THE POTENTIAL ROLE OF SLOW SAND FILTRATION
IN REDUCING ROTAVIRAL DIARRHOEA
IN LESS DEVELOPED COUNTRIES

BY

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OCTOBER 1989

SUBMITTED IN PART FULFILLMENT FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
ABSTRACT

A slow sand filter system was developed which was suitable both for experimental purposes and full scale community water supply. The performance of the system was enhanced by the incorporation of pretreatments such as gravel prefiltration, sub-sand prefiltration and direct protection of filters by the incorporation of synthetic fabric layers. The system was used to examine aspects of the biological and physico-chemical nature of Slow Sand Filtration with particular reference to the removal of Rotavirus and a variety of bacterial and viral indicators.

The results of the developmental phase and experiments conducted with operational filters and in the laboratory confirmed the importance of biological mechanisms, in particular adsorption to biological surfaces present in the upper horizons of slow sand filters. It was observed that both the efficiency and pattern of removal of Rotavirus in Slow Sand Filtration were similar to those observed for faecal indicator bacteria, bacteriophage and turbidity i.e. colloidal clay and other particles. Thus it was concluded that negatively charged particulate colloidal entities appear to behave similarly despite differences in size and surface characteristics and that Rotavirus is no more or less likely to pass through slow sand filters than any other particle. Thus, a well operated slow sand filter may be expected to achieve a reduction in Rotavirus densities of $2 \log_{10}$ orders of magnitude.

The principal mechanisms of removal of Rotavirus in the upper horizons would appear to be transportation by diffusion and adsorption to biofilms and biomass. Microbial predation is not likely to play a dominant role in the removal of Rotavirus from the aqueous phase but may be important in inactivation.

A detailed examination of the incidence, prevalence and seasonal occurrence of rotaviral and other diarrhoeas in less developed countries led to the observation that unlike some of the bacterial pathogens, transmission of Rotavirus does not have a strong association with water quality. Hence
the case for low level waterborne transmission and thereby maintenance of endemicity in the community remains entirely theoretical. Moreover, bearing in mind the likelihood that slow sand filters will be operated sub-optimally in many cases, a moderate reduction in Rotavirus densities eg 1 - 2 log_{10} units may not be significant in terms of diarrhoeal disease risk, particularly for children and especially in the rural sector. In view of these observations and other factors eg the low infective dose of the virus and the undoubted pre-eminence of person-to-person spread, it was concluded that as a single process, Slow Sand Filtration is probably not capable of significantly reducing the incidence of rotaviral diarrhoea in less developed countries.
ACKNOWLEDGEMENTS

The author wishes to thank the following institutions for financial and practical support during the course of this Study:

United Kingdom Overseas Development Administration
University of Surrey
OXFAM
World Health Organization
Panamerican Health Organization
Thames Water Authority
British Geological Survey

A special debt of gratitude is owed to Dr Barry Lloyd for his original inspiration and subsequent unfailing enthusiasm for the work described in this thesis. The author also wishes to record thanks to Professor Jim Bridges, Director of the Robens Institute, Mr Leonard Kail, Secretary of the University of Surrey and Dr Michael Butler of the Department of Microbiology for maintaining faith in the eventual completion of the work. In addition a number of people have assisted by providing vital technical support in several aspects of the work. They are Helen Adams, Tim Baker, Jamie Bartram, Terry Fieldus, Mauricio Pardon, Andy Rickards, Dr Helen Skilton and Chris Symonds. Finally I wish to thank Verity Larby for exceptional skill and dedication in the typing and preparation of this thesis.

David Wheeler
7 October 1989
LIST OF FIGURES

FIGURE 1 Father Thames introducing his offspring to the fair city of London. Cartoon from *Punch* (1856) depicting an overt association between cholera and pollution of the River Thames.

FIGURE 2 Mistaking cause for effect. Cartoon from *Punch* (1849) depicting perceived association between cholera and the public drinking water supply.

FIGURE 3 Burning of clothing during the cholera epidemic of 1831-32. Illustration from *The History of the Cholera in Exeter* (1849) by T Shapter showing the preoccupation with atmospheric influences of the Miasma.

FIGURE 4 Seasonality of diarrhoea reporting to St Joseph’s Hospital, Roma, Lesotho (1974). The air temperature pattern correlates well with that of rainfall. Adapted from Feachem et al (1978).


FIGURE 6 Relationship between a disease index based on the product of incidences of three potentially water borne diseases (watery diarrhoea, typhoid and hepatitis) and a water quality index based on the addition of percentage fail rates for water samples on two criteria (presence of coliforms and absence of chlorine residual) in 26 districts of Lima, Peru (1981-82). Adapted from Lloyd et al (1989).

FIGURE 7 Seasonality of reporting of diarrhoeal disease to the Lewanika Hospital, Mongu, Zambia (1986-87). Adapted from Wheeler & Utkilen (1987).


FIGURE 9 Dose-response relationship for young children at various levels of exposure to an array of enteric pathogens. Adapted from Esrey et al (1985).

FIGURE 10 Circular slow sand filters in La Sirena, Cali, Colombia.

FIGURE 11 Sectional view of slow sand filter design, Universidad del Valle, Colombia.

FIGURE 12 Optimal limits for sand size grading for slow sand filters.

FIGURE 13 Basic components of an outlet controlled conventional slow sand filter (Visscher et al, 1987)

FIGURE 14 Basic components of an inlet controlled conventional slow sand filter (Visscher et al, 1987)

FIGURE 15 Rectangular plan conventional slow sand filter (Visscher et al, 1987)

FIGURE 16 Circular plan conventional slow sand filter (Visscher et al, 1987).
FIGURE 17 Construction and dimensions (mm) of the protected slow sand filter and flow controller.

FIGURE 18 Mode of operation of flow controller showing position of float at start (A) and end (B) of filtration run.

FIGURE 19 Calibration curve for turbidity measurements by extinction compared with nephelometric standards.

FIGURE 20 Protected slow sand filters with sub-sand prefiltration. Experimental system configuration I.

FIGURE 21 Protected slow sand filters with gravel prefiltration. Experimental system configuration II.

FIGURE 22 Protected slow sand filters with reservoir storage. Experimental system configuration III.

FIGURE 23 Protected slow sand filters with gravel prefiltration. Experimental system configuration IV.

FIGURE 24 Consumption of dissolved oxygen (mg/l) by a protected slow sand filter system subsequent to commissioning.

FIGURE 25 Dissolved oxygen levels in supernatant water, filtered water and water re-aerated by flow controller subsequent to routine maintenance of a protected slow sand filter operated at 0.20 m/h.

FIGURE 26 Dissolved oxygen levels in supernatant water, filtered water and water re-aerated by flow controller subsequent to routine maintenance of a protected slow sand filter operated at 0.32 m/h.

FIGURE 27 Correlation between reduction in density of thermotolerant coliforms and an index of ciliate population (Skilton, 1983) in a protected slow sand filter.

FIGURE 28 Maturation curves for the reduction in density of thermotolerant coliforms by protected slow sand filters operated at 0.16 m/h and 0.32 m/h.

FIGURE 29 Maturation curve for the reduction in density of thermotolerant coliforms by protected slow sand filters operated at 0.32 m/h.

FIGURE 30 Maturation curve for the reduction in density of thermotolerant coliforms by protected slow sand filters operated at 0.20 m/h.

FIGURE 31 Recovery of microbiological efficiency with respect to reductions in density of thermotolerant coliforms by protected slow sand filters operated at 0.20 m/h.

FIGURE 32 Maturation curve for the reduction in density of thermotolerant coliforms by horizontal gravel prefilters operated at 0.50 m/h.

FIGURE 33 Maturation curves for the reduction in density of thermotolerant coliforms by vertical (downflow) gravel prefilters and protected slow sand filters in combination.

FIGURE 34 Reductions in density of thermotolerant coliforms by sub-sand prefilters in cold water conditions (water temperature <10 °C) with respect to time.
FIGURE 35  Reductions in density of thermotolerant coliforms by sub-sand prefilters in cold water conditions (water temperature \(<10^\circ\text{C}\)) with respect to head loss.

FIGURE 36  Reductions in density of thermotolerant coliforms by sub-sand prefilters in warm water conditions (water temperature \(>10^\circ\text{C}\)) with respect to time.

FIGURE 37  Reductions in density of thermotolerant coliforms by sub-sand prefilters in warm water conditions (water temperature \(>10^\circ\text{C}\)) with respect to head loss.

FIGURE 38  Cumulative reductions in density of thermotolerant coliforms through a three-stage horizontal gravel prefilter employing gravel media of 40 mm (0-8 ft), 20 mm (8-16 ft) and 10 mm (16-24 ft) in series.

FIGURE 39  Performance curves for reductions in density of thermotolerant coliforms by gravel prefilters at flow velocities of 0.50 - 10 m/h.

FIGURE 40  Performance curves for reductions in density of faecal streptococci by gravel prefilters at flow velocities of 0.50 - 10 m/h.

FIGURE 41  Cumulative reductions in dissolved oxygen (mg/l) through a three-stage horizontal gravel prefilter employing gravel media of 40 mm (0-8 ft), 20 mm (8-16 ft) and 10 mm (16-24 ft) in series.

FIGURE 42  Reductions in density of thermotolerant coliforms in a water treatment system comprising sedimentation chamber, gravel prefilter and protected slow sand filters by unit process and by cumulative total in dry season conditions.

FIGURE 43  Reductions in density of thermotolerant coliforms in a water treatment system comprising sedimentation chamber, gravel prefilter and protected slow sand filters by unit process and by cumulative total in wet season conditions.

FIGURE 44  Reductions in turbidity in a water treatment system comprising sedimentation chamber, gravel prefilter and protected slow sand filters by unit process and by cumulative total in dry season conditions.

FIGURE 45  Reductions in turbidity in a water treatment system comprising sedimentation chamber, gravel prefilter and protected slow sand filters by unit process and by cumulative total in wet season conditions.

FIGURE 46  Physical maturation of a horizontal gravel prefilter with respect to reduction of turbidity.

FIGURE 47  Physical maturation of a vertical (downflow) gravel prefilter with respect to efficiency of turbidity removal.

FIGURE 48  Physical maturation of sub-sand prefilters with respect to reduction of turbidity in cold water conditions (water temperature \(<10^\circ\text{C}\)) and time.

FIGURE 49  Physical maturation of sub-sand prefilters with respect to reduction of turbidity in cold water conditions (water temperature \(<10^\circ\text{C}\)) and head loss.

FIGURE 50  Physical maturation of sub-sand prefilters with respect to reduction in turbidity in warm water conditions (water temperature \(>10^\circ\text{C}\)) and time.
FIGURE 51 Physical maturation of sub-sand prefilters with respect to reduction in turbidity in warm water conditions (water temperature > 10°C) and head loss.

FIGURE 52 Cumulative reductions in turbidity through a three stage horizontal gravel prefilter employing gravel of 40 mm (0-8 ft), 20 mm (8-16 ft) and 10 mm (16-24 ft) in series.

FIGURE 53 Performance curves for reductions in turbidity by gravel prefilters at flow velocities of 0.50 - 10 m/h.

FIGURE 54 Difference in rates of head loss increase in slow sand filters protected by six layers of synthetic fabric compared with a conventional unprotected slow sand filter.

FIGURE 55 Accumulated head loss at various depths within the sand bed (as a percentage of total head loss at 300 mm), compared with silt penetration (as a volumetric percentage).

FIGURE 56 Recovery of microbiological efficiency of protected slow sand filters following routine maintenance and skimming with respect to reductions in densities of thermotolerant coliforms.

FIGURE 57 Recovery of microbiological efficiency of protected slow sand filters following routine maintenance and skimming with respect to reductions in densities of faecal streptococci.

FIGURE 58 Percentage removal of thermotolerant coliforms with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.

FIGURE 59 Percentage removal of faecal streptococci with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.

FIGURE 60 Percentage removal of 37°C/24h total plate count organisms with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.

FIGURE 61 Percentage removal of 20°C/72h total plate count organisms with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.

FIGURE 62 Percentage removal of Serratia marcescens bacteriophage with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.

FIGURE 63 Percentage removal of Erwinia carotovora bacteriophage with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.

FIGURE 64 Percentage removal of Escherichia coli K12 bacteriophage with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.

FIGURE 65 Percentage removal of Enterobacter cloacae bacteriophage with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.

FIGURE 66 Percentage removal of Bacillus licheniformis bacteriophage with respect to depth in protected slow sand filter operated at a filtration velocity of 0.28 m/h.

FIGURE 67-70 Percentage of total Serratia marcescens bacteriophage recovered with respect to time for sampling points at depths of 0, 100, 200 and 500 mm in a protected slow sand filter.
FIGURES 71-74  Percentage of total *Erwinia carotovora* bacteriophage recovered with respect to time for sampling points at depths of 0, 100, 200 and 500mm in a protected slow sand filter.

FIGURES 75-78  Percentage of total *Escherichia coli* K12 bacteriophage recovered with respect to time for sampling points at depths of 0, 100, 200 and 500 mm in a protected slow sand filter.

FIGURES 79-82  Percentage of total *Enterobacter cloacae* bacteriophage recovered with respect to time for sampling points at depths of 0, 100, 200 and 500 mm in a protected slow sand filter.

FIGURES 83-86  Percentage of total *Bacillus licheniformis* bacteriophage recovered with respect to time for sampling points at depths of 0, 100, 200 and 500 mm in a protected slow sand filter.

FIGURE 87  Attenuation of indigenous Rotavirus with respect to depth of sand below a wastewater lagoon.

FIGURE 88  Attenuation of indigenous Rotavirus with respect to depth of sand below a wastewater irrigation channel.

FIGURE 89  Attenuation of indigenous faecal streptococci with respect to depth of sand below a wastewater lagoon.

FIGURE 90  Attenuation of indigenous faecal streptococci with respect to depth of sand below a wastewater irrigation channel.

FIGURE 91  Attenuation of indigenous thermotolerant coliforms with respect to depth of sand below a wastewater lagoon.

FIGURE 92  Attenuation of indigenous thermotolerant coliforms with respect to depth of sand below a wastewater irrigation channel.

FIGURE 93  Mean levels of simian Rotavirus SA11 in the supernatant and fabric - sand interface of column protected slow sand filters operated at a filtration velocity of 0.20 m/h.

FIGURE 94  Percentage removal of simian Rotavirus SA11 with respect to depth in column slow sand filters operated at a filtration velocity of 0.20 m/h.

FIGURE 95  Adsorption of simian Rotavirus SA11. Control for sterile substrates.

FIGURE 96  Adsorption of simian Rotavirus SA11 in contact with sterile acid-washed filter sand.

FIGURE 97  Adsorption of simian Rotavirus SA11 in contact with sterile clean filter sand.

FIGURE 98  Adsorption of simian Rotavirus SA11 in contact with sterile clean 10 mm gravel.

FIGURE 99  Adsorption of simian Rotavirus SA11 in contact with sterile clean polypropylene fabric.

FIGURE 100  Adsorption of simian Rotavirus SA11. Control for non-sterile substrates.
FIGURE 101 Adsorption of simian Rotavirus SA11 in contact with sand and schmutzdecke from a conventional full scale slow sand filter.

FIGURE 102 Adsorption of simian Rotavirus SA11 in contact with sand and schmutzdecke from an experimental protected slow sand filter.

FIGURE 103 Adsorption of simian Rotavirus SA11 in contact with polypropylene fabric and schmutzdecke from an experimental protected slow sand filter.

FIGURE 104 - 107 Seasonality of human Rotavirus infections in Ecuador (adapted from Suzuki et al, 1981), Bangladesh (adapted from Stoll et al, 1982), India (adapted from Broor et al 1985) and Kenya (adapted from Mutanda et al, 1986).

FIGURE 108 Possible inter-relation of modes of transmission and maintenance of Rotavirus in the community.

FIGURE A.I.1 Schematic layout of four protected slow sand filters showing inlets, connections, common overflows and filtered water outlets.

FIGURE A.I.ii Cross-section of protected slow sand filter.

FIGURE A.I.iii Cross-section through under-drainage system of protected slow sand filter.

FIGURE A.II.1 Calibration curve for turbidity measurements by extinction compared with nephelometric standards.

FIGURE A.II.ii Performance curve for a portable bacteriological incubator developed at the University of Surrey compared with incubation in a conventional water bath.

FIGURE A.II.iii Performance curve for a portable bacteriological incubator developed at the University of Surrey compared with incubation in a commercial field-portable incubator.
LIST OF PLATES

PLATE I  Experimental protected slow sand filter installation at the University of Surrey Manor Farm field station.

PLATE II  Experimental protected slow sand filter installation at Thames Water Authority Hampton Water Treatment Works.

PLATES III & IV Experimental protected slow sand filter installations in the communities of San Buenaventura, Canta Valley and San Vicente de Azpitia, Mala Valley, Peru.

PLATES V & VI Column protected slow sand filters and raw water source.
CONTENTS

SECTION 1: INTRODUCTION

1.1 The Microbiological Quality of Drinking Water 1
1.2 Water Quality and Health in Less Developed Countries 6
1.3 Viruses as Agents of Waterborne Disease 19
1.4 The Advent of Slow Sand Filtration 26

SECTION 2: THE DEVELOPMENT OF AN EXPERIMENTAL PROTECTED SLOW SAND FILTRATION SYSTEM

2.1 General Criteria 30
2.1.i Filtration media 33
2.1.ii Filtration rate 35
2.1.iii Filter covering 36
2.1.ivPrefiltration 36
2.1.v Summary 40
2.2 System Development 41
2.3 Materials and Methods 49
2.3.i Bacteriological parameters 50
2.3.ii Chemical parameters 52
2.3.iii Physical parameters 52
2.4 Results and Data Presentation 57
2.4.i Microbiological maturation phenomena 64
2.4.ii Microbiological performance of systems 77
2.4.iii Physical performance of systems 83
2.4.iv Operational characteristics 97

SECTION 3: OBSERVATIONS ON THE REMOVAL OF VIRUSES BY FILTRATION THROUGH SAND

3.1 General Observations 101
3.2 The Removal of Viruses by Depth - Related Phenomena in Slow Sand Filtration 105
3.3 Materials and Methods 110
3.3.i Bacteriological parameters 110
3.3.ii Bacteriophage 110
3.3.iii Rotavirus - selection of techniques 112
3.3.iv Protected slow sand filter columns 119
3.4 Results 121
SECTION 4: ADSORPTION OF VIRUSES TO SURFACES IN SLOW SAND FILTRATION

| 4.1 General Observations | 143 |
| 4.2 Viruses as Colloids in Electrostatic Interactions | 143 |
| 4.2.i Transport mechanisms in filtration | 146 |
| 4.2.ii Virus adsorption to soils | 148 |
| 4.2.iii Virus adsorption to marine and estuarine sands and sediments | 151 |
| 4.2.iv Models to index virus behaviour | 152 |

| 4.3 Materials and Methods | 156 |
| 4.4 Results | 158 |

SECTION 5: DISCUSSION

| 5.1 Rotavirus and Rotaviral Infection: General Observations | 164 |
| 5.1.i Prevalence of rotaviral infection in industrialised countries | 167 |
| 5.1.ii Prevalence of rotaviral infection in less developed countries | 169 |

| 5.2 The Development of an Experimental Protected Slow Sand Filtration System - Discussion of the Results of Section 2. | 174 |
| 5.3 Observations on the Removal of Viruses by Filtration Through Sand - Discussion of the Results of Section 3. | 180 |
| 5.4 Adsorption of Viruses to Surfaces in Slow Sand Filtration - Discussion of the Results of Section 4. | 188 |
| 5.5 The Seasonality of Rotaviral Infection | 191 |

REFERENCES

APPENDIX I: COMPONENTS AND ASSEMBLY OF THE EXPERIMENTAL PROTECTED SLOW SAND FILTRATION SYSTEM

APPENDIX II: MEDIA AND DEVELOPMENT OF METHODS USED IN THE STUDY
For Andrea
"so long as the filter is a mere heap of sand on to which water is poured from above and abstracted from beneath, so long as he scorns microbes and shrugs his shoulders at biology, the engineer does not rise above the intellectual level of a foreman."

A Kemna, 1899
1.1 THE MICROBIOLOGICAL QUALITY OF DRINKING WATER

"I beg you will consider my doctrine, that all pump water is poisonous"

William Lambe (1803)

The history of public water provision in Western Europe has frequently been characterised by healthy scientific debate and no little public controversy. From the middle of the 18th century to the latter part of 19th, much of the scientific discussion was concerned with the impact on human health of physically or organically contaminated drinking water (Hardy, 1984). However, following the unequivocal demonstration of a waterborne transmission route for cholera (Snow, 1854) and the isolation of the causative agent for cholera by Robert Koch (Feachem, 1982), this discussion turned on the essential need to ensure the microbiological safety of domestic drinking water. This precipitated major advances in water treatment practice, most notably the introduction of routine disinfection of public water supplies.

The revelation that pollution of traditional sources of water could lead directly to epidemic disease resulted in a degree of justifiable unease on the part of consumers dwelling in the sprawling urban squalor of mid-nineteenth century Europe. But the scientific demonstration of a health risk associated specifically with water merely served to confirm the suspicions of the radical press and the early public health campaigners who were quite capable of recognising the undesirability of contamination of public water sources by the faecal waste from cholera victims (Figures 1 and 2).
In 1828, John Wright (then editor of the Parliamentary record Hansard) had anonymously published and distributed a polemical attack on drinking water quality in London which led to the establishment of a Royal Commission and the principle that water for human consumption should at all times be "wholesome" - a term which has since been incorporated into virtually every piece of United Kingdom legislation relating to water (Wheeler, 1986). Together with Sir Francis Burdett, a Radical Member of Parliament, Wright is generally credited with precipitating public disquiet on such a scale that one contemporary remarked: "the whole town was in convulsions under the notion that they should be poisoned with filthy water" (Lipschutz, 1968).

From the perspective of this Study, the Royal Commission of 1828 was especially important because it marked the first official recognition of the importance of both water quantity and quality in the UK. However, the definition of water quality and its relation to health is difficult to establish on objective criteria even in the latter part of the 20th Century (US National Research Council, 1977; World Health Organization, 1978; Wheeler, 1987). In 1828, it was impossible.
Boy, "I say, Tommy, I'm blow'd if there isn't a man a turning on
the Cholera."

FIGURE 2 Mistaking cause for effect. Cartoon from Punch (1849)
depicting perceived association between cholera and the public
drinking water supply.

Consequently, for nearly two hundred years, water suppliers, scientists, legislators and consumers
have been locked into arguments about water quality and health which owe little to established
epidemiological fact and everything to subjective, often sensory, perception.

For example, in the 19th century, distinguished chemists such as Justus Liebig, Thomas Clarke
and even leading sanitationists such as Sir Edwin Chadwick were able to dispute any direct link
between organic (sewage) pollution of drinking water and disease. Chadwick was a major
proponent of an alternative theory which assumed transmission via an ethereal quasi-atmospheric
Miasma. Hence, the elimination of the smell attributable to cholera victims and their environment
led to a preoccupation with burning the clothes and belongings of the dead. And it is at least
arguable that the construction of municipal trunk sewers owed as much to the desire on the part of
social engineers like Chadwick to remove the Miasma with its associated smell and "occult
influences" as to the need to improve the living conditions of the urban poor (Figure 3).
Similarly, the Great Stink of 1856, when sheets drenched in chloride of lime were hung from the windows of the Palace of Westminster to exclude the smell of the fermenting river below, is popularly considered to have been of considerably more influence in focussing the minds of politicians on the need for improvements than any scientific evidence of a health risk associated with the pollution.

Today, taste, smell, and appearance (colour, turbidity etc) are considered legitimate sensory criteria for judging drinking water quality (World Health Organization, 1984a). Furthermore, some attention has been devoted to establishing those criteria which the public apply in their assessment of natural waters (Dinius, 1981; Moser, 1984; Herzog, 1985). Thus it is interesting to note that in his Essay on Waters (1756), Charles Lucas defined water as "pure and simple" which satisfies all five senses viz: colourless, odourless, tasteless, soft to the touch, and even noisiest "when poured out of one vessel into another". Significantly, Lucas also pointed to the limitations of sensory perception and emphasised the need for complementary objective evidence of purity based on physical, chemical and medicinal properties.
This Study recognises that the principal factor which determines the acceptability of community water supplies in both developing and industrialised countries remains the perception of consumers. However, it must be recognised that freedom from microbiological or chemical agents of disease is only rarely the most important determinant in the public perception of water services. In all societies, quantity, cost and reliability almost always exert more influence in the design and implementation of water supply schemes than the prospect of improved quality.

The Study is concerned with an examination of the way in which a technology which was developed to improve only the physical and aesthetic quality of water is able to eliminate microbiological agents whose potential role in waterborne disease has yet to be fully recognised. In this sense, it falls within a tradition of research in water quality and health which owes as much to scientific postulation and debate as it does to experimentation and observation.
1.2 WATER QUALITY AND HEALTH IN LESS DEVELOPED COUNTRIES

"All the evidence proving the communication of cholera through the medium of water, confirms that with which I set out, of its communication in the crowded habitations of the poor, in coalmines and other places, by the hands getting soiled with the evacuations of the patients, and by small quantities of these evacuations being swallowed with the food....."

John Snow (1854)

Despite universal acceptance of the multifactorial nature of faecal-oral infection established by Snow more than one century ago, there remains a degree of uncertainty regarding the relative importance of those factors which contribute to morbidity and mortality attributable to waterborne diarrhoeal disease. In the best traditions of the debates of the 18th and 19th centuries, much of the discussion today surrounds the two ancient rivals: the quality and the quantity of the drinking water supply. Other factors describing the level of service of a water supply ie coverage, cost and continuity have only recently been discussed individually in the same context as quality and quantity (Pardon & Wheeler, 1986; Lloyd et al, 1987), but they have been merged for a number of years in the concept of "availability" (Feachem, 1983). Important variables in disease incidence which should not be overlooked include: immunity, nutritional status and level of hygiene awareness. However, these factors have little to do with the water supply per se, they relate more to the status and behaviour of the consumers.

There is some evidence to suggest that improvements in water quality alone may not give rise to measurable improvements in health (Feachem et al, 1978; Briscoe, 1978; Blum & Feachem, 1983). This observation would not be surprising in communities where faecal-oral transmission
routes were primarily person-to-person or foodborne. For example, when Feachem and co-workers (1978) investigated the phenomenon of "typhoid villages" in Lesotho (1971-1974) where typhoid was widely believed to be associated with poor water quality, they found no evidence that the disease was primarily waterborne. Furthermore, the study questioned the link between wet season peaks of diarrhoeal disease and typhoid and contamination levels in drinking water, since the peaks also occurred in villages with improved supplies and in a city with a chlorinated supply.

In the Lesotho study, it was concluded that "village water supplies, as they have been constructed and used in Lesotho, have not led to reductions in disease", and a number of complementary interventions were therefore proposed. Although it was recognised that water quality data were not always adequate (particularly for certain types of supply), there was a general impression that for most sources, differences in bacterial water quality between wet and dry seasons were not great. A five-fold increase in contamination levels in the wet season for unimproved sources was not considered significant.

The phenomenon of seasonality of faecal-oral infections is well known. Similarly the influence of climate, (particularly rainfall), on water quality is also well understood. These two observations could lead to an assumption that environmental factors can play a major role in wet season peaks in the incidence of certain waterborne diseases. However, caution must be observed in order to avoid over-extending this assumption. It is entirely possible that the flushing effect of rainfall is capable of reducing densities of certain enteric pathogens whilst simultaneously elevating the densities of others. Another consideration is that of survival or maintenance in the environment. For example, there is some evidence to suggest that the seasonality of cholera is very closely linked to fluctuations in estuarine salinity in some areas (Miller et al, 1982). Equally, in areas where a wet season coincides with cool conditions, the survival of many pathogens will be substantially enhanced. In contrast, where a wet season coincides with warmer temperatures, environmental persistence may not be maximal.
Clearly, a number of inter-relating factors need to be considered before categorical conclusions can be drawn on the association between climate, water quality and disease transmission. In this context, it is interesting to contrast seasonal data from the Lesotho study with similar information from Peru (Lloyd et al, 1989) and Zambia (Wheeler & Utkilen, 1987).

Feachem and co-workers concluded that the obvious seasonality in diarrheal disease reporting in Lesotho was due to factors other than water quality (Figure 4). Exactly the opposite conclusion was drawn by Lloyd and co-workers for data from the Lima-Callao conurbation in Peru (Figure 5).

![Figure 4](image_url)  
**Figure 4** Seasonality of diarrhoea reporting to St Joseph's Hospital, Roma, Lesotho (1974). The air temperature pattern correlates well with that of rainfall. Adapted from Feachem et al (1978).

In the case of Lesotho, the health data were derived from mostly rural populations depending on water sources of variable quality and reporting to a single hospital (St Joseph’s, Roma). The Lima-Callao data were derived from an exclusively urban and peri-urban population of several millions served by a variety of piped and tankered water sources. The factor which proved most convincing in sustaining the waterborne transmission hypothesis for seasonality in Lima-Callao was the demonstration of an unequivocal correlation between water quality (indexed by thermotolerant coliform and residual chlorine data) and health (indexed by the product of disease incidence for three potentially waterborne diseases) in 26 districts of the Peruvian capital (Figure 6).
FIGURE 6 Relationship between a disease index based on the product of incidences of three potentially water borne diseases (watery diarrhoea, typhoid and hepatitis) and a water quality index based on the addition of percentage fail rates for water samples on two criteria (presence of coliforms and absence of chlorine residual) in 26 districts of Lima, Peru (1981-82). Adapted from Lloyd et al (1989).

The population size and the relative reliability of both water quality and health data in a large metropolitan centre lend substantial credibility to the assertion that a principal factor in the elevation of numbers of cases of diarrhoea, typhoid and hepatitis during periods of higher monthly average air temperatures is the coincidence of these temperatures with periods of poor water quality.

It may be argued that the two populations in Lesotho and Peru were entirely dissimilar and that the absence of detailed water quality data in the Lesotho study invalidates any real comparison. However, recent data from two separate populations in the Western Province of Zambia (Wheeler & Utkilen, 1987) do provide some basis for drawing more general conclusions. Figure 7 illustrates the seasonality of diarrhoeal disease reporting to the Lewanika Hospital, Mongu of a largely urban and peri-urban population.
The quality of piped water provided for the majority of this population (estimated number : 29,000) was very good, but the peri-urban dwellers (estimated number : >10,000) did not necessarily have access to the piped supply and therefore relied on water sources of indeterminate quality. Figure 8 depicts the seasonal reporting of diarrhoeal disease to 20 rural health centres in the same province. Water quality data are available for the rural areas and ratios of bacterial water quality (indexed by thermotolerant coliform density) can be derived (Table 1).
The data described in Table 1 show that for water sources in seven health centre areas, the ratios of wet season to dry season contamination ranged from 1.00 to 41.3 (median = 3.34). The median ratios of contamination between unprotected and protected sources were 4.25 (wet season) and 1.42 (dry season). These comparisons provide strong evidence for the principle that in rural areas depending on a variety of protected and unprotected sources, contamination levels in the wet season tend to be higher than in the dry season. Furthermore, the median ratio for the Zambian sources (3.34) was not dissimilar to that observed in the Lesotho study (5.4).
<table>
<thead>
<tr>
<th>HC</th>
<th>FC(WET) : FC(DRY)</th>
<th>FC(WET) : FC(WET)</th>
<th>FC(DRY) : FC(DRY)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>unprotected</td>
<td>unprotected</td>
<td>unprotected</td>
</tr>
<tr>
<td>1</td>
<td>3.67*</td>
<td>5.32</td>
<td>0.66</td>
</tr>
<tr>
<td>2</td>
<td>41.3</td>
<td>19.9</td>
<td>0.28</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td>9.22*</td>
<td>9.19</td>
</tr>
<tr>
<td>4</td>
<td>6.05</td>
<td>9.22</td>
<td>1.57*</td>
</tr>
<tr>
<td>5</td>
<td>2.66</td>
<td>4.25</td>
<td>1.42</td>
</tr>
<tr>
<td>6</td>
<td>2.55</td>
<td>3.17</td>
<td>1.42</td>
</tr>
<tr>
<td>7</td>
<td>3.34</td>
<td>1.70</td>
<td>1.00*</td>
</tr>
</tbody>
</table>

**MEDIAN**

|               | 3.34 | 4.25 | 1.42 |

* TABLE 1. Ratios of geometric mean thermotolerant coliform (FC) densities in seven health centre (HC) areas in Western Province, Zambia in wet season (WET) and dry season (DRY) for unprotected (unprot) and protected (prot) water sources.

* Ratio contains geometric mean based on 4 or less samples.

Clearly, the seasonality of diarrhoeal disease reporting in the urban/peri-urban situation in Zambia (Figure 7) was much stronger than that observed in the rural sector (Figure 8). In this sense, it bears a closer comparison with the seasonality of reporting in Lima-Callao. In addition, the two urban/peri-urban relationships both derive from populations where a substantial proportion benefit from a relatively reliable and high quality supply, but a significant number (mostly associated with the peri-urban areas) are dependent on a generally low level of sanitary provision and a water supply of questionable quality.
Similarly, the observations on the rural sector in Zambia are quite comparable with those in the Lesotho study.

Ironically, conclusions from the Lima-Callao and Lesotho studies, though superficially contrary, are both confirmed by comparisons with the relevant Zambian data. The unifying hypothesis may be simply stated:

In isolated rural communities, elevations in levels of bacterial contamination in small water supplies during the wet season may actually be very modest. There is simply not a large volume of contaminated material waiting to be washed into the sources to sustain high levels of faecal microorganisms for long periods in those sources. In contrast, in urban situations where a proportion of the population may be living in overcrowded, insanitary and badly serviced housing, the likelihood of substantially elevated microbiological contamination of drinking water sources and drinking water in the home is proportionately greater. Thus seasonality in disease reporting is more likely to have an association with waterborne transmission in urban environments.

Since the publication of the Lesotho work, it has become common currency for many working in rural water supply schemes in developing countries to assert that "water quality is unimportant" or at least that "water quantity is more important than quality" (author's experience). This point of view represents a gross oversimplification of the arguments of Feachem and his co-workers. In a later attempt to provide a league table of the comparative importance of various possible interventions, water quality was accorded 11 points compared with 18 for water availability (Feachem, 1983).

However, at no time has Feachem missed an opportunity to stress the multifactorial nature of faecal-oral infection and thus the need for an approach to the improvement of water supply and
sanitation which encompasses all aspects of hygiene. Thus it is encouraging that a new and more sophisticated theoretical model has emerged which proposes a "threshold-saturation" dose-response relationship for individuals exposed to pathogens transmitted by the faecal-oral route (Shuval et al, 1981; Esrey et al, 1985). In particular, the model of Esrey, Feachem and Hughes, provides a useful framework for considering the impact of individual interventions on ingested dose and thus on the incidence of infection of children by individual agents of diarrhoeal disease. The model is reproduced in Figure 9.

![Dose-response relationship for young children at various levels of exposure to an array of enteric pathogens.](image)

**FIGURE 9** Dose-response relationship for young children at various levels of exposure to an array of enteric pathogens. Adapted from Esrey et al (1985).
Important theoretical implications of this model are:

1) At high levels of pathogen dose (E-F), an intervention which results in only moderate reductions in pathogen load may have little impact on the incidence of either severe or mild diarrhoea.

