

# **GUIDELINES FOR ROUTINE MONITORING OF MEMBRANE PERFORMANCE FOR POTABLE WATER PRODUCTION IN SMALL WATER TREATMENT PLANT**

**Report to the  
WATER RESEARCH COMMISSION**

**by**

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# EXECUTIVE SUMMARY

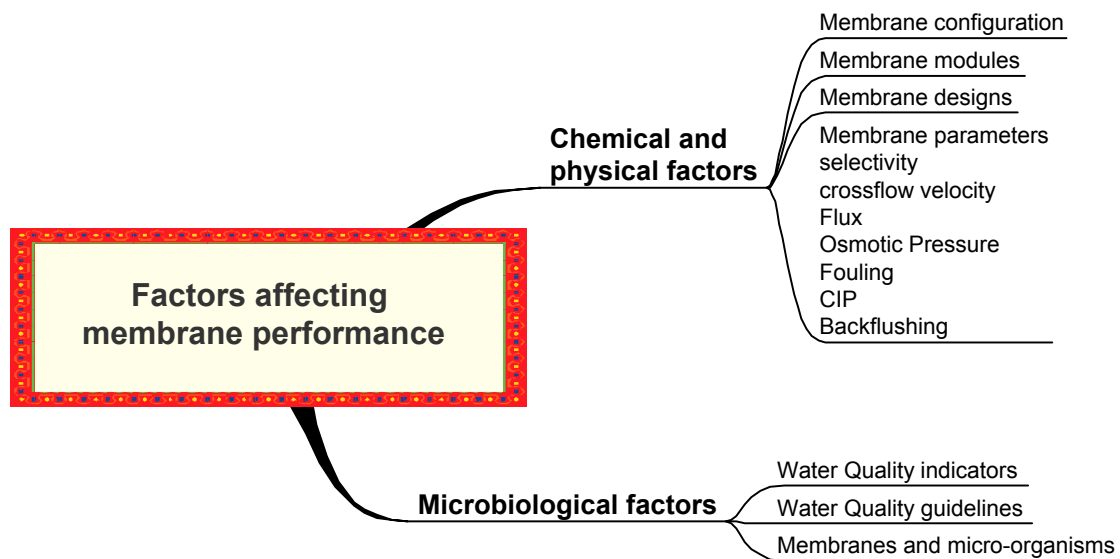
## STUDY BACKGROUND

Membrane filtration technology for potable water production is proving to be a suitably good alternative to conventional water treatment techniques. It offers the potential of improved removal efficiencies compared to conventional methods; it is suitable for operation in small areas and in the long-term can be economically viable. This technology offers the water industry a simple one or two-step treatment barrier to secure compliance with all of the new safety standards being recommended worldwide.

Membrane performance needs to be assessed routinely, especially if membrane abnormalities are suspected, or if new membranes are installed and/or the plant is restarted after shutdown. Protocols for testing membrane performance based on removal of microorganisms will ensure proper functioning of the ultrafiltration (UF) and microfiltration systems and reduce risks of infection by contamination of drinking water. Standard turbidity measurements have been shown to be ineffective in detecting significant numbers of microorganisms that pose a health risk. The rejection profile of the membranes for differently sized microorganisms such as bacteriophages (23 to 80 nm), bacteria (0.5 to 5  $\mu\text{m}$ ), protozoan cysts (5 to 15  $\mu\text{m}$ ) and yeasts (>10  $\mu\text{m}$ ) would be characteristic for each membrane type.

The aim of the research was to evaluate membrane membrane performance based on its efficiency in removing microorganisms and subsequently to set up protocols for guiding membrane users for evaluating membrane performance in potable water treatment.

Physical characteristics of membrane, chemical factors of membrane and influent water and the organic compounds in the water influence membrane performance. These parameters need be taken in cognizance when assessing membrane functioning and are outlined.



Furthermore, it has been shown that many of the alternatives to microbiological testing of water are not sensitive enough to detect low, but significant numbers of microorganisms that pose a health risk. This report describes the research undertaken to determine the performance characteristics of UF membranes using bacteriological tests and to develop a set of protocols for membrane users.

This study involved three different rig systems i.e., pilot scale system at the Wiggins process plant, a laboratory system and a bench-scale system with polysulphone membranes that were manufactured by the Institute of Polymer Science (IPS). The parameters that affect membrane performance were investigated using bacterial counts in the feed and permeate. The performance characteristic of the membranes was investigated by evaluating the removal of bacteria, bacteriophages, *Giardia* cysts and *Cryptosporidium* oocysts by UF. A final protocol that provides a guideline for membrane users was drawn based on:

- a) Determining membrane characteristics of the test plants during standard operating conditions;
- b) Evaluating cleaning strategies and determining the effect of biofilms on the membrane performance;

- c) Establishing the potential of membranes to remove conventional water-borne bacteria, parasites and viruses; and
- d) Evaluating rapid and simple detection techniques for field use.

Biological tests were conducted to determine the efficacy of clean-in-place (CIP) and backflushing. Further experiments were conducted to study the effect of fouling, and operating the system under cross-flow and dead-end operations. The relationship between microbial tests and flux was also investigated. Membranes were also challenged with bacteriophages (23 to 80 nm), bacteria (0.5 to 5  $\mu\text{m}$ ), protozoan cysts (5 to 15  $\mu\text{m}$ ) and yeasts (1-10  $\mu\text{m}$ ). Depending on the objective the test feed and permeate samples were tested total coliforms and heterotrophic bacteria.

**Microbial removal efficiency** of membranes under routine operations show that newer membranes “double skinned” provide better product water than the previous “single skinned” membranes.

**Cleaning of membranes during routine operations** show that CIP treatment (50 mg/l) was effective in removing all coliforms, faecal streptococci and reduced heterotrophic bacteria to levels acceptable for potable water production despite the quality of the feed water.

**Membrane integrity or bubble point test** is a good indicator for abnormalities for pinholes in the module or any burst capillaries on the membranes.

**Backflushing of membrane** showed that the bacterial counts were higher after backwash than before for all the different modules and membranes studied. There is a window period after backwash cycle that needs to be determined for different membranes

**Operating the membranes under crossflow and dead-end operations** showed that fluid flow in the dead-end mode is better suited for potable water production as potable water was achieved faster with dead-end mode of operation. However, flux values decrease drastically with dead-end production due to the development of the fouling layer.

**Membrane performance** of the Wiggins membranes (which were three years old) when operating under constant pressure and in cross flow mode gave 99.9 % rejection of faecal streptococci, total coliforms and faecal coliforms. Heterotrophic bacteria, however, were present. The laboratory-scale rig with membrane E31/1713 showed a 99.93% to 99.97% rejection of heterotrophic bacteria. All the experiments conducted on the bench-scale rig also showed 99.9% rejection. In all the membranes tested, potable water was produced after a short period of time i.e. once the fouling layer developed. This suggests that the fouling layer assists in the filtration process.

**Challenge tests** based on sizes of microorganisms showed that UF membranes, if intact, could act as absolute barriers for cysts and oocysts. However, bacteriophages, which are very small, e.g. MS2, are not removed completely and the removal of different organisms is influenced by the MWCO of the membrane.

These results have provided substantial information related to the membrane performance from a microbiological perspective. Based on these results a protocol for membrane users and suppliers (prior to use) for total quality checks and quality assurance systems (in-use testing) using simple techniques is recommended. Our findings have been significant in that the supplier of the membranes (IPS, Stellenbosch) has recognised the importance of microbiological performance testing.

The goal of this study was i) to develop microbiological criteria to assess membrane performance; ii) to develop protocols for membrane users to ensure quality of the membrane after installation, during processing and during cleaning; and iii) to compare different microbiological tests to chemical indicator tests and find simple methods for determining water quality, and finally to recommend protocols for implementation to membrane users for potable water production. Through these goals this study enabled the development of a protocol that can be used in conjunction with turbidity to monitor microbiological performance of a membrane. These protocols can be used to ensure quality of the membrane after installation, during processing and during cleaning.

Through this research, several B Tech and M Tech students were trained and research results were published in peer-reviewed journals and conference proceedings (Appendix 1).

## **FUTURE RESEARCH**

This protocol developed through this study could be extended to testing its efficacy for microfiltration and other industrial processes in which membranes are utilized, which would then lead to a general protocol for all membrane manufacturers and users.

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|----------------------------------------------------|-------------|
| <b>EXECUTIVE SUMMARY</b>                           | <b>ii</b>   |
| <b>ACKNOWLEDGEMENTS</b>                            | <b>vii</b>  |
| <b>TABLE OF CONTENTS</b>                           | <b>viii</b> |
| <b>LIST OF FIGURES</b>                             | <b>xiii</b> |
| <b>LIST OF TABLES</b>                              | <b>xv</b>   |
| <b>LIST OF ACRONYMS</b>                            | <b>xiv</b>  |
| <b>CHAPTER 1: INTRODUCTION</b>                     | <b>1</b>    |
| 1.1 Study Overview                                 | 1           |
| <b>CHAPTER 2: BACKGROUND LITERATURE</b>            | <b>3</b>    |
| 2.1 Membrane Technology and Water                  | 4           |
| 2.2 Membrane Modules                               | 6           |
| 2.3 Systems Design                                 | 7           |
| 2.4 Parameters Affecting Membrane Performance      | 9           |
| 2.4.1 Selectivity                                  | 9           |
| 2.4.2 Crossflow velocity                           | 10          |
| 2.4.3 Flux                                         | 10          |
| 2.4.4 Temperature                                  | 10          |
| 2.4.5 Fouling                                      | 11          |
| 2.4.6 Rejection                                    | 14          |
| 2.5 Microbiological Aspects of Water Treatment     | 14          |
| 2.6 Microbiological Water Quality Indicators       | 16          |
| 2.7 Microbiological Water Quality Guidelines       | 18          |
| 2.8 Membranes and Microorganisms                   | 22          |
| 2.8.1. Factors affecting removal of microorganisms | 23          |

|                                                                                                      |           |
|------------------------------------------------------------------------------------------------------|-----------|
| 2.8.1.1 Size of organism                                                                             | 23        |
| 2.8.1.2 Biofilms                                                                                     | 24        |
| 2.8.2 The role of membranes in removing microorganisms                                               | 25        |
| <b>CHAPTER 3: DESCRIPTION OF ULTRAFILTRATION SYSTEMS<br/>INVESTIGATED</b>                            | <b>29</b> |
| 3.1 Process Plant System (Wiggins)                                                                   | 29        |
| 3.2 Laboratory-Scale System                                                                          | 30        |
| 3.3 Bench-Scale System                                                                               | 32        |
| <b>CHAPTER 4: CALIBRATION AND OPTIMIZATION OF OPERATING<br/>PARAMETERS</b>                           | <b>35</b> |
| 4.1 Determining Initial Flux                                                                         | 35        |
| 4.2 Membrane Integrity Test (bubble point test)                                                      | 37        |
| 4.3 Clean in Place                                                                                   | 40        |
| 4.4 Backflushing                                                                                     | 41        |
| <b>CHAPTER 5: EVALUATION OF MEMBRANE PERFORMANCE USING<br/>MICROBIOLOGICAL TESTS</b>                 | <b>50</b> |
| 5.1 Sampling                                                                                         | 51        |
| 5.2 Microbiological Tests                                                                            | 52        |
| 5.2.1 Plate count technique (heterotropic counts)                                                    | 52        |
| 5.2.2 Membrane filtration technique for total coliforms, faecal<br>coliforms and faecal streptococci | 55        |
| 5.3 Relationship between Microbial Tests and Turbidity                                               | 58        |
| 5.4 Relationship between Total Counts, Coliforms Counts, Faecal<br>Coliforms and Faecal Streptococci | 61        |
| 5.5 Relationship between Flux and Bacterial Counts                                                   | 62        |

|                                                                                                   |                                                        |                |
|---------------------------------------------------------------------------------------------------|--------------------------------------------------------|----------------|
| <b>5.6</b>                                                                                        | <b>Fouling</b>                                         | <b>64</b>      |
| 5.6.1                                                                                             | Effect of fouling on bacterial numbers                 | 64             |
| 5.6.2                                                                                             | Ultrastructural analysis                               | 66             |
| <b>5.7</b>                                                                                        | <b>Comparison of Dead-end and Crossflow Operations</b> | <b>68</b>      |
| <b>5.8</b>                                                                                        | <b>Rapid Method of Analysis</b>                        | <b>70</b>      |
| <br><b>CHAPTER 6: PERFORMANCE CHARACTERISTICS OF MEMBRANES<br/>USING MICROBIAL CHALLENGE TEST</b> |                                                        | <br><b>73</b>  |
| <b>6.1</b>                                                                                        | <b>Bacterial Challenge</b>                             | <b>73</b>      |
| <b>6.2</b>                                                                                        | <b>Bacteriophage Challenge</b>                         | <b>74</b>      |
| 6.2.1                                                                                             | Somatic phages                                         | 75             |
| 6.2.2                                                                                             | F-RNA coliphage                                        | 75             |
| <b>6.3</b>                                                                                        | <b>Protozoan Challenge Tests</b>                       | <b>79</b>      |
| <b>6.4</b>                                                                                        | <b>Yeast Challenge Test</b>                            | <b>81</b>      |
| <br><b>CHAPTER 7: CONCLUSIONS</b>                                                                 |                                                        | <br><b>82</b>  |
| <br><b>REFERENCES</b>                                                                             |                                                        | <br><b>86</b>  |
| <br><b>PROTOCOL</b>                                                                               |                                                        | <br><b>95</b>  |
| <br><b>APPENDIX 1</b>                                                                             |                                                        | <br><b>115</b> |
| <br><b>APPENDIX 2</b>                                                                             |                                                        | <br><b>118</b> |

## List of Figures

|             |                                                                                                                                                 |    |
|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Figure 2-1. | Selected filtration processes for water treatment and size range of various materials found in raw waters                                       | 5  |
| Figure 2-2. | Ultrafiltration module designs                                                                                                                  | 8  |
| Figure 2-3. | Effect of operating variables on the performance of microfiltration and ultrafiltration                                                         | 9  |
| Figure 2-4. | Pore clogging and development of a fouling layer                                                                                                | 12 |
| Figure 3-1. | Schematic representation of Pilot -scale ultrafiltration system at the Process Evaluation Facility, Wiggins Waterworks, Durban                  | 30 |
| Figure 3-2. | Schematic representation of the laboratory-scale system                                                                                         | 32 |
| Figure 3-3. | Schematic representation of the bench-scale system                                                                                              | 33 |
| Figure 4-1. | Change in flux with differential pressure for membrane 1713-11 M1 and 1713-11 M2 (bench-scale rig)                                              | 37 |
| Figure 4-2. | Heterotrophic counts of permeate for extended analysis after backflush with single skinned membranes                                            | 44 |
| Figure 4-3. | Backflush experiments carried out on 5 successive days on the Wiggins Process Plant with membrane E111                                          | 45 |
| Figure 4-4. | Backflush experiments conducted on the Wiggins rig (membrane E111) for an extended period after backflushing for determination of window period | 45 |
| Figure 4-5. | Backflush experiments (1 min every 48 min) at the Wiggins Process Plant with membrane E101                                                      | 46 |
| Figure 4-6. | Backflush experiment (1 min every 72 min) at the Wiggins Process Plant with membrane E101                                                       | 46 |
| Figure 4-7. | Backflush procedures over 3 days for extended periods after backflushing to determine the window period                                         | 48 |
| Figure 5-1. | Schematic presentation of the protocol for the plate count technique                                                                            | 54 |
| Figure 5-2. | Schematic representation of the membrane filtration technique                                                                                   | 57 |
| Figure 5-3. | Graph of feed and permeate heterotrophic plate count and turbidity measurements for membrane 769-00 on the laboratory-scale system              | 60 |

|             |                                                                                                                                                                                                        |    |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Figure 5-4. | Graph of feed and permeate heterotrophic plate count and turbidity measurements for membrane 1713-11 on the laboratory-scale system                                                                    | 60 |
| Figure 5-5. | Correlation between heterotrophic plate count, differential pressure and flux measurements in the permeate during the filtration period with a spike of $3.4 \times 10^5$ cells/ml                     | 63 |
| Figure 5-6. | Scanning electron micrograph of polysulphone membrane (a) after a CIP; (b) 6 days after a filtration cycle; (c) biofilm formation inside the capillary with higher magnifications shown in (d) and (e) | 67 |
| Figure 5-7. | Heterotrophic counts of feed (● - crossflow and ▼ - dead-end) and permeate (▽ - dead-end and ○ - crossflow)                                                                                            | 69 |
| Figure 7-1. | Results of membrane challenged with different size organisms                                                                                                                                           | 85 |

## List of Tables

|            |                                                                                                             |    |
|------------|-------------------------------------------------------------------------------------------------------------|----|
| Table 2-1. | Crossflow membrane configuration comparison design                                                          | 6  |
| Table 2-2. | Common indicator microorganisms, typical methods and uses in water quality management                       | 17 |
| Table 2-3. | Comparison of microbiological parameters used for drinking water guidelines and standards                   | 20 |
| Table 2-4. | Size – range of waterborne microorganisms                                                                   | 24 |
| Table 3-1. | Characteristics of membranes and modules used                                                               | 34 |
| Table 4-1. | Membrane integrity using the bubble point test                                                              | 39 |
| Table 5-1. | Sampling regiments for the different rigs                                                                   | 52 |
| Table 5-2. | Media used, with their respective temperature and incubation periods                                        | 58 |
| Table 5-3. | Microbiological results of membranes during standard operations                                             | 62 |
| Table 5-4. | Flux and heterotrophic plate count results for the Wiggins Process Plant                                    | 65 |
| Table 5-5. | Flux and heterotrophic plate count results for the laboratory-scale system with the double skinned membrane | 65 |
| Table 5-6. | Minimum detection limits of conventional methods versus rapid commercial kits                               | 72 |
| Table 5-7. | Field tests results of conventional methods versus rapid commercial kits                                    | 72 |
| Table 6-1. | Microorganisms used for challenge tests                                                                     | 74 |
| Table 6-2. | Physical characteristics of bacteriophages                                                                  | 74 |
| Table 6-3. | Bacteriophage removal (PFU/ml)                                                                              | 77 |
| Table 6-4. | Reduction of <i>C. parvum</i> oocysts and <i>G. lamblia</i> cysts                                           | 81 |

## List of Acronyms

4-methylumbelliferyl-b-D-glucuronide (MUG)  
Clean-in-place (CIP)  
Colicount (CC)  
Colilert (CL)  
Colony-forming units (CFU)  
Define substrate technology (DST)  
Faecal coliform count (FCC)  
Faecal streptococcal count (FSC)  
Generally regarded as safe (GRAS)  
Heterotrophic Plate Count (HPC)  
Institute of Polymer Science (IPS)  
Microfiltration (MF)  
Molecular weight cut-off (MWCO)  
Nanofiltration (NF)  
O-nitro-phenyl -b-D-galactopyronoside (ONPG)  
Poly-methyl- methacrylate (PMMA)  
Polyvinyl chloride (PVC)  
Reverse osmosis (RO)  
Semi-solid yeast extract glucose agar (ssTYGA)  
Sulfite reducing clostridia (SSRC)  
Total coliform count (TCC)  
Total organic carbon (TOC)  
Tryptone yeast glucose broth (TYGB)  
Ultrafiltration (UF)

# CHAPTER 1

## INTRODUCTION

### 1.1 Study Overview

A large proportion of the world's under-developed population lack access to microbiologically - safe drinking water. It is also important to develop, implement and maintain low cost, low technology, water treatment systems. Membrane technology potentially offers the water industry a simple one or two-step treatment barrier to secure compliance with all of the new standards that are being recommended worldwide. These regulations are determined by microbiological standards. Thus, for any membrane system it is important to evaluate the membrane performance based on removal of microorganisms. Such a protocol is necessary to ensure proper functioning of the ultrafiltration (UF) system and reduce risks of infection due to contamination of drinking water. It has been shown that the alternatives to microbiological testing of water are not sensitive enough to detect low, but significant numbers of microorganisms that pose a health risk. This report describes the research undertaken to determine the performance characteristics of UF membranes using bacteriological tests and to develop a set of protocols for membrane users.

This study involved three different rig systems i.e., pilot scale system at the Wiggins process plant, a laboratory system and a bench-scale system with polysulphone membranes that were manufactured by the Institute of Polymer Science (IPS). The parameters that affect membrane performance were investigated using bacterial counts in the feed and permeate. The performance characteristic of the membranes was investigated by evaluating the removal of bacteria, bacteriophages, *Giardia* cysts and *Cryptosporidium* oocysts by UF. A final protocol that provides a guideline for membrane users was drawn based on:

- a) Determining membrane characteristics of the test plants during standard operating conditions;

- b) Evaluating cleaning strategies and determining the effect of biofilms on the membrane performance;
- c) Establishing the potential of membranes to remove conventional water-borne bacteria, parasites and viruses; and
- d) Evaluating rapid and simple detection techniques for field use.

The literature in chapter two gives an overview of membrane technology, membrane modules, and the factors that affect membrane performance such as selectivity, crossflow velocity, flux, temperature, fouling and rejection. The microbiological aspects which are important for membrane users are discussed by briefly overviewing indicators of water quality, guidelines that needs to be followed to assure potable water quality, and the microbiological size rejection capacity of membranes. Chapter three provides details of the UF systems investigated. Chapter four discusses calibration and optimization of operating parameters. Chapter five discusses the performance characteristics of the membrane using microbiological tests and in chapter six the membrane performance is evaluated by challenging the membranes with bacteriophages, bacteria, protozoan cysts and oocysts and yeasts. Conclusions and recommendations for future research can be found in chapter seven. The final part of the report is a protocol that can be used for the routine operation of membranes when producing potable water.

## **CHAPTER 2**

### **BACKGROUND LITERATURE**

Safety of drinking water is a worldwide concern, as contaminated water has a great impact on human health (Geildrich, 1996). Drinking of non-treated or improperly treated water is a major cause of illness in developing countries. Demand for potable water is constantly rising, necessitating investigations of alternative methods of purification. Membrane filtration technology is a suitably good alternative to conventional water treatment techniques (Cabassud *et al.*, 1991): it offers the potential of improved removal efficiencies compared to conventional methods; it is suitable for operation in small areas and in the long-term can be economically viable. This technology offers the water industry a simple one or two-step treatment barrier to secure compliance with all of the new safety standards being recommended worldwide. The IPS, University of Stellenbosch, has developed low cost capillary membranes that show potential for the production of potable water in remote areas, and for the removal of contaminants from industrial waters. This technology has been used to set up pilot-scale rigs at the Wiggins Water Works (Durban), Stellenbosch, Hermanus, George, Pietermaritzburg, Monvillia, Hanglip, Windhoek Municipality, Paradyskloof and Stanger. These systems are monitored by flux and pressure and the water quality is assessed by turbidity readings and periodic bacterial counts.

Membrane performance needs to be assessed routinely, especially if membrane abnormalities are suspected, or if new membranes are installed and/or the plant is restarted after shutdown. Standard turbidity measurements have been shown to be ineffective in detecting significant numbers of microorganisms that pose a health risk. The rejection profile of the membranes for differently sized microorganisms such as bacteriophages (23 to 80 nm), bacteria (0.5 to 5  $\mu\text{m}$ ), protozoan cysts (5 to 15  $\mu\text{m}$ ) and yeasts (>10  $\mu\text{m}$ ) would be characteristic for each membrane type. Once these profiles are evaluated, routine testing of membrane performance will involve analyzing microbial rejection profiles during different operational conditions. Protocols for testing membrane performance based on removal of microorganisms will ensure

proper functioning of the UF system and reduce risks of infection from contamination of drinking water.

In this study, the aims were to assess the membrane performance during routine use, to determine the relationship between membrane fouling and the effectiveness of clean-up using microbial tests. The results will be used to develop standard guidelines for membrane users and suppliers (prior to use) and for total quality checks and quality assurance systems (in-use testing).

## **2.1 Membrane Technology and Water**

Membrane technology potentially offers the water industry a simple one or two-step treatment barrier to secure compliance with all of the new standards that are being recommended worldwide. A membrane filtration process refers to a pressure driven separation of the components of a fluid mixture by selective permeation through a membrane separating the retentate (concentrate) stream from the permeate stream. Permeate passes through the membrane whilst the stream retained by the membrane is called the *retentate*. A pressure difference between the feed and permeate creates a driving force for the separation. The membrane resistance and pressure driving force determine the rate of transfer across a membrane.

Membrane technology usually is categorised as Microfiltration (MF), Ultrafiltration (UF), Nanofiltration (NF) and Reverse Osmosis (RO). They are in descending order of pore size (Figure 2-1). In UF, the driving force for fluid transport across the membrane is a gradient in pressure (Li, 1982). Clean water (permeate) is forced through the porous membrane while the waste that is retained by the membrane (concentrate) becomes more concentrated. The process is therefore capable of separating molecular or colloidal materials dissolved or suspended in a liquid phase (Li, 1982).

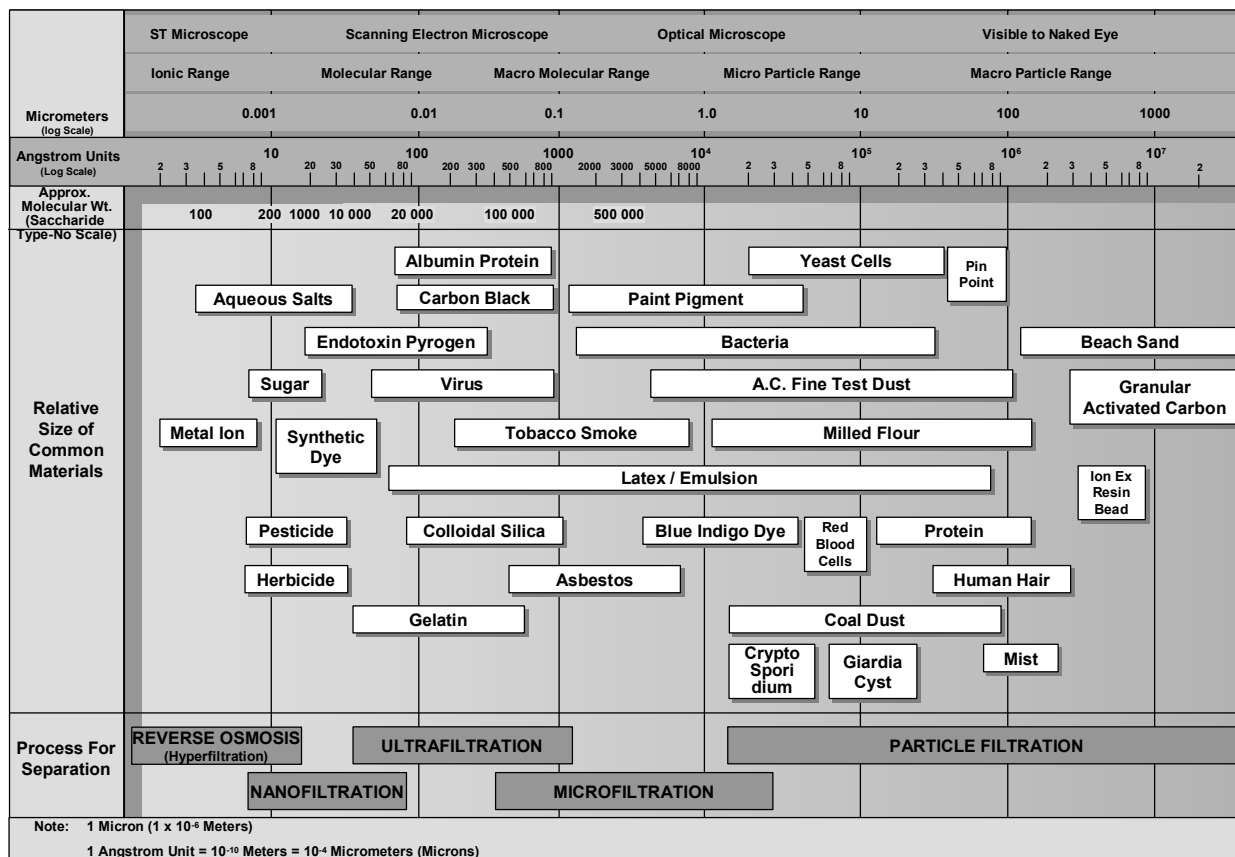


Figure 2-1. Selected filtration processes for water treatment and size range of various materials found in raw waters (Lipp *et al.*, 1998)

The choice of membrane is determined by the specific application objective (Table 2-1) (Gould, 1995). Commercially available organic UF membranes are produced from a number of polymers, including cellulose acetate, polyacrylates and polysulphones (Porter, 1983). For water treatment cellulose acetate are not generally used because they are mechanically weak and more thermally and chemically unstable than most of the alternative polymers. Furthermore, cellulose acetate is susceptible to bacterial attack (Strathmann, 1984). Polysulphone membranes on the other hand have excellent chemical stability and are not attacked in strong acid or alkali solutions, even at elevated temperatures. These membranes can withstand a pH range of 0.5 to 13, temperatures to 85°C (185°F), and 25 mg/L of free chlorine on a continuous basis. Hollow fibre polysulphone UF membranes used for potable water UF are often used in municipal applications to produce potable water. Pore dimensions typically in the 1 to 100 nm range characterise membranes used for UF. For UF the cut-off threshold ranges between 1,000 and 100,000 daltons (Bilstad *et al.*, 1994). UF membranes will remove numerous contaminants,

such as bacteriophages, *Giardia*, *Cryptosporidium*, and coliforms and will reduce other contaminants, such as turbidity and total organic carbon (TOC) from diverse water sources, including surface waters. Membranes that are used for UF are characterized by the molecular mass of a compound that is not able to pass through the membrane. In theory, a significant amount (90%) of compounds having a molecular mass greater than the molecular mass/weight cut-off (MWCO) would pass through the membrane and report to the permeate. It should be noted that the MWCO designation is somewhat misleading because a molecule having a molecular mass less than the membrane MWCO may still be retained by the membrane because its three dimensional shape will not allow it to pass through the membrane pores and vice versa (Reed and Dudley, 1997). For the successful application of UF as an efficient mass-separation process, the design of the module used to contain the membrane and the layout of the system in which the module is installed are as important as the selection of the proper membrane (Strathmann, 1984).

