



National Library  
of Canada

Bibliothèque nationale  
du Canada

Acquisitions and  
Bibliographic Services Branch

Direction des acquisitions et  
des services bibliographiques

395 Wellington Street  
Ottawa, Ontario  
K1A 0N4

395, rue Wellington  
Ottawa (Ontario)  
K1A 0N4

*Your file* *Voire référence*

*Our file* *Notre référence*

## NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

## AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

**Canada**

**THE HOLLOW FIBER DIFFUSION SYSTEM :  
A NOVEL METHOD FOR THE *IN SITU* SURVIVAL  
STUDIES IN THE AQUATIC ENVIRONMENT**

A Thesis Submitted to the School of Graduate Studies

University of Ottawa

In Partial Fulfillment of the Requirement for the Degree of Doctor of  
Philosophy

Department of Microbiology and Immunology, Faculty of Medicine

**Loh Chi Leong**

© Loh Chi Leong, Ottawa, Canada. 1994



National Library  
of Canada

Acquisitions and  
Bibliographic Services Branch

395 Wellington Street  
Ottawa, Ontario  
K1A 0N4

Bibliothèque nationale  
du Canada

Direction des acquisitions et  
des services bibliographiques

395, rue Wellington  
Ottawa (Ontario)  
K1A 0N4

*Your file* *Votre référence*

*Our file* *Notre référence*

THE AUTHOR HAS GRANTED AN IRREVOCABLE NON-EXCLUSIVE LICENCE ALLOWING THE NATIONAL LIBRARY OF CANADA TO REPRODUCE, LOAN, DISTRIBUTE OR SELL COPIES OF HIS/HER THESIS BY ANY MEANS AND IN ANY FORM OR FORMAT, MAKING THIS THESIS AVAILABLE TO INTERESTED PERSONS.

L'AUTEUR A ACCORDE UNE LICENCE IRREVOCABLE ET NON EXCLUSIVE PERMETTANT A LA BIBLIOTHEQUE NATIONALE DU CANADA DE REPRODUIRE, PRETER, DISTRIBUER OU VENDRE DES COPIES DE SA THESE DE QUELQUE MANIERE ET SOUS QUELQUE FORME QUE CE SOIT POUR METTRE DES EXEMPLAIRES DE CETTE THESE A LA DISPOSITION DES PERSONNE INTERESSEES.

THE AUTHOR RETAINS OWNERSHIP OF THE COPYRIGHT IN HIS/HER THESIS. NEITHER THE THESIS NOR SUBSTANTIAL EXTRACTS FROM IT MAY BE PRINTED OR OTHERWISE REPRODUCED WITHOUT HIS/HER PERMISSION.

L'AUTEUR CONSERVE LA PROPRIETE DU DROIT D'AUTEUR QUI PROTEGE SA THESE. NI LA THESE NI DES EXTRAITS SUBSTANTIELS DE CELLE-CI NE DOIVENT ETRE IMPRIMES OU AUTREMENT REPRODUITS SANS SON AUTORISATION.

ISBN 0-612-00612-3

Canada



UNIVERSITÉ D'OTTAWA  
UNIVERSITY OF OTTAWA

**T**he steps of a man are from the Lord,  
and he establishes him in whose way he delights;  
though he fall, he shall not be cast headlong,  
for the Lord is the stay of his hand.

—— Psalm 37 verse 23 -24 ——

*Dedicated to my parents*

## **Acknowledgments**

I wish to sincerely thank Professor Syed A. Sattar, my thesis supervisor, for his invaluable guidance, encouragement and support throughout this project. I also would like to thank Sue Springthorpe and all the students and staff in Professor Sattar's laboratory for their assistance, encouragement and help. I would also like to thank Dr. William Robertson, Dr. Campbell Wyndham and Dr. Ken Dimock for their advice and guidance during this project.

I am very grateful to the International Development Research Centre (IDRC) for their financial support for my studies and field work. Financial support for the work was also provided by Health Canada.

The field work in Canada could not be possible without the cooperation and help of the Britannia Water Treatment Facility, Ms. Dale Lynch and Mr. Ron Rook of the Regional Municipality of Ottawa-Carleton, and Mr. and Mrs. P. Garel-Jones, all of whom I gratefully acknowledge here.

I would like to thank Professor S. K. Lam and Professor Y. F. Ngeow of the Department of Medical Microbiology, University of Malaya, for their help and encouragement during the field work in Malaysia. I am grateful to Dr. C. W. Wang of the Department of Biochemistry, University of Malaya, for providing media and equipment for the field work. I would also like to thank the Forest Research

Institute of Malaysia (FRIM) for allowing me to carry out my field work within their boundaries.

I am grateful to Dr. John Austin and Mr. Gilles Bergeron of Health Canada, Tunney's Pasture, for their technical assistance in obtaining electron micrographs of the hollow fibers and the coliphages.

The field work involved much improvisation and long hours. In that respect, I wish to thank Denis Laframboise, Neil Armstrong, Chris Rainer and Graham Ashford for their assistance and specifically for the enjoyable sessions of brainstorming and problem-solving at the field sites.

I realize that I could not have achieved anything without the support of my family and so I thank my parents for their love, patience and guidance through the years that has led me to this point. I also thank my wife, Hoong, for her love, support and confidence in me and also for helping me type this thesis.

Finally, I thank God for watching over me, guiding me, strengthening me, providing for me, going before me and making my path straight, I thank Him for all and everything.

## Abstract

The Hollow Fiber Diffusion (HFD) system is a novel approach for the *in situ* study of the survival of bacteria and viruses in the aquatic environment. The HFD system employs a tangential flow, hollow fiber cartridge with a large area ( $7 \times 10^3$  cm<sup>2</sup>) of exchange surfaces for diffusion.

When compared with diffusion chambers, the HFD system responded significantly faster and more accurately to changes in pH, Eh, nutrient concentrations and to the presence of disinfectants in the external aqueous environment. The  $T_{90}$  diffusion of low molecular weight substrates was 0.6 h for the HFD system but was 4.2 h for the diffusion chamber. The rate of diffusion or equilibration could be further improved by increasing the flow rate through the HFD system or reducing the volume of the sample reservoir.

The HFD system was compatible with all test bacteria and viruses with the possible exception of tailed coliphages. The inactivation of tailed phages by the HFD system can be reduced or eliminated using a slower flow rate or larger diameter hollow fibers. Tailed coliphage inactivation in the HFD system was not apparent in natural waters.

Neither adsorption of microorganisms to the hollow fiber membrane surfaces nor colonization of those surfaces was found to be a significant problem during its use in natural waters. A protocol for the decontamination and reuse of

the hollow fiber cartridges using hydrogen peroxide was developed and applied successfully.

The results of trials of the HFD system at five field sites suggests that the HFD system permits "real-time" accommodation to changes in the physicochemical parameters of the external aqueous environment which can influence the survival of microorganisms. Differences in the survival of microorganisms in the HFD system and in batch samples were shown. The HFD system demonstrated regrowth of *Escherichia coli* in the Rideau River which is an eutrophic, temperate river. It also demonstrated a diurnal inactivation pattern for *Enterococcus durans* with the slower decay of *E. durans* numbers in the hours of darkness. For the other water sources tested (the Ottawa River, the Kennedy Burnett Stormwater Ponds, the Gombak River and the Kroh River), the general order of survival of test microorganisms was MS-2 coliphage > poliovirus > phage B > *E. coli* and *E. durans*. In the Rideau River, the order of survival was *E. coli* > poliovirus > MS-2 coliphage > *E. durans*. Surprisingly, there was no significant difference in the survival of microorganisms in the two equatorial rivers compared with the survival of the organisms in the oligotrophic, temperate Ottawa River.

The HFD system will be very useful in studying the survival and natural ecology of microorganisms in the aquatic environment. It can be applied to model the behavior of water quality indicators, pathogenic organisms and genetically engineered microorganisms. It also has potential for ecotoxicological studies,

monitoring for toxins or pollutants in the environment and for the in-line monitoring of the efficiency of water treatment processes such as chlorination.

## Table of Contents

Acknowledgments.....	iv
Abstract .....	vi
Table of Contents.....	ix
List of Tables.....	xiv
List of Figures.....	xv
1. Introduction .....	1
2. Review of the Literature .....	6
2.1 Survival of microorganisms in the aquatic environment.....	7
2.1.1 Microbial indicators of fecal pollution.....	7
2.1.1.1 Fecal indicator microorganisms.....	8
2.1.1.2 Fate of fecal indicator microorganisms in the aquatic environment.	10
2.1.2 Survival of pathogens in the aquatic environment.....	14
2.1.3 Monitoring and control of pollutants in the aqueous environment.....	17
2.1.4 Survival of genetically engineered microorganisms (GEMs).....	19
2.2 Factors affecting microbial survival in water.....	20
2.2.1 Abiotic factors .....	20
2.2.1.1 pH.....	20
2.2.1.2 Oxygen .....	21
2.2.1.3 Light.....	21
2.2.1.4 Temperature.....	23
2.2.1.5 Sediment and suspended solids .....	24
2.2.1.6 Nutrients and chemical composition of water and other factors.....	24
2.2.2 Biotic factors .....	25
2.2.3 Viability and dormancy .....	27
2.3 Experimental systems for the study of survival of microorganisms in the aquatic environment .....	28
2.3.1 Batch study systems.....	28
2.3.2 Dialysis sac systems.....	29

2.3.3 Membrane filter diffusion chambers .....	30
<b>3. Objectives.....</b>	<b>32</b>
<b>4. General Materials and Methods.....</b>	<b>35</b>
4.1 Bacteria and preparation of bacterial stocks .....	36
4.2 Coliphages .....	37
4.2.1 Somatic coliphage stock.....	37
4.2.2 MS-2 stock .....	38
4.3 Cells for the culture of mammalian viruses .....	39
4.4 Viruses .....	40
4.5 Enumeration of microorganisms .....	41
4.5.1 Enumeration of bacteria .....	41
4.5.2 Enumeration of somatic coliphages .....	42
4.5.3 Enumeration of F-specific phages .....	44
4.5.4 Plaque assays for viruses.....	44
4.6 Survival studies with samples of mixed microorganisms .....	45
4.6.1 Seeding of water with multiple organisms.....	46
4.6.2 Assaying for organisms from mixed culture samples .....	46
4.6.3 .....	48
4.6.3 Controls .....	49
4.6.4 Statistical and mathematical analysis.....	49
<b>5. Characterization of the Hollow Fiber Diffusion system and evaluation of its potential use for microbial survival studies.....</b>	<b>51</b>
5.1 Introduction .....	52
5.2 Materials and methods .....	55
5.2.1 The Hollow Fiber Diffusion (HFD) System.....	55
5.2.1.1 The HFD cartridge.....	56
5.2.1.2 Connecting tubing and peristaltic pump .....	56
5.2.1.3 Internal reservoir .....	58
5.2.2 Protocol for setting up the HFD system.....	58
5.2.3 Protocol for re-use of the HFD cartridge .....	60
5.2.4 Protocol for HFD system integrity check.....	61
5.2.5 Characterization of the HFD system .....	62
5.2.5.1 Adsorption studies.....	62
5.2.5.2 Survival studies to investigate the effects of the inherent properties of the HFD system.....	63

5.2.5.3 Coliphage inactivation studies.....	64
5.2.5.4 Characterization of the diffusion properties of the HFD system.....	65
5.2.5.4.1 Enzyme assays for ONPG and PNPG.....	66
5.2.5.5 Disinfection studies.....	67
5.2.6 Diffusion chambers.....	68
5.2.7 Comparisons between the HFD system and the diffusion chamber.....	71
5.2.7.1 pH and Redox Equilibration Experiments.....	71
5.2.7.2 Equilibration of the concentration of ONPG.....	73
5.2.7.3 Laboratory plug-flow studies.....	73
5.2.7.4 Response to disinfectants.....	74
5.2.8 Preliminary laboratory based microcosm survival studies using the HFD system.....	75
5.3 Results.....	75
5.3.1 Adsorption of microorganisms to the surfaces of the HFD system.....	75
5.3.2 Inherent properties of the HFD system affecting microbial survival.....	77
5.3.3 Effect of flow rates on the survival of coliphage B in the HFD system....	79
5.3.4 Equilibration of the HFD system to the external environment.....	81
5.3.5 Response of the HFD system to disinfectants in the external reservoir...	83
5.3.6 Comparisons between the HFD system and the diffusion chamber.....	86
5.3.6.1 pH and Eh equilibration.....	86
5.3.6.2 Response to disinfectants in the external reservoir.....	86
5.3.6.3 Diffusion rates of sugar analogues.....	89
5.3.7 <i>E. coli</i> survival in laboratory-simulated nutrient plug flow studies.....	89
5.3.8 The application of the HFD system in laboratory-based river water microcosm studies.....	94
5.3.8.1 Ottawa River water.....	94
5.3.8.2 Rideau River water.....	96
5.4 Discussion.....	98
<b>6. The Application of the Hollow Fiber Diffusion System for <i>in situ</i> Survival Studies.....</b>	<b>104</b>
6.1 Introduction.....	105
6.2 Materials and methods.....	105
6.2.1 Temperate Field Sites.....	105
6.2.1.1 Rideau River (site TA).....	106
6.2.1.1.1 Experimental set-up at site TA.....	106
6.2.1.2 Ottawa River (site TB).....	107
6.2.1.2.1 Experimental set-up at site TB.....	108
6.2.1.3 Kennedy-Burnett Stormwater Management Pond (site TC).....	109
6.2.1.3.1 Experimental set-up at site TC.....	110
6.2.2 Equatorial field sites.....	110
6.2.2.1 Gombak River (site EA).....	110

6.2.2.1.1 Experimental set-up at site EA.....	112
6.2.2.2 Kroh River Tributary (site EB).....	112
6.2.2.2.1 Experimental set-up at site EB.....	113
6.2.2.3 Modifications of the HFD system design for field work in Malaysia.....	113
6.2.2.3.1 HFD system internal reservoir.....	114
6.2.2.3.2 Configuration of the internal reservoir and hollow fiber cartridge components .....	116
6.2.2.3.3 The portable pump box.....	116
6.2.2.3.4 Power supply .....	118
6.2.2.3.5 Sampling procedure .....	118
6.2.2.3.6 Control of volume changes and gas production.....	118
6.2.3 Batch controls.....	122
6.2.4 Enumeration of microorganisms .....	122
6.3 Results.....	123
6.3.1 Survival of test microorganisms in the Rideau River (Site TA).....	123
6.3.1.1 HFD system results.....	123
6.3.1.2 Batch sample results.....	128
6.3.2 Survival of test microorganisms in the Ottawa River (Site TB) .....	129
6.3.2.1 HFD system results.....	133
6.3.2.2 Batch samples results.....	134
6.3.3 Survival of test microorganisms in the Kennedy-Burnett Stormwater Pond (Site TC).....	134
6.3.3.1 HFD system results.....	135
6.3.3.2 Batch sample results.....	135
6.3.4 Survival of test microorganisms in the Gombak River (Site EA) .....	135
6.3.4.1 HFD system results.....	139
6.3.4.1.1 Eh levels and bacterial survival.....	141
6.3.4.2 Batch sample results.....	141
6.3.5 Survival of test microorganisms in the Kroh River (Site EB).....	146
6.3.5.1 HFD system results.....	147
6.3.5.1.1 Eh levels and bacterial survival.....	147
6.3.5.2 Batch sample results.....	153
6.4 Discussion.....	154
<b>7. The effect of surface runoffs and changes in redox potential on the survival of bacteria in water .....</b>	<b>165</b>
7.1 Introduction .....	166
7.2 Materials and Methods.....	167
7.2.1 Protocol for Redox potential studies .....	167
7.2.2 Protocol for soil filtrate studies .....	170

7.2.2.1 Soil types.....	170
7.2.2.2 Preparation of soil filtrates.....	171
7.2.2.3 Studies into the bacterial response to soil filtrates .....	171
7.3 Results.....	172
7.3.1 The effect of rapid Eh changes on bacterial survival in the laboratory.....	172
7.3.2 Bacterial regrowth experiments .....	176
7.4 Discussion.....	179
<b>8. General Discussion .....</b>	<b>181</b>
<b>9. Conclusions .....</b>	<b>191</b>
<b>10. References .....</b>	<b>195</b>
<b>11. Appendices .....</b>	<b>208</b>
<b>12. Publications and Presentations .....</b>	<b>217</b>

## List of Tables

Table 2.1	Criteria for fecal indicator microorganisms.	9
Table 5.1	Comparison of times required for the diffusion of ONPG from the internal reservoirs of the HFD system and the diffusion chamber.	91
Table 5.2	The inactivation rate constants ( $K_i$ ) for microorganisms in Ottawa River water in a laboratory-based microcosm study.	96
Table 6.1	The survival and decay patterns of test microorganisms in the HFD system during <i>in situ</i> field studies.	155
Table 6.2	The survival and decay patterns of test microorganisms in batch systems during <i>in situ</i> field studies.	158

## **List of Figures**

<b>Fig. 4.1</b>	<b>The protocol for the seeding, processing and enumeration of a mixed culture of microorganisms.</b>	<b>48</b>
<b>Fig. 5.1</b>	<b>Schematic representation of the HFD system.</b>	<b>53</b>
<b>Fig. 5.2</b>	<b>Design of the hollow fibre cartridge.</b>	<b>57</b>
<b>Fig. 5.3</b>	<b>Design of the diffusion chamber.</b>	<b>69</b>
<b>Fig. 5.4</b>	<b>The effect of pH on the recovery of microorganisms from consecutive dead volume passages through the HFD system internal circuit.</b>	<b>76</b>
<b>Fig. 5.5</b>	<b>The effect of the inherent properties and characteristics of the HFD system on the survival and recovery of microorganisms.</b>	<b>78</b>
<b>Fig. 5.6</b>	<b>Factors that affect the survival and recovery of coliphage B in the HFD system.</b>	<b>80</b>
<b>Fig. 5.7</b>	<b>The different responses of the HFD system to pH changes in the external circuit due to different types of acids or bases.</b>	<b>82</b>

- Fig. 5.8 The equilibration of ONPG and PNPG concentrations between the internal and external circuits of the HFD system. 84
- Fig. 5.9 The response of microorganisms in the HFD system to exposure to chlorine in the external reservoir. 85
- Fig. 5.10 The comparative responses of the HFD system and the diffusion chamber to changes in the pH and Eh in the external environment. 87
- Fig. 5.11 The response of *E. coli* in the HFD system and in the diffusion chamber to the presence of chlorine in the external reservoir. 88
- Fig. 5.12 The rate of equilibration of ONPG concentration for the HFD system and the diffusion chamber. 90
- Fig. 5.13A The response of bacteria in the HFD system and in the diffusion chamber to exposure of transient nutrient spikes in the external reservoir. 92
- Fig. 5.13B The response of bacteria in the HFD system and in the diffusion chamber to repeated exposure to short transient nutrient spikes in the external reservoir. 93

Fig. 5.14	The survival of microorganisms in the HFD system during laboratory-based microcosm studies using Ottawa River water.	95
Fig. 5.15	The survival of microorganisms in the HFD system during laboratory-based microcosm studies using Rideau River water.	97
Fig. 6.1	The design of the internal reservoir of the HFD system used for field work in Malaysia.	115
Fig. 6.2	The schematic representation of the set-up of the HFD system during field work in Malaysia.	117
Fig. 6.3	The use of counterbalancing hydrostatic pressure to maintain a constant fluid level in the internal reservoir.	121
Fig. 6.4	The survival of microorganisms during <i>in situ</i> studies at the Rideau River (Site TA).	124
Fig. 6.5	The comparison of the survival of microorganisms during <i>in situ</i> studies at the Rideau River (Site TA) in the HFD system and in filter-sterilized and non-sterilized batch samples.	125
Fig. 6.6	The survival of bacteria during <i>in situ</i> studies at the Ottawa River (Site TB).	130

Fig. 6.7	The survival of viruses during <i>in situ</i> studies at the Ottawa River (Site TB).	131
Fig. 6.8	The results of field study 3 at the Ottawa River (Site TB).	132
Fig. 6.9	The survival of microorganisms during <i>in situ</i> studies at the Kennedy-Burnett Stormwater Ponds (Site TC).	136
Fig. 6.10	The survival of bacteria during <i>in situ</i> studies at the Gombak River (Site EA).	137
Fig. 6.11	The survival of viruses during <i>in situ</i> studies at the Gombak River (Site EA).	138
Fig. 6.12	Physico-chemical parameters of the river water during the fifth <i>in situ</i> survival study at the Gombak River (Site EA).	140
Fig. 6.13A	Physico-chemical parameters of the river water during the first <i>in situ</i> survival study at the Gombak River (Site EA).	142
Fig. 6.13B	The survival of <i>E. coli</i> during the first <i>in situ</i> survival studies at the Gombak River (Site EA).	143
Fig. 6.14A	Physico-chemical parameters of the river water during the second <i>in situ</i> survival study at the Gombak River (Site EA).	144

Fig. 6.14B	The survival of bacteria during the second <i>in situ</i> field study at the Gombak River (Site EA).	145
Fig. 6.15	The survival of bacteria during <i>in situ</i> studies at the Kroh River tributary (Site EB).	148
Fig. 6.16	The survival of viruses during <i>in situ</i> studies at the Kroh River tributary (Site EB).	149
Fig. 6.17	The survival of native bacteria during <i>in situ</i> survival studies at the Kroh River tributary (Site EB).	150
Fig. 6.18A	Physico-chemical parameters of the river water during the second <i>in situ</i> survival study at the Kroh River tributary (Site EB).	151
Fig. 6.18B	The survival of microorganisms during the second <i>in situ</i> survival study at the Kroh River tributary (Site EB).	152
Fig. 7.1	The redox potential (Eh) of ferric chloride solutions.	169
Fig. 7.2A	The effect of changes in Eh on the survival of bacteria (experiment 1).	173
Fig. 7.2B	The effect of changes in Eh on the survival of bacteria (experiment 2).	174
Fig. 7.2C	The effect of changes in Eh on the survival of bacteria (experiment 3).	175

Fig. 7.3	The effect of stormwater runoff on bacterial survival in water.	177
Fig. 7.4	The effect of soil filtrates on bacterial survival in water.	178

## List of Abbreviations

<b>BOD :</b>	Biochemical oxygen demand
<b>cfu :</b>	Colony-forming units
<b>cm :</b>	Centimeter
<b><i>E</i> :</b>	External
<b>EBSS :</b>	Earle's balanced salt solution
<b>Eh :</b>	Redox potential
<b>g :</b>	Grams
<b>GEM :</b>	Genetically-engineered microorganism
<b>h :</b>	Hours
<b>HAV :</b>	Hepatitis A Virus
<b>HFD :</b>	Hollow Fiber Diffusion
<b><i>I</i> :</b>	Internal
<b>IMRI :</b>	Industrial Membrane Research Institute
<b><i>k</i> :</b>	Diffusion coefficient
<b>K :</b>	Clearance
<b><i>Ki</i> :</b>	Decay or inactivation coefficient

<b>L :</b>	<b>Liter</b>
<b>M :</b>	<b>Molar</b>
<b>min :</b>	<b>Minutes</b>
<b>mL :</b>	<b>Milliliter</b>
<b>mM :</b>	<b>Millimolar</b>
<b>mV :</b>	<b>Millivolt</b>
<b>M.W. :</b>	<b>Molecular weight</b>
<b>ONPG :</b>	<b>o-nitrophenyl-<math>\beta</math>-D-galactopyranoside</b>
<b>pfu :</b>	<b>Plaque-forming units</b>
<b>PNPG :</b>	<b>p-nitrophenyl-<math>\alpha</math>-D-glucopyranoside</b>
<b>PSf :</b>	<b>Polysulfone</b>
<b>PV :</b>	<b>Poliovirus</b>
<b>rev :</b>	<b>Revolutions</b>
<b>TMP :</b>	<b>Transmembrane pressure</b>
<b>TPB :</b>	<b>Tryptose phosphate broth</b>
<b>TYGB :</b>	<b>Tryptone-yeast extract-glucose broth</b>
<b>UV :</b>	<b>Ultraviolet light</b>

w/v : Weight for volume ( $\text{g} \cdot 100 \text{ mL}^{-1}$ )

$\mu\text{m}$  : Micrometer

## 1. Introduction

Most non-polluted freshwaters and marine waters are oligotrophic. The indigenous or autochthonous microorganisms are adapted for survival, growth, and multiplication under such conditions. In contrast, other microorganisms may be introduced or transported into a particular aquatic ecosystem. This may be deliberate such as in the agricultural applications of genetically-engineered microorganisms (GEMs) or inadvertent such as coliforms from a sewage leak into an aquifer or the transport of soil microorganisms into a body of water by rain.

The survival of such introduced microorganisms in the aquatic environment and their responses to stresses or availability of nutrients is relevant to considerations of public health, ecotoxicology and the assessment of risks associated with the release of GEMs into the environment. In microbiological water quality monitoring, for example, the selection of an indicator microorganism for fecal pollution is based on certain criteria (Bonde, 1977). Ideally, the indicator microorganism should not be indigenous to unpolluted waters and should survive longer than potential pathogens. In recent years, the reliability of the most widely used group of indicator organisms, fecal coliforms and *Escherichia coli*, has been challenged especially for tropical waters by reports of *E. coli* regrowth in the aquatic environment (Carrillo *et al.*, 1985; Lopez-Torres *et al.*, 1987; Koujima, 1992) and its presence in pristine waters and soil (Fujioka *et al.*, 1988; Rivera *et al.*, 1988; Perez-Rosas and Hazen, 1989; Hardina and Fujioka, 1991). The possibility of long-term survival of pathogenic microorganisms which were previously considered transient in the aquatic environment (Chao *et al.*, 1987;

Valdes-Collazo *et al.*, 1987; Perez-Rosas and Hazen, 1988; Islam *et al.*, 1989; Jimenez *et al.*, 1989, Koul and Panhotra, 1989) is also of concern in assessing health risks.

However, the detection of high numbers of indicator organisms in pristine waters, cannot by itself prove that these microorganisms could persist since such data is open to the interpretation that there is an unknown source of fecal contamination (Feachem, 1974). Ultimately, some kind of *in situ* survival study needs to be conducted to resolve this issue.

Ecotoxicology in the aquatic environment is concerned with the impact of various pollutants on the ecosystem. This may be predicted from the survival of single or multiple species of microorganisms in microcosm tests (Pritchard and Bourquin, 1984; Niederlehner *et al.*, 1990; Cairns *et al.*, 1992). The survival and efficiency of certain bacteria in breaking down or mineralizing pollutants are important to considerations of the use of bioremediation (Kandel *et al.*, 1992; Pipke *et al.*, 1992; Hopkins *et al.*, 1993).

Similarly, information on the survival of GEMs in the aquatic environment would greatly help in the risk-benefit assessment for the release of such microorganisms into the environment (Lynch, 1990; Pipke *et al.*, 1992; Smit *et al.*, 1992; Wagner-Dobler *et al.*, 1992).

Most studies of microbial survival in aquatic systems have been carried out in batch samples (Geldreich *et al.*, 1968; Flint, 1987; Amy and Hiatt, 1989,,

Wright, 1989; Hosny *et al.*, 1990; Sorensen, 1991; Winstanley *et al.*, 1991; Barcina *et al.*, 1992; Kandel *et al.*, 1992). These are essentially artificial closed systems in which the exhaustion of substrates and the accumulation of inhibitory metabolites occur. Closed systems are also unable to respond to or effectively simulate the constantly changing conditions of the natural aquatic ecosystem.

The shortcomings of the batch sample method for studying microbial survival led to the use of dialysis chambers. These studies make use of dialysis sacs (Beard and Meadowcroft, 1935; Dutka, 1973; Chao *et al.*, 1988; Sagy and Kott, 1991) and membrane filter diffusion chambers (McFeters and Stuart, 1972; McFeters *et al.*, 1974; Vasconcelos and Swartz, 1976; Lessard and Sieburth, 1983; Rhodes and Kator, 1988; Perez-Rosas and Hazen, 1989; Terzieva and McFeters, 1991).

While differences in microbial survival have been noted between the batch system and the dialysis chamber systems (Lessard and Sieburth, 1983; Perez-Rosas and Hazen, 1988; McFeters and Terzieva, 1991), the dialysis chamber systems still require several hours to equilibrate with the external environment due to slow diffusion. For example, the time ( $T_{90}$ ) required for the diffusion of 90% of sucrose from within dialysis sacs has been reported as 7.5 h while the  $T_{90}$  for sucrose from diffusion chambers fitted with 0.015  $\mu\text{m}$  porosity polycarbonate membranes was 85 h (LaBelle and Gerba, 1980). Fouling of membranes leading to clogged pores is also a problem (Vargo *et al.*, 1975; Lessard and Sieburth, 1983).

This present study involves the development, characterization and field testing of a novel system for *in situ* microbial survival studies in the aquatic environment which is based on the use of polysulfone hollow fibers as the surfaces for diffusion and exchange. This Hollow Fiber Diffusion (HFD) system was then employed in microbial survival studies in both temperate and equatorial surface waters. The survival studies focused on a number of bacterial and viral indicators of fecal pollution which are important to considerations of microbiological water quality monitoring and public health.

## **2. Review of the Literature**

## **2.1 Survival of microorganisms in the aquatic environment.**

There is substantial literature on the survival of microorganisms in both fresh and marine water. However, it is often difficult to compare the results of these studies because of a lack of standardization in experimental design, and differences in the strains of microorganisms, enumeration techniques and media used. The different experimental designs used are discussed in greater detail in Section 2.3. In this section, some key areas where the survival of introduced or allochthonous microorganisms is an important consideration will be summarized.

### **2.1.1 Microbial indicators of fecal pollution**

Although less of a problem in developed nations, waterborne diseases remain a major global health problem with more than 250 million cases reported each year; it is estimated that 10 million deaths result from waterborne diseases annually (Barabas, 1986).

The monitoring of microbiological water quality for the assessment of potential health risk is based on the association of the transmission of waterborne-diseases with the contamination of that water by human feces. This was first demonstrated during a cholera outbreak in London, England, in which households that were struck by the disease were found to use the same fecally-contaminated well on Broad Street to draw drinking water (Snow, 1885).

It is not practical to routinely monitor water for the presence of specific pathogens as this would be too costly and time-consuming. Pathogens are also often present only intermittently and in low numbers. Therefore, microbial

indicators of fecal contamination are used instead. In doing so, certain assumptions are made regarding the relationship between the indicator organisms and potential pathogens in the aquatic environment. The criteria for the selection of fecal indicator microorganisms are summarized in Table 2.1.

#### 2.1.1.1 Fecal indicator microorganisms

Many microorganisms and groups of microorganisms have been proposed as fecal indicators. As early as the 1890's Klein and Houston (1898), used the term "bacteria of indication" for coliforms, fecal streptococci and *Clostridium perfringens*.

The coliform bacteria group included bacteria from the genera *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella*. However, it has been shown that not all the bacteria in the coliform group are exclusively associated with feces. In particular, *Citrobacter* and *Enterobacter* species were found to exist as autochthonous organisms in soil and on vegetation (Taylor, 1951) and could regrow especially in waters receiving organic load (Hendrick and Morrison, 1967; Dutka *et al.*, 1969). As a result, fecal coliforms or thermo-tolerant coliforms, a subset of the total coliform than bacterial indicators at representing the health risk from viral pathogens in water since viruses are generally believed to survive better than bacteria in the aquatic environment, are more resistant to disinfection processes and have a different distribution pattern in the environment. Of the enteroviruses, poliovirus (PV) was commonly used for this purpose and was

**Table 2.1** Criteria for fecal indicator microorganisms.  
(Adapted from Dutka, 1973; Bonde, 1977 and Olivieri, 1982).

1.	The indicator should always be present when the pathogens are present but absent from clean, uncontaminated water.
2.	The indicator should be present in greater numbers than the pathogens.
3.	The indicator should not be able to proliferate to any greater extent than the pathogens in the natural environment.
4.	The indicator should be at least as resistant as pathogens to disinfection, water treatment processes and to conditions in the aqueous environment.
5.	The indicator should be easy to isolate, identify without ambiguity and be enumerated.

particularly suitable where live attenuated poliovirus vaccine had been used in the population (Coin *et al.*, 1965; Payment *et al.*, 1979).

Among the bacteriophages, three groups are being actively investigated and used as fecal indicators. These are somatic coliphages (Kenard and Valentine, 1974; Kott *et al.*, 1974, Hernandez *et al.*, 1991), F-specific RNA bacteriophages (Cornax *et al.*, 1991; Reali *et al.*, 1991; Havelaar, 1993) and *Bacteriodes fragilis* phages (Cornax *et al.*, 1991; Havelaar, 1993).

#### **2.1.1.2 Fate of fecal indicator microorganisms in the aquatic environment**

As mentioned earlier, a fecal indicator should ideally conform to a number of criteria (Table 2.1). If pathogens were significantly more resistant to conditions in the aquatic environment than the indicators, then the monitoring for that indicator would underestimate the potential health risk. If, on the other hand, an indicator in the aquatic environment was capable of prolonged survival relative to the pathogens or is able to proliferate significantly or was even found to be autochthonous in a particular ecosystem, it would be again of little use in estimating the health risk from the consumption of or contact with the water.

Most early studies demonstrate that *E. coli* and fecal coliforms survive at least as well as bacterial pathogens in the aqueous environment (Geldreich *et al.*, 1968; McFeters *et al.*, 1974) and that the organisms generally did not survive very long. For example, the time for a 50% reduction in the recovery of fecal coliforms and fecal streptococci ( $T_{50}$ ) was determined to be 18.4 h and 22.2 h respectively in ground water (McFeters *et al.*, 1974). Generally, certain types of enteric viruses

survive better than bacteria and both survive better in ground-water than in surface water. A rapid loss of culturability of *E. coli* has also been reported for river water (Hosny *et al.*, 1990; Rhodes and Kator, 1990), lake water (Scheuerman *et al.*, 1988; Brettar and Hoefle, 1992) and marine water (Bonfont *et al.*, 1990; Cornax *et al.*, 1991; Sorensen, 1991).

However, other studies have indicated that *E. coli* is capable of surviving for long periods and may be even capable of re-growth in the aquatic environment (Hendricks and Morrison, 1967; Deaner and Kerri, 1969; Dutka, 1973; Carrillo *et al.*, 1985; Lopez-Torres *et al.*, , 1987). In some cases, the prolonged survival was dependent on the presence of sediment or aquatic plants in the study microcosm (Perez-Rosas and Hazen, 1988). It has also been shown that *E. coli* could survive for much longer periods of time in a temperate lake receiving thermal effluent (Gordon and Fliermans, 1978).

The higher temperatures of tropical waters have long been believed to encourage high coliform loads in polluted waters (Deaner and Kerri, 1969; Dutka, 1973; Evison and James, 1978). However, more recently, there is evidence from *in situ* studies with diffusion chambers that *E. coli* may persist even in pristine, unpolluted tropical waters (Lopez-Torres *et al.*, 1987; Perez-Rosas and Hazen, 1989). This is of great concern as it challenges the rationale for the use of *E. coli* as fecal indicators in tropical waters. Some researchers even suggest that *E. coli* may be part of the autochthonous community in the tropics, having been isolated in large numbers from water, soil and even leaf surfaces of epiphytic

bromeliads in pristine areas (Hazen, 1988; Rivera *et al.*, 1988; Hardina and Fujioka, 1991). As a result, Hazen (1988) has recommended alternative indicators such as *Bifidobacterium* or *C. perfringens* for the tropics.

The assay systems for many potential fecal indicators require costly media and equipment as well as highly trained personnel and normally require 24 - 48 h. These economic and logistical factors are major problems for the limited resources of many developing nations. Therefore, somatic coliphages have been put forward as an alternative indicator for water quality monitoring because of the relative simplicity, low cost and speed of their detection and enumeration assay (Loh *et al.*, 1988; Hernandez *et al.*, 1991; Toranzos, 1991).

However, not enough is known about the natural ecology of somatic coliphages. Particularly, it is not clear if coliphages are capable of significant replication in the environment. Wiggins and Alexander (1985) demonstrated in batch culture systems that a levels of host bacteria in excess of  $7 \times 10^3$  cfu. mL<sup>-1</sup> were required before an increase in the coliphage population could be observed. Such high levels of host bacteria do not exist in the aquatic environment except in highly contaminated waters. The implication therefore is that somatic coliphages are unlikely to replicate significantly in the environment.

Seely and Primrose (1980), however, made a distinction between environmental coliphages and coliphages of fecal origin in surface waters. This distinction was made on the basis of the different temperature ranges for productive infection for different phage isolates. They noted that some phages had

high temperature requirements and would be unable to replicate in the colder temperate waters. These coliphages were reasoned to be of fecal origin. However, other coliphages required the lower temperatures found in the temperate surface waters and were therefore more likely to be of environmental rather than fecal origin.

The issue is further complicated by the claim that indigenous *E. coli* is present in high numbers in pristine tropical watersheds and according to Hazen (1988) this may make it possible for coliphages to also survive and grow in such waters. This, he suggested, makes both *E. coli* and coliphages poor fecal indicators in tropical waters. However, Hernandez *et al.*, (1991) and Toranzos (1991), failed to isolate somatic coliphages from pristine or bromeliad waters that nevertheless had high levels of fecal coliforms. Ngeow *et al.*, (1990) found fecal coliforms, fecal streptococci and somatic coliphages in pristine and polluted waters from an isolated equatorial rainforest watershed. Somatic coliphages however correlated best with the sanitary information at each site with a mean level of 7 plaque forming units (pfu). 100 mL<sup>-1</sup> for pristine sites and 48 pfu. 100 mL<sup>-1</sup> for sites with human activity. In contrast, the levels of fecal coliforms and fecal streptococci between the pristine and polluted sites were very similar.

While the host receptor sites for somatic coliphages are located on the bacterial cell body, the receptor sites of F specific bacteriophages, such as MS-2 phage, are located on the sex-pili. This property was originally believed to make F-specific bacteriophages very specific indicators of fecal contamination since sex-

pili are believed to form at temperatures above 30°C which would be found in the intestinal environment of warm-blooded animals and not in environment (Havelaar and Pot-Hogeboom, 1989). However, studies have shown that F-specific RNA bacteriophages are present in sewage and wastewaters in numbers much higher than predicted from the level of input fecal contamination. This suggests environmental replication of these organisms in wastewater, although the mechanism by which this occurs is still unclear (Havelaar *et al.*, 1990).

Clearly, a lot more information about the natural ecology of indicator organisms in the aquatic environment, particularly in the tropics, is required to make informed decisions about the appropriate fecal indicators and to assess the significance of microbiological data on water quality. It is important to bear in mind that conclusions drawn from laboratory-based studies are sometimes in conflict with *in situ* observations and that microorganisms may behave differently in different aquatic ecosystems.

### **2.1.2 Survival of pathogens in the aquatic environment**

As with the fecal indicator microorganisms, most waterborne pathogens are considered to be of fecal origin and are allochthonous or transient in the aquatic environment. A number of batch and microcosm studies support this view. For example,  $T_{50}$  values of 7.2 h and 6.0 h are reported for *Vibrio cholerae* and *Salmonella typhi*, respectively, in groundwater (McFeters *et al.*, 1974). *Shigella* species survived slightly better with  $T_{50}$  values of 22.4 to 26.8 h. The die-off rate constants (represented as  $\log_{10} N_T/N_0$  where  $N_T$  is the concentration of organisms

after 24 h and  $N_0$  is the initial concentration) of viruses such as poliovirus 1, Coxsackievirus and rotavirus in groundwater was reported as 0.046 - 0.77, 0.19 and 0.36 day<sup>-1</sup>, respectively. This compares with die-off rate constants of 0.13 to 0.22 day<sup>-1</sup> for *S. typhimurium* and 0.16 to 0.32 for *E. coli* (Gerba and Bitton, 1984). Survival of bacteria and viruses were generally reported to be less in surface waters than in groundwater. For example, poliovirus 1 had decay rates of 0.0019 h<sup>-1</sup> in groundwater and 0.031 h<sup>-1</sup> in river water (Bitton *et al.*, 1983; O'Brien and Newman, 1977).

Both the study of the survival of pathogens in the aquatic environment and the study of the relative survival of pathogens to fecal indicator microorganisms are important. If the pathogen survives better than the fecal indicator organisms, some other means must be used to measure the risk of transmission of the pathogen in the water. Many studies compare directly the survival of a pathogen with that of an indicator organism such as *E. coli*. This has been done for *Salmonella* (McFeters *et al.*, 1974; Rhodes and Kator, 1988; Jimenez *et al.*, 1989; Wright, 1989; Chung and Yu, 1990), *Shigella* (McFeters *et al.*, 1974), *Aeromonas* (McFeters *et al.*, 1974; Chung and Yu, 1990), *Vibrio cholerae* (Perez-Rosas and Hazen, 1988; Perez-Rosas and Hazen, 1989; Chung and Yu, 1990), *Campylobacter jejuni* (Korhonen and Martikainen, 1991) and *Yersinia enterocolitica* (McFeters and Terzieva, 1991). Under certain conditions, *Aeromonas* (McFeters *et al.*, 1974; Chung and Yu, 1990), *Shigella* (McFeters *et al.*, 1974), *Vibrio cholerae* (Perez-

Rosas and Hazen, 1988) and *Salmonella* (Rhodes and Kator, 1988) survived longer than *E. coli* in the aquatic environment.

Occasionally, a relatively uncharacterized pathogen may gain prominence as a health concern. In such events, it is important to determine all possible modes of transmission of the pathogen. Studies on survival of the pathogen in the water environment would help determine if significant risk exists for waterborne transmission. For example, the presence of the human immunodeficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS), in body fluids and excretions of infected individuals raised fears on the possibility of its survival in wastewaters. However, studies, showed a 2 to 3 log reduction in infectivity within 48 h which makes HIV survival in raw and treated wastewaters significantly less than that of poliovirus (Casson *et al.*, 1992; Moore, 1993).

Another interesting aspect of pathogen survival in the aquatic environment is illustrated by *V. cholerae*. In certain tropical countries there appears to be regular periodic cholera outbreaks. In Bangladesh, for example, cholera epidemics occur twice a year but while *V. cholerae* was easily isolated from surface waters during epidemic periods, the bacteria could not be isolated during inter-epidemic periods (Islam *et al.*, 1990). It is believed that the bacteria persist in the inter-epidemic period in some environmental reservoir or microhabitat. Studies are being pursued in microcosm systems to study the effect of aquatic plants on *V. cholerae* survival and toxin production (Islam *et al.*, 1989; Islam, 1990; Islam *et al.*, 1990). *V. cholerae* has also been shown to survive and multiply in laboratory

microcosms due to the presence of the fresh water amoeba, *Naegleria gruberi* (Thom *et al.*, 1992).

Other researchers using diffusion chambers in *in situ* experiments found *V. cholerae* to survive longer in both tropical marine and tropical fresh water environments than might be predicted from batch and closed microcosm systems (Perez-Rosas and Hazen, 1988; Perez-Rosas and Hazen, 1989). These survival studies and the recovery of *V. cholerae* from pristine waters in Puerto Rico has led the investigators to suggest that *V. cholerae* may be indigenous to tropical waters.

When all these reports are taken into consideration, it is clear that more *in situ* and microcosm studies are required to determine the survival and natural ecology of pathogens in the water environment. This would allow an informed re-evaluation of the health risk posed by certain pathogens. The persistence of *V. cholerae* in the tropical coral reef environment means the threat of bacterial contamination of fish and shellfish may be more significant than previously appreciated (Perez-Rosas and Hazen, 1988). Understanding the natural ecology, associations with other flora and fauna and environmental reservoirs of pathogens such as *V. cholerae* could also furnish us the means of breaking the cycle of persistence of the pathogens in the environment.

### **2.1.3 Monitoring and control of pollutants in the aqueous environment**

Ecotoxicology is concerned with the effects of pollutants and toxins on ecosystems. Pollutants may affect an ecosystem in one of two ways. Firstly, a pollutant may not affect an organism directly but alters the physical and chemical

characteristic of the ecosystem. An example of this would be the eutrophication of inland and coastal waters by inorganic nutrients from sewage and agricultural fertilizers. The altered physical and chemical properties of the ecosystem would then affect the distribution of species in that ecosystem.

Secondly, pollutants may be in the form of toxins that are capable of killing or injuring organisms. Their effect on a particular ecosystem may depend on susceptible species, length of exposure and dose. Pesticides, dioxins and polychlorinated biphenyls (PCBs) are examples of such pollutants.

In assessing the risk posed by a chemical to the environment, it is important to know the amount that is likely to be released into the environment, its fate in the environment and its effects on individual organisms and hence on the ecosystem (Korte, 1977). In considering the fate of the pollutant, it is important to consider the tendency of the pollutant to disperse in the environment, its ability to persist and whether it is converted or broken down. These factors and the biological response depend not only on the pollutant's characteristics but also on the characteristics of that particular ecosystem (Roberts and Marshall, 1980). For example, the rate of hydrolysis of water is affected by pH. Hence predictions for one ecosystem may not be valid for others.

As with other survival studies, the effect of pollutants can be studied using batch systems, flow through systems like the chemostat, microcosms or *in situ* incubation systems (Atlas and Bartha, 1981). In the batch and flow-through systems, both single species tests and multiple species tests can be carried out.

Microcosms attempt to duplicate *in situ* field studies but are obviously limited in their ability to represent all the possible factors

The survival of microorganisms with the ability to degrade pollutants is also of interest. The survival of such microorganisms in the aqueous environment may enable them to be used in bioremediation processes (Kandel *et al.*, 1992; Pipke *et al.*, 1992; Hopkin *et al.*, 1993).

#### **2.1.4 Survival of genetically engineered microorganisms (GEMs)**

The application of GEMs into the environment offers great possibilities. These organisms may carry genes for enzymes or proteins that will enable degradation of pollutants or carry out other useful tasks. However, there are also risks involved in releasing GEMs into the environment. Before a GEM can be released into the environment, certain criteria should be met (Smit *et al.*, 1992). The GEM should not survive better than the wild-type organism nor should its distribution in the environment be any different. Transfer of the heterologous genetic element should not occur. Finally, its effect on ecosystem functioning and diversity should be known.

As a result, many studies have been carried out and study systems designed for examining GEM survival in the aquatic environment (Steffan *et al.*, 1989; Awong *et al.*, 1990; Chao and Feng, 1990; Pipke *et al.*, 1992; Sobecky *et al.*, 1992; Wagner-Dobler, 1992).

## **2.2 Factors affecting microbial survival in water**

A large number of abiotic and biotic factors have been determined to affect bacterial and viral survival in water. However, the factor that plays the most prominent role in killing or removing an organism from a particular water body may vary between water types and between locations. Among the abiotic properties of the water environment that might affect microbial survival are exposure to sunlight, temperature, pH, redox potential, osmotic pressure, sediment and chemical composition. Biotic factors may include predation and direct competition or the presence of toxins, antibiotics or growth factors.

### **2.2.1 Abiotic factors**

#### **2.2.1.1 pH**

Most microorganisms survive well within a particular range of pH but are not viable outside that range (Atlas and Bartha, 1981). *E. coli* survival in water was found to be optimal for a pH range of 5.5 and 7.5 but dropped sharply outside this range (McFeters and Stuart, 1972). With some exceptions (acidophilic bacteria), most microorganisms are unable to tolerate extreme pH values.

Other than a direct effect on the microorganisms and their enzymes and proteins, pH also affects the solubility of gases, such as carbon dioxide in water, the availability of certain nutrients and may potentiate the effect of toxins. Curtis *et al.*, (1992) reported that fecal coliforms did not survive well at elevated pH values (pH 8-9) and that in some undetermined way the elevated pH potentiated damage

to the fecal coliforms from exposure to light. A possible mechanism was that the elevated pH encouraged the photochemical production of oxygen radicals.

Elevated pH levels are known to induce a number of genes in various bacteria such as heat shock genes and sodium-proton antiporter genes (Slonczewski, 1992). The induction of such genes may have a protective effect against environmental stress and strains lacking these genes may be at a disadvantage.

#### **2.2.1.2 Oxygen**

Anaerobic organisms are unable to survive in the presence of significant levels of oxygen. Anaerobic bacteria such as *Bacteriodes fragilis* may be able to grow in anaerobic conditions of animal intestinal tracts but do not survive long in most aerated surface waters. Aerobic and facultatively anaerobic bacteria are less affected by oxygen but even so, the formation of free radicals and hydrogen peroxide from dissolved oxygen have been implicated in reducing the culturability of fecal coliforms (Curtis *et al.*, 1992).

#### **2.2.1.3 Light**

Exposure to sunlight has long been known to cause the die-off and loss of culturability of microorganisms. The survival of bacteria such as *E. coli* has been reported to be substantially affected by exposure to light in freshwater (Barcina *et al.*, 1986; Davies and Evison, 1991; Barcina *et al.*, 1992), estuarine (Rhodes and Kator, 1990) and marine waters (de Vicente *et al.*, 1988, Bonnefont *et al.*, 1990; Davies and Evison, 1991). There is at least one report that salinity potentiates the

inactivating effect of sunlight such that it is more pronounced in seawater than it is in freshwater (Davies and Evison, 1991). Viruses in the aquatic environment are also inactivated by sunlight. Bitton *et al.* (1979) showed a 90% reduction of poliovirus 1 within 3 h in groundwater exposed to sunlight whereas the virus was more stable in the dark.

Solar radiation includes ultraviolet light radiation, visible light radiation and infrared radiation with wavelengths in the ranges of 1 to 320 nm, 320 to 800 nm and 800 to 100,000 nm, respectively. The shorter wavelengths have more energy and penetrating power. Ultraviolet light (UV) and blue lights can penetrate further into aquatic habitats than red light. Infrared radiation has very low energy and penetrating power and is usually considered in microbial ecology only in respect to its ability to affect environmental temperature (Atlas and Bartha, 1981).

It is well known that UV can be used to sterilize water and surfaces. UV induces dimerization of thymine bases in DNA leading to progressive damage of the microorganism's genetic material (Hanawalt *et al.*, 1979). Davies and Evison (1991), demonstrated in their study system, that UV was the major component of sunlight responsible for loss of *E. coli* and *Salmonella* in both freshwater and seawater. Others feel that given the penetrating power of UV, the effects of turbidity and the movement of water, UV is not a significant factor in microbial survival in the natural environment (Wilson *et al.*, 1983).

Other researchers have found that it is the visible light component and not the UV that most affects bacterial recovery from the aquatic environment (Barcina

*et al.*, 1989; Curtis *et al.*, 1992). The effect of the visible light has been reported to be mediated through the photosensitized production of hydrogen peroxide and singlet oxygen in water (Arana *et al.*, 1992; Curtis *et al.*, 1992). A decrease in the uptake of amino acids (Bailey *et al.*, 1983) and of glucose (Barcina *et al.*, 1989) by bacteria was observed in the presence of visible light. It has been suggested that visible light causes bacteria to enter a non-culturable but viable, dormant state (Barcina *et al.*, 1989).

#### 2.2.1.4 Temperature

Generally, bacterial survival in the aquatic environment is inversely proportional to the temperature of the water. This has been demonstrated for *E. coli* (McFeters and Stuart, 1972; Flint, 1987) and pathogens such as *Aeromonas hydrophila*; *Yersinia enterocolitica* and *Vibrio cholerae* (Chao *et al.*, 1988b; Franco *et al.*, 1990; Varma and Iyer, 1990). McFeters and Stuart (1972) found that temperature significantly affected bacterial survival over the temperature range of 5°C to 15°C but became less of a factor at higher temperatures. Occasionally, environmental conditions may be suitable for regrowth or multiplication of the bacteria. In such cases, higher temperatures result in a higher bacterial level in water (Koul and Panhotra, 1989). For viruses, temperature is considered one of the major determinants of survival with higher temperatures reducing the survival time of viruses (Bitton, 1978; Sattar, 1981).

#### **2.2.1.5 Sediment and suspended solids**

Enteric bacteria are frequently found in higher numbers in aquatic sediment than in the overlaying waters (Sherer *et al.*, 1992). The presence of sediment has been shown to increase survival of enteric bacteria and pathogens in freshwater and seawater (Perez-Rosas and Hazen, 1988; Marino and Gannon, 1991; Sherer *et al.*, 1992).

Viruses are often adsorbed onto suspended solids or colloidal material in water and have been shown to be protected in such a state (Bitton and Mitchell, 1974; Babich and Stotsky, 1980). Adsorption to clay protected viruses from photoinactivation (Bitton *et al.*, 1979), as well as inactivation by temperature and salinity (LaBelle and Gerba, 1980).

#### **2.2.1.6 Nutrients and chemical composition of water and other factors**

Allochthonous bacteria are capable of regrowth in the presence of sufficient organic load in the water. This has been noted in waters receiving pulp and paper mill effluents (Hendrick and Morrison, 1967; Dutka *et al.*, 1969) and effluents from other factories such as a rum distillery (Valdes-Collazo *et al.*, 1987). Gauthier *et al.*, (1990) showed that *E. coli* from a phosphate rich source such as sewage survived better in seawater. *Aeromonas hydrophilia* will multiply in seeded seawater to a level proportional to the concentration of organic matter in the water (Araujo *et al.*, 1990).

There are many other organic and inorganic substances present in water that may affect microbial survival. The effect of any one substance or ion may vary

according to the microorganism. *E. coli* in stream water survives better at higher levels of inorganic ions (McFeters and Stuart, 1972). For avian cholera, it was more specifically higher calcium and magnesium levels that increased survival (Price *et al.*, 1992). Coliform numbers along a polluted stretch of the Gallinas River was correlated to phosphate and sulfate levels while higher chloride levels were associated with lower coliform numbers (Brasfeld, 1972) but higher chloride levels were correlated with higher fecal streptococci numbers.

In marine waters, the high salt and high osmotic pressure are other factors that microorganisms would have to contend with (Munro *et al.*, 1989). Under conditions of osmotic stress, non-halophilic bacteria may survive longer if they are able to accumulate organic osmolyte from their environment (Munro *et al.*, 1989; Bakhronf *et al.*, 1992; Flatau *et al.*, 1992). The survival of some viruses is adversely affected by the salinity of marine waters (Lo *et al.*, 1976).

### **2.2.2 Biotic factors**

The survival of an allochthonous microbial species may be affected by its interactions with the indigenous microflora. When enteric bacteria were seeded into sample of surface water that were either untreated or were filter sterilized, it was found that often the enteric bacteria survived longer in the filter sterilized water (Flint, 1987; Amy and Hiatt, 1989; Koul and Panhotra, 1989; Korhonen and Martikainen, 1991; Tashiro *et al.*, 1991). A similar phenomenon may also be observed for viruses (Sattar *et al.*, 1985). This suggest that either predation or competition or possibly both may be occurring.

Phagotrophic protozoa and some flagellates are known to feed on bacteria (Ehrlich, 1985; Rhodes and Kator, 1988; Gonzales *et al.*, 1990). There are also predatory bacteria like the *Bdellovibrio* which penetrate susceptible bacterium and enter into the periplasmic space where they feed on cellular components. The host range of *Bdellovibrio* includes enteric bacteria and pathogens. Bacteriophages may also reduce levels of their host bacteria in the environment.

