ppm MLSS, 10 hours flow through time and 5°C (inoculation 0 - 96 hrs).

Table 4.25. f2 titres ($\log_{10}$ pfu/ml) during continuous, dual-level inoculation of the model plant at 2000 ppm MLSS, 10 hours flow through time and 15°C, (also represented in Fig. 4.17).

TIME		TITRES INF	MLS	MLL	EFF
- 48 hrs.		2.70	2.60	2.00	2.45
- 24		3.15	2.60	2.48	1.48
0	(preinoc. 2.70)	4.85	2.60	1.70	1.30
2		5.04	3.50	4.15	2.90
4		4.96	3.00	4.07	3.48
8		5.00	3.57	4.07	3.98
22		4.96	3.84	3.60	4.32
24	(preinoc. 3.30)	5.28	3.45	4.28	4.32
46		5.38	3.75	5.02	4.26
52	(preinoc. 3.68)	8.11	3.80	4.74	5.48
70		8.05	6.45	7.15	7.74
78	(preinoc. 3.52)	8.03	6.59	7.24	7.35
94		8.18	6.67	7.48	7.52
102		8.05			
INOCULATION STOPPED					
103		3.43	6.70	6.81	7.44
118		3.48	6.01	6.02	5.30
126		3.26	6.11	5.32	4.65
140		3.41	6.15	4.75	4.63
149		3.32	6.21	5.72	3.78
168		3.40	5.98	5.23	4.44
192		3.36	4.59	4.46	3.39
8 - 46 hours	$\bar{x}$	5.11*	3.68	4.54	4.24
	s	4.80*	3.27	4.67	3.73
			Total ML $\bar{x}$ =	4.60	

Table 4.25. (continued).

70 - 102 hours	$\bar{x}$	8.09**	6.58	7.31	7.57
	s	7.26**	6.00	6.93	7.22
			Total ML $\bar{x}$ =	7.38	

Recoveries 8-46 hours

Inoculum $10^{9.57}$ pfu/ml, Expected titre = $\frac{10^{9.57}}{2500}$ = $10^{6.17}$ pfu/ml.

Recovery from INF = $\frac{10^{5.11}}{10^{6.17}}$ % = 8.71 %

$\frac{EFF}{INF}$ % = 13.49%, $\frac{ML}{INF}$ % = 30.90 %, $\frac{MLS}{ML}$ % = 12.02%.

Recoveries 70-102 hours

Inoculum $10^{11.81}$ pfu/ml, Expected titre = $\frac{10^{11.81}}{2500}$ = $10^{8.41}$ pfu/ml,

Recovery from INF = $\frac{10^{8.09}}{10^{8.41}}$ % = 47.86 %

$\frac{EFF}{INF}$ % = 30.20 %, $\frac{ML}{INF}$ % = 19.70 %, $\frac{MLS}{ML}$ % = 15.70 %

MLS regression coefficients:

from 8-46 hours + 42, from 70-94 hours +73417, from 103-192 hours -37369

* From zero time to 46 hours, ** from 52-102 hours.

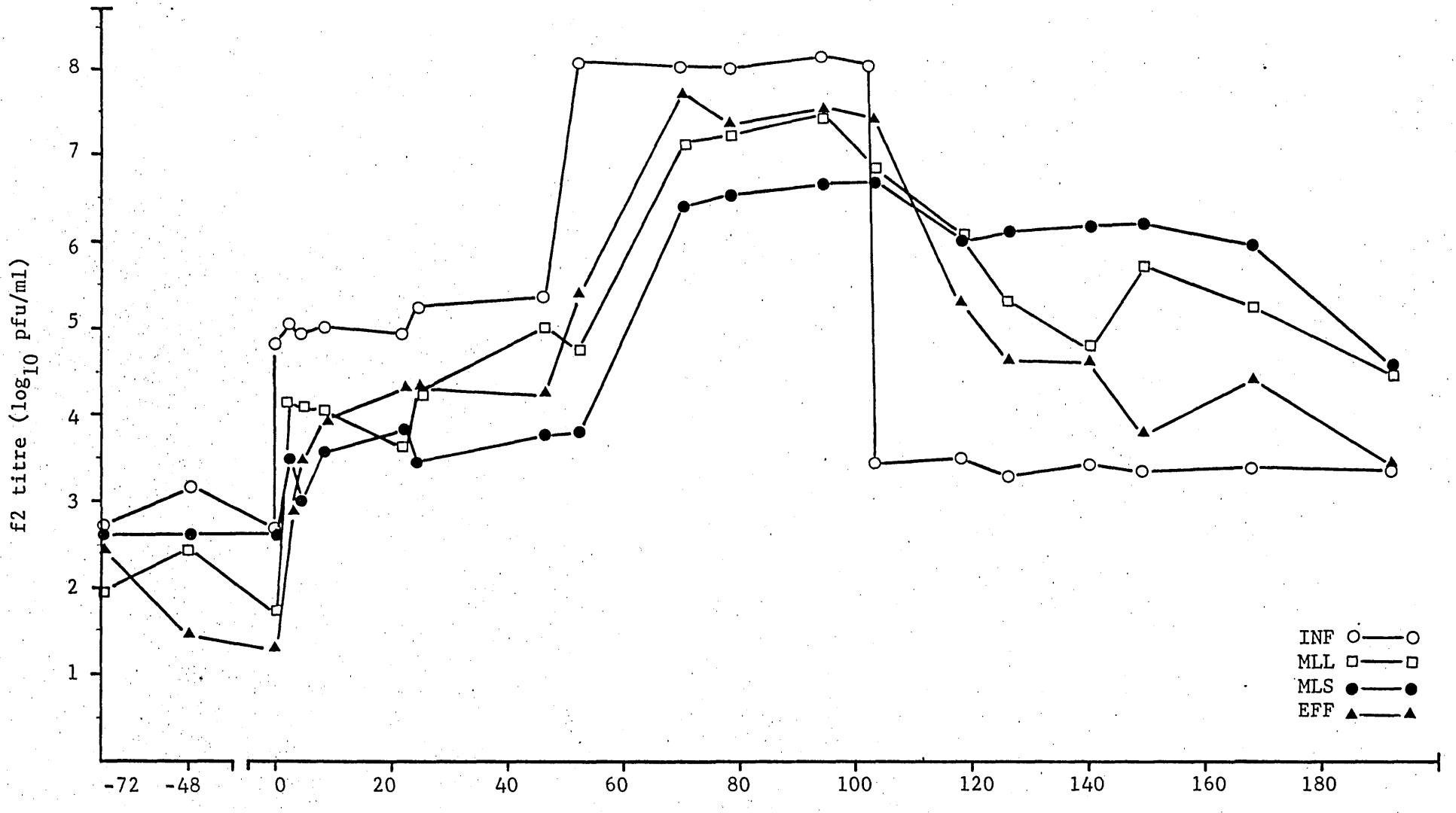


Fig. 4.17. Distribution of f2 coliphage during continuous, dual-level inoculation of the model plant at 2000 ppm MLSS, 15°C and 10 hours flow through time.
(Normal inoculation 0 - 46 hrs, high 46 - 102 hrs).

As observed in other experiments, a fraction of the inoculated f2 coliphage was detected in the influent and in this case it was 8.71 per cent. The plant could, however, remove 86.51 per cent of the detected f2 coliphage. The total mixed liquor titres represented 30.90 per cent of the total influent and the MLS titres represented 12.02 per cent of the total ML titres.

The same general pattern of distribution of f2 coliphage was maintained, except for being at higher titres, in all the model plant fluids and their fractions. Plateaux titres, as in the case of lower level inoculation period of this same experiment, were achieved rapidly after inoculation began within eight hours for the regular inoculation level and within eighteen hours for the higher inoculation level.

The efficiency of the model plant in removing f2 coliphage was observed to be reduced to 69.80 per cent, yet the recovery of f2 coliphage from the influent was 47.86 per cent. The proportion of the detected influent f2 coliphage in total mixed liquor titres was reduced to 19.70 per cent and the proportion of virus on the MLS was 15.70 per cent that of the total ML titres, being only slightly higher than during regular level inoculation.

The regression coefficients of MLS titres during regular inoculation (8-46 hours) was +42, and that during high level inoculation (70-94 hours) was +73417, while that after inoculation stoppage was -37369. These regression coefficients indicate high and much much higher uptake of f2 coliphage by the solids, and comparatively moderate decline after inoculation stoppage.

4. Recovery of poliovirus from sewage, effluent, mixed liquor and sludge

Results of experiments on the behaviour of poliovirus in sewage, activated sludge and the activated sludge sewage treatment process are presented below. Although the assay procedure involved direct inoculation of sewage, effluent and resuspended sludge solids onto tissue culture; no adverse effects were observed on the assay or on the plaque formation, size or shape. Contamination was largely absent, as it occurred only in about 2-3 per cent of the assays set up, in which case the sample concerned was assayed again.

A. Recovery of inoculated poliovirus from sewage and effluent

Recovery of inoculated poliovirus from natural and autoclaved sewage and sewage effluent collected from the Guildford sewage treatment plant is summarized in table 4.26. The maximum recovery recorded was 32.36 per cent and it appears from the figures that recovery from autoclaved sewage and effluent was somewhat greater than that from the natural (not autoclaved) samples. There also appears to be a general decline in titres in the later samples after a slight increase. In general, recovery from autoclaved sewage and effluent appeared to be greater than recoveries from natural sewage or effluent.

Table 4.26. Recovery of inoculated poliovirus from autoclaved and natural sewage and effluent (titres $\log_{10}$pfu/ml).

	Sewage		Effluent	
Time	Autoclaved	Natural	Autoclaved	Natural
0 hrs.	5.01 (27.54%)*	4.92 (22.39%)	4.96 (24.55%)	4.81 (17.38%)
7 hrs.	5.06 (30.90%)	4.96 (30.20%)	4.96 (24.55%)	4.82 (17.78%)
24 hrs.	5.08 (32.36%)	5.08 (32.36%)	4.98 (25.70%)	4.80 (16.98%)
48 hrs.	5.01 (27.54%)	4.92 (22.39%)	4.91 (21.88%)	4.76 (15.48%)
	$\bar{x}$ 29.55%	26.83%	24.17%	16.90%

* per cent of calculated initial titre of 5.57 $\log_{10}$pfu/ml.

Table 4.27. Recovery of inoculated poliovirus from autoclaved sewage (titres $\log_{10}$pfu/ml).

Time (hrs)	Titre	Recovery%*
zero	3.85	14.45
17	4.10	25.70
24	3.00	2.04

* calculated from initial calculated titre of 4.69.

Another experiment, represented in table 4.27 , shows recovery of poliovirus from autoclaved sewage. The same trend as above was apparent in the recovery values with a maximum recovery after initial sampling and a decline towards the last sample. Results from both tables 4.26 and 4.27 stress the low and variable recoverability of poliovirus from sewage and effluent.

B. Recovery of poliovirus from mixed liquor and sludge

The recovery of poliovirus from mixed liquor samples was investigated by resuspending the solids fraction in calf serum in PBS and treating the resuspended solids ultrasonically. Results of an experiment investigating the effect of serum concentration and the duration of ultrasonic treatment is presented in table 4.28.

The recorded percentage recoveries in table 4.28 are of replicate samples at zero-time after inoculating the mixed liquor with poliovirus and thorough mixing. The percentage recovery obtained by resuspending the solids in 10 per cent calf serum in PBS with one minute ultrasonic treatment showed the maximum recovery rate of 75.85 per cent. When coupled with the average titre of the mixed liquor fraction it amounted to 81.58 per cent total recovery. In general, the table showed that the effect of ultrasonic treatment increased virus recovery at 1-5 minutes but longer times reduced it. Similarly the effect of serum again giving a peak recovery at about 10 per cent concentration.

