THE IMPACT OF DISINFECTION PROCESSES ON BIOFILM FORMATION IN POTABLE WATER DISTRIBUTION SYSTEMS

by

MAGGY NDOMBO BENTEKE MOMBA

SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE

DOCTOR OF PHILOSOPHY

DEPARTMENT: MICROBIOLOGY AND PLANT PATHOLOGY

in the

FACULTY OF BIOLOGY AND AGRICULTURAL SCIENCES

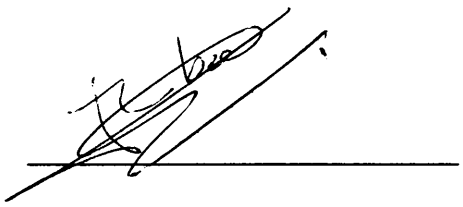
UNIVERSITY OF PRETORIA

SOUTH AFRICA

1997

DECLARATION

I, the undersigned, certify the thesis hereby submitted to the University of Pretoria for the degree of Ph.D and the study contained herein is my own original work and has not previously been submitted at another University for any degree.

Signature: 

Date: _____

THE IMPACT OF DISINFECTION PROCESSES ON BIOFILM FORMATION IN POTABLE WATER DISTRIBUTION SYSTEMS

by

MAGGY NDOMBO BENTEKE MOMBA

PROMOTOR: PROF T.E. CLOETE

CO-PROMOTOR: Dr R. KFIR

DEPARTMENT OF MICROBIOLOGY AND PLANT PATHOLOGY

DEGREE: Ph.D (Microbiology)

SUMMARY

In this study, surface and ground water were used to evaluate the impact of disinfection processes (chlorination, chloramination, ozonation, UV irradiation and hydrogen peroxide) on bacterial regrowth and biofilm formation in potable water distribution systems using laboratory-scale units. Disinfection was carried out using disinfectant concentrations which were as close as possible to those used in practise. The parameters that were used to evaluate the bactericidal effectiveness of each disinfectant were coliform bacteria, heterotrophic plate count and total bacteria. Membrane filter and standard spread plate procedures were used to enumerate coliform and heterotrophic plate count bacteria respectively. The epifluorescence direct count involving DAPI was used to enumerate total bacteria. The scanning electron microscopy

technique was used to visualize biofilm formation on stainless steel and cement coupons.

The microbial disinfection efficacy of all disinfectants was found to be equal in the elimination of coliform bacteria in surface water although recovery of coliforms occurred 24 h after disinfection in all treated water systems with the exception of chloraminated water systems. For the groundwater, all disinfectants were found to remove coliform bacteria within the first hours of disinfection with the exception of hydrogen peroxide. Complete removal of coliform bacteria by hydrogen peroxide occurred only 48 h after disinfection.

In surface water systems, more than 99 % (average kill percentage) of heterotrophic bacteria were removed by all the disinfection processes. Chloramination and hydrogen peroxide disinfection, however, resulted in a higher effective disinfection (average 99.99 %) although a longer period of time (between 24 and 72 h for chloramination and 72 h for hydrogen peroxide) was required to achieve this kill percentage. In groundwater systems, more than 99.90 % (average kill percentage) heterotrophic bacteria were removed by all the disinfectants with the exception of hydrogen peroxide (99.88 % average kill percentage). However, ozone was highly effective within the first 2 h as shown by the average kill percentage of 99.999 % of heterotrophic bacteria. These results led to the conclusion that the microbial disinfection efficacy was greater when using groundwater than when using surface water.

The phenomenon of bacterial regrowth was linked to the absence of concentrations of disinfectant residuals. In both water sources, bacterial regrowth occurred earlier in chlorinated, ozonated and UV treated water than in chloraminated and hydrogen peroxide treated water. Significantly higher heterotrophic bacteria counts were noted in chlorine, ozone and UV treated waters than either in chloramine or hydrogen peroxide treated waters. The greater persistence of monochloramine and hydrogen peroxide residuals was found to inhibit bacterial regrowth in both test waters. Chloramination of groundwater resulted in a lower regrowth rate (average

for source water 1 and 2: 0.057 h^{-1}, generation time 13.87 h^{-1}) than surface water (average for source water 1 and 2 -0.085 h^{-1}, generation time 11.55 h). Disinfection of groundwater with hydrogen peroxide resulted in a higher growth rate (average for source water 1 and 2 - 0.063 h^{-1}, generation time 12.58 h) than that of surface water (average for source water 1 and 2 - 0.035 h^{-1}, generation time 20.45 h). At the end of the study period, significant higher heterotrophic bacteria counts were found in surface water ($P = 0.0439$) than in groundwater ($P = 0.0036$).

Even in the presence of significant concentrations of disinfectant residuals (hydrogen peroxide: 13.20 - 16.5 $mg\ell^{-1}$ for surface water, 12.5 - 19.0 $mg.\ell$ for groundwater; monochloramine: 1.0 - 1.5 $mg\ell^{-1}$ for surface water, 0.8 - 1.0 $mg.\ell^{-1}$ for groundwater; chlorine: 0.2 - 0.5 $mg\ell^{-1}$ for surface water, 0.5 $mg.\ell^{-1}$ for groundwater) biofilm formation occurred on stainless steel and cement coupons within the first day of exposure. Although, these disinfectant concentrations did not prevent the formation of biofilms on coupons, biofilm regrowth was limited in laboratory-scale units with a disinfectant residual. Therefore monochloramine and hydrogen peroxide were found to be much more effective in controlling the growth of biofilm in laboratory-scale units than any of the other disinfectants.

Whereas the cement coupons had much lower viable bacteria in the initial treated waters than the stainless steel coupons, the relative difference between the two piping materials diminished with a prolonged exposure time. Attached bacteria were therefore significantly higher on cement coupons than on stainless steel coupons. A comparison between treated groundwater and treated surface water showed that treated surface water initially had the lowest attached bacteria counts (cement and stainless steel coupons) than treated groundwater. This difference, however, diminished by prolonged exposure time. Attached viable bacteria counts

appeared to be similar in both water sources, whereas significantly higher total bacteria counts were found on coupons exposed to treated groundwater.

Monochloramine was effective in preventing the attachment of coliform bacteria during early biofilm formation.

This investigation revealed that bacterial regrowth or biofilm regrowth is related to the water sources, (surface water or groundwater), the types of disinfectant, the piping materials as well as to the exposure time and mostly the presence orabsence of a disinfectant residual.

DIE UITWERKING VAN VERSKILLENDE ONTSMETTINGSPROSESSE OP DIE VORMING VAN BIOFILMS IN DRINKWATERVERSPREIDINGSNETWERKE

deur

MAGGY NDOMBO BENTEKE MOMBA

STUDIELEIER: PROF T.E. CLOETE

MEDESTUDIELEIER: Dr R.KFIR

DEPARTEMENT MIKROBIOLOGIE EN PLANTPATHOLOGIE

KWALIFIKASIE: Ph.D (Mikrobiologie)

OPSOMMING

Die uitwerking van verskillende ontsmettingsprosesse (chlorering, chlooraminering, osonering, UV bestraling en waterstofperoksied) op bakteriese nagroei en die vorming van biofilms in drinkwatersverspreidingsnetwerke, vir beide grond en oppervlakwater, is tydens hierdie studie op laboratoriumskaal geevalueer. Tydens ontsmetting is konsentrasies aangewend wat algemeen in die praktyk gebruik word. Koliforme bacterieë, totale heterotrofiese plaattellings en totale bakteriese tellings is gebruik om die doeltreffendheid van die onderskeie ontsmettingsprosesse te bepaal. Koliforme bakterieë en totale heterotrofiese plaattellings is onderskeidelik met behulp van die membraanfiltrasie en spreiplaattegniek bepaal. Totale bakteriese telling is bepaal met epifluoresensie mikroskopie en DAPI as kleurstof. Skandeerelktronmikroskopie is gebruik om biofilms op die vlekvrye staal en sementkoepons te visualiseer.

Al die ontsmettingsprosesse was ewe doeltreffend in terme van die verwydering van koliforme bakterieë uit oppervlakwater. Na 24 uur is koliforme bakterieë egter weer in al die sisteme opgespoor met die uitsondering van die sisteem wat met chlooramien behandel was. Binne ure na behandeling van grondwater met die verkillende ontsmettings prosesse kon geen koliforme bakterieë opgespoor word nie, met die uitsondering van die grondwater wat behandel was met waterstofperoksied. In hierdie sisteem is koliforme bakterieë slegs verwyder 48 uur na behandeling.

Die ontsmettingsprosesse het almal ten minste 99% (gemiddelde dodingspersentasie) van die heterotrofiese bakterieë in oppervlak water verwyder. Chlooramien en waterstofperoksied het na 'n langer tydperk (tussen 24 en 72 uur vir chlooramien en 72 uur vir waterstofperoksied) selfs beter verwydering getoon (99.99 %). Tydens die ontsmetting van grondwater is 99.90 % van die heterotrofiese bakterieë deur die verkillende prosesse verwyder, behalwe vir watertofperoksied, wat 'n laer verwydering van 99.88 % getoon het. Osoon was baie doeltreffend met 99.999 % verwydering van die heterotrofiese bakterieë binne die eerste twee ure na behandeling. Hieruit kon afgelei word dat die ontsmettingprosesse meer doeltreffend was vir die verwydering van heterotrofiese bakterie in grondwater as in oppervlak water.

Die voorkoms van bakteriese nagroei kon toegeskryf word aan die afwesigheid van die ontsmettingsmiddel. Nagroei het die vinnigste plaasgevind in beide grond- en oppervlakwater wat met chloor, UV en osoon behandel was. Die vlakke van heterotrofiese bakterieë was betekennisvol hoër in hierdie sisteme as in die sisteme wat met chlooramien en waterstofperoksied behandel is. Die residuvlakke van chloramien en waterstofperoksied wat oor 'n langer typerk waargeneem is, het bakteriese nagroei in albei waterbronne onderdruk. Die nagroeitempo in gechlooramineerde grondwater was laer (gemiddeld van 0.057 h^{-1}; verdubbe-

lingstyd van 13.87 h) as in die geval van oppervlak water (gemiddel van 0.085 h^{-1}; verdubbelingstyd 11.55 h). Die ontsmetting van grondwater met waterstofperoksied het gelei to 'n hoër groeitempo (gemidded van 0.063 h^{-1} , verdubbelingstyd van 12.58 h) as vir die behandelde oppervlakwater wat op dieselsde wyse ontsmet is (gemiddeld van 0.035 h^{-1}; verdubbelingstyd van 20.45 h). Aan die einde van die studie was die heterotrofiese bakteriese telling in oppervlak water betekennisvol hoër (P = 0.0439) as grondwater (P = 0.0036).

Biofilmvorming het, ten spyte van die teenwoordigheid van ontsmettingsmiddelresidu (waterstofperoksied:13.2 - 16.5 $mg\ell^{-1}$ vir oppervlakwater, 12.5 - 19 $mg\ell$ vir grondwater; monochloramien: 1.0 - 1.5 $mg\ell^{-1}$ vir oppervlakwater, 0.8 - 1.0 $mg\ell^{-1}$ vir grondwater, chloor: 0.2 - 0.5 $mg\ell^{-1}$ vir grondwater) binne die eerste dag plaasgevind op beide die vlekvrye staal en sementkoepons. Hoewel biofilmvorming nie voorkom is by hierdie konsentrasies nie, was groei wel onderdruk deur die teenwoordigheid van 'n residu. Chlooramien en waterstofperoksied was dus meer doeltreffend in die beheer van biofilmvormasie as enige van die ander ontsmettingsprosesse.

Hoewel die aanvanklike lewensvatbare tellings laer was vir die die sementkoepons het die verskil in tellings tussen die sementkoepons en die vlekvrye staalkoepons stelselmatig verminder met verlengde blootstelling. Die sessiele bakterie tellings was uiteindelik betekenisvol hoër op die sementkoepons as op die vlekvrye staalkoepons. Die getal bakterieë op sement en vlekvrye staalkoepons was aanvanklik laer in die geval van oppervlakwater maar die verskil het na verlengde blootstelling verminder. Die lewensvatbare bakteriese tellings was soortgelyk vir grond en oppervlakwater, maar die totale bakteriese tellings was egter hoër op die koepons wat aan behandelde grondwater blootgestel was.

Monochlooramien kon die vestiging van koliforme tydens vroeë biofilmvorming voorkom.

Hierdie studie het getoon dat daar 'n verwantskaps bestaan tussen bakteriese nagroei en die vorming van biofilms en die tipe waterbron (oppervlak of grondwater), die tipe onsmettingmiddel, die pypmateriaal so wel as die typperk van blooslling en residu van die ontsmettingssmiddel.

ACKNOWLEDGMENTS

A word of special acknowledgement and appreciation is addressed to the following persons and institutes for their contribution to the successful completion of this thesis:

To my GOD, one and only CREATOR of all opportunities, for mercy and without His strength, this study would not have been possible.

Prof. T.E. Cloete, Head of the Department of Microbiology and Plant Pathology, University of Pretoria and Supervisor of this study, for his excellent guidance, support and everlasting advice.

Dr R.Kfir, Technology Management, Council for Scientific and Industrial Research (C.S.I.R), Co-supervisor of this study, for her patience, guidance, hard work and for always maintaining this study in very high standards.

Mr S.N.Venter, Department of Microbiology and Plant Pathology, University of Pretoria, for his valuable advice and his technical assistance.

Mr A. Hall, Department of Microbiology and Plant Pathology, University of Pretoria, for his help in teaching me to work with the Electron microscopy.

Mr R.J. Grimbeek, Department of Statistics, University of Pretoria, for his help in the statistics lay-out.

Mrs J.H. Owen, Information Technology, University of Pretoria, for her help in the statistical analyses.

Mr M. Gioia, UV Water Systems, for providing a UV system for the disinfection of water samples.

Mr W. Barker , Building Technology - C.S.I.R. Pretoria, for providing the facility to produce the cement coupons.

Rand Water, for providing the surface water sample during the study period.

Department of Microbiology and Plant Pathology, University of Pretoria, for the provision of a bursary for two years.

Division of Environmentek, C.S.I.R -Pretoria, who made this investigation possible not only for funding the research, but also for providing a modern laboratory for the realisation of different experiments and for technical support.

Finaly to my son, Trésor Bumba for his patience and moral support as well as to my Parents for their encouragement and moral support throughout the entire study.

CONTENTS

PART I

INTRODUCTION, LITERATURE REVIEW,

MATERIALS AND METHODS

CHAPTER 1

GENERAL INTRODUCTION

In spite of progress in science and engineering, the microbiology of potable water remains an important concern worldwide. The major water quality objectives in water supplies consist of the removal of microorganisms which cause the so-called "waterborne" diseases and the prevention of contamination of drinking water by these organisms during storage and distribution. This goal can be achieved by the use of disinfection processes which include chlorination, chloramination, ozonation, UV irradiation, or hydrogen peroxide.

Disinfection processes play an important role in preparing potable water from surface water. Although treated drinking water generally contains low numbers of culturable heterotrophic bacteria, bacterial numbers can increase considerably in distribution systems (De Constantin *et al.*, 1986; LeChevallier *et al.*, 1987). The problem of bacterial regrowth in potable water supplies is not new. In 1930, the American Water Works Associations Committee on Water Supply reported on the problem of the regrowth of *Bacillus coli* in drinking water systems (Anon, 1930). Since then, it has been one of the most debated and most studied subjects of the water industry. Drinking water may harbour a large variety of bacteria (Geldreich *et al.*, 1972; Herman, 1978; Reasoner and Geldreich, 1979; LeChevallier *et al.*, 1980). The isolation of representatitves of 31 biotypes of *P. fluorescens* and 14 biotypes of *P. putida* from various types of drinking water in the Netherlands demonstrates the complexity and diversity of the heterotrophic bacterial community in drinking water (van der

Kooij, 1979). The presence of coliforms in drinking water distribution systems has also attracted much attention in the United States (Martin *et al.*, 1982; Wierenga, 1985; McFeters *et al.*, 1986; Geldreich and Rice 1987).

Over the past decade, considerable progress has been made in determining the cause of bacterial regrowth in drinking water distribution systems. On the one hand, there are a number of contributing mechanisms, which include contamination of the water due to mechanical failures of distribution systems (Clark *et al.*, 1993), growth of planktonic bacteria in the water (LeChevallier *et al.*, 1984) and the recovery of dormant bacteria or bacteria injured during treatment (McFeters *et al.*, 1986; LeChevallier *et al.*, 1988; Watters *et al.*, 1989). On the other hand, the conditions which enhance growth, include the nature and concentration of biodegradable coumpounds in freshly prepared drinking water (van der Kooij *et al.*, 1982, 1992; Joret *et al.*, 1991; Bernhard and Wilhems, 1985), the kind of piping material (Taylor, 1947; LeChevallier *et al.*, 1987; Roger *et al.*, 1994), the water temperature (LeChevallier *et al.*, 1996), the residence time of water in the distribution system and the residual disinfectant (LeChevallier *et al.*, 1980; van der Wende and Characklis, 1988; Gibbs *et al.*, 1990).

The potential for bacterial growth was observed during distribution of drinking water disinfected with chlorine (Earnhardt, 1980; Reilly and Kippen, 1983; Olivieri *et al.*, 1985; LeChevallier *et al.*, 1987a), chloramine (Watters *et al.*,1989), ozone (Dietlicher, 1970; Snoek, 1970; Stalder and Klosterkötter, 1976) and UV irradiation (Hengesbach *et al.*, 1993). LeChevallier *et al.* (1996) concluded that the occurrence of coliform bacteria within a distribution system is dependent upon a complex interaction of chemical, physical, operational, and engineering parameters. The authors reported that no one factor could account for all the coliform occurrences, and one must consider all of the parameters described above in devising a solution to the regrowth problem. However, aftergrowth in oligotrophic environments such

as potable water distribution systems depends mainly on the threshold concentration of assimilable organic carbon (van der Kooij *et al.*, 1982).

Chlorine is the most widely used disinfectant for the chemical treatment of water. However, survival and regrowth of bacteria in drinking water is generally accepted as an undesirable side-effect of disinfection by chlorination (Olivieri *et al.*, 1985). It has also been suggested that residual chlorine-resistant bacteria may arise as a result of chlorination (Farkas-Himsley, 1964; Leyval *et al.*, 1984), although other investigators have found no evidence for this (Haas and Morrison, 1981). Experiments with ozone, chloramine and chlorine have shown that regrowth occurs in water when the disinfectant residuals disappeared (Clark *et al.*, 1994). These disinfectants may react with organic material in source water to form nutrients that allow regrowth. Hengesbach *et al.* (1993) reported that any UV degradation of dissolved organic carbon is linked to the question of bacterial regrowth potential. The above investigations led to the conclusion that the effect of disinfection processes on bacterial regrowth is still not clearly understood.

Attention has also been drawn to various aspects related to biofilm formation in potable water distribution systems. Studies have shown that regrowth or biofilm formation in potable water systems occur mainly due to the presence of nutrients (van der Wende and Characklis, 1990), and that bacteria embedded in matrices (extracellular polymer substances) in biofilms are protected from disinfectants (Fletcher and Marshall, 1982; LeChevallier *et al.*, 1988). The presence of complex biofilms leads to continuous contamination of the water phase resulting from the release of the attached growing biomass (Paquin *et al.*, 1992).

Field investigations have described bacterial diversity within established drinking water biofilms and the physical-nutritional factors that influence their growth (LeChevallier *et al.*, 1987). Laboratory investigations have addressed factors promoting bacterial survival and

regrowth in potable water (Characklis, 1988; LeChevallier *et al.*, 1988; Watters *et al.*, 1989; LeChevallier *et al.*,1990, Matthieu *et al.*, 1992, Hengesbach *et al.*, 1993). Studies on the disinfectant most effective in the inactivation of established biofilms have also been conducted (LeChevallier *et al.*, 1988). However, the impact of disinfection processes on biofilm formation in drinking water distribution systems is still debatable.

Still now, most investigations have been concentrated on the formation of biofilm in treated surface water distribution systems. No investigation has been reported with complete information on the formation of biofilm after the disinfection of groundwater. Although initially derived from an aquifer, well water does not flow and remains in close contact with the well's inner surface. Consequently, a dense attached microflora develops, and its composition is greatly influenced by the type of wall surface or by the diameter of the pipe, i.e. the well volume (Kölbel-Boelke *et al.*, 1988a). The presence of complex biofilms will lead to continuous contamination of the groundwater resulting from the release of attached growing bacteria.

To limit the accumulation of bacteria in potable water and the formation of biofilms in potable water distribution systems, two parameters must be taken into account: the disinfection efficiency and the presence of the disinfectant residual. However, researchers have shown that less reactive, more persistent, monochloramine maintained a longer disinfectant residual through distribution systems and could penetrate the biofilm more effectively, and thus control biofilm organisms better than free chlorine (LeChevallier *et al.*, 1990; van der Wende and Characklis, 1990). Maintenance of a chlorine residual does not eliminate all bacteria in a water distribution system. Lechevallier *et al.* (1990) showed that biofilms grown on iron pipes treated with free chlorine doses as high as 4 $mg\ell^{-1}$ (3 $mg\ell^{-1}$ residual) for two weeks did not show a significant change in viability, but if treated with 4 $mg\ell^{-1}$ of monochloramine for two

weeks, the biofilms exhibited a more than 3 log die-off. The study performed by Clark *et al.* (1994) showed that, after various combinations of ozonation, chlorination and chloramination, microorganisms increased on fixed pipe surfaces when disinfectant residuals disappeared. A recent study performed by Lund and Ormerod (1995) showed that no biofilm was formed from chlorinated water containing a residual of 0.04 - 0.05 $mg.\ell^{-1}$. Most prolific regrowth was found in ozonated water, followed by the control (microstrained) water. UV irradiated water showed considerably less production of biofilm.

Although the causes of bacterial regrowth or biofilm formation in potable water distribution systems are well described, the deterioration of potable water quality after disinfection still causes a problem for water utilities. This study aims to compare a number of disinfectants commonly used in the South Africa potable water industry for their efficacy in rendering high quality potable water after the retention of water in the water distribution systems under South African conditions. The role that disinfection processes play on the deterioration of the water quality in treated water distribution systems was therefore investigated emphasizing disinfection efficacy, presence of disinfectant residual, bacterial regrowth or biofilm formation on different piping materials.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Water quality problems have intensified through the ages in response to the increased growth and concentration of populations in industrial centres. Problems have often been viewed as the inevitable consequence of community development and have sometimes even been accepted as evidence of affluence and progress.

Accordingly, many centuries ago, when populations were low and widely dispersed, there were few water quality problems. Nevertheless, they did occur when people congregated in cities and produced large flows of wastewater without safe and sanitary means for disposing of them. Those conditions often resulted in the health of the population being affected (deadly epidemics of cholera, typhoid fever and other waterborne diseases).

It is an established fact that the distribution system is often vital in determining the quality of potable water. The Water Decade has been preoccupied with the construction and expansion of water supplies, and it is only in its latter part that more attention has been given to the investigation, protection and control of the installations which supply drinking water (Lloyd and Bartram, 1991).

Drinking water contamination, caused by bacterial growth in storage or distribution systems remains one of the difficulties experienced with water supplies. In the last two

decades, microbial process technology has developed rapidly. Attention has been drawn to various aspects related to biofilm formation in drinking water distribution systems (LeChevallier *et al.*, 1987; LeChevallier *et al.*, 1991; Lund and Ormerod, 1995). While water produced in the treatment plant may be of high biological quality, the treated water may be subject to conditions in the distribution system that adversely affects it (O'Connor *et al.*, 1975). Research has been intensified throughout the world and several investigators have shown the multiplication of microorganisms along the distribution systems which results in the deterioration of the bacteriological quality of drinking water, the development of odour or colour as well as the acceleration of the phenomenon of corrosion within the pipework (Allen *et al.*, 1980; Olson *et al.*, 1981., Tuovinen and Hsu, 1982, Nagy and Olson, 1985).

To prevent bacterial growth in drinking water during distribution in the system, many water utilities have to use disinfection processes which include chlorination, chloramination, ozonation, ultraviolet irradiation, hydrogen peroxide or a combination of one of these disinfectants with another. Nevertheless, it has been shown that these substances are easily biodegradable and that most cannot inhibit biofilm formation in distribution systems (Glaze, 1987; Gilbert, 1988; Lund and Omerod, 1995). Biofilm on surfaces exposed to drinking water in distribution systems may well be the breeding ground for planktonic bacteria since up to 1000 sessile microorganisms may be present for each planktonic cell which is detected. The occurrence of biofilms or encrustations that harbour various types of microorganisms have been described extensively (van der Kooij and Zoetemann, 1978; Allen 1980, LeChevallier *et al.*, 1987). The most alarming results are the presence and multiplication of pathogenic and opportunistic pathogens such as *Pseudomonas, Mycobacter, Klebsiella, Aeromonas* and *Legionella spp.* occurring within the biofilms (Engel *et al.*, 1980; Wadowsky *et al.*, 1982; Burke *et al.*, 1984).

2.2 SOURCES OF POTABLE WATER SUPPLIES

Most potable water supplies are derived from surface water and groundwater sources. The choice of the best available raw source water as a drinking water supply is based on a number of factors which generally include: 1) adequate quantity through the year, 2) water quality that is amenable to treatment, 3) and some protective measures of the watershed from domestic, industrial and agriculture pollution (Office of Drinking Water, 1978). These sources of pollution introduce a wide range of potentially infectious agents to waters that may ultimately be used in the water supply (Smith and Twedt, 1971, US Environmental Protection Agency, 1971).

2.2.1 Surface water sources

Surface water originates as direct runoff which flows over impermeable or saturated surfaces and collects in reservoirs and streams. Surface water can be divided into rivers, streams, lakes, small dams and ponds.

Surface water quality is subjected to frequent, dramatic changes in microbial quality as a result of a variety of activities on a watershed. These changes are caused by discharges of municipal raw waters or treated effluents at specified point source locations into receiving waters (rivers, lakes) or by stormwater runoff into the drainage basin at non-point locations all over the watershed. Surface water quality is also influenced by the occasional recirculation of organisms in bottom sediments. There is an approximate 100 to 1 000-fold increase in faecal coliform bacteria in bottom sediments as compared to the overlying water (van Donsel and Geldreich, 1971). Recirculating pathways for pathogens in bottom sediments of lakes and reservoirs are another concern, especially where these water resources are located in temperate

zones in which pronounced seasonal changes in water temperature occur (Geldreich, 1990). Lakes are by far the most suitable surface water source for potable water. The quality of lake water is improved by self-purification through aeration, bio-chemical processes and the settling of suspended solids. The water can be very clear and of low organic content. Human and animal pollution usually only presents a health hazard near the shore. Apart from this, lake water is generally contain lower numbers of pathogenic bacteria and viruses than other surface water sources. However, algae may be present, especially near the surface. A surface water source must either be well protected or treated before it can be used for drinking (Lamb, 1985).

2.2.2 Groundwater sources

Groundwater sources consist of seepage ditches, infiltration drains, wells and boreholes. Throughout the ages groundwater has formed a substantial source of potable water in most parts of the world. In fact, nowadays some countries and many regions of the world still rely exclusively on well and borehole supplies. It has been estimated that global groundwater is more than 2 000 times as plentiful as all the fresh surface water combined (Pojasek, 1977). Urban groundwater is potentially as valuable as any other water sources, both as an environmental agent and as a water source (Lerner and Tellam, 1992).

For many small communities groundwater is the main water supply source and rural water supplies are obtained from wells and springs. In Switzerland, groundwater is the basis for the provision of small and medium sized cities and contributes 83% towards Switzerland's water supply, which shows its great importance (Schmalz, 1984; Trüeb, 1984). In the US, groundwater makes up nearly 25% of all water used, and about one half of the population relies on underground sources for their drinking water (Craun, 1984). Over 95% of rural domestic supplies are derived from subterranean aquifers (Pojasek, 1977). Although groundwater only

contributes about 15% to South Africa's water supply at present, it is of great importance to note that more than 280 towns and villages derive their water supply from groundwater. This represents towns and settlements spread over about 65% of South Africa (Kok and Somonis, 1989). Table 1 shows surface and groundwater use as a percentage of potable water in different countries.

Groundwater has long been considered to be of unquestionable excellence because the soil barrier is often effective in providing isolation of this high-quality source water from surface pollutants. Groundwater derived from deep aquifers are generally of good bacteriological quality because vertical percolation of water through soil results in the removal of much of microbial and organic pollution. By contrast, the waters from shallow wells are frequently grossly polluted with a variety of waste from surface runoff. Any study of the groundwater microbiota in the water-saturated zone should include both the sediment and the groundwater, because substantial differences have been found between the microbial communities of these two habitats (Kölbel-Boelke and Hirsch, 1989). Samples collected from the subsurface sediments and the aquifer water should be undisturbed and uncontaminated. However, it is very difficult to obtain representative samples of sediments, because many of the commonly used sampling methods cause drastic changes of the groundwater microflora (Rusterholtz and Mallory, 1994) and can lead to artifacts. Sediments and groundwater from wells differ in their microbial community structure (Hirsch *et al.*,1992).

The data of groundwater microbiological investigations commonly originate from the water present in the relatively large, open pores between sand-sized mineral grains. Water in these large pores is subject to less surface tension than water in small pores. It therefore represents the main part of the pumped sample, in addition to microorganisms which may be attached to smaller particles that were pumped up. The large -pore habitat, which is

predominantly populated by free-living rather than attached bacteria, may, however, exhibit much less community diversity and activity than the sediment as a whole. The lower density and diversity in groundwater relative to sediments might thus be the result of a sampling artifact (Chapelle, 1993). Reports of some waterborne outbreaks and unsatisfactory coliform results in groundwater have led to the recommendation to disinfect many well waters and springs used for drinking water (Lippy and Waltrip, 1984; Craun, 1985).

Table 2.1. Potable surface and groundwater use in different countries (Rimmer and Theron, 1990)

Country	Surface water %	Groundwater %
Italy	7	93
Federal Rep. of Germany	29	71
Belgium	30	70
Netherlands	35	65
France	50	50
Swenden	54	46
America (USA)	75	25
England and Wales	90	10
South Africa	85	15

2.3 HEALTH RISKS ASSOCIATED WITH DRINKING WATER MICROBIAL FLORA

Effective purification of drinking water is an important consideration in the prevention of waterborne disease and it transmission. Unfortunately, due to the contamination of source

surface or groundwater, treatment , storage and/or distribution are not an easy task.

The microbial flora of a potable water reflects the microflora characteristics of the raw water source (Taylor, 1958; Victoreen, 1970). These may be broadly classified into four groups: bacteria, viruses, protozoa and helminths. Only the bacteria group was investigated in this study. Table 2 summarizes major infectious agents found in contaminated potable water.

Epidemiological studies have implicated drinking water supplies as one of the important vehicles in the direct transmission of the infectious agents of several diseases. Morbidity resulting from the injection of contaminated water persists. Since the mid-1960s, the available epidemiological evidence has demonstrated a dramatic increase of morbidity in the United States (Craun, 1986). Nineteen outbreaks were reported in 1971, involving 5 182 individuals, and 32 outbreaks of waterborne diseases with 11 435 cases were reported in 1978 (Craun, 1980). During the period from 1971-1978, a total of 224 waterborne outbreaks of disease were reported in the United States. Two deaths and 48 193 illnesses were associated with these outbreaks in which water was epidemiologically implicated as the vehicle of transmission (Craun, 1981). Between 1986 and 1988, 24 states and Puerto Rico reported 50 outbreaks of illness due to the consumption of drinking water affecting an estimated 25 846 people (Levine et *al.*, 1990). During 1989 and 1990, 16 states reported 26 microbiological waterborne outbreaks which caused the illness of 4 288 people (Herwaldt *et al.*, 1992). In addition, during a 2-year period from 1991 to 1992, 17 states and territories reported 34 464 outbreaks affecting an estimated 17 people (Moore *et al.*, 1993). More recently, *Cryptosporidium* has been implicated as the causative organism in the largest documented outbreak of waterborne disease in the United States, affecting an estimated 403 000 individuals (MacKenzie *et al.*, 1994). Moreover, it should be noted that the incidence of waterborne outbreaks of unknown etiology and those caused by pathogens such as *Campylobacter sp,* is also increasing in the United States

Table 2.2. Major infectious agents found in contaminated drinking waters worldwide

(Geldreich, 1990)

Bacteria	**Viruses**	**Protozoa**	**Helminths**
Campylobacter jejuni	Adenovirus (31 types)	*Balantidium coli*	*Ancylostoma*
Enteropathogenic *E. coli*	Enteroviruses (71 types)	*Entamoeba histolytica*	*Strongyloides stercoralis*
Salmonella (1700 spp)	Hepatitis A	*Giardia lambia*	*Teania* (spp.)
Shigella (4 spp)	Norwalk agent	*Cryptosporidium*	*Ascaris lumbricoides*
Vibrio cholerae	Reovirus		*Necator americanus*
Yersinia enterocolitica	Rotavirus		Echinococcus granulosis
	Coxsackie virus		

(Mcfter, 1990). At the same time, some experts believe that as many as 90% of the outbreaks are unreported (LeChevallier *et al.*, 1984). Waterborne diseases represent one of the most important causes of illness within developing countries. Approximately half the world's population experiences diseases that are a direct consequence of drinking polluted water. Such illnesses are the primary cause of infant mortality in many Third World countries, since many people fail to associate their illness with contaminated drinking water (US Environmental Protection Agency, 1976).

Pathogenic and toxigenic microbiological agents in drinking water have long been known to cause disease and death in consumers (Craun, 1986). The health risks associated with these pathogens have been established, which range from viral and bacterial gastro-enteric diseases to infections such as hepatitis A and giardiasis.

Overall, the strategies devised early in the twentieth century to control waterborne microbial disease have changed very little. However, waterborne diseases are now known to be caused by a much broader variety of microbes than just enteric bacteria, and the diseases encompass a variety of health effects. Some of the diseases and their causative agents include fatal pneumonia or pontiac fever caused by *Legionella pneumophila* and similar bacteria, hepatitis caused by virus types A and E, cardiomyopathies caused by coxsackieviruses, incurable and fulminating gastroenteritis caused by *Cryptosporidium parvum* in AIDS patients and neurotoxicity caused by blue-green algae.

Most heterotrophic bacteria isolated from distribution systems have been related to human secondary opportunistic pathogens. These include:

- *Pseudomonas aeroginosa* - may cause eye and ear infections as well as infections of wounds and burns. This bacterial species was found to be responsible for serious post-operative infections in tonsillectomy patients and in hospital burn units (Louwbury *et al.*, 1970). *P.*

aeruginosa produce an enterotoxin which results in severe diarrhoea (Anon, 1984).

- *Aeromonas hydrophila* - reported as a bacterium capable of causing disease in man (Hauson, 1977). *Aeromonas* spp. also poses an increased risk for patients undergoing renal dialysis. Some strains of this bacterium produce enterotoxins causing severe gastroenteritis (Rosner, 1964). It is also reported that *Aeromonas* spp. is responsible for acute diarrhoea in children and traveller's diarrhoea in adults (Moyer, 1987).

- *Enterobacter cloacae* - an opportunistic bacteria frequently isolated from man in urine, sputum from the respirator tract, puss and occasionally blood and spinal fluid. Its important increase in hospitals, especially in intensive care units, emergency units and urology units has been reported (Krieg and Holt, 1984).

- *Klebsiella pneumonia* - is one of the dominant coliforms in water distributions (Clark *et al.*, 1982; Geldreich and Rice, 1987). The bacteria grow in sediments which provide a level of protection from disinfection (Martin *et al.*, 1982). It can also be considered as an indicator of faecal pollution.

- *Flavobacterium* spp - are recognized to be responsible for neonatal meningitis, meningitis in adults, bacteraemia and respiratory tract infections. These bacterial species are known as opportunistic pathogens which attack the seriously ill (Krieg and Holt, 1984).

- Other bacteria - *Moraxella, Acinetobacter, Bacillus, Alcaligenes* and *Achromobacter* have been isolated from drinking water distribution systems. All these bacteria are known as human opportunistic bacterial pathogens.

- Fungi - growing in the water distribution system has been reported to be a health hazard, resulting in the formation of mycotoxins and allergies. Acute toxins such as mycotoxins have been associated with the growth of fungi on food. Exposure to fungal spores causes respiratory symptoms during hot baths (Atterholm *et al.*, 1977).

Increased awareness of the breadth and magnitude of waterborne microbial disease suggests that new and improved strategies are needed to reduce the health risk from microbes in drinking water. Increasing or modifying present conventional treatment practices, especially disinfection, is likely to reduce such health risks.

One approach by the water treatment industry to provide safe drinking water and prevent outbreaks of waterborne disease, is the concept of multiple barriers. This includes the environmental protection of surface and groundwater quality at source, multiple treatment technologies applied at the utility (including coagulation, flocculation, sedimentation, filtration and disinfection) as well as distribution system management and protection.

2.4 TREATMENT METHODS FOR POTABLE WATER

The purpose of water treatment is to improve water quality. Important aspects to be addressed when considering any treatment method are the impact on human health, possible aesthetic objections, the formation of corrosion or scale in pipelines, bacterial regrowth in finished water or any other problems in household, commercial or industrial use of the water. The goal of potable water treatment is both to make the water safe for human consumption and to produce a finished product that is attractive and pleasing to the consumer. The first step in determining the type and extent of water treatment needed is to assess the quality of the raw water. As most water supplies are derived from surface and groundwater sources, specific analyses should include those for quality characteristics and constituents that are specifically of concern to the end-user. Additional analyses may by conducted for other known or suspected constituents which may be important for the various uses of the water. Comparison of the characteristics of the raw water with the set guidelines required for the finished water will indicate whether

there are any deficiencies that must be corrected. When no deficiencies exist or are likely to occur, no treatment is necessary. When deficiencies are found, appropriate types of treatment must be undertaken to correct them. There are treatment processes capable correcting most water quality problems.

Disinfection of surface waters is often more difficult because of fluctuating water quality. Almost all surface supplies are assumed to require treatment at least for the removal of turbidity, colour, and microorganisms. Conventional treatment most commonly used includes the chemical and physical processes of coagulation, flocculation, gravity separation, rapid sand filtration and disinfection.

As noted in section 2.2, groundwater has long been considered to be of unquestionable excellence because the soil barrier is often effective in isolating it from surface pollutants. Treatment is therefore mostly nonexistent or limited to water hardness reduction, taste and odour removal, or disinfection. Groundwater derived from aquifers is low in turbidity and color, generally of good bacteriological quality, and has no other significant defects or potential quality problems, making processes for removing constituents unnecessary. No corrective treatment is indicated, but sufficient contact time in a storage tank must be allowed for adequate mixing with all portions of the supply. As it is frequently grossly polluted with a variety of waste from surface runoff, water from shallow wells is acceptable for use only after treatment. Moreover, failure to periodically drain and clean water supply holding tanks to remove sediments will provide opportunities for heterotrophic bacterial colonization and allow biofilm development. These organisms may not only be an interference factor in coliform detection but form a population of unique organisms that may create undesirable taste, odour or degradation reactions when introduced into food products, beverages and pharmaceuticals (Geldreich *et al.*, 1972). The following section of the review will deal with more specific

methods.

2.4.1 Pre-disinfection

2.4.1.1 Pre-chlorination

The first step in treatment could be chlorination. Addition of the disinfectant at this point provides a maximum time of contact between the chemical and the organisms, assuming efficient bactericidal action. Chlorination at this point also sometimes enhances the subsequent removal of tastes and odours, iron, manganese and colour by oxidising some organics in the raw water. Furthermore, the addition of chlorine inhibits growth of algae and other objectional organisms during the several hours required for water to pass through the treatment plant (Lamb, 1985). Nevertheless recent investigations have noted that the early addition of chlorine can maximize the production of trihalomethanes (THMs). For this reason, there is a movement to postpone this step to a later point in the treatment, after most of the humics, which are precursors of the formation of THMs, have been removed (Singer et *al.*, 1981).

Some water utilities have considered the use of advanced oxidation processes (Glaze and Kang, 1988) in which increased production of highly reactive hydroxyl radicals is promoted. One such process involves the addition of hydrogen peroxide and ozone, referred to as the Peroxone process. The use of ozone or peroxone is being considered for a variety of reasons: as an alternative oxidant to chlorine in order to reduce halogenated organic by-products, for removing objectionable tastes and odours, for oxidizing reduced metals and specific synthetic organics, for potential benefits during coagulation and filtration, and to increase the biodegradability of organic matter for subsequent treatment. Preozonation also enhances the flocculation of suspended particles in surface waters (Glazes, 1987). However, investigations have shown that preoxidation with ozone and peroxone does not allow for a reduction in

coagulant doses (combined alum and cationic polymer) for good treatment, and sometimes causes increased filter run lengths resulting from head loss or delayed turbidity breakthrough. Peroxone is moderately more effective than ozone alone in eliminating odours in filtered waters (Tobiason *et al.*, 1992).

2.4.1.2 Coagulation

Conventional treatment of surface water usually begins with rapid dispersion of a coagulant by introducing a metal salt (aluminum sulphate, iron sulphate, ferric sulphate or ferric chloride) or a synthetic organic polymer into the flow stream followed at once by violent agitation. This is commonly referred to as initial, rapid, or flash-mixing. The purpose of the coagulant is to destabilize or neutralize the predominantly negative charge on the suspended solids and to form floc particles. These chemicals react with alkalinity in the water to form a gelatinous precipitate which is effective in promoting the coagulation of small suspended particles into larger ones that separate more rapidly in the settling basin. Alum also changes other characteristics of the particles and enhances their removal during sand filtration. It is desirable to disperse the coagulant in as short a time as possible. If softening is carried out, lime is added and dispersed before the coagulant is added. Proper introduction of coagulants into the water is very important. Reaction between the water and the chemical takes less than one second, so a weak or delayed mixing procedure results in wastage of coagulant or poor performance (Coad, 1990). The mode of chemical application can have a significant influence on subsequent solids removal, even with an optimum reactor design and proper mixer selection. These chemicals contribute to the removal of humic substances by the process of flocculation.

2.4.1.3 Flocculation

The importance of flocculation has been underrated in many designs. During the flocculation process, the water is gently mixed to provide opportunities for particle contacts, which promotes agglomeration of smaller particles into larger floc particles that are removed by sedimentation or filtration. The primary consideration in flocculation design is the provision of optimum velocity gradients. Too high velocity gradients will shear floc particles. Too low velocity gradients will fail to provide sufficient agitation to allow flocculation to become complete. Floc may be broken up at points of increased agitation in locations such as conduits or other points of restricted flow. Chemical agents such as activated silica or polyelectrolytes are sometimes used in order to increase floc strength, especially during cold water conditions.

2.4.1.4 Sedimentation

This operation is customarily used after coagulation and flocculation, and before sand filtration. Sedimentation ensures the removal of most turbidity and microorganisms, leaving only a few particles in the liquid. Sedimentation basins remove the bulk of the larger sized particles that settle by gravitation.

2.4.1.5 Filtration

Filtration is the most important process in the solids removal chain. Rapid filtration is used to remove suspended solids that have passed through the sedimentation stage, and to prepare the water for disinfection. Properly designed and operated filters are capable of removing suspended particles as well as some colloidal-sized particles, resulting in an overall solids removal by filtration in excess of 99%. The remaining suspended particles are removed and accumulated in the interstices between the sand grains until they have been largely filled.

Slow sand filtration provides suitable conditions for the growth of bacteria which will biodegrade organic compounds. In this process, uncoagulated water is applied to a sand bed about 1 m deep at a filtration rate (approach velocity) of about 0,1 m/h. Removal of microorganisms is aided by biological processes that occur on and in the filter bed, where a microbial ecosystem becomes established with extended use. On the top of the media, a biological active scum layer (*Schmutzdecke*) builds up and assists in filtration. As the water enters the *Schmutzdecke*, biological action breaks down some organic matter, and inert suspended particles may be physically strained out of the water. The water then enters the top layer of sand where physical straining and biological action occur and attachment of particles onto the sand grain surfaces takes place. Also some sedimentation may occur in the pores between the media grains where velocity is sufficiently slow (Logsdon, 1990). A chlorinated backwash is included in the water treatment to reduce bacterial growth. This step often results in increased growth of bacteria in the water distribution system as more organic matter enters the system (Kfir and Kott, 1989). Rapid filtration does not make the water safe to drink, but poor filtration can reduce the effectiveness of the subsequent disinfection by allowing cysts and bacteria shield inside the floc, to pass into the distribution system (Coad, 1990).

2.4.1.6 pH and lime treatment

The addition of lime after filtration reduces the acidity that results from adding alum as well as the corrosiveness of finished water to the distribution system and to plumbing in homes. An increase in pH by lime addition was shown to effectively eliminate coliform growth in water distribution systems (Martin *et al.*, 1982).

2.4.2 *Disinfection*

Amongst the various treatment barriers between the consumer and waterborne diseases, disinfection occupies a key position, as it is indispensable for the prevention of waterborne infectious diseases. Disinfection of drinking water can be regarded as the single most significant public heath measure of this century. The destruction of pathogens in drinking water has dramatically reduced the incidence of waterborne diseases in all industrialized countries. On the other hand, the lack of disinfection contributes to the death of millions of children and adults in developing countries every year. The disinfection step is introduced to remove bacteria or other microorganisms which escape prior treatment processes. It usually consists of the use of an oxidative process which, although it removes microorganisms, may also enhance the formation of easily biodegradable organic substances - these can be easily utilized by microorganisms as an energy source and promote biofilm formation in distribution systems (Anon, 1984; Gilbert 1988; Glaze, 1987).

The presence of a disinfectant residual in the distribution system also helps to maintain water quality by preventing the growth of nuisance microorganisms. Many chemical and physical agents have been studied with regard to their potential as drinking water disinfectants. This section deals with the processes generally used in the drinking water industry, e.g. chlorination, chloramination, ozonation, ultraviolet radiation and the addition of hydrogen peroxide.

2.4.2.1 *Chlorination*

Chlorination is the addition of chlorine directly to the water. Chlorine is available in three common forms: liquid (sodium hypochlorite - NaOCl) , powder [calcium hypochlorite - $Ca(OCl_2)$] and liquified compressed gas (Cl_2). Its disinfectant effect is influenced by the pH,

and is lowest in the alkaline range where the hypochlorite ion is the dominant component of free chlorine. Ammonia, and particularly organic nitrogen compounds, weaken the effect of chlorine as they bind with free chlorine to form inorganic and organic chloramines. The chlorine dose is related to the chlorine demand of a water. The chlorine demand of a water is therefore the amount of chlorine required to react with organic substances as well as other substances which may occur in the water. It is thus the difference between the amount of chlorine added and the residual after a certain contact period. The chlorine demand varies according to the amount of chlorine added and the temperature of the water. Turbid water and water containing algae, for example, will have a greater chlorine demand than clear water (Wium and Coetzee, 1985). The procedure of killing germs by chlorination depends very much on the pH, and within the permissible pH range of 6.5 - 9.5 the disinfectant effect of chlorine decreases as the pH increases.

Hypochlorous acid is generally considered to be a destructive, non-selective oxidant which reacts with all biological molecules. However, it became clear that HOCl has a specific target site of destruction, which is in conflict with the selective theory (Thomas *et al.*,1986). The hypochlorous ion reacted rapidly with cytosine (C) and adenine (A), slowly with guanine (G) and hardly at all with uracil (U). However, the hypochlorite ion reacted specially with G and to a minor extent with C and A, but not at all with U. In *E. coli*, HOCl terminates adenosine-triphosphate (ATP) production by inhibiting transport of the fermentative substrate, glucose and respiratory succinate, while simultaneously inactivating the membrane-localised proton-translocating ATP-synthetase, which is of utmost importance during respiration (Barrette *et al.*,1989). Two mechanisms intervene in the rapid ATP-hydrolysis by HOCl activity. On the one hand, there is a direct attack on a membrane-bound ATP-dependant enzyme, e.g. ATP-synthetase (Fillingame, 1980) or transport protein. Inhibition of ATP-synthetase involves

complete interruption of oxidative phosphorylation, which could account for ATP depletion in respiring cells. On the other hand, there is an attack upon a membrane-bound protein not necessitating ATP itself, but resulting in ATP hydrolysis by an ATP-dependant system that attempts to compensate for metabolite imbalances caused by damage (Barrette *et al.*, 1987).

The action site of HOCl involves electron-rich or functional groups located within the cellular envelope (Albrich and Hurst, 1982). Molecules that possess highly nucleophilic sites are the primary target (Barrette *et al.*, 1987) and these include porphyrins and hemes, ferredoxin-like iron-sulphur centres, purine and pyrimidine bases, conjugated polyene amines and sulphydryl groups (Albrich *et al.*, 1981; Hurst *et al.*, 1991). Due to HOCl's selective preference for two-electron, or atom transfer pathways, the electrophilic chlorine atom must be capable of nascent bond formation with the oxidation site (Held *et al.*, 1978). Alteration of a cell's trans-membrane metabolite transport capabilities due to HOCl action might be the consequence of the lysis of the plasma membrane. Lysis of the plasma membrane renders the cell incapable of maintaining any concentration gradients (Albrich *et al.*, 1986). However, Barrette *et al.*(1987) showed that HOCl -oxidised membranes retained their impermeability to small molecules and ions, eliminating membrane lysis as a possible mechanism for transport loss. The selective inactivation of membrane-localised transport proteins may be an alternative mechanism of action. The inability to accumulate metabolites by proton-coupled support mechanisms cannot be ascribed to the loss of activity in the cell since HOCl-oxidised bacteria maintain metabolite concentration gradients. An alternative explanation for this inhibition is that the membrane-localised transport proteins react directly with $HOCl^-$ due to the HOCl susceptible sulphydryl groups carried by these proteins (Albrich *et al.*,1981).

Research has showed that the cytosol does not seem to be a primary target site for HOCl destruction, since HOCl sensitive biomolecules located in the cytosol are not attacked until the

bacteria are exposed to concentration levels several fold higher (Albrich *et al.*, 1981; Barrette *et al., 1987*). Nevertheless, HOCl activity results in extensive hydrolysis of the cytosolic nucleotide phosphoanhydride bond in *E.coli,* which leads to the irreversible decline of adenylate energy to low levels (Barrette *et al.*, 1987).

Early damage of bacterial DNA has also been observed (Haas and Engelbrecht, 1980) although the high resistence of bacterial spores and acid-fast bacteria to chlorine has been revealed. Being small and electrically neutral, hypochlorous acid would easily be able to penetrate the cell wall. This brings the powerful oxidizing agent into immediate contact with the cell membrane which plays a vital role in cell metabolism. Interference with the activities of membrane-bound enzymes would also seem likely, the primary mode of action being oxidation of the sulphydryl and thioether groups (Dennis *et al.*, 1979; Venkobachar *et al.*, 1977).

The chlorination of drinking water is one of the biggest successes of sanitation achieved this century. Since the practice of disinfecting drinking water began in 1902, chlorine has been used as the principal disinfectant (Norman *et al.*, 1980). The factors contributing to the widespread use of chlorine include its relatively low cost, ease of application, proven reliability, detectability, and familiarity with its use (White, 1972). The main advantage of disinfecting with chlorine is the development of an easily measured residual which remains in the water after disinfection, and protects water in storage and in the distribution system. Decades of experience have shown that prescribed levels of residual chlorine remaining in the water after a prescribed time of contact assure the consumer that the water is safe to drink. Chlorine in the form of hypochlorous acid (HOCl) is an effective disinfectant against both bacteria and viruses. It also provides long-term protection of the water distribution network against contamination and microbial aftergrowth. However, an important disadvantage of

chlorination is the formation of many toxic and partially mutagenic or even carcinogenic chlorinated organics. Chlorine can react with organic matter to form organohalogenated compounds (Doré, 1989). Chloroform and certain other trihalomethanes are formed principally during chlorination. The magnitude of their production depends on the concentration of precursor in the influent water, contact time, as well as water quality factors such as pH and temperature (Symons *et al.*, 1981). When free chlorine is used as a post-disinfectant, an increase of disinfection by-products (DBPs, THMs and chloral hydrate) is observed in the distribution system (Meyer *et al.*, 1993). The formation of DBPs is linked to the consumption of chlorine and to the residual DOC of the finished water (Meyer *et al.*, 1993). It is also reported that the addition of chlorine at a sufficiently high concentration will kill most bacteria but the chlorine residual soon disappears in a distribution system with a high chlorine demand, allowing bacterial regrowth. The initial chlorine dose is limited by customer complaints and the formation of trihalomethanes (THMs) (Gibbs, *et al.*, 1990). Such products can also be found after application of alternative disinfectants, but most probably only to a much lesser extent (Stroebel and Dieter, 1990).

Disinfection of biofilm organisms by chlorine (or chloramine) is very difficult. A level of free chlorine residuals as high as 1-5 $mg.\ell^{-1}$ may not penetrate biofilms (LeChevallier *et al.*, 1988) and inactive bacteria. Mechanically scrapping, pigging or flushing are ineffective in the control of biofilm problems. Release of bacteria from biofilms through changes in shear forces or oxidative processes may lead to increased heterotrophic plate counts (HPC) and, in some cases, coliform occurrence and *Aeromonas* sp. isolation (some strains of which may be pathogenic). To control this bacterial contamination of potable water, high levels of residual disinfectant are generally effective. However, excessive doses of disinfectant can result in the formation of chloro-organic by-products (which pose a health hazard) and unacceptable tastes.