Predation and lysis by bacteriophages have in some instances been shown to be the major cause of decline in bacterial populations (Chao *et al.*, 1988 a; Chao *et al.*, 1988 b; Rhodes and Kator, 1988; Marino and Gannon, 1991; Sorensen, 1991). However, others found predation to be of secondary importance (Barcina *et al.*, 1986; Gonzalez *et al.*, 1993). Schueurman *et al.*, (1988) found the decline of bacteria in lake water to be the result of predation by protozoa and not by *Bdellovibrio* or bacteriophages nor by the action of soluble toxins.

The effect of toxins , antibiotics and other non-filterable biotic factors were shown to significantly reduce bacterial populations in other studies (Chao *et al.*, 1988 a; Chao *et al.*, 1988 b; de Vicente *et al.*, 1988).

Bacterial survival may also be affected by the indigenous flora by direct competition for nutrients. Bacteria may also be associated with protozoa or amoebae in a way that actually enhances survival in aquatic environments. *V. cholerae* was found to survive and multiply in the presence of *Naegleria gruberi* but not in the absence of the amoeba (Thom *et al.*, 1992). Attachment to surfaces

of aquatic plants may also protect bacteria in the aquatic environment (Islam *et al.*, 1989).

Although there are some conflicting studies, the majority of the studies suggest that bacteria and algae can inactivate viruses in water (Block, 1983, Fujioka *et al.*, 1980).

### **2.2.3 Viability and dormancy**

The survival of bacteria in the environment is normally measured by the formation of colonies on standard culture media. The viability of bacteria is defined as their ability to multiply and form colonies on an agar medium (Valentine and Bradfield, 1954; Kurath and Morita, 1983). In recent times, standard media have been modified or made less selective to recover stressed or injured bacteria that would not otherwise have been enumerated (LeChevallier *et al.*, 1982; Calabrese and Bissonnette, 1990). Bacteria that had lost culturability on solid media can be resuscitated by the addition of selected nutrients (Roszak *et al.*, 1984).

As a result of the above observations, viable cells were redefined as all culturable or recoverable cells, while cells that are morphologically intact and metabolically active but do not multiply are called somnicells (Roszak and Colwell, 1987; Barcina *et al.*, 1989). Roszak and Colwell (1987) describe the "somnicell" stage as the equivalent of a "spore" stage for non-spore-forming bacteria.

While the question of a dormant stage for non-sporulating bacteria is still considered controversial, there is mounting evidence that some environmental factors such as sunlight which were considered to cause cell death may in fact be inducing a dormant state (Barcina *et al.*, 1989). Dormancy may account for seasonal distribution patterns of animal and plant pathogens that are not considered to survive in soil or water (Gray, 1976). Xu *et al.*, (1982) stressed the need for re-evaluating the survival of pathogens such as *V. cholerae* in the aquatic environment in view of the possibility of dormant states for those bacteria.

### **2.3 Experimental systems for the study of survival of microorganisms in the aquatic environment**

#### **2.3.1 Batch study systems**

A large number of survival studies have been carried out with batch systems (Geldreich *et al.*, 1968; Flint, 1987; Wright, 1989; Hosny *et al.*, 1990; Sorensen, 1991; Winstanley *et al.*, 1991; Barcina *et al.*, 1992; Kandel *et al.*, 1992). The batch system essentially involves the study of the survival microorganisms in samples of water contained in flasks or some other suitable container. Batch systems may be as small as a few milliliters of water in a test-tube (Wright, 1989) or as large as several hundred liters in glass tanks (Harte *et al.*, 1980). Batch systems are essentially closed systems which are usually under artificial laboratory conditions.

Since it is possible to alter conditions in batch systems in a controlled manner in the laboratory, such systems are suitable to study the effect of individual

parameters on microbial survival in water. In this way, the effects of illumination (Barcina *et al.*, 1992) on microbial survival was studied with illuminated and darkened batch systems. Similarly the role of predation was studied using filtered or autoclaved water in comparison with untreated water in batch systems (Flint, 1987; Hosny *et al.*, 1990; Morgan *et al.*, 1993) or by the addition of eukaryotic inhibitors (Sorensen, 1991).

However, being basically a closed system, batch cultures are artificial and do not necessarily portray what happens *in situ*. Winstanley *et al.*, (1991) demonstrated that there was a large variability in survival studies carried out with batch samples obtained at different times. This underlines the constantly changing nature of water in the environment. This concern led to the use of dialysis chambers for microbial survival studies which would allow some interaction and exchange between the study system and the external environment.

### 2.3.2 Dialysis sac systems

The early systems employed the use of dialysis sacs as a diffusion barrier between the organism being studied and the outside environment (Jordan *et al.*, 1904; Beard and Meadowcroft, 1935; Zobell, 1936). Dialysis sacs continue to be used for *in situ* survival studies ( Dutka, 1973; Chao *et al.*, 1988a; Sagy and Kott, 1990; Jounene *et al.*, 1991).

Dialysis sacs are made of membranes that have small pore diameters (0.003  $\mu\text{m}$ ), relative thickness and poor diffusion (McFeters and Stuart, 1972; Vasconcelos and Swartz, 1976).  $T_{90}$  for the diffusion of sucrose was found to be 7.5 h (LaBelle

and Gerba, 1980). These disadvantages led to the development of membrane filter diffusion chambers.

### 2.3.3 Membrane filter diffusion chambers

McFeters and Stuart (1972) developed the membrane-filter diffusion chamber as an improvement over the use of dialysis tubing for *in situ* survival studies. This design consisted of two 0.45  $\mu\text{m}$  pore size membrane sheets sandwiched between two perspex retainers with a central spacer between the sheets. This design resulted in a 20 mL internal reservoir with a total membrane surface area for exchange of 56.8  $\text{cm}^2$  and a surface area to volume ratio of 2.84. This diffusion chamber had diffusion rates for inorganic ions, total carbon and glucose which was twice as fast as for dialysis sacs.

Initially, cellulosic membranes were used but these were found to have problems such as rapid fouling (Vargo *et al.*, 1975; Lessard and Sieburth, 1983) and becoming perforated by cellulose-digesting bacteria (D. Lavoie, M.S. Thesis, University of Rhode Island, Kingston, 1975). As a result, these were replaced in some studies by polycarbonate membrane filters (Vasconcelos and Swartz, 1976; Lessard and Sieburth, 1983).

For the diffusion chambers described by McFeters and Stuart (1972), the  $T_{90}$  for the equilibration of ions and low molecular weight substrates like dextran was reported to be between 9.5 to 48 h using 0.4  $\mu\text{m}$  pore size membrane filters. Vasconcelos and Swartz (1976) found similar equilibration rates for glucose and fluorescein with  $T_{90}$  of 17.5 and 48 h, respectively, using polycarbonate filters.

They found that the introduction of internal agitation within the chamber reduced the  $T_{90}$  for glucose and fluorescein to 9.5 and 14.5 h, respectively. When 0.015  $\mu\text{m}$  porosity polycarbonate filters (capable of retaining viruses) were used, the  $T_{90}$  for sucrose was estimated at 85 h (LaBelle and Gerba, 1980).

The design of these diffusion chambers limited the internal volume and was still relatively slow to equilibrate with the outside environment. Lessard and Sieburth (1983) attempted to improve diffusion chamber design to overcome some of those problems. Their design had a 5 L growth chamber, 0.4  $\mu\text{m}$  porosity polycarbonate membranes and stirring blades incorporated in the chamber to keep cells suspended, clean surfaces of fouling debris and to encourage diffusion. Equilibration for salinity and dissolved organic carbon was reached within 2 h for this design.

Diffusion chambers have been used in flow through and static renewal systems (McFeters and Stuart, 1972; McFeters *et al.*, 1974, Awong *et al.*, 1990; McFeters and Terzieva, 1991) and in *in situ* studies (Vasconcelos and Swartz, 1976; Valdes-Collazo *et al.*, 1987; Rhodes and Kator, 1988; Jimenez *et al.*, 1989; Perez-Rosas and Hazen, 1989).

### **3. Objectives**

The primary objective of this work was to develop a novel system, the Hollow Fibre Diffusion (HFD) system for the *in situ* study of the survival of bacteria and viruses in the aquatic environment based on the adaptation and use of hollow fiber technology. This involved system design, laboratory characterization and testing and finally adaptation of the system for field studies. It was hoped that the HFD system would be useful in studying the natural ecology of microorganisms in the water environment, their interactions with each other and the effect of environmental factors on their survival. The potential of the HFD system to be used as an in-line water treatment monitoring system for disinfection efficiency was also investigated.

The specific objectives of this study were as follows :-

1. To develop a protocol for the recovery and enumeration of *E. coli*, *E. durans*, somatic coliphages, F-specific bacteriophage (MS-2), poliovirus and hepatitis A virus from a mixed culture.
2. To develop the prototype design and protocol for the HFD system.
3. To characterize the HFD system with regards to its physico-chemical properties, diffusion rates of solutes and its biocompatibility with test microorganisms.
4. To develop and evaluate a protocol for the decontamination and re-use of the HFD systems hollow fibre cartridges.

5. To compare the performance of the HFD system and the diffusion chambers.
6. To use the HFD system to study the survival of the mixed microbial culture in laboratory microcosms.
7. To investigate the possibility of using the HFD system as an in-line system for monitoring water treatment disinfection efficiency.
8. To develop and adapt the HFD system for *in situ* field studies.
9. To use the HFD system to study the survival of test microorganisms *in situ* in temperate and equatorial waters.

## **4. General Materials and Methods**

#### 4.1 Bacteria and preparation of bacterial stocks

For bacterial survival studies, an *Escherichia coli* and an *Enterococcus durans* strain were used. They were chosen as representatives of the fecal coliform and the fecal streptococcus groups, respectively. The *E. coli* strain, designated *E. coli* F, was isolated from human fecal material. Its identity was confirmed using API-20E strips (Analytab Products; Plainsview, N.Y.). The *E. durans* strain was an environmental isolate which was kindly provided by G. Jordan of the Provincial Public Health Laboratories (Ottawa, Ontario, Canada): its identity was confirmed using MicroScan Neg BP Combo Type 5 panel (Baxter Healthcare Corp., MicroScan Division, West Sacramento, CA).

The bacterial hosts used for enumeration of somatic coliphages and F-specific coliphages were *E. coli* C (ATCC No : 13706) and *Salmonella typhimurium* WG49, respectively. The *S. typhimurium* WG49 strain (NCTC 12484) was kindly provided by Dr. A. H. Havelaar of the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.

All bacterial strains were maintained on tryptic(ase) soy agar (TSA; Difco, Detroit, MI) slopes and as frozen cultures. Frozen bacterial cultures were prepared by growing the bacteria in tryptic(ase) soy broth (TSB; Difco) with 10% glycerol (BDH, Inc., Toronto, Ontario, Canada). A 1.0 mL aliquot of an overnight culture of the bacteria was inoculated into 100 mL of the TSB-glycerol medium. This was then incubated at 35°C with shaking (150 rev.min<sup>-1</sup>; Lab-line Instruments Inc. Orbital Shaker Model No: 3520, Melrose Park, Ill). During the middle of the

exponential growth phase of the culture (4 hours after inoculation for *E. durans* and 3 hours for all other bacteria), the bacterial suspension was placed into test-tubes in 5 or 10 mL aliquots and kept frozen at  $-70^{\circ}\text{C}$  until ready for use.

## 4.2 Coliphages

A somatic coliphage, designated coliphage B and an  $F^{+}$  specific RNA coliphage, MS-2, were used in survival studies. Coliphage B was a fecal isolate provided by R. Ahmed of the Provincial Public Health Laboratories, Ottawa, Ontario, Canada. MS-2 phage was also provided by Dr. A. H. Havelaar.

### 4.2.1 Somatic coliphage stock

The *E. coli* C host was grown in TSB modified with the inclusion of 10 mM ammonium nitrate and 0.5 mM strontium nitrate (BDH, Toronto, Ontario, Canada). The cations were added to enhance the adsorption of phages to bacteria by reducing the charge boundary layer (Havelaar and Hogeboom, 1983). First, a 1.0 mL aliquot of an overnight *E. coli* C culture was transferred into 100 mL of fresh modified TSB. The culture was then incubated at  $35^{\circ}\text{C}$  in an orbital shaker ( $150 \text{ rev. min}^{-1}$ ). After 3 h of incubation, a coliphage B plaque from a previously infected bacterial lawn was isolated and picked from an agar plate with a sterile glass Pasteur pipette and transferred into the culture of *E. coli* C. The formation of phage plaques on a host bacterial lawn and its use in titering phage suspensions are described in section 4.5.2. After incubation for 24 h, the culture was centrifuged at  $3000 \text{ rev. min}^{-1}$  (International Equipment Co. Model HN, Rotor No: 215, Needham Heights, Mass.) for 20 min to pellet out the bacteria and cell debris.

The supernatant was decanted into a sterile flask. Chloroform was then added (0.1 mL per 100 mL of supernatant) to kill the remaining bacteria. The supernatant was stored at 4°C. This coliphage stock was then titered, and such stocks had a titer of approximately  $10^8$  pfu.mL<sup>-1</sup>.

#### 4.2.2 MS-2 stock

The MS-2 stock was raised using the *S. typhimurium* WG49 host. A frozen culture of the WG49 host culture was streaked onto a McConkey agar plate to screen and select host bacteria that still retained the F'42 *lac* :: Tn5 plasmid essential for the formation of the F or sex pilus which carry the receptors for F-specific RNA bacteriophages (Havelaar and Hogeboom, 1984). After incubation at 37°C for 24 h, a single red (lactose-fermenting) colony was selected and inoculated into 5 mL of typtone-yeast extract-glucose broth (TYGB) and incubated overnight at 37°C. A 1.0 mL inoculum of the overnight culture was then added to 100 mL of fresh TYGB medium. The culture was then incubated at 37°C with gentle swirling. As in the preparation of coliphage B stock, an MS-2 plaque was transferred from an agar plate into the host culture. After 24 h, the culture was centrifuged and its supernatant processed in the way described for coliphage B stock preparation. The MS-2 coliphage stock preparations had a titer of about  $10^{10}$  pfu/mL. The formation of MS-2 plaques and its use in the enumeration of MS-2 phages in suspensions are described in section 4.5.3.

### 4.3 Cells for the culture of mammalian viruses

Vero and FRhK-4 cells were cultivated and maintained in 75 cm<sup>2</sup> plastic culture flasks (Costar, Cambridge, MA). FRhK-4 cells was provided by Dr. M.D. Sobsey of the University of North Carolina, Chapel Hill, North Carolina. Vero cells were obtained from Dr. Pierre Payment (Institut Armand-Frappier, Montreal, Quebec, Canada). The growth medium was prepared from 500 g of powdered Eagle's minimum essential medium (Gibco, Grand Island, NY) resuspended in 4.5 L of double-distilled deionized water. The medium was filter sterilized and dispensed in 450 mL portions into sterile glass bottles.

For Vero cells, the medium was supplemented with 13.5 mL of a 7.5% solution of sodium bicarbonate (BDH), 5 mL of a 200 mM L-glutamine solution (Gibco), 5.0 mL of a 10 mg.mL<sup>-1</sup> kanamycin sulfate stock (Gibco) and 50 mL of heat-inactivated fetal bovine serum (FBS; Gibco). For FRhK-4 cells, the medium was similar except that only 7.5 mL of sodium bicarbonate was used and the medium was further supplemented with 5 mL of 1.5 M HEPES (N-2-hydroxyethyl piperazine-N-2-ethanesulfonic acid; Gibco), 5.0 mL of a 10 mM non-essential amino acid solution (Gibco) and 0.5 mL of a 50 mg. mL<sup>-1</sup> gentamycin sulfate preparation (Cidomycin; Roussel, Montreal, Quebec, Canada). Maintenance medium was prepared for both cell lines in the same way but had a FBS concentration of only 2 %.

Cells were harvested by washing the cell monolayer with calcium and magnesium free phosphate buffer saline (PBS), followed with incubation at 37°C

for 10 min with 1.0 mL of a trypsin-EDTA mixture (Gibco). The cells were then used for seeding new flasks or culture plates.

#### 4.4 Viruses

The Sabin strain poliovirus (PV) type 1 was obtained from Dan McCloud at the Laboratory Center for Disease Control (Ottawa, Ontario, Canada) and the HM-175 strain of hepatitis A virus (HAV) was obtained from Dr. M.D. Sobsey of the University of North Carolina. Both these viruses required handling at biosafety containment level 2 according to the Laboratory Biosafety Guidelines (1990).

Stock virus was prepared by infecting susceptible cell monolayers at a multiplicity of infection (MOI) of 0.01. PV was inoculated on to monolayers of Vero cells and allowed to adsorb to the cells for 60 min at 37°C. The infected monolayers were then kept in maintenance medium. After 24 h, virus cytopathology was visible in the cell monolayers. The monolayers were then frozen (-20°C) and thawed three times. The resultant culture fluid was centrifuged for 15 min at 1000 g. The supernatant containing the virus was then concentrated ten-fold by polyethylene glycol hydroextraction (Ramia and Sattar, 1979). Basically, 100 mL of supernatant was placed within lengths of sealed, sterilized dialysis tubing (Spectrum, Los Angeles, CA), which were then placed in a plastic tray and covered with polyethylene glycol (M.W. 8000; Matheson, Coleman and Bell, Norwood, Ohio). After 24 h at 4°C, with almost all the liquid extracted, the residue in the dialysis tube was resuspended in 10 mL of Earle's Balanced Salt Solution (EBSS, Gibco). The virus concentrate was then filtered through a 0.2 µm

pore size syringe membrane filter (Nalge Co., Rochester, N.Y.). The virus stock were then dispensed in 0.5 mL aliquots and stored at  $-70^{\circ}\text{C}$ . The titer of the PV stocks were approximately  $10^6$  pfu.mL<sup>-1</sup>.

HAV was propagated in FRhK-4 cells and a virus stock was prepared in the same way as for the PV stock except that HAV required longer times of incubation (Mbiti, 1993). After HAV was applied to the FRhK-4 cell monolayers, it was allowed to adsorb for 90 min at  $37^{\circ}\text{C}$  before the addition of the maintenance medium. These monolayers then had to be incubated until approximately 70-80% of the cells were affected by virus cytopathology which was typically after 4 - 5 days. The titer of the HAV stocks were approximately  $10^7$  pfu.mL<sup>-1</sup>.

#### **4.5 Enumeration of microorganisms**

##### **4.5.1 Enumeration of bacteria**

Enumeration of bacteria from experimental samples were carried out by a spreadplate method. In most cases, a series of dilutions were made in phosphate buffer diluent (pH 7.2, 0.3 mM) supplemented with magnesium sulfate as described in Standard Methods (APHA, 1992) and a 0.1 mL inoculum was placed on to selective agar and spread evenly with an alcohol sterilized glass spreader. When bacterial numbers were too low to be detected by this method, a membrane filtration method was employed. This was done by filtering 100 mL of the water sample onto 0.45  $\mu\text{m}$  porosity Millipore cellulose acetate filters. To determine the levels of indigenous *E. coli* or *Enterococcus* in environmental samples, 10 mL or 100 mL volumes of the water samples were filtered through the membrane filters.

These filters were then placed on top of the appropriate medium and colonies enumerated after incubation.

For *E. coli* enumeration, mT7 agar (Difco) plates were inoculated and incubated at 35°C (LeChevallier *et al.*, 1982). The mT7 medium is a selective medium for the enumeration of fecal coliform bacteria like *E. coli* and it usually gives better recovery of injured organisms. This is due to the replacement of bile salts used in other selective media with tergitol 7 and polyoxyethylene ether W-1 which allows selectivity to be maintained while encouraging the growth of injured or stressed *E. coli* bacteria. After 24 h, *E. coli* colonies which would be yellow in color, were counted.

KF Streptococcus agar (Difco) was used for *Enterococcus* enumeration. Inoculated plates were incubated at 35°C for 48 h. The incorporation of a final concentration of 0.01% sodium triphenyltetrazolium chloride salt (TPTZ, Sigma) into the medium enabled the easy enumeration of *Enterococcus* as small red colonies.

#### **4.5.2 Enumeration of somatic coliphages**

The enumeration of both coliphage B and indigenous somatic coliphages from environmental samples was carried out using a modification of the single-agar layer (SAL) APHA 919C method described in the Standard Methods (APHA, 1985). For the enumeration of low concentrations of indigenous somatic coliphages from environmental samples, a 5.0 mL sample was added to 5.5 mL of medium. For the enumeration of coliphage B from seeded water samples, a 0.1 mL sample was

added to 10.4 mL of assay medium. In both cases, the final volume of sample and medium combined was 10.5 mL.

The enumeration medium was basically TSB which had been modified by the addition of ammonium nitrate, strontium nitrate and agar (Que-Bact, Quelab Laboratories, Inc., Montreal, Quebec, Canada) for a final concentration of 10 mM, 0.5 mM and 0.8% (w/v), respectively, after the addition of the water sample. The formulated medium was dissolved and the agar liquefied by boiling. The liquefied medium was then dispensed either in 5.5 mL aliquots or 10.4 mL aliquots into test-tubes or universal bottles and autoclave sterilized (121°C; 15 min).

The medium was liquefied before use by boiling. It was then allowed to cool to 50°C before the sample was added. A 1.0 mL inoculum of a thawed frozen *E. coli* C preparation was also added and the mixture was poured onto a petri dish. Once the agar had set, the plates were incubated in an inverted position at 35°C. Plaques were enumerated after 24 h. Four replicates were carried out for each environmental sample. A total of 20 mL of each sample was processed in this way and the number of plaques were added up and multiplied by a factor of 5 to give the number (pfu.100 mL<sup>-1</sup>) of the coliphages in the sample.

For experimental samples with seeded coliphage B, 0.1 mL of sample was added to test-tubes with 10.4 mL of medium. Samples were assayed in triplicate with the mean number of plaques (pfu.mL<sup>-1</sup>) being calculated.

#### 4.5.3 Enumeration of F-specific phages

The assay was a modification of the method described by Havelaar and Hogeboom (1984). A freshly reselected lactose positive colony from a McConkey agar plate was inoculated into 5 mL of TYGB medium and incubated overnight at 37°C. A fresh host culture was then prepared by inoculating 1.0 mL of the overnight culture into 100 mL of TYGB medium. This was then incubated at 37°C for 5 h. After that, the culture was stored on melting ice until used.

Test-tubes containing 3.0 mL of the TYGB soft agar overlay (0.8% agar) were liquefied by boiling and maintained at 50°C. Into each tube, 0.1 mL of sample and 1.0 mL of the WG49 host culture were added. The mixture was then poured evenly onto a TYGB agar plate. After the overlay had set, the plates were incubated inverted at 37°C for 24 h. All samples were titrated in triplicate with dilutions in phosphate buffer diluent and the mean number of plaques (pfu.mL<sup>-1</sup>) was calculated.

#### 4.5.4 Plaque assays for viruses

Virus concentrations were determined by plaque assays using 12-well cell culture plates (Costar). Dilutions of the virus sample were made in EBSS. The cell monolayers were washed with EBSS twice and then the 0.1 mL virus inoculum was placed on each well and allowed to adsorb in a CO<sub>2</sub> incubator at 37°C. A set of three wells was used to titrate each dilution of the sample; three 10-fold serial dilutions were thus plated. Both negative and positive controls were also carried out on each cell culture plate.

For PV samples, the adsorption period was for at least 30 min at the end of which the cell monolayers were overlaid with maintenance medium with 0.75% agarose (type II; Sigma). The plates were then sealed in laminated plastic bags (Dazey Corporation, Industrial Airport, KS) and incubated at 37°C for 24 - 36 h.

For HAV samples, 90 min was allowed for adsorption of the virus to the cell monolayer before the addition of the overlay. The overlay consisted of maintenance medium with 0.75% agarose and 26 mM magnesium chloride (BDH). The plates were then incubated, sealed in bags for 8 days at 37°C.

For both PV and HAV titrations, the monolayers were then fixed by the addition of 2.0 mL of a 3.7% solution of formaldehyde (BDH) in normal saline to each of the wells. After 24 h, the formaldehyde and the agarose overlay were removed and the cell monolayers stained with a 1% crystal violet solution for about 5 minutes. The plaques were visible after the excess crystal violet was washed off in running tap water. The mean titer was determined from three replicates.

#### **4.6 Survival studies with samples of mixed microorganisms**

Mixed cultures of *E. coli* F, *E. durans*, coliphages and PV were used to seed water samples for survival studies. On occasion, *Pseudomonas* and HAV were also used in these studies. It was determined that neither coliphage B or MS-2 could infect and replicate in any of the bacteria used in the survival studies and that no microorganism adversely affected the assay for another microorganism (see section 4.6.2).

#### **4.6.1 Seeding of water with multiple organisms**

Frozen stock cultures of exponential phase bacteria were thawed. These bacterial cultures were centrifuged (3000 rev.; 15 min) and washed with phosphate diluent twice. Finally, the bacterial pellet was resuspended in phosphate diluent and 0.1 mL of the bacterial suspension was added for each 100 mL of water sample. The resultant bacterial concentration in the sample was between  $10^5$  -  $10^6$  cfu.mL<sup>-1</sup>.

For coliphages and viruses, stock cultures were first diluted in the phosphate buffer diluent and EBSS, respectively. Then, 0.1 mL aliquots of the diluted stock cultures were added for each 100 mL of water sample to give coliphage and virus concentrations of  $10^4$  -  $10^5$  pfu.mL<sup>-1</sup>.

Unless otherwise stated, these microbial concentrations in the seeded water samples were used for all survival studies and HFD system characterization experiments.

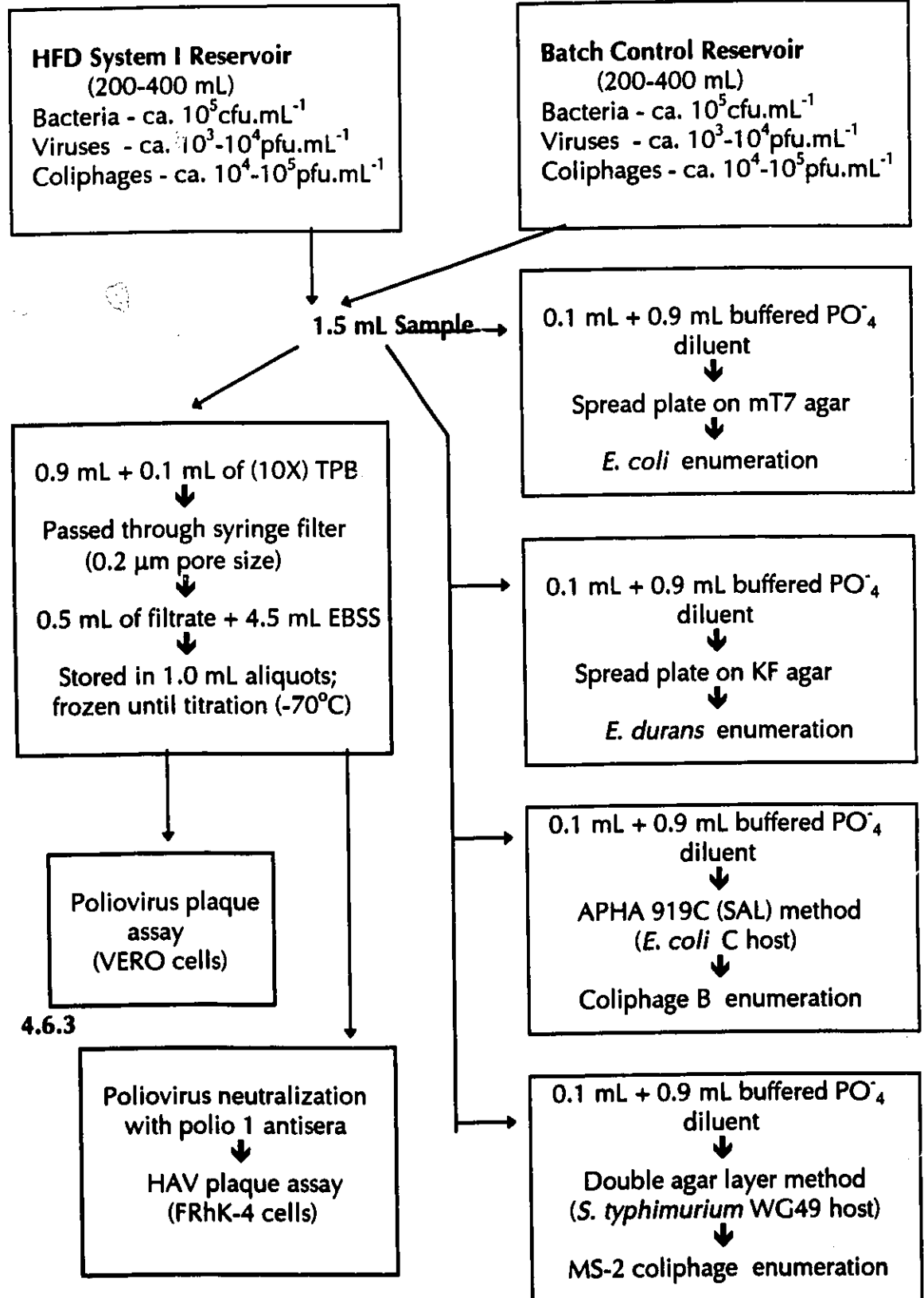
#### **4.6.2 Assaying for organisms from mixed culture samples**

The methods for assaying the numbers of individual types of organisms have been described earlier. However, preliminary experiments had to be carried out to determine whether the mixing of microorganisms could interfere with any of the individual assay. Stock suspensions of each organism ( $10^5$  cfu or pfu.mL<sup>-1</sup>) were prepared in phosphate buffer diluent. These suspensions were then mixed in equal amounts for every possible paired combination of organisms. Controls were prepared by mixing the stock suspensions with an equal amount of phosphate buffer diluent. The organisms were then assayed in the normal way for every

control and each of the mixed suspensions. In this way, it was determined that the mixing of the organisms did not in anyway affect the recovery and enumeration of the individual organisms due to any interference with the assays used. The protocol for processing samples in mixed culture samples is outlined in Fig. 4.1. Basically, a 1.5 mL sample was taken and processed. For bacterial and coliphage assays, 0.1 mL of sample was added to 0.9 mL of phosphate buffer diluent. A series of dilutions was then prepared and the enumeration of the microorganisms carried out as previously described. Occasionally, when bacterial numbers were expected to be very low, this dilution step was not required and 0.1 mL samples were plated directly on agar plates. With HAV and PV, however, additional steps were used to remove the bacteria from the mixture. This was done by first mixing 0.9 mL of sample with 0.1 mL of a (10x) TPB broth. The mixture was then passed through a syringe filter (0.2  $\mu$ m pore size). Then 0.5 mL of the filtrate was added to 4.5 mL of EBSS and stored frozen (-70°C) in 1.0 mL aliquots until ready for titration. Titration was carried out as described earlier. Before HAV could be titrated, the PV in the sample had to be neutralized. This was carried out by using polio 1 MAb pp12/A42A1 which was obtained from Dr. Pierre Payment (Institut Armand-Frappier, Montreal, Quebec, Canada). The antibody was added to the samples at a 1:500 dilution and left for 30 min at room temperature. The sample could then be used for HAV enumeration as previously described (Section 4.5.4).

Figure 4.1

**Figure 4.1 The protocol for the seeding, processing and enumeration of a mixed culture of microorganisms.**



## Controls

Both positive and negative controls were included for all bacterial and viral assays to ensure that there were no problems with the assay systems. Positive controls consisted of a dilution of the stock culture of the organism while the negative control consisted of an inoculum of sterile diluent (which was phosphate buffer for the bacteria and phages and was EBSS for the viruses).

### 4.6.4 Statistical and mathematical analysis

For survival studies, the titres at each sampling time ( $N_t$ ) were normalized by expressing them as a percentage of the original titre ( $N_0$ ) at the start of the experiment. The decay or inactivation rates ( $K_i$ ) were determined from the slopes of the linear regression of log-linear plots of the normalized data against time. This was based on the relationship  $\log N_t / N_0 = -K_i t$  which is derived from Chick's law which states that the number of cells that die per unit time is proportional to the number of cells surviving at that time (Mara, 1974; Lessard and Sieburth, 1983). Often the rate of decay altered during the course of the study.  $K_i$  was calculated for each period that showed a different slope on the log-linear plots.

Similarly, the coefficient of diffusion of substrates across the HFD system and diffusion chamber membranes was calculated from the formula  $\log C_t / C_0 = -kt$  where  $C_t$  is the concentration of the substrate at time,  $t$ ;  $C_0$  is the initial concentration and  $k$  is the coefficient of diffusion. The time required for substrate exchange was determined from  $k$  (Gameson and Saxon, 1967; Lessard and

Sieburth, 1983). For example, the time required for 50% ( $T_{50}$ ) and 90% ( $T_{90}$ ) diffusion was calculated by the equations;  $T_{50} = \log 2 / k$  and  $T_{90} = \log 10 / k$ .

Clearance ( $K$ ) is a measure of the volume that is cleared of a particular solute per unit time and is a coefficient that is often used to characterize the rate of diffusion across membranes of a hollow fiber cartridge (Schultz and Gerhardt, 1969; Cogan and Garovoy, 1985).  $K$  is calculated from the permeability coefficient,  $P_m$ , and the total surface area of exchange,  $A_m$ .  $P_m$  is calculated from the diffusion coefficient  $k$ . The relationships between these coefficients are given below;

$$1) P_m = 2.303 k / (1/V_1 + 1/V_2) \cdot A_m \text{ where } V_1 \text{ and } V_2 \text{ are the external and internal reservoirs used.}$$

$$2) K = P_m \cdot A_m$$

The Student's t-test was used to determine any significant differences in data means. All graphs were plotted using the SigmaPlot Scientific Graphing System for Windows Version 1.02 and statistical analysis carried out using SigmaStat for Windows Version 1.0 (Jandel Scientific, Corte Madera, CA). In all graphs, error bars represent the standard deviation of the results.

**5. Characterization of the Hollow Fiber Diffusion system and  
evaluation of its potential use for microbial survival studies**

## 5.1 Introduction

The HFD system may be considered to consist of three compartments; (1) the internal reservoir and circuit (which includes the lumina of the hollow fibers), (2) the hollow fiber cartridge, and (3) the external or environmental circuit (which includes the fluid-filled space surrounding the fibers) (Fig. 5.1). The hollow fiber cartridge links the internal circuit to the external circuit, allowing exchange and diffusion across the surfaces of the hollow fibers.

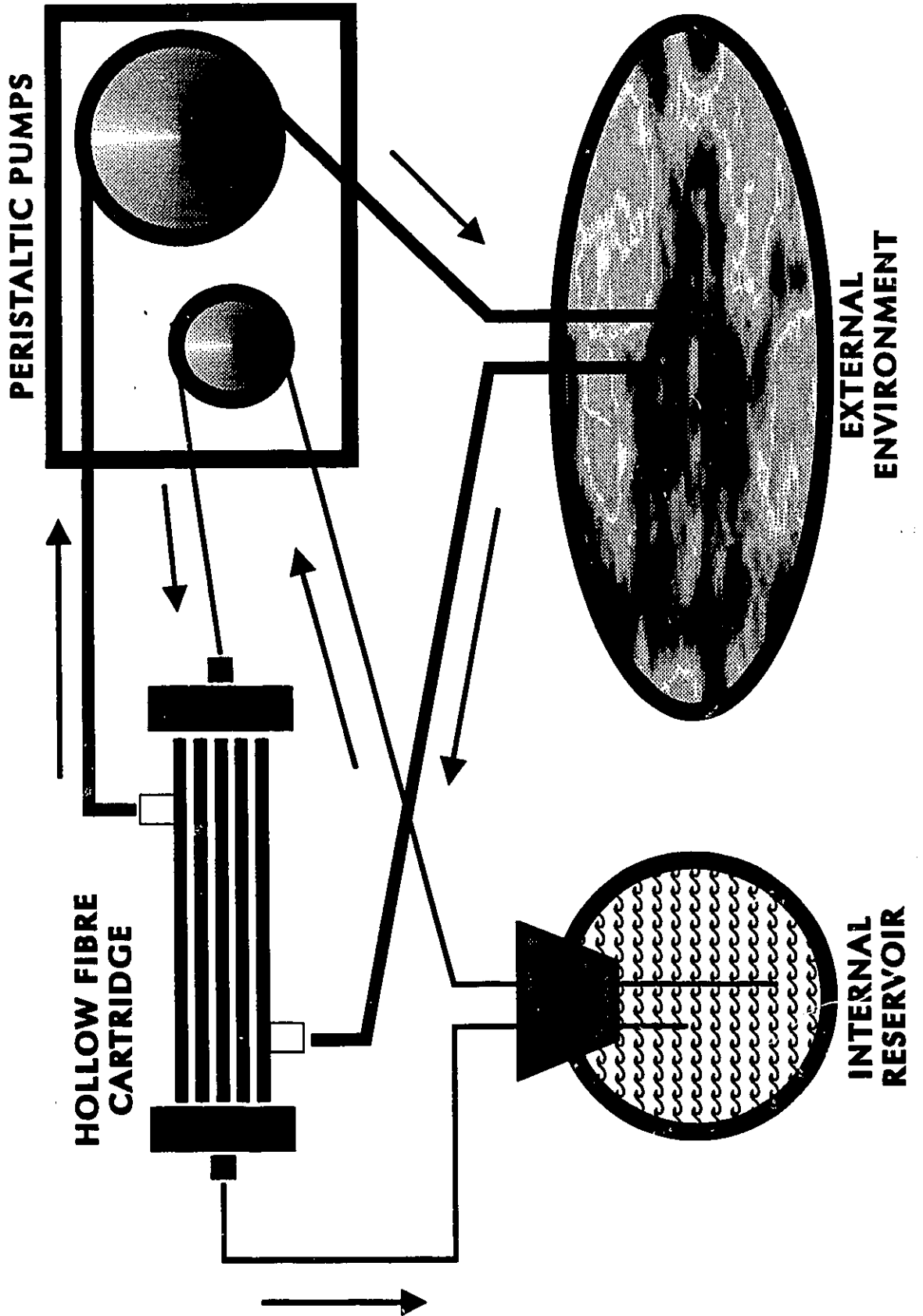
Hollow fibers are generally defined as tubular-shaped membranes with an internal diameter of less than 500  $\mu\text{m}$  (Mulder, 1991). The hollow fibers used in this study were 200  $\mu\text{m}$  in diameter. At this size, the fibers were self-supporting and would not collapse without external pressure.

The hollow fibers are made from polymers which are spun from specially shaped spinnerets through processes referred to as wet spinning, melt spinning or dry spinning. The hollow fibers used in the HFD system in this study consisted of polysulfone (PSf) whose structure is given in Appendix B1.

The formation of the pores in the hollow fiber matrix is achieved through the use of solvents. Dimethylacetamide (DMAC) and dimethylformamide (DMF) are commonly used solvents for PSf. With a 10 - 20% PSf concentration and water as a non-solvent, instantaneous demixing occurs which result in porous membranes. Alternatively, non-porous membranes may be produced using i-propanol as a solvent.

**Fig. 5.1 :** Schematic representation of the HFD system.

The Hollow Fiber Diffusion (HFD) system consists of an internal reservoir, a hollow fiber cartridge and a peristaltic pump. The liquid in the internal reservoir is circulated by the peristaltic pump through an internal circuit consisting of the internal lumen of the hollow fibers and connecting tubing. The outside of the hollow fibers are bathed with water from the external reservoir or environment which is brought into the hollow fiber cartridge by the action of the peristaltic pump on the external circuit. Exchange of low molecular weight solutes and gases between the two circuits occur across the hollow fiber surfaces. The internal and external circuits run countercurrent through the hollow fiber cartridge.



The polysulfone fibers used in the HFD cartridge had an asymmetrical porous structure. The main body of the fiber consists of a spongy matrix with relatively large pores but the inside top layer of the fiber has considerably smaller pores. This "skin" layer with the small pores limits the size of particles that can diffuse through the fiber membrane. The diffusion of solutes between the internal and external circuits of the HFD system is limited by the thickness of this skin layer and not by that of the other layer with the much larger pores.

Hollow fibers have been used in a number of applications. These include cell culture and cell product harvesting (Tiebout, 1990; Gorter *et al.*, 1993); kidney dialysis (Gotch and Keen, 1985; Ifediora *et al.*, 1992), reverse osmosis, desalination (Jackson and Jones, 1991); concentration of microorganisms (Kuwabara and Harvey, 1990), intravenous oxygenation (Hattler *et al.*, 1992; Kallis *et al.*, 1992), medical humidifying (Hanssler *et al.*, 1992) and as a wearable blood glucose-sensor (Schoonen *et al.*, 1990). Other potential usages of hollow fibers include the experimental encapsulation of dopamine secreting cells for the treatment of Parkinson's disease (Aebischer *et al.*, 1991) and suggested hemopurification systems based on the inclusion of kidney cells and reticuloendothelial cells in hollow fibers (Ota, 1991).

In all the above applications, the conditions for use of the hollow fiber cartridges involved establishing deliberate gradients for treatment of fluid streams, for nutrition of cell cultures or the harvesting of products. Our purpose was to avoid a net pressure differential between the internal and external compartments of

the system so that solutes smaller than the nominal pore size can pass freely in either direction across the membranes. This allowed us to investigate the possibility of using the hollow fiber cartridge as part of a system for *in situ* microbial survival studies. Preliminary testing included studies on the biocompatibility of the HFD components and the possibility of significant microbial adsorption to the large surface area available in the hollow fibers of the HFD system. Diffusion characteristics of solutes in the HFD system were also studied. The performance and characteristics of the HFD system was also directly compared with diffusion chambers.

The HFD system can be potentially of use in two key areas; as a system for studying microbial survival and ecology in the natural aquatic environment and as a system for monitoring for toxins and disinfectants. Some preliminary microbial survival studies were carried out using the HFD system in laboratory microcosms to evaluate the system and to identify potential problems. The feasibility of using the HFD system for studying disinfection efficiency of water treatment processes was studied by following the exposure of the HFD system to chlorine and chloramine T.

## **5.2 Materials and methods**

### **5.2.1 The Hollow Fiber Diffusion (HFD) System**

The HFD system consists of a hollow fiber dialyzer unit, connecting tubing, a peristaltic pump, an internal sample reservoir (IR) and an external or

environmental reservoir (ER) (Fig. 5.1). The system has an internal (*I*) circuit and an external (*E*) circuit which are circulated by peristaltic pumps.

#### 5.2.1.1 The HFD cartridge

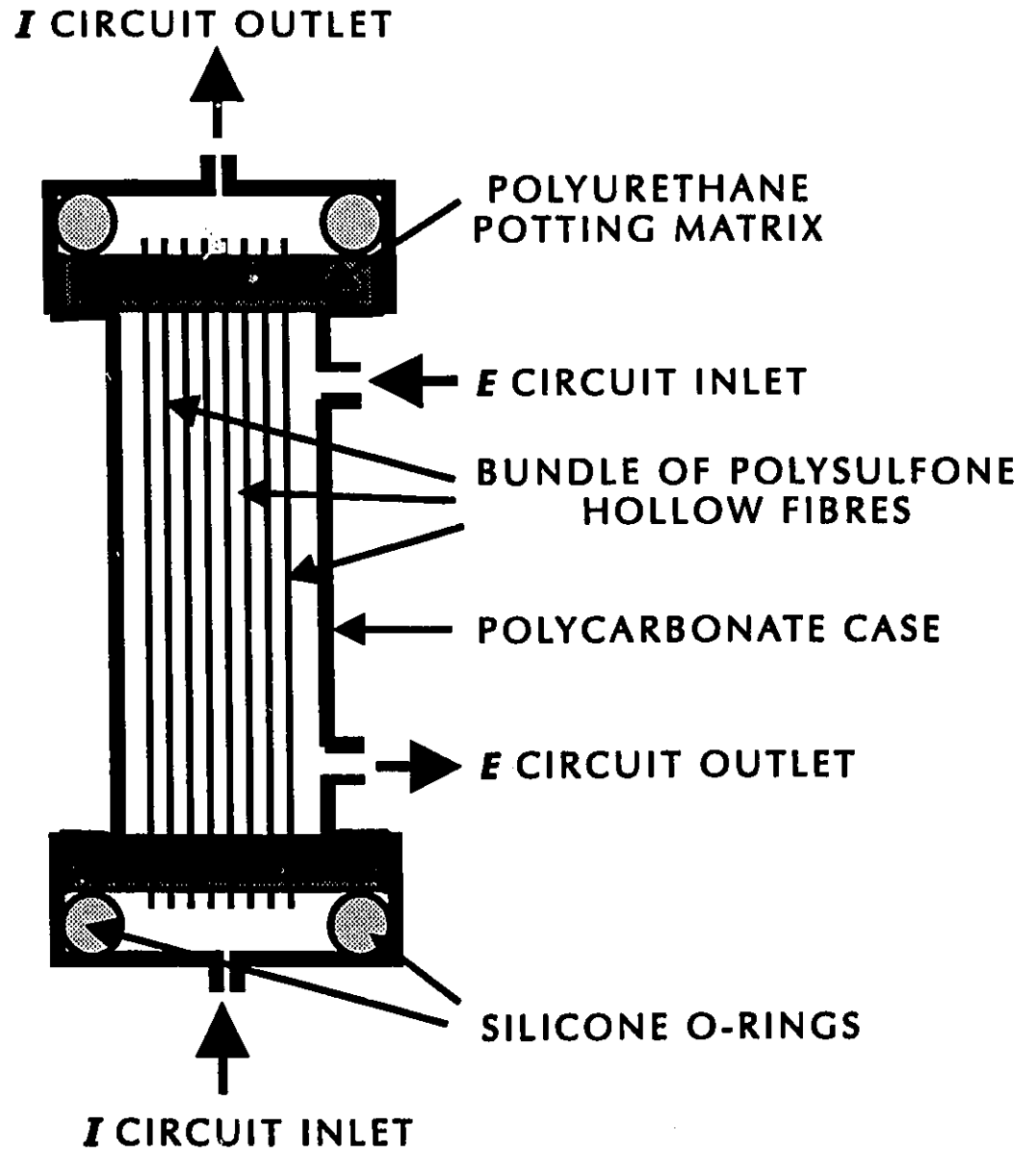
Unless otherwise stated, the hollow fiber dialyzer cartridge used was the Hemaflow F4 (Fresenius AG, Bad Homburg, Germany). The unit consisted of a bundle of 7000 polysulfone hollow fibers in a polycarbonate housing (Fig 5.2). The potting compound used to anchor the hollow fibers to the ends of the cartridge and separate the *E* and *I* circuits, consisted of polyurethane. The fibers had an internal diameter of 200  $\mu\text{m}$  and a wall thickness of 40  $\mu\text{m}$ . Silicone O-rings sealed the ends of the cartridge. Short lengths of rubber tubing were attached to the *E* circuit inlet and outlet ports. The rubber tubing was then connected to the silicone tubing of the *E* circuit by a polypropylene adapter. The cartridge possessed  $7.0 \times 10^3 \text{ cm}^2$  surface area for diffusion and exchange. Its priming or dead volume was 44 mL. The cartridges were obtained pre-sterilized with ethylene oxide.

#### 5.2.1.2 Connecting tubing and peristaltic pump

Two sets of tubing (Masterflex; Cole-Parmer Instrument Co., Chicago) were used. The first set was used to connect up the *I* circuit and had an internal diameter of 3.1 mm. The second set was used to connect up the *E* circuit and had an internal diameter of 6.4 mm. Both silicone and norprene tubing were used with the system. The norprene tubing was found to be more resistant to wear when used with the peristaltic pump. The connecting tubing lengths were variable depending on the experimental conditions. For all preliminary characterization of

**Fig. 5.2 : Design of the hollow fiber cartridge.**

The hollow fiber cartridge consists of a bundle of polysulfone hollow fibers bound at the two ends by a polyurethane potting matrix and housed in a polycarbonate case.



the HFD system, the lengths used were such that the total priming or dead volume of the *I* circuit, including the cartridge hollow fibers and the connecting tubing, was 55 mL. Tubing lengths had to be increased for microbial survival studies in the field and in experiments where the HFD system was directly compared with diffusion chambers. The tubing were set-up with a peristaltic pump (Cartridge pump series 400, Cole-Parmer). The tubing were sterilized by autoclaving before use.

#### **5.2.1.3 Internal reservoir**

The internal reservoir consisted of a 500 mL wide-mouth Duran bottle which was equipped with a rubber bung. Two glass tubes were put through the bung and had their ends in the reservoir bent away from one another. The height of the tubes in the reservoir were also adjusted so that one tube reached all the way to the bottom of the reservoir while the other was placed to end just below the expected water level in the reservoir. The *I* circuit connecting tubings were attached to the glass tubes so that liquid would be drawn out of the reservoir through the tube opening near the bottom of the reservoir and returned to the reservoir through the other tube. The entire reservoir was sterilized by autoclaving.

#### **5.2.2 Protocol for setting up the HFD system**

With the HFD cartridge in a vertical position, the peristaltic pump was started to pump water into the external compartment of the cartridge. This was done with the inlet at the base and the outlet at the top of the cartridge. In this way, the compartment would fill properly from bottom to top. Occasionally, air

bubbles were trapped in the compartment and had to be removed by either flushing the system at a high  $500 \text{ mL}\cdot\text{min}^{-1}$  rate or by pinching and releasing the connecting tubing to cause a build-up and release of water pressure.

Once the *E* circuit compartment was properly filled, it was then flushed with 1.0 L of water. The water used for flushing would be the water that would be used for the experiment i.e. distilled water or phosphate buffer for laboratory characterization experiments and river water for *in situ* survival studies in the river. This action of flushing was meant to remove sterilization residues and to reduce adsorption of particles to surfaces.

After the pre-treatment of the *E* circuit, the *I* circuit was similarly filled with filter sterilized river water or buffer with the peristaltic pump set to give a  $150 \text{ mL}\cdot\text{min}^{-1}$  flow rate. Air trapped in the cartridge ends was removed by the action of clamping and releasing the connecting tubing. As the water filled the *I* circuit, the first 300 mL of the water to pass through the circuit was discarded.

The ends of the connecting tubing were then placed into a bottle containing 300 mL of the same water. This was allowed to cycle at  $300 \text{ mL}\cdot\text{min}^{-1}$  for ten minutes. Then the bottle was removed and replaced with another bottle with fresh 300 mL water. This was repeated a third time during which the *E* circuit was also flushed with water.

Finally, the ends of the *I* circuit connecting tubing were attached to the internal reservoir and the *E* circuit was exposed to the external reservoir or environmental compartment.

### 5.2.3 Protocol for re-use of the HFD cartridge

The normal procedure employed for the decontamination and reuse of cartridges involved the use of hydrogen peroxide. At the end of an experiment, the excess liquid in the cartridge was removed. Then both the *E* and *I* circuits were made to circulate a 400 mL solution of hydrogen peroxide (10%) for 24 h. The solution was then replaced with a fresh batch of hydrogen peroxide solution and this was similarly circulated for another 24 h. The cartridge was then removed and stored in a clean, dark place until required for use.

Just before the cartridge was to be reused, the cartridge was flushed with 1.0 L of sterile distilled water. This process was repeated three times to remove any excess hydrogen peroxide or other residues.

The efficiency of this process to decontaminate the cartridge was tested using *E. coli* F or coliphage B or a suspension of both in the *I* circuit of the HFD system for 24 h. After that, the decontamination procedure was carried out, followed by the distilled water washes. Each of the 1.0 L washes was collected separately and screened for *E. coli* and coliphages. *E. coli* was screened by filtering the water through a 0.2 µm porosity membrane filter and incubating the filter on mT7 agar. Coliphage B was assayed as previously described after an amplification step. The survival of microorganisms in the reused cartridges and

new cartridges were also compared to determine if any residual peroxide or other factor may affect microbial survival in the reused cartridges.

#### 5.2.4 Protocol for HFD system integrity check

An important characteristic for the HFD system for its use in *in situ* microbial survival studies, is the integrity of the system to retain the organisms being studied within its internal circuit. A protocol was therefore developed for testing the system and ensuring the system would not lose or release study microorganisms into the external reservoir or environment. This was done in one of two ways. The first test employed the use of high molecular weight Blue Dextran (M.W. 2,000,000; Sigma). The HFD system with the hollow fiber cartridge to be tested was set-up with an internal circuit and external reservoir volumes of 200 mL and 400 mL. A solution of blue dextran was placed in the *I* circuit. The system was run for 24 h and then 3 mL samples from both the internal and external reservoirs were taken. The samples were examined in a spectrophotometer for absorption at 320 nm ( $E_{320}$ ). Integrity of the system was determined to be intact because the  $E_{320}$  for the internal reservoir did not drop over the 24 h and the  $E_{320}$  for the external reservoir sample did not increase in that time.

The second test was carried out using a biological indicator which in this case was coliphage B. The HFD system cartridge to be tested was set-up with a 200 mL coliphage B ( $10^4$  pfu.mL<sup>-1</sup>) suspension in phosphate buffer diluent placed in the *I* circuit and a 400 mL phosphate buffer diluent external reservoir. The system was then allowed to run for 24 h. The external reservoir was then detached

from the system. If there was a breach of system integrity, it would be expected that coliphage B would be found in the external reservoir. The sensitivity of the test was increased by carrying out an amplification step for coliphage B before assaying. This was done by adding 5 mL of an exponential phase culture of *E. coli* C and 40 mL of a 10x concentrated modified APHA 919C medium to the external reservoir and then incubating the mixture for 24 h at 37<sup>0</sup>C. Then 20 mL of the mixture was assayed for coliphage B using the standard APHA 919C procedure.

## **5.2.5 Characterization of the HFD system**

### **5.2.5.1 Adsorption studies**

Tests were carried out to determine if significant loss of seeded organisms would result due to adsorption to the hollow fiber membrane surfaces or to the associated tubing. The HFD system was set up as described before except for the *I* circuit. The internal reservoir was seeded with a mixed culture of PV, *E. coli* F and coliphage B. The microbial suspension was drawn by the peristaltic pump through the hollow fiber cartridge. However, after passage through the cartridge, the microbial suspension was collected into sterile containers rather than being returned into the internal reservoir. In this way, samples of consecutive dead volume passages of the *I* circuit were collected and the microorganisms present were enumerated. The dead volume for the system consisted of the volume in the hollow fiber lumen, the cartridge ends and the connecting tubings and was calculated to be 55 mL. The average contact time of each dead volume sample with the surfaces as it went through the HFD system was 1.5 min. The number of

organisms recovered from each dead volume sample was compared with numbers assayed in the internal reservoir prior to passage through the HFD system.

Phosphate buffer was used for both the internal and external reservoirs. Since adsorption of microorganisms to surfaces or particulates is known to be influenced by pH (Bitton, 1980), the experiment was repeated using phosphate buffer diluent adjusted to pH 5, 6, 7 and 8. The phosphate buffer was prepared as described in the Documenta Geigy Scientific Tables (Diem and Lentner, 1970). Basically, a stock solution of 66.7 mM monopotassium phosphate ( $\text{KH}_2\text{PO}_4$ ) and a stock solution of 66.7 mM disodium phosphate ( $\text{Na}_2\text{HPO}_4$ ) were prepared. Different amounts of the two stock solutions were mixed to obtain the various pH (Appendix A2). The solutions were then diluted to give a 0.3 mM phosphate buffer. Finally, 0.25 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  was added to each 1.0 L of the buffer in accordance to the formulation of the phosphate buffer diluent. The adsorption experiments were also carried out using Ottawa River water.