Table 4.28. Effect of duration of ultrasonic treatment and calf serum concentration on poliovirus recovery from mixed liquor*. (titres $\log_{10}$pfu/ml).

Serum conc. in PBS	Length of ultrasonic treatment				
	zero minutes	one minute	5 minutes	10 minutes	20 minutes
0	4.14**	4.35	4.28	4.27	4.31
	(19.50%)***	(31.62%)	(26.91%)	(26.30%)	(28.84%)
10%	4.24	4.73	4.68	4.49	4.41
	(24.54%)	(75.85%)	(67.60%)	(43.65%)	(36.31%)
50%	4.35	4.71	4.08	4.48	4.42
	(31.62%)	(72.44%)	(16.98%)	(42.66%)	(37.15%)

Calculated initial titre 4.85 $\log_{10}$pfu/ml whole sample.

* Mixed liquor solids concentration 3760 ppm.

** MLS titres, (MLL titres average 3.58, average of 5 MLL samples assayed).

*** Recovery from MLS fraction alone, MLL constitutes 5.37%.

The 10 per cent serum and one minute ultrasonic treatment gave a solids to liquid titres ratio of approximately 14:1. This distribution of poliovirus between the solids and liquid fractions of mixed liquor seemed to take place very quickly as the samples were - for practical purposes - considered to be zero-time samples.

5. Behaviour of poliovirus in the model plant

Initially, the model plant was run on distilled water at pH 7 and inoculated with poliovirus at a final calculated titre of 4.32 $\log_{10}$pfu/ml. The poliovirus was monitored over 200 hours and results are summarized in table 4.29 and figure 4.18. The average recovery from the "influent reservoir" was 42.66 per cent of the calculated influent values. Titres reached plateaux values at around 24 hours and maintained them until inoculation stoppage when the titres declined to non-detectable after 100 hours in a smooth manner. All samples from the aeration tank and "effluent" were very close in their pattern of variation and in titre values.

The behaviour of poliovirus in the model plant run on activated sludge and autoclaved influent sewage was studied by inoculating the aeration tank with a single inoculation and monitoring poliovirus titres in influent, mixed liquor and effluent. Results of this experiment are presented in table 4.30 and figure 4.19. It may be observed from these results that mixed liquor and effluent titres peaked at 20 minutes and 2 hours respectively. Titres then quickly began to decline with the MLS assuming a higher titre than that of MLL and effluent. The calculated total ML

Table 4.29. Titres (log_{10}pfu/ml) during continuous inoculation of the model plant (run on distilled water) with poliovirus I at 15°C, 10 hours flow through time (also represented in figure 4.18).

Time	Titres (INF)+	(ML)	(EFF)
zero	3.90	-	-
30 min.		2.30	-
2 hrs.		3.00	2.30
22		4.00	4.23
24	3.79	3.68	3.76
45	4.09	3.97	4.12
72		4.19	4.08
77	3.98	4.11	3.95
93	3.93	3.89	3.75
95		4.00	3.96
INOCULATION STOPPED			
100		4.04	3.90
116		3.78	3.45
124		3.70	3.24
150		2.70	2.48
200		2.00	2.00
$\bar{x}$ *	3.95	4.00*	4.01*
s	3.35	3.54	3.61

* from 22-95 hours

+ calculated influent titre 4.32.

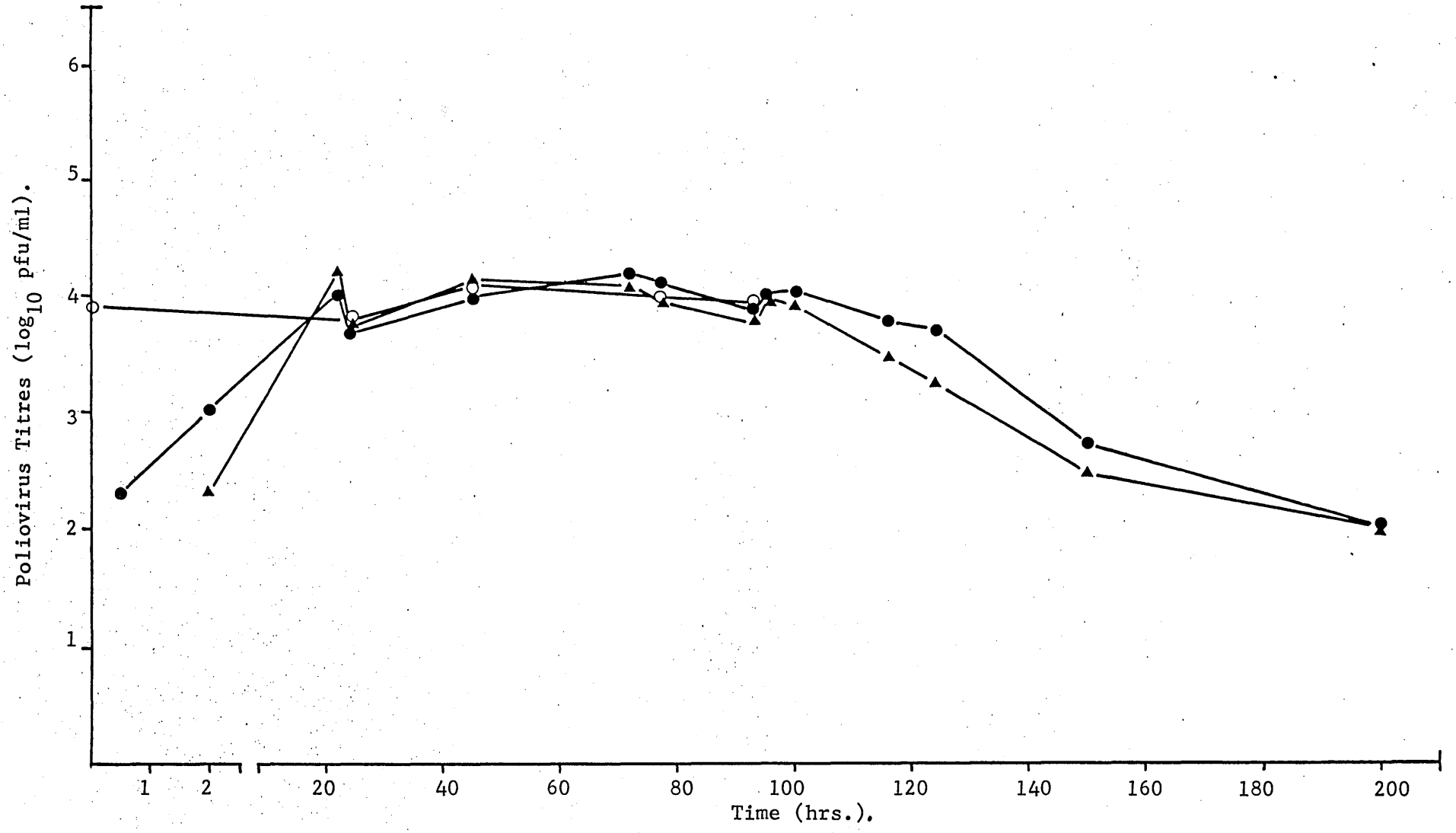


Fig. 4.18. Behaviour of poliovirus in model plant run on distilled water at pH 7, 15°C and 10 hours flow through time ("Influent" o——o, "ML" ●——● and "effluent"▲——▲ titres) (inoculation 0 - 95 hrs).

Table 4.30. Titres ($\log_{10}TCID_{50}$/ml) during single inoculation of the model plant with poliovirus I at 15°C, 10 hours flow through time and 2000 ppm MLSS (also represented in figure 4.19).

Time	Titres		
	MLL	MLS	EFF
0-time	4.72	4.40	-
10 min.	4.50	3.35	3.79
20	4.73	4.27	3.63
40	4.53	3.80	4.05
1 hr.	4.40	4.19	4.05
2 hrs.	3.76	3.85	4.21
4 hrs.	3.10	3.60	3.30
9 hrs.	2.87	3.03	2.73
23 hrs.	2.80	2.95	2.50
48 hrs.	1.96	2.88	-

Expected titre in ML $\frac{10^{8.31}}{10^{3}} = 10^{5.31}$

Recovery $\frac{10^{4.72} + 10^{4.40}}{10^{5.11}} = \frac{10^{4.89}}{10^{5.31}} = 38.02\%$

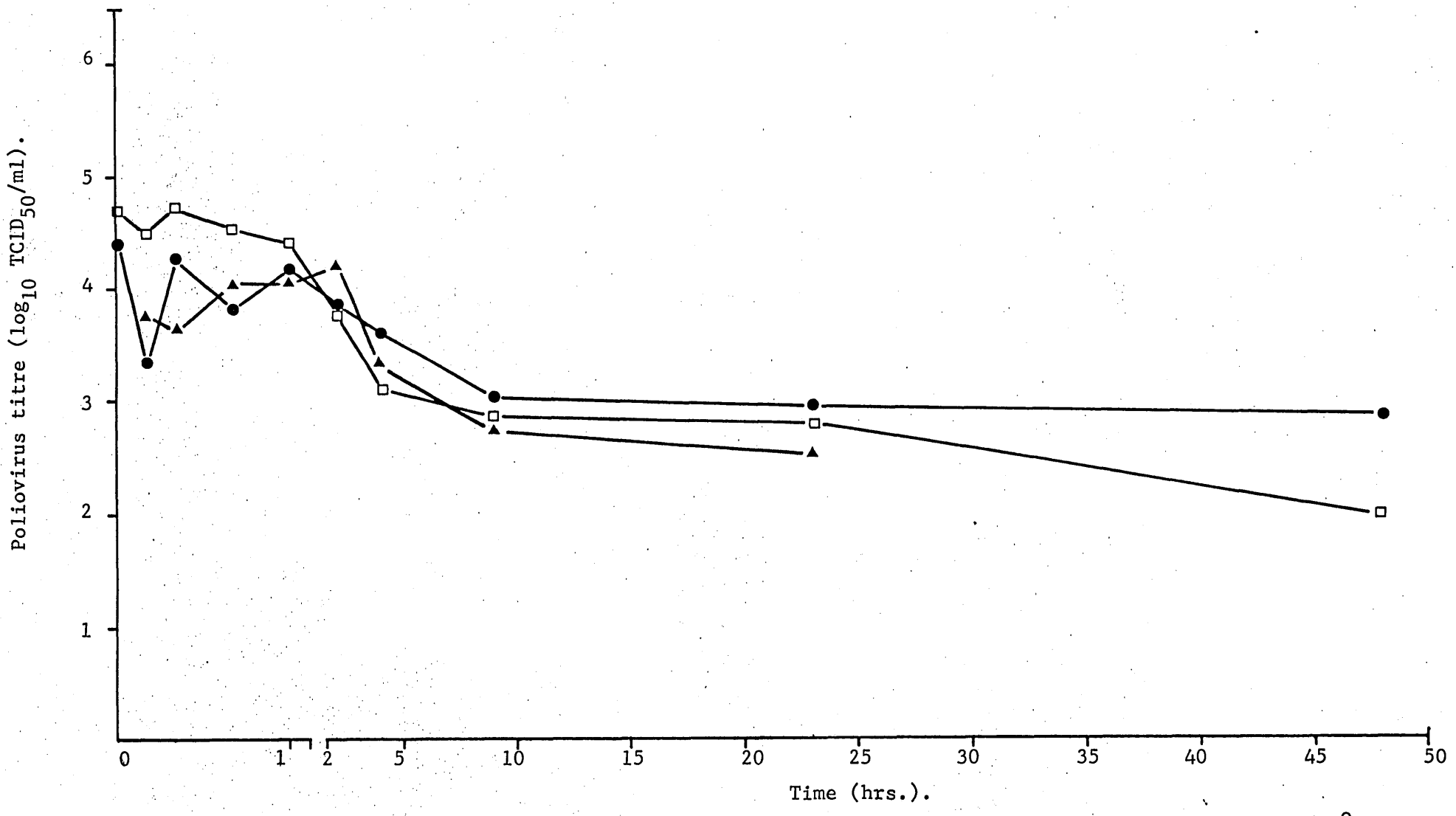


Fig. 4.19. Distribution of poliovirus during single inoculation experiment with autoclaved sewage at 15°C, 10 hour flow through time and 2000 ppm MLSS. (MLS ●——●, MLL □——□ and EFF ▲——▲).

titre, $10^{5.31}TCID_{50/ml}$, and atpeak recovery value was 38.02 per cent. The virus distribution between the MLL and MLS fractions was about equal within the first hour of the experiment but by the 4th hour after inoculation, the greater bulk of the virus, 75.97 per cent, was associated with the MLS fraction. This state of distribution of poliovirus persisted until the other titres become non-detectable and maybe longer.