2.4.2.2 Chloramination

The process of chloramination has usually been referred to as combined residual chlorination or the ammonia-chlorine process (combination of free chlorine and ammonia). The total chlorine residual is a term used to characterize the sum of the combined and free chlorine residuals. The process is based upon the reaction of hypochlorous acid with ammonia. Monochloramine is the predominant species in chloraminated water and is produced rapidly in the pH range 7-9 and mostly readly whith the molar ratio Cl_2:N ≤ 1:1 or 5:1 by mass. The mechanisms of bacterial inactivation have been postulated to be similar to those of free chlorine (Olivieri *et al.*, 1980). Monochloramine can enter the cell and react with proteins and nucleic acid (Jacangelo *et al.*,1991).

Chloramines were most popular in the U.S. in 1930's but their usage decreased upon the discovery of breakpoint chlorination and its better disinfection ability (Stacha and Pontius, 1983). They are more commnly used as a secondary disinfectant, especially in high chlorine demand waters where they can provide bactericidal protection, control of algae, and bacterial after-growth in lieu of free chlorine which would be exhausted in the extremities of the distribution systems. Chloramines have also been used in place of free chlorine residual to minimize taste (White, 1972). Recent studies have shown that monochloramine has a greater bacterial effect than had previously been thought. Its high stability produces good prevention against bacterial regrowth, particularly in distribution networks. Studies in the United States have generally concluded that utilities using chloramine have been able to meet the coliform standard in distribution systems (Kreft *et al.*, 1985) although a number of bacterial genera have been identified in systems that use chloramines as well as in systems that use free chlorine (Ridgway and Olson, 1981). Combined residuals are longer-lasting in distribution systems than

free chlorine residuals. For points in the distribution system where contact times are long, such as dead ends, there may be benefits in changing from free chlorine to chloramines. A related issue is cross-contamination through direct cross-connections or back siphonage. On the one hand it has been demonstrated that free chlorine (as HOCl or OCl^{-1}) is a better disinfectant over a shorter time than chloramines (Kreft *et al.*,1985). On the other hand, a chloramine residual has been reported to be more effective over a long period of time than free chlorine because of its persistence (Kruse *et al.*, 1981).

Chloramination has been useful for balancing the conflicting goals of disinfection and the control of disinfection by-products (DBP). Water treatment by chloramination has emerged as the most popular alternative disinfection process because of the appreciable reduction of THMs during the treatment. However chloramines offer both advantages and disadvantages over free chlorine.

Advantages include limiting DBPs, greater residual persistence, lower corrosivity (to some materials) and less noticeable taste and odours than free chlorine. Although chloramines produce insignificant quantities of THMs, they do produce some chlorinated organic by-products as measured by organic halogen (Symons, 1980). Experience gained over years of use has showed that if sufficient concentrations and contact times are provided, chloramines can be an effective primary disinfectant for waters having pH values less than 8.0 (Shull, 1981). Although chloramine treatment may not be effective in all systems, many utilities are rediscovering the use of chloramine as a primary or secondary disinfectant in their quest to reduce THMs.

The disadvantages include limited disinfection capabilities and longer contact times compared to chlorine. It is well established that chloramines are less effective bactericides and viricides than either hypochlorous acid or hypochlorite ion (American Water Work Association,

1971). Neither enteric viruses nor protozoan cysts are effectively inactivated by chloramine over short periods of time (10 min.) at low dosage (1 - 2 $mg.\ell^{-1}$) (American Water Work Association, 1982). To further complicate issues, chloramination is limited to a pH range of 6 to 9 for disinfection. Chloramines improperly applied can lead to nitrification problems in the distribution systems. Health effects of chloramines have been mentioned by Kreft *et al.* (1985) as well as by Daniel *et al.* (1993). Since chloramines (and other disinfectants as well) function as oxidants, most research on the health effects of chloramines has been directed at the oxidative effect on blood. No effects were generally observed, but for kidney dialysis patients, a need for caution is required since chloramines have been found to induce haemolytic anaemia. Other health implications of the application of chloramines seem to be connected with the proteolytic degradation of chloramines, the consequence of which is freely available chlorine, which, although not in the same degree as for chlorination, is able to form toxic by-products (Anon, 1981).

2.4.2.3 Ozonation

Ozone is a triatomic molecule, being an allotropic modification of diatomic oxygen. It is partially soluble in water and has a characteristic penetrating odour, readily detectable at concentrations as low as 0,01 to 0,05 ppm. Although many water treatment plants throughout the world still utilize ozone primarily for disinfection, most modern plants rely on ozone to perform one or more functions. Applications of ozonation now include oxidation of inorganic and organic materials, flocculation and micro-flocculation for removal of turbidity or suspended solids, and recently the promotion of aerobic biological processes conducted in filter and adsorption media (Rice *et al.*, 1981).

The only method of commercial importance used for ozone generation in water treatment and disinfection is a process which uses a strong alternating current electric field to produce corona discharge in the feed gas, which is usually air that has been filtered and dried (Nebel, 1983). The usual ozone dosage rate for bacterial disinfection in water treatment plants is 1,5 to 2 $mg.\ell^{-1}$ (Rice *et al.*, 1981). A contact time of five minutes is adequate in clear water < 1 NTU (Yapijakis, 1978). Where pre-ozonation treatment is nonexistent or when loadings of ozone-demanding organic or inorganic materials are high, as during pollution episodes, ozone doses much larger than 2 $mg.\ell^{-1}$ can be required to attain the desired degree of bacterial disinfection. In addition, the bacterial action of ozone is little affected by changes in temperature and the disinfecting action of ozone is virtually instantaneous (Rice *et al.*, 1981). France increases the ozonation time to at least 8 min. Contact times of 12 min are sometimes employed (Rice *et al.*, 1981).

Although the inhibitory and lethal effects of ozone on pathogenic organisms have been observed since the latter part of the nineteenth century, the mechanisms for these actions have not yet been satisfactorily elucidated. Ozone is a strong germicide needing only a few micrograms per liter for measurable action. At a concentration of $1 g.m^{-3}$ O_3 at 1 °C, ozone rapidily inactivates coliform bacteria, *Staphylococcus aureus* and *Aeromonas hydrophila* (Lohr and Gratzek, 1984). Ozone 's bactericidal effects centers on disruption of envelope integrity through peroxidation of phospholipids and lipoproteins. There is evidence for interaction with proteins as well (Mudd *et al.*, 1969). In one study (Ishizaki *et al.*, 1987) exploring the effect of ozone on *E.coli,* evidence was found for ozone's penetration of the cell membrane, reacting with cytoplasm substances and converting the closed circular plasmid DNA to open circular DNA, which would presumably lessen the efficiency of bacterial proliferation.

Ozonation has long been a proven technology for water disinfection and is particularly

well established in Europe, Canada, and the countries of the former Soviet Union. The earliest experiments on the use of ozone as a germicide were conducted in France by Meritens, who showed that even dilute ozonized air could effect the sterilization of polluted water (Rice *et al.*, 1981). In 1891, Frölich reported the bactericidal proprieties of ozone from pilot tests conducted at a drinking water treatment plant in Martinikenfeld, West Germany. Ozone is applied at over 1 300 plants around the world (e.g. Budapest, Düsseldorf, Los Angeles, Montreal, Moscow, Paris and Zürich) as disinfectant (Schalekamp, 1988) and oxidant. In South Africa, ozone is used at full scale by the Western Transvaal Regional Water Company (Pietersen *et al.*, 1993).

In tests in which ozone has been compared with chlorine, ozone has been shown to be superior to chlorine as a coagulant (Prendiville, 1986). It also appears to form much smaller amounts of mutagens than either chlorine or chlorine dioxide. In addition, when ozone decomposes, it generates radical intermediates that have much greater oxidizing power than ozone itself (Glaze, 1987). The specific advantages of ozone are the improvement of odours and taste as well as microflocculation effects (Strobel and Dieter, 1990). As an oxidant, it can oxidize many nuisance compounds or potential toxicants in water supplies (Glaze, 1987).

Nevertheless, ozone suffers from two major limitations as an alternative to chlorine. Firstly, it is unstable in water. For example, at pH 8, which is typical of many drinking-water supplies, its half-life is less than one hour, which is too short to ensure that a residual disinfectant capacity will remain at the far reaches of a large distribution system. Despite its short lifetime, however, ozone is particularly effective against recalcitrant microorganisms. It is more effective than chlorine for the removal of Giardia, viruses and cation forms of algae (Glaze, 1987). Secondly, ozone reacts with natural organic substances to produce low-molecular-weight oxygenated by-products that are generally more biodegradable than their

precursors. These substances will promote biological growth in a distribution system ("regrowth"), further limiting the disinfection efficacy of ozone. For these reasons, ozone should be used in combination with other disinfectants that maintain an active residual for longer periods and it should be combined with some method of filtration for removing biodegradable material (Glaze, 1987). Another disadvantage of ozone is that it cannot be stored on site. Generation capacity must be equal to the peak rate of use resulting in an expensive installation (Stacha and Pontius, 1983). High investment costs are associated with the required equipment, and a high degree of expertise is required to serve the plant.

2.4.2.4 Ultraviolet irradiation

Ultraviolet light is a non-chemical form of disinfection. Water to be disinfected passes through a chamber where it is exposed to ultraviolet light with a given germicidal efficiency. The lamps must be kept clean so that the organisms are exposed to the full intensity of the ultraviolet light. In practice, UV irradiation is produced by mercury-vapour lamps that emit UV-C rays. The killing effect and thus the disinfecting effect therefore depends on the total dosage or quantity of energy input from the mercury-vapour lamps applied accross the target surface. This energy input is denominated in $W.m^{-2}$, and the dosage is determined by multiplying the energy input by the time in seconds that the dosage is applied. The necessary dosage for the killing of microorganisms depends on the morphology of the specific microorganism. In general, bacteria with thick cell enclosures are harder to destroy than bacteria with thin cell enclosures. Algae are harder to kill than bacteria, and require approximately 100 times more energy for destruction (van der Westhuizen, 1995). Moreover, not all microorganisms that are exposed to UV irradiation are inactivated or killed instantly or simultaneously, but only a portion of the actual live quantity is inactivated at certain time

intervals. The irradiation dosage to achieve an average destruction of the different kinds of microorganisms present in a certain system will differ for each application. A specific degree of disinfection, but not sterilisation, can thus be achieved once the required dosage has been determined (van der Westhuizen, 1995).

The efficiency of UV-irradiation also depends on the quality of the water, especially in relation to the turbidity of the water, agglomeration of microorganisms and organic and inorganic dissolved substances. Suspended particles in the water absorb UV rays, and thus the effective dosage is reduced. Filtration prior to UV irradiation is therefore essential. The organisms at the centre of the bundle or lump of agglomerated organisms cannot be reached efficiently by the photons. Most organic components (i.e. humic acids) absorb UV irradiation, as do certain inorganic salts. The more substances there are present in the water the lower is the transmittance of the water for UV irradiation (van der Westhuizen, 1995).

The reduction of germs through UV irradiation is achieved when photons with a wavelength in the UV-C band or region, i.e. 200 nm to 280 nm, hit sensitive and biologically essential components of a living cell such as RNA or DNA molecules. These high-energy photons, most effective at a wavelength of 253,7 nm, destroy the vital RNA or DNA molecules, thereby killing the hazardous bacteria without the addition of chemicals. Upon adsorption of a UV quantum, two adjacent pyrimidine nucleobases can dimerize (e.g. formation of thymine dimers (Sakiß *et al.*, 1976). Such lesions can present the replication of the genome of a cell. The cell can then no longer propagate (reproductive cell death) although it may still be metabolically active. Any persisting metabolism is not a matter of concern because the ability of a pathogen to propagate is a precondition of its infectivity (von Sonntag and Schuchmann, 1992).

Ultraviolet (UV) radiation's germicidal properties had been recognized for many years before the first attempt was made to use UV for disinfection of water in 1919 (Oda, 1969). Before chlorination became the choice method in the United States, UV disinfection was used by at least four water authorities, the largest serving about 12 000 people (Yapijakis, 1978). UV irradiation was abandoned in favour of chlorination because of high operation costs and maintenance problems, and also because of the improved residual protection afforded by the application of chlorine. As research has developed cheaper, more efficient and more reliable UV light source, interest in the disinfection of drinking water by UV rays has been growing in recent years. More than 2 000 communal waterworks in Europe disinfect their drinking water by means of UV (Angehrn, 1984) and since 1984, over three hundred systems have been installed at municipal wastewater treatment plants in North America (Maarschalkerweerd *et al.*, 1990).

The rapid diffusion of UV disinfection technology in drinking water supply has always been hampered by the lack of residual. In distribution stations, however, this poses no serious problems as the interval between disinfection and point-of-use obviates the need for a residual. The main advantages of UV irradiation are related to the non introduction of foreign substances to the water and no formation of by products, low maintenance and easy operation, operating costs restricted to energy input and high operational security. As previously noted, a disadvantage is that no long-term residual germicidal effect occurs. As a consequence, effectivity cannot be verified immediately by measuring a residual level, as is the case with chemical oxidising agents. UV irradiation may be supplemented by a disinfectant (one of the chemical methods such as chlorine) with a remnant effect.

2.4.2.5 Hydrogen Peroxide

Hydrogen peroxide is a weakly acid, clear colourless liquid, miscible with water in all proportions. It is a physiological compound, which forms two stable and toxicologically inert decomposition products (water and oxygen). The formation of toxicologically relevant side-products seems improbable, a deterioration of taste and odour of the treated water does not occur and it would provide permanent protection (Strobel and Dieter, 1990). It is commercially available in aqueous solutions over a wide concentration range. In South Africa, hydrogen peroxide is produced by Alliance Peroxide under the trade name Hyprox.

The application of hydrogen peroxide as a disinfectant is not difficult, but the amounts required present a problem (Weber, 1972). Although it is a strong oxidant, it has proven a poor disinfectant. Moreover, three to four hours is the shortest practical time period for peroxide disinfection at concentrations of 1,5 - 5% (Yapijakis, 1978). According to South Africa 's Department of Health Clearance, hydrogen peroxide can be used to disinfect drinking water at a dosage (final disinfection stage) below to 17 ppm (100% hydrogen peroxide) and the residual in the consumable water must be below 5 ppm (100% hydrogen peroxide). At these concentration levels, hydrogen peroxide produces an evident taste that must be removed after the contact period.

Hydrogen peroxide itself is not reactive and therefore not bactericidal. It has to be converted to radicals, such as the hydroxyl radical ($OH^{\cdot}$), which react with cell components such as nucleic acids, proteins and lipids (Halliwell and Gutteridge, 1992). Hydrogen peroxide can act as a weak oxidising agent and will attack thiol groups of proteins or reduced glutathione. It can also react directly with some keto acids (Halliwell and Gutteridge, 1990). Most significantly, hydrogen peroxide will react with reduced iron or copper ions to generate hydroxyl radicals in the Fenton reaction (Cadenas, 1989). The generation of $OH^{\cdot}$ upon reaction

of Fe^{2+} or Cu with hydrogen peroxide (H_2O_2) might be consistent with the concept of site-specific damage (Cadenas, 1989). The reactivity of $OH^{\cdot}$ is due to its very high electrode potential. Because of the reactivity, the average diffusion distance of an $OH^{\cdot}$ radical is only a few nanometers and thus its effect on any biomolecule will depend largely on the location of its formation (Farr and Kogoma, 1991).

Hydrogen peroxide is a disinfectant with bactericidal and sporocidal properties (Toledo *et al.*, 1973), and is effective against a broad range of bacteria, including many which have become resistant to chlorine-based chemicals, such as *Pseudomonas aeruginosa* (Reimann,1992). The antimicrobial action of hydrogen peroxide may involve impingement of surface membranes through $OH^{\cdot}$ formation, oxidation of protein sulphydryl groups and double bonds. ` Bacterial surfaces exposed to hydrogen peroxide have also exhibited err17 April 1998osion of protective polysaccharide coatings and cleavage of cell chains (Baldry and Fraser, 1988). As it also has a sporocidal effect, hydrogen peroxide causes lysis of spores which includes damage to the spore coat, oxidative cortex hydrolysis or germination-like changes due to activation of cortex lytic enzymes (King and Gould, 1969; Foster and Johnstone, 1987).

Hydrogen peroxide can traverse bacterial membranes to gain access to the inside of the cell where it can cause DNA damage and oxidation of respiratory chain compounds (Hasset and Cohen, 1995). It can react with myeloperoxidase (or other peroxidases) in the presence of a halogen such as chloride to form HOCl and with HOCl to form the highly reactive singlet oxygen (Rosen and Klebanoff, 1985).

It has been reported that *E.coli* is killed in two ways by hydrogen peroxide (Imlay and Linn, 1986). The first mechanism (mode I killing) requires relatively low concentrations of hydrogen peroxide and active metabolism. It is suggested that DNA is the side of lethal oxidising damage. DNA-repair deficient cells are particularly susceptible to mode I killing.

The second mechanism (mode II killing) requires a higher concentration of hydrogen peroxide, and targets metabolically inactive cells, with DNA not to be excluded. Furthermore, mode II killing occurs in repair-proficient cells. This second mechanism appears to function on the basis that thiourea, a hydroxyl radial-scavenger, prevents mode I but not mode II killing by hydrogen peroxide. It has been reported that nicotinamine adenine dinucleotide (NAD) degradation and ATP depletion caused by hydrogen peroxide preceded cell death by several hours. In addition hydrogen peroxide has distinct targets with the exception of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) which is inactivated by both oxidants (Schraufstätter *et al.*, 1990).

Hydrogen peroxide is used to prevent and control bacterial activity. It has effective bactericidal action in swimming pools and controls biofouling. Since hydrogen peroxide systems require a higher residual concentration for effective sanitation than chlorine, it is uneconomical to consider it as a direct replacement in large-scale drinking water treatment. Realistic applications are found in recirculating systems and special cases where chlorine usage is undesirable or uneconomical, for example waters with a high ammonia content. However, hydrogen peroxide is used on a large scale in conjunction with other disinfectants such as UV irradiation and ozone, particularly the latter. In this case, it is known as the peroxone process which is becoming popular in many parts of the world. Hydrogen peroxide has been also used in wastewater treatment for many years, and its use as a disinfectant in conjunction with peracetic acid has been suggested (Anon, 1979).

In fact, hydrogen peroxide provides a long-lasting residual in water, which is stable towards sunlight and temperature changes. If a high peroxide residual is necessary for bacteria destruction, the final water can be run through an activated column which very effectively kills the peroxide residual. At higher concentrations hydrogen peroxide is capable of destroying

bacterial spores and can therefore be used to provide sterile conditions (Reimann, 1992). In addition, hydrogen peroxide may be used to destroy the chlorine taste (Yapijakis, 1978).

2.4.3 Resistance of bacteria to disinfectants

Studies have shown that bacteria can survive, multiply and develop resistance to disinfectants in the distribution systems(Ridgway and Olson, 1981, 1982, Olivieri *et al.*, 1985; LeChevallier *et al.*, 1987,1988). For many bacteria, the sensivity to disinfectants is lower after nutrient limitation (Pyle and McFeters, 1989; Matin and Harakeh, 1990; Stewart and Olson, 1992a). Limitation or depletion of nutrients affects cell surface properties and membrane functions such as proton motive force (Brown *et al.*, 1990) which may be involved in resistance mechanisms. Changes in the content and composition of lipids, lipopolysaccharides, purines and cations in the outer and cytoplasmic membrane may be related to susceptibility changes of gram-negative bacteria. The type of carbon source may also influence cell physiology and preservative sensibility. Al-Hiti and Gilbert (1980) found that *Pseudomonas aerogina* required three times more phosphate when glycerol was replaced by sodium citrate as the sole carbon source. In that study, the greatest changes in sensivity of preservatives were observed for cultures grown on a different carbon source rather than depletion of carbon nitrogen or phosphate.

Bacterial survival following chlorination has been observed in water in the presence of supposedly adequate residual concentration of disinfectant residual (LeChevallier *et al.*, 1984; Mathieu *et al.*, 1992). Experience has shown that maintenance of a chlorine residual cannot be relied on to totally prevent bacterial occurrences. It has also been suggested that chlorine-resistant bacteria may arise as a result of chlorination (Farkas-Himsley, 1964; Ridgway and Olson, 1982; Leyval *et al.*, 1984), although other investigators found no evidence for this

(Haas and Morrison, 1981).

Survival and regrowth of bacteria in water are now generally accepted as an undesirable side-effects of disinfection by chlorination (Olivieri *et al.*, 1985), although aftergrowth in oligotrophic environments such as potable water distribution systems depends on a threshold concentration of assimilable organic carbon (van der Kooij, 1992). Moreover, chlorine has been the most widely recognized cause of injury to coliform bacteria in drinking water (Bissonnette *et al.*, 1977; Beauchat, 1978; Camper and McFeters, 1979) although other biocides and factors such as low concentrations of metals (e.g. copper and zinc), temperature extremes and interaction with other bacteria (LeChevallier and McFeters, 1985; LeChevallier *et al.*, 1988) may also contribute. Both the chlorine and the time of exposure have been shown to influence the degree of injury in coliform organisms (Camper and McFeters, 1979; McFeters and Camper, 1983). McFeters *et al.* (1986) found that enteropathogenic and indicator bacteria become injured in drinking water with exposure to sublethal levels of various biological, chemical and physical factors.

Factors influencing resistance to halogen disinfectants may include cell aggregation, surface adhesion, spore formation and protective capsules (Ridgway and Olson 1982; Leyval *et al.*, 1984; Pyle and McFeters, 1989). Experiments have shown that surface attachment of bacteria provided the greatest increase in disinfectant resistance. Attachment of high nutrient grown, unencapsulated *Klebsiella pneumonia* to glass microscope slides afforded the microorganisms as much as a 150-fold increase in disinfection resistance (LeChevallier *et al.*, 1988). The age of the biofilm, bacterial encapsulation and previous growth conditions (e.g. growth medium and growth temperature) increased chlorine resistance from two- to 10-fold (LeChevallier *et al.*, 1988). The choice of disinfectant residual also influenced the type of resistance mechanisms. LeChevallier *et al.* (1988) found that disinfection by free chlorine was

affected by surfaces, age of the biofilm, encapsulation and nutrient effects, whereas disinfection by monochloramine was only affected by surfaces. This research showed that these resistance mechanisms were multiplicative (e.g. the resistance provided by one mechanism could be multiplied by the resistance provided by a second).

At present, little quantitative information is available regarding resistance mechanisms of bacterial regrowth to other biocides. But the main fact is that regrowth of bacteria or biofilm formation in drinking water distribution systems occurs at the expense of nutrients in water (van der Wende and Characklis 1990; Clark *et al.*, 1994). Experiments with ozone have shown that ozone may also react with organic material in source water to form nutrients that allow regrowth in the distribution system and increase the growth of biofilm on internal surfaces of distribution pipes (Clark *et al.*, 1994). After studying various combinations of ozone, chlorination and chloramination, Clark *et al.* (1994) reported that microorganisms increased in the bulk phase of the water and on fixed pipe surfaces when disinfectant residual disappeared. Dissolved organic carbon (DOC) decreased, providing a nutrient source for this biological growth. The biofilm cells are continuously seeded with planktonic organisms entering the system via breakthrough. Biofilm cells are generally less susceptible to disinfectants than planktonic cells. As a consequence, excessive viable cell numbers may accumulate in bulk from detachment of cells from the biofilm in areas where insufficient disinfectant residual is maintained (van der Wende and Characklis, 1990). Certain disinfectants may have properties more conducive to controlling biofilm populations. Less reactive, more persistent compounds, such as chloramine, maintain a higher disinfectant residual throughout the distribution system and may penetrate the biofilm more effectively, and thus, control biofilm organisms better than free chlorine (van der Wende and Characklis, 1990). The potential for bacterial regrowth has also been determined in UV-irradiated drinking water

(Hengesbach *et al.*, 1993). Results showed that as the low-molecular weight components of the total DOC determine the degree of its metabolic availability to bacteria, any UV degradation of DOC is linked to the question of bacterial regrowth potential (Hengesbach *et al.*, 1993). As disinfectant resistance may increase after nutrient-restricted growth, it is also possible that growth on different carbon sources may affect disinfectant susceptibility. If differences in sensitivity are related to particular carbon sources, certain physiological mechanisms may be implicated, e.g. repression of sensitivity by glucose which can be alleviated by the addition of cyclic AMP would suggest catabolite repression (Magasanik and Neidhart, 1987).

2.5 POTABLE WATER DISTRIBUTION SYSTEMS

The water distribution system is a network of pipes and appurtenances that delivers finished water to the consumers. The function of the distribution system is to deliver treated water to each consumer when desired, in needed quantities, at acceptable pressure and with unimpaired quality. Therefore the total water quality must be satisfactory upon delivery to the consumers and not merely when it leaves the treatment plant.

Water distribution systems differ widely in their size and distances, depending on the area which they serve, the size of the population and the distances the water is carried from its source. Transmission lines and pumps must be provided to move the flow from the source to the treatment plant and subsequently from there to the distribution system in the community. These differences in size and distances sometimes result in the degradation of the finished water quality. The closer the water can be brought to the house, the higher the domestic use and the lower the risk of contamination between source and user, thus the greater the potential

impact. Although the main objective is to bring to the consumer potable water of a quality that is identical to that of the water leaving the treatment plant, research has proved that water can deteriorate in the system between the point of treatment and consumption (Clark *et al.*, 1993).

The difficulties of maintaining water quality in transmission and distribution systems are legion. The problems of water quality control in distribution systems are of two kinds. Probably the most prevalent are taste, odour, and dirty water. The other problem is deterioration of bacteriological quality. In fact, the deterioration of water quality in a distribution system is usually attributed to three phenomena: 1) biofouling, due to the proliferation of microorganisms, which causes tastes, odours, and dirty water, with loss of carrying capacity of pipes and sometimes severe corrosion; 2) chemical and electrolytic corrosion, resulting in undesirable end products, such as metallic and brackish tastes as well as failure of hot water heaters and residence water piping; 3) appearance of coliform organisms in the distribution system, indicating recontamination of an otherwise safe water.

One of the most important distribution system characteristics for preventing biological or other contamination of water is the maintenance of constant internal pressure at all points. Most distribution systems may have leaks, and adequate pressure ensures that flow is always outward from the pipes. Low or zero pressures at any point could permit polluted water outside the pipes to enter them at leaks, contaminating the drinking water before its delivery to the consumer (Lamb, 1995).

It is also important to note that potable water resources may contribute somehow or other to the proliferation of bacteria in distribution systems. Impounded surface waters that are allowed to produce growth of algae and are not filtered will contain organic matter that could be used as a carbon source once the algae die in the distribution systems (White, 1992). Although filtration removes solid material, the soluble nutrients (such as proteinaceous matter,

nitrates, phosphates, sulphur, ferrous and manganese compounds and many others) still persist even in low quantities. Groundwaters are low in dissolved oxygen, a situation that contributes to anaerobic conditions, and are more likely to contain significant quantities of soluble iron and sulphur compounds, which provide energy for the two most offensive species of bacteria (iron and sulphur) that infest water distribution systems.

2.6 DETERIORATION OF POTABLE WATER QUALITY IN DISTRIBUTION SYSTEMS

Treated drinking waters generally contain low numbers of culturable heterotrophic bacteria. However, bacterial numbers can increase considerably through distribution systems (De Constantin *et al.*, 1986; LeChevallier *et al.*, 1987). The reason why bacterial numbers increase through distribution systems is not fully understood. There may be a number of contributing mechanisms which include contamination of the water due to mechanical, chemical and biological failure of distribution systems. This section deals only with bacterial contamination due to mechanical and biological failure of distribution systems.

2.6.1 Mechanical failures of distribution systems

There is increasing evidence that finished water may undergo substantial changes in quality while being transported through the distribution system to the consumer. Many mechanisms can introduce bacteria into drinking water during distribution. These include open reservoirs, enclosed reservoirs to which chlorine is not added, breakages due to new constructions that may disturb the existing distribution system, mains breaks (which may become an increasing problem as the distribution system ages), back pressure, dead-ends in

mains with stagnant water and lining materials that protect bacteria but may release bacteria into the drinking water when mains are disturbed (Rossie, 1975).

2.6.1.1 Reservoirs

Uncovered distribution reservoirs are recognized by public health and water utilities as a weak link in the protection of potable drinking water, prior to delivery to the consumer (Committee of Control of Water Quality, Report, 1983). Monitoring studies carried out by the Metropolitan Water District of South California demonstrated the degree of water quality change associated with the open storage of potable water. Although results showed no proven health hazard associated with the water stored in the open reservoirs selected during the study, they did show a deterioration in water quality of varying degrees within different reservoirs (Committee of Control of Water Quality, Report, 1983). In this case uncovered reservoirs may receive inputs of hazardous pollution from wild birds (especially gulls), flying insects and algal growth due to daylight (Jones *et al.*, 1978).

In South Africa, supply tanks were often found to be open at the top. Lids, when provided for tanks, were hardly ever in their proper closed position. Many instances of birds, rodents, insects, larvae, lizards and leaves having been found in storage tanks were reported from all over South Africa (Anon, 1961). Also, considerable quantities of dust were found to have penetrated into tanks, especially those without covers. It is obvious that the presence of decomposing organic matter will pollute the water, with a possibility perhaps of making it harmful to humans, depending upon the pollutants and the degree of pollution. Dust can be a carrier of numerous germs which could be pathogenic, and possibly eggs of intestinal parasites. There can be no doubt that water in storage tanks can easily be contaminated and often is.

Contamination of water after the first heavy rainfall can occur in roof reservoirs of apartment buildings which are not completely covered. *E. coli* and other coliforms of different origins are responsible for such contamination (Anon, 1984). Nevertheless, the fact remains that a major step in reducing overall system vulnerability to degraded water quality would be to cover and secure all open distribution reservoirs. Often if the tanks or reservoirs are underground, possibly in close proximity to sewage, cross contamination due to sewage overflow may occur. As the distribution system itself is the final barrier in preventing outbreaks of waterborne disease on the one hand, and may contribute to water quality deterioration on the other (Clark, *et al.*, 1993), it is therefore recommended that reservoirs for storage of potable water be situated and built in such a way as to lower the risk of sewage or any other contaminants from being introduced into the water distribution system (Augoustinos *et al.*, 1992).

2.6.1.2 Design and integrity of systems

Contamination of potable water can be induced via cross connection as well as via back siphonage. The integrity of the distribution system is often an important issue in systems with old pipe networks, cross connection potential and flow reversals. Thus every water system has its unique strengths and weaknesses and would require careful customizing and frequent monitoring (Geldreich, 1989). As noted in section 2.5, the maintenance of constant internal pressure at all points is one of the most important ways for preventing biological or other contamination of water in distribution systems. Reliability of the system, including constant maintenance of pressure, requires careful attention to the interconnection of pipes to provide closed loops. This allows the flow to approach any point in the network from more than one direction and helps ensure continued water supply and the maintenance of pressure everywhere in the event of pipeline failures.

Reduction of the water flow pressure in the system may result in back siphonage (flow against the normal flow direction of the water supply network). Therefore substantial microbial contamination may result, depending on the system lay out. The relatively slow flow rate along pipe walls facilitates a build-up of biofilms. Although chlorine will attack biofilms, it cannot prevent bacterial growth on pipe walls (LeChevallier, 1988). It is very important to recognize that as the population increases, so will the size and complexity of the system and the potential for distribution network contamination by cross connections and back siphonage (Geldreich, 1989).

2.6.2 Biological failure of distribution systems

In particular, a number of microorganisms, including filamentous iron bacteria and sulfate-reducing bacteria, have been linked to changes in iron concentration in water distribution systems (Starkey, 1945). Sulphate bacteria have been recognised as the most significant contributors to so-called Microbiologically Induced Corrosion (MIC). They have been branded as the most troublesome microorganisms to control because of their anaerobic growth potential underneath biological slimes or biofilms. This makes them inaccessible to chemical biocides which are commonly dosed into open and closed recirculating cooling water systems. They play a dual role in causing corrosion: firstly, they act as cathodic depolarisers and secondly, they produce corrosive hydrogen sulphide. The cathodic depolarisation is thought of as a biological catalyst, involving the enzyme hydrogenase present in the sulphate-reducing bacterial cell. Bacterial action reduces the sulphate present in the water to sulphide, using the hydrogen to form an iron sulphide precipitate (von Holy, 1987). Sulphate-reducing bacteria are associated with open pitting or gouging on stainless steel; on nickel and nickel alloys they are said to produce conical pits with concentric rings or steps; in cast iron

corrosion, graphitisation (removal of the metallic iron leaving a graphite skeleton) occurs due to the activities of sulphate reducers. Four main predisposing factors to microbiological corrosion by sulphate-reducing bacteria have been identified: high levels of available sulphate, anaerobic conditions, areas of low or no flow and the presence of biological slime or other deposits.

2.7 BIOFILM FORMATION IN POTABLE WATER DISTRIBUTION SYSTEMS

The biological activity in distribution systems for drinking water, and the formation of biofilms in pipes and on submerged surfaces of storage tanks and other constituent parts of networks have been thorougly studied from the microbiological point of view (Martin *et al.*, 1982; Bernhard and Liesons, 1988; Rogers *et al.*, 1994).

The occurrence of excessive bacterial populations in distribution systems is referred to as events of blooms in the literature. To describe the process contributing to the increase in microbial numbers between the point of entry into the distribution system and the final point of consumption, the water utility industry uses the terms "regrowth" and "aftergrowth". The term regrowth is used when disinfected injured bacteria originating at the point of water treatment are recovered. Therefore the expression "injured organisms" can be defined as a form of reversible injury. The term aftergrowth consequently denotes microbial growth in the distribution system. In water distribution systems, the bacteria can be introduced by the mechanism of breakthrough for bacteria passing through the disinfection process (or injured bacteria) in the treatment plant or by growth within the distribution system (Characklis, 1988).

In aqueous systems, bacteria are attracted to surfaces which they readily colonize (Marshall, 1992). The term "Biofilm" is used to describe a layer of microorganisms in an

aquatic environment held together in a polymetric matrix attached to a substratum such as pipes, tubercules or sediment deposits (van der Wende and Characklis, 1990). The matrix consists of organic polymers that are produced and excreted by the biofilm microorganisms and are referred to as extracellular polymetric substances (EPS). The chemical structure of the EPS varies among different types of organisms and is also dependent on environmental conditions. Biofilms sometimes form continuous, evenly distributed layers but are often quite patchy in appearance. Biofilms in water distribution systems are thin, reaching maximum thicknesses of perhaps a few hundred micrometers (van der Wende and Characklis, 1990). Biofilms from natural environments are generally heterogenous, frequently containing more than one distinct micro-environment. In addition, the film often contains organic or inorganic debris from external sources. Inorganic particles may result from the adsorption of silt and sediment and the precipitation of inorganic salts or corrosion products (van der Wende and Characklis, 1990).

Not only attached bacteria can be apparent in finished drinking water, but also unattached bacteria (also called "planktonic cells"). The contribution of planktonic bacterial growth in the water to the increase in bacterial numbers in distribution systems is difficult to assess because of the problem of separating it from bacteria coming from the pipe biofilm. The concentration of planktonic cells in the distribution system may increase as a result of planktonic growth and the contribution of biofilm erosion and biofilm sloughing (van der Wende *et al.*, 1989).

Bacteria attached to surfaces are ubiquitous in natural and man-made environments. The ability of many bacteria to attach to a variety of surfaces may often be a deciding factor in their survival, especially in low-nutrient environments. The level of bacterial contamination of the drinking water results from two phenomena: firstly, the influx of cells introduced by the water,

which have not been eliminated by the treatment process; and secondly, the growth and continual erosion process of the fixed biomass by the flow of water (Mathieu *et al.*, 1993).

Attachment is a first step in the process of microbial colonization of any surface and may initially limit the rate of the process (Escher and Characklis, 1988). Biofilm development is a result of successful attachment and subsequent growth of microorganisms on a surface. Under suitable conditions a biofilm develops, initially through the accumulation of organic matter on the metal surface which is then colonized by bacteria (Wolfaardt and Archibald 1990). Bacteria subsequently develop into a consortium of species within a polysaccaride matrix which imparts the slimy nature to the biofilm. As the slime layer thickens, micro-environmental changes take place within the biofilm as a result of the activities of the bacteria.

The formation of glycocalyx has been reported to be critical for cells to attach to exposed surfaces and survive shear forces (Ridgway and Olson, 1981). Moreover, cells which are continually intimately associated with interior walls of water mains may be less susceptible to chemical disinfection processes due to boundary layer effects and the secretion of extracellular coatings (Baylis, 1930). As a result, the extent to which microbes become associated with the inner surfaces of pipes or with suspended particulate matter within the water column could significantly enhance their survival and regrowth potential in distribution lines and reservoirs (Ridgway and Olson, 1981).

A number of investigators have provided evidence that bacteria colonize pipe surfaces with reported bacterial densities ranging from 10 to 1 x 10^9.cm^{-2} (LeChevallier *et al.*, 1987; Donlan and Pipes, 1988; Haudidier *et al.*, 1988; van der Wende *et al.*, 1989). Opheim *et al.* (1988) reported that flow changes and dropping of weights on pipe surfaces increased the numbers of bacteria in the water column.

Most studies of bacterial numbers in water distribution systems have used plate counts to estimate bacterial numbers. However, plate counts give only a limited estimate of the total bacterial population. And often coliforms in an injured state do not form colonies on M. Endo agar Les but may be recovered on a special medium developed for injured coliforms (LeChevallier *et al.*, 1983). Brazos and O'Connor (1989) found that epifluorescence microscopy total counts in the water entering a distribution system were high ($3.2 \times 10^5 .m\ell^{-1}$) and remained relatively constant through the distribution systems. The total counts for each of five sampling points correlated perfectly with the total counts of the final treated water, even though there was a seasonal variation in all the counts. According to these authors, bacteria originating from the source water or their successor cells (regrowth bacteria) made a much greater contribution to the planktonic population of the distributed water than bacteria originating from the pipe surfaces (aftergrowth bacteria).

2.7.1 Factors determining regrowth/aftergrowth

Bacterial regrowth or aftergrowth in water distribution systems is a common phenomenon which can result in the occurrence of high numbers of bacteria in drinking water. If penetration of bacteria from outside can be excluded, the presence of high numbers of bacteria can then be attributed to cell recovery after treatment or disinfection, regrowth on pipe surfaces and in pipe sediment, recontamination via open reservoirs and/or cross-connections (Power *et al.*, 1980) as noted in section 2.6. The small number of non-pathogenic bacteria which can survive the water treatment process or bacteria already present in the distribution system provide a seed which will multiply in the distribution system given the right conditions for growth. The conditions which will enhance growth include the nature and concentration of biodegradable compounds in the freshly prepared drinking water, the kind of piping

material, the water temperature, the residence time of the water in the distribution system and the disinfectants (chlorine).

2.7.1.1 Nature and concentration of biodegradable compounds

Microorganisms can be characterised on the basis of the compounds that they use as energy requirement (inorganic or organic hydrogen), carbon requirement (inorganic or organic carbon) and the kind of hydrogen acceptor (oxygen, nitrate, sulphate, carbon dioxide, or organic C).

Microorganisms giving most aftergrowth, and in particular the bacteria which contribute to the colony counts of drinking water are of a heterotrophical nature, which means that they use organic carbon compounds as a C-source and energy source. In addition, most of them use oxygen as a hydrogen acceptor. Approximately 50% of the absorbed organic carbon is converted into CO_2 (dissimilation) to satisfy the energy requirements of the cells and 50% are used for the new cellular material (assimilation). The compounds of C, N, and P are then required in an approximate ratio of C : N : P = 100 : 10 : 1. In general, compounds that may serve as a source of N are present in concentrations varying from a few tenths of a milligram to a few milligrams of N (mostly in the form of nitrate) per litre, and phosphates are present in concentrations between a few tenths of a microgram and a few hundred micrograms of P per litre (van der Kooij *et al.*, 1982).

In most environments, only a small fraction of dissolved organic carbon is actually susceptible to microbial attack, the rest consisting made of refractory organic compounds, generally called "humic substances", which are not available for bacterial growth (Barber, 1968; Ogura, 1977). Biodegradable organic matter can be broken down into two separate constituents both of which can be determined: Biodegradable Dissolved Organic Carbon

(BDOC) and Assimilable Organic Carbon (AOC).

Biodegradable Dissolved Organic Carbon can be defined as the fraction of the organic content of a water sample that can potentially be biologically oxidised during water treatment (Easton and Jago, 1991). The degree of bacterial regrowth is responsible for the overall content of biodegradable organic carbon which partly consists of particulate and dissolved organic carbon and is largely determined by the origin of the drinking water produced. It has been reported that water from lakes and reservoirs contains a much higher concentration of DOC than groundwater (Bernhardt and Classen, 1993). According to Bernhard and Wilhems (1985), treated water containing a DOC concentration below 1 mg.ℓ^{-1} is not prone to regrowth. Joret et *al.*(1991) reported that *E. coli, Klebsiella pneumonia* and *Enterobacter cloacae* can regrow in river water samples with a 3.2 mg.ℓ^{-1} initial DOC and a 1.4 mg.ℓ^{-1} initial BDOC, but are unable to multiply in finished water samples with initial DOCs of 0.4 mg.ℓ^{-1} and 0.8 mg.ℓ^{-1} and BDOCs of <0.1 and 0.1 mg.ℓ^{-1} respectively.

Assimilable Organic Carbon can be determined as the fraction of the biodegradable organic carbon that can be converted into new cellular material (assimilation). This can therefore give an indication of the regrowth potential of a water sample (van der Kooij et *al.*, 1982), but can measure only carbon converted to biomass. The concentration of easily assimilable organic carbon compounds is usually a small part of the dissolved organic carbon (DOC) concentration, particularly when the water has been exposed to biological processes for a long time, e.g. groundwater (van der Kooij *et al.*, 1982). van der Kooij (1992) regards a concentration of AOC in drinking water of 10 μg acetate-C equivalent.ℓ^{-1} or less as acceptable. At such low concentration, heterotrophic bacteria will not reproduce in drinking water. Under the given conditions, the number of bacteria remains below 100 CFU.mℓ^{-1}. In drinking water such characteristics can be regarded as "biologically stable". If the AOC concentration is to

be reduced to 10 $\mu g.\ell^{-1}$ by means of biological filtration processes, the dissolved organic carbon content in the raw water must be moderate.

Total organic carbon (TOC) has not been found to be a good predictor of bacterial regrowth (Rizet *et al.*, 1982) because the ratio of organic nutrients to TOC is not a constant (van der Kooij *et al.*, 1982; Hascoet *et al.*, 1986). In the system, the value of AOC is often 4 - 20% of the TOC (Kfir and Kott, 1989).

2.7.1.2 Plumbing materials

Pipe materials for water supply and distribution can generally be classified into one of three generic types, i.e. cementitious, metallic or plastic. A wide range of pressure pipe materials is available within these categories, and such materials are used in varying proportions in countries throughout the world. During the age of water conveyance, many types of material have been used. Often the material used was indigenous to the area and in early times included timber and clay. In today's world, the choice has been widened to include concrete, cast iron, steel and more recently plastic.

It is well known that there is a relationship between the material used for the construction of potable water distribution systems and the quality of water (Taylor, 1947; Le Chevallier, *et al.*, 1987; Rogers *et al.*, 1994). As stated previously, for growth to occur, necessary elements must be available to the organisms in its food supply or in the water, otherwise they cannot build new cell materials. Therefore, biofilms may be encouraged to develop on the surface of a plumbing material if that material is able to supply nutrients for bacterial growth. That is why in the United Kingdom, before plumbing materials are permitted for use, their influence on water quality is examined according to British Standard BS6920 (Anon, 1988). This ensures that the material does not contribute to poor water quality

by producing unacceptable tastes or odours, releasing chemicals or encouraging microbial growth.

There are a number of examples of how different materials result in the biofilm growth of different microorganisms. The hydrophobic-hydrophillic nature of the surface is known to affect the attachment of aquatic bacterial species to surfaces (Fletcher and Marshall, 1982). Experiments have shown that most pipe surfaces in distribution systems contain biofilms with bacterial densities as high as 10^9 bacteria.cm^{-2} (Olson, 1982).

Concrete pipes for conveyance of surface waters have been used in the United Kingdom, United States and Germany to some extent for almost 100 years, but it was only in the 1920s that their use was extended to sewers (Perkins, 1968). The involvement of microorganisms in the deterioration of concrete has been considered for a number of years, but only recently have attempts been made to understand the basis of microbial mediated decay of concrete. Moreover, the major portion of the work carried out to date on biofilm formation on concrete and biodeterioration of concrete pipes has been conducted on sewage systems and marine environments (Stott, 1986; Kulpa and Baker, 1990) as well as underground concrete (Tazawa *et al.*, 1994). The process is believed to be a complex one involving several different groups of microorganisms and either inorganic or organic acids. One type of microbial deterioration involves the sulphur cycle mediated by anaerobic and aerobic bacteria. In fact, *Thiobacillus* spp and Anaerobic Sulphate Reducing Bacteria (ASRBS) have been identified as the bacteria responsible for the degradation of concrete in cooling water systems, fire-water systems or sewer pipe (Sand *et al.*, 1984; Bibb, 1985; Kulpa and Baker, 1990; Poulton and Nixon, 1992). An investigation conducted by Poulton and Nixon (1992) showed microbial attachment of bacteria on concrete while no deterioration effect was noted.

Plumbing material such as wooden pipes, cast iron or steel pipes with a protective lining made either of bitumen or coal was used for the construction of potable water distribution systems until the 1950s. During this period these materials could influence the microbial quality of potable water (Taylor, 1947; Burman and Colbourne, 1976). Cast iron substrata, in the form of a cylinder, was directly installed into the drinking water mains through a corporation valve in order to examine biofilm formation under *in situ* conditions (Donlan *et al.*, 1994). The study revealed biofilm formation at a steady state within 30 days under warm water conditions (15 - 25 °C).

Historically, mild steel protected with thin-film organic coatings has been used as the material for fabrication of pipework in industrial water systems. Research work carried out by Poulton and Nixon (1992) reported that uniform microbiological attachment occurred on mild steel, epoxy coated mild steel, mortar and concrete although no deleterious effect on the concrete and mortar was noted. It has been widely reported that in an aqueous system, microorganisms will attach themselves to available surfaces, forming biofilms or biofouling deposits. Discrete colonies within the biofilms or biofouling deposits on metal or cement structures can result in degradation of material. This can result in the deterioration of water quality in the distribution system.

Copper has also been used as piping material for distribution systems. Scanning Electron Microscopy (SEM) has revealed two distinct layers: a layer of extracellular polymeric substance in direct contact with the copper and a second layer consisting of bacteria which were not embedded in the extracellular polymeric substance, in the direction of the luminary. Some of the microorganisms of this layer showed holes. Bacteria in direct contact with disinfection solution showed a rough thick end surface indicating the existence of capsule substances (Exner *et al.*, 1983).

From the 1950s, synthetic materials were used in the construction of potable water distribution systems. This material was used as a positive control surface for biofouling. However, it was not known whether the surface would encourage the growth of pathogens including *L. pneumophila* (Rogers *et al.*, 1994).

Polyethylene (PE) and Polybutylene (PB) have been adapted for use as pipe and tubing material for fluid conveyance (Crowson and Chambers, 1985). Because of their many advantageous properties, such as resistance to chemicals and corrosion, electrical non-conductivity, competitive cost, flexibility and ease of handling, storage and installation, these piping materials have gained widespread acceptance and use within the water industry (Crowson and Chambers, 1985). Despite their many advantages, they also contribute to biofilm formation in drinking water. A study conducted by Rogers *et al.* (1994) showed that the materials commonly used in the construction of water systems encourage the development of biofilm.

2.7.1.3 Residence time and residual chlorine

Water quality varies in distribution systems in accordance with residence time and distance from the treatment plant. Water quality is affected by the age of the water in the system. Examples of this include the decrease in chlorine residuals that occurs in most systems and the formation of trihalomethanes. Both of these phenomena are functions of time. Using a booster chlorination, Gibbs and his co-workers (1990) observed a quick reduction in bacterial number, but regrowth occurred when the residual chlorine was quickly decreased with retention in the distribution system. Lechevallier *et al.* (1980) noted that dead-end distribution lines in which no free residual chlorine could be detected contained 23 times the number of standard plate count (SPC) compared with distribution lines with a free chlorine residual. van der

Wende and Characklis (1988) revealed that biofilm accumulation varied with time and distance from the inlet in the RotoTorque system.

2.7.1.4 Temperature

The ability of bacteria to grow and survive over a wide range of temperatures has been demonstrated. LeChevallier et *al.* (1980) noted that several trends were apparent in the seasonal distribution and species diversity of the SPC population present in distribution water. For example, there was greater species diversity in the warmer period than during the cold winter months. After incorporating *L. pneumonia* into both the planktonic and biofilm phases of the model system at 20, 40 and 50 °C, Rogers and his co-workers (1994) noted the overall trend of growth was temperature related although this growth was also related to piping material.

2.7.2 Monitoring of biofilms in the distribution systems

The development of microbial biofilms in flowing-water systems has been the subject of many investigators, usually to elucidate a large number of undesirable effects concomitant with growing biofilms. This results in increased flow resistance, induction and acceleration of corrosion processes, decreasing heat exchanger capacity, and clogging of filters. Therefore studies have been directed at monitoring adhesion processes, biofilm formation and the subsequent advantages of this mode of growth. The following have been designed for the *in situ* development and monitoring of sessile microbial population in water distribution systems.

2.7.2.1 Robbins device (RD)

The Robbins device, named after its originator (Costerton and Robbins, 1980), consists

of a metal stud that can be inserted and withdrawn through a small cylinder. The design of the cylinder allows it to be inserted directly into a pipe or a bypass system, so that the surface of the stud makes up a part of the metal surface available for colonization in a system to be sampled. An equally important finding was that the construction material did not significantly influence the colonization.

2.7.2.2 Modified Robbins device (MRD)

Also developed by Robbins, the MRD has been used extensively in conjunction with batch cultures and used to study bacterial colonization and biofilm formation (Ruseska *et al.*, 1982; Nickel *et al.*, 1985). The design was constructed for the purpose of colonizing a large number of surfaces in a reproducible manner for multi-sampling in tabular devices such as catheters and pipelines. However, the system has been criticised because of the lack of growth rate control of planktonic bacteria colonizing the surface (Brown *et al.*, 1988).

2.7.2.3 Chemostat-coupled modified Robbins device

The combination of a chemostat and an MRD was designed in order to provide a large number of sample surfaces for monitoring both the formation and control of biofilms over extended periods of time. This technique proved to be successful for studying bacterial adhesion and biofilm formation in tubular devices by bacterial population at controlled and low growth rates (Jass *et al.*, 1995).

2.7.2.4 Pedersen device

Named after its originator, Pedersen (1982), the Pedersen device consists of microscope cover slips (60 x 24 x 0.15 mm) fitted into acrylic plastic holders forming two parallel test

piles, each with room for 19 slips. The Pedersen device was developed in order to study microbial biofilms in flowing-water systems with special reference to the flow conditions in electrochemical concentration cells. The absorbances of the slides are measured at 590 nm and plotted against time to give microbial biofilm development.

2.7.2.5 Spectrophotometric monitoring of biofouling

Apparatus such as the Robbins and the Pedersen devices have proved useful in the study of undisturbed portion of the biofilm. Unfortunately, these techniques rely on the culturing of the organisms after removal of the biofilm from the metal studs, or a scanning electron microscopy investigation of the biofilm (Costerton *et al.*, 1987; Brözel 1990). Counting bacteria by making use of culture media is not only time consuming, but also the growth of the diversity of bacteria species involved in biofouling cannot be supported by the composition of a single culture medium. Because of this, the modified Pedersen device involving the spectrophotometric monitoring of biofouling was developed by Jacobs *et al.* (1996).

A flow-through glass tube allowing a continuously circulating batch culture was designed to study biofouling spectrophotometrically. The direct absorbance measurements of bacteria attached to a glass tube directly quantifies the rate of colonization. Spectrophometry has proved to be an easy, inexpensive and reliable alternative to techniques requiring laborious counting of microorganisms, e.g. the 4',6 - diamino - phenylindole (DAPI) technique, for quantification of biofouling (Jacobs *et al.*, 1996). In addition, the adhesion of bacteria on the glass surface can be seen with the naked eye. Because of these advantages the modified Pedersen device involving the spectrophotometric monitoring of biofouling was used in the present study.

2.7. 2. 6 *Some novel approaches to monitoring biofilm population in potable water systems.*

A number of new monitoring techniques are currently under investigation, and include: reflectance using fibre optic sensors, differential turbidity measurement, courier transforming infrared spectrometry (FTIR), resonance raman spectrometry and electrochemical monitoring. The main problem with most of these techniques is the detection level which is superior to 10^5 cells/ml.

In any process, it is important to monitor possible contamination so that corrective action can be instigated as rapidly as possible. The ideal monitors should be non-destructive so that they will not inhibit or damage the biofilm. Thus the sensors will provide a true picture of the problem. If the biosensors can be placed in supply lines or in the system ahead of purification/disinfection systems then treatments can be modified to maintained the desired level of purity. Most of these monitoring techniques, reviewed by Mittelman (1995), are discussed briefly in the present section.