2) At moderate/high levels of pathogen dose (D - E), only the incidence of severe diarrhoea may be reduced.

3) At moderate/low levels of pathogen dose (B - D), both severe and mild diarrhoea incidence may be reduced, but at different rates.

The proposers of the model recognised its tentative and simplified nature. However, they were able to point to a number of areas where the model appeared verifiable by established fact.

For example, some impact studies dealing with the effect of improved water supply and sanitation on diarrhoeal disease have failed to demonstrate a positive effect on morbidity (Blum & Feachem, 1983). This may be expected (in terms of the model) if pathogen dose decreases only between points F and D on the graph. The model also predicts that studies which concentrate on the impact of improvements on severe diarrhoea are more likely to show a positive impact. This is confirmed by the few studies which have been restricted to impact on mortality (either total mortality or that due specifically to diarrhoeal disease) rather than morbidity.

Impact studies which have failed to demonstrate any reduction in morbidity have all had demonstrable defects in methodology (Esrey et al., 1985). But taking data from all identified studies (53 in total), it was demonstrated that the overall reduction in diarrhoeal disease morbidity from all interventions was 22 per cent (median value). When only selected "best studies" were taken into account the overall median reduction in morbidity was 27 per cent, and the reduction in
total mortality was 30 per cent. Significantly, the median reduction values (all studies) in diarrhoeal disease morbidity for specific interventions of particular interest to this Study were:

- **Water Quality (n = 9)**: 16 per cent
- **Water Availability (n = 17)**: 25 per cent
- **Water Quality plus Water Availability (n = 8)**: 37 per cent

The model is also verified indirectly by the demonstration of the importance of other factors eg breast feeding and literacy (educational status) in reducing the incidence of diarrhoeal disease. For example, non-breast-fed children are likely to be further to the right on the horizontal axis, and in some circumstances may therefore expect to obtain greater benefit from certain interventions than breast-fed children.

A further important implication of the model for this Study is that it predicts that reductions in pathogen loading for organisms with a high infective dose eg cholera (ID$_{50}$ : $10^8$ - $10^{11}$) will be much more likely to result in improvements in diarrhoeal disease incidence than reductions in doses of agents with low infective doses eg enteric viruses, protozoa and *Shigella*. Cited in support of this prediction was the observation that the incidence rate for Rotaviruses in children under two years of age is 0.3 - 0.4 episodes per annum both in Bangladesh and Winnipeg, Canada.

However, the effects of rotaviral infection in Bangladesh and Winnipeg are not equivalent. It has been estimated that nearly half of all infant mortality due to diarrhoeal disease in Bangladesh is caused by rotaviral infection (ICDDRB, 1979). Thus, the severity of the infection leads to an impact on health which is not experienced in industrialised countries. Therefore it is important to qualify the implication of the model for improvements in water supply and sanitation and their likely impact on diseases with a low infective dose:
Unless reductions in pathogen loading are very large (perhaps several orders of magnitude), interventions which attempt to improve water supply or sanitation may not result in a measurable impact on the incidence of illness caused by agents with a low infective dose.

Close scrutiny of some impact studies confirms the validity of this qualified implication. For example an investigation of the impact of chlorination of wells which only resulted in approximately ten-fold improvements in bacterial water quality was described by Trivedi and co-workers (1971). During the period of chlorination, the incidence of infectious hepatitis (low infective dose) was not reduced to the same extent as gastroenteritis and dysentery which both have potentially higher infective doses. However, as previously described, more effective disinfection, such as that practiced in large areas of metropolitan Lima (which may be expected to reduce microbial loadings by several orders of magnitude), has been demonstrated to result in water quality improvements which may be directly correlated with a disease index which includes the incidence of hepatitis (Lloyd et al, 1989).
1.3 VIRUSES AS AGENTS OF WATERBORNE DISEASE

"Koch's discovery of the comma-bacillus alters nothing"

M Von Pettenkofer (1884)

Diarrhoeal disease is the single most important health problem in less developed countries (Snyder & Merson, 1982). Diarrhoea, or acute malabsorption in the gut is caused by a very wide variety of microbial agents of which bacteria, protozoa and human enteric viruses comprise the most important groups. This Study is particularly concerned with the enteric viruses - a group which contains a number of recently recognised pathogens with undefined relative importance and unknown principal transmission routes. In this context, the relatively recent recognition of certain enteric viruses and their postulated association with waterborne faecal-oral infection may be likened to the discovery of the "comma-bacillus" *Vibrio cholerae*. A very high proportion of waterborne disease outbreaks in industrialised countries are of unknown aetiology (Lippy & Waltrip, 1984). And although there is a considerable amount of information relating to the relative frequency with which different agents are detected in the stools of diarrhoea patients in developing countries, this information does not necessarily reflect those proportions which result from waterborne infection.

Thus it is not possible to say with any certainty that human enteric viruses are responsible for a high proportion of waterborne infections in developing countries. But equally, because of methodological difficulties, it is not possible to refute the hypothesis.

A useful review of the relevance of human excreted viruses to sanitation and disease is available (Feachem et al, 1983) A similar classification was provided by Goyal (1984), and the two are combined in Table 2.
<table>
<thead>
<tr>
<th>Virus Group</th>
<th>Family</th>
<th>Size and Composition</th>
<th>Number of types</th>
<th>Disease or symptoms caused</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterovirus</td>
<td>Picornaviridae</td>
<td>About 20-30 nanometers diameter, Single-stranded RNA in a protein shell</td>
<td>3</td>
<td>Poliomyelitis, meningitis, fever</td>
</tr>
<tr>
<td>Poliovirus</td>
<td></td>
<td></td>
<td>2/23</td>
<td>Herpangina, respiratory disease, meningitis, fever</td>
</tr>
<tr>
<td>Coxsackievirus A</td>
<td></td>
<td></td>
<td>6</td>
<td>Myocarditis, congenital heart anomalies, meningitis, respiratory disease, pleurodynia, rash, fever.</td>
</tr>
<tr>
<td>Coxsackievirus B</td>
<td></td>
<td></td>
<td>34/31</td>
<td>Meningitis, respiratory disease, rash, diarrhoea, fever.</td>
</tr>
<tr>
<td>Echovirus</td>
<td></td>
<td></td>
<td>4</td>
<td>Meningitis, exanthema, respiratory disease, acute hemorrhagic conjunctivitis, fever.</td>
</tr>
<tr>
<td>New enteroviruses</td>
<td></td>
<td></td>
<td>30</td>
<td>Respiratory disease, eye infection</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Adenoviridae</td>
<td>About 70-80 nanometers diameter, Double-stranded DNA in a protein shell</td>
<td></td>
<td>Respiratory disease, eye infection</td>
</tr>
<tr>
<td>Faecal Adenovirus</td>
<td>Reoviridae</td>
<td>About 75 nanometers diameter, Double-stranded RNA in a double protein shell</td>
<td>27</td>
<td>Gastro-enteritis</td>
</tr>
<tr>
<td>Reovirus</td>
<td></td>
<td></td>
<td>3</td>
<td>Not clearly established</td>
</tr>
<tr>
<td>Hepatitis A virus</td>
<td>Picornaviridae</td>
<td>About 24-29 nanometers diameter, Single-stranded RNA</td>
<td>1</td>
<td>Infectious hepatitis</td>
</tr>
<tr>
<td>Non-A Non-B Hepatitis</td>
<td></td>
<td></td>
<td>17</td>
<td>Infectious hepatitis</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>Reoviridae</td>
<td>About 70 nanometers diameter, Double-stranded RNA in a double protein shell</td>
<td>2</td>
<td>Vomiting and diarrhoea</td>
</tr>
<tr>
<td>Astrovirus</td>
<td></td>
<td>About 28 nanometers diameter</td>
<td>27</td>
<td>Gastro-enteritis</td>
</tr>
<tr>
<td>Calicivirus</td>
<td></td>
<td>About 35-40 nanometers diameter, Single-stranded RNA in a protein shell</td>
<td>17</td>
<td>Vomiting and diarrhoea</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>Coronaviridae</td>
<td>Between 20 and 220 nanometers diameter, Pleomorphic with petal-shaped projections 20 nanometers long, Single-stranded RNA in protein shell and lipid envelope.</td>
<td>37</td>
<td>Common cold</td>
</tr>
<tr>
<td>(Includes Enteric Coronavirus)</td>
<td></td>
<td></td>
<td>17</td>
<td>Gastro-enteritis</td>
</tr>
<tr>
<td>Norwalk agent and other small round viruses</td>
<td></td>
<td>About 20-35 nanometers diameter</td>
<td>39</td>
<td>Vomiting and diarrhoea</td>
</tr>
</tbody>
</table>

The overwhelming majority of data relating to the occurrence, persistence and distribution of enteric viruses in the environment concern the enteroviruses. This group is classified as a sub-group of the Picornaviridae (Melnick et al., 1979), and is responsible for a variety of infections of the respiratory tract, the central nervous system and organs such as the eye and the heart. However, with the possible exception of Poliovirus, the entire group is hardly ever associated with waterborne infection. Between 1913 and 1953 there were only eight suspected outbreaks of enteroviral waterborne disease; the number of cases in each outbreak was relatively low (median = 63) and the aetiology was not proven (Morris, 1986). Because the group are relatively amenable to isolation from the aquatic environment, this observation implies that the enteroviruses are not the principal agents of 'non-bacterial gastroenteritis' resulting from waterborne outbreaks. This non-association, combined with the absence of data relating to more likely agents of waterborne viral disease, has inevitably reduced the impact of water virology on water engineering practice.

In contrast, despite the lack of suitable methodologies for detecting hepatitis viruses in water, there is little doubt about their role in waterborne disease. The diagnosis of the infection is related to readily recognised symptoms and (usually) a specific agent, and thus it has been possible to link hepatitis with waterborne transmission for many years. In fact there have been at least fifty outbreaks involving Hepatitis A virus since 1895 and these have resulted in numbers of cases ranging between 6 and 800 (Morris, 1986). Confirmed cases of waterborne Hepatitis A infection in the United States constitute approximately 0.4 per cent of all infections (Feachem et al., 1983). Hepatitis A virus is now also classified as a member of the Picornaviridae (Skidmore & Tadros, 1976). In addition, a less well defined agent of infectious hepatitis described as Non-A Non-B Hepatitis virus has been confirmed serologically as the responsible agent in three particularly large outbreaks of waterborne disease since 1955. These outbreaks resulted in 29,300, 2,572 and 1,169 cases.
Clearly, the above observations (the lack of an association between enteroviruses and waterborne disease and the explicit association of waterborne hepatitis with just two agents), do not provide an adequate explanation for the high proportion of waterborne outbreaks for which there is no demonstrable aetiology. Thus, substantial efforts have been devoted to the identification of other agents (including bacteria, protozoa and viruses) which may be responsible for these outbreaks.

Among those human enteric viruses recently postulated to have a waterborne transmission route (based on symptomatic and serologic evidence), Norwalk agent and other small, round viruses appear to have some importance. Indeed, they have been suggested as likely agents of gastroenteritis mediated by recreational contact with polluted water (Cabelli, 1980), and may be a major cause of waterborne disease outbreaks in many parts of the world. Morris (1986) listed ten outbreaks of waterborne Norwalk-like infection between 1976 and 1982 in the United States and Australia involving between 6 and 1,500 cases (median = 217). Furthermore, Morris claimed indirect epidemiological evidence for 8 Norwalk-like waterborne outbreaks in the United Kingdom between 1950 and 1982. Other candidates for waterborne viral gastroenteritis include Astrovirus, Calicivirus, Coronavirus (Banatvala, 1981), and Adenovirus types 4 and 41.

However, the viral agents which have the strongest demonstrated association with diarrhoeal disease and which have been clearly implicated in a number of waterborne outbreaks are the Rotaviruses. Between 1977 and 1982/3 there were at least nine outbreaks of waterborne rotaviral gastroenteritis and in all but one outbreak the agent was identified in stool specimens. Outbreaks of waterborne disease involving Rotavirus have a very wide geographical distribution and are usually associated with a large number of cases. Examples, with the number of cases, include: Sweden - 3,172 (Lycke et al, 1978), USSR - 173 (Zamotin et al, 1981), Malaysia - 600 (Lam 1982), Brazil - 850 (Sutmoler et al, 1982), United States - approximately 500 per annum (Murphy et al, 1983), United States - 41 (Hopkins et al, 1984), German Democratic Republic - 11,600 (Walter et al, 1984), and China - 5942 and 7369 (Tao et al, 1984).
The Rotaviruses are members of the Reoviridae group; there are at least two serotypes, approximately 70 nm in diameter and they comprise a double stranded RNA genome in a physically characteristic protein shell. Rotavirus was first described in the early 1970s (Bishop et al, 1973; Flewett et al, 1973), and since that time has become generally recognised as a significant cause of gastroenteritis.

In industrialised countries, Rotavirus is responsible for approximately half of all hospitalisations due to diarrhoea in children. The prevalence of Rotavirus infection in hospitalised children in various countries has been reviewed by Feachem et al (1983): Rates varied from 39 - 71 per cent in a Washington hospital depending on season (Brandt et al, 1979), to 40 per cent in Bangladesh (Black et al, 1979), 41 per cent in Venezuela (Viera de Torres et al, 1978), 25 per cent in Mexico (Espejo et al, 1978), 31 per cent in Tanzania (Brookfield et al, 1979), and 14-41 per cent in Kenya (Mutanda, 1980). It should be noted that there is evidence to suggest that because of its severity for young children Rotavirus is associated with a higher proportion of hospitalisations than it is with total infections (Spencer et al, 1980).

Even before human Rotavirus (HRV) was isolated from aquatic environments, its association with non-bacterial waterborne disease outbreaks was suspected. Reasons for this were summarised by Vaughn et al (1986):

i) no human population is devoid of HRV antibodies;

ii) Rotavirus is responsible for many cases of acute, endemic and epidemic diarrhoeal disease of both children and adults;

iii) numbers of virus excreted during infection are very large;

iv) there is little attenuation of the virus during conventional sewage treatment; and

v) Rotavirus is relatively stable in aquatic systems.
In fact, it is now known that Rotavirus is distributed ubiquitously in the aquatic environment. Despite the relatively recent development of methods for the isolation and enumeration of Rotavirus from water (in 1983, Feachem et al were still able to say "the virus cannot be routinely isolated from water"), the virus has been demonstrated in virtually every part of the water cycle. A summary of isolations is included in the comprehensive review of all viruses detected in water by Bitton and co-workers (1985). Rotavirus has been found in drinking water, even after conventional treatment (Smith & Gerba, 1980; Deetz et al, 1984) as well as during outbreaks of waterborne disease (Murphy et al, 1983). The virus has been isolated from river water (Epp, 1984), wastewater (Hejkal et al, 1982) and sediments (Rao & Melnick, cited by Bitton et al, 1985).

It has long been assumed that the primary transmission route for human Rotavirus, (and indeed most other viruses implicated in faecal-oral infections including hepatitis), is the direct person-to-person transfer of faecal contamination. At present, there is no evidence to contradict this belief. However, the potential for waterborne transmission of Rotavirus exists, and there is direct evidence of large outbreaks affecting both children and adults. In addition, there is an unquantifiable theoretical risk of low-level non-epidemic transmission arising from the contamination of drinking water by human enteric viruses (World Health Organization, 1979). This risk arises from the assumed low infective dose of human Rotavirus, and the large number of asymptomatic cases.

The saturation-threshold dose-response curve for faecal-oral infection proposed by Esrey et al (1985) and illustrated in Figure 9 indicates that for agents with a low infective dose, improvements in water supply and sanitation need to be substantial in order to obtain measurable reductions in disease.
In particular, it follows that for agents such as human Rotavirus which are not primarily waterborne, the contribution of moderate improvements in water quality may be irrelevant to the overall incidence of the disease.

To place this implication in perspective, it should be noted that it may indeed be possible to maintain a cycle of re-infection and endemicity within a community which is constantly exposed to low level contamination of drinking water by Rotaviruses. Thus, although the primary transmission route for cases of acute Rotavirus diarrhoea may be person-to-person, its maintenance in the population could be assisted by continuous low level waterborne infection.

There is some evidence to suggest that infective doses for some human enteric viruses may not be as low as previously concluded from volunteer studies. For example, it has been demonstrated that the number of viruses administered to volunteers may be substantially higher than is suggested by existing detection systems which are unable to distinguish between single and aggregated virus (Ward & Akin, 1984).

Notwithstanding these caveats, it is reasonable to conclude that on the basis of current knowledge, moderate improvements in water quality should not be expected to result in noticeable impacts on the incidence of rotaviral diarrhoea. Thus, in planning water supply improvements in developing countries where much diarrhoeal disease is rotaviral, the need to assure substantially enhanced water quality may be unavoidable. There appears to be no case for the philosophy that "some improvement is better than none". In the absence of appropriate methods of disinfection, there is only one technology which is capable of reliably improving bacteriological water quality by several orders of magnitude. The process is Slow Sand Filtration. To date it has not been shown that the process is capable of removing human Rotavirus from water with the same efficiency as it is known to remove bacterial indicators and pathogens.
1.4 THE ADVENT OF SLOW SAND FILTRATION

"In the construction of filter beds, water works engineers have certainly never been guided by an acquaintance with the habits of microorganisms and yet by carefully improving their methods, so as to secure the removal of visible suspended matter, they have hardly less successfully, although unconsciously, attacked the invisible particles, and reduced them to an extent which is surprising."

P F Frankland (1886)

The history of the development of the Slow Sand Filtration process has been reviewed by Lloyd (1974). The earliest references to filtration through granular media preceed the birth of Christ. However, the first documented technical descriptions of processes which incorporated sand filtration and which were reproducible in domestic applications were to be found in the late 17th and 18th centuries. By the beginning of the 19th century, filtration through sand was beginning to show promise in community water supply in the United Kingdom, and by the end of that century a process recognisable as Slow Sand Filtration had proven sufficiently effective to find widespread application in urban water supply in Northern Europe (Baker, 1949), with approximately 20 million people benefiting from the process (Hazen, 1896).

The first large scale sand filter in London was constructed by James Simpson for the Chelsea Water Company in 1829. Simpson claimed nothing more than a filter capable of removing suspended particles from impounded river water. The previous year, the same Royal Commission which had accepted water quality as a valid cause for public concern concluded that filtering water through a sand bed was an appropriate and inexpensive means of removing such matter from drinking water sources. The mode of operation of sand filters was believed to be simply that of mechanical sieving (Buffle, 1984). Bearing in mind that these particles and 'animalcules' were
considered to have no relation to health (this official point of view prevailed well into the second half of the 19th century), the principal perceived benefits of sand filtration at the time were evidently predominantly aesthetic.

In 1852, sand filtration made its first appearance in UK legislation when its application became mandatory in Metropolitan London. The Metropolis Water Act stipulated that "Every company shall effectually filter all water supplied by them within the Metropolis for domestic use, before the same shall pass into the pipes for distribution."

Thus by 1884, the seven London water companies applying sand filtration were employing filtration rates, media and depths of media which were consistent with a process which may be described today as Slow Sand Filtration (SSF). Average flow rates varied between 0.061 and 0.102 m\(^3\)/m\(^2\)/h; media were various types of fine sand overlaying gravel drainage; and sand depths varied between 0.61 and 1.37 m (Bolton & Scratchley, 1888).

It should be noted that the science of microbiology played no part in the development of the process. Only in 1886 was it reported by Percy F Frankland that sand filtration reduced bacterial counts in the River Thames by 97.9 per cent: "...even the strongest advocate of sand filtration could not have reasonably anticipated that filtration through a few feet of material could effect this remarkable reduction in the number of micro-organisms" (Baker, 1949).

It was not until the Hamburg cholera epidemic of 1892 that the ability of Slow Sand Filtration to provide protection against waterborne epidemic diseases was fully recognised (van de Vloed, 1955). And the first thorough review of the biological populations present in functional slow sand filters was not presented to an audience of water engineers until the very end of the 19th Century (Kemna, 1899). However, by that time the design and operating principles of the process had long been established by empirical factors bearing little or no relation to the microbiological dimension or to the immense public health potential of Slow Sand Filtration.
The development of sand filtration in this century has been characterised by a significant divergence in water treatment practice between North America and Europe (Lloyd, 1974, Logsdon & Fox, 1988). The development of small sand filters employing coarse media and rapid flow velocities by Patrick Clark in the United States resulted in the virtual eclipse of Slow Sand Filtration in that country. Thus by 1940 there were only 100 slow sand filters in the US compared with 2275 rapid sand filters (Baker, 1949). The scepticism with which most 20th century US water engineers viewed such a low technology, labour intensive process as Slow Sand Filtration led to increased reliance on the processes of chemical coagulation, rapid sand filtration and disinfection in most industrialised countries. It has even been proposed that existing slow sand filters might usefully be converted or replaced by direct dual media filters with automatic cleaning (Willis, 1972).

However, more enlightened commentators in the US have long argued for an appropriate role for Slow Sand Filtration, particularly in small communities where constant maintenance of automated plants is difficult to guarantee (Mariner, 1946). And in recent years, US interest in the process has gained considerable momentum (Logsdon & Fox, 1982; Cleasby et al, 1984; Fox et al, 1984; Bellamy et al, 1985; Logsdon & Fox 1988), the previous disinterest in Slow Sand Filtration giving way to an active consideration of its merits. Notwithstanding this renewed interest, the number of functional Slow Sand Filtration facilities in the US remains small. In a recent survey only forty-seven slow sand filter works were identified (Slezak & Sims, 1984). The majority of these plants served communities of less than 10,000 persons and most were more than fifty years old. In general, plants were operated at flow rates of less than 0.25 m²/m²/h.

Part of the reason for the reassessment of Slow Sand Filtration in the United States is the increasing recognition of the inherent disadvantages of automated 'fail-safe' treatment plants. Most importantly, in small community supplies, breakdowns can result in outbreaks of waterborne disease (Craun & Gunn, 1979). Moreover, some of the processes which are used in automated plants may have potential hazards. There is evidence of breakthrough by pathogens, (Keswick et
al, 1984), production of excessive quantities of chlorinated organic compounds in treated waters (Collins & Eighmy, 1988), and carry-over of potentially harmful constituents eg aluminium from alum coagulants (Wheeler, 1986). Hence, as a result of the introduction of new regulations in the United States, it is estimated that approximately 1,000 slow sand filters may need to be constructed for the treatment of surface waters (Logsdon & Fox, 1988).
SECTION 2

THE DEVELOPMENT OF AN EXPERIMENTAL PROTECTED SLOW SAND FILTRATION SYSTEM
2.1 GENERAL CRITERIA

The design criteria for conventional slow rate sand filtration in less developed, or non-industrialised countries have been described in some detail by several authorities, most notably the International Reference Centre (IRC) for Community Water Supply and Sanitation (van Dijk & Oomen, 1978). Other reviews have been provided by the Asian Institute of Technology (Thanh & Pescod, 1976), and the Centro Panamericano de Ingenieria Sanitaria y Ciencias del Ambiente - CEPIS (Perez & de Vargas, undated). A definitive statement on the planning, design, construction, operation and maintenance of Slow Sand Filtration for community water supply based on the IRC seven-country Research and Demonstration Project on Slow Sand Filtration has been published (Visscher et al, 1987).

Some of the most interesting work undertaken under the aegis of the IRC Research and Demonstration Project was conducted in India, Thailand, and more recently, Colombia (Figures 10 and 11). Other countries involved were Jamaica, Sudan, Ghana and Kenya.

FIGURE 10 Circular slow sand filters in La Sirena, Cali, Colombia.
The research conducted in India by the National Environmental Engineering Research Institute (NEERI) was undertaken in two phases. The first phase was concerned with laboratory and field validation of the operational characteristics of Slow Sand Filtration (NEERI, 1977). From a microbiological perspective, some important observations were recorded; among these were the effect of filtration velocity on dissolved oxygen levels, and the effect of shading and intermittent operation on performance.

Flow rates of between 0.1 and 0.3 m/h were observed to result in very similar bacteriological quality in treated waters. However, at the lowest flow rate of 0.1 m/h, dissolved oxygen levels in treated waters dropped to less than 1.0 mg/l on occasion. Thus it was concluded that higher flow rates may be preferable in tropical climates in order to avoid anaerobiosis and consequent reduction in both the microbiological and organoleptic quality of the filtrate. Higher flow rates were also noted to increase the overall volume output of filters although at a cost of reducing the average length of filtration runs.
By shading experimental filters and running them on a continuous flow regime, it was observed that oxygen levels in the supernatant and filtered waters remained relatively constant. This was assumed to be due to the inhibition of algal growth in water overlying the filter. When there was no shading or when filtration was discontinuous, dissolved oxygen levels in the treated water varied, and in the case of discontinuous operation bacteriological quality declined.

The second phase of the NEERI research was devoted to a demonstration of Slow Sand Filtration technology in four villages with populations in the range 1300 - 12700 persons (Sundaresan & Paramasivam, 1982). These demonstrations were integrated with other activities in each location, including community motivation and hygiene education. The installations were reviewed with respect to design and economic criteria as well as microbiological and physico-chemical performance. Although raw water quality in three of the installations did not represent a major challenge to the process, the slow sand filters nevertheless provided filtrates of reliable bacteriological and physico-chemical quality.

Experience in India and in other collaborating countries in the IRC programme of research has allowed the establishment of some general parameters for slow sand filter design (Heijnan & White, 1981). Firstly, there is a need for flexibility in allowing for population growth or increased water usage when planning future demand for community water supplies. Thus a Slow Sand Filtration system should lend itself to augmentation towards the end of a 10 - 15 year design period by the addition of extra filtration area. A minimum provision of two filters ensures continuity of treatment during maintenance and cleaning. For villages of less than 1000 persons, prefabricated units are likely to be more economic than filters constructed conventionally on site using concrete, masonry or ferrocement. Optimal use should be made of gravity flow in order to minimise the need for pumping. In addition, there are a number of specific recommendations which can be made with respect to such factors as filter media, flow rates, covering and prefiltration.
2.1.i Filtration Media

There is broad agreement that the minimum depth of media in slow sand filters should be 0.6 - 0.7m. In their review of Slow Sand Filtration research in Colombia, Visscher & Galvis (1987) noted that sand size may be related to the efficiency of removal of total coliforms. In fact it has been proposed (Bellamy et al., 1985) that increasing sand diameter may have a linear inverse relationship with the percentage average removal of coliforms. However, it seems clear from experience in a number of countries, that provided the effective size* of filter medium remains within the range 0.15 - 0.3 mm and the coefficient of uniformity** is within the range 2.0 - 3.0, the process will operate optimally, and within those ranges little difference in bacteriological quality of filtrates will be observed. In many cases, clean builder-grade sand is chosen as a substitute for specially selected and graded media. However, the ideal distribution of sand grain sizes may be calculated and expressed graphically (Figure 12).

![Figure 12 Optimal limits for sand size grading for slow sand filters.](image)

* Effective size is the sieve opening through which 10 per cent of grains will pass ($d_{10}$).

** Coefficient of uniformity is the ratio of the sieve opening through which 60 per cent of grains will pass to the effective size ($d_{60} : d_{10}$).
The use of builder-grade sand has been demonstrated to function at least as efficiently as optimally graded material (Pardon et al, 1983). In fact, longer filtration runs may result from the application of coarser sands than are conventionally recommended (NEERI, 1977; Bowles et al, 1983). However, for Slow Sand Filtration to be effective, one of the most important criteria remains the selection of a relatively uniform, clean sand with a low silt content.

The selection of the principal filter medium may often be governed by the local availability of appropriate materials. In rural areas of less developed countries this will invariably be ungraded sand eg from a river bed or other deposit. However, other options have been examined. A dual filtration system based on shredded coconut husks and burnt rice husks has been described (Frankel, 1972; Frankel, 1974). The concept has been developed with apparently satisfactory results (Thanh & Pescod, 1976; Frankel, 1981; Barnes & Mampitiyarachichi, 1983). Multi-medium filters have also been used which employed gravel, sand and charcoal (Merchant, undated) as well as more conventional dual medium filters (Renade et al, 1976).

Notwithstanding other possibilities, sand is usually the preferred principal medium. However, there are methods for optimising its efficiency as a biological filtration medium. The use of natural fabrics in water treatment practice was first suggested in 1703 (Baker, 1949). Experience in less developed countries, in Britain and in Belgium with synthetic fabrics or foam overlaid on the surface of a slow sand filters has confirmed the potential of direct protection in enhancing the filtration process (Lloyd et al, 1983; Bridges, 1985; Mbwette & Graham, 1987; Mbwette & Graham, 1988; Vochten et al, 1988).

Recent research in the United States (McNair et al, 1987) has shown that the incorporation of an ammonium - selective zeolite, clinoptilolite, as an 80 mm layer above the sand may permit higher flow rates and enhanced removal of Giardia without adversely affecting schmutzdecke formation.

Such observations have led to the concept of Protected Slow Sand Filtration (PSSF) which forms an integral part of the design of the experimental system developed for this Study.
2.1.ii Filtration Rate

There is now general acceptance of the fact that flow rates for Slow Sand Filtration may rise to 0.3 m/h without substantial deterioration in the microbiological or physico-chemical quality of filtrates. In fact, higher flow rates tend to reduce oxygen consumption (depletion) and yield higher overall outputs of water per filter run (Paramasivam et al, 1981). Nevertheless, a law of diminishing returns applies, because at high flow rates the increased frequency of cleaning eventually results in an unacceptably high proportion of time when the filter is unproductive (Kerkhoven, 1979). Despite this, flow rates as high as 0.6 m/h have been proposed on economic grounds (Agrawal et al, undated). This analysis would appear to take little account of the biological nature of the Slow Sand Filtration process.

The United Kingdom Thames Water Authority currently operates several of its largest slow sand filter treatment works with filtration rates of 0.2 - 0.3 m/h. This represents a doubling of the design rate in some cases. Huisman and Wood (1974) considered flow rates of between 0.1 and 0.4 m/h to be normal, and reported no significant difference in efficiency of filtration at rates of 0.1, 0.25 and 0.45 m/h.

The maintenance of a constant filtration rate is also an important factor in biological efficiency. Fluctuations in filtration velocity can result in disturbance of biological communities and thus every effort should be made to incorporate some sort of flow control either at the inlet or the outlet of the sand filter. Ideally, such flow control should be automatic or self-adjusting in order to minimise the need for operator intervention (Pardon et al, 1983).
2.1.iii Filter Covering

The effect of covering or shading on the efficiency of Slow Sand Filtration is not great (NEERI, 1977). However, there is no doubt that on a small scale it is advisable. A small filter is very vulnerable to direct pollution eg by avian defaecation. This factor has been postulated to be one of the probable causes of poor filter performance following the cleaning of conventional slow sand filters (Burman, 1962). Covering also prevents some heat loss in severe cold conditions - this is an important consideration for small scale installations subject to freezing. Covering also obviates the proliferation of unicellular algae in the supernatant water thereby preventing premature blockage. Experience in the Netherlands indicates that the covering of slow sand filters can allow a four-fold increase in filtration rates in some circumstances (Houghton, 1970).

2.1.iv Prefiltration

Because Slow Sand Filtration is both a physico-chemical and biological process, designs should take account of the need for stability and continuity of operation. For example, a major consideration in the effective application of Slow Sand Filtration is that the loading of influent suspended solids should not be so great as to cause premature blockage of filters. Because the process depends on biological mechanisms which may take several days to become established, short filter runs are unproductive.

For this reason, many authorities consider a mean influent turbidity of 10 NTU (nephelometric turbidity units) to be the maximum for cost-effective and efficient operation of Slow Sand Filtration systems. Where raw water turbidities regularly exceed 10 NTU, some form of pretreatment is necessary (Lloyd et al, 1986).
The various means of pretreatment employed in industrialised countries include reservoir storage, chemical coagulation, rapid sand filtration, micro-straining and infiltration. Two technologies which are not conventionally employed on a large scale are gravel filtration and pumped abstraction through sand. However, these two methods of water clarification have both been proposed as possible options for small community water supply systems subject to high raw water turbidities (Lloyd et al 1983; Wegelin, 1986; Wegelin, 1988). In addition, both pretreatments have potential for the microbiological improvement of influent waters.

Horizontal coarse medium gravel filtration was employed prior to sand filtration by John Gibb in Paisley, Scotland, as early as 1804 (Baker, 1949). A 23 metre trench, 2.4 x 1.2 m in section, provided roughing filtration and was operated at a flow rate of 0.43 m/h. Since then, the concept of roughing filtration has been somewhat neglected in industrialised countries, reliance being placed increasingly on reservoir storage, rapid sand filtration, and latterly in this century, micro-straining and chemical clarification. However, interest in the process of gravel filtration is now increasing, especially in developing countries.

Sevilla (1971) investigated the function of roughing filters with respect to optimum filtration rates and influent turbidity limits on a laboratory scale. It was concluded that a "series filtration" system comprising prefiltration and Slow Sand Filtration was most appropriate for the minimisation of labour and training costs in surface water treatment. Kuntschick (1976) described the efficiency of full-scale horizontal gravel filters operating at velocities of approximately 1.5 - 20 m/h. Even at such high flow rates, substantial reductions in suspended solids loadings were achieved. Attention was drawn to the low frequency of maintenance (up to 60 months between cleaning) and the phenomenon of a maturation process which took approximately 14 days.
Lower flow rates were recommended by van Dijk and Oomen (1978); using filter bed depths of approximately 1 m and lengths of between 4 and 10 m, filtration rates of between 0.4 and 1.0 m/h were considered appropriate. The use of alternating coarse and fine gravel has been recommended. Thanh (1978) employed an alternating series of gravel media and obtained turbidity removals of approximately 50 per cent and coliform reductions of approximately 80 per cent in filters 6 x 2 x 0.8 m operated at 1.5 m/h. More recently, Thanh and Hettiaratchi (1982) whilst retaining the principle of variation in gravel size, recommended lower flow rates of 0.5 m/h for influent turbidities in the range 15 - 50 NTU and 0.3 m/h for turbidities of up to 150 NTU. Turbidity and coliform reductions of 60 - 70 per cent and 80 per cent respectively were predicted.

Hofkes (1983) suggested flow rates of 0.5 - 1.0 m/h for longitudinal gravel filtration employing different gravel sizes in series. CEPIS (1982) investigated the viability of horizontal prefiltration in Latin America and proposed a flow rate of 0.5 m/h for a three stage 10 m filter using decreasing sizes of gravel in each section.

A detailed series of laboratory and field trials was undertaken by the University of Dar es Salaam, Tanzania (Wegelin, 1980; Mbwette & Wegelin, 1982). This research confirmed that pretreatment by horizontal gravel filtration could successfully prolong slow sand filter run lengths. In addition, it was demonstrated that efficiency improved both with increasing filter length and decreasing filtration rate. These important observations imply that retention time may well be the most critical factor in optimal performance of horizontal gravel filtration. In general, the smaller the aggregate size, the more efficient the removal of turbidity. However, the use of three or four separate zones of filter medium with grain sizes in the range 2 - 40 mm were recommended for ease of operation and overall efficiency.
Vertical filtration through gravel has also been investigated, and three layer beds of 2 m and 0.8 m depth have been recommended by the International Reference Centre for Community Water Supply and CEPIS respectively. Cleaning of vertical gravel filters is particularly simple, requiring only a rapid drain-down to effect a gravity wash of the media. Similar recommendations have been made for downflow filters employing a matrix of pebbles and sand (Ives & Rajapakse, 1988).