Another single inoculation experiment done on the model plant at 15°C, 2000 ppm MLSS and 10 hours flow through time is represented in figure 4.20 and table 4.31. As seen also in the previous experiment, the titres of poliovirus in the MLL, MLS and effluent reached peak values very quickly, and were very close to one another during the first two hours. By the fourth hour, the bulk of the virus, 91.82 per cent, was associated with the MLS fraction and persisted there until decline began at 24 hours. Solids titres became non-detectable between 48 and 72 hours. The total recovered virus in this experiment was 20.57 per cent at 10 minutes and 36.62 per cent at 20 minutes.

A single inoculation experiment done at 12.5 hours flow through time, 15°C and 2000 ppm MLSS is represented in table 4.32 and figure 4.21. An additional feature of this experiment is the sampling and assay of return sludge in both its fractions, solids and liquid. Both MLS and RSS titres were similar but peaked at different times. Other features were essentially

Table 4.31. Poliovirus titres ($\log_{10}TCID_{50}$/ml) monitored during single inoculation experiment in model plant at 2000 ppm MLSS, 15°C and 10 hours flow through time (also represented in figure 4.20).

	Titres		
Time	MLS	MLL	EFF
pre-inoc.	-	-	-
10 min.	4.21	4.80	3.41
20	4.92	4.63	4.30
40	4.39	4.63	4.06
1 hr.	4.77	4.30	3.93
2	4.03	3.96	3.80
4	4.35	3.30	3.30
8	4.22	3.51	-
24	4.17	3.30	-
48	2.88	-	-
72	-	-	-

Calculated titre = 5.60

Recovery of virus at 10 min. $\frac{10^{4.91}}{10^{5.60}}$ % = 20.57%

Recovery of virus at 20 min. $\frac{10^{5.16}}{5.60}$ % = 31.61%

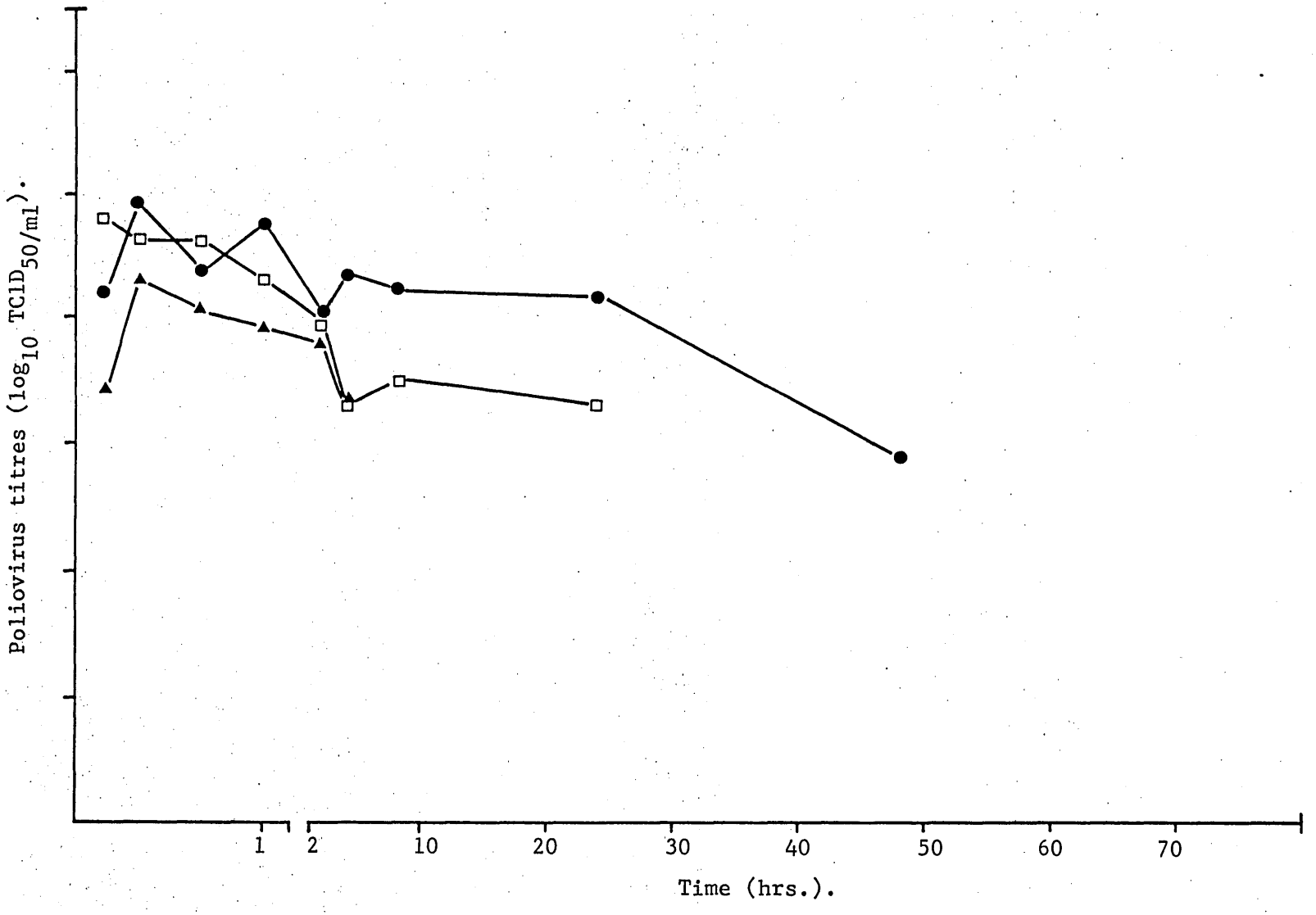


Fig. 4.20. Distribution of poliovirus during single inoculation experiment in model plant at 15°C, 2000 ppm MLSS, and 10 hrs. flow through time (MLS ●——●, MLL □——□ and EFF ▲——▲ titres).

similar to other experiments with poliovirus and these included early disappearance of effluent titres, persistence of solids titres and concentration of the virus on the solids fraction (93.93% associated with MLS at 2 hours). Similar concentration of poliovirus was observed on the RSS and amounted to 89.70 per cent at 2 hours.

The objective of the next experiment was to study the removal of poliovirus by the model plant at 6000 ppm MLSS, 10 hours flow through time and 25°C (figure 4.22 and table 4.33). The pattern of distribution and behaviour of poliovirus I were essentially the same as the previous experiment except for the initial recovery from mixed liquor which amounted here to 5.40 per cent of the calculated titre. The distribution of poliovirus between the MLL and MLS was also affected with 48.85 per cent of the total virus in the MLS at 2 hours. This proportion of the virus persists on the solids long after the virus disappears from the effluent.

The next experiment was aimed at studying the performance of the model plant under prolonged continuous inoculation at 2000 ppm MLSS, 15°C and 10 hours flow through time (Figure 4.23 and table 4.34). During the first few hours the virus titres steadily increased to plateaux values at about eight hours when it became apparent that the bulk of the virus (about 85%) was associated with the MLS. Effluent titres represented only a small fraction, 0.4 per cent, of the total mixed liquor titres.

Table 4.32. Poliovirus titres ($\log_{10}$ $TCID_{50}$/ml) monitored during single inoculation of the model plant at 2000 ppm MLSS, 15°C and 12.5 hours flow through time (also represented in figure 4.21).

	Titres				
Time	MLL	MLS	RSL	RSS	EFF
pre-inoc.	-	-	-	-	-
zero	4.63	4.13	-	-	-
20 min.	3.73	4.40	-	4.20	3.65
1 hr.	3.71	4.41	3.42	4.06	2.50
2 hr.	3.06	4.25	3.58	4.52	2.13
5 hr.	3.24	4.10	3.50	4.20	2.50
20 hr.	2.89	4.30	3.17	4.60	2.37
25 hr.	2.50	4.23	2.71	4.05	-
30 hr.	2.30	3.67	3.10	4.00	-
45 hr.	-	3.69	2.49	3.60	-
53 hr.	-	3.10	3.30	3.97	-

Calculated titre = $\frac{10^{8.63}}{10^{3}} = 10^{5.63}$

Recovery zero time $\frac{10^{4.75}}{10^{5.63}} = 13.16\%$

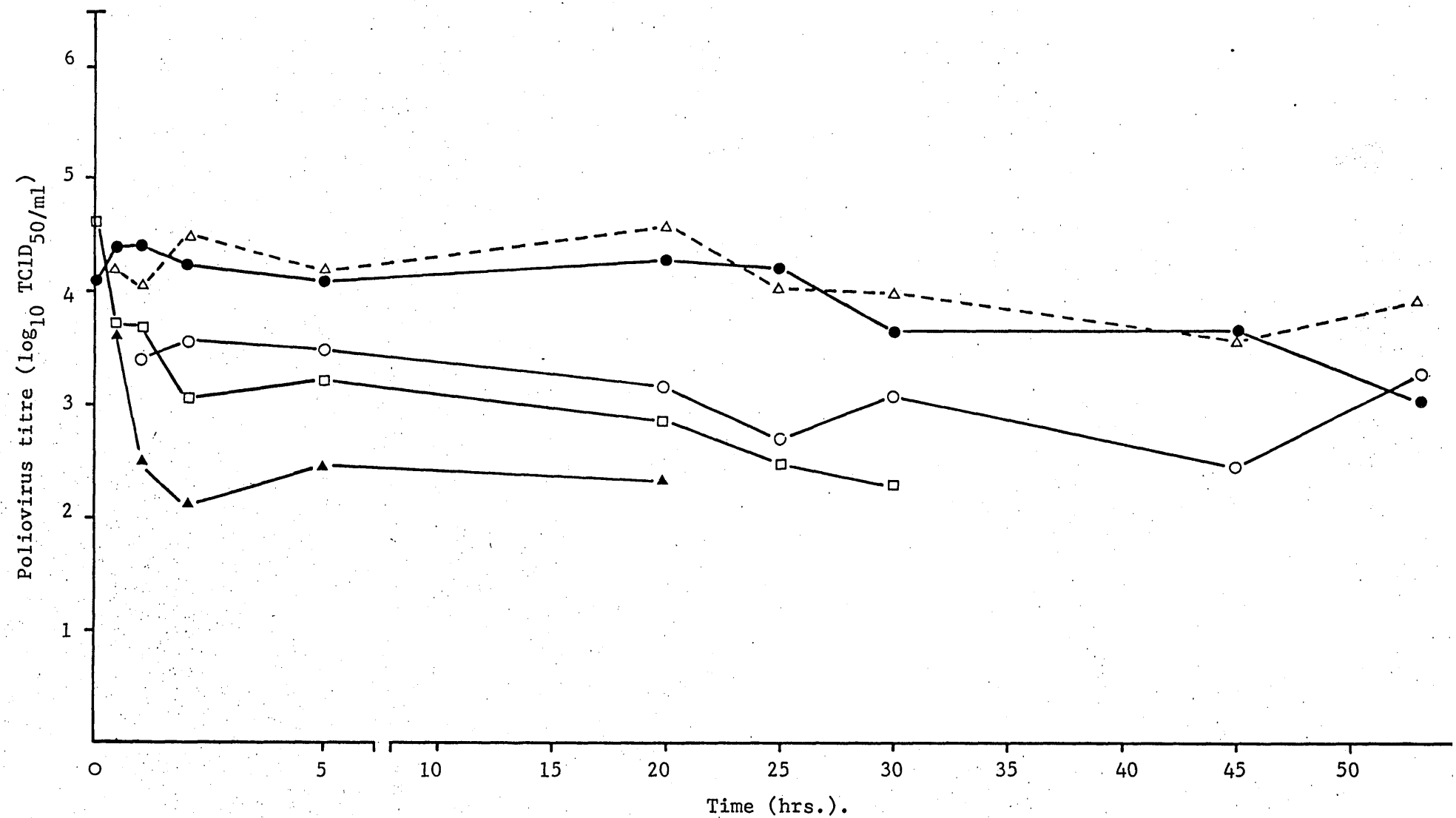


Fig. 4.21. Distribution of poliovirus during single inoculation experiment in model plant at 15°C, 2000 ppm MLSS and 12.5 hrs. flow through time (RSS △---△, MLS ●——●, RSL ○——○, MLL □——□ and EFF ▲——▲ titres).