Reflectance using fibre optic sensors - Optical fibres can be incorporated into water systems. There are small, flexible, electrically as well as chemically passive and can be placed in suitable sites. Once immersed into a water system, biofilms will develop not only on the walls but also on the tip of an optical fibre. In many cases, this an unwanted effect, e.g., if a fibre is supposed to measure parameters of the water phase. Then, it is referred to as "fouling" (Cloete, 1997). However, this can be used as an indication for the development of fouling layers. The sensor tip can be integrate into a surface to be monitored. The crucial part of the sensor system is the probe head. It has to be able to transmit light of a chosen wavelength to the surface to which the biofilm is attached, illuminate a well defined measuring area, collect the reflected light and transmit it to a photodector without changing its light upon

the measured intensity of the backscattered light and display a long term stability. This technology is in the early stages of development, but potentially very useful.

Differential turbidity measurement device (DTM) - Commercial turbidity measurement devices are based on the measurement of light reflected by particles in suspension. The data can be overlayed by signals resulting from particles deposited on the measuring window of the device. Again this is fouling effect (Cloete, 1997). It can be directly measured if the signal of a device with a clean surface is subtracted. The deposit is developing on both the light source and the detector windows, which increases the intensity of the signal caused by the deposit. As the window material is glass, it valid that deposition on glass is measured and that at least in the first stages of deposit development a non-representative surface is monitored. Thus, the principle seems to be more suitable for systems with a high fouling potential like paper production. In such cases, the first layers of the deposit will mask the original surface, quenching selective effects between material and microorganisms or particles.

FTIR - Courier transforming infrared spectrometry (FTIR operated in the attenuated total reflectance mode) allows the detection of bacterial biofilms as they form on a crystal of zinc selenide or germanium (Nichols *et al.*, 1985). The amide stretching of the proteins and ether stretch of the carbohydrates are clearly detectable when bacteria attach to surfaces. Often the nutrients attracted to the surface from the bulk phase are also clearly indicated by their infrared fingerprint. If the water system contained IR 'windows', biofilm formation could be monitored and an indication of the chemical nature of the contamination would be apparent. Periodically, some mechanism for cleaning the 'window' would need to be provided. A detection limit of c. 5 x 10^5 cells per cm^2 *Caulobacter crescentus* cells was obtained using a germanium substratum monitored in attenuated total reflectance (ATR) flow cell (Nivens *et al.*, 1993a). This non-destructive technique holds promise as an online monitoring tool for bacterial

colonization in purified water systems.

The technique also provides information on the nutritional status of the biofilm microbes as the formation of the endogenous storage polymer poly ß-hydroxyalkanoate (PHA) can be detected in the spectra. PHA forms during conditions of unbalanced growth wen some critical shortage prevents bacterial cell division (Mittelman, 1995). This ability to monitor biomass signature compounds may be of particular utility in assessing chemical treatment efficacies against purified water system foulants.

*Resonance Raman spectro*scopy - Laser which provide high power at frequencies between 190 and 269 nm can differentially activate components in the bacterial cell walls and cytoplasm and produce a shift in the wavelength of the scattered photons which can be detected as a resonance Raman spectrum (Manoharan *et al.*, 1990). With a highly focussed pulsed laser it is possible to detect a single cell. Selective excitement of bacterial cellular components can be achieved using a tunable laser. By progressively shortening the incident wavelength, nucleic acids, calcium dipicolinate in endospores, tyrosine and tryptophan in the proteins, and the other aromatic amino acids can be distinguished by their vibronic fingerprint spectra. The patterns are specific for groups of bacteria and can be utilized for identification . Information on the nutritional status of the bacteria can be obtained (Dalterio *et al.*, 1987) as they pass through UV transmissive capillaries.

In the presence of precious metal microstructures, the electron plasmon can markedly potentiate the Raman scattering of molecules intimately associated with the surface in surface enhanced Raman spectroscopy (SERS). In theory, it should be possible to use this technique to detect non-destructively the attachment of bacteria or bacterial derived products to the metal microbases.

Fluorometry - The use of fluorometry for on-line monitoring of biofilm development

has been described for monitoring of antifouling (Mittelman *et al.*, 1993) and uncoated, stainless steel surfaces (Khoury *et al.*, 1992). Reduced DNA compounds along with the aromatic amino acids (for example tyrosine and tryptophan) can be monitored as indicators of cell energy charge and biomass, respectively. As with FTIR and quartz crystal microgravimetry (described below), this technique holds promise for application to monitoring of purified water system fouling. Detection limits remains relatively high, however, in the range of ca. 10^5 cells per cm^2 .

Electrochemical monitoring - If electrically isolated electrodes can be incorporated into a stainless steel system it is possible to monitor biofilm formation and persistence electrochemically. Electrochemical methods have, for the most part, been applies towards monitoring of microbial influenced corrosion of metallic substrata. However, potential exists for application of electrochemical monitoring to purified water system biofilm detection.

There are two types of electrochemical monitoring systems. In the first system, the potential between a standard electrode and a working electrode is monitored. The open circuit potential becomes more negative as the biofilm forms on the electrode surface. The potential shifts as metabolic activity and/or biomass associated with the biofilm changes (Mittelman *et al.*, 1992).

The effects of the biofilm may also be monitored on the same coupon using a frequency spectrum of the noise (electrochemical noise). Perturbation response methods may also be utilized to show changes in the surface chemistry as a result of biofilm activity. In electrochemical impedance spectroscopy, a small sinusoidal potential is induced in the system over 5 decade frequency range and the induced changes in current and phase shift angle determined (Dowling *et al.*, 1988).

The presence of material of intrinsic viscosity significantly different from that of the water can be detected as a change in frequency of a vibrating quartz crystal microbalance (QCM) technology. The surface can also be used as an electrode to produce both the microbalance and electrochemical signals (Deakin and Buttry, 1989). This technique was applied to monitoring biofilm formation in a simulated purified water system for the space station project. Detection limits of c.10^5 cells per cm^2 were obtained using an AT-cut quartz crystal (Nivens *et al.*, 1993b).

Significant insight into the nature of contamination of ultra pure water can be gained from destructive analysis of biofilms or filter retentates recovered from the system. The most important contaminants of pure water systems are Gram negative bacteria lipopolysaccharide (LPS) products from these organisms. Novitsky (1984, 1987), has reviewed the significance and detection of endotoxins in semiconductor grade and other ultrapure waters. Lack of sensitivity appears to be the greatest problem for the semiconductor industry. It is unlikely that bacterial LPS contributes significantly to the total oxidizable carbon load in purified water systems (Mittelman, 1995).

2.8 TECHNIQUES AND MEDIA FOR ENUMERATION OF BACTERIA FROM POTABLE WATER DISTRIBUTION SYSTEMS

The classical evaluation of methods that rely on the culturing of organisms suffer from several serious deficiencies. Organisms from extreme environments (e.i ultrapure water) are notoriously difficult to culture and the standard plate s will not give an accurate estimate. Not only do the microorganisms not grow out readily on attempted isolation, but the growth of may oligotrophic organisms is extremely slow. This delay is clearly not acceptable for process

waters in which continual is essential.

Several of the classical microscopic techniques, lack sensitivity. They require at least 10^3 organisms per ml in small samples for reliable detection (Mittelman, 1995). Concentration by centrifugation or filtration with vital staining and microscopic examination requires that the water systems be sampled (thereby creating a potential contamination), is time consuming, and requires trained operators for monitoring. Usually type (s) of bacteria involved in the contamination are not readily determined with the microscopy examination. It is possible to identify specific microbes using fluorescent labelled probed. Gene probes for ribosomal RNA which can differentiate between pro- and eucaryotes and the gamma, beta and alpha group of bacteria has also now found its way into biofilm studies. Data generated remains to be of academic importance in terms of biofouling control. However, these techniques require that the organisms commonly contaminating the system be known so that antibody or gene probes can be developed. Despite these efficiencies, the recoveries associated with direct counting techniques are often significantly greater than those obtained with cultural techniques.

Direct count techniques with applications for bacterial biofilms in purified waters have been described by a number of investigators (Mittelman *et al.*, 1987; Newby, 1991; Patterson *et al.*, 1991). Rodriguez *et al.* (1992) have described a direct count technique employing two different fluorochromes for assessing *in situ* bacterial activity. Yu and McFeters (1993) employed this technique to assess bacterial viability on stainless steel surface in nutrient limited environments. Both the total and the respiring fraction of attached bacteria may be measured using this technique. Adams *et al.* (1991) described the application of direct count techniques in ozonated waters. These techniques may prove particularly useful in estimating populations of damaged and viable, but non-culturable bacteria in biofilms. CTC staining, together with epifluorescence microscopy is a technique used successfully and worthwhile investigating, for

determining live biomass in water systems (activated sludge/biofilms etc) with a short space of time. ATP monitoring for biomass determination is gaining popularity and already forms an integral part of an on-line monitoring device used in the Netherlands for biofilm formation. The late ATP monitoring systems have many improvements over the older systems and have become useful tools for monitoring microbial biomass in natural systems. The following parts of this section describe specific methods and media used for enumeration of specific group of bacteria from potable water samples as well as from biofilms.

2.8.1 Coliform bacteria

Two methods for the microbiological analyses of potable water are specified by the 17th edition of Standard Methods for the examination of Water and Wastewater (1989): the Most Probable Number (MPN) method and the membrane filter (MF) method. The MF technique has gained wide acceptance because of its simplicity, rapidity and precision. This method also gives definitive results. However, factors such as membrane filter type (McFeters and Stuart, 1972; Sladek *et al.*, 1975), high numbers of noncoliform bacteria (Clark and Pagel, 1977; Geldreich *et al.*, 1978; LeChevallier *et al.*, 1980; Clark, 1980), and turbidity (Geldreich *et al.*, 1978; LeChevallier *et al.*, 1981) may severely influence the sensitivity of the procedure. Moreover, the medium specified for use with the membrane, m-Endo agar or m-Endo LES agar, has several shortcomings, including low recoveries of injured coliforms (McFeters and Stuart, 1972; McFeters and *et al.*, 1982, LeChevallier *et al.*, 1983), poor detection and differentiation of coliforms from noncoliforms (Schiff *et al.*, 1970; Dutka, 1973; Evans *et al.*, 1981) . Therefore , a new medium. termed m-T7 agar, has been developed for the improved recovery of injured coliforms from drinking water (LeChevallier *et. al.*, 1983) . This new membrane filter medium has been found to be superior to m-Endo agar, especially for the

isolation of injured coliforms from drinking water (LeChevallier *et al.*, 1983). The MF procedure for faecal coliforms generally includes m-FC agar medium. However. m-T7, designed to improve recoveries of injured total coliforms, was also evaluated for its effectiveness as a faecal coliform medium (LeChevallier *et al.*, 1984). When compared to m-FC agar, verification rates for faecal coliforms isolated on m-T7 agar averaged 89.0%, whereas verification rates for m-FC agar averaged 82.8%. According to the authors, both media isolated similar faecal coliform population.

2.8.2 Heterotrophic plate count

The heterotrophic plate count may be determined by pour plate count, spread plate, or membrane filter method (APHA, -AWWA-WPCF, 1989). The pour plate method's greatest asset is its simplicity and ease of use. However, it has two drawbacks that make it the least desirable method for enumerating bacteria in water. Bacteria in low-nutrient aquatic environments are generally considered to be physiologically stressed. As such, they have been shown to be susceptible to secondary stress caused by warming due to the melted agar tempered to 43°C to 46°C (Klein and Wu, 1974). Klein and Wu (1974) showed that warming either by melted, tempered agar or no pre-warming agar spread plates to 45°C caused significantly decreased recoveries of bacteria compared to counts obtained by spread plate method. They concluded that pour plate method is not suitable for enumeration of heat-sensitive and possibly nutritionally stressed heterotrophic bacteria in water. Moreover, bacteria are submerged in the agar in the pour plate method and the colonies tend to be smaller and do not show colony morphology characteristics that may be useful in identification.

The spread plate procedure is no more difficult than pour plate, but does require preparation of plates ahead of time and the use of a sterile bent plates spreading rod to

distribute the sample over the surface of the agar. The procedure is more tedious than the pour plate procedure. The spread plate for heterotrophic plate count provides conditions that are more conducive to growth of bacteria in water pour plate method. Consequently, the spread plate almost invariably yields higher colony counts than does the pour plate method. Since all bacteria in the sample are planted on the surface of the agar medium, their growth is not constrained by being surrounded by the agar. Thus, it is easy to observe differences in colony shape, texture, pigmentation and other characteristic. A disadvantage that the spread plate method shares with the pour plate method is that the maximum sample volume that can be analysed is 1.0 ml using conventional culture dishes.

Heterotrophic plate count provides an approximate enumeration of total numbers of viable bacteria that may yield useful information about the water quality and may provide supporting data on the significance of coliform test results (APHA-AWWA-WPCF, 1989). Bacterial plate count examination of drinking water is used as a measure of the effectiveness of water treatment processes such as coagulation, filtration, and disinfection and as an indication of distribution system cleanliness, rather than for assessing safety or potability.

Plate count agar is presently the recommended medium for the standard bacterial plate count of water and wastewater. However, plate count agar does not permit the growth of many bacteria that may be present in treated potable water supplies. A new medium, termed R2A agar, has been developed for use in heterotrophic plate count analyses of bacteria isolated from potable water samples (Reasoner and Geldreich, 1985). Results from parallel studies with spread, membrane filter, and pour plate procedures showed that R2A medium yield significantly higher bacterial counts than did plate count agar (Reasoner and Geldreich, 1985). As a tool to monitor heterotrophic bacterial populations in water treatment processes and in treated distribution water R2A spread or membrane filter plates incubated at 28°C for 5 to 7

days is recommended. These conditions provide adequate time for pigment development and for growth of slow-growing bacteria (Reasoner and Geldreich, 1985).

2.8.3 Total bacteria

Epifluorescence direct counting is one of the important methods for the evaluation of bacterial counts in naturals waters as well as in biofilms. Early studies were done with acridine orange [3,6-bis (dimethylamino) acridium chloride], but this fluorescent dye was replaced by 4', 6-diamidino-2-phenylindole (DAPI), which has more stable fluorescence (Porter and Feig, 1980). The DAPI is believed to be very specific for DNA and it thus used to count total (including nonviable and viable but nonculturable) bacteria in water (Porter and Feig, 1980).

DAPI is generally believed to bind to DNA preferentially at AT-rich regions within the minor groove of B-DNA in solution (Manzini *et al.*, 1983; Larsen *et al.*, 1989; Barcellona and Gratton, 1990) . Footprinting experiments at low binding ratios indicate that DAPI prevents the cleavage of DNA at AT sequences of 3 or 4 bp, implying that DAPI binds to sites with the poly [$d(G\text{-}C)_2$], presumably in the major groove of the polynucleotide. Matsuzawa and Yshikawa (1994) suggest that DAPI molecules interact with DNA via the phosphate groups along the DNA strand. The DAPI bound to these sites seems to be governed by the pH of the medium. DAPI exists as a protonated form with a fluorescent emission maximum near 490 nm and as an unprotonated form with a fluorescent emission maximum at 440 nm (Szabo *et al.*, 1986). Hence, the maximum fluorescence of the DAPI is between 440 nm (blue) and 490 nm (blue-green), depending on wether the DAPI is bound inside or outside the minor groove of DNA (Kubista *et al.*, 1987; Barcellona and Gratton, 1990, Mazzini *et al.*, 1992).

2.9 MICROBIAL ECOLOGY OF BIOFILMS

Biofilm serves as a focal point where bacterial and protozoal populations interact. The microbial flora of a finished water reflects the microbial flora characteristics of the raw water source (Taylor, 1958; Victoreen, 1970). These microbial flora include saprophyte, mycobacteria, *Salmonella*, zoogleal strains, bacteriophages, amoebas, rotifers, nematodes, algae, fungi and actinomycetes (Willis, 1957). The pioneering bacterial population modifies the surface conditions to enable bacterial succession to take place.

Potable water of good bacteriological quality is generally regarded as that containing less than one total coliform per 100 ml of water sample. Therefore the number of many other microorganisms common to the flora of finished water far exceeds the number of coliform. Although no single medium, temperature, or choice of incubation time will ensure recovery of all organisms present in water, selected members of the bacterial population can be measured accurately and their numbers related to water treatment efficiency, quality deterioration in the reservoir or distribution lines, or possible suppression in coliform detection.

Although the detection of coliform bacteria is the primary concern in potable water quality measurements, attention has been directed at controlling the general bacteria population (Geldreich *et al.*, 1972). A large variety of different heterotrophic bacteria ranging from potentially pathogenic bacteria to coliform bacteria to different types of aquatic bacteria have been isolated from the biofilm both in chlorinated and non-disinfected water distribution systems (LeChevallier *et al.*, 1987; Colbourne *et al.*, 1988). The presence of *E.coli, Pseudomonas Aeromonas, Artrobacter, Caulobacter, Klebsiella Bacillus, Enterobacter, Citrobacter, Acinetobacter, Prosthescomicrobium, Alcaligenes, Serratior* and *Actinolegionella* has been reported (van der Kooij and Zoetemann, 1978; Martin *et al.*, 1982; Olson, 1982;

Olivieri *et al.*, 1985; Herson *et al.*, 1987; Ridgway and Olson, 1981; Schindler and Metz, 1991). The iron bacterium *Galionella* has been observed both in water samples and attached to pipe surfaces by the scanning electron microscopy (Ridgway and Olson, 1981). Other bacteria generally associated with distribution system biofilm include *Moraxella*, *Achromobacter, Flavobacterium* (LeChevallier, *et al.*, 1987), *Cytophaga,* iron bacteria and sulphate reducers (Touvinen *et al.,* 1980; Ridgway and Olson, 1981). Attachment of pathogenic bacteria such as *Yersinia enteroliticor*, *Salmonella typhimurium* and enterotoxigenic *E. coli* to particles of granular activated carbon has been noted in water distribution system (Camper *et al.*, 1985).

CHAPTER 3

MATERIALS AND METHODS

3.1 WATER SOURCES

Surface water and groundwater were used as test waters. Partially treated surface water was collected from a water treatment plant (Rand Water Board). The partial treatment included: coagulation (70 mg. ℓ^{-1} lime, 2.2 mg. ℓ^{-1} sodium silica followed by 1.5 mg. ℓ^{-1} $FeCl_3$ for source water 1 and 80 mg.ℓ^{-1} lime, 2.5 mg.ℓ^{-1} sodium silica followed by 1.5 mg.ℓ^{-1} $FeCl_3$ for source water 2), flocculation, sedimentation and filtration. Before filtration, the pH of the raw water was adjusted from 11 to 8.4 with CO_2. Partially treated surface water was used as a control during these experiments. Groundwater was collected from wells in a rural area. Untreated groundwater was used as a control during these experiments.

3.2 SAMPLING OF TEST WATERS

Test waters were collected in 4 x 100 ℓ clean polyethylene drums, and transferred into 6 x 50 ℓ polyethylene drums. Before use, the drums were washed with detergent solution, rinsed with hot water and distilled water, and then disinfected with 5 mg. ℓ^{-1} chlorine for 24 h, and dechlorinated with sodium thiosulphate (ca 17.5 mg.ℓ^{-1}) to neutralize any residual disinfectant. Sterile distilled water was then passed through the drums for 12 h, after which a total

microbiological count was done in order to check the sterility of the drums.

3.3 LABORATORY-SCALE UNIT

The laboratory scale unit consisted of 6 x 50 ℓ polyethylene drums (Fig. 3.1). Each container was used as a batch reactor.

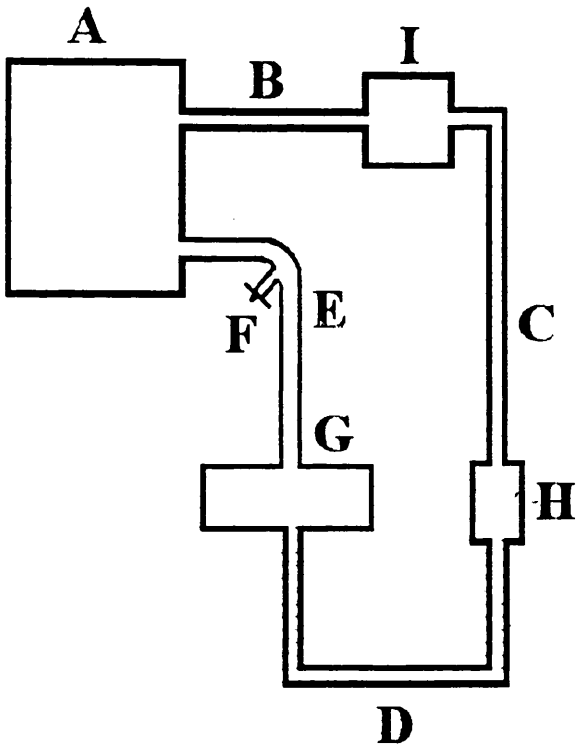


Fig. 3.1. Schematic diagram of the laboratory scale unit: (A) a sterile drum, (B), (C), D), (E) latex tubing to allow water circulation, (F) a tap to allow sampling, (G) Pedersen device, (H) flow- through glass tube, and (I) a peristaltic pump.

The batch reactor was attached to a peristaltic pump, which in turn was connected in series to a Pedersen device and a flow-through glass tube (Fig. 3.1). Water circulation (the control and the disinfected waters) was facilitated at a flow rate of 2.8 $\ell.h^{-1}$.

3.4 DISINFECTION PROCESSES

No neutralization of any of the disinfectants used for the source water, with the exception of samples on which microbiological analysis were conducted, was done during the experimental period.

3.4.1 Chlorination

Chlorine stock solution was prepared from sodium hypochlorite (commercial bleach). To obtain a 100 $mg.\ell^{-1}$ concentration, 2 mℓ sodium hypochlorite was diluted in 1 ℓ distilled water. This stock solution was standardized before use to determine the correct chlorine concentration. Standardization of chlorine was done according to Wium and Coetze (1985). Chlorinated water was produced by addition of stock solution to give a 2 $mg.\ell^{-1}$ and 2.5 $mg.\ell^{-1}$ free chlorine solution, for source water 1 and 2 respectively.

3.4.2 Chloramination

Chloramination was accomplished using 2 $mg.\ell^{-1}$ (for source water 1) and 2.5 $mg.\ell^{1}$ (for source water 2) monochloramine solution. The monochloramine solution was prepared by reacting the chlorine solution with a NH_4Cl solution at a NH_4:Cl mass ratio of 4 : 1. When preparing a monochloramine solution, the ammonium sulphate [$(NH_4)_2SO_4$] (Protea Laboratory) was added first and then the sodium hypochlorite (commercial bleach). The concentration of monochloramine was determined after 24 h.

3.4.3 Ozonation

Ozonated test water was produced by using an ozonator (Degremont) consisting of a

tube-type oxygen-fed water-cooled unit. Ozonation was carried out in a batch bubble column reactor with a volume of 1 ℓ . The ozone was applied at 2.07 mg.ℓ^{-1} for source water 1 and 2.65 mg.ℓ^{1} for source water 2, for a contact time of 5 min.

3.4.5 UV Irradiation

An ultraviolet irradiation unit (Willand UV systems, represented in South Africa by UV Water Systems (Pty) Ltd) able to disinfect water at the flow rate of 6 ℓ/min, at a 107 m Ws/cm^2 dosage, was used.

\

3.4.6 Hydrogen peroxide

The water was disinfected with hydrogen peroxide at a concentration of 20.70 mg.ℓ^{-1} for source water 1 and 17.73 mg.ℓ^{-1} for source water 2 (50% H_2O_2, Alliance Peroxide).

3.5 MICROBIOLOGICAL ANALYSES

3.5.1 Water sample analyses

Sample frequency is indicated in the results and was the same for all experiments. Chlorinated, chloraminated and ozonated samples were collected in sterile bottles previously rinsed with MilliQ water, which contained sodium thiosulphate (ca 17.5 mg.ℓ^{-1}) in order to neutralize any residual disinfectant.

Coliform bacteria - Coliform bacteria were enumerated by the membrane filter procedure using a filter with a 0.45 μm pore size (APHA-AWWA-WPCF, 1989). Membranes (Whatman) were placed on m-Endo Les agar (Difco) for the enumeration of total coliform bacteria (Grabow, 1990). Injured coliforms were recovered by placing the membranes on m-

T7 agar (Difco) (LeChevallier *et al.*, 1983). Faecal coliforms were determined according to standard methods using m-FC agar (Merck) (APHA-AWWA-WPCF, 1989). MacConkey Agar No 3 (Oxoid) was used as a confirmation test for coliform bacteria. Each determination was performed in triplicate.

Heterotrophic plate count (HPC) bacteria - HPC bacteria were enumerated by the standard spread plate procedure using R2A agar (Difco), incubated at 28°C for 7 d (Reasoner and Geldreich, 1985). Analyses were carried out in triplicate.

Total bacteria - The total bacteria numbers were determined using epifluorescence direct count procedures, involving DAPI (Boehringer Mannheim GmbH) according to Kepner and Pratt (1994). 9 mℓ samples were collected in a sterilized tube and preserved with 1 mℓ buffered gluteraldehyde (GTA). When bacterial numbers could not be enumerated immediately, fixed samples were stored at refrigerated temperatures (4°C in the dark) for a maximum of 3 weeks. Slides were normally prepared within a few days of collection, in order to avoid a decrease in bacteria numbers during storage.

Polycarbonate nucleopore filters (0.2 μm size - 25 mm diameter) were stained before use, by soaking for 24 h in an Irgalan black (IB) solution in a sterile petri dish. The filters were then rinsed twice in sterile MilliQ water. Backing filters were placed on the filter tower apparatus support, prewetted with 1 mℓ sterile fresh distilled water. A cover slip was placed on the rewetted black membrane filter. Aliquots of 0.50 mℓ of each sample were poured into a sterile glass tube and 0.1 mℓ DAPI (1μg.mℓ^{-1} working concentration) was finally added. Staining was performed in a darkened room for 7 min. Immediately following stain addition, funnel contents were swirled to promote thorough mixing of stain and sample. DAPI was reacted with the samples for 3 min, while the filter funnel contents were occasionally swirled. Funnel contents were drawn through the filter tower under low vacuum (<30 mm Hg[<4.0

KPa]). Membrane filters were rinsed with 1 mℓ sterile fresh MilliQ water to remove excess stain, enhancing image contrast. The vacuum was continued until all liquid was removed. The vacuum was then released, the filter tower apparatus was disassembled and the membrane filter was removed with a forceps.

The number of bacteria were counted within a minimum of 20 microscope fields. Two slides were used for each determination. The equation of Schmidt (1974) was used to calculate the number of bacteria in the original sample:

Bacteria (cells per mℓ) = (Nf x A x D)/aV

Nf = average number of cells per microscope field

A = effective filtering area (320 mm^2)

D = dilution factor

a = area of microscope field (49 x 10^{-6} mm^2)

V = volume of sample passed through filter (mℓ)

Bacteria numbers were also determined for IB stained filters, as a control, and substracted from the final calculated numbers.

Calculation of the kill percentage and the regrowth rate of bacteria

The kill percentage (Brözel and Cloete, 1991) and the regrowth rate of bacteria (Stanier *et al.*, 1976) were calculated using the following formula:

(1)

$$\%\,kill = 100 - \frac{Survivorcount}{Initial\ count} \times 100$$

(2)

$$\textit{Regrowth rate } (\mu, \textit{ expressed by time}^{-1}) = \frac{\ln X_1 - \ln X_0}{t_1 - t_0}$$

X_0 = the initial bacteria count (mass.volume^{-1}).

X_1 = the bacteria count after disinfection at time t_1 (expressed by hour).

t_0 = time zero t_1 = after a specific time following disinfection

The generation time (g, expressed in hours) was derived from the above equation (2):

(3) $\mu = \ln 2/g \rightarrow g = \ln 2/\mu$

3.5.2 Biofilm analyses

Biofilm experiments were conducted for the control (untreated water) and disinfected water distribution systems (laboratory-scale units). For surface water, two series of experiments were done during the study period: the first series involved the analyses of biofilms on stainless steel coupons using DAPI-epifluorescence microscopy (for enumeration of attached total bacteria) in surface water-source water 1, whereas the second series involved the analyses of biofilms on stainless steel and cement coupons using the standard spread plate procedure (for enumeration of HPC bacteria) and the DAPI-epifluorescence microscopy in surface water-source water 2. As to the surface water both types of coupons as well as both techniques were used for biofilm analyses. Coupons were withdrawn 24 h after disinfection and weekly for a period of 22 d for cement coupons and 38 d for stainless steel coupons.

Biofilm monitoring - Biofilm monitoring was done using a spectrophotometric method. The purpose of a flow-through glass tube was for biofilm monitoring, using spectrophotometric analysis (Jacobs *et al.*, 1996). The modified Pedersen device was used to allow biofilm formation to be studied (Fig. 1). For this purpose, 20 stainless steel coupons, the size of

microscope slides, were installed vertically into the Pedersen device. On both sides of the device, a total of 8 cement coupons 25 x 25 x 10 mm were installed. The coupons were used for staining (stainless coupons 50 x 25 x 1 mm, cement coupons 25 x 25 x 10 mm) and the enumeration of heterotrophic attached bacteria, as well as for the Scanning Electron Microscopy (SEM) studies. Prior to use, the coupons were cleaned in a detergent solution, rinsed first in tap water and disinfected with chlorine at 5 $mg.\ell^{-1}$ for 24 h, dechlorinated with sodium thiosulfate ($\pm$ 17.5 $mg.\ell^{-1}$) and flushed with sterile distilled water to remove any residues. The Merck SQ 118 photometer was adjusted to a wavelength of 550 nm. An absorbance measurement of bacteria attached to the flow-through glass tube was obtained simultaneously with a measurement of the absorbance of bacteria in the water sample in the flow-through glass tubes. The absorbance measurements were determined at the same frequency as the attached bacteria on the coupons.

Total cell densities - The colonized coupons (stainless steel and cement) were aseptically removed from the Pedersen device. Attached bacteria were enumerated directly from the stainless steel coupons or after their release from the coupons. For cells enumerated directly from the coupons, 0.2 mℓ DAPI (1 $\mu g.m\ell^{-1}$ - working concentration) (Boehringer Mannheim GmbH) was added to the stainless steel coupons (50 x 25 x 1 mm). Staining was carried out in a dark room for 7 min. Coupons were rinsed with sterile MilliQ water. The total bacterial cells were evaluated by using a microscopic counting technique and attached bacteria were counted from the coupons with a minimum of 20 microscope fields and expressed in $cells.cm^{-2}$. The following equation was used to calculate the number of cells:

Bacteria density ($cells.cm^{-2}$) = Nf/a

Nf = average number of cells per microscope field

a = area of microscope field (49 x 10^{-6} mm^2)

Attached bacteria were released from the stainless steel or cement coupons by 2 min sonication (Bandelin Sonorex RK 255S) in 20 ml sterile MilliQ water. The total bacterial cells were evaluated by using epifluorescence direct count procedures, involving DAPI (Boehringer Mannheim GmbH) according to Kepner and Pratt (1994). The number of bacteria was counted within a minimum of 20 microscope fields and expressed in cells.cm^{-2}. Two slides were used for each determination. The following equation was used to calculate the bacterial density:

Bacteria density (cells.cm^{-2}) = Nf x A x D/av x surface of coupons.

Bacterial densities obtained from appropriate blanks were subtracted from the final calculated densities.

Heterotrophic attached bacteria - Attached bacteria were released from the stainless steel and cement coupons by 2 min sonication in 20 ml sterile MilliQ water. Heterotrophic bacteria were enumerated by the standard spread plate procedure using R2A agar (Difco), incubated at 28° C for 7 d (Reasoner and Geldreich, 1985). Analyses were carried out in triplicate. The following equation was used to calculate the number of HPC bacteria (cfu. cm^{-2}):

cfu.cm^{-2} = N x D/ Surface of coupons

N = average number of colonies

D = dilution factor

Visualization of biofilm formation - The colonized stainless steel and cement coupons

were aseptically removed from the Pedersen device and put into sterile bottles containing 2.5% gluteraldehyde in a phosphate buffer (GTA). The biofilm formation was vizualized done by scanning electron microscopy (SEM).

Effect of washing on the removal of biofilm from the coupons - To investigate the effect of washing on the detachment of biofilm from the stainless steel coupons, biofilm cells (22, 30 and 37 days) were counted using the epifluorescence microscopy technique without removing the excess of the DAPI solution and after rinsing the coupons with MilliQ water. Attached bacteria were counted from the coupons with a minimum of 20 microscope fields and expressed in cells.cm^{-2}. Statistical analyses (ANOVA - section 3.7) were done to determine whether there was any difference between the two treatments.

3.6 PHYSICO-CHEMICAL ANALYSES

3.6.1 Determination of disinfectant residuals

Chlorine and chloramine - Concentrations of free chlorine and monochloramine residuals were measured using the N,N-diethyl-p-phenylenediamine (DPD, Sigma) ferrous titrimetric method (APHA- AWWA- WPCF, 1989). 5 mℓ of each reagent (phosphate buffer solution and DPD indicator solution) were mixed in a 150 mℓ titration flask. A 100 mℓ water sample was further added. Free chlorine was measured by titration with standard ferrous ammonium sulphate (FAS) until the red colour disappeared (reading A). Monochloramine was then measured by adding 0.5 mg potassium iodide crystal (KI, Merck) to the mixture. Titration was carried out until the red colour disappeared (reading B). Free chlorine concentrations were represented by A and expressed in mgCl.ℓ$^{-1}$. The monochloramine concentrations were obtained by reading B from A, and expressed in mg.ℓ$^{-1}$.

Ozone - The residual disinfectant ozone was measured by using the indigo colorimetric method (APHA-AWWA-WPCF, 1989). Two 100 mℓ volumetric flasks each containing 10 mℓ indigo reagent II (for the range of 0.05 - 0.50 mg $O_3.\ell^{-1}$) were filled to mark with distilled water (blank) and sample. Absorbance was determined at 600 ± 5 nm as soon as possible, but within 4 h. Results were expressed in mg $0_3.\ell^{-1}$ and calculated by the following formula:

$mgO_3.\ell^{-1}$ = 100 x D4/f x b x V

D4 = difference in absorbance between sample and blank

b = path length of cell, cm

V = Volume of samples, mℓ (normally 90 mℓ)

f = 0.42

Hydrogen peroxide - The hydrogen peroxide (H_2O_2) residual was determined using the RQflex reflectometer (Reflectoquant 16974, Merck).

3.6.2 pH , temperature, turbidity, total suspended solids

Temperature, pH, total suspended solids and turbidity were determined according to standard methods (APHA-AWWA-WPCF, 1989). Tables 3.1 summarizes the physico-chemical values of disinfected and untreated (control) surface water, whereas Tables 3.2 illustrates those of groundwater during the experimental period.

3.7 STATISTICAL ANALYSES

To compare variations in treatments, ANOVA ($\alpha = 0.05$) was applied to the bacteria counts with the latter as the dependant variable. The counts were transformed by taking logarithms

Table 3.1 The physico-chemical values (average) of surface water in different distribution systems.

Parameter	Surface water - source water 1					
	control	chlorine	chloramin	hyd.perox	ozone	UV
pH	8.0	7.9	8.0	8.1	7.9	7.9
T°C	23.0	23.0	23.0	23.0	23.0	23.0
NTU	3.0	3.7	4.9	5.7	5.6	7.0
SS *mg/ℓ*	4.0	6.0	6.0	4.0	5.0	5.0
Parameter	Surface water - source water 2					
	control	chlorine	chloramin	hyd.perox	ozone	UV
pH	8.6	8.5	8.7	8.7	8.5	8.5
T°C	17.0	17.0	17.0	17.0	17.0	17.0
NTU	3.3	3.5	3.5	2.4	3.3	5.0
SS *mg/ℓ*	3.3	3.7	3.7	2.5	2.8	3.9

Table 3.2 The physico-chemical values (average) of groundwater water in different distribution systems.

Parameter	Groundwater - source water 1					
	control	chlorine	chloramin	hyd.perox	ozone	UV
pH	7.9	7.9	7.7	7.6	8.2	7.8
T°C	24.0	24.0	24.0	23.0	24.0	24.0
Turb	1.2	1.8	1.4	1.2	1.2	1.4
SS *mg/ℓ*	5.1	5.0	4.8	4.9	5.8	4.5
Parameter	Groundwater - source water 2					
	control	chlorine	chloramin	hyd.perox	ozone	UV
pH	7.9	7.8	7.8	7.9	8.3	7.9
T°C	25.0	24.0	25.0	24.0	24.5	25.0
Turb	1.8	2.4	1.9	2.4	2.3	2.0
SS *mg/ℓ*	8.3	7.5	8.0	11.8	10.5	7.0

base 10 (LCount) in order to stabilize the variance. The number of days from ouset of the experimentation were included as a co-variate. If significant factors (e.g. treatments) were found, then the multiple comparison technique based on Least Squares Means (LSMEANS) was applied to establish which particular levels of a factor are different, in terms of the bacteria counts.

PART II

INFLUENCE OF DISINFECTION PROCESSES ON THE MICROBIAL QUALITY OF SURFACE WATER AND GROUNDWATER DISTRIBUTION SYSTEMS USING A LABORATORY-SCALE UNIT

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS

SECTION I INFLUENCE OF DISINFECTION ON THE MICROBIAL QUALITY OF SURFACE WATER DISTRIBUTION SYSTEMS USING A LABORATORY SCALE UNIT.

4.1.1 *Control water (raw water)*

4.1.1.1 Water sample analyses

Total bacterial numbers determined using DAPI- epifluorescence microscopy.

Source water 1 - No specific increase in bacteria number was observed although some minor fluctuations took place from time to time (Fig. 4.1). The cell number during the experimental period of the study remained between 5.42×10^7 and $2.08\ 10^8$ cells.mℓ^{-1}.

Source water 2-Although some fluctuations were noted, the cell number ranged between 3.07×10^7 and 2.61×10^8 cells.mℓ^{-1} during the study period (Fig. 4.1).

Heterotrophic plate count (HPC)

Source water 1- An 1 log cfu.mℓ^{-1} increase took place over the study period of 38d (Fig. 4.1). The average growth rate was $0.14\ h^{-1}$ (generation time 4.95h) between the first day and the 14th day.

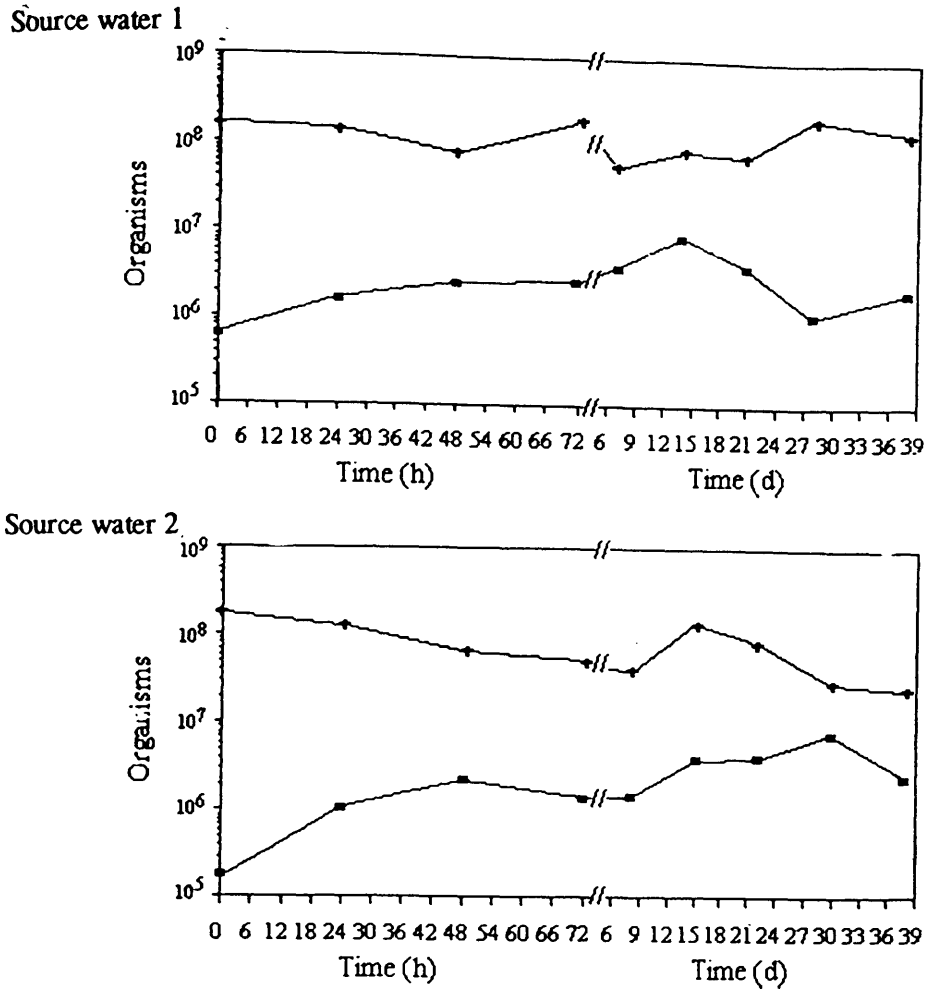


Fig. Fig. 4.1 Number of total bacteria (TB - epifluorescence) and heterotrophic plate count (HPC) bacteria in the raw water distribution system.

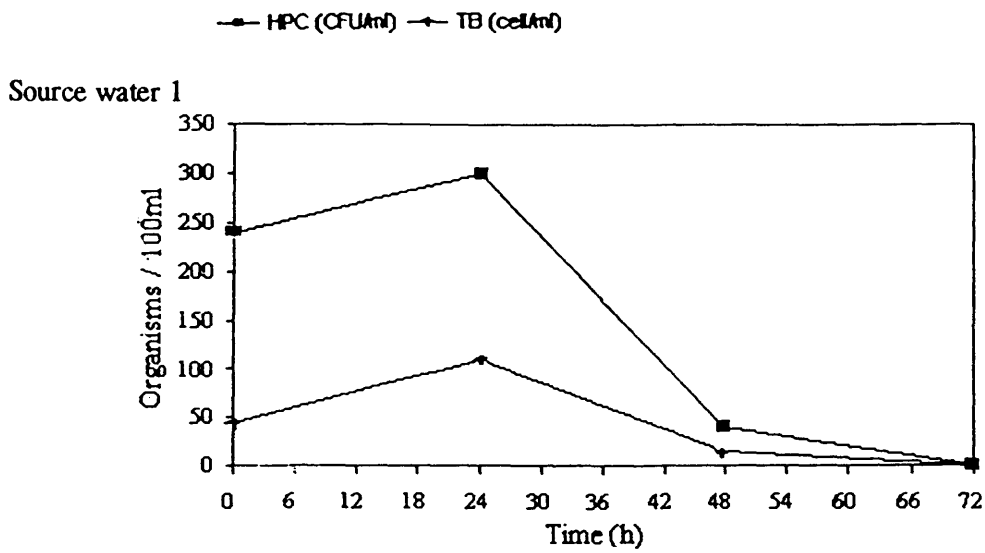


Fig. 4.2 The total number of coliform bacteria in the raw water distribution system.

Source water 2 - An 1 log cfu.mℓ$^{-1}$ increase was also found over the study period of 38 d (Fig. 4.1). Between 1 and 29 d, the average growth rate was 0.22 h^{-1} (generation time 3.15 h).

Coliform bacteria

Source water 1 - Within 24 h, an initial total coliform of 240 cfu/100 mℓ increased to 300 cfu/100 mℓ, and faecal coliform numbers increased from 45 cfu/100 mℓ to 110 cfu/100 mℓ after which a decrease occurred. Complete elimination of coliform in the raw water distribution system occurred after 72 h (Fig. 4.2).

Source water 2 - No coliform bacteria were detected in the raw water (control).

4.1.1.2 Biofilm analyses

Biofilm monitoring

Source water 1 - The adhesion of bacteria on the glass surface could be seen with the naked eye within 1 d after exposure. An increase in the absorbance measurements occurred between 1 and 14 d, after which a decrease was noted over the remainder of the study period. The absorbance measurement ranged between 1.40 and 2.75 during the experimental period (Fig. 4.13).

Source water 2 - The attachment of bacterial cells to the glass tube was obvious within 1d after exposure (Fig. 4.14). Although some fluctuations took place from time to time, the absorbance measurements ranged between 0.53 and 0.63.

Attached total bacteria numbers determined using epifluorescence microscopy

Source water 1

Stainless steel coupons - Within 1 d, the total number of cells attached to the stainless steel coupons amounted to 7 log cell.cm^{-2}. In spite of some fluctuations, this bacteria count remained constant for a period of 38 d (Fig. 4.15).

Source water 2

Stainless steel coupons - Total cells attached to the stainless steel coupons amounted to 7 log cell.cm^{-2} after 1 d of exposure. Attached bacteria increased progressively from 7 log to 8 log cells.cm^{-2} between the first day and the 36th day (Fig. 4.16).

Cement coupons - The adhesion of attached total cells to cement coupons resulted in 7 log cells.cm^{-2} after 1 d exposure. An exponential phase progressively occurred between 1 d and 15 d, and the bacteria reached 8 log cells.cm^{-2}, after which the number of attached bacteria remained constant at at 7 log cell.cm^{-2} for the remainder of the study period (Fig. 4.17).

Attached heterotrophic plate count (HPC)

Source water 2

Stainless steel coupons - The formation of micro-colonies on stainless steel coupons occurred after 1 d of exposure with a bacterial density of 1 log cfu.cm^{-2} (Fig. 4.18). Attached viable bacteria progressively increased to 7 log cfu.cm^{-2} and resulted in a mature biofilm after 15 d. At this level, no specific increase was noted for the rest of the study period (Fig. 4.18).

Cement coupons - The adhesion of viable bacteria on cement surfaces resulted in a bacterial community of 2 log cfu.cm^{-2} within 1 d after disinfection (Fig. 4.19). Attached HPC increased progressively to 7 log cfu.cm^{-2} between 1 d and 15 d, after which the number of viable bacteria remained constant.

4.1.2 *Disinfection of surface water by chlorination*

4.1.2.1 Water sample analyses

Residual Disinfectant

Source water 1 - An initial concentration of 3.5 mg.ℓ^{-1} free chlorine was reduced to 1.5 mg.ℓ^{-1} residual free chlorine within 20 min. This free chlorine concentration further decreased to 0.5

mg.ℓ^{-1} within 24 h. Residual free chlorine completely disappeared in the system after 48 h (Fig. 4.3).

Source water 2 - A reduction in free chlorine concentration from 2.5 mg.ℓ^{-1} to 1.2 mg.ℓ^{1} occurred within 20 min. The residual free chlorine concentration progressively decreased to 0.2 mg.ℓ^{-1} during the next 24 h and completely disappeared after 48 h (Fig. 4.3).

Total bacterial numbers determined using DAPI- epifluorescence microscopy

Source water 1 - 49 % of cells were killed by chlorine within 20 min. 24 h after chlorination of water, 91.75 % of cells were killed (Table 3). The phenomenon of the regrowth was apparent between 24 h and 48 d (regrowth rate 0.029 h^{-1}, generation time 23.90 h) (Fig. 4.3).

Source water 2 - 86 % of cells were killed by chlorine within 20 min. After 24 h, chlorine exhibited a kill percentage of 88.65 % (Table 4.1). The number of bacteria increased between 48 h and 8 d (growth rate 0.011 h^{-1}, generation time 63.01 h) (Fig. 4.3).

Heterotrophic plate count (HPC)

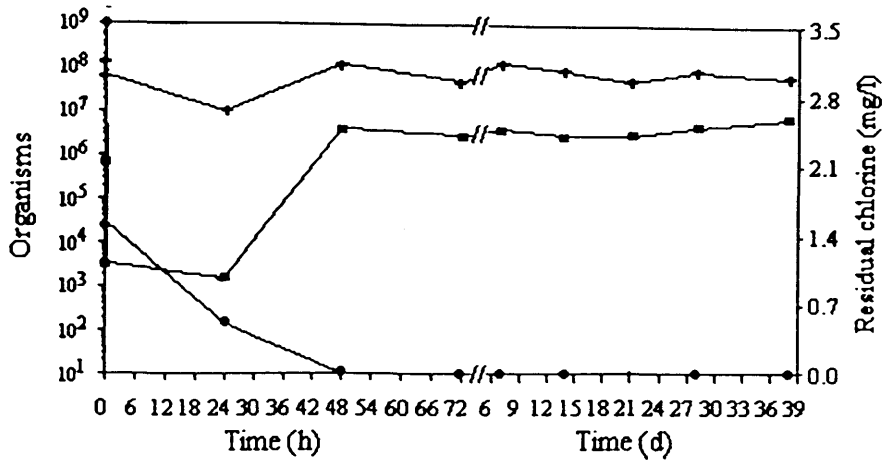
Source water 1 - 99.52 % of the bacterial community were killed by chlorine within 20 min. After 24 h, the kill percentage increased to 99.76 %, after which bacterial regrowth took place between 24 h and 48 h. The regrowth rate between 24 h and 48 h was 0.168 h^{-1} and the generation time 4.13 h, after which the bacterial number remained constant for the rest of the experimental period (Fig. 4.3).

Source water 2 - 99.94 % of the bacterial community were killed by chlorine within 20 min after which regrowth occurred between 20 min and 3 d. The regrowth rate was 0.176 h^{-1} and the generation time was 3.94 h (Table 4.1). No increase in bacteria number was noted over the remainder of the study period (Fig. 4.3).

Coliform bacteria

Source water 1 - No coliform bacteria were detected 20 min after chlorination. However,

Source water 1



Source water 2

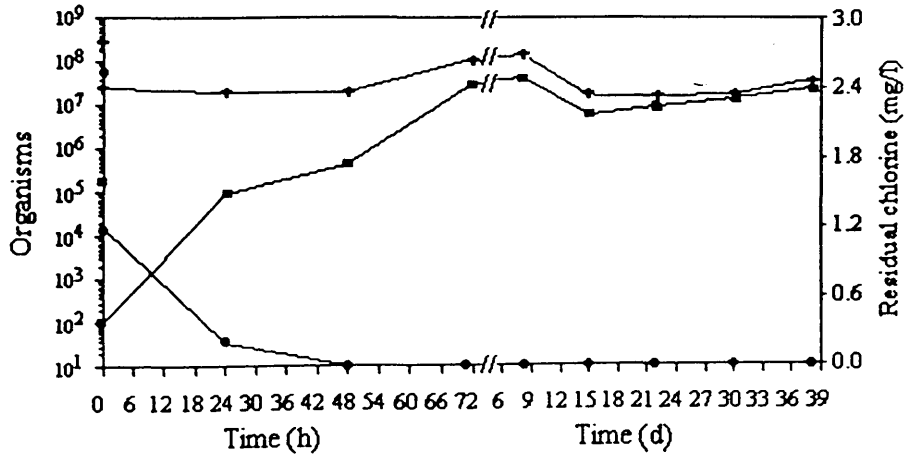


Fig. 4.3 Number of total bacteria (TB - epifluorescence) and heterotrophic plate count (HPC) bacteria in the chlorinated water distribution system.

HPC (CFU/ml) TB (cell/ml) Residual chlorine

Source water 1

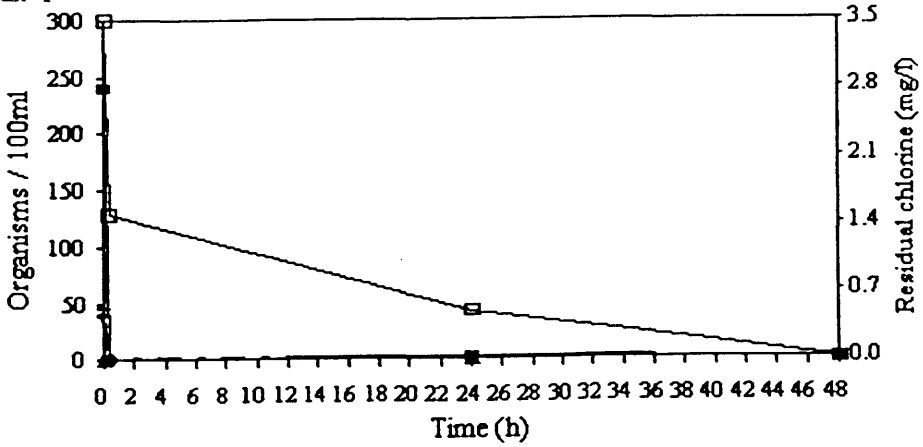


Fig. 4.4. The total number of coliform bacteria in the chlorinated water distribution system.

Total coliform Fecal coliform Injured coliform Residual chlorine

Table.4.1 Characteristic of potable surface water after disinfection.