Table 2-1. Crossflow membrane configuration comparison design (Paulson, 1995)

| Characteristic           | Spiral-wound | Fibres                  | Tubular             | Plate & Frame |
|--------------------------|--------------|-------------------------|---------------------|---------------|
| Cost                     | Low          | Low                     | High                | High          |
| Packing density          | High         | UF-High<br>RO-Very High | Low                 | Moderate      |
| Pressure capability      | High         | UF-Low<br>RO-High       | UF-Low<br>RO-Medium | High          |
| Membrane polymer choices | Many         | Few                     | Few                 | Many          |
| Fouling resistance       | Fair         | UF-Good<br>RO-Poor      | Very Good           | Fair          |
| Cleanability             | Good         | UF-Very Good<br>RO-Poor | Very Good           | Good          |

## 2.2 Membrane Modules

Membranes themselves are either formed as flat sheets, hollow fibres, or coated tubes. Membranes must be configured into modules to manage the flow conditions and to support the membrane under the required hydraulic pressures. Flat sheet

configurations include the plate-and-frame (Figure 2-2a) and spiral-wound design (Figure 2-2b); the latter predominates all forms including tubular (Figure 2-2c) and hollow fibre (Figure 2-2d). The plate-and-frame design allows a variety of feed and permeate, but is a high cost approach and finds only niche applications (Paulson, 1995). Of the different membrane geometries (Table 2-1) the capillary type offers a viable technology for effecting clarification and disinfection filtration to provide potable water in a single step operation.

Capillary membranes have relatively high surface area to volume packing densities, are self-supporting and have the additional hydrodynamic advantage of open-flow passages offered by tubular type membranes (Jacobs *et al.*, 1997). The capillary membrane module system consists of a large number of membrane capillaries with a diameter in the range of 0.5 to 1.5 mm. The feed solution is passed down the centre of the capillary and the filtrate permeates the wall of the capillary (Strathmann, 1984). Hollow fibres of greater lumen diameter (0.5 to 2 mm) are used in UF processes. They can handle high solids loading without plugging and can be backflushed to remove foulant layers. Since they are self-supporting homogenous fibres, they are limited by the tensile, compressive and flexural strengths of the membrane material, which is porous. This limits the operating pressure and flow rates to less than those of spiral wounds (Paulson, 1995).

## **2.3 Systems Design**

The manufacturer generally assembles membrane systems on a frame or skid. They can be tested at the factory and shipped ready to operate as soon as they are plumbed in and wired (Paulson, 1995). A simple design includes a pump to provide the driving pressure and crossflow velocity required, the membrane elements in a housing, connecting plumbing, control valve(s) and instruments. Pressure gauges and motor controls are required, but the degree of instrumentation and other controls can and does vary tremendously.

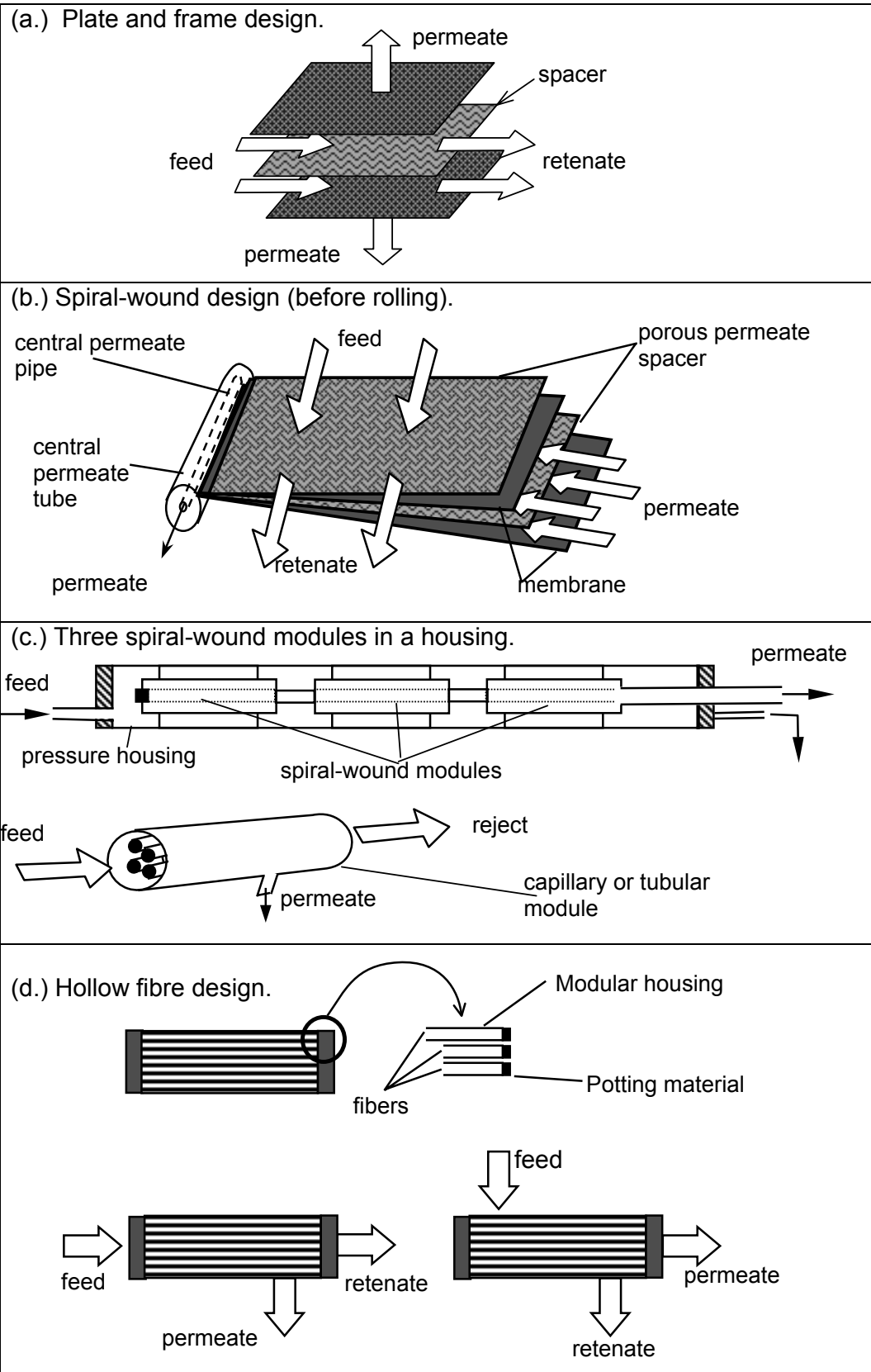


Figure 2-2. Ultrafiltration module designs

Everything from simple on/off conductivity sensor lights to complex, sophisticated PLC and computer-controlled systems are employed today for crossflow membrane machines. Roughing prefilter cartridges are usually included to reduce membrane fouling.

## 2.4 Parameters Affecting Membrane Performance

In any simple membrane filtration process, the water stream passing through the membrane is called the *permeate*, whilst the stream retained by the membrane is called the *retentate*. A difference in pressure between the feed and permeate creates a driving force for the separation. The performance of the membrane is based in selectivity and permeate flux. Factors that determine the flux of species across a membrane and factors that determine membrane performance are: pressure driving force, membrane selectivity and the operating characteristics. The effects of these are illustrated in Figure 2-3.

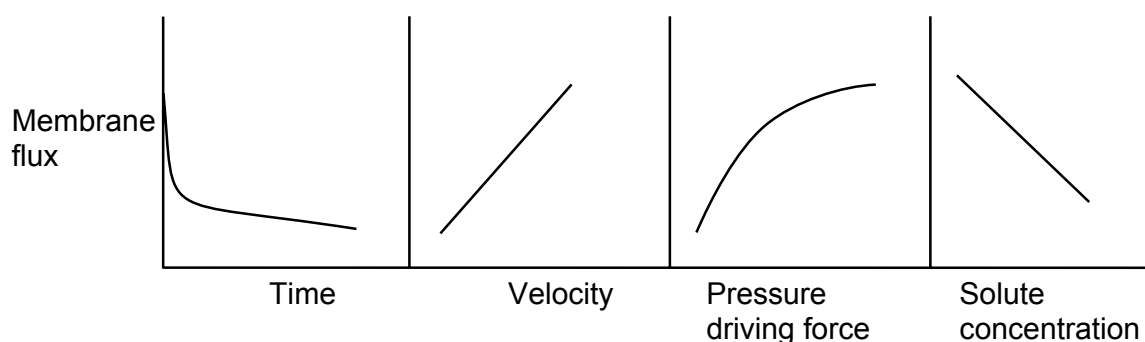


Figure 2-3. Effect of operating variables on the performance of microfiltration and ultrafiltration

### 2.4.1 Selectivity

Selectivity indicates the ability of the membrane to retain (reject) a specific entity. Which is usually indicated by the rejection: (R)

$$R = \frac{C_F - C_p}{C_F} = 1 - \frac{C_p}{C_F}$$

$C_F$  = Concentration in feed

$C_p$  = Concentration in permeate

This selectivity is based on the pore size distribution (MWCO) of the membrane. The UF is chosen such that the cut-off size is significantly smaller than the size of the contaminants to be removed. Thus for all practical purposes, the rejection in a real system approaches 100%.

#### **2.4.2 Crossflow velocity**

The most important design considerations are pressure and crossflow velocity. Higher-pressure causes higher permeate throughput (membrane flux) and would thus increase efficiency, except that it also causes more severe fouling by retained substances. Higher crossflow velocity reduces fouling, so a balance of flow and pressure must be achieved. The optimum balance varies by membrane type and especially by feed solution characteristics. The strength of the membrane element and hardware determines the maximum hydraulic pressure and crossflow rate that can practically be applied (Paulson, 1995).

#### **2.4.3 Flux**

Flux (permeate rate per unit area of membrane) is directly proportional to *effective* pressure. Thus with a pure water feed stream, increasing pressure proportionally increases permeate output, and the pressure limit would be based on the membrane/element/hardware strength and pump limitations. In actual practice, the increased fouling which results from increased flux and the practical limit of the crossflow, which mitigates this fouling, determine the optimum pressure (Paulson, 1995).

#### **2.4.4 Temperature**

Temperature also influences flux due to viscosity reduction; the warmer the feed stream the higher the throughput. Of course the higher the flux, the higher the fouling rate. Arriving at the optimum balance of pressure, recovery, temperature and crossflow rate is an engineering art, and will vary with each feed source as long as proper design of crossflow velocity and recovery is considered, however, increasing temperatures up to the limits of the membrane and system will tend to increase system efficiency. Cold feed sources require larger systems (increased membrane).

Decreasing solution viscosity has the same effect as increasing temperature (viscosity is the determinant for the temperature effect on flux) (Paulson, 1995).

#### **2.4.5 Fouling**

Membrane fouling is a phenomenon whereby substances in raw water, such as suspended inorganic particles, bacteria, bacteriophages, and organic molecules (e.g., humic substances) may either adsorb into the membrane pores or plug the pores, and in many cases are deposited onto the membrane surface (Figure 2-4) (Laine *et al.*, 1991; Gandihon and Tambo, 1992; Laine *et al.*, 1989; Bersillon, 1989; Clark and Jucker, 1993 and Weisner and Chellam, 1992). Foulants can be classified into four categories: adsorbed organic compounds, biological growth, metallic (hydroxides, and particulate matter. Particulate foulants can be classified further into suspended matter, microorganisms, and colloids. Fouling can be reduced by pre-treatment of the feed water. Conventional pre-treatment for surface water sources (i.e. coagulation, sedimentation and filtration) is used for the removal of fouling material; however, this is not completely effective in the removal of small colloids. The extent to which UF membranes are capable of retaining colloidal particles depends on the UF pore size expressed as a molecular cut off, surface morphology (e.g. porosity) and the nature of the membrane material (e.g. hydrophilicity) (Boerlage *et al.*, 1997).

The ratio of permeate to feed volume also affects the fouling rate and is known as “recovery.” Recovery is measured on both the individual element basis and for the entire machine. Feedwater applications usually run at 75 to 80% machine recovery, with 90% of the practical upper limit.

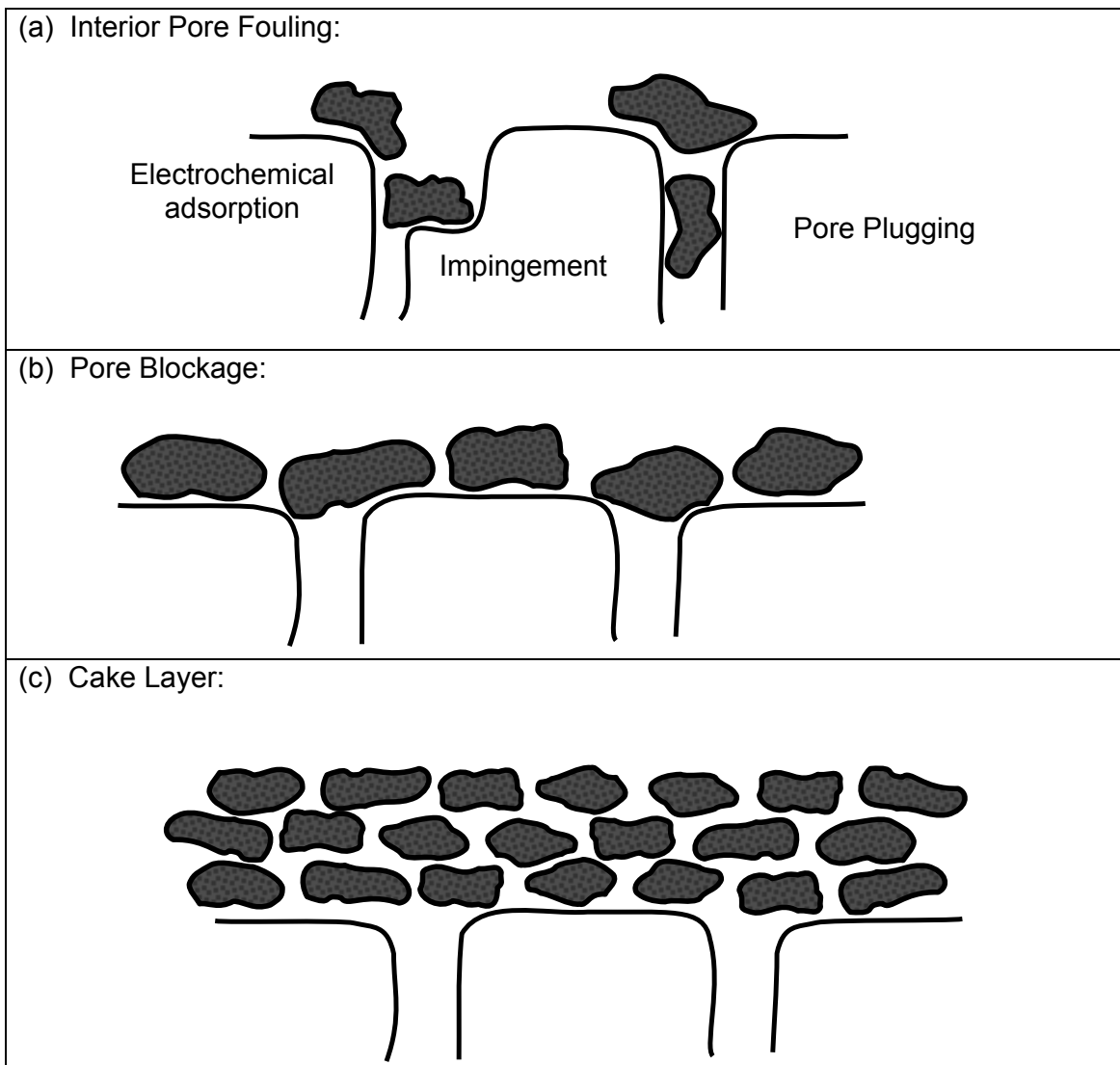


Figure 2-4. Pore clogging and development of a fouling layer

Some UF applications have relatively high fouling rates. Fouling is defined as irreversible deposition of material onto or into the membrane, causing loss of flux and altered rejection. The overall permeability decay is caused by pore clogging and or by the formation of a cake on the membrane surface (Cabassud *et al.*, 1991). With a constant pressure pump, membrane fouling will result in a flux decline, while in a constant flow rate filtration, fouling will cause an increase in pressure drop across the membrane (Bicknel *et al.*, 1985). Fouling is particularly a problem in the case where the feed solution is of biological origin (Aptel and Clifton, 1983). To date, fouling is the most important factor that has limited the use of membrane technology for the

removal of microorganisms from water (Jacangelo, 1990). Flux is a function of both pore size and pore density. As fluid passes through a membrane, pores become blocked with particles and therefore the pore density is reduced, resulting in a drop in the filtration rate (Beeby, 1989). Another cause of the flux decline is the accumulation of fouling material either inorganic (McCuthan and Johnson, 1970) or organic (Baileu *et al.*, 1974; Winfield, 1979), on the membrane surface. In addition to flux decline, fouling alters rejection, reduces plant efficiency, shortens membrane life, and increases operating pressure and cleaning frequency (Madaeni, 1999).

The hydraulic cleaning including backwashing (Li *et al.*, 1996) is the most important method for reducing membrane fouling. However, this technique interrupts the continuous filtration process and cannot maintain the membrane filtration operating at high permeate flux (Chai *et al.*, 1998). Effective cleaning relies on a backwash surge of sufficient flow and pressure to remove contaminants impacted upon the filter surface. Depending on particle size and shape, the mesh size of the filter media, and the pressure and flow rate of the filtrate, solids will build up in various ways on the filter surface. Automating a backwashing filter system eliminates the cost of system shutdown, reduces maintenance costs, and allows controlled, “hands-off” operation (Visser, 1984).

Nevertheless, over long periods of operation, membrane fouling is generally not totally reversible by the hydraulic backwash procedure. As the number of filtration cycles increase, so the irreversible fraction of membrane fouling increases. In order to obtain the desired production flow rates, or flux, an increase in transmembrane pressure is required. When this pressure reaches a maximum allowed by the mechanical resistance of the membrane, chemical cleaning of the membrane is required for the membrane to regain most of its initial permeability. Regardless of the membrane system used, chemical cleaning is typically cumbersome and requires the shutdown of the unit being washed for several hours. This results in a reduction of the overall plant capacity, and produces a waste that may be difficult to dispose of. There are also concerns that repeated cleaning may affect the membrane life. Chemical cleaning should thus be limited to a minimum or avoided (Crozes *et al.*, 1997).

If the flux cannot be reinitiated to its full capacity by cleaning, via backflushing or some other method, the fouling can lead to eventual membrane replacement (Hodson, 1997).

#### **2.4.6 Rejection**

There are two mechanisms commonly attributed to particulate removal, sieve retention and adsorption sequestration (Tanny *et al.*, 1979). In sieve retention the porous media acts as a barrier for particle penetration. The particles are retained on the membrane surface and form a cake that grows in thickness as the filtration progresses. The second mechanism involves the entry and capture of the particles into the membrane matrix. Membranes are highly effective in removing biological colloids either larger or smaller than the membrane pores. For non-biological colloids, high rejection of particles smaller than the membrane pore size has also been reported. The rejection of small particles depends on membrane loading. It also depends on particle size, membrane pore size and membrane thickness (Ho and Sirkar, 1992). However under favourable conditions, the rejection can be very high even at high loading. Operating conditions, e.g. transmembrane pressure and flux can also affect the rejection. In a test for the effect of pressure on the passage of microorganisms, Leahy and Sullivan (1978) used *Pseudomonas diminuta* and observed that for those membranes whose pore sizes allow passage of *Pseudomonas diminuta*, the transmission is pressure dependant being higher at higher transmembrane pressure. Flux and rejection are interdependent (Waters and Fane, 1981) with the general expectation of enhancing the rejection by reducing the flux.

### **2.5 Microbiological Aspects of Water Treatment**

Untreated water sources such as surface waters (streams, rivers, lakes, etc.) or unprotected open wells are the vehicle for waterborne bacterial diseases such as cholera and typhoid fevers. Disinfection of water dramatically reduces the incidence of these diseases. Untreated water may also play a role in the transmission of water-washed viral enteric diseases such as hepatitis (hepatitis A virus and non-A non-B hepatitis agents), gastroenteritis (rotaviruses, Norwalk and Norwalk like viruses), as

well as an unknown number of ill-defined diseases caused by the other enteric viruses (adenoviruses, astroviruses, coxsackieviruses and echoviruses). The faecal-oral route is probably the major route for transmission of these bacterial and viral diseases as well as of many parasitic diseases in poor sanitary conditions. An improvement of water quality and water usage for improving sanitary conditions should result in a decrease of waterborne as well as water-washed diseases (Feachem *et al.*, 1983).

Since the mid-70's, when both *Cryptosporidium parvum* and *Giardia intestinalis* became recognised as important pathogenic protozoans of man, with the potential to cause diarrhoeal disease, considerable attention and resources have been directed at unravelling the epidemiology of these diseases and limiting the spread of the organisms (Smith *et al.*, 1995). Attention to the potential health hazard posed by *Giardia* and *Cryptosporidium* in South African water supplies has, until recently, been relatively low key. In recent years urban and rural settlements along waterways (rivers and dams) have been increasing in South Africa. Such settlements are often informal and have no infrastructure, resulting in increased levels of pollution of drinking water sources. With respect to potable water, 13% of the South African supplies tested were found to be positive, with 12% containing *Giardia* cysts. Local attention to the problem has increased in recent years. Harding and Genthe (1998) found the following in an investigation of water in the Cape Metropolitan Area, 31 samples were collected between August and December 1997, i.e. during spring. Of the 17 samples of raw potable water collected from 10 impoundments, 9 (53%) were positive for *Giardia* and 2 (12%), both from the same source, positive for *Cryptosporidium*. The mean number of *Giardia* cysts was 1.04 per litre. The incidence here was comparable to the 55% reported for South African surface water, although the mean level was lower (1.04 vs 3.0 per litre). The presence of *Cryptosporidium* was only detected in the samples collected from the upper and lower Steenbras Dams during December 1997. None of the treated waters, all processed via treatment plants utilizing coagulation, flocculation and filtration, contained *Giardia* or *Cryptosporidium*.

All the samples collected from the Lourens River, which provides part of the supply to the towns of Somerset West and Strand, were positive for *Giardia*, and one for *Cryptosporidium*. Monitoring of the treated water revealed a continual breakthrough of cysts. Both of the treatment plants were elderly, slow sand-filtration units. Ten samples of various recreational and other surface waters were collected during the same period. Of these, 50% were positive for *Giardia*, and none for *Cryptosporidium*. The occurrence of the protozoan parasites in a high proportion of the country's surface water indicates a need for extra care when these surface waters are used as a source of potable water in purification plants. The failure of purification systems to remove or inactivate cysts or oocysts will facilitate further spreading of *giardiasis* and *cryptosporidiosis* via the faecal-oral route. This study has identified a clear need for the development of water quality guidelines for the levels of *Giardia* cysts and *Cryptosporidium* oocysts in different types of usage with special reference to potable water quality (Kfir *et al.*, 1997).

## **2.6 Microbiological Water Quality Indicators**

The recognized bacterial indicators for assessing water quality are bacteria of the *Enterobacteriaceae* family defined as the total coliform bacteria and the faecal coliform bacteria. The coliform bacteria are gram-negative rods, aerobic or facultative anaerobic, nonspore forming, rapid-lactose-fermenting with gas formation within 48 h at 35°C. Some bacteria from this group are indigenous to soil and waters. This made necessary the use of a more stringent indicator for faecal contamination in many situations. The faecal coliform bacteria are members of the coliform group of bacteria usually (but not always) found in the feces of warm-blooded animals: they have the characteristics of the coliform group, but will also produce gas within 24 h at 44.5°C. The presence of faecal coliform bacteria in water is indicative of contamination by faecal material and is therefore considered indicative of a health risk because many enteric pathogens (bacterial, viral and parasitic) are present in faeces. Furthermore, the significance of the coliform group density has been established as an indication of the degree of pollution and thus the sanitary quality of water (Feachem *et al.*, 1983).

The preferred methods for assessing water quality, using as indicators the members of the coliform group and the faecal coliform group, are the membrane filtration technique and the multiple-tube fermentation techniques (APHA *et al.*, 1989).

Both are quantitative methods with high levels of sensitivity and they can be used to evaluate all types of waters including treated drinking waters, recreational waters and untreated drinking waters. The membrane filtration technique is usually more expensive and requires more equipment than the multiple-tube fermentation technique. Table 2-2 summarizes the number and type of tests investigated. This table also provides references to the standard testing procedures.

Table 2-2. Common indicator microorganisms, typical methods and uses in water quality management

| Indicator Organism                                         | Use in Water Quality Management                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Reference                          |
|------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| Standard plate count / Heterotrophic plate count organisms | <b>Refers</b> to all microorganisms which produce visible colonies on non-selective medium after incubation at 35°C for 48 h. Indicator of the general microbiological quality of water.<br><b>Used</b> for monitoring of efficiency of water treatment and disinfection processes, of after growth in water distribution systems, and of the quality of swimming pool water.                                                                                                                                                                                                                                          | Grabow (1983, 1984)<br>Kfir (1989) |
| Total coliform bacteria (total coliforms)                  | <b>Refers</b> to all bacteria which produce colonies with a typical metallic sheen within 20 to 24 h of incubation at 35°C on m-Endo agar. Gives an indication of the general sanitary quality of water, since this group includes bacteria of faecal origin. However, many of the bacteria in this group may originate from growth in the aquatic environment.<br><b>Used</b> to evaluate quality of drinking water and related waters, e.g. swimming pool water.                                                                                                                                                     | Grabow (1983, 1984)<br>Kfir (1989) |
| Faecal coliform bacteria (faecal coliforms)                | <b>Refers</b> to all bacteria which produce typical blue colonies on m-FC agar with 20 to 24 h of incubation at 44.5°C, and comprises members of the total coliform group which are capable of growth at elevated temperature. Indicator of probable faecal pollution of water since this group is much more closely associated with faecal pollution than the broader total coliform group. Some faecal coliforms may not be of faecal origin.<br><b>Used</b> to evaluate the quality of wastewater effluents, river water, seawater at bathing beaches, raw water for drinking water supply and recreational waters. | Grabow (1983, 1984)<br>Kfir (1989) |

| Table 2-2. (cont): Common indicator microorganisms, typical methods and uses in water quality management |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                             |
|----------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|
| Indicator Organism                                                                                       | Use in Water Quality Management                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Reference                                                   |
| <i>Escherichia coli</i> ( <i>E. coli</i> )                                                               | <b>Refers</b> to faecal coliforms which are indole-positive at 44.5°C, and generally consists only of <i>E.coli</i> which is almost definitely of faecal origin from warm-blooded animals.<br><b>Used</b> to evaluate the possible faecal origin of total and faecal coliforms, usually when these are isolated from drinking water.                                                                                                                                                                                                                                                   | Grabow (1983, 1984)<br>Kfir (1989)                          |
| Faecal streptococci (enterococci)                                                                        | <b>Refers</b> to bacteria which produce typical reddish colonies on m-Enterococcus agar after 48 h incubation at 35°C. These bacteria often appear in human and animal faeces, but in lower numbers than total or faecal coliforms, and are more resistant than coliform bacteria. Enterococci comprise a sub-group of faecal streptococci, being considered to include predominantly faecal streptococci of proven faecal origin.<br><b>Used</b> in evaluation of treatment processes and recreational waters.                                                                        | Kfir (1989)<br>De Wet <i>et al.</i> , (1991)                |
| Clostridia (presumptive <i>Clostridium perfringens</i> )                                                 | <b>Refers</b> to bacteria which produce typical black colonies on TSC (yolk-free tryptose-sulphate-cycloserine) agar when incubated anaerobically at 45°C for 20 h. The group includes <i>C. perfringens</i> , but is likely to also include other species of <i>Clostridium</i> . <i>C. perfringens</i> is almost conclusive proof of faecal pollution. These bacteria produce spores which are resistant to treatment processes and other unfavorable conditions.<br><b>Used</b> to indicate remote faecal pollution and to assess efficacy of treatment and disinfection processes. | Burger <i>et al.</i> (1989)<br>Grabow (1984)<br>Kfir (1989) |
| Coliphages                                                                                               | <b>Refers</b> to viruses which have bacterial hosts (bacteriophages) and which produce a visible plaque in a double-layer-agar plaque assay using <i>E. coli</i> strain as host and incubation at 35°C for 16 h.<br><b>Used</b> as indicators of the incidence and behavior of human enteric viruses in the evaluation of drinking water. Also serves as an indicator of the presence of host bacteria.                                                                                                                                                                                | Grabow (1984)<br>Kfir (1989)                                |

## 2.7 Microbiological Water Quality Guidelines

Water quality guidelines and standards recommended by various authorities are summarised in Table 2-3, and show that they are similar in that they intend to ensure the minimum risk of infection. However, they differ in detail because of considerations such as economic and technical capabilities, and perceptions of acceptable risks of infection.