The use of river bed infiltration, usually employing gravity percolation and collection via a network of underdrains is well known (van Dijk & Oomen, 1978; Thanh & Hettiaratchi, 1982). Infiltration through sand is used in full scale conventional water treatment in Europe, and information on the bacteriological and virological effectiveness of this form of pretreatment is available (Kool, 1979).

Methods for increasing water availability from a small river bed site by sub-sand abstraction using a pump have been proposed (Cansdale, 1982). Unless sited in a fairly fast flowing river, these systems are not usually self-cleaning and require maintenance on a periodic basis ie scraping or backwashing to overcome blockage. There is little evidence that sub-sand abstraction can be successfully applied in small community supplies in less developed countries. However, provided that flow rates are comparable with those employed in Slow Sand Filtration, and if pumping is continuous, worthwhile improvements in the physical and microbiological quality of surface water may be achieved (Wheeler, 1978; Smet & Wheeler, 1988).
2.1.v. Summary

In summary, the key features of slow sand filter design for small communities in rural areas of less developed countries are:

i) Protection by pretreatments where turbidities exceed 10 NTU.
ii) A flow rate of 0.1-0.3 m/h.
iii) A minimum filtration medium bed depth of 0.6 m.
iv) Appropriate filtration medium.
v) Covering to prevent the ingress of contamination and light.
vi) Automatic or self-adjusting constant flow control.
vii) Modular design to allow extensions.
2.2. SYSTEM DEVELOPMENT

The conventional design of a slow sand filter is illustrated in Figures 13 and 14. Flow control may be effected either at the inlet by means of a V-shaped inlet weir, or at the outlet by means of a valve or floating weir (constant flow) arrangement. Filters may be rectangular or circular in plan (Figures 15 and 16), but common features include a minimum of two filtration tanks, under-drainage, inlet or outlet flow regulation, filter medium and supernatant water. Filter tanks are usually constructed of concrete, ferrocement or masonry, or a combination of these.

The majority of Slow Sand Filtration demonstration treatment plants worldwide have served village populations of more than 500 people. In India, designs were for populations of between 1300 and 12700 (Sundaresan & Paramasivam, 1982). In Colombia, the promotion of the technology by the Universidad del Valle and IRC was proposed in communities with projected populations of between 1500 and 4500 (Visscher & Galvis, 1987). Thanh (1978) described the construction of a demonstration plant comprising horizontal roughing filtration and Slow Sand Filtration for a design population of 967 persons.

One of the reasons why research on Slow Sand Filtration has been restricted to 'pilot scale' filters is that conventional slow sand filters are generally considered to be concrete or masonry structures of considerable size. However, many of these pilot studies, including those of Lloyd (1974), NEERI (1977), Bowles et al (1983), Cleasby et al (1984) and McNair et al (1987), have employed filters of dimensions suitable for small community water supply. Several other studies, including those concerned with the removal of enteric viruses by sand filtration (Lefler & Kott, 1974; Poynter & Slade, 1977; McConnell et al, 1984), have been conducted on a laboratory scale using relatively narrow cylinders of perspex or glass.

There are a number of possible disadvantages in employing experimental systems which are of substantially different dimensions to those facilities they are designed to simulate. Flow
A: raw-water inlet valve
B: valve for drainage of supernatant water layer
C: valve for back-filling the filter bed with clean water
D: valve for drainage of filter bed and outlet chamber
E: valve for regulation of the filtration rate
F: valve for delivery of treated water to waste
G: valve for delivery of treated water to the clear-water reservoir
H: outlet weir
I: calibrated flow indicator

FIGURE 13 Basic components of an outlet controlled conventional slow sand filter (Visscher et al, 1987)

A: valve for raw-water inlet and regulation of filtration rate
B: valve for drainage of supernatant water layer
C: valve for back-filling the filter bed with clean water
D: valve for drainage of filter bed and outlet chamber
E: valve for delivery of treated water to waste
F: valve for delivery of treated water to the clear-water reservoir
G: inlet weir
H: calibrated flow indicator

FIGURE 14 Basic components of an inlet controlled conventional slow sand filter (Visscher et al, 1987)
FIGURE 15 Rectangular plan conventional slow sand filter (Visscher et al, 1987)

FIGURE 16 Circular plan conventional slow sand filter (Visscher et al, 1987).
Characteristics may be different and the potential for edge-effects (streamlining of water between the filter medium and the walls of the filter), is much greater for narrow filters constructed from plastic materials than for larger structures made of concrete. Constant flow control is not readily achieved on a small scale, and maintenance procedures may not be equivalent. Thus, the experimental system developed for the purpose of this study was of a scale suitable for application in water supplies of rural communities in less developed countries (Lloyd et al, 1985; Lloyd et al, 1986; Lloyd et al, 1988).

Adopting the general design and construction criteria listed in 2.1 (above), a prototype Protected Slow Sand Filtration (PSSF) system was developed. This system represented the principal test facility for observing the microbiological performance of sand filters, and a brief description of the main components and their development is provided in the following paragraphs.

Having conducted preliminary investigations with two sand filters of 0.8 m² filtration area for a period of 7 months (Wheeler et al, 1983), the first design scale slow sand filter (1.5 m² filtration area) was commissioned. The module was approximately cubic (dimensions 4 x 4 x 4 ft), constructed from medium density polyethylene (MDPE), and supported by a rigid frame. The tank was a commercially available unit (WCB Rotomoulding Ltd, Stalybridge, UK) and although it could be modified to permit further experimentation on the desired scale, it was not entirely appropriate on grounds of cost, shape, and structural rigidity.

Following consultation with the manufacturers, it was apparent that the rotation moulding process could provide an MDPE tank to precise design specifications at relatively low unit cost. The first units commissioned as protected slow sand filters were evaluated at the University of Surrey Manor Farm field station (Figure 17, Plate I). Subsequently, two units were installed at the Hampton Water Treatment Works of the Thames Water Authority to permit long term investigations of filter efficiency (Plate II). Concurrently nine units were installed in three geographical zones in Peru in order to provide data relating to operational efficiency under rigorous field conditions which included poor raw water quality (Plates III and IV).
FIGURE 17 Construction and dimensions (mm) of the protected slow sand filter and flow controller.

The requirement for automatic flow control to obviate the need for the daily adjustment of flow rates and thus allow for filter blockage, was met by the design of a constant flow controller based on the principle of the floating weir (Figure 18). These devices were manufactured in the Biological Services Unit of the University of Surrey from standard plumbing parts and fittings. Constant flow controllers were initially installed to coincide with the evaluation of the first purpose-designed PSSF units at the University of Surrey field station. For the remainder of the research programme flow controllers were commissioned from Pendar Environmental Ltd of Bridgwater, UK.

Synthetic filter fabrics were included as a protection for the sand bed from the early stages of experimentation. The materials eventually selected for inclusion in the PSSF package were needle-punched polypropylene fabrics of two different densities. However a variety of coarse fabrics were obtained from commercial suppliers and several were evaluated for physical and microbiological efficiency before final selection was made. Complete technical specifications of the experimental plant thus developed are provided in Appendix 1.
FIGURE 18 Mode of operation of flow controller showing position of float at start (A) and end (B) of filtration run.
PLATE I  Experimental protected slow sand filter installation at the University of Surrey Manor Farm field station.

PLATE II  Experimental protected slow sand filter installation at Thames Water Authority Hampton Water Treatment Works.
PLATES III & IV Experimental protected slow sand filter installations in the communities of San Buenaventura, Canta Valley and San Vicente de Azpitia, Mala Valley, Peru.
2.3. MATERIALS AND METHODS

The evidence from a large number of studies of Slow Sand Filtration suggests that the best indicators of filter performance may be restricted to a relatively small number of microbiological, chemical and physical parameters. These parameters provide information on filter efficiency and operational characteristics, but it should be noted that they may not adequately describe the functional mechanisms which determine the removal of enteric viruses by the process.

The tests which were undertaken routinely to demonstrate the performance of the experimental Protected Slow Sand Filtration system during its development are listed below together with their relevance to operational efficiency:

<table>
<thead>
<tr>
<th>BACTERIOLOGICAL PARAMETERS</th>
<th>RELEVANCE*</th>
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</thead>
<tbody>
<tr>
<td>Reduction in Thermotolerant (Faecal) Coliforms</td>
<td>1, 2</td>
</tr>
<tr>
<td>Reduction in Faecal Streptococci</td>
<td>1, 2</td>
</tr>
<tr>
<td>37°C General Plate Count (Variation with Depth)</td>
<td>3</td>
</tr>
<tr>
<td>22°C General Plate Count (Variation with Depth)</td>
<td>4</td>
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<table>
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<tr>
<th>CHEMICAL PARAMETERS</th>
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<tbody>
<tr>
<td>Depression in Dissolved Oxygen</td>
<td>1, 3, 5</td>
</tr>
<tr>
<td>Alteration of pH</td>
<td>-</td>
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</tbody>
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<table>
<thead>
<tr>
<th>PHYSICAL PARAMETERS</th>
<th>RELEVANCE</th>
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</thead>
<tbody>
<tr>
<td>Reduction in Turbidity</td>
<td>5, 6</td>
</tr>
<tr>
<td>Head Loss</td>
<td>7</td>
</tr>
<tr>
<td>Size Grading of Filter Medium</td>
<td>8</td>
</tr>
<tr>
<td>Silt Analysis of Filter Medium</td>
<td>9</td>
</tr>
<tr>
<td>Filterability</td>
<td>6, 10</td>
</tr>
<tr>
<td>Suspended Solids</td>
<td>6, 10</td>
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*See Overleaf
RELEVANCE OF TEST

1. General microbiological performance including filter maturity
2. Hygienic quality of filtered water
3. Indirect indication of microbiological performance
4. Indication of non-attached heterotrophic populations
5. Aesthetic quality of filtered water
6. Indication of physical performance
7. Indication of progress of filter run
8. Characteristic affecting progress of filter run
9. Characteristic affecting progress of filter run
10. Indication of likely progress of filter run

2.3.i. Bacteriological Parameters

Thermotolerant (faecal) coliforms were enumerated by the standard membrane filter technique recommended in the United Kingdom (Anon, 1983), the United States (Anon, 1985) and by the World Health Organization (1984a). An appropriate volume of water was drawn under vacuum through a sterile membrane filter of nominal pore size 0.45 μm and diameter 47 mm (Gelman Sciences, UK). By this process bacteria are retained within the pores formed by a mesh of linked cellulose nitrate and cellulose acetate ester fibres. The membrane was transferred onto a nutrient broth pad which had been soaked in an excess of recovery medium (Membrane Lauryl Sulphate Broth, Oxoid, UK). The membrane was then incubated in a moist environment at 30°C for four hours followed by 44°C for 14-18 hours. Colonies of thermotolerant coliforms were recognised by their yellow colour due to the production of acid from lactose in the presence of phenol red.
Enumeration of thermotolerant coliforms at field installations (including those in Peru) required the development of a portable membrane filtration and incubation system. The system was produced initially in the Biological Services Unit workshop of the University of Surrey and was validated with reference to conventional incubation systems (Appendix II).

Faecal streptococci were enumerated by a similar membrane filter technique to that employed for thermotolerant coliforms. Membranes were incubated on MF Enterococcus Agar (Gibco Europe) at 37°C for 4 hours followed by 44°C for 44 hours. Colonies of faecal streptococci were recognised by their pink or red colour attributable to the reduction of 2,3,5-triphenyl tetrazolium chloride in the medium.

General plate counts at 37°C and 22°C were not conducted according to standard methods. These methods require the mixing of water samples of 1.0 ml with 15 ml of molten agar at a temperature of 45-50°C. They are designed to provide an index of microbiological stability in public water supplies, but in no way do the tests provide a reliable picture of the microbiological populations present (Kool, 1979). The recovery medium, Yeast Extract Agar (Oxoid) does not support the growth of fastidious micro-organisms, the aerobic incubation eliminates the anaerobic fraction of the population, and the use of molten agar can result in substantial loss of viability of vegetative cells. For the latter reasons, it was decided to employ a modified spread plate technique based on the use of an automatic spiral diluter (Gilchrist et al, 1972). Aerobic incubation on Yeast Extract Agar at 37°C and 22°C for 24 and 72 hours respectively was retained in order to provide the basis for comparison with other studies.
2.3.ii. Chemical Parameters

Dissolved oxygen measurements were made using pre-calibrated portable instrumentation in order that direct readings could be made on-site. This obviated the need for specific measures in sample preservation. Instruments used for dissolved oxygen measurement provided readings in per cent saturation (EIL, UK) which were then converted to mg/l with reference to water temperature and atmospheric pressure, or directly in mg/l (Horiba, Japan). In both cases, calibration was undertaken on a daily basis with reference to a standard saturated solution of oxygen in distilled water.

pH was measured with a conventional pH probe and meter which was calibrated immediately before use both in the laboratory (EIL, UK) or in the field (Horiba, Japan).

2.3.iii. Physical Parameters

Turbidity is a measure of the ability of suspended particulate matter in a water sample to deflect light, and may be quantified either by extinction methods (traditionally by blocking the light from a candle placed at increasing distance from the water sample) or nephelometry (ie the capacity to deflect light towards an electronic sensor placed at 90° to the light path). All turbidity measurements quoted in this Study were made using conventional nephelometric instrumentation (Fisher HF, Canada and Hach, US). However, in preparation for overseas field analysis, a new method of turbidimetric analysis was developed based on light extinction. Contrary to published information which suggests that there is no relationship between turbidity measured by nephelometry and turbidity measured by extinction, it was observed that by taking the logarithm of results obtained by both methods, a linear relationship can be derived (Figure 19).

This relationship permits for the first time an accurate calibration of the extinction method in the range 5 - 2000 TU where TU (Turbidity Units) are equivalent to NTU (Nephelometric Turbidity Units) based on formazine standards (Anon, 1985). The experimental work which demonstrated the relationship is described in Appendix II.
Head loss is a function of the degree of blockage of a filter, and increases with respect to time as material occludes the interstices of the medium. It is a measure of the reduction of water height (head) above the filter in comparison with the natural water level downstream and it is also related to flow rate; ie filter resistance increases with water velocity. In the case of slow sand filters and horizontal gravel pre-filters, head loss was measured by the insertion of manometer tubes directly into the media. The manometer tube ends were protected from ingress of granular filter media by a sleeve of coarse fabric which exerted no resistance to flow. Where filtration through sand was induced by pumping (in sub-sand pre-filtration), head loss was measured in terms of vacuum pressure by standard pressure guages placed on the suction lines between filter and pump.

FIGURE 19 Calibration curve for turbidity measurements by extinction compared with nephelometric standards.
Size grading of filter media was undertaken in order to provide a record of the suitability of the media for filtration. As has been noted, the size grading is not now considered as critical for filter performance as previously thought. However, it is clear that uniformity and effective size remain of some importance. Size grading of sand samples was accomplished by first drying a representative sample of approximately 200 g sand to constant weight in a hot air cabinet. The dried sample was then evenly dispersed and a 100 g sample weighed to an accuracy of 0.01 g on a top loading balance. The 100 g sample was then passed through a stacked series of pre-weighed British Standard sieves (Endecotts, UK) with a ten-minute period of vigorous shaking. Sieves were individually re-weighed with their sand fractions as well as the final fraction which passed all sieves. The weights of each fraction were calculated, and provided the weight of all fractions totalled 100 g ± 0.1 g, the result was accepted and the cumulative weights were plotted on a size grading graph similar to that illustrated in Figure 12.

Silt analysis is an empirical measure of the fraction (percentage by volume) of silt and other particulate matter which has been trapped in the filter medium. The test may be applied at different depths in the filter media thereby giving a quantitative comparison of the proportion of such matter which has been removed at each stage of the filter. The test is based on a traditional British waterworks test designed to assess the suitability of sand media for continued Slow Sand Filtration after cleaning (Anon, 1950). For efficient filtration, sand media should not have a silt fraction greater than 5 per cent by volume.

A sample of filter medium was placed in a 1000 ml measuring cylinder to a depth approximating in volume to 300 ml. The measuring cylinder was filled with clean water to a volume of exactly 1000 ml. The measuring cylinder was then inverted rapidly at least twenty times in order to dislodge particulate matter from the medium. With the material thus mixed and dissociated, the measuring cylinder was placed on a flat surface and left for a period of up to 24 hours. After 5 minutes and 24 hours, the volumes of sand (lower fraction of material which settles immediately) and silt (upper fraction of material which settles over a longer period) were noted. The fraction of
silt was expressed as a percentage of the volume of the sand fraction.

For silt assays of filter fabrics, a sample of fabric 100 x 100 mm was cut from the fabric layer and placed in a strong walled glass bottle. 500 ml clean tap water was added and the bottle shaken vigorously for 3 minutes. The water was then decanted into an Imhoff cone. The washing was repeated two times and water decanted into the same cone. After a period of five further minutes for settlement, the total volume of liberated silt was quantified.

Filterability is a test designed to establish the filter-blocking potential of raw waters. In this sense, it may be indirectly related to turbidity (and indeed suspended solids); however, the relationship is unlikely to be a quantifiable one unless the source of turbidity is very constant. Filterability tests have been described (Wegelin, 1986). The test employed in this Study was based on a modification of a standard method employed in British water engineering practice (Anon, 1950). A membrane filter was wetted and placed in a filtration assembly with the vacuum line to the filter switched off and a pressure of 25 inches of mercury ("Hg) established. A 100 ml volume of distilled deionised water was placed in the filter manifold and the vacuum applied. The time taken for the water to pass the filter was noted. The vacuum line was switched off, a second membrane was placed in the assembly and wetted, and the procedure repeated with 100ml of test water. Filterability was expressed as:

\[
\text{Filterability} = \frac{\text{Time taken for distilled water} \times 100}{\text{Time taken for test water}}
\]
Suspended solids analysis was undertaken according to standard methods (Anon, 1985). A predetermined volume of water was filtered through a preweighed filter paper (Whatman GF) stored dry in a dessicator. The paper was dried in a hot air oven to constant weight and the solids extracted from the original water sample calculated on the basis of weight per unit volume (mg/l).

2.3.iv. Sampling Procedures

Samples for analysis taken from field installations were collected in sterile 500 ml or 1000 ml bottles (Schott, FRG). Bottles were cleaned individually in non-ionic detergent and rinsed in hot water twice and distilled water three times before sterilisation. In certain cases and for certain parameters, analysis was undertaken in the field using portable equipment. In all cases analysis was undertaken within four hours of sample collection. All bacteriological analyses undertaken in UK field trials were conducted in duplicate.
2.4. RESULTS AND DATA PRESENTATION

Many of the results in this Section are expressed in the conventional form for Slow Sand Filtration research ie with respect to time after commencement of filtration runs. This convention is also applied to data from prefiltration systems. Results are also expressed in other ways eg performance with respect to vacuum pressure or head loss in sand prefiltration, performance with respect to depth and biological populations in Protected Slow Sand Filtration and performance with respect to season in Peruvian field installations. Where regression analyses are quoted they represent the analysis which provides the highest correlation coefficient in a comparison of linear, logarithmic, exponential and power regressions.

The four configurations of field installation are described in Figures 20-23. They comprised:

I Slow Sand Filtration with sub-sand prefiltration (UK)
II Slow Sand Filtration with/without gravel prefiltration (UK)
III Slow Sand Filtration with reservoir storage (UK)
IV Slow Sand Filtration with gravel prefiltration (Peru)

Raw water quality data for the periods of experimentation in each case are described in Tables 2.4.1 - 2.4.4.

Configurations I and II were evaluated at the Manor Farm field station of the University of Surrey. Configuration III was evaluated at the Hampton Water Treatment Works of the Thames Water Authority. Configuration IV was evaluated in the community of San Vicente de Azpitia, Mala Valley, Peru.
FIGURE 20  Protected slow sand filters with sub-sand prefiltration. Experimental system configuration I.

FIGURE 21  Protected slow sand filters with gravel prefiltration. Experimental system configuration II.
FIGURE 22 Protected slow sand filters with reservoir storage. Experimental system configuration III.

FIGURE 23 Protected slow sand filters with gravel prefiltration. Experimental system configuration IV.
<table>
<thead>
<tr>
<th></th>
<th>RANGE</th>
<th>MEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermotolerant Coliforms</td>
<td>37.5 - 51000</td>
<td>6977</td>
</tr>
<tr>
<td>per 100 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faecal Streptococci</td>
<td>10 - 222000</td>
<td>24221</td>
</tr>
<tr>
<td>per 100 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turbidity</td>
<td>3.1 - 83.5</td>
<td>23.2</td>
</tr>
<tr>
<td>NTU</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.4.1. Raw water quality during testing of experimental system configuration I.

<table>
<thead>
<tr>
<th></th>
<th>RANGE</th>
<th>MEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermotolerant Coliforms</td>
<td>30 - 44500</td>
<td>11480</td>
</tr>
<tr>
<td>per 100 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faecal Streptococci</td>
<td>22.5 - 53500</td>
<td>8848</td>
</tr>
<tr>
<td>per 100 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turbidity</td>
<td>5 - 100</td>
<td>16.2</td>
</tr>
<tr>
<td>NTU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>10.8 - 16</td>
<td>14.2</td>
</tr>
<tr>
<td>mg/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspended Solids</td>
<td>5.8 - 53.4</td>
<td>28.1</td>
</tr>
<tr>
<td>mg/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filterability</td>
<td>50.3 - 83.8</td>
<td>62.7</td>
</tr>
</tbody>
</table>

Table 2.4.2. Raw water quality during testing of experimental system configuration II.
<table>
<thead>
<tr>
<th></th>
<th>RANGE</th>
<th>MEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermotolerant Coliforms per 100 ml</td>
<td>6 - 360</td>
<td>55.9</td>
</tr>
<tr>
<td>Faecal Streptococci per 100 ml</td>
<td>3 - 277.5</td>
<td>36.3</td>
</tr>
<tr>
<td>Turbidity NTU</td>
<td>1.15 - 4.30</td>
<td>2.23</td>
</tr>
<tr>
<td>Dissolved Oxygen mg/l</td>
<td>5.2 - 10.3</td>
<td>7.61</td>
</tr>
<tr>
<td>Filterability</td>
<td>34.4 - 90.2</td>
<td>72.2</td>
</tr>
</tbody>
</table>

Table 2.4.3. Raw water quality during testing of experimental system configuration III.

<table>
<thead>
<tr>
<th></th>
<th>MEAN</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>WET SEASON</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermotolerant Coliforms per 100 ml</td>
<td>1059</td>
<td>37</td>
</tr>
<tr>
<td>Turbidity NTU</td>
<td>197.3</td>
<td>111</td>
</tr>
</tbody>
</table>

|                                |            |     |
| DRY SEASON                     |            |     |
| Thermotolerant Coliforms per 100 ml | 330        | 14  |

Table 2.4.4. Raw water quality during testing of experimental system configuration IV.
Results of experiments 2.1 - 2 XIV are presented below as fourteen separate investigations in four categories (Sections 2.4.i - 2.4.iv). In some cases, experiments include data from different experimental configurations.

**LIST OF EXPERIMENTS**

**2.4.i. Microbiological Maturation Phenomena**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.I</td>
<td>Maturation indexed by oxygen depletion</td>
</tr>
<tr>
<td>2.II</td>
<td>Maturation indexed by biological populations</td>
</tr>
<tr>
<td>2.III</td>
<td>Maturation indexed by the microbiological efficiency of protected slow sand filters</td>
</tr>
<tr>
<td>2.IV</td>
<td>Maturation indexed by the microbiological efficiency of gravel prefilters</td>
</tr>
<tr>
<td>2.V</td>
<td>Maturation indexed by the microbiological efficiency of sub-sand prefilters</td>
</tr>
</tbody>
</table>

**2.4.ii. Microbiological Performance of Systems**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.VI</td>
<td>Microbiological performance of the experimental protected slow sand filters</td>
</tr>
<tr>
<td>2.VII</td>
<td>Microbiological performance of the gravel prefiltration system</td>
</tr>
</tbody>
</table>
2.4.iii. Physical Performance of Systems

Experiment 2.VIII : Removal of suspended matter by fabrics and sand in protected slow sand filters

Experiment 2.IX : Removal of low level turbidity by protected slow sand filters

Experiment 2.X : Removal of high level turbidity by prefilters and protected slow sand filters

Experiment 2.XI : Improvement of physical performance with respect to time

Experiment 2.XII : Relationship of physical performance to particle size in gravel prefiltration

2.4.iv. Operational Characteristics

Experiment 2.XIII : Protection of physical performance of slow sand filters by synthetic fabrics

Experiment 2.XIV : Protection of microbiological performance of slow sand filters by synthetic fabrics
2.4.1. Microbiological Maturation Phenomena

Experiment 2.1: Maturation indexed by oxygen depletion.

The biological nature of Slow Sand Filtration is revealed directly by the depletion of oxygen which is observed between raw (influent) and treated (effluent) waters. Oxygen levels in raw waters vary diurnally, particularly where there is exposure to light in the presence of algal populations. In order to observe the progress of depletion of dissolved oxygen by a clean (non-biologically mature) slow sand filter using a non-prefiltered water source, configuration III (UK) was observed for a period of 23 days subsequent to commissioning. Samples were taken at approximately the same time of day (0900 - 10.30) on sixteen occasions, and a logarithmic pattern of oxygen consumption was observed. After a period of ten days, a consistent reduction of 2 - 4 mg/l of oxygen was noted between the supernatant water in the slow sand filter and the outlet from the constant flow controller (Figure 24).

![Graph showing consumption of dissolved oxygen (mg/l) by a protected slow sand filter system subsequent to commissioning.](image-url)

**FIGURE 24** Consumption of dissolved oxygen (mg/l) by a protected slow sand filter system subsequent to commissioning.
In conventional full-scale slow sand filters, cleaning results in the discarding of biological populations together with sand removed from the filter. However, routine maintenance of filters protected by fabric allows biological populations to be preserved, both in the sand and in those layers of fabric which do not require cleaning. In order to observe the effect of routine maintenance and of filter medium occlusion on a biologically mature system, closer examination of the phenomenon of oxygen depletion was undertaken using configuration II (UK) without gravel prefiltration. Dissolved oxygen levels were measured in supernatant water in covered filters, in water before it had passed the flow controller, and in water downstream of the flow controller, on fifteen occasions subsequent to routine maintenance (cleaning) of two slow sand filters. Sampling was undertaken at approximately the same time of day on each occasion (08.30 - 10.00).

It was noted (Figures 25 and 26) that oxygen depletion of several mg/l was established within a day of routine maintenance of two parallel protected slow sand filters operated at 0.32 m/h. Levels of dissolved oxygen in the water leaving the flow controller were consistently higher by at least 1 mg/l for the first twenty days of filtration due to the re-aerating effect of the flow control mechanism. However, as noted in Figure 24, the establishment of stable microbiological populations in the sand and fabric of the filter resulted in a relatively consistent depletion of oxygen of 2 - 4 mg/l between supernatant water and the outlet of the flow controller, and thus 3 - >6 mg/l across the filter media for the majority of the filter run. But in the latter part of the run (after 23 days in Figures 25 and 26), there was a noticeable convergence of dissolved oxygen levels in influent and effluent waters. This seems to provide evidence of a moderate diminution of biological activity in the filter, which at constant rates of water flow results in less respiratory activity and thus less oxygen consumption.
FIGURE 25 Dissolved oxygen levels in supernatant water, filtered water and water re-aerated by flow controller subsequent to routine maintenance of a protected slow sand filter operated at 0.20 m/h.

FIGURE 26 Dissolved oxygen levels in supernatant water, filtered water and water re-aerated by flow controller subsequent to routine maintenance of a protected slow sand filter operated at 0.32 m/h.
Experiment 2.II: Maturation indexed by biological populations.

Concurrent work described elsewhere (Wheeler *et al.*, forthcoming) allowed the confirmation of observations relating to dissolved oxygen with reference to populations of ciliated protozoa and reductions in densities of thermotolerant coliforms by slow sand filters in experimental configuration I (downstream of sub-sand prefiltration). Figure 27 illustrates the increase in numbers of ciliated protozoa which were observed in the sand and fabric protecting a slow sand filter with respect to time, and the correlation of these numbers with the elimination of faecal bacteria by the filtration system. Thermotolerant coliform densities were assayed with respect to time and observations on ciliate populations were made by Skilton (1983). A clear linear relationship emerged between the logarithm of coliform reductions and the logarithm of protozoal populations.

![Graph showing correlation](image)

**FIGURE 27** Correlation between reduction in density of thermotolerant coliforms and an index of ciliate population (Skilton, 1983) in a protected slow sand filter.
Experiment 2.III: Maturation indexed by the microbiological efficiency of protected slow sand filters.

Clearly, the logarithmic observations relating to oxygen depletion, ciliate populations and thermotolerant coliform reductions provide strong circumstantial confirmation of the intimate link between biological activity and the removal of bacterial indicators of the hygienic quality of water by the experimental Protected Slow Sand Filtration system. To further investigate this connection, maturation rates of parallel slow sand filters operated at two flow rates were compared with respect to reductions in thermotolerant coliform densities.

Figure 28 illustrates maturation curves for filters operated at 0.16 and 0.32 m/h (configuration II without gravel prefiltration). Data were taken from two experiments which required the complete replacement of filter media before commencement of filtration. Thus the curves relate to true maturation rather than recovery of efficiency subsequent to routine maintenance. Thermotolerant coliform densities were assayed at intervals over a thirteen day period.

![Maturation curves for filters operated at 0.16 m/h and 0.32 m/h.](Image)

**FIGURE 28** Maturation curves for the reduction in density of thermotolerant coliforms by protected slow sand filters operated at 0.16 m/h and 0.32 m/h.
If the observation of thermotolerant coliform reductions is extended to longer periods, for example 30 days in configuration II (Figure 29) or 64 days in configuration III (Figure 30), the logarithmic relationship is preserved with no reduction in the very high level of statistical significance (\( p < 0.001 \) in each case). As in all experiments of this type, thermotolerant coliform densities were assayed in duplicate on frequent occasions throughout filter runs. Data for Figure 29 were obtained from two separate filtration runs, and for Figure 30 from five separate filtration runs.

**FIGURE 29** Maturation curve for the reduction in density of thermotolerant coliforms by protected slow sand filters operated at 0.32 m/h.
The purpose of direct protection of sand filtration by synthetic fabrics was to reduce the physical burden of influent turbidity on sand media and to preserve biological populations during routine maintenance. The microbiological advantage of this protection may be observed with reference to Figures 30 and 31. When reductions in faecal indicator bacteria are plotted with respect to time subsequent to commissioning with clean media, a 90 per cent (one log_{10}) reduction was typically obtained within 3 - 10 days, and a 99 per cent (two log_{10}) reduction in > 25 days (Figure 30).

To examine the recovery of efficiency following routine maintenance of fabric-protected slow sand filters, parallel filters in configuration III were observed over a six day period following cleaning on three separate occasions. Filters were cleaned by draining down, skimming of sand to a depth of 2 cm, washing of upper two layers of fabric, but replacement of four lower layers of fabric with biological populations relatively intact. In each case, the entire process occupied less
than 30 minutes. Results from two filters with duplicate analyses of thermotolerant coliform densities were combined to provide the data on which Figure 31 was based. Under these circumstances, recovery of 90 per cent efficiency with respect to coliform reductions was obtained in less than 24 hours and a 99 per cent reduction was obtained in just over 120 hours (five days).

FIGURE 31 Recovery of microbiological efficiency with respect to reductions in density of thermotolerant coliforms by protected slow sand filters operated at 0.20 m/h.
Experiment 2.IV: Maturation indexed by the microbiological efficiency of gravel prefilters.

The maturation phenomena observed in the Protected Slow Sand Filtration process provide a comprehensive background against which to consider the biological mechanisms which may impact on the removal of enteric viruses by the process. However, slow sand filter units are not the only elements of a community water supply which are capable of reducing microbiological contamination of raw waters. Thus it is important to examine the maturation phenomena which occur in other treatments, i.e., prefiltration, which contribute to the overall efficiency of the water treatment system and which may also help explain the mode of action of biological processes in filtration.

To examine the microbiological maturation of gravel filtration, a horizontal gravel prefilter with dimensions 1 x 1 x 8 ft and medium size 10 - 12 mm was operated at a flow rate of 0.5 m/h (configuration II) for a period of ten days subsequent to commissioning. Thermotolerant coliform densities were assayed in duplicate samples on seven occasions throughout this period and plotted with respect to time. The logarithmic relationship is depicted in Figure 32.

![Figure 32: Maturation curve for the reduction in density of thermotolerant coliforms by horizontal gravel prefilters operated at 0.50 m/h.](image)

\[ r = 0.980 \]

\[ p < 0.001 \]
The maturation of vertical gravel filters in combination with protected slow sand filters is described in Figure 33. The data derive from Peruvian field trials employing downflow gravel prefiltration (configuration IV). It is noteworthy that in conditions of high raw water turbidity and the absence of re-aeration between prefiltration and Protected Slow Sand Filtration, the reduction in thermotolerant coliform densities by the prefilter exceeded that for the slow sand filter for a period of 20 days after initial commissioning of the plant.

FIGURE 33 Maturation curves for the reduction in density of thermotolerant coliforms by vertical (downflow) gravel prefilters (---) and protected slow sand filters in combination (----).
Experiment 2.V: Maturation indexed by the microbiological efficiency of sub-sand prefilters.

In order to investigate maturation in sub-sand prefiltration, the effluent from three prefilters was monitored frequently throughout experimentation with configuration I systems. This required a substantial degree of maintenance, including the backwashing of filter beds on a weekly or biweekly basis. This process necessitated a vigorous disruption of the sand medium by a pumping procedure which fluidised the bed and dislodged compacted particulate and organic matter in the top few centimetres of the sand. The procedure may be likened to the vigorous cleaning of rapid (non-biological) sand filters in conventional water treatment. Thus the maintenance of sub-sand filters may be reliably concluded to have dramatically reduced any microbiological populations which were capable of improving the bacteriological quality of the raw water in the manner of a slow sand filter.

Improvements in bacteriological performance of sub-sand prefilters were monitored on a frequent basis for a period of one year. In each case, water temperature, day of filter run and head loss (indexed by vacuum pressure on the suction line to the pump) were noted (together with turbidity, dissolved oxygen and other microbiological parameters). Figure 34 illustrates the relationship between reduction in thermotolerant coliform density and filter run length in days when water temperatures were lower than 10°C. Figure 35 illustrates the same data plotted with respect to vacuum pressure (head loss). In both cases a linear relationship provides the best description of the data.

The equivalent data for water temperatures in excess of 10°C are depicted in Figures 36 and 37. In both cases a logarithmic regression provides the best fit for the data. The contrast in these relationships in different seasonal conditions provides indirect evidence of a biological factor which exerts more impact on the process of sub-sand prefiltration in warm water compared with cold water conditions.
FIGURE 34 Reductions in density of thermotolerant coliforms by sub-sand prefilters in cold water conditions (water temperature <10 °C) with respect to time.