#### **5.2.5.2 Survival studies to investigate the effects of the inherent properties of the HFD system.**

The survival and recovery of *E. coli* F, somatic coliphage B, MS-2 phage, PV and HAV in the HFD system and a closed batch control was followed and compared under conditions that were as identical and as constant as possible. The object of the experiment was to determine whether the HFD system inherently increased or reduced microorganism recovery for reasons other than changes in water quality and properties. The organisms were suspended in sterile phosphate

buffer (pH 7.2) and placed into the HFD *I* circuit. The volume of the HFD system *I* circuit was 200 mL and the *I* circuit was operated at 40 mL.min<sup>-1</sup>. Similarly, another 200 mL of the microbial suspension was placed in a loosely capped wide-mouth Duran bottle. This was used as the closed batch control. The HFD system external circuit was made up of 400 mL phosphate buffer. The experiment was also carried out using dechlorinated tap water instead of the phosphate buffer.

### 5.2.5.3 Coliphage inactivation studies

Based on the results of the survival studies above, it became apparent that some characteristic of the HFD system, as operated, significantly reduced the survival of coliphage B when compared to its survival in the closed batch control. Experiments were carried out to determine the reason for this difference.

The two components of the HFD system that may possibly be affecting the coliphage B survival was the peristaltic pump and the hollow fiber cartridge. Firstly, the effect of the pump and its peristaltic action was studied. Two bottles with 200 mL coliphage B suspension in phosphate buffer were prepared. The liquid of one of the bottles was circulated through a 0.3 m length of the 3.1 mm internal diameter silicon tubing using the peristaltic pump. Essentially, this represented the set-up of the HFD system but without the hollow fiber cartridge on-line. The survival and recovery of coliphage B in the test and control systems were followed over a time period.

Based on the results of these experiments, it was decided to concentrate on studying the effects of changing in turn, certain parameters of the HFD system *I*

circuit complete with the hollow fiber cartridge. These parameters were the flow rate through the *I* circuit, the diameter of the hollow fibers and the suspended solid load of the coliphage suspension. The *I* circuit flow rate was altered over the 20 mL.min<sup>-1</sup> to 45 mL.min<sup>-1</sup> range. The diameter of the hollow fibers was changed by replacing the Fresenius cartridge with a custom made cartridge with larger diameter hollow fibers. This cartridge was made for our study by the Industrial Membrane Research Institute (IMRI). A comparison between the properties of the two cartridges is provided in Appendix B2.

The effect of suspended solids on coliphage recovery from the HFD system was examined by using river water with high levels of suspended solids. The control used the same river water that had been filtered to remove suspended solids.

Coliphage B was also characterized by examination under the electron microscope to attempt to determine if any characteristic of the phage made it particularly susceptible to the inactivation factors involved.

#### **5.2.5.4 Characterization of the diffusion properties of the HFD system**

The HFD system was characterized with regards to the diffusion of low molecular weight solutes. Diffusion studies were based on the use of chromogenic substrates that could be distinguished enzymatically. Two substrates were used in this study; they were o-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG) and p-nitrophenyl- $\alpha$ -D-glucopyranoside (PNPG). Both substrates had the identical molecular weight of 301.3 and were similar in structure. The concentration of

each sugar analogue in a mixed sample could be distinguished by the incubation of the sample with the appropriate enzyme.

$\beta$ -galactosidase (*E. coli*; Sigma) would specifically cleave ONPG with the release of the nitro-phenol chromophore which could then be measured spectrophotometrically at 410 nm. PNPB concentration could similarly be determined using  $\alpha$ -glucosidase (Sigma).

#### 5.2.5.4.1 Enzyme assays for ONPG and PNPB

Enzyme stocks of  $\alpha$ -glucosidase and  $\beta$ -galactosidase were prepared with an activity of 50 units.mL<sup>-1</sup> (each unit being able to catalyze 1.0  $\mu$ mole of substrate each minute under optimal conditions at 37°C incubation).  $\alpha$ -glucosidase stocks were prepared in 0.1 M phosphate buffer (pH 6.8) while  $\beta$ -galactosidase stocks were prepared in a 2.2 M ammonium sulfate solution (pH 7.3). The stocks were aliquoted in 0.2 mL amounts into vials and stored at -70°C.

A vial of enzyme stock was thawed and diluted 10-fold before use in an assay. The  $\alpha$ -glucosidase assay was carried out using 0.1 mL of the enzyme, 0.1 mL of the sample and 2.8 mL of phosphate buffer (0.1 M; pH 7.0). This was mixed and incubated in a water bath at 37°C for 5 minutes. The mixture was then placed into cuvettes and the absorbance at 410 nm was determined.

The  $\beta$ -galactosidase assay was more involved, requiring activation of the enzyme. A 0.1 mL enzyme aliquot was mixed with 0.1 mL of 3.36 M 2-mercaptoethanol solution, 0.1 mL of a 0.03 M magnesium chloride solution and

2.6 mL of sodium phosphate buffer (0.1 M; pH 7.0). The mixture was incubated for 3 min at 37°C to activate the enzyme. Then a 0.1 mL sample was added and the mixture was incubated for another 5 min before absorbance at 410 nm was determined.

For these diffusion studies, the HFD system *I* and *E* circuits had volumes of 200 mL and 400 mL respectively. The flow rates of the *I* circuit and *E* circuit were 30 mL.min<sup>-1</sup> and 200 mL.min<sup>-1</sup>, respectively.

To determine the absolute exchange rates across the HFD system compartments in the absence of an osmotic gradient, an experiment was carried out with ONPG in the *E* circuit and PNPG was placed in the *I* circuit in near equimolar concentrations (i.e. starting concentrations of 10 - 12 mM of sugar analogues in both compartments). Samples were taken from both *I* and *E* circuits at various time intervals and the concentration of ONPG and PNPG determined by incubation with  $\beta$ -galactosidase and  $\alpha$ -glucosidase, respectively.

Another experiment was carried out, to study the rate of diffusion across the HFD system in the absence of an equivalent substrate on the other side of the membrane. PNPG (1.0 mM) was placed in the *E* circuit and its diffusion into the *I* circuit was followed.

#### 5.2.5.5 Disinfection studies

Bacterial suspension in the internal reservoirs of the two systems were exposed to disinfectants by placing the two systems in an external reservoir

consisting of chlorinated tap water. Samples were taken and added to buffer with sodium thiosulfate to neutralize the excess chlorine. The survival and recovery of the bacteria by spread plate method was then followed to study the response of the systems to the presence of the chlorine.

#### 5.2.6 Diffusion chambers

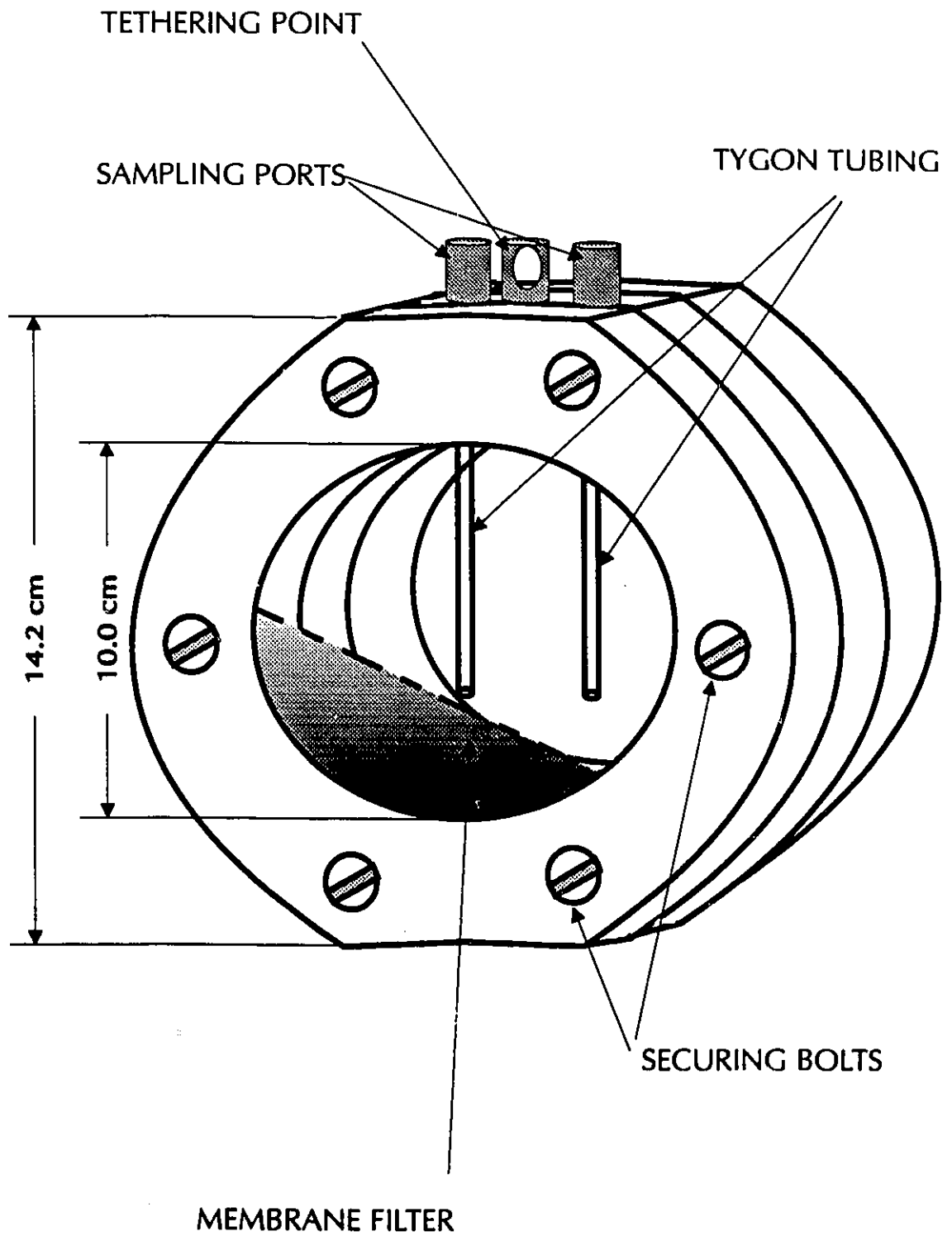
Diffusion chambers used were obtained from Montana State University (Bozeman, Montana) and were similar in design to those used by McFeters and Stuart (1972). The chambers were constructed of three Perspex parts fitted with O-rings, two lengths of tygon tubing and stainless steel sample ports (Fig. 5.3).

The diameter of the internal lumen of the chambers was 10 cm. The total surface area for exchange was determined to be 157.1 cm<sup>2</sup> with an internal lumen volume of 100 mL. Cellulose acetate membrane filters (HAWP 142 50; Millipore) of 0.45 µm pore size and 14.2 cm diameter were used with the chambers. In some experiments, dialysis membrane sheets were used instead of membrane filters.

The three perspex parts of the diffusion chambers were sterilized by autoclaving. The membrane filters or the dialysis membrane sheets were placed into a plastic container, submerged in distilled water and autoclave sterilized. When required for use, the chamber was assembled by placing the filter membranes between the perspex parts, securing the parts tightly using the nuts and bolts on the chambers.

**Fig. 5.3 : Design of the diffusion chamber.**

The assembled diffusion chamber consists of two membrane filters sandwiched between three perspex discs and held in place by bolts and nuts. This results in an interior lumen of approximately 100 mL which is bound by the membrane filters on two sides. Access to the lumen is provided by two lengths of tygon tubing attached to luer-lock sampling ports mounted through the middle perspex disc.



After the chambers had been assembled, the water sample was placed into the internal lumen through the sample ports, using a sterile syringe. As the membrane filters used were not rigid or supported, the 100 mL of water sample had to be added into the chamber rapidly and then the chamber immediately suspended in a large external reservoir. The chamber was suspended so that the membrane surfaces were fully submerged while the sample ports were above the water surface. Microorganisms were seeded into the internal lumen of the diffusion chamber via the sample ports.

During microorganism survival and equilibration studies, samples were collected through the sample ports. Sampling was carried out using a luer lock syringe which was attached to one of the sampling ports. First, 2 mL of liquid was drawn into the syringe and then expelled back into the port. This was repeated twice to improve mixing of the bacterial suspension within the chamber lumen. Finally, another 2 mL of liquid was drawn into the syringe and the plunger removed to allow a 0.1 mL aliquot to be taken for analysis or enumeration of microorganisms.

The external reservoir in which the chambers were submerged had a volume of 10 L. Circulation of the water in the external reservoir was enhanced by placing the reservoir on a four-place magnetic stirrer.

## 5.2.7 Comparisons between the HFD system and the diffusion chamber

### 5.2.7.1 pH and Redox Equilibration Experiments

For equilibration experiments, the same 10 L distilled water external reservoir was used for both the diffusion chamber and the HFD system. The diffusion chamber was suspended in the external reservoir as described previously while the HFD system's *E* circuit tubing circulated water from the same reservoir. The pH and Eh in the external reservoir was continuously measured and recorded using a datalogging unit (Datasonde 3; Hydrolab Corp., Austin, TX).

The HFD system was set-up with an *I* circuit volume of 250 mL. The flow rate through the *I* and *E* circuits were 15 mL.min<sup>-1</sup> and 180 mL.min<sup>-1</sup> respectively. These settings allowed the experiments to reproduce the conditions employed by the portable field HFD system. The internal reservoir was modified to allow a pH or redox probe to be placed directly into the reservoir.

The diffusion chamber had a luer-lock syringe attached to its sampling port. For pH measurements 3 mL of water was drawn up into the syringe barrel and expelled back into the internal lumen. This was done twice to encourage some mixing within the lumen. Then, the 3 mL sample was drawn up into the syringe barrel and the plunger removed to allow access for the pH probe. In this way, the pH of the diffusion chamber was monitored.

A different monitoring procedure for the diffusion chamber had to be carried out for the redox equilibration experiments. This was necessary because the shape and size of the redox probe prevented it from being placed in the sample

syringe barrel. For redox measurements, a 5 mL sample had to be removed and placed in a small plastic container. The redox probe could then be applied to the sample. The act of withdrawing such a large sample (5% of total volume) would obviously enhance any equilibration of the redox since simultaneously 5 mL would be drawn from the external reservoir into the internal lumen through the filter membranes of the chamber. In order to minimize this effect, multiple diffusion chambers were used. Samples were taken in pairs at different time points with sample collection rotated between the diffusion chambers in each set. No more than three samples were taken from any one diffusion chamber. The time points for sampling were divided into an early set (0-10 min), a late set (20-60 min) and an overlapping middle set (10-30 min). For each time set, a different pair of diffusion chambers were used. The experiments were repeated three times and the mean Eh measurements for each time point were then recorded.

For these experiments, both the external and internal reservoirs were filled with double distilled water. Changes in external reservoir pH was effected by the addition of dilute acetic acid to drop the pH or by the addition of sodium bicarbonate to raise it. The pH in the diffusion chamber, HFD internal reservoir and the external reservoir was then monitored as described above.

Changes in the Eh of the external reservoir was achieved by the addition of sodium thiosulfate which lowered the Eh. Monitoring of the changes in Eh in the different systems were then carried out as described above.

### 5.2.7.2 Equilibration of the concentration of ONPG

The HFD system and diffusion chambers were set-up in a similar manner as was described for the pH equilibration experiments. The one difference was that the ONPG solution was used in the internal reservoir and lumen of the two systems and the 10 L external reservoir consisted of phosphate buffer (pH 7.2).

### 5.2.7.3 Laboratory plug-flow studies

An exponential phase *E. coli* F culture was centrifuged, washed twice with phosphate buffer and then resuspended in distilled water. The *E. coli* suspension was then added to the internal reservoirs of the HFD system and the diffusion chamber to give a final concentration in the  $10^5$  cfu.mL<sup>-1</sup> range. Both systems were then provided with the same 10 L distilled water external reservoir as described above. Survival of the organism was followed by taking 0.1 mL samples from the two systems at different times for spread plate assay on mT7 agar. After 23 h, tryptose phosphate broth (TPB) was added to the external reservoir to give a one-hundredth strength solution. After a one hour exposure, the two systems were removed and flushed with fresh distilled water. The two systems were then placed in a "wash" reservoir for 1 h before finally being transferred to a fresh external reservoir. This was done to attempt to simulate the passage of a transient nutrient plug through a water body such as could occur in the natural environment. Later, at 72 h, a two hour spike of nutrients (TPB) in the external reservoir was similarly carried out. The time in the "wash" reservoir was proportionally increased to 2 h. Throughout, the *E. coli* numbers in the systems were monitored to determine the effect of the transient nutrient availability in the external reservoir. The experiment

was also carried out using dialysis membranes instead of cellulose acetate membrane filters on the diffusion chambers.

In another experiment, the effect of shorter but repeated transient spikes of nutrient on bacterial survival was studied. This was carried out using TPB as before but in this case, the systems were exposed to the external nutrients for 10 min.

The systems were then washed with distilled water and placed in fresh clean water for 20 min before the next 10 min nutrient spike was introduced. This cycle of 10 min nutrient spike followed by a 20 min "wash" was repeated 4 times within a 2 h period. As before, *E. coli* survival and recovery in the internal reservoir of the two systems was followed before, during and after that treatment.

#### **5.2.7.4 Response to disinfectants**

The response of the two systems to exposure to disinfectants was studied by the addition of chloramine T in the external reservoir for a final concentration of 0.03 ppm. The diffusion chambers were fitted with dialysis membranes so as to retain the viruses included in the study. Samples were taken at various time intervals and added to buffer with sodium thiosulfate to neutralize excess chloramine. Enumeration of microorganisms was then carried out as previously described.

### **5.2.8 Preliminary laboratory based microcosm survival studies using the HFD system**

The HFD system was used to carry out survival studies in laboratory-based microcosms. Essentially, 15 L of river water was used as the external reservoir which was meant to simulate the large, external environment of the river, complete with indigenous organisms. These experiments were carried out using water collected from the Ottawa River in winter and the Rideau River in summer.

As previously described, the test microorganisms were seeded into the 200 mL HFD *I* circuit and into the 200 mL batch control. Samples were collected at various times for the enumeration of test organisms. A variation was carried out with Rideau River water in that autoclave sterilized river water was also seeded for use in a second set of the HFD system and batch controls. This was done to attempt to study the direct biotic effects of natural flora on test microorganism survival.

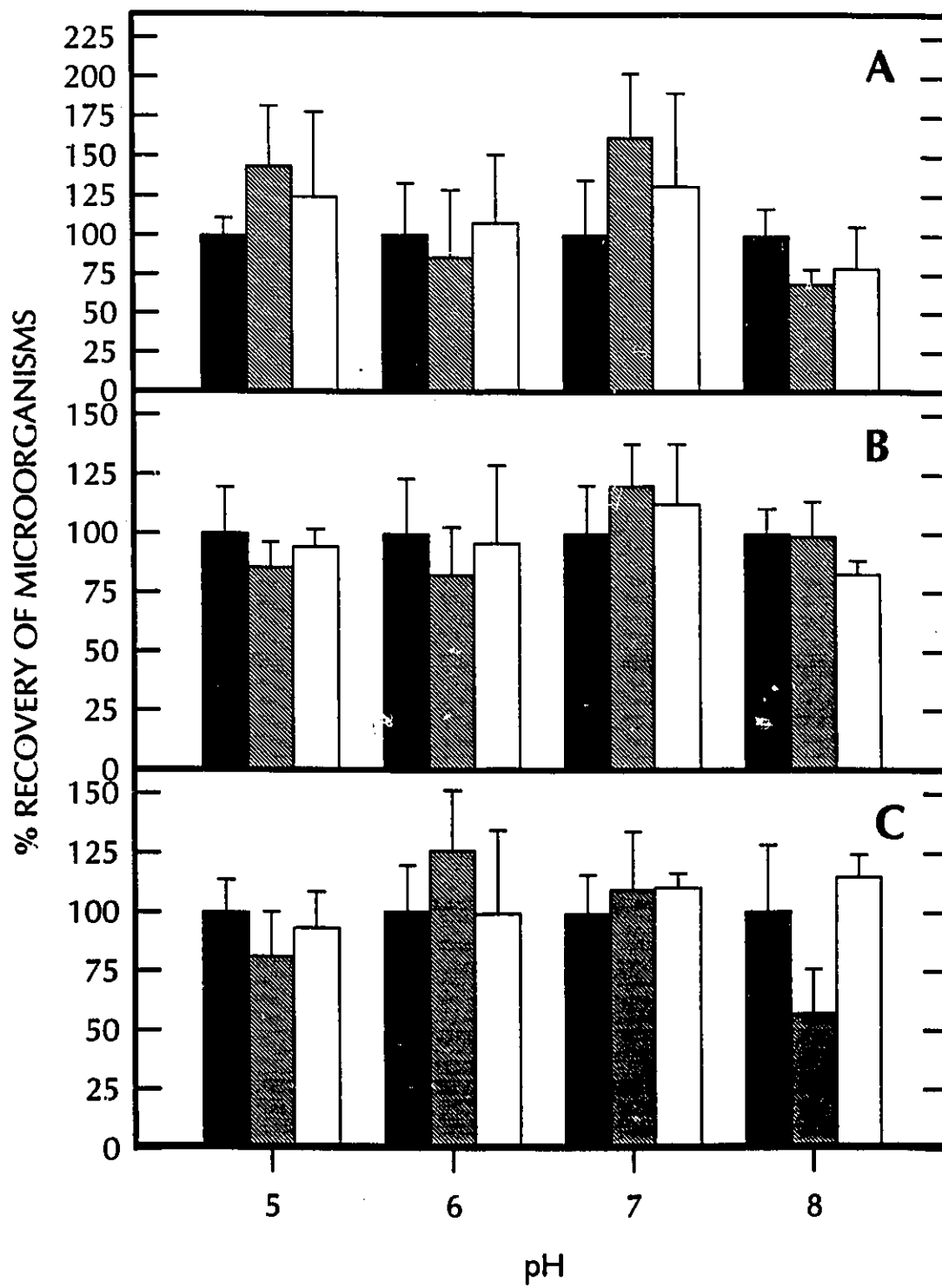
## **5.3 Results**

### **5.3.1 Adsorption of microorganisms to the surfaces of the HFD system**

The possible adsorption of *E. coli* F, coliphage B and PV to the materials in the HFD system was examined using suspensions of these organisms in 0.3 mM phosphate buffer, adjusted over the range of pH 5 to 8. There was no significant drop in the titre of any of the microorganisms between consecutive dead-volume passages through the HFD system (Fig. 5.4). Similarly, no drop in titre of the microorganisms was observed when Ottawa River water (pH 6.8) was used instead

**Fig. 5.4 :** The effect of pH on the recovery of microorganisms from consecutive dead volume passages through the HFD system / circuit.

The recovery of three microorganisms, PV (A); *E. coli* F (B) and coliphage B (C), was followed. The organisms were suspended in phosphate buffers adjusted to the various pH values and the mixture was passaged through the HFD system. ■ Initial titre; ■ first dead volume passage; □ second dead volume passage.



of phosphate buffer. PV and HAV also showed no significant loss in titre between consecutive dead-volume passages through the HFD system when suspended in dechlorinated tap water (pH 6.8).

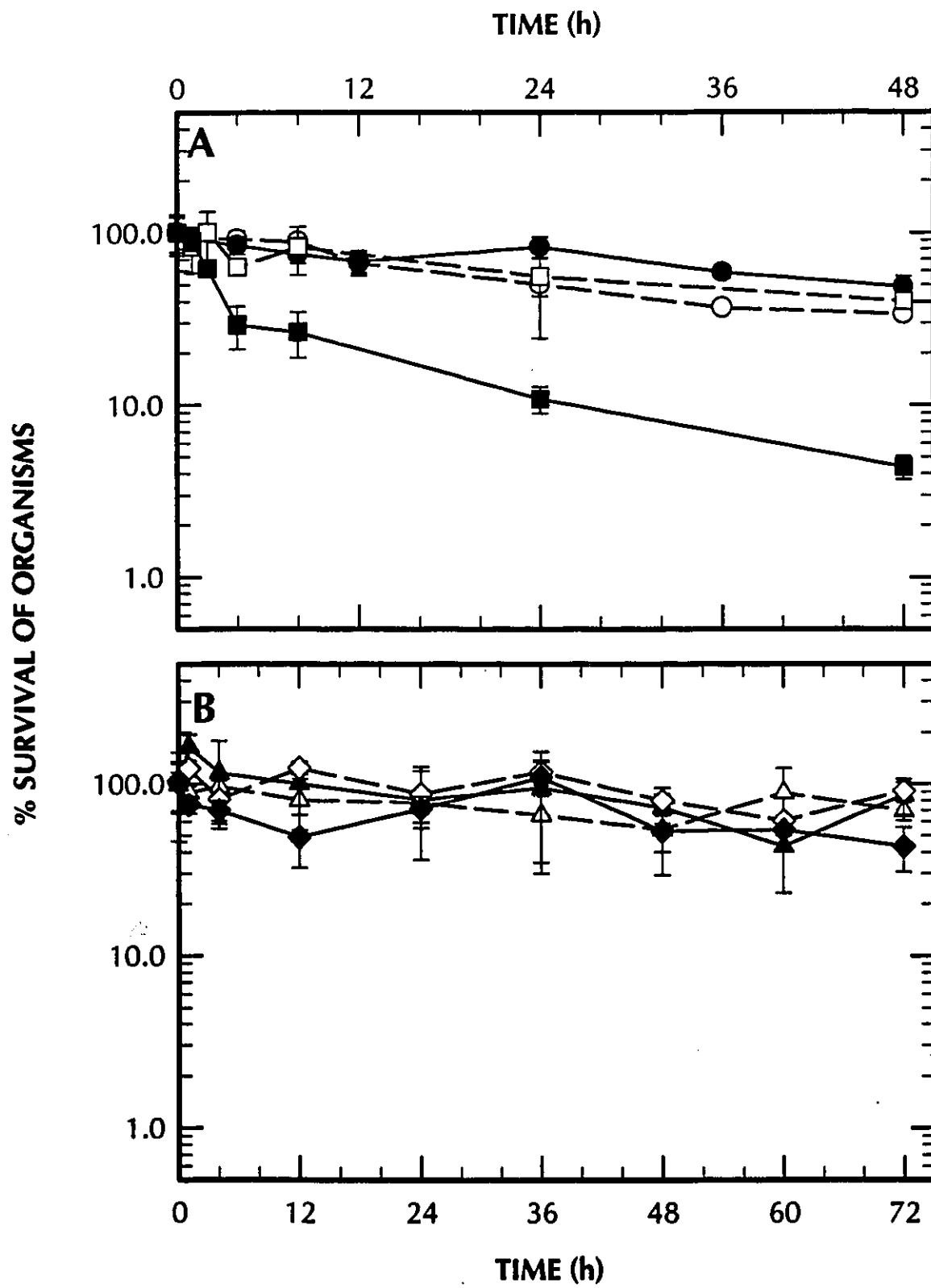
Adsorption of particles to surfaces is rapid and pH dependent (Bitton, 1980). However, no significant adsorption of microorganisms to the HFD system surfaces was evident under all the test conditions examined.

### **5.3.2 Inherent properties of the HFD system affecting microbial survival**

By studying the survival of microorganisms in the HFD system under controlled laboratory conditions in which changes in water characteristics and biotic effects were minimized, it was hoped to identify any inherent properties of the HFD system that affected microbial survival (Fig. 5.5). Under such conditions, it was found that *E. coli* survival was slightly but significantly higher after 24 h in the HFD system as compared to a static batch control. The inactivation of coliphage B was more rapid in the HFD system such that its survival was significantly different from the static batch control by 8 h ( $p < 0.05$ ). The inactivation curve of coliphage B in the HFD system appears to be biphasic with a rapid drop in phage numbers within the first 8 h followed by a period in which the inactivation rate appears very similar to that observed for the coliphage in the batch control. With PV and HAV, there was no significant difference in their survival in the HFD system and in the batch control.

**Fig. 5.5 :** The effect of the inherent properties and characteristics of the HFD system on the survival and recovery of microorganisms.

Bacteria and viruses were suspended in 0.3 mM phosphate buffer and placed in both the HFD system (solid symbols) and in a closed batch control (open symbols). Both systems were then exposed to identical and constant conditions and the microorganisms were enumerated over a period of time: (●) *E. coli*, (■) somatic coliphage B, (▲) HAV, (◆) PV.



### 5.3.3 Effect of flow rates on the survival of coliphage B in the HFD system

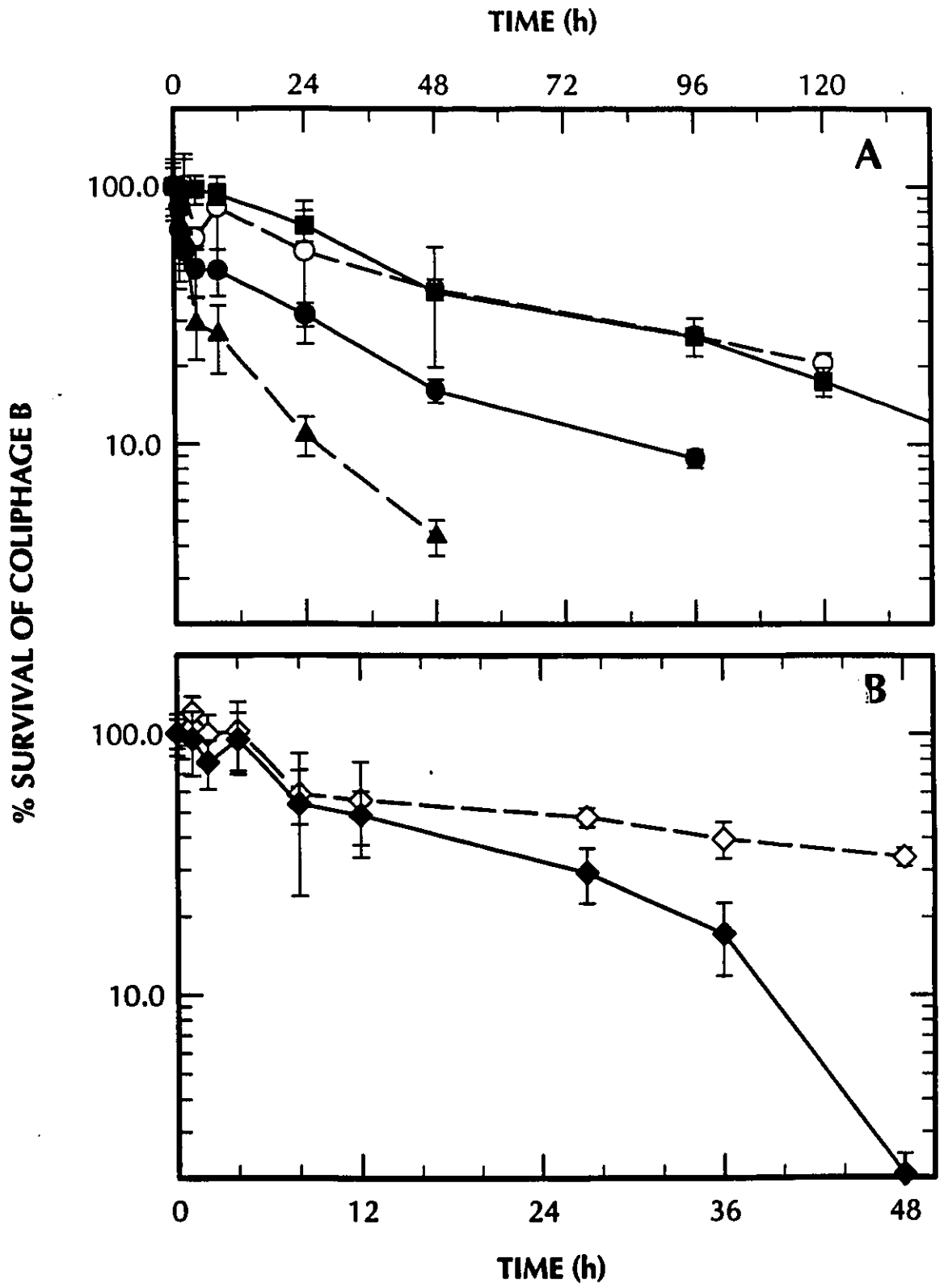
Coliphage B survival in phosphate buffer was studied while being circulated through the HFD system at different *I* circuit flow rates (Fig. 5.6-A). The flow rates used were 20 mL.min<sup>-1</sup> and 45 mL.min<sup>-1</sup>. As before, the survival curve of coliphage B in the HFD system was biphasic in nature. The initial inactivation rates were very similar;  $K_i = 0.078$  over the first 8 h at 45 mL.min<sup>-1</sup> and  $K_i = 0.074$  over the first 4 h at 20 mL.min. However, secondary inactivation rates were  $K_i = 0.019$  and  $K_i = 0.008$  for flow rates of 45 and 20 mL.min<sup>-1</sup> respectively. When the coliphage B suspension was circulated through the HFD circuit from which the hollow fiber cartridge had been removed, there was no significant difference in the survival of the coliphage in the HFD system and the batch control up to 120 h (Fig. 5.6-A). The inactivation rate in the absence of the hollow fiber cartridge was  $K_i = 0.007$  and in the batch control was  $K_i = 0.004$ . There was also no biphasic inactivation curve for either system.

Using the IMRI cartridge in place of the Fresenius cartridge (with a flow rate of 45 mL.min<sup>-1</sup>), there was no significant difference in coliphage survival between the HFD system and the batch control for up to 12 h (Fig. 5.6-B). After that, coliphage numbers in the HFD system appeared to drop more rapidly. This was, however, coincident with the appearance of a white precipitate in the HFD system. It is believed that the precipitate was leaching out of the IMRI fibers and that this was affecting coliphage recovery.

**Fig. 5.6:** Factors that affect the survival and recovery of coliphage B in the HFD system.

**A** - the Fresenius hollow fiber cartridge; (○) static control, (■) HFD system circulating at  $45 \text{ mL}\cdot\text{min}^{-1}$  without hollow fiber cartridge in line, (●) HFD system circulating at  $20 \text{ mL}\cdot\text{min}^{-1}$ , (▲) HFD system circulating at  $45 \text{ mL}\cdot\text{min}^{-1}$ .

**B** - the IMRI hollow fiber cartridge. Closed symbols - HFD system fitted with IMRI hollow fiber cartridge circulating at  $45 \text{ mL}\cdot\text{min}^{-1}$ ; open symbols - static batch sample.



The results of these experiments suggest that both flow rate and fiber diameter could have affected the coliphage inactivation/ survival. Electron microscopic examination of coliphage B revealed it to be a tailed phage.

#### 5.3.4 Equilibration of the HFD system to the external environment

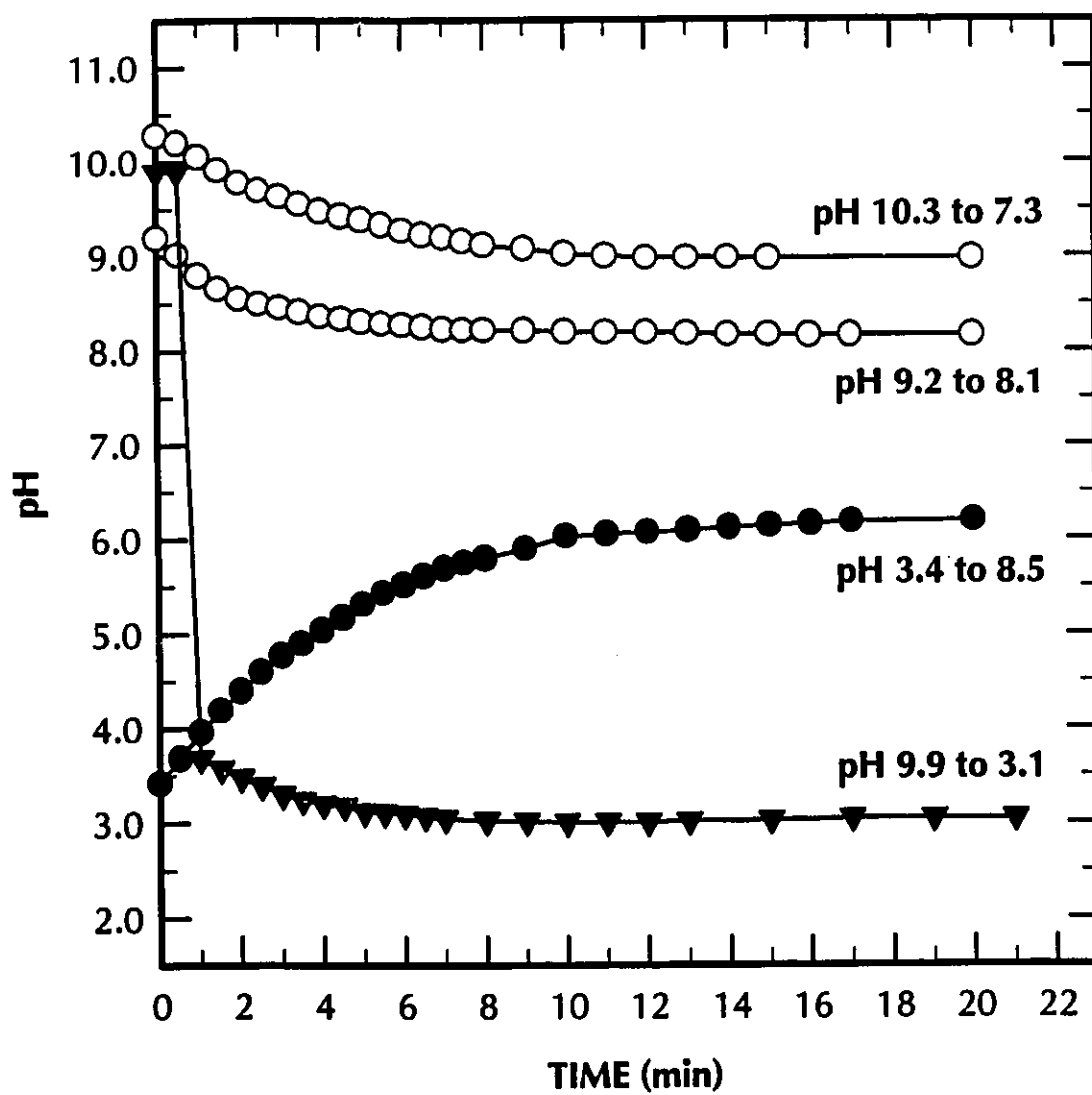
The rate at which the HFD system equilibrated with the external environment was studied in the laboratory. The equilibration of pH and the concentration of low molecular weight solutes (ONPG and PNPG) were used. In this experiment, the flow rates of the *I* and *E* circuits were 30 mL.min<sup>-1</sup> and 200 mL.min<sup>-1</sup> respectively.

With pH, it was found that rate of equilibration of the HFD system to changes in the external reservoir was dependent on the chemical agent being used (Fig. 5.7). If a weak organic acid such as acetic acid was used to change the external reservoir pH, the equilibration of the HFD system to that change was rapid; i.e. within 5 min. The same rapid equilibration was evident with weak alkaline solutions such as sodium bicarbonate.

However, if a strong mineral acid with a strong disassociation coefficient such as hydrochloric acid or a strong base like sodium hydroxide was used, the rate of change in pH in the HFD system was not as rapid (Fig. 5.7). Also, with the highly dissociated acids and bases, the HFD system never quite equilibrated with the external reservoir. Hence, despite a pH change of 10.3 to 7.3 in external

**Fig. 5.7:** The different responses of the HFD system to pH changes in the external circuit due to different types of acids or bases.

(○) dilute hydrochloric acid, (●) sodium hydroxide, (▼) acetic acid.



reservoir, the pH in the HFD system's *I* circuit only dropped to pH 9.0 even after 1 h.

A similar inability of the HFD system to equilibrate was observed when strongly ionic solutions were used. When potassium chloride was added to the external reservoir, the HFD system was unable to fully equilibrate its concentration in the *I* circuit in a manner similar to that seen with the strong mineral acids. The diffusion of sugar analogues ONPG and PNPG was followed for the HFD system in the presence and absence of a net osmotic pressure (Fig. 5.8). The diffusion of ONPG and PNPG under isotonic conditions was rapid with the concentrations equilibrating in the two compartments within 10 - 15 min. From the data for PNPG, it was calculated that the diffusion coefficient was  $k = 0.0395$  and the  $T_{90} = 10.8$  min. The clearance for PNPG was calculated to be  $K = 28.9$  mL.min<sup>-1</sup>.

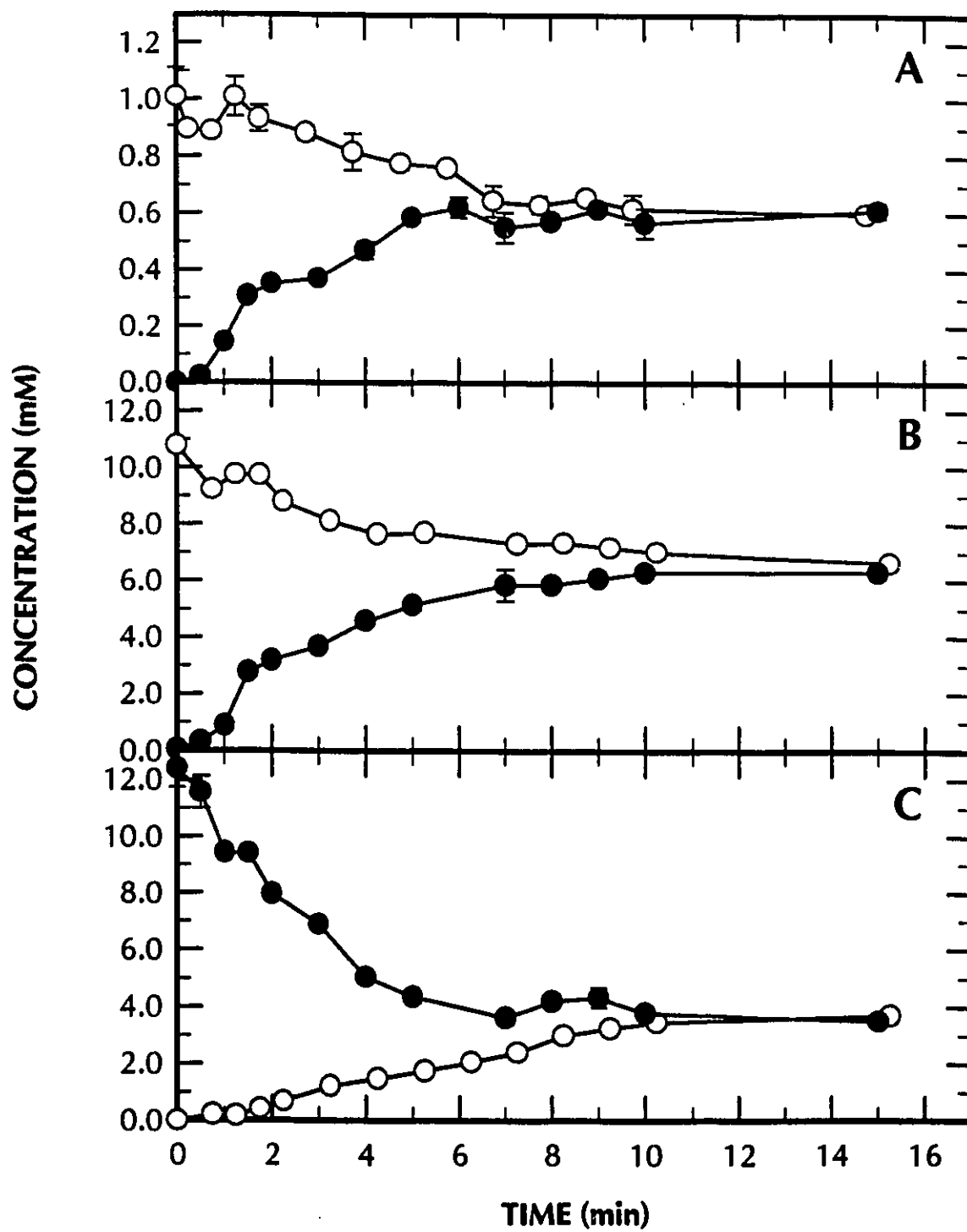
In another experiment, the diffusion of PNPG down a concentration gradient but against an osmotic gradient was followed. Here too, equilibration was reached rapidly; within 7 min (Fig. 5.8-A).

### 5.3.5 Response of the HFD system to disinfectants in the external reservoir

Fig. 5.9 illustrates the rapid response of the HFD system to chlorine in the external reservoir. The effect of the chlorine was seen to affect the microorganisms rapidly. There was a lag period before any drop; for *E. coli* that lag period was about 30 min but for the other organisms it was about 10 min. The inactivation

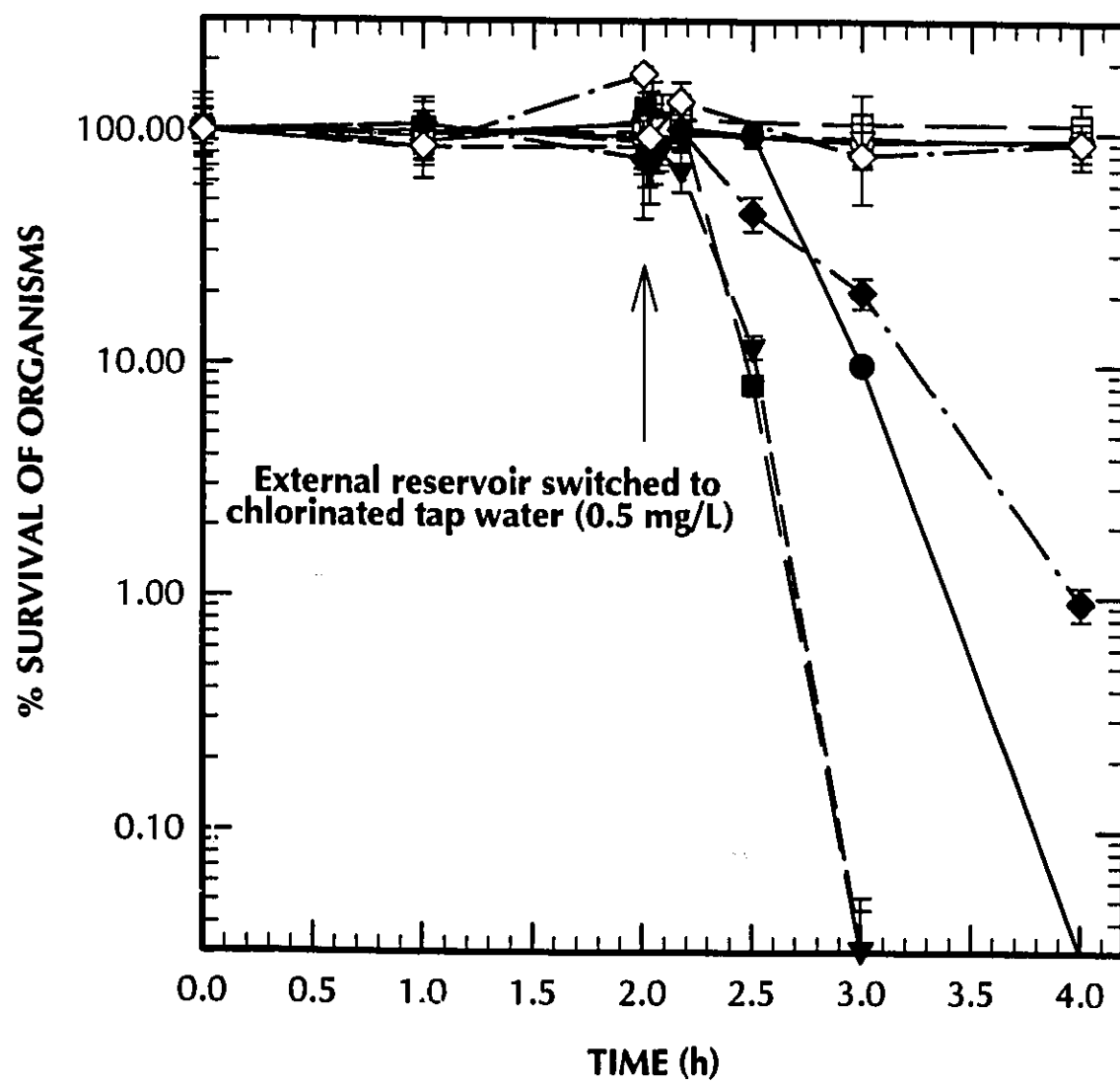
**Fig. 5.8 :** The equilibration of ONPG and PNPG concentrations between the internal and external circuits of the HFD system.

(A) PNPG was placed in the internal circuit of the HFD system and its diffusion out in to the external circuit was followed. (B) and (C) ONPG was placed in the external circuit and PNPG was placed in the internal circuit in equimolar concentrations and the diffusion of the sugar analogues in the absence of an osmotic gradient was followed. (B) ONPG and (C) PNPG. (●) concentration in the internal circuit, (○) concentration in the external circuit.



**Fig. 5.9 :** The response of microorganisms in the HFD system to exposure to chlorine in the external reservoir.

Two HFD systems are carried out simultaneously using distilled water in the internal and external circuits. Closed symbols - at 2 h, the 10 L external reservoir containing distilled water was replaced with chlorinated tap water containing 0.5 mg.L<sup>-1</sup> total chlorine. Open symbols - control in which the external reservoir was not changed. (●) *E. coli*, (■) *E. durans*, (▼) MS-2 phage, (◆) PV.



constants,  $K_i$  for *E. coli*, *E. durans*, MS-2 phage and PV were 4.98, 4.42, 4.12 and 1.10 respectively.

### 5.3.6 Comparisons between the HFD system and the diffusion chamber

The flow rates for the *I* and *E* circuits employed in these experiments were 15 mL.min<sup>-1</sup> and 180 mL.min<sup>-1</sup> respectively.

#### 5.3.6.1 pH and Eh equilibration

When parameters such as pH and redox were altered, the  $T_{90}$  values for HFD system equilibration were 2 min and 22 min, respectively (Fig. 5.10).

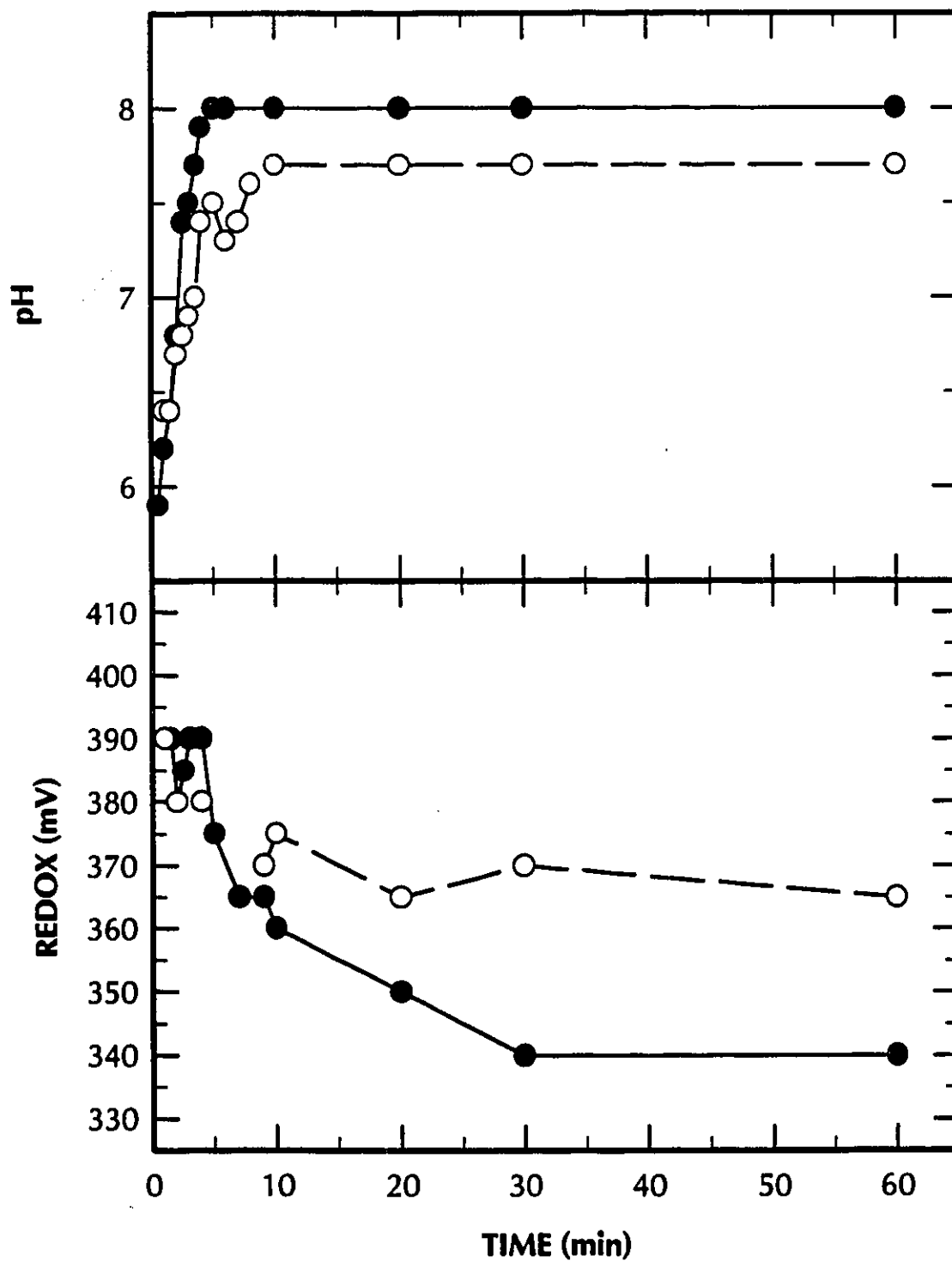
However, with the diffusion chambers, pH and Eh values never completely equilibrated with the external reservoir during the course of the experiment. When the pH of the external reservoir was raised from pH 5.9 to 8.0, the pH within the diffusion chamber failed to rise beyond pH 7.7. Similarly, when the Eh of the external reservoir was changed from 410 mV to 340 mV, the Eh within the diffusion chamber only dropped to 365 mV.

#### 5.3.6.2 Response to disinfectants in the external reservoir.

Fig. 5.11 illustrates the experiment in which the HFD system and a diffusion chamber were first exposed to the presence of nutrients in the external reservoir over an 8 h period and then were similarly exposed to chloramine T in the external reservoir at a latter time. In this case, the diffusion chamber was fitted with dialysis membranes. *E. coli* in the HFD system responded rapidly to nutrients in the external reservoir and had increased 10-fold in number by the end of that 8 h nutrient spike.