The guidelines state that drinking water must not contain waterborne pathogens. More specifically, *E. coli* or thermotolerant coliforms should not be present in 100 ml samples of drinking water at any time, for any type of water supply, treated or untreated, piped or unpiped. In the case of large supplies, where sufficient numbers of samples are examined, total coliforms are acceptable in the distribution system in a maximum of 5% of samples taken throughout any 12-month period.

If guideline values are exceeded, immediate investigative action must be taken, including repeat testing, and thorough inspection of the treatment plant and its operation, the raw water source, and general hygiene of the water distribution system.

Because routine monitoring techniques are not available for bacteriophages and protozoa of health significance, the *Guidelines* recommend protection of the source and treatment techniques to ensure their absence. The degree of treatment required is a function of the nature (ground or surface water) and level of faecal contamination of the source.

Detection and enumeration of indicator bacteria are of primary importance in the microbiological quality control of water. In particular, coliforms and *E. coli* are used by many in the water supply industry as a criterion of operational parameters and indicators for faecal pollution. Included in the SABS specifications and other South African guidelines for assessing the quality of drinking water are total and faecal coliforms with confirmation of *E.coli*. There are two methods commonly applied for the enumeration of these indicator bacteria from drinking water. The multiple tube fermentation provides a most-probable-number analysis after growth of coliforms in liquid medium. The MF technique enumerates total coliforms on the surface of agar by providing a colony-forming unit (CFU) per 100 ml count (SABS Standard Methods 221, 1990; APHA, AWWA, and WPCF, 1992). These methods are approved for regulatory monitoring purposes (Le Chevallier *et al.*, 1983; Covert 1985; Standard Methods 1992; SABS 221-1990).

Table 2-3: Comparison of microbiological parameters used for drinking water guidelines and standards

| Country<br>Microbiological<br>parameter | South Africa 2002<br>SABS Specifications 241                                                                                                                                                     | South Africa 1990<br>DNH & PD* Guidelines<br>proposed by Aucamp &<br>Vivier**   | South Africa 1996<br>DWA & F## Water quality guidelines domestic<br>use (suggested guidelines –2 <sup>nd</sup> edition)                                                                                                                                                                                                                                                                                                                                                                                                | WHO International<br>Guideline 1993                             |
|-----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|
| Heterotrophic Plate<br>Count            | Minimum allowable counts per ml is a 100 counts per ml for 95% of the samples, and a maximum of 1 000 counts per ml for 4% of the samples and a count of 10 000 per ml for 1% of the sample.     |                                                                                 | 0-100 counts per ml indicates negligible risk of microbial risk of infection.<br><br>100-1000 counts per ml indicates inadequate treatment, post treatment contamination or after growth in the water distribution system. Slight risk of microbial risk of infection.<br><br>> 1000 counts per ml indicates poor treatment, post-treatment contamination or definite after growth in the water distribution system. Increased risk of infectious disease transmission.                                                |                                                                 |
| Total coliforms                         | Minimum allowable counts per 100 ml is 0 counts per 100 ml for 95% of the samples, and a maximum of 10 counts per 100 ml for 4% of the samples and a 100 counts per 100 ml for 1% of the sample. | 0/100 ml for max. level for no risk; 5/100 ml max. level for insignificant risk | 0-5 counts per 100 ml indicates negligible risk of microbial risk of infection.<br><br>5-100 counts 100 per ml indicates inadequate treatment, post-treatment contamination or after growth in the water distribution system. Slight risk of microbial risk of infection with occasional exposure.<br><br>> 100 counts per 100 ml indicates poor treatment, post-treatment contamination or definite after growth in the water distribution system. Significant and increased risk of infectious disease transmission. | 0/100 ml in 95% of samples in a year in the distribution system |
|                                         |                                                                                                                                                                                                  | 100/100 ml max. for risk                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 0/100 ml in treated water entering the distribution system      |
| Faecal coliforms                        | Minimum allowable counts per 100 ml is 0 counts per 100 ml for 95% of the samples, and a maximum of 1 counts per 100 ml for 4% of the samples and a 10 counts per 100 ml for 1% of the sample.   | 0/100ml; 1/100 ml & 20/100 ml for the e levels of risk                          | 0/100ml; 1/100 ml & 20/100 ml for the 3 levels of risk                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 0/100 ml                                                        |

|                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|
| <i>E. coli</i>                                                                                                                                                                                                                                                                                                                                                                                                                            | Minimum allowable counts per 100 ml is 0 counts per 100 ml for 95% of the samples, and a maximum of 0 counts per 100 ml for 4% of the samples and 1 count per 100 ml for 1% of the sample.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                 |
| Other indicators and pathogen                                                                                                                                                                                                                                                                                                                                                                                                             | <p><b>Somatic coliphages:</b><br/>Minimum allowable counts is 0 counts per 10 ml for 95% of the samples, and a maximum of 1 count per 10 ml for 4% of the samples and a 10 counts per 10 ml for 1% of the sample.</p> <p><b>Enteric virus:</b><br/>Minimum allowable counts per 100 l is 0 counts per 100 l for 95% of the samples, and a maximum of 1 count per 100 l for 4% of the samples and 10 counts per 100 l for 1% of the sample.</p> <p><b>Protozoan parasites:</b><br/>(<i>Giardia/Cryptosporidium</i>)<br/>Minimum allowable counts per 10 l is 0 counts per 10 l for 95% of the samples, and a maximum of 0 count per 10 l for 4% of the samples and 1 counts per 10 l for 1% of the sample.</p> | <p><i>Clostridium perfringens:</i><br/>0.100 ml; 1/100 ml &amp; 100/100 ml for the 3 levels of risk;<br/>Coliphages:<br/>0/100 ml; 10/100 ml &amp; 100/100 ml;<br/>Enteric viruses:<br/>0.10 l; 1.10 l &amp; 10.10 l for the 3 levels of risk</p> | <p><b>Coliphages:</b><br/>0-1 counts per 100 ml indicate that there is no sewage pollution and negligible risk of viral infection is indicated.<br/>1-10 counts per 100 ml indicates slight probability of sewage pollution. A very slight risk of viral infection is indicated for continuous exposure, but negligible risk is expected for short occasional exposure.<br/>10-100 counts per 100 ml indicate probable sewage pollution. A low risk of viral infection is indicated with continuous exposure. Minimal effects expected for occasional exposure.<br/>&gt; 100 indicate significant sewage pollution and increasing and increasing risk of viral infection as coliphage levels increases.<br/><b>Protozoan parasites:</b><br/>&gt; 1 cyst or oocyst per 10 ml indicates that there may be a risk of protozoan parasite infection for continuous short or occasional exposures.</p> |                                                                 |
| Sampling                                                                                                                                                                                                                                                                                                                                                                                                                                  | According to population size served                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Depends on water source, treatment and size of population serve |
| <p>*DNH &amp; PD – Department of National Health and Population Development<br/> ** Guidelines based on a 3 tier system: The 1<sup>st</sup> tier is the ideal level; the 2<sup>nd</sup> tier is the lowest quality of water acceptable under normal circumstances; the 3<sup>rd</sup> tier values should not occur for more than 2 consecutive days;<br/> <sup>###</sup> Based on a tiered system ranging from ideal to unacceptable.</p> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                 |

However, a complete analysis for coliforms with confirmation of *E.coli* requires 72 h for a result according to the SABS Standard Method. Several countries have changed the manner of reporting total coliforms from numbers per 100 ml of sample to a presence-absence (P/A) form of reporting the proportion of samples that are positive. This prompted the re-examination of traditional methods. New media have been developed for direct and simultaneous detection of coliforms and *E. coli* without confirmation. This new methodology is a refinement of defined substrate technology (DST) applied in clinical laboratories. DST is unique because it directs the metabolism of the target bacteria to specific indicator nutrients. The formula for the new media is in a stable powder form that can be added directly to the sample or vice versa. In the new methodology, two active substrates, o-nitro-phenyl- $\beta$ -D-galactopyranoside (ONPG) and 4-methylumbelliferyl- $\beta$ -D-glucuronide (MUG), are combined simultaneously to detect total coliforms and *E. coli* (Oslon *et al.*, 1991). Total coliforms produce the enzyme  $\beta$ -galactosidase, which hydrolyses ONPG and thereby releases o-nitrophenol, which produces a yellow colour. *E. coli* produces the enzyme  $\beta$ -glucuronidase, which hydrolyses MUG to form a fluorescent compound.

## **2.8 Membranes and Microorganisms**

In general, the particle exclusion sizes of microfiltration membranes in pure water systems range from 0.05 to 5  $\mu\text{m}$  and for UF it ranges from as low as 0.005 to 0.05  $\mu\text{m}$ . The efficiency of the membrane to remove microorganisms range in size from 0.027 microns for poliovirus to protozoan cyst which are 1:3 microns. The removal of these microorganisms (without pre-treatment, particle attachment, or adsorption to the membrane) is specific to a particular membrane and its exclusion size distribution. If membranes are considered as physical strainers that are not influenced by water quality and membrane or microbial surface characteristics, then any microorganisms larger than the largest exclusion size of the membrane will be removed, and this smaller will pass through. Thus, there are several factors that influence the removal of microorganisms through membranes.

## **2.8.1 Factors affecting removal of microorganisms**

In crossflow modules and systems the potential exists for seal leaks, membrane flaws, and back-contamination. Crossflow membrane systems are capable of significant microbial reductions, but their long-term performance is based on both diligent operation and good design. Critical factors that influence the removal of organisms depends on size of organism, biofilm formation and biofilm removal.

### **2.8.1.1 Size of organism**

The removal of particles including biological and non-biological colloids by membranes depends on many factors including membrane pore size. In the disinfection of water with membrane pore size is critical (Leahy and Sullivan, 1978) and therefore membrane pore diameter should be smaller than microorganism size. The pathogenic organisms, which are of potential risk in water, can be summarized as:

- a) Viruses, e.g. hepatitis A
- b) Bacteria
- c) Protozoa, helminths, etc.

Bacteriophages are the smallest organisms of concern to the water community, ranging from 0.01 to 0.025  $\mu\text{m}$ , followed by bacteria (0.5 to 10  $\mu\text{m}$ ) and protozoan cysts and oocysts (3 to 15  $\mu\text{m}$ ). The pore sizes for UF and MF range from 0.01 to 5  $\mu\text{m}$ . Through examination of the sizes of the target organisms and the range of membrane pore sizes (Table 2-4), it is apparent that removal of these organisms is specific to the particular membrane and its pore size distribution, when considering the membrane as a simple physical barrier (Jacangelo *et al.*, 1997).

Table 2-4. Size – range of waterborne microorganisms (Jacobs *et al.*, 1997)

| Waterborne microorganisms                                        | Size (µm)         |
|------------------------------------------------------------------|-------------------|
| Protozoans                                                       |                   |
| <i>Giardia lamblia</i>                                           | 5-15 by 10-20     |
| Ovoid cyst                                                       | 6 by 10           |
| <i>Entamoeba histolytica</i>                                     | 15 by 25          |
| Cyst                                                             | 10 by 25          |
| Yeasts, Fungi                                                    | 1-10              |
| ( <i>Salmonella</i> , <i>Shigella</i> , <i>Legionella</i> , etc) |                   |
| Spherical bacteria (cocci)                                       | 0.5-4             |
| Rod-shaped (bacilli)                                             | 0.3-1.5 by 1-10   |
| <i>Escherichia coli</i> (human faeces)                           | 0.5 by 2.0        |
| Rod shaped curved (vibrios)                                      | 0.4-2.0 by 1.0-10 |
| Spiral shaped (spirilla)                                         | < 50 in length    |
| Filamentous                                                      | < 100 in length   |
| Viruses                                                          | 0.01-0.025        |

#### 2.8.1.2 Biofilms

If microbial levels are not controlled, they will eventually form biofilms. Established bacteria of most types found in water secrete a polysaccharide-containing slime (glycocalyx), which enhances the ability of a bacteria cell to adhere to a slime. Bacteria grow and multiply faster when attached than when free floating. Attached cells form a larger colony. The slime layer helps adhere other bacterial cells and nutrients, which float past, and also acts as a protective layer, which resists chemical penetration. This is known as a biofilm.

The size, complexity, and resistance to sanitization of the colony grows within this biofilm which is very difficult to penetrate using typical sanitizing agents. They also become a source of recontamination when sanitizing steps do not completely remove the biofilm. A single routine sanitization usually only affects the top layer of the biofilm, so viable bacteria deep in the biofilm will quickly recontaminate the system and high bacteria levels will be seen again within a few days.

To destroy an established biofilm, repetitive sanitizing cycles are usually required. The first step uses a normal biocidal agent. The second step uses a high pH solution, usually sodium hydroxide, to help digest and remove the top layer of bacteria killed by the biocide. Fresh biocide is then reintroduced to the system to kill the next bacterial layer, again followed by caustic. This biocide/caustic cycle may need to be repeated several times until the entire biofilm is removed. For a well-established biofilm, 5 or 10 cycles are commonly required.

### **2.8.2 The role of membranes in removing microorganisms**

In any membrane the removal of particles is based on sieve retention and adsorption sequestration. The rejection is based on particle size, membrane pore size and membrane thickness and operating parameters. The passage of bacteria through membranes with smaller nominal pore sizes has been reported. The penetration of bacteria 0.25 to 0.3  $\mu\text{m}$  in diameter by 0.6 to 1.0  $\mu\text{m}$  in length through 0.22  $\mu\text{m}$  membranes have been observed (Pall *et al.*, 1980). This phenomenon can be explained due to the availability of some openings in the membrane larger than the nominal pore size or imperfections or inferior installation of the membrane (Jacangelo *et al.*, 1989). Bacterial deformation is suggested by Pall *et al.* (1980) to explain their passage through membranes with smaller pore size. They presumed that at high pressure 0.3  $\mu\text{m}$  diameter *Pseudomonas diminuta* organisms deform to pass through 0.2  $\mu\text{m}$  diameter holes of the Nucleopore membrane. Considering that cell walls are non-deformable (Brock and Madigan, 1991) and the presence of some openings as large as 0.298  $\mu\text{m}$  in the 0.2  $\mu\text{m}$  Nucleopore membranes (Stamm, 1971), a better explanation is the passage of 0.3  $\mu\text{m}$  diameter bacteria through these openings. Another possible explanation is changes in the size of the microorganisms. The size of an organism changes as the organism goes through the various growth phases (Leahy and Sullivan, 1978). During the transition from the growth phase to the stationary phase, cells become smaller because they divide faster than they grow so they may pass through the membrane when they are smaller than their normal size. If waterborne microorganisms and pathogens are to be retained by a membrane, the dimensions of the pores in the membrane must be smaller than those of the organisms.

Simonetti and Schroeder (1984) studied the bacterial passage as a function of time. They selected *Pseudomonas diminuta* as a model organism and used different types of 0.2 µm membranes both cartridge and flat disc configurations. Cartridges made of cellulose, polypropylene and polysulphone membranes retained *Pseudomonas diminuta* completely. Results of flat disc membranes indicated that cellulose, polypropylene and polysulphone filters are capable of retaining *Pseudomonas diminuta*.

Cabassud *et al.*, (1991) carried out pilot plant experiments to clarify water by hollow fibre UF membranes. Coliforms, faecal coliforms, *spectro* and *clostridium* were present in the raw water. No bacteria were detected in the ultrafiltered water.

Tests were conducted on a membrane filtration system used as a substitute for conventional sand filtration systems for the removal of turbidities from drinking water. This membrane system permitted obtaining better water qualities with lower coagulant dosage levels as compared to a sand filtration system. For instance, the turbidity, total bacteria and coliform bacteria were removed completely (Oe *et al.*, 1996). Hofman *et al.*, (1998) investigated the removal of microorganisms by the UF process, heterotrophic plate count (HPC) at 22°C and 37°C were measured in the feed water and the ultrafiltrate. In the feed water, median values of 1 000/ml and 285/ml were found for, respectively, HPC22 and HPC37. After UF these values were lowered to median values of, respectively, 93/ml and 25/ml. In other words a reduction factor of approximately one log<sub>10</sub> unit was achieved. To make a more accurate estimation of the elimination capacity, indicator organisms as coliforms, thermotolerant coliforms and sulfite reducing clostridia (SSRC) were monitored. The results showed that a log<sub>10</sub> reduction of at least 2.5 to 3.5 can be realized, based on the median concentrations.

The integration of UF into conventional drinking water treatment for a better particle removal was investigated by Lipp *et al.*, (1998). A pilot-scale UF plant was operated for 2 years with sand filtered and microstrained surface water. It appeared that the feed water quality did not affect final product water quality in relation to particle removal. The UF process was very effective in removing particles from the sand

filtered water; less than 1 particle/ml were detected in the ultrafiltrate. UF achieved a particle removal of more than 99%.

Botes *et al.*, (1998) presented the results of a 15 m<sup>2</sup> low-pressure capillary membrane UF pilot investigation. The membrane filtration system was able to remove greater than 95% turbidity, 92 to 97% apparent colour, 97 to 99% iron and 60 to 85% humic substances present in the feed. All faecal and other coliform bacteria could also be removed while the HPC was reduced to an acceptable level.

Hirata and Hashimoto (1998), who conducted an experimental assessment of the efficacy of MF and UF for *Cryptosporidium* removal made the following conclusions, firstly in the crossflow mode filtration carried out at an oocyst concentration of 10<sup>6</sup> oocysts/l in the influent, no oocysts were found in the filtrate of either the MF or UF and the estimated removal efficiency was >7log<sub>10</sub> for both membranes. Secondly, in the MF challenge at a higher level up to 10<sup>8</sup> oocysts/l in the influent, the removal efficiency was still >7log<sub>10</sub> in both the crossflow and dead-end modes, although some oocysts appeared in the filtrate (as high as about 6log<sub>10</sub> after the correction with the recovery rate of testing method). And finally, both MF and UF are conclusively recommended processes for the treatment of highly polluted source waters as a single process for producing safe (below an annual risk 10<sup>-4</sup>) drinking water from highly polluted source waters.

Belfort *et al.*, (1974) used a cellulose acetate hollow fibre membrane with 30 000 MWCO to concentrate poliovirus - 1 from 1 l of water. The study revealed an average virus rejection of 76%. A similar study was conducted by Katzenelson and Rotem (1975) using an asymmetric polysulphone membrane with 10 000 MWCO to concentrate enteric human viruses from 50 L of tap and deionized water.

No virus (100% removal) was detected in the permeate in either water samples. Removal of virus using polysulphone hollow fibre membrane with 10 000 MWCO proved virus reduction by 5 orders of magnitude in the feed for volumes as large as 100 l (Belfort *et al.*, 1974).

Urase *et al.*, (1994) conducted research by evaluating virus removal by MF and UF processes using coliphage QB (23 nm) in the virus challenge test. The primary factor investigated for virus removal efficiency was on the basis of membrane configuration (crossflow and dead-end) and surface deposits by feed solution.

Feed solutions used were poly- methyl- methacrylate (PMMA) and pond water. Crossflow conditions with UF membranes using PMMA resulted in 90% virus rejection. This gradually increased to 99% when pond water was used.

However, Urase *et al.*, (1996) conducted another study by evaluating quantitatively virus retention in a very high retention range. Results revealed that QB penetrated all types of membranes including UF and NF membranes.

Dead-end flow with MF showed results fairly similar to UF except that virus rejection was lower probably due to cut off size of the membrane. The study concluded that both membranes under cross and dead-end flow leaked virus to a certain extent when employing QB as a tracer and that the major part of the phage were adsorbed onto the surface deposit.

Oshima *et al.*, (1995) evaluated virus removal with phages T1 pp7, poliovirus, and found very efficient removal of phages T1, PP7 and polio virus using hollow fibre UF under crossflow conditions with MWCO 13 000 to 6 000.

## **CHAPTER 3**

### **DESCRIPTION OF ULTRAFILTRATION SYSTEMS INVESTIGATED**

Three different UF systems were used in this study, each differing primarily by the number and length of the UF membranes used and its associated size implication for the UF systems. A pilot-plant at Wiggins was operational for two years prior to the commencement of this project. A laboratory-scale and bench-scale system was constructed by students from the Departments of Biotechnology and Chemical Engineering, at the Durban Institute of Technology. The membranes and module for the three systems are described in Table 3-1.

#### **3.1 Process Plant System (Wiggins)**

The pilot-scale UF system (Figure 3-1) is located at the Process Evaluation Facility, Wiggins Water Works, in Durban. Feed water from the Inanda Dam is stored in a 1 000 l tank. A feed pump draws this water through a sand filter and a 100  $\mu\text{m}$  feed strainer during two pre-filtration steps. The filtered feed passes through a rotameter, which is controlled at a flow rate of 600 l/h. The feed pressure is controlled at 215 to 220 kPa. The pilot plant consists of three UF membrane modules arranged in parallel. Each module contains 1 200 membranes. The feed enters the tube side of the membrane modules and permeate is drawn from the shell side of the modules and passes through a permeate rotameter. Permeate is collected in a permeate tank which is also used for backwashing the system. During the backwash step, fouling is removed by reversing the direction of the permeate flow. During normal filtration, part of the reject water leaving the tube side of the module is sent to drain while the majority is recycled and joins the feed water via recycle pumps. The recycle flow rate of 1 500 l/h is much higher than the feed flow rate. The permeate can be produced using one of two operating conditions, i.e., by operating the plant at constant feed pressure or at constant permeate

flux. When one of these conditions is kept constant, fouling of the membranes is observed by monitoring the other variable. For the duration of the study, the permeate was produced at a constant operating pressure and the post contact columns for the water treatment were bypassed. The resultant differential pressure across the membranes was in the range of 100 to 110 kPa. Periodically, the system is cleaned using a 50 mg/l sodium hypochlorite solution that is passed through the tube side of the modules.

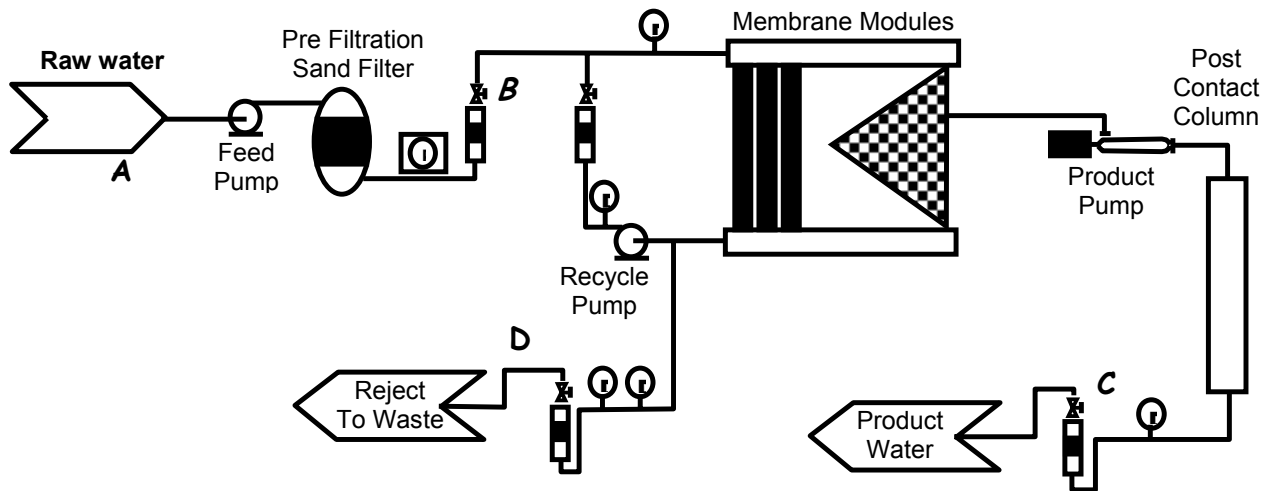


Figure 3-1. Schematic representation of pilot-scale ultrafiltration system at the Process Evaluation Facility, Wiggins Waterworks, Durban

### 3.2 Laboratory-Scale System

A schematic diagram of the laboratory system is shown in Figure 3-2. This mimics the UF pilot plant at the Wiggins Water Works. The unit is operational in crossflow mode at constant feed pressures. The system consists of a membrane module, a positive displacement pump, two feed tanks (20 l and 60 l), two flow meters (to monitor feed and reject flow rate), feed and permeate pressure gauges, three cumulative meters (to monitor volume of feed, permeate and reject), three sample valves (feed, reject and permeate), a diaphragm to adjust feed pressure, a dosing port, a peristaltic pump (for injection of microbiological samples through the dosing port) and a Varispeed

inverter (speed control pump). All pipes and fittings are made of polyvinyl chloride (PVC) except the permeate line leaving the module, which consists of 5 mm flexible tubing with a clamp to adjust the permeate pressure. The valves V5 and V6 allow for the reject to be directed into the tank of choice. Valves V3 and V4 are used to drain the feed tank and permeate/clean-in-place (CIP) tank respectively. An air release valve was fitted above membrane the module to release trapped air, in order to carry out backflush procedures. The module consists of 50 capillaries with a filtration length of 0.4 m<sup>2</sup> and a total membrane area of 0.0754 m<sup>2</sup>. The burst pressure of the capillaries was 1 400 kPa with its rejection being 92%, as stated by the supplier.

During filtration the feed water is fed from a 60 l tank. V5 is opened to allow feed from the feed tank (TO1) to enter the pump and V6 is closed. To achieve recycling, V1 is opened and reject is directed into the feed tank (TO1) and permeate is collected in the permeate tank (TO2). An air release valve was retro-fitted above the membrane module to aid in backflushing.

During backflushing minor adjustments were implemented in order to accomplish this procedure. An elbow joint is attached to the bottom of the membrane module and the permeate line is closed off by a nipple. V4 is opened and V3 is closed in order to direct the feed through the elbow joint and fill the membrane module from the bottom thereby creating a reversal of flow from the shell side to the tube side. The water used for backflushing is contained in the permeate tank (TO2) and fed to the membrane by opening V2 and closing V1. The flow rate during a backflush is maintained at a maximum of 150 l/h with the differential pressure varying between 140 to 160 kPa. In order to maintain the required pressure, DV1 is opened fully and V7 opened slightly to allow for a trickle. In order to fill up the module faster with the backflush water, the nipple closing off the permeate line is slightly opened to released trapped air bubbles.