FIGURE 35 Reductions in density of thermotolerant coliforms by sub-sand prefilters in cold water conditions (water temperature <10 °C) with respect to head loss.
FIGURE 36 Reductions in density of thermotolerant coliforms by sub-sand prefilters in warm water conditions (water temperature >10°C) with respect to time.

FIGURE 37 Reductions in density of thermotolerant coliforms by sub-sand prefilters in warm water conditions (water temperature >10°C) with respect to head loss.
2.4.ii. Microbiological Performance of Systems

Experiment 2.VI: Microbiological performance of the experimental protected slow sand filter system.

The overall microbiological efficiency of protected slow sand filter modules may be calculated in several ways. If the first days of a filtration run are excluded, then it is possible to quote an overall percentage performance for the mature filter. Although it may be argued that overall performance should take into account all results, whether from mature or non-mature facilities, this may give a misleading picture unless results are weighted with respect to time. Thus, in this sub-section, all results describing microbiological efficiency are based on means obtained after 90 per cent removal of thermotolerant bacteria is first achieved. At this point filters were considered to be mature.

Typical results from a single filtration run achieved by protected slow sand filters in configuration III are described in Table 2.4.5.

<table>
<thead>
<tr>
<th>Overall Reductions in Faecal Indicator Bacteria</th>
<th>Thermotolerant Coliforms</th>
<th>Faecal Streptococci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Percentage</td>
<td>&gt; 97.9</td>
<td>&gt; 97.9</td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Standard Dev</td>
<td>1.80</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Table 2.4.5. Reductions in densities of faecal indicator bacteria by protected slow sand filters served by reservoir stored water.
Experiment 2.VII: Microbiological performance of the gravel prefiltration systems.

Data were obtained for gravel prefilters operated in the vertical (upflow) mode (Table 2.4.6) and with horizontal flow (Figure 38).

### Overall Reductions in Faecal Indicator Bacteria

<table>
<thead>
<tr>
<th>Thermotolerant Coliforms</th>
<th>Faecal Streptococci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Percentage</td>
<td>74.1</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
</tr>
<tr>
<td>Standard Dev</td>
<td>10.3</td>
</tr>
</tbody>
</table>

Table 2.4.6. Reductions in densities of faecal indicator bacteria by vertical (upflow) gravel prefiltration served by surface water.

**FIGURE 38** Cumulative reductions in density of thermotolerant coliforms through a three-stage horizontal gravel prefilter employing gravel media of 40 mm (0-8 ft), 20 mm (8-16 ft) and 10 mm (16-24 ft) in series.
To examine the bacteriological efficiency of horizontal gravel prefiltration, three pilot filters of dimensions 8 x 1 x 1ft containing gravel media of 40 mm, 20 mm and 10 mm in sequence were operated at a flow rate of 0.5 m/h for a period of two months. Following a period of maturation, samples were taken on seven occasions at the outlet from each filter. The overall mean reductions in density of thermotolerant coliforms are illustrated in Figure 38. It is apparent that at low flow rates, substantial overall improvements in the bacteriological quality of water were obtained through sequential coarse filtration.

In order to further establish the character of microbiological improvements through coarse media, the three experimental horizontal gravel prefilters were operated in parallel at flow rates increasing between 0.5 and 10 m/h. Data were obtained subsequent to maturation and are plotted for thermotolerant coliforms and faecal streptococci in Figures 39 and 40. The performance characteristics for 10 mm and 20 mm media were similar, providing significant logarithmic relationships for flow velocities 0.5 - 10 m/h for both bacteria.

FIGURE 39 Performance curves for reductions in density of thermotolerant coliforms by gravel prefilters at flow velocities of 0.50 - 10 m/h.
FIGURE 40 Performance curves for reductions in density of faecal streptococci by gravel prefilters at flow velocities of 0.50 - 10 m/h.

The performance characteristic for 40 mm gravel was entirely different, exhibiting a peak efficiency at 2 - 4 m/h. These observations tend to confirm the presence of biological mechanisms of removal of faecal indicator bacteria at low flow rates for media sizes 10 and 20 mm. These mechanisms would appear particularly effective in 10 mm gravel with efficiencies approaching 80 per cent at flow velocities less than 1.0 m/h.

However, at flow rates greater than 2.0 m/h, and for media of size 40 mm, the mechanism of removal of bacteria may not be anything other than physical sedimentation and impaction, with consequent low relative efficiency of removal.
To confirm the observation that most effective biological activity in gravel filtration occurs in media of size less than 20 mm, dissolved oxygen levels were monitored for a period of one month in filters operated in series. Following maturation, samples were taken from eight sample ports in the three stage prefilter on five occasions. Results are plotted in Figure 41 and illustrate unequivocally that little reduction in dissolved oxygen occurred in the 40 mm gravel section.

However a significant linear relationship was obtained for oxygen depletion with respect to filter length in both 20 mm and 10 mm media.

FIGURE 41 Cumulative reductions in dissolved oxygen (mg/l) through a three-stage horizontal gravel prefilter employing gravel media of 40 mm (0-8 ft), 20 mm (8-16 ft) and 10 mm (16-24 ft) in series.
The performance of protected slow sand filters in combination with vertical (downflow) gravel prefiltration in configuration IV is depicted in Figures 42 and 43. The system was commissioned in February 1985 and following biological maturation of both gravel prefILTER and slow sand filters, the bacteriological and physico-chemical performance of the units was monitored for a period of one year. Data are divided into those obtained in the dry season (Figure 42) and those obtained in the wet season (Figure 43). It was observed that in the dry season, the relative contributions to reductions in thermotolerant coliform densities were 63.5 and 92.7 per cent for the gravel prefILTER and four protected slow sand filters respectively. In the wet season, the contributions were 74.1 per cent for the gravel prefILTER and 91.1 per cent for the slow sand filters.

**FIGURE 42** Reductions in density of thermotolerant coliforms in a water treatment system comprising sedimentation chamber, gravel prefILTER and protected slow sand filters by unit process (S - G - P) and by cumulative total (s - g - p) in dry season conditions.
FIGURE 43 Reductions in density of thermotolerant coliforms in a water treatment system comprising sedimentation chamber, gravel prefilter and protected slow sand filters by unit process (S - G - P) and by cumulative total (s - g - p) in wet season conditions.

2.4.iii. Physical Performance of Systems

Experiment 2.VIII: Removal of suspended matter by fabrics and sand in protected slow sand filters.

In order to examine the enhancement of physical performance by filter fabric protection, a number of investigations were conducted on operational slow sand filters. The most frequently applied indices of physical performance of slow sand filters are reductions in turbidity (NTU), suspended solids (mg/l) and filterability. However, a useful index of the comparative contribution of different horizons of the filter to overall removal of suspended material is the volumetric silt test. Parallel protected slow sand filters were operated for a period of 28 days with two types of fabric protection (configuration II without gravel prefiltration). One filter employed a double layer of
polyester/polyvinylchloride/polyamide fabric (specific surface 1671 m²/m³; porosity 0.98; thickness 14 mm). The other contained a double layer of the first fabric plus a fabric of different density (specific surface 2545 m²/m³; porosity 0.97; and thickness 15 mm). The second fabric was a spray-bonded polyester/polyvinyl acetate material. The filters were drained and samples taken of filter fabrics and sand. The volume of silt trapped in each layer of the fabric and the top 2 cm of sand in the sand filters was calculated and related to the cross-sectional area of filter media. Results are expressed as volume of silt (litres by 5 minute settlement test from three washings) in Table 2.4.7.

<table>
<thead>
<tr>
<th>Fabric Combination</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume of Silt (1/m² medium)</td>
<td>per cent of total</td>
</tr>
<tr>
<td>Upper Fabric</td>
<td>10.39</td>
<td>43.9</td>
</tr>
<tr>
<td>Lower Fabric</td>
<td>2.90</td>
<td>12.3</td>
</tr>
<tr>
<td>Sand (top 2 cm)</td>
<td>10.36</td>
<td>43.9</td>
</tr>
<tr>
<td>Total</td>
<td>23.65</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 2.4.7. Distribution of silt and suspended material in filter fabrics and sand subsequent to a filtration run of 28 days in parallel filters protected by an identical double layer of fabric (combination A) and a double layer of fabrics of differing density (combination B).
To investigate the effect of flow velocity on the penetration of suspended solids into fabrics, two parallel filters (configuration II without gravel prefiltration) were operated for a period of 12 days at flow rates of 0.23 m/h and 0.34 m/h. Both filters were protected by six layers of a polyester/polyvinyl acetate spray-bonded fabric (specific surface 2545 m²/m³; porosity 0.97; and thickness 15 mm). At the termination of the experiment, filters were drained and samples taken of each fabric layer. Results are depicted in Table 2.4.8.

<table>
<thead>
<tr>
<th>Filter Layer</th>
<th>Volume of Silt (1/m² medium)</th>
<th>Per cent of total</th>
<th>Volume of Silt (1/m² medium)</th>
<th>Per cent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.49</td>
<td>87.8</td>
<td>9.89</td>
<td>95.1</td>
</tr>
<tr>
<td>2</td>
<td>0.72</td>
<td>11.5</td>
<td>0.41</td>
<td>4.0</td>
</tr>
<tr>
<td>3</td>
<td>0.05</td>
<td>0.8</td>
<td>0.03</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0.04</td>
<td>0.3</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0.03</td>
<td>0.3</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>6.26</td>
<td>100</td>
<td>10.40</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2.4.8. Distribution of silt and suspended material in six layers of filter fabric subsequent to a filtration run of 12 days in parallel slow sand filters operated at 0.23 m/h (Filter 1) and 0.34 m/h (Filter 2).
At the higher flow rate, silt penetration was marginally greater with respect to depth (detectable penetration to layer five compared with layer three at the lower flow rate). However, the maximum deposition (95.1 per cent of total deposition in layer one of Filter 2) occurred at the higher flow rate with only 4 per cent of total deposition in the second layer. This observation implies that deposition in the top layer is largely a passive process linked more to total volume of water filtered than to flow rate. In contrast, the spread of deposition across successive layers of fabric may be linked to water velocity, with lower flow rates resulting in more substantial breakthrough to a second layer, but higher flow rates carrying small quantities of detectable solids to subsequent layers.

Finally, to investigate the benefit of increasing tortuosity of flow through fabric media, the effect of alternating fabric densities was investigated. Parallel slow sand filters were protected by three layers of polyester/polyvinyl chloride/ polyamide spray-bonded fabric (specific surface 1671 m\(^2/m^3\), porosity 0.98; thickness 14 mm) and three layers of polyester/ polyvinyl acetate spray-bonded fabric (specific surface 2545 m\(^2/m^3\); porosity 0.97; thickness 15 mm). In Filter 1, fabrics were alternated, in Filter 2, three layers of the coarser fabric overlaid three of the denser fabric. Filters were operated for a complete filtration run (22 days) at a flow rate of 0.32 m/h. At the end of the filtration run, filters were drained and samples taken of fabrics and the top 5 cm of sand in the filter. Results of silt analyses of fabrics and sand depicted in Table 2.4.9.
Table 2.4.9. Distribution of silt and suspended material in six layers of filter fabric and sand in two filters protected by coarse (c) and dense (d) fabrics in alternating format (Filter A) and simple format (Filter B).

<table>
<thead>
<tr>
<th>Fabric Layer</th>
<th>Volume of Silt (l/m² medium)</th>
<th>A per cent of total</th>
<th>Volume of Silt (l/m² medium)</th>
<th>B per cent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(c)</td>
<td>19.4</td>
<td>49.4</td>
<td>17.6</td>
<td>40.5</td>
</tr>
<tr>
<td>2(d)</td>
<td>8.9</td>
<td>22.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2(c)</td>
<td></td>
<td></td>
<td>2.9</td>
<td>6.6</td>
</tr>
<tr>
<td>3(c)</td>
<td>1.7</td>
<td>4.4</td>
<td>1.5</td>
<td>3.5</td>
</tr>
<tr>
<td>4(d)</td>
<td>0.9</td>
<td>2.3</td>
<td>1.1</td>
<td>2.0</td>
</tr>
<tr>
<td>5(c)</td>
<td>0.01</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5(d)</td>
<td></td>
<td></td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>6(d)</td>
<td>0.03</td>
<td>0.07</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Sand (top 5 cm)</td>
<td>8.33</td>
<td>21.1</td>
<td>20.4</td>
<td>46.9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>39.27</strong></td>
<td><strong>100</strong></td>
<td><strong>43.52</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Clearly, the alternation of fabric densities in Filter 1 resulted in substantially improved silt retention and, consequently, reduced penetration of silt into the top 5 cm of the sand bed (8.33 l/m² versus 20.4 l/m²).
Experiment 2.IX: Reduction of turbidity by experimental protected slow sand filters in conditions of low raw water turbidity.

The physical performance of prefilters and protected slow sand filters was assessed primarily by their capacity to reduce turbidity in raw waters. Attempts to correlate turbidity with suspended solids and with filterability proved unsuccessful, due mostly to the variability in derivation of suspended matter with respect to seasonal factors. However, turbidity provides a useful and direct index of the filter-blocking potential of inorganic solids (i.e., clay and other particulate or colloidal minerals) and for this reason, tends to be employed as one of the key parameters for selecting unit processes for the treatment of surface waters. The ability of turbidity measurements to adequately reflect the filter-blocking potential of organic suspended matter (especially unicellular algae) is in doubt. However, because the principal source of suspended matter in surface waters in less developed countries tends to be inorganic due to the impact of heavy rains and consequent erosion of soils, turbidity was selected as the index of suspended material most relevant to this Study.

In order to investigate the physical efficiency of protected slow sand filter systems in reducing low raw water turbidity, experimental configuration III was monitored for a period of two months subsequent to filter maturation. In experiments with configuration III, raw water was reservoir-stored river water with a uniformly low turbidity, but typically, a constant presence of suspended algal populations. Samples were taken twice weekly for turbidimetric analysis. Results, together with results for other physico-chemical parameters are illustrated in Table 2.4.10.
Overall Reductions in Physico-Chemical Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean Percentage</th>
<th>pH</th>
<th>Dissolved Oxygen</th>
<th>Conductivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turbidity</td>
<td>80.1</td>
<td>0.34</td>
<td>2.63</td>
<td>0.021</td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>9</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Standard Dev</td>
<td>4.554</td>
<td>0.064</td>
<td>0.82</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Table 2.4.10 Reductions in physico-chemical parameters by protected slow sand filters (0.20 m/h) served by reservoir stored water.

Experiment 2. X: Removal of high level turbidity by prefilters and protected slow sand filters.

In order to investigate the physical performance of both protected slow sand filters and prefiltration in conditions of high raw water turbidity, configuration IV was monitored for a period of sixteen months. Samples were taken for turbidimetric analysis on a regular basis. The period included two wet weather seasons. Samples were taken downstream of the gravel prefilter sedimentation chamber, the prefilter itself and the protected slow sand filters. The flow rates for the gravel prefilter and protected slow sand filters were 0.2 m/h and 0.15 m/h respectively. Results are described in bar-chart form in Figures 44 and 45. In the first wet season, when mean influent turbidity was 217 NTU (n = 55; Standard Dev = 340), the respective contributions to turbidity reduction by the sedimenter, gravel filter and protected slow sand filters were 21.7, 79.4 and 65.8 per cent respectively. The total cumulative reduction in turbidity was 94.5 per cent.
FIGURE 44 Reductions in turbidity in a water treatment system comprising sedimentation chamber, gravel prefilter and protected slow sand filters by unit process (S - G - P) and by cumulative total (s - g - p) in dry season conditions.

FIGURE 45 Reductions in turbidity in a water treatment system comprising sedimentation chamber, gravel prefilter and protected slow sand filters by unit process (S - G - P) and by cumulative total (s - g - p) in wet season conditions.
In the second wet season, the individual contribution of protected slow sand filtration to reductions in turbidity increased to 81.7 per cent (compared with 32.6 and 81.3 per cent for the sedimenter and gravel prefilter respectively). Mean raw water quality was 178 NTU (n = 56, Standard Dev = 250). The total cumulative reduction in turbidity for all three processes in these conditions was 97.7 per cent.

The reason for the increase in percentage efficiency observed both in the gravel prefilter and protected slow sand filters in configuration IV in the second period of high (wet season) mineral turbidity loading may be related to long term physical maturation phenomena. The first wet season immediately followed the commissioning of the plant; thus both prefilters and protected slow sand filters were not mature, either in a microbiological or physical sense.

Experiment 2. XI: Improvements of physical performance with respect to time.

To observe physical maturation phenomena in greater detail, all prefiltration systems employed in the study were monitored closely in the first few days of their operation. Results of turbidity reductions during maturation of prefilters are described in Figure 46 for horizontal gravel prefiltration, Figure 47 for vertical (downflow) gravel prefiltration, and Figures 48 - 51 for sub-sand prefiltration.

All prefilters exhibited an increasing efficiency with respect to time. In the case of horizontal gravel prefiltration (Figure 46), efficiencies of 50 - 80 per cent were established within eight days. With vertical (downflow) gravel prefiltration in very adverse raw water conditions, similar performance was obtained within the same period.
FIGURE 46 Physical maturation of a horizontal gravel prefilter with respect to reduction of turbidity.

FIGURE 47 Physical maturation of a vertical (downflow) gravel prefilter with respect to efficiency of turbidity removal.
FIGURE 48 Physical maturation of sub-sand prefilters with respect to reduction of turbidity in cold water conditions (water temperature <10°C) and time.

FIGURE 49 Physical maturation of sub-sand prefilters with respect to reduction of turbidity in cold water conditions (water temperature <10°C) and head loss.
FIGURE 50 Physical maturation of sub-sand prefilters with respect to reduction in turbidity in warm water conditions (water temperature > 10°C) and time.

FIGURE 51 Physical maturation of sub-sand prefilters with respect to reduction in turbidity in warm water conditions (water temperature > 10°C) and head loss.
The performance of sub-sand prefiltration provides some indication of solely physical maturation in cold water conditions (Figures 48 and 49) where modest but relatively reliable reductions of $>0.5 \log_{10}$ were obtained within ten days of filtration commencing (or when head loss measured by vacuum pressure was $> 20''\text{Hg}$). In warm water conditions (Figures 50 and 51), overall efficiency was higher, with reductions in turbidity exceeding the efficiency of gravel prefiltration ie $> 90$ per cent or $1.0 \log_{10}$. However, this performance was not attained on a reliable basis before filtration runs were terminated due to filter blockage. The reason for enhanced efficiency in warm water conditions by sub-sand prefiltration may be related to qualitative changes in suspended material. In warm water (summer conditions) turbidity was mainly organic in nature and thus of a larger particle size than in cold water/winter conditions.

Experiment 2. XII: Relationship of physical performance to particle size in gravel prefiltration.

The physical efficiency of filtration with respect to size of medium and flow rate in prefilters was investigated in configuration II, employing horizontal gravel prefiltration. Gravel filters were operated in series to obtain data depicted in Figure 52. This figure illustrates that the derivation of cumulative efficiencies of $> 60$ per cent removal in turbidity may depend on a significant contribution by several media sizes. The effect of flow rate on turbidity removal was investigated by operating the three filters in parallel (Figure 53). Two clear observations which can be made from these data are that over the flow range 0.5 - 10 m/h, efficiency is inversely related to media size, and that efficiency falls logarithmically with respect to increasing flow rate for all media sizes.
FIGURE 52 Cumulative reductions in turbidity through a three stage horizontal gravel prefilter employing gravel of 40 mm (0-8 ft), 20 mm (8-16 ft) and 10 mm (16-24 ft) in series.

FIGURE 53 Performance curves for reductions in turbidity by gravel prefilters at flow velocities of 0.50 - 10 m/h.
2.4.iv. Operational Characteristics

Experiment 2 XIII: Protection of physical performance of slow sand filters by synthetic fabrics.

The operational performance of slow sand filters is usually described in terms of filter run length, and thus in terms of rate of increase in filter blockage or head loss. In areas of the world where raw water turbidities can be very high, Slow Sand Filtration without some form prefiltration or other protection may be incapable of treating that water because the filter medium will block too frequently to permit stable biological filtration to become established. In order to investigate the ability of filter fabrics applied directly to the surface of slow sand filters to extend filter run length, two parallel filters were operated (configuration III) with and without fabric protection for a series of five filtration runs. After the termination of each run, fabrics were transferred from one filter to the other in order to eliminate any systematic bias between filters. Filters were operated at 0.20 m/h. Fabrics were in alternating sequence, and comprised three layers each of polypropylene needle felted material of specific surface 10609 m²/m³, porosity 0.87 and thickness 4.5 mm, and specific surface 10615 m²/m³, porosity 0.82 and thickness 8 mm. Results are depicted in Figure 54 and demonstrate the unequivocal benefit of fabric protection in terms of slowing the rate of increase in head loss compared with an unprotected filter.

![Figure 54](image-url)
The insertion of manometers at the interface of fabric layers and sand showed that even at the end of filtration runs, there was no observable head loss across filter fabrics. This may be attributed to the loose network of fibres which afforded no resistance to flow whilst contributing significantly to the removal of silt and other suspended material. In contrast, due to the penetration of a proportion of suspended particles into the sand bed, conventional head loss increases were noted with respect to depth. To correlate these effects, manometers were inserted at different depths through parallel protected slow sand filters in configuration I. Filters were operated for a period of three months (five complete filtration runs) during which manometer readings were taken at least three times per week. At the termination of filter runs, filters were drained and samples taken of sand at different depths before being subjected to silt analysis.

Results of these experiments are summarised in Figure 55. A clear obverse logarithmic association was observed which demonstrates the direct relationship between head loss and silt penetration in sand - a phenomenon which did not extend to filter fabrics.

![Diagram](image)

**FIGURE 55** Accumulated head loss at various depths within the sand bed (as a percentage of total head loss at 300 mm), compared with silt penetration (as a volumetric percentage).
Experiment 2. XIV: Protection of microbiological performance of slow sand filters by synthetic fabrics.

To assess the comparative benefits of fabric protection during routine maintenance and conventional skimming of slow sand filters, two parallel filters (configuration II) were stopped in mid-filtration run and drained down. One filter was skimmed, a process which involved the removal of the top 2cm of sand before replacement of filter fabrics (the top two fabrics having been cleaned). The other filter was momentarily 'backflushed' with clean water to release head loss due to compaction of the bed before replacement of fabrics (two layers having also been cleansed).

Results are described in Figure 56 and 57. Recovery was more rapid for both thermotolerant coliforms and faecal streptococci for the filter which was not skimmed.

![Figure 56](image_url)  // Replace image_url with the actual URL to the image

**FIGURE 56** Recovery of microbiological efficiency of protected slow sand filters following routine maintenance (●---●) and skimming (■---■) with respect to reductions in densities of thermotolerant coliforms.
FIGURE 57 Recovery of microbiological efficiency of protected slow sand filters following routine maintenance (○—○) and skimming (■—■) with respect to reductions in densities of faecal streptococci.
SECTION 3

OBSERVATIONS ON THE REMOVAL OF VIRUSES
BY FILTRATION THROUGH SAND
3.1 GENERAL OBSERVATIONS

A comprehensive review of the impact of a number of water treatments on the removal of indicator bacteria, pathogenic bacteria, viruses and protozoa from natural waters has been presented by Kool (1979). Unlike many reviews of this type, due recognition was given to processes such as dune infiltration and conventional Slow Sand Filtration in the removal of bacteriophage and some human enteric viruses (enteroviruses). Kool summarised data from a number of sources and concluded that infiltration through sand dunes was capable of reducing titres of bacteriophage and enteric viruses by four log\(_10\) units; this compared with a reduction through slow sand filters of 1 - 2 log\(_10\) units and through rapid sand filtration of 0 - < 1 log\(_10\) units.

It was noted that dune infiltration through 10 m of sand and a detention time of 8 days would simultaneously reduce thermotolerant coliform densities by > 2 log\(_10\) units and 37°C and 22°C colony counts by 4 log\(_10\) units. In comparison with sand filtration by this method, other processes prove reasonably effective. Under well controlled circumstances, bacteria, bacteriophage and enteroviruses are removed by chemical coagulation and flocculation by 1 - 3 log\(_10\) units, by ozonisation: 3 - >5 log\(_10\) units, and by reservoir storage : 1 - 3 log\(_10\) units.

In their review of the impact of water treatments on viruses, Lloyd and Morris (1982) considered coagulation to be an effective chemical procedure for the removal of enteric viruses. Percentage reductions using aluminium-based coagulants were reported; they were 99.99 per cent for Poliovirus 1, 93.3 per cent for simian Rotavirus (SA 11), 79 - 85 per cent for Echovirus 7, and 57 - 99.9 per cent for T2 and MS2 coliphages. Reported reductions in virus titre using ferric salts were 99.9 per cent for Poliovirus 1 and 92 - 99.4 per cent for f2 coliphage. In comparison with the potential of well controlled chemical coagulation, the removal of viruses by rapid sand filtration was noted to be poor.
According to this review, improvement of virus attenuation by rapid sand filtration may be achieved by the addition of alum floc or the presence of calcium cations. Removal of T4 coliphage by a rapid sand filtration plant was 0 - 87 per cent. However, Slow Sand Filtration was observed to be substantially more efficient, with reductions of 95 - 100 per cent in Poliovirus 1 and 99.75 - 99.996 per cent in MS2 coliphage being achieved in some studies.

As discussed in Section 2, the usual microbiological criteria applied to slow sand filter performance relate to bacteriological parameters - in particular those of the faecal indicator bacteria ie thermotolerant coliforms (or Escherichia coli) and faecal streptococci. However, some work has been undertaken on the removal of specific microorganisms of public health significance, including protozoa (Logsdon et al, 1981; Bellamy et al, 1985; McNair et al, 1987) and helminths (Kawata, 1982). Studies specific to the removal of enteric viruses by sand filtration are relatively few in number.

Work conducted by Robeck et al (1962) confirmed the ability of slow sand filters to reduce titres of Poliovirus 1 by 83 - 98 per cent. Lower flow rates were found to provide greater efficiencies of Poliovirus removal. However, removals were still relatively low (69 per cent) at moderate conventional flow velocities ie 0.15 m/h.

Using perspex cylinders of dimensions 200 x 40 mm, Lefler and Kott (1974) were able to simulate the transport of coliphage and Poliovirus through dune sand. Although these experiments were not continuous flow in nature, and thus not strictly comparable with Slow Sand Filtration, some interesting observations were made. For example, it was noted that bivalent calcium and magnesium cations at concentrations of 0.001 M and 0.01 M significantly increased adsorption whereas monovalent sodium and trivalent iron cations had no such effect. Elimination was greatest in the upper portions of the sand columns. Elution of viruses from sand columns was not totally effective even after 10 washings with pH 10.5 buffer.

*Attenuation is used here to mean non-recoverability which may be due to any mechanism of removal or inactivation.*
Detailed work was conducted by Poynter and Slade over a period of years using perspex slow sand filter columns of surface area 0.09 m\(^2\) (1977). At flow rates of 0.2 - 0.5 m/h and water temperatures of 5 - 18°C, reductions in viruses and bacteria were as follows: Poliovirus 1: 98.25 - 99.99 per cent, *Escherichia coli*: > 88.0 - > 98.6 per cent, 37°C colony count: 81.2 - 93.7 per cent, and 22°C colony count: 94.5 - 98.4 per cent.

The effect of flow rate and sand depth on efficiency suggested that to a certain extent, a reduction in efficiency caused by higher flow rates might be mitigated by increasing sand depth. The observation that reductions in Poliovirus 1 titres were consistently similar to or greater than reductions in *Escherichia coli* densities led to the conclusion that the mechanism of removal was essentially similar, and was predominantly biological.

Subsequent observations by the Thames Water Authority (Slade, 1978) showed that full scale filters (0.337 hectare) reduced enterovirus levels in impounded river water with a consistently higher efficiency than *Escherichia coli*. Sixteen samples taken from two filters over a ten week period (water temperatures 6 - 9°C) showed that reductions in enterovirus titres in conventional Slow Sand Filtration were high (97.1 - 99.8 per cent) but not necessarily equivalent to those observed in pilot scale experiments.

However, from the perspective of this Study some of the most relevant experiments on virus removal by slow sand filtration have been conducted in the United States using Reovirus as a model (McConnell *et al.*, 1984). Reoviruses are closely related to Rotaviruses and they are readily isolated from raw sewage at comparable concentrations i.e. \(10^2 - 10^5\) viruses per litre. Thus it is at least arguable that Reoviruses provide a relevant comparative index for Rotavirus removal in water treatment. Using radioactively labelled Reovirus and glass columns of dimensions 3 m x 150 mm, a number of important observations were made.

At a flow rate of 0.2 m/h, greatest removal (more than 65 per cent) occurred in the top 350 mm of...
the sand bed. At no time was Reovirus detected deeper than 1.2 m in the sand bed. However, between depths of 0.3 and 1.0 m, Reovirus continued to be removed as a relatively constant proportion of the influent virus titre.

In contrast to the studies of Poynter and Slade (1977), there was no significant difference in removal between clean and aged (mature) sands. Furthermore, there was no difference in the pattern of removal with depth for clean or aged sands, and graded sands were no more efficient than ungraded sands in removing Reovirus. Due to the high overall removal efficiencies obtained (more than 4 log₁₀ units), Reovirus densities in effluents did not allow absolute comparisons to be made between clean and aged (mature) sands. A summary of results with respect to sand type is illustrated in Table 3.1.1.

<table>
<thead>
<tr>
<th>SAND TYPE</th>
<th>PER CENT REOVIRUS RECOVERED</th>
<th>In Effluent</th>
<th>In Sand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graded - Clean</td>
<td>3.3</td>
<td>96.7</td>
<td></td>
</tr>
<tr>
<td>Ungraded - Clean</td>
<td>5.2</td>
<td>94.8</td>
<td></td>
</tr>
<tr>
<td>Ungraded - Aged (with schmutzdecke)</td>
<td>5.9</td>
<td>94.1</td>
<td></td>
</tr>
<tr>
<td>Ungraded - Aged (without schmutzdecke)</td>
<td>4.9</td>
<td>95.1</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1.1. Percentage of added ¹²⁵I-labelled Reovirus detected in effluent and sand samples collected from slow sand filter columns containing various sand types (McConnell et al 1984).

Thus, it was concluded that biological activity within sand horizons was not primarily responsible for elimination of viruses. Indeed, because infectious virus was undetectable in sand samples extracted at the end of filter runs from non-mature columns, it was clear that either elution was ineffective or some other factor had inactivated the viruses.
3.2 THE REMOVAL OF VIRUSES BY DEPTH-RELATED PHENOMENA IN SLOW SAND FILTRATION

It has been noted in several reviews that the mechanisms of transport and removal of microorganisms (usually bacteria) by the Slow Sand Filtration process are biological, physical and physico-chemical (van de Vloed, 1955). However, because of the small size of viruses, some physical mechanisms of transport and removal are clearly of little importance, even if viruses are present as aggregates; these include straining and interception. In addition, gravitational sedimentation is not considered to be a major factor in the removal of particles of less than 1 μm, e.g., colloids, from aqueous solution. Thus, unless there is considerable association with suspended particulate matter, the three factors of greatest potential significance in the elimination of viruses in slow sand filters are microbial predation, adsorption to biomass or biofilms and adsorption to non-biological surfaces.

Of these three factors, empirical observations such as those of Poynter and Slade (1977) would suggest that two biologically-related phenomena: microbial predation and adsorption to biomass/biofilms have the greatest influence, particularly in those upper horizons of slow sand filters where mixed populations of bacteria, fungi, algae and scavenging protozoa and metazoa proliferate. However, it should be noted that the experiments of McConnell and co-workers (1984) do not support this view.

Although their relative importance may vary, adsorption to both biological and non-biological surfaces may theoretically play a role at all depths of the filter. Adsorption to surfaces is discussed in greater detail in Section 4. However, there is considerable evidence to suggest that virus adsorption to microbial biomass and biofilms may be a particularly important factor in removal in the upper horizons of slow sand filters. Hence this factor is examined here in the context of depth-related mechanisms of removal.
The association between bacteria and protozoa has been understood for some time (Ball, 1969). And the presence of a "jelly-like coat" around sand grains has long been postulated as an adsorbent for both solutes and colloids passing through slow sand filters (van de Vloed, 1955; Lloyd, 1974). In nature, biofilms are noted for their porosity and adsorptive properties. Thus, it is entirely possible that extracellular polymers associated with the microbial colonisation of sand play a very important role in providing local binding sites for viruses. Empirical evidence strongly supports this hypothesis. For example, experiments have shown that the efficiency of filtration is improved in the presence of polymers capable of enhancing bridging between colloidal particles and filter media (Sehn & Gimbel, 1983).

It has been noted by Corpe (1980) that virus adsorption to cells is mediated by cell fractions which can be experimentally inactivated by proteases. Thus mucoproteins, glycolipoproteins and lipoproteins are all candidates for virus receptors. The affinity of bacteriophage for peptidoglycans and other polymers such as lipopolysaccharides was also noted. Fenner et al (1974) demonstrated that the possession of a lipoprotein envelope enhanced the infectivity of Herpes virus and concluded that this was due to the facilitation of rapid attachment and subsequent merging of virus and cell membranes.

Adsorption of enteroviruses and bacteriophage to biological surfaces in sewage floc has been investigated in detail. Balluz and Butler (1979) showed that Poliovirus 1 adsorbed strongly to bacterial floc in a bench-scale activated sludge plant. Following sedimentation of this floc, the amount of virus remaining in the aqueous effluent of the plant was only 0.2 per cent of the original inoculum. In contrast, another member of the Picornaviridae, f2 coliphage, was adsorbed and removed much less efficiently. More than 31 per cent of the original coliphage inoculum remained in the aqueous phase after sedimentation. Later work demonstrated that other enteroviruses, including Coxsackie B5 and Echovirus 1 adsorbed more or less as efficiently as Poliovirus to sewage floc (Bates & Goddard, 1981). Drury and Wheeler (1982) demonstrated that
high proportions of *Serratia marcescens* bacteriophage may be adsorbed by autoclaved activated sludge floc and subsequently removed from the aqueous phase by sedimentation. Gerba and co-workers (1980) obtained 67 - 99.8 per cent adsorption of six enteroviruses to activated sludge floc.

Observations on full scale slow sand filters have confirmed that immediately after commissioning or cleaning, improvements in the bacteriological quality of filtrates may take several days to become established with reliable reductions in excess of 90 per cent (Burman, 1962). Thus the concept of a maturation period has been developed. Clearly, maturation is both microbiological and physico-chemical. The former requires the establishment of vigorous populations of protozoa, metazoa, fungi, bacteria and algae (Bellinger, 1979). The latter requires the establishment of appropriate physico-chemical properties throughout the filter medium in order to facilitate adsorption (Section 4).

However, the removal of the top few centimetres of biologically active sand and biomass during conventional cleaning (‘skimming’) results in poor bacteriological removals for several days despite the presence of up to 1 metre depth of physico-chemically conditioned sand and associated biofilms.