Parameter	Time	Residual ($mg\ell^{-1}$)	% kill		Regrowth rate (h^{-1})		Generation time(h)		Time for regrowth	
			TB	HPC	TB	HPC	TB	HPC	TB	HPC
CAW *S1*	20 min	1.50	49.00	99.52						
	24 h	0.50	91.75	99.76	0.029	0.168	23.09	4.13	24-48 h	24-48 h
S2	20 min	1.20	86.00	99.94						
	24 h	0.20	88.65		0.011	0.176	63.01	3.94	48 h-8 d	20'-72 h
CMW *S1*	20 min	2.00	48.65	98.28						
	24 h	1.50	85.08	99.71						
	48 h	1.20		99.95						
	72 h	0.80		99.99	*	0.13	*	5.33	*	72-7 d
S2	20 min	1.20	88.26	96.78						
	24 h	1.00	89.38	99.99						
	48 h	0.80	89.38	99.99	*	0.039	*	17.77	*	8-22 d
OW *S1*	2 h	0.52	80.95	99.87						
	24 h	0.00			0.020	0.178	34.00	3.89	2-72 h	2-48 h
S2	2 h	0.02	98.53	99.81						
	24 h	0.00			0.059	0.152	11.75	4.56	2-72 h	2-72 h

Parameter		Time	Residual ($mg\ell^{-1}$)	% kill		Regrowth rate (h^{-1})		Generation time(h)		Time for regrowth	
				TB	HPC	TB	HPC	TB	HPC	TB	HPC
UVW	*S1*	2 h	**	91.67	99.84	0.117	0.194	5.92	3.57	2-24 h	2-48 h
		24 h	**								
	S2	2 h	**	98.53	99.93	0.076	0.161	9.12	4.31	2-48 h	2-72 h
		24 h	**								
HPW	*S1*	24 h	13.20	18.25	86.95						
		48 h	10.15	52.30	99.53						
		72 h	3.00	80.95	99.99	0.002	0.041	346.57	16.91	72 h-28 d	72 h-14 d
	S2	24 h	16.50	75.79	99.93						
		48 h	13.00	94.16	99.95						
		72 h	4.90		99.99	0.016	0.029	43.32	23.90	48h-8d	72 h-22 d

CAW = chlorinated water
CMW = chloraminated water
OW = ozonated water
UVW = UV irradiated water
HPW = hydrogen peroxide water

* = no specific regrowth
** = no residual
' = minute
TB = total bacteria
HPC = heterotrophic plate count

S1 = source water 1
S2 = source water 2

recovery of coliform bacteria (2 total, 2 faecal, 3 injured coliform/100 mℓ) occurred within 24 h after chlorination, but were not detected during the remainder of the study period (Fig.4.4).

4.1.2.2 Biofilm analyses

Biofilm monitoring

Source water 1 - Bacterial cells attached to the glass tube surface were apparent within 1d after chlorination (Fig. 4.13). Absorbance measurements increased from 0.54 to 1.91 nm between 1d and 7d after which a decrease occurred between 7 and 14 d (1.63 nm). No specific increase in the absorbance values was detected over the remainder of the study period (Fig. 4.13).

Source water 2 - The adhesion of attached bacteria on the glass tube surfaces was visible within 1d. Absorbance measurements gradually increased and reached a value of 0.68 nm within 36d (Fig.4.14). During the experimental period, absorbance measurements ranged between 0.54 and 0.68 nm.

Total bacteria number determined using DAPI epifluorescence microscopy

Source water 1

Stainless steel coupons - The initial adhesion of total bacteria on the stainless steel coupons occurred with a cell density reaching 7 log cells.cm^{-2} within 1 d (Fig. 4.15) after which the number of bacteria remained constant although some fluctuations took place from time to time during the experimental period.

Source water 2

Stainless steel coupons - The attachment of cells to the stainless steel coupons was detected after 1 d of exposure in a chlorinated water distribution system with a cell density of 6 log

cells.cm^{-2} (Fig. 4.16). Between 1 d and the rest of the exposure time, the attached bacteria number increased to 7 log cells.cm^{-2} (Fig. 4.16).

Cement coupons - The adhesion of total cells was observed on the cement coupons after 1 d of exposure with a bacterial density of 6 log.cells.cm^{-2}, after which a typical increase was visible, up to 7 log cells.cm^{-2} during the remainder of the experimental study (Fig. 4.17).

Heterotrophic plate count (HPC)

Source water 2

Stainless steel coupons - The adhesion of viable bacteria on stainless steel coupons was visible with a bacterial community of 2 log cfu.cm^{-2} within 1 d after exposure of the coupons in a chlorinated water system (Fig. 4.18). Attached HPC numbers increased progressively up to 7 log cfu.cm^{-2} within 15 d, after which bacterial density remained constant for the rest of the study period (Fig. 4.18).

Cement coupons - The attachment of heterotrophic bacteria on cement occurred within 1 d with 2 log cfu.cm^{-2} after exposure. The number of bacteria increased to 7 log cfu.cm^{-2} within 15 d and remained stable over the remainder of the study period (Fig. 4.19).

4.1.3 *Disinfection of surface water by chloramination*

4.1.3.1 Water samples analyses

Residual disinfectant

Source water 1 - Within 20 min, a 3.5 mg.ℓ^{-1} initial monochloramine concentration was reduced to 2 mg.ℓ^{-1}. Residual monochloramine disappeared in the system after 7 d (Fig. 4,5).

Source water 2 - The monochloramine concentration decreased from 2.5 to 1.2 mg.ℓ^{-1} within 20 min after disinfection. Between 20 min and 15 d, the residual monochloramine concentration gradually declined to 0 mg.ℓ^{-1} (Fig. 4.5).

Total bacterial numbers determined using DAPI- epifluorescence microscopy

Source water 1 - 48.65% of cells were killed by monochloramine within 20 min. After 24 h chloramination, the kill percentage increased to 85.08 %, after which no specific increase rate was observed during the remainder of the study period (Fig. 4.5).

Source water 2 - 88.26 % of cells were killed by monochloramine within 20 min, after which no specific increase rate was noted over the remainder of the study period (Table 4.1, Fig. 4.5). *Heterotrophic plate count (HPC)*

Source water 1 - 98.28 % of heterotrophic plate count bacteria were killed by monochloramine within 20 min after disinfection and 99.99 % within 72 h (Table 4.1), after which regrowth occurred between 72 h and 7 d, and bacteria count remained stable for the rest of the study period (Fig. 4.5). Between 72 h and 7 d, the regrowth rate was 0.13 h^{-1} (generation time 5.33 h).

Source water 2 - 96.78 % of heterotrophic plate count bacteria were killed by monochloramine during the first 20 min and 99.99 % after 24 h (Table 4.1). Bacterial regrowth was apparent between 8 and 22 d, after which bacteria no specific increase in bacteria number occurred during the remainder of the study period (Fig. 4.5). The regrowth rate was 0.039 h^{-1} (generation time 17.77 h) between 8 and 22 d.

Coliform bacteria

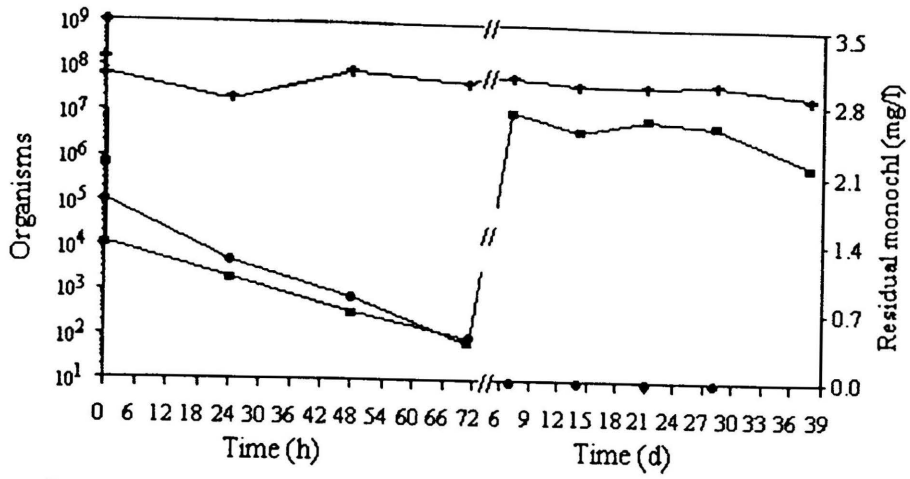
Source water 1- No coliform bacteria were detected during the experimental period (Fig. 4.6).

4.1.3.2 Biofilms analyses

Biofilm monitoring

Source water 1 - Bacteria adhered to the glass tube surface from the first day after the initiation of the experiment (Fig. 4.13). Although some fluctuations were noted from time to

Source water 1



Source water 2

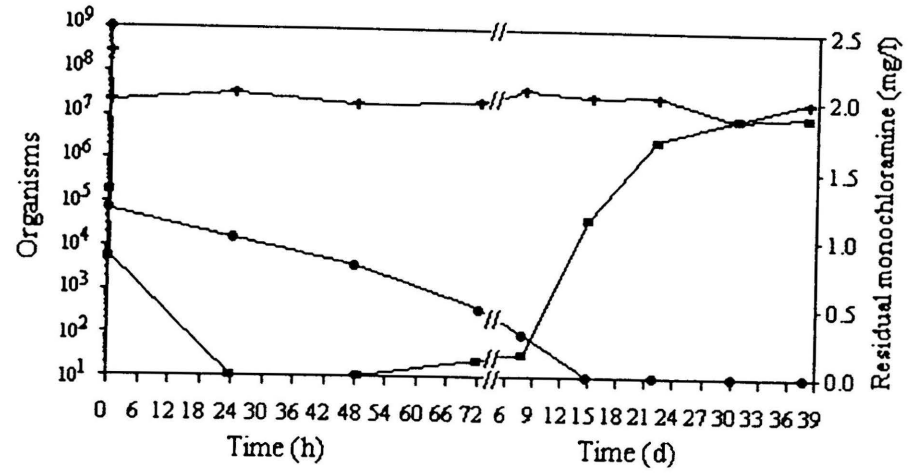


Fig. 4.5 Number of total bacteria (TB - epifluorescence) and heterotrophic plate count (HPC) bacteria in the chloraminated water distribution system.

HPC (CFU/ml) TB (cell/ml) Residual monochl

Source water 1

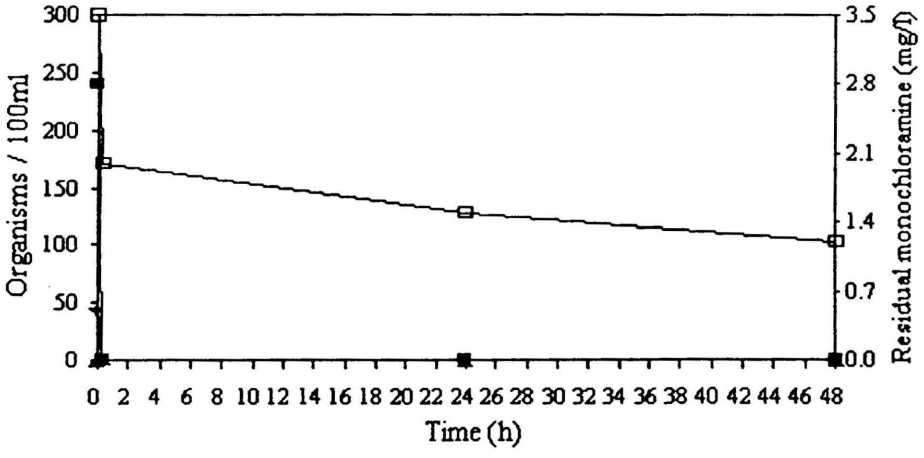


Fig. 4.6 The total number of coliform bacteria in the chloraminated water distribution system.

Total coliform Fecal coliform Injured coliform Residual monochl.

time, the absorbance measurements remained between 1.35 and 2.09 (Fig. 4.13).

Source water 1 - The adhesion of attached bacteria on the glass tube surface occurred within 1d with a value of 0.51 absorbance (Fig. 4.14). An increase in absorbance measurement was noted between 1d and 8d, after which a decrease occurred between 8d and 37d. The absorbance measurements ranged between 0.51 and 0.66 nm for a period of 38d.

Total bacteria number determined using DAPI-epifluorescence microscopy

Source water 1

Stainless steel coupons - A density of 7 log cells.cm^{-2} was detected on the stainless steel coupons within 1 d after disinfection, after which no specific increase was noted over the remainder of the study period (Fig. 4.15).

Source water 2

Stainless steel coupons - The biofilm formation on the stainless steel coupons started from the first day after disinfection, with a bacterial density of 6 log cells.cm^{-2} which gradually increased to 8 log cells.cm^{-2} between 1 d and 37 d (Fig. 4.16). This gradual increase could be also seen on Plates 5-6.

Cement coupons - Biofilm formation on cement coupons started with a cell density of 6 log cells.cm^{-2} which increased to 7 log cells.cm^{-2} within 8 d (Fig. 4.17). At this level, the cell density remained constant over the rest of the study period.

Heterotrophic plate count (HPC)

Source water 2

Stainless steel - The adhesion of HPC on the stainless steel coupons was visible within the first day after exposure in a chloraminated water system (Fig. 4.18). This resulted in a bacterial community of 2 log cfu.cm^{-2} which gradually increased to 7 log cfu.cm^{-2} within 37 d.

Cement coupons - Bacteria adhered to the cement coupons within 1 d after disinfection (Fig.

4.19). Between 1 d and 15 d, the number of bacteria increased from 1 log to 6 log cfu.cm^{-2} which remained constant for the rest of the study period (Fig. 4.19).

4.1.4 *Disinfection of surface water by ozonation*

4.1.4.1 Water samples analyses

Residual disinfectant

Source water 1- A depletion of ozone concentration occurred from 2.64 mg.ℓ^{-1} to 0.52 mg.ℓ^{-1} within 2 h and disappeared after 24 h (Fig. 4.7).

Source water 2 - The ozone concentration decreased from 2.60 to 0.02 mg.ℓ^{-1} within 2 h after disinfection and disappeared after 24 h (Fig. 4.8).

Total bacterial numbers determined using DAPI- epifluorescence microscopy

Source water 1 - Within 2 h, 80.95 % of cells were killed by ozone, after which bacterial numbers increased between 2 h and 72 h (regrowth rate 0.020 h^{-1}, generation time 34 h), and fluctuations took place during the remainder of the study period (Fig. 4.7).

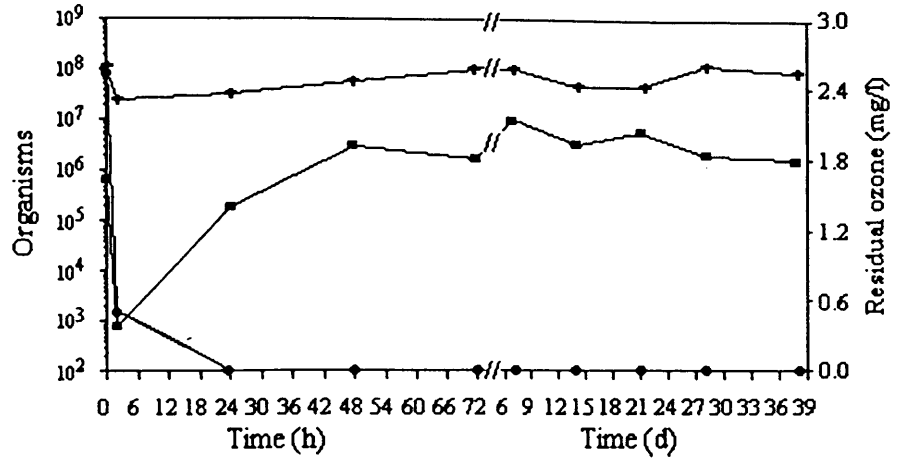
Source water 2 - 98.53 % of cells were killed by ozone within 2 h. Bacterial regrowth was noted between 2 h and 72 h (regrowth rate, 0.059 h^{-1} , generation time 11.75 h) (Table 4.1) after which no specific increase was observed over the remainder of the study period (Fig. *4.7)*

Heterotrophic plate count (HPC)

Source water 1 - 99.87 % of the bacterial community were killed by ozone. Regrowth took place between 2 h and 48 h, after which some fluctuations were noted over the remainder of the study period of 38 d (Fig. 4.7). Between 2 h and 48 h, the regrowth rate was 0.178 h^{-1} (generation time 3.89 h).

Source water 2 - 99.81 % HPC were killed by ozone within 2 h, after which bacterial regrowth occurred between 2 h and 72 h reaching the plateau after 8 d (Fig. 4.7). The regrowth rate

Source water 1



Source water 2

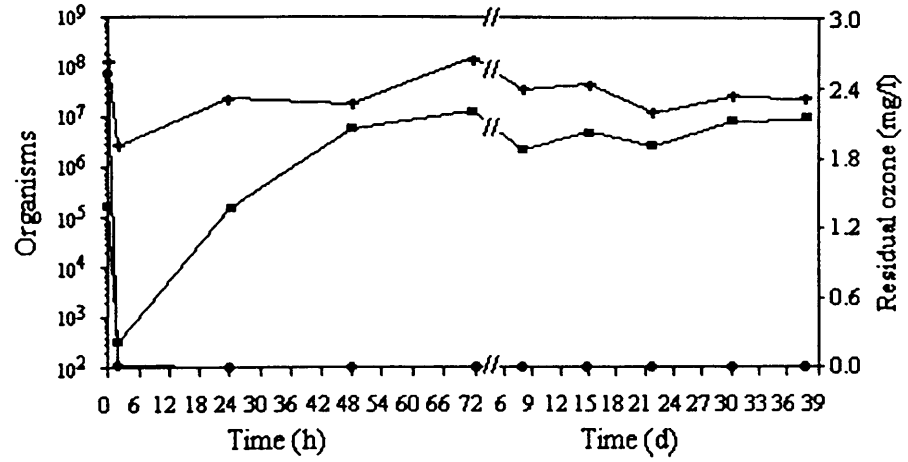


Fig. 4.7 Number of total bacteria (TB - epifluorescence) and heterotrophic plate count (HPC) bacteria in the ozonated water distribution system.

HPC (CFU/ml) TB (cell/ml) Residual ozone

Source water 1

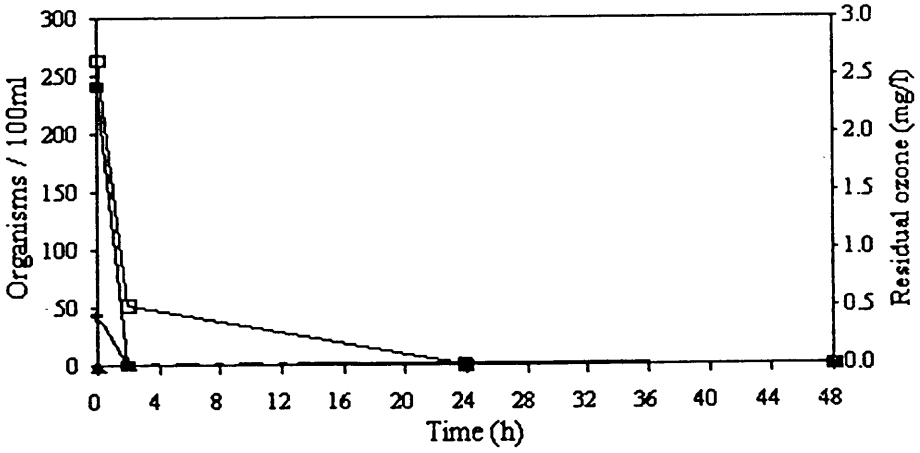


Fig. 4.8 The total number of bacteria in the ozonated water distribution system.

Total coliform Fecal coliform Injured coliform Residual ozone

between 2 h and 72 h was 0.152 h^{-1} and the generation time was 4.56 h (Table 4.1).

Coliform bacteria

Source water 1 - No coliform bacteria were noted after 2 h ozonation. However, recovery of coliform bacteria (2 cfu/100 ml of total and injured coliform) occurred within 24 h after ozonation, but were not detected thereafter (Fig. 4.8).

4.1.4.2 *Biofilms analyses*

Biofilm monitoring

Source water 1 - Biofilm formation on the glass tube surface was apparent within the first day after disinfection. Between 1 and 7 d, the absorbance measurement increased from 0.39 to1.82 after which no specific increase was observed over the rest of the study period (Fig. 4.13).

Source water - The adhesion of bacteria on the glass tube surface was observed from the first day after disinfection (Fig. 4.14). In spite of some fluctuations, the absorbance measurement ranged between 0.49 and 0.66 over the remainder of the experimental.

Total bacteria number determined using DAPI epifluorescence microscopy

Source water 1

Stainless steel coupons - The adhesion of cells on the stainless steel coupons was observed after 1 d of exposure in the ozonated water system, with a bacterial community of 7 log cells.cm^{-2} , after which some fluctuations took place over the next 21 d. These resulted in an increase of bacteria count to 8 log cells.cm^{-2}. Between 21 d and 38 d, bacteria count decreased again to 7 log cells.cm^{-2} (Fig. 4.15).

Source water 2

Stainless steel coupons - The formation of biofilm on the stainless steel coupons within the first day occurred at a bacterial cell density of 6 log cells.cm^{-2}, which gradually increased to

8 log cells.cm^{-2} after 38 d (Fig. 4.16). This gradual increase of cells could be observed on Plates 7-8.

Cement coupons - An initial cell density of 6 log cells.cm^{-2} was detected on the cement coupons within 1 d of exposure in ozonated water. Between 1 d and 8 d, bacteria numbers increased to 7 log cells.cm^{-2}, which marked the formation of a mature biofilm (Fig. 4.15).

Heterotrophic plate count (HPC)

Source water 2

Stainless steel coupons - Within the first day, a bacterial count of 2 log cfu.cm^{-2} was noted on the stainless steel coupons exposed to the ozonated water (Fig. 4.18). This bacterial density gradually increased to 7 log cfu.cm^{-2} between 1 d and 15 d, after which no increase in bacteria number was observed.

Cement coupons - Viable bacteria adhered to the cement coupons with a bacterial community of 2 log cfu.cm^{-2} after the first day of exposure (Fig. 4.19). An increase occurred between 1 d and 15 d, and the attached HPC reached 7 log cfu.cm^{-2}. This figure remained stable over the remainder of the study period.

4.1.5 Disinfection of surface water by UV irradiation

4.1.5.1 Water samples analyses

Total bacterial numbers determined using DAPI- epifluorescence microscopy

Source water 1 - 91.67 % of cells were killed within 2 h, after which bacterial regrowth was apparent between 2 h and 24 h (regrowth rate 0.117 h^{-1}, generation time 5.92 h). Some fluctuations took place from time to time during the remainder of the study period, and the count of bacteria ranged between 7 and 8 log cells.mℓ$^{-1}$ (Fig. 4.9).

Source water 2 - During the first 2 h after disinfection, 98.53 % of cells were killed by UV

irradiation (Table 4.1). The bacterial number increased between 2 and 48 h (regrowth rate 0.076 h^{-1}, generation time 9.12 h) after which bacteria no specific increase was noted over the remainder of the study period (Fig. 4.9).

Heterotrophic plate count (HPC)

Source water 1 - 99.84% heterotrophic bacteria were killed by UV irradiation after the first 2 h of the disinfection (Table4.1). Bacterial regrowth occurred between 2 h and 48 h (regrowth rate 0.194 h^{-1}, generation time 3.57 h), after which bacteria number remained constant during the rmainder of the experimental period (Fig. 4.9).

Source water 2 - Disinfection of surface water by UV irradiation resulted in a kill percentage of 99.93 % within 2 h (Table 4.1). Regrowth was noted between 2 h and 72 h, after which bacteria number remained constant for the next 38 d of the study period (Fig. 4.9). The regrowth rate between 2 h and 72 h was 0.161 h^{-1} (generation time 4.31 h).

Coliform bacteria

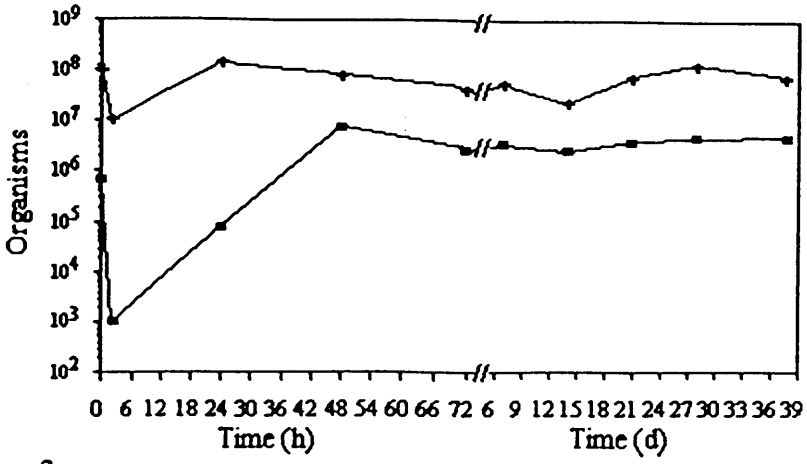
Source water 1 - 99.58 % coliform bacteria were killed by UV irradiation within 2 h after disinfection. However, recovery of coliform bacteria increased the number of bacteria to 4 cfu/100 mℓ within 24 h, after which no coliform bacteria were detected during the remainder of the study period (Fig. 4.10).

4.1.5.2 Biofilm analyses

Biofilm monitoring

Source water 1 - The adhesion of bacteria on glass tube surface exposed to UV system was visible with a value of 1.33 within 1 d, after which an increase occurred between 1 and 7 d (1.93). A levelling off of the absorbance measurement was observed between 14 and 38 d (Fig. 4.13).

Source water 1



Source water 2

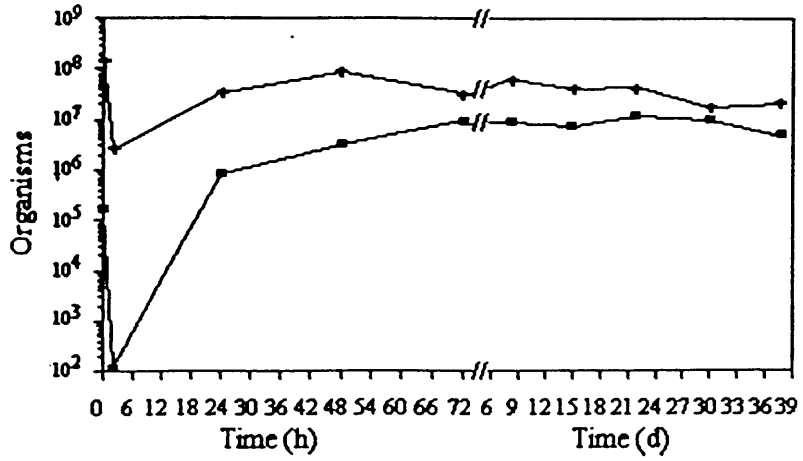


Fig. 4.9 Number of total bacteria (TB - epifluorescence) and heterotrophic plate count (HPC) bacteria in the UV irradiated water distribution system.

HPC (CFU/ml) TB (cell/ml)

Source water 1

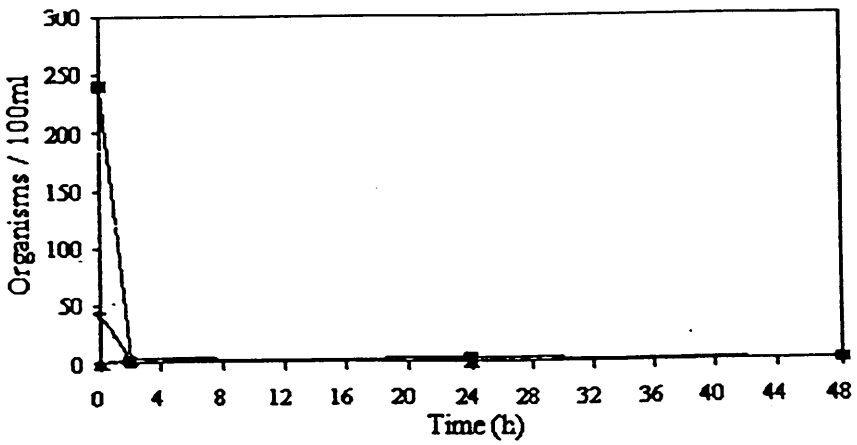


Fig. 4.10 The total number of bacteria in the UV irradiated water distribution system.

Total coliform Fecal coliform Injured coliform

Souce water 2 - Formation of biofilm on the glass tube surface was apparent within the first day with an absorbance measurement of 0.80, after which some fluctuations took place and aborbance measurements ranged between 0.56, and 0.79 (Fig. 4.14).

Total bacteria number determined using DAPI epifluorescence microscopy

Source water 1

Stainless steel coupons - A bacterial community of 7 log cells.cm^{-2} was noted on the stainless steel coupons after 1 d of exposure in a UV water distribution system. During the study period, bacteria number remained at this level (Fig. 4.15).

Source water 2

Stainless steel coupons - Within 1 d, the total bacteria count was 6 log cells. cm^{-2}. This number progressively increased to 8 log cells.cm^{-2} between 1 d and 38 d and marked a levelling off of the adhesion rate over the remainder of the study period (Fig. 4.16). Gradual increase of biofilm cells could seen on Plates 9 -10.

Cement coupons - The formation of biofilms on the cement coupons was detected within 1 d and reached 7 log cells.cm^{-2}, after which the bacteria number progressively increased to 8 log cells. cm^{-2} between 1 and 8 d. A levelling off of the bacteria adhesion rate was noted over the remainder of the study period (Fig. 4.17).

Heterotrophic plate count (HPC)

Source water 1

Stainless steel coupons - Within 1 d, the adhesion of HPC bacteria on the stainless steel coupons reached a density of 2 log cfu.cm^{-2}, after which the bacteria number progressively increased to 7 log cfu.cm^{-2} between 1 d and 15 d. No specific increase in bacterial count was noted over the remainder of the study period (Fig. 4. 18).

Cement coupons - The attachment of viable bacteria on the cement surface occurred within the

first day after exposure in a UV water system, and resulted in a bacterial community of less than 1 log cfu.cm^{-2}. Between 1 and 15 d, the bacteria increased to 7 log cfu.cm^{-2} and the attached HPC reached a plateau over the remainder of the study period (Fig. 4.19).

4.1.6 Disinfection of surface water with hydrogen peroxide

4.1.6.1 Water samples analyses

Residual disinfectant

Source water 1 - A gradual depletion of hydrogen peroxide from 20.77 mg.ℓ^{-1} to 3 mg.ℓ^{1} occurred within 72 h. The residual hydrogen peroxide completely disappeared after 7 d (Fig. 4.11).

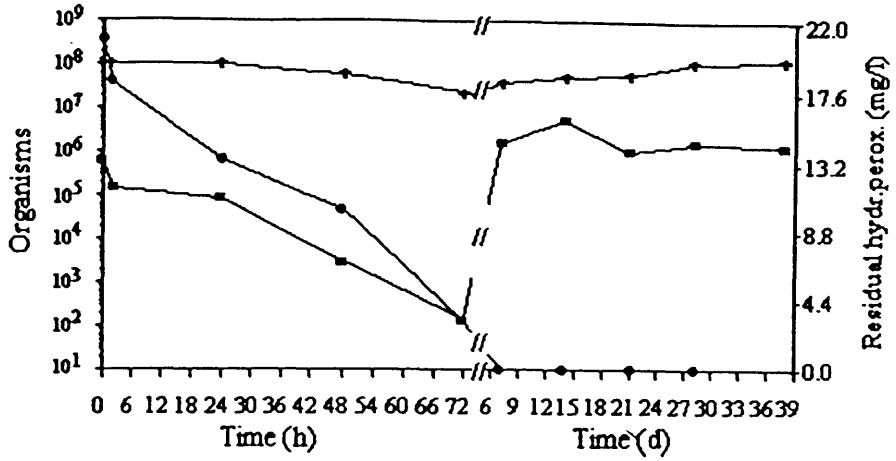
Source water 2- The hydrogen peroxide concentration decreased from 19.43 to 16.5 mg.ℓ^{-1} within 24 h. Between 24 h and 22 d, the residual hydrogen peroxide concentration progressively declined to 0 mgℓ^{-1} (Fig. 4.11).

Total bacterial numbers determined using DAPI- epifluorescence microscopy

Source water 1 - 18.25 % of cells were killed by hydrogen peroxide within 24 h. After 72h, the kill percentage increased to 80.95 % (Table 4.1). Between 72 h and 28 d, bacterial regrowth was apparent (Fig. 4.11). The regrowth rate was 0.002 h^{-1} and the generation time was 346.57 h.

Source water 1 - 75.79 % of cells were killed by hydrogen peroxide during the first 24 h after disinfection (Table 3). Hydrogen peroxide exhibited a kill percentage of 94.16 % after 48 h. Bacterial regrowth occurred between 48 h and 8 d, after which bacteria reached the stationary phase over the remainder of the study period of 38 d (Fig. 4.11). The regrowth rate was 0.016 h^{-1} (generation time 43.32 h) between 48 h and 8 d.

Source water 1



Source water 2

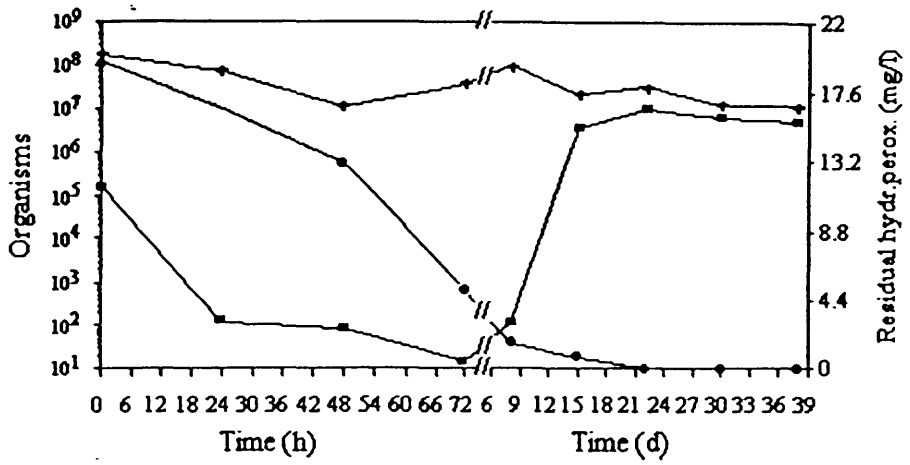


Fig. 4.11. Number of total bacteria (TB - epifluorescence) and heterotrophic plate count (HPC) bacteria in the hydrogen peroxide water distribution system.

HPC (CFU/ml) TB (cell/ml) Residual hydr.pero

Source water 1

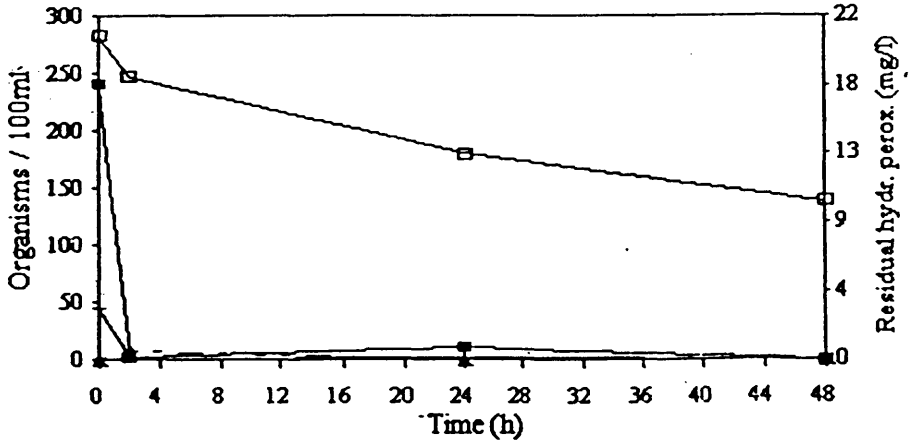


Fig. 4.12. The total number of coliform bacteria in the hydrogen peroxide water distribution system.

Total coliform Fecal coliform Injured coliform Residual Hyd. perox

Heterotrophic plate count (HPC)

Source water 1 - 86.95 % of heterotrophic bacteria were killed by hydrogen peroxide during the first 24 h after disinfection. Within 72 h, 99.98 % of bacteria were killed, after which the phenomenon of regrowth occurred between 72 h and 14 d, and the bacteria reached the plateau over the study period of 38 d. The regrowth rate was 0.041 h^{-1} and the generation time was 16.91 h between 72 h and 14 d (Table 4.1).

Source water 2 - 99.93 % of heterotrophic bacteria were killed within 24 h after disinfection. The kill percentage increased to 99.99 % within 72 h, after which bacterial regrowth was apparent between 72 h and 22 d, and between 22 d and 38 d the bacteria reached the plateau (Fig. 4.11). The regrowth rate was 0.029 h^{-1} (generation time 23.90 h) between 72 h and 22 d.

Coliform bacteria

Source water 1 - 99.17 % of coliform bacteria were killed by hydrogen peroxide after the first 2 h of disinfection. Recovery of coliform bacteria occurred within 24 h after disinfection, none were detected during the remainder of the study period (Fig. 4.12).

4.1.6.2 Biofilm analyses

Biofilm monitoring

Source water 1 - Formation of biofilm on the glass tube surface was obvious within 1d after disinfection (Fig. 4.15), In spite of some fluctuations, absorbance measurements ranged between 0.36 and 2.01 during the experimental period of 38 d (Fig. 4.13).

Source water 2 - The adhesion of bacteria on the glass tube surface was visible within the first day after disinfection. In spite of some fluctuations, absorbance measurements were between 4.41 and 0.70 (Fig. 4.14).

Total bacteria number determined using epifluorescence microscopy

Source water 1

Stainless steel coupons - After an exposure time of 1 d, cells adhered to the stainless steel coupons with a density of 7 log cells.cm^{-2}. Although some fluctuations took place, no specific increase was noted over the remainder of the study period (Fig. 4.15).

Source water 2

Stainless steel coupons - An initial number of attached cells of 6 log cells.cm^{-2} was detected within 1 d of exposure in hydrogen peroxide treated water. In spite of some fluctuations, the bacteria number increased to 8 log cells.cm^{-2} between 1 d and 38 d (Fig. 4.16). This could clearly be seen on Plates 11-12.

Cement coupons - A bacterial density of 6 log cells.cm^{-2} was noted on the cement coupons within 1 d after disinfection. This number increased to 7 log cm^{-2} between 1 d and 8 d, after which the bacteria reached the plateau (Fig. 4.17).

Heterotrophic plate count (HPC)

Source water 2

Stainless steel coupons - Within the first day, a 2 log cfu.cm^{-2} bacterial community adhered to the stainless steel coupons exposed to the hydrogen peroxide treated water (Fig. 4.18). This number gradually increased to 6 log cfu.cm^{-2} between 1 d and 15 d, after which the bacteria reached the plateau over the remainder of the study period (Fig. 4. 18).

Cement coupons - The attachment of HPC bacteria on the cement coupons occurred within 1 d with a bacterial community of 1 log cfu.cm^{-2}, after which this number progressively increased to 7 log cfu.cm^{-2} between 1 d and 22 d (Fig. 4.19).

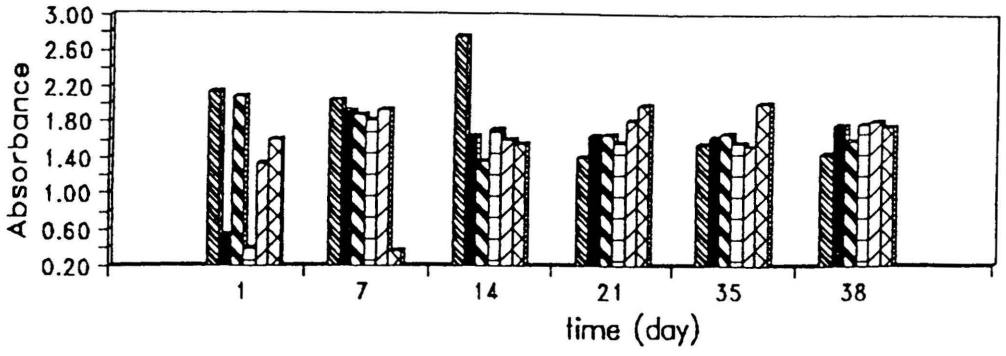


Fig. 4.13. Measurement of bacterial cells attached to flow-through glass tubes during the experimental period - source water 1.

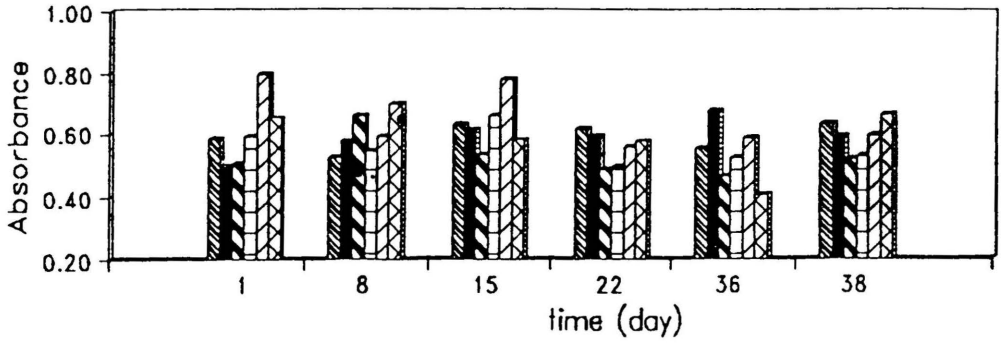
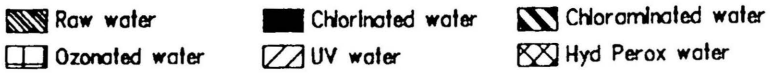


Fig. 4.14. Measurement of bacterial cells attached to flow-through glass tubes during the experimental period - source water 2.



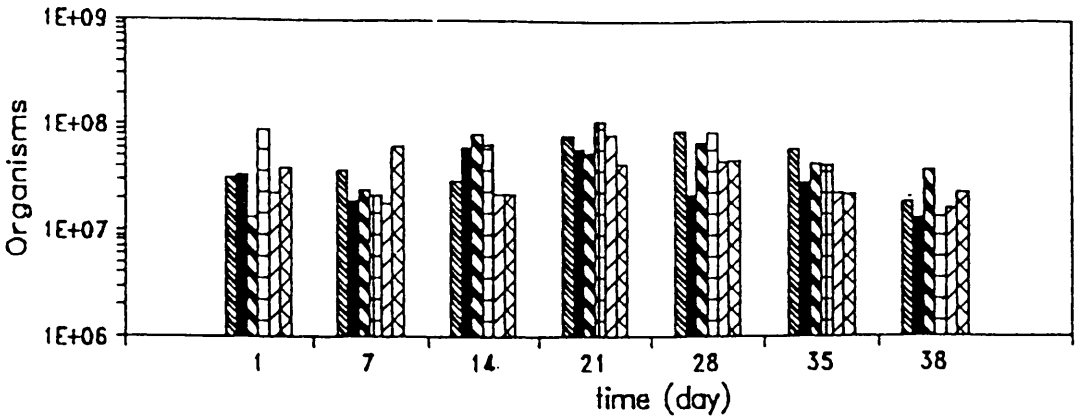


Fig. 4.15. Total bacteria (epifluorescence) attached to stainless steel coupons during the experimental period - source water 1.

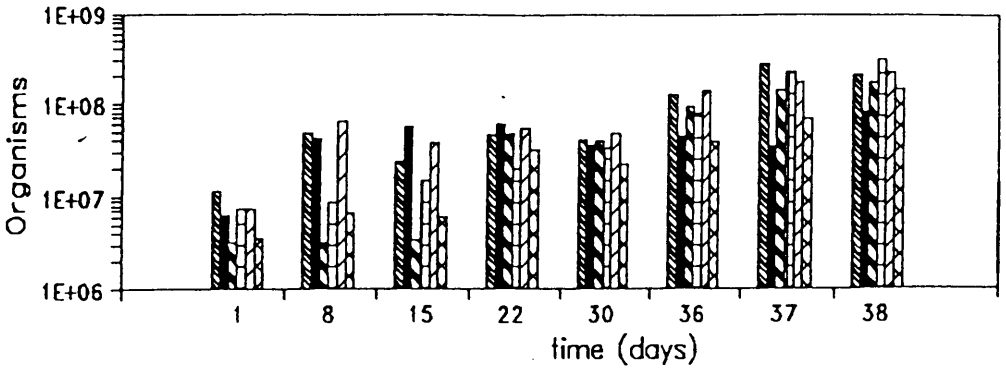


Fig. 16. Total bacteria (epifluorescence) attached to stainless steel coupons during the experimental period - source water 2.

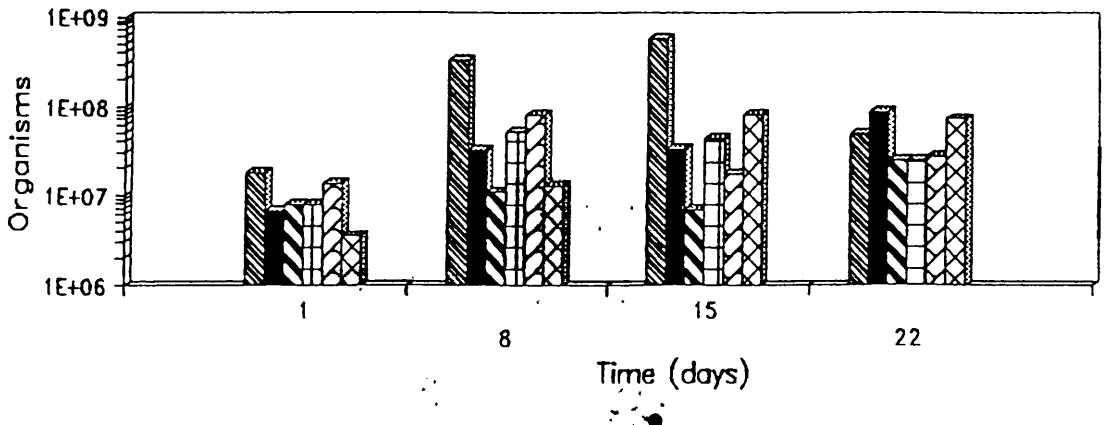
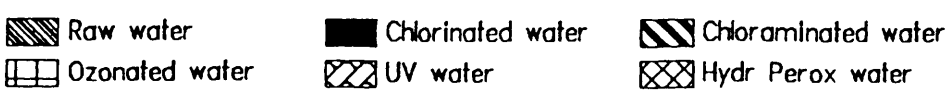


Fig. 17. Total bacteria (epifluorescence) attached to cement coupons during the experimental period - source water 2.



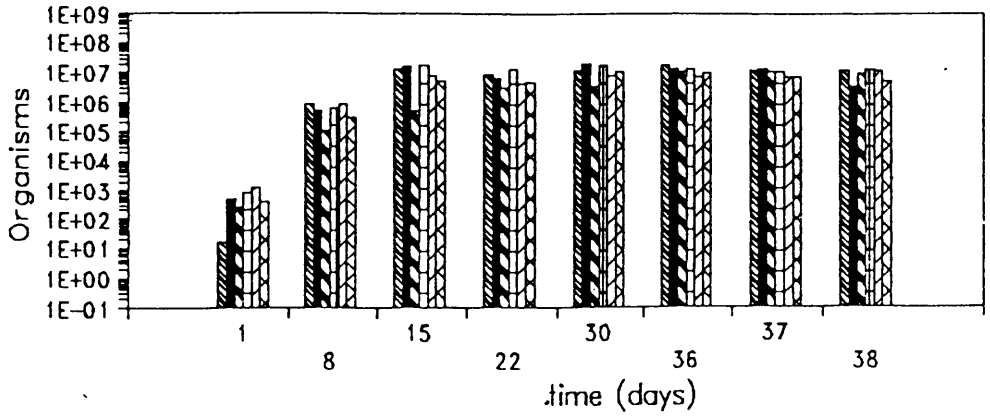


Fig. 18. Heterotrophic plate count bacteria attached to stainless steel coupons during the experimental period - source water 2.

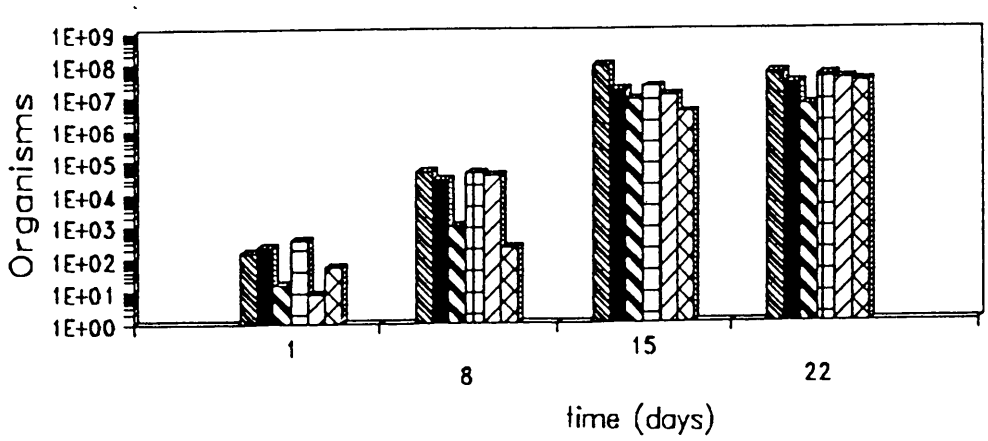


Fig. 19. Heterotrophic plate count bacteria attached to cement coupons during the experimental period - source water 2.

RAW
Chlorinated water
Chloraminated water
Ozonated water
UV water
Hydr Perox water

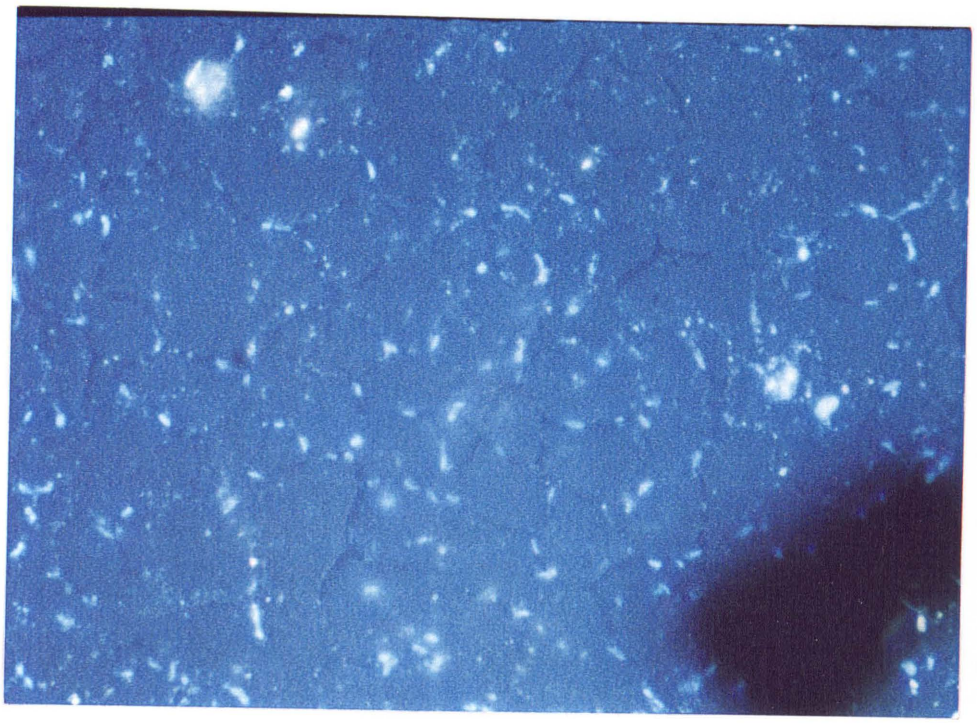


Plate 1

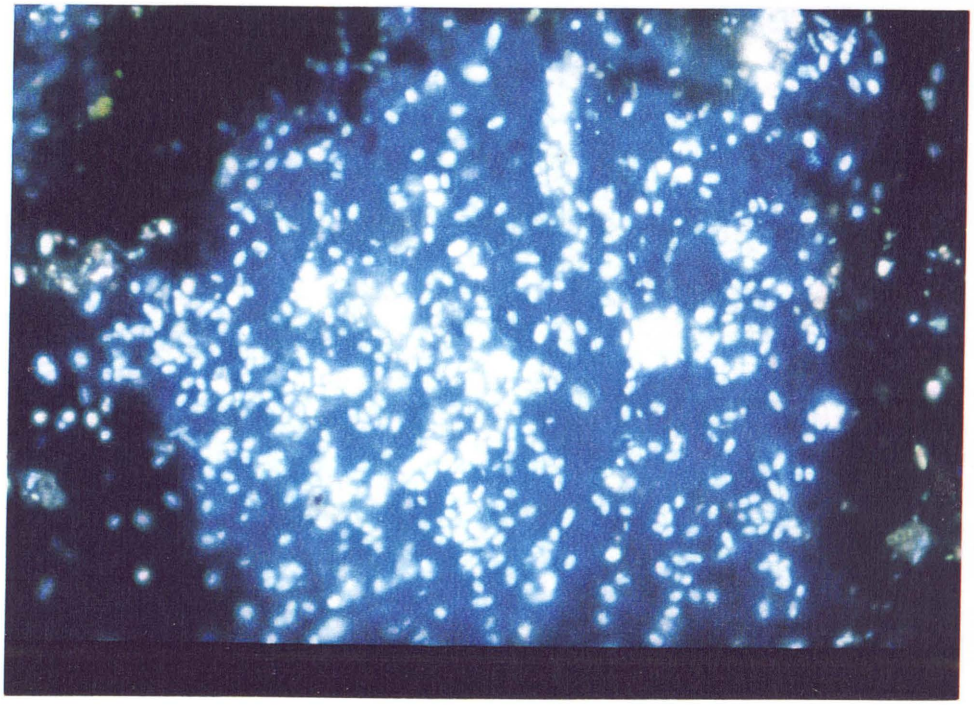


Plate 2

Plates 1 - 2 Bacteria (epifluorescence) attached to stainless steel coupons after 7 d (Plate 1) and 38 d (Plate 2) exposure to the raw water (control).