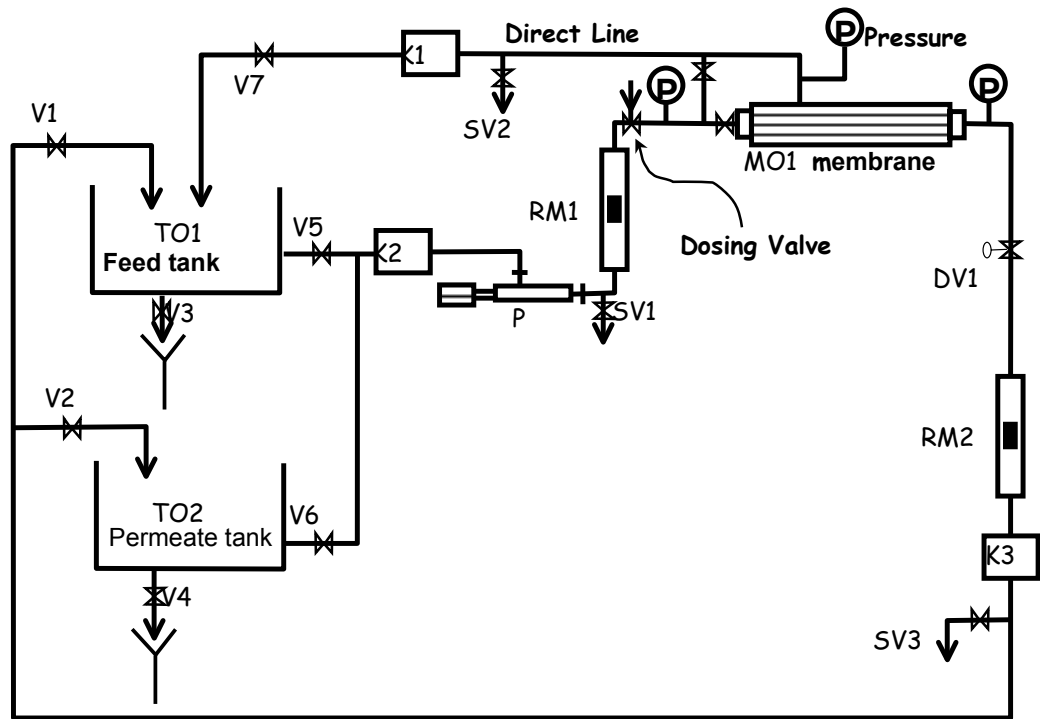


Figure 3-2. Schematic representation of the laboratory-scale system

### 3.3 Bench-Scale System

The unit (Figure 3-3) is operational on both crossflow and dead-end mode at differential pressures. It consists of a 5 l glass Schott bottle with three side arms as the feed tank and a 1 l Erlenmeyer flask as the permeate tank. The pressure drop is controlled by tightening or loosening the clamp on both the feed and permeate lines. This low-pressure system is fitted with a peristaltic pump (Watson Marlow 313S) that is controlled by a variable speed drive of 50 rpm per min and the permeate is pumped out of the shell side of the steel membrane module. The reject water is recycled back into the feed tank. The membrane module houses 5 capillaries. Both the reject and permeate lines are interrupted by plastic connecting adaptors which serve as sampling ports.

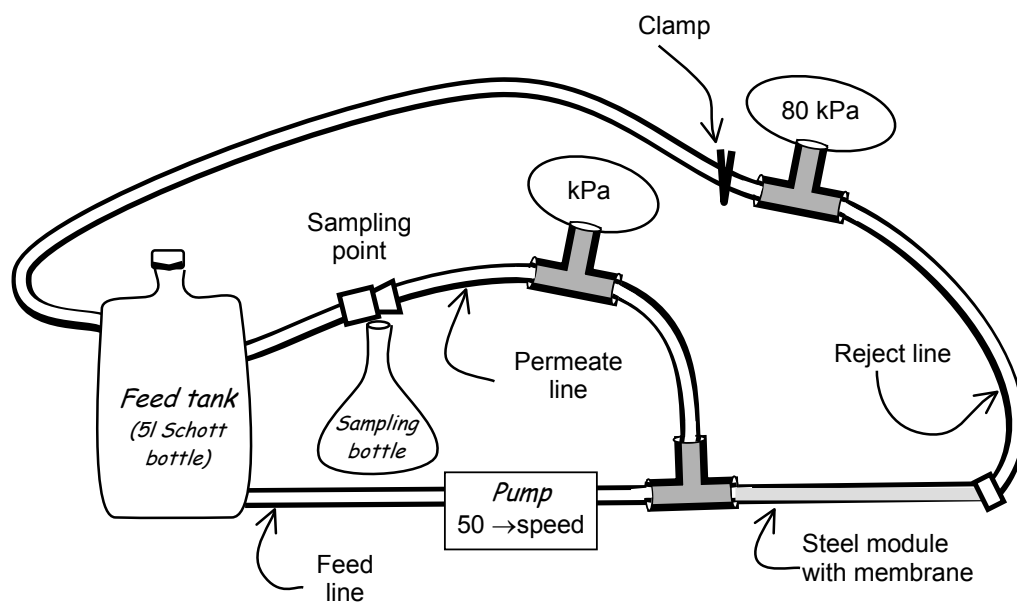


Figure 3-3. Schematic representation of the bench-scale system

Table 3-1. Characteristics of membranes and modules used

|                              | <b>Wiggins</b>                                                                      | <b>Laboratory-scale system</b>                             | <b>Bench-scale system</b>                                |
|------------------------------|-------------------------------------------------------------------------------------|------------------------------------------------------------|----------------------------------------------------------|
| <b>Membrane Type</b>         | Single skinned membrane E111 replaced with E101 which was a double skinned membrane | E31/1713M1 and M2 - double skinned Polysulphone membranes  | 1713-11<br>1713-05<br>769-00<br>769-01<br>798-02         |
| <b>Module</b>                | 3 modules in parallel each having 1200 capillary membranes                          | 1 module with 50 capillary membranes                       | 1 module with 5 capillary membranes                      |
| <b>Burst pressure</b>        | 2 bar                                                                               | 1. 4 bar                                                   | 2 bar                                                    |
| <b>Operating Mode</b>        | Filtration with automated backflush,                                                | Filtration, manual backflush                               | Filtration, manual backflush                             |
| <b>Filtration Mode</b>       | Crossflow operating mode<br>Reject recycled via pump and re-circulation loop        | Crossflow operating mode<br>Reject recycled into feed tank | Crossflow and dead end<br>Reject recycled into feed tank |
| <b>Feed Tank</b>             | 1000 l Tank                                                                         | 20 + 60 l Tank                                             | 5 l Schott Bottle                                        |
| <b>Differential Pressure</b> | 100 and 110 kPa                                                                     | 80 and 100 kPa                                             | 80 and 100 kPa                                           |
| <b>Flow rate</b>             | 600 l/h                                                                             | 100 l/h                                                    | 40 l/h                                                   |
| <b>CIP</b>                   | 50 mg/l sodium hypochlorite                                                         | 50 mg/l sodium hypochlorite                                | 50 mg/l sodium hypochlorite                              |

## **CHAPTER 4**

### **CALIBRATION AND OPTIMIZATION OF OPERATING PARAMETERS**

In designing a UF system for potable water production, some of the most important factors to consider are the feed water characteristics that enter the system and the quality of the finished water leaving the treatment process. The type of membrane selected, temperature, pressure and flux often dictates this. To ensure long-term operation of an UF system, flow, temperature and pressure are interrelated and must be monitored to ensure the correct operation of the system.

#### **4.1 Determining Initial Flux**

Pure water flux determines the flux through an "unfouled" membrane thereby representing maximum flux values and maximum production capacity. As the membrane fouls, the flux decreases accordingly and the decrease in flux can be compared to the pure water flux. A pure water flux is conducted with every new membrane in order to establish optimum flux values at differing transmembrane pressures. Water is run through the membrane under crossflow conditions at various allotted pressures.

##### Procedure

- i) The pump was calibrated to deliver sufficient, (permeate) output. A Watson Marlow 313S pump with Marprene II tubing (902.0064.02400) was tested at different speeds to determine flowrate. Three arbitrarily pump speeds (50, 80 and 100) were chosen and the flow rate was measured (using a measuring cylinder and stop watch method).
- ii) Prior to determining the pure water flux, each new membrane was flushed for about 3 h with distilled water.
- iii) The feed tank was filled with sterile distilled water and pumped through the membrane at selected feed flowrates.

- iv) Pressure differences of 80 kPa, 100 kPa and 150 kPa were selected on the basis that they do not exceed the burst pressures of the membranes.
- v) Permeate was collected at each pressure setting in a graduated cylinder. The permeate flow was recorded for 1 min.
- vi) The volumetric flowrate was determined using the equation:

$$\frac{\text{Permeate volume}}{\text{Time}}$$

**Time**

- vii) The initial flux was calculated using the equation below.

$$\text{Initial flux} = \frac{\text{Volumetric flow rate}}{\text{Membrane area}^*} = \text{l/hm}^2$$

$$\text{*Membrane area: } A = \pi d l N$$

d: diameter of membrane, m

l: length in metres, m

N: no. of capillaries

## Results

Experimental results (Figure 4-1) of the pure water flux on the bench-scale for two different membranes (1713-11 M1 and 1713-11 M2) that were manufactured in the same batch show a variation in flux at the same pressure difference. This result suggests that the pure water flux of every change in module or membrane need to be determined. The initial flux will influence the volumetric flow rate through the membrane. It is therefore imperative that the initial flux is calibrated prior to starting a rig or if membranes are replaced, changed or altered.

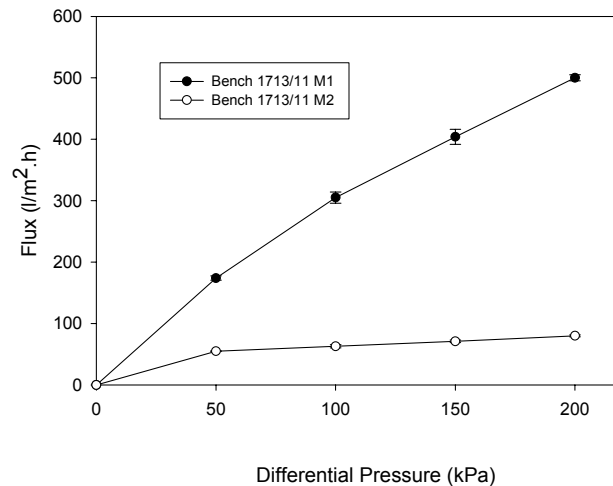


Figure 4-1. Change in flux with differential pressure for membrane 1713-11 M1 and 1713-11 M2 (bench-scale rig)

## 4.2 Membrane Integrity Test (bubble point test)

To reduce the risk of passage of microorganisms into the filtrate, it is important to check that the membranes are intact and are continuing to provide a barrier between the feedwater and the permeate or product water. Breakage of fibres or loss of system integrity can lead to passage of pathogens into the receiving water. Membrane integrity can be suspected of being compromised after high turbidity values, bacterial counts or flux values of the permeate. If this is suspected, Bubble Point Testing is a quick, direct measurement of membrane integrity.

### Procedure

- i) This test was carried out by removing the membrane module from the rig.
- ii) The permeate line was connected via silicone tubing to an oxygen cylinder, which is used to pressurize the shell side of the module.
- iii) A clamp was used to suspend the membrane vertically with one end of the membrane being immersed in water.
- iv) The opposite end of the module was sealed by constricting the attached tubing with a clamp.

- v) The oxygen was allowed to enter the module via the permeate outlet at varying pressures (80 kPa, 100 kPa and 120 kPa). A clamp attached after the pressure gauge on the tubing controlled the pressure. Oxygen was filled in from the shell side of the membrane through to the tube side i.e. in the opposite direction of flow.
- vi) The pressure was monitored for a period of 10 min. The pressure was varied/adjusted by opening and closing the valve on the cylinder.

The presence of bubbles and a decrease in pressure indicated the presence of pinholes and/or a compromised membrane.

### Results

Experiments conducted on all membranes at different pressure (Table 4-1) showed that when the membranes are intact, there are no bubbles at any of the pressures. If the membranes are compromised, depending on the membrane abnormality a positive bubble test will be evident. Compromised membrane integrity was indicated in membrane 1713-11 M2. Upon the isolation of abnormality and plugging of the membrane the bubble test results were negative.

Table 4-1. Membrane integrity using the bubble point test

| Pressure<br>(kPa) | Wiggins Process Plant           |   |                           |   | Laboratory-scale  |   |                   |   |                                            |   |                                        |   | Bench-scale |   |                                                  |   |
|-------------------|---------------------------------|---|---------------------------|---|-------------------|---|-------------------|---|--------------------------------------------|---|----------------------------------------|---|-------------|---|--------------------------------------------------|---|
|                   | E 111<br>3 year old<br>membrane |   | E101<br>Double<br>skinned |   | E 1713-11<br>(M1) |   | E 1713-11<br>(M1) |   | Membrane<br>1713-11<br>(M2)<br>Compromised |   | Membrane<br>1713-11<br>(M2)<br>Plugged |   | 769-11      |   | 1713-11<br>1713-05<br>769-00<br>769-01<br>798-02 |   |
|                   | B                               | P | B                         | P | B                 | P | B                 | P | B                                          | P | B                                      | P | B           | P | B                                                | P |
| 80                | ×                               | × | ×                         | × | ×                 | × | ×                 | × | ×                                          | × | ×                                      | × | ×           | × | ×                                                | × |
| 100               | ×                               | × | ×                         | × | ×                 | × | ×                 | × | ✓                                          | ✓ | ✓                                      | × | ✓           | × | ×                                                | × |
| 150               | ×                               | × | ×                         | × | ×                 | × | ×                 | × | ✓                                          | × | ✓                                      | × | ×           | × | ×                                                | × |

B = Bubbles

✓ = bubbles present

×

= no bubbles

P = Pressure change

### 4.3 Clean in Place

A clean in place (CIP) strategy is part of the design of any UF system. Generally, a membrane is considered clean when the initial water flux has been restored. A CIP is carried out prior to every sample run or when there is a significant decline in flux due to fouling. Depending on the membrane material, chemicals such as sodium hypochlorite, detergents and disinfectants are used. In our study all the membranes were cleaned using the recommended sodium hydroxide.

#### Procedure

CIP with sodium hypochlorite

- i) To effect the CIP 50 mg/l solution of sodium hypochlorite was fed into the system as per normal operating condition (filtration mode), and recycled for a period of 24 h or less depending on the extent of fouling.
- ii) This was followed by flushing the system using 10 mg/l sodium hypochlorite solution.
- iii) The residual chlorine was then removed with distilled water, which is fed through the system for 2 h.
- iv) Samples were collected from the permeate line and analysed for heterotrophic bacteria.

#### Results

Bacteriological analysis of permeate water after CIP treatment (50 mg/l NaOCl) with membranes E101 at the pilot plant in Wiggins, membrane E31/1713 on the laboratory module and membranes 1713-11 M1, 1713-11 M2 and 769-00 on the bench-scale module showed that quality of water produced immediately after clean-up was excellent. Water is always of potable quality.

#### **4.4 Backflushing**

Over long periods of operation, membrane fouling is generally not totally reversible by the hydraulic backwash procedure. As the number of filtration cycles increase, the irreversible fraction of membrane fouling also increases. In order to obtain the desired production flow rates, or flux, an increase in transmembrane pressure is required. When this pressure reaches a maximum allowed by the mechanical resistance of the membrane, chemical cleaning of the membrane is required for the membrane to regain most of its initial permeability. Regardless of the membrane system used, chemical cleaning is typically cumbersome and requires the shutdown of the unit while being washed for several hours. This results in a reduction of the overall plant capacity, and produces a waste that may be difficult to dispose of. There are also concerns that repeated cleaning may affect the membrane life.

To date, fouling of membranes is the most important factor that has limited the use of membrane technology for the removal of microorganisms from water (Jacangelo, 1990). Depending on particle size and shape, the mesh size of the filter media, and the pressure and flow rate of the filtrate, solids will build up in various ways on the filter surface. Hydraulic cleaning including backflushing (Li *et al.*, 1996) is the most important method for reducing membrane fouling. However, this technique interrupts the continuous filtration process (Chai *et al.*, 1998). Effective cleaning relies on a backwash surge of sufficient flow and pressure to remove contaminants impacted upon the filter surface.

##### Procedure

In this study the backwashing of different membranes was assessed using bacterial counts as an indicator to monitor its effectiveness. The membranes were operated under reverse filtration conditions, whereby clean filtered distilled water was forced back through the membrane under pressure. Permeate was collected once normal filtration had resumed and bacterial were counted in the permeate.

Permeate and reject samples from the pilot plant at Wiggins which had membrane E111 (single skinned) was tested for total HPC. For HPCs, a pour-plate technique using plate count agar (Merck) was used. For permeate samples, 1 ml of an undiluted sample, and dilutions of 1:10 and 1:100 were plated out in duplicate. For the reject samples, dilutions of 1:10, 1:100 and 1:1 000 were plated out in duplicate. The plates were incubated at 37°C for 48 h and colonies were thereafter counted using a colony counter.

Further analysis of the backwashing was carried on the improved membrane configuration i.e., double skinned membrane E101 at a specific backflush regimen of a 1 min backflush every 48 min. Approximately 200 ml of the permeate and reject samples were collected immediately before and after a backflush in sterile bottles and stored at 4°C. The samples were thereafter transported on ice for analysis. The samples were tested for total coliforms and heterotrophic bacteria. For the total coliform tests, aliquots of permeate and reject samples were filtered through 0.45 µm membrane filters (Millipore) in duplicate. The membranes were subsequently placed on M.Endo agar (Merck) and incubated for 24 to 48 h at 37°C. Colonies with a green metallic sheen were counted visually.

To determine the quality of the water produced after an extended period after backwashing the newly installed double skinned membrane (E101) was investigated. The main objectives were to determine if backflushing was responsible for producing a temporary deterioration in water quality and to find the time period required before potable levels could be reached. To ascertain the levels of bacteria in the permeate in a 3-h period after a backflush, the UF system was spiked with contaminated water from a wastewater treatment plant. Initially, 12.5 l of the spike was added and thereafter 0.75 l was added every 15 min. The filtration period was carried out for 3 h for a fouling layer to develop. A 10 min backflush was then carried out. For the last 2 min of the cycle, the recycle pump was switched off to allow the entire reject to drain off. Samples of the feed, permeate, reject and backflush water were taken prior to

the backflush. Permeate and reject samples were taken after the backflush at the following time intervals: immediately, 5; 30; 60; 90; 120; 150 and 180 min. Serial dilutions of the samples were made and the heterotrophic bacteria enumerated as described above.

## Results

At the Wiggins pilot plant, backflushes are carried out at different time intervals and for different periods of time depending on the rate of the flux decline. Samples taken after a backflush using a single skinned polysulphone configuration membranes showed that after a backflush the bacterial levels in the permeate were considerably high and water is of poor quality. It was found that after a backflush there was an increase in bacterial numbers for about an hour and a gradual decrease after approximately 2 to 3 h (Figure 4-2). These high counts could be indicative of deformities in the membrane used, or it could indicate that the biofilm layer enhances UF. Bucklin *et al.* (1991) observed higher bacterial counts during the post backwash period in experiments conducted using municipal drinking water filters. Logsdon and Rice (1985) found higher levels of injured coliforms in the treated water immediately following a filter backwash suggesting that injured coliforms (which are generally smaller in size) could penetrate the filter undetected in the first 10 min of filter operation.

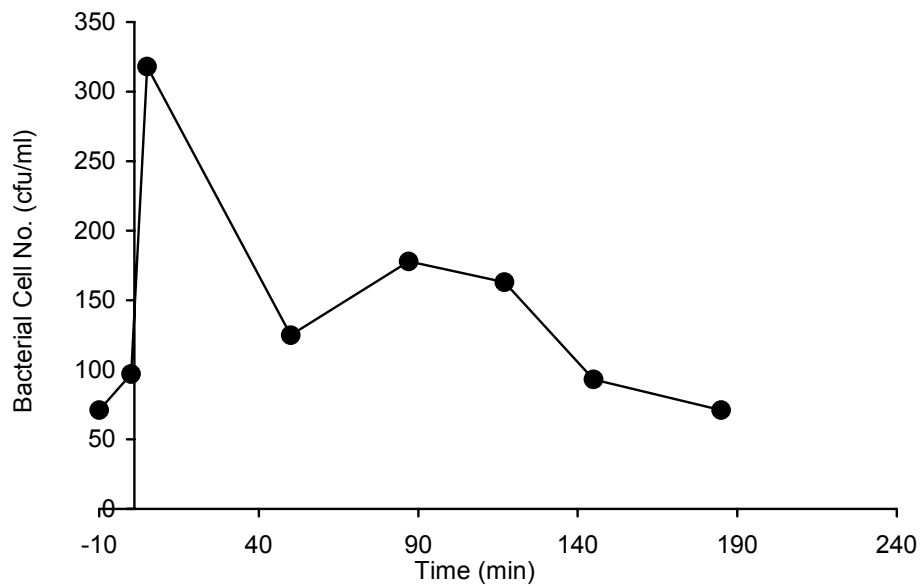


Figure 4-2. Heterotrophic counts of permeate for extended analysis after backflush with single skinned membranes

When using the double skinned membrane E101, five backflush experiments gave higher counts in the permeate after the backflush procedure than the feed. These results are shown in Figure 4-3. A similar trend was observed (Figure 4-4) when a different backflush regimen was carried out indicating that this increase in bacterial counts after a backwash treatment is indeed a phenomenon of the backflushing procedure. The time taken for the counts to stabilise (Figure 4-5 and 4-6) is shorter with the improved membrane (E101) than that observed with the older single skinned membrane (Figure 4-2).

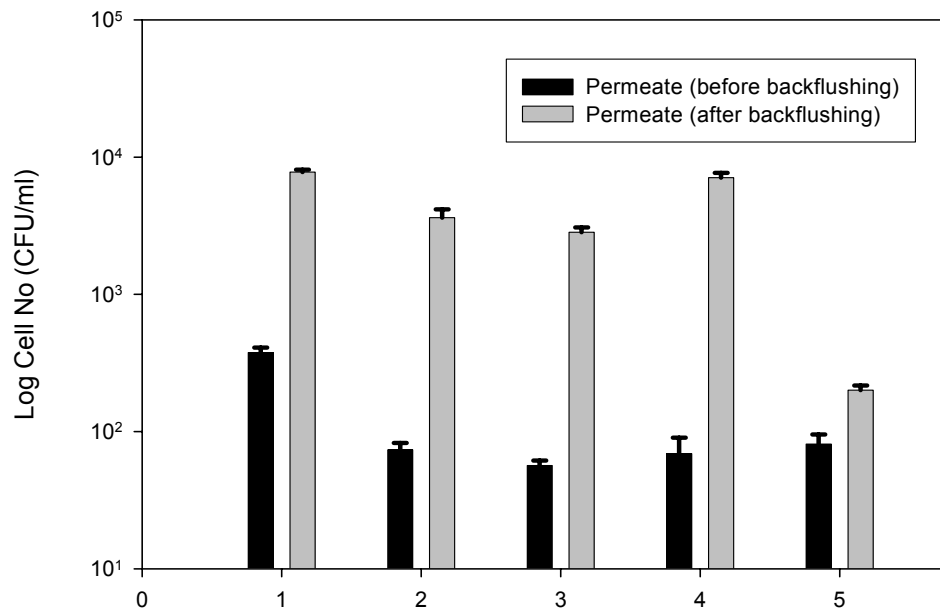


Figure 4-3. Backflush experiments carried out on 5 successive days on the Wiggins Process Plant with membrane E111.

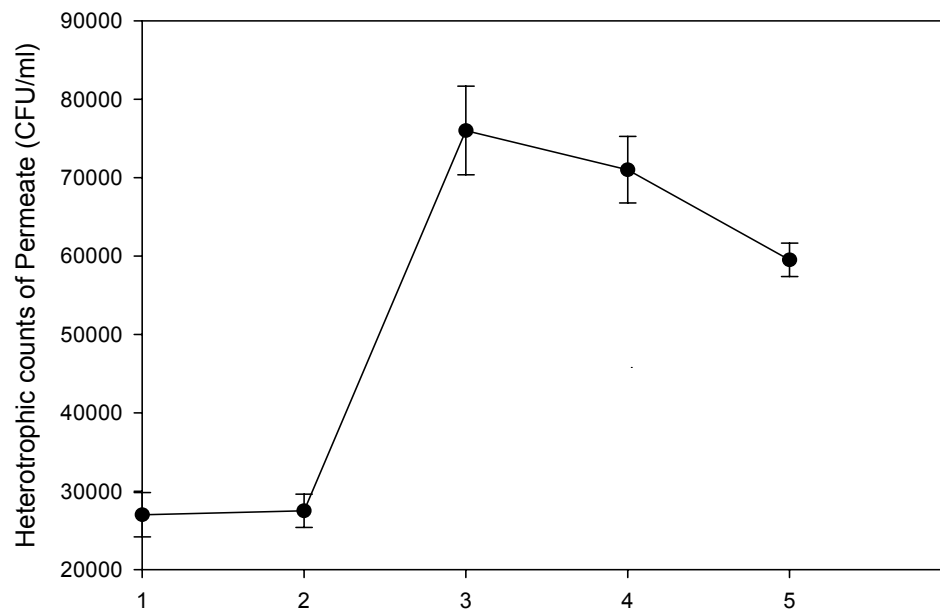


Figure 4-4. Backflush experiments conducted on the Wiggins rig (membrane E111) for an extended period after backflushing for determination of window period

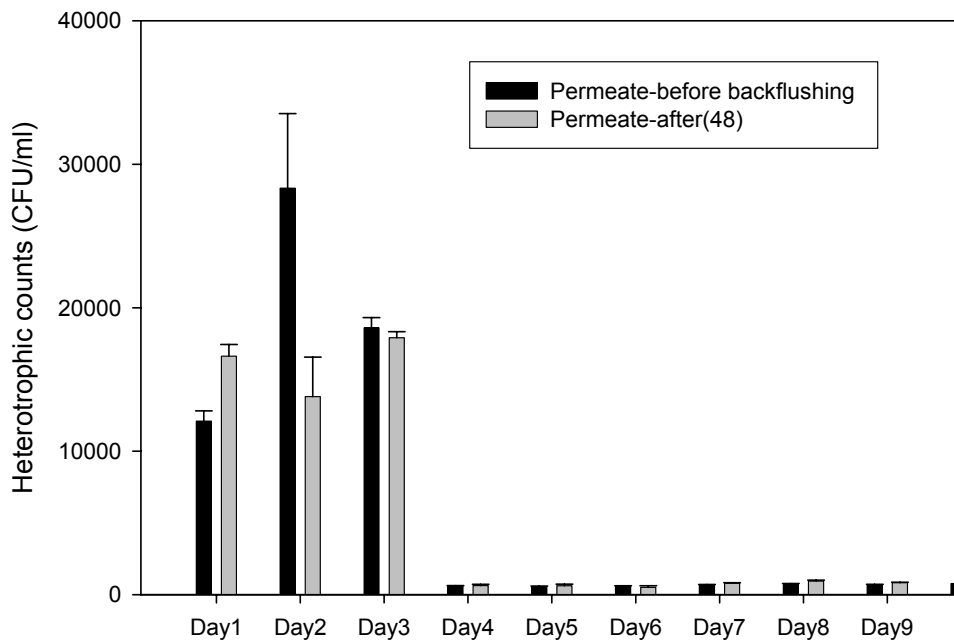


Figure 4-5. Backflush experiments (1 min every 48 min) at the Wiggins process plant with membrane E101

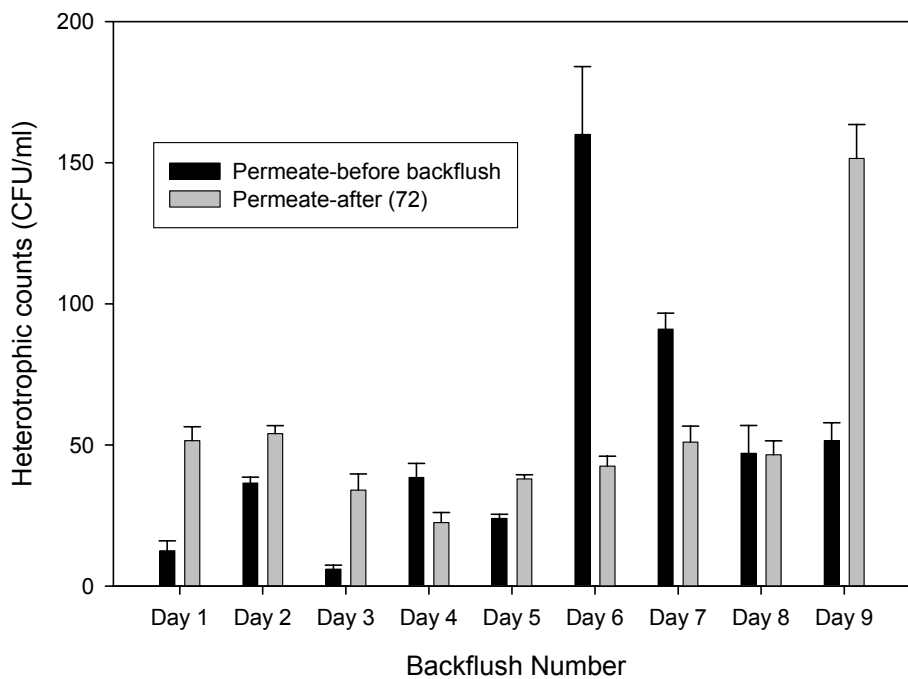


Figure 4-6. Backflush experiments (1 min every 72 min) at the Wiggins process plant with membrane E101

The effect of backflush regimens on all membranes show that the double skinned membrane has better retention capabilities than the older membrane (single skinned). However immediately after backflushing there is a temporary deterioration in water quality. A possible explanation for this is that as the fouling layer develops any abnormal pores present become smaller because the fouling material is deposited into and onto the membrane. When these pores become smaller the membrane is capable of greater retention and after a backflush this fouling layer is removed as the membrane is cleaned, so the abnormal pores return to their normal size and more bacteria are able to pass through. During the backwash procedure, the permeate dislodges the biofilm from the inside of the capillary, and if burst capillaries are present in the membrane, the bacteria will pass through to the shell side into the permeate resulting in higher counts.