In studying the inter-relation of protozoal populations and bacterial densities in interstitial water of slow sand filters, Lloyd (1974) demonstrated 90 per cent removal of coliform bacteria and 37°C standard plate count bacteria in the top 5 - 8 cm of sand. In contrast, 90 per cent elimination of 22°C standard plate count bacteria was not achieved in the top 23 cm. Feeding experiments demonstrated a considerable capacity for bacterial consumption by ciliated protozoa, and it was confirmed that protozoal populations were largely restricted to the upper horizons of the sand bed. It was concluded that either the mechanism of removal of 22°C plate count organisms was less effective or that the heterotrophic, saprophytic bacteria were dividing and maintaining populations at depth within the filter. Of these two hypotheses, the latter seems more likely.
It may be concluded therefore, that under normal circumstances, most of the activity of slow sand filters with respect to the removal of bacteria occurs within the uppermost horizons of the filter where microfauna and flora are most abundant. Thus, it is most likely that for bacteria, it is in this area that both predation and adsorption are most effective. This hypothesis was examined and empirically confirmed in experiments described in Section 2.

There have been relatively few studies involving operational slow sand filters which have properly examined virological removal with respect to depth. The majority of studies relating to similar phenomena eg adsorption and desorption to mineral or organic material in granular matrices, have been designed to examine the impact of wastewater recharge on aquifers. These studies are described in Section 4 in the context of mechanisms of adsorption of viruses to surfaces.

The relative contribution of biological factors to the removal of microorganisms may be indirectly assessed by establishing the pattern of removal of bacterial indicators with respect to depth in an operational filter. The pattern of removal may be compared with that obtained for indicator viruses which may be expected to exhibit behaviour similar to enteric viruses such as Rotavirus on the basis of their physical characteristics.

Thus, experiments described in this Section concern the removal of bacteria, bacteriophage and Rotavirus with respect to depth in sand. These phenomena were investigated to provide comparative data on the relative importance of two depth-related biological factors: predation by microfauna and adsorption to biomass in the removal of viruses through sand.
Experiments were undertaken in the United Kingdom and in Peru. They were:

Experiment 3.I  :-  The removal of indicator bacteria by experimental protected slow sand filters

Experiment 3.II :-  The removal of bacteriophage by experimental protected slow sand filters

Experiment 3.III :-  The removal of indigenous Rotavirus by experimental protected slow sand filters

Experiment 3.IV :-  The removal of indigenous Rotavirus through sand and anaerobic biomass beneath a wastewater lagoon and irrigation site

Experiment 3.V  :-  The removal of simian Rotavirus by protected slow sand filter columns
3.3. MATERIALS AND METHODS

3.3.i. Bacteriological Parameters

Faecal indicator bacteria in water samples were assayed according to standard procedures described in Section 2. The faecal indicator groups examined for attenuation with depth were thermostolerant coliforms and faecal streptococci. Plate count bacteria (37°C and 22°C) were enumerated by the spiral plate technique (see also Section 2). Thermostolerant coliforms and faecal streptococci in sand samples were assayed by a modified most probable number (MPN) technique. Sand was weighed in three series of five wet weights (5 x 1.0 g, 5 x 0.1 g and 5 x 0.01 g) and placed in sterile 25 compartment replidishes (Sterilin). To these sub-samples was added Minerals Modified Glutamate Broth (Oxoid) or Kanamycin Aesculin Azide Broth (Oxoid) for the primary isolation of thermostolerant coliforms and faecal streptococci respectively. Dishes were incubated at 44°C for 24 hours. Compartments showing growth were subcultured into confirmatory media: Brilliant Green Bile Broth for thermostolerant coliforms and Kanamycin Aesculin Azide Agar (Oxoid) for faecal streptococci and incubated at 44°C for a further 24 hours. Quantitative MPN results were computed from standard statistical tables (Anon, 1983).

3.3.ii. Bacteriophage

Five bacteriophage (bacterial viruses) were employed in the investigation. They were selected primarily on the basis of their size, each having a head with cross-sectional area within the range ± 50 per cent of the cross-sectional area of Rotavirus particles. Bacteriophage were drawn from three groups of the Tikhonenko (1970) classification, and possessed tails which varied in size between < 20 nm and 150 nm. The sources of the bacteriophage have been described together with various applications (Skilton, 1987; Wheeler et al, 1988; Skilton & Wheeler, 1988; Skilton & Wheeler, 1989) and their characteristics are summarised in Table 3.3.1.
<table>
<thead>
<tr>
<th>Bacteriophage</th>
<th>Classification</th>
<th>Dimensions (nm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Head</td>
<td>Tail</td>
<td></td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>III</td>
<td>50 x 50</td>
<td>&lt; 20</td>
<td></td>
</tr>
<tr>
<td><em>Erwinia carotovora</em></td>
<td>III</td>
<td>75 x 75</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli K12</em></td>
<td>IV</td>
<td>50 x 50</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>V</td>
<td>100 x 70</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus licheniformis</em></td>
<td>V</td>
<td>70 x 70</td>
<td>150</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.3.1  Morphological characteristics of bacteriophage used in experiment 3.II (classification according to Tikhonenko, 1970).

Bacteriophage were assayed using a modification of the soft agar overlay technique of Adams (1959). All bacteriological products were obtained from Oxoid and chemicals were obtained from BDH (Analar Grade). The basal medium was Blood Agar Base and the soft agar overlay was a simple formulation of Nutrient Broth (1.2 g/l), Purified Agar (5.5 g/l) and sodium chloride (7.0 g/l).

Sub-sample volumes of 0.1 ml were pipetted onto 90 mm petri dishes of basal medium. To each sample was added 0.5 ml of an overnight culture of host bacterium grown in a rich nutrient broth comprising Brain Heart Infusion (20 g/l), Cassein Hydrolysate (20 g/l), potassium dihydrogen orthophosphate (5.0 g/l) and glycerol (2 per cent in distilled water). To the mixture of sample and
host was added 3.0 ml molten soft agar at a temperature of 45°C. The plate was swirled vigorously to ensure thorough mixing of the overlay. The petri dish was then placed in a clean environment with the lid ajar for a period of five minutes in order to promote rapid setting and avoid condensation forming on the underside of the lid (drops of condensation can interfere with the assay). Finally, dishes were reassembled, inverted and incubated at the optimum growth temperature for the host. Plaque formation was usually within 6 - 12 hours; following enumeration of plaques, results were expressed as plaque forming units (pfu) per ml.

Bacteriophage were prepared from confluently lysed soft agar overlays. An aliquot of 0.1 ml of a high titre of bacteriophage ( > 10^8 per ml) was inoculated onto basal medium and 0.5 ml host bacterium added. To this 3.0 ml of molten soft agar was added as above and the overlay allowed to set before incubation. Following confluent lysis, the overlay was removed and mashed with 15 ml of rich nutrient broth (formula as above) in a sterile universal bottle. The mash was freeze-thawed three times (-20°C - +20°C) to disrupt bacterial cells and thus maximise the liberation of bacteriophage. The preparation was clarified by centrifugation at 7500 g for 25 minutes before filtration through a 0.45 μ membrane filter. Further purification or concentration was deemed unnecessary because of the rapid dispersion expected in the experimental system.

3.3.iii. Rotavirus - Selection of Techniques

A number of viruses of considerable public health significance are not amenable to routine detection in water. They include Hepatitis A and Norwalk-type viruses. Until recently, there were no suitable methods for quantifying Rotaviruses in environmental samples. However, current techniques for detecting Rotaviruses may now be divided into three categories:

i) those that detect viral components eg antigens or RNA;

ii) those that detect morphologically distinct particles; and

iii) those that detect replicating virus either directly or indirectly eg cell culture followed by immunochemical staining.
Methods for the detection of Rotavirus in clinical samples have been available for a number of years, for example electron microscopy and enzyme linked immunosorbent assay (ELISA). The sensitivity of commercial ELISA tests has been shown to be approximately $10^6$ particles per ml for simian Rotavirus SA11 and $10^7$ particles per ml for human Rotavirus HRV (Rubenstein & Miller, 1982). Similar (or slightly less) sensitivity has been reported for electron microscopy (Steinmann, 1981; Walter et al, 1982). The two methods have been shown to have a relatively high degree of correlation, with 88.7 per cent agreement on positive samples and 91.95 agreement on negative samples (Birch et al, 1983).

Enhancement of the sensitivity of ELISA is feasible, for example by the use of fluorogenic reagents (Yolken & Stopa, 1979). However, it is clear that neither ELISA nor electron microscopy is capable of routinely detecting Rotaviruses in the concentrations in which they occur in natural waters. Furthermore, both techniques detect characteristics which are common to live and inactivated virus. Therefore, they do not necessarily provide a relevant quantification of health risk if samples are found to be positive.

The development of sensitive quantitative tests for Rotavirus in environmental samples has depended on the application of appropriate techniques for the concentration, cultivation and detection of live virus.

CONCENTRATION

Concentration procedures used in this study comprised filtration, elution and chemical flocculation steps.

i) Filtration

Initial concentration of Rotavirus was accomplished by adsorption onto epoxy-bound glass fibre
depth filters (Balston 100-12-C). Water was placed in sterilised pressure vessels of 10 litre capacity. Ten litre samples were adjusted to pH 3.5 by the addition of sterile hydrochloric acid (1.0 M). pH was measured using a probe which had been thoroughly cleaned and calibrated in sterile buffers immediately prior to the conditioning of samples. A predetermined volume of sterile aluminium chloride was added to provide for a final concentration of 0.5 mM in the sample. Water was forced under positive pressure from a nitrogen cylinder through the filter housing (outside to inside). No advantage was conferred by recirculating the filtrate and thus it was discarded. Flow through the filter was adjusted to ensure an overall mean flow rate of no more than 100 ml per minute.

ii) Elution

Rotavirus was eluted from filters and from sand samples using 3 per cent Beef Extract solution (Oxoid) at pH 9.5. Elution from filters was achieved using a volume of eluent in the range 200 - 400 ml. Eluent was first used to soak the filter for a period of 60 minutes before being passed through filter cartridges three times under positive pressure and at a mean flow rate not exceeding 20 ml per minute. This procedure was found to substantially enhance recovery. Elution from sand was accomplished by placing 100 g sand sample in the base of a sterile 1 litre flask and adding 500 ml beef extract eluent. A sterile magnetic stirrer was introduced, and the sand and eluent agitated for a period of sixty minutes. Eluents from sand were decanted.

iii) Flocculation

Because of reported variations in the efficiency of beef extract flocculation (Morris, 1986), eluted virus was precipitated by a process of enhanced organic flocculation. Eluents were placed in sterile 500 ml or 1 litre flasks and sterile Skim Milk concentrate (Oxoid) was added and dispersed to provide for a final concentration of 1.5 per cent by weight. The pH of the flask contents was then adjusted to pH 3.5 by the addition of a predetermined volume of hydrochloric acid (1.0 M). A sterile magnetic stirrer was introduced and the flocculating mixture agitated slowly for a period of
30 minutes. The mixture was then centrifuged in 200 ml or 500 ml polypropylene bottles at 3000 g for 30 minutes at 4°C. Supernatants were discarded and precipitated pellets dissolved in the minimum volume of a 0.15 M solution of sodium dihydrogen orthophosphate (pH 9.2). This volume was typically 10 ml per 200 ml original eluent.

CULTIVATION

Rotavirus was cultivated and detected using a foetal rhesus monkey cell line (MA104) donated originally by the Department of Microbiology, University of Warwick. The cells permit the cultivation of both human and simian Rotaviruses, the latter with cytopathic effect and subsequent cell death.

Cells from a confluent 25 cm² growth flask (Nunc) were stored following dispersion in 1.5 ml of growth medium (Appendix II) containing double strength foetal calf serum and 7.5 per cent dimethyl sulphoxide (BDH). Cells were stored in sterile ampoules (Nunc) in liquid nitrogen (-190°C) following a series of temperature reductions over a period of 4 hours. Cells were resuscitated by rapid defrosting in warm water at 25 - 30°C. The warm cell suspension was transferred to a 25 cm² growth flask containing 8 ml of growth medium. Cells were incubated for 2 hours at 37°C in 5 per cent carbon dioxide to allow for attachment. After this period unattached cells were discarded and those which attached were washed three times in Phosphate Buffered Saline (Oxoid). Fresh growth medium was then added. Within 48 - 72 hours of incubation at 37°C cells reached confluence. The health of the culture was visually assessed at least twice daily.

Cells were usually passaged in a ratio of between 1 : 4 and 1 : 6 following 3 - 4 days incubation in normal growth medium. Cells were washed in three changes of PBS and subsequently stripped in a trypsin-EDTA solution (Appendix II). Cells were seeded at an approximate concentration of 50,000 cells per ml, adjusted if necessary following a haemocytometer density estimate.
Simian Rotavirus was cultivated in 25 cm$^2$, 80 cm$^2$ and 175 cm$^2$ growth flasks (Nunc). A confluent 3-4 day culture of MA104 cells was washed in three changes of PBS and 10, 30 or 100 ml of serum-free growth medium was added (Appendix II). Following incubation for 24 hours at 37°C, the cells were washed three times in PBS and fresh serum-free medium containing 10 μg per ml trypsin was added together with 0.5 - 5.0 ml virus suspension. Extensive cytopathic effect including cell lysis occurred within 48 - 72 hours incubation at 37°C.

Viruses were harvested by freeze-thawing (-20°C - +20°C) to disrupt remaining cells. Debris was removed by centrifugation at 7500 g for 25 minutes. The supernatant was filtered through a sterile 0.45 μ membrane filter and centrifuged again at 11,000 g for sixty minutes at 4°C. Finally, dispersed virus was dialysed in visking tubing which had been previously soaked for three hours in serum albumin and washed in PBS. Dialysis was against three changes of PBS in a volume ratio of 200 : 1 over a period of 24 hours.

DETECTION

Rotaviruses in aqueous samples were usually enumerated by visual counts of fluorescent foci in multiwells. Most Probable Number counts were also made.

i) Visual Counts

MA104 cells in 80 cm$^2$ bottles (Nunc) were washed three times in PBS before being stripped in trypsin - EDTA solution. Cells were suspended in growth medium (Appendix II) to yield a concentration of 10$^5$ cells per ml. 4.0 ml of suspension were added to each compartment of a six well multiwell plate (Nunc). Cells were grown to confluence over a period of 24 - 48 hours and then sheets were washed three times in PBS before being bathed in serum free medium for 24 hours (Appendix II) to eliminate the influence of any residual anti-Rotavirus antibodies in the foetal calf serum.
Before application of samples to the cell sheets, medium was drained off and replaced by 0.2 - 3ml fresh serum-free medium containing 10 μg per ml trypsin. To this was added 0.1 - 0.5ml of sample (or sample dilution), in order to provide a complete immersion of the cell sheet. Failure to observe extreme care in this procedure was shown to result in fraying or destruction of the cell sheet. The sample was incubated for a period of 60 minutes in an atmosphere of 5 per cent carbon dioxide and > 95 per cent humidity (37°C) to allow attachment of virus to susceptible cells. Following this period, 3-4 ml of fresh serum-free growth medium was added, and the plate was incubated for a further 24 hours at 37°C. No advantage was conferred in centrifuging samples to encourage virus attachment to cell sheets.

Cell sheets were then washed gently in PBS before being fixed for 15 minutes in approximately 2.0 ml of methanol at 4°C. After pouring off the methanol, cell sheets were air dried at ambient temperature. In this state, the fixed cells could be stored indefinitely, but in practice staining was invariably undertaken within two hours.

The staining procedure comprised two steps. Following washing in PBS, 0.3 ml of a 1:60 dilution of bovine anti-Rotavirus serum (from a gnotobiotic calf) was added to each cell sheet. The plate was incubated for 30-60 minutes in an atmosphere of > 95 per cent humidity (37°C) and each cell sheet was then washed for ten minutes in three changes of PBS. The second step required the addition of 0.3 ml of a 1:60 dilution of lapine anti-bovine serum conjugated with fluorescein isothiocyanate (Wellcome MF-13) followed by a further incubation period of 30-60 minutes. Finally, each well was bathed in three further ten minute changes of PBS to remove all traces of excess reagent before being air-dried. No advantage was conferred by using reagents in concentrations of 1:40.

Cell sheets were scanned using incident UV light in a Leitz microscope. Replication of Rotavirus in individual cells was readily visualised by characteristic cytoplasmic staining, invariably
observed as unmistakeable apple-green foci forming horseshoe or annulus shapes around the cell nucleus. The use of positive and negative controls together with careful contrasting of observed staining with cell morphologies observed under normal transmitted light ensured that false positives were eliminated. Counts were related to the volume of concentrate and subsequently to the volume of original sample and quoted as fluorescent focus forming units (fffu) per litre.

ii) Most Probable Number Counts

Confluent MA104 cells in 48 - 72 hour cultures in 80 ml bottles (Nunc) were bathed in serum-free growth medium for three periods of 30 minutes before being incubated for a further period of 24 hours in the serum-free medium. Cells were then washed three times in PBS before being stripped using a trypsin-EDTA solution of 0.025 per cent trypsin, and suspended in serum-free growth medium to yield a concentration of $2 \times 10^5$ cells per ml.

Samples were prepared in decimal dilutions (0.1 ml into 0.9 ml PBS) and pipetted in 0.1 ml volumes together with 0.1 ml of cell suspension and 0.1 ml double strength serum-free medium (containing 20 μg per ml trypsin) into microtitre wells. Five wells were set up for each dilution of sample. Microtitre plates were then sealed with an adhesive acetate sheet and incubated at $37^\circ C$ in 5 per cent carbon dioxide. After three days incubation, microwells showing cytopathic effects were noted and 100 μl of supernatants were sub-cultured onto 24-place multiwell plates containing healthy MA104 cells growing in serum-free medium. After 24 hours incubation, cell sheets were fixed and stained to confirm the presence of large numbers of fluorescent foci attributable to Rotavirus infection. The confirmed MPN count was obtained by reference to standard statistical tables.
3.3.iv. Protected Slow Sand Filter Columns

Two column protected slow sand filters were constructed using standard 6" plumbing fittings (Osma, UK). The columns were approximately 1.6 m in height and provided for sample ports at various depths within the filter (Plates V and VI). Sample ports were also provided for the supernatant (untreated) and downstream (treated) waters. Each sample port was protected by a fabric sleeve in the same fashion as sample ports in configuration III of the experimental slow sand filter system (Section 2). The columns comprised 200 mm supernatant, six layers of polypropylene fabric and 1.0 m clean sand taken from the sand cleaning plant of Surbiton Water Treatment Works (Thames Water Authority). Drainage was effected through 10 mm shingle and flow was regulated by twice daily adjustment of taps downstream of the filters. Flow rate was maintained at the equivalent of 0.2 m/h.

The raw water source was a domestic pond populated by amphibia, principally the common newt *Triturus vulgaris*. Raw water quality was monitored on a weekly basis for iron, nitrates and ammonia using a LASA Aqua photometer (Dr Lange, FRG), pH and faecal indicator bacteria. In general, raw water nitrate levels did not exceed 10 mg/l and ammonium levels did not exceed 1 mg/l. Iron levels were consistently < 0.2 mg/l and pH remained relatively stable in the range 7.4 - 7.6. In all respects the raw water appeared suitable for Slow Sand Filtration and this was confirmed by the typical maturation and performance exhibited with respect to removal of faecal indicator bacteria (see Table 3.3.2). The columns were operated for a period of eight weeks in total.

<table>
<thead>
<tr>
<th>Days of Operation</th>
<th>Percentage Reduction in Faecal Coliform Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>99.47</td>
</tr>
<tr>
<td>21</td>
<td>99.55</td>
</tr>
<tr>
<td>35</td>
<td>99.98</td>
</tr>
</tbody>
</table>

Table 3.3.2. Bacteriological performance of protected slow sand filter columns.
PLATES V & VI Column protected slow sand filters and raw water source.
3.4 RESULTS

Experiment 3.1: The removal of indicator bacteria by the experimental protected slow sand filter system.

To investigate the attenuation of indicator bacteria with respect to depth, parallel protected slow sand filters were operated for a period of more than 12 months at a flow rate of 0.28 m/h (experimental configuration III). Samples of supernatant and interstitial water were taken in duplicate from a mature filter protected by six layers of polypropylene fabric. Sampling ports enabled depth samples to be taken at 0 mm (the fabric-sand interface), 20 mm, 100 mm, 200 mm and 500 mm (the filter outlet). Samples were assayed within four hours of collection and the mean results of a large number of sampling occasions are summarised in Figures 58 to 61.

![Graph showing percentage removal of thermotolerant coliforms with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.](image_url)

FIGURE 58 Percentage removal of thermotolerant coliforms with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.
Figure 58 illustrates the attenuation of thermotolerant coliforms with depth. Marginally more than 50 per cent of thermotolerant coliforms were eliminated through the six layers of polypropylene fabric. The removal rate increased to 88 per cent at a depth of 100 mm in the sand and > 98 per cent at the filter outlet (500 mm).

Similar results were obtained for faecal streptococci (Figure 59). However, in the case of the relatively larger, gram positive organisms, more attenuation occurred in the first 100 mm of sand compared with the fabric layers. Nonetheless, mean overall elimination was equivalent for faecal streptococci at 100 mm (88 per cent), 200 mm (> 96 per cent) and 500 mm (> 98 per cent).

**FIGURE 59** Percentage removal of faecal streptococci with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.
Figure 60 depicts the slight increase and subsequent reduction in density of 37°C plate count organisms in interstitial water with respect to depth. In order to avoid sampling these organisms at the fabric-sand interface where populations may be subject to atypical influences, the first sample port was placed at a depth of 20 mm in the sand bed. It is noticeable that there was no reduction in numbers of these bacteria between the supernatant water and interstitial water at a depth of 100 mm in the sand bed. If anything, there was a slight, but relatively uniform increase. Below a sand depth of 100 mm, there was a marked attenuation with the result that at the filter outlet (500 mm), the overall reduction was 74 per cent.

**FIGURE 60** Percentage removal of 37°C/24h total plate count organisms with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.
The results for 22°C plate count organisms (Figure 61) demonstrate an approximate doubling of bacterial populations between the supernatant water and interstitial water at a depth of 100 mm. This increase in numbers appears to be approximately uniform across both fabric and sand horizons of the slow sand filter. However, below 100 mm, there was a marked decline in densities, resulting in similar overall reductions to those observed for 37°C plate count bacteria.

FIGURE 61 Percentage removal of 22°C/72h total plate count organisms with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.
Experiment 3.II.: The removal of bacteriophage by the experimental protected slow-sand filter system.

Attenuation of bacteriophage with respect to depth was investigated in an intensive experiment. Parallel slow sand filters protected by six layers of polypropylene fabric were operated at a flow rate of 0.28 m/h for a period of ten days to establish full biological maturity (reflected by a stable reduction of > 98 per cent in densities of thermotolerant coliforms). Preparations of five bacteriophage were simultaneously introduced into one filter in the following total numbers:

- *Serratia marcescens* bacteriophage \(1.54 \times 10^9\)
- *Erwinia carotovora* bacteriophage \(1.52 \times 10^{10}\)
- *Escherichia coli K12* bacteriophage \(3.96 \times 10^8\)
- *Enterobacter cloacae* bacteriophage \(1.51 \times 10^9\)
- *Bacillus licheniformis* bacteriophage \(1.24 \times 10^8\)

Samples were taken from the supernatant (untreated) water and from interstitial water at the following depths in the sand bed: 0 mm (the fabric-sand interface), 100 mm, 200 mm and 500 mm (the filter outlet). Samples were taken at 10 minute intervals for 130 minutes, at 15 minute intervals for the following 165 minutes, 30 minute intervals for the following 240 minutes and on five occasions in the subsequent 24 hours.

The density of each bacteriophage passing each sampling point was related to flow, and the total number of each was then expressed as a percentage of the original inoculum. Results are described in Table 3.4.1 and depicted in Figures 62 to 66. Overall attenuations ranged from 99.23 per cent for *Bacillus licheniformis* bacteriophage to 99.84 for *Erwinia carotovora* bacteriophage.
<table>
<thead>
<tr>
<th>Bacteriophage</th>
<th>Depth in Sand Bed (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0*</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>53.4</td>
</tr>
<tr>
<td>Erwinia carotovora</td>
<td>22.3</td>
</tr>
<tr>
<td>Escherichia coli K12</td>
<td>49.3</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>63.7</td>
</tr>
<tr>
<td>Bacillus licheniformis</td>
<td>46.4</td>
</tr>
</tbody>
</table>

Table 3.4.1. Percentage of bacteriophage remaining at successive sand depths in a slow sand filter protected by six layers of fabric. * Depth 0 represents the fabric-sand interface.

![Graph showing percentage removal of Serratia marcescens bacteriophage](image)

**FIGURE 62** Percentage removal of *Serratia marcescens* bacteriophage with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.

Original Inoculum $1.54 \times 10^9$ pfu
Total Recovered $9.62 \times 10^6$ pfu
FIGURE 63 Percentage removal of Erwinia carotovora bacteriophage with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.

FIGURE 64 Percentage removal of Escherichia coli K12 bacteriophage with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.
FIGURE 65 Percentage removal of *Enterobacter cloacae* bacteriophage with respect to depth in a protected slow sand filter operated at a filtration velocity of 0.28 m/h.

Original Inoculum $1.51 \times 10^9$ pfu  
Total Recovered $9.86 \times 10^6$ pfu

FIGURE 66 Percentage removal of *Bacillus licheniformis* bacteriophage with respect to depth in protected slow sand filter operated at a filtration velocity of 0.28 m/h.

Original Inoculum $1.24 \times 10^8$ pfu  
Total Recovered $9.50 \times 10^5$ pfu
FIGURE 67-70. Percentage of total *Serratia marcescens* bacteriophage recovered with respect to time for sampling points at depths of 0, 100, 200 and 500 mm in a protected slow sand filter.
FIGURES 71-74 Percentage of total Erwinia carotovora bacteriophage recovered with respect to time for sampling points at depths of 0, 100, 200 and 500mm in a protected slow sand filter.
FIGURES 75-78 Percentage of total *Escherichia coli* K12 bacteriophage recovered with respect to time for sampling points at depths of 0, 100, 200 and 500 mm in a protected slow sand filter.
FIGURES 79-82 Percentage of total Enterobacter cloacae bacteriophage recovered with respect to time for sampling points at depths of 0, 100, 200 and 500 mm in a protected slow sand filter.
FIGURES 83-86 Percentage of total *Bacillus licheniformis* bacteriophage recovered with respect to time for sampling points at depths of 0, 100, 200 and 500 mm in a protected slow sand filter.
The total number of each bacteriophage passing each point in the filter (0, 100, 200 and 500 mm) was calculated, and the proportion of that total passing per minute was expressed as a percentage. The results are depicted in Figures 67 to 86, and illustrate the recovery pattern of each with respect to time after introduction of the bacteriophage to the supernatant water. The traces appear partially biphasic, but this is probably due to imperfect mixing in the supernatant water rather than any retardation effect within the fabric layers. Where recoveries were very low, for example Bacillus licheniformis and Escherichia coli K12 bacteriophage at depths 200 and 500 mm, the traces were not especially descriptive.

Experiment 3.III: The removal of indigenous rotavirus by the experimental protected slow sand filter system.

The removal of naturally occurring Rotavirus was observed in a slow sand filtration system served by water of relatively good microbiological quality. Parallel slow sand filters protected by six layers of polypropylene fabric were operated for a period of six months at a flow rate of 0.28 m/h (experimental configuration III). The period coincided with a winter period October - March when Rotavirus densities levels in raw waters receiving sewage effluents would be expected to be maximal.

Duplicate 5 litre samples of both influent and treated waters were taken from the protected slow sand filtration system on six separate occasions. Samples were concentrated and assayed for the presence of indigenous Rotavirus within six hours of collection.

At no time was Rotavirus detected in water treated by the slow sand filtration system. However, this is unsurprising because indigenous Rotavirus was only detected on one out of six occasions from the raw water (reservoir impounded River Thames water), and then only in very low numbers. Results are summarised in Table 3.4.2.
<table>
<thead>
<tr>
<th>Sampling Occasion</th>
<th>Fluorescent Focus Forming Units per Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw Water</td>
</tr>
<tr>
<td>1</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>2</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>5</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>6</td>
<td>&lt; 2</td>
</tr>
</tbody>
</table>

Table 3.4.2. Results of monitoring of protected slow sand filters for the removal of indigenous Rotavirus present in reservoir stored River Thames water.

Experiment 3.IV: The removal of indigenous rotavirus through sand and anaerobic biomass beneath a wastewater lagoon and irrigation site.

The difficulty of securing a stable experimental system served by a raw water source with high levels of indigenous Rotavirus, led to the investigation of alternative systems which could provide comparable experimental data. The most appropriate system was considered to be a wastewater application site close to Lima, Peru: San Juan de Miraflores. The site occupies 20 hectares dedicated to 21 shallow, unlined lagoons and 375 hectares of flood-irrigated agricultural land and woodland. This former desert land receives 360 litres per second of raw sewage from three low-income residential areas in southern Metropolitan Lima. As a result, the permeable aeolian and alluvial sands (mostly 0.1 - 0.3 mm in diameter) allowed wastewater to penetrate at a rate of 0.07 - 0.10 m/day below lagoons and 0.03 - 0.05 m/day below irrigation channels (Geake et al,
Although the percolation rates were comparable, there was an important difference between microbial penetration through lagoon floors and penetration through irrigation channels. The former required passage through an anaerobic sludge of settled biomass approximately 50 - 100 mm in depth prior to transport through sand, whereas the latter represented direct application to the sand.

Samples were taken from sand horizons to a depth of 18 metres below the lagoons and irrigation channels. Results were calculated per 100 g wet weight of sand. Results are described in Figures 87 - 92.

In the case of penetration below lagoons, there was a $4 \log_{10}$ reduction in thermotolerant coliforms and faecal streptococci following infiltration through 5 m of sand. Rotavirus was not detected in levels greater than 60 fffu per 100g more than 100 mm below the anaerobic sludge.

Attenuation of faecal indicator bacteria through sand below irrigation channels was comparable to that below lagoons (3-4 $\log_{10}$ units). But in contrast with results obtained below lagoons, Rotavirus was detected at levels of between $10^3$ and $10^4$ fffu per 100g to a depth of 7.5 metres. Rotavirus was undetectable (less than 20 fffu per 100 g) below 12.5 metres.
FIGURE 87 Attenuation of indigenous Rotavirus with respect to depth of sand below a wastewater lagoon.

FIGURE 88 Attenuation of indigenous Rotavirus with respect to depth of sand below a wastewater irrigation channel.
FIGURE 89 Attenuation of indigenous faecal streptococci with respect to depth of sand below a wastewater lagoon.

FIGURE 90 Attenuation of indigenous faecal streptococci with respect to depth of sand below a wastewater irrigation channel.
FIGURE 91 Attenuation of indigenous thermotolerant coliforms with respect to depth of sand below a wastewater lagoon.

FIGURE 92 Attenuation of indigenous thermotolerant coliforms with respect to depth of sand below a wastewater irrigation channel.
Having established two protected slow sand filter columns with bacteriological efficiencies typical of conventional slow sand filters ie >99 per cent reductions in densities of thermotolerant coliforms (Table 3.3.2), preparations of simian Rotavirus SA11 were added to both on two occasions. Approximately 100 ml of the same virus stock suspension were added to the supernatant water above each column and samples were abstracted from the supernatants, all sample ports and the two filter outlets at intervals of 10 - 20 minutes for a period of 200 minutes. Samples were stored at -20°C before analysis both by plaque assay and indirect immunofluorescence in multiwell dishes.

Results from the plaque assay proved unsatisfactory due to methodological difficulties and were discarded. However, the immunofluorescent assay proved very reliable, even after prolonged storage of samples (12 months), and mean levels of Rotavirus in the supernatant water and the sample port placed at the fabric - sand interface are presented in Figure 93.
Levels of Rotavirus passing further into the filter columns were too low to express graphically. Thus all results have been integrated in terms of average total virus recovered for each sampling point, taking into account concentration detected, time and total volume of flow. These results, together with the percentage elimination rates they represent are contained in Table 3.4.3 and Figure 94.

It may be seen that in a period when reductions in densities of thermotolerant coliforms were of the order of 2-3 log_{10} units, the elimination of simian Rotavirus across the slow sand filter columns was very similar i.e. 99.6 per cent. Moreover, the pattern of removal of simian Rotavirus very closely paralleled that observed for indication bacteria and viruses in previous experiments (3.1 and 3.11).

<table>
<thead>
<tr>
<th>Depth in Sand Bed (mm)</th>
<th>Average Total Rotavirus Recovered*</th>
<th>Average Percentage Rotavirus Remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant</td>
<td>1,984,000</td>
<td>100</td>
</tr>
<tr>
<td>0 (Fabric-Sand Interface)</td>
<td>1,200,000</td>
<td>60.5</td>
</tr>
<tr>
<td>250</td>
<td>46,600</td>
<td>2.4</td>
</tr>
<tr>
<td>450</td>
<td>39,200</td>
<td>2.0</td>
</tr>
<tr>
<td>650</td>
<td>&lt;8,000+</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>850</td>
<td>&lt;8,000+</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>Outlet</td>
<td>7,800+</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 3.4.3  Reduction in titres of simian Rotavirus SA11 through two protected slow sand filter columns operated at 0.2 m/h.

*Average of four experiments
+Results close to the limit of detection
FIGURE 94 Percentage removal of simian Rotavirus SA11 with respect to depth in column slow sand filters operated at a filtration velocity of 0.20 m/h.
SECTION 4

ADSORPTION OF VIRUSES TO SURFACES IN SLOW SAND FILTRATION
4.1 GENERAL OBSERVATIONS

A number of characteristics of natural aquatic systems have been examined in order to assess their relative importance in facilitating adsorption of viruses to surfaces. Typically, studies have devoted particular attention to such factors as pH, the presence of organic material (e.g., total organic carbon), and the presence of inorganic ions (e.g., by conductivity, salinity or with reference to specific anions or cations in solution).

In addition, a number of physico-chemical concepts have been applied in order to describe the mechanisms of interaction between viruses and surfaces. These include the zeta potential of the surface, the cationic exchange capacity of the surface (CEC), the anionic exchange capacity (AEC) of the surface, and the isoelectric point of the virus (pI).

4.2 VIRUSES AS COLLOIDS IN ELECTROSTATIC INTERACTIONS

Edwards and Monke (1967) noted that natural silica carries a net negative charge due to lattice imperfections. Using acrylic cylinders of 4" diameter containing silica of effective size 0.35 mm and coefficient of uniformity of 1.43, they were able to demonstrate that a typical slow sand filtration medium had a natural zeta potential of -133 mV in distilled water. Zeta potential is the electrical potential at the surface shear plane with respect to a point far out in the bulk liquid. It may be derived both from electrophoretic theory and from concepts of streaming potential.

However, following normal bacterial colonisation of silica at the top of the column, zeta potentials were observed to be +850 mV at the top and -200 mV at the bottom. Furthermore, it was observed that the column very quickly reached its isoelectric point with the passage of water of pH 7.0, thereby enhancing the rate of deposition of colloidal clay. This was reflected in an increase in head loss across the column of 15" in 40 hours. But when water of pH 5.1, 7.8 and 8.4 was passed, deposition was retarded and head loss increased by only 4" after 60 hours.
The above observations are noteworthy because there is now a consensus that the adsorption of viruses to surfaces in aquatic systems is most appropriately described in terms of colloid chemistry (Bitton, 1975; Schaub & Sagik, 1975; Yeager & O'Brien, 1979; Globa, 1985). This approach provides a theoretical framework which supports several empirical observations such as the improvement of adsorption by the lowering of pH or by the introduction of divalent cations.