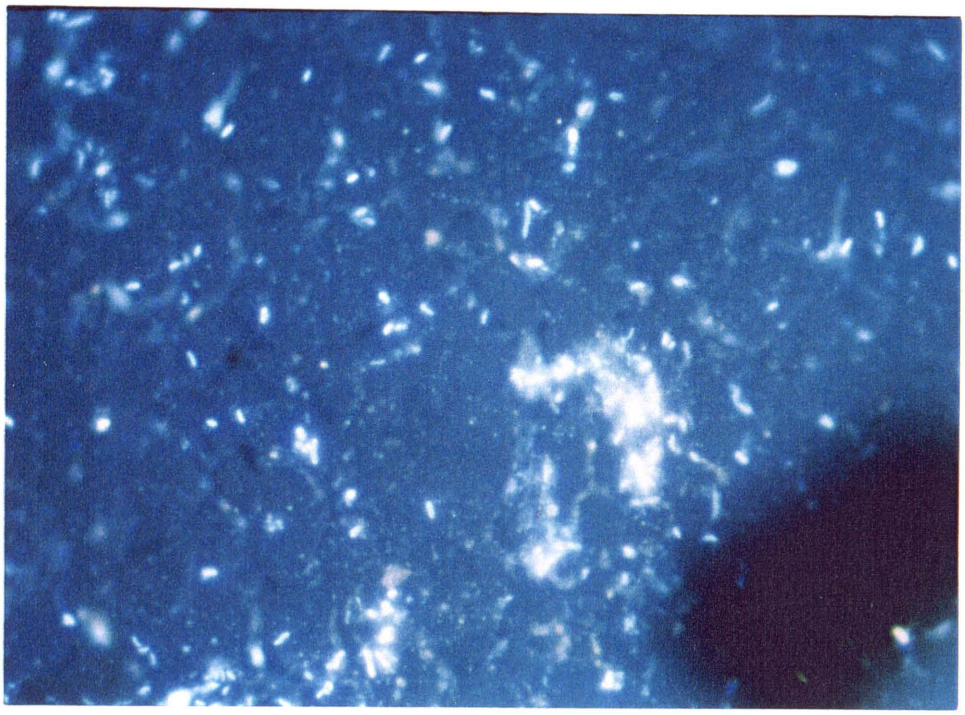


Plate 3

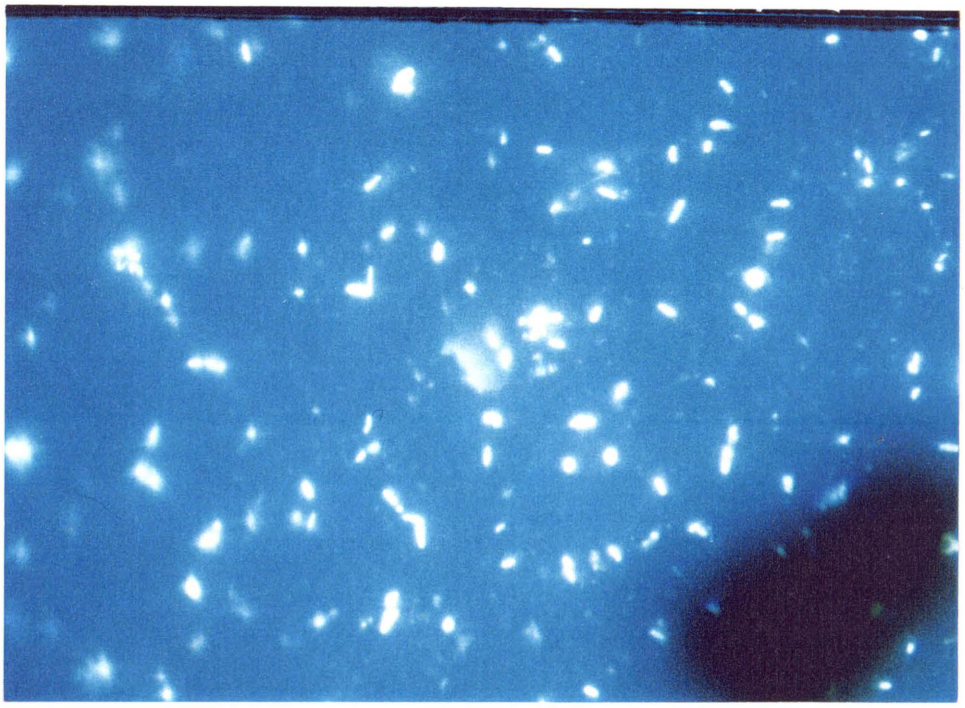


Plate 4

Plates 3 - 4 Bacteria (epifluorescence) attached to stainless steel coupons after 7 d (Plate 3) and 38 d (Plate 4) exposure to the chlorinated water.

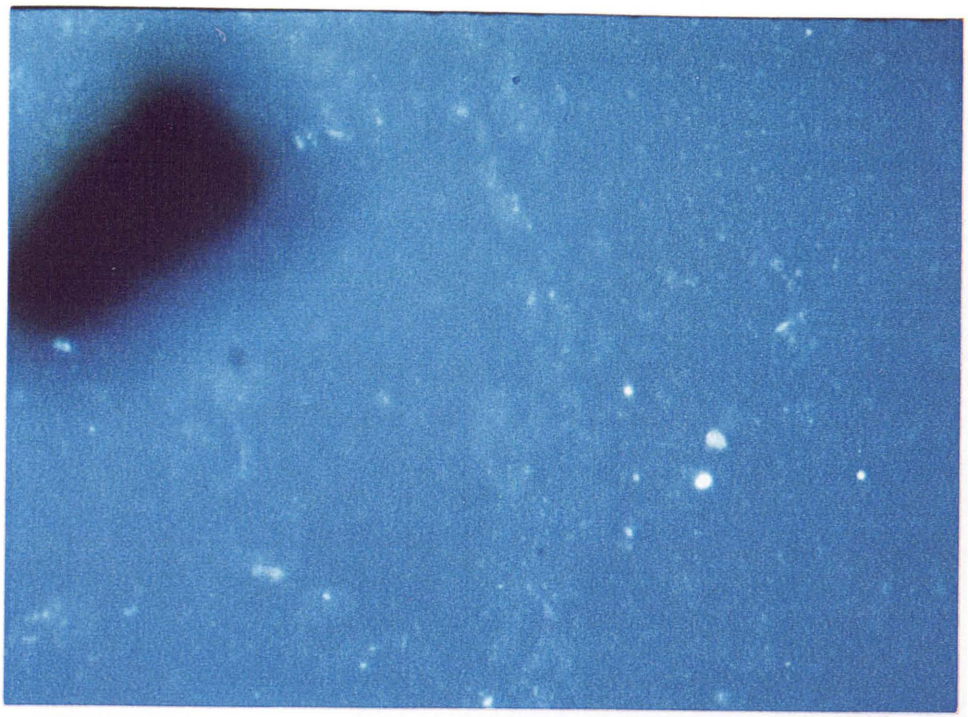


Plate 5

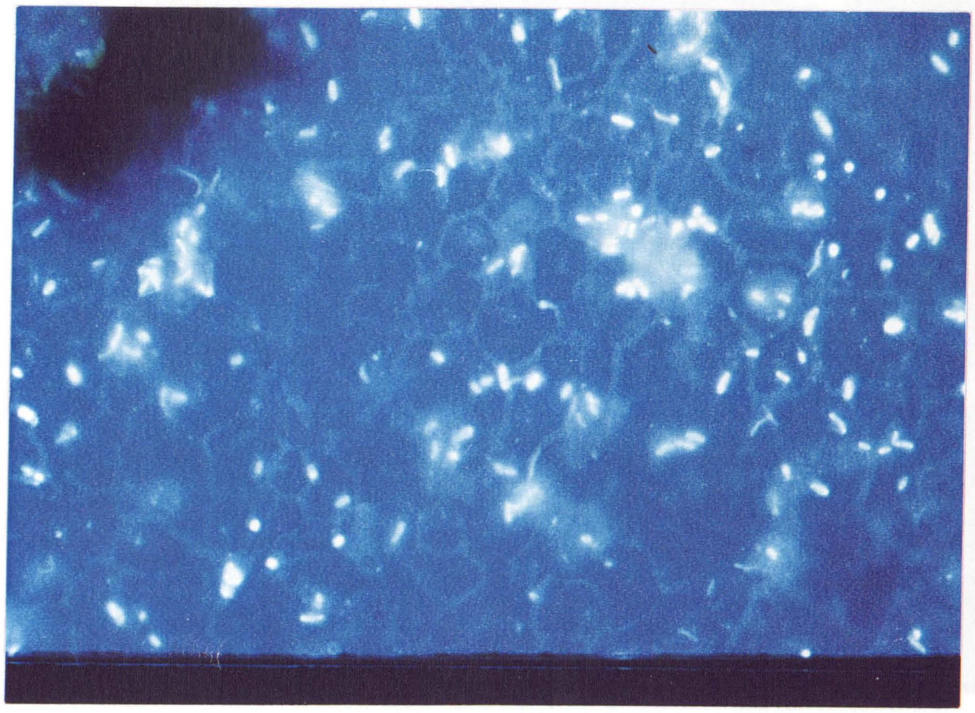


Plate 6

Plates 5 - 6 Bacteria (epifluorescence) attached to stainless steel coupons after 7 d (Plate 5) and 38 d (Plate 6) exposure to the chloraminated water.

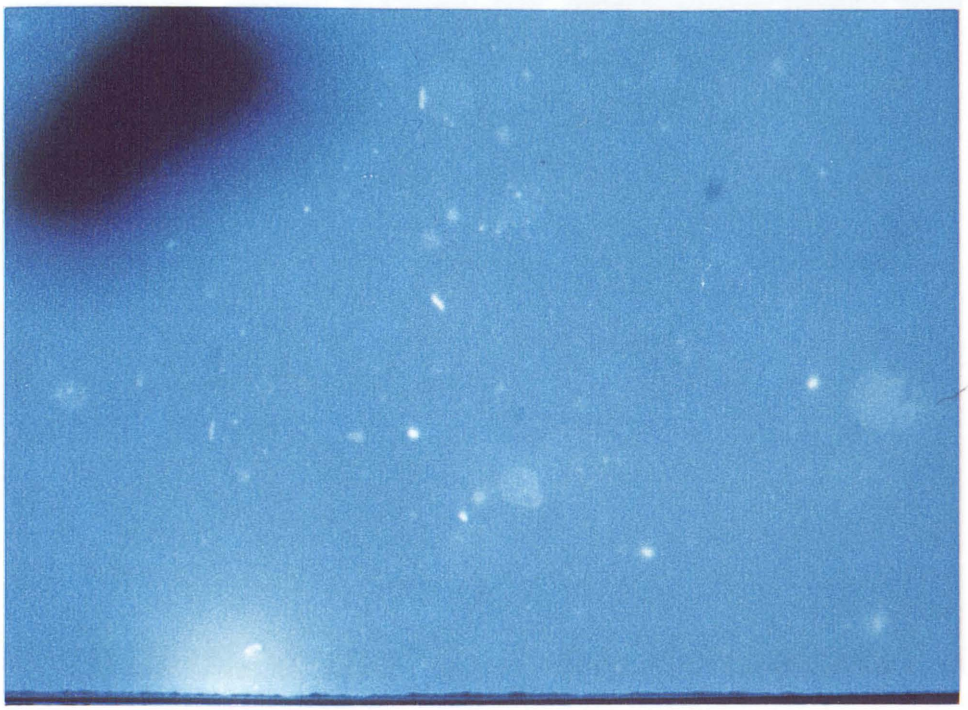


Plate 7

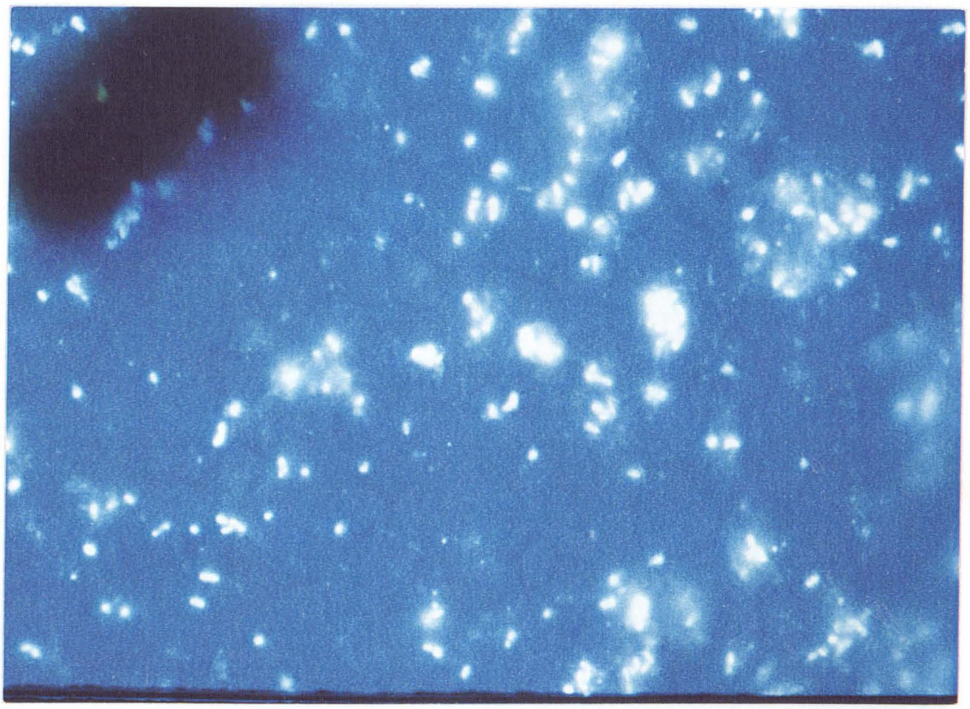


Plate 8

Plates 7 - 8 Bacteria (epifluorescence) attached to stainless steel coupons after 7 d (Plate 7) and 38 d (Plate 8) exposure to the ozonated water.

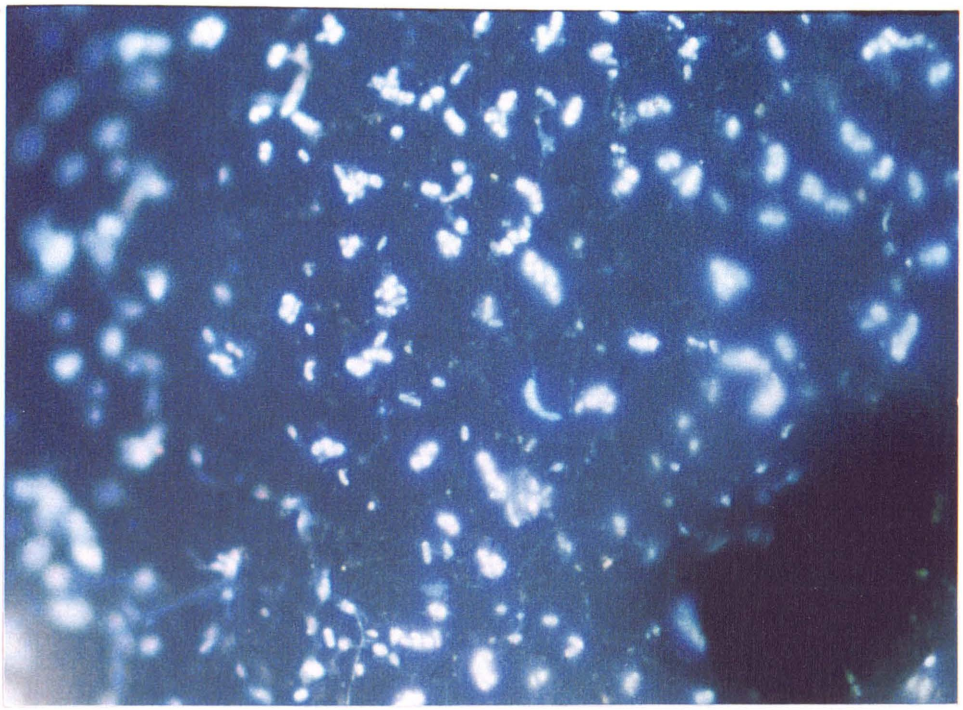


Plate 9

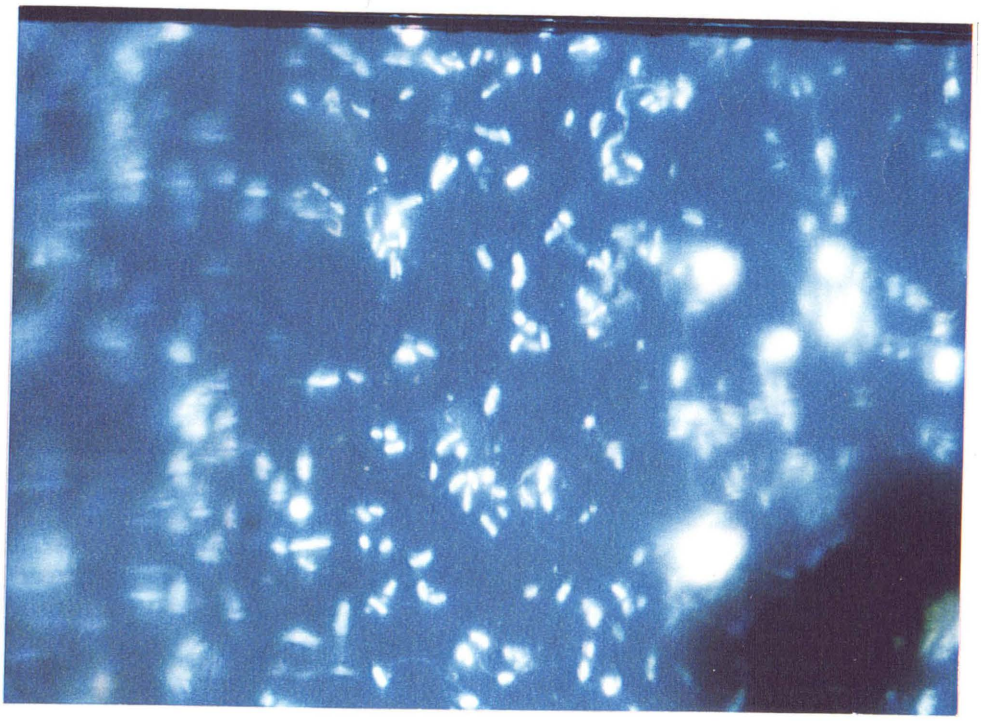


Plate 10

Plates 9 - 10 Bacteria (epifluorescence) attached to stainless steel coupons after 7 d (Plate 9) and 38 d (Plate 10) exposure to the UV treated water.

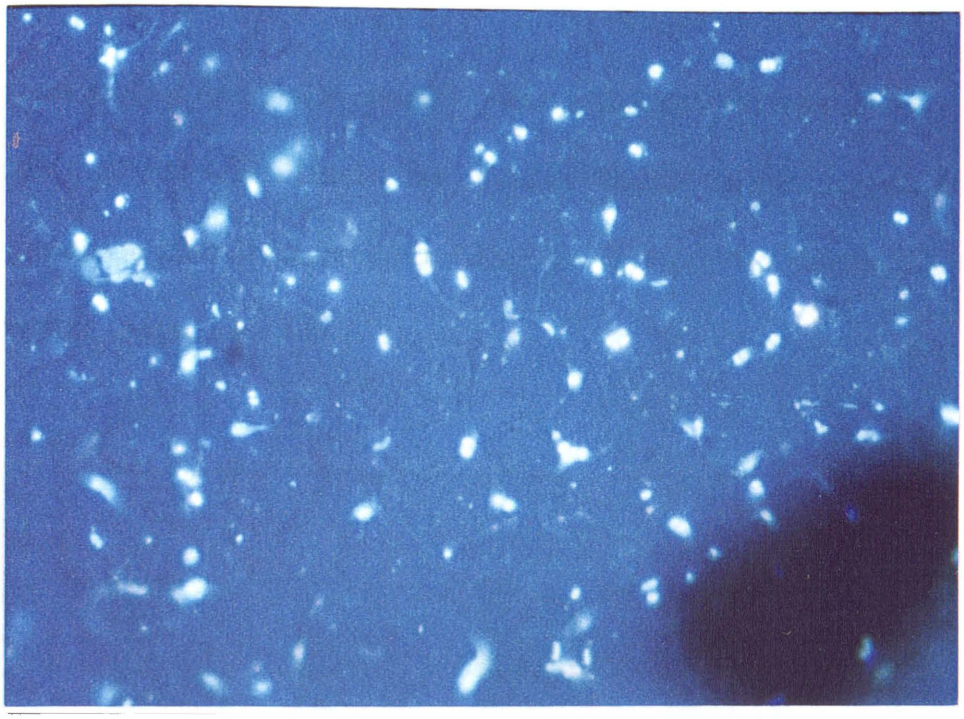


Plate 11

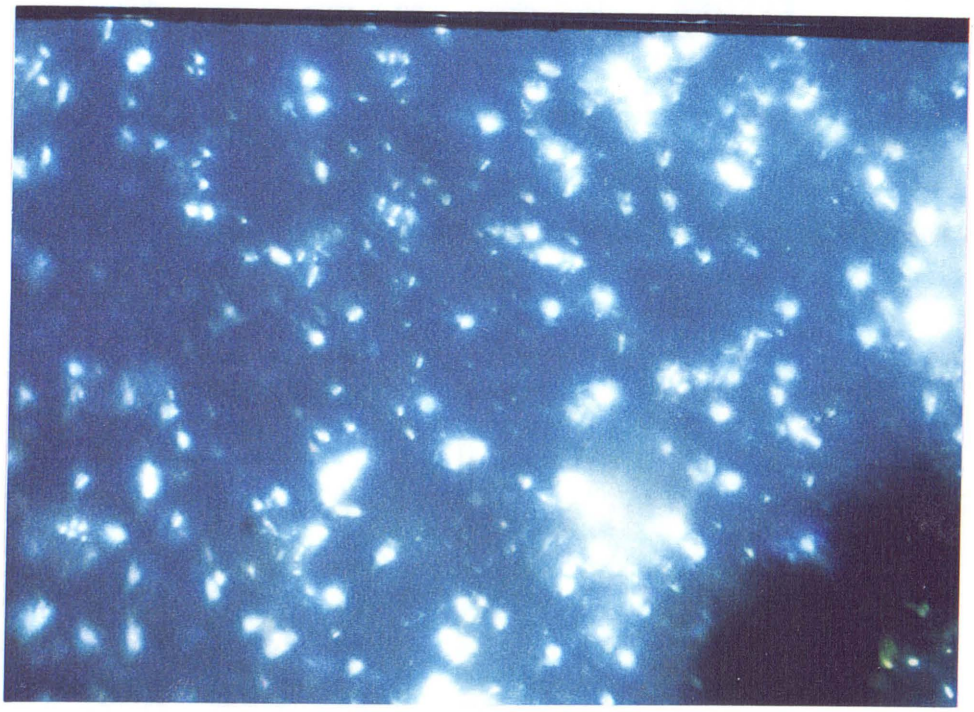


Plate 12

Plates 11 - 12 Bacteria (epifluorescence) attached to stainless steel coupons after 7 d (Plate 11) and 38 d (Plate 12) exposure to the hydrogen peroxide treated water.

4.1.7 Influence of washing on the removal of biofilms on stainless steel coupons.

Figs. 4.20 and 4.21 illustrate the effect of washing on the removal of biofilms. Attached bacteria numbers from non-washed coupons were significantly higher than those from washed coupons (Appendices 3.6 -3.7).

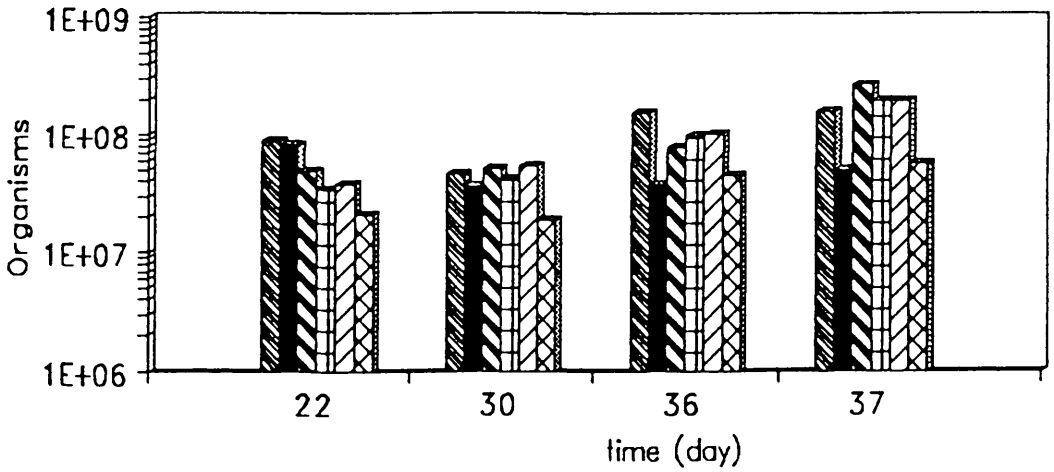


Fig. 4.20. Total bacteria (epifluorescence) attached to stainless steel coupons without washing the coupons.

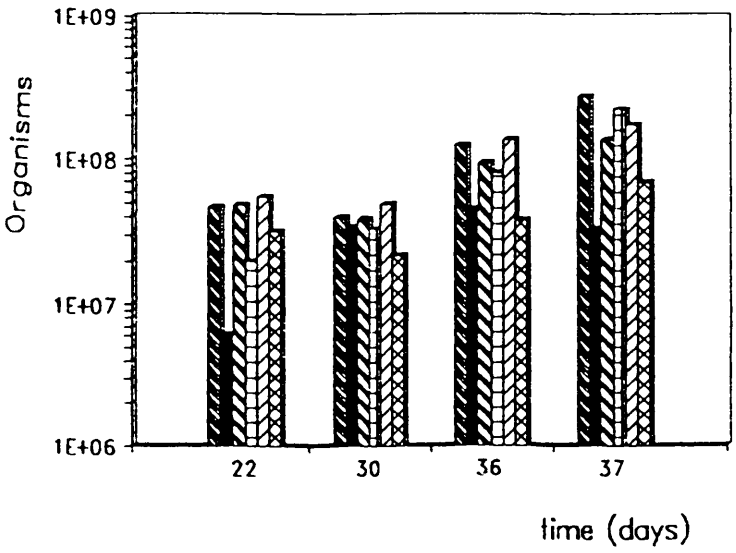
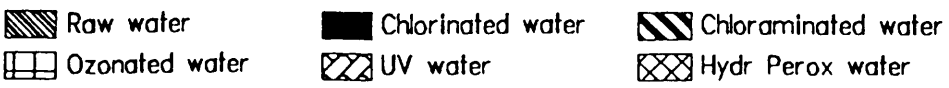


Fig. 4.21. Total bacteria (epifluorescence) attached to stainless steel coupons coupons after washing the coupons.



4.1.8 Scanning electron microscopy study

Observations with SEM indicated a decrease of bacteria on stainless steel coupons exposed to different water distribution systems (source water 1 and 2). The absence of biofilm was noted on stainless steel coupons from chloraminated water (Plate 15) and hydrogen peroxide treated water (Plates 18). The formation of biofilms was obvious on coupons from raw water (Plate 13) and chlorinated water (Plate 14). No uniform biofilms were noted on coupons from ozonated water (Plate 16). A small number of cells was observed on coupons from UV treated water (Plate 17). The SEM observation revealed the formation of biofilms on cement coupons exposed to different waters (Plates 19 - 24). All of these plates derived from source water 1.

4.1.9 Statistical analyses

4.1.9.1 Water sample data

Total bacteria - Total bacteria counts were significantly higher ($P = 0.0220$ during the first 3 d, $P = 0.0001$ over a period of 38 d) in source water 1 (mean = 7.45×10^{-7} cells.mℓ$^{-1}$ during the first 3 d, 1.08×10^{-8} cells.mℓ$^{-1}$) compared to source water 2 (mean = 4.45×10^{-7} cells.mℓ$^{-1}$ during the first 3 d, 5.08×10^{-7} cells.mℓ over a period of 38 d). Significant differences also existed between treatments (Appendix 1.1, 1.3, 1.4 and 1.6). This significant differences were only found between untreated water ant treated water (Appendices 1.3 and 1.6). During the first 3 days, total bacteria counts were significantly higher in raw water than in chlorinated, chloraminated, ozonated and UV water (Appendix 1.3), and after an exposure time of 38 d, raw water had significantly higher total bacteria count than any other disinfection method (Appendix 1.6).

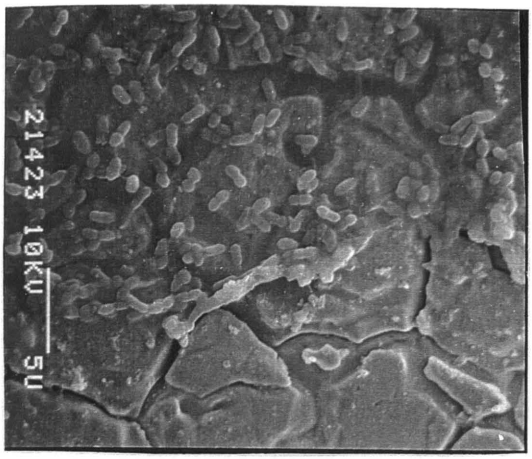


Plate 13

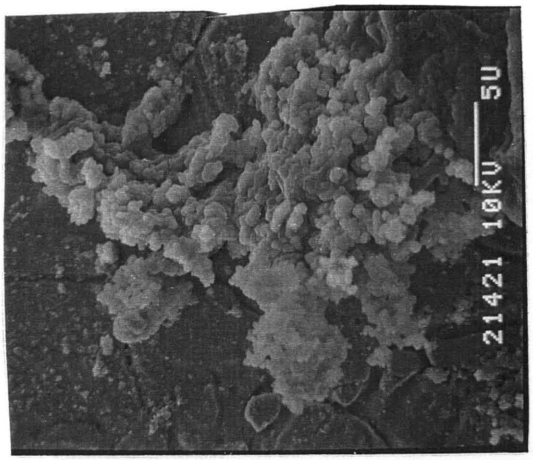


Plate 14

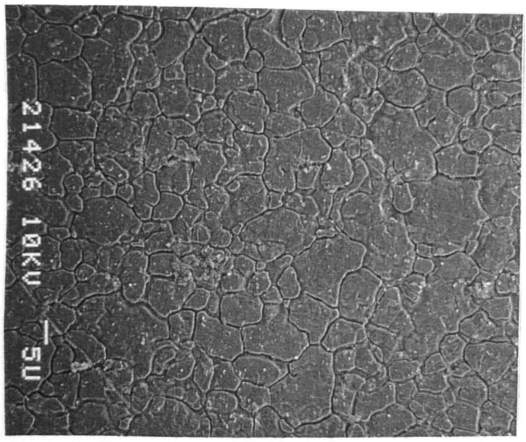


Plate 15

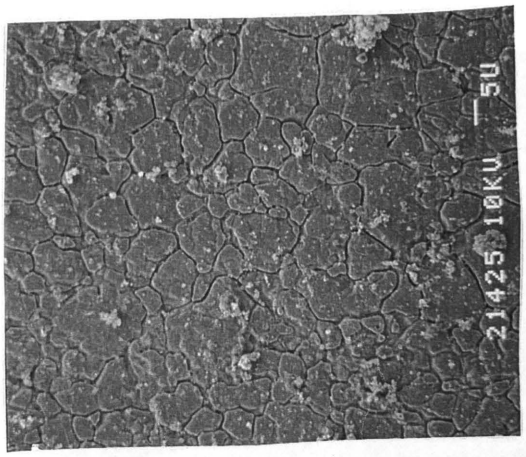


Plate 16

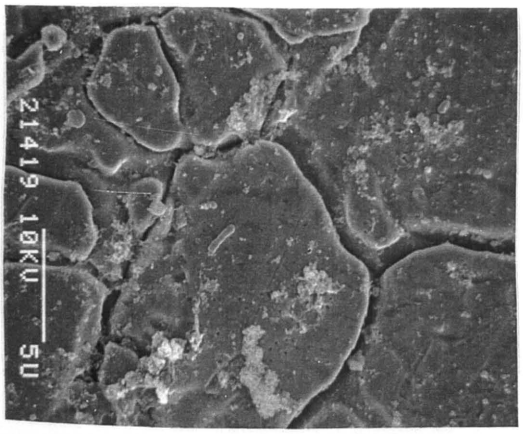


Plate 17

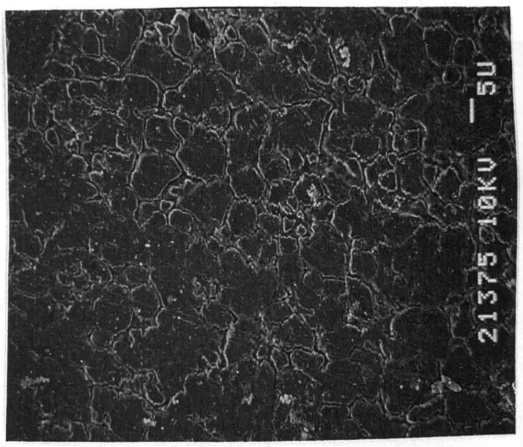


Plate 18

Plates 13 - 18. SEM of attached bacteria on stainless steel coupons exposed to the control (Plate 13), chlorinated water (Plate 14), chloraminated water (Plate 15), ozonated water (Plate 16), UV water (Plate 17), hydrogen peroxide treated water (Plate 18) after 38 days - Source water 1.

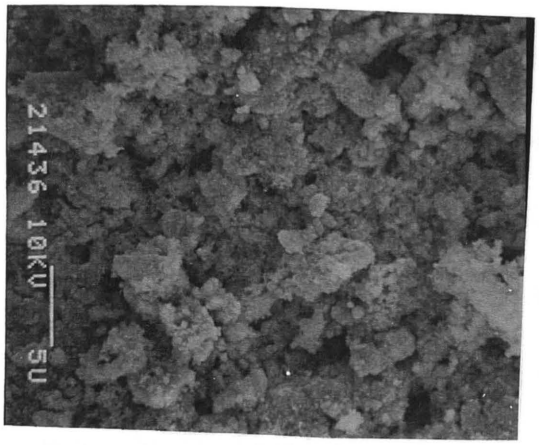


Plate 19

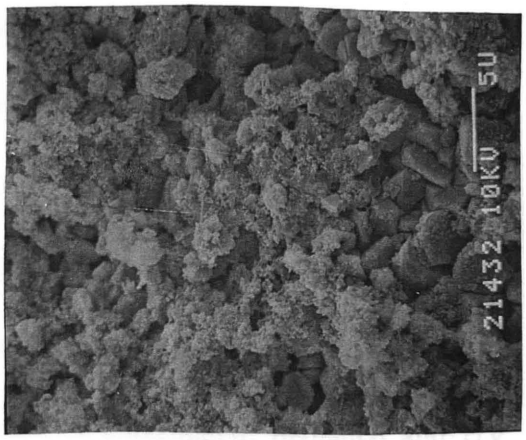


Plate 20

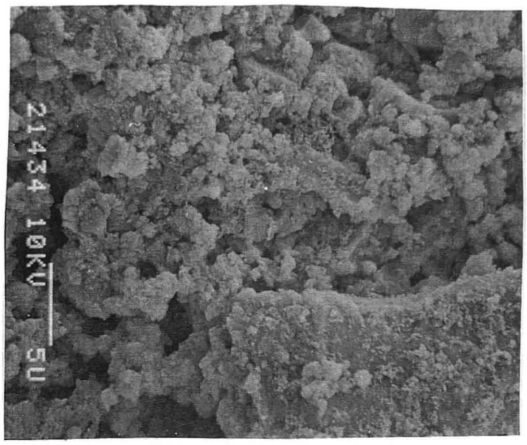


Plate 21

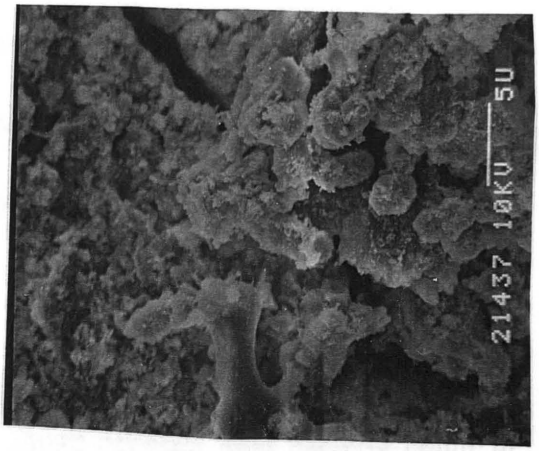


Plate 22

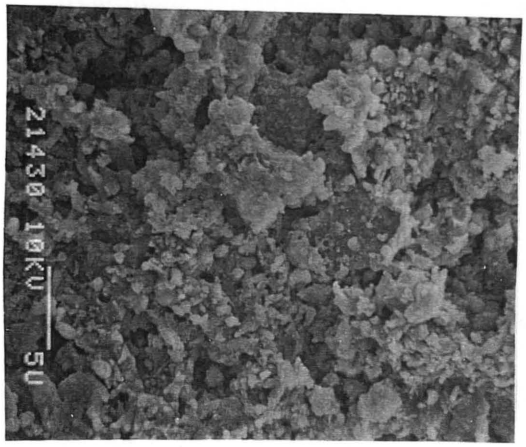


Plate 23

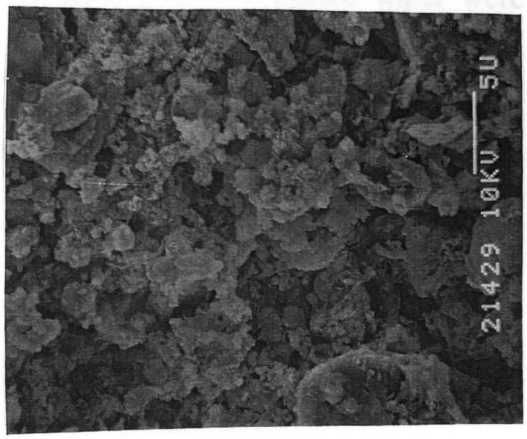


Plate 24

Plates 19 - 24. SEM of attached bacteria on cement coupons exposed to the control (Plate 19), chlorinated water (Plate 20), chloraminated water (Plate 21), ozonated water (Plate 22), UV water (Plate 23), hydrogen peroxide water (Plate 24) within 38 days.

Heterotrophic plate count bacteria - Heterotrophic bacteria numbers did not differ significantly between source water 1 and source water 2, but significant differences did exist between treatments (Appendix 1.1, 1.2, 1.4 and 1.5). During the first 3 d, chloraminated water had significantly lower heterotrophic bacteria counts than ozonated and UV irradiated water. During a period of 38 d, heterotrophic bacteria numbers in chloraminated water and hydrogen peroxide disinfected water were significantly lower than in chlorinated, ozonated and UV irradiated water. A prolonged exposure time increased the number of bacteria. Bacteria numbers in raw water (control) did not differ significantly from chlorinated, ozonated and UV water, but raw water exhibited significantly higher heterotrophic bacteria counts than chloraminated and hydrogen peroxide disinfected water.

4.1.9.2 Biofilm data

Total bacteria - Significant differences were found between total bacteria count in treatments (Appendices 3.2, 3.3) as follows: the total bacteria in UV treated water were significantly higher than in chloramine treated water (Appendix 3.3). The control (raw water) had significantly higher total bacteria counts than the disinfected waters. Significant differences in total bacteria were also found between coupons stainless steel and cement coupons (Appendices 3.2, 3.4). Attached total bacteria counts were significantly higher on the cement coupons (average mean = 6.66×10^7 cells.cm^{-2}) than on the stainless steel coupons (average mean = 2.53×10^7 cells.cm^{-2}). The attached total bacteria counts on the stainless steel coupons did not differ significantly between source water 1 and source water 2 (Appendix 3.5).

Heterotrophic plate count - Heterotrophic bacteria numbers did not significantly differ between treatments. No significant differences in heterotrophic bacteria count were found between stainless steel and cement coupons (Appendix 3.1)

SECTION II INFLUENCE OF DISINFECTION ON THE MICROBIAL QUALITY OF GROUNDWATER DISTRIBUTION SYSTEMS USING A LABORATORY SCALE UNIT

4.2.1 Control water (raw water)

4.2.1.1 Water sample analyses

Total bacterial numbers determined using DAPI- epifluorescence microscopy

Source water 1 - Within the first 24 h, the bacterial number increased from 7 log to 8 log cells.mℓ$^{-1}$, after which some minor fluctuations were noted between 24 h and 35 d (Fig. 4.22).

Source water 2 - Within the first 8 d, the number of total bacteria was 7 log cells.mℓ$^{-1}$ after which some minor fluctuations took place from time to time over the rest of the study period (Fig. 22).

Heterotrophic plate count (HPC)

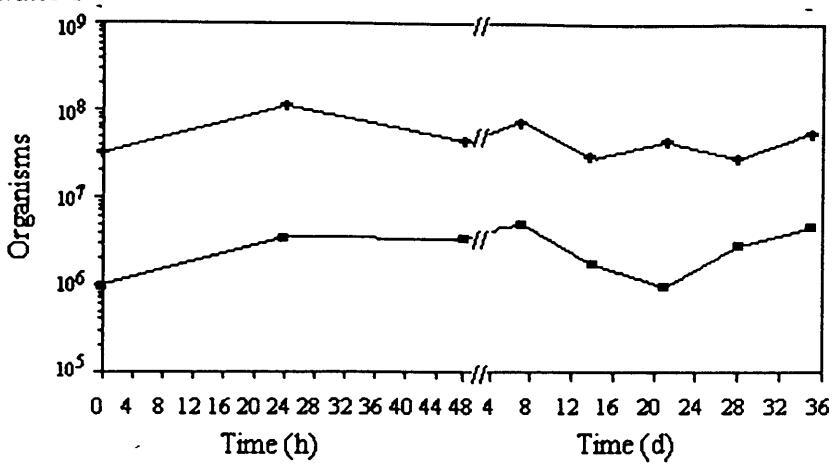
Source water 1 - Within the first 7 d, the bacteria count increased from 5 to 6 log cfu.mℓ$^{-1}$ after which some fluctuation took place from time to time over the remainder of the study period (Fig. 22). The average growth rate was 0.01 h^{-1} (generation time 69.31 h) within 7 d.

Source water 2 - The bacteria count increased from 4 to 6 log cfu.mℓ$^{-1}$ within the first 48 h (average growth rate 0.084 h^{-1}, generation time 8.25 h). Between 48h and 36 d some fluctuations took place and bacteria count ranged between 5 and 6 log cfu.mℓ$^{-1}$ (Fig. 22).

Coliform bacteria

Source water 1 - An initial number of 71 cfu/100 mℓ total coliform bacteria was detected in the raw water. Within the first day, this number increased to 113 cfu/100 mℓ, after which a decrease occurred between 1 and 7 d, and total coliform bacteria completely disappeared for the remainder of the study period (Fig. 23). Faecal coliform numbers increased from 20 to

Source water 1



Source water 2

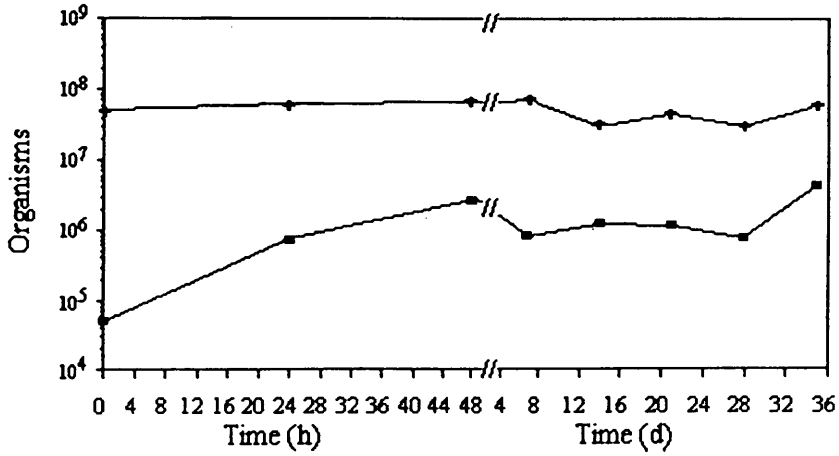
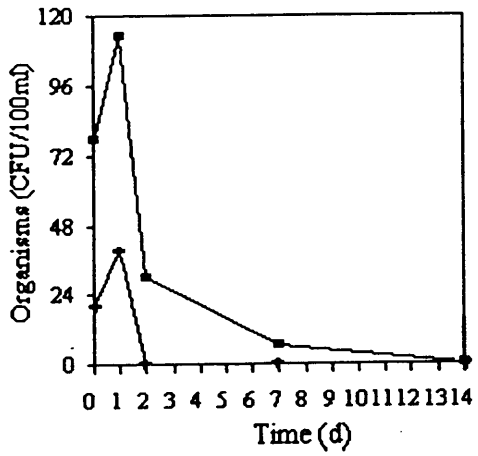


Fig. 4.22. Number of total bacteria (TB - epifluorescence) and heterotrophic plate count (HPC) bacteria in the raw water distribution system. —■— HPC (CFU/ml) —•— TB (cell/ml)

Source water 1



Source water 2

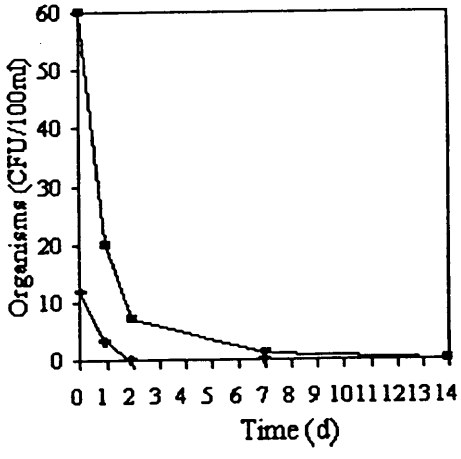


Fig. 4.23. The total number of coliform bacteria in the raw water distribution system

40 cfu/100 mℓ within 1 d, after which a decrease occurred and no faecal coliform were detected over the remainder of the study period (Fig. 23).

Source water 2 - Initial counts of 60 cfu/ 100 mℓ total coliform bacteria and 12 cfu/100 mℓ faecal coliform decreased to 7 cfu/100 mℓ and to 3 cfu/100 mℓ within 7 d and completely disappeared for the remainder of the study period (Fig. 23). Faecal coliform numbers decreased from 12 cfu/100 mℓ to 3 cfu/100 mℓ within 1 d. A complete elimination of faecal coliform in the raw water distribution system occurred after 2 d (Fig. 23).

4.2.1.2 Biofilm analyses

Biofilm monitoring

Source water 1

Bacteria adhered to the glass surface within the first day of the experiment. the absorbance measurements ranged between 0.53 and 0.60 during the study period (Fig. 4.30).

Source water 2

Within the first day, the adhesion of bacteria on the glass surface was obvious. The absorbance measurements ranged between 0.39 and 0.84 during the study period (Fig. 4. 31).

Attached total bacteria numbers determined using epifluorescence microscopy

Source water 1

Stainless steel coupons - The attachment of total bacteria to the stainless steel coupons was noted 1 d after exposure with a cell density of 8 log cells.cm^{-2} (Fig. 4.31). Although some fluctuations took place from time to time, the attached total cells ranged between 7 and 8 log. cells.cm^{-2} for a period of 35 d.

Cement coupons - The adhesion of total cells on the cement coupons resulted in 7 log cell.cm^{-2} after 1 d of exposure (Fig. 4.32). An exponential phase progressively occurred between 1 and

14 d, and the bacteria reached 8 log cells.cm^{-2}, after which the bacteria number remained constant over the rest of the study period (Fig. 32).

Source water 2

Stainless steel - The biofilm formation on the stainless steel coupons started from the first day of exposure, with a bacterial density of 7 log cells.cm^{-2} which marked a levelling off of the adhesion rate over the remainder of the study period (Fig. 4.31).

Cement coupons - The initial adhesion of total bacteria on the cement coupons occurred at a cell density of 7 log cells.cm^{-2} after 1 d of exposure. An increase up to 8 log cells.cm^{-2} was noted within 14 d, which marked a levelling off of the adhesion rate over the remainder of the study period (Fig. 4.32).

Attached heterotrophic plate count (HPC)

Source water 1

Stainless steel coupons - After one day of exposure, the formation of micro-colonies on the stainless steel coupons occurred with a bacterial density of 6 log cfu.cm^{-2} (Fig. 4.33). At this level, attached HPC reached the plateau (Fig. 4.33).

Cement coupons - The adhesion of viable bacteria on the cement surfaces resulted in a bacterial community of 5 log cfu.cm^{-2} within 1 d of disinfection (Fig. 4.34). Attached HPC increased to 7 log cfu.cm^{-2} between 1 and 7 d after which a decrease in the bacteria count (6 log cfu.cm^{-2}) occurred between 21 and 35 d.

Source water 2

Stainless steel coupons - Viable bacteria adhered to the stainless steel coupons with a bacterial community of 5 log cfu.cm^{-2} within the first day of exposure (Fig. 4.33). An increase occurred between 1 and 7 d, and the attached HPC reached 6 log cfu.cm^{-2}. In spite of some fluctuations, no specific increase in bacteria count was found over the remainder of the experimental period.

Cement coupons - The adhesion of viable bacteria on the cement coupons was obvious from the first day of exposure, with a bacterial density of 3 log cfu.cm^{-2} which increased to 7 log cfu.cm^{-2} between 1 and 28 d (Fig. 4.34).

4.2.2 Disinfection of groundwater by chlorination

4.2.2.1 Water sample analyses

Disinfectant residual

Source water 1 - A reduction in free chlorine concentration from 2 mg.ℓ^{-1} to 1.25 mg.ℓ^{1} occurred within 20 min. The residual free chlorine concentration progressively decreased to 0.5 mg.ℓ^{-1} during the next 24 h and completely disappeared after 48 h (Fig. 4.24).

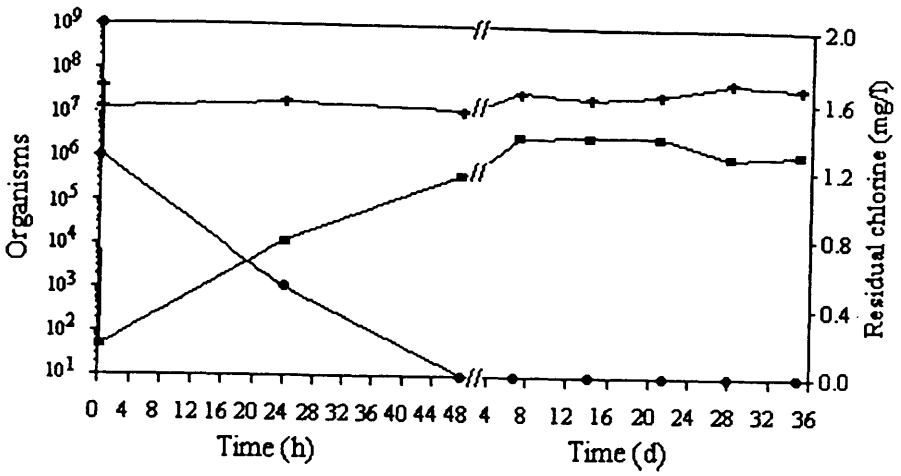
Source water 2 - An initial concentration of 2.5 mg.ℓ^{-1} free chlorine was reduced to 1.4 mg.ℓ^{-1} residual free chlorine within 20 min. This free chlorine concentration further decreased to 0.5 mg.ℓ^{-1} within 24 h. Residual free chlorine concentration could be detected after 7 d (Fig. 4.24).

Total bacterial numbers determined using DAPI- epifluorescence microscopy

Source water 1 - Chlorine exhibited a kill percentage of 61.98 % after 20 min (Table 3). Although no specific increase was noted during the next 24 h, the regrowth rate was 0.006 h^{-1} and the generation time was 115.52 h between 20 min and 7 d (Table 4.2).

Source water 2 - 68.19 % of cells were killed by chlorine within 20 min. 24 h after disinfection, chlorine exhibited a kill percentage of 87.67 %. Bacterial regrowth was apparent within 7 d after which bacteria reached the plateau over the remainder of the study period (Fig. 4.24). The regrowth rate was 0.012 h^{-1} (generation time 57.76 h) between 24 h and 7 d.

Source water 1



Source water 2

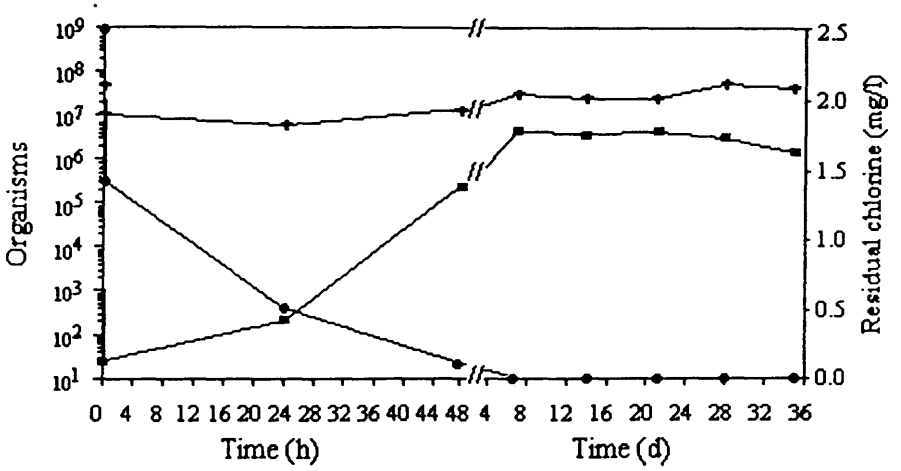


Fig. 4.24 Number of total bacteria (TB - epifluorescence) and heterotrophic plate count (HPC) bacteria in the chlorine treated water. HPC (CFU/ml) TB (cell/ml) Residual monochl

Table 4.2. Characteristics of potable groundwater after disinfection.