Another possible reason for the temporary elevation in bacterial counts immediately after a backflush, is the possibility that bacteria present in the permeate used for the backflush, are impacted on the shell side of the membrane. Upon normal filtration, these bacteria become dislodged and are responsible for the high count after a backflush. This was investigated by determining the number of bacteria in the permeate that was used during the backflush. The results showed that the bacterial concentration in the permeate was low. However, even at a low bacteria count, during long backwash steps, significant number of bacteria can accumulate on the shell side of the membranes and contribute to this phenomenon.

To determine the window period after a backwash cycle during which time water may be unsafe, three experiments were conducted over three different days. A window period of 12 to 15 min was identified during which the water needs to be discarded (Figure 4-7). Results of this study suggests that the permeate approximately 15 to 20 min after a backflush should be discarded. Hence, membrane users need to be cautioned that immediately after a backflush there could be a window period during which time the water should

be discarded. Similar results were also noted by Bucklin *et al.* (1991). They incorporated a filter-to-waste period immediately following the backwash.

Due to unavailability of the pilot-scale rig, these two possibilities could not be explored further. Two experiments could be used to determine the origin of the bacteria. Firstly, characteristic bacteria (either coloured bacteria or antibiotic-resistant bacteria) that may be easily traced through the UF system could be inoculated either in the feed tank or in the permeate tank. After a backflush, it would then be possible to determine if these bacteria either penetrated the membrane or contributed significantly to the increased counts seen after a backflush by accumulation on the shell side of the membrane. Alternatively, chlorination of the permeate tank would reduce the bacterial count to near zero and this would be useful in determining if the permeate water contributed to the increased counts.

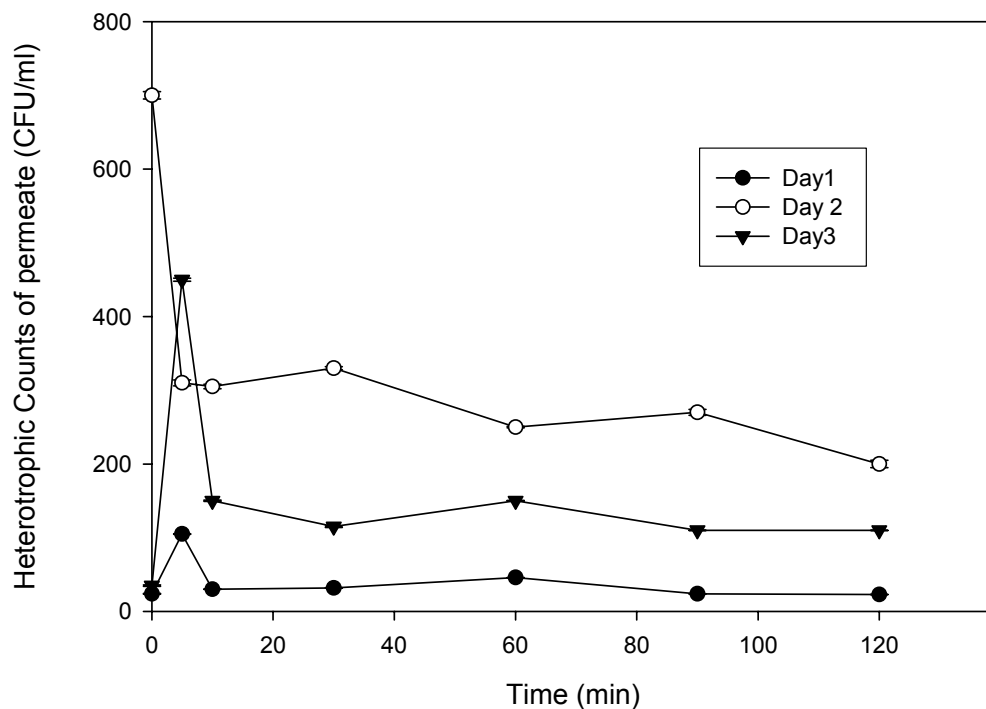


Figure 4-7. Backflush procedures over 3 days for extended periods after backflushing to determine the window period

From the observations of this study and that of others every UF system should establish bacterial counts after a backflush to prevent leakage of bacteria into the water and to avoid seeding the distribution system. If necessary, a filter to waste strategy for an appropriate time period should be implemented. Alternatively, the water could be chlorinated, especially if it is going to be distributed via a reticulation system.

## CHAPTER 5

### EVALUATION OF MEMBRANE PERFORMANCE USING MICROBIOLOGICAL TESTS

To reduce risk of passage of microorganisms into the filtrate, it is critical to assure that the membranes are intact and continuing to provide a barrier between the feedwater and the permeate or product water. Breakage of fibres or loss of system integrity will allow passage of pathogens into the receiving water. Routinely measuring the turbidity of permeate is used to monitor membrane performance, as it is a low cost and simple method. However, turbidity measurements are generally not sensitive enough to detect changes in particles at low densities unless one uses very sensitive turbidimeters (Jacangelo *et al.*, 1991). Biological tests represent more sensitive methods for monitoring membrane performance. They can be used to conduct measurements while the membrane module is in place, and is relatively cost effective and is the legislated guideline to assure potable quality water to the consumer.

These tests enumerate microorganisms occurring in the UF system during operation to provide an accurate determination of membrane performance in producing water of an acceptable quality. These tests can be performed for the following reasons:

- a) to routinely test permeate quality at selected intervals;
- b) to randomly test water at various ports in the UF system;
- c) to assess efficiency of chemical cleaning before and after a CIP regimen;
- d) to determine the optimum backwash length by monitoring the reject at intervals during a backwash; and to assess permeate quality before and after a backwash and for a short period after a backwash to determine whether a filter-to-waste regimen is necessary immediately after a backwash.

This chapter discusses the results of biological tests used for monitoring membrane performance whilst operating the UF system at various standard operating parameters. Experiments were conducted on membranes used for Wiggins Process

Plant, the laboratory rig and the bench-scale rig to compare relationships between; (a) turbidity measurements and bacterial counts; (b) between heterotrophic counts and coliform bacteria; (c) between flux and bacterial counts; (d) effect of fouling and bacterial counts; (e) the performance of the membrane under dead end and crossflow operations; and (f) a comparison of conventional microbiological tests to some commercially available rapid test.

## 5.1 Sampling

Total heterotrophic counts, total coliforms, faecal coliforms, faecal streptococci and heterotrophic bacteria in permeate and feed water samples were determined using the Membrane Filtration Technique and Plate Count Technique according to SABS Methods (1990). Sampling strategy was based on type of test and below is general protocol followed.

|                                                                                                                                                                                                                                                                                           |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <u>Sampling ports:</u>                                                                                                                                                                                                                                                                    |
| Those are identified based on the type of test employed.                                                                                                                                                                                                                                  |
| <u>Sampling:</u>                                                                                                                                                                                                                                                                          |
| <ul style="list-style-type: none"><li>• Open the sample port and allow void volumes to drain.</li><li>• Collect the sample in a sterile container.</li><li>• Place the sample on ice and transport to the laboratory.</li><li>• Close the container immediately after sampling.</li></ul> |
| <u>Bacteriological analyses:</u>                                                                                                                                                                                                                                                          |
| Conduct analyses as soon as possible after receipt of the samples.                                                                                                                                                                                                                        |



### Procedure

For routine testing of membranes, samples were analysed at:

- random intervals during a period of operation that involved no backflush or CIP procedure;
- before and after approximately 1 h after the UF system was cleaned with 50 mg/l NaOCl for approximately 1 h;

- c) before and after the backwash to determine what effect it would have on the number of bacteria in the permeate; and
- d) at different time intervals after the backwash to determine when the permeate quality stabilizes and meets the potable water limits.

The sampling methods for the three UF systems used in this study is shown in Table 5-1.

Table 5-1. Sampling regimens for the different rigs

|                                | <b>Wiggins<br/>Process plant</b> | <b>Laboratory-<br/>scale</b> | <b>Bench-<br/>scale</b>     |
|--------------------------------|----------------------------------|------------------------------|-----------------------------|
| <b>Sample bottle</b>           | 500 ml Sterile Schott bottle     | 250 ml Sterile Schott bottle | 25 ml Sterile Schott bottle |
| <b>Storage before analysis</b> | Cooler box with ice              | Within 2 h of collection     | Within 2 h of collection    |
| <b>Sampling points</b>         | Permeate<br>Reject<br>Feed       | Permeate<br>Feed             | Permeate<br>Feed            |

## 5.2 Microbiological Tests

Total number of microorganisms was enumerated using the plate count technique and the total coliforms, faecal coliforms and faecal streptococci were enumerated with the membrane filtration method.

### 5.2.1 Plate count technique (heterotropic counts)

The plate count technique estimates the total number of viable heterotrophic bacteria in water.

- a) Permeate and feed samples were tested for heterotrophic bacteria.
- b) For HPC, a pour-plate technique using plate count agar (Merck) was used.
- c) Samples were diluted and plated out in duplicate.

- d) The plates were incubated at 37°C for 48 h and colonies were counted using a colony counter

### Basic Protocol

#### Sample dilution:

- Dilute the water sample appropriately using 10-fold dilutions depending on the expected bacterial load.

A dilution greater than  $10^{-9}$  is unnecessary. A physiological saline solution (0.85% NaCl) or a similar isotonic solution is recommended for maintaining cell viability.

#### Plating out:

- Thoroughly mix 0.1 ml or 1 ml volumes of the dilutions with molten plate count agar and pour into sterile petri dishes.

The dilutions chosen to plate out again, depends on the expected bacterial load.

Replicates of this plating procedure are recommended for accuracy of the bacterial counts.

#### Incubation:

- After the agar has hardened, invert the plates and incubate for 48 h at 37°C.

#### Calculation:

- Count colonies arising from single cells using a colony counter.  
Only plates having well-spaced colonies and more than 30 colonies are enumerated.
- The bacterial count in the original sample is calculated by multiplying the average colony count on the plates by the total dilution factor.

A detailed protocol for this procedure is shown schematically in Figure 5-1.

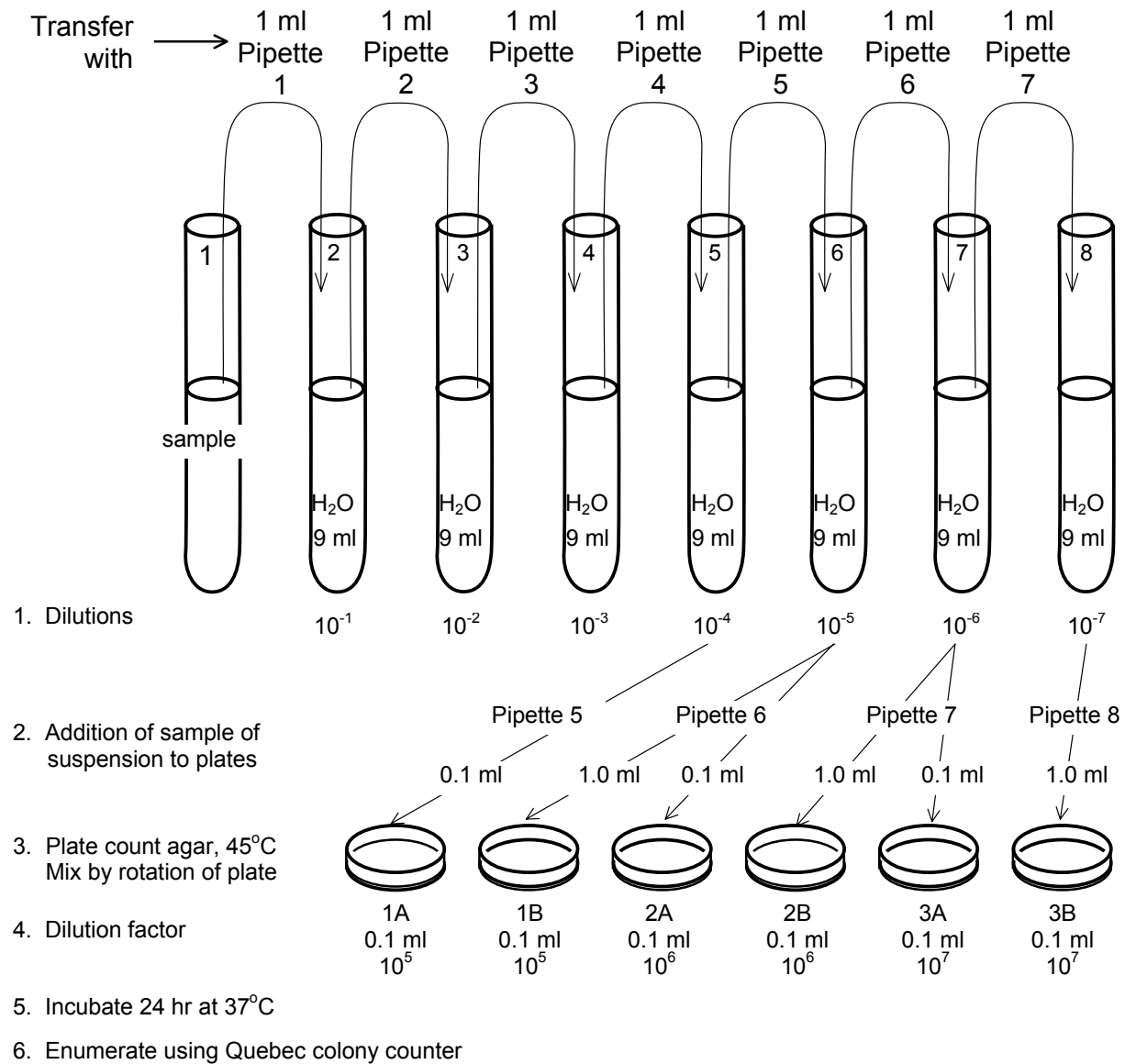


Figure 5-1. Schematic presentation of the protocol for the plate count technique

### **5.2.2 Membrane filtration technique for total coliforms, faecal coliforms and faecal streptococci**

The membrane filter method enables a count of viable total coliforms, faecal coliforms and faecal streptococci present in a given sample of water. Bacteria-tight membrane filters capable of retaining microorganisms larger than 0.45  $\mu\text{m}$  are frequently used for analysis of water. These filters offer several advantages over the conventional, multiple-tube method of water analysis:

- a) results are available in a shorter period of time,
- b) larger volumes of sample can be processed, and
- c) because of the high accuracy of this method, the results are readily reproducible.

In the membrane filtration technique, a measured volume of water is filtered, under vacuum, through a 0.45  $\mu\text{m}$  membrane filter. Bacteria are retained on the surface of the membrane, which is placed on a suitable selective medium in a sterile petri dish and incubated at an appropriate temperature. If coliforms, faecal coliforms or faecal streptococci are present in the water, sample, characteristic colonies form that can be counted directly.

Water samples are diluted prior to the membrane filtration if high bacterial counts are expected. Alternatively, if low counts are expected, large volumes can be filtered to obtain an accurate estimate of the bacterial load.

The procedure is outlined below and diagrammatically shown in Figure 5-2. The different media used and their incubation regimens is shown in Table 5-2.

**Procedure:** (for schematic representation, see Figure 5-2)

Label dilution sample tubes:

Label four 90 ml water blanks with the source of the water sample and intended dilution ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$ ).

Perform dilutions:

Aseptically perform a 10-fold serial dilution of the assigned undiluted water sample using the four 90 ml water blanks to effect dilutions:  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$ .

Label Petri dishes:

Arrange 15 Petri dishes containing appropriate selective media into three sets of five plates. Label each set as follows:

1. For total coliform count (TCC):  
Undiluted,  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  &  $10^{-4}$ .
2. For faecal coliform count (FCC):  
Dilutions as described in Step 1.
3. For faecal streptococcal count (FSC):  
Dilutions as described in Step 1.

This plating procedure is duplicated and the average count per plate for each dilution is reported.

Aseptically assemble the membrane filter unit:

- Unwrap and insert the sintered glass filter base into the neck of a one-litre side-arm suction flask.
- With sterile forceps, place a sterile membrane filter disc, grid side up, on the sintered glass platform.
- Unwrap and carefully place the funnel section of the apparatus on top of the filter disc. Using the filter clamp, secure the funnel to the filter base.
- Attach a rubber hose from the side-arm on the vacuum flask to a vacuum source.

Filtration process for highest dilutions:

- Starting with the highest sample dilution ( $10^{-4}$ ), pipette 20 ml of the dilution into the funnel.
- Start the vacuum.
- When the entire sample has been filtered, wash the inner surface of the funnel with 20 ml of sterile water.
- Disconnect the vacuum, unclamp the filter assembly, and with sterile forceps remove the membrane filter.
- Place it on the agar medium in the Petri dish labelled TCC:  $10^{-4}$ .
- Aseptically place a new membrane on the platform, reassemble the filtration apparatus, and repeat '*Filtration process*' twice,
- Add these filter discs to the Petri dishes labelled FCC:  $10^{-4}$  and FSC  $10^{-4}$ .

Filtration process for other dilutions:

Repeat '*Filtration Procedure*', using 20 ml of the  $10^{-3}$ ,  $10^{-2}$ , and  $10^{-1}$  dilutions and the undiluted samples for each of the three sets (TCC; FCC & FSC).

Incubation:

Incubate the plates in an inverted position as follows:

- TCC and FSC plates for 24 h at  $37^{\circ}\text{C}$ .
- FCC plates at  $44^{\circ}\text{C}$  for 24 h.

Calculation:

Count the number of colonies.

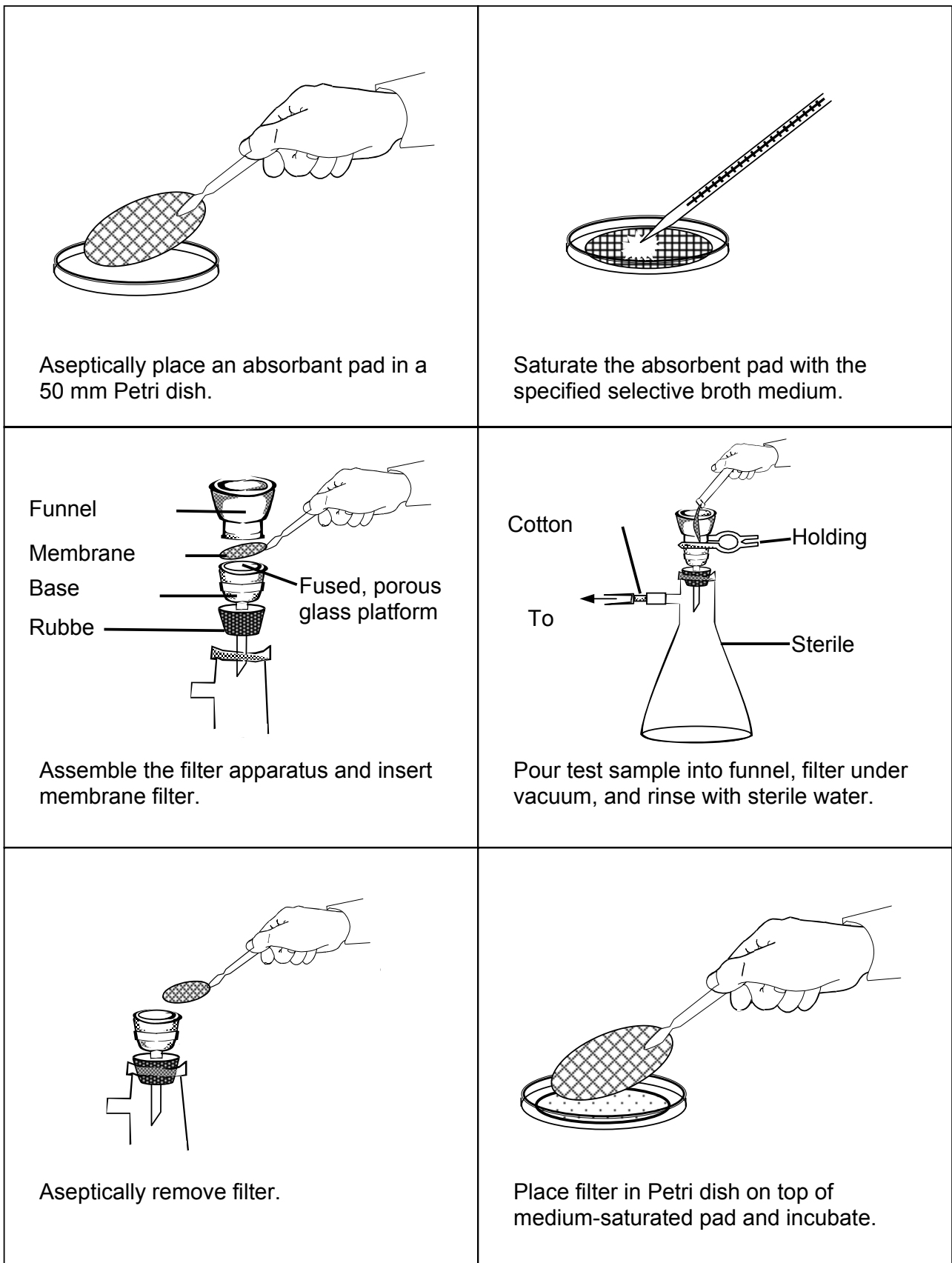


Figure 5-2. Schematic representation of the membrane filtration technique

Table 5-2. Media used, with their respective temperature and incubation periods

| Agar                     | Selective for                          | Incubation T°                   | Incubation Period |
|--------------------------|----------------------------------------|---------------------------------|-------------------|
| m-Endo LES               | total coliform bacteria                | $35 \pm 0.5^{\circ}\text{C}$    | 18 to 24 h        |
| m-FC                     | faecal coliforms                       | $44.5 \pm 0.25^{\circ}\text{C}$ | 18 to 24 h        |
| Membrane<br>enterococcus | faecal streptococci                    | $35 \pm 0.5^{\circ}\text{C}$    | 48 h              |
| Plate count              | total viable heterotrophic<br>bacteria | $37^{\circ}\text{C}$            | 48 h              |

### 5.3 Relationship between Microbial Tests and Turbidity

There are numerous methods to estimate membrane performance. Of these tests, one that is used routinely is the measurement of the **turbidity values** of the permeate.

The advantages of this test are that:

- a) it is simple to perform;
- b) it is a rapid test with results available within minutes;
- c) it is an inexpensive test, assuming a turbidity meter is available;
- d) an increase in turbidity correlates well with an increase in microbial numbers.

While these factors are desirable for routine/daily testing, membrane users need to be cognisant of the drawback of turbidity measurements in assessing membrane performance.

The disadvantages include:

- a) a lack of specificity for estimating microorganisms, since any particulate will contribute towards turbidity;

- b) poor sensitivity in detecting low numbers of microorganisms or a slight increase in microbial levels, that would nevertheless pose a serious health risk; and
- c) poor quantitative method as microbial counts cannot be estimated.

It is therefore imperative to supplement turbidity tests with regular microbiological testing of the permeate to determine whether the water conforms to the legislated specifications.

#### Procedure

The relationship between turbidity and bacterial counts were conducted on feed and permeate samples using membranes 769-00 and 1713-11A on the bench-scale rig.

#### Results

The results (Figures 5-3 and 5-4) showed that when bacterial counts were high, as in the feed samples for both membranes, a good correlation was seen which was not as evident when a low number of organisms were present. Although turbidity measurements are not as sensitive as bacterial counts they can be used as guideline for monitoring the operation. This is further supported by the work of Pryor *et al.*, (1998) who performed three pilot studies in different areas of South Africa, demonstrating that drinking water (0.5 NTU turbidity standards) particulate and colloidal turbidity can be reduced to acceptable levels with membranes.

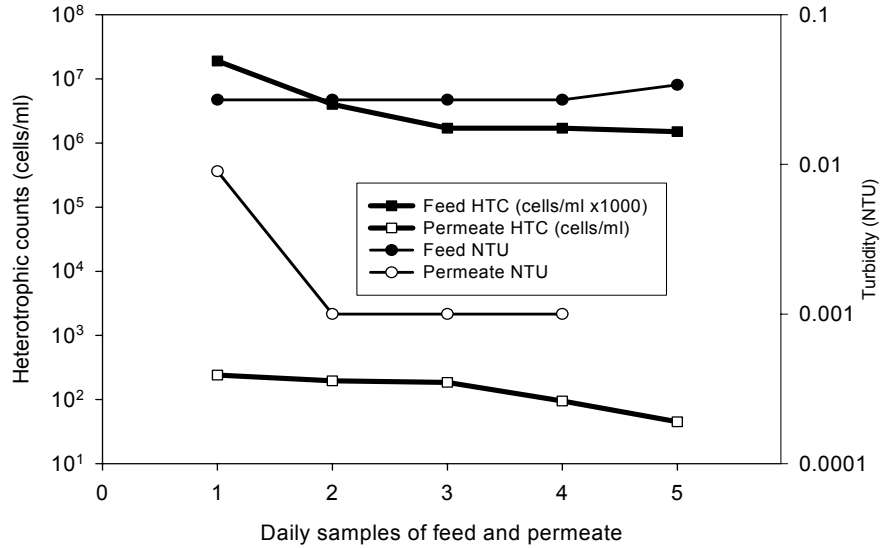


Figure 5-3. Graph of feed and permeate heterotrophic plate count and turbidity measurements for membrane 769-00 on the laboratory-scale system

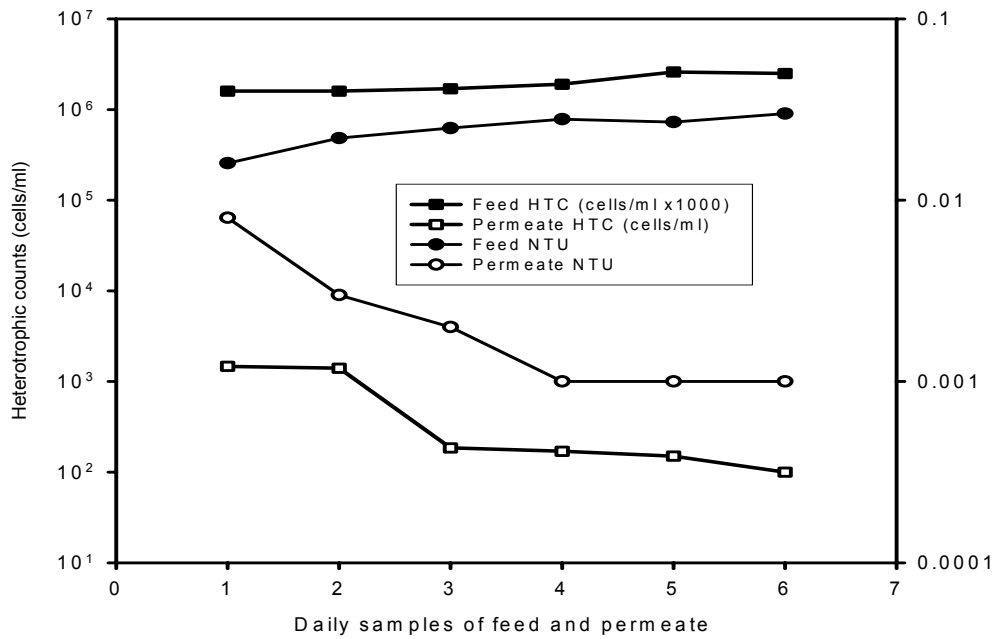


Figure 5-4. Graph of feed and permeate heterotrophic plate count and turbidity measurements for membrane 1713-11 on the laboratory-scale system

#### **5.4 Relationship between Total Counts, Coliform Counts, Faecal Coliforms and Faecal Streptococci**

##### Procedure

Evaluation was performed over a two-week period on the Wiggins plant with new membranes (double skinned). The operating conditions were as previously described i.e., constant pressure, crossflow mode and no backwash. These membranes were challenged with both high and low numbers of organisms.