Colloids in water adopt a charge which reflects the dissociation of ionisable groups on the colloid surface or the association of ions from the surrounding medium. Thus at normal pH, and in the absence of cations, colloids do not aggregate but remain in suspension. To encourage aggregation between similarly charged colloidal particles, or between a colloid and a negatively charged surface, the net negative charges of the colloids must be overcome and the overall interaction energy (the sum of electrostatic repulsion and van der Waals attraction) must be reduced. Rutter and Vincent (1984) distinguished between short range and long range forces (the latter including those contributing to overall interaction energy). They noted that in low concentrations of electrolytes, the free energy barrier of long range forces would be substantially greater than in a solution of intermediate or high concentrations of electrolyte.

The electrical double layer around viruses in aqueous suspension has been described (Gerba, 1984). The carboxyl and amino groups on the protein surface of the virus ionise and attract a dense layer of counter-ions with a net positive charge (the Stern layer). Outside this layer is a more diffuse area of net negative charge (the Gouy layer). Together, the layers maintain the electrical stability of the colloidal particle in suspension. It is generally acknowledged that the incorporation of simple non-hydrated cations such as calcium (Ca$^{2+}$), magnesium (Mg$^{2+}$) or sodium (Na$^{+}$) results in an overall compression of the double layer. This allows otherwise non-attractive surfaces into sufficiently close proximity to permit physical forces ie van der Waals attraction to be exerted.
There is evidence to suggest that divalent cations are more effective than monovalent cations in enhancing the adsorption of colloids (Bitton, 1975). This may be due to a more efficient compression of the double layer. However, it has also been postulated that the formation of salt bridges between surfaces of similar charge results directly from the cross-complexation of divalent and trivalent ions such as calcium, iron and aluminium.

The role of pH is also extremely important in influencing the efficiency of adsorption between surfaces of similar charge in natural waters. A reduction in pH will have an equivalent effect to the introduction of monovalent cations. Because the isoelectric point of most viruses in water is less than 7.0, it is axiomatic that viruses will adsorb to surfaces more efficiently in acid waters than at neutral or higher pH.

Zerda and co-workers (1985) examined the effect of modifying the surface charge of silica and concluded that electrostatic forces are of considerable importance in determining virus-surface interactions. Silica was given a positive surface charge by refluxing with organosilanes. This process produced silica grains carrying primary and quaternary amine groups. Further reaction of the primary amines with 1 per cent succinic anhydride solution resulted in the carboxylation of these groups and the production of silica grains with a high net negative charge. Adsorption studies were undertaken in 1.5 ml micro-centrifuge tubes with 0.5 ml of 0.1 M ionic strength buffer of pH 4.0, 6.4, or 8.5 with 3 mg of charge-modified silica. 5 μl of virus was added, the tube vortexed and agitated at 300 - 400 rpm and samples extracted over periods of 2 - 60 minutes. Samples were then centrifuged at 12,000 rpm for 1 minute and supernatants assayed.

Viruses had isoelectric points in the range 3.9 - 7.1 and included Poliovirus 1, MS2 coliphage, T2 coliphage, Reovirus 1 and Reovirus 3. It was demonstrated that viruses adsorbed well to negatively charged silica at pH values below their isoelectric point (pI), and to positively charged silica at pH values above their isoelectric point. At values close to their pI, viruses adsorbed to all
silica types, although with less efficiency. Thus it was concluded that knowledge of both the
isoelectric point of the virus and the charge on the surface could be used to predict the adsorption
characteristics of viruses. Alternatively, knowledge of the adsorption behaviour could be used to
describe the surface charge conditions on the virus.

Corpe (1980) noted that extracellular polymers eg lipoprotein and lipopolysaccharide, are very
important in the formation of cell bridges and thus the promotion of floc formation. Such flocs are
undoubtedly very influential in the entrapment of colloids both in activated sludge sewage
treatment and slow sand filtration (Section 3). Of equal importance may be the observation that
extracellular fractions incorporate a complement of counter-ions to the net bacterial surface charge
(Robb, 1984). Other mechanisms of surface force attraction have been considered (Ives &
Gregory, 1967). These include hydration and mutual adsorption. However, neither of these is
likely to be significant in the adsorption of viruses to surfaces. Hydration is more likely to
increase colloid stability and thereby decrease adsorption, and mutual adsorption is unlikely to be
an important phenomenon when numbers of viruses in natural systems are so low.

4.2.i Transport Mechanisms in Filtration

Physical impaction is particularly important in the adsorption of colloidal particles because at
near-neutral pH and over short distances ie 0.2 - 0.3 nm, electrostatic repulsion will predominate
over the attractive chemical interactions of hydrogen bonding and covalent bonding and the
physical attraction of van der Waals forces.

Thus, whatever the mechanism of adsorption, the establishment of intimate contact between the
adsorbing particle and the solid surface is likely to be of especial importance in the removal of
non-motile organisms such as enteric viruses during filtration. Transport mechanisms include
straining, gravity, hydrodynamics, interception and diffusion (Ives & Gregory, 1967). However,
in the filtration of small colloidal particles, only hydrodynamics and diffusion are thought likely to play a significant role.

Hydrodynamic effects rely on the laminar nature of flow between streamlines of different velocity passing grain surfaces. The velocity of flow at the surface will be zero, whilst the velocity in the centre of the pore will be maximum. There is a tendency for particles to migrate from streamlines of high velocity to those with lower velocity, thereby bringing them within interception distance of the grain surface. This effect has been likened to the deposition of material on the inside curve of a river, and the forces to those causing the curved flight of a spinning tennis ball (Ives, 1980). Hydrodynamic action is described in terms of the Reynolds’ number (Re) of the filter, thus:

\[
Re = \frac{v D p}{\mu}
\]

where:

- \(v\) is the approach velocity of filtration
- \(D\) is the grain diameter
- \(p\) is the mass density of the fluid
- \(\mu\) is the dynamic viscosity of the fluid

Hydrodynamics are most important where particles are irregularly shaped ie not spherical.

Diffusion relates to the natural movement of colloidal particles caused by the thermal energy of water molecules. The movement is random and is opposed by the viscous drag of the liquid. Thus, the total effect is described by the Stokes-Einstein coefficient of diffusion (\(B\)):

\[
B = \frac{RT}{3H \mu d}
\]
where:

\[ R \text{ is the Boltzmann’s constant } (1.38 \times 10^{-23} \text{ J} \cdot \text{K}^{-1}) \]

\[ T \text{ is the Kelvin (absolute) temperature} \]

\[ \mu \text{ is the dynamic viscosity of the fluid} \]

\[ d \text{ is the particle diameter} \]

Diffusion is particularly important in determining the movement of very small particles. Thus it is potentially the predominant transport mechanism involved in the impaction of viruses to surfaces in natural environments. Adsorption of bacteriophage to bacteria follows first order kinetics and thus may also be ascribed primarily to diffusion (Bitton, 1975).

Valentine and Allison (1959) investigated the rate at which latex particles of equivalent size to viruses came into collision with surfaces and concluded that the phenomenon could be described entirely in terms of Brownian motion i.e. diffusion. External physical forces such as shaking the suspension were considered irrelevant because they would not disturb the static fluid layer of 0.1 - 1.0 mm around the solid surface.

Although the transport and adsorption mechanisms are relatively well understood, as yet there is no general explanation for the observed uniformity or indeed, non-uniformity between viruses in their response to different environments. The two environments in which virus adsorption has been studied in most detail are soils, and marine or estuarine sands and sediments.

4.2.ii Virus Adsorption to Soils

Despite markedly different isoelectric points, Poliovirus and Reovirus have been observed to exhibit very similar behaviour in adsorbing to a variety of soil types (Sobsey et al., 1980). In contrast, different efficiencies were noted for enterovirus strains in their adsorption to both organic and inorganic substrates (Gerba et al., 1980). Using Poliovirus 1, Echoviruses 1, 7 and 29 and Coxsackie viruses B3 and B4, Gerba and co-workers observed adsorption rates of 0 - 99.9 per cent
to sandy loam soil. These differences were considered reasonable due to the different isoelectric points and hence electronegativity of the viruses in natural waters.

In a study of Poliovirus adsorption to 34 different minerals and soils (Moore et al, 1981), it was established that substrate surface area and pH are not likely to be limiting factors in virus adsorption in normal environments. Adsorption equilibria between Poliovirus and Ottowa sand were reached within 1 hour at a total of $2.5 \times 10^6$ pfu virus per mg sand. The two factors of greatest importance were considered to be the presence of organic material and available surface charge. Studies were conducted on a batch basis using polypropylene containers and a synthetic freshwater medium.

In experiments which bear closer comparison with the Slow Sand Filtration process, Funderberg and co-workers (1981) examined the transport of Poliovirus 1, Reovirus and $\Phi x 174$ coliphage through saturated soil columns. It was found that the variables most significantly correlated with Poliovirus removal were cation exchange capacity (CEC), total organic carbon (TOC) and percentage of clay. When CEC exceeded 23 meq per 100 g of soil, 99 per cent of Poliovirus was removed in 330 mm of soil column. Similar results were obtained with Reovirus, but coliphage removal appeared to be related most strongly to total organic carbon in the soil and residence time in the columns.

These observations were similar to those of Goyal and Gerba (1979). Although they found no specific correlation between bacteriophage adsorption and cation exchange capacity or surface area, Goyal and Gerba showed that a number of chemical and physical parameters were potentially important. These included percentage clay, percentage sand, percentage organic matter, total phosphorus, resin-extractable phosphorus, total iron, total aluminium, and exchangeable magnesium. However, each of these characteristics was correlated with the adsorption of only three (at most) of a wide range of enteric viruses and bacteriophage and no
general model was proposed.

The adsorption of bacteriophage and other viruses to clays has been investigated. It was found that pretreatment of clay with negative ions reduced the adsorption of coliphage but not Reovirus or Herpes virus (Stotzky et al, 1981). Thus it was concluded that Reovirus could bind to negative sites. Subsequent studies with T1 and T7 coliphage and kaolinite and montmorillonite clays confirmed a particular affinity for anion exchange sites on the part of T1 coliphage (Schiffenbauer & Stotzky, 1982). A degree of adsorption still occurred when all cationic sites were blocked with a polyanionic salt (SMP). Differences in behaviour between the two bacteriophage were ascribed to differences in amino acid configuration on the virus capsomers.

Duboise and co-workers (1976) confirmed that elution of Poliovirus from soil columns was much greater following the addition of distilled water compared with the continued passage of sewage effluent. It was concluded that desorption of viruses was strongly favoured by a reduction in the electrical conductivity of solutions surrounding sand or soil grains. Migration of viruses through columns was likely to be enhanced by a decreasing ionic gradient.

The presence of organic material in water can result in competition for binding sites and a consequent reduction in adsorption efficiency (Lipson & Stotzky, 1984). Schaub and Sorber (1977) demonstrated that in the presence of autoclaved primary sewage effluent, the adsorption of both Poliovirus 1 and f2 coliphage to soil was poor. Thus they concluded that it was doubtful that adsorption would play a major role in retaining bacteria and viruses in the soil beneath wastewater recharge sites.

It has been demonstrated that during intermittent loading of 100 mm columns of sands and organic soils with wastewater, relatively high adsorption (greater than 95 per cent) of both Poliovirus and Reovirus may occur (Sobsey et al, 1980). Consistent with the findings of Duboise and co-workers (1976), solutions of low ionic strength resulted in the elution of a significant quantity of virus.
These observations are consistent with adsorption theory, but imply that in this case, adsorption was reversible ie not covalent.

4.2.iii Virus Adsorption to Marine and Estuarine Sands and Sediments

A number of studies which have examined the adsorption of enteric viruses to marine and estuarine sands and sediments have been concerned with the development of elution techniques to quantify viruses of public health significance in the environment. Relatively few have examined adsorption phenomena per se.

Labelle and Gerba (1979) observed that with some exceptions, Enteroviruses adsorb readily to estuarine sediments. More than 99 per cent of Poliovirus 1, Coxsackie B3 and simian Rotavirus SA11 adsorbed to sediment at salinities of up to 35 g/kg. However, Echovirus adsorbed less well. A contrasting observation was made by Wyn-Jones and Edwards (1982) who found that Echovirus 11 showed greater affinity for mud and freshwater sediments than either Coxsackie or Poliovirus.

Differences in adsorption to Mississippi estuarine sediments were observed for Coxsackie 31, Echovirus 11 and Poliovirus 1 (Johnson et al., 1984). It was further noted that elution of virus from sediments was progressively more difficult as clay content increased. These observations confirmed earlier experiments (Tsai et al., 1983) which demonstrated 92 - 95 per cent adsorption of Poliovirus and Coxsackie virus to Mississippi sediment within one minute of contact. Adsorption was found to be unaffected by the method of sterilisation of the sediment.

Another factor which has been examined is the protecting effect of sediments on adsorbed virus. Using survival chambers, Labelle and Gerba (1982a) showed that the presence of natural sediment could enhance the survival of Poliovirus 1 by a factor of more than 100-fold. This effect was confirmed in subsequent studies (Labelle & Gerba, 1982b) which demonstrated that sediment
protects Poliovirus from inactivation both by microbiological and physical factors eg temperature. It was suggested that adsorption may also stabilise the virus capsid against antagonistic chemical influences such as heavy metals, salinity and enzymatic attack. This suggestion accords with the view of Stotzky and co-workers (1981) who concluded that the presence of clay increased the resistance of both coliphage and Reovirus to "abiological inactivation" by lysozymes.

Thus it may be concluded that as with adsorption to soils and clays, virus adsorption to marine and estuarine sediments is both rapid and relatively efficient. However, there is not necessarily any uniformity in the behaviour of members of the enterovirus group in adsorption to surfaces in the environment.

4.2.iv Models to Index Virus Behaviour

Farrah and co-workers (1978) concluded that Poliovirus could not be used as a model for simian Rotavirus SA11 adsorption because it was $2 \log_{10}$ units more efficient in adsorbing to aluminium hydroxide flocs in tap water and $1 \log_{10}$ unit more efficient in adsorbing to sewage floc. However, it was noted that because of its physical similarities, SA11 was a suitable model for human Rotavirus adsorption and that Reovirus was another candidate.

This view was confirmed by Gerba and co-workers (1980) who noted that Poliovirus 1 was not a good model for the adsorption of other enteroviruses, to say nothing of other human enteric viruses. They observed that six enteroviruses exhibited widely differing adsorption rates to a number of substrates including activated sludges (67 - 99.8 per cent), estuarine sediment (87 - > 99.99 per cent) and sandy loam soil (0 - 99.9 per cent).

The earlier studies of Goyal and Gerba (1979) described above had suggested that there may be significant differences in the adsorption characteristics of wild strains compared with reference
strains of the same virus due to differences in protein configurations on the capsid. It was observed that the adsorption of simian Rotavirus SA11 to sandy loam was relatively low (51.6 per cent after 30 minutes of batch adsorption) compared with six Coxsackie viruses (96 - 99.99 per cent), three Polioviruses (98 - 99.9 per cent) and eighteen Echoviruses (14 - 99.99 per cent). The best adsorbers to nine soil types were Poliovirus and T4 coliphage; the least efficient adsorbers were f2 coliphage and Echovirus 1.

Thus it is clear that no single enterovirus or bacteriophage can adequately index the general behaviour of all viruses. For this reason, a number of studies have devoted particular attention to the construction of mathematical models for the adsorption of specific viruses in defined environments. In this way a more fundamental understanding of adsorption phenomena may be established.

In studies by Lance and co-workers (1976) the mechanisms of virus adsorption and desorption to natural granular material were partially elucidated. Using PVC columns of calcareous sand of dimensions 2500 x 100 mm and flooding them with sewage at flow rates of 150 - 55 mm per day for a period of 27 days, it was demonstrated that efficiencies of Poliovirus 1 removal were consistently greater than 4 log units. Most importantly, it was noted that reductions of 2 log units were obtained in the top 20 mm of the column. Below 50 mm, virus removal was proportional to virus concentration and followed the conventional relationship:

\[
\frac{dC_v}{dx} = -kC_v
\]

where:

\( C_v \) is the virus concentration detected

\( x \) is the column depth

\( k \) is the removal constant
Because this relationship did not hold for the top 20 mm of the columns, it was concluded that in this horizon factors other than adsorption were operating. It was also noted that flooding columns with deionised water led to desorption but that this could be prevented by the addition of calcium chloride.

The studies of Moore and co-workers (1981) relied on an assumption that a monolayer of hexagonally packed virus on a sand surface would constitute $2.3 \times 10^{10}$ particles per mg to calculate that their experimental observations indicated that maximum virus adsorption occurred at 1 per cent coverage of surface area. In their survey of different substrates, it was shown that materials with a high pi which were free of organic material were the best adsorbents for Poliovirus. Even in the absence of interference from organic material, clays with a low pi did not make good adsorbents. It was found that at low virus concentrations, adsorption followed the Freundlich isotherm, whereas at higher concentrations a Langmuir isotherm provided a better description of the data.

The coverage of substrate surface area by adsorbents is not necessarily a constant factor and may be governed by flow regime. Thus, in quiescent systems, high saturation densities of up to 45 per cent of surface area may be achieved (Fletcher, 1977). In contrast, in laminar flow conditions, coverage is likely to be substantially less, for example 1 - 5 per cent (Powell & Slater, 1983). Furthermore, where adsorbents are of a similar charge, they may inhibit successive adsorption. Using negatively charged latex particles and negatively charged glass as the adsorption surface, Dabros and van der Ven (1983) demonstrated that particles adsorbed weakly, oscillating about a fixed position and excluding adhesion of further particles within an area of some 20 - 30 times the particle cross section. Clearly, adsorption phenomena are not subject to simplistic analysis.

There are four principal classes of adsorption isotherm which describe the attachment of particulates or solutes to surfaces (Giles et al, 1960). The Langmuir isotherm is an "L" curve which is indicative of flat adsorption of particles to the surface. Other isotherms are described as
"S" curves (indicative of a vertical orientation of adsorbent), "H" curves (high affinity), and "C" curves (constant partition). "L" curves depict a situation where there is progressively less chance of a bombarding particle finding an adsorption site as particles are taken up from solution. Thus to increase adsorption rate, the concentration of adsorbent must be increased. This also applies to the later stages of "S" and "H" curves. However, in the early stage of an "S" curve, the opposite occurs due to co-operative adsorption. It is thought that "H" curves are special cases of "L" curves, their verticality deriving principally from the rapid adsorption observed with solutes such as ionic micelles and polymeric molecules.

In this study, two principal experiments were designed to model the adsorption of Rotavirus to surfaces in Slow Sand Filtration. They were:

I Adsorption of simian Rotavirus to sterile and non-sterile substrates in glass shake flasks;

II Adsorption of simian Rotavirus and bacteriophage to substrates in polypropylene centrifuge tubes.

However, owing to time constraints and the non-availability of a reliable antiserum to Rotavirus in the UK, the second of these experiments could not be undertaken. The experimental design is thus presented as a recommendation for future work.
4.3 MATERIALS AND METHODS

Simian Rotavirus SA11 was prepared and assayed in identical fashion to methods described in Section 3.

Experiment 4.1 Adsorption of simian Rotavirus to sterile and non-sterile substrates in glass shake flasks

Untreated water was collected from the Hampton Water Treatment Works (Thames Water Authority) and sterilised by autoclaving at 121°C for 20 minutes. A volume of 22 ml SA11 virus stock solution was added to 375 ml sterilised raw water and agitated slowly for a period of 60 minutes on a rotary shaker in an ambient temperature of 18°C. An aliquot of 5 ml of this stabilised virus suspension was removed and stored at 4°C for subsequent assay.

Except in the case of controls, substrates were taken from functional protected slow sand filters and conventional slow sand filters. 75 ml volumes of virus suspension were added to 250 ml flat-bottomed glass flasks containing pre-weighed quantities of substrate (100g wet weight sand or 20g wet weight fabric). Flasks and contents were vigorously mixed and supernatants immediated sampled. Thereafter, flasks were agitated gently on a rotary shaker and supernatants sampled on six further occasions over a period of 20 hours.

Simian Rotavirus SA11 densities were assayed by immunofluorescence, with counts of fluorescent foci in ten representative microscope fields being extrapolated to obtain the original density in supernatants.
Experiment 4.II Adsorption of simian Rotavirus and bacteriophage to substrates in polypropylene centrifuge tubes - Recommendations for future work

Raw water should be obtained from the Hampton Water Treatment Works and three sterile stock solutions prepared (pH 6, pH 7 and pH 8). Simian Rotavirus SA11, Serratia marcescens bacteriophage and K12 coliphage should be suspended in the three stock solutions in decimal dilutions to provide a range of virus concentrations.

Oak Ridge centrifuge tubes should be selected for adsorption studies due to their non-adsorptive properties, and a range of substrates should be introduced into the tubes in weights in the range 0.1 - 1 g. Substrates should include:

i Sterile polypropylene fabric;
ii Sterile acid-washed sand;
iii Sterile clean sand;
iv Polypropylene fabric and schmutzdecke;
v Sand and schmutzdecke;
vi Polypropylene fabric and schmutzdecke with protozoa inactivated by low temperature storage;
vii Sand and schmutzdecke with lipoproteins and lipopolysaccharides pre-digested with enzymes.

Adsorption experiments should be conducted over periods of one hour, followed by centrifugation and assay of supernatants to quantify levels of non-adsorbed virus. Viral stock solutions at various concentrations and ranges of substrate weights should then be used to construct adsorption isotherms in the conventional manner. Isotherms should be elaborated for the pH range indicated, namely pHs 6, 7 and 8, in order to explore adsorption phenomena over the normal pH range for natural waters.
4.4 RESULTS

Experiment 4.1: Adsorption of simian Rotavirus to sterile and non-sterile substrates in glass shake flasks

Experiments were conducted in two series - one for the sterile substrates and another for non-sterile substrates extracted from operational filters. Results are expressed in graphical form (Figures 95-103). In the case of sterile substrates, both the control and the experimental flasks exhibited similar initial removals of simian Rotavirus from the aqueous phase: approximately one log$_{10}$ unit in one hour. It was only in the longer term that adsorption efficiencies diverged with Rotavirus titres in the aqueous phase remaining approximately constant in the control flask over the period 1 - 20 hours (Figure 95) but decreasing by 2 log$_{10}$ units over the same period for sterile acid-washed filter sand and clean filter sand (Figure 96 & 97). In contrast sterile 10 mm gravel and polypropylene fabric did not significantly adsorb virus from the aqueous phase in the period 1 - 20 hours (Figures 98 & 99).

In the adsorption experiments with non-sterile substrates Rotavirus exhibited somewhat different behaviour with very little removal from the aqueous phase in the control flask in the first hour (Figure 100). Over the entire 20 hours, adsorption in the control flask was negligible. In contrast removal from the aqueous phase was rapid for the three non-sterile substrates and was greater than 2 log$_{10}$ units over the full 20 hour period in all cases. Sand and schmutzdecke from a conventional slow sand filter appeared the most effective in removing viruses from the aqueous phase after a period of two hours (Figure 101) but removal by sand and schmutzdecke, and polypropylene and schmutzdecke from a protected slow sand filter were remarkably similar after periods of 1 and 20 hours (Figures 102 & 103).
FIGURE 95 Adsorption of simian Rotavirus SA11. Control for sterile substrates.

FIGURE 96 Adsorption of simian Rotavirus SA11 in contact with sterile acid-washed filter sand.
FIGURE 97 Adsorption of simian Rotavirus SA11 in contact with sterile clean filter sand.

FIGURE 98 Adsorption of simian Rotavirus SA11 in contact with sterile clean 10 mm gravel.
FIGURE 99 Adsorption of simian Rotavirus SA11 in contact with sterile clean polypropylene fabric.

FIGURE 100 Adsorption of simian Rotavirus SA11. Control for non-sterile substrates.
FIGURE 101 Adsorption of simian Rotavirus SA11 in contact with sand and schmutzdecke from a conventional full scale slow sand filter.

FIGURE 102 Adsorption of simian Rotavirus SA11 in contact with sand and schmutzdecke from an experimental protected slow sand filter.
FIGURE 103 Adsorption of simian Rotavirus SA11 in contact with polypropylene fabric and schmutzdecke from an experimental protected slow sand filter.
SECTION 5

DISCUSSION
DISCUSSION

5.1 ROTAVIRUSES AND ROTAVIRAL INFECTION: GENERAL OBSERVATIONS

Human Rotaviruses have been generally accepted as the single most important cause of diarrhoea in infants requiring hospital treatment (WHO, 1982). However, in the sixteen years since the virus was first described (Bishop et al., 1973), it has not proven possible to elucidate with any certainty the comparative importance of various modes of transmission of the human strains. In contrast, the pathology and biochemical characterisation of the virus have been the subject of detailed investigation. As a result, there is substantial agreement on a broad range of basic details (Blacklow & Cukor, 1981; Holmes, 1983).

Rotaviruses are now classified as members of the Reoviridae family. They are icosahedral, approximately 70 nm in diameter, and in their complete form have a double-shelled capsid and thirty-two capsomers radiating from a central core. The double capsid is vital for infectivity (Shirley et al., 1981); incomplete (rough) particles possessing only one capsid are observed both in stool specimens and in cell culture but they are not capable of initiating infection. The majority of Rotaviruses have a common group antigen located on the inner capsid shell irrespective of the species from which the virus was isolated. However, since 1983, atypical Rotaviruses (sometimes called pararotaviruses) which do not possess a group antigen have been isolated with increasing frequency (Pedley et al., 1986). These include a number of avian and mammalian (non-human) strains but an important example of such isolations was a human pathogen responsible for a large waterborne outbreak in China (Tao et al., 1984). As a result, the classification of human Rotaviruses has been rationalised into sub-groups and serotypes (WHO, 1984b). It is now possible to cultivate representatives of two sub-groups and four serotypes of human Rotavirus in cell culture (Beards & Flewett, 1984). Rotaviruses contain a genome of double-stranded RNA with eleven segments varying in molecular weight up to $2 \times 10^6$. 
Rotavirus strains have been differentiated by neutralisation, complement fixation, ELISA and polyacrylamide gel electrophoresis (Gerba et al, 1985). Serotype antigens are associated with the outer capsid protein which is a glycosylated polypeptide of mass 36,000. Sub-group antigens are located on the major inner capsid polypeptide which has a mass of 46,000. Of interest to this study is the fact that the principal model used in adsorption experiments, simian Rotavirus SA11 falls into the same classification (serotype 3, sub-group I) as a number of important human Rotaviruses, including the Ito, Nemoto and Yo strains. Holmes (1983) considered simian Rotavirus SA11 to be the most likely eventual choice for a type species.

In humans, exposure to Rotavirus gives rise to disease, asymptomatic infection and carriage (ie passive infection with no detectable antibody response). Primarily a disease of children, Rotavirus gastroenteritis is characterised by an incubation period of 1 - 7 days (usually less than two) before the onset of watery diarrhoea, vomiting, and generally, temperature elevation. In studies in Bangladesh and Brazil, vomiting was associated with 71 and 68 per cent of cases and fever with 53 and 65.6 per cent of cases respectively (Stoll et al, 1982; Linhares et al, 1983). Symptoms persist for 5 - 7 days with virus shedding detectable both before the onset of the illness and several days thereafter. Most cases are observed in children between 6 and 24 months old, with peak incidence occurring in the period 9 - 12 months (WHO, 1980).

The early perception of Rotavirus infection as a disorder largely confined to children has persisted. However, it is now becoming clear that asymptomatic infection is very common in older children and adults (Hrdy, 1987) and that Rotaviruses are implicated in travellers’ diarrhoea (Bolivar et al, 1978, Ryder et al, 1981) and severe gastroenteritis of the very old (Cubitt & Holzel, 1980; Echeverria et al, 1983).

Asymptomatic infection and virus carriage also occur in children (Cameron et al, 1984). Champsaur and co-workers (1984) monitored hospital admissions over an 11 month period and detected asymptomatic infection in 2 per cent of neonates, 20 per cent of children between 1 and 6
months old, and 37 per cent of children between 7 and 24 months old. In contrast, carriage was detected in 27 per cent of neonates, 19 per cent of children between 1 and 6 months old, and 14 per cent of children between 7 and 24 months old. The presence of detectable antibodies was not observed to prevent re-infection. In fact, sequential infection by Rotaviruses is known to occur in infants (possibly due to different serotypes) and intra-familial transfer of the agents appears to be efficient, especially among children (Senturia, 1986). Volunteer studies using adult subjects failed to demonstrate a correlation between levels of serum antibody to Rotavirus and susceptibility to infection (Ward et al., 1986).

The implications of the above observations for water treatment practice are profound. Firstly, it is possible that adults may provide an asymptomatic reservoir for rotaviral infection. Thus it cannot be assumed that children are always the source of Rotaviruses introduced into a family (WHO, 1980). Secondly, if the infectious dose of non-attenuated virus in a susceptible individual is as low as 10 particles (Ward et al., 1986), then the presence of low numbers of human Rotavirus in drinking water supplies would represent a significant public health threat. Thus there remains the possibility that low level infection and re-infection of adults and children may occur through drinking water and that amplification of the agent may occur in the community in the absence of characteristic waterborne outbreaks (Graham et al., 1987). Hrdy (1987) listed five principle sources of rotaviral infection of adults, two of which (environmentally mediated and travellers’ diarrhoea) had strong associations with waterborne transmission. This postulated epidemiology may be complex, but it is undoubtedly feasible, particularly in regions of the world where disinfection is not routinely practised.

In the first, introductory Section to this study, a possible waterborne mode of transmission for human Rotaviruses was raised in the context of the importance of Rotavirus as an agent of worldwide morbidity and mortality from diarrhoeal disease. Global statistics for rotaviral infection were presented in terms of prevalence rates in various countries. Such statistics may do little to establish the comparative importance of various modes of transmission of human
Rotavirus, except to confirm the probable pre-eminence of person-to-person spread within institutions (Holmes, 1983). However, it is useful to consider literature on the prevalence of rotaviral infection since it does confirm the universality of the organism as well as both similarities and differences in its aetiology worldwide. In fact, the aetiology of rotaviral infection has been reviewed in considerable detail (Kapikian et al, 1978; Holmes, 1983; Ho et al, 1988.)

5.1.i Prevalence of Rotavirus Infection in Industrialised Countries

There have been numerous studies of hospital populations in order to determine the prevalence of rotaviral infection in children and adults presenting with symptoms of acute gastroenteritis in industrialised countries. In a prospective study of the general ward of a children’s hospital in Montreal, Canada, Rotavirus and/or Calicivirus was associated with 20 out of 41 infants with diarrhoea (Spratt et al, 1978). In a three year study of viruses associated with diarrhoea in outpatients reporting to a paediatric clinic in Osaka, Japan, Rotavirus was detected in 30.9 per cent of all cases and 67.4 per cent of virus positive cases (Oishi et al, 1985). Of the total of six virus groups identified, only Rotavirus exhibited seasonality, with peak incidence occurring in cooler months. A similar observation was made in a three year study in north-east Scotland between 1982 and 1984 where 708 human Rotavirus infections were recorded (Cash et al, 1986). The majority of cases occurred in winter months, 83 per cent being children below the age of five.

During a study of hospital patients in Ohio, US, Rotavirus was identified in 40 per cent of cases of nosocomial infection and incidence was highest on wards where the majority of children were less than two years of age (Pacini et al, 1987). In a three year study in Rome, Rotavirus infection was demonstrated in 29.3 per cent of children hospitalised due to acute diarrhoea (Donelli et al, 1988). Other viruses eg. Enteroviruses, Adenoviruses and small round viruses were detected in a much smaller proportion of cases. The median age of patients with Rotavirus infection was 17, 10 and 11.5 months in 1983, 1984 and 1985 respectively, but only in the winter of 1982/3 was there an increased incidence associated with season.
In addition to studies of general hospital and outpatient populations, some attention has been devoted to the prevalence of Rotavirus infection in specific risk groups such as neonates in special care nurseries. In a 15 month study in Melbourne, Australia, 28 per cent of babies admitted to a special care nursery within two hours of birth subsequently developed diarrhoea. It was concluded that because excretion of a Reovirus-like particle was temporally related to symptoms of diarrhoea, Rotavirus was likely to be an important cause of endemic diarrhoea in such nurseries (Cameron et al, 1978). In a twelve month study in Maastricht, Netherlands, 15.4 per cent of neonates in a referral nursery were found to be positive for Rotavirus and one quarter of those cases had symptoms of diarrhoea (Walther et al, 1984). No seasonality was observed, but breast-fed neonates were less frequently infected than those fed on milk substitutes. Similar studies of Rotavirus infection in neonate nurseries have been conducted in the UK (Bryden et al, 1982), the US (McCarroll & Vogel, 1983), Israel (Shif et al, 1983) and Sweden (Grillner et al, 1985).

Another focus of concern for Rotavirus infection in industrialised counties is the potential for outbreaks of gastroenteritis in day care centres. In a 19 month prospective study of children attending twenty day care centres in Houston, US, there were fifteen outbreaks of diarrhoea involving a total of 195 cases (Pickering et al, 1981). Rotavirus was only implicated in two of the outbreaks and only in children less than three years of age. Secondary attack rates within families were 21 per cent for Shigella, 17 per cent for Giardia, and 15 per cent for Rotavirus. Thus it was concluded that day care centres could play an important role in the transmission of diarrhoea in the US. In a subsequent 15 month study, the annual rate of human Rotavirus infection in day care centres was 0.55 episodes per child per year with diarrhoea being exhibited in 40 per cent of cases (Bartlett, et al, 1988). Of 45 outbreaks of diarrhoea in the day centres nine were attributable to human Rotavirus. It was noted that the profile of infection in the day care centres paralleled that observed within the community. Outbreaks of rotaviral infection in adult hospital populations including cardiac and geriatric risk groups have also been investigated (Holzel et al, 1980; Abbas & Denton, 1987).
Nelson (1985) has noted that figures for prevalence and incidence of Rotavirus infection should be treated with caution since asymptomatic colonisation of the gut is very common in early infancy, and the presence of Rotavirus antigen may only have a causal association with disease in half the cases where it is detected. Nevertheless, given the constraints of low rates of presentation to physicians and non-availability of diagnostic tests for all possible agents of diarrhoeal disease, human Rotavirus may be correctly assumed to be the greatest single cause of gastroenteritis in hospital and outpatient populations in industrialised countries.

5.1.iii Prevalence of Rotavirus Infection in Less Developed Countries

The prevalence of Rotavirus infection in developing countries has been studied in defined populations e.g. patients presenting with symptoms of diarrhoea, as well as in more formal prospective epidemiological investigations of hospital and village communities. In addition, there have been a number of retrospective investigations of outbreaks of rotaviral diarrhoea.

In a cross-sectional survey of 80 children with acute gastroenteritis in Taiwan, 75 were tested for the presence of "reovirus-like agents" by direct faecal examination and by complement fixation and immunofluorescence tests (Echeverria et al, 1977). Fifty-six per cent of those tested were positive for the presence of Reovirus-like agents - a much higher proportion than proved positive for the presence of enterotoxigenic E. coli (ETEC). It was concluded that the Reovirus-like agent was the major cause of paediatric enteritis in Taiwan in 1975.