Parameter		Time	Residual ($mg\ell^{-1}$)	% kill		Regrowth rate (h^{-1})		Generation time(h)		Presence of regrowth	
				TB	HPC	TB	HPC	TB	HPC	TB	HPC
CAW	*S1*	20 min	1.25	61.98	99.995	0.006	0.067	115.52	10.35	20' - 24 h	20'-7d
	S2	20 min	1.40	68.19	99.949						
		24 h	0.50	87.67	99.760	0.012	0.060	57.76	11.55	24 - 7 d	20' - 7 d
CMW	*S1*	20 min	1.70	49.20	99.961						
		24 h	0.80	79.14	99.995						
		48 h	0.50	91.12		0.009	0.077	77.16	9.00	48 h - 14 d	24 h- 7 d
	S2	20 min	2.00	50.89	99.760						
		24 h	1.00	80.52	99.970	0.005	0.037	138.63	18.73	24 h-14 d	4 8 h - 1 4 d
		48h	0.70		*						
OW	*S1*	2h	0.22	69.74	99.999	0.007	0.315	99.02	2.20	2 h - 14 d	2 h - 48 h

Parameter		Time	Residual ($mg\ell^{-1}$)	% kill		Regrowth rate (h^{-1})		Generation time(h)		Presence of regrowth	
				TB	HPC	TB	HPC	TB	HPC	TB	HPC
OW	*S2*	2h	0.30	59.05	99.998	0.004	0.088	173.29	7.88	2 h-14 d	2 h - 7 d
UVW	*S1*	2 h	**	84.69	99.999	0.101	0.302	6.86	2.30	2 h-24 h	2 - 48 h
	S2	2 h	**	73.36	99.920	0.005	0.224	138.63	3.09	2 h-14 h	2 - 48 h
HPW	*S1*	2 h	20.70	*	97.474						
		48 h	18.10	16.93	99.973	0.006	0.041	115.52	17.33	2 h-7 d	48 h - 14 d
	S2	2 h	13.10	45.73	98.283						
		48 h	10.70	79.32	99.784	0.011	0.085	63.01	8.15	2 h-7 d	48 h - 7 d

CAW = chlorinated water UVW = UV irradiated water TB = total bacteria *** = no residual disinfectant

CMW = chloraminated water HPW = hydrogen peroxide water * = no specific growth ' = minute

OW = ozonated water HPC = heterotrophic plate count ** = no kill of bacteria S1, S2 = source water 1 and 2

Heterotrophic plate count (HPC)

Source water 1 - 99.995 % of the bacterial community were killed by chlorine within 20 min after which regrowth occurred between 20 min and 7 d. The regrowth rate was 0.067 h^{-1} and the generation time was 10.35 h (Table 4.2), after which the bacteria reached the plateau for the rest of the study (Fig. 4.24). *Source water 2* - 99.949 % of the bacterial community were killed by chlorine within 20 min, after which bacterial regrowth took place between 20 min and 7 d. The regrowth rate was 0.060 h^{-1} and the generation time 11.55 h, after which the bacteria reached the plateau over the remainder of the experimental study (Fig. 4.24).

Coliform bacteria

Source water 1 and source water 2 - No coliform bacteria were detected 20 min after chlorination.

4.2.2.2 Biofilm analyses

Biofilm monitoring

Source water 1 - Bacteria adhered to the glass surface within the first day of exposure in chlorinated water distribution system (Fig. 4.30). Although some minor fluctuations took place from time to time, the absorbance measurements ranged between 0.41 and 0.66 during the study period of 35d.

Source water 2 - The adhesion of bacteria on the glass surface tube was obvious within 1 d of exposure (Fig. 4.30). Although fluctuation took place from time to time, the absorbance measurements ranged between 0.31 and 0.58 during the study period.

Total bacteria number determined using DAPI epifluorescence microscopy

Source water 1

Stainless steel coupons - The attachment of cells to the stainless steel coupons was observed

after 1 d of exposure in a chlorinated water distribution system, with a cell density of 6 log cells.cm^{-2} (Fig. 4.31). Between 1 and 7 d, the attached bacteria number increased to 7 log cells.cm^{-2}. In spite of some fluctuations, this density marked a levelling off of the adhesion rate over the remainder of the study period.

Cement coupons - Adhesion of total cells was observed on the cement coupons after 1 d of exposure with a bacterial density of 7 log cells.cm^{-2}. Between 1 and 14 d, a typical increase was visible, up to 8 log cells.cm^{-2}, after which the bacteria reached the plateau over the remainder of the study period (Fig. 4.32).

Source water 2

Stainless steel coupons - Within 1 d, the total cells attached to the stainless steel coupons amounted to 9.67 x 10^{6} cells.cm^{-2}, after which the bacteria increased to 7 log cells.cm^{-2}. This bacteria count remained stable over the remainder of the study period (Fig. 5.31).

Cement coupons - The initial adhesion of total bacteria to the stainless steel coupons occurred with a cell density of 7 log cells.cm^{-2} within 1 d (Fig. 5.32), after which a typical increase was visible, up to 8 log cells.cm^{-2} over the remainder of the study period.

Heterotrophic plate count (HPC)

Source water 1

Stainless steel coupons - The adhesion of viable bacteria on the stainless steel coupons was visible with a bacterial community of 5 log cfu.cm^{-2} within 1 d of exposure in a chlorinated water system (Fig. 4.33). Attached HPC numbers increased progressively up to 6 log cfu.cm^{-2} within 14 d, after which the bacteria reached the plateau over the remainder of the study period.

Cement coupons - Attachment of heterotrophic bacteria to the cement occurred within 1 d of exposure with 4 log cfu.cm^{-2} (Fig. 4. 34). The number of bacteria increased to 7 log cfu.cm^{-2}

within 14d. In spite of some fluctuations, the attached heterotrophic bacteria count remained constant for the rest of the experimental period.

Source water 2

Stainless steel coupons - Within the first day, viable bacteria attached to the stainless steel coupons amounted to 4 log cfu.cm^{-2} (Fig.4.33). Between 1 and 7 d, the number of heterotrophic bacteria increased to 6 log cfu.cm^{-2}, which was maintained over the remainder of the study period.

Cement coupons - Heterotrophic bacteria adhered to the cement coupons within 1 d of exposure with a bacterial density of 3 log cfu.cm^{-2} (Fig. 4.34), which gradually increased to 7 log.cfu.cm^{-2} between 1 and 14 d. Although some minor fluctuations occurred over the remainder of the study period, attached viable bacteria reached the plateau at this level.

4.2.3 Disinfection of groundwater by chloramination

4.2.3.1 Water sample analyses

Disinfectant residual

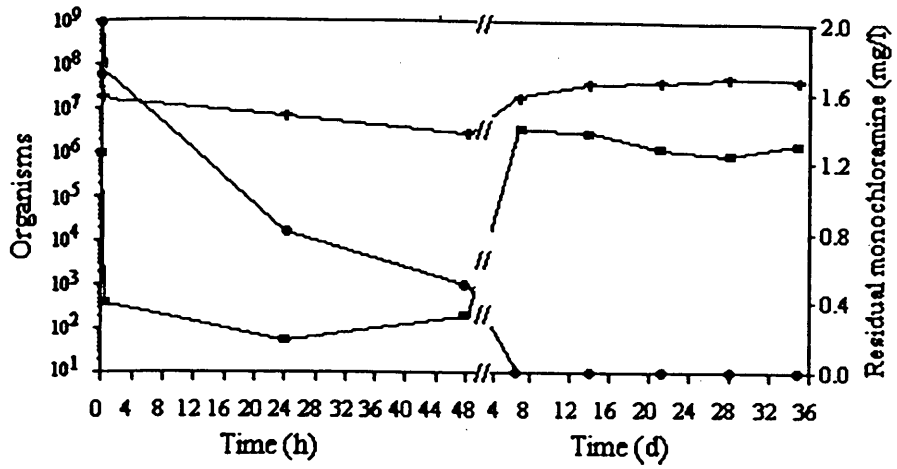
Source water 1 - The monochloramine concentration decreased from 2 to 1.7 mg.ℓ^{-1} within 20 min after disinfection. Between 20 min and 7 d, the residual monochloramine concentration gradually declined to 0 mg.ℓ^{-1} (Fig. 4.25).

Source water 2 - Within 20min, an 2.5 mg.ℓ^{-1} initial monochloramine concentration was reduced to 2 mg.ℓ^{-1} (Fig. 4.25). Residual monochloramine disappeared in the system after 14 days.

Total bacterial numbers determined using DAPI- epifluorescence microscopy

Source water 1 - 49.20 % of cells were killed by monochloramine within 20 min and 91.120 % within 48 h, after which bacterial regrowth occurred between 48 h and 14 d. The regrowth

Source water 1



Source water 2

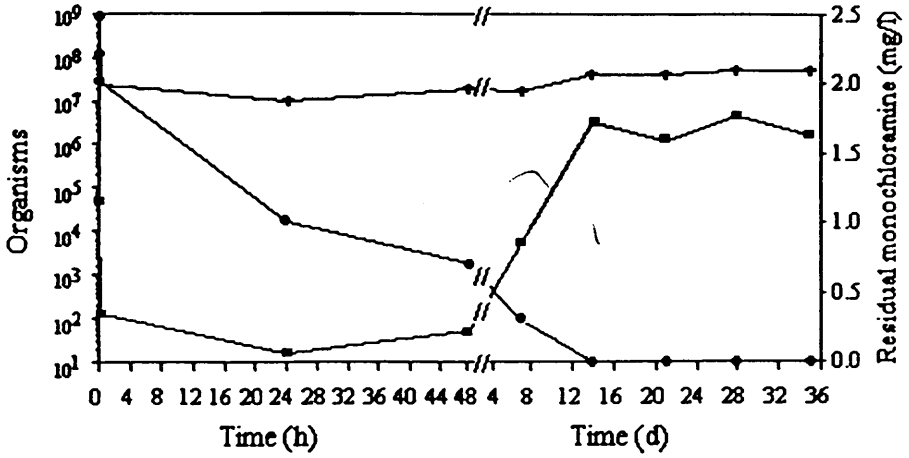


Fig. 4.25 Number of total bacteria (TB - epifluorescence) and heterotrophic plate count (HPC) bacteria in the chloramine treated water.

HPC (CFU/ml) TB (cell/ml) Residual monochl

rate was 0.009 h^{-1} and the generation time was 77.02 h (Table 4.2, Fig. 4.25). Between 14 and 35 d, the bacteria numbers remained constant.

Source water 2 - 50.89 % of cells were killed by monochloramine within 20 min. After 24 h chloramination, the kill percentage increased to 80.52 %, after which the phenomenon of regrowth was observed between 24 h and 14 d (regrowth rate 0.005 h^{-1}, generation time 138.63 h). Between 14 and 35 d, bacteria reached the plateau (Fig.4.25).

Heterotrophic plate count (HPC)

Source water 1 - 99.961 % of heterotrophic plate count bacteria were killed by monochloramine during the first 20 min and 99.995 % after 24 h (Table 4.2). Bacterial regrowth was apparent

between 24 h and 7 d, after which the bacteria count remained constant (Fig. 4.25). The regrowth rate was 0.077 h^{-1} (generation time 9 h) between 24 h and 7 d.

Source water 2 - 99.760 % of heterotrophic plate count bacteria were killed by monochloramine within 20 min after disinfection and 99.970 % within 24 h (Table 4.2). Bacterial regrowth was apparent between 48 h and 14 d, after which some fluctuations took place over the remainder of the study period of 35 d (Fig. 4.25). Between 48 h and 14 d, the regrowth rate was 0.037 h^{-1} (generation time 18.73 h). *Coliform bacteria*

Source water 1 and Source water 2 - No coliform bacteria were detected within 20 min after chloramination.

4.2.3.2 Biofilm analyses

Biofilm monitoring

Source water 1 - The adhesion of bacteria on the flow-through glass tube was visible within the first day with an absorbance measurement of 0.39 (Fig.4.30). Although some fluctuation

took place from time to time, the absorbance measurements ranged between 0.38 and 0.63 during the study period.

Source water 2 - Within 1 d, the attachment of bacteria to the flow-through glass tube was apparent with an absorbance measurement of 0.61 (Fig.4.30), after which some fluctuations took place, and the absorbance measurement ranged between 0. 40 and 5.90 durin the study period.

Total bacteria number determined using DAPI epifluorescence microscopy

Source water 1

Stainless steel coupons - Biofilm formation on the stainless steel coupons started from the first day of disinfection, with a bacterial density of 7 log cells.cm^{-2} which increased to 8 log cells.cm^{-2} between 1 and 28 d (Fig. 4.31).

Cement coupons - Biofilm formation on the cement coupons started with a cell density of 7 log cells.cm^{-2} which increased to 8 log cells.cm^{-2} within 14 d (Fig. 4.32). Although the bacteria count fluctuated from time to time, no specific increase was noted over the rest of the study period.

Source water 2

Stainless steel coupons - A density of 6 log cells.cm^{-2} was detected on the stainless steel coupons within 1 d after disinfection, after which the number of bacteria increased up to 8 log cells.cm^{-2} within 14 d (Fig. 4.31). A decrease in bacteria count took place over the remainder of the study period and the bacteria count was 7 log cells.cm^{-2} .

Cement coupons - An initial density of 2.73 x 10^{7} cells.cm^{-2} was recorded on the cement coupons within 1 d of disinfection. Between 1 and 14 d, an exponential phase progressively occurred, and bacteria reached 8 log cells.cm^{-2}, after which some fluctuations took place from time to time over the rest of the study period (Fig. 4.32).

Heterotrophic plate count (HPC)

Source water 1

Stainless steel coupons - Adhesion of HPC on the stainless steel coupons was visible within the first day of exposure of the coupons in a chloraminated water system (Fig. 4.33). This resulted in a bacterial community of 4 log cfu.cm^{-2} which increased to 7 log cfu.cm^{-2} within 28 d.

Cement coupons - Heterotrophic plate count bacteria adhered to the cement coupons within 1 d of disinfection (Fig. 4.34). Between 1 and 21 d, the number of bacteria increased from 4 log to 7 log cfu.cm^{-2} which marked the levelling off of the adhesion rate over the remainder of of the study period.

Source water 2

Stainless steel coupons - Viable bacteria adhered to the stainless steel coupons within the first day of disinfection. Between 1 d and 21 d, the number of bacteria increased from 4 to 6 log cfu.cm^{-2} (Fig. 4.33). At this level, the bacteria counts remained constant for the rest of the study.

Cement coupons - An initial heterotrophic count of 8.8 x 10 cells.cm^{-2} was recorded on the cement coupons after 1 d of exposure in a chloraminated water distribution system. This count increased progressively and the bacteria reached 8 log cfu.cm^{-2} within 21 d, which remained stable for the rest of the study period (Fig. 4.34).

4.2.4 Disinfection of groundwater by ozonation

4.2.4.1 Water sample analyses

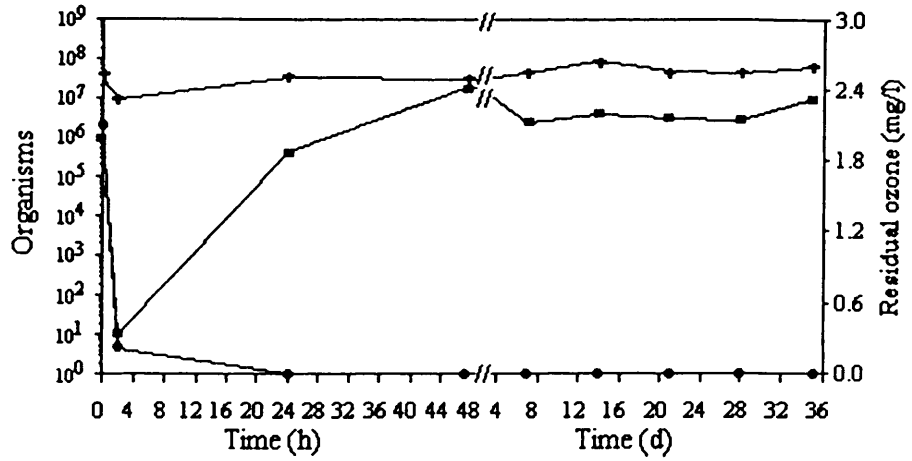
Disinfectant residual

Source water 1 - The ozone concentration decreased from 2.07 to 0.22 mg.ℓ^{-1} within 2 h after

disinfection and disappeared after 24 h (Fig. 4.26).

Source water 2 - A depletion of ozone concentration from 2.65 mg.ℓ^{-1} to 0.30 mg.ℓ^{-1} occurred within 2 h and disappeared after 24 h (Fig. 4.26).

Source water 1



Source water 2

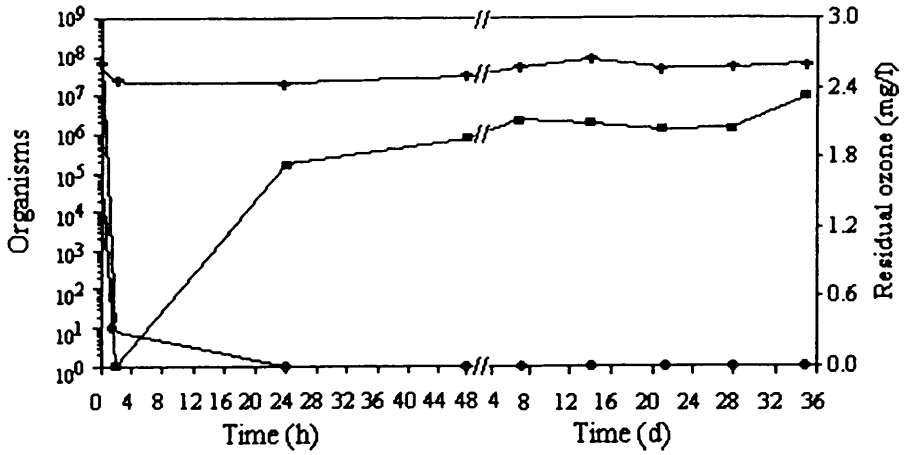


Fig. 4.26 Number of total bacteria (TB - epifluorescence) and heterotrophic plate count (HPC) bacteria in the ozone treated water. HPC (CFU/ml) TB (cell/ml) Residual ozone

Total bacterial numbers determined using DAPI- epifluorescence microscopy

Source water 1 - 69.74 % of cells were killed by ozone within 2 h. Bacterial regrowth was

noted between 2 h and 14 d after which the bacteria reached the stationary phase at 4.57 x 10^7 cells.mℓ^{-1} (Fig. 4.26). The regrowth rate between 2 h and 14 d was 0.007 h and the generation time was 99.02 h (Table 4.2) after which bacterial reached the plateau for the remainder of the study period.

Source water 2 - Within 2 h, 59.05 % of cells were killed by ozone, after which the bacterial numbers increased between 2 h and 14 d (regrowth rate 0.004 h^{-1}, generation time 173.29 h) after which no specific increase was noted over the rest of the study period (Fig. 4.26).

Heterotrophic plate count (HPC)

Source water 1 - 99.999 % HPC were killed by ozone within 2 h, after which bacterial regrowth occurred between 2 h and 48 h, reaching the plateau over the remainder of the study period (Fig. 4.26). The regrowth rate between 2 h and 48 h was 0.315 h^{-1} and the generation time was 2.20 h (Table 4.2).

Source water 2 - 99.998 % of the bacterial community were killed by ozone. Regrowth took place between 2 h and 7 d, after which the bacteria reached the plateau at 1.77 x 10^6 cfu.mℓ^{-1} (Fig. 4.26). Between 2 h and 7 d, the regrowth rate was 0.088 h^{-1} (generation time 7.88 h).

Coliform bacteria

Source water 1 and 2 - The absence of coliform bacteria were observed after 2 h ozonation.

4.2.4.2 Biofilm analyses

Biofilm monitoring

Source water 1 - The formation of biofilm on the flow-through glass tube within 1 d occurred with an aborbance value of 0.47 after which some fluctuations took place (Fig. 4.30). During the study period of 35 d, the absorbance measurements ranged between 0.47 and 0.58.

Source water 2 - The adhesion of bacteria on the glass surface tube was apparent with an initial

absorbance measurement of 0.51 after which fluctuations took place over the remainder of the study period (Fig. 4.30).

Total bacteria number determined using DAPI - epifluorescence microscopy

Source water 1

Stainless steel coupons - The formation of biofilm on the stainless steel coupons within the first day occurred at a bacterial cell density of 7 log cells.cm^{-2}, which gradually increased to 8 log cells.cm^{-2} after 28 d (Fig. 4.31).

Cement coupons - An initial cell density of 6 log cells.cm^{-2} was detected on the cement coupons within 1 d of exposure in ozonated water. Between 1 and 14 d, bacteria numbers increased to 8 log cells.cm^{-2}, which marked the levelling off of the adhesion rate over the rest of the study period (Fig. 4.32).

Source water 2

Stainless steel coupons - Adhesion of cells on the stainless steel coupons was observed after 1 d of exposure in the ozonated water system, with a bacterial community of 7 log cells.cm^{-2}, which remained stable over the remainder of the study period (Fig. 4.31).

Cement coupons - A bacterial community of 7 log cells.cm^{-2} adhered to the cement coupons after the first day of exposure (Fig. 4.32). An increase occurred between 1 and 14 d, and the attached total bacteria reached 8 log cells.cm^{-2} which marked the levelling off of the adhesion rate over the rest of the study period .

Heterotrophic plate count (HPC)

Source water 1

Stainless steel coupons - Within the first day, a bacterial count of 5 log cfu.cm^{-2} was noted on the stainless steel coupons exposed to the ozonated water (Fig. 4.33). This bacterial density gradually increased to 6 log cfu.cm^{-2} between 1 d and 7 d, after which the attached HPC

reached the plateau for the rest of the study.

Cement coupons - Viable bacteria adhered to the cement coupons with a bacterial community of 4 log cfu.cm^{-2} after the first day of exposure (Fig. 4.34). An increase occurred between 1 and 7 d, and the attached HPC reached 7 log cfu.cm^{-2}. Although some fluctuations occurred over the remainder of the study period, no specific increase was noted.

Source water 2

Stainless steel coupons - An initial count of 4 log cfu.cm^{-2} adhered to the stainless steel coupons within 1 d of disinfection. The number of HPC increased up to 6 log cfu.cm^{-2} between 1 d and 7 d, after which some fluctuations took place over the remainder of the study period (Fig. 4.33).

Cement coupons - Within the first day, a bacterial count of 3 log cfu.cm^{-2} was noted on the stainless steel coupons exposed to the ozonated water (Fig. 4.34). This bacterial density gradually increased to 7 log cfu.cm^{-2} between 1 and 14 d, after which the attached HPC reached the plateau for the rest of the experimental period.

4.2.5 Disinfection of groundwater by UV irradiation

4.2..5.1 Water sample analyses

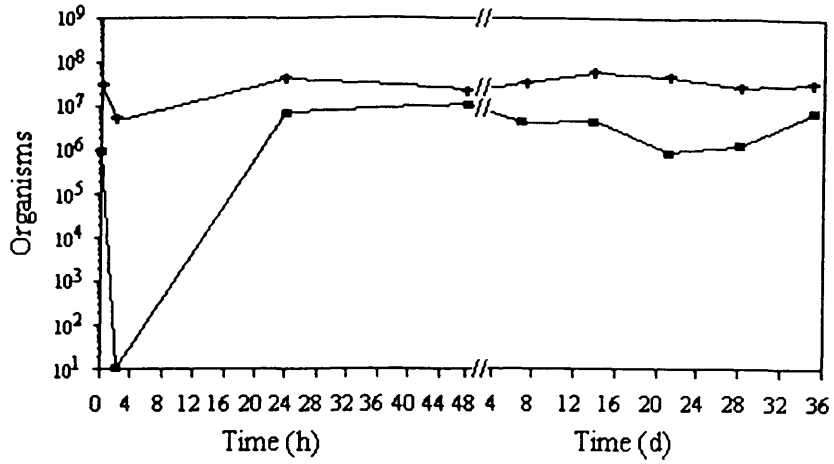
Total bacterial numbers determined using DAPI- epifluorescence microscopy

Source water 1 - During the first 2 h after disinfection, 84.69 % cells were killed by UV irradiation (Table 3). The bacterial number increased between 2 and 24 h (regrowth rate 0.101 h^{-1}, generation time 6.86 h) after which no specific increase was noted over the remainder of the study period (Fig.4.27)

Source water 2 - 73.36 % cells were killed within 2 h. Bacterial regrowth was apparent between 2 h and 14 d (regrowth rate 0.005 h^{-1}, generation time 138.63 h) after which the

bacteria number declined during the remainder of the study period (Fig. 4.27).

Source water 1



Source water 2

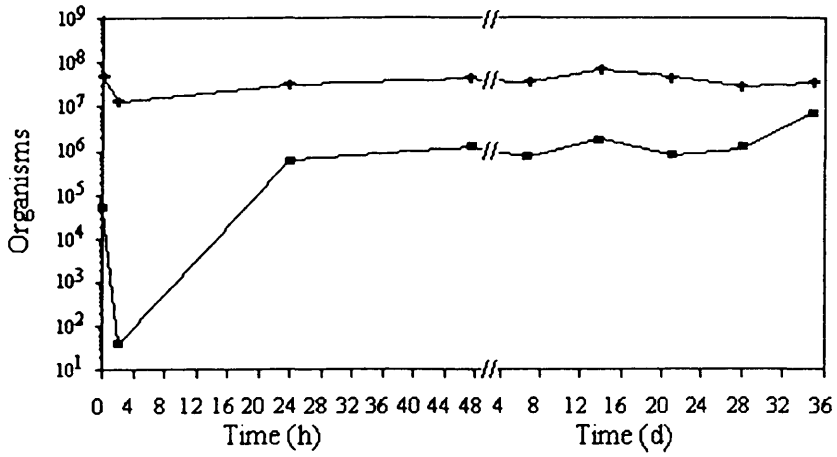


Fig. 4.27. Number of total bacteria (TB - epifluorescence) and heterotrophic plate count (HPC) bacteria in the UV irradiated water. —■— HPC (CFU/ml) —◆— TB (cell/ml)

Heterotrophic plate count (HPC)

Source water 1 - Disinfection of surface water by UV irradiation resulted in a kill percentage of 99.999 % within 2 h (Table 3). Regrowth was noted between 2 and 48 h, after which the

bacteria number declined between 14 and 28 d (Fig. 4.7). The regrowth rate between 2 and 48 h was 0.302 h^{-1} (generation time 2.30 h).

Source water 2 - 99.919 % of heterotrophic bacteria were killed by UV irradiation after the first 2 h of disinfection (Table 4.2). Bacterial regrowth occurred between 2 h and 48 h (regrowth rate 0.224 h^{-1}, generation time 3.09 h), after which some fluctuations took place during the remainder of the experimental period (Fig. 4.27).

Coliform bacteria

Source water 1 and source water 2 - 100 % of coliform bacteria were killed by UV irradiation within 2 h.

4.2.5.2 Biofilm analyses

Biofilm monitoring

Source water 1 - Bacteria adhered to the glass surface within the first day of the experiment. During the study period, the absorbance measurements ranged between 0.50 and 0.62 although some fluctuation took place from time to time (Fig. 4.30)

Source water 2 - The adehsion of bacteria on the flow-through glass tube was obvious within the first day of the experiment with an initial absorbance measurement of 0. 52. Although some fluctuation took place from time to time, the absorbance measurement ranged between 0. 41 and 0. 62 (Fig. 4.30)

Total bacteria number determined using DAPI epifluorescence microscopy

Source water 1

Stainless steel coupons - Within 1 d, the total bacteria count was 7 log cells. cm^{-2}. This number progressively increased to 8 log cells.cm^{-2} between 1 and 21 d and marked a levelling off of the adhesion rate over the remainder of the study period (Fig. 4.31).

Cement coupons - The formation of biofilms on the cement coupons was detected within 1 d with a cell density of 6 log cells.cm^{-2} (Fig. 4.32), after which the bacteria number progressively increased to 8 log cells. cm^{-2} between 1 d and 14 d. A levelling off of the bacteria adhesion rate was noted over the remainder of the study period.

Source water 2

Stainless steel coupons - A bacterial community of 7 log cells.cm^{-2} was noted on the stainless steel coupons after 1 d of exposure of coupons in a UV water distribution system. In spite of some fluctuations, no specific increase was observed over the remainder of the study period (Fig. 4.31).

Cement coupons - A bacterial community of 7 log cells.cm^{-2} adhered to the cement coupons after the first day of exposure (Fig. 4.32). An increase occurred between 1 and 14 d, and resulted in 8 log cells.cm^{-2} which marked the formation of a mature biofilm over the remainder of the study period.

Heterotrophic plate count (HPC)

Source water 1

Stainless steel coupons - Within 1 d the adhesion of HPC bacteria on the stainless steel coupons reached a density of 5 log cfu.cm^{-2}, after which the bacteria number progressively increased to 6 log cfu.cm^{-2} between 1 and 14 d. At this level, the bacteria reached the plateau over the remainder of the study period (Fig. 4.33).

Cement coupons - The attachment of viable bacteria on the cement surfaces occurred within the first day after exposure of the coupons in a UV water system, and resulted in a bacterial community of 4 log cfu.cm^{-2}. Between 1 and 7 d, the bacteria increased to 7 log cfu.cm^{-2} and the attached HPC reached a plateau over the remainder of the study period (Fig. 4. 34).

Source water 2

Stainless steel - Within the first day, a bacterial count of 5 log cfu.cm^{-2} was noted on the stainless steel coupons exposed to the UV treated water (Fig. 4.33). This bacterial density increased to 6 log cfu.cm^{-2} between 1 d and 7 d, after which some fluctuations took place over the remainder of the study period.

Cement coupons - Viable bacteria adhered to the cement coupons with a bacterial community of 3 log cfu.cm^{-2} after the first day of exposure (Fig. 4.34). An increase occurred between 1 and 7 d, and resulted in 7 log cfu.cm^{-2}. Although some fluctuations occurred over the remainder of the study period, no specific increase was noted.

4.2.6 Disinfection of groundwater with hydrogen peroxide

4.2.6.1 Water sample analyses

Residual disinfectant

Source water 1 - The hydrogen peroxide concentration decreased from 20.70 to 20 mg.ℓ^{-1} within 2 h. Between 2 h and 21 h, the residual hydrogen peroxide concentration declined to 0 mgℓ^{-1} (Fig. 4.28).

Source water 2 - A gradual depletion of hydrogen peroxide from 17.73 mg.ℓ^{-1} to 3 mg.ℓ^{1} occurred within 72 h. The residual hydrogen peroxide completely disappeared after 14 d (Fig. 4.28).

Total bacterial numbers determined using DAPI- epifluorescence microscopy

Source water 1 - Hydrogen peroxide exhibited a kill percentage of 16.93 % after 48 h. No specific increase (regrowth rate 0.006 h^{-1}, generation time 115.52 h) in bacteria number was noted over the remainder of the study period of 35 d (Fig. 4.28).

Source water 2 - 45.73 % cells were killed by hydrogen peroxide within 24 h. After 48 h, the

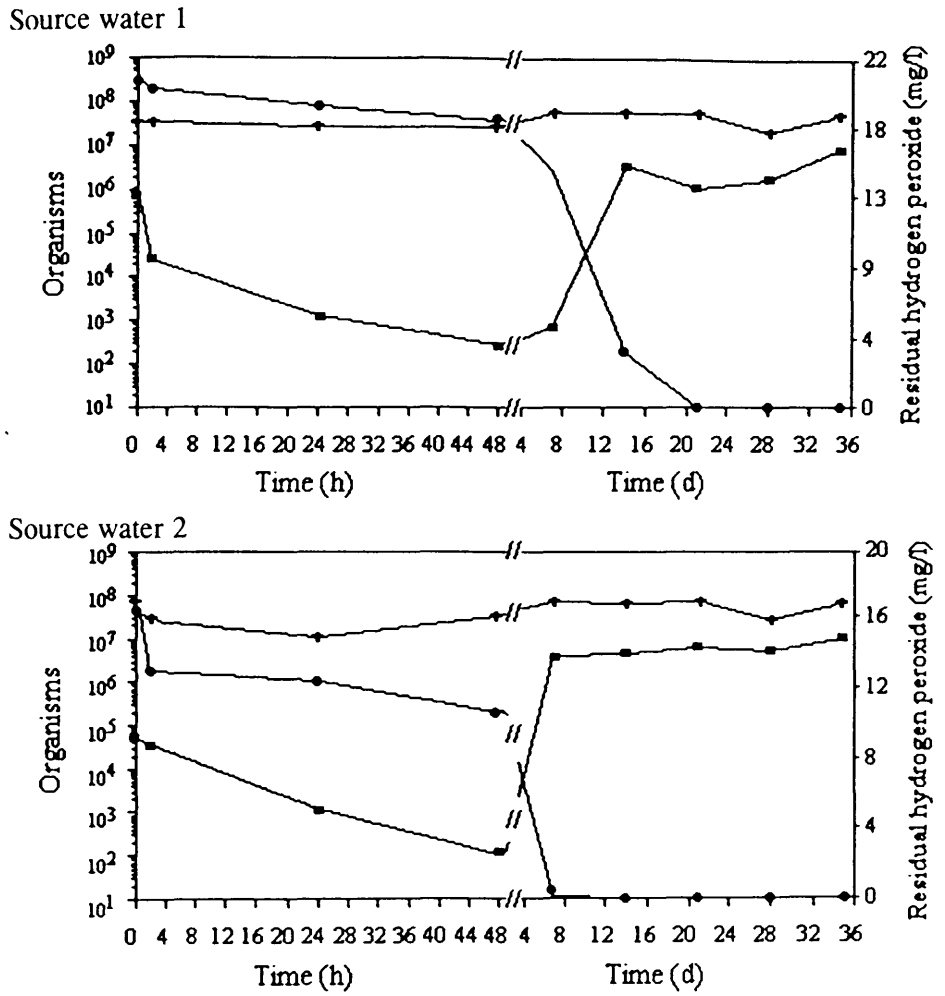


Fig. 4.28. Number of total bacteria (TB - epifluorescence) and heterotrophic plate count (HPC) bacteria in the hydrogen peroxide treated water.

HPC (CFU/ml) TB (cell/ml) Residual hydr.pero

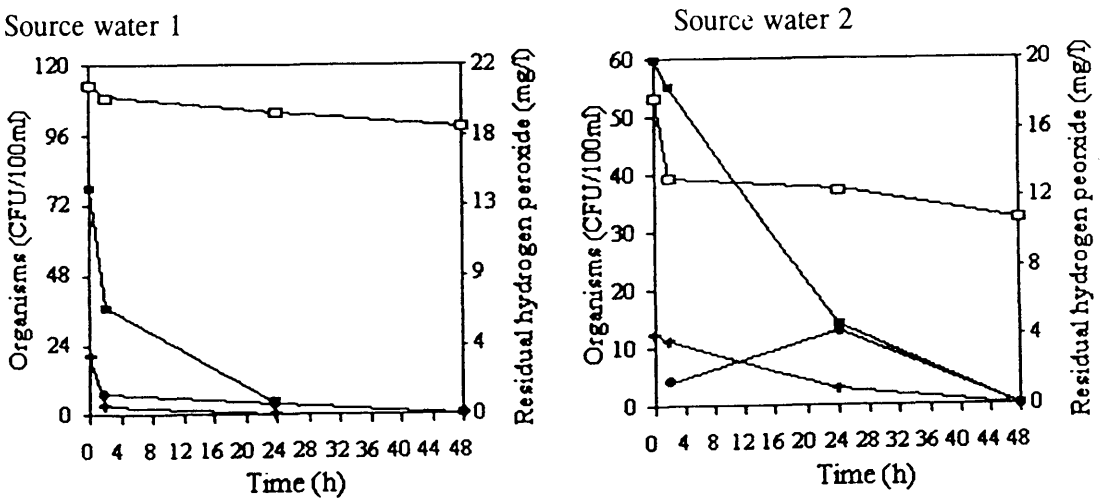


Fig. 4. 29. The total number of coliform bacteria in the hydrogen peroxide treated water

Total coliform Fecal coliform Injured coliform Residual Hyd. perox

kill percentage increased to 79.32 % (Table 3). Between 48 h and 7 d, bacterial regrowth was apparent, after which the bacteria number remained constant for the rest of the experimental period (Fig.4.28). The regrowth rate was 0.011 h^{-1} and the generation time was 63.01 h.

Heterotrophic plate count (HPC)

Source water 1 - 97.47 % of heterotrophic bacteria were killed within 2 h after the disinfection of the water by hydrogen peroxide. The kill percentage increased to 99.97 % within 48 h, after which bacterial regrowth was apparent between 48 h and 14 d, and between 14 d and 28 d bacteria number declined (Fig. 4.28). The regrowth rate was 0.041 h^{-1} and the generation time 17.33 h between 48 h and 14 d .

Source water 2 - 28.28 % of heterotrophic bacteria were killed by hydrogen peroxide during the first 2 h after disinfection. Within 48 h, 99.78 % bacteria were killed, after which the phenomenon of regrowth occurred between 48 h and 7 d, and no specific increase was noted over the remainder of the study period (Fig. 4.28). The regrowth rate was 0.085 h^{-1} and the generation time was 8.15 h between 48 h and 7 d (Table 4.2).

Coliform bacteria

Source water 1 - 91.67 % coliform bacteria were killed by hydrogen peroxide during the first 2h after disinfection. The number of coliform bacteria declined within 24 h, and none were detected during the remainder of the study period (Fig. 4.29).

Source water 2 - Hydrogen peroxide exhibited a kill percentage of 48.05 % coliform bacteria within 2 h. Complete elimination of coliform bacteria occurred after 48 h (Fig. 4.29).

4.2.6.2. Biofilm analyses

Biofilm monitoring

Source water 1 - The attachment of bacteria to the flow-through glass tube was apparent within

the day after exposure in hydrogen peroxide treated water. During the study period, the absorbance measuremnt ranged between 0.43 and 0.61 (Fig. 4.30).

Source water 2 - Bacteria adhered to the flow-through glass surface 1 d after exposure in hydrogen peroxide treated water. In spite of some fluctuations, the absorbance measurement ranged between 0.33 and 065 during the study period (Fig. 4.30),

Total bacteria number determined using epifluorescence microscopy

Source water 1

Stainless steel coupons - An initial number of attached cells of 7 log cells.cm^{-2} was detected on stainless steel coupons within 1 d of exposure in hydrogen peroxide treated water (Fig. 4.31). Between 1 and 28 d, this bacteria count progressively increased to 8 log cells.cm^{-2}.

Cement coupons - A bacterial density of 7 log cells.cm^{-2} was noted on the cement coupons within 1 d of disinfection. This number increased to 8 log cells.cm^{-2} between 1 and 14 d, after which the bacteria reached the plateau over the remainder of the experimental period (Fig. 4.32).

Source water 2

Stainless steel - After an exposure time of 1 d, a bacterial density of 7 log cells.cm^{-2} adhered to the stainless steel coupons. No specific increase was noted over the remainder of the study period (Fig. 4.31).

Cement coupons - A bacterial density of 7 log cells.cm^{-2} was noted on the cement coupons within 1 d of disinfection. This number gradually increased to 8 log cells.cm^{-2} between 1 and 14 d, after which the bacteria reached the plateau (Fig. 4.32).

Heterotrophic plate count (HPC)

Source water 1

Stainless steel coupons - Within the first day, a 5 log cfu.cm^{-2} bacterial community adhered

Source water 1

Source water 2

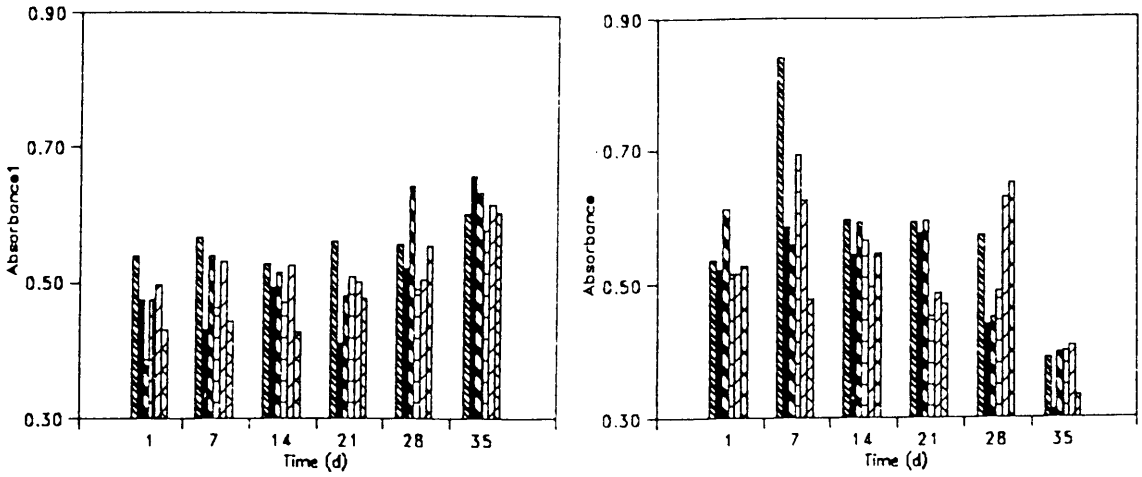


Fig. 4.30 Measurement of bacterial cells attached to flow-through glass tubes during the experimental period for source water 1 and 2.

Source water 1

Source water 2

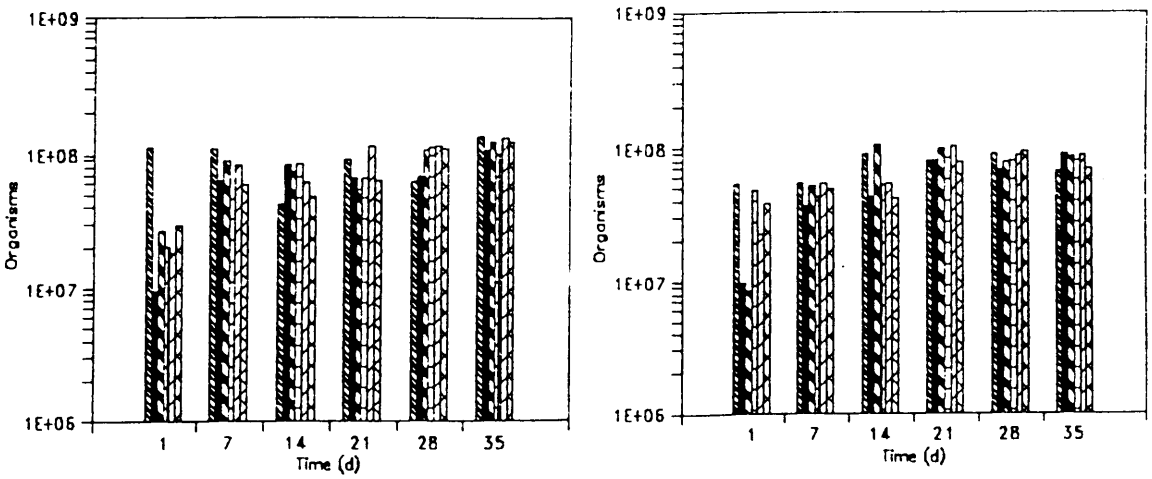
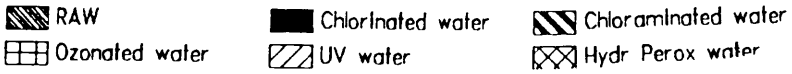


Fig. 4.31. Total bacteria (epifluorescence) attached to stainless steel coupons during the experimental study - source water 1 and 2.



Source water 1 Source water 2

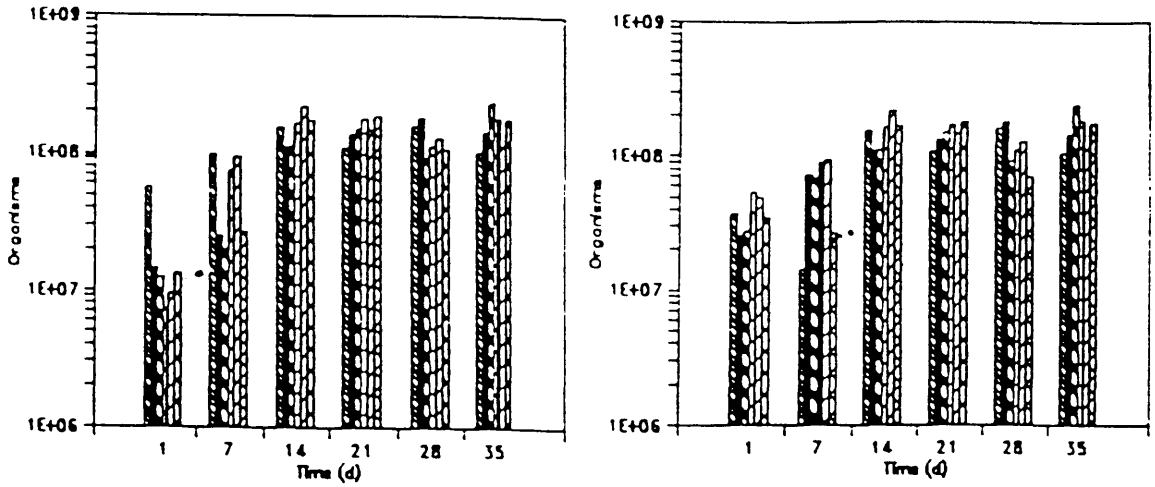


Fig. 4.32. Total bacteria (epifluorescence) attached to cement coupons during the experimental study - source water 1 and 2.

Source water 1 Source water 2

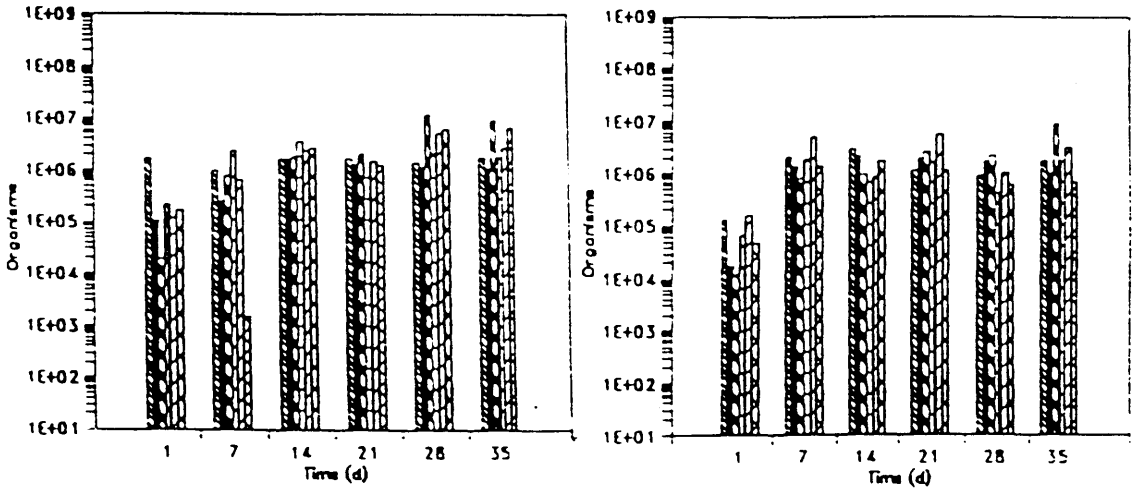


Fig. 4.33. Heterotrophic plate count bacteria attached to stainless steel coupons during the experimental study - source water 1 and 2 .

RAW Chlorinated water Chloraminated water
Ozonated water UV water Hydr Perox water

Source water 1 Source water 2

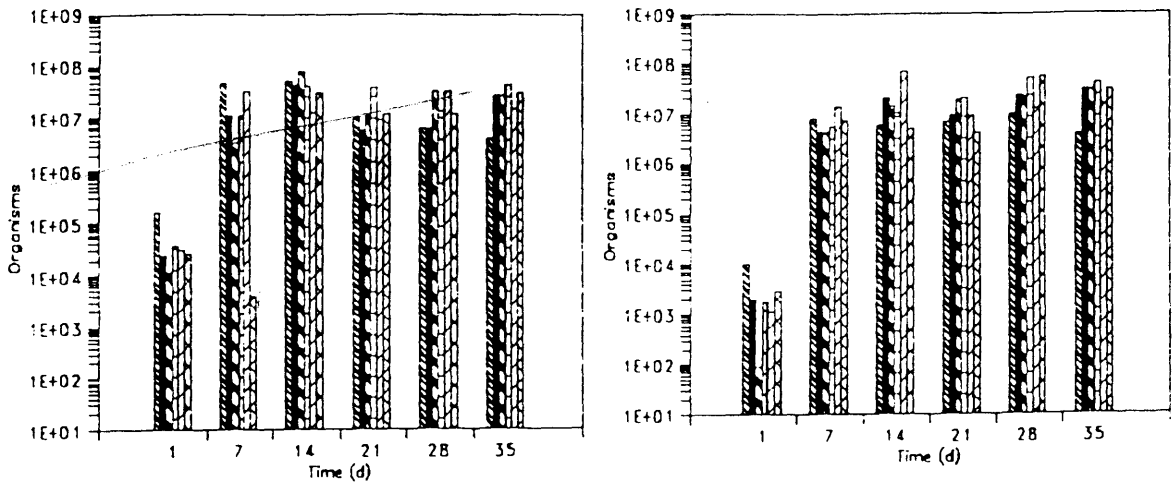


Fig. 4.34. Heterotrophic plate count bacteria attached to cement coupons during the experimental study - source water 1 and 2 .

to the stainless steel coupons exposed to the hydrogen peroxide treated water (Fig. 4.33). This number decreased to 3 log cfu.cm^{-2} during the next 7 d, after which an increase occurred between 7 and 14 d and the bacteria number reached 6 log cells.cm^{-2} which marked the levelling of the adhesion rate over the remainder of the study period (Fig. 4.33).

Cement coupons - The attachment of HPC bacteria on the cement coupons occurred within 1 d with a bacterial community of 4 log cfu.cm^{-2}, after which it decreased progressively to 3 log cfu.cm^{-2} between 1 and 7 d (Fig. 4.34). An increase from 3 to 7 log cfu.cm^{-2} occurred between 7 d and 14 d, and the bacteria number remained constant at this level .

Source water 2

stainless steel coupons - A bacterial density of 4 logs cfu.cm^{-2} was noted on the cement coupons within 1 d after disinfection. This number increased to 6 log cfu.cm^{-2} between 1 and 7 d, after which the bacteria reached the plateau for the rest of the study period (Fig. 4.33).

Cement coupons - After an exposure time of 1 d, viable bacteria adhered to the cement coupons with a density of 3 log cfu.cm^{-2}. Between 1 and 28 d, this number increased to 7 log

cfu.cm^{-2} (Fig. 4.34).

4.2.7 Scanning electron microscopy study

SEM observations indicated a decrease of bacteria on stainless steel coupons exposed to different water distribution systems (Source water 1). Similar observation was also noted for stainless steel coupons from source water 2. The absence of biofilm cells was noted on the stainless steel coupons from all the disinfected water distribution systems (Plate 26 -30), whereas some cells

were visible on the stainless steel coupons from the control (Plate 25). Although no uniform biofilms were noted on the cement coupons, the SEM observations revealed the formation of biofilms on cement coupons exposed to different waters (Plates 31 - 36).

4.2.8 Statistical analyses

4.2.8.1 Water sample data

Bacteria numbers (heterotrophic bacteria and total bacteria) did not differ significantly between source water 1 and source water 2, but significant differences did exist between treatments (Appendix 2.1 and 2.3).