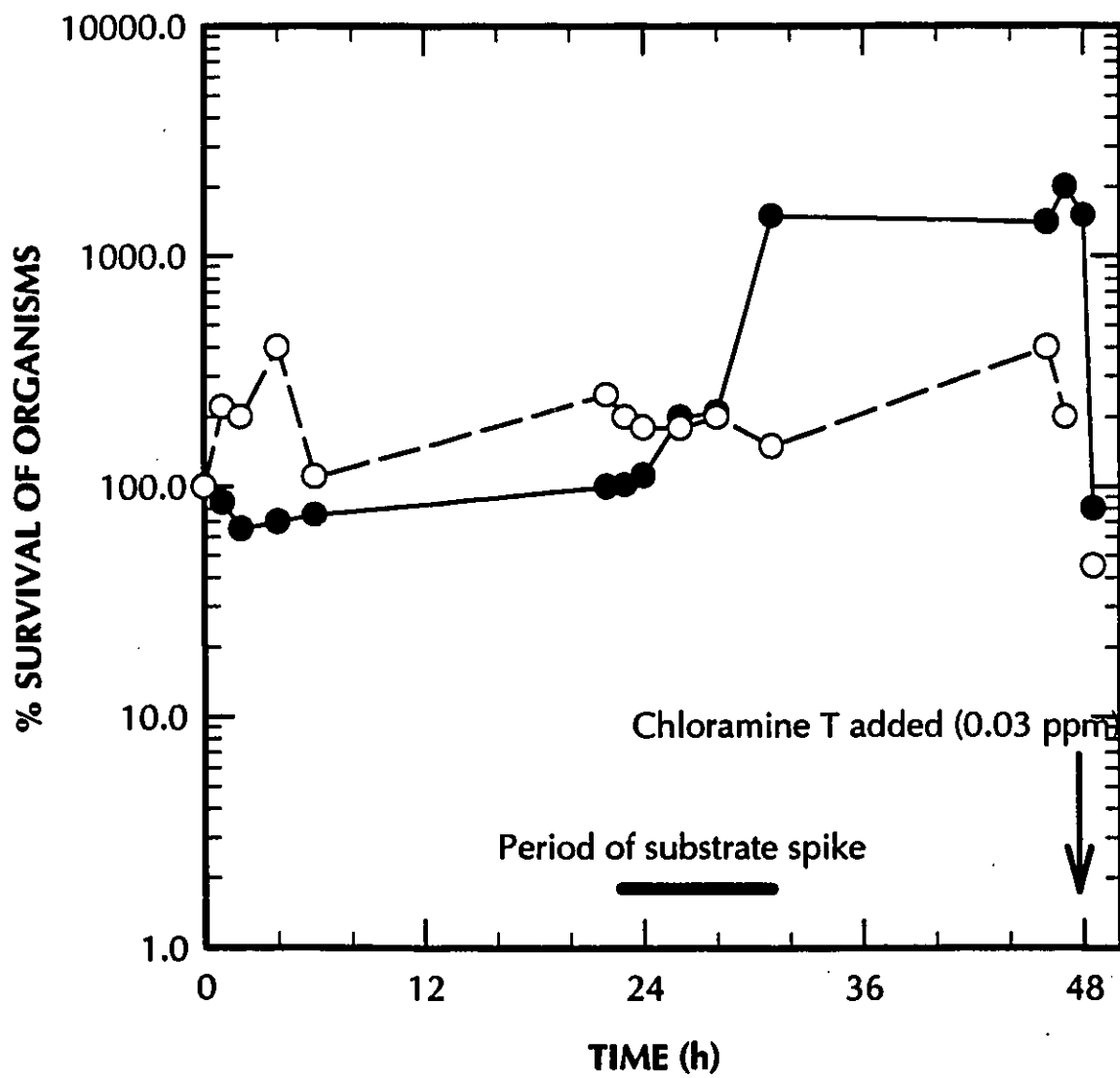
**Fig. 5.10 :** The comparative responses of the HFD system and the diffusion chamber to changes in the pH and Eh in the external environment.

The diffusion chamber was submerged in the same 10 L external reservoir that was being used by the HFD system. The pH and Eh of the external reservoir was altered using acetic acid and sodium thiosulfate respectively. The pH in the external reservoir was altered from 5.8 to 8.0; while in the Eh experiment, the Eh was altered from 390 mV to 340 mV. The pH (A) and Eh (B) of the internal lumen of the two systems was then followed. Closed symbols - HFD system, open symbols - diffusion chamber.



**Fig. 5.11 :** The response of *E. coli* in the HFD system and in the diffusion chamber to the presence of chlorine in the external reservoir.

The HFD system and the diffusion chamber were placed into the same 10 L external reservoir. Tryptose phosphate broth was added to the reservoir so that both systems were exposed to a 1/ 1000 strength TPB between 22 - 30 h. The external reservoir was then replaced with fresh 10 L of distilled deionized water. At 48 h, chloramine T was added to give a final concentration in the external reservoir of 0.03 ppm. Closed symbols - HFD system; open symbols - diffusion chamber.



### 5.3.6.3 Diffusion rates of sugar analogues

The diffusion of ONPG out of the internal reservoirs of the HFD system and the diffusion chamber and into a 10 L external reservoir of phosphate buffer was followed. The results are shown in Fig. 5.12. Initially the flux of ONPG out of the reservoirs was rapid in both systems. However, the flux of ONPG out dropped off much more rapidly in the diffusion chamber. The diffusion coefficients for ONPG in the HFD system and the diffusion chamber were  $k = 0.028$  and  $0.004$  respectively. The clearance for ONPG in the HFD system was  $12.6 \text{ mL}\cdot\text{min}^{-1}$ . The differences in the amount of ONPG that had diffused out in the two systems are illustrated in Table 5.1.

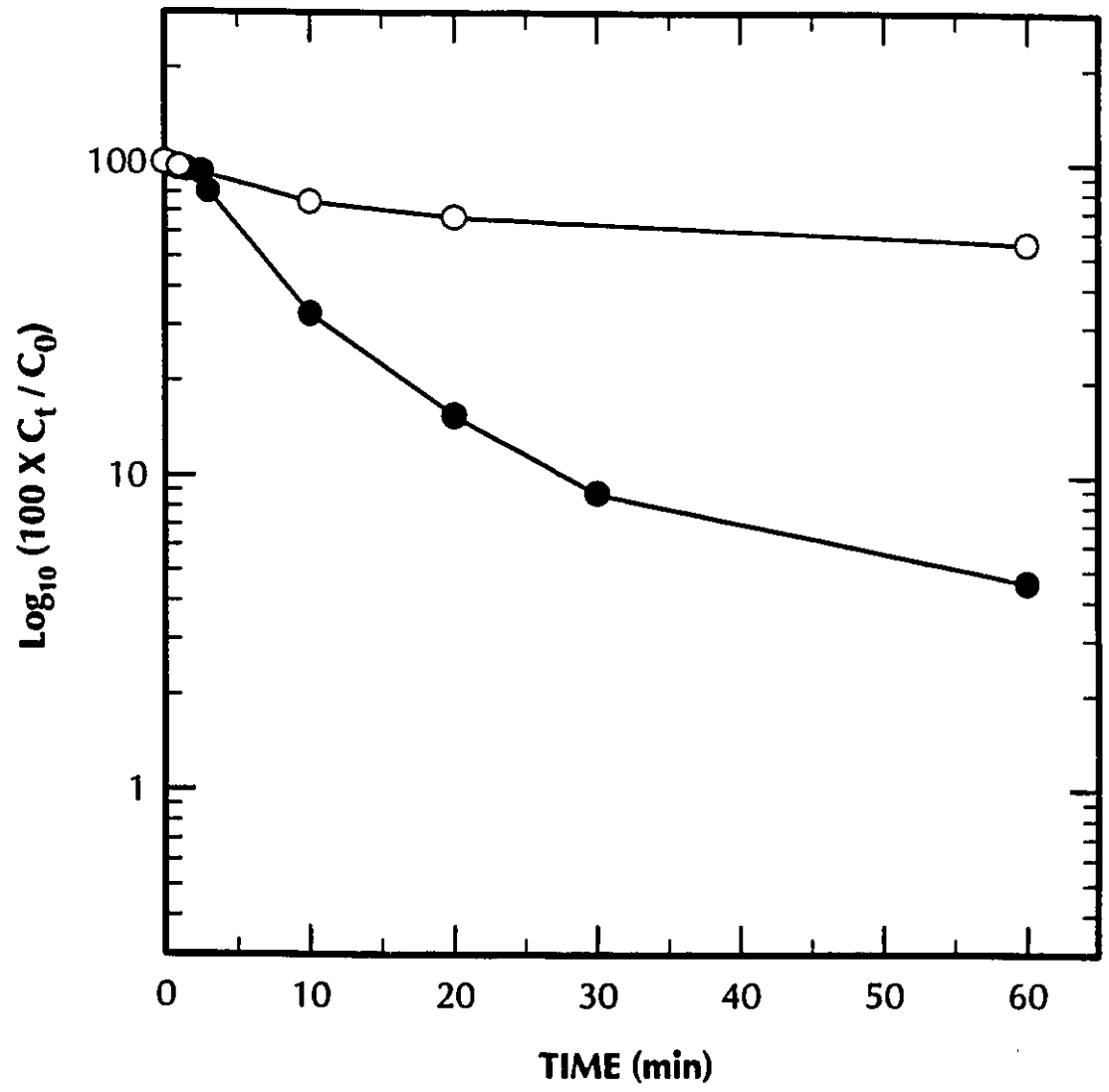
However, the *E. coli* in the diffusion chamber did not seem to respond to the 8 h nutrient spike. Both systems responded rapidly to chloramine T in the external reservoir with *E. coli* numbers dropping almost immediately.

### 5.3.7 *E. coli* survival in laboratory-simulated nutrient plug flow studies

There is a difference in the response of *E. coli* in the HFD system and in the diffusion chamber to the transient presence of nutrients in the external reservoir (Fig. 5.13A and 5.13B). In Fig. 5.13A, it can be seen that both systems respond to the nutrient spikes but generally the *E. coli* population in the HFD system responded more rapidly. With a 1 h nutrient exposure, *E. coli* numbers in the HFD system peaked 5 h after the initiation of the nutrient spike with a net increase of 62% over pre-exposure levels, while in the diffusion chamber, the numbers peaked after 7 h with a net increase of only 18.5%. *E. coli* numbers were also observed to drop off rapidly after peaking in the HFD system but did not do so in

**Fig. 5.12 :** The equilibration of ONPG concentration for the HFD system and the diffusion chamber.

ONPG was placed in the internal reservoirs of the two systems and its concentration in the internal reservoirs was monitored. A log-linear plot of the % ONPG remaining in the internal reservoirs.  $C_t$  - concentration of ONPG at time,  $t$ .  $C_0$  - initial concentration of ONPG. (●) HFD system, (○) diffusion chamber.



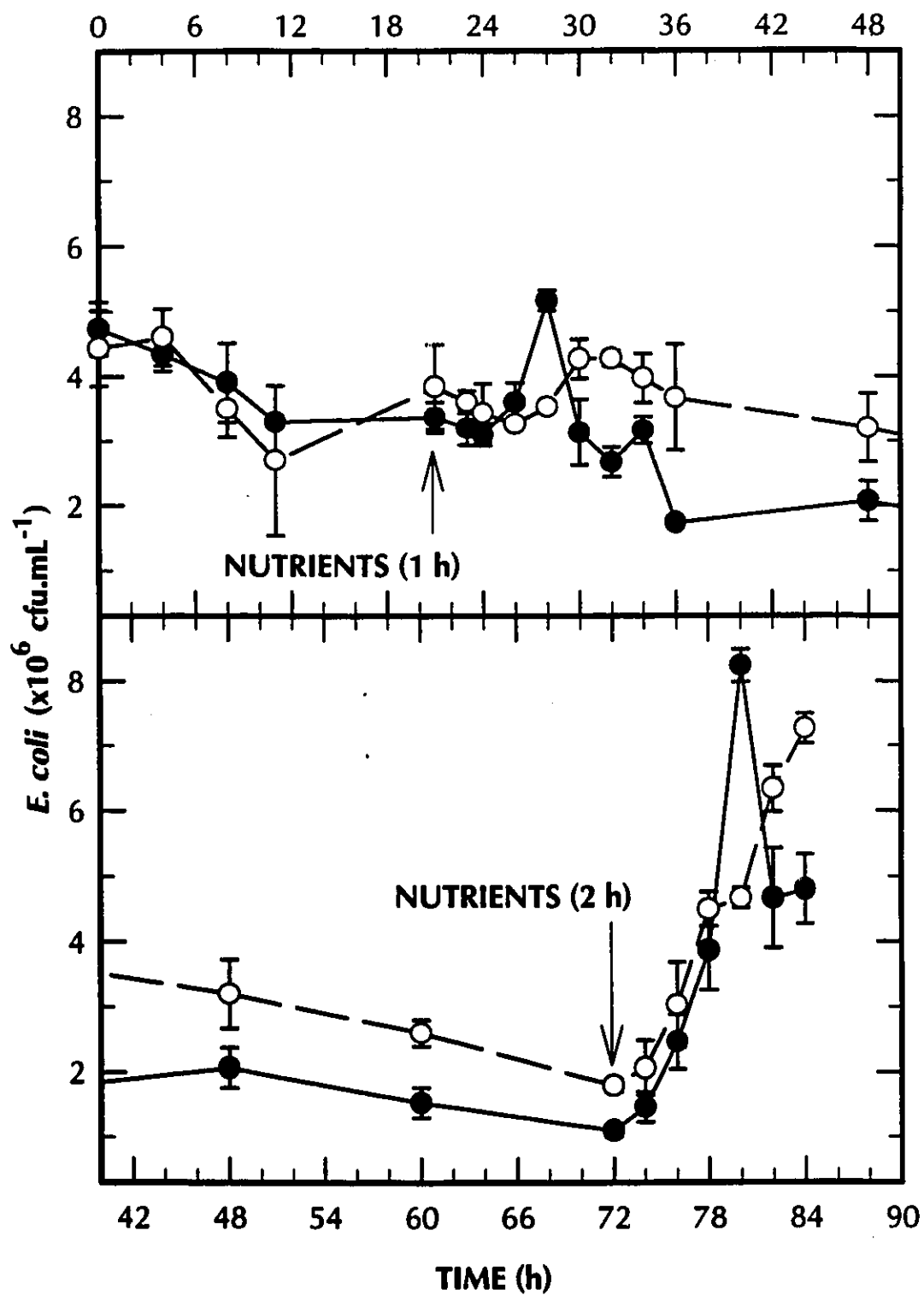
**Table 5.1 Comparison of the times observed to be required for the diffusion of ONPG from the internal reservoirs of the HFD system and diffusion chamber.**

	DIFFUSION CHAMBER		HFD SYSTEM	
	Observed*	Calculated*	Observed*	Calculated*
T <sub>20</sub>	6	24	3	3.5
T <sub>30</sub>	13	39	4	5.5
T <sub>40</sub>	42	55.5	5.5	7.9
T <sub>50</sub>	90	75.3	8	10.8
T <sub>90</sub>	>90	250	27	36

\*The observed times were determined from the graph of actual experimental results. The calculated times were determined from the estimated diffusion coefficients. (HFD system,  $k=0.028 \text{ h}^{-1}$ ; diffusion chamber,  $k=0.004 \text{ h}^{-1}$ ).

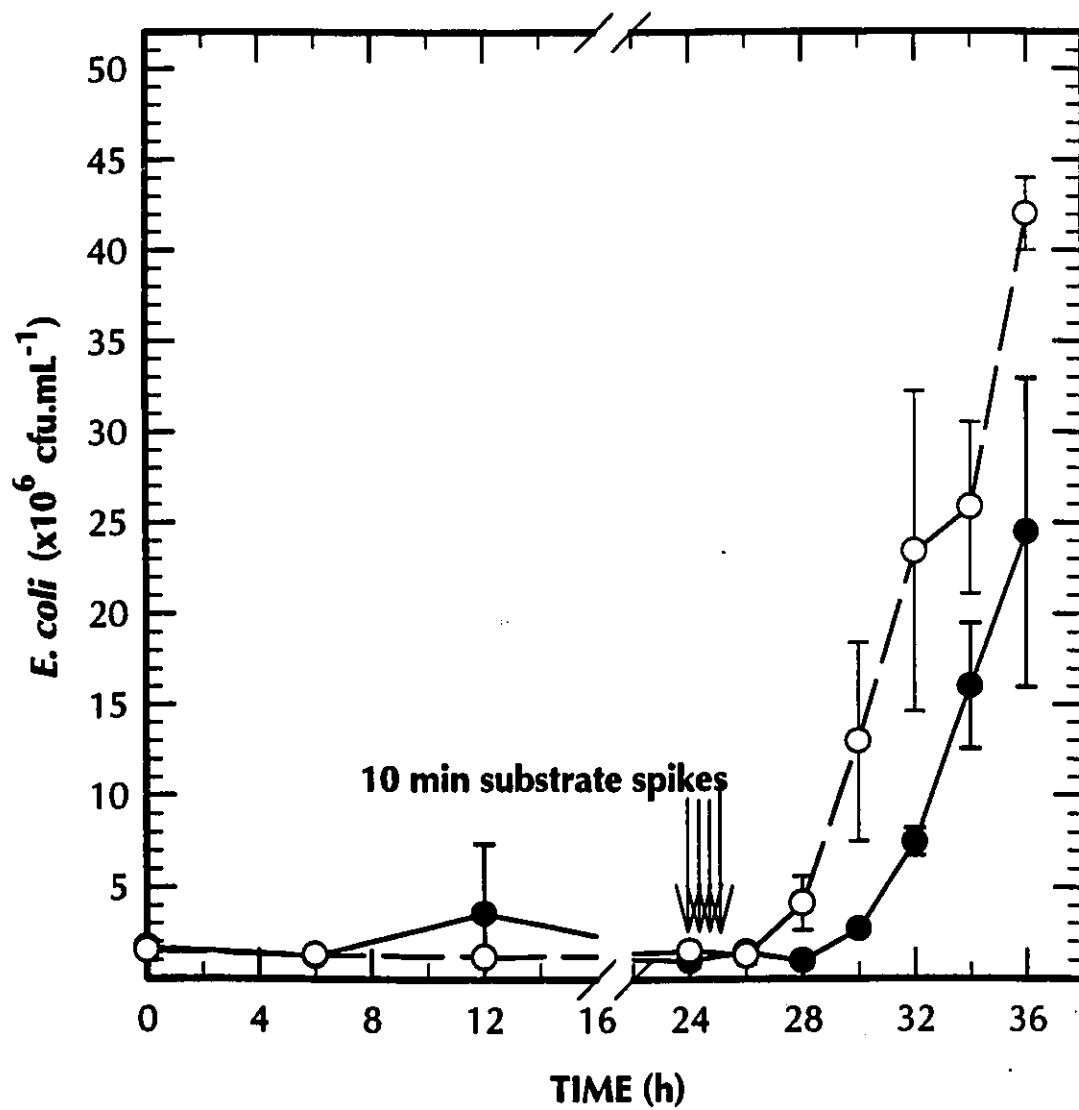
**Fig. 5.13A :** The response of bacteria in the HFD system and in the diffusion chamber to exposure of transient nutrient spikes in the external reservoir.

The HFD system and the diffusion chamber were placed into the same 10 L external reservoir. Both systems were transiently exposed to 1/100 strength tryptic phosphate broth for one hour and two hour periods by changing the external reservoir. Closed symbols - HFD system; open symbols - diffusion chamber.



**Fig. 5.13B :** The response of bacteria in the HFD system and in the diffusion chamber to repeated exposure to short transient nutrient spikes in the external reservoir.

The HFD system and the diffusion chamber were exposed to four cycles of 10 min substrate spikes (1/100 TPB solution) in the external reservoir followed by a 20 min wash with fresh clean distilled deionized water. Closed symbols - HFD system; open symbols - diffusion chamber.



the diffusion chamber. A similar pattern was observed with the 2 h nutrient exposure. The *E. coli* population peaked after 8 h in the HFD system but only after 12 h in the diffusion chamber. The increase in *E. coli* population over the levels before the nutrient spike was 650% and 300% for the HFD system and the diffusion chamber, respectively. The observed increases in *E. coli* numbers were found to be significant ( $p < 0.05$ ). However, when dialysis sac membranes were used with the diffusion chamber, no significant rise in *E. coli* numbers could be detected even after an 8 h exposure to a nutrient spike (Fig. 5.12).

When the nutrient spikes were short but frequent (10 min spike and 20 min wash or flushing cycles), the *E. coli* in the diffusion chamber responded more rapidly with an increase of 2700%, 12 h after the initiation of the nutrient spike cycle (Fig. 5.13B). In the HFD system, the *E. coli* population had increased by 2200% in that same time.

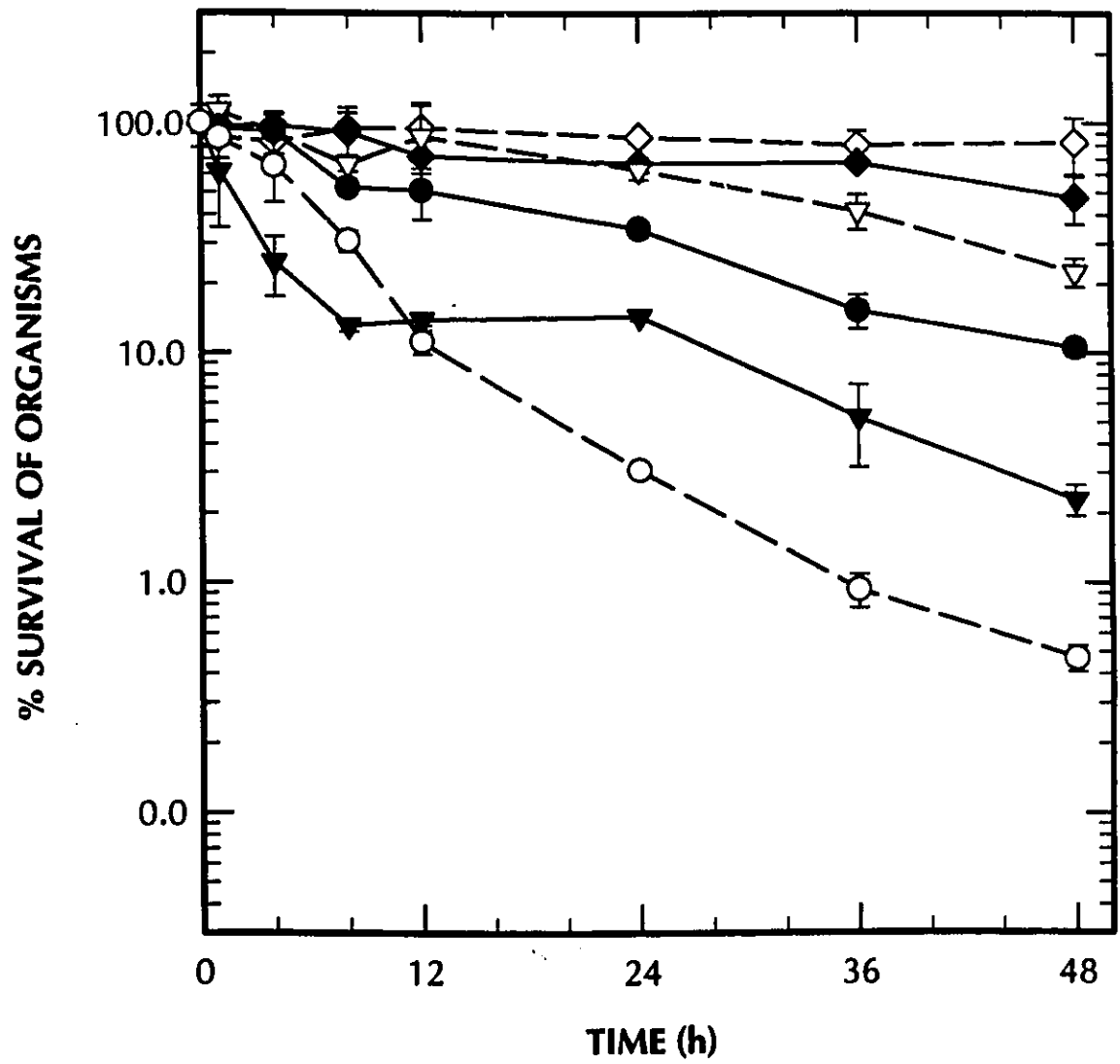
### **5.3.8 The application of the HFD system in laboratory-based river water microcosm studies**

#### **5.3.8.1 Ottawa River water**

Survival studies with Ottawa River water in laboratory-based microcosm studies with the HFD system show the order of survival of the organisms to be PV > *E. coli* > coliphage B (Fig. 5.14). In the batch control, the order of survival was PV > coliphage B > *E. coli*. The *K<sub>i</sub>* values for the microorganisms in the two systems are shown in Table 5.2. The study was repeated a number of times and the results were very reproducible.

**Fig. 5.14: The survival of microorganisms in the HFD system during laboratory based microcosm studies using Ottawa River water.**

The HFD system was exposed to a 10 L reservoir of Ottawa River water. (●) *E. coli*, (▼) somatic coliphage B, (◆) poliovirus. Closed symbols - HFD system; open symbols - batch sample.



**Table 5.2** The decay rate constants ( $K_i$ ) for microorganisms in Ottawa River water in a laboratory-based microcosm study.

	HFD SYSTEM	BATCH SAMPLE
PV	0.006 h <sup>-1</sup>	0.001 h <sup>-1</sup>
Phage B	0.027 h <sup>-1</sup>	0.013 h <sup>-1</sup>
<i>E. coli</i>	0.021 h <sup>-1</sup>	0.051 h <sup>-1</sup>

#### 5.3.8.2 Rideau River water

In this microcosm study, the effect of predation on microbial survival was looked at by using autoclave sterilized and non-sterilized river water in the internal reservoir of the HFD system and in the batch samples. Over the first 8 h, there was very little difference in the survival of *E. coli* between either the batch samples or the HFD systems, whether or not the water had been pre-sterilized (Fig. 5.15). The most rapid decline in *E. coli* numbers was observed in the sterile batch sample and in the non-sterile HFD system up to 38 h. An apparent regrowth was then observed in both the sterile batch sample and the sterile HFD system, whereas in the non-sterile batch sample and HFD system the *E. coli* continued to decline.

**Fig. 5.15** The survival of microorganisms in the HFD system during laboratory-based microcosm studies using Rideau River water.

(A) *E. coli*, (B) somatic phage B and (C) PV. Test microorganisms were suspended in both untreated, non-sterilized and in autoclave sterilized Rideau River water and placed in the internal reservoir of the HFD system and in a batch control. The HFD system was then exposed to a 10 L reservoir of fresh Rideau River water. (●) non-sterilized / HFD, (■) non-sterilized / batch control, (▲) sterilized / HFD, (▼) sterilized / batch control.



Coliphage B interestingly did not demonstrate the biphasic inactivation in the HFD system (Fig. 5.15) which had been previously observed in phosphate buffer and water from the Ottawa River. Loss of coliphage B in the non-sterile batch sample was significantly different from that of the other systems ( $p < 0.05$ ) after 36 h. PV showed no significant drop in numbers in sterilized river water but had a significant drop in numbers in both systems in non-sterilized river water.

#### 5.4 Discussion

The HFD system demonstrated very rapid diffusion of low molecular weight solutes and equilibration of pH and Eh as well as a rapid response to disinfectants. When compared directly under the experimental conditions described, the diffusion coefficient,  $k$ , for ONPG was 0.028 and 0.004 for the HFD system and the diffusion chamber, respectively. This means that the  $T_{90}$  value (calculated from  $k$ ) for ONPG for the HFD system was 36 min (the observed  $T_{90}$  value was 27 min), while the  $T_{90}$  value for the diffusion chamber was 250 min or 4.2 h. It is interesting to note that when the flow rate of the *I* circuit was  $15 \text{ mL}\cdot\text{min}^{-1}$ , the clearance for ONPG or PNPG was  $12.6 \text{ mL}\cdot\text{min}^{-1}$  and when the flow rate was  $30 \text{ mL}\cdot\text{min}^{-1}$ , the clearance for PNPG was estimated at  $29 \text{ mL}\cdot\text{min}^{-1}$ . The rate of clearance was approximately equivalent to the flow rate of the *I* circuit. This suggests an almost instantaneous equilibration across the hollow fiber membranes. The rate of equilibration of the internal reservoir with the external reservoir with regards to the concentration of low molecular weight solutes may therefore be

increased by either increasing the flow rate of the *I* circuit or reducing the total volume of the *I* circuit.

The HFD system equilibrated rapidly for parameters like pH and redox with  $T_{90}$  values of 2 min and 22 min, respectively. The relationship of pH change to time was linear until equilibration was reached which suggests a logarithmic relationship between hydrogen ion diffusion and time. A logarithmic relationship between Eh and time does not exist and is suggestive that additional factors are at work in the equilibration of Eh than just the diffusion of molecules. The equilibration of Eh was slower than might be anticipated.

It was noted however, that the HFD system failed to fully equilibrate for strongly ionic species such as mineral acids strong bases and strongly ionic salts such as potassium chloride. This did not occur with weak electrolytes (e.g. acetic acid and sodium bicarbonate). This problem was even more pronounced with diffusion chambers where failure to equilibrate was seen for pH and Eh even when weakly ionic species such as acetic acid were involved.

Clearly, there is a problem with the passage of charged ions or species through the membranes. This phenomenon is well recognized for certain membranes and is especially true for cellulose acetate membranes (Tuwiner, 1962). Ionic species such as the ammonium ion are reported to be exceptions to this phenomenon and it is believed that this is due to its ability to pass through the membrane by making and breaking hydrogen bonds with the membrane matrix compound.

The retention of charged ions would also cause a boundary effect with the repulsion of other ions away from the membrane surface. This may be another problem with the use of cellulose acetate membranes for diffusion chambers.

The polysulfone hollow fibers may be inherently less retentive for strong electrolytes and the tangential flow of fluid through the fibers may reduce any boundary effect. Together, this may result in the better performance of the HFD system with regards to diffusion of electrolytes.

The large surface area for exchange ( $7 \times 10^3 \text{ cm}^2$ ) in the hollow fibers may be responsible for the rapid diffusion rates of the HFD system but it was feared that it would also cause significant adsorption of test microorganisms. This was not found to be a problem for the pH range of 5 to 8 which is representative of the pH encountered in most natural waters.

As it turned out, there was a problem with an apparent inactivation of coliphage B as a result of passage through the hollow fibers of the HFD system. This inactivation was directly correlated to the flow rate through the hollow fibers and was demonstrated to be not because of adsorption to surfaces or the action of the peristaltic pumps. It was characterized by a rapid drop in coliphage numbers in the first 8 h followed by a decay rate that was more similar to that of a static control. It was found that the rapid inactivation was reduced or absent if the water had a high organic load or if the IMRI cartridge with the larger diameter fibers (0.8 mm) was used instead of the Fresenius Cartridge (0.2 mm). No inactivation occurred with other viruses tested including the MS-2 phage.

The evidence suggests that the mechanism for coliphage B inactivation due to the HFD system was shearing forces. Such shearing forces occur in the flow of fluids through narrow tubes and increase with increasing flow rates and decreasing fiber diameter. Coliphage B is a tailed phage and may be more susceptible to the shearing forces than a non-tailed phage such as MS-2.

The HFD system responded more rapidly to the presence of nutrients in the environment than diffusion chambers. This was demonstrated by the growth of *E. coli* in the two systems after exposure to nutrient spikes in the external environment. This is not unexpected based on the comparative rate of diffusion of the sugar analogues, ONPG and PNPG.

With 1 h and 2 h nutrient spikes, the *E. coli* in the HFD system responded more rapidly and had a higher net increase in numbers. However, with the shorter 10 min spike / 20 min wash cycles (Fig. 5.13B), *E. coli* had a higher net increase in the diffusion chamber. This is likely due to the "bottle" effect of the diffusion chamber. Within the 10 min nutrient spike, more nutrients enter the HFD system than the diffusion chamber but during the wash, almost all the nutrients are removed from the HFD system but only a portion of the nutrients are removed in the diffusion chamber. The repeated nutrient spike / wash cycles allowed a significant amount of nutrients to remain in the diffusion chamber throughout resulting in the higher level of *E. coli* growth.

When cellulose acetate membranes are used with the diffusion chamber, the pore sizes are too large to retain viruses. The HFD system, however is capable

of retaining viruses in the study reservoir. For the diffusion chambers to be able to do so, it would have to be equipped with either dialysis membranes or the 0.015  $\mu\text{m}$  pore size polycarbonate membrane filters. However, when dialysis membranes were used with the diffusion chamber, the *E. coli* in diffusion chamber showed no response to nutrients in the external reservoir even after 8 h of exposure..

The microcosm studies with Ottawa River water and Rideau River water demonstrate differences in the relative survival of test microorganisms in the HFD system and in batch samples. In the Ottawa River microcosm, *E. coli* survived much better in the HFD system than in the batch sample ( $K_i = 0.021$  and  $0.051$ , respectively).

In the Rideau River microcosm experiments, the decline in *E. coli* was most rapid in the sterilized batch sample and in the non-sterilized HFD system. If batch systems were used alone, it may be concluded that predation was not a major cause of *E. coli* decline. However, the opposite conclusion may be inferred from the HFD results. Both coliphage B and PV appear to be significantly affected by predation. Regrowth of *E. coli* in pre-sterilized HFD and batch systems was probably due to the utilization of nutrients freed during the autoclave process.

The HFD system has been demonstrated to respond more rapidly to changes in the external environment than diffusion chambers. The HFD system more closely resembles a system that exposes test organism to "real-time" changes in the aquatic environment. Caution may be required to interpret results involving tailed phages. However, the inactivation of tailed phages due to the passage through the

hollow fibers can be reduced or eliminated by increasing fiber diameter and/or reducing  $J$  circuit flow rates.

**6. The Application of the Hollow Fiber Diffusion System  
for *in situ* Survival Studies**

## 6.1 Introduction

Ultimately, the HFD system was meant to be used in *in situ* field studies on microbial survival. Towards this aim, a series of field trips were carried out to test the HFD system identifying and solving potential problems related to its field application and to obtain data on the survival of test microorganisms at those sites.

The HFD system had to be adapted to make it more portable and easy to set up. Other changes were carried out to reduce the need for constant monitoring during use by incorporating a system for volume control and gas to escape from the *I* circuit.

Three temperate and two equatorial surface water sites were used to field test the HFD system. The sites were chosen to give the widest range of surface water types possible, from oligotrophic to eutrophic from clear to turbid. It was also meant to obtain comparative data on the survival of microorganisms in temperate and equatorial waters.

## 6.2 Materials and methods

### 6.2.1 Temperate Field Sites

The HFD system was employed at three sites in and around Ottawa, Ontario, Canada, for *in situ* microbial survival studies. Two of the sites were river sites; one was on the eutrophic Rideau River (site TA) while the other was on the oligotrophic Ottawa river (site TB). The third site chosen was the Kennedy-Burnett Stormwater Management Pond (site TC).

### **6.2.1.1 Rideau River (site TA)**

The field site selected was located on the west bank of the Rideau River upstream of Mooney's Bay. The HFD system was installed on a riverside dock on the premises located at 1 Stephanie Avenue. The *in situ* microbial survival studies were carried out during the summer months of June to August, 1991. At the site, the Rideau River was sluggish with an almost undetectable flow. The river receives drainage from a mixture of urban and agricultural lands and from stormwater management ponds upstream from the site. The river was visibly eutrophic with an abundance of algae and weeds. The river is also used extensively by recreational boaters. The river water temperature at the site during the study period was in the 20°C - 28°C range.

#### **6.2.1.1.1 Experimental set-up at site TA**

Using a peristaltic pump (cartridge pump series 400, Cole Parmer), river water was collected 3 m from the edge of the river bank and 0.3 m below the surface. The water sample was placed in 250 mL volumes into two glass vessels, one was used as a closed batch control while the other was used as the internal reservoir for the HFD system.

The HFD system was set-up essentially in the same manner as in laboratory experiments. Both the batch control and the HFD system internal reservoir were placed into a wire rack which was equipped with floats. The wire rack construction was maintained in place by wooden guide-bars but allowed to rise

and fall with the river water level. This arrangement permitted the water levels in the vessels to be maintained at the level of the river water.

Both vessels were shielded from direct sunlight. The HFD system tubing was surrounded with expanded polyurethane foam to protect the tubing from direct sunlight and to insulate the tubing from heat. Both the HFD system cartridge and the peristaltic pump were placed into a wooden "dog-house" construction which similarly protected both from the elements. An extension electric cable was strung from a nearby house to provide power to run the pump.

#### **6.2.1.2 Ottawa River (site TB)**

Site TB was at the Britannia Water Purification Plant on the Ottawa River. The HFD system was set-up in a laboratory at the facility, using the plant input water supply of raw Ottawa River water. This water was collected from the middle of the Ottawa River and flows continuously from a sampling tap in the laboratory. The water at this point had not yet received any treatment and was normally used for routine chemical and microbiological analysis. Experiments were carried out at the site during the spring of 1992.

The Ottawa River at this site is a wide fast-flowing river with low levels of suspended solids and organic load. Rapids upstream ensure relatively high vertical mixing in the river.

#### 6.2.1.2.1 Experimental set-up at site TB

Since the experiments at this site were carried out in early spring, the river water temperature was 2 - 4°C. Two experiments were conducted using the river water at its low ambient temperature.

River water was used to suspend the study microorganisms in the HFD system internal reservoir and the batch control. Both vessels were then placed into a large plastic container (the experimental working reservoir) which was filled with river water. This enabled the temperature of the vessels to be maintained at that of the river water. The water in the experimental working reservoir was constantly renewed by fresh river water from the plant sampling tap at the rate of 1.0 L min<sup>-1</sup>. The HFD system's *E* circuit also circulated water from this container in what was essentially a flow-through system.

Preliminary results from the two early experiments suggested that the effect of water properties on microbial survival was limited by the preserving nature of the low ambient river water temperature. Since, the objective was to test the HFD system as a means of studying microbial survival and response to changing water qualities, it was decided to artificially raise the river water temperature. This was done with the use of the condensing unit from a water distiller. River water from the plant sampling tap was made to flow through the condenser's inner glass tubing coil before entering the experimental working reservoir. The outside of the condenser's coil was bathed in warm water which would raise the temperature of the river water as it passed through the condenser unit. The rate of flow of the

warm water through the system was adjusted to cause the river water entering the working reservoir to be approximately 20°C. For the most part, this arrangement proved fairly reliable although some temperature fluctuations did occur due to unavoidable variations in the warm water supply at the plant.

#### **6.2.1.3 Kennedy-Burnett Stormwater Management Pond (site TC)**

The Kennedy-Burnett Stormwater Management Pond is located at Strandherd Road in Nepean, Ontario, Canada. This pond essentially served as a holding pond for stormwater from newly developed housing in the community of Barrhaven. The pond consisted of two elongated rectangular shaped cells which were operated in series, separated by a bridge and culverts. The pond was about 8 m wide and of a trapezoid cross-sectional profile with a water depth of 0.6 - 1.0 m. It empties into a small creek and subsequently into the Jock River. Naturalization of the pond had led to differences between its two cells. Sedimentation had caused the first cell to be generally shallower than the second one but it had a narrow, deeper middle channel. The first cell also had a substantial growth of cattails. Site C was located near the effluent end of the second cell of the pond.

Experiments were carried out at the site during the summer of 1992. The beginning of the summer was very wet and the pond for the most part was operating in a flow through mode. The frequent rain caused it to exceed capacity and overflow, greatly reducing the retention time from the prescribed 72 h. Towards the end of the season though, the pond level had fallen and the pond operated under dry weather flow conditions.

#### **6.2.1.3.1 Experimental set-up at site TC**

The experimental set-up was similar to that described for site A except that a modification had to be incorporated as a means of placing and retaining the HFD system and the batch control in the mid-channel of the stormwater pond. This was achieved using a floating platform to house all the equipment. The platform could be maintained at mid-channel or brought to the side of the pond for sample collection through the use of a pulley system.

The power supply for running the peristaltic pump and incubators was available in a small shed at the site.

#### **6.2.2 Equatorial field sites**

Field applications of the HFD system were carried out at two sites in Malaysia. The choice of sites was partly dictated by considerations of accessibility, security and logistics. However, the two chosen sites were located on two very different types of rivers. The Gombak River (site EA) is a polluted river while the Kroh River (site EB) represents a pristine equatorial river.

##### **6.2.2.1 Gombak River (site EA)**

Site EA was within the perimeter of the University of Malaya's Field Research Center (Pusat Penyelidikan Luar or PPL) which is located in the Gombak District, approximately 25 km northeast of the capital city of Kuala Lumpur. The site was at the headwaters of the Gombak River and the watershed area consisted primarily of "disturbed" equatorial rainforest; i.e. the area had been previously logged within the last 20 years.

The PPL is used exclusively for rainforest research and for the training of biologists. There is a collection of buildings at the PPL for use as sleeping quarters and laboratories.

The PPL site can be reached by a tarred road which closely parallels the route of the river. Site EA was located approximately 200 m from the road at the base of the hill from the main PPL compound. The river at this site was approximately 30 m wide and up to 1.5 m deep and fast-flowing. The site was also well-shaded by trees and other vegetation. The river basin was sandy and rocky.

The Gombak River may receive pollution from a number of sources. The PPL site had a septic tank but its overflow enters the river about 100 m downstream of the site EA. However, about 300 m upstream of site EA, a small stream joins the Gombak River. This small stream passes by a small aboriginal village of about 10 families. It is likely that contamination from the villagers may be entering the river. The villagers are known to defecate near the stream. *E. coli* and *Enterococcus* counts in the stream were found to be occasionally higher than that of the main river. Some 10 km further upstream, some small tracts of forest had been cleared for small flower farms and for the construction of electrical pylons. Runoff from these sites is likely to contribute to the high levels of turbidity seen after rainfall. The soil at these locations is primarily laterite clay which colors the water reddish-brown. Typical of many rivers in Malaysia, the river water at site EA becomes very turbid immediately after rainfall but later clears.

#### **6.2.2.1.1 Experimental set-up at site EA**

For this site, a very different HFD system set-up was used. The modifications made to the system were designed to make the system more portable and easier to set-up at different sites while reducing the need to monitor volume changes in the internal reservoir. This modified design is described in section 6.2.2.3.

The HFD system internal reservoir and hollow fiber cartridge were placed into a plastic holder which was then suspended from the middle of a child's polystyrene, donut shaped, swimming float. When placed in the water the assembly floated, holding the internal reservoir just below the water surface and submerged the hollow fiber cartridge to a depth of 0.6 m.

#### **6.2.2.2 Kroh River Tributary (site EB)**

This pristine field site was located within the Bukit Lagong Forest Reserve at the Forest Research Institute of Malaysia (FRIM). FRIM is the premier research institution involved in equatorial rainforest research and development throughout Malaysia. The entire Kroh River headwaters and watershed are located within FRIM boundaries. The entire area is set aside for scientific study and there is no human habitation or commercial activity within the forest reserve.

Site EB was located on a small tributary of the Kroh River. The stream at the site was about 3 m wide and was flowing down a steep rocky incline. The depth of the stream varied from 0.1 to 0.3 m at the site. The water was highly oxygenated from falling over rocks. The water was clear except during periods of

heavy rain when the water turned turbid for a short while. The site was heavily shaded by natural vegetation.

#### **6.2.2.2.1 Experimental set-up at site EB**

The HFD system was set up differently than at site EA. The hollow fiber cartridge could not be submerged below the *I* reservoir due to the shallow nature of the stream at the site. The cartridge was therefore located on a rock near the site together with the pump box and batteries. This made it necessary for regular checks and manual manipulations of the flow rates to maintain a constant volume of water within the *I* circuit. The *I* reservoir was bathed in stream water to keep the *I* circuit temperature constant with the temperature of the stream.

#### **6.2.2.3 Modifications of the HFD system design for field work in Malaysia**

Field work in Malaysia posed several problems that had to be considered in the design of the HFD system. Unlike the earlier field experiments in Canada, the possible field sites in Malaysia were more remote and difficult to access. Power to run the peristaltic pump had to be considered since the sites were without electrical power supply. Lack of technical support and man-power also made it important to make the system as maintenance free as possible while being operated.

With those objectives in mind, modifications were made to the design of the internal reservoir, the configuration of the reservoir and the hollow fiber cartridge, the peristaltic pumps and a portable power supply were incorporated. These modifications are described in detail below.

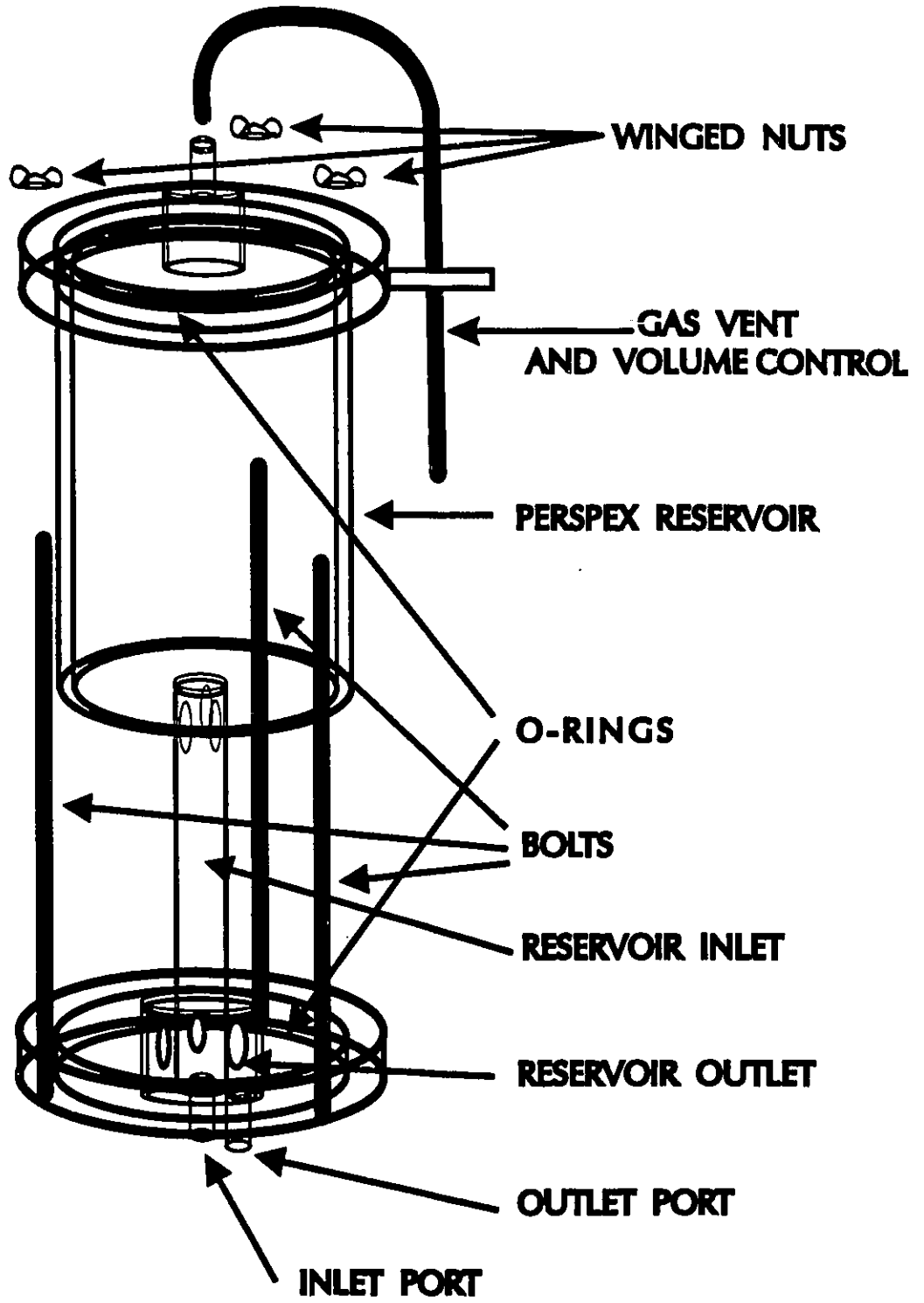
#### 6.2.2.3.1 HFD system internal reservoir

The resulting internal reservoir is represented in (Fig. 6.1). The reservoir consisted of three perspex pieces held together with bolts and winged nuts. The bottom perspex disc held the stainless steel inlet and outlet ports for the reservoir. The inlet port opened into a 10 cm long perspex tube which had four outlet holes located about 0.5 cm from the opposing, sealed end. The outlet port opened into a small perspex chamber (2.5 cm in diameter and 2.5 cm high) which surrounded the base of the inlet tube. This outlet chamber had four holes which were positioned so as to be offset from the holes of the inlet tube. This arrangement had been determined to provide the best circulation and mixing of water when the *I* circuit was being run.

The long cylindrical middle piece was then placed on top of the bottom disc. This perspex piece made up the walls of the reservoir. Finally, the top perspex disc was placed on top and the three parts held in place by winged nuts. A water-tight seal was ensured by silicone O-rings located in the seat of both the upper and lower disc.

The upper disc also had a metal port. A piece of noreprene tubing connected this port to another metal tube at the edge of the upper disc. The open end of the metal tube was adjusted to be in line with the desired level of the water inside the reservoir. This configuration of the tubing and metal tube resulted in an inverted U-shaped arrangement that was similar to that of a manometer.

**Fig. 6.1 :** The design of the internal reservoir of the HFD system used for field work in Malaysia.



#### **6.2.2.3.2 Configuration of the internal reservoir and hollow fiber cartridge components**

The internal reservoir was suspended so as to be just submerged below the water surface. The hollow fiber cartridge was submerged in a vertical orientation below the level of the reservoir at a depth of 0.6 m. Water in the *I* circuit was pushed through the hollow fiber cartridge from the bottom to the top while in the *E* circuit, water was drawn through the cartridge in a counter current direction. The field set-up is illustrated in Fig.6.2.

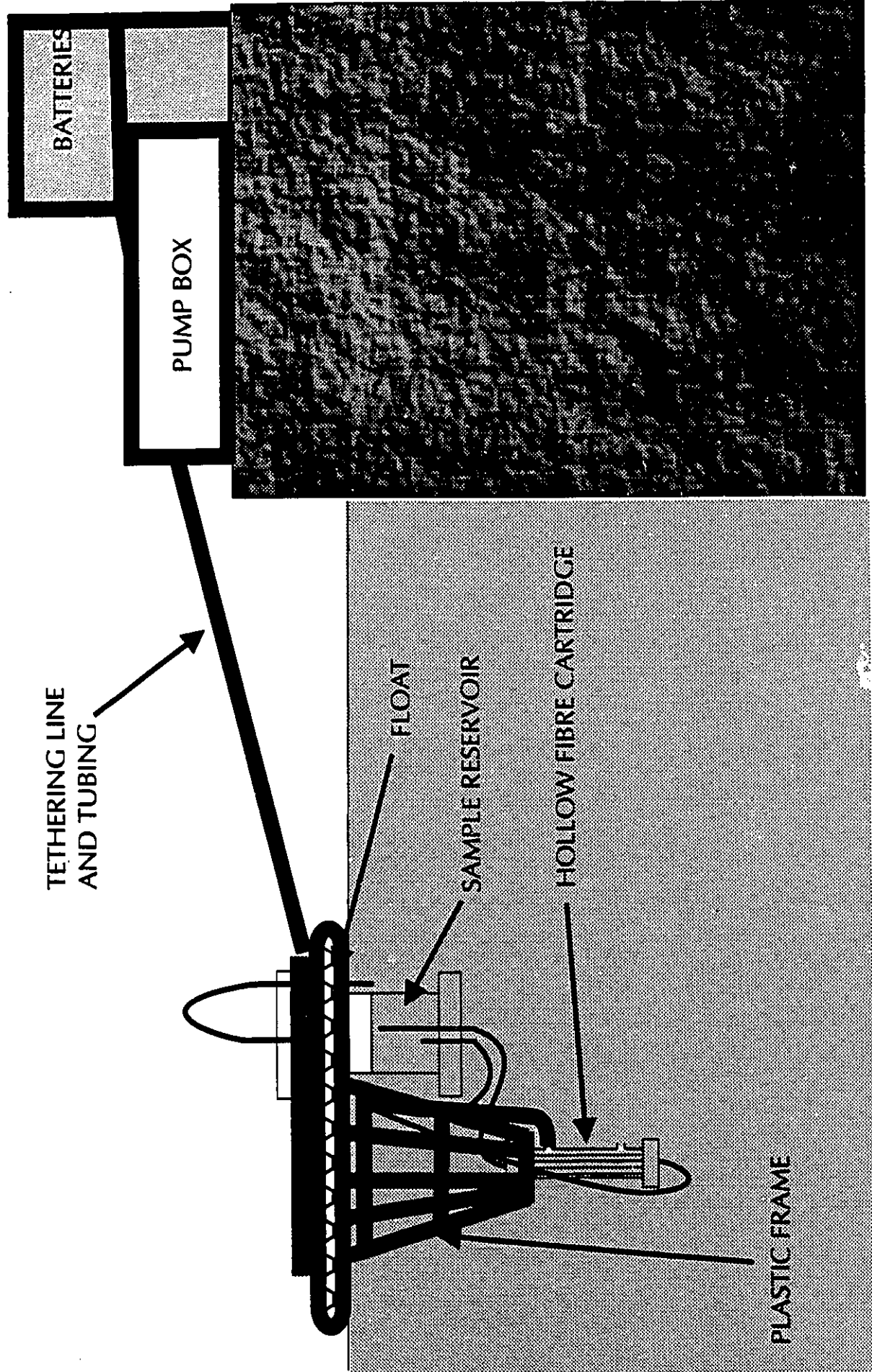
#### **6.2.2.3.3 The portable pump box**

Two small peristaltic pumps (Omegaflex™ OEM pumps, Models FPU106 and FPU109, OMEGA Engineering, Inc., Stamford,CT) were housed in a plastic box. The two pumps' electrical circuits were connected in parallel and each pump was equipped with a separate switch. A small glass sampling port with a self-sealing silicone gasket was placed in the *I* circuit before the *I* circuit pump.

The pump box was very portable and rugged. On arrival at a field site, the *I* and *E* circuit tubing were passed through holes in the side of the box and connected to the peristaltic pumps. After the power supply was connected to the pumps' electrical circuit, the pump box was operational. The housing of the box protected the pumps and tubing from rain and direct sunlight.

**Fig. 6.2 :** The schematic representation of the set-up of the HFD system during field work in Malaysia.

The hollow fiber cartridge was submerged in the river water from a polystyrene float. The sample reservoir was also partially submerged from the same float. The float was anchored to shore by tethering lines. The sample reservoir and hollow cartridges were connected to the peristaltic pumps in the pump box by connecting tubing. The in-line sample port was also located in the pump box. The pumps were powered by two 12 V lead acid batteries.



#### 6.2.2.3.4 Power supply

Electrical power to run the peristaltic pumps was provided by two 12 V lead acid car batteries which were connected in series to provide a combined D.C. voltage of 24 V.

#### 6.2.2.3.5 Sampling procedure

A syringe with a needle was used to obtain samples from the *I* circuit. The needle was inserted into the sample port through the silicone gasket. A 2.0 mL volume of water was drawn into the syringe and then expelled. This was repeated three times before finally a 2.0 mL sample was taken. This sample was then processed as previously described for the enumeration of mixed microorganisms.

#### 6.2.2.3.6 Control of volume changes and gas production

Gas may be released from the water in the reservoir due to photosynthetic activities of algae or a drop in gas solubility of the water due to temperature changes. This could result in the accumulation of such gases or gas bubbles within the *I* circuit which would interfere with the flow of water through the circuit.