In a study of 702 hospitalised infants and children in Guayaquil, Ecuador, Rotavirus was detected in 21.1 per cent of stools. There was no significant seasonal variation in incidence but the frequency of Rotavirus infection among children with acute diarrhoea was slightly elevated during the dry and cooler months (Suzuki et al, 1981).
Comprehensive surveillance undertaken in Bangladesh between 1979 and 1980 included examination of 3550 patients with diarrhoea attending the International Centre for Diarrhoeal Diseases Research hospital in Dacca (Stoll et al, 1982). For all age groups, enterotoxigenic E coli (ETEC) was the most important pathogen (20 per cent versus 19 per cent for Rotavirus). However, among the 0-5 year age group, Rotavirus was the agent most frequently associated with symptoms of gastroenteritis, and in the 0-1 year age group, 35 per cent of diarrhoeal cases were associated with Rotavirus (versus 25 per cent for Campylobacter jejuni and 20 per cent for ETEC). Rotavirus infection was characterised by watery diarrhoea in 77 per cent of cases, vomiting in 71 per cent of cases and moderate - severe dehydration in 19 per cent of cases (n = 635). The seasonality of Rotavirus infection was entirely different to that observed for the bacterial pathogens Shigella, ETEC and Vibrio cholerae, the peak incidence of Rotavirus being associated with the cooler months of January - March. Of the four pathogens, only V cholerae and Shigella showed increases in incidence during the monsoon season of September - December.

No marked seasonality of Rotavirus infection was detected in a study of 369 diarrhoeic children below the age of six in Belem, Brazil (Linhares et al, 1983). Rotavirus was detected in 33.1 per cent of the children. In 45.1 per cent of the Rotavirus positive specimens no other pathogen was detected. In a study of hospital outpatients in Costa Rica between 1976 and 1979, cases were examined within four days of the onset of symptoms. Rotaviruses were the most common agents, associated with 45.3 per cent of diarrhoeic children compared with 13.4 per cent for ETEC (heat stable toxin producers only) and 8.1 per cent for Shigella (Mata et al, 1983a). Over a 5.5 year period, the peak incidence of Rotavirus was associated with the dry and cooler months of December and January, a pattern which did not coincide with that of the bacterial pathogens. Of 248 paediatric hospital patients between the age of one and sixty months who were examined in Bangkok, Thailand, 29 per cent were demonstrated to be positive for Rotavirus (Luisirirotchanakul et al, 1984). In two related studies of 126 inpatients and 352 outpatients in Durban, South Africa, Rotavirus was demonstrated in 20 per cent and 34 per cent of cases respectively. All patients in the two studies were children (Mackenjee et al, 1984). A large study
of pathogens associated with diarrhoea was conducted in the Central African Republic between 1981 and 1982 (Georges et al, 1984). Nearly twelve hundred children less than 15 years old were investigated. Rotavirus was the most frequently isolated pathogen in children less than 18 months old and a peak in the incidence of infections was detected during the dry season.

Bhan and co-workers (1987) examined 204 patients with acute diarrhoea below the age of five in a slum community near New Delhi, India. The most frequently isolated agents were ETEC (23 per cent) and Rotavirus (20.6 per cent). Detection rates for Rotavirus were highest in the 0-6 months age group (40.0 per cent) whereas incidence of ETEC infection was highest in the second and third years of life (26.1 and 29.1 per cent respectively).

A number of prospective epidemiological studies have been undertaken in less developed countries both with adults and children. In a study involving 165 students from the United States, Venezuela and Mexico visiting a Mexican university, a significantly greater number of diarrhoeic students had Rotavirus in their stools compared with control students (25 versus 12 per cent). In the case of students newly arrived from the US, the difference was even more marked: 26 versus 3 per cent of controls (Bolivar et al, 1978). In a similar study of Panamanian travellers in Mexico, 23 were observed to experience a total of 27 episodes of diarrhoea (Ryder et al, 1981). The most commonly identified agent was Rotavirus (26 per cent) followed by Norwalk agent (15 per cent) and Campylobacter fetus (11 per cent).

A cohort of 45 Mayan Indian children in Guatamala was observed from birth to three years of age. Weekly faecal specimens were stored and analysed by ELISA (5891 extracts were analysed in total). Infection with Rotavirus was rare in intensively breast-fed infants, but incidence was greatest in the age range 6-18 months (Mata et al, 1983b). Rotaviruses were associated with only 10 per cent of total diarrhoeal episodes with an incidence rate of 0.8 episodes per child per annum. Outbreaks were generally associated with the months September - December and repeat infections
with Rotavirus were common. In a cross-sectional study of 150 diarrhoeic patients in the age range 1-60 months in Damman, Saudi Arabia, 39.6 per cent of the worst affected age range (7-12 months) gave positive ELISA tests for Rotavirus compared with 7.5 per cent for the control group (Huq et al, 1987).

In a longitudinal cohort study involving 56 infants born in a Mexican village during a single year, faecal specimens were taken fortnightly for the first two years of life (Cravioto et al, 1988). Isolation of Rotaviruses was significantly higher in diarrhoeic infants compared with controls. A similar community-based prospective study was carried out in Ibadan, Nigeria (Oyejide & Fagbami, 1988). Of 131 neonates initially recruited, 77 per cent completed one year of follow-up. The incidence of all diarrhoeas was 3.2 episodes per child per annum and the mean total duration of diarrhoea in the first year of life was 16 days per child per annum (range 3-34 days). Of the 280 episodes of diarrhoea investigated 7.7 per cent showed evidence of Rotavirus infection by ELISA tests.

A cohort study in Bangui, Central African Republic, was conducted with 111 children from birth to two years of age (Georges-Courbot et al, 1988). At least one infection with Rotavirus was experienced by 34.2 per cent of children by the age of 6 months and 27 per cent actually presented with Rotavirus-associated diarrhoea before the age of two. In a study lasting more than three years in Belem, Brazil, 80 children were followed through 441 diarrhoeal episodes of which 8.2 per cent were associated with Rotavirus (Linhares et al, 1989). The frequency of all diarrhoeal infections was 2.3 episodes per child per annum. Other studies of the epidemiology of Rotavirus infection have been undertaken in Laos and Indonesia (Urasawa et al, 1981; Albert et al, 1982), Papua, New Guinea (Albert et al, 1983), India (Samantaray et al, 1982; Broor et al, 1985; Panigrahi et al, 1985; Bhan et al, 1987; Malik et al, 1987), Chile (Spencer et al, 1983), Brazil (Trabulsi et al, 1988) and Morocco (Tazi-Lakhsassi et al, 1988). In most cases these studies were concerned with the definition of serotypes and electropherotypes of Rotavirus involved in diarrhoeal disease.
Apart from two major outbreaks of waterborne epidemic rotaviral disease (Tao et al, 1984), there have been a number of other investigations of outbreaks of Rotavirus in less developed countries. In 1977 an explosive outbreak of Rotavirus diarrhoea occurred in an isolated community of Tiriyo Indians in Brazil (Linhares et al, 1981). Diarrhoea was reported by 70 per cent of 224 of the Indians, and although children had the most pronounced symptoms, all age groups were affected. The only postulated cause of such a rapid spread of infection was the use of a river for both personal bathing and cleaning of utensils resulting in the consumption of contaminated food or water.

Two outbreaks of Rotavirus-associated diarrhoea occurred in 1983 and 1984 in Guangxi Region, China, involving a total of 19,007 cases (Wang et al, 1985). Rotaviruses were detected in 44.1 per cent of stools examined by immune electron microscopy and it was concluded that the outbreaks were due to non-group A Rotaviruses on the basis of their migration in polyacrylamide gel electrophoresis (PAGE). In a later epidemic involving neonates in Zhao Tong Regional Hospital, Yunnan Province, China, Rotaviruses were detected in 66.7 per cent of cases by RNA PAGE and in 72.7 per cent of cases by electron microscopy (Dai et al, 1987). The strain involved in the outbreak did not possess the common group A antigen but was considered similar to the serogroup B (Chinese adult) Rotaviruses. The cause of the outbreaks was not established.

The combined evidence of all the above studies serves to confirm that Rotavirus is the single most important agent of gastroenteritis and diarrhoeal disease in infants in less developed countries. In some studies, the prevalence of Rotavirus infection was very high and there can be little doubt that repeat infections occur with sufficient frequency to ensure that the incidence of infection by Rotavirus remains high and that within the principal risk group the infection may often be considered endemic.
Evidence from prospective studies involving adults and outbreaks of Rotavirus diarrhoea in China and elsewhere leave little doubt that epidemic transmission of Rotavirus may also occur. However, in most cases the principal route of transmission is not even postulated, still less investigated, and authors generally restrict themselves to a discussion of the prevalence and/or incidence of infection. Occasionally, seasonal variations of prevalence and incidence are included in discussions of rotaviral infection in less developed countries. This information may provide the most important indirect evidence of the relative importance of the waterborne transmission route for human Rotavirus in less developed countries and it is discussed in detail in the final part of this discussion (5.5). However, before drawing conclusions on the central question of this Study it is necessary to discuss the findings of the experimental sections in some detail.

5.2 THE DEVELOPMENT OF AN EXPERIMENTAL PROTECTED SLOW SAND FILTRATION SYSTEM - DISCUSSION OF THE RESULTS OF SECTION 2.

The first five experiments described in Section 2 (2.1 - 2.V) relate to microbiological maturation phenomena in protected slow sand filters and in pretreatment systems employing sand, gravel and filter fabrics. Experiment 2.1 demonstrated a clear logarithmic relationship (p <0.05) between oxygen consumption and time in a newly commissioned protected slow sand filter system (Figure 24). Similar patterns of increasing oxygen consumption were obtained for protected slow sand filters subsequent to routine maintenance (Figures 25 & 26), illustrating the essentially biological nature of the maturation process and hence the need to allow adequate time for the establishment of active aerobic microbiological and biological communities within slow sand filters.

A correlation between the establishment of active populations of aerobic fauna and the reduction in densities of indicators of faecal pollution was unequivocally demonstrated in Experiment 2.1I. A strong linear relationship (p<0.001) was observed between the logarithm of the ciliate population observed in sand profile samplers by Skilton (1983), and the logarithm of the reduction in density of thermotolerant coliforms in an operational filter (Figure 27).
The above two observations: oxygen depletion with respect to time, and the direct relationship between ciliate populations and microbiological performance are most important. Although they do not establish causal associations in either case, they do provide direct support for the hypothesis that the characteristic logarithmic nature of microbiological performance with respect to time after commissioning or cleaning a slow sand filter is due more to the accumulation of microbial biomass and/or the establishment of active biological populations, than to the physico-chemical conditioning of the filter medium. In this respect they confirm many of the conclusions of Lloyd (1974) regarding the role of biological populations in slow sand filters.

Maturation curves are depicted in results from Experiment 2.III. Protected slow sand filters with widely differing raw water qualities all exhibited characteristic logarithmic maturation of microbiological performance over periods of 13, 30 and 64 days after commissioning with high correlation coefficients and levels of significance (p<0.001) in all cases (Figures 28-30). In addition, logarithmic recovery occurred (p<0.1) - although over a much shorter time period - when microbiological performance was recorded directly after filter cleaning (Figure 31).

The above observations are not wholly surprising. For many years slow sand filters have been understood to require a ripening or maturation period and despite the novelty of some of these observations, it is no more than reassuring to note the essential mathematical correspondence between such phenomena as oxygen depletion, populations of ciliate protozoa and microbiological performance (as indexed by reductions in density of thermotolerant coliforms) in operational filters. But to what extent is this correspondence reproduced in other forms of filtration, for example filtration through coarse sand or gravel? And to what extent are microbiological and/or biological phenomena able to contribute to the overall efficiency of such treatments? Experiments 2.IV and 2.V illustrate the fact that the reliability and overall performance of coarse medium filtration may not be as great as that of protected slow filters, particularly where flow rates are
significantly higher than conventional rates for Slow Sand Filtration. Nevertheless, logarithmic maturation phenomena were still observed (eg Figures 32 & 33). Most interestingly, in warm water conditions reductions in densities of thermotolerant coliforms through sub-sand prefilters were observed to follow a log-log relationship with respect to time (Figures 36 & 37) whereas in cold water conditions (where water temperatures were <10°C and ciliate populations would not be expected to establish themselves within short periods), a simple logarithmic relationship was obtained (Figures 34 & 35).

This suggests that logarithmic maturation phenomena may not be dependent on the growth of biological populations eg ciliate protozoa and metazoa, alone and raises the possibility that other processes, microbiological or physico-chemical, may be operating. Clearly it is unlikely that the logarithmic maturation of microbiological performance in very coarse media or in relatively rapid filtration through sand in low water temperatures is due to phenomena identical to those presumed to be important in conventional Slow Sand Filtration. Two of the most important prerequisites for the establishment of active populations of protozoa and metazoa in slow sand filters are the availability of nutrients and dissolved oxygen. These provide the basis for the establishment of biofilms and microbial biomass in and among which the biological populations eg ciliates graze and proliferate. Such phenomena are bound to be logarithmic (provided nutrients are not limiting) due to the nature of microbiological growth.

However, in coarse filtration or rapid sand filtration, the deposition of suspended colloidal material may also have a logarithmic dimension with respect to time, irrespective of strictly biological factors. The more material that is deposited, the more conditions are improved for subsequent sedimentation and adsorption of both organic and inorganic particulate matter. Since coliform bacteria may be treated as colloids for the purpose of this argument, there would appear to be absolutely no reason why coliform removal should not exhibit logarithmic maturation in both gravel and sub-sand filtration. Indeed, it is quite probable that adsorption of bacteria to oxides, organic macromolecules, clay minerals, other microorganisms eg algae, and sand may be
quite sufficient to account for both the overall efficiencies and the logarithmic nature of maturation phenomena observed in experiments 2.VI and 2.V. Thus it may be concluded that in favourable circumstances the absence of those biological populations which are involved in scavenging and predation in slow sand filters may not preclude high efficiencies of removal of microbial contaminants.

Experiments 2. VI and 2. VII serve to illustrate the difference in absolute microbiological performance of protected slow sand filters and coarse medium (gravel) filtration. Whereas the slow sand filters typically returned mean percentage reductions of faecal indicator bacteria greater than $1 \log_{10}$ unit (Table 2.4.5, Figures 42 & 43), vertical and horizontal gravel filters gave typical mean reductions of thermotolerant coliforms in the range 70-85 per cent (Table 2.4.6, Figures 38-43). Notwithstanding this overall difference, some features of coarse medium filtration do provide useful points of reference for comparison with the equivalent performance of slow sand filters. For example, the microbiological performance of horizontal gravel filters operated at flow rates of between 0.5 and 10 m/h demonstrated that in the absence of predation (clearly not a significant factor at the higher rates) significant reductions in the densities of faecal microorganisms still occur. Moreover, the phenomenon occurs with sufficient uniformity to generate significant plots of performance for gravel sizes of 10 and 20 mm (Figures 39 & 40). The logical conclusion which may be drawn from these observations is that in coarse medium filtration, biological mechanisms of removal may be restricted to the lowest flow rates ie $<1$ m/h and the smaller media sizes. Moreover, these mechanisms are unlikely to include predation as a major component but are probably restricted to adsorption phenomena involving impaction of colloidal particles on biomass and biofilms. The presence of respiring biomass in coarse medium filters was indirectly confirmed for media size 10 and 20 mm (but not 40 mm) by the demonstration of oxygen depletion with respect to length of filter (Figure 41).

The physical performance of water treatments developed in this Study is described in Experiments 2. VIII - 2. XII. An important feature of the experimental sand filter system was the incorporation
of synthetic fabrics in order to enhance both the physical and microbiological performance of Slow Sand Filtration. Fabrics were shown to be very efficient in the entrapment of suspended material defined as ‘silt’ - a generic term which may be assumed to include both inorganic (eg clay) and organic (eg algal) components. Tables 2.4.7 and 2.4.8 illustrate the efficiency of fabrics in removing suspended particulate matter from water prior to Slow Sand Filtration. However, perhaps the most important observation relates to the value of increasing the tortuosity of flow by alternating the type, and specifically the density of successive layers of fabric overlaid on the sand surface (Table 2.4.9). Detailed consideration of the implications of this observation is not the primary concern of this Study. But relating the removal of essentially colloidal turbidity to the removal of faecal microorganisms, it is clear that a relatively open matrix ie synthetic fabrics, can provide an appropriate environment for aggregation, sedimentation, deposition and adsorption of very small particles (silt particles are mostly of the order of $10^{-8} - 10^{-5}$ m in diameter).

In his review of colloids in the aquatic environment, Tipping (1988) placed clay minerals such as illite, kaolinite and montmorillonite in an intermediate size range ($10^{-8} - 10^{-6}$ m) compared with viruses ($10^{-8} - 10^{-7}$ m) and bacteria ($10^{-6} - 10^{-5}$ m). Thus it is at least arguable that because of the net negative surface charge of clay minerals, viruses and bacteria in colloidal suspension in natural waters, they may be expected to behave with some degree of similarity with respect to their transport and removal by adsorption in slow sand filters. Indeed, the closer proximity in size of colloidal clay and viruses may even make turbidity a better index of viral removal in filtration than bacteria. With this in view, experiments which examine the physical efficiency of filtration systems may provide further indirect evidence of the likely capacity of slow sand filters to remove enteric viruses from water.

It has long been observed that in full scale water treatment works, the removal of turbidity and faecal bacteria are often equivalent and may be related to viral disease risk (Hudson, 1962). Thus it is interesting to record that very similar performance was obtained for turbidity removal by slow sand filters and vertical gravel prefilters as was noted for bacteriological reductions (Experiments
Low flow rates in coarse medium filters (in this case 0.2 m/h) clearly permit the operation of some biologically related phenomena. Hence it is also interesting that reductions in turbidity (79.4 and 81.3 per cent in the dry and wet seasons respectively) were marginally higher than the reductions in density of thermotolerant coliforms (63.5 and 74.1 per cent for dry and wet seasons).

When the removal of turbidity with respect to time was examined in coarse media filters (Experiment 2.XI), the similarity with maturation phenomena observed for bacterial removal is quite striking. Efficiencies of turbidity removal increased logarithmically in horizontal and vertical gravel filtration (Figures 46 & 47) as indeed they did for sub-sand filtration in both cold and warm water conditions (Figures 48 - 51). Efficiencies of turbidity removal with respect to distance and flow velocity in horizontal gravel filtration were very similar to efficiencies of removal of thermotolerant coliforms and faecal streptococci in the same circumstances (Figures 52 & 53).

Experiments 2.XIII - 2.XIV dealt with the enhancement in efficiency of Slow Sand Filtration - both with respect to physical and microbiological factors - by the incorporation of synthetic fabric layers. The overall conclusions which may be drawn from these experiments are that i) fabrics extend filter run lengths by minimising the penetration of filter-blocking material ie silt to the sand bed with negligible contribution to head loss; and ii) filter fabrics afford some continuity for microbiological and biological populations during and between filter maintenance events. The net result of these effects is that operational efficiency is significantly increased.

In summary, experiments in Section 2 established that:

1) Irrespective of filter medium type, size or flow rate, logarithmic maturation phenomena are observed for oxygen consumption, and the removal of bacterial indicators and turbidity;
2) With coarse medium filtration and sand filtration at high flow rates it is most unlikely that biological predation is the primary factor responsible for maturation phenomena, particularly in cold water temperatures; hence it may be postulated that maturation phenomena in slow sand filters can be accounted for largely in terms of adsorption to microbial biomass and deposited organic and inorganic colloidal particles in the uppermost horizons of the sand bed;

3) Patterns of removal of turbidity (mostly colloidal clay minerals, biological aggregates and algae) through sand filters and coarse medium filters are very similar to patterns of removal of bacterial indicators; hence it is possible that both may provide useful indications of the likely behaviour of other colloidal particulates, including enteric viruses, in slow sand filters; and

4) The physical and microbiological performance of slow sand filters may be significantly enhanced by allowing silt deposition to occur in a synthetic fabric matrix placed above the sand bed.

5.3 OBSERVATIONS ON THE REMOVAL OF VIRUSES BY FILTRATION THROUGH SAND - DISCUSSION OF THE RESULTS OF SECTION 3

Experiment 3.1 was concerned with the removal of indicator bacteria with respect to depth in a protected slow sand filter. It is clear from Figures 58 and 59 that synthetic fabrics played a useful role in the removal of both thermotolerant coliforms and faecal streptococci. Patterns of removal were similar for both organisms with the overall rate of removal being >98 per cent in each case. The observations are certainly consistent with those of Lloyd (1974) who noted 90 per cent reductions in coliform bacteria in the uppermost 5-8 cm of medium in a conventional slow sand filter. In this Study, thermotolerant coliform and faecal streptococcus removal rates were both 88 per cent at a depth of 100 mm in the sand bed, suggesting that the beneficial effect of the
incorporation of synthetic fabrics may have been offset by the slightly higher flow rates employed. Results for total plate count bacteria (Figures 60 & 61) diverged from those recorded by Lloyd, there being no overall decline in 37°C counts in the top 100 mm. In the case of 22°C counts there was a marked increase in numbers between 20 and 100 mm followed by a decline in counts between 100 and 500 mm in the sand bed. These results indicate the presence of stable and replicating heterotrophic bacterial populations in the fabric and upper horizons of the sand filters rather than any lack of efficiency of removal. One possible explanation for the difference in results obtained by Lloyd may be the use in this Study of the spiral plate technique for enumeration of plate count organisms. It is possible that the standard pour plate technique employed by Lloyd resulted in heat shock to indigenous bacterial populations to a degree which was not suffered by more hardy immigrant plate count bacteria. The effect of this would have been to proportionately depress the counts of bacteria detected in interstitial water at depth within the filter with the result that real elevations in numbers may have been partially masked.

The establishment of patterns of removal for bacterial indicators through protected slow sand filters was principally to serve as a reference for subsequent investigations using live virus. The first of these investigations (Experiment 3.II) employed a range of bacteriophage as surrogates for Rotavirus. Bacteriophage were employed for their ease of handling and were selected on the basis of their similar size and likely physico-chemical characteristics compared with Rotavirus. Each bacteriophage had a head with a cross-sectional area ± 50 per cent that of Rotavirus. Moreover, although isoelectric points are not known for most of the bacteriophage used in this Study, it is unlikely that the pI of the majority of Rotavirus strains differs markedly from the range associated with most bacterial viruses ie 3.9 - 5.0 (Gerba, 1984). It may also be worth noting in this context that the pI for clays such as kaolinite and montmorillonite range from <2 - 4.6.

What may be readily observed from the results of experiment 3.II is that regardless of the bacteriophage size and type the removal efficiencies with respect to depth in a protected slow sand
filter were remarkably consistent (Table 3.4.1 and Figures 62-66). Moreover the percentage reductions across the fabric layers (average 53 per cent; range 36.3 - 77.7 per cent) were not dissimilar to reductions observed for thermotolerant coliforms and faecal streptococci in Experiment 3.1. Indeed the patterns and overall efficiencies of removal of bacteriophage through protected slow sand filters were indistinguishable from results obtained for the bacterial indicators.

It was the firm conviction of the author that experimental methods should at all times rely on test systems as representative as possible of operational sand filters. There are simply too many inter-relating factors in Slow Sand Filtration to attempt to control for all possible biological, microbiological, physico-chemical, chemical and hydraulic variables on a small scale in the laboratory. Similarly, the difficulties of Rotavirus detection and assay should not be used as justification for conducting experiments with radio-labelled or otherwise tagged virus in the environment. The use of live virus capable of replication was considered very important. Hence, enumeration of replicating virus following growth in cell culture and immunochemical staining was, despite its difficulty, the preferred method of assay in all experiments using Rotavirus.

Clearly, the single experiment which would satisfy both of the above criteria would be the isolation and quantification of naturally occurring Rotavirus in the raw and treated waters before, during and after conventional full scale Slow Sand Filtration. Thus a method was devised for the isolation of Rotavirus largely based on standard techniques for the concentration of enteroviruses and the detection of Rotavirus by immunofluorescence following replication in cell culture. This method is described in Section 3. However, the search for an appropriate UK slow sand filter works in which to monitor Rotavirus proved fruitless. The nearest equivalent was the experimental protected slow sand filter system established at Hampton Water Treatment Works which received raw water from a reservoir of impounded River Thames water. Because of the number of wastewater inputs to the River Thames, the impounded water could contain adequate numbers of Rotavirus and thus a programme of monitoring raw and treated water was adopted for
a period of six months. Experiment 3.IV was a partial success, since the concentration and
detection techniques developed for naturally occurring Rotaviruses in the aquatic environment
proved successful. These were the first isolations of Rotavirus from natural waters in the UK of
which the author is aware. However, numbers of Rotavirus in the impounded reservoir water were
too low to achieve isolations with the necessary frequency to enable a quantitative estimate of the
efficiency of Slow Sand Filtration in removing Rotavirus from water. Thus the experiment was
discontinued.

The search for an alternative means of conducting the experiment was inhibited by the
inadvisability of deliberately introducing large numbers of Rotavirus into an experimental facility
on an operational water treatment works. Meanwhile an opportunity arose to gain comparable
data on the elimination of naturally occurring Rotavirus through sand below a wastewater lagoon
and irrigation site. The site in question was in Peru and there was a safe assumption that levels of
Rotavirus in the lagoon water would be high. The results of Experiment 3.IV were interesting for
several reasons. Firstly they provided confirmation that adsorption rates for faecal indicator
bacteria following slow percolation through sand under wastewater lagoons were comparable to or
greater than those typically achieved in dune infiltration. Secondly whilst the adsorption rates for
indicator bacteria beneath the wastewater lagoons were similar to those observed below irrigation
channels, there was a marked difference in the comparative attenuation of naturally occurring
Rotavirus. Whereas Rotavirus adsorption decreased by $> 2 \log_{10}$ units within 1 metre of the base
of the lagoon, the reduction was only $1 \log_{10}$ unit within 7.5 metres of the base of irrigation
channels.

The only material difference between percolation beneath lagoons and irrigation channels was the
presence at the base of the wastewater lagoons of an anaerobic sludge of settled biomass and other
solids up to 100 mm in depth. Thus, before penetration into the aeolian and alluvial sands below
the facility, Rotaviruses in the lagoon wastewater had to pass through an environment replete with
lipoproteins, lipopolysaccharides, glycolipoproteins and mucoproteins - in short a matrix very well
suited to viral adsorption. In contrast, wastewater in the irrigation channels had relatively direct access to the sand below.

Although the diameters of the sand grains below the wastewater facility were not dissimilar to those recommended for slow sand filters (0.1-0.3 mm), the estimated penetration rates were considerably lower than slow sand filter flow velocities: 0.07 - 0.10 m/day below lagoons and 0.03 - 0.05 m/day below irrigation channels. It is unlikely that the marginally greater water velocities below irrigation channels compared with those below lagoons would have resulted in the considerable difference in penetration rates noted for Rotavirus. Thus it must be concluded that the observed difference was due in the main to the effect of percolation through settled biomass at the base of the lagoons. The main implication of this conclusion for the removal of Rotavirus by slow sand filters may be significant since there would have been no populations of aerobic protozoa in the biomass and thus no possibility of the type of biological scavenging which is normally associated with slow sand filters. In summary, therefore, it may be recorded that through a shallow depth of biomass and 2 m of sand similar to that employed in slow sand filtration, thermotolerant coliforms and faecal streptococci in wastewater were reduced in density by a factor of 1-2 log$_{10}$ units, and indigenous Rotavirus by just over 2 log$_{10}$ units, in the complete absence of scavenging protozoa and metazoa.

The above observations are not entirely consistent with those of Dizer and co-workers (1983) which were conducted from the perspective of virus adsorption to sand in aquifers. Using glass columns of diameter 5 cm packed with sand grains of diameter less than 2 mm, viruses were introduced into a continuous flow of water equivalent to 2.5 m / day. Viruses used were Poliovirus 1, Coxsackie B1, Coxsackie A9, Echo 7 and simian Rotavirus SA11. In all cases adsorption was restricted to the top few centimetres of columns (approximately 90 per cent adsorption of Rotavirus occurred in the top 2 centimetres). However, since results were expressed only in terms of percentages of total recoveries it is not possible to estimate precisely the degree of adsorption compared with total input. It was claimed that in experiments lasting up to 14 days the
rate of elution of virus did not exceed 0.1 per cent of the input per day. This would tend to imply that the adsorption of viruses to sand may be very efficient even in the absence of biomass. This was not the conclusion drawn from Experiment 3.IV where Rotavirus penetration and adsorption occurred to substantial depths below irrigation channels. However, it is possible that the high levels of dissolved organics present in wastewater had a significant influence with the result that virus adsorption was inhibited at all levels.

The final experiment in the series (3.V) was the most difficult to arrange and conduct, but it did provide, for the first time, direct evidence of the removal efficiency of Rotavirus by Slow Sand Filtration. The sand filters which were established for the purpose of the experiment were column filters with a recirculating system for raw and treated waters. This obviated any question of discharge of Rotavirus to the environment.

Because simian Rotavirus SA11 is probably the most appropriate type virus for the group (Holmes, 1983), it was chosen in preference to a human strain obtained from a faecal extract. The latter option had the obvious attraction of providing potentially high titres for dosing into the system, whereas simian Rotavirus may only be cultivated in the laboratory in titres of around $10^6$ per ml. Also it is arguable that a diluted faecal extract might be more representative of the kind of contamination which may be faced by slow sand filters in developing countries. However, the majority of experimental work conducted with Rotavirus in the environment has employed the SA11 strain. Moreover, its preparation and purification is a standard procedure which should result in a relatively standard suspension of intact virus particles. In contrast, faecally derived Rotavirus would be potentially less representative of the group and would not necessarily be either uniform or standard in its characteristics. Hence, the simian virus was selected to provide the definitive direct evidence of removal efficiency through Slow Sand Filtration.

As may be readily observed from Figure 93, levels of SA11 in the supernatant water decreased rapidly after addition of the stock suspension, and the peak level at the fabric-sand interface was
observed approximately 70 minutes after the initial dosing of the supernatant (mean of four experiments). Integrating the total recoveries for these samples together with the results from other sample ports with respect to time and total volume of water passing each sampling point, overall elimination rates were calculated. They are reflected in Figure 94 and Table 3.4.3. The clear outcome of this experiment is the observation that the removal pattern and overall efficiency of elimination of Rotavirus through protected slow sand filters did not differ significantly from equivalent observations for thermotolerant coliforms, faecal streptococci and five indicator viruses in previous experiments. This severely limits the scope for discussing differences in behaviour of the organisms examined due to their widely different sizes, structural conformations and physico-chemistry. If their behaviour is so similar, then clearly such factors as the differences between gram negative and gram positive bacteria, between rods and cocci, between tailed and non-tailed bacteriophage, between the isoelectric points of various viruses, and the differences due to glycoproteins on the surface of Rotaviruses and polysaccharides on the surface of bacteria, may all be irrelevant in the context of the microbiological performance of slow sand filters. In a well controlled process, reductions of the order of $2 \log_{10}$ units are apparently obtained for all of the organisms in question. Why should this be so? The answer probably lies in the sheer heterogeneity of surfaces which are found in the upper horizons of a slow sand filter - in particular within the schmutzdecke. The colloids of interest i.e. viruses and bacteria are merely aggregating with and adsorbing to the other colloids, inorganic and organic debris, biofilms, biomass and mineral surfaces which are present in such superabundance in slow sand filters.

The above observations are entirely consistent with the view of Kool (1979) who considered slow sand filters capable of removing equivalent orders of magnitude of bacteriophage, viruses and bacteria. The work of Robeck (1962) demonstrated reductions in enterovirus titres of 83-98 per cent and Slade (1978) showed reductions of 97.1 - 99.8 per cent in full scale filters. Similar ranges of reductions have been noted in the literature (Lloyd & Morris, 1982). Only experiments conducted on a smaller scale within columns have shown significantly higher rates of removal for
Poliovirus than for thermotolerant coliforms ie 2-4 $\log_{10}$ units compared with 1-2 $\log_{10}$ units (Poynter & Slade, 1977). In this case, an explanation may be found in the rather high isoelectric point exhibited by some Poliovirus strains (Gerba, 1984), a factor which would make those strains especially likely to adsorb in the pH range of most natural waters.

Hence, the only experiments which now appear to lack full explanation and partially contradict the observations of this Study and others described above are those of McConnell and co-workers (1984) which showed high levels of overall removal but which did not demonstrate any difference whatsoever in removal of Reovirus between filters with or without schmutzdecke. Apart from these experiments, there appears to be consensus that biological maturity is important in the removal of enteric viruses by slow sand filters. The question remains, to what extent is predation an important component in biological maturation and microbiological performance?

Following adsorption, the contribution of predation by free-living ciliate protozoa and metazoa to the elimination of viruses may be considerable. But this will not be reflected in the overall microbiological performance of filters because once adsorbed, these organisms will not readily desorb (except in the case of significant changes in the physico-chemistry of the influent water), and will eventually be inactivated eg by proteolytic enzymes. In contrast, the activity of sessile types eg *Vorticella* to the entrapment and immobilisation of transitory, unadsorbed viruses passing between sand grains will contribute directly to the overall efficiency of removal. However, compared with the efficiency of adsorption to organic material, clearly demonstrated in the absence of aerobic fauna in Experiment 3.IV, the contribution of sessile types would seem to be of lesser importance.
5.4 MECHANISMS OF ADSORPTION OF VIRUSES TO SURFACES IN SLOW SAND FILTERS - DISCUSSION OF SECTION 4

It has been demonstrated that even in the most unfavourable conditions ie distilled water and neutral pH, equilibrium between viruses and mineral surfaces is achieved within 60 minutes (Moore et al, 1981; Globa, 1985). Also, adsorption kinetics tend not to be affected by agitation (Globa, 1985). Thus, although the conditions in shake flask experiments were probably not optimal for adsorption, the time-scales adopted ie intensive sampling for 60 minutes and later at 1200 minutes were probably adequate, at least for comparative purposes.

The environment within shake flasks is different to that within slow sand filters where adsorption would be expected to occur primarily following transport by diffusion mechanisms. In shake flasks, the swirling of supernatant water on a rotary shaker is probably not capable of ensuring the kind of intimate contact between aqueous and solid phases which occurs in Slow Sand Filtration. Thus, the overall efficiencies and timescales for adsorption may not be considered strictly representative. Nevertheless the comparisons between different substrates and their adsorption characteristics are likely to provide for valid interpretation. Results were expressed graphically in Section 4.8. They are presented below in tabular form taking into account differences between experimental and control flasks.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Sterile Substrates</th>
<th>Non-Sterile Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid Washed Sand</td>
<td>Clean Polypropylene Fabric</td>
</tr>
<tr>
<td>10</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>60</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>1200</td>
<td>1.98</td>
<td>1.98</td>
</tr>
</tbody>
</table>

Table 5.4.1 Log reduction of simian Rotavirus SA11 densities in water in intimate contact with various substrates. NR = No Reduction
The results illustrate that compared with control flasks, sterile substrates effected no overall reduction in the titre of simian Rotavirus in the aqueous phase over periods of up to 60 minutes. Only after an extended period (1200 minutes) was a reduction noted for acid washed and clean sand (nearly $2 \log_{10}$ units). In contrast, non-sterile substrates exhibited adsorption effects ranging from 0.31 to 0.48 $\log_{10}$ units within 10 minutes. It is not conceivable that such reductions could have been achieved by biological predation. Reductions after 60 minutes were similar or greater to those noted after 10 minutes, and in each case were more than $2.5 \log_{10}$ units after 1200 minutes.