Table 4.33. Poliovirus titres ($\log_{10}$pfu/ml) monitored during single inoculation of the model plant at 6000 ppm MLSS, 10 hours flow through time and 25°C (also represented in figure 4.22).

Time	MLL	MLS	RSL	RSS	EFF
pre-inoc.	-	-	-	-	-
0-time	4.00	4.06	2.30	2.70	1.82
15 min.	3.95	4.15	3.65	3.58	-
30 min.	4.05	4.05	3.77	3.98	2.30
1 hr.	3.68	3.52	4.04	3.55	2.77
2 hr.	3.32	3.30	3.81	3.23	2.57
4 hr.	-	2.75	3.06	-	1.96
9 hr.	-	2.37	1.20	2.25	-
24 hr.	-	2.21	0.8	1.80	-
33 hr.	1.3	1.30	0.7	2.30	-
48 hr.	1.00	1.30	-	1.10	-
57 hr.	-	1.10	-	1.40	-
72 hr.	-	1.00	-	-	-
96 hr.	-	-	-	1.70	-

Inoculum $10^{8.60}$, calculated titre in ML $\frac{10^{8.60}}{10^{3}} = 10^{5.60}$

Recovery at 0-time $\frac{10^{4.00} + 10^{4.06}}{10^{5.60}}$ % = 5.40%

Recovery at 15 min. $\frac{10^{13.95} + 10^{4.15}}{10^{5.60}}$ % = 5.79%

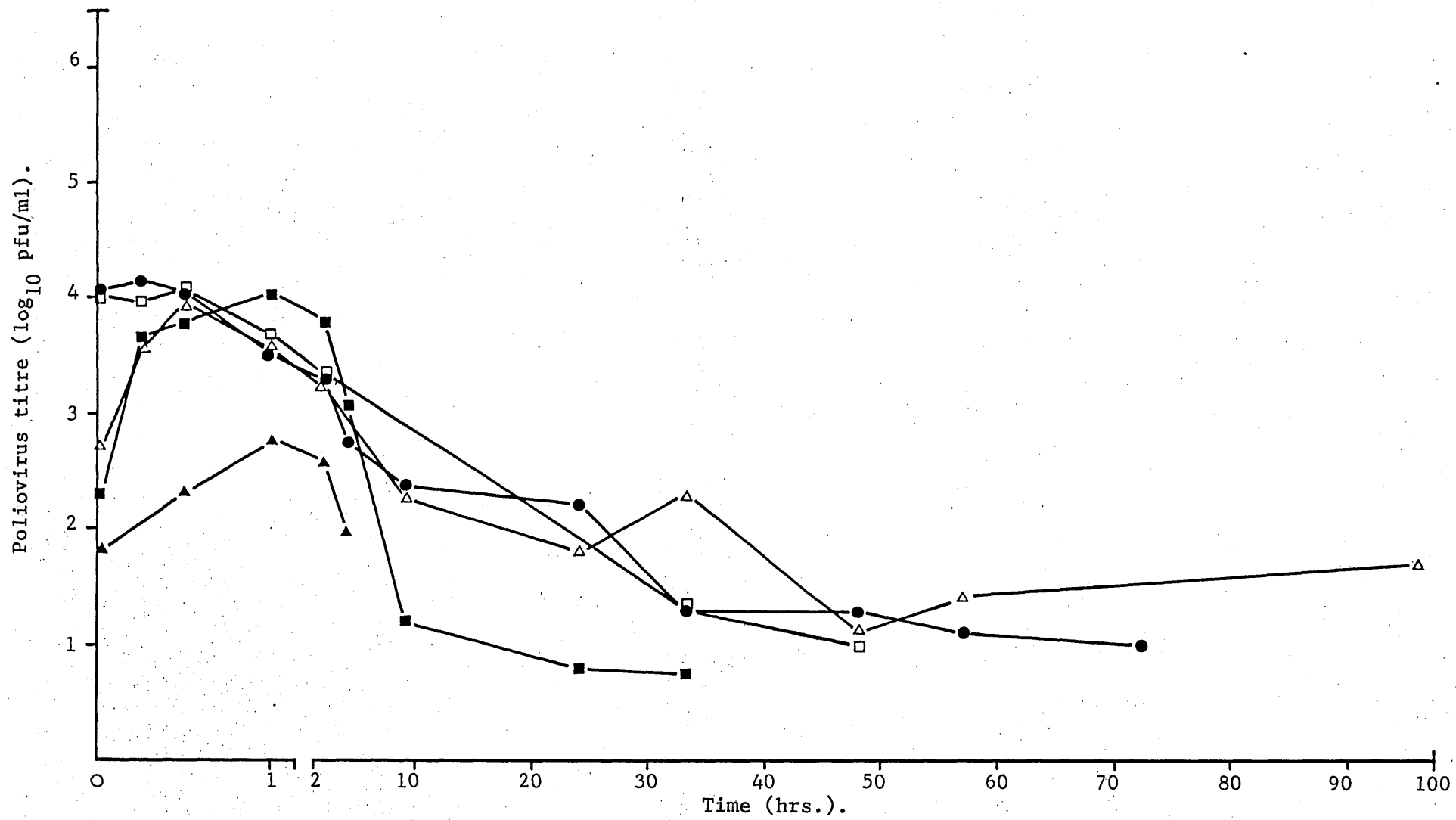


Fig. 4.22. Distributionof poliovirus during single inoculation experiment in model plant at 6000 ppm MLSS, 10 h flow through time and 25°C (RSS △——△, MLS ●——●, RSL■——■, MLL □——□ and EFF ▲——▲ titres).

As soon as inoculation was stopped, effluent titres fell sharply to non-detectable values. Mixed liquor liquid and solids fractions fell steadily but less sharply than the effluent. The regression coefficient of MLS titres between 8 hours and 16 days was +8.39, while that after inoculation stoppage was -25.48 indicating that the virus was released at a greater rate than it was taken up.

The behaviour of poliovirus and f2 coliphage under the various operating and loading conditions of the model plant is summarized in table 4.35. It can be seen from the table that the efficiency of the model plant in removing f2 coliphage ranged from 65.52 - 95.53 per cent over the range of operating conditions, with the removal during high virus loading near the lower end of that range at 69.80 per cent. The removal of poliovirus was, in general, much higher at 99.73 per cent. The distribution of f2 in the MLS and MLL fractions of mixed liquor ranged between 4.79:95.21 and 26.92:73.08 , while the distribution of poliovirus I exhibited a reversed trend being 84.86:15.14 in the MLS and MLL respectively.

The decline of f2 coliphage after inoculation stoppage in most experiments showed lesser decline than uptake by MLS, except in the case of the experiment in figure 4.15 (at $25^{o}C$) which showed an appreciable increase in the rate of decline as evidenced by the regression coefficient values.

Table 4.34. Poliovirus titres ($\log_{10}$ pfu/ml) monitored during prolonged, continuous inoculation experiment in the model plant at 2000 ppm MLSS, 15°C, 10 hours flow through time, (also represented in figure 4.23).

	Titres			
Time	INF	MLS	MLL	EFF
pre-inoc.	-	-	-	-
zero	4.70	-	-	-
5 min.		-	1.78	-
10		0.90	2.30	-
20		1.90	2.26	-
40		1.94	2.75	-
1 hr.		3.05	2.45	1.59
2		2.65	3.00	1.70
5		3.20	3.39	2.45
8		3.46	3.26	1.91
23	4.76	3.46	2.28	1.90
33	4.04	3.41	2.78	1.91
48	4.04	3.60	2.66	1.91
72		3.46	2.48	
96	4.20			2.00
120	4.68	3.45	3.12	
129	4.40	3.78	3.34	2.00
144	4.30	3.50	2.48	2.03
145	4.82			
153	4.70	3.96	2.58	2.00
168	4.45			
177	4.30		1.91	1.65
192	4.07	3.51	2.80	1.72
193	4.87	3.44	2.42	2.48
215	4.55	3.16	2.75	2.30
216	4.60			
223	4.50	3.72	3.17	2.07
239		4.07	2.74	
240	4.87			
263		3.64	3.23	1.79
264	4.70			
287	4.00	3.66	2.30	1.60
288	4.86			
296	4.19	4.10	2.35	2.00
311	4.14	3.83	3.00	2.00
312	4.86			
335	4.12	3.47	2.91	1.20
336	4.62			
359	4.12	3.44	2.88	2.00
360	4.81			
383	4.20			
INOCULATION STOPPED				

Table 4.34. (continued)

Time		INF	MLS	MLL	EFF
384		-	3.60	3.19	-
407			3.10	2.07	-
408		-			-
432			2.70	2.00	-
456		-		2.00	-
480					-
504			2.30	1.30	-
	$\bar{x}$*	4.56**	3.67	2.88	2.00
	s	4.36	3.46	2.78	1.79
			Total ML $\bar{x}$ =	3.74	

Calculated daily titre in Inf

$\frac{10^{8.63}}{2500} = 10^{5.23}$ recovery from INF 21.38%

$\frac{EFF}{INF}$ % = 0.27% $\frac{ML}{INF}$ % = 15.14 $\frac{MLS}{ML}$ % = 85.11