During the first 48 h, heterotrophic bacteria numbers in raw water (control) did not differ significantly from those in the chlorinated and UV irradiated waters, but the control exhibited significantly higher heterotrophic bacteria counts than chloraminated, hydrogen peroxide treated water and ozonated water (Appendix 2.2). A prolonged exposure time (35 d) resulted in significant difference in the bacteria counts (Appendix 2.3). The ozonated and UV irradiated water had significantly higher hetrotrophic bacteria than chlorinated, chloraminated and hydrogen peroxide water (Appendix 2.4). The count from chloramine treated water was

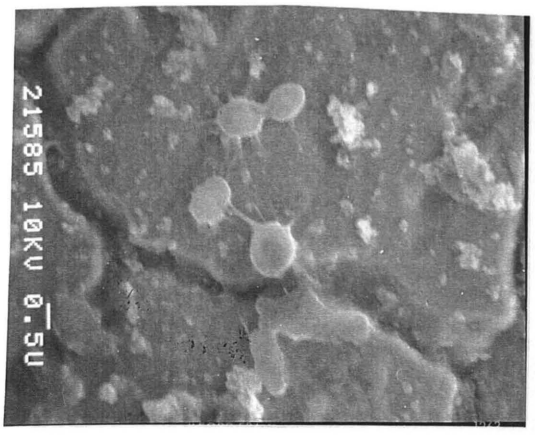


Plate 25

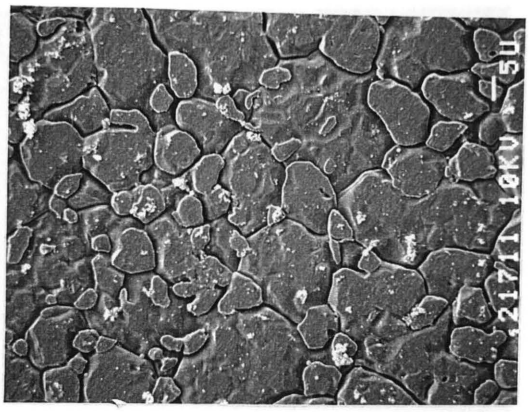


Plate 26

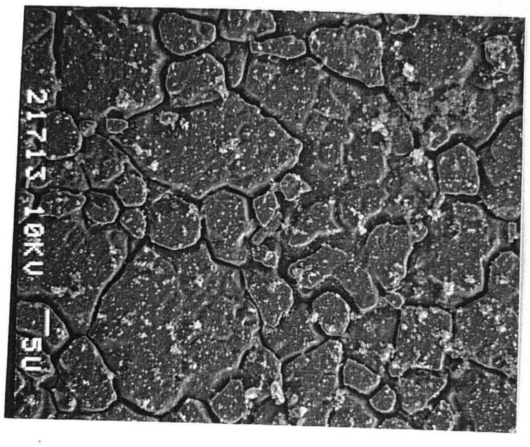


Plate 27

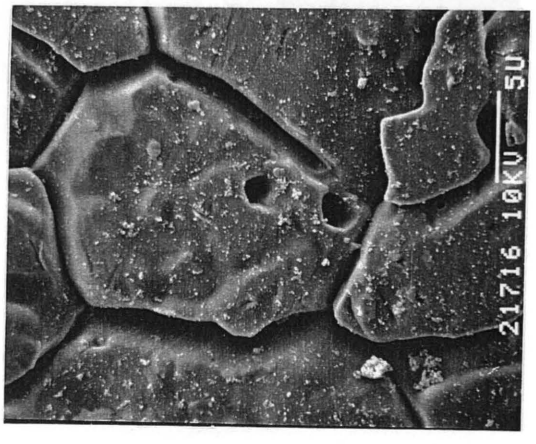


Plate 28

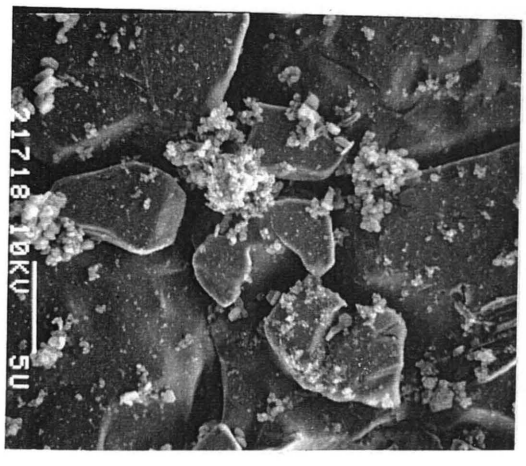


Plate 29

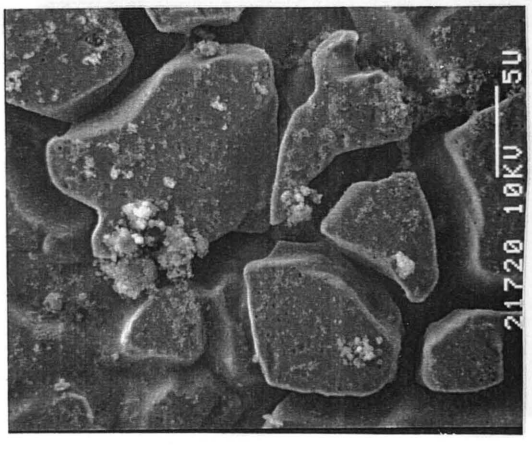


Plate 30

Plates 25 - 30. SEM photographs of bacteria attached to the stainless steel coupons exposed to the control (Plate 25), chlorinated water (Plate 26), Chloraminated water (3), ozonated water (Plate 27), UV treated water (Plate 29) and hydrogen peroxide treated water (Plate 30) after 35 days - source water 1.

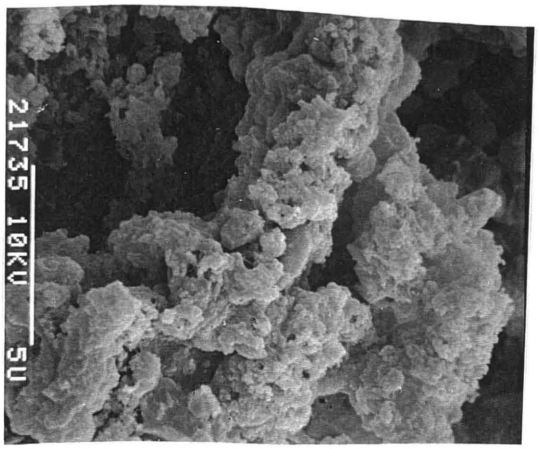


Plate 31

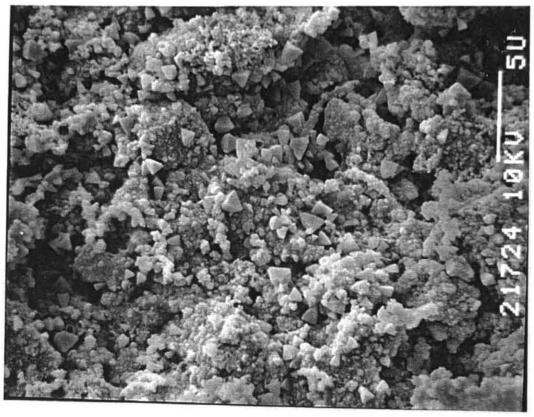


Plate 32

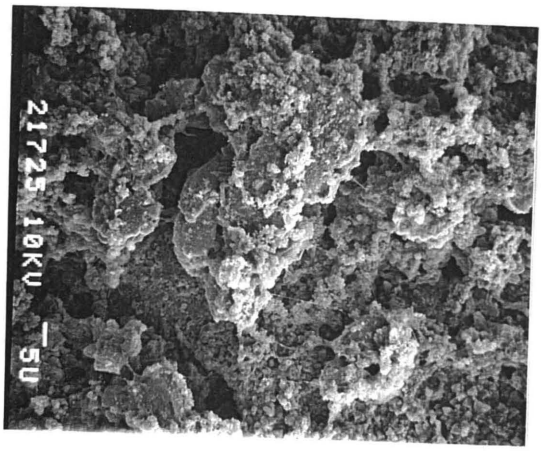


Plate 33

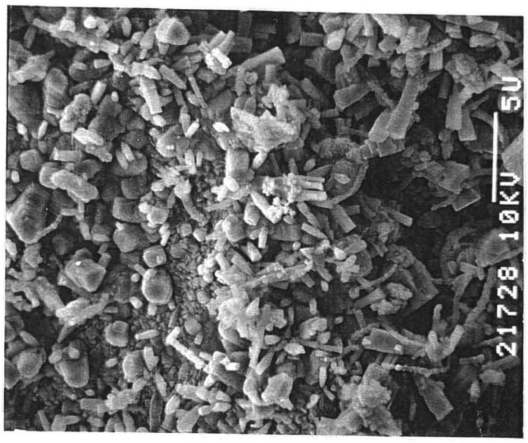


Plate 34

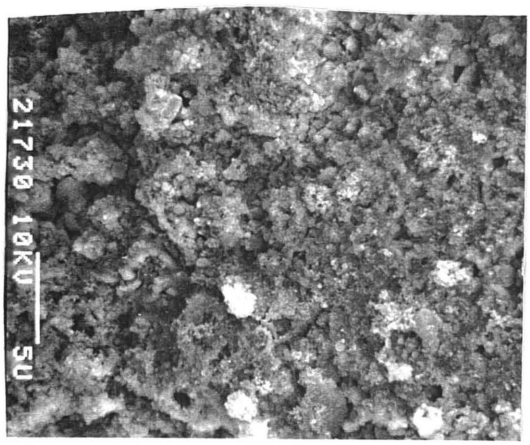


Plate 35

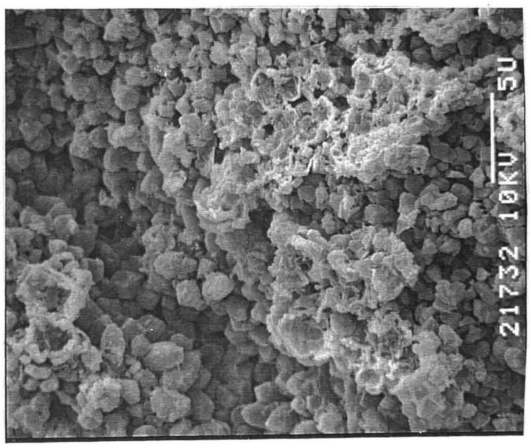


Plate 36

Plates 31 - 36. SEM photographs of bacteria attached to cement coupons exposed to the control (Plate 31), chlorinated water (Plate 32), chloraminated treated water (Plate 33), ozonated treated water (Plate 34), UV treated water (Plate 35) and hydrogen peroxide treated water (Plate 36) after 35 days - Source water 1.

significantly lower than ozonated and UV-irradiated during the entire period of the study (Appendix 2.2 and 2.4).

During a period of 35 d, total bacteria counts (epifluorescence) in the control did not differ significantly from those in the UV irradiated water, hydrogen peroxide and ozone treated water, but the control had a significantly higher count than chlorinated and chloraminated water. Total bacteria counts from chlorine treated water were, however, significantly lower than those from ozonated and raw water. Chloramine treated water had a significantly lower total bacteria count than hydrogen peroxide and ozone treated water (Appendix 2.5).

4.2.8.2 Biofilm data

During the study period, bacteria counts were calculated as a function of time (Appendices 4.1 - 4.3, 4.6 - 4.8). The bacteria numbers did not differ significantly between treatments (Appendices 4.1, 4.6). No significant differences in bacteria count were found between source waters 1 and 2 (Appendices 4.2, 4.7).

However, significant differences were found between heterotrophic bacteria attached to coupons (stainless steel and cement coupons) from source water 1 (Appendices 4.3 and 4.4). Significant differences were also found between coupons from source water 2 (Appendices 4.4 and 4.5). There was a significantly higher number of heterotrophic bacteria attached to the cement coupons than to the stainless steel coupons from source water and also from source water 2 (Appendices 4.4 - 4.5).

As regards the total bacteria counts, significant differences were found between coupons exposed to source water 2 (Appendix 4.8): the cement coupons exhibited significantly higher total bacteria counts than the stainless steel coupons (Appendix 4.9). The attached total bacteria counts on the coupons exposed to source water 1 did not differ significantly (Appendix 4.8).

DISCUSSION

Any investigation of the impact of disinfection processes on biofilm formation must take into account the concentrations of the disinfectants. In this study, chlorine, monochloramine, ozone, UV and hydrogen peroxide were applied with doses similar to those used in practise. The parameters that were used to evaluate the bactericidal effectiveness of each disinfectant were coliform bacteria, heterotrophic plate count and total bacteria. The most important factors such as pH, temperature, turbidity and suspended solids were also taken into account.

Coliform bacteria

All disinfectants were equally effective in the elimination of coliform bacteria. In groundwater, all bactericides were found to remove coliform bacteria within the first hours after disinfection with the exception of hydrogen peroxide (48 h after disinfection) (Fig. 4.29). As to the surface water, with the exception of choraminated treated water system, the presence of injured coliforms was obvious 24 h after disinfection whereas no coliform bacteria were noted within the first hours after disinfection (Figs. 4.4, 4.6, 4.8, 4.10. 4.12). The complete elimination of coliform bacteria occurred in all treated systems, 48 h after disinfection. This coincided with the regrowth of heterotrophic bacteria with the exception of hydrogen peroxide treated water(Fig. 4.12). Competition for limiting organic carbon could be responsible for this, as indicated by LeChevallier and McFeters (1985), who found a significant correlation between the initial level of heterotrophic bacteria and the rate of coliform decline. Although, all disinfectants were effective in the elimination of coliform bacteria, it could be noted that this removal was quick when using groundwater than when using surface water.

All disinfection processes were effective as shown by the average kill percentage of 99% of the heterotrophic bacteria (Tables 4.1 - 4.2, Figs. 4.1 -4.12, and Figs. 4. 22 -4.29). Greater than 99.90 % of heterotrophic bacteria were removed using chlorine, monochloramine, ozone and UV irradiation within the first hours after disinfection of groundwater (Table 4.2), whereas chlorine (source water 1) and UV (source water 1) achieved such a kill percentage when using surface water (Table 4.1). However, an 5 log cfu.ml^{-1} initial count was found in both test waters (Figs. 4.1 - 4.12, Figs. 4.22 - 4.29). This indicated that the effectiveness of chlorine, monochloramine, ozone and UV against heterotrophic bacteria was greater in groundwater than in surface water. Therefore, groundwater could be used as the main water source to disinfect and to supply to many small communities, given that rural water supplies are obtained from wells and springs.

Among all disinfectants used during this study, ozone showed the greatest germicidal effectiveness (average 99.999 %) against heterotrophic bacteria when using groundwater as the test water source. However, chloramination and hydrogen peroxide disinfection exhibited an average kill percentage of 99.99% of the heterotrophic bacteria although a longer period of time (between 24 h and 72 h for chloramination, 72 h for hydrogen peroxide disinfection process) was required to achieve this kill percentage when using surface water (Table 4.1, Figs. 4.5, 4.6). A comparison between source water 1 and source water 2 showed that in groundwater, ozone efficacy was similar and greater after 2 h in both sources water than any of the other disinfectants (Table 4.2). As to the surface water, chloramine and hydrogen peroxide were equally effective in source water 1 (S1) and in source water 2 (S2) (Table 4.1), although different periods of time (24 for S2 and 72 h for S1) were required by chloramine to achieve this kill percentage of 99.99 % . Although 99.879 % (average after 48 h, for groundwater) and 99.99 % (average for surface water) of heterotrophic bacteria were removed

using hydrogen peroxide disinfection, its bactericidal efficacy remained slower than that any of the other disinfection processes. Therefore, the use of this disinfectant as a primary disinfectant for potable water might not be recommended. Undoubtedly, all the disinfectants did progressively undergo a process of depletion throughout the systems. Whereas the decay occurred earlier in ozonated water (1 day for surface water and groundwater), residual disinfectant concentrations could be detected up to 2 days in chlorinated water (for surface water and groundwater), between 14 - 15 days in chloraminated water from surface, up to 7 days in chloramine and hydrogen peroxide treated water from groundwater, and between 7 - 22 d in hydrogen peroxide treated water from surface water. Consequently, this decay of residual disinfectants resulted in bacterial regrowth.

Bacterial regrowth, therefore, occurred earlier in the chlorinated, ozonated and UV irradiated water (Figs. 4.3, 4.7, 4.9, Figs. 4.24, 4.26, 4.27), than in the chloraminated water (Fig. 4.5 Fig. 4.25) and hydrogen peroxide treated water (Figs. 4.11, 4,28). However monochloramine and hydrogen peroxide exhibited lower growth rates (Table 4.1 and 4.2) than any of the other disinfectants. These observations were similar in source water 1 as well as in source water 2 for both test waters (surface water and groundwater). While monochloramine and hydrogen peroxide in surface water exhibited a greater residual persistence (7 d for monochloramine and hydrogen peroxide - source water 2), the regrowth rates were 0.039 h^{-1} generation time 17.77 h and 0. 029 h^{-1} (generation time 23.90 h) respectively and much lower than when there was a shorter residual persistence of monochloramine and hydrogen peroxide (72 h resulting in a regrowth rate of 0.13 h^{-1}, generation time 5.33 h, for monochloramine; regrowth rate of 0.041 h^{-1}, generation time 16.91 h for hydrogen peroxide - source water 1). Similar observations were also noted in the findings of study with groundwater (Table 4.2). A comparison between groundwater and surface water source showed that groundwater

chloramination resulted in lower growth rates (0.077 h^{-1} , generation time 9 h - source water 1; 0.037 h^{-1} , generation time 18.73 h - source water 2) than surface water chloramination, whereas disinfection with hydrogen peroxide resulted in an equal regrowth rate (0.041 h^{-1} , generation time 17 h) for surface water - S1 and groundwater - S1, and in a lower growth rate (0.29 h^{-1} , generation time 23.90 h) for surface water - S2 than for groundwater - S2 (0.085 h^{-1} , generation time 8.15 h). These findings were also confirmed statistically when using ANOVA ($\alpha = 0.05$) (Appendices 1.2, 1.5, 2.2, 2.4). Within the first hours after disinfection (72 h for surface water and 48 h for groundwater), significantly higher heterotrophic bacteria numbers were noted in ozone and UV treated surface waters than in chloramine (Appendix 1.2), whereas viable bacteria counts were significantly higher in ozone, chlorine and UV treated groundwater than in chloraminated water (Appendix 2.2). At the end of 38 d for surface water and 35 d for groundwater, significantly higher heterotrophic bacteria counts were noted in chlorine, ozone and UV treated surface water than either in chloramine or hydrogen peroxide treated water (Appendices 1.5), whereas the situation remained constant in treated groundwater (Appendix 2.4). Moreover, raw water did not significantly differ from chlorine, ozone and UV treated water as a result of regrowth in the treated water (groundwater and surface water). This demonstrated that the presence of an adequate disinfectant residual, whether it be monochloramine or hydrogen peroxide will enable water suppliers to make significant progress compliance with bacteriological water quality standards. The greater effectiveness of chloramine in controlling heterotrophic plate count bacteria than chlorine was also reported by Neden *et al.* (1992).

When comparing source water 1 and source water 2 in both test waters ($P = 0.2909$ for surface water, $P = 0.3347$ for groundwater), or different treatments in source water 1 and source water 2 (3 d after disinfection - $P = 0.8617$, 37 d after disinfection - $P = 0.8071$ for

surface water; 3 d after disinfection - P = 0.9989, 35 d after disinfection - P = 0.9218 for groundwater), ANOVA showed no significant difference in viable counts. This demonstrated that the phenomenon of bacterial regrowth was not influenced by the physico -chemical quality of potable water (Tables 3.1- 3.4).

The phenomenon of bacterial regrowth in potable water was therefore related to the absence of concentrations of disinfectant residual and the microbial efficacy of chloramine and hydrogen peroxide provided a longer concentration which inhibited bacterial regrowth in treated water. Clark *et al.* (1994), reported similar observations when using monochloramine. Regrowth of bacteria in drinking water distribution systems occurs at the expense of nutrients in water (van der Wende and Characklis 1990, Clark *et al.* , 1994). Experiments with ozone have showed that ozone may also react with organic material in source water to form nutrients that allow regrowth in the distribution system and increase the growth in the bulk phase of water (Clark *et al.* 1994). After studying various combinations of ozone, chlorination and chloramination Clark *et al.* (1994) reported that microorganisms increase in the bulk phase of the water when disinfectant residual disappeared. Dissolved organic (DOC) decreased, providing a nutrient source for this biological growth. The potential for bacterial regrowth has also been determined in UV-irradiated drinking water (Hengesbach *et al.*, 1993). Results showed that as the low-molecular weight component of the total DOC determine the degree of its metabolic availability to bacteria, any UV degradation of DOC is linked to the question of bacterial regrowth potential (Hengesbach *et al.*, 1993).

In our study, treated water quality also varied in laboratory-scale units in accordance with the age of water in the systems. Examples of this included the decrease of chlorine, ozone, chloramine and hydrogen residuals that occurred in surface water and groundwater systems. This phenomenon was function of time (Figs. 4.3, 4.5, 4.7, 4.11 for surface water

and Figs. 4.24 - 4.28 for groundwater). This was also statistically confirmed using ANOVA ($\alpha = 0.05$). Significant higher heterotrophic bacteria numbers ($P = 0.0001$ for surface water and groundwater) occurred 35 d (for groundwater) or 37 d (for surface water) after disinfection than 3 d after disinfection ($P = 0.0036$ for groundwater, $P = 0.0439$ for surface water). Gibbs and his co-workers (1990) observed a quick reduction in bacterial number, but regrowth occurred when the residual chlorine was decreased with the retention in the distribution system. LeChevallier *et al.* (1980) noted that dead-end distribution lines in which no free chlorine could be detected contained 23 times the number of standard plate count compared with distribution chlorine was decreased with retention in the distribution lines with a free chlorine residual. The findings of our study with surface water and groundwater suggests that a combination any of the disinfection processes using monochloramine or hydrogen peroxide would provide an effective treatment for inhibiting bacterial regrowth in drinking water as long as the residual chloramine or hydrogen peroxide concentrations persist.

Total bacteria

Counting of bacteria by the epifluorescence technique involving DAPI staining gave a lower percentage kills than by the standard plate procedure with R2A agar for all treatments (Tables 4.1 - 4.2). Although the findings of study with treated surface water (Figs. 4.3, 4.5, 4.7, 4.9. 4.11) as well as with treated groundwater (Figs. 4.24 - 4.29) revealed higher total bacteria counts than heterotrophic bacteria, the fluorescence was low indicating the possibility that these cells were actually dead. A recent study has shown that counting bacteria in drinking water samples by the epifluorescence technique involving DAPI staining was complicated by the fact that bacterial fluorescence varied with exposure of the cells to sodium hypochloride (Saby *et al.*, 1997). According to the authors, DAPI-stained bacteria (*Escherichia coli*) treated with sodium hypochlorite (5 or 15 $mg.\ell^{-1}$ chlorine) for 90 min was highly fluorescent (probably

due to cell membrane permeation and better DAPI fusion) than those treated at chlorine concentration greater than 25 mg.ℓ^{-1} chlorine. Although, during our study, the chlorine concentrations used for the treatment of water were lower (2 -3.5 mg.ℓ^{-1}) than those used by Saby *et al.* (1997), the bacteria, also had a low fluorescence. Baleux and Got (1996) also reported that exposing bacteria to UV irradiation causes a decrease in the fluorescence of bacteria stained with DAPI. According to Paquin *et al.* (1994), the fluorescence of bacterial suspensions in potable water disinfected with chlorine varied greatly, from bright blue fluorescing cells to poorly fluorescing bacteria. This was also noted during this study. The presence of the poorly fluorescent bacteria after DAPI staining could be interpreted as sign of dead cells (Saby *et al.*, 1997). Cell alterations caused by oxidative stresses (disinfectants such as sodium hypochlorite, chlorine dioxide, UV) led to a reduction in the fluorescence of DAPI-stained bacteria and generally occurred after the loss of viability of the bacteria (Saby *et al.*, 1997). The reduction in the fluorescence of DAPI-stained bacteria makes counting them more difficult and leads to the risk of underestimating the number of bacterial cells. This phenomenon has been reported by several groups of investigators on several occassions in studies on seawater and river estuary water (Kepner and Pratt, 1993; Suzuki *et al.*, 1993; Kepner and Pratt, 1994) and disinfectant water (Mathieu *et al.*,1992).

Biofilm formation

In all laboratory-scale units (groundwater and surface water), the adhesion of bacteria on the glass surfaces could be seen with the naked eye after an exposure time of 1 d. This was confirmed by direct absorbance measurements (Figs 4.13 -4.14 for surface water , Figs. 4.30 for groundwater). Bacteria also started to adhere to the coupons within the first day after disinfection (Figs.4.15 - 4.19, Figs. 4.31 -34).

Although biofilm formation on coupons (cement and stainless steel) was obvious in every

system (groundwater and surface water), the control (raw water) from groundwater source exhibited a higher viable bacteria count than the disinfected waters (Figs 4.33 - 4.34) within the first day. The lowest attached viable count in groundwater systems, however, was noted on coupons exposed to chloramine treated water (Figs 4.33 - 4.34). In the presence of 14.83 $mg.\ell^{-1}$ hydrogen peroxide, a decrease in viable count occurred on coupons exposed to source water 1, whereas the concentration of 0.20 $mg\ell^{-1}$ hydrogen peroxide resulted in the accumulation of viable bacteria on coupons exposed to source water 2 within 7 days. This demonstrated the inability of fixed viable bacteria to regrow in the presence of a significant concentration of disinfectant residuals and also showed that monochloramine and hydrogen peroxide residuals did limit the increase of fixed viable bacteria on the coupons.

Whereas the stainless steel coupons had much lower viable bacteria counts in the initial untreated surface water (raw water) than those from the initial treated surface waters, the relative difference between the two types of water diminished by prolonged exposure time (Figs. 4.15 - 4.19). No significant differences in viable bacteria count were therefore noted between the two types of waters (Appendix 3.1). However, the viable count yield was higher on the stainless steel coupons (Figs. 4.15, 4.16, 4.18) than on the cement coupons (Figs. 4.17,4.19) within the first 8 days in every surface water system. While the viable count from UV irradiated water was higher on the stainless steel coupons, a low yield was detected on the cement coupons after the exposure time of 1 day. Within 8 d, viable counts from the cement coupons were similar in chlorinated, ozonated and UV irradiated water whereas low counts were noted in hydrogen peroxide treated water and chloramine treated water. The lowest viable count was noted on cement coupons exposed to hydrogen peroxide treated water (Fig. 4.19). The susceptibility of bacteria in chloramine and hydrogen peroxide treated water could be related to effective disinfectant residual concentrations which could be detected up to 8 d

(for monochloramine) and 15 d (for hydrogen peroxide (Table 3). The effectiveness of residual monochloramine and hydrogen peroxide in controlling regrowth of bacteria in bulk phase was previously shown . This demonstrated that either of these disinfection processes would provide effective treatment which would inhibit bacterial regrowth in drinking water distribution systems as long as the residuals persist. This also showed the relationship that could be detected between the level of planktonic bacteria bulk phase and that of attached bacteria on coupons, and supports Mathieu *et al.* (1993) who reported that the level of bacterial contamination in drinking water resulted from the cells introduced in water, which have not been eliminated by the treatment process.

Even in the presence of significant residual disinfectant concentrations (12.5 - 19.0 $mg.\ell^{-1}$,hydrogen peroxide, 0.8 -1 $mg.\ell^{-1}$ monochloramine and 0.5 $mg.\ell^{-1}$ free chlorine; 16.50 $mg.\ell^{-1}$ hydrogen peroxide, 1 $mg.\ell^{-1}$ monochloramine and 0.2 $mg\ell^{-1}$ free chlorine, for groundwater and surface water respectively), attached bacteria were detected on the coupons (Figs. 4.15 - 4.19; Figs, 4.31 - 4.34, Plates 3 - 12, 19 -24, 31 -36). This experiment showed that the maintenance of a disinfectant residual in potable water could not be relied on to prevent bacteria adhesion on stainless steel coupons, cement coupons and glass surfaces.

On the one hand, our observations support van der Wende and Characklis (1990) who found using an experimental Roto Torque System (RTS), an accumulation of biomass in the system with a high chlorine concentration (0.8 $mg\ell^{-1}$, after exposure time of 17 and 38 d) as well as with a low chlorine concentration (0.2 $mg\ell^{-1}$ after exposure times of 17 and 45 d). On the other hand, this study is not in agreement with that of Lund and Ormerod (1995), who found that free chlorine as low as 0.05 $mg\ell^{-1}$ was sufficient and formation of biofilm to prevent sludge production on pipe walls.

Prolonged exposure time led to a change in the potable water distribution systems: a decay of

the disinfectant concentrations followed by an increase of attached viable counts on coupons in every systems (Plates 1 - - 2, Plates 3 - 4, Plates 5 - 6, Plates 7 - 8, Plates 9 -10, Plates 11 - 12). Whereas the cement coupons initially had much lower viable counts in the treated waters than the stainless steel coupons, the relative difference between the two plumbing materials was diminished by prolonged exposure time (groundwater and surface water). In groundwater, attached viable counts were therefore significantly higher on the cement coupons than on the stainless steel coupons (Appendices 4.3 - 4.5) . However, no significant difference was noted between treatments (P = 0.7880 - source water 1, P = 0.9271 - source water 2) nor between source waters (P = 0.9233 -stainless steel, P = 0.9026 cement coupons). As to the surface water, within 22 d chloraminated water revealed a somewhat lower average viable count (6 log cfu.cm^{-2} for cement coupons, 5 log cfu.cm^{-2} for stainless steel coupons) of biofilm cells than other treated waters (Figs. 4.18 - 4.19). One explanation is that there could have been an effective monochloramine residual present in the system. Although average viable count of biofilm cells (for all waters) from cement coupons appeared to be higher (1.40 x 10^7 cfu.cm^{-2}) than those from stainless steel coupons (7.88 x 10^6 cfu.cm^{-2}), ANOVA (α = 0.05) showed no significant difference (P = 0.8032) between the two (Appendix 3.1).

A comparison between treated groundwater and treated surface water revealed that treated surface water had the initial lowest attached viable bacteria counts (cement and stainless steel) (Figs. 4.15 - 4.19) than treated groundwater (Figs.4.31 - 4.34). However this difference diminished by prolonged exposure time. Attached viable bacteria counts, therefore, appeared to be similar in both water sources (averages = 6 log cfu..cm^{-2} - stainless steel coupons, 7 log cfu..cm^{-2} - cement coupons).

Counting bacteria on coupons (stainless steel and cement coupons) by the epifluorescence

techniques also revealed the effectiveness of chloramine and/or hydrogen peroxide in controlling the increase of total bacteria numbers on coupons.

For surface water source, chloramine and hydrogen peroxide treated waters had a lower count that any of the other disinfected water (Figs.15- 17). The lowest count in chloraminated water was found up to 15 d on both types of plumbing materials (Figs. 16 - 17 - source water 2). However during the study period of 38 d, ANOVA showed no significant difference in attached total bacteria between source water 1 and source water 2. The same observation was made for groundwater source as well. ANOVA ($\alpha = 0.05$) also confirmed that the attached total bacteria counts from the coupons were significantly higher in the control (raw water) than in disinfected waters for a period of 22 d (for cement coupons) and 38 d (for stainless steel coupons) (Appendix 3.3), and that the attached total bacteria count was significantly higher in UV irradiated water than in chloraminated water. A high number of biofilm cells in UV irradiated water could be related to the absence of disinfectant residuals. This study, is again not in agreement with the findings of Lund and Ormerod (1995), who reported a lower production of biofilm in UV irradiated water. Moreover, results of our study on biofilm formation showed that monochloramine and hydrogen peroxide residuals did limit the increase of attached bacterial density in the systems, which also support Mathieu *et al.* (1993) who reported that a residual disinfectant did not prevent the development of a specific microbial ecosystem (biofilm) at the liquid-solid interface. Therefore the presence of a complex biofilm leads to continuous contamination of the water phase resulting from the release of the attached growing biomass (Paquin *et al.*, 1992). ANOVA also showed that attached total bacteria were significantly lower on stainless steel coupons than on cement coupons during the study period (Appendix 3.4). Although significantly higher total bacteria counts were noted on cement coupons than on stainless steel coupons (Appendices 3.4 and 4.9) in both water sources

(surface water and groundwater), the highest total bacteria counts were found on coupons from groundwater (Appendices 4.9). This demonstrated the rapidity of bacteria to attach on coupons from groundwater source. In addition, these results also confirmed the importance of surface material on organism numbers, as previously seen with attached viable bacteria. It therefore appeared that the cement surface was capable of enhancing biofilm regrowth rather than only protecting it from the action of the disinfectants.

For groundwater, although the lowest counts on stainless steel and cement coupons were found in the chlorinated water (S1 and S2), chloraminated water (S2), and in ozonated water (S1) respectively, within the first day of exposure, no significant difference in total bacteria count was noted between source water 1 and source water 2 nor between treatments at the end of the study period (Appendix 4.6 - 4.7).

When the bacteria on the coupons were counted by the epifluorescence technique, a significant difference in total bacteria was found between washed stainless steel coupons (during the removal of stain prior epifluorescence microscopy) and non-washed stainless steel coupons (Appendices 3.6 - 3.7). The attached total bacteria from non-washed coupons were significantly higher than those from washed coupons. The removal of the stain did cause a decrease in the composition of the biofilm cells. On the one hand, this could be related to the reduction in the fluorescence of DAPI-stained bacteria which made counting of bacterial cells more difficult with the risk of undercounting. On the other hand, the removal of excess DAPI stain solution could also have caused the decrease of the bacteria count on washed coupons. This was also noted during the SEM, which includes a lot of washing processes. Whereas more cells were visible on Plates 1 - 12 (epifluorescence), fewer cells (Plates 16 - 17) or absence of cells (Plates 15 and 18) were observed during SEM.

This study showed that biofilm formation occurred on stainless steel and cement coupons in

laboratory scale units in the absence of disinfectant residuals as well as in the presence of significant disinfectant residuals. Regrowth rate of biofilm was therefore influenced by the disinfectant residuals. In the absence of a disinfectant residual concentration, a high increase in biofilm cells was detected, whereas a low increase was recorded in the presence of the residuals. Therefore monochloramine and hydrogen peroxide were found to be much more effective in controlling the growth of biofilm in laboratory scale units. High bacterial density (attached total bacteria) more successfully colonized the cement coupons than the stainless steel coupons. A decrease of attached bacterial density on stainless steel coupons was related to the washing process during the epifluorescence direct count involving DAPI and during the scanning electron microscopy study.

PART III

EXAMINATION OF THE BEHAVIOUR OF COLIFORM BACTERIA IN BIOFILMS ESTABLISHED IN LABORATORY-SCALE UNITS RECEIVING CHLORINATED CHLORAMINATED GROUNDWATER

CHAPTER 5

EXAMINATION OF THE BEHAVIOUR OF COLIFORM BACTERIA IN BIOFILMS ESTABLISHED IN LABORATORY-SCALE UNITS RECEIVING CHLORINATED AND CHLORAMINATED GROUNDWATER

5.1 INTRODUCTION

The formation and presence of biofilms in drinking water distribution systems have been reported frequently (Allen *et al.*, 1980; Ridgway and Olson, 1981; van der Wende and Characklis, 1990). As described by van der Wende and Characklis (1990), the biofilm is a layer of microorganisms in an aquatic environment, held together in a polymetric matrix attached to substrata such as pipes, tubercles or sediment deposits. The biofilm then serves as a focal point where bacterial and protozoal populations interact.

A large variety of different heterotrophic bacteria ranging from potentially pathogenic bacteria to coliform bacteria have been isolated from the biofilm in distribution systems receiving both chlorinated and non-disinfected water (LeChevallier *et al.*, 1987; Colbourne *et al.*, 1988). The coliform group of bacteria is used by many in the water industry as a criterion for operational parameters. They remain one of the traditional indicator organisms for the efficacy of treatment and the presence of bacterial pathogens. The increase in reported cases of coliform occurrence in chlorinated drinking water has been related to outbreaks of waterborne diseases in the United States (Craun, 1981; Lippy and Waltrip, 1984).

A number of chemical and physical factors common to drinking water distribution systems are known to cause a form of sublethal and reversible injury during disinfection.. These include chlorine and other biocides, low concentration of metals such as copper and zinc, extremes of temperature and pH and interactions with other bacteria (LeChevallier and McFeters, 1985). Several investigators have shown that coliforms could survive standard chlorine residuals with subsequent release into the distribution systems (McFeters and Camper, 1983, Olivieri *et al.*, 1985; McFeters *et al.*, 1986). Attachment of bacteria to surfaces and specific growth conditions of bacterial cultures have also been shown to increase the chlorine resistance of bacteria. Ridgway and Olson (1982) showed high correlations between numbers of viable bacteria in chlorinated water and numbers of bacteria attached to particles. A 150-fold increase in resistance to chlorine by *Klebsiella pneumonia* was observed when they were attached to microscope slide (LeChevallier *et al.*, 1988).

Research initiated by LeChevallier and co-workers (1987) indicated that coliforms in distribution systems originated from pipeline biofilms. Results showed that coliforms levels increased as the water moved from the treatment plant through the distribution systems. Computer modelling of hydraulic residence times showed that the increased coliform densities could not be accounted for by growth of the cells in the water column alone but must have come from biofilms in the distribution system. Results also showed that maintenance of 1 -2 $mg\ell^{-1}$ free chlorine residual was insufficient to eliminate these occurrences of coliforms. The collection of samples with and without sodium thiosulphate showed that coliform bacteria in the water column were not significantly affected by an additional hour of contact time with 0.75 $mg.\ell^{-1}$ free chlorine residual.

Most studies on the survival of coliform bacteria in drinking water distribution systems have been done using surface water. No information is available to date on the situation

pertaining to coliform bacteria in biofilm formed in groundwater distribution systems. In this study, water from a well was used to examine the behavior of coliform bacteria in the biofilm. *Escherichia coli* was used as a model organisms for coliform bacteria. As chlorine is the most widely used disinfectant for the chemical treatment of water, and monochloramine penetrates biofilms and inactivates bacteria more efficiently than hypochlorous acid, hypochlorite and chlorine dioxide (LeChevallier *et al.*, 1988), chlorine and monochloramine were used as disinfectants in this study.

5.2 MATERIAL AND METHODS

5.2.1 Biofilm apparatus

The laboratory-scale unit described in chapter 3 was also used for this study. For each system, 20 stainless steel coupons (75 x 25 x 1 mm) were used for the enumeration of attached viable bacteria and coliform bacteria.

5.2.2 Test organisms

Escherichia coli, isolated from the Rand Water treatment plant (South Africa), was obtained from the culture collection of the Water Quality Programme of the Division of Water, Environment and Forestry Technology of the Council for Scientific and Industrial Research (CSIR) in Pretoria, South Africa. The culture was stored on nutrient agar (Merck) at 4°C. When needed, 2 loops of *E. coli* were transferred into 100 mℓ nutrient broth (Merck) and aerobically cultured at 37°C for 24 h.

5.2.3 *Source water and disinfection*

Chlorine and monochloramine were used to disinfect water collected from the same well as our previous study. The preparation of the chlorine stock solution was done as described in chapter 3. Chloramination was accomplished using a 1.5 mg.ℓ^{-1} monochloramine solution.

No neutralization was done during the experimental period for any of the disinfectants used. For a period of 96 h, the concentrations of free chlorine and monochloramine residuals were measured using the N,N-diethyl-p-phenylenediamine (DPD, Sigma) ferrous titrimetric method (APHA- AWWA-WPCF, 1989).

5.2.4 *Biofilm formation*

The control and the 2 disinfected waters were transferred into experimental distribution systems designed for the study of biofilm production. Water circulation was facilitated at a flow rate of 2.8 $\ell.h^{-1}$. 24 h after disinfection, 100 mℓ of *E. coli* suspension (2.6 x 10^4 cfu.mℓ^{-1}) were inoculated into all three systems.

5.2.5 *Sampling and microbial analyses*

Stainless steel coupons were withdrawn 24 h after inoculation of *E. coli* and thereafter every day until the disinfectant concentrations had been completely eliminated in distribution systems. Attached bacteria were released from the stainless steel coupons by 2 min sonication (Bandelin Sonorex RK 255S) in 20 mℓ sterile MilliQ water.

Attached heterotrophic bacteria were enumerated by the standard spread plate procedure using R2A agar (Difco), incubated at 28°C for 7 days (Reasoner and Geldreich, 1985). Analyses were carried out in duplicate. The following equation was used to calculate the number of heterotrophic bacteria (cfu.cm^{-2}):

$cfu.cm^{-2}$ = N x D/surface of coupons.

N = average number of colonies

D = dilution factor

Escherichia coli were enumerated by the membrane filter procedure using a filter with a 0.45 μm pore size (APHA-AWWA-WPCF, 1989). Membranes (Whatman) were placed on m-FC agar (Merck) and incubated for 24 $\pm$ 2 h at 44.5 $\pm$ 0.2° C. Each determination was performed in triplicate. The above equation was also used to calculate the number of *E. coli* ($cfu.cm^{-2}$).

5.3 RESULTS AND DISCUSSION

Although different concentrations of free chlorine (2 $mg.\ell^{-1}$) and monochloramine (1.5 $mg.\ell^{-1}$) were used to produce the chlorinated and chloraminated waters respectively, the treated waters contained approximately the same concentration of disinfectant residual (0.7 $mg.\ell^{-1}$ for free chlorine and 0.8 $mg.\ell^{-1}$ for monochloramine) within 24 h. Over a period of 24 h, residual levels decreased in the systems, and no residual was present in the chlorinated water after 48 h (Table 5.1). Monochloramine residual could be detected up to 48 h (0.3 $mg\ell^{-1}$) and was only depleted after 72 h. The persistence of monochloramine residual in potable water distribution system was also found by other researchers (Mathieu *et al.*, 1992), as well as during our study with bacterial regrowth or biofilm formation in potable surface water and groundwater distribution systems (chapter 4).

Table 5.2 shows that the formation of biofilm (attached heterotrophic bacteria) on the stainless steel coupons could be noted in the three systems within 48 h. During the entire study

period (96 h), a higher attached heterotrophic count (4 log cfu.cm^{-2}) was found in the control than in the disinfected water systems. A lower attached heterotrophic count, however, was found in the monochloramine treated water system (3 log cfu.cm^{-2}) than in the chlorine treated water system (4 log cfu.cm^{-2}). This confirmed our findings with surface water and groundwater, showing the effectiveness of monochloramine in controlling the growth of biofilms in a laboratory-scale unit within the days after disinfection.

While the attachment of *E. coli* in early biofilm formation was obvious in the control and chlorinated water distribution systems, no *E.coli* were detected on the stainless steel coupons exposed to the monochloramine treated water for the whole of the study period (Table 5.2). However, 100 mℓ *E. coli* suspension (2.6 x 10^{6} cfu.100mℓ$^{-1}$) was inoculated 24 h after the circulation of disinfected waters in the distribution systems. At this time 0.7 mg.ℓ$^{-1}$ free chlorine and 0.8 mgℓ$^{-1}$ monochloramine were present in the potable water systems. The absence of attahed coliform in the chloramine treated water showed the effectiveness of monochloramine in preventing the attachment of *E. coli;* and the presence of attached coliform in the chlorine treated water showed the resistance of *E. coli* to chlorination and their attachment in young biofilms. This observation supported the findings of others showing the capability of coliform bacteria to survive high disinfectant doses of chlorine (Tracy *et al.*, 1966, LeChevallier *et al.*, 1984, 1988).

Within 72 h, a 240-fold increase of *E. coli* in a young biofilm was noted in the chlorinated water and a 37-fold increase was observed in the control. Attachment of bacteria to the surface, specific growth conditions of bacterial culture, as well as the disappearance of chlorine residual could explain this increase of *E. coli* in chlorinated water. The increase of *E. coli* in chlorinated water could also be accounted for by the regrowth of injured cells in the systems. Several investigators have shown that many coliforms survive standard chlorine

residuals as chlorine-injured cells, with subsequent release in distribution systems (McFeters and Camper, 1983; Oliveri *et al.*, 1985; McFeters *et al.*, 1986).

Within 96 h, a decrease occurred in the attached coliform bacteria count in the chlorinated water system (from 4.8 x 10^2 to 2 cfu.cm^2) as well as in the control system (from 3.32 x 10^2 to 1.88 x 10 cfu.cm^{-2}) whereas the count for attached heterotrophic bacteria remained approximately the same as the initial count (3 log.cm^{-2} for the chlorinated water and 4 log.cm^{-2} for the control). Competition for limiting organic carbon could be reponsible for this, as indicated by Lechevallier and McFeters (1985), who found a significant correlation between the initial level of heterotrophic bacteria and the rate of coliform decline.

In conclusion, this study showed the effectiveness of monochloramine in preventing the growth or attachment of *E. coli* in a young biofilm formation. While the attachment of *E. coli* in young biofilms was obvious on stainless steel coupons exposed to the control and chlorinated water distribution systems, no attached *E. coli* were noted on stainless steel coupons exposed to monochloramine treated water over a period of 96 h.

Table 5.1. Concentrations of the disinfectant residuals in potable water distribution systems.

Time	Residual disinfectant	
	Chlorine	Monochloramine
0 h	2	1.5
24 h	0.7	0.8
48 h	0.0	0.3
72 h	0.0	0.0

Table 5.2. The total number of attached heterotrophic and coliform bacteria (cfu.cm^{-2}) in different water distribution systems.

Time	Attached heterotrophic bacteria (average cfu.cm^{-2})		
	Control	Chlorinated water	Chloramin. water
48 h	9.33×10^4	8.36×10^3	1.78×10^2
72 h	4.30×10^4	5.69×10^3	8.89×10^2
96 h	1.80×10^4	1.78×10^3	4.00×10^2
	Attached *E. coli* (average cfu.cm^{-2})		
	Control	Chlorinated water	Chloramin. water
48 h	9	2	0
72 h	3.32×10^2	4.8×10^2	0
96 h	1.88×10^1	2	0

PART IV

CONCLUSIONS

CHAPTER 6

CONCLUSIONS

Disinfectant effectiveness

* The results of this study indicate that chlorine, monochloramine, ozone and UV were generally effective in the initial removal of coliform bacteria and reduction of heterotrophic bacteria in both surface and ground water. This effectiveness appeared to be greater in groundwater than in surface water.

* Ozone was very effective in the initial reduction of heterotrophic bacteria when using groundwater.

* This study confirms hydrogen peroxide as a weak disinfectant for the initial removal of coliform bacteria and initial reduction of heterotrophic bacteria. However, monochloramine and hydrogen peroxide were more effective in controlling heterotrophic bacteria levels if sufficient residual could be maintained.

* To make significant progress toward compliance with bacteriological groundwater quality standards, it is recommended that ozone be used as a primary disinfectant for the disinfection of groundwater with a short storage duration in the distribution system.

* The presence of an adequate disinfectant residual, whether it be monochloramine or hydrogen peroxide , will enable compliance with bacteriological water quality standards.

The effect of disinfectant Residual s on bacterial regrowth

* This study confirm the instability of ozone in terms of providing a residual effect. Chlorine was relatively unstable and also did not provide a sustainable residual. Monochloramine and

hydrogen peroxide gave a relatively stable residual.

* Monochloramine and hydrogen peroxide were found to be significantly better at attaining and maintaining a disinfectant residual than chlorine and ozone.

* The greater persistence of monochloramine and hydrogen peroxide residuals was found to inhibit bacterial regrowth in treated water systems.

* Based on the results of this study, chlorination, ozonation and UV irradiation could not provide effective treatment unless used in combination with a residual monochloramine or residual hydrogen peroxide concentration.

Biofilm formation

* Biofilm formation was obvious in the presence of significant disinfectant concentrations within the first day after disinfection.

* The cement coupons were found more capable of enhancing biofilm regrowth than the stainless steel coupons within a prolonged exposure time.

* Attached viable bacteria counts appeared to be similar in both water sources (surface water and groundwater), whereas attached total bacteria were found significantly higher on coupons exposed to groundwater than those exposed to surface water.

* Monochloramine was found more effective in preventing the attachment of coliform during the early biofilm formation than chlorine.

* It is therefore recommended that the maintenance of a disinfectant residual greatly would improve bacteriological conditions compared with little or no residual .

* Based on the results of this study, it is recommended that water suppliers must consider the origin (source) of intake water, the type of disinfectants, the piping material and the retention periods of water in distribution systems in devising a solution to the problem of bacterial regrowth or biolfim formation in potable water distribution systems.

REFERENCES

Adam J.C., Lytle M.S., Dickman D.G. and Bressler W.R. (1991) Use of direct viable count methodology with ozonation in drinking water. *Ozone Science Eng.* , **13**, 1-10.

Albrich J.M., McCarthy C.A. and Hurst J.K. (1981) Biological reactivity of hypochlorous acid. Implications for microbiocidal mechanisms of leukocyte myeloperoxidase. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 210-214.

Albrich J.M. and Hurst J.K. (1982) Oxidative inactivation of *Escherichia coli* by hypochlorous acid: rates and differentiation from other reaction sites. *FEBS Lett.* **144**, 157-161.

Albrich J.M., Gilbaugh III, J.H., Callahan K.B. and Hurst J.K. (1986) Effects of the putative neutrophil-generated toxin, hypochlorous acid, on membrane permeability and transport systems of *Escherichia coli*. *J.Clin. Invest.* **78**, 177-184.

Al-Hiti M.M.A. and Gilbert P. (1980) Changes in preservative sensitivity for the USP antimicrobial agents effectiveness test micro-organisms. *J. Appl. Bacter.* **49**, 119-126.

Allen M.J., Taylor R.H. and Geldreich E.E. (1980) The occurence of microorganisms in water main encrustation. *J. AWWA.*, **72**, 614.

American Water Works Association (1971) Water quality and treatment. 3rd Edition. *McGraw-Hill Book Company.*

American Water Works Association (1982). Disinfection Committee Report. *J. AWWA.* **74** (7), 376-380

Angehrn M. (1984) Ultraviolet disinfection of water. *Aqua* **2**, 109-115 Anonymous (1930) Bacterial aftergrowths in water ditribution systems. Report of the committee on water quality. *A. J. Pub. Health* **20,** 485-491.

Anonymous (1961) Water storage tanks. *N.B.R.I. Information sheet. C.S.I.R. technical note* No XD75. South Africa.

Anonymous (1979) Municipal effluent treatment with hydrogen peroxide. *Effluent Water Treat*. **19** (8-suppl), 6-11

Anonymous (1981) Treatment techniques for controlling THMs in drinking water. *US environmental Protection Agency (EPA)* **600** (2), 81-156.

Anonymous (1984) Drinking water microbiology. Committee on the challenges of modern society *Nato/CCMS, CCMS 128, Pilot Project Service 1984.*

Anonymous (1988) British Standard 6980. Suitability of non-metallic products for use in contact with water intended for human consumption with regard to their effect on the water quality. Growth of aquatic microorganisms. *British Standards Institution London.* Section 2-4

APHA (1989) *Standard Methods for the Examination of Water and Wastewater.* 17th edition. American Public Health Association, Washington, D.C.

Augoustinos M.T., Kfir R. and Venter S.N. (1992) Assessment of water quality problems due to microbial growth in drinking water distribution systems. *Report to the Water Research Commission.* Division of Water Technology, C.S.I.R. WRC Report N° 252/1/92.

Atterholm I., Hallbert T., Ganrot-Nortim K. and Ringertz O. (1977). Unexplained acute fever after a hot bath. *Lancet.* **ii**, 684-686.

Baldry M.G.C. and Fraser J.A.L. (1988) Disinfection with peroxygens. In: *Industrial biocide* (Edited by Payne K.R.), pp 91-116, John Wiley & Sons, Chichester.

Baleux B. and Got P. (1996) Apport de l'observation microscopique couplée à l'analyse d'images dans l'évaluation de la qualité bactériologique des eaux: approche cellulaire globale. *Techniques Sci. L'Eau* **91**, 430-436

Barber, R.T (1968). Dissolved organic carbon from deep waters resists microbial oxidation *Nature*. **220**, 274-275.

Barrette W.C., Albrich J.M. and Hurst J.K. (1987) Hypochlorous acid-promoted loss of metabolic energy in *Escherichia coli*. *Infect. Immun.* **55**, 2518-2525.

Barcellona M.L. and Gratton E. (1990) The fluorescence properties of a DNA probe. *Eur. Biophys. J.* **17** ,315-323.

Barrette W.C., Hannum D.M., Wheeler W.D. and Hurst J.K. (1989) General mechanism for the bacterial toxicity of hypochlorous acid: Abolition of ATP production. *Biochem.* **28**, 9172-9178.

Baylis J.R. (1930) Bacterial aftergrowth in water distribution systmes. *Wat. WKS. Sewer.* **77** (10), 335

Beauchat L.R. (1978) Injury and the repair of gram-negative bacteria with special consideration of the involvement of the cytoplasmic membrane. *Adv. Appl. Microbiol.* **23**, 219-243.

Bernhardt H. and Wilhelms A. (1985) Erfahrungen mit der Desinfektion von Trinkwasser durch Chlorodioxid. *38 Internat CFBEDEAU Konferenz - CEBEDOC*. Liège, 193-231.

Bernhard H. and Lieson H.U. (1988) Trinkwasserverkeimungen in Verteilungsnetzen durch Korrosionsschutz auf bitumenbasis. *Wasser. Abwasser GWF*. **129**(41), 28-129.

Bernhard H. and Classen J. (1993) Eutrophication control as an essential condition for an optimum disinfection. *Water Supply*. **11**(3), 89-108.

Bibb M. (1985). Bacterial corrosion in the South African power industry. In: *Proceeding International Conference* on Microbiologically Induced Corrosion, Washington.

Bissonnette G.K., Jezeski J.J., McFeters G.A. and Stuart D.G. (1977) Evaluation of recovery methods to detect coliform in water. *Appl. Environ. Microbiol.* **33**(3), 590.

Brazos B.J. and O'Connor J.T. (1989) Relative contribution of regrowth and aftergrowth of the number of bacteria in a drinking water distribution system. *In Proc. AWWA. Wat. Qual. Tech. Cont.*, Philadelphia, Pennysylvannia, Nov. 1989.

Brown M.R.W, Allison D.G. and Gilbert P. (1988) Resistance of bacterial biofilms to antibiotics: a growth-rate related effect? *J. Antimicrob. Chemother.* **22**, 777-780.