##### Results

These results showed that the Wiggins membranes (which were three years old) when operating under constant pressure and in crossflow mode gave 99.9% rejection of faecal streptococci, total coliforms and faecal coliforms. Heterotrophic bacteria, however, were present. This membrane showed a 99.93 to 99.97% rejection of heterotrophic bacteria as shown in Table 5-3, coliforms are reduced significantly in the permeate, although not to levels that are usually acceptable for potable water. The heterotrophic counts were high when coliforms were detected. Although membrane performance is evaluated by the heterotrophic counts it is ultimately the coliform count that determine whether the water is safe for consumption. Thus for routine testing of membrane it is essential that heterotrophic as well as coliform counts are carried out.

Table 5-3. Microbiological results of membranes during standard operations

| Routine testing with no backflushing or CIP – Wiggins (old membrane E 101) |      |           |          |                   |          |                      |          |               |          |
|----------------------------------------------------------------------------|------|-----------|----------|-------------------|----------|----------------------|----------|---------------|----------|
|                                                                            | Days | HPC /ml   |          | Coliforms / 100ml |          | Faecal coli / 100 ml |          | F. Str 100 ml |          |
|                                                                            |      | Raw water | Permeate | Raw water         | Permeate | Raw water            | Permeate | Raw water     | Permeate |
| Old membrane                                                               | 1    | 184       | 19       | 2                 | 0        | 0                    |          | 0             |          |
|                                                                            | 2    | 1 923     | 293      | 0                 | 13       |                      | 0        |               | 0        |
|                                                                            | 3    | 278       | 256      | 300               | 0        | 1                    | 0        | 0             | 0        |
|                                                                            | 4    | 316       | 132      | 11                | 0        | 1                    | 0        | 0             | 0        |
|                                                                            | 5    | 123       | 185      | 16                | 0        | 0                    | 0        | 0             | 0        |
|                                                                            | 6    | 15 667    | 500      | 300               | 0        |                      | 0        |               | 0        |

| Routine testing with no backflushing or CIP<br>Laboratory system with membrane E 1713-11 |      |           |           |                   |          |
|------------------------------------------------------------------------------------------|------|-----------|-----------|-------------------|----------|
|                                                                                          | Days | HPC / ml  |           | Coliforms / 100ml |          |
|                                                                                          |      | Raw water | Permeate  | Raw water         | Permeate |
| New membrane                                                                             | 1    | 700       | 20        | 19 500            | 1        |
|                                                                                          | 2    | 7 500     | 31        | 680               | 15       |
|                                                                                          | 3    | 7 700     | 1 265     | 114 500           | 19       |
|                                                                                          | 4    | 1 290     | 115       | 93 500            | 12       |
|                                                                                          | 5    | 5 050     | 4 150     | 30 250            | 25       |
|                                                                                          | 6    | 5 750     | 1 240     | 86 500            | 4        |
|                                                                                          | 7    | 965       | 3 675     | 64 250            | 4        |
|                                                                                          | 8    | No result | No result | 6 500             | 0        |

## 5.5 Relationship between Flux and Bacterial Counts

A module is rated at a certain permeate flux at a given standard or test conditions (temperature, pressure, feed solution composition and water recovery). An assessment of the experimental results indicated that the bacterial counts in the permeate decreases as the flux increased.

A correlation between HPC, differential pressure and flux in the permeate of samples from a laboratory-scale experiment over a 18 day period showed the critical flux value. The critical flux is the maximum flux below which there is a negligible flux decline. With a decrease in pressure drop, the flux decreases correspondingly, thereby reducing the filtration across the membrane and inevitably partial prevention

of further deposition of a fouling layer onto the membrane. This results in majority of the feed solution to flow past the membrane, lifting and transporting with it the already deposited fouling layer from the membrane. This results in the increase in flux obtained after the initial reduction of flux below the critical flux value. This trend was observed in our experiments (Figure 5-5). A possible explanation for the above trends lies in the partial removal of the fouling layer when a decrease in pressure drop occurs. The decrease in pressure drop results in a decrease in flux, which leads to the partial removal of the fouling layer. However, after the pressure drop was adjusted back to 100 kPa a greater increase in flux was noted due to the absence of the fouling layer.

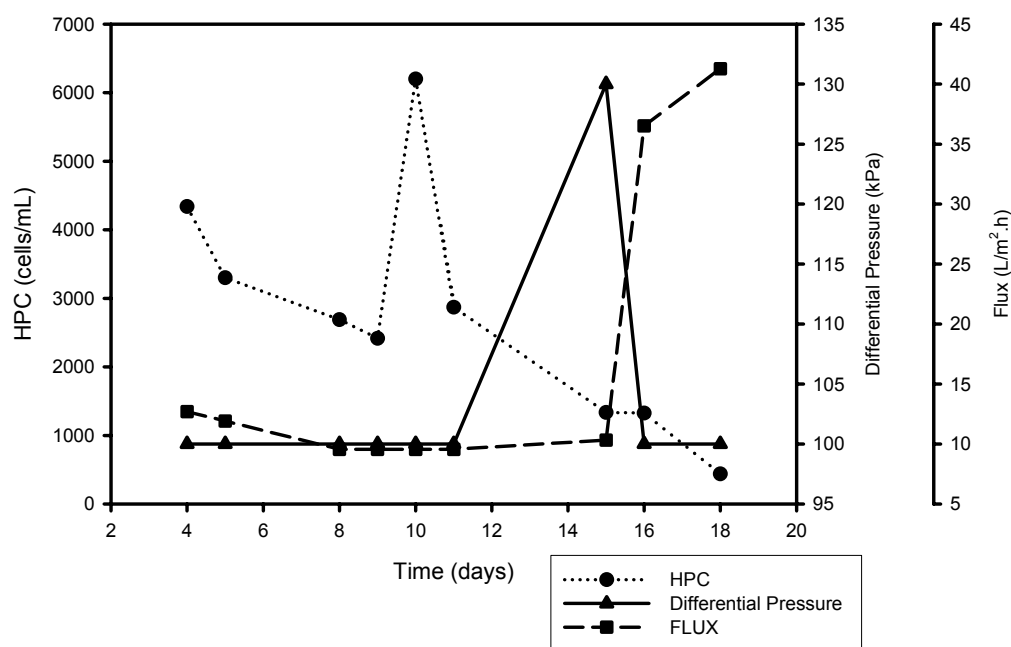


Figure 5-5. Correlation between heterotrophic plate count, differential pressure and flux measurements in the permeate during the filtration period with a spike of  $3.4 \times 10^5$  cells/ml

## 5.6 Fouling

Control of fouling is of utmost importance. Techniques involved are firstly pretreatment of feed, which can reduce the particulate density onto the membranes and therefore reduce fouling (Aptel and Clifton, 1983; Jacangelo, 1990). Secondly, by varying operating conditions, such as moderate pressure, crossflow and backwashing (Beeby, 1989). Thirdly, membrane regeneration, e.g., washing the membrane with chemicals, can also reduce fouling. Depending on the membrane material, chemicals such as sodium hydroxide, detergents and disinfectants can be used (Jacangelo, 1990).

### 5.6.1 Effect of fouling on bacterial numbers

#### Procedure

To evaluate whether fouling could be monitored using bacterial counts experiments were conducted on the Wiggins rig over a nine-day period and a laboratory rig over a six day period. The Wiggins rig was run in a filtration mode for nine consecutive days. During this period HPC and flux readings were taken on a daily basis.

#### Results

From the results in Table 5-4, one can see that there is a gradual decrease in flux over time indicating the development of a fouling layer. A corresponding decrease in HPC's should have occurred due to the fouling layer providing a secondary barrier, however the above results did not reflect such a trend. HPC counts fluctuated throughout the 9-day period with counts reaching up to  $1.2 \times 10^4$  cfu/ml. This exceeds potable water limits by 2 logs and was regarded as unacceptable.

A similar experiment carried out in the laboratory rig where 2 l of water with  $1.125 \times 10^7$  cells/ml was added to the feed tank. The system was operated in a crossflow mode at pressure difference of 100 kPa, a feed flow rate of 40 l/h over a 6-day period. The results (Table 5-5) show of the HPC of the permeate show a decline with time, reaching potable water quality after 6 days of filtration. Immediately after the spike 845 cfu/ml was recorded. Thereafter this value increased to 1 400 cfu/ml after 1 day of filtration.

Table 5-4. Flux and heterotrophic plate count results for the Wiggins Process Plant

| Sample     | Flow rate<br>(l/h) | Flux<br>(l/m <sup>2</sup> .h) | HPC<br>(cfu/ml) |
|------------|--------------------|-------------------------------|-----------------|
| Pure water | 9.92               | 131.52                        | n/a             |
| Day 1      | 8.38               | 111.10                        | n/a             |
| Day 2      | 7.49               | 99.30                         | 3 150           |
| Day 3      | 4.94               | 65.50                         | 710             |
| Day 4      | 4.25               | 56.35                         | 161             |
| Day 5      | 3.53               | 46.80                         | 12 000          |
| Day 6      | 3.23               | 44.28                         | 9 640           |
| Day 7      | 3.17               | 42.03                         | 8 850           |
| Day 8      | 2.28               | 30.23                         | 8 000           |
| Day 9      | 1.80               | 23.86                         | 8 550           |

Table 5-5. Flux and heterotrophic plate count results for the laboratory-scale system with the double skinned membrane

| Sample                     | Days                       |                            |                            |                           |                            |             |                          |
|----------------------------|----------------------------|----------------------------|----------------------------|---------------------------|----------------------------|-------------|--------------------------|
|                            | 0                          | 1                          | 2                          | 3                         | 4                          | 5           | 6                        |
| Perm<br>(cfu/ml)           | 845                        | 1 400                      | 1 470                      | 185                       | 170                        | 150         | 100                      |
| Feed<br>(cfu/ml)           | 1.125<br>x 10 <sup>7</sup> | 1.565<br>x 10 <sup>6</sup> | 1.595<br>x 10 <sup>6</sup> | 1.71 x<br>10 <sup>6</sup> | 1.925<br>x 10 <sup>6</sup> | 2.6 x<br>10 | 2.5 x<br>10 <sup>6</sup> |
| Log Red.                   | 5 log                      | 3 log                      | 3 log                      | 4 log                     | 4 log                      | 4 log       | 4 log                    |
| % Log<br>reduction         | 99.99                      | 99.91                      | 99.91                      | 99.99                     | 99.99                      | 99.99       | +99.99                   |
| Flux (l/m <sup>2</sup> /l) | 174.65                     | 134.96                     | 134.96                     | 127.02                    | 134.96                     | 119.08      | 111.14                   |
| Pressure<br>(Kpa)          | 100                        | 100                        | 100                        | 100                       | 110                        | 105         | 100                      |
| Temperature                | 26                         | 26                         | 26                         | 26                        | 26                         | 26          | 26                       |
| Feed (NTU)                 |                            | 0.016                      | 0.022                      | 0.025                     | 0.028                      | 0.030       | 0.027                    |
| Permeate<br>(NTU)          | 0.001                      | 0.008                      | 0.003                      | -0.002                    | 0                          | 0.001       | 0.002                    |

A possible explanation lies in the fact that the sample taken immediately after spiking the system represented a portion of the spike. The feed counts portray a different trend where a sharp decrease in counts (i.e. 1 log) was observed on Day 1 and thereafter counts seemed to increase gradually over the 6-day filtration. The 1 log reduction is attributed to the initial retention of the bacteria onto the membrane – which is the maximum due to the greater membrane surface for absorption. The gradual increase thereafter is due to the concentration of the feed by the reject which is being recycled.

### **5.6.2 Ultrastructural analysis**

#### Procedure

An ultrastructural analysis using scanning electron microscopy was carried out at the University of Natal Electron Microscope Unit. Cut sections of the membrane capillaries were freeze dried, then sputter-coated with gold for approximately 20 min and viewed in a Jeol JSM-840 SEM.

#### Results

The membrane showed a layer of bacterial flocs that penetrates the outer layer fouling layer that develops (Figure 5-6).

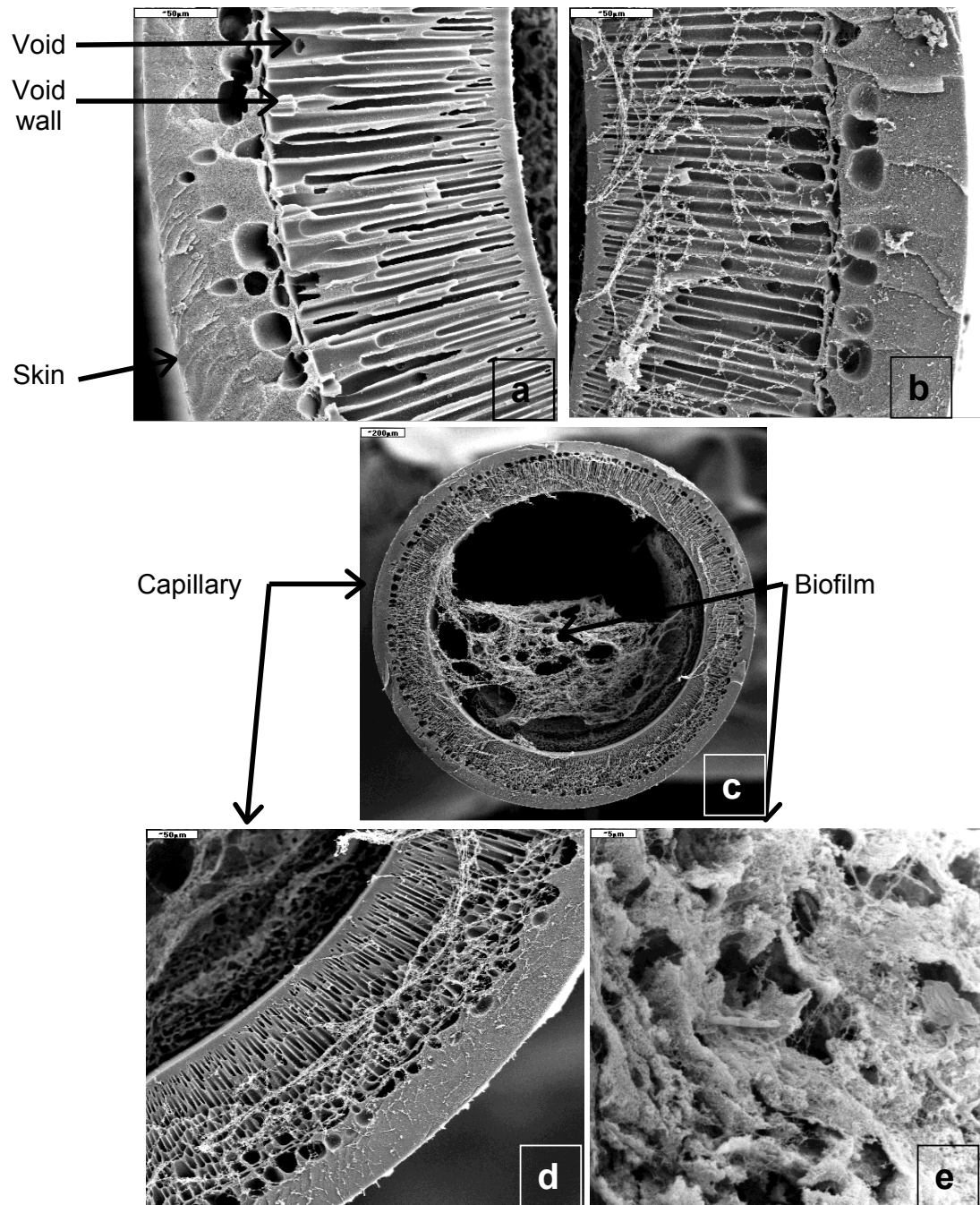


Figure 5-6. Scanning electron micrograph of polysulphone membrane (a) after a CIP; (b) 6 days after a filtration cycle; (c) biofilm formation inside the capillary with higher magnifications shown in (d) and (e)

## 5.7 Comparison of Dead-end and Crossflow Operations

### Procedure

Experiments were conducted with five different membranes (1713-11, 1713-05, 769-00, 798-02 and 769-01) on the bench-scale rig to establish the effect of crossflow and dead-end filtration. The membranes were spiked and the system was operated for 3 days with each membrane in dead-end and crossflow mode. HPC were conducted from permeate and feed samples.

### Results

The results of these experiments are shown in Figures 5-7a to e. It was found that fluid flow in the dead-end mode is better suited for potable water production. Potability was achieved faster with dead-end mode. However, flux values decrease drastically with dead-end production due to the development of the fouling layer. A gradual decrease is noticed for crossflow operation due to the recycling of the reject.

- a) A 99.99% rejection was obtained for dead-end filtration as opposed to crossflow filtration, averaged of 99.91%. This rejection is possibly due to the increased biofilm layer that develops during dead-end filtration, acting as a secondary filter.
- b) Flux values decreased drastically during dead-end filtration, as anticipated, due to the development of the fouling layer. A gradual decrease was observed for crossflow filtration.

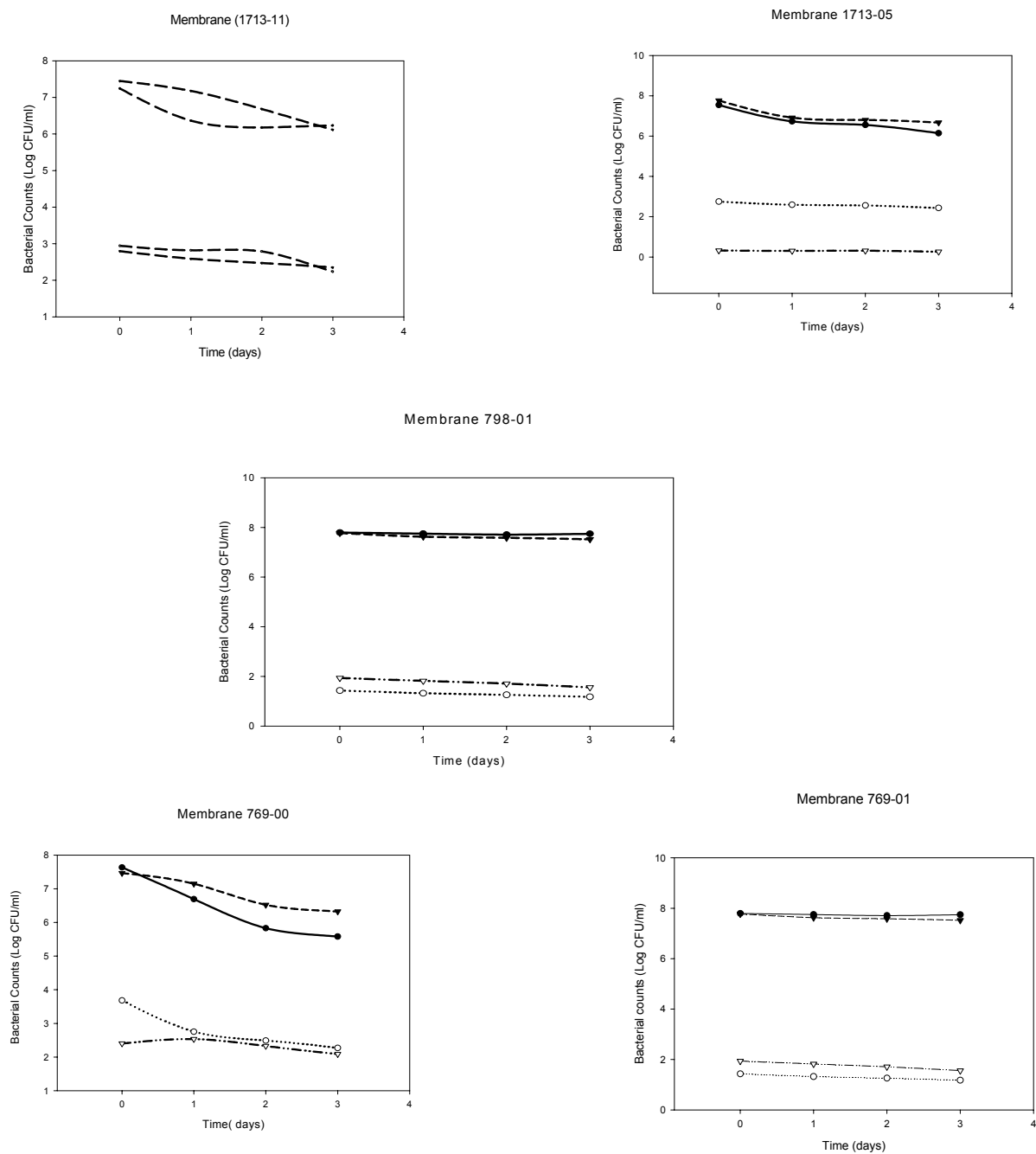


Figure 5-7. Heterotrophic counts of feed (● - crossflow and ▼ - dead-end) and permeate (▽ - dead-end and ○ - crossflow)

## 5.8 Rapid Method of Analysis

The aim of this study was compare the detection of low numbers of total coliform bacteria and *E. coli* in permeate samples using simple rapid kits i.e. Colilert (CL), Colicount (CC) and Petrifilm method with the SABS MF method.

With regard to the new methods, special attention was given to the following aspects:

- a) enumeration of 1 total coliform per 1 ml and 100 ml in a maximum of 24 h
- b) requirements for confirmatory tests
- c) sample inoculation method
- d) interpretation of results
- e) costs per test
- f) robustness of the test

### Procedure

Three test kits, i.e., CC, CL and Petrifilm were compared to conventional bacteriological tests for ease of use, simplicity and accuracy. The minimum detection limit of each kit was evaluated with an overnight culture of *E. coli* that was inoculated into each kit with counts ranging from 1 to  $10^9$  cells/ml. The use of these kits in field situations was evaluated at the Wiggins plant by sampling the permeate water during backwash.

### Coliform MF

Total coliforms were enumerated by the MF-method with m-Endo agar LES as prescribed in SABS Standard Methods 221-1990. Plates were incubated  $35 \pm 0.5^\circ\text{C}$  and read at 24 h. Colonies exhibiting the green metallic shine were picked and confirmed for gas production in lactose peptone water. A culture that produced gas within 24 to 48 h was considered positive for coliforms.

### Faecal coliform MF and confirmation of *E.coli*

Faecal coliform were enumerated by the MF method with m-FC agar. Plates were incubated at  $44.5 \pm 0.25^\circ\text{C}$  and read at 24 h. Suspect colonies exhibiting a blue centre with a translucent periphery were picked and confirmed with lactose peptone water.

A culture that produced gas within 48 h was considered positive for faecal coliforms. To confirm the presence of *E. coli* confirmed cultures of faecal coliforms were subcultured from the lactose peptone water to tryptone water and incubated at  $44.5 \pm 0.25^{\circ}\text{C}$  for 24 h. After incubation 0,3 to 0,5 ml Kovacs reagent were added to each tube to test for the formation of indole. The development of a red colour denotes the presence of indole and coliforms the presence of *E. coli* (SABS 221-1990).

#### Presence-Absence Test (CL)

The CL test reagents were in powder form in plastic pillows and screw-cap glass tubes, respectively. Each reagent was added to a 100 ml sample, which was shaken to dissolve the powder. The sample mixture was pipetted in 10 ml aliquots into 10 sterile glass tubes to quantify by means of the MPN method. Capped tubes were incubated for 24 to 28 h at  $35 \pm 0.5^{\circ}\text{C}$  to determine the concentration of total coliforms and *E. coli* per 100 ml, the number of positive tubes per sample was compared to the standard MPN probability tables.

CL tubes exhibiting a yellow colour and CC tubes exhibiting a dark red/purple (magenta) colour were considered confirmed total coliforms. Each coliform positive tube was checked for fluorescence using a long wavelength ultraviolet lamp (366nm). If fluorescence was exhibited the presence of *E. coli* was considered confirmed.

#### Results

The results show that all three kits were able to enumerate cells between 100 cells/ml to 1 cell/ml (Table 5-6). The results of the permeate samples during backwash confirmed that the numbers were higher after backwash than during backwash (Table 5-7). Although the Petrifilm yields the quickest results (8 h) it is expensive and requires microbiological knowledge as the inoculation has to be done under sterile conditions. The CL kit is not quantitative. The disadvantage of using conventional method for testing coliforms on M-Endo Agar by membrane filtration is that the metallic sheen colonies are often indistinguishable from other colonies. In comparison to the above, the CC kit is a simple dipstick method, that required no microbiological skills, is easy to operate, is fairly accurate and also gives an indication of heterotrophic bacteria that appear as white colonies. This test appears

to have potential for testing UF systems in remote locations where there is lack of a laboratory and skilled personnel.

Table 5-6. Minimum detection limits of conventional method versus rapid commercial kits method

| Tests                 | 100 Cell | 10 Cells | 1 Cell |
|-----------------------|----------|----------|--------|
| Petrifilm (N=5)       | 70       | 8        | 1-2    |
| Coli-Count (N=5)      | 55       | 7        | 2      |
| MF Conventional (N=5) | 88       | 9        | 2      |

Table 5-7. Field tests results of conventional method versus rapid commercial kits

| Test 1          | RAW WATER               |                          | PERMEATE                |                          |
|-----------------|-------------------------|--------------------------|-------------------------|--------------------------|
|                 | After backwash (CFU/ml) | Before backwash (CFU/ml) | After backwash (CFU/ml) | Before backwash (CFU/ml) |
| Petrifilm       | 65                      | 110                      | 0                       | 0                        |
| Coli Count      | 50                      | 80                       | 0 Coli<br>2 HTB         | 0 Coli<br>54 HTB         |
| Coli-Lert       | +ve Yellow              | +ve Yellow               | -ve (no change)         | Colour change 1 in 3     |
| Conventional MF | 58                      | 116                      | 0 Coli<br>70 HTB        | 0 Coli<br>96 HTB         |

| Test 2          | RAW WATER               |                          | PERMEATE                |                          |
|-----------------|-------------------------|--------------------------|-------------------------|--------------------------|
|                 | After backwash (CFU/ml) | Before backwash (CFU/ml) | After backwash (CFU/ml) | Before backwash (CFU/ml) |
| Petrifilm       | 45                      | 95                       | 0                       | 0                        |
| Coli Count      |                         | 34                       | 0 Coli<br>2 HTB         | 0 Coli<br>2 HTB          |
| Cili-Lert       | +ve Yellow              | +ve Yellow               | -ve (no change)         | Colour change 1 in 3     |
| Conventional MF | 44                      | 124                      | 0 Coli<br>67 HTB        | 0 Coli<br>99 HTB         |

## CHAPTER 6

### PERFORMANCE CHARACTERISTICS OF MEMBRANES USING MICROBIAL CHALLENGE TEST

Microbial challenge tests are performed using specific microorganisms of known size to determine the ability of the membrane to reject these particles. These challenge tests are best suited to laboratory or pilot-scale UF systems when new membranes are installed or changed. If used in larger UF systems, one has to consider the large inoculation volumes required, cost of the test organisms if they are being purchased and persistence of the microorganisms in the potable water UF systems. Microorganisms that can be used in these challenge tests include:

- a) bacteriophages
- b) bacteria
- c) protozoan (oo)cysts
- d) yeasts

To assess the removal of various organisms by a variety of locally manufactured polysulphane membranes without the impact of water quality will reveal the specific size exclusion distribution. This can be achieved by challenging the membranes with differently sized organisms ranging from bacteriophages, bacteria, protozoan cysts and oocysts under conditions that favour microbial passage. Based on the size of those organisms one could predict any abnormality of the membrane.

#### 6.1 Bacterial Challenge

##### Procedure

The test organism used was *E.coli HB 101 DH5  $\alpha$*  - for the bacterial spike. Sterile distilled water (2 l) was spiked with different concentrations of the organism for the different experiments. The spike was prepared by inoculating 50 ml of nutrient broth with the organism and running a shake flask at 150 rpm and 37°C for 24 h. The broth was subsequently centrifuged for 15 min at 3 000 rpm. The supernatant was discarded and the pellet was resuspended in 15 ml of sterile distilled water. The concentration of cells was measured by diluting 1 ml of suspension and using the

pour plate technique. Colonies were counted and multiplied by the dilution factor to obtain cells per ml.

## 6.2 Bacteriophage Challenge

### Procedure

The host strains and bacteriophage that were employed during this study were obtained from various sources as shown in Table 6-1 and their characteristics are shown in Table 6-2. Permeate samples are taken at 1 h intervals over a period of 4 to 5 h. All samples are collected in sterile bottles. Commercial media was supplied by Difco and Biolab and was prepared and sterilized according to the manufacturer instructions unless stated otherwise. The preparation of all media is shown in Appendix 2.