These observations clearly demonstrate the contribution of adsorption to biological surfaces in the removal of simian Rotavirus in slow sand filters. The results are consistent with those of the depth experiments described in Section 3 in that sub-optimal conditions for adsorption (shake flasks) still provide for significant reductions in simian Rotavirus from aqueous suspension (approaching $1 \log_{10}$ unit in 60 minutes in the case of sand and schmutzdecke from a conventional slow sand filter).

It is also interesting to note that the removal efficiency of the 20g (wet weight) combination of fabric and schmutzdecke of $0.46 \log_{10}$ units in 10 minutes was equivalent to that achieved by 100g (wet weight) of sand and schmutzdecke from a conventional filter. Although surface areas of substrates (and thus weight) were unlikely to be rate limiting in these experiments, this observation goes some way in explaining the efficiency of filter fabrics in the removal of bacteria, bacteriophages, viruses and other suspended matter noted in Section 3. If adsorption occurs with this efficiency, then clearly both the hydraulic and bio-physico-chemical conditions which exist in fabric filtration are appropriate for the removal of colloidal entities from the aqueous phase.

In their study of Rotavirus binding to animal cells Keljo and Smith (1988) observed that the number of binding sites available for Rotavirus was 13,000 per cell. Clearly this represents a substantial capacity on the part of individual mammalian cells and one which is probably capable
of reproduction by organic and inorganic surfaces in natural aquatic environments where saturation densities of 1 per cent of surface area or greater have been demonstrated for enteric viruses (Moore et al., 1981; Powell & Slater, 1982).

It is somewhat unfortunate that the overwhelming majority of adsorption experiments which have been conducted have been concerned with mineral substrates and have only considered interaction with organic macromolecules as potential competition for binding sites. Thus the presence of organic matter, humic substances and protein in the aqueous phase is usually considered inhibitory to effective adsorption (Schaub & Sorber, 1977; Goyal & Gerba, 1979; Moore et al., 1981; Lipson & Stotzky, 1984). From the perspective of this Study, the presence of biofilms and biomass in the upper horizons of slow sand filters appears to be a major contributory factor in the efficiency of removal of Rotavirus. The adsorption to biomass in wastewater treatment described in Section 3 provides ample justification for more detailed general investigation of the potential contribution of adsorption to biological surfaces in water treatment. This point has also been stressed by Globa (1985).

Experiments proposed in Section 4.3 should provide considerable insight into the general mechanisms and efficiency of adsorption of Rotavirus to surfaces. The nature and extent of adsorption to substrates found naturally in slow sand filters would also help elucidate the nature of attachment to specific macromolecular components of biofilms and biomass. This should be confirmed by controlled experiments involving the enzymatic digestion of these components. These experiments would provide the basis for a fundamental understanding of adsorption phenomena between viruses and surfaces in Slow Sand Filtration.
5.5 THE SEASONALITY OF ROTAVIRAL INFECTION

There is some consensus that in temperate and cooler climates such as those of Europe and North America, peak incidences of Rotavirus infection tend to be associated with winter months ie when it is cool and when rainfall and relative humidity are highest. Seasonal peaks in incidence of rotaviral diarrhoea have been noted in Japan (Oishi et al, 1985), Scotland (Cash et al, 1986) and Italy (Donelli et al, 1988), although not in a study conducted in the Netherlands (Walter et al, 1984).

In tropical climates such as those encountered in less developed countries, seasonal peaks in rotaviral incidence also tend to be associated with cooler seasons, but in many cases such conditions are characterised by low rainfall and low relative humidity. Studies in southern India (Paniker et al, 1982), Bangladesh (Stoll et al, 1982), Costa Rica (Mata et al 1983a), the Central African Republic (Georges et al, 1984), the Punjab (Broor et al, 1985) and Gambia (Rowland et al, 1985; Hanlon et al, 1987), have all shown some seasonality in the incidence of rotaviral diarrhoea.

In a 14 month study of 776 diarrhoeic children attending a hospital in Nairobi, Kenya, two clear seasonal peaks in Rotavirus incidence were demonstrated (Mutanda et al, 1986). Both of these peaks were associated with periods of low humidity, one in months with warm temperatures, the other in months with cooler temperatures. These observations confirmed the findings of an earlier study (Mutanda et al, 1984) which demonstrated unequivocal seasonality and a correlation between peak rotavirus incidence and minimum humidity in Nairobi (p<0.01). No correlation was found with rainfall. In each of the above cases, peak incidences were associated with the cooler, drier months. However, in contrast, little or no seasonality was observed in studies in Ecuador (Suzuki et al, 1981) northern India (Samantary et al, 1982; Panigrahi et al, 1985) and Brazil (Linhares et al, 1983). Thus, Holmes (1983) was probably correct in concluding that in tropical countries, reported variations in seasonal incidence are not consistent either geographically or temporally.
In Section 1 of this Study, the seasonal variation of diarrhoeal disease incidence in developing countries was described and it was observed that in rural areas where populations are relatively sparsely distributed there may only be a weak association between rainfall, water quality and incidence of diarrhoea. In contrast, in urban and peri-urban populations dependent on water supplies which are significantly affected by seasonal influences, the correlation between water quality and incidence of diarrhoea is much more pronounced. Hence it was concluded that "seasonality in disease reporting is more likely to have an association with waterborne transmission in urban environments." The greater general vulnerability of poor urban communities to infectious disease compared with their rural counterparts has been noted (Harpham, 1986).

There have been four detailed studies of the seasonality of Rotavirus incidence in urban environments of less developed countries. They were conducted in Guayaquil, Ecuador (Suzuki et al, 1981), Dacca, Bangladesh (Stoll et al, 1985) and Nairobi, Kenya (Mutanda et al, 1986). Data from these studies have been adapted and are summarised in Figures 104-107.

It may immediately be observed that in no case was the seasonal pattern of rotaviral incidence particularly pronounced. In Guayaquil the dry season incidence appeared marginally higher than the wet season incidence; in Dacca incidence decreased in the early months of the wet season and increased only at the end of the wet season and the end of the dry season. In Chandigarh, Rotavirus incidence was generally higher in the wet season but the main period of increasing incidence occurred during the dry season. In Nairobi peak incidence occurred in the two dry seasons and levels decreased at the start of both wet seasons.
FIGURE 104 - 107 Seasonality of human Rotavirus infections in Ecuador (adapted from Suzuki et al, 1981), Bangladesh (adapted from Stoll et al, 1982), India (adapted from Broor et al 1985) and Kenya (adapted from Mutanda et al, 1986).
Thus it appears that the incidence of Rotavirus diarrhoea in the urban sector of less developed countries has no association with periods of the year when drinking water would be expected to be of lowest quality. Furthermore, since the correlation between water quality and all diarrhoeas is less strong in the rural sector than in the urban sector (Section 1), there is even less reason to suppose that Rotavirus transmission is strongly associated with water quality in rural areas of developing countries. If the primary mode of transmission of Rotavirus in developing countries is not water how may the peaks of incidence in dry seasons in Ecuador, Kenya and elsewhere eg the Central African Republic and Costa Rica, be explained?

Work conducted in Canada has shown that Rotaviruses suspended in faecal material survive well on non-porous materials at lower temperatures and at lower humidities (Sattar et al, 1986). Moe and Harper (1983) demonstrated that bovine Rotavirus aerosols are stable at low and high (but not medium) relative humidity. This has led some to postulate a possible airborne dimension to Rotavirus transmission where incidence is enhanced during periods of low humidity eg in dry seasons (Mutanda et al, 1984; Mutanda et al, 1986).

The possibility of an airborne - respiratory transmission route has also been raised by Santosham and co-workers (1985) following an investigation of a community - wide epidemic in a population of White Mountain Apache Indians in the United States. The high attack rate and speed of spread of this outbreak were similar to phenomena observed in an outbreak in the Truk Islands (Foster et al, 1980) where airborne transmission was discussed together with the possibility of multiplication of the virus in the respiratory tract. Also, in a study of Rotavirus epidemiology in a town in northern India (Malik et al, 1987), a transmission route other than faecal - oral infection eg via droplets was postulated. In this study Rotavirus infection occurred with similar frequencies in all social -economic groups and appeared to be unrelated both to water source and type of sanitary facility.
The airborne theory may be superficially attractive and it does have interesting historical associations - most particularly with the Miasma theory of early nineteenth century Europe. However, a more plausible explanation of increases in incidence of Rotavirus during cool dry seasons in less developed countries is that if the virus is spread primarily by the person - person route, its survival and incidence will be greater during periods of cooler weather and water shortage when hand washing and general cleaning is less frequent. Thus it is interesting to note that in the studies in Bangladesh (Stoll et al, 1982) and India (Broor et al, 1985), Rotavirus incidence increases steadily in the last four months of the dry season when water shortages would be expected to be most acute. In addition, where incidence peaks in the dry season (as in the studies in Ecuador and Kenya) it may be argued that the early dry season increase is due to water shortage and the late dry season decrease to short - term immunity. Strong support for the generally assumed pre-eminence of person - person spread is lent by the observation that Rotavirus survives well on environmental surfaces (Keswick et al, 1983) and that it is possible to detect the virus from both surfaces and the hand washings of teachers and attendants of children (Samadi et al, 1983).

In Section 1 the possibility of low level transmission of Rotavirus via contaminated drinking water was discussed and related to the possibility of subsequent amplification in the community by direct person - person spread. It is interesting to note that this hypothesis (as applied to all viruses and represented in Figure 108) was discussed by an international forum of water microbiologists in 1982 (IAWPRC, 1983). The outcome was thought important enough to record the division of expert opinions numerically. Of the sixteen water microbiologists who considered the question, eleven thought it unnecessary to include the low level transmission hypothesis in the estimation of health risks of viruses in water. Two were undecided and only three had sufficient faith in the theory to consider it an important dimension in risk assessment.
Finally, the question of infective dose must be addressed. In Section 1 the dose response curve proposed by Esrey et al. (1985) was considered of sufficient conceptual merit to conclude that "unless reductions in pathogen loading are very large .... interventions which attempt to improve water supply or sanitation may not result in a measurable impact on the incidence of illness caused by agents with a low infective dose." The main implication of this statement for the reduction of Rotavirus morbidity is that even if it could be shown that low level waterborne transmission is important to the maintenance of Rotavirus endemicity, improvements in water quality would need to be substantial in order to reduce its incidence in the community.

In this Study it has been shown that Rotavirus is removed by the process of Slow Sand Filtration with an efficiency equivalent to the removal of indicator bacteria and viruses. The pattern and mechanisms of removal also appear to be similar with greatest removal in the upper horizons due mostly to adsorption to biological surfaces. In well operated slow sand filter facilities, a reliable
two log\(_{10}\) reduction in titre may be achieved. In the context of the model of Esrey and co-workers (1985), this reduction may not be enough, and taking into account the reality that slow sand filters are likely to be operated sub-optimally, it may be concluded that the introduction of Slow Sand Filtration as a single treatment is unlikely to reduce either the epidemic or endemic incidence of Rotavirus diarrhoea in less developed countries - especially in the rural sector.

Furthermore, the evidence for low level waterborne transmission of Rotavirus remains theoretical and there appears to be no association between season (and thus water quality) and rotaviral incidence in less developed countries.

Thus it must be recorded that the widespread application of Slow Sand Filtration in less developed countries is unlikely to have a major impact on the morbidity of Rotavirus infection. If, in the future, evidence for the low level waterborne transmission of Rotavirus is forthcoming and this can be rationalised with the apparent lack of any direct association with poor water quality, treatments such as Slow Sand Filtration may have an important role to play. However, reductions in titre of only 1-2 log\(_{10}\) units would in all probability not be significant in this context and disinfection with typical reductions of the order of 4 log\(_{10}\) units may well represent a more effective barrier to the waterborne transmission of Rotavirus.
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APPENDIX I

COMPONENTS AND ASSEMBLY OF THE EXPERIMENTAL PROTECTED SLOW SAND FILTRATION SYSTEM
<table>
<thead>
<tr>
<th>Component number</th>
<th>Description</th>
<th>Material*</th>
<th>Amount required per PSSF unit</th>
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<tbody>
<tr>
<td>1</td>
<td>Filter tank</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Filter tank lid</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Filter fabrics (coarse)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Filter fabrics (dense)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>Fabric mesh</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Cross piece</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>a) Perforated pipe, 1.1/4&quot;or 3m</td>
<td>3</td>
<td>3m</td>
</tr>
<tr>
<td>8</td>
<td>Plain cap, 1.1/4&quot;</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>Plain bush, 1.1/4&quot;</td>
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<td>1</td>
</tr>
<tr>
<td>10</td>
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<td>3 mm soft washer</td>
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<td>Ball float, 5&quot;</td>
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<td>14</td>
<td>Threaded socket, 2&quot;</td>
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</tr>
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<td>19</td>
<td>Plain elbow, 1.1/2&quot;</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>Concave spacer, 1.1/2&quot;</td>
<td>3/6</td>
<td>2</td>
</tr>
<tr>
<td>21</td>
<td>Convex spacer, 1.1/2&quot;</td>
<td>3/6</td>
<td>2</td>
</tr>
<tr>
<td>22</td>
<td>3mm soft washer</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>23</td>
<td>Threaded nipple, 1.1/2&quot;</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>24</td>
<td>Threaded reducing bush, 1.1/2&quot; x 1.1/4&quot;</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>25</td>
<td>Drainage pipe, 6&quot;</td>
<td>3</td>
<td>1.2m</td>
</tr>
<tr>
<td>26</td>
<td>Cap for drainage pipe, 6&quot;</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>27</td>
<td>Tank connector, 3/4&quot;</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>28</td>
<td>Threaded cap &amp; seal, 3/4&quot;</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>29</td>
<td>Three way T valve, 1.1/2&quot;</td>
<td>3/6</td>
<td>1</td>
</tr>
<tr>
<td>30</td>
<td>Y piece connector</td>
<td>3/6</td>
<td>1</td>
</tr>
<tr>
<td>31</td>
<td>Jubilee clip</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>32</td>
<td>Flexible hose</td>
<td>4</td>
<td>10m</td>
</tr>
<tr>
<td>33</td>
<td>a) Inlet valve, 1.1/2&quot; with hose unions, or 3/8</td>
<td>3/8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>b) Ball valve, 1.1/2&quot;</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>34</td>
<td>Outlet valve, 1.1/2&quot; with hose unions</td>
<td>3/8</td>
<td>1</td>
</tr>
<tr>
<td>35</td>
<td>Tank connector with hose fitting, 1.1/2&quot;</td>
<td>3/6</td>
<td>2</td>
</tr>
<tr>
<td>36</td>
<td>Tank connector, threaded fitting, 1.1/2&quot;</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>37</td>
<td>Threaded cap and seal, 1.1/2&quot;</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Table A.I.1 Components, materials and amounts required for the PSSF system.
*MATERIAL*

1. Medium density polyethylene (black) or equivalent eg GRP
2. Polypropylene, nylon or equivalent
3. UPVC
4. Approved synthetic for contact with drinking water
5. Polyethylene
6. Nylon
7. Alloy
8. Brass

FIGURE A.I.i Schematic layout of four protected slow sand filters showing inlets (I), connections (C), common overflows (O) and filtered water outlets (F).
2 ASSEMBLY OF THE EXPERIMENTAL PROTECTED SLOW SAND FILTRATION SYSTEM

2.1 Filter Tanks

2.1a Filter tanks (1) were made of materials suitable for intimate contact with water and they incorporated a ridge to support the filter fabric layers. Tanks required four types of orifice to be cut.

A) Inlet
B) Overflow / Interconnection
C) Backwash overflows (side drains)
D) Underdrainage

2.1b Appropriately sized hole saws and a drill (usually electric) were used for cutting through polyethylene tanks.

2.1c PSSF units were usually installed on a concrete plinth with a very slight slope to prevent the ponding of water. An overflow waste channel was also constructed.

2.2 Underdrainage

2.2a The objective of the underdrainage system was to ensure a uniform and free flow of water through the filter. Where perforated or slotted drainage pipe (7) was not available, holes were drilled in standard UPVC pressure pipe. In this case, a thickwalled pipe was selected which remained strong when it was drilled. 6 mm holes were drilled at intervals of 50 mm at an angle of 30 degrees below the horizontal.

2.2b An underdrainage "cross" was constructed which fitted tightly into the base of the filter. The three ends of the cross not leading to the outlet were capped. Components were not cemented until all lengths and configurations had been verified. Four lengths of perforated pipe (7) were cut and inserted into the cross piece (6). Caps (8) were placed on three ends of the cross and the cross fitted tight in the filter base.

2.2c The arm of the cross leading to the outlet was cut to allow the bush (9) and threaded nipple (10) to be inserted through the outlet orifice. There was sufficient thread on the inside of the orifice to install a backnut (11) and washer(s) and sufficient thread on the outside of the orifice to install a backnut and washer(s) and leave a further 50 mm of thread beyond the backnut for the attachment of hose.

2.2d When the underdrainage configuration was verified as satisfactory, and a perfect seal was made at the orifice, the caps (8) were cemented onto the blank ends of the perforated pipe. Ensuring that all drainage holes were oriented downwards, the perforated pipe sections were cemented into the cross piece (6).

2.2e The bush (9), nipple (10) and internal backnut (11) and washer(s) (12) were arranged in position, and the underdrainage cross was cemented to the outlet assembly. The cemented components were left for at least one hour before the underdrainage cross was tightened in place. One or two washers were used on both sides of the tanks to ensure a perfect seal at the outlet orifice.
2.3 Filter Bed Support Medium

2.3a A layer of gravel was installed in the base of the filter to act as a support for the sand bed and to prevent blockage of the underdrainage.

2.3b Coarse gravel (20 - 40 mm diameter) was placed around the underdrainage cross until it was completely covered. The overall depth of this layer was 50 - 60 mm.

2.3c Pea gravel or shingle (6-10 mm diameter) was spread over the first layer to a further depth of 60 - 80 mm.

2.4 Filter Sand and Fabric

2.4a The careful installation and preparation of the sand bed was the key to effective biological treatment in the PSSF unit. It was not considered essential to use very carefully graded sand, but care was observed in the selection of the medium. The sand was not too fine - this would have blocked too quickly. The sand was relatively uniform and coarse - a good quality builder grade sand was found ideal. Some marine sands are perfectly acceptable after several days washing to remove salts. Both builder grade and marine sands were used in this study.

2.4b The key criteria for sand intended for slow sand filtration are that the medium should be relatively coarse (the sieve size which retains 10 per cent by weight should be between 0.15 and 0.35 mm), and it should be relatively uniform in size (the ratio of sieve size passing 60 per cent by weight to the sieve passing 10 per cent by weight should not exceed a factor of 2). These criteria are expressed as the Effective Size ($P_{60\%}$) and the Coefficient of Uniformity ($P_{60\%}/P_{10\%}$). The procedure for defining these values depends on a granulometric size analysis by weight (Section 2).

2.4c The best quality sand available was placed into the filter to a level 50 mm above the filter fabric support ridge.

2.4d Filter fabrics provided protection for the sand bed, extending filter run lengths and improving biological efficiency. After the sand bed had been thoroughly cleaned, it was remixed with a spade. Then, three layers of coarse fabric (3) and three layers of more dense fabric (4) were installed in alternating layers. A dense layer was placed next to the sand bed and a coarse layer was placed at the top of the fabric pile. During the evaluation of filter fabrics, other combinations were used.

2.4e Fabrics were anchored in place with a suitable frame of non-degradable mesh (5).
FIGURE A.I.ii Cross-section of protected slow sand filter.

FIGURE A.I.iii Cross-section through under-drainage system protected slow sand filter.
APPENDIX II

MEDIA AND DEVELOPMENT OF METHODS USED IN THE STUDY
### 1. MEDIA

**MA104 Growth Medium**

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Minimal Essential Medium (with Earles Salts) x 10</td>
<td>50</td>
</tr>
<tr>
<td>2.</td>
<td>Foetal Calf Serum</td>
<td>50</td>
</tr>
<tr>
<td>3.</td>
<td>Non-Essential Amino Acids x 100</td>
<td>5</td>
</tr>
<tr>
<td>4.</td>
<td>L-Glutamine (200 mM) x 100</td>
<td>5</td>
</tr>
<tr>
<td>5.</td>
<td>MEM Vitamins Solution x 100</td>
<td>5</td>
</tr>
<tr>
<td>6.</td>
<td>Penicillin - Streptomycin Solution (10,000 u + 10,000 µg/ml)</td>
<td>5</td>
</tr>
<tr>
<td>7.</td>
<td>Sodium Bicarbonate (7.5 per cent)</td>
<td>12.5</td>
</tr>
<tr>
<td>8.</td>
<td>Distilled/Deionised Water</td>
<td>370</td>
</tr>
</tbody>
</table>

Items 1 and 3-7 from Gibco - Europe  
Item 2 from Sera Lab or Gibco - Europe  
All constituents prepared sterile.

**Serum-Free Medium**

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Minimal Essential Medium (with Earles Salts) x 10</td>
<td>50</td>
</tr>
<tr>
<td>2.</td>
<td>Non-Essential Amino Acids x 100</td>
<td>5</td>
</tr>
<tr>
<td>3.</td>
<td>L-Glutamine (200 mM) x 100</td>
<td>5</td>
</tr>
<tr>
<td>4.</td>
<td>MEM Vitamins Solution x 100</td>
<td>5</td>
</tr>
<tr>
<td>5.</td>
<td>Penicillin - Streptomycin Solution (10,000 u + 10,000 µg/ml)</td>
<td>5</td>
</tr>
<tr>
<td>6.</td>
<td>Sodium Bicarbonate (7.5 per cent)</td>
<td>15</td>
</tr>
<tr>
<td>7.</td>
<td>Distilled/Deionised Water</td>
<td>415</td>
</tr>
<tr>
<td>8.</td>
<td>Trypsin (2.5 mg/ml)</td>
<td>2</td>
</tr>
</tbody>
</table>

Items 1-6 and 8 from Gibco - Europe  
All constituents prepared sterile.  
Constituent 8 only included during assay procedures.
Plaque Assay Medium

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Minimal Essential Medium (with Hanks Salts) x 10</td>
<td>100</td>
</tr>
<tr>
<td>2.</td>
<td>Non-Essential Amino Acids x 100</td>
<td>10</td>
</tr>
<tr>
<td>3.</td>
<td>L-Glutamine (200 mM) x 100</td>
<td>10</td>
</tr>
<tr>
<td>4.</td>
<td>MEM Vitamins Solution x 100</td>
<td>10</td>
</tr>
<tr>
<td>5.</td>
<td>Penicillin - Streptomycin Solution (10,000 u + 10,000 µg/ml)</td>
<td>10</td>
</tr>
<tr>
<td>6.</td>
<td>Sodium Bicarbonate (7.5 per cent)</td>
<td>20</td>
</tr>
<tr>
<td>7.</td>
<td>Neutral Red (1.0 mg/ml)</td>
<td>15</td>
</tr>
<tr>
<td>8.</td>
<td>Distilled/Deionised Water</td>
<td>325</td>
</tr>
<tr>
<td>9.</td>
<td>Trypsin (2.5 mg/ml)</td>
<td>4</td>
</tr>
<tr>
<td>10.</td>
<td>DEAE - Dextran (1.0 per cent)</td>
<td>2</td>
</tr>
<tr>
<td>11.</td>
<td>Purified Agar (2.4 per cent)</td>
<td>500</td>
</tr>
</tbody>
</table>

Items 1-6 and 9 from Gibco - Europe
Item 7 from BDH
Item 10 from Sigma
Item 11 from Oxoid

Constituents 1-8 prepared sterile and brought up to 45°C.
Constituent 11 added molten at 45°C.
Constituents 9 and 10 filter sterilised and added as final components.

Trypsinisation Solution

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Trypsin (1:250)</td>
<td>0.5 mg/ml</td>
</tr>
<tr>
<td>2.</td>
<td>EDTA</td>
<td>0.2 mg/ml</td>
</tr>
<tr>
<td>3.</td>
<td>Sodium Chloride</td>
<td>0.85 mg/ml</td>
</tr>
</tbody>
</table>

Items 1-3 from Gibco-Europe as lyophilised products.

Constituents made up to volume in EBSS, Earle’s Balanced Salt Solution without Calcium and without Magnesium (from Gibco-Europe).
Rich Nutrient Medium

1. Brain Heart Infusion 20g/l
2. Casein Hydrolysate (acid) 20g/l
3. Yeast Extract 1g/l
4. Potassium Dihydrogen Orthophosphate 5g/l
5. Magnesium Sulphate (hydrated) 1g/l
6. Glycerol 20 ml

Items 1-3 from Oxoid
Items 4-6 from BDH

Constituents prepared in 980 ml distilled water, adjusted to pH 7.2 and autoclaved at 121°C for 14 minutes.

Soft Agar Overlay

1. Nutrient Broth 11.2 g/l
2. Purified Agar 5.5 g/l
3. Sodium chloride 7 g/l

Items 1 and 2 from Oxoid.
Item 3 from BDH.

Constituents prepared in 1000 ml distilled water, adjusted to pH 7.2 and autoclaved at 121°C for 15 minutes.
2. DEVELOPMENT OF TEST METHODS

2.1 Stability of MA104 Monolayers in Serum Free Medium Containing Trypsin

1. Seven flasks of confluent MA104 cells (25 cm², Nunc) were washed three times in PBS. Monolayers were then incubated for 24 hours in serum free medium. Following this period of incubation, serum free medium containing concentrations of trypsin in the range 0-250 µg/ml was added to flasks.

2. Cell monolayers were inspected for a period of four days and the percentage of each monolayer showing evidence of deterioration or destruction was estimated. Results are depicted below.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>0</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&lt;1</td>
</tr>
<tr>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&lt;1</td>
<td>5</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>48</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&lt;1</td>
<td>5</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>92</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td>15</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Table A.II.1 Percentage of cell monolayers showing evidence of deterioration in the presence of trypsin.

Conclusions

The observations confirmed that no discernible damage occurred in cell monolayers at concentrations of up to 200 µg/ml trypsin over periods of up to 24 hours. Thus it was concluded that concentrations of 10-25 µ/ml of trypsin could be routinely used in MA104 assays for Simian Rotavirus SA11.
2.2 Stability of MA104 Monolayers in Multiwell Plates During Infection and Replication of Simian Rotavirus SA11

1. MA104 cells were grown to confluence in three six place multiwell plates (Nunc). Monolayers were washed three times in PBS and 3 ml of serum free medium was then added to each well.

2. Plates were reincubated at 37°C for 24 hours before being washed a further three times in PBS.

3. 0.5 ml of serum free medium containing 25 μg/ml of trypsin was added to each well followed by 100 μl of a dilution of simian Rotavirus SA11 stock. Samples were incubated for 60 minutes at 37°C in >95% relative humidity.

4. To each well was added 4 ml of either serum free medium, serum free medium containing 25 μg/ml of trypsin or normal growth medium containing 10 per cent foetal calf serum.

5. Following incubation for 24 hours, cells were washed three times in PBS and fixed in 50 per cent ice-cold methanol followed by 100 per cent ice-cold methanol. The percentage of each monolayer remaining intact after fixing was estimated and results are depicted below.

<table>
<thead>
<tr>
<th>Dilution of Simian Rotavirus SA11 Stock</th>
<th>10^0</th>
<th>10^-1</th>
<th>10^-2</th>
<th>10^-3</th>
<th>10^-4</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFM</td>
<td>95</td>
<td>100</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>SFM+Trypsin</td>
<td>70</td>
<td>80</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>NGM</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

Table A.II.2 Percentage of cell monolayers remaining intact following infection and replication of simian Rotavirus SA11 in serum free medium (SFM), serum free medium with 25 μg/ml trypsin (SFM + Trypsin) and normal growth medium (NGM) containing 10 per cent foetal calf serum.

Conclusions

The observations confirmed that the proportion of cell monolayer remaining intact after fixation was high, even at relatively high concentrations of virus (10^-2 dilution of stock approximated to 10^4 fluorescent forming foci per ml).
2.3 Efficiency of Eluates in Detaching Enteric Viruses from Glass Fibre Depth Filters

1. A $10^9$ per ml (approx) suspension of poliovirus was serially diluted in PBS to provide dilutions of $10^{-4} - 10^6$ of suspension.

2. 9 ml of each diluted suspension were added to 10 litres of distilled water which was then acidified to pH 3.5 and sufficient aluminium chloride was added to give a final concentration of 0.005 M.

3. 10 litre solution containing poliovirus were then filtered through glass fibre depth filters (Balston 100-12-C) under positive pressure.

4. Three types of eluent were employed:
   - 3 per cent Beef Extract (Oxoid)
   - 3 per cent Beef Extract plus 3 per cent glycine
   - 3 per cent Beef Extract plus 3 per cent Skim Milk (Oxoid)

5. Eluents and filtrate were diluted tenfold in PBS to give concentrations of $10^0$, $10^{-1}$ and $10^{-2}$ for virus assay. Poliovirus was assayed by a modified MPN technique in BGM cells in microtitre plates (Nunc). Wells showing cytopathic effect were scored after 2, 4 and 7 days. The best comparative results between confluent growth and cytopathic effect were observed after 4 days and the most relevant are recorded below.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Eluent</th>
<th>Poliovirus per ml Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-8}$</td>
<td>BE/G</td>
<td>&lt;2</td>
</tr>
<tr>
<td>$10^{-7}$</td>
<td>BE/G</td>
<td>&lt;2</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>BE/G</td>
<td>&lt;2</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>BE/G</td>
<td>17</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>BE/G</td>
<td>230</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>BE/SM</td>
<td>230</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>BE</td>
<td>540</td>
</tr>
</tbody>
</table>

Table A.II.3 Recovery of poliovirus through elution of glass fibre filters with beef extract (BE), beef extract plus glycine (BE/E) and beef extract plus skim milk (BE/SM).

Conclusions

It was concluded that no particular advantage was gained by incorporating glycine or skim milk into 3 per cent beef extract eluent. However, due to reported difficulties in flocculating some grades of extract, the incorporation of skim milk after elution in order to enhance flocculation was still considered desirable. The concentration of skim milk used in the flocculation of beef extract eluents was 1.5 per cent.
2.4 Calibration of an Extinction Method for Performing Field Analysis of Turbidity

1. Formazine turbidity standards were prepared according to US standard methods in distilled water in the range 20-2000 NTU. Twelve unlabelled water samples were then prepared for turbidities of 20, 50, 100, 400, 1000, and 2000 NTU.

2. Acrylic tubes of approximate length 400 mm with a black circle scored and painted on the base were then used to define the point at which each water sample obscured the black circle.

3. Four volunteers were requested to pour the unknown water samples into the acrylic tubes and (without straining their eyesight) measure the height of water each sample needed to obscure the mark on the base of the tube.

4. Results of turbidity and distances in log NTU and log centimetres are illustrated below.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Log NTU</th>
<th>Subject</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>log cm</td>
</tr>
<tr>
<td>1</td>
<td>2.6</td>
<td>0.51</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>0.94</td>
</tr>
<tr>
<td>3</td>
<td>1.3</td>
<td>1.49</td>
</tr>
<tr>
<td>4</td>
<td>3.3</td>
<td>0.15</td>
</tr>
<tr>
<td>5</td>
<td>3.0</td>
<td>0.30</td>
</tr>
<tr>
<td>6</td>
<td>2.6</td>
<td>0.53</td>
</tr>
<tr>
<td>7</td>
<td>1.7</td>
<td>1.16</td>
</tr>
<tr>
<td>8</td>
<td>1.3</td>
<td>1.41</td>
</tr>
<tr>
<td>9</td>
<td>3.3</td>
<td>0.10</td>
</tr>
<tr>
<td>10</td>
<td>3.0</td>
<td>0.30</td>
</tr>
<tr>
<td>11</td>
<td>1.7</td>
<td>1.10</td>
</tr>
<tr>
<td>12</td>
<td>2.0</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Table A.II.4 Results of calibration exercise for turbidity by an extinction method in an acrylic tube using four subjects (A-D) and twelve unknown water samples.
5. Results were assimilated into regression analyses which gave the following results.

<table>
<thead>
<tr>
<th>Subject</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>2.269</td>
<td>2.286</td>
<td>2.271</td>
<td>2.190</td>
<td>2.254</td>
</tr>
<tr>
<td>Slope</td>
<td>-0.658</td>
<td>-0.652</td>
<td>-0.654</td>
<td>-0.630</td>
<td>0.649</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>-0.997</td>
<td>-0.992</td>
<td>-0.993</td>
<td>-0.995</td>
<td>-0.994</td>
</tr>
</tbody>
</table>

Table A.II.5 Regression of calibration exercise.

Conclusions

As a result of the above analyses, a calibration curve was constructed which gave a straight line relationship between log NTU and log distance for extinction. This unique observation allows, for the first time, the quotation of extinction turbidity units in the equivalent of formazine standards (previously only used for nephelometric calibrations).

In a comparative study of particle counting versus nephelometry in water treatment plant control, Beard and Tanaka (1977)*, concluded that particle counting was a more sensitive measure of performance and a more reliable means of quantifying suspended solids than nephelometry. It was noted that studies tend to indicate no reliable correlation between suspended solids and turbidimetric measurements due to variations in particle shape, refractive index and the wavelength of light used in the measurement. However, in remote locations, particle counting is clearly not a viable technique, and thus a more field-appropriate method for turbidity measurement than nephelometry is required.

FIGURE A.II.i Calibration curve for turbidity measurements by extinction compared with nephelometric standards.
2.5 Calibration of a Bacteriological Incubator Developed for Field Analysis of Thermotolerant Coliforms.

1. Replicate samples of surface water derived from a lake at the University of Surrey were passed through three separate GN6 membrane filters (Gelman, US) and placed on nutrient pads soaked in Membrane Lauryl Sulphate Broth (Oxoid, UK).

2. Following the standard period of resuscitation, (Anon, 1983), one pad was incubated in a canister placed in a water bath at 44°C. A second pad was placed in the incubator of a commercial field testing kit (Millipore, US), and a third pad was placed in the bacteriological incubator developed at the University of Surrey.

3. Conventional incubation periods were allowed, and thermotolerant coliform colonies were then counted according to standard procedures.

4. Counts were plotted of the University of Surrey incubator system versus both the commercial kit and the standard method based on water bath incubation (Figures A.II.ii and A.II.iii).

Conclusions

Regression analysis showed no significant difference between incubation systems tested. Temperature stability of the University of Surrey incubator system was acceptable (+ 0.2°C), and thus the incubator was considered sufficiently reliable to be included in the programme of evaluation of protected slow sand filters in Peru.
FIGURE A.II.ii Performance curve for a portable bacteriological incubator developed at the University of Surrey compared with incubation in a conventional water bath.

FIGURE A.II.iii Performance curve for a portable bacteriological incubator developed at the University of Surrey compared with incubation in a commercial field-portable incubator.