* from 8 hrs. to 16 days

** from 0 time to 16 days.

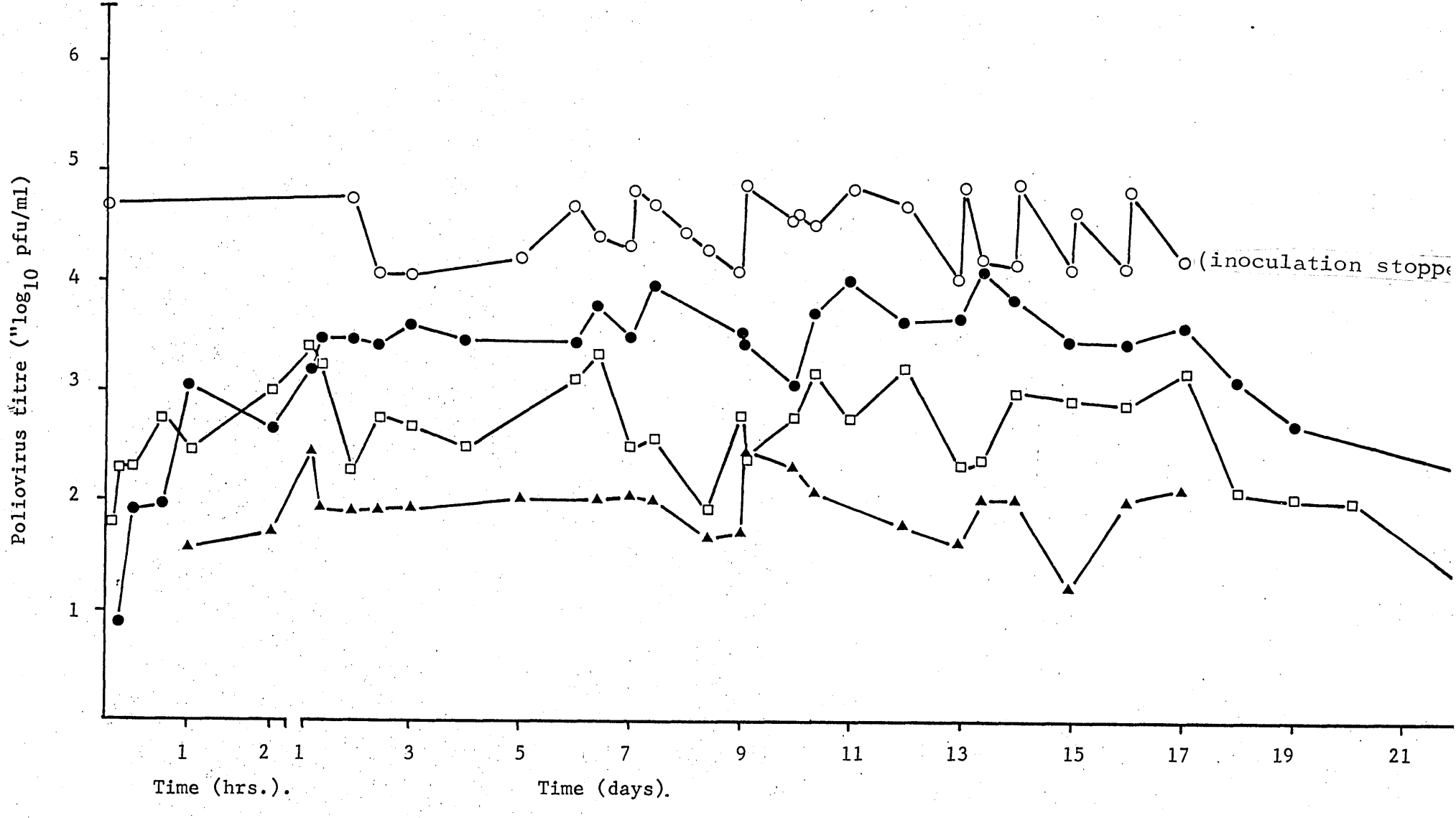


Fig. 4.23. Distribution of poliovirus during continuous inoculation experiment in model plant at 15°C, 2000 ppm MLSS and 10 hours flow through time (INFO○——○, MLS●——●, MLL□——□ and EFF▲——▲ titres).

Table 4.35. The influence of functional conditions of the model plant and virus load on the behaviour of f2 coliphage, and poliovirus I under same conditions.

Plant Conditions	MLSS (ppm), flow through time (h), and temperature (°C)	% distribution of virus MLS:MLL	% recovery of virus from influent	% virus in effluent $\frac{EFF}{INF}$ %	%removal of virus across plant	MLS titres regression co-efficient during, and after inoculation	Figur Refer Numbe
Different Flow Rates	2000, 10, 15*	21.88 : 78.12	56.23	11.22	88.78	+ 299, - 121	4.1(
	2000, 5.4, 15*	15.85 : 84.15	32.36	10.47	89.53	+ 65, - 45	4.1
	4000, 10, 15*	4.79 : 95.21	89.31	35.48	64.52	+ 485, - 328	4.1
	4000, 5.4, 15*	15.85 : 84.15	69.18	23.44	76.56	+ 113, - 126	4.1
Different MLSS	2000, 10, 15	21.88 : 78.12	56.23	11.22	88.78	+ 299, - 121	4.1(
	4000, 10, 15	4.79 : 95.21	89.31	35.48	64.52	+ 485, - 328	4.1
	6000, 10, 15*	26.92 : 73.08	51.29	13.80	86.20	+ 665, - 512	4.1
Different Temperatures	4000, 10, 5*	7.94 : 92.06	45.71	12.59	87.41	+ 21, - 14	4.1
	4000, 10, 15	4.79 : 95.21	89.31	35.48	64.52	+ 485, - 328	4.1
	4000, 10, 25*	23.99 : 76.01	54.95	15.85	84.15	+ 253, - 622	4.1
Different Virus Loads	2000, 10, 15 (Low)*	36.31 : 63.69	59.98	4.47	95.53	- 45, - 37	4.9
	2000, 10, 15 (Med.)*	12.02 : 87.98	8.71	13.49	86.51	+ 42, —	4.1
	2000, 10, 15 (High)*	15.70 : 84.30	47.86	30.20	69.80	+ 73417, -37369	4.1
Different Viruses	2000, 10, 15 (f2)	21.88 : 78.12	56.23	11.22	88.78	+ 299, - 121	4.1(
	2000, 10, 15 (polio)	84.86 : 15.14	21.38	00.27	99.73	+ 8, - 25	4.2
Average for experiments with f2		18.13 : 81.87	$\bar{x}$ =51.56 s =21.32	$\bar{x}$=17.10 s= 9.62	$\bar{x}$=84.35 s= 8.96		

* Results of indicated experiments are averaged.

Table 4.36. Summary of single inoculation experiments with Poliovirus I in the model plant

Experimental conditions MLSS, flow through time, Temp.	MLS:MLL ratio	Persistence of MLS titres	ML peak titre recovery %	Figure reference number
2000, 10, 15 (autoclaved sewage).	75.97:24.03 (at 4th hour)	48 + hours	38.02%	4.19
2000, 10, 15	91.82:8.18 (at 4th hour)	48 + hours	31.61%	4.20
2000, 12.5, 15	93.93:6.07 (at 2nd hour) (RSS 89.70:10.30 at 2nd hour)	53 + hours	13.16%	4.21
6000, 10, 25	61.31:38.69 (RSS 45.98:54.02 at 15 minutes)	72 + hours (lower titres)	5.79%	4.22

Shortly after inoculation was stopped, effluent titres fell sharply to non-detectable values. Mixed liquor liquid and solids fractions fell steadily but less sharply than the effluent. The regression coefficient of MLS titres between 8 hours and 16 days was + 8.39, while that after inoculation stoppage was - 25.48 indicating that the virus was released or lost at a greater rate than it was taken up.

The behaviour of poliovirus and f2 coliphage under the various operating and loading conditions of the model plant is summarized in table 4.35. It can be seen from the table that the efficiency of the model plant in removing f2 coliphage ranged from 65.52 - 95.53 per cent over the range of operating conditions, with the removal during high virus loading near the lower end of that range at 69.80 per cent. The removal of poliovirus was, in general, much higher at 99.73 per cent. The distribution of f2 in the MLS and MLL fractions of mixed liquor ranged between 4.79:95.21 and 26.92:73.08, while the distribution of poliovirus I exhibited a reversed trend being 84.86:15.14 in the MLS and MLL respectively.

The decline of f2 coliphage after inoculation stoppage in most experiments showed lesser decline than uptake by MLS, except in the case of the experiment in figure 4.15 (at $25^{o}C$) which showed an appreciable increase in the rate of decline as evidenced by the regression coefficient values.

Poliovirus also showed the same trend in greater decline after inoculation stoppage, although the difference between the regression coefficients of MLS titres during inoculation and after inoculation stoppage was small. The removal of poliovirus across the plant, however, registered the highest values when compared to rates of removal of f2 across the plant.

The persistence of poliovirus in the model plant after single inoculation experiments was prolonged as long as monitoring was carried out (table 4.36). Persistence was observed in these experiments for as long as 72 hours but not shorter than 48 hours after inoculation (figures 4.19, 4.20, 4.21 and 4.22). The ratios of MLS:MLL titres varied from 61.31:38.69 at 25^{o}C to 93.93 : 6.07 at 15^{o}C.

V. DISCUSSION

The behaviour of f2 coliphage and poliovirus in the model plant confirmed the general observation that sewage treatment by activated sludge reduces virus titres (Berg, 1973a; 1973b; Grabow, 1968; Kollins, 1966; Schwartzbrod et al., 1973). However it is clear that such removal of viruses by the activated sludge process is complex and reflects on the nature of each of the viruses concerned and on the nature of the activated sludge floc surfaces.

Activated sludge floc surfaces are covered with slimes of polyesters and polysaccharides which are essentially neutral except for one component of the polysaccharides, glucuronic acid, which is the main ionogenic moeity (Forster, 1971; Steiner, McLaren and Forster, 1976; Wallen and Davis, 1972). Viruses in general, have a nucleic acid core with a protein coat and some are enveloped, thus the first encounter between a virus particle and activated sludge is likely to be between the virus coat or envelope with the polysaccharide mucilage. Poliovirus and f2 coliphage, which are both picornaviruses, are similar enough to one another in many respects (Table 5.1) except for the complete lack of histidine from f2 protein (Olivieri et al., 1975; Zinder, 1965). The charge on such viruses at pH around neutral is negative (Grabow, 1968; Tenney and Stumm, 1965), but of course, there are degrees of negativity depending on the particular configurational

Table 5.1. Characteristics of poliovirus and f2 coliphage. (modified from Olivieri et al., 1975).

Criteria	Poliovirus	f2 coliphage
Nucleic acid	RNA	RNA
Capsid symmetry	cubic	cubic
Envelope	none	none
Capsid diametre	27-30 nm	20-25 nm
Virion wt x 10^6 daltons	5.5 - 6.8	3.0
Nature of nucleic acid		
Number of strands	1	1
Shape	linear	linear
Molecular wt x 10^6 daltons	2.5 - 2.7	0.7 - 1.2
Nucleotides	6000	3300
Nature of protein		
Number of different polypeptides	4	2
Amino acids not found	-	Histidine
pH stability	3-10	3-10
Stability ($50^{\circ}C$, 30 min)	Cationic stabilized	Stable
Biological properties		
Host	Primate cell	Male *E. coli*
Adsorption	cell surface	F pili-sides
Entrance into cell	Virion	RNA
Release	Rupture of surface vacuoles	Lysis

presentation of the charged residues in the protein moeity of the virus. Thus it may be argued, that the behaviour of virus in the activated sludge process especially with respect to the association with activated sludge solids would be dictated by the nature of the protein coat, its iso-electric point, its structure, its configuration as well as the abundancies or rarities of neutral, acidic, basic, hydrophilic or hydrophobic amino acids in it. Furthermore, since the overall character of the virus could well be influenced by the structure and configuration of the nucleic acid and its interaction with the protein coat, it is possible, then, that no two viruses would behave alike in activated sludge. Such differences in the behaviour, were, indeed clear from the results. Forinstance, the association of poliovirus with the mixed liquor solids was immediate and reached about 85 per cent, whereas, a much lower per centage - about 18 per cent of f2 associated with the solids. A corollary of the increased association of poliovirus with solids was the high removal of virus -at least 99 per cent- across the plant because the solids were retained in the plant as dictated by the process design. The gradual and lower degree of association between f2 coliphage and mixed liquor solids manifested itself in lower rates of removal, about 84 per cent, which incidentally agrees with the rates reported by Safferman and Morris (1976), contradicting those given by Naparstek et al. (1976) who reported only 11.1 per cent removal, but the dubious validity of the latter authors' results was raised in the review of the literature towards the beginning of this thesis.

The distribution of f2 coliphage and poliovirus between the mixed liquor solids and liquid fractions had interesting implications. On average, the ratio for experiments with medium virus loading f2 coliphage was about 16:84 with a range from about 5-27 per cent for the solids, and for the liquid fraction consequently 95-73 per cent. For poliovirus, the distribution during continuous inoculation was in the ratio of about 85:15 for the solids and liquid fractions respectively.