Table 6-1. Microorganisms used for challenge tests

| CULTURE                                                                                           |                                  | SOURCE                                                     |
|---------------------------------------------------------------------------------------------------|----------------------------------|------------------------------------------------------------|
| SOMATIC COLIPHAGES                                                                                |                                  |                                                            |
| <b>Host:</b> <i>Eschericia coli</i> C WG5 (ATCC 700078)<br>i.e. Naladixic acid resistant mutant.  | <b>Phage:</b> T1 (ATCC 11303-B)  | Department of Medical Virology,<br>University of Pretoria. |
| <b>Host:</b> <i>Eschericia coli</i> B (ATCC12139)                                                 | <b>Phage:</b> T4 (ATCC13206)     |                                                            |
| <b>Host:</b> <i>Salmonella typhimurium</i> WG49 (ATCC14028) i.e. Naladixic acid resistant mutant. | <b>Phage:</b> MS2 (ATCC15597-B1) | Department of Medical Virology,<br>University of Pretoria. |

Table 6-2. Physical characteristics of bacteriophages

| Bacteriophage | Diameter (nm) | Shape       | Genome |
|---------------|---------------|-------------|--------|
| T1            | 50 nm         | Icosahedral | dsDNA  |
| T4            | 80 nm         | Polyhedral  | dsDNA  |
| MS2           | 23-25 nm      | Spherical   | dsRNA  |

Analyses were designed to determine bacteriophage rejection by the membrane over time. The efficiency of the membrane performance was expressed either by its Rejection (R), Rejection percentage (%R) or reduction in log orders. The average rejection (R) of a membrane to one (excellent rejecting membrane).

$$\text{Rejection (R)} = 1 - C_p / C_f$$

$C_p$  = concentration of bacteriophage in permeate

$C_f$  = concentration of bacteriophage in feed

### **6.2.1 Somatic phages**

The somatic phages were detected using the Plaque Assay method (Grabow, 1997). A required number of test tubes containing top agar were liquified by immersion into a water bath at a temperature of 48°C. 300 µl calcium chloride was added to the top agar followed by 1 ml of test sample and 0.5 ml of host culture. Top agar was gently mixed and without delay pored over the bottom layer of phage agar plate. The top layer was distributed evenly and allowed to solidify on a perfectly horizontal cool surface. This procedure was done in triplicate. The inverted plates were incubated overnight at 35 to 37°C. Plaques were visible as circular disc-like clearings through the lawn of bacteria. The total number plaques observed on all three plates were averaged and multiplied by a factor of ten to give results in PFU 10 ml<sup>-1</sup>.

### **6.2.2 F-RNA coliphage**

The Double Layer Plaque Assay Method for F-RNA (male specific) coliphages was followed (Grabow, 1997). A required number of test tubes containing top agar (ssTYGA) was liquified by immersion into a water bath at 48°C and 33 µl of calcium glucose was added to top agar. A 1 ml volume of permeate sample was then added to the top agar followed by 1 ml of host culture. The top agar mixture poured with minimum delay onto bottom agar (TYGA) in 90 mm phage agar plate and distributed evenly and allowed to solidify on a horizontal cool surface. Unlike plaques formed by somatic coliphages, the plaques formed by F-RNA coliphages are smaller and more opaque than clear.

### *Preparation of working culture*

One vial of the stock organism was thawed and added to 50 ml of tryptone yeast extract broth in a 250 ml flask. This was incubated in a 37°C shaking waterbath. A 3 ml sample aliquot was at time 0 and used as a blank for spectrophotometric analysis ( $A_{560}$ ). The absorbance was read at 20 min intervals and once it approached 0.7 absorbance, 1 ml of host was added to 2.5 ml of top agar and 1 ml positive control. This host, top-agar and positive control were then poured onto the bottom agar layer in petri dish. The plates were incubated overnight at 37°C in an inverted position. Following incubation, the absorbance where the most number of plaques were observed was noted. Cultures used in future used a predetermined absorbance was used.

### Results

The laboratory system and the bench-scale system were operated at constant feed, flow rate and flux under crossflow conditions. The membrane removal efficiency was tested by challenged initially with *E. coli* and then by seeding the feed tank with bacteriophages. T4 (polyhedral bacteriophage with a diameter of 80 nm) was the largest of the three bacteriophages and the penetration of this bacteriophage indicates presence of large pores. T1 (an icosahedral bacteriophage with a diameter of 50 nm) was used as its presence can be used as an indicator of faecal pollution and MS2 is the smallest bacteriophage and its presence in the permeate is expected due to its size and shape. Bacteriophages MS2 (a spherical bacteriophage with a diameter of 23 to 25 nm) commonly used for bacteriophage challenges, closely resembles human enteric bacteriophages in their small size and shape. Their resistance to disinfection and non-pathogenicity to humans is an added advantage to their use in bacteriophage challenge tests.

Rejection profile of membrane E101 showed 99-100% rejection of T4, 2 fold log reduction for T1 and only a log reduction of < 1 for phage MS2, suggesting that the UF membrane was "leaky" to phages. The membrane performance improved after 2 to 3 h after which rejection increased over time (Table 6-3).

Table 6-3. Bacteriophage removal (PFU/ml)

| Time (h) | T4 PFU/ml         | T4 PFU/ml         | T1 PFU/ml          | T1 PFU/ml          | MS2 PFU/ml        | MS2 PFU/ml        |
|----------|-------------------|-------------------|--------------------|--------------------|-------------------|-------------------|
| 0        | $2.1 \times 10^8$ | $1.0 \times 10^7$ | $1.25 \times 10^6$ | $1.05 \times 10^6$ | $2.4 \times 10^7$ | $2.4 \times 10^7$ |
| 1        | $1.5 \times 10^6$ | $6.1 \times 10^5$ | $2.6 \times 10^6$  | $4.15 \times 10^6$ | $5.7 \times 10^6$ | $6.1 \times 10^6$ |
| 2        | $1.0 \times 10^4$ | $5.9 \times 10^4$ | $2.9 \times 10^6$  | $2.6 \times 10^5$  | $5.4 \times 10^6$ | $5.2 \times 10^6$ |
| 3        | $8.0 \times 10^5$ | $2.9 \times 10^5$ | $1.2 \times 10^6$  | $1.2 \times 10^6$  | $2.2 \times 10^6$ | $5.0 \times 10^6$ |
| 4        | 0                 | $1.5 \times 10^3$ | $1.1 \times 10^5$  | $1.0 \times 10^5$  | $9.9 \times 10^5$ | $4.3 \times 10^6$ |

In the laboratory-scale system showed that bacteriophage T4 was completely removed after 4 h in one experiment and a 3.8 log reduction was observed in another experiment. Phage T1 showed a similar trend, however, after 4 h, there was only a 2-fold log reduction and only a one log reduction of bacteriophage MS2 (Table 6-3). The results suggest that the UF membrane will be “leaky” to bacteriophage during the first 2 to 3 h after which rejection increases over time. Bacteriophage removal efficiency for MS2 achieved with UF was less than 1 log removal. This trend was unexpectedly low compared to other UF studies, but consistent with results obtained during studies done by Iranpur (1997). As with the other bacteriophage tested, bacteriophage removal efficiency in UF appeared to increase moderately with time. These results could aid in estimating the size of the pores in the membrane and indicates that membranes have rejection capacity of 80 nm, once a biofilm has been established in the membranes.

The most important parameter to examine when assessing the performance of a bacteriophage rejection by membranes is the Rejection value (R). For this study rejection efficiency estimates were based on the comparison of bacteriophage concentration in the feed with the bacteriophage concentration in the permeate. In keeping with the objective of assessing the capillary UF system to remove bacteriophage of different sizes, a noticeable reduction in bacteriophage concentration over time was observed in all experiments (results not shown).

A very similar profile was observed with a study done by Madaeni *et al.*, (1995). For these data the bacteriophage rejection was obtained by analysis of the permeate collected at 1 h intervals. T4 results indicate that with estimated equivalent diameter

(for complicated bacteriophage) the module show a good agreement with bacteriophage rejection. This method proved adequate for 8-log removal order. The average rejection value (R) was 1, which indicated a very good rejection capability of the membrane.

The R-value indicated that some of the bacteriophages being removed from the feed solution were possibly due to:

- a) adsorption onto flow system surfaces (inside and outside the tubes, reservoirs);
- b) lumping due to bacteriophage flocculation and aggregation onto themselves;
- c) intrusion into and adsorption onto the internal membrane surface;
- d) inactivation of bacteriophages after 4 h;
- e) bacteriophage diameter larger than the pore diameter of the membrane;
- f) for T4 no bacteriophage was detected in the permeate (i.e. bacteriophage rejection was complete after 4 to 5 h). This means that after 4 h of filtration, potable water would be free of any somatic phages in that size range.

During the second set of phage challenges, T1 (50 nm) was employed and as in the case of T4 phage removal was time dependent. However, during the second run there was a decrease in rejection. Initially the phage rejection was high probably due to adsorption of the bacteriophage on and within the membrane (Fane, 1996). Once the most accessible adsorption sites have been accommodated the phage was more easily transmitted through the membrane. The subsequent gradual increase was believed to be due to slow changes in permeability of the pores. An average of 2-log reduction was observed for this set of runs. The average rejection for all 3 runs ranged from 67 to 99%. This indicated an average reduction value of 90%, which was fairly good considering the size of the phages.

Concluding from the results a very good rejection of phage by the membrane could possibly be due to a well-known phenomenon, concentration polarisation to be responsible for phage aggregation and subsequent adsorption onto the membrane surface. However, concentration polarisation can be reduced by increasing the feed

flow rate inside the fibre, which in turn will increase the shear at the solution membrane interface.

### 6.3 Protozoan Challenge Tests

#### Procedure

*Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts were purchased from Labretoria (Pretoria, South Africa). The oocysts and cysts were cultured by Parasitology Research Labs, (LLC, USA) by the biological model, C57/B16 mice and Biologic model, Mongolian gerbil culture method respectively.

In the laboratory system 40 l of distilled water was placed in the 70 l feed tank and fed through the system as per normal operating conditions at two different pressures. Pressures of 80 kPa and 100 kPa were chosen as they fell within the acceptable range of less than 100 kPa.  $\sim 1,2 \times 10^6$  cysts or oocysts were injected directly into the feed line, just before the module, via the dosing port using the peristaltic pump (Watson-Marlow 101F). After 2 min, sampling began. A 10 l water sample was collected over a 3 h period. Analysis was carried out immediately.

#### *Immunofluorescence assay for the detection of oocysts*

The immunofluorescence assay was carried out using a *Crypto/Giardia*-Cel I.F. test kit that was manufactured by Cellabs Pty Ltd (Brookvale, Australia). 20  $\mu$ l of the concentrated water sample was placed on fluorescent microscope slides (Syva MicroTrak) in triplicate. The sample was allowed to completely air-dried and fixed with acetone (99%) for 5 min and air-dried. The labelled antibody reagent (25  $\mu$ l) was added onto the fixed sample and positive control slide.

The slides were incubated in a humidified chamber at 37°C for 30 min. Following incubation, the slides were washed with phosphate buffered saline (Oxoid). Slides were drained and allowed to dry in a 37°C incubator for 5 to 10 min.

A drop of mounting fluid (Cellabs Pty Ltd – Brookvale, Australia) was added to the slide, and a cover slip was placed over it. Samples were examined using a Nikon

Fluoro-phot, 78564 fluorescent microscope with a Nikon camera attached. Samples were viewed at a maximum excitation of 490 nm and emission wavelength of 530 nm, initially at X100 magnification and then at X400 magnification.

- a) Identification of presumptive oocysts and cysts. *Cryptosporidium* oocysts were identified due to bright green fluorescence of the spherical oocysts (4 to 6 µm in size) and the presence of a suture line. *Giardia* cysts were identified by bright green fluorescence of the 5 to 15 µm wide ovoid cysts.
- b) Determination of oocyst and cyst numbers in sample. Once the sample was stained with the immunofluorescent stain, it was viewed by fluorescence microscopy and systematically counted for cysts and oocysts. The following calculation was used to determine the number of protozoa per 10 l of sample.

Results are reported as oocysts-cysts/10 l.

$$N \times (V / v)$$

where:

N = number of oocysts/cysts

V = total volume of pellet (ml)

v = volume examined (ml)

## Results

In the Wiggins system the evaluation of *Giardia lamblia* cyst (8 to 12 µm) and *Cryptosporidium parvum* oocyst (4 to 6 µm) removal by membranes showed a 97% removal of oocysts at a differential pressure of 100 kPa and 99% at 80 kPa. For *Giardia* cysts at 100 kPa removal was 99% and 100% at 80 kPa. The actual numbers are shown in Table 6-4. The higher rejection of *Giardia* cysts is consistent with its bigger size. Based on the size exclusion, a 100% removal by the membrane is expected under all operating conditions, but these results indicate that some leakage does occur, and this may be due to the fact that the above experiments were conducted at the Wiggins Process Plant where the membranes were three years old. The operating conditions influence the removal efficacy of the membranes.

Table 6-4. Reduction of *C. parvum* oocysts and *G. lamblia* cysts

|                          | 100 kPa       | 80 kPa        | Initial Count    |
|--------------------------|---------------|---------------|------------------|
| <b><i>C. parvum</i></b>  | 950 oocysts/l | 312 oocysts/l | 30 000 oocysts/l |
| <b><i>G. lamblia</i></b> | 375 cysts/l   | 175 cysts/l   | 30 000cysts/l    |

The lab system the showed a 98.4% rejection at 100 kPa and 99.8% at 80 kPa. Their absolute removal by the membrane is thus expected. Further confirmation of removal was seen upon using another membrane in the laboratory-scale system. 100% rejection was noted for *Giardia lamblia* cysts and *cryptosporidium parvum* oocysts.

## 6.4 Yeast Challenge Test

### Procedure

1. The organism *Saccharomyces cerevisiae* is ideal for use in UF systems as it is non-pathogenic, has bacteria-like cultural characteristics and has generally regarded as safe (GRAS) status.
2. A spike of known concentration was prepared and inoculated in the UF system.
3. Samples were collected from the permeate line and the total number of yeasts were enumerated by serial dilutions on Malt Extract Agar incubated overnight at 37°C.

### Results

Both the laboratory-scale and Wiggins rig showed 100% rejection when spiked with  $1.4 \times 10^6$  cell/ml. This indicates that for routine monitoring yeast cells could be used instead of protozoan cells, as these require storage and are difficult to culture for spiking. Yeast cells, particularly *S. cerevisiae* are easy to grow and are readily available and are non-pathogenic.

## CHAPTER 7

### CONCLUSIONS

Standard turbidity measurements are inadequate to ensure safe potable water production and cannot detect significant numbers of microorganisms that pose a health risk. A protocol is necessary to ensure proper functioning of the UF system and reduce risks of infection due to contamination of drinking water. Membrane performance needs to be assessed routinely, especially if membrane abnormalities are suspected, or when new membranes are installed/plant is restarted after shutdown. Experiments were conducted to determine the microbiological performance of polysulphone membranes. Based on the results, a protocol for testing various parameters to assess the **membrane performance** is documented.

**Microbial removal efficiency** of membranes under routine operations show that newer membranes provide better product water than the previous “single skinned” membranes.

- a) The Wiggins membranes (which were three years old) when operating under constant pressure and in crossflow mode gave 99.9% rejection of faecal streptococci, total coliforms and faecal coliforms. Heterotrophic bacteria, however, were present.
- b) The laboratory-scale rig (model) has a single displacement pump and does not have a recycle loop, or a pre-treatment step. The module used on the lab scale consists of 20 capillaries with a path length of 0.5 m. This membrane showed a 99.93 to 99.97% rejection of heterotrophic bacteria.
- c) Five different membranes tested under crossflow and dead-end operations gave a 99.99% rejection of bacteria.

**Cleaning of membranes during routine operations** show that CIP treatment (50 mg/l) was effective in removing all coliforms, faecal streptococci and reduced heterotrophic bacteria to levels acceptable for potable water production despite the quality of the feed water.

**Membrane integrity or bubble point test** is a good indicator for abnormalities for pinholes in the module or any burst capillaries on the membrane. The modules were pressurized at 50, 100 and 120 kPa, with oxygen and observed for any changes in the pressure over a 10 min period. A decrease in the pressure or presence of bubbles would signify deformities in the membrane. Our results indicated that the membranes were not compromised nor had any pinhole defects.

**An assessment of backflushing membrane** showed that the bacterial counts were higher after backwash than before for all the different modules and membranes studied. There is a window period after backwash cycle that needs to be determined for different membranes. We have previously reported that with the single skinned membranes, a period of about 185 min is required before the permeate levels stabilized. In the three experiments performed with double-skinned membranes, it was found that the bacterial counts in the permeate after a backflush are greatest in the first 10 min, after which the levels drop and stabilize. This provides the strongest evidence that the newer membranes have superior bacterial retention capabilities.

A total of 17 backflushes were analyzed where bacterial levels were determined before and after backflush. In all these experiments except for two, there was an increase in bacterial numbers in the permeate after the backflush. It is apparent that the same pattern of increased bacterial counts in the permeate after a backflush which was observed previously with the older single-skinned membrane was also present with the newer double skinned membranes. This observation has also been observed by other researchers.

**Rapid Methods** for field use tested three test kits, i.e. CC, CL and petrifilm and compared them to conventional bacteriological tests for ease of use, simplicity and accuracy. The results showed that the CC kit satisfies the above criteria. This test appears to have potential for testing UF systems in remote locations where there is a lack of a laboratory and skilled personnel as it correlates with the conventional method of coliform detection, it is robust, and can be performed by non-microbiological personnel.

**Challenge tests** based on sizes of microorganisms showed that UF membranes, if intact, can act as absolute barriers for cysts and oocysts. However, bacteriophages which are very small, e.g. MS2, are not removed completely and the removal of different organisms are influenced by the MWCO of the membrane.

- a) **Bacterial challenge tests** (conducted on membrane 1 and 2):

**Membrane 1:** described as “pre-skinless” was spiked with  $2.9 \times 10^4$  cells/ml. Potable water was produced 3 h after operation as the fouling layer developed. This suggests that the fouling layer assist in the filtration process.

**Membrane 2:** described as E31/1713. This spike was maintained at  $3 \times 10^5$  cells/ml. A decline in flux values did not give a corresponding decline in bacterial counts and a relationship between heterotrophic counts and flux cannot be used in protocol development.

- b) The **membrane removal efficiency for bacteriophages** (T4, 80 nm; T1, 50 nm; MS2, 23 to 25 nm) suggested that the UF membrane was “leaky” to virus during the first 2 to 3 h after which rejection increased over time.
- c) The **evaluation of *Giardia lamblia* cysts** (8 to 12  $\mu\text{m}$ ) and *Cryptosporidium parvum* oocysts (4 to 6  $\mu\text{m}$ ) removal by membranes showed a 98.4% rejection at 100 kPa and 99.8% at 80 KPa. The membrane molecular cut off is 10 to 30 000 daltons and the oocysts and cysts used in the challenge test were well above this size limit. Their absolute removal by the membrane was thus expected.

A summary of these results on the three rigs (Figure 7-1) clearly show that, when there is a fault in the membrane or system, there will be a leakage of organisms which are 5 to 15  $\mu\text{m}$  in diameter.

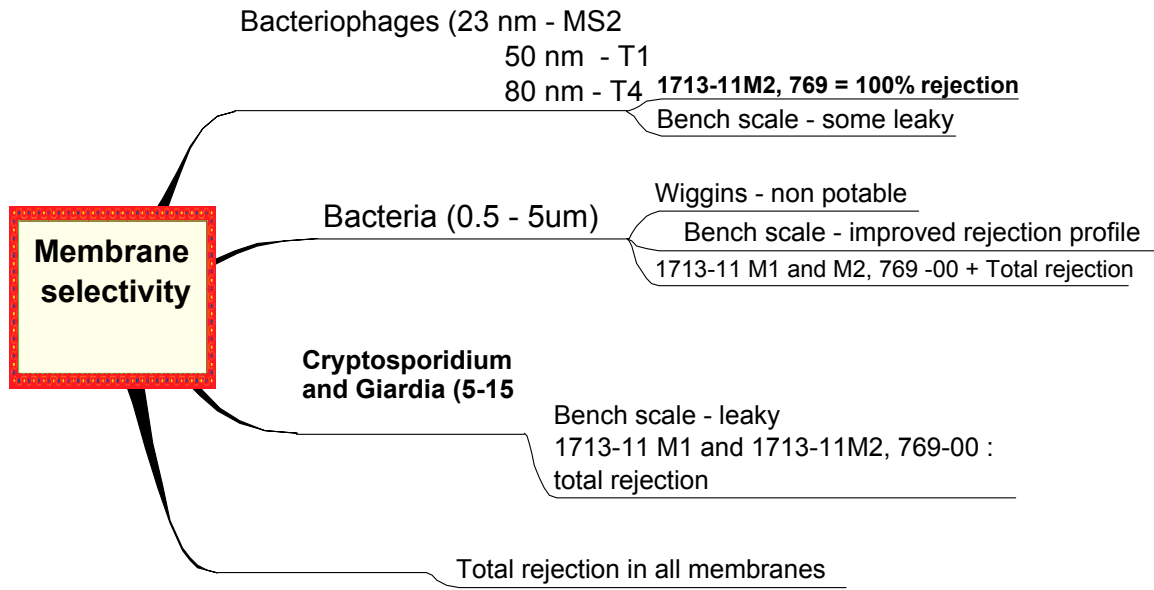


Figure 7-1. Results of membrane challenged with different size organisms

The goal of this study was i) to develop microbiological criteria to assess membrane performance; ii) to develop protocols for membrane users to ensure quality of the membrane after installation, during processing and during cleaning; and iii) to compare different microbiological tests to chemical indicator tests and find simple methods for determining water quality, and finally to recommend protocols for implementation to membrane users for potable water production. Through these goals this study enabled the development of a protocol that can be used in conjunction with turbidity to monitor microbiological performance of a membrane. These protocols can be used to ensure quality of the membrane after installation, during processing and during cleaning.

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**Guidelines for routine monitoring  
of membrane performance for  
potable water production in  
small water treatment plants**

## Structure of Guide

This protocol is divided into 3 parts:

### Part 1

|                                           |    |
|-------------------------------------------|----|
| General introduction to testing membranes | 97 |
|-------------------------------------------|----|

### Part 2

|                                                                                              |     |
|----------------------------------------------------------------------------------------------|-----|
| Protocol for characterisation of new membranes or system calibration for start up operations | 101 |
|----------------------------------------------------------------------------------------------|-----|

### Part 3

|                                     |     |
|-------------------------------------|-----|
| Protocol for daily system operation | 111 |
|-------------------------------------|-----|

### Part 4

|                                                               |     |
|---------------------------------------------------------------|-----|
| Recommended microbial challenge tests for membrane developers | 104 |
|---------------------------------------------------------------|-----|

### Abbreviations

|                            |     |
|----------------------------|-----|
| Faecal coliform count      | FCC |
| Faecal streptococcal count | FSC |
| Total coliform count       | TCC |
| Ultrafiltration            | UF  |

## Part 1

### **GENERAL INTRODUCTION TO TESTING MEMBRANES**

The protocols described in this manual are intended for testing the performance of ultrafiltration (UF) and microfiltration membranes, used in the production of potable water, at various stages of the membrane lifecycle. This manual focuses on physical and microbial tests for evaluating membrane performance.

The physical tests include turbidity measurements, which is used to routinely monitor membrane performance, calibrating the initial water flux to determine maximum flux values of the membrane either during normal operation or at various transmembrane pressures and the bubble point test to ascertain membrane integrity. Microbiological test to monitor membrane performance include the plate count technique for heterotrophic plate counts and the membrane filtration technique for coliform counts. Detailed methodologies for these methods are in the WRC report (obtainable from the WRC offices).

There are numerous methods to estimate membrane performance. Of these tests, one that is used routinely is the measurement of the **turbidity values** of the permeate.

The advantages of this test are that:

- a) it is simple to perform;
- b) it is a rapid test with results available within minutes;
- c) it is an inexpensive test, assuming a turbidity meter is available; and
- d) an increase in turbidity correlates well with an increase in microbial numbers.

While these factors are desirable for routine/daily testing, membrane users need to be cognisant of the drawback of turbidity measurements in assessing membrane performance.

The disadvantages include:

- a) a lack of specificity for estimating microorganisms, since any particulate will contribute towards turbidity;
- b) poor sensitivity in detecting low numbers of microorganisms or a slight increase in microbial levels, that would nevertheless pose a serious health risk; and
- c) not a quantitative method as microbial counts cannot be estimated.

It is therefore imperative to supplement turbidity tests with regular microbiological testing of the permeate to determine whether the water conforms to the legislated specifications.

In this manual two additional non-microbiological tests have been included, since they are important in calibrating for microbiological tests. These two tests are preliminary tests that should, preferably, be performed prior to microbiological rejection tests. The first of these tests, the **Pure water flux determination**, is carried out on new or “unfouled” membranes to determine maximum flux values for the membrane at various transmembrane pressures. With subsequent potable water production and deposition of a fouling layer, this initial value decreases gradually. Comparison of flux values with the initial value provides an indication of the level of fouling of the membranes.

The second non-microbiological test, the **Bubble-point test**, is used specifically to confirm the presence of pinholes, burst capillaries or otherwise compromised membranes. This test can be used on new membranes but is more often used in testing membranes suspected of being compromised. Compromised membrane integrity can be suspected after high turbidity values, bacterial counts or flux values of the permeate. After individual burst capillaries are identified and plugged, membrane integrity can be restored.

The microbiological tests in this manual are divided into two broad categories depending on when they are used. The first category describes testing membrane performance using bacteria present in the feed and UF system. Consequently, these tests are conducted while the system is operating and does not require interruption of the filtration process. These generally use the **plate count technique** for enumerating the total number of heterotrophic bacteria and the **filtration technique** for enumerating coliforms. In essence, these tests can constitute a quality control procedure to monitor membrane performance in rejecting the resident microbial flora.

The second category of microbial tests describes microbial challenge tests where membranes are challenged with known concentrations of a certain microbial species. To obtain an accurate estimate of the membrane's performance, these tests should be carried out with little or no competing microorganisms. Hence, they are most suited to laboratory-scale or pilot-scale testing of new membranes. With new membranes, the test yields a "rejection profile" of differently sized microorganisms. These could include challenging the membrane with different sizes of bacteriophages, bacteria, protozoa and/or yeast, depending on the membranes pore size that is being tested. When a membrane is suspected of being compromised, the test is repeated and the rejection profiles are compared.

## Suggested Tests

### **Turbidity values:**

Routine test for membranes in use in operating system  
(Increased turbidity correlates with an increase in microbial numbers)

### **Non-microbiological tests:**

- Calibration of Initial Water Flux  
(To determine maximum flux values for a new or unfouled membrane at various trans-membrane pressures)
- Bubble point test  
(When compromised membranes integrity i.e. the presence of pinholes, burst capillaries, is suspected)

### **Microbiological tests:**

To monitor membrane performance

To determine the rejection profile of a membrane for bacteria present in feed (resident microbial flora):

- Plate count technique for heterotrophic plate counts
- Membrane filtration technique for coliform counts

To determine the rejection profile of a new membrane:  
(Known concentration of a certain microbial species is used to challenge membrane)

- Bacteriophage challenge tests
- Somatic phages
- F-RNA coliphage test
- Protozoan challenge test
- Yeast challenge test

## **Part 2**

### **PROTOCOL FOR CHARACTERISATION OF NEW MEMBRANES OR SYSTEM CALIBRATION FOR START UP OPERATIONS**

#### **Calibration of Initial Water Flux**

Pure water flux determines the flux through an "unfouled" membrane thereby representing maximum flux values and increased production capacity. As the membrane fouls, flux decreases according. Therefore, subsequent water flux determinations can be used to monitor membrane fouling.

A pure water flux is conducted with every new membrane in order to establish optimum flux values at differing transmembrane pressures.

**Procedure:**

- Fill feed tank with sterile distilled water.
- Flush each membrane with distilled water for approximately 3 hours.
- Operate UF system.

**Differential pressures:**

- Select about 3 differential pressures settings (not exceeding membrane burst pressure).
- At each of the suitable differential pressure settings collect permeate in graduated cylinder for a set period of time.

**Calculations:**

- Calculate the volumetric flow rate:

$$\frac{\text{Permeate volume}}{\text{Time}}$$

- Calculate the surface area of membrane:

$$A = \pi d l N$$

Where: d = diameter of membrane (mm)

l = length in meters (m)

N = no. of capillaries

- Calculate the initial water flux:

$$\frac{\text{Volumetric flow rate}}{\text{Membrane area}}$$

## Bubble Point Test

To reduce the risk of passage of microorganisms into the filtrate, it is critical to assure that the membranes are intact and continue to provide a barrier between the feed water and the permeate or product water. Breakage of fibres or loss of system integrity can lead to passage of pathogens to the receiving water. If this is suspected, Bubble Point Testing is a quick, direct measurement of membrane integrity. However, the membrane module has to be removed off-line to conduct the test.