Experience with the HFD system in the laboratory and in the field in Canada also indicated that there was a need to monitor the volume in the *I* circuit. Usually, there was a net volume gain or loss for the *I* circuit as it passes through the hollow fiber cartridge. This volume change resulted from actual filtration of water across the hollow fiber surfaces driven by the transmembrane pressure (TMP). The TMP is not constant but changes along the length of the hollow fiber cartridge due to flow resistance in the fibers and the *E* circuit path in the cartridge.

The direction and scale of the volume flux was affected by flow-rate, the direction of flow of the circuits through the hollow fiber cartridge and hydrostatic pressure.

The volume of the *I* circuit could be maintained at a relatively constant level by regular manual manipulation of flow-rates and direction of flow of both the *I* and *E* circuits as was done in the laboratory and in the field in Canada. However, for the Malaysian field work, it was decided to incorporate some form of automatic, self-compensatory mechanism in the HFD system to maintain the *I* circuit volume.

Initially, an attempt was made to link a volume or level sensor to some electrical switch to allow automatic adjustments of flow rate or direction of flow to maintain the *I* circuit. Two types of level sensors were considered. The first employed the use of a long stemmed glass float. The internal reservoir had to incorporate a sintered glass neck at the top in which the sintered stem of the glass float could rise or fall. As the level of water in the reservoir changed, the float would rise or fall accordingly and activate the appropriate electrical switch. This design was constructed but while it appeared to work in the laboratory, the stem of the glass float turned out to be too fragile for use in the field.

The second sensor considered employed the use of ultrasound to determine water level. The sensors emit a sound wave that is reflected from the water surface and redetected by the sensors. The water level can then be ascertained by the time between the emission and detection of the sound wave. A number of

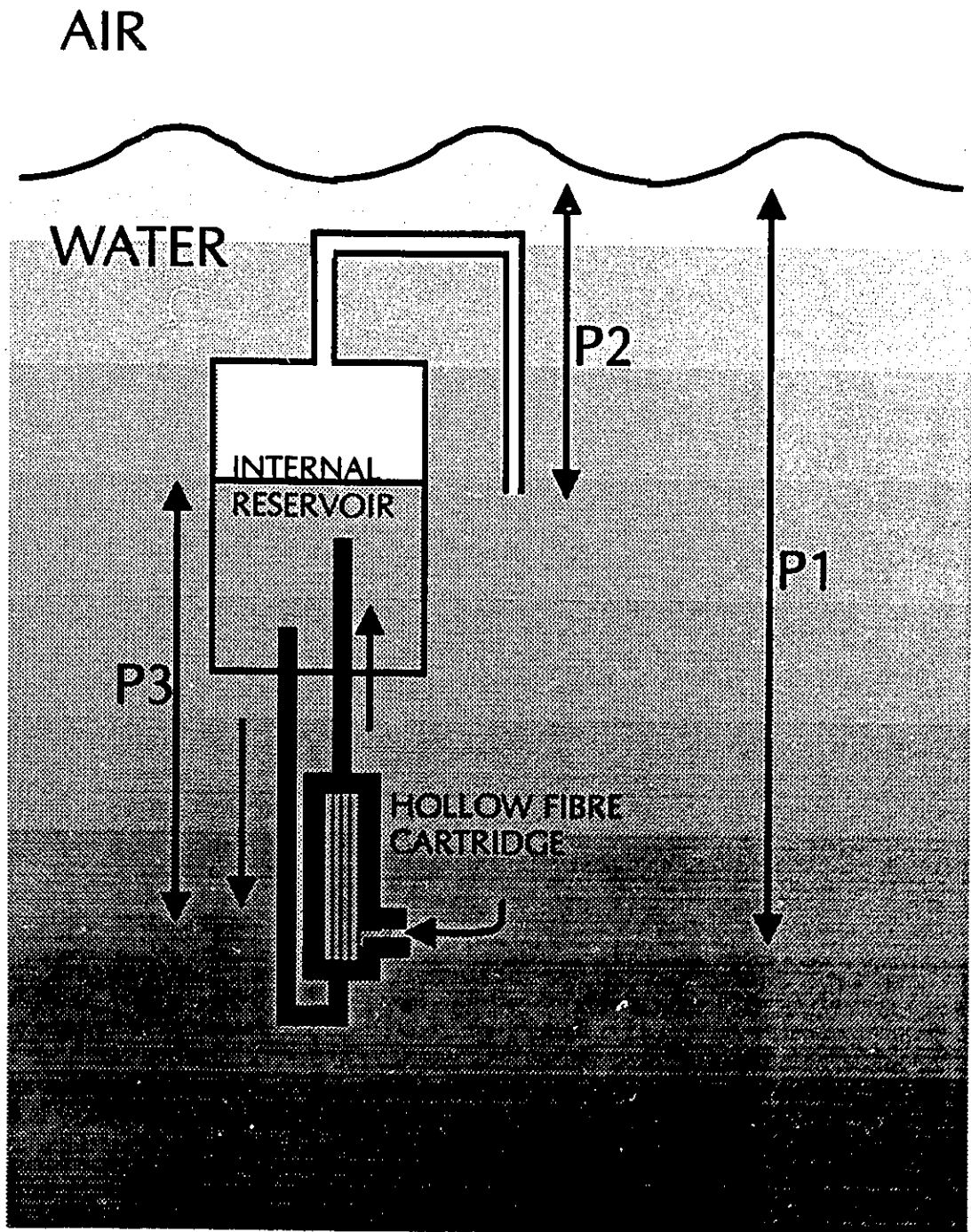
commercially produced ultrasound level sensors are available for detection of chemical levels in tanks or drums and for use in aquariums. However, since it is difficult to raise large amounts of the viruses used in these studies, it was important to limit the volume of the internal circuit. The volume used during the field work in Malaysia was 350 mL. The commercial ultrasound sensors were physically too large to be incorporated into reservoirs for such small volumes. While it is possible to construct an ultrasound level sensor with smaller dimensions, there was insufficient time to do so before the start of the Malaysian field trips.

Finally, it was decided to put aside the use of level sensors and to develop an alternative design which was based on the use of counterbalancing hydrostatic pressure. Key to the functioning of this design was the inclusion of the inverted U-shaped tubing at the top of the *I* reservoir.

With the *I* reservoir submerged in the water and the hollow fiber cartridge submerged about 0.6 m deeper than the *I* reservoir, the hydrostatic pressure will act across the hollow fiber membrane surfaces to cause a net influx of liquid into the *I* circuit. This would result in an increase of volume of water in the *I* circuit and an associated rise in the water level in the *I* reservoir. The water level would however only rise until it was level with the end of the inverted U-shaped tubing that was open to the external water. At that point, the hydrostatic pressures acting at both ends of the air column trapped in the U-shaped tubing would be identical, ensuring no rise in the level of water in the *I* reservoir. This is represented in Fig. 6.3 where  $P_1 = P_2 + P_3$ .

**Fig. 6.3 :** The use of counterbalancing hydrostatic pressure to maintain a constant fluid level in the internal reservoir.

The design allowed the level of water in the internal reservoir of the HFD system to be adjusted and maintained by altering the level of the open end of the inverted U-shaped tubing. The level of the water in the reservoir was maintained by counteracting hydrostatic pressures such that  $P_1 \approx P_2 + P_3$ .



The arrangement also allows any excess gas released into the *I* reservoir from the water to escape by bubbling out of the inverted U-shaped tubing. In this way both the dispersal of excess gases and *I* circuit volume control was achieved.

### **6.2.3 Batch controls**

Batch controls were carried out in parallel to the HFD system in all the field experiments. This was done to provide a comparison of the HFD system which is in dynamic equilibrium with the external environment with the other extreme, that of a closed system. In all cases, the batch controls consisted of a suspension of test microorganisms in 250 mL of freshly collected river water that was placed in a 500 mL wide-mouthed bottle. The bottles were loosely capped to allow exchange with the atmosphere and placed in the river or water next to the HFD system reservoir. In this way, the batch samples were maintained at the temperature of the water and exposed to the same ambient light conditions as the HFD system.

### **6.2.4 Enumeration of microorganisms**

The samples were processed as previously described (Section 4.6.2) for the enumeration of organisms from mixed culture samples. All samples were plated onto mT7 and/or KF agar and incubated at the field sites. Coliphage B enumeration was also carried out at the sites. Other samples for PV and MS-2 enumeration were processed and then kept at 4°C for up to 48 h. The PV samples were then stored at -70°C until assayed. The MS-2 samples were assayed upon returning to the laboratory at the end of the field study.

## 6.3 Results

### 6.3.1 Survival of test microorganisms in the Rideau River (Site TA)

Data for the *in situ* survival of microorganisms in the Rideau River are presented in Fig. 6.4 and Fig 6.5. All experiments were carried out over a 48 h period. In some cases, data points apparently end before that period. This was because the level of test organisms had in fact fallen below the range of detection and so no data was available. This was especially true for *E. durans* data at this site. The one exception was with *E. coli* in the HFD system in Fig. 6.5-A, in this case, *E. coli* numbers after 30 h could not be determined due to the overgrowth of the mT7 plates by *Proteus*.

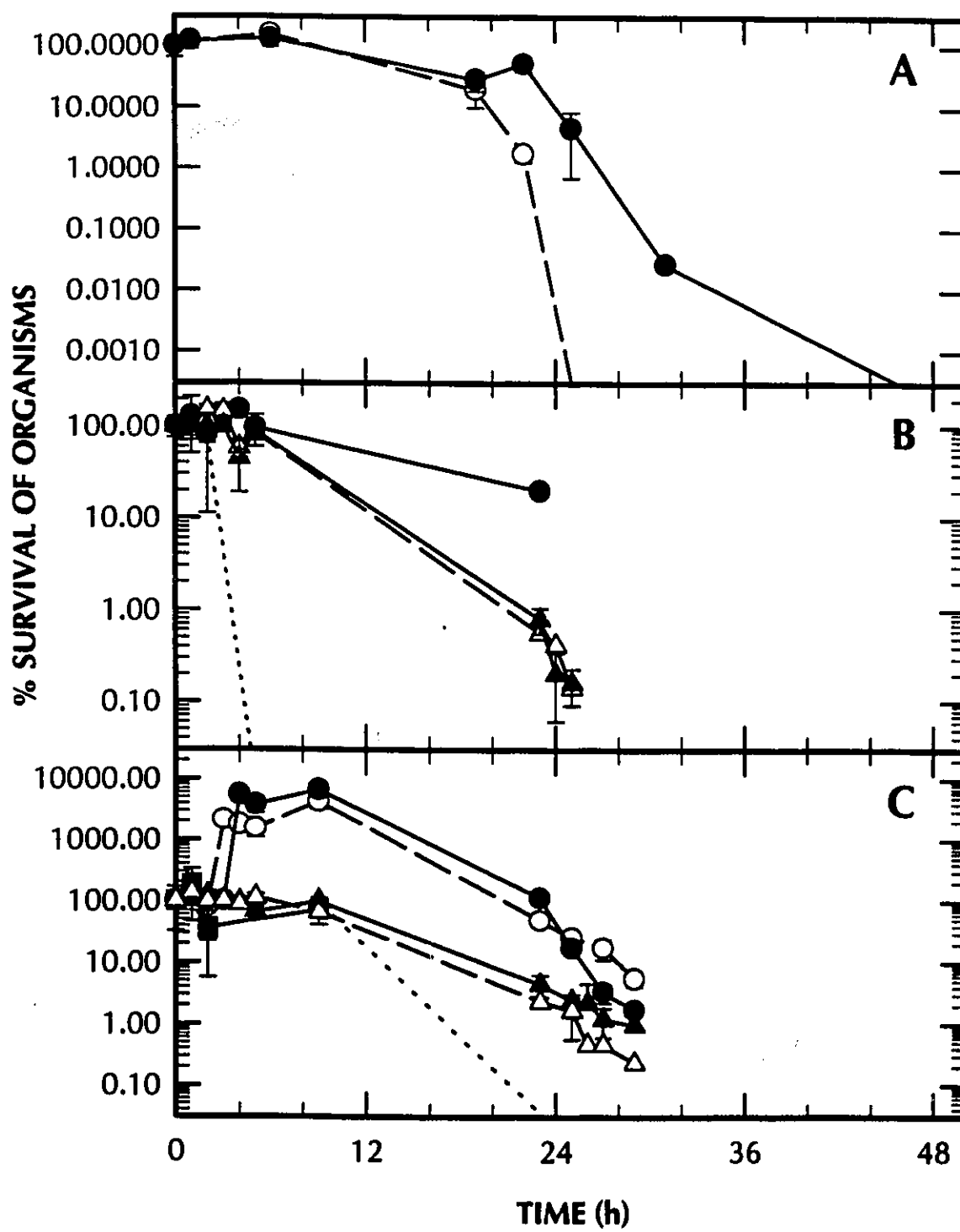
There was quite a large variation in the survival of test microorganisms at different times within the same field study and also between field studies. Both rapid inactivation and the regrowth or multiplication of numbers was observed for some microorganisms. Generally, all test microorganisms in the HFD system survived at least as well and often better than those test organisms in the batch systems.

#### 6.3.1.1 HFD system results

The general order of survival of the test microorganism in the HFD system site TA was *E. coli* > PV > MS-2 > *E. durans*. The longer *E. coli* survival was actually due to the ability of *E. coli* to grow. This was evident during the third and fourth field studies (Fig. 6.4-C and Fig. 6.5-A). During field study 3, *E. coli* growth in the HFD system began almost immediately and was paralleled by a

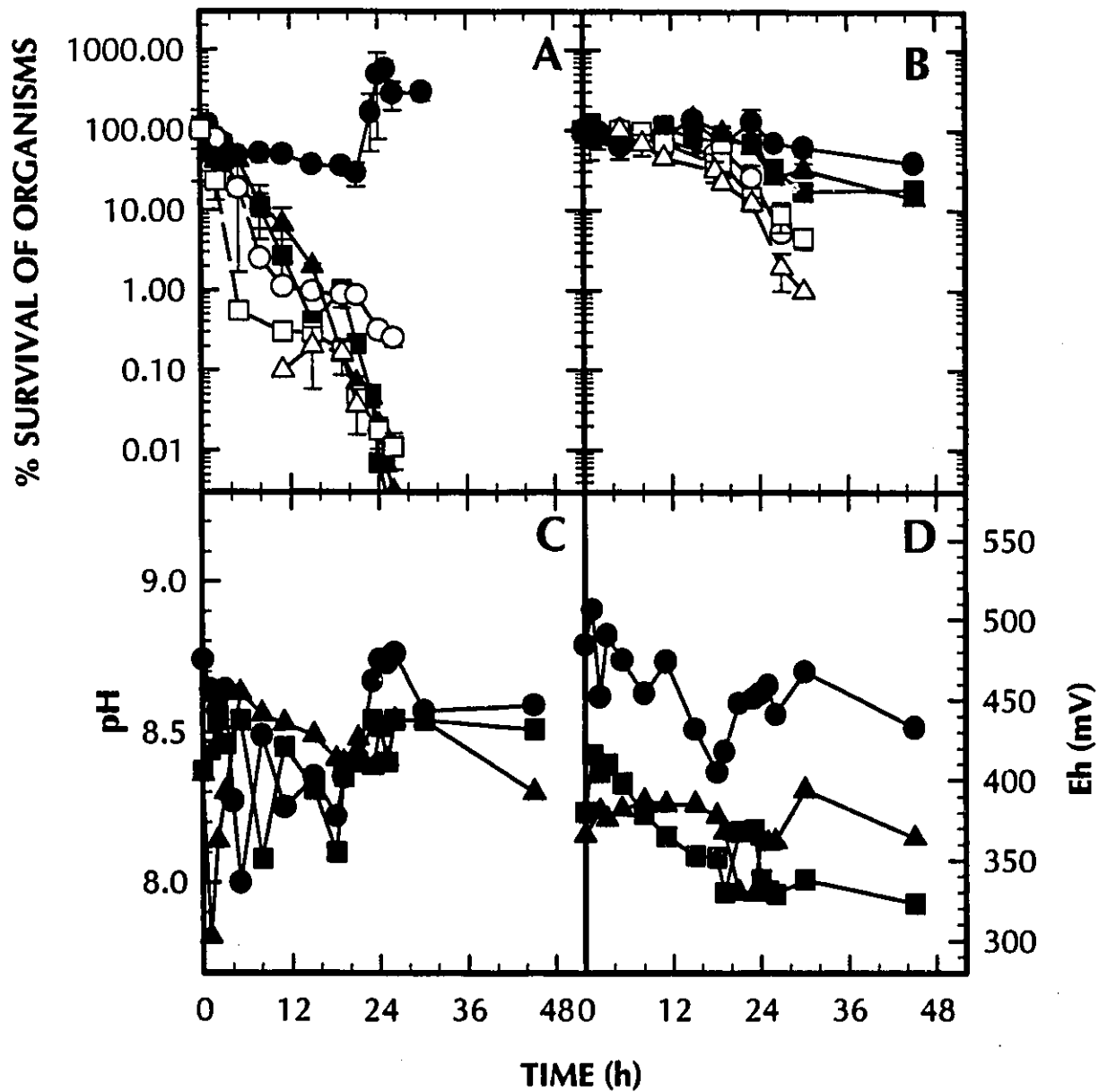
**Fig. 6.4 :** The survival of microorganisms during *in situ* studies at the Rideau River (Site TA).

A - first field trip; B - second field trip; C - third field trip. (●) *E. coli*, (■) *E. durans*, (▲) poliovirus. Closed symbols - HFD system; open symbols - batch sample. For panels B and C, ----- indicates that the *E. durans* numbers fell out of the detection range of the dilutions used for bacterial enumerations.



**Fig. 6.5 :** The comparison of the survival of microorganisms during *in situ* studies at the Rideau River (Site TA) in the HFD system and in filter-sterilized and non-sterilized batch samples.

For all graphs, (●) HFD system, (■) non-sterilized batch sample, (▲) filter-sterilized batch sample. **A** - bacteria, Closed symbols - *E. coli*; open symbols - *E. durans*. **B** - viruses, Closed symbols - poliovirus; open symbols - coliphage MS-2. **C** - pH; **D** - Eh.



growth in the batch control of similar magnitude and duration. This suggests that nutrients and other factors required for *E. coli* growth was present in the original grab sample of river water used in the reservoirs of the HFD and batch systems.

During field study 4, *E. coli* declined slowly ( $K_i = 0.024$ ) over the first 20 h but then its numbers increased rapidly, between 21 and 25 h (Fig. 6.5). By 25 h, it had increased by 1850% over its numbers at 20 h. This spectacular growth of *E. coli* in the HFD system could be linked to a number of observations. Firstly, *E. coli* regrowth was coincident with an increase in pH of the river water at the site (Fig. 6.5). Secondly, the regrowth began at 9.00 am and was therefore coincident with increasing sunlight and water temperatures. Thirdly, the biochemical oxygen demand (BOD) of the river water immediately upstream of the field site peaked at  $4 \text{ mg.L}^{-1}$  about 3 - 5 hours before the onset of *E. coli* regrowth. The background BOD measured throughout the rest of the field study; both before and after that time point was less than  $1 \text{ mg.L}^{-1}$ . Finally, a thunderstorm occurred in parts of the watershed upstream of site TA about 12 h before the onset of *E. coli* regrowth.

Some times, *E. coli* numbers showed no significant change over several hours. While at other times, *E. coli* numbers showed a sharp decline. The range of  $K_i$  values for *E. coli* was 0.000 to 0.202.

*E. durans* was included only in field study 3 and 4 (Fig. 6.4-C and Fig. 6.5). In each case, *E. durans* declined more rapidly than *E. coli*. During field study 3, *E. durans* was detected in the HFD system and in the batch sample until 9 h and 2 h, respectively. After that, the numbers fell below the dilution sampling range and

failed to be detected. This rapid drop in *E. durans* in both the systems may be associated to the growth of *E. coli* and other unidentified bacteria in the systems over the first 24 h. The unidentified bacteria formed a number of atypical colonies on mT7 agar and were observed to be increasing in number over that period.

During field study 4 (Fig. 6.5), *E. durans* decline seemed to follow a diurnal pattern. There was an initial 11 h period of decline ( $K_i = 0.190$ ), followed by a "plateau" phase ( $K_i = 0.007$ ) lasting 8 h and finally a second phase of rapid decline ( $K_i = 0.087$ ) over the next 11 h period. The plateau phase coincided with the hours of darkness at the site and lower water temperatures of 23 - 24°C which compares with the daytime water temperatures of 25 - 27°C.

The survival of MS-2 phage was only followed during field study 4 (Fig. 6.5). There was little difference in the survival of MS-2 between the HFD system and the batch samples. The  $K_i$  value for MS-2 phage at site TA during the period of the study ranged from 0.000 to 0.133.

During field study 2 and 3, PV was stable in the HFD system for the first 3 and 5 h respectively (Fig. 6.4). Similar periods of stability were observed for the batch samples. In the HFD system, PV declined rapidly after this period with  $K_i$  values of 0.102 to 0.124.

There was no stable period during field study 4 (Fig. 6.5). Instead, PV showed a slower but constant decline over the entire study period ( $K_i = 0.003$ ).

HAV was included and recovered from each of the HFD and batch samples used during studies at site TA. However, the titres recorded for  $t = 0$  were between 100- to 1000-fold lower than had been expected from the virus stock used to seed the water. The standard deviations of the titres were also very large. The titres were considered to be unreliable.

#### 6.3.1.2 Batch sample results

The general order of survival of the test microorganisms in batch samples of Rideau River water was PV > MS-2 phage > *E. coli* > *E. durans*. *E. coli* did not survive as well in batch samples as in the HFD system. However, during field study 3, *E. coli* regrew in the batch sample as well as the HFD system (Fig. 6.4-C). The *Ki* values determined for *E. coli* in the batch samples were between 0.032 - 2.040.

*E. durans* was detected for up to 2 h after the start of field study 3 but after that it declined too rapidly to be detected. During field study 4, the diurnal pattern of inactivation seen with HFD system was also seen with the batch samples (Fig. 6.5). There was a plateau phase (*Ki* = 0.031) sandwiched between two periods of rapid decline. The range of *Ki* values for *E. durans* in batch samples was 0.000 to 0.290.

The survival of MS-2 coliphage in batch samples during field study 4 (Fig. 6.5), indicated two periods of inactivation with a short period of stability in between for both the sterilized and non-sterile batch samples. For the non-sterile

batch sample, the  $K_i$  values were 0.033, 0.000 and 0.082 while for the sterilized batch sample it was 0.037, 0.000 and 0.159.

The PV survival pattern in the batch systems were fairly similar to its survival in the HFD system. The  $K_i$  values for PV in batch samples were 0.000 to 0.123.

As in the HFD system, HAV recovery was much lower than expected and the titres were considered unreliable.

There was no difference between the survival of test organisms in a non-sterilized and a sterilized batch sample.

### **6.3.2 Survival of test microorganisms in the Ottawa River (Site TB)**

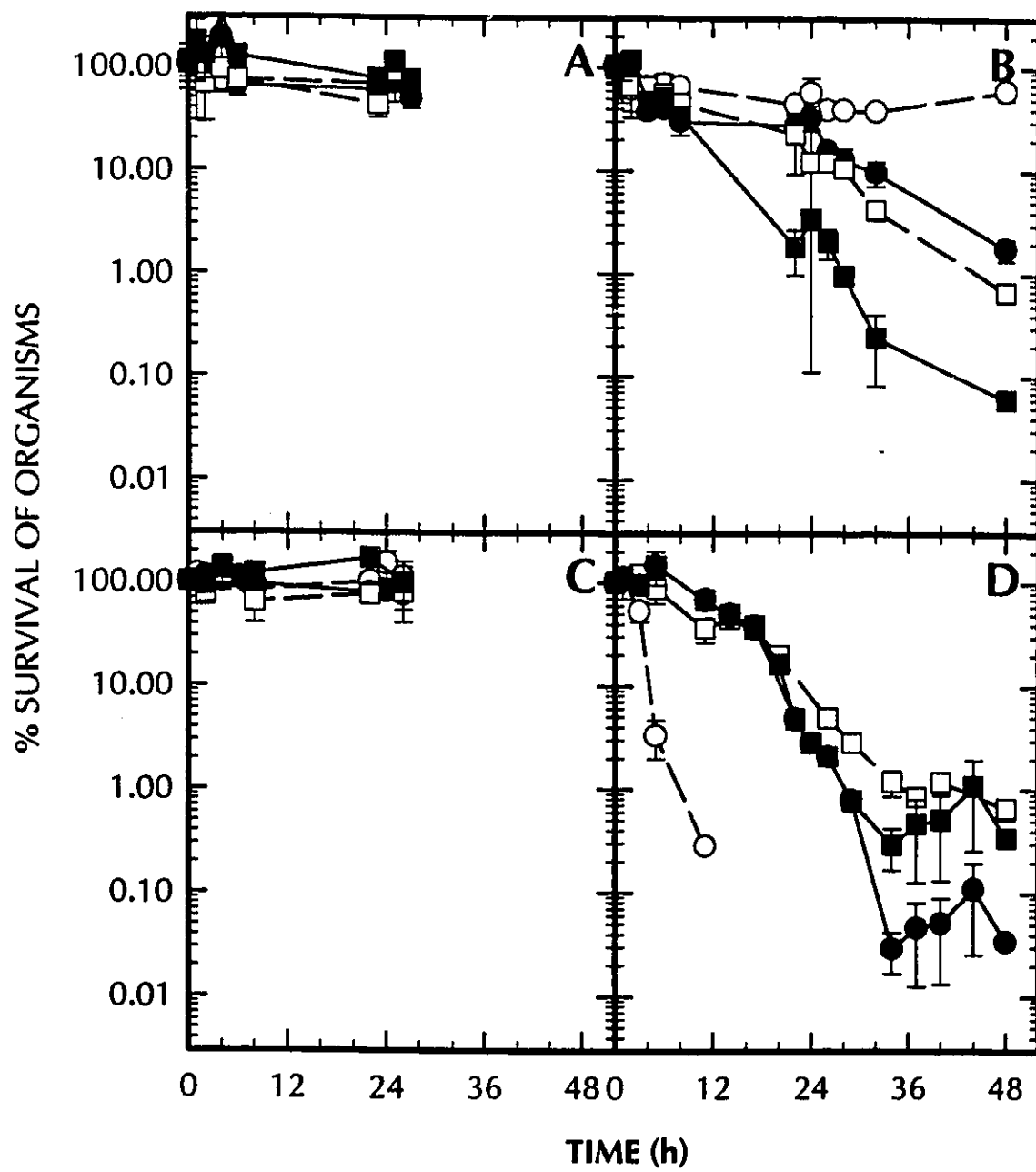
Five field studies were carried out at site TB (Fig. 6.6, 6.7 and 6.8). Field study 1 and 3 were carried out at the ambient river water temperature of 2-4°C. The remaining field studies were carried out at about 20°C.

Occasionally, when the Britannia Water Purification Plant's main intake pump is shut down, a backwash of chlorinated river water enters the Ottawa River. This resulted in chlorine being taken in inadvertently by the study system intake, leading to chlorine in the experimental reservoirs during field study 3 (Fig. 6.8) and field study 5 (Fig. 6.6-D and 6.7-D).

The presence of chlorine during the later part of field study 3 was not determined directly but was represented by the spike in the Eh readings at 44 - 46 h (Fig. 6.8). It was determined that the plant's main intake pump had been shut down at 43 h and after consultation with plant personnel, it was believed that the

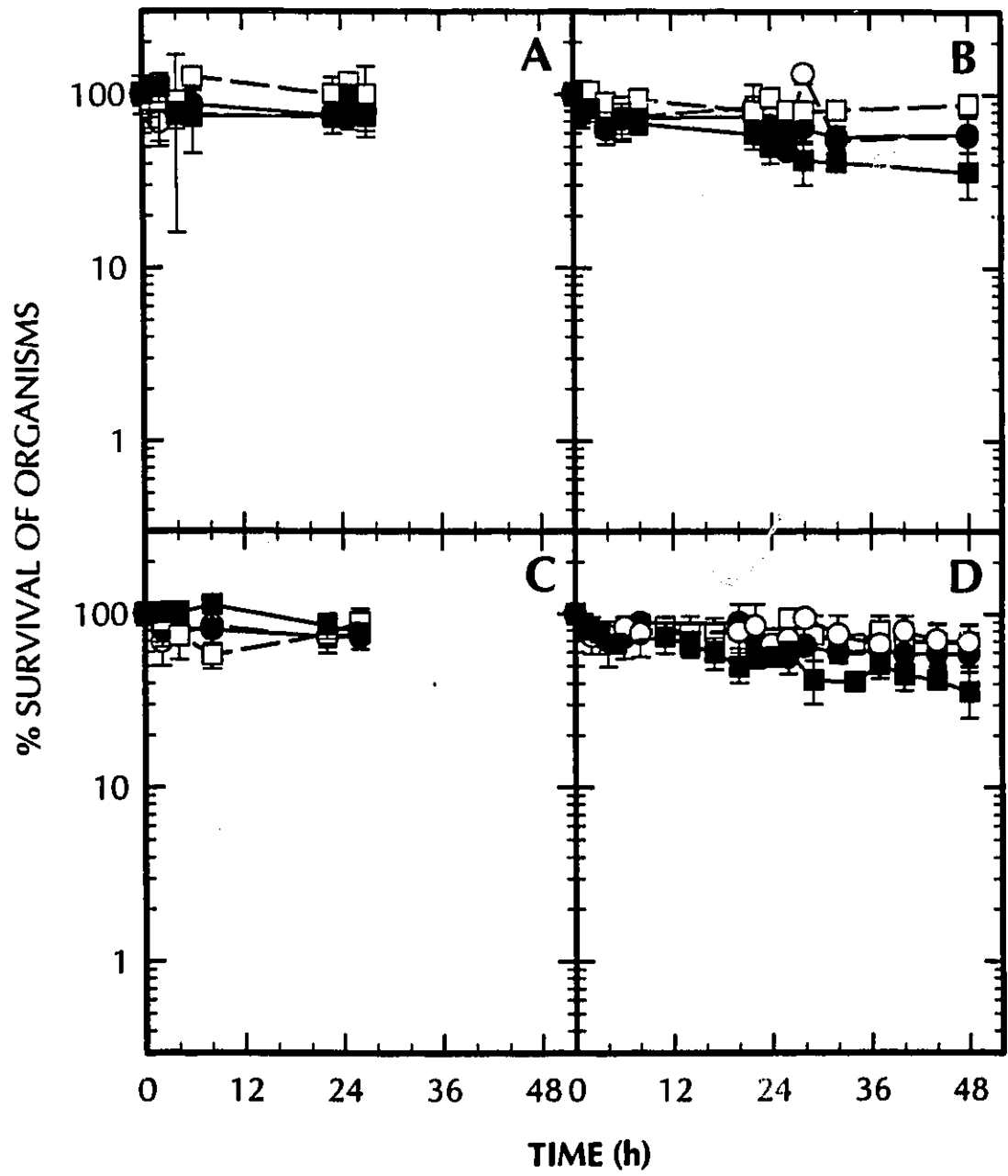
**Fig. 6.6 :** The survival of bacteria during *in situ* studies at the Ottawa River (Site TB).

**A** - first field trip; **B** - second field trip; **C** - fourth field trip; **D** - fifth field trip. (●) *E. coli*, (■) *E. durans*. Closed symbols - HFD system; open symbols - batch sample.



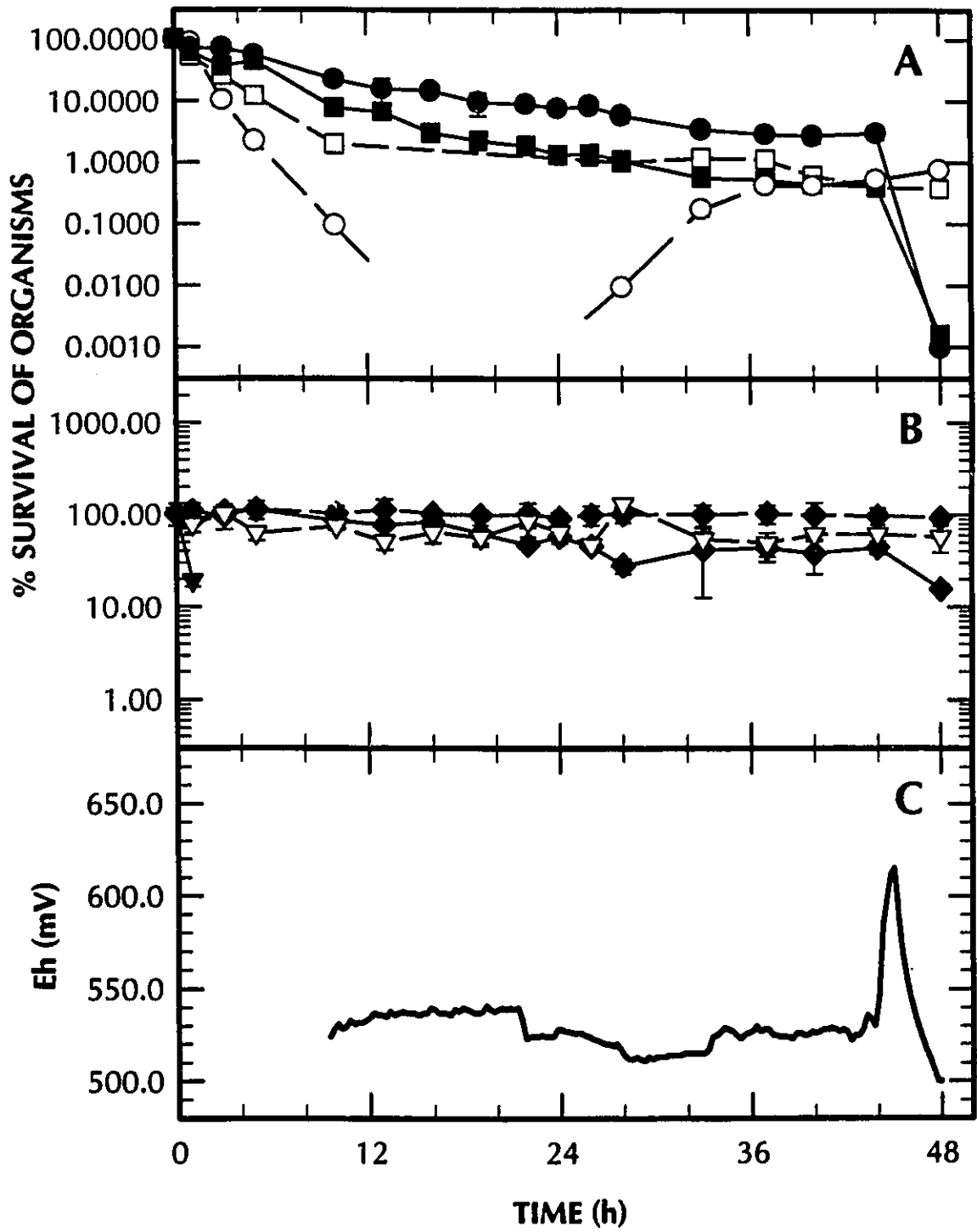
**Fig. 6.7 :** The survival of viruses during *in situ* studies at the Ottawa River (Site TB).

**A** - first field trip; **B** - second field trip; **C** - fourth field trip; **D** - fifth field trip. (●) MS-2 phage, (■) PV. Closed symbols - HFD system; open symbols - batch sample.



**Fig. 6.8 :** The results of field study 3 at the Ottawa River (Site TB).

**A** - bacteria, **B** - viruses, **C** - Eh. (●) *E. coli* (■) *E. durans*, (▼) MS-2 phage, (◆) PV. Closed symbols - HFD system; open symbols - batch samples.



observed effects was a result of a chlorine backwash into the Ottawa River. During field study 5, a similar but smaller peak in Eh values was observed. In this case, chlorine levels were monitored and was detected in the experimental reservoir (0.04 -0.09 ppm total and 0.02 -0.04 ppm free chlorine) from 19 to 20 h and again from 23 to 48 h (0.02 - 0.08 ppm total and 0.02 - 0.06 ppm free chlorine).

#### 6.3.2.1 HFD system results

At 2 - 4°C, there was no significant change in numbers of test microorganisms in the HFD system. However, at 20°C decline of microorganisms was observed. This was also true for the batch samples. At 20°C, the general order of survival was MS-2 phage > PV > *E. coli* > *E. durans*. It was observed that *E. durans* was more resistant to chlorine than *E. coli* while MS-2 and PV were more resistant than the bacteria.

There were generally no multiple phases or diurnal patterns of inactivation; nor was there regrowth of bacteria as had been seen in the Rideau River. This is not unexpected since the experiments were being performed within the Britannia Water Treatment Plant and were therefore not directly exposed to factors such as sunlight that change through the day. The inactivation constants,  $K_i$ , of *E. coli*, *E. durans* MS-2 and PV was 0.027 - 0.037, 0.027 - 0.073, 0.004 - 0.007 and 0.003 - 0.008, respectively. During field study 3, the MS-2 in the HFD system decreased very rapidly but the data was not available for  $K_i$  estimation. The inactivation constant,  $K_i$ , for *E. coli*, *E. durans* and PV due to chlorine at 44 -48 h of field study 3 was 0.874, 0.874 and 0.604, respectively while that for the low chlorine levels

between 19 and 48 h in field study 5 for *E. coli*, *E. durans*, MS-2 and PV was 0.095, 0.046, 0.004 and 0.006, respectively.

#### 6.3.2.2 Batch samples results

At 2 - 4°C, there was no significant change in microorganism numbers in batch samples. At 20°C however, the general order of survival of test microorganisms was PV > MS-2 phage > *E. durans* > *E. coli*. On one occasion, *E. coli* demonstrated regrowth after 28 h (field study 3; Fig. 6.6-C). The inactivation constant, *K<sub>i</sub>*, determined for *E. coli*, *E. durans*, MS-2 and PV was 0.001 - 0.252, 0.032 - 0.103, 0.002 - 0.043 and 0.001 - 0.002, respectively.

#### 6.3.3 Survival of test microorganisms in the Kennedy-Burnett Stormwater Pond (Site TC).

Seven field studies were carried out at this site. However, in the initial few experiments, the bacterial die-off was so rapid that bacterial numbers fell below the level of detection almost immediately and were no longer detected by 2 h. The reasons for this rapid drop are unclear but on at least one occasion, the incomplete removal of hydrogen peroxide, used in decontamination of hollow fiber cartridges may have been responsible. This occurred due to the rapid turnaround and reuse of the cartridges. These early experiments were carried out during heavy rainfall and pond overflow conditions when the Eh was generally higher (Eh = 500 - 650 mV). It is possible that some factor(s) in the water during the early experiments may have caused the rapid inactivation of test microorganisms. Field study 6 and 7 were the only experiments that provided reliable bacterial results with a slower rate of decay. These were also the only field

studies carried out under non-overflow conditions and the initial Eh was generally lower (400 -550 mV). The results of field study 6 and 7 are presented in Fig. 6.9.

#### **6.3.3.1 HFD system results**

In the HFD system, *E. durans* generally survived longer than *E. coli*. Both organisms had periods of stability and periods of rapid decay. *Ki* values for *E. coli* and for *E. durans* were in the range of 0.000 - 0.482 and 0.000 - 0.152, respectively.

#### **6.3.3.2 Batch sample results**

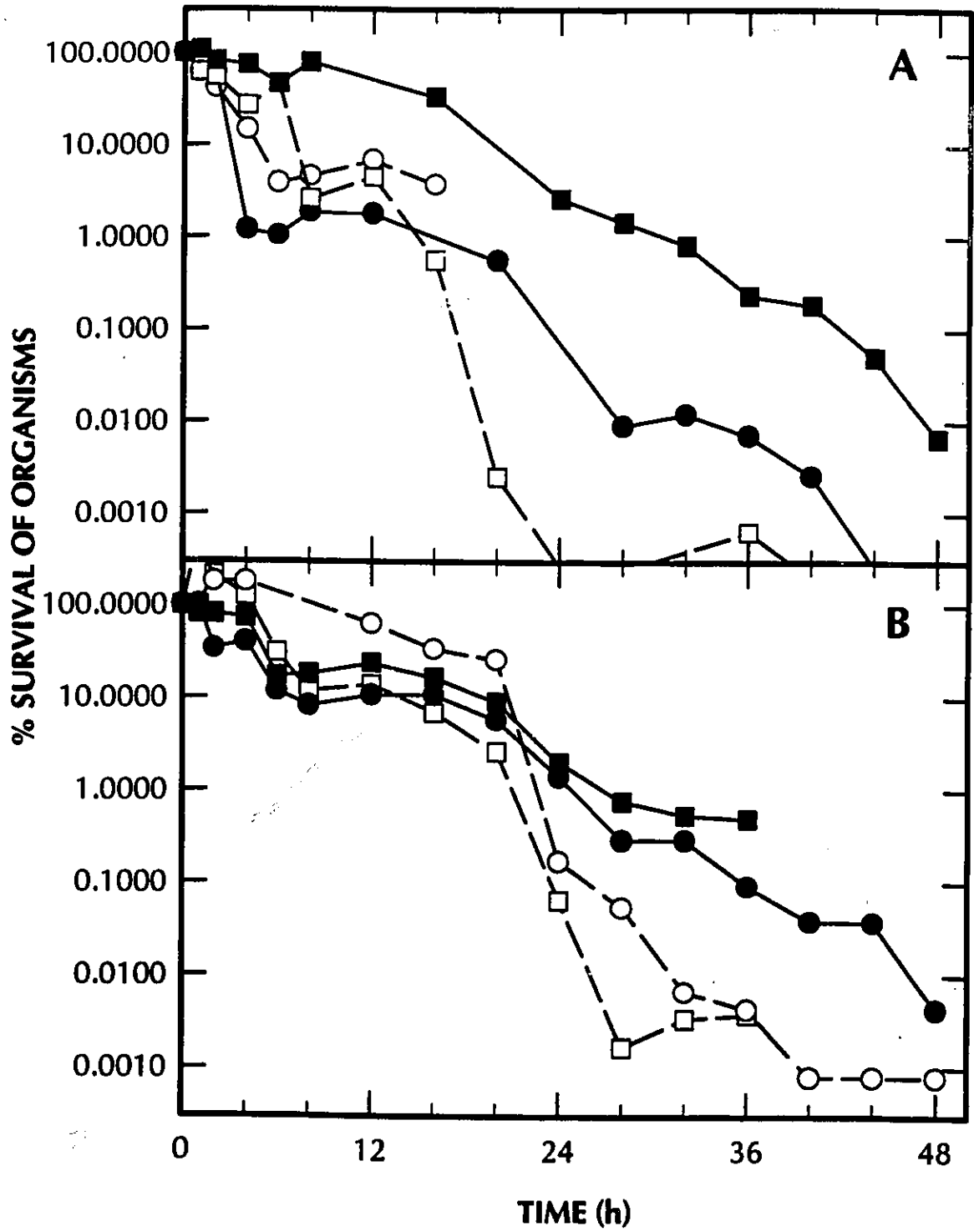
Like the HFD system, the bacteria showed varying decay rates at different periods. However, unlike the HFD system, during field study 7, both *E. coli* and *E. durans* demonstrated an increase of up to 330% and 100% in cfu within the first 4 h. This was then followed by a rapid decline. *Ki* values for *E. coli* and for *E. durans* were in the range of 0.000 - 0.152 and 0.000 - 0.256, respectively.

#### **6.3.4 Survival of test microorganisms in the Gombak River (Site EA)**

There were five field study trips to site EA on the Gombak River. Field study 3 had to be abandoned due to flash-flooding. The results of the other four field studies are shown in Fig. 6.10 and 6.11. Viral survival studies were only included in field study 4 and 5.

**Fig. 6.9 :** The survival of microorganisms during *in situ* studies at the Kennedy-Burnett Stormwater Ponds (Site TC).

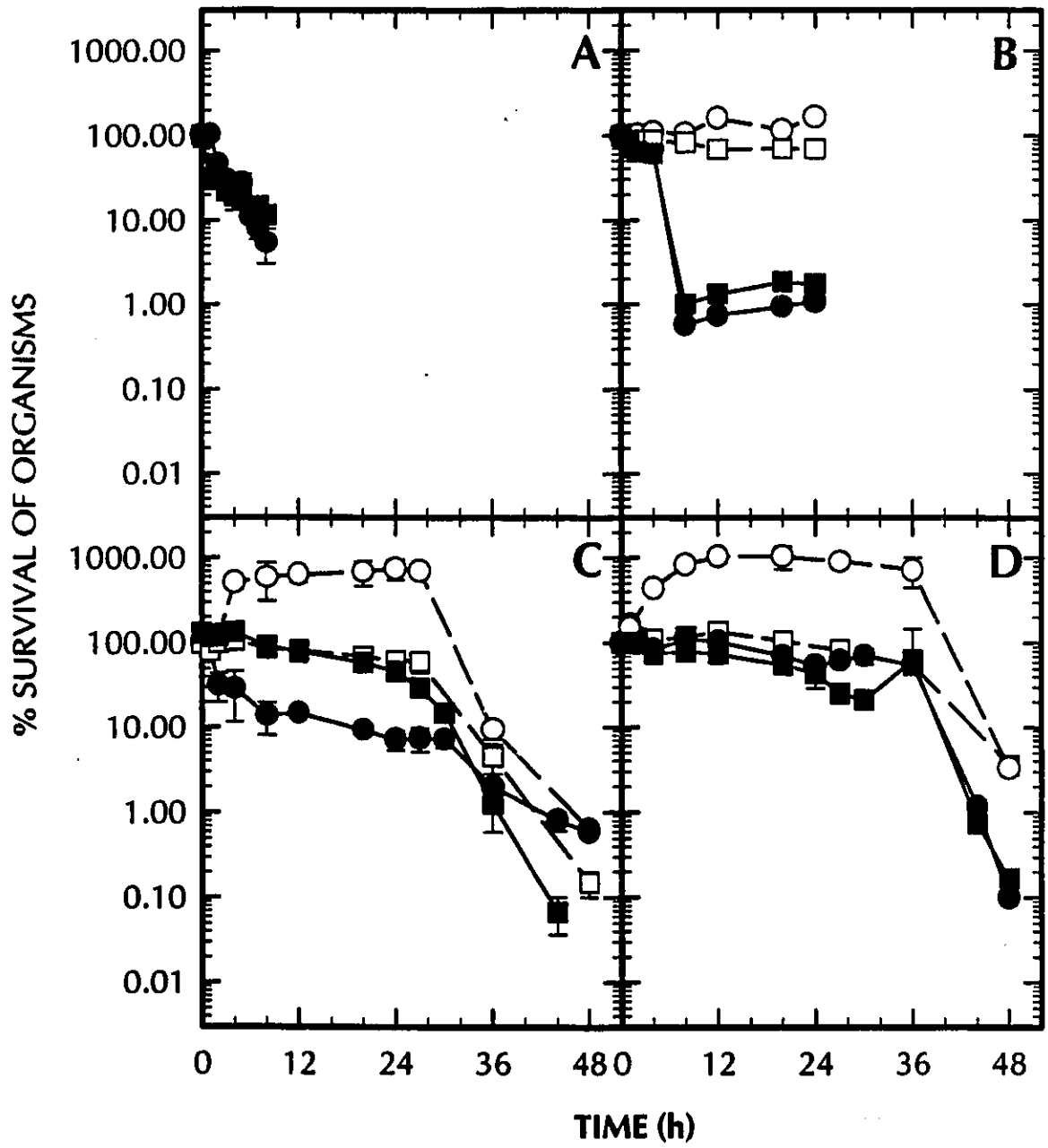
**A** - field study 6, **B** - field study 7. . (●) *E. coli* (■) *E. durans*.  
Closed symbols - HFD system; open symbols - batch samples.



**Fig. 6.10 :** The survival of bacteria during *in situ* studies at the Gombak River (Site EA).

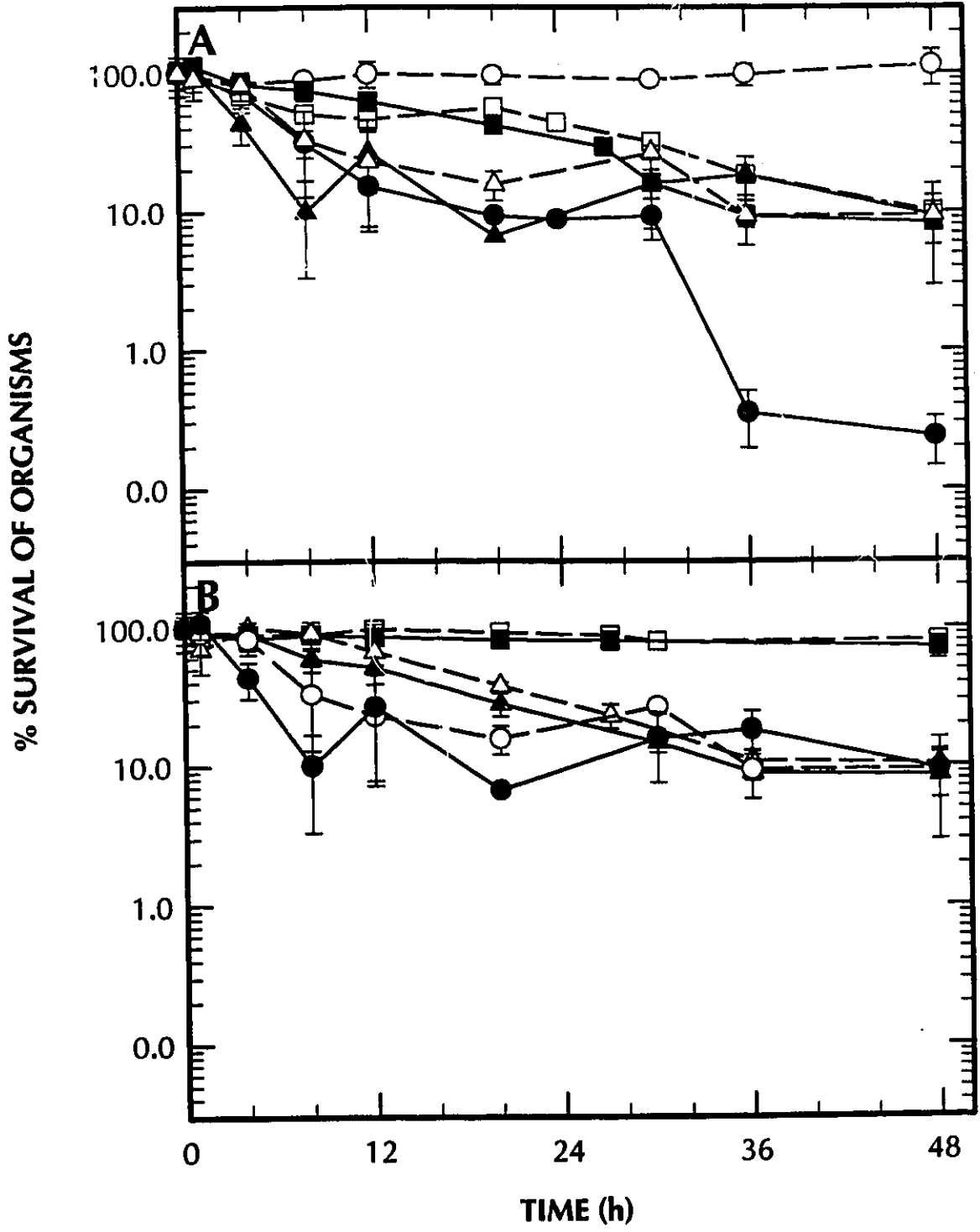
A - first field trip; B - second field trip; C - fourth field trip; D -fifth field trip. (●) *E. coli* , (■) *E. durans*. Closed symbols - HFD system; open symbols - batch sample.





**Fig. 6.11 :** The survival of viruses during *in situ* studies at the Gombak River (Site EA).

**A** - fourth field trip; **B** - fifth field trip. (●) somatic coliphage B, (■) MS-2 coliphage, (▲) poliovirus. Closed symbols - HFD system; open symbols - batch sample.



#### 6.3.4.1 HFD system results

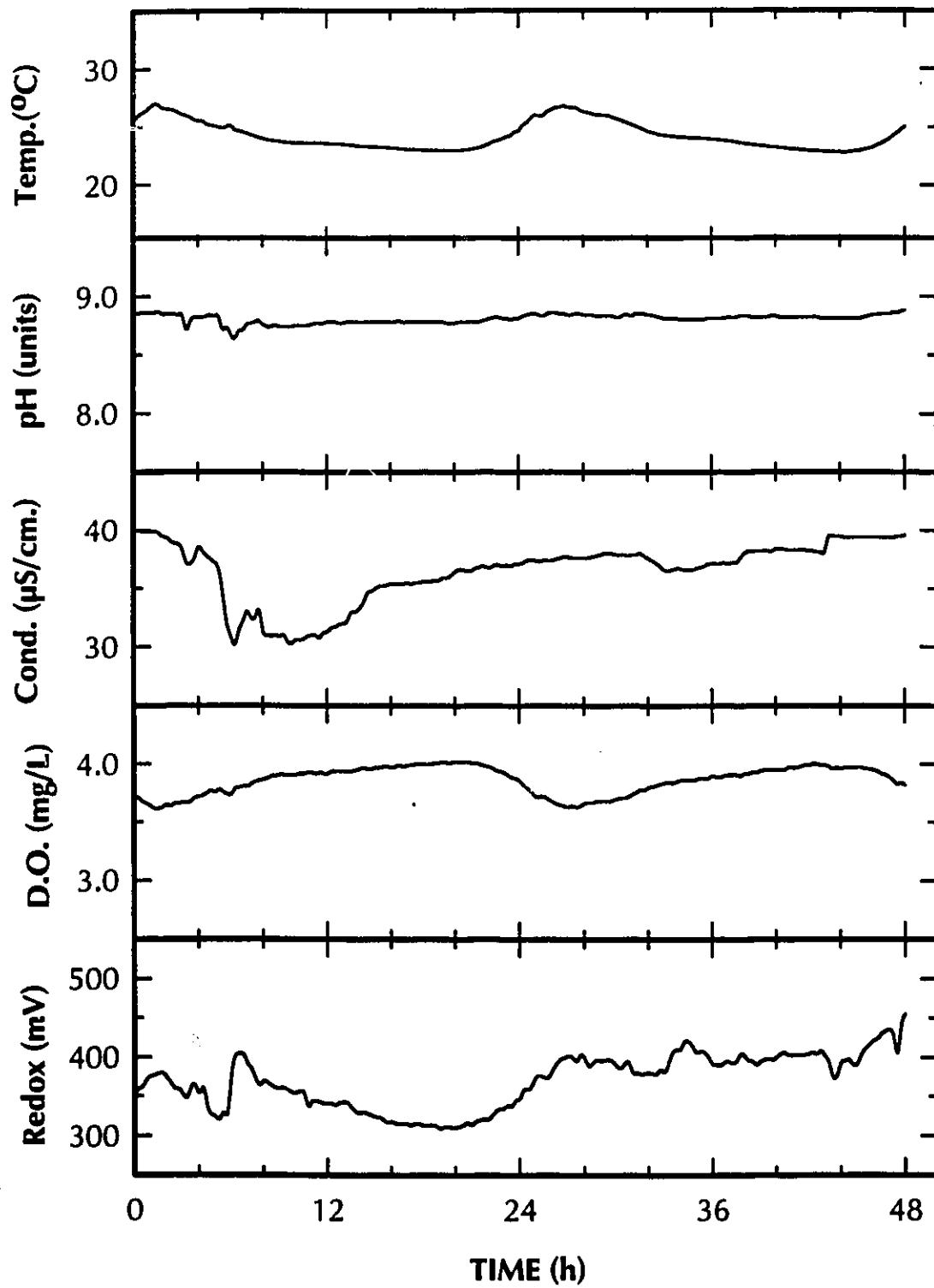
The situation at the Gombak River was similar to that seen at the Rideau River; different rates of inactivation of test organisms were observed at different periods within a field study and also between field studies. However, unlike the situation at the Rideau River, no regrowth of test bacteria was seen. The general pattern of survival was MS-2 phage > PV > phage B > *E. durans* and *E. coli*.