Since the distribution of virus between the solids and liquid phases of mixed liquor seemed to follow a particular pattern for each virus, it implied a state of equilibrium which presumably depended upon several factors. These factors included the concentrations of the reactants (i.e. virus titre and solids concentration), temperature, activity of the reactants, the rate of dilution and the nature of the reactant(s). One of the reactions which could take place between viruses and activated sludge was adsorption of the virus onto the sludge surfaces and such adsorption may be one of two types - physical or chemical. Chemical adsorption probably does not apply to the relationship between virus and activated sludge because this is an essentially irreversible phenomenon accompanied by a change in the nature of the reactants, which could result with inactivation of the bulk of the virus. Physical adsorption, on the other hand is reversible and not accompanied by a permanent change in the nature of the reactants (Marshall, 1976).

Evidence in favour of physical adsorption was fairly good. Forinstance, the recovery of increasing amounts of f2 coliphage in the repeatedly extracted samples of activated sludge on the one hand, and the presence of elevated titres of virus in the mixed liquor liquid after the inoculation was stopped indicated the reversibility of adsorption. Furthermore, the very fact that virus was detected in the mixed liquor solids implied that chemical adsorption had not occurred because it would be likely to result in inactivation of the virus. Physical adsorption would also increase as the concentration of the reactants increased, and it was shown that the uptake of f2 coliphage increased with increased suspended solids concentration in the plant, also similar striking increases in adsorption were observed in the so-called dual-level inoculation experiment.

The physical adsorption of virus onto activated sludge is not surprising since viruses presumably have an innate tendency to adsorb to surfaces thus reflecting their need to parasitise host cells. This innate tendency, was apparently overlooked by Clarke et al. (1961) as a possible mechanism for the removal of virus during activated sludge treatment otherwise they would surely have not only monitored the mixed liquor liquid fraction, but the solids fraction as well. Kelly, Sanderson and Neidl (1961), also did not monitor the mixed liquor solids and, furthermore claimed that all the "missing" virus had been inactivated. Malina et al. (1974), Malina et al. (1975) and Ranganathan, Malina and Sagik (1975) considered that all the virus removed across activated sludge sewage

treatment was "inactivated", basing their conclusion on the statement that activated sludge "constantly synthesizes new microbial films during oxidation of organic substrates" and that "the biological solids also exhibit a capacity for permanently inactivating adsorbed virus with subsequent release of degraded viral components." It is regretted that their statement was not based on any observation or experimental finding. The conclusions by these workers that "sludge" inactivated virus possibly reflected their lack of a sensitive method for recovering infective virus from sludge solids - although it is more likely that they did not consider it as a likely source of virus.

The hypothesis that virus is simply removed by adsorption fits both the behaviour of f2 and poliovirus I in the model plant and the interaction of both viruses with activated sludge, which, as a process of dynamic equilibrium, was affected by the operating conditions of the plant. Variations in the operating conditions did not seem, on the basis of recovered titres of virus, to affect the performance of the plant, but an analysis of the uptake of f2 by the regression coefficient (slope) of the plateaux titres of mixed liquor solids which occurred during the continuous inoculation experiments showed positive values. Forinstance, lowering the flow through time from 10 to 5.4 hours in the plant lowered the regression coefficient from +299 to +65 and from +485 to +113 while increases the mixed liquor suspended solids concentration from 2000 to 4000 and 6000 ppm resulted in an almost proportionate increase in the regression coefficents from +299 to +485 to +665. Temperature

exerted a noticeable effect on f2 coliphage uptake with an optimum at 15°C (regression coefficient +485) while the uptake was lower at 5°C and moderate at 25°C (regression coefficients +21 and +253 respectively). Highly increased uptake was observed by increasing the inoculum resulting in a regression coefficient of +73417.

Applying the same analysis to mixed liquor solids titres after inoculation had stopped revealed negative regression coefficients for all experiments, thus indicating that the trend for association was reversed. In all experiments but two the absolute value of the regression coefficient in the decline phase was smaller than that of the uptake which clearly indicated persistence of a proportion of the solids associated virus. Those exceptions (higher absolute values of the regression coefficients of the decline phase) may be due to dilution in one case and to thermal death coupled with increased "physical and kinetic activity" of the virus in the other. Thermal death could also have been responsible for the decreased uptake at 25°C, while lowered physical activity of the virus and physical and biological activity of the activated sludge at 5°C could have resulted with lowered absolute value for the regression coefficient. Thus, the association of f2 coliphage with mixed liquor suspended solids seem to be favoured by greater opportunity for and longer duration of contact with the solids. This is clear from the increased uptake with higher solids concentrations and lower flow through times. The effect of

temperature seems to function along the same principle in such a way that lower temperature decreased chances of contact due to decreased kinetic (and probably electric) activities of the virus and the mixed liquor solids. The reverse of this effect seemed to operate at higher temperatures, with an optimum at 15°C and decline at 25°C that may have been due to thermal death.

The previous analysis of the ways that association of virus with solids is achieved by a process of dynamic equilibration necessitated the introduction of the concept of a constant of equilibrium. Such a constant, however, could not be calculated at this stage due to lack of accurate information about the nature of activated sludge, the absolute virus titres associated with solids, and the factors which influence decline in virus titres including inactivation for the duration of the experiments .

It is the persistence of the solids titres for long periods running into 90 hours after inoculation was stopped that suggested the formation of a somewhat stable virus-solids complex. The solids probably protect the virus to a certain extent and, of course, keep it in the model plant because the sludge is continuously recycled. Furthermore, it is probable that the establishment of a kinetic and dynamic equilibrium of distribution of virus between the mixed liquor liquid and solids would necessarily result in the presence of virus in the mixed liquor liquid and effluent after inoculation had stopped. It is not improbable that the activity of activated sludge with the constant renewal and evolution of its microbial surfaces

played an important role in the establishment of this equilibrium and, from another point of view, in the ultimate inactivation of the residual virus on the mixed liquor solids. It was with similar speculative reasoning that Malina et al. (1974 and 1975) and Ranganathan et al. (1975) referred to all the virus removed during activated sludge treatment as "inactivated" virus; whether this was justifiable or not is questionable since sufficient proof was not provided for or against inactivation. It is for this reason that the word removal is used, advisably, in this work.

The regression coefficients are based on the mixed liquor solids titres, but these values are only as accurate as the values of the titres themselves and it is important here to note that the total recoveries of f2 coliphage from the newly inoculated mixed liquor ranged between as little as about 54 per cent to no more that about 77 per cent. This partial recovery of f2 coliphage from inoculated mixed liquor, although probably adequate for the study of the behaviour and distribution of f2 coliphage in activated sludge treatment, implies a certain loss of virus titre due to such undetermined factors as aggregation, adsorption to suspended and colloidal matter, and inactivation (Bitton, 1975; Bitton and Mitchell, 1974; Gerba and Schaiberger, 1975; Lund, 1971; Lund and Ronne, 1973). Of these factors, only aggregation and adsorption to solids satisfy the conditions of a dynamic equilibrium, and thus partly explain the partial loss in titre. Furthermore, since the mixed liquor solids were assayed by direct inoculation each solids particle could have had more than one plaque forming unit

and could be read as one plaque. The direct inoculation of solids (Buras, 1974; Lund, 1971), however, coupled with the resuspension of inoculated solids in 10 per cent calf serum during ultrasonic treatment improved virus recoveries satisfactorily. It is worth noting that recent reports indicate that the highest recoveries of solids - associated viruses occurred when the solids were resuspended in 10 per cent protein (beef extract or calf serum) with ultrasonic treatment (Sattar and Westwood, 1976; Subrahmanyan, 1977; Wellings, Lewis and Mountain, 1976). In fact, the method of resuspension in 10 per cent serum at pH's around 7.2-7.4 was reported to give maximum recoveries which matched recoveries at elevated pH's with lower (3 per cent) serum concentrations (Sattar and Westwood, 1976; Subrahmanyan, 1977). The removal of f2 coliphage across the plant is, therefore, thought to be a combination of the factors of adsorption, aggregation, and almost definitely with a certain degree of inactivation.

The validity of the removal and distribution results of f2 and poliovirus is verified by the standardization experiments. The observation that f2 associates to a lesser extent with mixed liquor (and sewage) solids supports its higher initial recoveries from sewage (67-117%) than poliovirus (14-32%). Higher recoveries were observed, however, for both viruses a few hours after inoculation than at zero time; and these recoveries were significantly higher for f2. This temporary loss in titre could be due to an initial aggregation or over-association with the sewage solids due to the sudden exposure of the virus to the sewage environment. The quilibrium distribution of virus in the sewage probably followed later, as evidenced by the lack of the same effect with filtered sewage.

The recovery of both viruses from mixed liquor solids was also highest with the method (resuspension in 10% serum with ultrasonic treatment) than the other procedures tested. The validity of the comparison of the behaviours of the two viruses rested in the employment of the same method of treatment of samples prior to assay.

The marked differences in the behaviour of f2 coliphage and poliovirus in activated sludge treatment suggest that f2 is not a suitable indicator for viral pollution of waters. If f2 does not behave like polio, it is not likely to behave like, say, the rota-viruses or hepatitis A agent. Ranganathan et al. (1974) had also dismissed f2 coliphage as a suitable index on basis of its different survival patterns in laboratory scale activated sludge plant.

If any generalizations are to be made on the survival patterns of different viruses and their behaviour in sewage treatment and in the water environment, they can only be made after adequate understanding of their many properties, not only biological but, probably also the biochemical. Forinstance, the nature of the protein coat, including the distribution and nature of the amino acids and especially their interaction. Studies on the removal or survival of viruses by extracts or pure cultures of one or more of the various organisms from activated sludge may be very informative. The interaction of different viruses, say f2 and poliovirus inoculated together into the plant, could shed more light on the effect of one virus on the behaviour of the other when both would be present in the system. In any case, it seems that there is no alternative at

present but to study the fate or behaviour of each virus individually or in conjunction with other viruses in activated sludge treatment. The two most urgent viruses to be studied would be those of infectious gastroenteritis or rotaviruses and hepatitis A which are excreted in large numbers in the faeces of clinically ill people or animals and thus may, not surprisingly, be transmitted in sewage and other waters.

VI. REFERENCES

Appleton, H. and Pereira, M. (1977). A possible virus aetiology in outbreaks of food-poisoning from cockles. The Lancet, April 9, 780-781.

Baghdasaryan, G. A. and Kazantzeva, V. A. (1965). A study of sewage decontamination from the cytopathogenic enteroviruses in sewage treatment plants. Gig. Sanit. 37(7), 14-17.

Baker, M. N. (1948). The Quest for Pure Water. Amer. Wat. Wks. Assoc. Inc., New York.

Bancroft, P. M., Engelhard, W. E. and Evans, C. A. (1957). Poliomyelitis in Huskerville (Lincoln) Nebraska. J. Amer. Med. Assoc. 164, 836-839.

Berg, G. (1971). Integrated approach to the problem of viruses in water. J. San. Eng. 97 (SA 6), 867-882.

Berg, G. (1973a). Removal of viruses from sewage, effluents and waters. I. A review. Bull. WHO 49, 451-460.

Berg, G. (1973b). Removal of viruses from sewage, effluents and waters. 2. Present and future trends. Bull. WHO, 49, 461-469.

Berger, B. B. (Chairman, Committee on Environmental Quality Management of the Sanitary Engineering Division). (1970). Engineering evaluation of virus hazard in water. J. San. Eng. Div., ASCE. 96 (SA 1), 111-150.

Bitton, G. (1975). Adsorption of viruses onto surfaces in soil and water. Water Research 9, 473-484.