### Procedure:

#### Setting up:

- Remove the membrane module from the UF rig.
- Connect the permeate line via silicone tubing, if possible, to an oxygen cylinder, which is used to pressurize the shell side of the module.

#### Suspend module:

- Vertically suspend one end of the membrane module in water.
- Seal the opposite end of the module by constricting the attached tubing with a clamp.

#### Vary oxygen pressures:

- Allow oxygen into the shell side of the module via the permeate line at varying pressures.
- At each selected pressure, monitor the pressure for 10 min.

#### Observation:

- Bubbles and decrease in pressure indicate a compromised membrane.

### Recommendation

Individual capillaries that are compromised need to be identified and sealed/plugged to restore membrane integrity. The test needs to be repeated to verify this.

### **Part 3**

## **PROTOCOL FOR DAILY SYSTEM OPERATION**

### **Microbiological Tests to Monitor Membrane Performance**

These tests enumerate microorganisms occurring in the UF system during operation to provide an accurate determination of membrane performance in producing water of an acceptable quality. These tests can be performed for the following reasons:

- a) to routinely test permeate quality at selected intervals;
- b) to randomly test water at various ports in the UF system;
- c) to assess efficiency of chemical cleaning before and after a clean-in-place regimen;
- d) to determine the optimum backwash length by monitoring the reject at intervals during a backwash; and
- e) to assess permeate quality before and after a backwash and for an short period after a backwash to determine whether a filter-to-waste regimen is necessary immediately after a backwash.

#### **Sampling methodology:**

|                                                                                                                                                                                                                                                                                           |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <u>Sampling ports:</u>                                                                                                                                                                                                                                                                    |
| Those are identified based on the type of test employed.                                                                                                                                                                                                                                  |
| <u>Sampling:</u>                                                                                                                                                                                                                                                                          |
| <ul style="list-style-type: none"><li>• Open the sample port and allow void volumes to drain.</li><li>• Collect the sample in a sterile container.</li><li>• Place the sample on ice and transport to the laboratory.</li><li>• Close the container immediately after sampling.</li></ul> |
| <u>Bacteriological analyses:</u>                                                                                                                                                                                                                                                          |
| Conduct analyses as soon as possible after receipt of the samples.                                                                                                                                                                                                                        |

## Plate Count Technique for Heterotrophic Plate Counts

The plate count technique estimates the total number of viable heterotrophic bacteria in a water sample.

### Sample dilution:

- Dilute the water sample appropriately using 10-fold dilutions depending on the expected bacterial load.

A dilution greater than  $10^{-9}$  is unnecessary. A physiological saline solution (0.85% NaCl) or a similar isotonic solution is recommended for maintaining cell viability.

### Plate out:

- Thoroughly mix 0.1 ml or 1 ml volumes of the dilutions with molten plate count agar and pour into sterile petri dishes.

The dilutions chosen to plate out again, depends on the expected bacterial load.

Replicates of this plating procedure are recommended for accuracy of the bacterial counts.

### Incubation:

- After the agar has hardened, invert the plates and incubate for 48 hours at  $37^{\circ}\text{C}$ .

### Calculation:

- Count colonies arising from single cells using a colony counter.  
Only plates having well-spaced colonies and more than 30 colonies are enumerated.
- The bacterial count in the original sample is calculated by multiplying the average colony count on the plates by the total dilution factor.

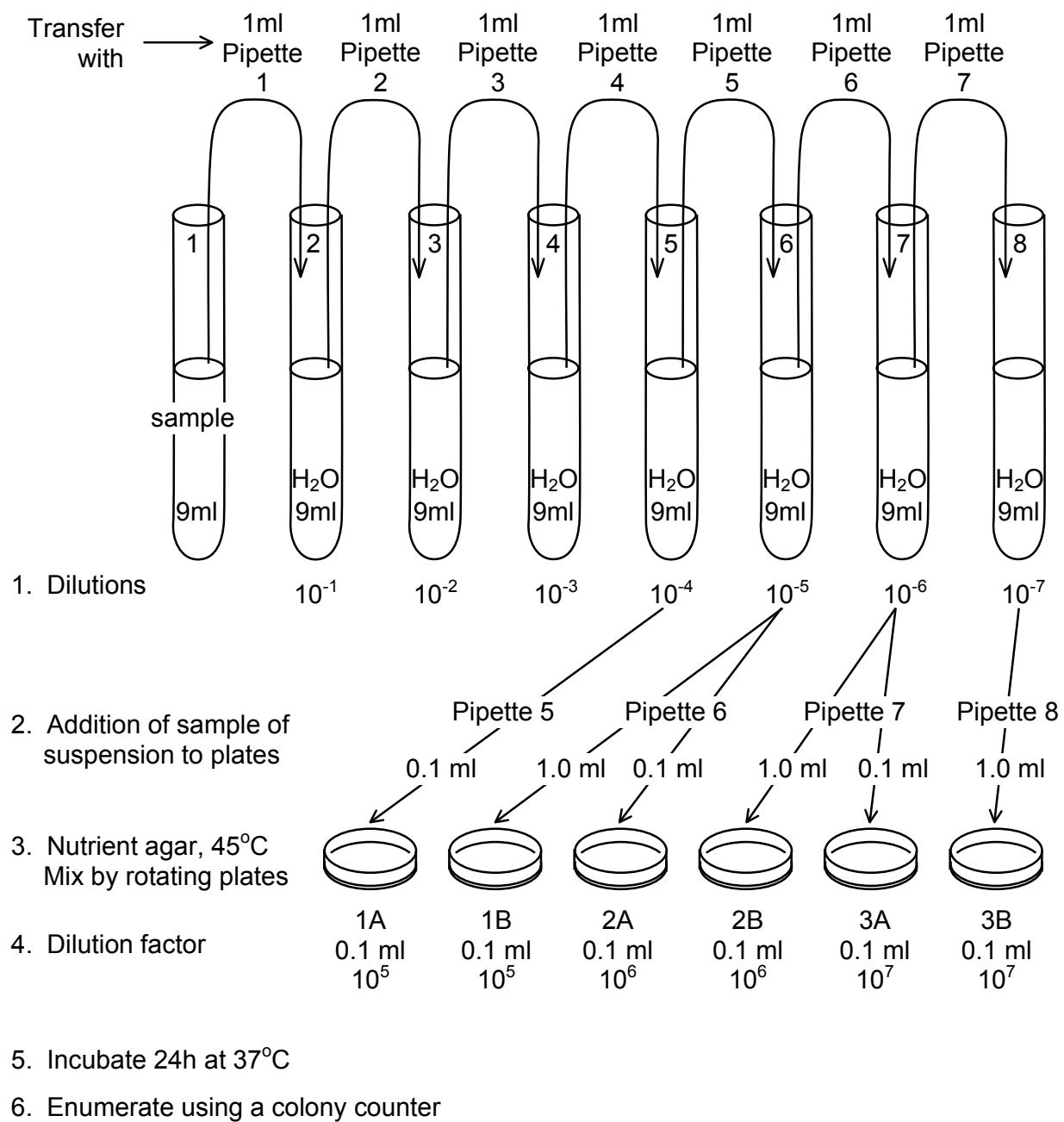
### Recommendation

The minimum allowable heterotrophic plate count per is 100 counts per ml for 95% of the samples, and a maximum of 1 000 counts per ml for 4% of the sample and a count of 10 000 per ml for 1% of the sample. (SABS Specification 241:2002).

## **Membrane Filtration Technique**

In the membrane filtration technique, a measured volume of water is filtered, under vacuum, through a 0.45 µm membrane filter. Bacteria are retained on the surface of the membrane, which is placed on a suitable selective medium in a sterile petri dish and incubated at an appropriate temperature. If coliforms, faecal coliforms or faecal streptococci are present in the water, sample, characteristic colonies form that can be counted directly.

Water samples are diluted prior to the membrane filtration if high bacterial counts are expected. Alternatively, if low counts are expected, large volumes can be filtered to obtain an accurate estimate of the bacterial load.



**Procedure:** (for schematic presentation, see Figure 1)

Label dilution sample tubes:

Label four 90 ml water blanks with the source of the water sample and intended dilution ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$ ).

Perform dilutions:

Aseptically perform a 10-fold serial dilution of the assigned undiluted water sample using the four 90 ml water blanks to effect dilutions:  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$ .

Label Petri dishes:

Arrange 15 Petri dishes containing appropriate selective media into three sets of five plates. Label each set as follows:

1. For total coliform count (TCC):  
Undiluted,  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  &  $10^{-4}$ .
2. For faecal coliform count (FCC):  
Dilutions as described in Step 1.
3. For faecal streptococcal count (FSC):  
Dilutions as described in Step 1.

This plating procedure is duplicated and the average count per plate for each dilution is reported.

Aseptically assemble the membrane filter unit:

- Unwrap and insert the sintered glass filter base into the neck of a one-litre side-arm suction flask.
- With sterile forceps, place a sterile membrane filter disc, grid side up, on the sintered glass platform.
- Unwrap and carefully place the funnel section of the apparatus on top of the filter disc. Using the filter clamp, secure the funnel to the filter base.
- Attach a rubber hose from the side-arm on the vacuum flask to a vacuum source.

Filtration process for highest dilutions:

- Starting with the highest sample dilution ( $10^{-4}$ ), pipette 20 ml of the dilution into the funnel.
- Start the vacuum.
- When the entire sample has been filtered, wash the inner surface of the funnel with 20 ml of sterile water.
- Disconnect the vacuum, unclamp the filter assembly, and with sterile forceps remove the membrane filter.
- Place it on the agar medium in the Petri dish labelled TCC;  $10^{-4}$ .
- Aseptically place a new membrane on the platform, reassemble the filtration apparatus, and repeat 'Filtration process' twice.
- Add these filter discs to the Petri dishes labelled FCC:  $10^{-4}$  and FSC  $10^{-4}$ .

Filtration process for other dilutions:

Repeat 'Filtration Procedure', using 20 ml of the  $10^{-3}$ ,  $10^{-2}$ , and  $10^{-1}$  dilutions and the undiluted samples for each of the three sets (TCC; FCC & FSC).

Incubation:

Incubate the plates in an inverted position as follows:

- TCC and FSC plates for 24 h at  $37^{\circ}\text{C}$ .
- FCC plates at  $44^{\circ}\text{C}$  for 24 h.

Calculation:

Count the number of colonies, multiply by the dilution factor and calculate the number of bacteria per 100 ml of permeate

### **Recommendation**

The number of bacteria in the permeate should not exceed the following limits:

#### Heterotrophic Plate Count

Minimum allowable counts per ml is a 100 counts per ml for 95% of the samples, and a maximum of 1 000 counts per ml for 4% of the samples and a count of 10 000 per ml for 1% of the sample.

#### Total coliforms

Minimum allowable counts per 100 ml is 0 counts per 100 ml for 95% of the samples, and a maximum of 10 counts per 100 ml for 4% of the samples and a 100 counts per 100 ml for 1% of the sample.

#### Faecal coliforms

Minimum allowable counts per 100 ml is 0 counts per 100 ml for 95% of the samples, and a maximum of 1 counts per 100 ml for 4% of the samples and a 10 counts per 100 ml for 1% of the sample

#### *E. coli*

Minimum allowable counts per 100 ml is 0 counts per 100 ml for 95% of the samples, and a maximum of 0 counts per 100 ml for 4% of the samples and 1 count per 100 ml for 1% of the sample.

## **Part 4**

### **RECOMMENDED MICROBIAL CHALLENGE TESTS FOR MEMBRANE DEVELOPERS**

Microbial challenge tests are performed using specific microorganisms of known size to determine the ability of the membrane to reject these particles. Microbial challenge tests should be carried out on a plant-scale UF system. However, the membrane user needs to consider the following factors:

- a) testing is usually only necessary if the number of microorganisms in the UF system is extremely low;
- b) the number of microorganisms in the re-circulation loop is usually far greater than the spike in the feed tank and represents a greater challenge to the membranes;
- c) test microorganisms must be carefully chosen as they may enter the permeate and will persist in the UF system for extended periods due to re-circulation; and
- d) a post membrane step such as chlorination may be required to disinfect the permeate.

These challenge tests are best suited to laboratory or pilot-scale UF systems. If used in larger UF systems, one has to consider the large inoculation volumes required, cost of the test organisms if they are being purchased and persistence of the microorganisms in the potable water UF systems. Microorganisms that can be used in these challenge tests include:

- a) bacteriophages
- b) bacteria
- c) protozoan (oo)cysts
- d) yeasts

The following challenge tests for the different microorganisms vary only in the preparation of the microorganisms before inoculation into the UF system and the different methods used to enumerate these microorganisms. They essentially behave as particles in the UF system, albeit living (except for the protozoan cysts). After each microorganism is inoculated and permeate

samples analysed after filtration, log reduction values can be determined for each new membrane.

### **Bacteriophage Challenge Tests**

Both somatic coliphages and F-specific RNA phages can be used as test particles. F-specific RNA phages are generally smaller than somatic coliphages but assays for somatic coliphages are easier to perform.

### **Somatic phages**

Once inoculated in the UF system, the somatic phages are detected using the Double Agar Layer Plaque Assay method (Grabow, 1997).

|                                                                                                                                                                                                                                    |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <u>Add sample:</u>                                                                                                                                                                                                                 |
| To test tubes containing molten top agar, add calcium chloride, water sample and the host <i>E. coli</i> culture.                                                                                                                  |
| <u>Mixing:</u>                                                                                                                                                                                                                     |
| Mix the top agar and without delay pour over the bottom agar layer of phage agar plate. The top layer is distributed evenly and allowed to solidify on a perfectly horizontal, cool surface. This procedure is done in triplicate. |
| <u>Incubation:</u>                                                                                                                                                                                                                 |
| The inverted plates are incubated overnight at 35 to 37°C.<br>Plaques are visible as circular disc-like clearings in the lawn of bacteria.                                                                                         |
| <u>Calculation:</u>                                                                                                                                                                                                                |
| The total number plaques observed on all three plates is averaged and multiplied by the dilution factor to yield results in PFU.10 ml <sup>-1</sup> .                                                                              |

## F-RNA coliphage analysis

| F-RNA coliphage analysis                                                                                                                                                                     |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Once inoculated in the UF system, F-RNA phages are enumerated using the Double Layer Plaque Assay Method for F-RNA (male specific) coliphages (Grabow, 1997).                                |
| <u>Add sample:</u><br>To test tubes containing molten top agar, add calcium glucose, the water sample and the <i>Salmonella typhimurium</i> host culture.                                    |
| <u>Mixing:</u><br>Mix the top agar mixture and poured with minimum delay onto bottom agar in phage agar plates and distributed evenly.<br>Allowed to solidify on a horizontal, cool surface. |
| <u>Incubation:</u><br>Incubate overnight at 35 to 37°C.<br>Unlike plaques formed by somatic coliphages, the plaques formed by F-RNA coliphages are smaller and more opaque.                  |
| <u>Calculation:</u><br>The total number plaques observed on all three plates is averaged and multiplied by the dilution factor to yield results in PFU/10 ml <sup>-1</sup> .                 |

| Bacterial challenge test                                                                                                                                                                                                                     |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <u>Prepare spike:</u><br>Prepare a bacterial spike ranging from 10 <sup>3</sup> to 10 <sup>6</sup> cells/ml.                                                                                                                                 |
| <u>Inoculation and sample collection:</u><br>Inoculate into UF system.<br>Collect samples at the various sampling points at specific intervals.                                                                                              |
| <u>Calculation:</u><br>Since only a single bacterial species is used, membrane filtration and the use of selective media is not necessary. Bacteria in the samples can therefore be enumerated using the HPC method as described previously. |

### Protozoan Challenge Test

#### Test organisms:

Test organisms used in these challenge tests are *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts. They can be purchased from suppliers at a fixed cyst concentration.

#### Spike and inoculation:

After a spike of known concentration is prepared, it is inoculated into the UF system.

#### Collect samples:

Collect samples at the various sampling points at specific intervals.

#### Calculation:

Cysts are concentrated by precipitation and enumerated using immunofluorescence microscopy after preparing samples using a *Crypto/Giardia*-Cell IF test kit.

### Yeast challenge test

#### Test organism:

The organism *Saccharomyces cerevisiae* is ideal for use in UF systems as it is non-pathogenic, has bacteria-like cultural characteristics and has GRAS status.

#### Spike and inoculation:

After a spike of known concentration is prepared it is inoculated in the UF system.

#### Sample collection:

Collect samples at the various sampling points at specific intervals.

#### Calculation:

Yeasts can be enumerated by plating on an agar medium or by counting diluted samples in a microscope counting chamber.

**Recommendation**

When membranes are challenged with known concentrations of a certain microbial species the test yields a “rejection profile” of differently sized microorganisms depending on the different sizes of bacteriophages, bacteria, protozoa and/or yeast used, and on the membranes pore size that is being tested. When a membrane is suspected of being compromised, the test is repeated and the rejection profiles are compared.

## **APPENDIX 1**

### **OUTPUTS THAT HAVE EMANATED FROM THIS RESEARCH WORK**

#### **Conference Proceedings**

Hoosman Z, Govinden R, Permaul K, and Odhav B. (2001). Analysis of backflushing of ultrafiltration membranes during potable water production. Proceedings of the 4th WISA-MTD Symposium, p. 43-45.

Hoosman Z, Govinden R, Odhav B, and Permaul K. (2000). The effect of hydraulic defouling on ultrafiltration membranes. Proceedings of the 13th Annual Symposium of the SASM (Natal Branch).

Reddy P, Govinden R, Odhav B, and Permaul K. (2000). Detection of bacteria in ultrafiltration permeates. Proceedings of the 13th Annual Symposium of the SASM (Natal Branch).

Singh N, Permaul K, Singh S, and Odhav B. (1999). Bacterial removal by polysulphone ultrafiltration membranes. Proceedings of the 3rd WISA-MTD Symposium.

Singh N, Singh S, Permaul K, and Odhav B. (1999). Potable water production by capillary ultrafiltration. 15 October, ML Sultan Technikon, Durban. Proc. 12th Annual Symposium of the South African Society for Microbiology (Natal Branch), p. 11.

Leku T, Singh S, Permaul K, and Odhav B. (1999). Evaluation of rapid and simple techniques for field testing of water quality. 15 October, ML Sultan Technikon, Durban. Proc. 12th Annual Symposium of the South African Society for Microbiology (Natal Branch), p. 11.

Persadh N, Rathbone PA, and Odhav B. (1999). Assessment of coliphages as an indicator of fecal pollution in water. 15 October, ML Sultan Technikon, Durban. Proc. 12th Annual Symposium of the South African Society for Microbiology (Natal Branch), p.1.

Mzinyane M, and Odhav B. (1998). A microbiological and chemical assessment of the causeway canal in Fynland, Durban. 5 - 8 July, Durban. Proc. 10th Biennial Congress of the South African Society of Microbiology, p. 48.

Pienaar W, Karodia IE, and Odhav B. (1997). An investigation on the bacteriological quality of the water in the Durban Bay. 15-17 October, Durban. Proc. Joint Kwa-Zulu Natal Biochemistry and Microbiology Symposium, p. 33.

Mzinyane M, Karodia IE, and Odhav B. (1997). The occurrence and detection of waterborne bacterial pathogens and chemical pollutants at causeway canal. 15-17 October, Durban. Proc. Joint Kwa-Zulu Natal Biochemistry and Microbiology Symposium, p. 30.

***Summary of students trained as part of this programme***

- 4 B Tech students (1999)
- 2 B Tech (2000)
- 1 M Tech (2000)
- 2 M Tech (2001)

Hoosman Z, Govinden R, Permaul K, Odhav B Proceedings of the 4<sup>th</sup> WISA-MTD Symposium, 2001.

Analysis of backflushing of ultrafiltration membranes during potable water production.

Hoosman Z, Govinden R, Permaul K, Odhav B. Proceedings of the 13<sup>th</sup> Annual Symposium of SASM, 2000

The effect of hydraulic defouling on ultrafiltration membranes

Reddy P, Govinden R, Permaul K, Odhav B. Proceedings of the 13<sup>th</sup> Annual Symposium of SASM, 2000

Detection of bacteria in ultrafiltration permeates

Singh N, Permaul K, Odhav B. Proceedings of 3<sup>rd</sup> WISA-MTD Symposium, 1999

Bacterial removal by polysulphone ultrafiltration membranes

Leku T, Singh S, Permaul K, Odhav B. 12 Annual Symposium of SASM, 1999

Evaluation of rapid and simple techniques for field testing of water quality

Persadh N, Rathbone PA, Odhav B. 12 Annual Symposium of SASM, 1999

Assessment of coliphage as indicators of faecal pollution

Mzinyane M, Odhav B. 10 Biennial Congress of SASM, 1999

A microbiological and chemical assessment of the causewayyy canal in Fynland.

Pienaar P, Karodia I, Odhav B. Joint Kwa-Zulu Biochemistry and Microbiology Symposium, 1999

An investigation on the bacteriological quality of the Durban Bay

**B Tech Students Graduated:**

- Padayachee 2000/B Tech
- Singh 2000/B Tech
- Pienaar 2000/B Tech
- Leku 2001/B Tech
- Moodley 2001/B Tech
- Reddy 2001/B Tech
- Hoosman 2001/B Tech

**Workshop Attendance:**

**Workshops on microbiological aspects of membranes**

Microbiological tests for water (6)

Chemical engineering and microbiological principles exchanged

**Workshop on chemical aspects of membranes**

Engineering aspects of membrane technology (8)

Chemical engineering and microbiological principles exchanged

**WRC Meeting (2000 & 2001)**

WRC Meeting of microbiological aspects of membranes (15 & 17)

WRC Steering committee meeting to discuss progress and future of projects

## **APPENDIX 2**

### **1. Tryptone yeast glucose broth (TYGB)**

TYGB was prepared by adding 10 g trypticase peptone, 1 g of yeast extract agar and 8 g of NaCl to 1 L of distilled water. After sterilisation by autoclaving, 10 ml sterile calcium glucose solution (3%  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 10% glucose) was added aseptically to the medium. 50 ml quantities of media was dispensed into sterile 250 ml bottles and stored at 4°C in the dark.

### **2. Tryptone yeast extract agar (bottom agar)**

TYGA was prepared by adding 14 g of Bacto agar, 13 g of tryptone, 8 g of NaCl and 1.5 g of glucose to 1 l of distilled water. After sterilisation by autoclaving, 10 ml of calcium glucose was added to the solution and poured into 90 mm petri dishes. Plates were stored at 4°C.

### **3. Semi-solid yeast extract glucose agar (ssTYGA) (top agar)**

SsTYGA was prepared as for basal medium, but only 6.5 g of agar was added. Nalidixic acid was added to a final concentration of 25 µg/ml. 2.5 ml volumes were distributed into sterile test tubes and stored at 4°C.

## **MEDIA FOR SOMATIC PLAQUE ASSAYS**

### **1. Nutrient broth**

16 g of nutrient broth was added to 1 l of distilled water. After sterilisation by autoclaving, 50 ml quantities were distributed into sterile 250 ml bottles and stored at 4°C.

### **2. Phage bottom agar**

Phage bottom agar was prepared by adding 14 g of Bacto agar, 13 g of tryptone, 8 g of NaCl and 1.5 g of glucose to 1 l of distilled water. The ingredients were dissolved by heating and then autoclaved. Media was poured gently into 90 mm petri dishes and stored at 4°C.

### **3. Phage top agar**

Phage top agar was prepared as for top agar but only 8 g of agar was added. 5 g of  $\text{Na}_2\text{CO}_3$  and 1 g of  $\text{MgCl}_2$  was added to 1 l of distilled water and sterilised by autoclaving. Media was poured into 90 mm petri dishes and stored at 4°C.

### **GROWTH AND MAINTENANCE OF *escherichia coli* (strain C, B)**

A lyophilised host culture was rehydrated in 5 ml of nutrient broth using a Pasteur pipette. The suspension was transferred to 50 ml of nutrient broth and incubated for 18 h at 37°C, while shaking at 100 rpm. After addition of 10 ml of glycerol and thorough mixing, 1.5 ml aliquots were dispensed into plastic vials and stored at -70°C. These vials were kept as stock cultures for preparation of working cultures.

Working cultures were prepared by thawing one vial of stock culture at RT. A loopful of this culture was streaked onto a MacConkey agar plate to obtain single colonies. Plates were incubated at 37°C for 24 h. Three to five lactose positive colonies were selected and inoculated into 50 ml of nutrient broth. The flask was inoculated for 5 h at 37°C while shaking at 100 rpm. After addition of 10 ml of glycerol and thorough mixing, 1.2 ml aliquots were dispensed into plastic vials and stored at -70°C. These vials were kept for preparation on inocula for plaque assays.

Inocula for plaque assays were prepared as follows: one vial of working culture was thawed at RT and 0.5 ml was added to 50 ml of nutrient broth in conical flask. The flask was incubated at 37°C while shaking at 100 rpm/min for up to 3 h. At a turbidity corresponding to a cell density of approximately  $10^8$  cfu/ml, the inoculum culture was taken from the incubator and quickly cooled on ice. The host strain was used within 2 h.

### **Preparation of Phage Stock Cultures**

Phages were propagated by the plate and broth methods (Grabow *et al.*, 1998). Briefly, phages were added to the host culture near the end of the exponential phase to ensure that almost every cell is simultaneously infected. The suspensions were centrifuged at  $3000 \times g$  for 20 min after an overnight incubation. The supernatant was removed and chloroform was added for cell lysis. Serial dilutions were carried out to determine the titre of the phage suspension.

For further amplification, an appropriate dilution of the phage suspension was inoculated with a culture of bacterial host into tubes containing phage top agar and poured onto phage bottom agar plates. Following an 18 h incubation step, top agar was homogenised by vigorous shaking and then centrifuged at 3000 x *g* for 20 min. After centrifugation, the supernatant containing the phages was removed and chloroform (10% v/v) was added. The lysates were stored at 4°C.

### **Growth and maintenance of *S. Typhimurium* WG49**

A lyophilised culture was rehydrated in 5 ml of growth medium (TYGB) by using a Pasteur pipette; the suspension was transferred to 50 ml of TYGB and incubated for 18 h at 37°C, while shaking at 100 rpm/min after addition of 10 ml of glycerol and thorough mixing, 1.5 ml aliquots were dispensed into vials and stored at -70°C. These vials were kept as stock culture for preparation of working cultures.

Working cultures were prepared by thawing one vial of stock culture at RT. A loopful of this was streaked onto a plate of McConkey agar to obtain single colonies. Plates were incubated at 37°C for 18 h. Three to five lactose positive colonies were selected and inoculated into 50 ml of TYGB. The flask was inoculated for 5 h at 37°C while shaking at 100 rpm. After addition of 10 ml of glycerol and thorough mixing, 1.2 ml aliquots were dispensed into plastic vials and stored at -70°C. These vials were kept for preparation of inoculate for plaque assays. Inocula for plaque assays were prepared as follows: A stock culture was thawed at RT and inoculated on McConkey agar plates. Plates were incubated at 37°C for up to 3 h. At a turbidity corresponding to a cell density of approximately 10<sup>8</sup> cfu/ml, the inoculum culture was taken from the incubator and quickly cooled on ice. The host strain was used within 2 h.

## **2. Concentration of oocysts and cysts in water sample**

Concentration of oocysts and cysts was carried out according to the procedure obtained from Umgeni Water, Pietermaritzburg (C. JarmeySwan, personal communication).

The 10 l sample was collected in a flat-bottomed plastic bucket. 100 ml of 1 M calcium chloride and 100 ml 1 M sodium bicarbonate were added to the sample and

mixed well. The pH was adjusted to 10 using 10 M sodium hydroxide solution. The sample was allowed to flocculate overnight. The supernatant was removed using vacuum aspiration. The residue was dissolved using 200 ml of 10% sulphamic acid and shaking vigorously for 15 s. The pH was then adjusted to 6 using 10 M NaOH. The liquid was decanted into 50 ml centrifuge tubes and the bucket was rinsed with 200 ml 0.1% Tween 80 and the fluids added to the centrifuge tubes. The tubes were centrifuged at 3000 x *g* for 10 min. The supernatant were removed, leaving a residue of 20 ml in total. The pellets were resuspended and transferred to a 50 ml centrifuge tube. The tube was centrifuged at 3000 x *g* for 10 min and the supernatant removed leaving a 5 ml residue. The pellet was resuspended and analysis by immunofluorescence assay was carried out immediately.