The range of *K<sub>i</sub>* values determined for *E. coli*, *E. durans*, phage B, MS-2 phage and PV in the HFD system were 0.010 - 0.519, 0.014 - 0.447, 0.012 - 0.0237, 0.002 - 0.025 and 0.000 - 0.037, respectively. *E. coli* and *E. durans* survival was very similar in all field studies except field study 3 in which *E. durans* survived better over the initial 24 h but then declined more rapidly over the next 24 h. Rapid and large drops in the numbers of both bacteria were encountered which were contrasted by periods of little or no decline.

During field study 4, phage B appears to have periods of rapid decline (*K<sub>i</sub>* = 0.070 and 0.237) followed by plateaus with less rapid decline (*K<sub>i</sub>* = 0.012 and 0.014). Two periods of each were recorded (Fig. 6.11). The periods of slower decline occurred in the hours after midnight to just after noon. (All field experiments at all the sites were started at noon). The reason for this pattern is unclear but the physico-chemical data of the river water (Fig. 6.12) shows that the period of rapid phage B decline roughly corresponds to periods of rapidly dropping river water temperature and rapidly increasing dissolved oxygen levels. In contrast, the periods of slower decline of phage B roughly corresponded to periods

**Fig 6.12 :** Physico-chemical parameters of the river water during the fifth *in situ* survival study at the Gombak River (Site EA).

Parameters recorded by the Datasonde datalogger include temperature, pH, the specific conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ ), dissolved oxygen (D.O.;  $\text{mg}\cdot\text{L}^{-1}$ ) and the redox potential (Eh; mV). The period shown in the figure corresponds to the period of the microbial survival study during the field study.



of relatively stable or increasing temperature and stable or decreasing dissolved oxygen levels. This phenomenon however was not repeated in field study 5. In this case, the  $K_i$  for phage B was determined to be 0.017.

MS-2 phage had  $K_i$  values of 0.025 and 0.002 for field studies 4 and 5, respectively. PV showed a  $K_i$  of 0.017 for 0 - 36 h but then did not decline for 36 - 38 h of study 4. During field study 5, the  $K_i$  value for PV was 0.037.

#### 6.3.4.1.1 Eh levels and bacterial survival

A comparison of the survival data of the bacteria and the measured physico-chemical parameters of the river water suggests a link between a rapid increase in Eh and a rapid drop in bacterial populations (Fig. 6.13A, 6.13B, 6.14A and 6.14B). For field study 2 (Fig. 6.14B), the Eh rose from 256 mV at 7.5 h to 319 mV at 10.25 h. Both *E. coli* and *E. durans* numbers had dropped almost two orders of magnitude at 8 h as compared to the previous sampling point at 4 h.

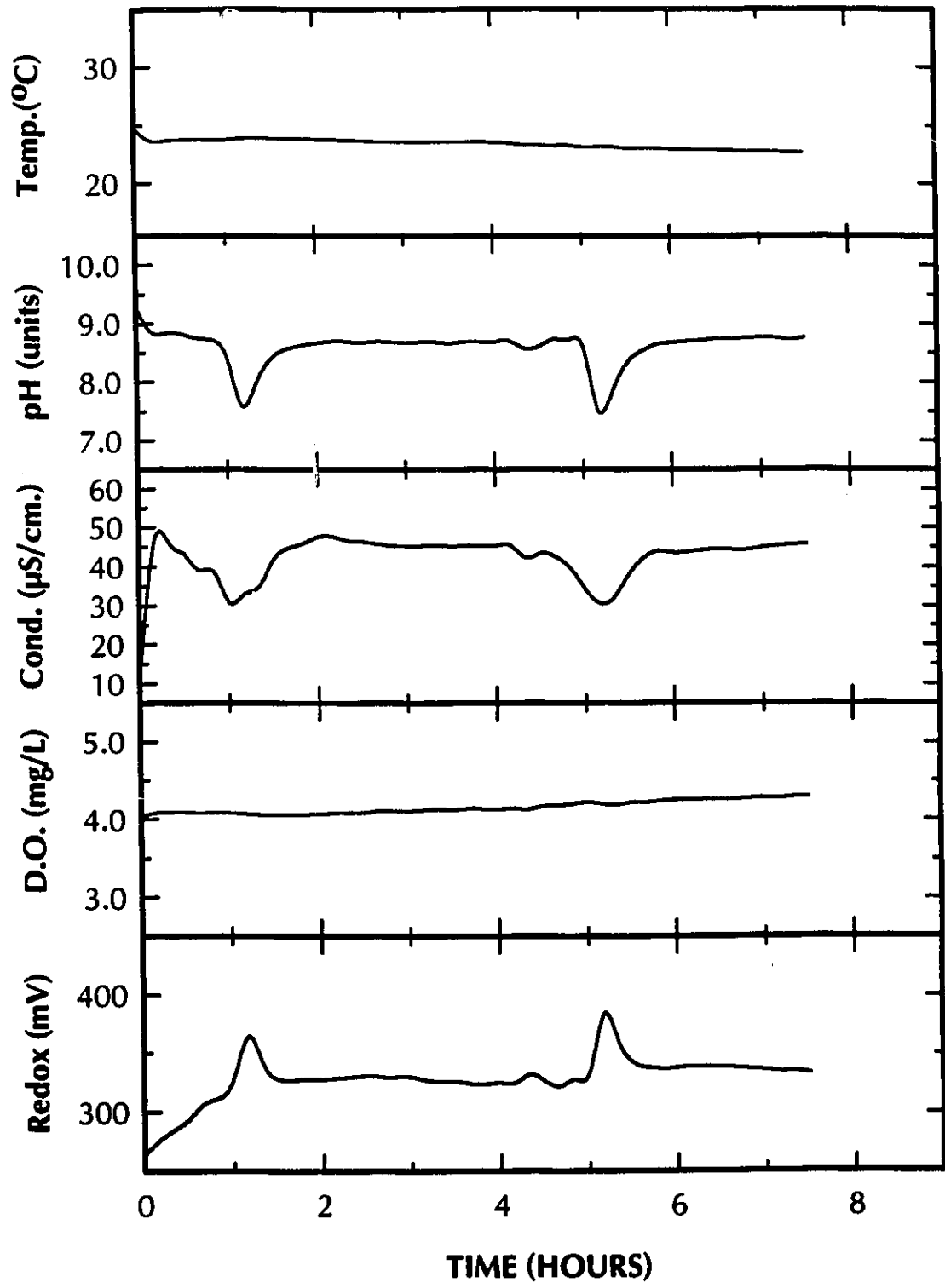
It must however be noted that on other occasions, increases in Eh were not accompanied by drops in bacterial levels (field study 4; Fig. 6.10-C and Fig. 6.12) and similarly a drop in bacterial levels did not occur in concert with a rise in Eh (field study 5).

#### 6.3.4.2 Batch sample results

The viruses showed a constant rate of decline but the bacteria did not in the batch samples. *E. coli* demonstrated regrowth in field studies 2, 4 and 5

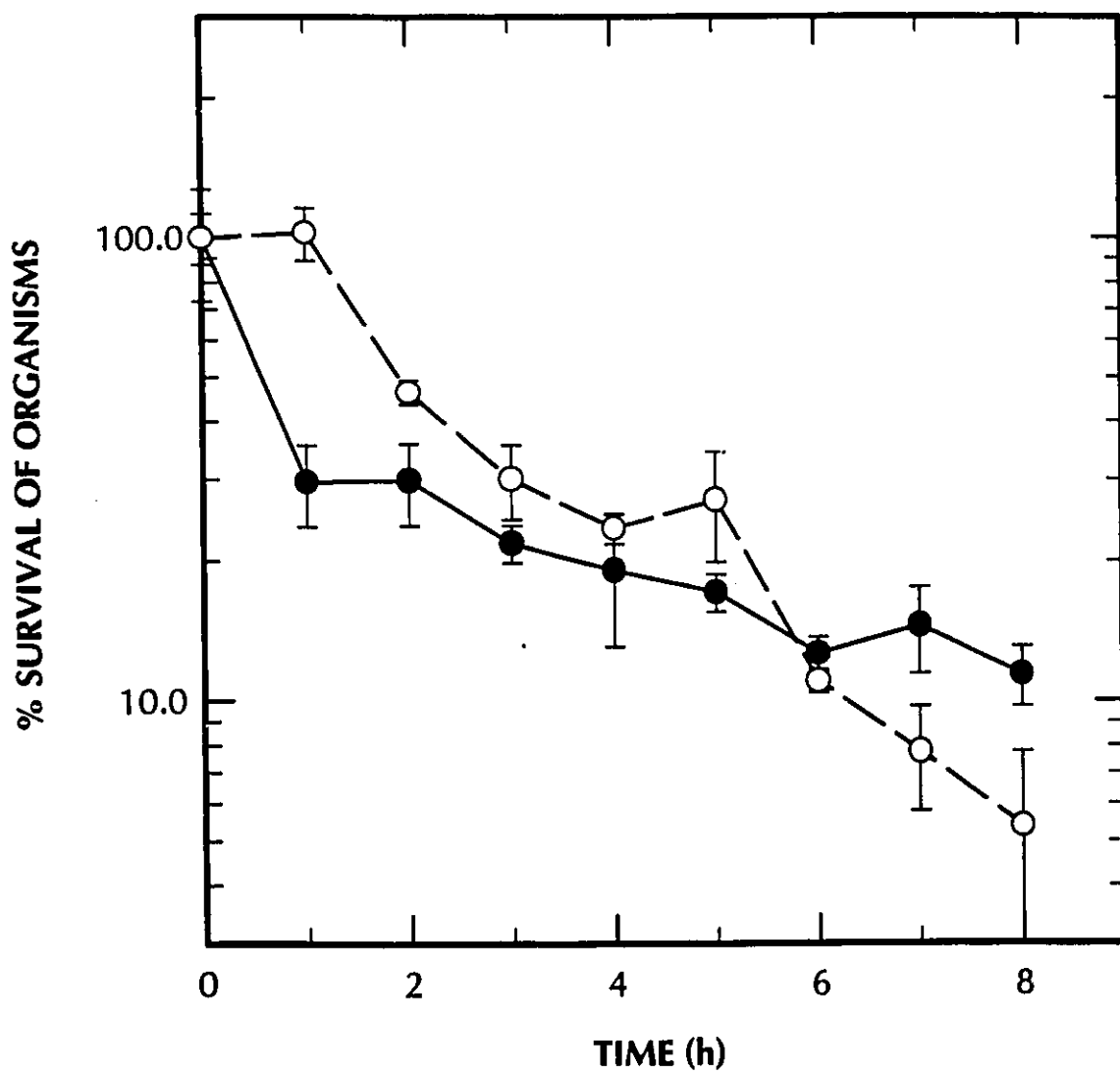
**Fig. 6.13A :** Physico-chemical parameters of the river water during the *in situ* survival study of the first field trip to the Gombak River (Site EA).

Parameters recorded by the Datasonde datalogger include temperature, pH, the specific conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ ), dissolved oxygen (D.O.;  $\text{mg}\cdot\text{L}^{-1}$ ) and the redox potential (Eh; mV). The period shown in the figure corresponds to the period of the microbial survival study in the field.



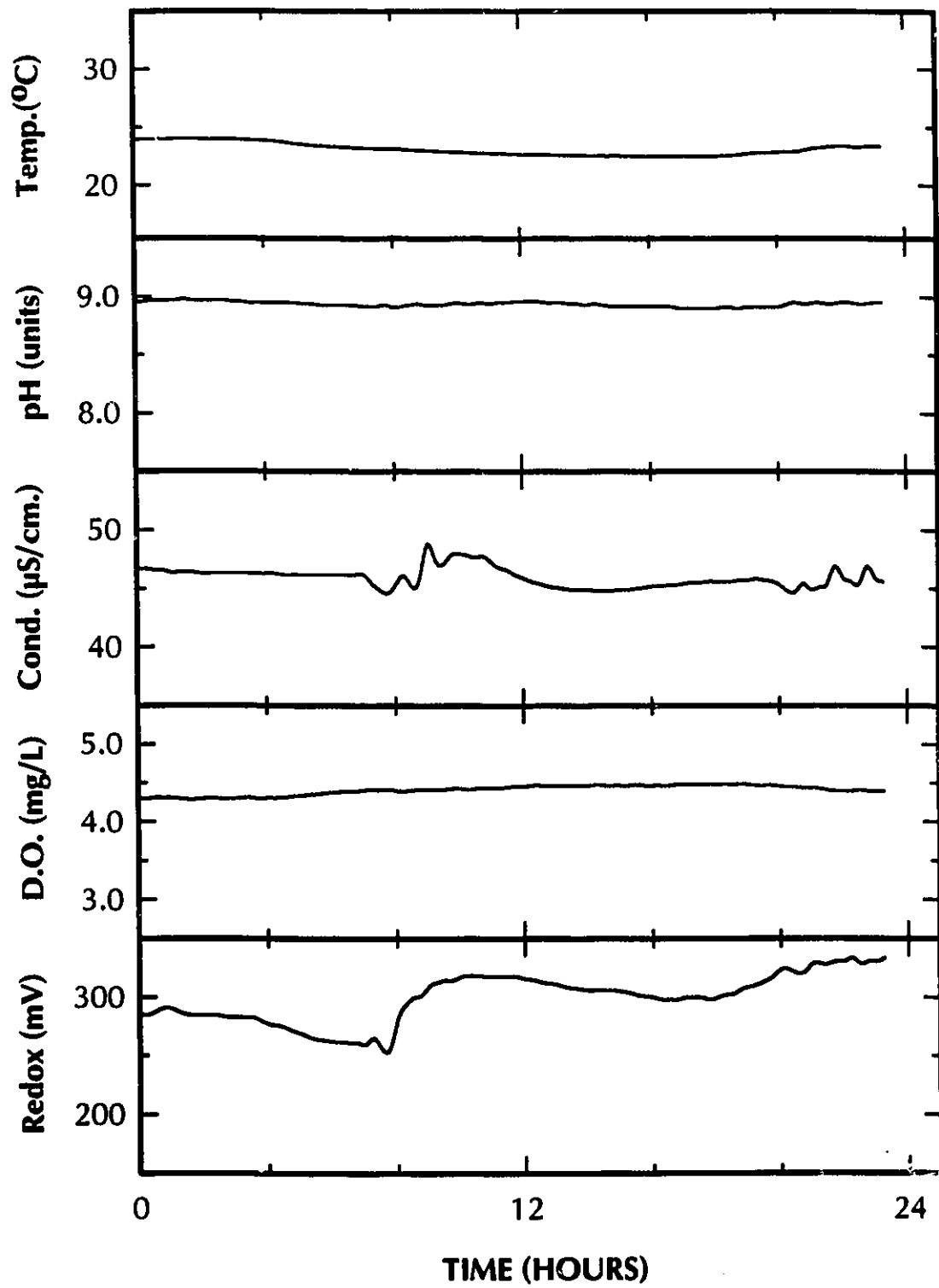
**Fig. 6.13B :** The survival of *E. coli* during the first *in situ* survival studies at the Gombak River (Site EA).

Closed symbols - HFD system, Open symbols - batch sample.



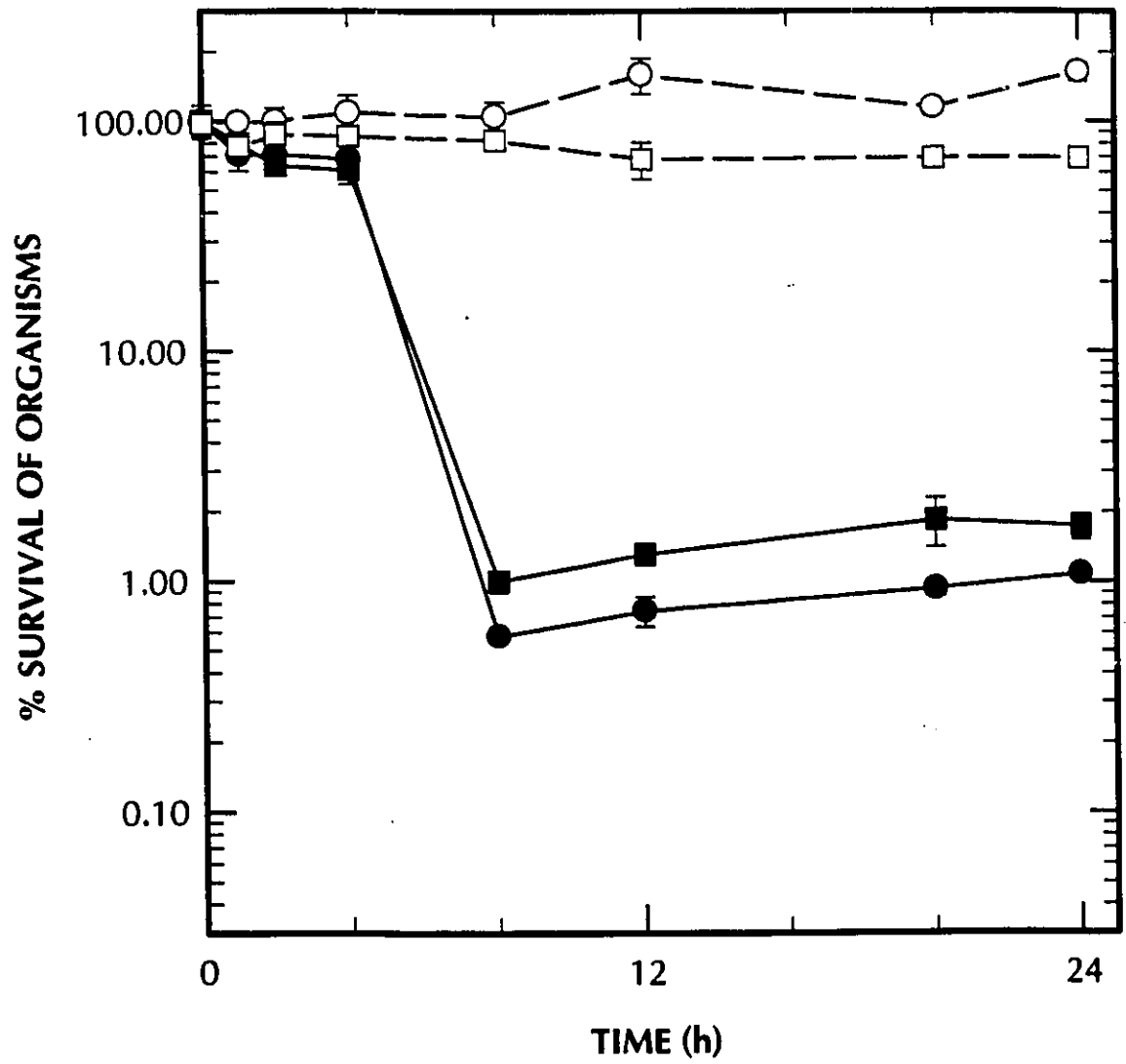
**Fig. 6.14A :** Physico-chemical parameters of the river water during the *in situ* survival study of the second field trip to the Gombak River (Site EA).

Parameters recorded by the Datasonde datalogger include temperature, pH, the specific conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ ), dissolved oxygen (D.O.;  $\text{mg}\cdot\text{L}^{-1}$ ) and the redox potential (Eh; mV). The period shown in the figure corresponds to the period of microbial survival study in the field.



**Fig. 6.14B :** The survival of bacteria during the second *in situ* field study at the Gombak River (Site EA).

(●) *E. coli*, (■) *E. durans*. Closed symbols - HFD system, Open symbols - batch sample.



(Fig. 6.10). The general order of survival of test organisms in Gombak River batch samples was the viruses > *E. coli* > *E. durans*.

*E. coli* regrowth was as high as 940% over the level at the start of the study (field study 5; Fig. 6.10). However, there was often a precipitous drop in *E. coli* numbers after a period of growth. The  $K_i$  values for *E. coli* at those times were in the range of 0.142 - 0.195.

*E. durans* did not demonstrate regrowth but had periods of little or no decline which corresponded in time with the period of *E. coli* growth. Similarly, *E. durans* showed rapid drops at times corresponding to similar drops in *E. coli* numbers. From this, it is tempting to speculate that the presence of some nutrient sustained *E. durans* and allowed *E. coli* to multiply but the viability of both bacteria was lost rapidly when that nutrient ran out. Alternatively, conditions may have been initially suitable for *E. coli* and *E. durans* survival but some parameter such as a microbial toxin increased within the closed system to cause a rapid loss of the bacteria.

Phage B, MS-2 and PV had inactivation constants,  $K_i$ , in the range of 0.000 - 0.021, 0.002 - 0.018 and 0.021 - 0.028, respectively.

### 6.3.5 Survival of test microorganisms in the Kroh River (Site EB)

The survival of the test organisms at site EB was very complex with constantly changing patterns within and between the five field studies. Regrowth of the bacteria was also observed. The survival data are

represented in Fig. 6.15, 6.16, 6.17 and 6.18B. Field study 5 (Fig. 6.17) was different than the rest in that native *E. coli* and enterococci in a sample of contaminated water taken downstream of the site was used instead of water seeded with the laboratory cultures of *E. coli* and *E. durans*.

#### 6.3.5.1 HFD system results

The general pattern of survival of test organisms was MS-2 > PV and phage B > *E. durans* > *E. coli*. The patterns of survival of the two bacteria were relatively similar to each other in all field studies at site EB. For both, there were periods of relative stability and periods of rapid loss. The *Ki* values for *E. coli* and *E. durans* in the five field studies were in the ranges of 0.000 - 0.478 and 0.000 - 0.319 respectively. The *Ki* value for the native *E. coli* was 0.017. The native enterococcus numbers increased up to 160% higher than the initial level between 4 -12 h. The survival pattern of the viruses at site EB showed no consistency between field studies and also showed periods of relative stability and periods of rapid loss. The range of *Ki* values for all field studies for phage B, MS-2 phage and PV were 0.000 - 0.179, 0.020 - 0.036 and 0.000 - 0.304, respectively.

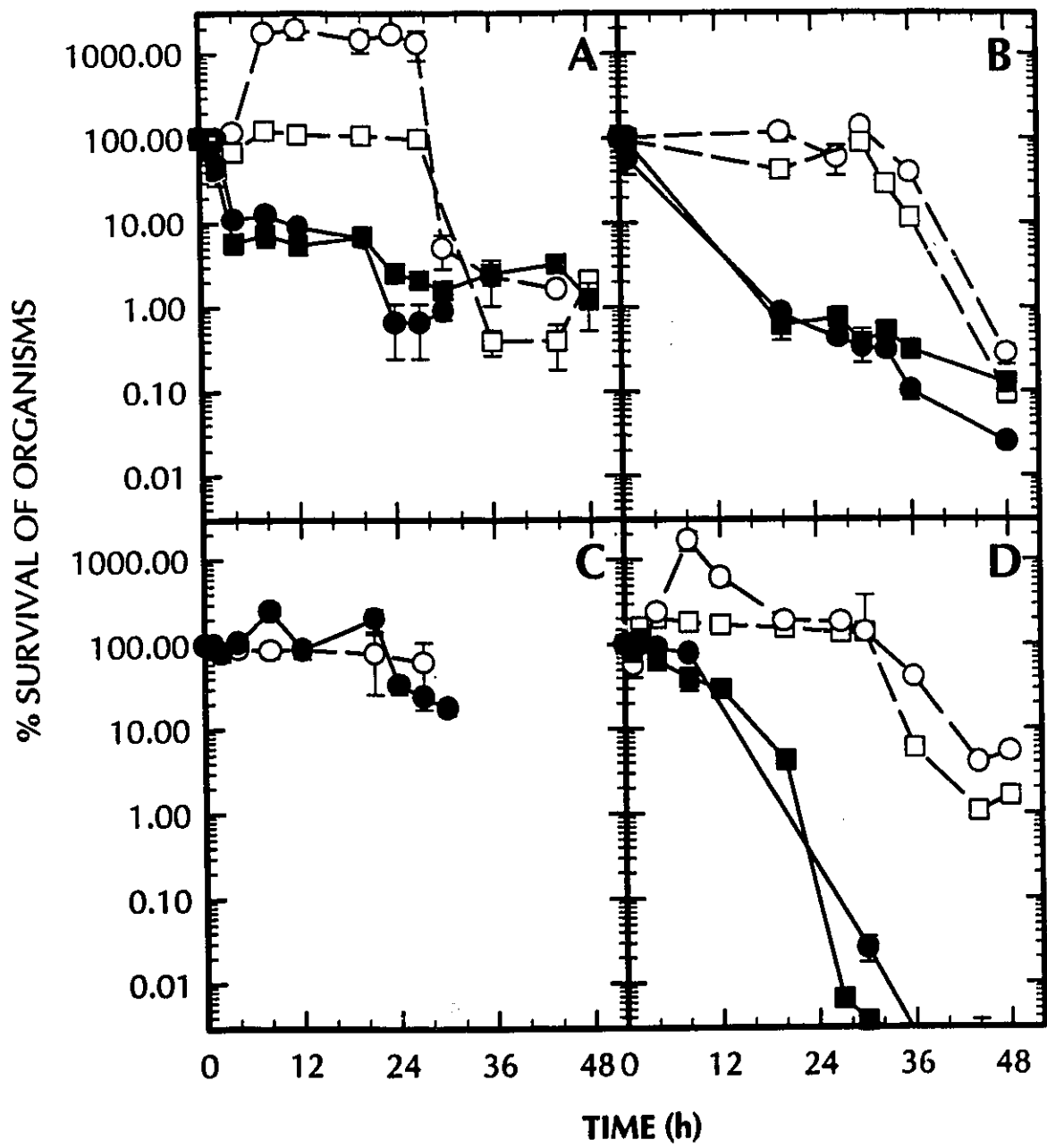
##### 6.3.5.1.1 Eh levels and bacterial survival

Once again, a rapid drop in bacterial levels was temporally correlated with rising Eh. This was the case for field study 2 (Fig. 6.18A and 6.18B). In this case, there was also a rapid change in specific conductivity at 6 h.

However once again, the results of another study (field study 4), showed a rapid drop in bacterial numbers. The Eh levels over that period were fairly stable.

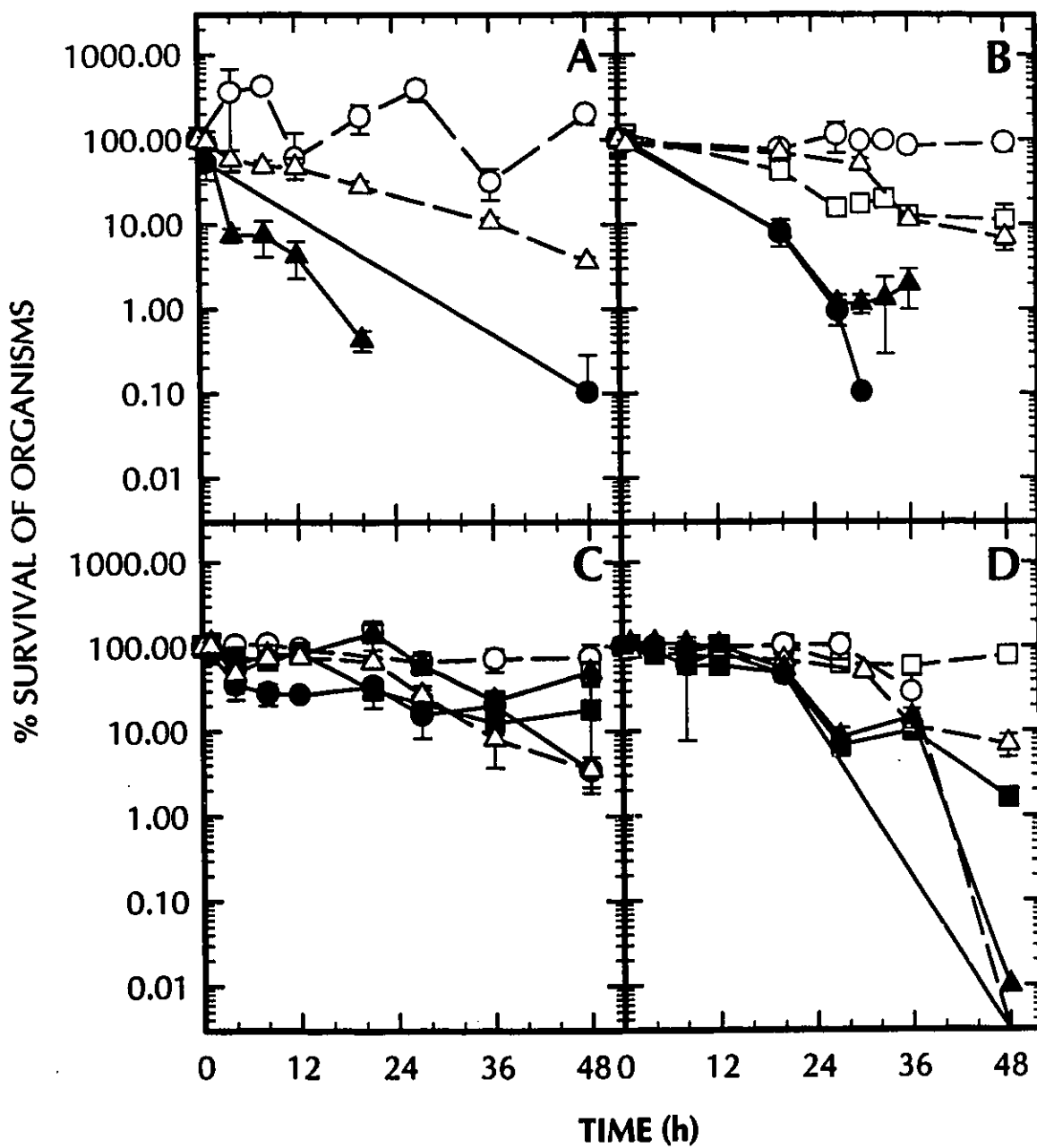
**Fig. 6.15 :** The survival of bacteria during *in situ* studies at the Kroh River tributary (Site EB).

**A** - first field trip; **B** - second field trip; **C** - third field trip; **D** - fourth field trip. (●) *E. coli* , (■) *E. durans*. Closed symbols - HFD system; open symbols - batch sample.



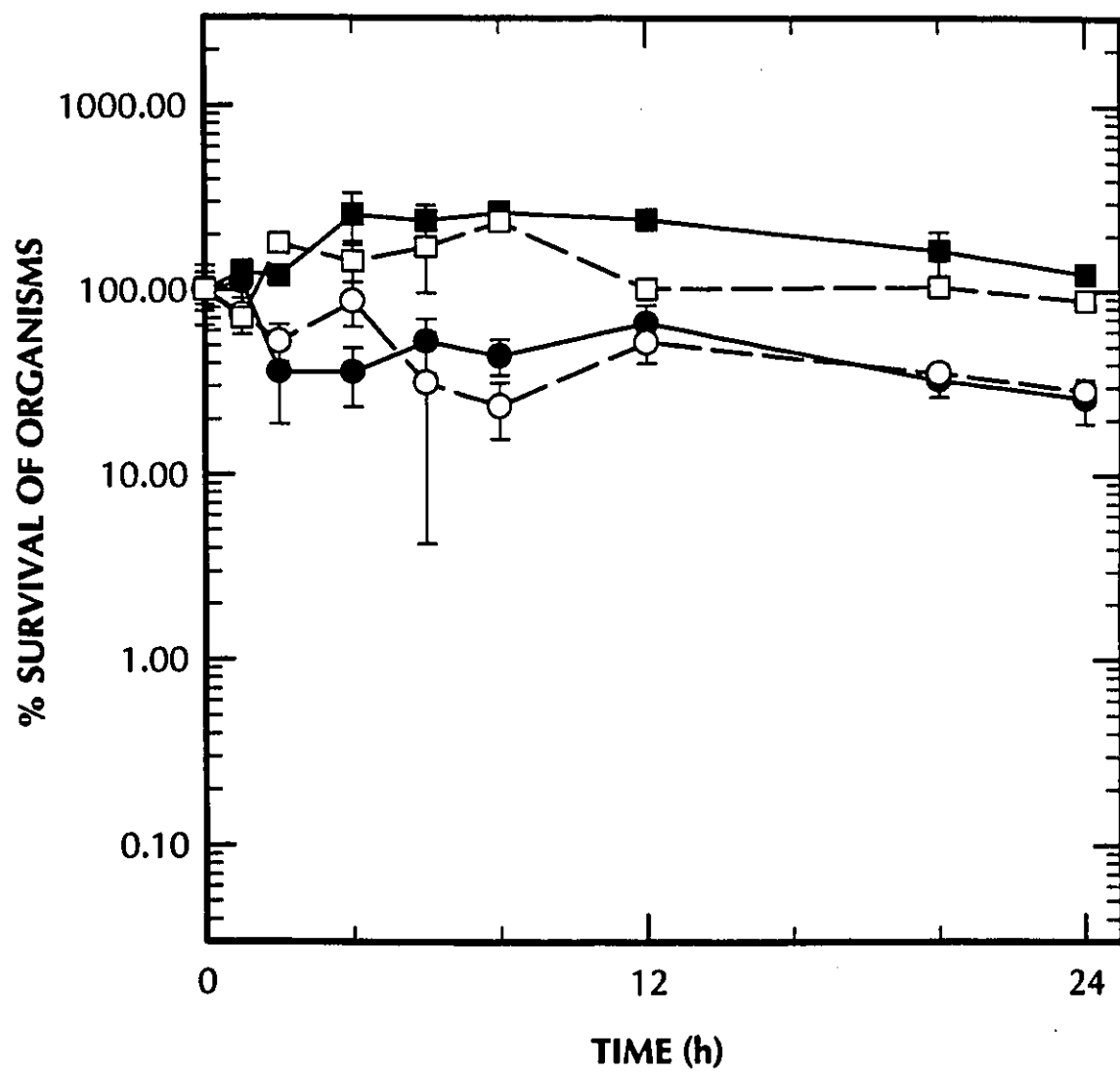
**Fig. 6.16 :** The survival of viruses during *in situ* studies at the Kroh River tributary (Site EB).

**A** - first field trip; **B** - second field trip; **C** - third field trip; **D** - fourth field trip. (●) somatic coliphage B, (■) MS-2 coliphage, (▲) poliovirus. Closed symbols - HFD system; open symbols - batch sample.



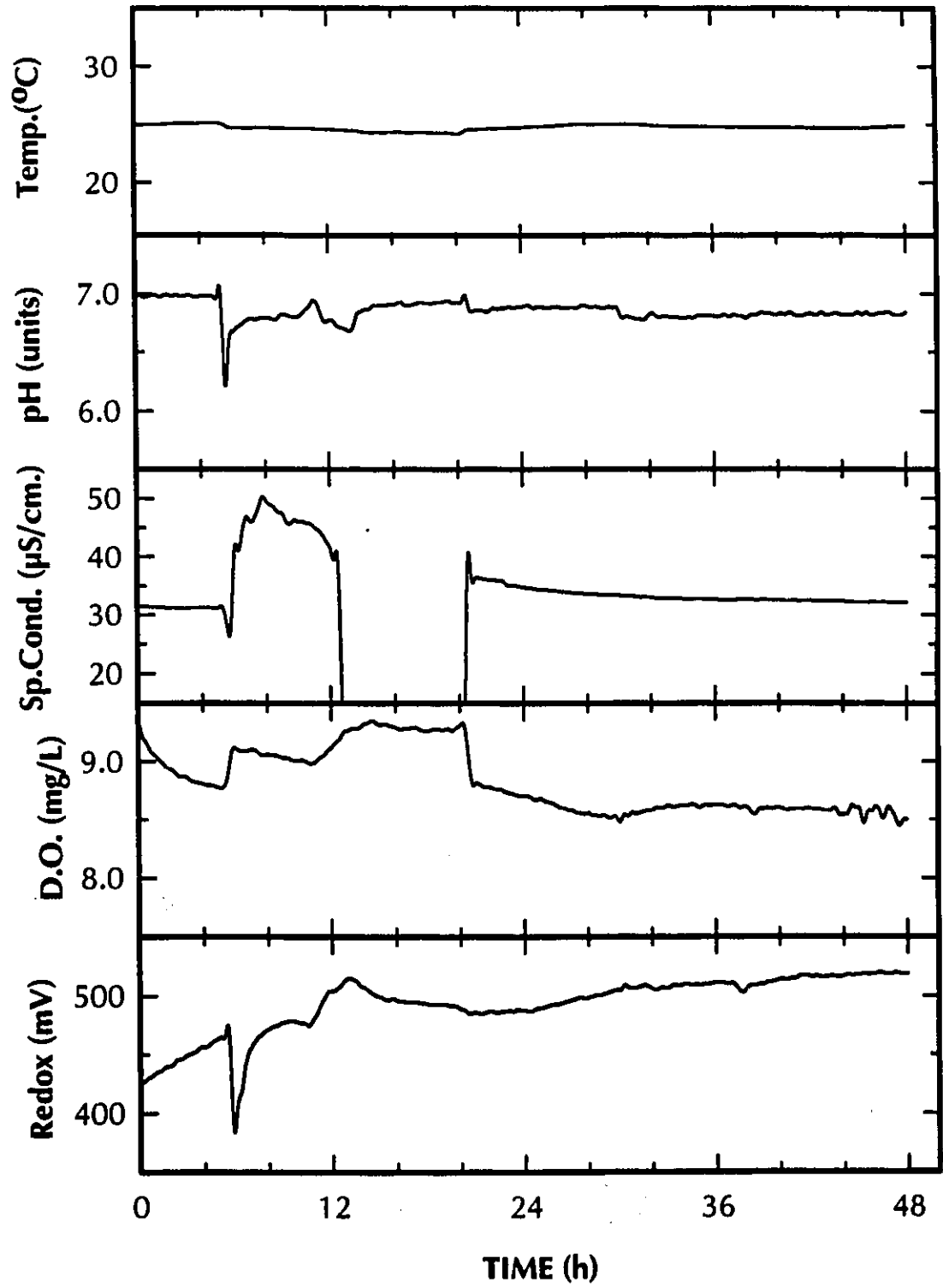
**Fig. 6.17 :** The survival of native bacteria during *in situ* survival studies at the Kroh River tributary (Site EB).

(●) native *E. coli* , (■) native enterococci. Closed symbols - HFD system; open symbols - batch sample.



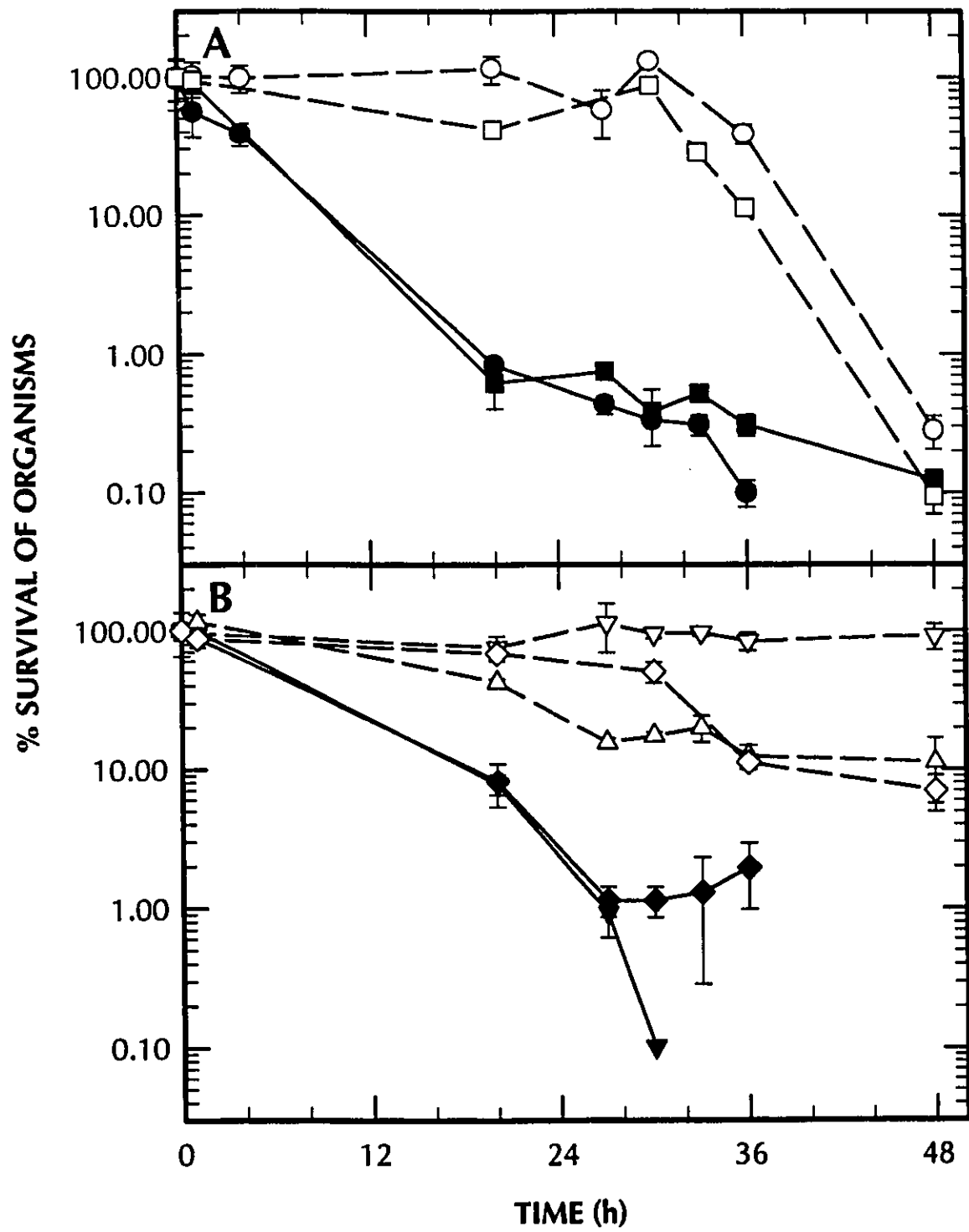
**Fig. 6.18A :** Physico-chemical parameters of the river water during the second *in situ* survival study at the Kroh River tributary (Site EB).

Parameters recorded by the Datasonde datalogger include temperature, pH, the specific conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ ), dissolved oxygen (D.O.;  $\text{mg}\cdot\text{L}^{-1}$ ) and the redox potential (Eh; mV). The period shown in the figure corresponds to the period of the microbial survival study in the field. The loss of specific conductivity measurements and the rise of D.O. levels between 13 h to 20 h is believed to be due to the exposure of the electrodes to the air when the datalogger was disturbed by fast-flowing water.



**Fig. 6.18B :** The survival of microorganisms during the second *in situ* survival study at the Kroh River tributary (Site EB).

**A** - bacteria, **B** - viruses. (●) *E. coli*, (■) *E. durans*, (▼) coliphage B, (◆) PV. Closed symbols - HFD system; open symbols - batch sample.



### 6.3.5.2 Batch sample results

Regrowth of *E. coli* occurred during two studies while regrowth of enterococci occurred in three studies. *E. coli* regrowth started almost immediately in field studies 1 and 4 increasing by 1800% in 12 h and 1600% in 8 h, respectively.

In study 4, *E. durans* increased by 100% at 4 h while in study 1, *E. durans* grew by 480% between 44 h and 48 h. The native enterococci increased by 140% by 8 h in study 5. The range of *K<sub>i</sub>* values for *E. coli* and *E. durans* in batch samples were 0.006 - 0.806 and 0.000 - 0.165, respectively. The *K<sub>i</sub>* value for native *E. coli* was 0.017.

With the exception of study 1, phage B showed a constant slow decline. *K<sub>i</sub>* values for phage B were 0.000 - 0.246. During study 1 however, there appeared to be cycles of growth for phage B (Fig. 6.16-A). Phage B numbers appeared to increase and then decrease in cycles of approximately 12 h duration. It was noted that study 1 had the highest regrowth of the test bacteria. It may be that a suitable native host may have also been able to grow to numbers high enough to allow phage B replication. The native *E. coli* count in the river water at the start of the study was 100,000 cfu. 100 mL<sup>-1</sup>.

The range of *K<sub>i</sub>* values for MS-2 and PV in the batch samples were 0.003 - 0.023 and 0.006 - 0.047, respectively.

#### 6.4 Discussion

The HFD system was successfully adapted for use in *in situ* field survival studies. Its design evolved over the course of the experiments to make the system more portable and needing less supervision. The problem of volume control of the *I* circuit was solved by using balancing hydrostatic pressure (Fig. 6.2). However, this required the hollow fiber cartridge and *I* reservoir to be submerged in the river. This is not always possible as at site EB where the stream was not deep enough or perhaps where the water was too swift or turbulent. Eventually, it would be advisable to develop a system of volume control based on electronic level detection and flow rate and flow direction control. This adaptation would make the system more versatile.

The field studies pointed out differences in the HFD system and batch samples in studying microbial survival. The results are summarized in Table 6.1 and Table 6.2.

One major difference is that batch samples often showed regrowth of *E. coli* with Rideau River, Ottawa River, Gombak River and Kroh River water while *E. coli* regrowth was only seen twice in the Rideau River water in the HFD system. On one occasion of *E. coli* regrowth in the HFD system in the Rideau River, there was a nearly identical regrowth of *E. coli* in the batch sample (Fig. 6.4). The regrowth occurred near the beginning of the experiment for both systems suggesting that the original river water grab sample used in the reservoirs of the two systems may have already contained the necessary nutrients or factors for regrowth.

Table 6.1 The survival and inactivation patterns of test microorganisms in the HFD system during *in situ* field studies.

SITE	ORGANISM	<i>Ki</i> range	GENERAL ORDER OF SURVIVAL AND GENERAL OBSERVATIONS
TA			<i>E. coli</i> > PV > MS-2 > <i>E. durans</i>
	<i>E. coli</i>	0.000 - 0.202	a) Different rates of inactivation were observed at different times within a single field study and between studies. b) Regrowth occurred in two of four field studies.
	<i>E. durans</i>	0.007 - 0.190	a) Different rates of inactivation were observed at different times within a single field study and between studies. b) Drop in <i>E. durans</i> population during field study 3 was too rapid for <i>Ki</i> estimation. c) A diurnal pattern of inactivation suspected.
	MS-2 phage	0.000 - 0.133	a) Different rates of inactivation were observed at different times within a single field study and between studies.
	PV	0.000 - 0.124	a) Different rates of inactivation were observed at different times within a single field study and between studies. b) In two of three field studies, there was an initial 3 - 5 h of stability before a relatively rapid decline. c) In one field study, there was a relatively slow decline over the entire study period.

Table 6.1 (continued)

TB			MS-2 > PV > <i>E. coli</i> > <i>E. durans</i>
	<i>E. coli</i>	0.027 - 0.037	a) No inactivation observed at 2-4°C. b) In the presence of chlorine (0.08 ppm), <i>Ki</i> = 0.095 - 0.874.
	<i>E. durans</i>	0.027 - 0.073	a) No inactivation observed at 2-4°C. b) In the presence of chlorine (0.08 ppm), <i>Ki</i> = 0.046 - 0.874.
	MS-2 phage	0.004 - 0.007	a) No inactivation observed at 2-4°C. b) In the presence of chlorine (0.08 ppm), <i>Ki</i> = 0.004
	PV	0.003 - 0.008	a) No inactivation observed at 2-4°C. b) In the presence of chlorine (0.08 ppm), <i>Ki</i> = 0.006 - 0.604.
TC			<i>E. durans</i> > <i>E. coli</i>
	<i>E. coli</i>	0.000 - 0.482	a) Different rates of inactivation were observed at different times within a single field study and between studies. b) Drop in population was often too rapid for <i>Ki</i> determination.
	<i>E. durans</i>	0.000 - 0.152	a) Different rates of inactivation were observed at different times within a single field study and between studies. b) Drop in population was often too rapid for <i>Ki</i> determination.
EA			MS-2 > PV > Phage B > <i>E. durans</i> and <i>E. coli</i>
	<i>E. coli</i>	0.010 - 0.519	a) Different rates of inactivation were observed at different times within a single field study and between studies.

Table 6.1 (continued)

EA	<i>E. durans</i>	0.014 - 0.447	a) Different rates of inactivation were observed at different times within a single field study and between studies.
	Phage B	0.012 - 0.237	a) Different rates of inactivation were observed at different times within a single field study and between studies.
	MS-2 phage	0.002 - 0.025	
	PV	0.000 - 0.037	a) Different rates of inactivation were observed at different times within a single field study and between studies.
EB			MS-2 > PV and Phage B > <i>E. durans</i> > <i>E. coli</i>
	<i>E. coli</i>	0.000 - 0.478	a) Different rates of inactivation were observed at different times within a single field study and between studies.
	<i>E. durans</i>	0.000 - 0.319	a) Different rates of inactivation were observed at different times within a single field study and between studies.
	Phage B	0.000 - 0.179	a) Different rates of inactivation were observed at different times within a single field study and between studies.
	MS-2 phage	0.020 - 0.036	a) Different rates of inactivation were observed at different times within a single field study and between studies. b) During field study 2, the MS-2 titre declined too rapidly for $K_i$ estimation.
	PV	0.000 - 0.304	a) Different rates of inactivation were observed at different times within a single field study and between studies.

Table 6.2 The survival and inactivation patterns of test microorganisms in batch samples during *in situ* field studies.

SITE	ORGANISM	<i>Ki</i> range	GENERAL ORDER OF SURVIVAL AND GENERAL OBSERVATIONS
TA			PV > MS-2 > <i>E. coli</i> > <i>E. durans</i>
	<i>E. coli</i>	0.032 - 2.040	a) Different rates of inactivation were observed at different times within a single field study and between studies. b) Regrowth occurred in one of four field studies.
	<i>E. durans</i>	0.000 - 0.290	a) Different rates of inactivation were observed at different times within a single field study and between studies. b) Drop in <i>E. durans</i> population during field study 3 was too rapid for <i>Ki</i> estimation. c) A diurnal pattern of inactivation suspected.
	MS-2 phage	0.000 - 0.159	a) Different rates of inactivation were observed at different times within a single field study and between studies.
	PV	0.000 - 0.123	a) Different rates of inactivation were observed at different times within a single field study and between studies b) In two of three field studies, there was an initial 3 - 5 h of stability before a relatively rapid decline was noted. c) In one field study, there was a relatively slow decline over the entire study period.

Table 6.2 (continued)

TB			PV > MS-2 > <i>E. durans</i> > <i>E. coli</i>
	<i>E. coli</i>	0.001 - 0.252	a) No inactivation observed at 2-4°C. b) Regrowth occurred in one of three field studies at 20°C.
	<i>E. durans</i>	0.032 - 0.103	a) No inactivation observed at 2-4°C.
	MS-2 phage	0.002 - 0.043	a) No inactivation observed at 2-4°C.
	PV	0.001 - 0.002	a) No inactivation observed at 2-4°C.
TC			<i>E. durans</i> > <i>E. coli</i>
	<i>E. coli</i>	0.000 - 0.482	a) Different rates of inactivation were observed at different times within a single field study and between studies. b) Drop in population was often too rapid for <i>K<sub>i</sub></i> determination.
	<i>E. durans</i>	0.000 - 0.256	a) Different rates of inactivation were observed at different times within a single field study and between studies. b) Drop in population was often too rapid for <i>K<sub>i</sub></i> determination.
EA			MS-2, PV, Phage B > <i>E. coli</i> > <i>E. durans</i>
	<i>E. coli</i>	0.142 - 0.195	a) Regrowth occurred in all field studies.
	<i>E. durans</i>	0.000 - 0.124	a) Different rates of inactivation were observed at different times within a single field study and between studies.
	Phage B	0.000 - 0.021	

Table 6.2 (continued)

EA	MS-2 phage	0.002 - 0.018	
	PV	0.021 - 0.028	
EB			Variable due to regrowth of bacteria and phage B
	<i>E. coli</i>	0.006 - 0.806	a) Different rates of inactivation were observed at different times within a single field study and between studies. b) Regrowth occurred in two of five field studies.
	<i>E. durans</i>	0.000 - 0.165	a) Different rates of inactivation were observed at different times within a single field study and between studies. b) Regrowth occurred in three of four field studies.
	Phage B	0.000 - 0.246	a) Different rates of inactivation were observed at different times within a single field study and between studies. b) Cycles of growth and decline of approx. 12 h duration way observed in one field study.
	MS-2 phage	0.003 - 0.023	
	PV	0.006 - 0.047	a) Different rates of inactivation were observed at different times within a single field study and between studies.

On the second occasion of *E. coli* regrowth in the HFD system in the Rideau River, there was no similar regrowth in the batch sample (Fig. 6.5) and it occurred towards the end of the field study. In this case, the HFD system appeared to be responding to a plug-flow of nutrients going past the field site on the Rideau River. This conclusion is supported by physico-chemical data indicating a transient increase in BOD and pH levels and by observations of rainfall upstream of the site. It seems likely that stormwater run-off from the agricultural lands upstream of site TA may have caused a plug-flow of nutrients past the site which was utilized by the *E. coli* in the HFD system. The batch sample being a closed system clearly is unable to respond to such changes in river conditions. Fig. 6.5 also demonstrates how different the values of certain parameters such as pH and Eh become in the HFD system and in the batch sample. The pH and Eh levels in the HFD system were found to be almost identical with the constantly changing levels in the river. However, in the batch samples, the values were different with the Eh in particular almost 100 mV lower than that in the river. Filter-sterilized batch sample had higher Eh values than the non-sterilized batch sample suggesting that the lower Eh levels in the batch samples may be due to the metabolic activity of heterotrophic organisms. The Eh effects and the frequent regrowth of bacteria in batch samples appear to be the result of the "bottle" effect of the system.

Regrowth of bacteria did not occur in all batch samples from a particular site. This may stress the difficulty in relying on a few batch sample studies to draw conclusions on the survival of organisms since a lot depends on the nature of the

original grab sample and grab samples obtained at different times showed a large variability (Winstanley *et al.*, 1991). Moreover, different limiting factors may be responsible for bacterial regrowth on different occasions and at different sites.

The batch sample studies at the Kroh River (Site EB) showed regrowth of *E. coli*, *E. durans* and possibly of phage B whereas no regrowth was seen in the HFD system. This was the only occasion that a possible regrowth of phage B was seen. It may be argued that this shows that phage B is capable of multiplying in tropical waters and therefore would be an unsuitable indicator organism (Hazen, 1988). However, it is known that the batch sample on that occasion had an unusually high level of indigenous coliform bacteria. Under the closed conditions of the batch sample, it appears that the level of indigenous bacteria may have even increased further and created an artificial condition conducive for phage replication that did not occur with the HFD system.