Bitton, G. and Mitchell, R. (1974). Effect of colloids on the survival of bacteriophages in sea water. Water Research 8, 227-229.

Bradley, D. J. (1977). Health aspects of water supplied in tropical countries (p.3-17). In Water, Wastes and Health in Hot Climates (Eds. Feachem, R., McGarry, M. and Mara, D.). John Wiley and Sons, London.

British Drug Houses, The. (undated a). Ammonia determination by Nessleriser methods. Publication number 586 A/1.0/0271.

British Drug Houses, The. (undated b). Nitrates (phenoldisulphonic acid method) determination by Nessleriser methods. Publication number 688A/1.0/1171.

Buras, N. (1974). Recovery of viruses from waste-water and effluent by the direct inoculation method. Water Research 8, 19-22.

Buras, N. and Kott, Y. (1966). Bacteriophage as an indicator for the estimation of water pollution. Israel J. Med. Sci. 2, 660-661.

Bush, A. F. and Isherwood, J. D. (1966). Virus removal in sewage treatment. J. San. Eng. Div., ASCE, 92 (SA 1), 99-107.

Butterfield, C. F. (1935). Studies of sewage purification. II. A zooglea-forming bacterium isolated from activated sludge. Publ. Hlth. Rep., Wash. 50, 671-684.

Cannon, R. E. (1977). Personal communication.

Carlson, H. J., Ridenour, G. M. and McKhann, C. F. Jr. (1943). Effect of the activated sludge process of sewage treatment on poliomyelitis virus. Amer. J. Publ. Hlth. 33, 1083-1087.

Carstens, E. M. J., Coetzee, O. J., Malherbe, H. H. and Harwin, R.M. (1965). Bacteriophage of Serratia marcescens as an index of human virus survival during sewage purification. CSIR (S. Afr. Counc. Sci. Indust. Res.) Research Report 241, 1-17.

Chang, S. L. (1968). Waterborne viral infections and their prevention. Bull. WHO 38, 401-414.

Christie, A. E. (1966). Virus reduction in the waste stabilization pond. Ontario Water Resources Commission, Division of Research, publication number 9, pp19

Cipolla, C. M. (1976). Public Health and the Medical Profession in the Renaissance, Cambridge University Press, Cambridge.

Clarke, N. A. and Chang, S. L. (1975). Removal of enteroviruses from sewage by bench-scale rotary-tube trickling filters. Applied Microbiol. 30, 223-228.

Clarke, N. A., Stevenson, R. E., Chang, S. L. and Kabler, P. W. (1961). Removal of enteroviruses from sewage by activated sludge treatment. Amer. J. Publ. Hlth. 51, 1118-1129.

Cliver, D. O. (1971). Viruses in water and wastewater: Effects of some treatment methods (p.149-158). In Proceedings of the 13th Water Quality Conference, (Eds. Snoeyink, V. and Griffin, V.) Univ. of Illinois, Urbana.

Cliver, D. O. (1975). Virus association with wastewater solids. Environ. Letters 10, 215-223.

Cohen, J. and Shuval, H. I. (1973). Coliforms, fecal coliforms, and fecal streptococci as indicators of water pollution. Water, Air, Soil pollution 2, 85-95.

Coin, L., Menetrier, M. L., Labonde, J. and Hannoun, M. C. (1964). Modern microbiological and virological aspects of water pollution. Proceedings of the 2nd International Conference on Water Pollution Research, Tokyo. (Vol. 1, 1-18).

Cotteral, J. A. and Norris, D. M. (1969). Septic tank systems. J. Sanit. Eng. Div. ASCE 95, 715-746.

Curds, C. R. (1969). An illustrated key to the British freshwater ciliated protozoa commonly found in activated sludge. Water Pollution Technical Paper number 12, HMSO, London.

Curds, C. R., Cockburn, A. and Vandyke, J. M. (1968). An experimental study of the role of ciliated protozoa in the activated sludge process. J. Inst. Wat. Pollut. Cont. 67, 312-329.

Curds, C. R. and Fey, G. J. (1969). The effect of ciliated protozoa on the fate of E. coli in the activated sludge process. Water Research 3, 853-867.

Dennis, J. M. (1959). 1955-56 infectious hepatitis epidemic in Delhi, India. J. Amer. Wat. Wks. Assoc. 51, 1288-1296.

Department of The Environment (1972). Analysis of Raw, Potable and Waste Waters. HMSO, London.

Dhillon, T. S., Chan, Y. S., Sun, S. M. and Chau, W. S. (1970). Distribution of coliphages in Hong Kong sewage. Appl. Microbiol. 20, 187-191.

Dias, F. F. and Bhat, J. V. (1965). Microbial ecology of activated sludge II. Bacteriophages, Bdellvibrios, coliforms and other organisms. Appl. Microbiol. 13, 257-261.

Dibdin, W. J. (1907). The Purification of Sewage and Water. The Sanitary Publishing Co. Ltd., London.

Dolin, R., Blacklow, N. R., Dupont, H., Formal, S., Buscho, R. F., Kasel, J. A., Chames, R. P., Hornick, R. and Chanock, R. M. (1971). Transmission of acute infectious non-bacterial gastroenteritis to volunteers by oral administration of stool filtrates. J. Inf. Dis. 123, 307-312.

Douglas, J. (1975). Bacteriophages. Chapman and Hall, London.

Dulbecco, R. (1952). Production of plaques in monolayer tissue cultures by single particles of an animal virus. Proc. Natl. Acad. Sci. U.S. 38, 747-752.

Dutka, B. J. (1973). Coliforms are an inadequate index of water quality. J. Env. Hlth. 36, 39-46.

England, B., Leach, R. E., Adame, B. and Shiosaki, R. (1967). Virologic assessment of sewage treatment at Santee, Calif. (p.401-418). In Transmission of Viruses by the Water Route (Ed. Berg, G.). Interscience Publishers, New York.

Fattal, B. and Nishmi, M. (1977). Enterovirus types in Israel sewage. Water Research 11, 393-396.

Feachem, R. G. (1977). Infectious disease related to water supply and excreta disposal facilities. Ambio 6, 55-58.

Feachem, R., McGarry, M. and Mara, D. (1977). Water, Wastes and Health in Hot Climates. John Wiley and Sons, London.

Fenner, F., McAuslan, B. R., Mims, C. A., Sambrook, J. and White, D. O. (1974). The Biology of Animal Viruses. Academic Press, New York.

Fish, H. (1973). Principles of Water Quality Management. Thunderbird Enterprises, Ltd., Harrow.

Flewett, T. H. (1977). Acute non-bacterial infectious gastroenteritis, An essay in comparative virology. Recent Adv. Clin. Virol. 1, 151-169.

Forster, C. F. (1971). Activated sludge surfaces in relation to the sludge volume index. Water Research 5, 861-870.

Geldriech, E. E. and Kenner, G. A. (1969). Concepts of fecal streptococci in stream pollution. J. Wat. Pollut. Cont. Fed. 41, R336-R352.

Genetelli, E. J. (1971). Biological waste treatment (p.415-449). In Water and Water Pollution Handbook, Vol. 1. (Ed. Ciaccio, L. L.). Marcel Dekker, New York.

Gerba, C. P. and Schaiberger, G. E. (1975). Aggregation as a factor in loss of viral titre in sea water. Water Research 9, 567-571.

Gilcrease, F. W. and Kelly, S. M. (1954). Significance of the coliform test in relation to intestinal virus pollution of water. J. New England Wat. Wks. Assoc. 68, 255-277.

Golterman, H. L. (1971). Methods for Chemical Analysis of Fresh Waters. IBP (International Biological Program) Handbook number 8, third printing. Blackwell Scientific Publications, Oxford.

Gordon, I., Ingraham, H. S. and Korns, R. F. (1947). Transmission of epidemic gastroenteritis to human volunteers by oral administration of fecal filtrates. J. Exp. Med. 86, 409-422.

Grabow, W. O. K. (1968). The virology of waste water treatment. Water Research 2, 675-701.

Hajenian, H. (1977). Personal communication.

Hammer, M. J. (1975). Water and Waste-Water Technology. John Wiley and Sons, Inc., New York.

Hawkes, H. A. (1963). The Ecology of Waste Water Treatment. Pergamon Press, Oxford.

Hines, D. A., Bailey, M., Ousby, J. C. and Roesler, F. C. (1975). The ICI deep shaft aeration process for effluent treatment. I. Chem. E. Symposium Series number 41 (pp10).

Howard, J., Lloyd, B. and Webber, D. (1975). The design and testing of a sanitation and sewage treatment unit for disasters and long term use. Oxfam technical paper.

Hughes, D. E. and Stafford, D. A. (1976). The microbiology of the activated-sludge process. CRC Critical Reviews in Environmental Control 6, 233-257.

Hunter, J. V. and Heukelekian, H. (1965). The composition of domestic sewage fractions. J. Wat. Pollut. Cont. Fed. 37, 1142-1163.

Isherwood, J. D. (1965). The fate of Coxsackie virus A-13 in water reclamation. Amer. J. Publ. Hlth. 55, 1945-1952.

Jawetz, E., Melnick, J. L. and Adleberg, E. A. (1974). Review of Medical Microbiology (11th Edition). Lange Medical Publications, Los Altos, Calif..

Jones, E. W. (1966). The history and development of sewage, its collection and treatment in England and Wales. J. Roy. Inst. Publ. Hlth. 29, 34-44.

Jordan, W. S., Gordon, I. and Dorrance, W. R. (1953). A study of illness in a group of Cleveland families. VII. Transmission of acute non-bacterial gastroenteritis to volunteers: evidence of two different etiologic agents. J. Exp. Med. 98, 461-475.

Katz, M. and Plotkin, S. A. (1967). Minimal infective dose of attenuated poliovirus for man. Amer. J. Publ. Hlth. Assoc. 57, 1837-1840.

Kelly, S. and Sanderson, W. W. (1959). The effect of sewage treatment on viruses. Sew. Ind. Wastes 31, 683-689.

Kelly, S., Sanderson, W. W. and Neidl, C. (1961). Removal of enteroviruses from sewage by activated sludge. J. Wat. Pollut. Cont. Fed. 33, 1056-1062.

Kollins, S. A. (1966). The presence of human enteric viruses in sewage and their removal by conventional sewage treatment methods. Adv. Appl. Microbiol. 8, 145-193.

Kott, Y., Roze, N., Sperber, S. and Betzer, N. (1974). Bacteriophages as viral pollution indicators. Water Research 8, 165-171.

Krejs, G. J., Gassner, M. and Blum, A.L. (1974). Epidemiology of infectious hepatitis. Clinics in Gastroenterology 3, 277-303.

Laak, R. and McLean, D. M. (1967). Virus transfer through a sewage disposal unit. Can. J. Publ. Hlth. 58, 172-176.

Lighthart, B. and Oglesby, R. T. (1969). Bacteriology of an activated sludge wastewater treatment plant - a guide to methodology. J. Wat. Pollut. Cont. Fed. 41, R267-R281.

Little, G. M. (1954). Poliomyelitis and Water supply. Can. J. Publ. Hlth. 45, 100-102.

Lockett, W. T. (1954a). The evolution of the activated sludge process. J. Inst. Sew. Purif. 3, 189-193.

Locket, W. T. (1954b). The development of the process using diffused air, with special reference to the Manchester pioneering work. J. Inst. Sew. Purif. 3, 194-210.

Loveday, R. (1975). Statistics, second course in statistics, (2nd Edition). Cambridge University Press, London.

Ludzak, F. J. (1960). Laboratory model activated sludge unit. J. Wat. Pollut. Cont. Fed. 32, 605-609.

Lund, E. (1971). Observations on the virus binding capacity of sludge (p.$I24/_1$ - $I24/_5$). Proceedings of the 5th International Conference on Water pollution Research, 1970.