The HFD system and the batch sample provide significantly different survival profiles of organisms. In the Rideau River, for example, the order of survival of the test organisms in the HFD system was *E. coli* > PV > MS-2 > *E. durans* but in the batch samples it was PV > MS-2 > *E. coli* > *E. durans*. In the Kroh River, the HFD system showed a relatively consistent order of survival of MS-2 > PV and phage B > *E. durans* > *E. coli*. However, no consistent order of survival was evident from the batch sample results due to the large variability observed between samples.

In analyzing all the HFD results, differences and similarities between the different water bodies were observed. The order of survival of organisms was more or less identical in the Ottawa River, the Gombak River and the Kroh River with the order being MS-2 phage > PV > phage B > *E. coli* and *E. durans*. In the Rideau River, however, *E. coli* survived better than the viruses and *E. durans*. The Rideau River also stands out as the only site where regrowth of test bacteria was seen in the HFD system.

It is perhaps significant that the HFD system failed to show regrowth of *E. coli* in either equatorial rivers in light of the reports of high levels of the bacteria in pristine tropical waters (Hazen, 1988; Rivera *et al.*, 1988; Hardina and Fujioka, 1991). Perhaps the laboratory strain, *E. coli* F, isolated from temperate waters was unable to adapt to the conditions in the equatorial waters to enable its regrowth. When a native strain of *E. coli* was used, it was found that the  $K_i = 0.017$  which was lower than the  $K_i$  values for the *E. coli* F.

Native enterococci were observed to increase in numbers; this was not seen with the *E. durans* which was also an isolate from temperate waters. It may therefore be important to repeat the experiments with more isolates from equatorial waters to determine the potential of regrowth of those bacteria in the warmer equatorial waters.

The HFD system responded well to changes in pH, Eh, BOD levels and the presence of disinfectants in the river water as demonstrated by changes in bacterial survival in the system. The relationship between higher BOD levels and bacterial

regrowth is clearly associated by the presence of nutrients and organic load in the river as indicated by the BOD measurements. However, it was also noted that often but not always, a rapid drop in bacterial numbers was temporally associated with a rapid rise in Eh values. The increase in Eh levels was in the 100 mV range. Site TC also presented many problems with very poor *E. coli* and *E. durans* recovery during the initial field studies. Interestingly, the Eh levels were higher during the early field studies at site TC than in the later studies in which bacterial survival was much improved. It is not clear though whether the rising Eh levels were directly affecting the bacteria or were just indicative of another factor such as algal activity. It was observed that the changing Eh levels could occur in the absence of changes in dissolved oxygen levels.

One final observation was an apparent diurnal pattern of inactivation for *E. durans* in the Rideau River. This was observed in both HFD and batch samples. It is not certain what may be affecting the *E. durans* survival but the decay was slower during the hours of darkness when temperatures were lower. It seems possible that sunlight or temperature or perhaps both may be the major factor affecting *E. durans* survival.

**7. The effect of surface runoffs and changes in redox potential on the survival of bacteria in water**

## 7.1 Introduction

As mentioned earlier in Chapter 6, changes in redox potential and the biochemical oxygen demand (BOD) of the surface water was temporally associated with significant changes in *E. coli* and *E. durans* survival in the HFD system.

Redox potential refers to the oxidation-reduction potential which is a system's affinity for electrons relative to that of hydrogen gas. Redox potential is measured as the electromotive force generated by the system with respect to the standard hydrogen electrode. The redox potential or Eh of the hydrogen ion-hydrogen atom couple at 1 M concentration and 1 atmosphere pressure respectively is arbitrarily defined as 0 volts.

The redox potential is temperature and pH dependent and is greatly influenced by oxygen levels. Surface waters in equilibrium with atmospheric oxygen have theoretical Eh values of +800 mV but in reality, the effect of heterotrophic activity keeps most aerobic natural waters at 400 - 500 mV (Atlas and Bartha, 1981). Bacteria are known to have pH and Eh tolerance ranges for growth, hence aerobic bacteria cannot grow at low Eh levels while vegetative cells of anaerobic bacteria cannot survive at high Eh levels. In this study, we attempted to study the effect of the changes in Eh on *E. coli* and *E. durans* survival.

The availability of nutrients in the aquatic environment clearly affects heterotrophic bacterial populations. Under eutrophic conditions especially when industrial sewage and animal pollution add to the organic content of the water, bacterial numbers may be expected to be high (Hendricks and Morrison, 1967;

Dutka *et al.*, 1969; Valdes-Collazo *et al.*, 1987; Araujo *et al.*, 1990). Non-polluted freshwater and marine waters are generally considered to be oligotrophic with approximately 10 mg of organic carbon per liter (Morita, 1992).

In Chapter 6, an incident was described on the Rideau River in which *E. coli* regrowth was observed after a rise in the biochemical oxygen demand that was believed to be due to a plug-flow of stormwater run-off from the agricultural lands up river. An attempt was made in the laboratory to confirm this by inducing *E. coli* growth in river water samples by exposure to stormwater run-offs and soil filtrates.

## **7.2 Materials and Methods**

### **7.2.1 Protocol for Redox potential studies**

Redox potential was measured using a hand-held pocket size ORP tester (Oakton ORP Testr<sup>™</sup>; Cole Parmer). This meter uses an Ag-AgCl reference electrode and so all readings were adjusted with the addition of 220 mV to bring the values in line with the hydrogen reference standard. Calibration of the meter was carried out using freshly prepared quinhydrone solutions.

For the manipulation of the redox potentials of bacterial suspensions used in experiments, ferric chloride was used to raise the Eh while sodium thioglycollate was used to lower it. A stock solution of 1% ferric chloride hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ; BDH) was prepared fresh for each experiment and filter sterilized through 0.2  $\mu\text{m}$  pore size membrane filters. Different dilutions of the ferric chloride stock in distilled water was prepared and the Eh of those solutions was measured and

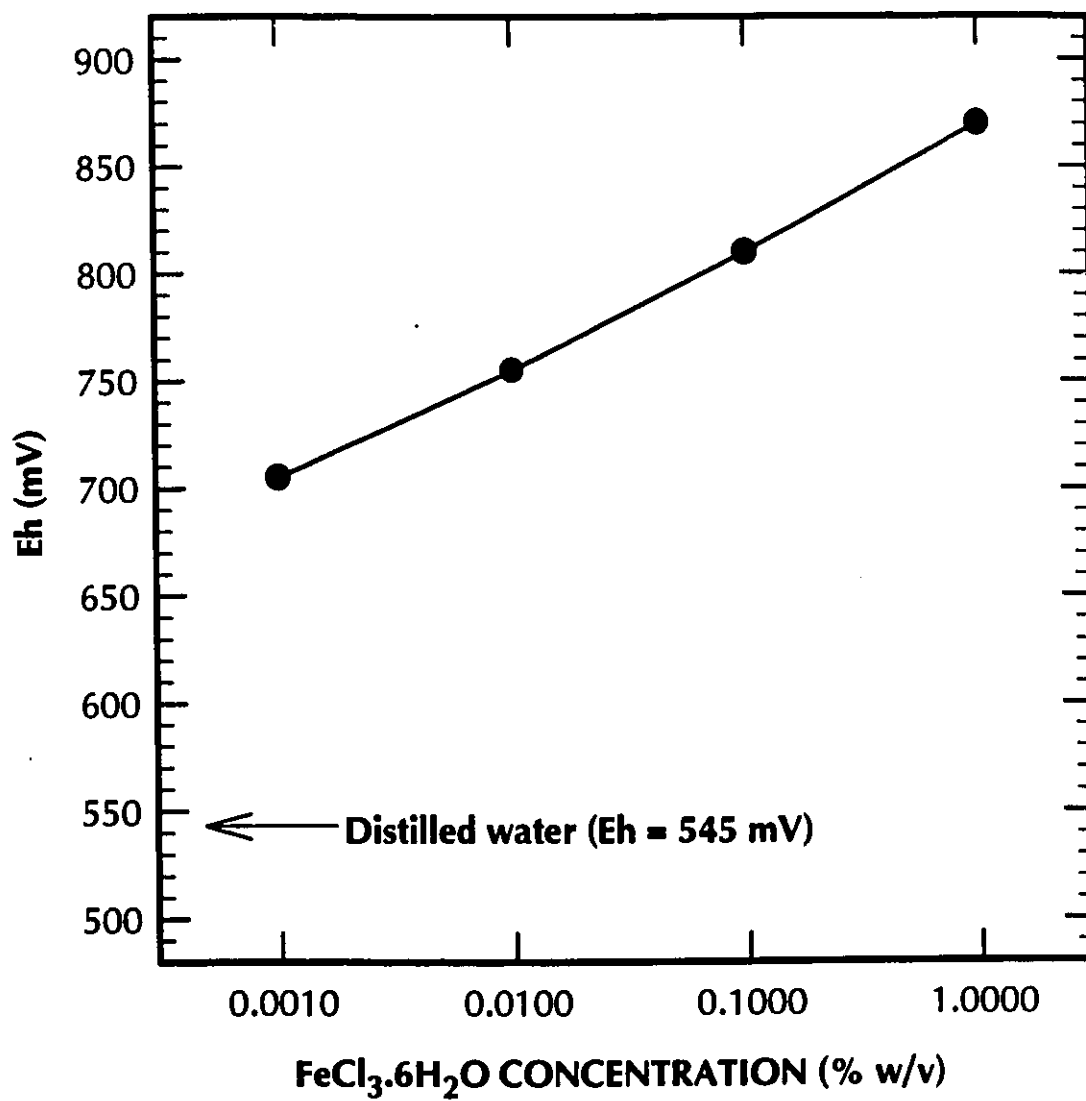
compared with that of the distilled water used (Fig. 7.1). Based on those results, it was decided to add 0.1 - 0.2 mL of the ferric chloride stock to each 100 mL of bacterial suspensions in distilled water to obtain an Eh increase in the range desired. Sodium thioglycollate was prepared from the neutralization of thioglycollate acid with sodium hydroxide and 0.1 mL of the solution was added per 100 mL of bacterial suspension to bring the Eh down to the desired level.

Distilled water was filter sterilized and dispensed in 100 mL aliquots into sterile bottles. Four sets of bottles were prepared in this way. Each set consisted of three bottles to which *E. coli* was added to one bottle, *Enterococcus* was added to another, and both bacteria were added to the third bottle. The bacterial inoculum added gave a final concentration of  $10^4 - 10^5$  cfu.mL<sup>-1</sup>.

Two sets of bottles were used as a control group in which no redox manipulation was carried out. The Eh of the bacterial suspensions in the remaining two sets of bottles were altered at various time points by either the addition of ferric chloride or sodium thioglycollate.

All bottles were incubated at ambient temperature. One set of bacterial suspensions in each of the control and redox manipulated groups was used for bacterial enumeration. Samples were taken at different times and the bacterial numbers enumerated on mT7 and KF agar plates.

**Fig. 7.1:** The redox potential (Eh) of ferric chloride solutions.



The remaining sets of bacterial suspensions were used for Eh measurements at different time points. The bacterial suspension was poured into a sterile 250 mL beaker and the Eh was measured using the redox meter. Between samples, the beaker and the redox probe were washed twice with sterile water and ethanol.

### **7.2.2 Protocol for soil filtrate studies**

Different soil samples were collected and used to prepare separate soil filtrates or eluents. The resultant filtrate was then used in batch culture and HFD system experiments with *E. coli* F and *E. durans* to determine if the bacteria could utilize the substrate present in the filtrate and regrow.

#### **7.2.2.1 Soil types**

A total of four soil types were used in these experiments. The first soil type (Soil 1) used was a silty soil obtained in autumn from the banks of the Rideau River. The next soil type (Soil 2) used was actually a commercially available potting soil (Home Hardware, St. Jacob, Ontario). The third and fourth soil samples were collected from a farm to the west of Ottawa in early spring. Both of these agricultural soils were of a sandy loam type and had had fertilizer applied to them the previous autumn. However, the third soil sample (Soil 3) had received chemical fertilizer while the latter soil (Soil 4) had received applications of animal fecal waste.

### 7.2.2.2 Preparation of soil filtrates

The filtrates were prepared based on the protocol for preparation of the AOAC soil extract medium (Beloian, 1990). Basically, a 50% (w/v) suspension of a soil sample was prepared in rainwater and then filtered. This was done by measuring 300 g of the soil and mixing it with 600 mL of rainwater. The mixture was left for 1 h at room temperature while being stirred by a magnetic stirrer. The mixture was then allowed to settle for 30 min. With most of the sediment and soil at the bottom of the vessel, the soil eluate was carefully decanted into another flask.

For experiments with the HFD system, the resultant eluate was used directly into the *E* circuit. However, for the batch culture studies, the soil eluate was first sterilized. This was done by passing the soil eluate through a 15 cm diameter filter paper (Whatman No 1; W & R Balston Ltd, England) in a Buchner funnel and then through a 0.2  $\mu\text{m}$  pore size membrane filter. The filtrate was then used in the batch culture experiments.

### 7.2.2.3 Studies into the bacterial response to soil filtrates

The different soil filtrates were first screened to determine if the filtrate would support regrowth or may have toxic or inhibitory material that could reduce bacterial numbers. This screening was done by adding the *E. coli* and *E. durans* into 100 mL of the filter sterilized filtrate. Samples were taken throughout a 24 h period to determine the bacterial numbers. As a control a bacterial suspension in distilled water or rain water was also followed over the 24 h.

After this preliminary screening experiment, the HFD system was set-up with a 250 mL *I* circuit and a 500 mL *E* circuit. Initially, sterile distilled water was used in both circuits with the *I* circuit seeded with *E. coli* and *E. durans* ( $10^4$  cfu.mL<sup>-1</sup> each). A closed batch control was also set-up. The bacterial survival in both systems was followed for 20 h. Then the *E* circuit reservoir was replaced with 500 mL of soil filtrate. The HFD system was exposed to the soil filtrate for varying amounts of time such as 1 h, 2 h, 12 h and 24 h. After such exposure, the *E* circuit reservoir was once again replaced with fresh distilled water. The bacterial survival was followed for another 48 h after initial exposure to the soil filtrates.

### 7.3 Results

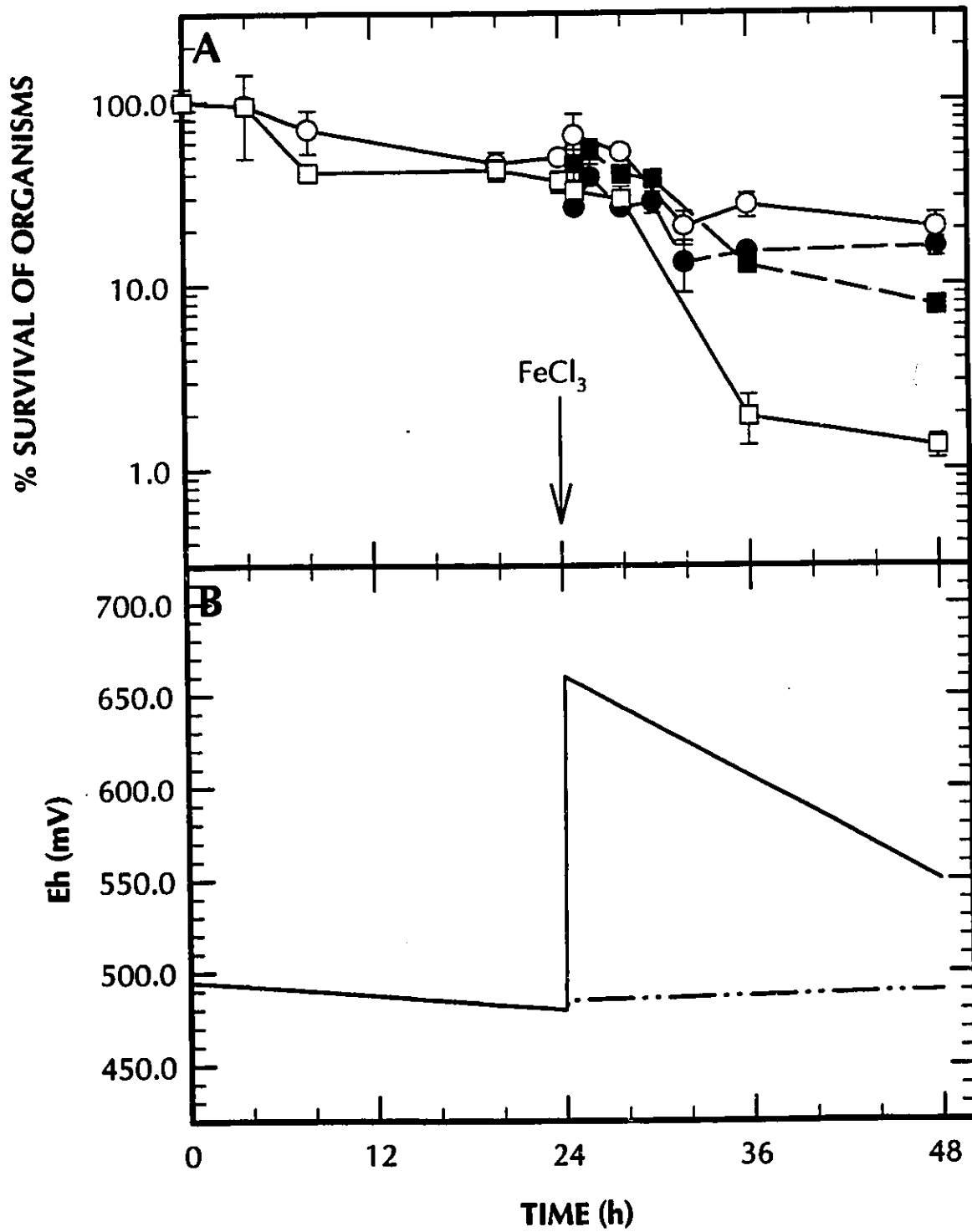
#### 7.3.1 The effect of rapid Eh changes on bacterial survival in the laboratory

The result of the three experiments are shown in Fig. 7.2A - Fig. 7.2C. In these experiments it was found that the response of *E. coli* and *E. durans* to changes in Eh levels was partly affected by whether the organism was present in a single culture suspension or in a mixed culture suspension. Experiments 1 and 2 indicate that *E. coli* and *E. durans* suffer a significant drop ( $p < 0.05$ ) in numbers almost immediately after the change in Eh due to the addition of ferric chloride when the organisms were in single suspension but not when they are present as a mixture. In experiment 3, however, only *E. durans* in single suspension demonstrated a significant drop in numbers ( $p < 0.05$ ) when the Eh was raised. The drop occurred when the Eh was raised at 20 h but did not occur when the Eh was raised earlier at 6 h. It may be that the metabolic state (i.e. exponential phase or stationary phase) or level of stress may modulate the effects of changes in Eh. In all

**Fig. 7.2A:** The effect of changes in Eh on the survival of bacteria (experiment 1).

*E. coli* and *E. durans* were placed into sterile deionised distilled water as a mixed suspension culture. In experimental samples, the Eh was then altered by the addition of ferric chloride ( $\text{FeCl}_3$ ).

A - bacteria, (●) *E. coli*, (■) *E. durans*, Closed symbols - redox change experiments; open symbols - controls; B - redox potential (Eh). Dotted line represents Eh of control.

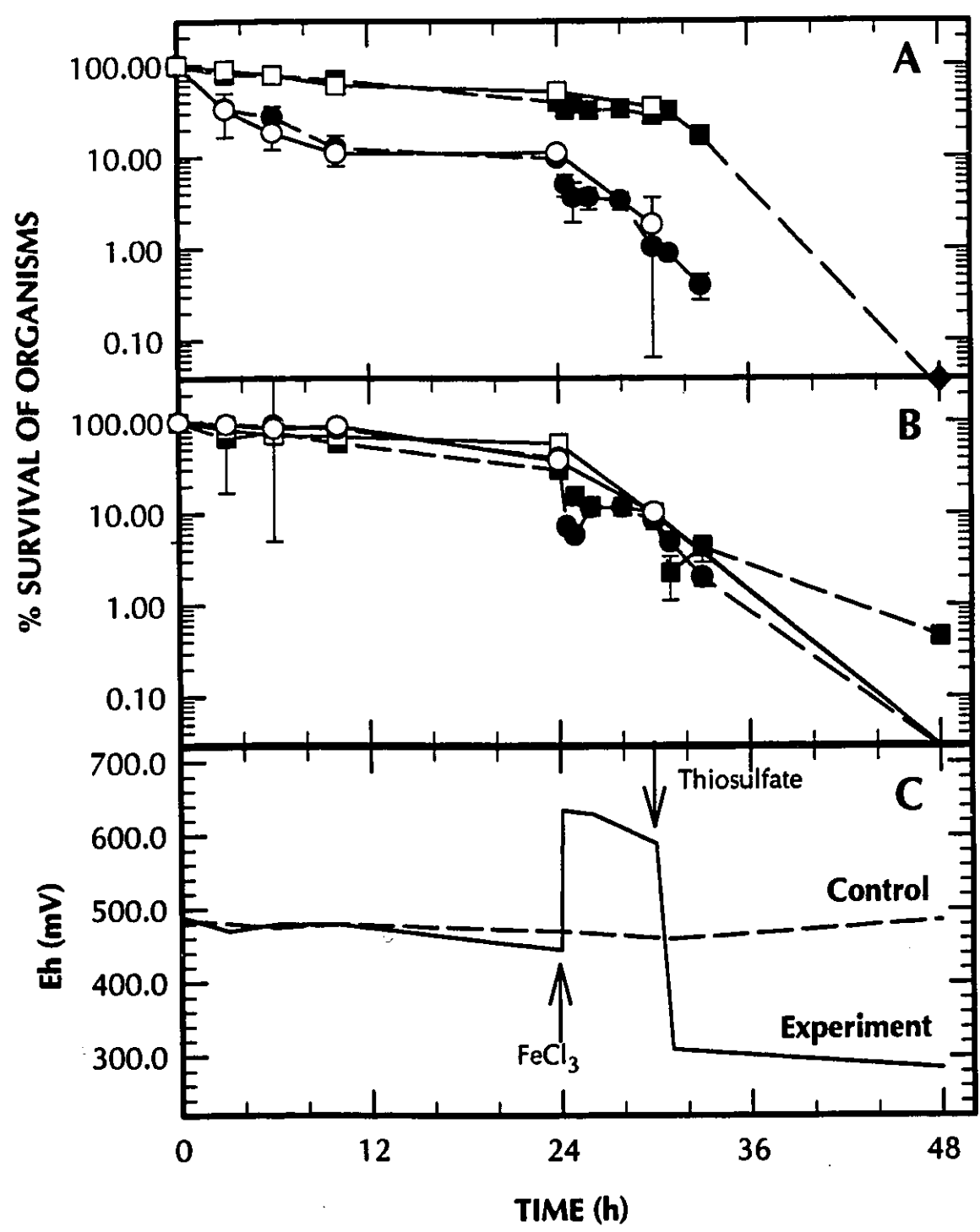


**Fig. 7.2B:** The effect of changes in Eh on the survival of bacteria (experiment 2).

*E. coli* and *E. durans* were placed into sterile deionized distilled water either in a single microorganism suspension or a mixed suspension. In experimental samples, the Eh was then altered by the addition of ferric chloride ( $\text{FeCl}_3$ ) or thioglycollate.

**A** - *E. coli* ; **B** - *E. durans*; **C** - redox potential (Eh).

(●) single microorganism suspension, (■) mixed microorganism suspension; Closed symbols - redox change experiments; open symbols - controls

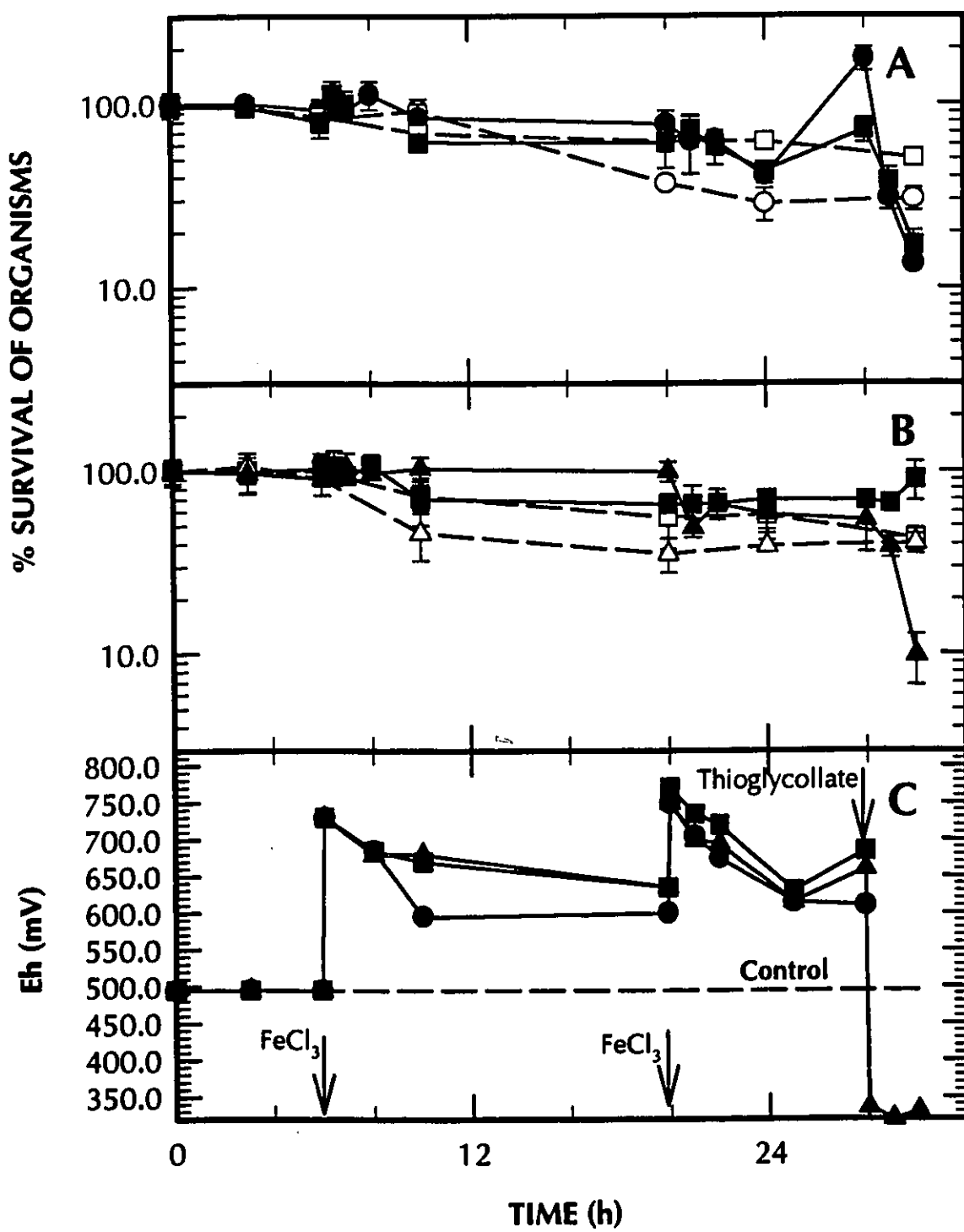


**Fig. 7.2C: The effect of changes in Eh on the survival of bacteria (experiment 3).**

*E. coli* and *E. durans* were placed into sterile deionized distilled water either in a single microorganism suspension or a mixed suspension. In experimental samples, the Eh was then altered by the addition of ferric chloride ( $\text{FeCl}_3$ ) or thioglycollate.

**A - *E. coli* ; B - *E. durans*; C - redox potential (Eh).**

**(●) single microorganism *E. coli* suspension, (■) mixed microorganism suspension, (▲) single microorganism *E. durans* suspension; Closed symbols - redox change experiments; open symbols - controls**



these cases, the drop in bacterial populations was by about 50 - 75%. In a fourth experiment, the Eh was raised only at 6 h and in this case no effect was seen in any of the bacterial cultures.

The effect of rapidly lowering the Eh by the addition of thioglycollate apparently caused a rapid decline in *E. coli* in both single and mixed suspensions during experiment 3. However, *E. durans* in the same experiment experienced a rapid drop only in single culture. There was no evident effect of rapid Eh drop in experiment 2 on the survival of bacteria.

### 7.3.2 Bacterial regrowth experiments

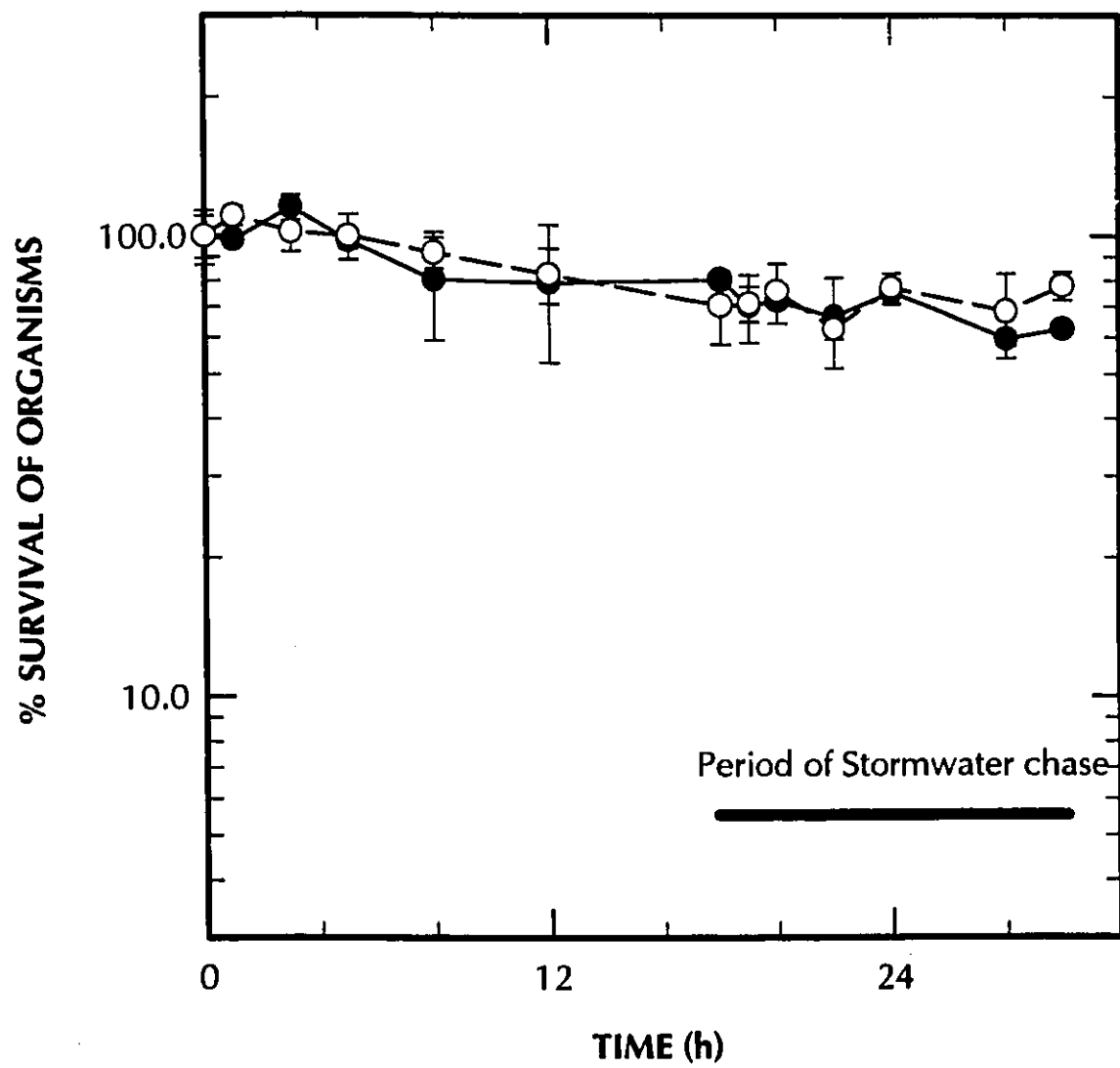
Several experiments were carried out in which the external reservoir of the HFD system was replaced by 10 L of freshly collected stormwater runoff. However, *E. coli* numbers were never seen to increase as a result of exposure to the stormwater (Fig. 7.3).

With the soil filtrates, batch culture studies showed a 100 - fold increase in *E. coli* numbers with soil 2 and soil 4 but not with soil 1 or soil 3 after a 24 h exposure (Fig. 7.4). The soil filtrates from soil 2 and 4 were then used to spike the HFD system external reservoir. The results were disappointing in that only with soil filtrate 2 (potting soil) was there an increase in the *E. coli* numbers and that was after a 12 h exposure. With *E. durans*, exposure to the soil filtrates may even cause a more rapid decline in numbers.

**Fig. 7.3 :** The effect of stormwater runoff on bacterial survival in water.

*E. coli* was placed in the *I* circuit of two HFD systems. One setup was then exposed to stormwater in the external reservoir after 18 h while the other remained exposed to distilled water and acted as the control.

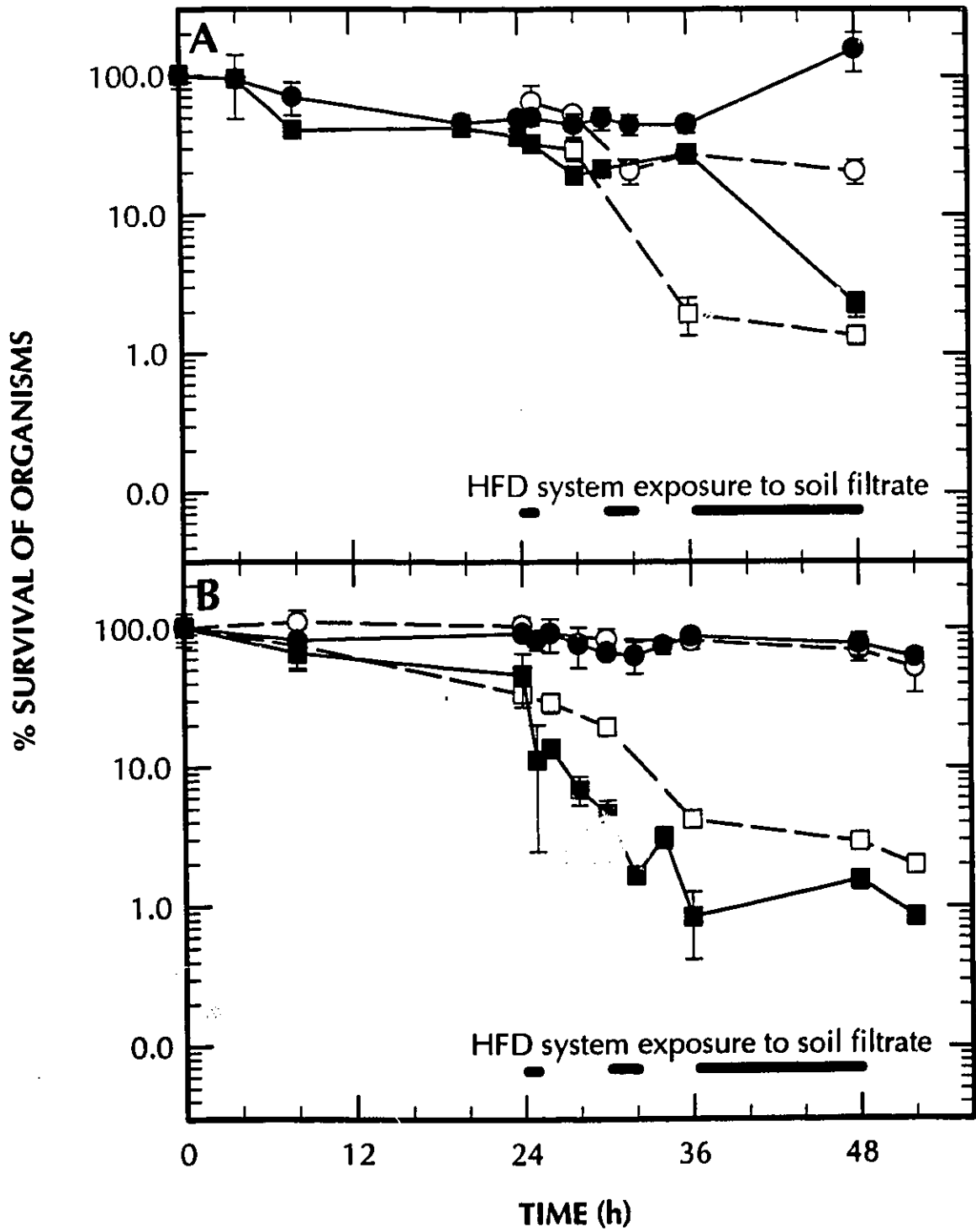
Closed symbols - exposed to stormwater, open symbols - control.



**Fig. 7.4 :** The effect of soil filtrates on bacterial survival in water.

*E. coli* and *E. durans* were placed into the HFD system. The HFD system was then exposed to soil filtrates in the external reservoir for 1, 2 and 12 h periods.

**A** - commercial potting soil filtrate, **B** - filtrate of agricultural soil to which manure had been applied previously. (●) *E. coli*, (■) *E. durans*. Closed symbols - HFD system exposed to soil filtrates, open symbols - batch control.



#### 7.4 Discussion

These preliminary experiments on the rapid changes in redox potential, indicate that there may be some effect on *E. coli* and *E. durans* survival. The mechanism by which a rapid decline in population of the bacteria is effected by Eh changes is unclear. It would seem that the effect was more marked in single organism suspensions, suggesting that each type of bacterium receives some protection by the presence of the other. The metabolic activities of the two types of bacteria may in some way compliment each other to allow both organisms to better cope with the stress imposed by a rapidly changing Eh.

The size of the drop in bacterial numbers in these experiments was one to two orders of magnitude smaller than that observed in the *in situ* field studies when rapid changes in Eh occur. Perhaps Eh needs to act in concert with other factors. From the laboratory experiments, it was also noted that changing the Eh at 6 h had no effect on *E. coli* numbers but changing the Eh at 24 h did. Perhaps therefore, the effect of Eh is potentiated when an organism is stressed. After 24 h in a starvation-survival situation, perhaps the *E. coli* was less capable at coping with the additional stress of Eh levels. During *in situ* field studies, the bacteria may have been under stress from a larger number of factors than in the laboratory experiments.

Attempts to cause bacterial regrowth by using stormwater and soil filtrates to simulate a nutrient plug-flow in the HFD system met with only limited success. The stormwater and three of the four soil filtrates used were unable to cause a

significant regrowth of *E. coli* or *E. durans* in the HFD system. Only soil filtrate from commercial potting soil was able to cause an approximately two fold increase in bacterial numbers and that was only after the HFD system had been exposed to the filtrate for 12 h. In batch systems, the agricultural soil filtrate (with manure applied) was also able to cause *E. coli* regrowth but only after a 24 h exposure.

Clearly, these soil filtrates were unable to reproduce the large regrowth seen in the Rideau River due to a short transient (less than 4 h) exposure to a nutrient plug-flow. It should be remembered though that most soils lack readily utilizable substrate (Williams, 1985). Often the substrates need to be freed by other microorganisms for the use of heterotrophs. The range of soils to be tested should be increased and soils where there is microbial degradation of vegetation should be given particular attention.

These experiments demonstrate how difficult it is to reproduce conditions that occur in nature in a laboratory-base microcosm study. However, the collection of more field data may help in the designing of such laboratory-based studies and hopefully enable us to study the interaction of factors that affect microbial survival in the aquatic environment.

## **8. General Discussion**

In recent years, some established ideas about the survival of certain microorganisms in the aquatic environment have been increasingly challenged. Organisms such as *E. coli* and fecal coliforms are used as indicators of fecal pollution because it was believed that these organisms were present in feces but could not survive long in the aqueous environment (Geldreich *et al.*, 1968; McFeters *et al.*, 1974; Waite, 1985). However, some recent studies suggest that *E. coli* may be able to survive for long periods and even regrow in the aquatic environment (Deaner and Kerri, 1969; Carrillo *et al.*, 1985; Lopez-Torres *et al.*, 1987). Some researchers even suggest that *E. coli* may be autochthonous in the tropical environment (Hazen, 1988; Rivera *et al.*, 1988; Hardina and Fujioka, 1991).

Even the notion of bacterial death is being challenged. Instead it is proposed that a large proportion of the population of any gram-negative bacteria under conditions of starvation-survival in the aqueous environment may persist in the form of unculturable but metabolically active somnicells (Roszak and Colwell, 1987; Barcina *et al.*, 1989). These are essentially dormant and non-dividing cells but may be resuscitated by the introduction of certain select nutrients (Roszak *et al.*, 1984). The possibility that such a strategy for persistence may exist forces us to reevaluate the potential for survival of certain pathogens like *V. cholerae* in the aquatic environment (Gray, 1976; Xu *et al.*, 1982).

Clearly not enough is known about the survival and natural ecology of many microorganisms in the aquatic environment. At the same time, such

knowledge is important to considerations of public health, toxicology and the assessment of risks associated with the release of GEMs into the environment.

The survival of microorganisms is either studied in batch samples or in systems using either dialysis sacs or diffusion chambers with membrane filters. Often the survival of microorganisms in a particular water may be different depending on the study system used (Perez-Rosas and Hazen, 1988; McFeters and Terzieva, 1991).

It is generally accepted that the batch samples do not provide reliable information on the survival of microorganisms in the environment. This is because batch systems are essentially closed systems, under artificial conditions and cannot adequately represent the constantly changing conditions that occur in nature. Moreover, heterotrophic activity in nonsterile batch samples can rapidly alter the water characteristics.

Dialysis sacs were used to enable interaction and exchange between the study system and the external environment. While an improvement over the use of batch samples, dialysis sacs and diffusion chambers require an unacceptably long period of time to respond to changes in the external environment with  $T_{90}$  for the diffusion of sugars in the range of 7.5 to 85 h (Vasconcelos and Swartz, 1976; McFeters and Stuart, 1972; LaBelle and Gerba, 1980). Such slow diffusion rates are unlikely to suit survival studies (LaBelle and Gerba, 1980).

The primary objective of this work was to develop the Hollow Fiber Diffusion system, a novel approach to the *in situ* study of microbial survival in water. The HFD system is based around the use of a tangential flow hollow fiber system which allows a more rapid diffusion and equilibration rates between the study reservoir and the external aquatic environment.

The hollow fibers used in the study have a large total surface area of  $7 \times 10^3$  cm<sup>2</sup>. The tangential circulation of the *I* circuit of the HFD system and the large surface area / volume ratio of 159.1 cm<sup>-1</sup> combine to ensure rapid diffusion. This compares with the surface area / volume ratio of 1.6 for the diffusion chambers used for comparison in this study. The asymmetrical porous structure of the polysulfone fibers also increases the permeability of the fibers since only the thin inner "skin" layer is rate limiting for the diffusion of solutes (Mulder, 1991).

The HFD system was characterized and compared with the diffusion chambers. It was found that diffusion and equilibration rates for pH, Eh and low molecular weight substrates were substantially better in the HFD system. Under the conditions used in this study, the HFD system equilibrated to changes in pH and Eh rapidly with T<sub>90</sub> values of 2 min and 22 min respectively. In contrast, diffusion chambers with cellulose acetate membranes failed to completely equilibrate during the study period. This failure to equilibrate for charged species like the H<sup>+</sup> ion may be a particular characteristic of cellulose acetate membranes (Tuwiner, 1962). The polysulfone membranes of the HFD system did not appear to

have problems with charged ionic species except when the compound was a particularly strong electrolyte like hydrochloric acid or potassium chloride.

This characteristic of the HFD system is not believed to be a major obstacle to its use for *in situ* survival studies. Changes in concentration of strong electrolytes do not readily occur in nature except perhaps changes in salinity in estuarine environments. In such cases, the results obtained from the HFD system may need to be qualified. However, more work needs to be done to determine if this would be a problem for estuarine environments.

For survival studies, it is particularly important to follow the response of microorganisms to the presence of nutrients and carbon sources that may encourage growth of the organism. In this study, we determined the  $T_{90}$  diffusion of low molecular weight sugar analogues to be 0.6 h ( $k = 0.028$ ) and 4.2 h ( $k = 0.004$ ) for the HFD system and the diffusion chamber, respectively. This compares with  $T_{90}$  values of 9.5 to 17.5 h reported for diffusion chambers in other studies. (McFeters and Stuart, 1972; Vasconcelos and Swartz, 1976). For diffusion chambers with 0.015 porosity membranes capable of retaining viruses just as the HFD system, the  $T_{90}$  for diffusion of sucrose was estimated at 85 h (LaBelle and Gerba, 1980). Lessard and Sieburth (1983) managed to design a diffusion chamber that equilibrated for dissolved organic carbon within 2 h. However, they were only able to do this by using a large 5 L chamber and stirring blades.

The rate of equilibration in the HFD system can be further improved. The clearance of ONPG and PNPG through the HFD system was determined to be  $K =$

29 mL.min<sup>-1</sup> and 13 mL.min<sup>-1</sup> for *I* circuit flow rates of 30 mL.min<sup>-1</sup> and 15 mL.min<sup>-1</sup>, respectively. This implies that exchange within the hollow fibers was almost instantaneous and that the rate of equilibration was limited by the flow rate of the *I* circuit. Therefore, increasing the flow rate and reducing the *I* circuit volume would enable the HFD system to equilibrate even more rapidly.

Conditions in the natural aquatic environment are constantly and rapidly changing. The HFD system responds to those changes within minutes and is therefore a better system for *in situ* survival studies than diffusion chambers. The HFD system responds at a rate that better simulates "real-time" changes and its effect on test microorganisms.

The HFD system also was able to respond to changes in negative factors such as the presence of disinfectants. This was demonstrated clearly in laboratory studies and also at the Ottawa River field site. This response of the HFD system was again more rapid than the diffusion chamber to the presence of chloramine T, suggesting that the HFD system may have a useful role in in-line toxicity monitoring or in studying the disinfection of drinking water in the distribution system.

The action of the peristaltic pumps and the properties of the hollow fibers themselves did not affect the survival of most of the test organisms. Adsorption to the surfaces of the HFD system also did not appear to be a problem. The one exception in our study was the tailed phage B. Coliphage B suffered a rapid rate of inactivation during the first 8 h of passage through the system. Under the electron

microscope, it was determined that phage B was a member of the family Myoviridae which possesses contractile tails and tail fibers. It is believed that the shearing forces encountered in the hollow fibers may cause loss or damage to the fragile tail structures. MS-2 phage (family Leviviridae) which is a non-tailed cubic phage was not similarly inactivated. The inactivation of coliphage B was reduced by decreasing the flow rate or increasing the diameter of the fibers. The inactivation of phage B by the HFD system also did not occur in the presence of suspended solids. It is therefore possible to adjust the HFD system to enable survival studies with tailed phages.

Colonization of the hollow fibers by microorganisms after use with river water was not seen during examination of the fibers under the electron microscope (Appendix C1). However, in very turbid water, some clogging of the fiber surfaces may occur. A protocol for hollow fiber cartridge decontamination, sterilization and reuse was developed based on the use of a 10% hydrogen peroxide solution. This proved to be a very efficient and effective way of reusing cartridges. Under the electron microscope, the fibers themselves showed no signs of damage by peroxide treatment and membrane integrity checks indicated no leakage resulting from the treatment. However, the cartridge needs to be well flushed to remove residual peroxide that could otherwise interfere with microbial survival.

The HFD system was successfully adapted for use in field studies. Changes and modifications to the prototype were made to improve portability and reduce maintenance. Volume control of the *I* reservoir was maintained using

counterbalanced hydrostatic pressure in a design that also allowed the escape of gases from the *I* reservoir. This system of volume control requires the submerging of the hollow fiber cartridge and the *I* reservoir. Since this is not always possible, it may be necessary to further modify the system to incorporate an electronic level sensor and control the *I* circuit volume through the adjustment of flow rates.

The HFD system was used in *in situ* field studies at three temperate and two equatorial sites. The system was shown to respond rapidly to *in situ* changes in pH, Eh, temperature, dissolved oxygen, total dissolved solids and organic loads. The rapid response to these changes were on occasion marked by a rapid growth or decline of the bacteria in the system.

On different occasions, the passage of a plug-flow of nutrients in the Rideau River caused the *E. coli* numbers to increase in the HFD system while the presence of chlorine in the Ottawa River water quickly resulted in a rapid drop of bacterial and viral numbers in the HFD system.

During the *in situ* survival studies, differences were seen between the HFD system and the batch samples. Batch samples showed regrowth of bacteria at least once for all the sites except site TC. Even coliphage B appeared to multiply in one batch sample from the Gombak River. In contrast, the HFD system only demonstrated regrowth in Rideau River water which was the most eutrophic of all the sites studied.

The survival pattern for test microorganisms according to the HFD system was similar for the Ottawa, Gombak and Kroh Rivers with MS-2 > PV > phage B > *E. coli* and *E. durans*. The Rideau River was different in that *E. coli* had the best survival. *E. coli* regrowth in equatorial waters was not demonstrated in our study using the HFD system. However, the study should be repeated using a locally isolated *E. coli* strain. Other interesting results include an apparent diurnal survival pattern for *E. durans* in the Rideau River.

In some cases, the "regrowth" of bacteria observed occurred after a period of decline and never exceeded the initial numbers at the start of the experiment. On those occasions, it is important to consider the possibility that the regrowth was apparent rather than real. In view of the proposed somnicell adaptation to starvation stress (Roszak and Colwell, 1987; Barcina *et al.*, 1989), it is possible that initially, the bacteria persisted but was non-culturable on the selective media used and that the apparent regrowth was just the bacteria reverting back to a culturable form under appropriate conditions. In this study, survival of bacteria was only followed by its ability to form colonies on agar medium and did not address the issue of somnicells. However, the HFD system may be a suitable system for the study of that phenomena. The ability of the HFD system to respond rapidly to changes in the external environment would allow the study of bacteria under near natural conditions and determine the natural ecology of the bacteria including whether somnicells are a naturally-occurring survival strategy. It may also enable

the identification of the factors that may trigger both the bacteria's entry into and recovery from this state.

The survival of microorganisms and particular of *E. coli* and *E. durans*, were often seen not to decline or increase constantly over the period of the study. Rather, the bacterial survival appeared to be influenced more by catastrophic or stimulative events; declining or increasing rapidly in short periods of time. It would be very useful to try to determine the nature of those events. The presence of nutrients in the water due to runoff from rainfall and rapid changes in Eh were identified from field studies as possible factors that greatly influence bacterial survival. However, the effect of those factors could not be satisfactorily demonstrated in laboratory experiments.

In conclusion, the HFD system was found to be an improvement over existing systems like diffusion chambers for the *in situ* study of microbial survival because of the rapid rate of equilibration of the system to the external environment. The HFD system performed well during field testing and colonization or clogging of the hollow fiber pores do not appear to be a problem. The HFD system allows the study of microbial responses to real-time changes in conditions in the aquatic environment. The system may be potentially used for the study of microbial survival and persistence (including GEMs), for the study of microbial ecology, for the study of the effect of pollutants in water and for the monitoring of disinfectants in the water treatment or distribution system.

## 9. Conclusions

1. A protocol for the recovery and enumeration of *E. coli*, *E. durans*, somatic coliphages, F-specific bacteriophage MS-2, poliovirus and hepatitis A virus from a mixed culture was successfully developed.
2. The HFD system for the study of microbial survival was developed based on the adaptation and use of hollow fibers as the surface for interaction between the study reservoir and the external environment.
3. The HFD system was found to equilibrate rapidly to changes in pH, Eh and solute concentrations in the external environment with the exception of strong electrolytes such as strong acids and bases and strongly ionic salts.
4. Colonization and adsorption of microorganisms to the HFD system surfaces were not found to be a problem over a 48 h period.
5. The HFD system did not inherently affect the survival or recovery of any test organism with the exception of tailed coliphage B. The inactivation of coliphage B by the HFD system occurred within the first 8 h and was found to be related to the passage of the organisms through the hollow fibers. The extent of this inactivation could be greatly reduced by either increasing the diameter of the fibers or by decreasing the flow rate through the fibers. The inactivation of coliphage B in this manner was not apparent in riverwater with suspended solids.
6. A protocol for the decontamination and reuse of hollow fiber cartridges was developed based on the use of 10% hydrogen peroxide treatment. The

treatment did not damage the integrity of the hollow fibers and with careful flushing, there was no toxic residue that could affect microbial survival studies.

7. The HFD system equilibrated to changes in the external environment faster than diffusion chambers. The HFD system was able to equilibrate in minutes while the diffusion chamber equilibrated within hours. The diffusion chamber with cellulose acetate membranes was also found to have problems allowing the diffusion of charged ions across. The diffusion chambers demonstrated a "bottle" - effect that was not seen in the HFD system resulting in bacterial responses to changes in the external environment that was different than those observed in the HFD system.
8. Microorganisms in the HFD system responded rapidly to the presence of chlorine and chloramine T in the external environment. This suggests that the HFD system could be potentially used for the in-line monitoring of disinfectants in the water treatment or distribution systems.
9. The HFD system was successfully adapted for field application. This involved some modifications in the design of the internal reservoir and the configuration of the system to allow for portability, to ensure a constant volume in the / circuit and to prevent the accumulation of gas bubbles in the circuit.

10. The survival of test microorganisms was followed *in situ* with the HFD system and with batch samples at three temperate and two equatorial sites. The general order of survival of test microorganisms in the HFD system was found to be MS-2 phage > PV > somatic coliphage B > *E. coli* and *E. durans*. The one exception was in the Rideau River where *E. coli* generally survived better than the other test organisms. The survival of organisms in batch samples was different from that observed in the HFD system. Differences were noted in the general order of survival of test organisms, the greater variability between samples, the relative importance of predation and the fact that regrowth of bacteria occurred in batch samples from all sites but only occurred in the HFD system at the Rideau River which was the most eutrophic site in the studies. Some factors that appear to be related either directly or indirectly to bacterial survival include changes in BOD and Eh.

## 10. References



- Aebischer, P., L. Wahlberg, P.A. Tresco and S.R. Winn. 1991. Macroencapsulation of dopamine-secreting cells by coextrusion with an organic polymer solution. *Biomaterials*. 12: 50-56.
- Amy, P.S. and H.D. Hiatt. 1989. Survival and detection of bacteria in an aquatic environment. *Appl. Environ. Microbiol.* 55: 788-793.
- APHA. 1985. Standard Methods for the Examination of Water and Wastewater. 16th Ed. American Public Health Association, American Water Works Association and Water Pollution Control Federation. Washington D.C.
- APHA. 1992. Standard Methods for the Examination of Water and Wastewater. 18th Ed. American Public Health Association, American Water Works Association and Water Pollution Control Federation. Washington D.C.
- Arana, I., A. Muela, J. Iriberry, L. Egea and I. Barcina. 1992. Role of hydrogen peroxide in loss of culturability mediated by visible light in *Escherichia coli* in a freshwater ecosystem. *Appl. Environ. Microbiol.* 58: 3903-3907.
- Araujo, R.M., R. Pares and F. Lucena. 1990. The effect of terrestrial effluents on the incidence of *Aeromonas* spp. in coastal waters. *J. Appl. Bacteriol.* 69: 439-444.
- Atlas, R.M. and R. Bartha. 1981. Microbial Ecology : Fundamentals and Applications. Addison-Wesley Publishing Co., Reading, Massachusetts.
- Awong, J., G. Bitton and G.R. Chaudhry. 1990. Microcosm for assessing survival of genetically engineered microorganisms in aquatic environments. *Appl. Environ. Microbiol.* 56: 977-983.
- Baas-Becking, L.M.G. and E.J.F. Wood. 1955. Biological processes in the estuarine environment. *Kon. Ned. Akad. Wetten. Proc. B.* 58: 160-181.
- Babich, H. and G. Stotsky. 1980. Reductions in inactivation rates of bacteriophages by clay minerals in lake water. *Water Res.* 14: 185-187
- Bailey, C.A., R.A. Neihof and D.S. Tabor. 1983. Inhibitory effect of solar radiation on amino acid uptake in Chesapeake Bay bacteria. *Appl. Environ. Microbiol.* 46: 44-49.
- Bakhrouf, A., M. Jeddi and M.J. Gauthier. 1992. Survival of *Salmonella paratyphi* B and *Pseudomonas aeruginosa* in seawater after incubation or washing in the presence of osmolytes. *Can. J. Microbiol.* 38: 690-693.
- Barbaras, S. 1986. Monitoring natural waters for drinking water quality. *WHO Stat. Q.* 39: 32-45.
- Barcina, I., I. Arana, J. Iriberry and L. Egea. 1986. Influence of light and natural microbiota of the Butrón river on *Escherichia coli* survival. *Antonie Van Leeuwenhoek.* 52: 555-566.
- Barcina, I., J.M. Gonzalez, J. Iriberry and L. Egea. 1989. Effect of visible light on progressive dormancy of *Escherichia coli* cells during the survival process in natural fresh water. *Appl. Environ. Microbiol.* 55: 246-251.
- Barcina, I., I. Arana, A.A. Fernandez, J. Iriberry and L. Egea. 1992. Survival strategies of plasmid-carrier and plasmidless *Escherichia coli* strains under illuminated and non-illuminated conditions, in a fresh water ecosystem. *J. Appl. Bacteriol.* 73: 229-236.

- Beard, P.J. and N.F. Meadowcroft. 1935. Survival and rate of death of intestinal bacteria in sea water. *Am. J. Publ. Hlth. Nat. Hlth.* 25: 1023-1026.
- Beloian, A. 1990. Disinfectants. pp.133-146. *In* K. Helrich (ed.), *Official Methods of the AOAC.* AOAC, Arlington.
- Bitton, G. 1978. Survival of enteric viruses. pp.273-299. *In* R. Mitchell (ed.), *Water Pollution Microbiology, Vol : 2.* John Wiley and Sons, New York.
- Bitton, G. 1980. Adsorption of microorganisms to surfaces. *In* Marshall KC and Bitton G.(ed.), *Adsorption of Microorganisms to Surfaces.* Wiley-Interscience, New York.
- Bitton, G. and R. Mitchell. 1974. Effect of colloids on the survival of bacteriophages in seawater. *Water Res.* 8: 227-229.
- Bitton, G., R. Fraxedas and G.E. Gifford. 1979. Effect of solar radiation on poliovirus: preliminary experiments. *Water Res.* 13: 225-228.
- Bitton, G., S.B. Farrah, R.H. Ruskin, J. Butner and Y.J. Chong. 1983. Survival of pathogenic and indicator organisms in groundwater. *Ground Water* 21: 405-410.
- Block, J.C. 1983. Viruses in environmental waters. pp.118-145. *In* G. Berg (ed.); *Viral Pollution of the Environment.* CRC Press, Boca Raton, Florida.
- Bonde, G.F. 1977. Bacterial indicators of water pollution. pp.273-364. *In* M.R. Droop and H.W. Jannasch (ed.), *Advances in Aquatic Microbiology.* Academic Press, London
- Bonnefont, J.L., Y.P. Martin and B. Guiennet. 1990. Experimental studies on the survival of fecal bacterial from urban sewage in seawater. *Water Res.* 24: 267-274.
- Brasfeild, H. 1972. Environmental factors correlated with size of bacterial populations in a polluted stream. *Appl. Environ. Microbiol.* 24: 349-352.
- Brettar, I. and M.G. Hoefle. 1992. Influence of ecosystematic factors on survival of *Escherichia coli* after large-scale release into lake water mesocosms. *Appl. Environ. Microbiol.* 58: 2201-2210.
- Cabelli, V.J. 1977. *Clostridium perfringens* as a water quality indicator. pp.65. *In* Dutka B.J. (ed.), *Bacterial Indicators / Health Hazards Associated with Water.* American Society for Testing and Materials, Philadelphia.
- Cairns, J., Jr., P.V. McCormick and S.E. Belanger. 1992. Ecotoxicological testing: small is reliable. *J. Environ. Pathol. Toxicol. Oncol.* 11: 247-263.
- Calabrese, J.P. and G.K. Bissonnette. 1990. Improved membrane filtration method incorporating catalase and sodium pyruvate for detection of chlorine-stressed coliform bacteria. *Appl. Environ. Microbiol.* 56: 3558-3564.
- Carrillo, M., E. Estrade and T.C. Hazen. 1985. Survival and enumeration of fecal indicators *Bifidobacterium adolescentis* and *Escherichia coli* in a tropical rain forest watershed. *Appl. Environ. Microbiol.* 50: 468-476.
- Casson, L.W., C.A. Sorber, R.H. Palmer, A. Enrico and P. Gupta. 1992. HIV survivability in wastewater. *Water Environ. Res.* 64: 213-215.
- Castillo, G.C. and B.A. Trumper. 1991. Coliphages and other microbial indicators in stabilization ponds. *Environ. Toxicol. Water Quality.* 6: 197-208.

- Chao, W.L. and R.L. Feng. 1990. Survival of genetically engineered *Escherichia coli* in natural soil and river water. *J. Appl. Bacteriol.* 68: 319-325.
- Chao, W.L., R.J. Ding and R.S. Chen. 1987. Survival of pathogenic bacteria in environmental microcosms. *Chinese J. Microbiol. Immunol.* 20: 339-348.
- Chao, W.L., R.S. Chen and C.L. Tai. 1988a. Factors affecting the survival of pathogenic bacteria in subtropical river water. *Chinese J. Microbiol. Immunol.* 21: 85-92.
- Chao, W.L., R.J. Ding and R.S. Chen. 1988b. Survival of *Yersinia enterocolitica* in the environment. *Can. J. Microbiol.* 34: 753-756.
- Chung, K.T. and F.P. Yu. 1990. Survival of *Aeromonas hydrophila* and *Escherichia coli* in aquatic environments. *Chinese J. Microbiol. Immunol.* 23: 181-188.
- Coin, L., M.L. Menetrier, J. Loboude and M.C. Hannoun. 1965. Modern microbiological and virological aspects of water pollution. In O. Jaag (ed.), *Advances in Water Pollution Research*. Pergamon Press, London.
- Cornax, R., M.A. Morinigo, M.C. Balebona, D. Castro and J.J. Borrego. 1991. Significance of several bacteriophage groups as indicators of sewage pollution in marine waters. *Water Res.* 25: 673-678.
- Curtis, T.P., D.D. Mara and S.A. Silva. 1992. Influence of pH, oxygen, and humic substances on ability of sunlight to damage fecal coliforms in waste stabilization pond water. *Appl. Environ. Microbiol.* 58: 1335-1343.
- Davies, C.M. and L.M. Evison. 1991. Sunlight and the survival of enteric bacteria in natural waters. *J. Appl. Bacteriol.* 70: 265-274.
- De Vicente, A., M. Aviles, J.J. Borrego and P. Romero. 1988. Die-off and survival of *Pseudomonas aeruginosa* in seawater. *Zentralblatt Fur Bakteriologie, Mikrobiologie Und Hygiene - Serie B, Umwelthygiene, Krankenhaushygiene, Arbeitshygiene, Praventive Medizin.* 186: 261-272.
- Deaner, D.G. and K.D. Kerri. 1969. Regrowth of faecal coliforms. *J. Am. Water Works Assoc.* 61: 465-468.
- Diem, K. and C. Lentner. 1970. *Documenta Geigy Scientific Tables*. 7th Ed. J.R. Geigy S.A., Basle, Switzerland.
- Dutka, B.J. 1973. Coliforms are an inadequate index of water quality. *J. Environ. Hlth.* 36: 39-46.
- Dutka B.J., J.B. Bell, P. Collins and J. Popplow. 1969. A bacteriological study of the Rainy River conducted for the Advisory Board on Water Pollution, Rainy River and Lake of the Woods International Joint Commission. Manuscript Rep. KR 69-1, Dept. of Nat. Health and Welfare, Public Health Division, Toronto, Ontario.
- Edberg, S.C., M.J. Allen, D.B. Smith and N.J. Kriz. 1990. Enumeration of total coliforms and *Escherichia coli* from source water by the defined substrate technology. *Appl. Environ. Microbiol.* 56: 366-369.
- Ehrlich, H.L. 1985. The position of bacteria and their products in food webs. pp.199-219. In Leadbetter ER and Poindexter JS.(ed.), *Bacteria in Nature*. Vol : 1, *Bacterial Activities in Perspective*. Plenum Press, New York.

- Evison, L.M. and A. James. 1975.** Bifidobacterium as an indicator of fecal pollution in water. *Prog. Water Tech.* 7: 57-60
- Evison, L.M. and A. James. 1978.** Microbiological criteria for tropical water quality. pp.30-51. *In* R. Feachem, M. McGarry and D. Mare (ed.), *Water, Wastes and Health in Hot Climates.* Wiley-Interscience, New York.
- Feachem, R. 1974.** Fecal coliforms and fecal streptococci in streams in the New Guinea Highlands. *Water Res.* 8: 367-374.
- Feng, P.C.S. and P.A. Hartman. 1982.** Fluorogenic assays for immediate confirmation of *Escherichia coli*. *Appl. Environ. Microbiol.* 43: 1320-1329.
- Fiksdal, L., J.S. Maki, S.J. LaCroix and J.T. Staley. 1985.** Survival and detection of *Bacteriodes* spp., prospective indicator bacteria. *Appl. Environ. Microbiol.* 49: 148-150.
- Flatau, G.N., R.L. Clement, M.J. Gauthier and D.C. Puel. 1992.** Effect of incubation of *Escherichia coli* cells with halophyte extracts on their subsequent survival in seawater. *Can. J. Microbiol.* 38: 838-842.
- Flint, K.P. 1987.** The long term survival of *Escherichia coli* in river water. *J. Appl. Bacteriol.* 63: 261-270.
- Franco, M.A., P.I. Santini, S. Korol, J. Moretton, N. Dominguez and M. D'Aquino. 1990.** *In vitro* survival of pathogenic microorganisms in recreational water. *Revista Latinamericana De Microbiologia* 32: 149-152.
- Fujioka, R.S., P.C. Loh and L.S. Lau. 1980.** Survival of human enteroviruses in the Hawaiian ocean environment : evidence for virus-inactivating microorganisms. *Appl. Environ. Microbiol.* 39: 1105-1110.
- Fujioka, R.S., K. Tenno and S. Kansako. 1988.** Naturally occurring fecal coliforms and fecal streptococci in Hawaii's freshwater streams. *Toxicity Assess.* 3: 613-630.
- Gameson, A.L.H. and J.R. Saxon. 1967.** Field studies on the effect of daylight on mortality of coliform bacteria. *Water Res.* 1: 279-295.
- Gauthier, M.J., G.N. Flatau and R.L. Clement. 1990.** Influence of phosphate ions and alkaline phosphatase activity of cells on survival of *Escherichia coli* in seawater. *Microb. Ecol.* 20: 245-252.
- Geldreich, E.E. and B.A. Kenner. 1969.** Concepts of fecal streptococci in stream pollution. *J. Water Pollut. Control Fed.* 41: R336-R352.
- Geldreich, E.E., L.C. Best, B.A. Kenner and D.J. Van Donsel. 1968.** The bacteriological aspects of stormwater pollution. *J. Water Pollut. Control Fed.* 40: 1861-1872.
- Gerba, C.P. and G. Bitton. 1984.** Microbial pollutants: their survival and transport pattern to groundwater. *In* G. Bitton and C.P. Gerba (ed.), *Groundwater Pollution Microbiology.* John Wiley and Sons, New York.
- Gonzalez, J.M., E.B. Sherr and B.F. Sherr. 1990.** Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. *Appl. Environ. Microbiol.* 56: 583-589.

- Gonzalez, J.M., J. Iriberry, L. Egea and I. Barcina. 1993. Characterization of culturability, protistan grazing, and death of enteric bacteria in aquatic ecosystems. *Appl. Environ. Microbiol.* 58: 998-1004.
- Gordon, R.W. and C.B. Fliermans. 1978. Survival and viability of *Escherichia coli* in a thermally altered reservoir. *Water Res.* 12: 343-352.
- Gorter, A., R.J. van de Griend, J.D. van Eendenburg, W.H. Haasnoot and G.J. Fleuren. 1993. Production of bi-specific monoclonal antibodies in a hollow-fibre bioreactor. *J. Immunol. Methods.* 161: 145-150.
- Gotch, F.A. and M.L. Keen. 1985. Dialyzers and delivery systems. pp.1-40. *In* M.G. Cogan and M.R. Garovoy (ed.), *Introduction to Dialysis.* Churchill Livingstone, New York.
- Gray, T.R.G. 1976. Survival of vegetative microbes in soil. *Symp. Soc. Gen. Microbiol.* 26: 327-364.
- Hanawalt, P.C., P.K. Cooper, A.K. Ganesan and C.A. Smith. 1979. DNA repair in bacteria and mammalian cells. *Ann. Rev. Biochem.* 48: 783-836.
- Hansler, L., W. Tennhoff and C. Roll. 1992. Membrane humidification - a new method for humidification of respiratory gases in ventilator treatment of neonates. *Arch. Diseases Childhood* 67: 1182-1184.
- Hardina, C.M. and R.S. Fujioka. 1991. Soil: the environmental source of *Escherichia coli* and enterococci in Hawaii's (USA) streams. *Environ. Toxicol. Water Quality.* 6: 185-196.
- Harte, J., D. Levy, J. Rees and E. Saegebarth. 1980. Making microcosms an effective assessment tool. *In* Giesy JP.(ed.), *Microcosms in Ecological Research.*
- Hattler, B.G., P.C. Johnson, P.J. Sawzik, F.D. Shaffer, M. Klain, L.W. Lund, G.D. Reeder, F.R. Waters, J.S. Goode and H.S. Borovetz. 1992. Respiratory dialysis. A new concept in pulmonary support. *ASAIO Journal.* 38: M322-M325.
- Havelaar, A.H. 1993. Bacteriophages as models of human enteric viruses in the environment. *ASM News* 59: 614-619.
- Havelaar, A.H. and W.M. Hogeboom. 1983. Factors affecting the enumeration of coliphages in sewage and sewage-polluted waters. *Antonie Van Leeuwenhoek.* 49: 387-397.
- Havelaar, A.H. and W.M. Hogeboom. 1984. A method for the enumeration of male-specific bacteriophages in sewage. *J. Appl. Bacteriol.* 56: 439-447.
- Havelaar, A.H. and W.M. Pot-Hogeboom. 1989. F-specific RNA-bacteriophages as model viruses in water hygiene: ecological aspects. *Water Sci. Technol.* 20: 399-407.
- Havelaar, A.H., K. Furuse and W.M. Hogeboom. 1986. Bacteriophages and indicator bacteria in human and animal faeces. *J. Appl. Bacteriol.* 60: 255-262.
- Havelaar, A.H., W.M. Pot-Hogeboom, K. Furuse, R. Pot and M.P. Hormann. 1990. F-specific RNA bacteriophages and sensitive host strains in faeces and wastewater of human and animal origin. *J. Appl. Bacteriol.* 69: 30-37.
- Hazen, T.C. 1988. Fecal coliforms as indicators in tropical waters: A review. *Toxicity Assess.* 3: 461-478.

- Hendrick, C.W. and S.M. Morrison. 1967. Multiplication and growth of selected enteric bacteria in clear mountain stream water. *Water Res.* 1: 567-576.
- Hernandez, D.E.A., M.L. Serra and G.A. Toranzos. 1991. Coliphages as alternate indicators of fecal contamination in tropical waters. *Environ. Toxicol. Water Quality.* 6: 131-144.
- Hopkins, G.D., L. Semprini and P.L. McCarty. 1993. Microcosm and *in situ* field studies of enhanced biotransformation of trichloroethylene by phenol-utilizing microorganisms. *Appl. Environ. Microbiol.* 59: 2277-2285.
- Hosny, I., Z.H.T. El, M. Fayez and A. Shaban. 1990. Bacteriological evaluation of underground waters in Egypt. *Egyptian J. Microbiol.* 25: 277-290.
- Ifediora, O.C., B.P. Teehan and M.H. Sigler. 1992. Solute clearance in continuous venovenous hemodialysis. A comparison of cuprophane, polyacrylonitrile, and polysulfone membranes. *ASAIO Journal.* 38: M697-M701.
- Isbister, J.D., J.A. Simmons, W.M. Scott and J.F. Kitchens. 1983. A simplified method for coliphage detection in natural waters. *Acta Microbiol. Polonica.* 32: 197-201.
- Islam, M.S. 1990. Increased toxin production by *Vibrio cholerae* O1 during survival with a green alga, *Rhizoclonium fontanum*, in an artificial aquatic environment. *Microbiol. Immunol.* 34: 557-563.
- Islam, S., B.S. Drasar and D.J. Bradley. 1989. Attachment of toxigenic *Vibrio cholerae* O1 to various freshwater plants and survival with a filamentous green alga, *Rhizoclonium fontanum*. *J. Trop. Med. Hyg.* 92: 396-401.
- Islam, M.S., B.S. Drasar and D.J. Bradley. 1990. Survival of toxigenic *Vibrio cholerae* O1 with a common duckweed, *Lemna minor*, in artificial aquatic ecosystems. *Trans. Roy. Soc. Trop. Med. Hyg.* 84: 422-424.
- Jackson, P.E. and W.R. Jones. 1991. Hollow-fiber membrane-based sample preparation device for the clean-up of brine samples prior to ion chromatographic analysis. *J. Chromatography* 538: 497-503.
- Jimenez, L., L. Mûniz, G.A. Toranzos and T.C. Hazen. 1989. Survival and activity of *Salmonella typhimurium* and *Escherichia coli* in tropical freshwater. *J. Appl. Bacteriol.* 67: 61-69.
- Jorden, E.O., H.L. Russel and H.R. Zeil. 1904. The longevity of the typhoid bacillus in water. *J. Infect. Dis.* 1: 641-689.
- Jouenne, T., L. Bertin, G. Charriere and G.A. Junter. 1991. Electrochemical assessment of *Escherichia coli* survival in natural water. *Water Res.* 25: 829-834.
- Kallis, P., N.M. al-Saady, E.D. Bennett and T. Treasure. 1992. Intravascular oxygenation with the IVOX. *Brit. J. Hosp. Med.* 47: 824-828.
- Kandel, A., O. Nybroe and O.F. Rasmussen. 1992. Survival of 2,4-dichlorophenoxyacetic acid degrading *Alcaligenes eutrophus* AEO106(pR0101) in lake water microcosms. *Microb. Ecol.* 24: 291-303.
- Katamay, M.M. 1990. Assessing defined-substrate technology for meeting monitoring requirements of the total coliform rule. *Am. Water Works Assoc. J.* 82: 83-87.

- Kenard, R.P. and R. S. Valentine.** 1974. Rapid determination of the presence of enteric bacteria in water. *Appl. Environ. Microbiol.* 27: 484-488.
- Kilian, M. and P. Bulow.** 1976. Rapid diagnosis of *Enterobacteriaceae*. I. Detection of bacterial glycosidases. *Acta Path. Microbiol. Scand. Sect. B.* 84: 245-251.
- Klein, E. and A.C. Houston.** 1898. Further reports on bacteriological evidence of recent and therefore dangerous sewage pollution of elsewhere potable waters. *Suppl. Report of Medical Officer to 28th Report App. B.* 8: 498-504.
- Korhonen, L.K. and P.J. Martikainen.** 1991. Survival of *Escherichia coli* and *Campylobacter jejuni* in untreated and filtered lake water. *J. Appl. Bacteriol.* 71: 379-382.
- Korte, F.** 1977. Occurrence and fate of synthetic chemicals in the environment. pp.235-246. *In* W.J. Hunter and J.G.P.M. Smeets (ed.), *The Evaluation of Toxicological Data for the Protection of Public Health.* Pergamon Press, Oxford.
- Kott, Y., N. Roze, S. Sperber and N. Betzer.** 1974. Bacteriophages as viral pollution indicators. *Water Res.* 8: 165-171.
- Koujima, I.** 1992. Studies of bacterial indicators for water pollution--growth of *Escherichia coli* and enterococci in limited nutrient conditions. *Japanese. J. Publ. Hlth.* 39: 278-283.
- Koul, K. and B.R. Panhotra.** 1989. Isolation, survival & transfer of R-plasmid of *Salmonella typhimurium* in Dal Lake of Kashmir (India). *Indian J. Med. Res.* 89: 138-143.
- Kromoredjo, P. and R.S. Fujioka.** 1991. Evaluating three simple methods to assess the microbial quality of drinking water in Indonesia. *Environ. Toxicol. Water Quality.* 6: 259-270.
- Kurath, G. and Y. Morita.** 1983. Starvation survival physiological studies of a marine *Pseudomonas* species. *Appl. Environ. Microbiol.* 45: 1206-1211.
- Kuwabara, J.S. and R.W. Harvey.** 1990. Application of a hollow-fiber, tangential-flow device for sampling suspended bacteria and particles from natural waters. *J. Environ. Quality.* 19: 625-629.
- LaBelle R.L. and C.P. Gerba.** 1980. Influence of estuarine sediment on virus survival under field conditions. *Appl. Environ. Microbiol.* 39: 749-755.
- Laboratory Biosafety Guidelines.** 1990. Health and Welfare, Canada and the Medical Research Council of Canada, Ottawa, Ont., Canada.
- LeChevallier, M.W., S.C. Cameron and G.A. McFeters.** 1982. New medium for improved recovery of coliform bacteria from drinking water. *Appl. Environ. Microbiol.* 45: 484-492.
- Lessard, E.J. and J.M. Sieburth.** 1983. Survival of natural sewage populations of enteric bacteria in diffusion and batch chambers in the marine environment. *Appl. Environ. Microbiol.* 45: 950-959.
- Lim, C.H. and K.P. Flint.** 1989. The effects of nutrients on the survival of *Escherichia coli* in lake water. *J. Appl. Bacteriol.* 66: 559-569.

- Lo, S., J. Gilbert and F. Hetrick. 1976. Stability of human enteroviruses in estuarine and marine waters. *Appl. Environ. Microbiol.* 32: 245
- Loh, C.L., M.S. Jangi, Y.C. Ho, Y.F. Ngeow and C.W. Wang. 1988. Development of a field test kit for coliphage detection in natural waters. *Toxicity Assess.* 3: 451-460.
- Lopez-Torres, A.J., T.C. Hazen and G.A. Toranzos. 1987. Distribution and *in situ* survival and activity of *Klebsiella pneumoniae* and *Escherichia coli* in a tropical rain forest watershed. *Curr. Microbiol.* 15: 213-218.
- Lynch, J.M. 1990. Longevity of bacteria: Considerations in environmental release. *Curr. Microbiol.* 20: 387-390.
- Mara, D.D. 1974. *Bacteriology for Sanitary Engineers*. Churchill-Livingstone, London.
- Marino, R.P. and J.J. Gannon. 1991. Survival of fecal coliforms and fecal streptococci in storm drain sediment. *Water Res.* 25: 1089-1098.
- Mbithi, J.J.N. 1993. Studies on the role of inanimate surfaces and hands in the spread of Hepatitis A virus and their chemical disinfection. (PhD. thesis, University of Ottawa, Ottawa, Ontario).
- McFeters, G.A. and D.G. Stuart. 1972. Survival of coliform bacteria in natural waters : field and laboratory studies with membrane-filter chambers. *Appl. Microbiol.* 24: 805-811.
- McFeters, G.A. and S.I. Terzieva. 1991. Survival of *Escherichia coli* and *Yersinia enterocolitica* in stream water: Comparison of field and laboratory exposure. *Microb. Ecol.* 22: 65-74.
- McFeters, G.A., G.K. Bissonnette, J.J. Jezeski, C.A. Thomson and D.G. Stuart. 1974. Comparative survival of indicator bacteria and enteric pathogens in well water. *Appl. Microbiol.* 27: 823-829.
- Moore, B.E. 1993. Survival of human immunodeficiency virus (HIV), HIV infected lymphocytes and poliovirus in water. *Appl. Environ. Microbiol.* 59: 1437-1443.
- Morgan, J.A.W., G. Rhodes and R.W. Pickup. 1993. Survival of nonculturable *Aeromonas salmonicida* in lake water. *Appl. Environ. Microbiol.* 59: 874-880.
- Mulder, M. 1991. *Basic Principles of Membrane Technology*. Kluwer Acad. Press. Dordrecht, Netherlands.
- Munro, P.M., M.J. Gauthier, V.A. Breittmayer and J. Bongiovanni. 1989. Influence of osmoregulation processes on starvation survival of *Escherichia coli* in seawater. *Appl. Environ. Microbiol.* 55: 2017-2024.
- Ngeow, Y.F., C.L. Loh, H.M. Zain, C.W. Wang and Y.C. Ho. 1990. Performance of standard tests for fecal contamination in Malaysian pristine waters. *Proc. Second Biennial Water Quality Symp : Microbiological Aspects.*: 302-310.
- Niederlehner, B.R., K.W. Pontasch, J.R. Pratt and J. Cairns, Jr. 1990. Field evaluation of predictions of environmental effects from a multispecies-microcosm toxicity test. *Arch. Environ. Contam. Toxicol.* 19: 62-71.
- O'Brien, R.T. and J.S. Newman. 1977. Inactivation of polioviruses and coxsackieviruses in surface water. *Appl. Environ. Microbiol.* 33: 334-340.

- Olivieri, V.P. 1982. Bacterial indicators of pollution. pp.21-41. In W.O. Pipes (ed.), Bacterial Indicators of Pollution. CRC Press Inc., Boca Raton, Florida.
- Ota, K. 1991. Biocompatibility and capability of haemopurification systems: review and future development. Nephrol. Dialysis Transplantation 6(Suppl 2): 86-90.
- Payment, P., Y. Larose and M. Trudel. 1979. Polioviruses as indicators of virological quality of water. Can. J. Microbiol. 25: 1212-1214.
- Perez-Rosas, N. and T.C. Hazen. 1988. *In situ* survival of *Vibrio cholerae* and *Escherichia coli* in tropical coral reefs. Appl. Environ. Microbiol. 54: 1-9.
- Perez-Rosas, N. and T.C. Hazen. 1989. *In situ* survival of *Vibrio cholerae* and *Escherichia coli* in a tropical rain forest watershed. Appl. Environ. Microbiol. 55: 495-499.
- Pipes, W.O. 1982. Bacterial indicators of pollution. pp.1-19. In W.O. Pipes (ed.), Bacterial Indicators of Pollution. CRC Press Inc., Boca Raton, Florida.
- Pipke, R., D.I. Wagner, K.N. Timmis and D.F. Dwyer. 1992. Survival and function of a genetically engineered Pseudomonad in aquatic sediment microcosms. Appl. Environ. Microbiol. 58: 1259-1265.
- Pokorny, J. 1988. Survival and virulence of salmonellae in water. J. Hyg. Epidemiol. Microbiol. Immunol. 32: 361-366.
- Price, J.I., B.S. Yandell and W.P. Porter. 1992. Chemical ions affect survival of avian cholera organisms in pond water. J. Wildlife Management. 56: 274-278.
- Pritchard, P.H. and A.W. Bourquin. 1984. The use of microcosms for evaluation of interactions between pollutants and microorganisms. pp.133-216. In Marshall KC.(ed.), Advances in Microbial Ecology. Vol. 7. Plenum Press, New York.
- Ramia, S. and S.A. Sattar. 1979. Second-step concentration of viruses in drinking and surface waters using polyethylene glycol hydroextraction. Can. J. Microbiol. 25: 87-92.
- Reali, D., S. Rosati, B. Pinto and M.R. Iavarone. 1991. On the use of phages as indicators of fecal pollution. Igiene Moderna 95: 820-834.
- Rhodes, M.W. and H. Kator. 1988. Survival of *Escherichia coli* and *Salmonella* spp. in estuarine environments. Appl. Environ. Microbiol. 54: 2902-2907.
- Rhodes, M.W. and H.I. Kator. 1990. Effects of sunlight and autochthonous microbiota on *Escherichia coli* survival in an estuarine environment. Curr. Microbiol. 21: 65-74.
- Rice, E.W., M.J. Allen and S.C. Edberg. 1990. Efficacy of beta-glucuronidase assay for identification of *Escherichia coli* by the defined-substrate technology. Appl. Environ. Microbiol. 56: 1203-1205.
- Rice, E.W., C.H. Johnson, D.K. Wild and D.J. Reasoner. 1992. Survival of *Escherichia coli* O157:H7 in drinking water associated with a waterborne disease outbreak of hemorrhagic colitis. Lett. Appl. Microbiol. 15: 38-40.
- Rivera, S.C., T.C. Hazen and G.A. Toranzos. 1988. Isolation of fecal coliforms from pristine sites in a tropical rain forest. Appl. Environ. Microbiol. 54: 513-517.

- Rivilla, R. and C.C. Gonzalez. 1989. Seasonal variations of pollution indicators in a wildfowl reserve (Donana National Park, Spain). *J. Appl. Bacteriol.* 67: 219-223.
- Roberts, J.R. and W.K. Marshall. 1980. Retentive capacity : an index of chemical persistence expressed in terms of chemical-specific and ecosystem-specific parameters. *Ecotoxicol. Environ. Safety.* 4: 158-171.
- Roszak, D.B. and R.R. Colwell. 1987. Survival strategies of bacteria in the natural environment. *Microbiol. Rev.* 51: 365-379.
- Roszak, D.B., D.J. Grimes and R.R. Colwell. 1984. Viable but nonrecoverable stage of *Salmonella enteritidis* in aquatic systems. *Can. J. Microbiol.* 30: 334-338.
- Sagy, M. and Y. Kott. 1990. Efficiency of rotating biological contactor in removing pathogenic bacteria from domestic sewage. *Water Res.* 24: 1125-1128.
- Sattar, S.A. 1981. Virus survival in receiving waters. pp. 91-108. *In* M. Goddard and M. Butler (eds.), *Viruses and Wastewater Treatment*. Pergamon Press, Toronto, Ontario.
- Sattar, S.A., R.A. Raphael and V.S. Springthorpe. 1985. Rotavirus survival in raw and treated waters and its health implications. *Water Sci. Tech.* 17: 7-14.
- Scheuerman, P.R., J.P. Schmidt and M. Alexander. 1988. Factors affecting the survival and growth of bacteria introduced into lake water. *Arch. Microbiol.* 150: 320-325.
- Schoonen, A.J.M., F.J. Schmidt, H. Hasper, D.A. Verbrugge, R.G. Tiessen and C.F. Lerk. 1990. Development of a potentially wearable glucose sensor for patients with diabetes mellitus: Design and *in vitro* evaluation. *Biosensors & Bioelectronics.* 5: 37-46.
- Seely, N.D. and S.B. Primrose. 1982. The isolation of bacteriophages from the environment. *J. Appl. Bacteriol.* 47: 1-15.
- Sherer, B.M., J.R. Miner, J.A. Moore and J.C. Buckhouse. 1992. Indicator bacterial survival in stream sediments. *J. Environ. Quality.* 21: 591-595.
- Slanetz, L.W. and C.H. Bartley. 1964. Detection and sanitary significance of fecal streptococci in water. *Am. J. Public Hlth.* 54: 609-613.
- Slonczewski, J.L. 1992. pH-regulated genes in enteric bacteria. *ASM News.* 58: 140-144.
- Smit, E., E.J.D. Van and V.J.A. Van. 1992. Risks associated with the application of genetically modified microorganisms in terrestrial ecosystems. *FEMS Microbiol. Rev.* 88: 263-278.
- Snow J. 1855. *On the Mode of Communication of Cholera.* 2. J. Churchill, London.
- Sobecky, P.A., M.A. Schell, M.A. Moran and R.E. Hodson. 1992. Adaptation of model genetically engineered microorganisms to lake water: Growth rate enhancements and plasmid loss. *Appl. Environ. Microbiol.* 58: 3630-3637.
- Sorensen, S.J. 1991. Survival of *Escherichia coli* K12 in seawater. *FEMS Microbiol. Ecol.* 85: 161-168.

- Steffan, R.J., A. Breen, R.M. Atlas and G.S. Sayler. 1989. Monitoring genetically engineered microorganisms in freshwater microcosms. *J. Industrial Microbiol.* 4: 441-446.
- Tashiro, K., Y. Kubokura, Y. Kato, K.I. Kaneko and M. Ogawa. 1991. Survival of *Yersinia enterocolitica* in soil and water. *J. Vet. Med. Sci.* 53: 23-28.
- Taylor, C.B. 1951. Coli-aerogenes bacteria in soils. *J. Hyg.* 49: 162-168.
- Thom, S., D. Warhurst and B.S. Drasar. 1992. Association of *Vibrio cholerae* with fresh water amoebae. *J. Med. Microbiol.* 36: 303-306.
- Tiebout, R.F. 1990. Tissue culture in hollow-fibre systems: implications for downstream processing and stability analysis. *Developments in Biological Standardization.* 71: 65-71.
- Toranzos, G.A. 1991. Current and possible alternative indicators of fecal contamination in tropical waters: a short review. *Environ. Toxicol. Water Quality* 6: 121-130.
- Tuwiner, S.B. 1962. Diffusion and Membrane Technology. American Chemical Society Monograph Series. Reinhold Publ. Corp., New York.
- Valdes-Collazo, L., A.J. Schultz and T.C. Hazen. 1987. Survival of *Candida albicans* in tropical marine and fresh water. *Appl. Environ. Microbiol.* 53: 1762-1767.
- Valentine, R.C. and J.R.G. Bradfield. 1954. The urea method for bacterial viability counts with the electron microscope and its relation to other viability counting methods. *J. Gen. Microbiol.* 11: 349-357.
- Vargo, G., P. Hargraves and P. Johnson. 1975. Scanning electron micrographs of dialysis tubes incubated in flowing seawater. *Marine Biol.* 31: 113-120.
- Varma, P.R.G. and T.S.G. Iyer. 1990. Viability of *Vibrio cholerae* in water. *Fishery Tech.* 27: 151-152.
- Vasconcelos, G.J. and R.G. Swartz. 1976. Survival of bacteria in seawater using a diffusion chamber apparatus in situ. *Appl. Environ. Microbiol.* 31: 913-920.
- Wagner-Dobler, I., R. Pipke, K.N. Timmis and D.F. Dwyer. 1992. Evaluation of aquatic sediment microcosms and their use in assessing possible effects of introduced microorganisms on ecosystem parameters. *Appl. Environ. Microbiol.* 58: 1249-1258.
- Waite, W.M. 1985. A critical appraisal of the coliform test. *J. Inst. Water Eng. Sci.* 39: 341-357.
- Wiggins, B.A. and M. Alexander. 1985. Minimum bacterial density for bacteriophage replication: implications for significance of bacteriophages in natural ecosystems. *Appl. Environ. Microbiol.* 49: 19-23.
- Williams, S.T. 1985. Oligotrophy in soil: fact or fiction? pp. 89-110. *In* M. Fletcher and G. Floodgate (eds), *Bacteria in the Natural Environment: The Effect of Nutrient Conditions.* Acad. Press, New York.
- Wilson G.S., A.A. Miles, M.T. Parker and H.M. Dick. 1983. Topley and Wilson's Principles of Bacteriology, Virology and Immunity. Vol : 1, General Microbiology and Immunity. 7th ed. Edward Arnold Ltd., London.

- Winstanley, C., J.A.W. Morgan, R.W. Pickup and J.R. Saunders. 1991.** Use of a *xylE* marker gene to monitor survival of recombinant *Pseudomonas putida* populations in lake water by culture on nonselective media. *Appl. Environ. Microbiol.* 57: 1905-1913.
- Wright, R.C. 1989.** The survival patterns of selected faecal bacteria in tropical fresh waters. *Epidemiol. Infect.* 103: 603-611.
- Xu, H.S., N. Roberts, F.L. Singleton, R.W. Attwell, D.J. Grimes and R.R. Colwell. 1982.** Survival and viability of non-culturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment. *Microb. Ecol.* 8: 313-323.
- Zobell, C.E. 1936.** Bacterial action of seawater. *Proc. Soc. Exp. Biol. Med.* 34: 113-116.

## 11. Appendices

## Appendix A1

### Tryptone- yeast extract-glucose broth (TYGB)

a)	<b>Basal medium</b>	
	Tryptone (Oxoid)	10.0 g
	Yeast extract (Difco)	1.0 g
	Sodium chloride (BDH)	8.0 g
	Distilled water	1000.0 mL
b)	<b>Calcium-glucose solution</b>	
	CaCl <sub>2</sub> .2H <sub>2</sub> O (BDH)	3.0 g
	Glucose (Fisher)	10.0 g
	Distilled water	100.0 g
c)	<b>Complete medium</b>	
	Basal medium	1000.0 mL
	Calcium-glucose solution	10.0 mL

The basal medium was prepared and autoclave-sterilized. The calcium-glucose solution was prepared separately and filter-sterilized through a 0.22  $\mu\text{m}$  membrane filter and added to the autoclaved basal medium. Tryptone-yeast extract-glucose agar was prepared by the adding agar to TYGB basal medium. After autoclaving, the basal

## Appendix A2

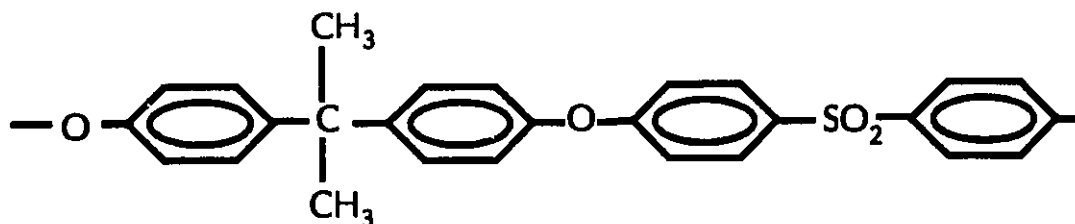
### Preparation of phosphate buffers

A 66.7 mM stock of  $\text{KH}_2\text{PO}_4$  and a 66.7 mM stock of  $\text{Na}_2\text{HPO}_4$  were prepared. The two stocks were mixed in varying amounts to make buffers of the appropriate pH and were then diluted to make 3 mM working buffers. These buffers were used for the experiment studying adsorption of microorganisms to surfaces of the HFD system.

pH	Volume (mL) of $\text{KH}_2\text{PO}_4$ stock	Volume (mL) of $\text{Na}_2\text{HPO}_4$ stock
5	99.2	0.8
6	88.9	11.1
7	41.3	58.7
8	3.7	96.3

## Appendix B1

### Polysulfone structure



## Appendix B2

### Characteristics of the Hemaflow F4 and the IMRI hollow fibers.

Characteristics	Hemaflow F4 hollow fibers	IMRI hollow fibers
Inner diameter ( $\mu\text{m}$ )	200	789
Thickness of membrane ( $\mu\text{m}$ )	40	336
Length (cm)	22.5	22.5
Number of fibers	7000	255
Total surface area ( $\text{cm}^2$ )	$7 \times 10^3$	$1.4 \times 10^3$

## Appendix C1

### Electron Micrographs

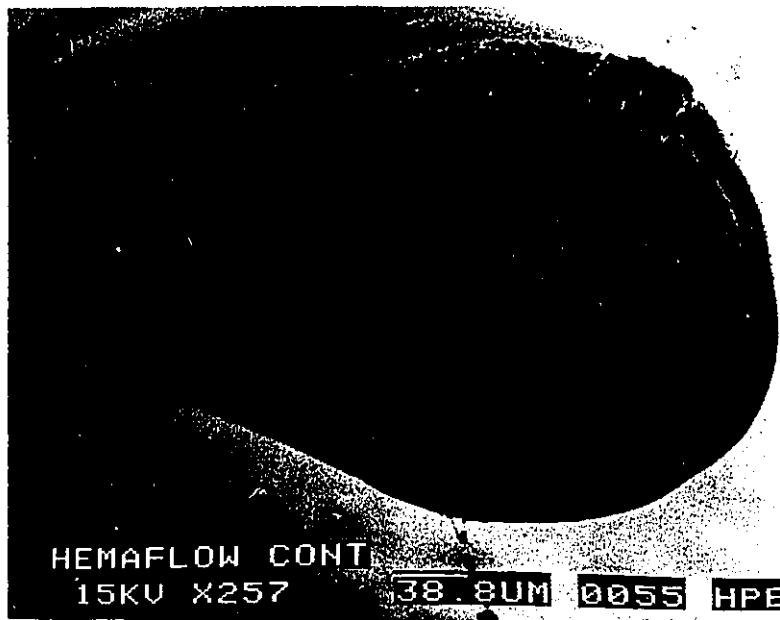
**a) Scanning Electron Micrograph (x257): Polysulfone hollow fiber. (overall view)**

The fibers are asymmetrical with a thin "skin" with small pores on the inner luminal surface and a spongy matrix with large pores on the outer side.

**b) Scanning Electron Micrograph (x2040): Inner skin of the polysulfone hollow fiber**

**c) Scanning Electron Micrograph (x282): IMRI, University of Ottawa custom-made hollow fibers. (cross-sectional view)**

This fiber shows a spongy matrix between two "skin"-layers. The presence of the two skin layers was for added structural strength but would also cause slower diffusion of solutes.



## Appendix C1

### Electron Micrographs

**d) Scanning Electron Micrograph (x2000): Polysulfone hollow fiber. (outer surface)**

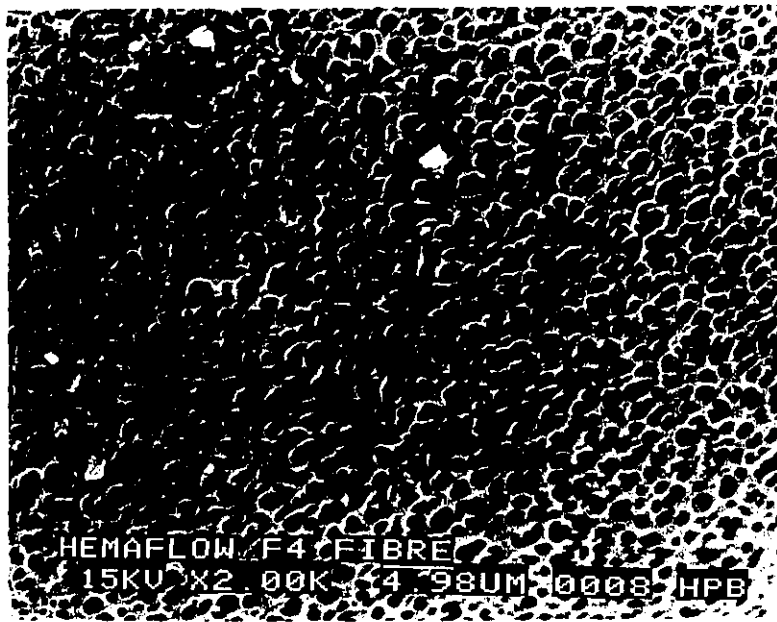
The outer surface demonstrates the spongy nature of most of the matrix of the polysulfone hollow fiber.

**e) Scanning Electron Micrograph (x5300): Polysulfone hollow fiber - after exposure to river water. (outer surface)**

The hollow fiber was recovered from a cartridge that had been exposed to very turbid river water seeded with bacteria for 48 h. A certain amount of debris and a few bacteria are visible. **DB** - dividing rod-shaped bacterium.

**f) Scanning Electron Micrograph (x2000): Polysulfone hollow fiber - after hydrogen peroxide treatment. (outer surface)**

The hollow fiber had been exposed to river water as previously described for Plate d and then was put through the hydrogen peroxide (10%) decontamination procedure. The procedure successfully removed debris and bacteria without any evident damage to the fiber integrity.



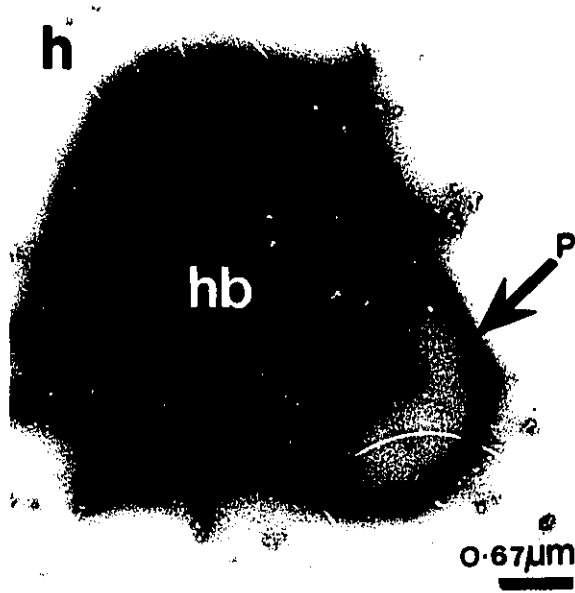
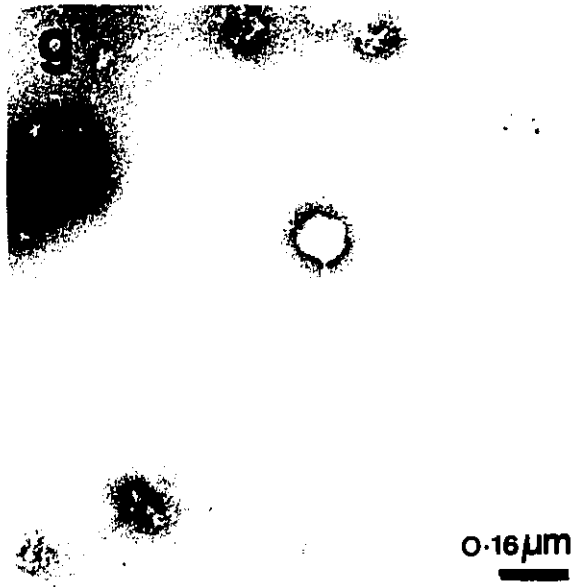
## Appendix C1

### Electron Micrographs

- g) Transmission Electron Micrograph (x62500): Somatic coliphage B.**
  
- h) Transmission Electron Micrograph (x14900): MS-2 coliphage.**

The MS-2 phages are seen together with the host bacterium, *Salmonella typhimurium* WG49. Some MS-2 phage are seen attached to the sex-pilus.

**hb** - host bacterium; **P** - sex pilus.



## Appendix C2

### Color Plates

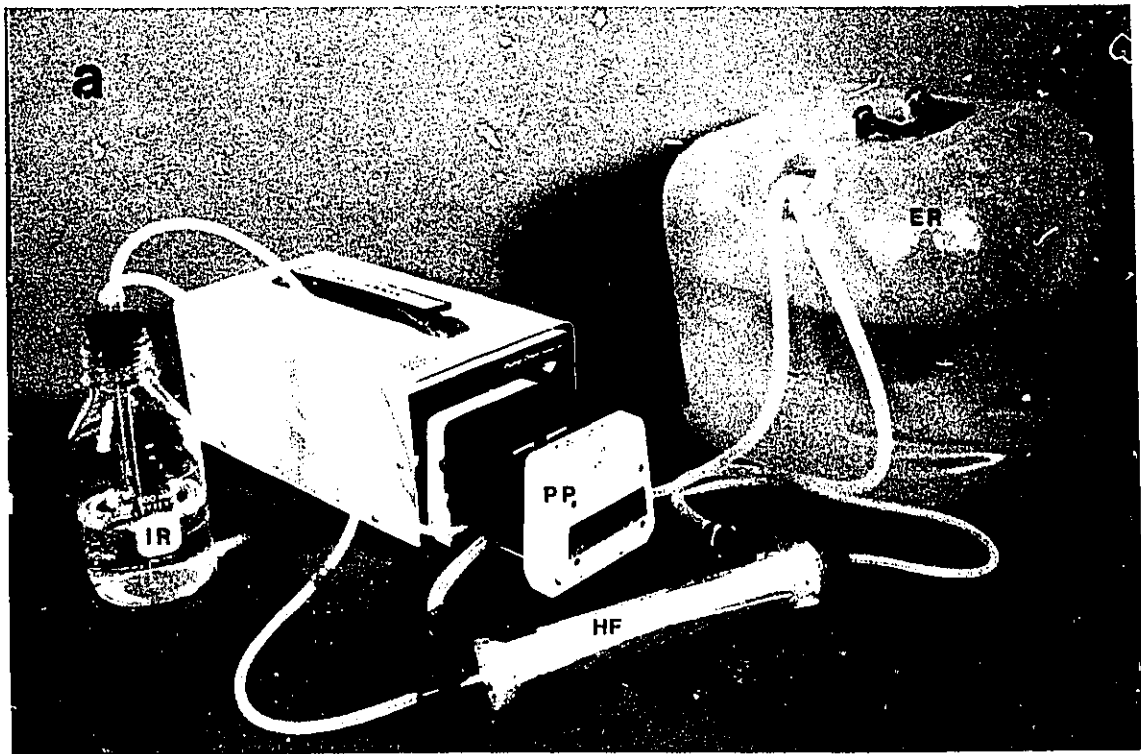
a) **The components of the HFD system.**

**ER** - External reservoir; **IR** - Internal reservoir; **HF** - hollow fiber cartridge and **PP** - peristaltic pump.

b) **The Ottawa River field site (TB) at the Britannia Water Treatment Plant.**

For experiments carried out during the late winter - early spring, the Ottawa River water was warmed up to about 20°C by passage through an adapted condenser which acted as a heat-exchange unit. The water then entered a large plastic container which was used as the experimental reservoir to which the HFD system was exposed.

**C** - adapted condenser unit; **DS** - Datasonde datalogging unit; **ER** - experimental reservoir; **WI** - the water inlet for pre-warmed Ottawa River water.



## Appendix C2

### Color Plates

**c) The Rideau River site (TA)**

The experiments were carried out from the side of a floating dock. The peristaltic pump and hollow fiber cartridge were housed in a small wooden shelter. The sample reservoirs were partly submerged in the river. River water was drawn through insulated tubing into the *E* circuit of the HFD system from 3 m out in the river.

S - sample reservoirs; WS - wooden shelter

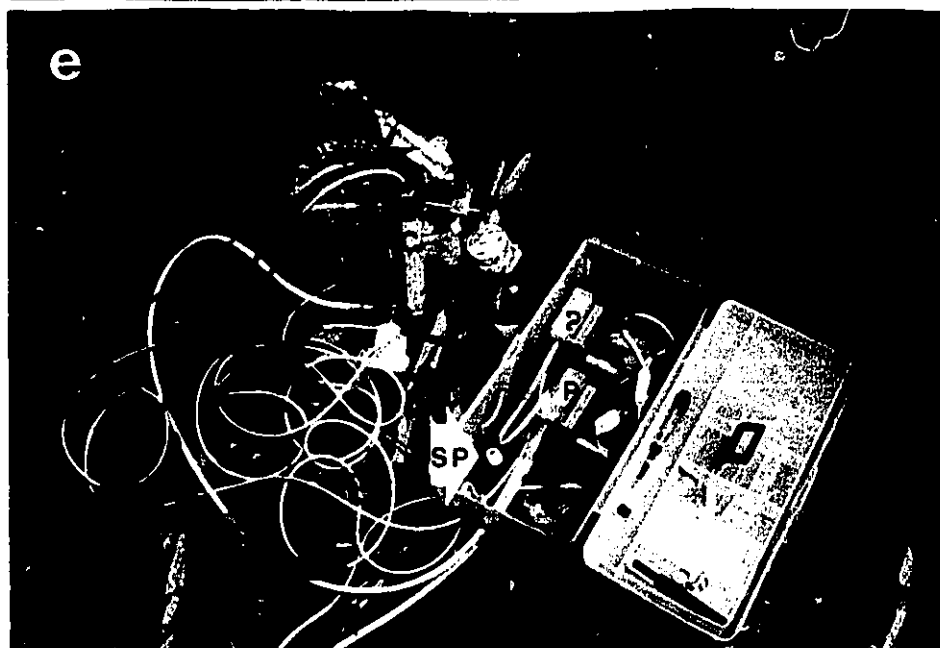
**d) The Gombak River site (EA) - after heavy rainfall.**

The HFD system was adapted for portability. The peristaltic pumps were housed in a plastic pump box and powered by two 12 V lead acid batteries. The pump box also houses the *I* circuit's sampling port. The sample reservoir and hollow fiber cartridge was submerged in the river from a float.

B - batteries under a plastic cover; P - pump box; S - sample reservoirs and hollow fibre cartridge.

**e) HFD system - apparatus for field work in Malaysia.**

C - hollow fiber cartridge; P - peristaltic pumps; S - sample reservoir; SP - sampling port.



## **12. Publications and Presentations**

### Publications

1. **Springthorpe, V.S., C.L. Loh, W.J. Robertson and S.A. Sattar.** 1993. *In situ* survival of indicator bacteria, MS-2 phage and human pathogenic viruses in river water. *Wat. Sci. Tech.* 27: 413-420.

### Papers under Preparation

1. **Loh, C.L., V.S. Springthorpe and S.A. Sattar.** The characterization of a hollow fiber system for the study of microbial survival in the aquatic environment: laboratory studies.
2. **Loh, C.L., V.S. Springthorpe and S.A. Sattar.** The field application of a hollow fiber system for microbial survival studies in aquatic environments.

### Presentations

1. **Springthorpe, V.S., Loh, C.L., Mbithi, J.N. and Sattar, S.A.** (April, 1991). The Ecodyne system: A novel technique for the *in situ* study of the survival of microorganisms in the water environment. International Workshop on 'Virology for the Water Supply in the Nineties', organized jointly by The Netherlands Waterworks Testing and Research Institute (KIWA Ltd.), The Netherlands Nat. Institute of Public Health and Environ. Protection (RIVM) and the International Water Supply Assoc.
2. **Springthorpe, V.S., Loh, C.-L. and Sattar, S.A.** (May, 1992). *In situ* survival of indicator bacteria, MS-2 phage and human pathogenic viruses in river water. International Symposium on 'Health-Related Water Microbiology', held in Washington, D.C.
3. **Springthorpe, V.S., C.L. Loh, W.J. Robertson and S.A. Sattar.** (April, 1993). A new tool for simulating natural water quality: Application in *in situ* microbiological studies. Presented at Am. Soc. Microbiol. Conf. on Water Quality in the Western Hemisphere, San Juan, Puerto Rico.
4. **Springthorpe, V.S., C.L. Loh and S.A. Sattar.** (Oct. 1993). Water disinfection *in situ* : A new experimental approach. Paper at the International Symp. on Chemistry and Biology of Municipal Water Treatment: Current Status and Future Directions. Can. Centre for Inland Waters, Burlington, Ontario.

5. **Loh, C.L., V.S. Springthorpe and S.A. Sattar.** (Feb. 1994). Characterization of a hollow fibre diffusion system for studies of microbial fate in aquatic ecosystems. Presented at the 29th Central Can. Symp. on Water Pollution Research., Can. Centre for Inland Waters, Burlington, Ontario.

#### **Patent Pending**

1. **Loh, C.L., V.S. Springthorpe and S.A. Sattar.** Hollow fiber monitoring system.