Lund, E., Hedstrom, C. E. and Jantzen, N. (1969). Occurrence of enteric viruses in wastewater after activated sludge treatment. J. Wat. Pollut. Cont. Fed. 41, 169-174.

Lund, E. and Ronne, V. (1973). On the isolation of virus from sewage treatment plant sludges. Water Research 7, 863-871.

McCabe, L. J. and Craun, G. F. (1975). Status of waterborne diseases in the U.S. and Canada: Committee Report. J. Am. Wat. Wks. Assoc. 67, 95-98.

McDermott, J. H. (1974). Virus problems and their relation to water supplies. J. Amer. Wat. Wks. Assoc. 66, 693-698.

McKinney, R. E. and Harwood, M. P. (1952). Fundamental approach to the activated sludge process. I. Floc-forming bacteria. Sew. Ind. Wastes 24, 117-223.

MacFarlane, D. E. and Sommerville, R. G. (1969). Vero cells (Cercopithecus aethiops kidney) growth characteristics and viral susceptibility for use in diagnostic virology. Archiv. fur die Gesamte Virus. 27, 379-385.

Mack, W. N., Frey, J. R., Riegle, B. J. and Mallmann, W. L. (1962). Enterovirus removal by activated sludge treatment. J. Wat. Pollut. Cont. Fed. 34, 1133-1139.

Malan, W. M. (1964). CSIR research report number 219, Council for Scientific and Industrial Research, South Africa (Through Mara, 1976).

Malherbe, H. H. and Strickland-Cholmley, M. (1967). Quantitative studies on viral survival in sewage purification processes (p.379-388). In Transmission of Viruses by the Water Route (Ed. Berg, G.). Interscience Publishers, New York.

Malina, J. F. Jr., Ranganathan, K. R., Moore, B. E. D. and Sagik, B. P. (1974). Poliovirus inactivation by activated sludge (p.95-106). In Virus Survival in Water and Wastewater Systems (Eds. Malina, J. F. Jr. and Sagik, B. P.) Water Resources Symposium number 7, Center for Research in Water Resourses, Univ. of Texas, Austin.

Malina, J. F. Jr., Ranganathan, K. R., Sagik, B. P. and Moore, B. E. (1975). Poliovirus inactivation by activated sludge. J. Wat. Pollut. Cont. Fed. 47, 2178-2183.

Mara, D. (1976). Sewage Treatment in Hot Climates. John Wiley and Sons, London.

Marshall, K. C. (1976). Interfaces in Microbial Ecology. Harvard Univ. Press, Cambridge, Mass..

Metcalf, T. G. (1971). Biologic parameters in water transmission of viruses (p.1-16). In Proceedings of the 13th Wat. Qual. Conf., (Eds. Snoeyink, V. and Griffin, V.). Univ. of Illinois, Urbana.

Mosley, J. G. (1967). Transmission of viral diseases by drinking water (p.5-23). In Transmission of Viruses by The Water Route (Ed. Berg, G.). Interscience Publishers, New York.

Naparstek, J. D. (1973). Virus removal by activated sludge using f2 coliphage model. Sc.M. Thesis, Johns Hopkins Univ. Baltimore, Md. (through Naparstek et al. 1976).

Naparstek, J. D., Kawata, K., Olivieri, V. P. and Sherman, V. R. (1976). Virus removal in an activated sludge plant. Wat. Sew. Wks. 123 (Reference number), R18-R20.

Nupen, E. M. (1970). The isolation of viruses from sewage and treated sewage effluents. Wat. Pollut. Cont. 60, 430-435.

Olivieri, V. P., Kruse, C. W., Hsu, Y. C., Griffiths, A. C. and Kawata, K. (1975). The comparative mode of action of chlorine, bromine and iodine on f2 bacterial virus (p.145-162). In Disinfection - Water and Wastewater. (Ed. Johnson, J. D.). Ann Arbor Science Publishers Inc. Ann Arbor, Mich..

Painter, H. A. (1971). Chemical, physical and biological characteristics of wastes and waste effluents (p.329-364). In Water and Water Pollution Handbook Vol. 1. (Ed, Ciaccio, L. L.). Marcel Dekker, Inc. New York.

Painter, H. A. (1977). Unpublished data obtained by personal communication.

Painter, H. A. and Viney, M. (1959). Composition of a domestic sewage. J. Biochem. Microbial Technol. Eng. 1, 143-162.

Pike, E. B. and Carrington, E. G. (1972). Recent developments in the study of bacteria in the activated-sludge process. J. Inst. Wat. Pollut. Cont. 71, 583-605.

Pike, E. B. and Curds, C. R. (1971). The microbial ecology of the activated sludge (p.123-147). In Microbial Aspects of Pollution (Eds. Sykes, J. and Skinner, F. A.) Society for Applied Bacteriology Symposium Series 1. Academic Press, London.

Pipes, W. O. (1966). The ecological approach to the study of activated sludge. Adv. Appl. Microbiol. 8, 77-103.

Plotkin, S. A. and Katz, M. (1967). Minimal infective doses of viruses for man by the oral route (p.151-166). In Transmission of Viruses by The Water Route (Ed. Berg, G.). Interscience Publishers, New York.

Poynter, S. F. B. and Slade, J. S. (1977). The removal of viruses by slow sand filtration. Progr. Wat. Technol. 9, 75-88.

Pretorius, W. A. (1962). Some observations on the role of coliphages in the number of Escherichia coli in oxidation ponds. J. Hyg. Camb. 60, 279-281.

Ranganathan, K. R., Malina, J. F. Jr. and Sagik, B. P. (1975). Inactivation of enteric viruses during biological wastewater treatment (p.669-675). In Advances in Water Pollution Research, Proceedings of the 7th International Conference, Paris, Sept. 1974. Pergamon Press, New York.

Ranganathan, K. R., Malina, J. F. Jr., Sagik, B. P. and Moore, B. D. (1974). The inactivation of poliovirus, bacteriophage of E. coli B and enteric bacteria in biological waste treatment systems. Technical report to the National Science Foundation, Research applied to National Needs, Univ. of Texas, Center for Research in Water Resources, CRWR - 111, EHE - 74 - 03 (pp.100).

Safferman, R. S. and Moris, M. E. (1976). Assessment of virus removal by a multi-stage activated-sludge process. Water Research 10, 413-420.

Sattar, S. A. and Westwood, J. C. N. (1976). Comparison of four eluents in the recovery of indigenous viruses from raw sludge. Can. J. Microbiol. 22, 1586-1589.

Scarpino, P. V. (1971). Bacterial and viral analysis of water and waste water (p.639-761). In Water and Water Pollution Handbook Vol. 2 (Ed. Ciaccio, L. L.). Marcel Dekker, Inc., New York.

Scarpino, P. V. (1975). Human enteric viruses and bacteriophages as indicators of sewage pollution (p.49-61). In Discharge of Sewage from Sea Outfalls (Ed. Gameson, A. L. H.). Pergamon Press, Oxford.

Shah, P. C. and McCamish, J. M. (1972). Relative chlorine resistance of poliovirus I and coliphages f2 and T2 in water. Appl. Microbiol. 24, 658-659.

Sherman, V. R., Kawata, K., Olivieri, V. P. and Naparstek, J. D. (1975). Virus removals in trickling filter plants. Wat. Sew. Wks. 122 (Reference number), R36-R44.

Schwartzbrod, L., Schwartzbrod, J., Doncoeur, F., Foliguet, J.-M. and Lautier, F. (1973). Virus et eaux. I. Enterovirus et traitement des eaux usées et des eaux destinées à l'alimentation humaine. Rev. Epidem. Med. Soc. et Santé Publ. 21, 99-114.

Sidwick, J. M. and Murray, J. E. (1976a). A brief history of sewage treatment - 1. Before the Royal Commission. Eff. Wat. Treat. J. 16, 65-66.

Sidwick, J. M. and Murray, J. E. (1976b). A brief history of sewage treatment - 2. The Royal Commission. Eff. Wat. Treat. J. 16, 193-199.

Sidwick, J. M. and Murray, J. E. (1976c). A brief history of sewage treatment - 3. The turn of the century. Eff. Wat. Treat. J. 16, 295-301.

Sidwick, J. M. and Murrary, J. E. (1976d). A brief history of sewage treatment - 4. Between the wars. Eff. Wat. Treat. J. 16, 403-409.

Singley, J. E. (1971). Chemical and physical purification of water and wastewater. P.365-414. In Water and water Pollution Handbook Vol. 1. (Ed. Ciaccio, L. L.). Marcel Dekker, Inc. New York.

Smedberg, C. T. and Cannon, R. E. (1976). Cyanophage analysis as a biological pollution indicator - bacterial and viral. J. Wat. Pollut. Cont. Fed. 48, 2416-2426.

Snow, J. (1936). Snow on Cholera. Oxford Univ. Press, Oxford.

Sobsey, M. D. and Cooper, R. R. (1973). Enteric virus survival in algal-bacterial wastewater treatment systems. I. Laboratory studies. Water Research 7, 669-685.

Southgate, B. A. (1969). Water: Pollution and Conservation. Thunderbird Enterprises, Harrow.

Steiner, A. E., McLaren, D. A. and Forster, C. F. (1976). The nature of activated sludge flocs. Water Research 10, 25-30.

Subrahmanyan, T. P. (1977). Examination of sewage and sewage sludge for enteroviruses, Vol. 2. Ontario Ministry of the Environment. Pollution Control Branch.

Tenney, M. W. and Stumm, W. (1965). Chemical flocculation of microorganisms in biological waste treatment. J. Wat. Pollut. Cont. Fed. 37, 1370-1388.

Thornton, A. and Zuckerman, A. J. (1975). The virus of acute diarrhoea. Nature 254, 557-558.

Treybal, R. E. (1968). Mass Transfer Operations (2nd Edition). McGraw-Hill, Kogakusha.

Utz, J. P. (1974). Viruria in man, an update. Progr. Med. Virol. 17, 77-90.

Vincent, J. L. (1963). Hygiene and Sanitation in Relation to Housing, Symposium proceedings, Commission for Technical Cooperation in Africa, London. (Through Mara, 1976).

Viswanathan, R. (1957). Infectious hepatitis in Delhi (1955-1956): Epidemiology. Indian J. Med. Res. 45(Suppl.), 1-29.

Wallen, L. L. and Davis, E. N. (1972). Biopolymers of activated sludge. Environ. Sci. Technol. 6, 161-164.

Ware, G. C. and Mellon, M. A. (1956). Some observations on the coli/coliphage relationship in sewage. J. Hyg. Camb. 54, 99-101.

Weibel, S. R., Dixon, F. R., Weidner, R. B. and McCabe, L. J. (1964). Waterborne-disease outbreaks 1946-1960. J. Amer. Wat. Wks. Assoc. 56, 947-957.

Wellings, F.-M., Lewis, A. L. and Mountain, C. W. (1976). Demonstration of solids-associated virus in wastewater and sludge. Appl. Environ. Microbiol. 31, 354-358.

Williams, A. R., Forster, C. F., and Hughes, D. E. (1971). Using an ultrasonic technique in the enumeration of activated sludge bacteria. Eff. Wat. Treat. J. 11, 83-86.

Williams, A. R., Stafford, D. A., Callely, A. G. and Hughes, D. E. (1970). Ultrasonic dispersal of activated sludge flocs. J. Appl. Bacteriol. 33, 656-663.

Wolf, H. W. (1972). The coliform count as a measure of water quality (p.333-345). In Water Pollution Microbiology (Ed. Mitchell, R.). Wiley-Interscience, New York.

Wolverton, B. and McDonald, R. C. (1976). Don't Waste Water weed. New Scientist, 71, 318-320.

Zinder, N. D. (1965). RNA phages. Ann. Rev. Microbiol. 19, 455-472.