Brown M.R.W, Collier P.J. and Gilbert P. (1990) Influence of growth rate on susceptibility to antimicrobial agents: modification of the cell envelope and batch and continuous cultural studies. *J.Antimicrob. Chemother.* **34**, 1623-1628.

Brözel V.S. (1990) Aspects of bacterial biofouling control in water systems. *MSc Thesis*, University of Pretoria, South Africa.

Brözel V.S. and Cloete T.E. (1991) Fingerprinting of commercially available water treatment bactericides in South Africa. *Wat. SA.* **17** (1), 57-66.

Burman N. and Colbourne J. (1976) The effect of plumbing material on water quality. *J. Inst. Plumb.* **3**, 12-13.

Burke V., Robinson J., Gracey M., Petersen D. and Partridge K. (1984) Isolation of *Aeromonas hydrophila* from a metropolitan water supply: seasonal correlation with clinical isolates. *Appl. Environ. Microbiol.* **48,** 361-366.

Cadenas E. (1989) Biochemistry of oxygen toxicity. *Ann. Rev. Biochem.* **58**, 79-110.

Camper A.K. and McFeters G.A. (1979) Chlorine injury and the enumeration of waterborne coliform bacteria. *Appl. Environ. Microbiol.* **37**(3), 633.

Camper A.K., LeChevallier M.W., Broadway S.C. and McFeters G.A. (1985) Growth and persistence of pathogens in granular activated carbon filters. *Appl. Environ.microbiol.* **50**, 1378-1382.

Chapelle F.H. (1993). *Groundwater microbiology and geochemistry*. John Wiley & Sons, Inc. New York, Chichester, Brisbane, Toronto, Singapore.

Characklis W.G. (1988) Bacterial population in potable water distribution systems. *In: Bacterial Regrowth in Distribution Systems*. Research Project for AWWA Foundation.

Clark J.A. and Pagel J.E. (1977). Pollution indicator bacteria associated with municipal raw and drinking water supplies. *Can. J. Microbiol.* **23,** 464-470.

Clark J.A. (1980) The influence of increasing numbers of non-indicator organisms upon the detection of indicator organisms by membrane filter and presence-absence test. *Can. J. Microbiol.***26,** 827-832.

Clark J.A., Burger C.A. and Sabatinos L.E. (1982) Characterization of indicator bacteria in municipal raw water, drinking water and new mains water. *Can. J. Microbiol.*, **28**, 1002-1013.

Clark R.M., Goodrich J.A. and Wimer L.J. (1993) Effect of the distribution system on drinking water quality. *J. Wat. SRT-Aqua*. **42**, 30-38.

Clark R.M., Lykins B.W, Block J.C., Wymer L.J. and Reasoner D.J. (1994) Water quality changes in a simulated distribution system. *J. Wat. SRT-Aqua* **43**(6), 263-277.

Cloete T.E., Brözel V.S., von Holy A. (1992) Practical aspects of biofouling control in industrial water systems. *Int. Biodegradation*. **29**, 299-341.

Cloete T.E. (1997) Technology Scan Report - Eskom on International Workshop on Industrial Biofouling and Biocorrosion -4 -5 Sptember 1997. Mulheim ander Ruhr - Germany.

Coad, A. (1990) Conventional water treatment. *In: Developing World Water. Water Treatment,* (Edited by WEDC Grosvenor Press International) 1981-1990. Hong Kong, 110-118.

Colbourne J.S., Dennis P.J., Trew R.M., Berry C. and Kesey G. (1988) Legionellis and public water supplies. *In Proc. Int. Conf. on water and wastewater microbiology*, Newport Beach, California.

Committee Report (1983) Deterioration of water quality in large distribution reservoirs (open reservoirs). Committee on control of water quality in transmission and distribution systems, Metropolitan Water District of Southern California, the Seattle (Wash.) Water Department and the Hackensack (NJ) Water Company. *J. AWWA.* **75** (6), 313-318.

Costerton J.W. and Robbins J.R. (1980) The Robbins device for the in situ development of sessile bacterial populations. *Patent.* Canada and USA.

Costerton J.W., Cheng K-J, Geesey G.G., Ladd T.I., Nickel J.C., Dasgupta M., Marrie T.J. (1987) Bacterial biofilms in nature and disease. *Ann. Rev. Microbiol.* **41,** 435-464.

Craun G.F. (1980) Disease outbreaks caused by drinking water. *J. WPCF.* **52**(6), 1833.

Craun G.F. (1981) Disease outbreaks caused by drinking water. *J. Water. Poll. Control Fed* **53,** 1134-1138

Craun G.F. (1984) Waterborne outbreaks of giardiasis: current status. In: *Giardia and Giardiasis* (Edited by Erlandsen S.L. and Meyers E.A.), 243-261. Plenum Press, New York.

Craun G.F. (1985) An overview of statistics on acute and chronic water contamination problems. In: *4th domestic water quality symposium*: Point-of-use treatment and its implications. Water Quality Association, Lisle, IL, 5-15.

Craun G.F. (1986) Waterborne diseases in the United States. *CRC Press, Inc.* Boca Raton, Florida, 295.

Crowson D.L. and Chambers R. (1985) Polyethylene and polybutylene pipe and tubing: A status report. *J. AWWA*. **77**, (11), 45-46.

Dalterio R.A., Nelson W.H., Britt D and Sperry J.F. (1987) An ultraviolet (242 nm excitation) resonance Raman study of live bacterial components. *Appl. Spectroscopy*. **41**, 417.

Daniel P., Zafer B. and Price S. (1993) Consideration in chloramine disinfection. *Wat. Supp*. **11**, (3-4), 189-202.

Deakin M.R. and Buttry D.A. (1989) Electrochemical applications of the quartz crystal microbalance. *Analyt. Chemist*. **61**, 1147-54.

De Constantin S., Pascal O., Block J.C. and Dollard M.A. (1986) La flore bacterienne dans les reseaux de distribution: comparaison de deux études. *Wat. Supp*. **4**, 19-215.

Dennis W.H., Olivieri V.P. and Kruse C.W. (1979) Mechanisms of disinfection: Incorporation of ^{36}Cl into f2 virus. *Wat. Res*. **13**, 363-369.

Dietlicher K. (1970) Wiederverkeimung ozonierter Schnellfiltrate in Rohrnetz. *Schriftenreihe Verein für Wasser-Boden-und Lufthygiene*. **31**, 1-16.

Donlan R.M. and Pipes W.O. (1988) Selected drinking water characteristic and attached microbial population density. *J. AWWA*. **80** (11), 70-76.

Donlan R.M., Pipes W.O. and Yohe T.L. (1994) Biofilm formation on cast iron substrata in water distribution systems. *Wat. Res*. **28** (6) 1497-1503.

Doré M. (1989) Chimie des oxydants et traitement des eaux. Technique et documentation, *Lacoisies*, Paris,350.

Dowling N.J.E., Stansbury E.E., White D.C., Borenstein S.W. and Danko J.C. (1988) On-line electrochemical monitoring of microbially induced corrosion. *In:* Microbial corrosion, pp. 5-17. Palo Alto, Calif.

Dutka, B.J. (1973) Coliform are an inadequate index of water quality. *J. Environ.Health* **36**, 39-46.

Easton and Jago (1991). Progress report on the measurement and significance of assimilable organic carbon. *Water Research Commission*, FR 0249.

Earnhardt K.B., Jr. (1980) Chlorine resistant coliform - the Muncie, Indiana experience. *Proc. AWWA. Wat. Qual. Tech. Conf.* Miami Beach, Fla. American Water Works Association.

Engel H.W.B., Berwald L.G. and Havelaar A.H. (1980) The occurence of *Mycobacterium kansasii* in tap water. *Tubercle* **61,** 21-26.

Escher A.R. and Characklis W.G. (1988) Microbial colonization of a smooth substratum: A kinetic analysis using Image Analysis. *Wat. Wastewat. Microbiol. Wat. Sci. Tech.* **20**,(11/12), 277-283.

Evans T.M., Waarvick C.E., Seider R.J., and LeChevallier M.W. (1981). Failure of the most-probable-number technique to detect coliforms in drinking water and raw water supplies. *Appl. Environ. Microbiol* . **41,** 130-138.

Exner M., Tuschewitzki G.J. and Thofern E. (1983) Observation of bacterial growth on a copper pipeline of a central disinfection dosage apparatus. *Zbl. Bkt. Hyg. I. Abt. Orig. B*. **177**, 170-181.

Farkas-Himsley H. (1964) Killing of chlorine-resistant bacteria by chlorine-bromine solutions. *Appl. Microbiol.* **12**, 1-6.

Farr S.B. and Kogoma T. (1991) Oxidative stress response in *Escherichia coli* and *Salmonella typhimurium. Microbiol. Rev.* **55**, 56-58.

Fillingame R.H. (1980) The proton-translocating pumps of oxidative phosphorylation. *Ann. Rev. Biochem.* 49, 1079-1113.

Fletcher M. and Marshall K.C. (1982) Bubble contact angle method for evaluation of subtratum interfacial characteristics and its relevance to bacterial attachment. *Appl. Environ. Microbiol*, **44,** 797-732.

Foster S.J. and Johnstone K. (1987) Purification and properties of a germination-specific cortex-lytic enzyme from spores of *Bacillus megaterium* KM. *Biochem. J.* **242**, 573-579.

Geldreich E.E., Nash D.H., Reasoner D.J. and Taylor R.H. (1972) The necessity of controlling bacterial populations on potable waters: Community water supply. *J. AWWA*. **64**, 596-602.

Geldreich E. E., Allen M.J. and Taylor R.H. (1978) Interferences to colifom detection in potable water supplies, p. 13-20. *In*:: C.W. Hendricks (ed), Evaluation of the microbiology standards for drinking water. U.S. Environmental Protection agency, Washington, D.C.

Geldreich E.E. and Rice E.W. (1987) Occurrence, significance and detection of Klebsiella in water systems. *J. AWWA* **79**, 74-80.

Geldreich E.E. (1989) Drinking water microbiology and new directions toward water quality enhancement. *Inter. J. Food Microbiol*. **9**, 295-312.

Geldreich E.E. (1990) Microbiology quality of source waters for water supply. In: *Drinking Water Microbiology: progress and recent developments* (Edited by McFeters G.A.), 3-31. Springer-Verlag. New York.

Gibbs R.A., Sutt J.E. and Croll B.T. (1990) Microbiological and trihalomethane responses to booster chlorination. *J. IWEM*. **4**(1), 131-139.

Gilbert E. (1988) Biodegradability of ozonation products as a function of COD and DOC elimination by the example of humic acid. *Wat. Res*. **92**, 123-126.

GLaze W.H. (1987) Drinking water treatment with ozone. *Environ. Sci. Technol.* **21**(3), 224-230.

Glaze W.H. and Kang J.W. (1988) Advanced oxidation processes for treating groundwater contaminated with TCE and PCE: laboratory studies. *J. AWWA* **80** (5), 57.

Grabow W.O.K., Burger J.S. and Nupen E.M. (1980) Evaluation of acid-fast bacteria, *Candida albicans*, enteric viruses and conventional indicators for monitoring wastewater reclamations. *Prog. Wat. Tech.* **12**, 803-817.

Grabow W.O.K. (1990) microbiology of drinking water treatment: Reclaimed wastewater. In: *Drinking Water Microbiology: progress and recent developments* (Edited by McFters G.A.), pp. 185-204. Springer-Verlag. New York.

Haas C.N. and Morrison E.C. (1981) Repeated exposure of *Escherichia coli* to free chlorine: production of strains possessing altered sensitivity. *Water Air , Soil Pollut.* **16**, 233-242.

Haas C.N.and Engelbrecht R.S. (1980) Physiological alterations of vegetative microorganisms resulting from chlorination. *J. Water. Poll. Control Fed.* **52**, 1976-1989.

Halliwel B. and Gutteridge JMC (1990) Role of free radicals and catalytic metal ions in human disease: on overview. *Methods Enzymol.* , 186, 1-85.

Hascoët M.C., Servais P. and Billen G. (1986) Use of biological analytical methods to optimize ozonation and GAC filtration in surface water treatment. *Annual Meeting, AWWA,* Denver, Col.

Hasset D.J. and Cohen M.S (1995) Rec A versus catalase: Whoo's on first ? *J. Clin. Invest.* **95**, 924 - 925.

Hauson P.G. (1977) Fresh water wound infection due to *Aeromonas hydrophila*. *Am. Med. Assoc.* **233**, 1053-1054.

Haudidier K., Paquin J.L., Français T., Harteman P., Grapin G., Colin F., Joudain M.J., Block J.C., Cheron J., Pascal O., Levi Y. and Miazga J. (1988) Biofilm growth in a drinking water network: A preliminary industrial pilot-plant experiment. *Wat. Sci. Tech.* **20**, 109-115.

Hengesbach B., Schoenen D., Hoyer O., Bernhardt H., Mark G., Schuchmann and von Sonntag C. (1993) UV disinfection of drinking water - The question of bacterial regrowth and the photolytic degradation of biogenic high-molecular-weight substances. *J. Water SRT-Aqua* **42** (1), 13-22.

Held A.M., Halko D.G. and Hust J.K. (1978) Mechanisms of chlorine oxidation of hydrogen peroxide. *J. Am. Chem. Soc.* 100, 5732-5740.

Herwaldt B.L., Craun G.F., Stokes S.L. and Juranek D.D. (1992) Outbreaks of waterborn disease in the United States: 1989 - 1990. *J. AWWA*. **84**(4), 129-135.

Herman L.G. (1978) The slow-growing pigmented bacteria: problems and sources. *Adv. Appl. Microbiol.* **23**, 155-171.

Herson D.S., McGonighe B., Payer M.A. and Baker K.H. (1987) Attachment as a factor in the protection of *Enterobacter cloacea* from chlorination. *Appl. Envir. Microbiol.* **53**, 1178-1180.

Hirsch P., Rades-Rohkohl E., Kolbel-Boelke J., Nehrkorn A., Schweisfurth R., Selenka F. and Hack A. (1992) Methods of studying groundwater microbiology: critical evaluation and method suggestions. In: *Progress in hydrogeochemistry* (Edited by Matthess G., Frimmel F.H., Hirsch P., Schulz H.D. and Usdowski E.), 325-333.Springer, Heidelberg.

Hoadley A.W. and Gould J. (1976) Disinfection. *J. Wat. Poll. Control. Fed.* **48**, 1166-1170.

Hurst J.K., Barrette W.C., Jr, Michel B.R. and Rosen H. (1991) Hypochlorous acid and myeloperoxidase-catalyzed oxidation of iron-sulfur clusters in bacterial respiratory dehydrogenases *Eur. J. Biochem* **220**, 1275-1282.

Imlay J.A. and Linn S. (1986) Bimodal patterns of killing of DNA-repair-defective or anoxically grown *Escherichia coli* by hydrogen peroxide. *J. Bacteriol.* **166**, 519-527.

Ishizaki K., Sawadaishi D., Miura K., Shinriki N. (1987) Effect of ozone on plasmid DNA of *Escherichia coli in situ*. *Wat. Res.I* **21** (7), 823-828.

Jacangelo J.G., Olivieri V.P. and Kazuyoshi K. (1991) Investigating the mechanism of inactivation of *Escherichia coli* B by monochloramine. *J. AWWA*. **73** (5), 40-44.

Jacobs L., De Bruyn E.E. and Cloete T.E. (1996) Spectrophotometric monitoring of biofouling. *Wat. Sci. Tech.* **34** (5-6), 533-540.

Jass J., Costerton J.W. and Lappin-Scott H.M. (1995) Assessment of a chemostat-coupled modified Robbins device to study biofilms. *J. Ind. Microbiol.* **15**, 283-289.

Jones F., Smith P. and Watson D.C. (1978) Pollution of a water supply catchment by breeding gulls and the potential environmental health implications. *J. Inst. Wat. Engineers Sci.* **32**, 465-482.

Joret J.C., Levi Y. and Volk C. (1991) Biodegradable dissolved organic carbon (BDOC) content of drinking water and potential regrowth of bacteria. *Wat. Sc. Tech.* **24** (2), 95-101.

Kepner R.L., Jr. and Pratt J.R. (1993) Effects of sediments on estimates of bacterial density. *Trans. Am. Microsc. Soc.* **112**, 316-330.

Kepner R.L., Jr. and Pratt J.R. (1994) Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. *Microbiol Rev.* **58**, 603-615.

Kfir R. and Kott Y. (1989) Microbial growth in water distribution systems: A literature review. *Division of Water Technology*, CSIR, Health Programme, Pretoria.

Khoury A.E., Nicholov R. Soltes S., Bruce A.W,, Reid G. and DiCosmo F. (1992). A priliminary assessment of *Pseudomonas aeroginosa* biofilm development using fluorescence spectroscopy. *International Bioterioration and Biodegradation* , **30**, 187-199.

King W.L and Gould G.W. (1969) Lysis of bacterial spores with hydrogen peroxide *J. Appl. Bacteriol.* **32**, 481-490.

Klein, D.A. and Wu, S. (1974) Stress: a factor to be considered in heterotrophic microorganin enumeration from aquatic environment. *Appl. Microbiol.* **27**, 427 - 431.

Kok T.S. and Simons J.J. (1989) Notes on geohydrological characteristics of the more important waterbearing formations of S.A. with special reference to possible groundwater pollution. Unpbl. *Report GH 3641, Directorate of Geohydrology*, Department of Water Affairs and Forestery, Pretoria.

Kölbel-Boelke J., Tienken B. and Nehrkorn A. (1988a) Microbial communities in the saturated groundwater environment. I. Methods of isolation and characterization of heterotrophic bacteria. *Microb. Ecol.* **16**, 17-29.

Kölbel-Boelke J. and Hirsch P. (1989) Comparative physiology of biofilm and suspended organisms in the groundwater environment. In*: Report of the Dahlem Workshop on structure and function of biofilms,* Berlin 1988, November 27 - December 2 (Edited by Charaklis W.G. and Wilderer P.A), 221-238 . John Wiley & Sons, Chichester, New York.

Kreft P., Umphres M., Hand J.M., Tate C., McGuire M.J. and Trussel R.R. (1985) Converting from chlorine to chloramines: A case study. *J. AWWA.* **77**(1), 38-45.

Krieg N. and Holt J.G. (1984) *Bergey's Manual of Systematic Bacteriology*, Vol. 1. London, William and Wilkins.

Kruse C.W., Snead M.C. and Olivieri V.P. (1981) Design of alternative disinfecting systems with special reference to water qualitry at the consumer's tap. In: *Proc. 23rd Public Wtr. Supply Eng. Conf., Eng. Publ. Off.*, Univ. of Illinois, Urbana.

Kubista M., Akerman B and Nordé B. (1987) Characterization of interaction between DNA and 4',6-diamidino-2-phenylindole by optical spectroscopy. *Biochemistry* **26** , 4545-4553.

Kulpa C.F. and Baker C. (1990) Involvement of sulfur-oxidizing bacteria in the concrete deterioration In: *Paul Klieger symposium on performance of concrete* (Edited by Whiting D.) USA. SP 122-17, 313-322.

Lamb J.C . (1985) Water quality and its control. In: *John Wiley & Sons, edit.* New York, USA, 384.

Larsen T.E., Goodsell D.S. Cascio D., Grzeskowiak K. And Dickerson R.E. (1989) The structure of DAPI bound to DNA. *J.Biomol. Struct. Dyn.* **7,** 477-491.

LeChevallier M.W., Seidler R.J. and Evans T.M. (1980) Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. *Appl. Environ. Microbiol.* **40**(5), 922-930.

LeChevallier W.M., Evans T.M. and Seidler R.J. (1981) Effect of turbidity on chlorination efficiency and bacterial persistence in drinking water supplies. *Appl. Environ. Microbiol.* **42**, 159-167.

LeChevallier M.W., Cameron J.C. and McFeters G.A. (1983) New medium for improved recovery of coliform bacteria from drinking water. *Appl. Environ. Microbiol.* **45**, 484- 492.

LeChevallier M.W., Hassenauer T.S., Camper A. L. and McFeters G.A (1984) Disinfection of bacteria attached to granular activated carbon. *Appl. Environ. Microbiol.* **48,** 918-923.

LeChevallier M.W. and McFeters G.A (1985) Interactions between heterotrophic plate count bacteria and coliforms organisms. *Appl. Environ. Microbiol.* **49**, 1338-1341.

LeChevallier M.W., Babcock T.M. and Lee R.G. (1987) Examination and characterization of distribution system biofilms. *Appl. Environ. Microbiol.* **53** (12), 2714-2724.

LeChevallier M.W., Cawthon C.D. and Lee R.G. (1988) Factors promoting survival of bacteria in chlorinated water supplies. *Appl. Environ. Microbiol.* **54**, 649.

LeChevallier M.W., Cawthon C.D. and Lee R.G. (1988) Mechanisms of bacterial survival in chlorinated drinking water water. *Wat. Sci. Tech.* **20** (11/12), 145-151.

LeChevallier M.W., Lowry C.D. and Lee R.G. (1990) Disinfecting biofilms in a model distribution system. *J. AWWA.* **82**, 87-99.

LeChevallier, M.W., Schulzand W. and Lee R.G. 1991. Bacterial nutrients in drinking water. *Appl. Environ. Microbiol.*, **57** (3), 857-862.

LeChevallier, M.W., Welch N.J. and Smith D.B. (1996) Full-scale studies of factors related to coliform regrowth in drinking water. *Appl. Environ. Microbiol.* **62**(7), 2201-2211

Levine W.C., Stephenson W.T. and Craun G.F. (1990) Waterborne disease outbreaks, 1986-1988. *MMWR CDC Surveil. Summ.* **39**(1), 1-13.

Lerner N.D. and Tellam J.H. 1992. The protection of urban groundwater from pollution. *Journal IWEM*. 28-37.

Leyval C., Arz., C., Block J.C. and Rizet M. (1984). *Escherichia coli* resistance to chlorine after successive chlorination. *Environ. Tech. Lett.* **5**, 359-364.

Lippy E.C. and Waltrip S.C. (1984) Waterborne disease outbreaks 1946-1980: a thirty-five-year perspective. *J. AWWA.* **76**, 60-67.

Lloyd B.J. and Bartram J.K. (1991) Surveillance solutions to microbiological problems in water quality control in developing countries. *Wat. Sci. Tech.* **24** (2): 61-75.

Logsdon G.S. (1990) Microbiology and drinking water filtration. In: *Drinking Water microbiology: progress and recent development* (ed. McFeters G.A.). Springer-Verlag. New York. 120-145.

Lohr A. and Gratzek J. (1984) Bactericidal and paraciticidal effects of an activated air oxidant in a closed aquatic system. *J. Aquaric. Aquat. Sci.* **4** (4 1/2), 1-8.

Louwbury E.J.L., Thom B.T., Lilly H.A., Rabb J. and Whittal A. (1970) Sources of infection with *Pseudomonas aeroginosa* in patients with tracheostomy. *J. Med. Microbiol.* **3**, 39.

Lund V. and Ormerod K. (1995) The influence of disinfection processes on biofilm formation in water distribution systems. *Wat. Res.*, **99**(4), 1013-1021.

Maarschalkerweerd J., Murphy R. and Sakamoto G. (1990) Ultraviolet disinfection in municipal wastewater treatment plants. *Wat. Sci. Tech* **22** (7/8), 145-152.

Mackenzie W.R., Hoxie N.H., Proctor M.E., Gradus M.S., Blair K.A., Peterson D.E., Kazmierczak J.J., Addiss D.G., Fox K.R., Rose J.B. and Davis J.P. (1994) A massive outbreak in Milwaukee of *Cryptosporium* infection transmitted through the public water supply. *New Engl. J. Med.* **33**(1), 161-167.

Magasanik B. and Neidhardt F.C. (1987) Regulation of carbon and nitrogen utilization in *Escherichia coli* and *Salomonella typhimurium.* In: *Cellular molecular biology* (Edited by Neidhardt F.C. *et al.*) **2**, 1318-1325. Washington DC, American Society for Microbiology.

Manzini G., Barcelona M.L., Avitabile M.,Quadrifoglio F. (1983) Interaction of diamidino-2-phenylindole (DAPI) with natural and synthetic nucleic acids. *Nucleic Acids Res.* **11**,8861-8876.

Manoharan R., Ghiamati E., Dalterio R.A., Britton K.A. and Nelson W.H. (1990) Ultraviolet resonance Raman spectra of bacteria. bacterial spores, protoplasts, and calcium dipicolinate. *J. Microbiolog. Meth.* **11**, 1-15.

Marshall K.C. (1992) Biofilm: An overview of bacterial adhesion, activity and control at surfaces. *ASM News*, **50**, 202-207.

Martin R.S., Gates W.H., Tobin R.S., Granthan D., Junorals R., Wolfe D. and Forestoll P. (1982) Factors affecting coliform bacteria growth in distribution systems. *J.AWWA*. **74**, 34-37.

Mathieu L., Paquin J.L., Hartemann P., Colin F. (1992) Paramètres controlant l'accumulation de bactéries dans les réseaux de distribution: approche expérimantale. Séminaire International. Matière organique biodégradable dans les réseaux de distribution, Nancy (Soumis à *Sci. de l'Eau*).

Mathieu L., Block J.C., Naillant J. and Reasoner D. (1993) Control of biofilm accumulation in drinking water distribution systems. *Wat. Supp*. **11**(3-4), 365-376.

Matin A. and Haraketi S. (1990) Effect of starvation on bacterial resistence to disinfectants. In: *Drinking Water Microbiology: progress and recent developments* (Edited by McFeters G.A.), 88-103. Springer-Verlag. New York.

Matsuzawa Y. And Yoshikawa K. (1994) Change of the higher order structure in a giant DNA induced by 4',6-diamidino-2-phenylindole as aminor groove binder and ethidium bromide as an intercalator. *Nucleosides Nucleotides* **13,** 1415-1423.

Mazzini A., Cavatoria P., Iori M., Favilla R. And Sartor G. (1992) The binding of 4',6-diamidino-2-phenylindole as a minor to bovine serum albumin. *Biophys. Chem.* **42**,

McFters G.A. and Stuart D.G. (1972) Survival of coliform bacteria in natural waters: field and laboratory studies with membrane filter chambers. *Appl. Microbiol.* ***24,*** 805-811

McFeters G.A., Cameron S.C. and LeChevallier M.W. (1982) Influence of diluents, media and membrane filters on the detection of injured waterborne coliform bacteria. *Appl. Environ. Microbiol.* ***43*** , 97 -103.

McFeters G.A. and Camper A.K. (1983) Enumeration of indicator bacteria exposed to chlorine. *Adv. Appl. Microbiol.* **20**, 177-193.

McFeters G.A., Kippin J.J. and LeChevallier M.W. (1986) Injured coliforms in drinking water. *Appl. Environ. Microbiol.* **51**, 1-5.

McFeters G.A. (1990) Enumeration, occurrence, and significance of injured indicator bacteria in drinking water, In: *Drinking Water Microbiology: progress and recent developments* (Edited by McFeters G.A.), pp. 478-492. Springer-Verlag. New York Inc.

Meyer T., Lykins B. and Hartemann P. (1993) Influence of biofilm on disinfection by-products in a distribution system. *Wat. Supp.* **11**,(3-4): 355-364.

Mittelman M.W. Islander R. And Platt R.M. (1987) Biofilm formation in a closed-loop purified water system. *Medical Device and diagnostics Industy* , **10**, 50-55; 75.

Mittelman M.W., Nivens, D.S, Low C. and White D.C. (1990) Differential adhesion, activity, and carbohydrate : protein ratios of *Pseudomonas atlantica* monocultures attaching to stainless steel in a linear shear gradient. *Microbiol . Ecol.* **19,** 269-278.

Mittelman M.W., Kohring L.L. and White D.C. (1992) Multipurpose laminar -flow adhesion cells for the study of bacterial colonization and biofilm formation. *Biofouling,* **6**, 39-51.

Mittelman M.W., Packard J., Arrage A.A., Bean S. L., Angell P and White D.C. (1993). Test systems for evaluating antifouling coating efficacy using on-line detection of biolimunescence and fluorescence in a laminar-flow environment. *J. Micriobiolog. Meth.* **18,** 1-10.

Mittelman M.W. (1995) Biofilm development in purified water systems. *In:* Microbial biofilms pp. 133-147. Lappin-Scott H.M and Costerton J.W. (Eds) Cambridge Univ. Press, Cambridge, U.K.

Moore A.C., Herwaldt B.L., Craun G.F., Calderon R.L., Highsmith A.K. and Juranek D.D. (1993) Surveillance for waterborne disease outbreaks - United States, 1991 -1992. *MMWR CDC Surveil. Summ.* **42**(5), 1-22.

Moyer N.B. (1987) Clinical significance of *Aeromonas* spp. isolated from patients with diarrhoea. *J. Clin. Microbiol.* **25**, 2044-2048.

Mudd J.B and Leavitt R., Ongun A., McManus T. (1969) Reaction of ozone with amino acids and proteins. *Atmos. Environ.***3**, 669-682.

Nagy L.A. and Olson B.H. (1985) Occurrence and significance of bacteria, fungi and yeasts associated with distribution pipe surfaces. *Wat. Supp.* **11**(3-4): 365-376.

Nebel C. (1983) Ozone. Kirk-Othmer's Encyclopedia of Chemical Technology, 2nd Edition, Wiley Interscience, New York, **16**, 683-713.

Neden D.G., Jones J.R., Smith J.R., Kirmeyer G.J. and Foust G.W. (1992) Comparing chlorination and chloramination for controlling bacterial regrowth. *J.AWWA.*, **84** , 80-88.

Newby P.J (1991) Analysis of high-quality pharmaceutical grade water by a direct epifluorescence filter technique microcoly method. *Ltters in Appl. Microbiol,* **13**,291-293.

Nichols P.D., Henson J.M, Guckert J.B., Nivens D.E. and White D.C (1985). Fourier transform-infrared spectroscopic methods for microbial ecology: analysis of bacteria, bacteria-polymer mixtures, and biofilms. *J. Microbiolog. Meth.* **17**, 199-213.

Nickel J.C., Wright J.B., Ruseska I., Marrie T.J., Whitfield C. and Costerton J.W. (1985) Antibiotic resistance of *Pseudomonas aeruginosa* colonising a urinary catheter *in vitro*. *Eur. J. Clin. Microbiol.* **4**, 213-218.

Nivens D.E, Chambers J.Q., Anderson T.R., Tunlid A., Smit J. And White D.C. (1993a) Monitoring microbial adhesion and biofilm formation by attenuated total reflection/Fourier transform infrared spectroscopy. *J.microbiol Meth.* **17,** 199-213.

Nivens D.E, Chambers J.Q., AndersonT.R . And White D.C. (1993b) Long-term, on-line monitoring of microbial biofilms using a quartz crystal microbalance. *Anal. chem.***65,** 65-69.

Norman T.S., Harms L.L. and Looyenga R.W. (1980) The use of chloramines to prevent trihalomethane formation in Huron, S.D., drinking water. *J.AWWA.* **72**(2), 176-180.

Novitsky T.Y (1984) Monitoring and validation of high purity water systems with the *Limulus* amebocyte lysate test for pyrogens. *Pharmaceutical Engineering,* **4,** 21-33.

Novitsky T.J. (1987) Bacterial endotoxins (pyrogens) in purified waters. *In:* Biological fouling of industrial water systems: a problem solving approach ed. M.W. Mittelman and G.G. Geesy, pp. 77-96. San Diego, Calif: Water Micro Associates.

O'Connor J.T., Hash L. and Edwards A.B. (1975) Deterioration of water quality in distribution systems. *J. AWWA.* **67**, 113-116.

Oda A. (1969) Ultraviolet disinfection of potable water supplies. In: *Ontario Water Resources Commission*. Paper No. 2012.

Office of Drinking water (1978) *Guidance for planning the location of water supply intakes downstream from municipal wastewater treatment facilities.* Contract No. 68-01-4473. Office of Water Supply. U.S. Environmental Protection Agency, Washington, DC.

Ogura N. (1977) High molecular weight organic matter in seawater. *Mar. Chem.* **5**, 534- 549

Olivieri V.P., Dennis W.H., Snead M.C., Richfield D.T. and Kruse C.W. (1980) Reaction of chlorine and chloramines with nucleic acids under disinfection conditions. In: *Water Chlorination: Environmental Impact and Health Effects* (Edited by Jolley, R.L, Brungs W.A, Cumming R.B. and Jacobs V.A.) **3**, 651-653. Ann Arbor Science Publishers, Inc., Ann Arbor, MI.

Olivieri V.P., Bakalian A.E., Bossung K.W. and Lowther E.D. (1985) Recurrent coiliforms in water didtribution systems in the presence of free residual chlorine. In: *Water chlorination: Chemistry, Environmental Impact and Health Effects* (Edited by Jolley, R.L., Bull R.J., Davis W.P., Katz S., Roberts M.H., Jr., and Jacobs V.A.), 651-666. Lewis Publishers, Inc., Chelsea, MI.

Olson B.H., Ridgway H.F. and Mears E.G. (1981) III Bacterial colonization of mortar-lined and galvanized-iron water distribution mains. In: *Proc. AWWA Ann. Conf.* St Louis, Missosuri. June.

Olson B.H. (1982) Assessment and implications of bacterial regrowth in water distribution systems. *EPA*. 600/52-82-072. U.S. Environmental Protection Agency.

Opheim D., Grochowski J. and Gaidish T. (1988) Monitoring the microbial content of biofilms in an artificial pipe network system. In: *Proc. AWWA. Wat. Qual. Tech. Conf.*, St Louis Missosuri. 913-929.

Paquin J.L., Block J.C., Haudidier K., Hartemann P., Colin F. and Levi Y. (1992) Effect of chlorine on the bacterial colonisation of a model distribution system. *Rev. Sci. de l'eau.* **5**, 399-414.

Paquin J.L., Queyroi F. and Hartemann P. (1994) Interférences des disinfectants sur le dénombrement des bactéries aquatiques par épifluorescence avec l'acridine et le DAPI, p.43. In: *Abstract of the "Le point sur la microbiology de l'environnement"* Meeting of the French Society of Microbiology 1994. Institut Pasteur. Paris, France.

Patterson M. K., Husted G.R., Rutkowski A. Mayett D.C. (1991) Bacteria: isolation, identification, and microscopic properties of biofilms in high-purity water distribution systems. *Ultrapure water,* **8** (4),18-24.

Pedersen K. (1982) Method for stuying microbial biofilms in flowing-water systems. *Appl. Environ. Microbiol.* **3**, 6-13.

Perkins, P.H. (1968) Concrete pipes - Development, design and construction practice. *Pipes and pipeline engineering convention.* 6 pages.

Pietersens J.W.D., Krüger M. and Willemse G.A. (1993) Treatment of eutrophic middle Vaal River water at the Western Transvaal Regional Water Company (WTRWC). *In: Proc. WISA Conf.* **3**, 285-294. Durban.

Pojasek R.B. (1977) How to protect drinking water resources. *Environ. Sc. Tech.* **11**(4), 342-347.

Porter K.G. and Feig Y.S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.25, 943-948.*

Poulton W.I.J. and Mixon M. (1992) Investigation into the degradation of mortar linings and concrete by microorganisms in industrial water systems. *Water Research Commision*

Report No 398, 39. S.A.

Power K., Cooper B., Watts H. and Nagy C.A. (1980) Aftergrowths in drinking water systems: An Australian perspective. *Wat. Sci. Tech.* **20**(11-12), 437-439.

Prendiville P.W. (1986) Ozonation at the 900-cfs Los Angeles water purification plant. *Ozone. Sci. Eng.* **8**(1), 77-93.

Pyle B.H. and McFeters G.A. (1989) Iodine sensitivity of bacteria isolated from iodine water systems. *Can. J. Microbiol.* **35**, 520-523.

Reasoner D.J. and Geldreich E.E. (1979) Significance of pigmented bacteria in water supplies. In: *Proc. AWWA. Wat. Qual. Tech. Conf.* 187-196.

Reasoner D.J. and Geldreich E.E. (1985) New medium for the enumeration and subculture of bacteria from potable water. *Appl. Environ. Microbiol.* **49**, 1-7.

Reilly J.K. and Kippen J.S. (1983) Relationship of bacterial counts with turbidity and free chlorine in two distribution systems. *J. AWWA.* **75** (6), 309.

Reimann R.H. (1992) Biocidal properties of hydrogen peroxide. In: *Alliance Peroxide Bulletins* **13.** 0, 3rd October, 2 pages.

Rice R.P., Robson C. M., Miller G.W. and Hill A.G. (1981) Use of ozone in drinking water treatment. *AWWA.* **73**(1),44-56.

Ridgway H.F. and Olson B.H. (1981) Scanning electron microscopy evidence for bacterial colonization of a drinking water distribution system. *Appl. Environ. Microbiol.* **41**(1), 974-987.

Ridgway H.F. and Olson B.H. (1982) Chlorine resistance pattern of bacteria from drinking water systems. *Appl. Environ. Microbiol.* **44**, 972-987.

Rimmer R. and Theron P.F. (1990) Experience in monitoring of groundwater at sanitary landfills. *Imiesa*, May 1990.

Rizet M., Fiessinger F. and Houel N. (1982) Bacterial regrowth in a distribution system and its relationship with the quality of the feed water: case studies. In: *Proc. AWWA. Wat. Qual. Tech. Conf.* 1199-1214. Miami Beach.

Rodriguez G.G., Philipps D., Ishiguro, K. And Ridgway H.F. (1992) Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. *Appl. Environ. Microbiol.* **58**, 1801-1808.

Rogers J., Dowsett A.B., Dennis P.J., Lee J.V. and Keevil C.W. (1994) Influence of plumbing materials on biofilm formation and growth of *Legionella pneumophila* in potable water systems. *Appl. Environ. Microbiol.* pp. 1832-1851.

Rosen H. and Klebanoff S.J. (1985) Oxidation of microbial iron-sulfur centers by the myeloperoxidase-H_2O_2-halide antimicrobial system. *Infect. Immun.* **47**, 613-618.

Rosner R. (1964) *Aeromonas Hydrophyla* as the biological agent in a case of some gastroenteritis. *Am. J. Clin. Path.* **42**, 402-404.

Rossie W.L.Jr. (1975) Control of water quality in transmission and distribution systems. *J. AWWA*, **67** (8), 425.

Ruseska I, Robbins J., Costerton J.W. and Lashen E.S. (1982) Biocide testing against corrosion causing oil-field bacteria helps control plugging. *Oil & Gas J.* **8**, 253-264.

Rusterholtz K.J. and Mallory L.M. (1994) Density, activity and diversity of bacteria indigenous to karstic aquifer. *Microb. Ecol.* **28**, 79-99.

Saby S., Sibille I.L., Mathieu L., Paquin J.L. and Block J.C. (1997) Influence of water chlorination on the counting of bacteria with DAPI (4',6-Diamidine-2-Phenylindole). *Appl. Environ. Microbiol.* **63**(4), 1564-1569.

Sakriß W., Gäb S. and Korte F. (1976) Beitrage zur ökologischen Chemie CXXVIII. Photo-oxidationsreaktionen von Metabenzthiazuran in Lösung. *Chemosphere* **5**, 339-348.

Sand W., E. Bock and D.C. White. (1984) Role of sulfur oxidizing bacteria in the degradation of concrete. Presented at: *Corrosion* 84, New Orleans, USA. Paper No 96.

Schalekamp M. (1988) One city's experience with ozone for treating lake waters. *Asian Water & Sewage* **19**, 24-26.

Schiff L.J., Morrison M. And Mayeux J.V. (1970) Synergistic false-positive coliform reaction on m-Endo MF medium. *Appl. Microbiol.* ***20*** , 778-781.

Schindler P.R.G. and Metz H. (1991) Coliform bacteria in drinking water from South Bavaria: identification by the API 20G system and resistance patterns. *Wat. Sci. Tech.* **24** (2), 81-84.

Schmalz R. (1984) Groundwater as a cornerstone of the water supply in Switzerland. *Aqua* **6**, 349- 353.

Schmidt E.L. (1974) Quantitative auto-ecological study of microorganisms in soil by immunofluorescence. *Soil Sci.* **118**,141-149.

Schraufstätter I.U., Browne K., Harris A., Hyslop P.A, Jackson J.H., Quehenberger O. and Cochrane C.G. (1990) Mechanisms of hypochlorite injury of target cells. *J. Clin. Invest.* **85**, 554-562.

Shull H.R. (1981) Experience with chloramines as primary disinfectants. *J.AWWA.* **73**(2), 101- 104.

Singer P.C., Barry J.J., Palen G.M. and Scrivner A.E. (1981) Trihalomethane formation in North Carolina drinking water. *J. AWWA.* **73**, 392-401.

Sladek K.J., Suslavich R.V., Sohn B.I. and Dawson F.W (1975) Optimum membrane structures for growth of coliform and faecal coliform organisms. *Appl. Microbiol.* **30**,685-691

Smith R.J. and Twedt R.M. (1971) Natural relationships of indicators and pathogenic bacteria in stream waters. *J. Wat. Poll. Contr. Fed.* **41**, 2200-2209.

Snoek, 1970. Enige chemische en bacteriologische aspecten van de toepassing van ozon bij de waterzuivering. *H_2O*. **3**, 108-110.

Stacha J.H. and Pontius F.W. (1983) An overview of water quality and treatment activities in the United States. *Australian Water and Wastewater Association*. Technical papers, Tenth federal convention, Sydney. p2-1 - p2-19.

Stalder K. and Klosterkötter W. (1976) Untersuchungen zur Wiederverkeimung von Trinkwasser nach Ozonbehandlung. *Zentralblatt. Bakt. Microbiol. Hyg. Series B*. **161**, 474-481.

Stanier R.Y., Adelberg E.A. and Ingraham J.L. (1976) General Microbiology. *The Macmillan Press* Ltd Publisher (4th ed.), 275-280. London and Basingstoke.

Starkey R.L. (1945) Transformation of iron by bacteria in water. *J.AWWA*, **37**(10), 963.

Stewart M.H. and Olson B.H. (1992a) Impact of growth condition on resistance of *Klebsiella pneumoniae* to chloramine. *Appl. Environ. Microbiol.* **58**, 2649-2653.

Stott J.F.D. (1986) Microbial corrosion - Hazard assessment and case histories. *H & V Engineer*. **60** No 682.

Strobel K. and Dieter H.H. (1990) Toxicological risk/benefit-aspects of drinking water chlorination and alternative disinfection procedures. *Z. Wasser-Abwasser-Forsch*. **23**, 152-162.

Suzuki M.T., Sherr E.B. and Sherr B.F. (1993) DAPI direct counting underestimates bacteria abundances and average cell size compared to AO direct counting. *Limnol.Oceanogr.* **38**, 1566-1570.

Symons J.M. (1980) Utilization of various treatment unit processes and treatment modifications for trihalomethane control. In: *Proc. Control of organic chemical contaminants in drinking water*, U.S. EPA, Washington, D.C.

Symons J.M., Tevens A.A., Clark R.M., Geldreich E.E., Love O.T., Jr. and DeMarco J. (1981) Treatment techniques for controlling trihalomethanes in drinking water. *U.S. EPA*. - 600/2-81-156, Cincinnati, OH, Esp.

Szabo A.C., Krajcarski D.T., Cavatorta P,, Masotti I. and Barcellona M.L. (1986) Excited state pKa behaviour of DAPI. A rationalisation of the fluorescence enhancement of DAPI in DAPI-nucleic acid complexes. *Photochem. Photobiol.* **44,** 143-150.

Taylor E.W. (1947) Bacteriology of water samples from water mains. *J. Appl. Bacteriol.* **10**, 34-58.

Taylor E.W. (1958) The examination of waters and water supplies. *The Blaskiston Co*. (7th ed.).

Tazawa E.I., Morinaga T. and Kawa K. (1994) Deterioration of concrete derived from metabolites of microorganisms. In*: Third International Conference on Durability of concrete*. (Edited by Malhotra V.M.), 1085-1093. Nice, France.

Tobiason J.E., Edzwald J.K., Schneider O.D., Fox M.B. and Dunn H.J. (1992) Pilot study of the effect of ozone and peroxone on in-line direct filtration. *J. AWWA*. 72-83.

Thomas E.L., Grisham M.B. and Jefferson M.M. (1986) Preparation and characterisation of chloramines. *Methods. Enzymol*. **132**, 569-585.

Toledo R.T., Escher F.E. and Ayres J.C. (1973) Sporocidal properties of hydrogen peroxide against food spoilage organisms. *Appl. microbiol.* **26**, 592-597.

Tracy H.W., Camarena V.M. and Wing F. (1966) Coliform persistence in highly chlorinated waters. *J.AWWA*. **58**, 1151-1159.

Trüeb E. (1984) Eau potable - Resources en Suisse. *Aqua* **6**, 347-348.

Tuovinen O.H., Button K.S., Tuovinen A., Carlson L., Mair D.M. and Yut A.L. (1980) Bacterial, chemical and mineralogical characteristics of tubercles in distribution pipelines. *AWWA*. **72**, 626.

U.S. Environmental Protection Agency, Office of Water Quality (1971) *Report on Missouri River water quality studies*. Regional Office, Kansas City, Missouri.

U.S. Environmental Protection Agency (1976). *National interim primary drinking water regulations*. Office of Water Supply EPA-570/9-76-003. Washington, D.C.

van Donsel D.J. and Geldreich E.E. (1971) Relationships of *Salmonellae* to fecal coliform in bottom sediments. *Wat. Res.* **5**, 1079-1087.

van der Kooij D., and Zoeteman B.C.J. (1978) Water quality in distribution systems. *IWSA 12th Congress, Kyoto, Proceedings*.

van der kooij D. (1979) Characterization and classification of fluorescent *Pseudomonas* isolated from tap water and surface water. *Antonie van Leeuwenhoek. J. Microbiol.* **45,** 225.

van der Kooij D., Visser A. and Hijnen W.A.M. (1980) Growth of *Aeromonas hydrophila* at low concentration of substrates added to tap water. *Appl. Environ. Microbiol.* **39,** 1198-1204.

van der Kooij D., Orange J.P. and Hijnen W.A.M. (1982) Growth of *Pseudomonas aeruginosa* in tap water in relation to utilization of substrates at concentrations of a few micrograms per liter. *Appl. Environ. Microbiol.* **44**, 1086-1095.

van der Kooij, D. 1992. Assimilable organic carbon as an indicator of bacterial regrowth. *J. AWWA* **84**, 57-65.

van der Wende E., Characklis, W.G. and Grochowski J. (1988) Bacterial growth in water distribution systems. *Wat. Sci. Tech.* **20**(11-12), 521-524.

van der Wende E., Characklis, W.G. and Smith, D.B. 1989. Biofilm and bacterial drinking water quality. *Wat. Res.* **23**(10), 1313-1322.

van der Wende E. and Characklis W.G. (1990) Biofilms in potable water distribution systems. In: *Drinking Water Microbiology: progress and recent developments* (Edited by McFeters G.A.), 249-268. Springer-Verlag. New York.

van der Westhuizen D. (1995) Disinfection by ultra violet irradiation. *Chem. Tech. Wat. Treat.* 5-7.

Venkobachar C., Iyengar L. and Prabhakara-Rao A.V.S. (1977) Mechanism of Disinfection: Effect of chlorine on cell membrane funtions. *Wat. Res.* **11**, 727 - 729.

Victoreen H.T. (1970) Panel discussion on bacteriological testing of potable waters. *AWWA Ann. Conf.* Washington.

von Holy A. (1987) New approches to microbiologically induced corrosion. *Wat. Sew. Effl.* **30**, 39-44.

von Sonntag C. and Schuchmann H.P. (1992) UV disinfection of drinking water and by-product formation - Some basic considerations. *J. Wat. SRT-Aqua.* **41**(2), 67-74.

Wadowsky R.M, Yee R.B., Mezmar L., Wing E.J. and Dowling N.J. (1982) Hot water systems as sources of *Legionella pneumophila* in hospital and nonhospital plumbing fixtures. *Appl.Environ. Microbiol.* **43,** 1104-1110.

Watters S.K., Pyle B.H., LeChevallier M.W. and McFeters G.A. (1989) Enumeration of *Enterobacter cloacae* after chloramine exposure. *Appl. Environ. Microbiol.* **55**(12), 3226-3228.

Weber W.J. (1972) Physicochemical process for water quality control. *John Wiley & Sons, Inc.* New York.

White G.C. (1972) *Handbook of Chlorination*. Van Nostrand Reinhold Co. New York, N.Y.

White G.S. (1992) *Handbook of chlorination*. Van Nostrand Reinhold Co. New York, NY.

Wierenga J.T. (1985) Recovery of coliforms in the presence of the free chlorine residual. *J. AWWA*. **77**(11), 83-88.

Willis A.T. (1957) Anaerobic bacillus in a treated water supply. *J. Appl. Bact*. **20**, 61.

Wilson C. (1950) Control of growth in California distribution system - Panel discussion. *J. AWWA*, **42** (9), 849.

Wium J.S and Coetzee J.(1985) Control of analyses for conventional water purification plants. *CSIR Technical Guide* K76. 1-17.

Wolfaardt G.M. and Archibald R. (1990) Microbially induced corrosion or biocorrosion in industrial water systems. *Tech. SA*. 1-7.

Yapijakis C. (1978) Rx for H_2O. A pure and simple guide to selecting drinking water disinfectants. *Wat. Wast. Eng*. **15**(5) 33-37.

Yu P. F., and McFeters G.A. (1993) Rapid *in situ* physiological assessment of bacteria in biofilm using, fluorescent probe. American Society for Microbiology. Annual Meeting pp 359. Atlanta, USA.

APPENDIX 1

STATISTICAL DATA FOR SURFACE WATER: WATER SAMPLES

1.1 Results of ANOVA for comparing heterotrophic bacteria and total bacteria for different water treatments, source waters 1 & 2, and the interaction of TRT and source waters (using the first 3 d as a co-variate).

Source of variation	DF		P	
	HPC	TB	HPC	TB
Source water 1 & 2	1	1	0.2909	0.0220
Treatment (TRT)	5	5	0.0175	0.0510
Source water 1 & 2 * TRT	5	5	0.8617	0.9044
3 days	1	1	0.0439	0.2331

HPC = heterotrophic bacteria DF = degree of freedom

TB = total bacteria P = exceedance probability

1.2 Multiple comparisons (LSMEANS) for heterotrophic bacteria between treatments based on the ANOVA results of Appendix 1.1

Treatment	LCount LSMEANS	Exceedance Probability LSMEANS					
		1	2	3	4	5	6
1. CMW	2.60	1 .	b	b	a	a	a
2. HPW	3.72	2 b	.	b	b	b	a
3. CAW	4.33	3 b	b	.	b	b	b
4. OW	4.69	4 a	b	b	.	b	b
5. UVW	4.87	5 a	b	b	b	.	b
6. RAW	6.01	6 a	a	b	b	b	b

CMW = chloraminated water HPW = water treated with hydrogen peroxide

CAW = chlorinated water UVW = UV irradiated water

OW = ozonated water RAW = raw water

1.3 Multiple comparisons (LSMEANS) for total bacteria between treatments based on the ANOVA results of Appendix 1.1

Treatment	LCount LSMeans	Exceedance probability - LSMeans					
		1	2	3	4	5	6
1 OW	7.28	1 .	b	b	b	b	a
2 CAW	7.48	2 b	.	b	b	b	a
3 UVW	7.49	3 b	b	.	b	b	a
4 CMW	7.52	4 b	b	b	.	b	a
5 HPW	7.72	5 b	b	b	b	.	b
6 RAW	8.06	6 a	a	a	a	b	.

OW = ozonated water CMW = chloraminated water

CAW = chlorinated water HPW = water treated with Hydrogen peroxide

UVW = UV irradiated water RAW = raw water

a = treatments with significant difference

b = no significant difference

LSMeans = Least Squares Means

LCount = count transformed by taking logarithms 10

1.4 Results of ANOVA for comparing heterotrophic bacteria and total bacteria for different water treatments, source waters 1 & 2, and the interaction of TRT and source waters (using 38 days (total days) as a co-variate).

Source of variation	DF		P	
	HPC	TB	HPC	TB
Source water 1 & 2	1	1	0.7368	0.0001
Treatment (TRT)	5	5	0.0005	0.0005
Source water 1 & 2 and TRT	5	5	0.8071	0.8914
Total Days (38)	1	1	0.0001	0.1180

HPC = heterotrophic bacteria DF = degree of freedom

TB = total bacteria P = exceedance probability

1.5 Multiple comparisons (LSMEANS) for heterotrophic bacteria between treatments based on the ANOVA results of Appendix 1.4.

Treatment	LCount LSMEANS	Exceedance Probability LSMEANS					
		1	2	3	4	5	6
1. CMW	5.02	1 .	b	a	a	a	a
2. HPW	5.15	2 b	.	a	a	a	a
3. OW	6.07	3 a	a	.	b	b	b
4. CAW	6.24	4 a	a	b	.	b	b
5. UVW	6.29	5 a	a	b	b	.	b
6. RAW	6.37	6 a	a	b	b	b	.

OW = ozonated water CMW = chloraminated water

CAW = chlorinated water HPW = water treated with hydrogen peroxide

UVW = UV irradiated water RAW = raw water

LSMeans = Least Squares Means Lcount = count transformed by taking logarithms 10

a = treatments with significant difference

b = no significant difference

1.6 Multiple comparisons (LSMEANS) for total bacteria between treatments based on the ANOVA results of Appendix 1.4.

Treatment	LCount LSMEANS	Exceedance Probability LSMEANS					
		1	2	3	4	5	6
1. CMW	7.59	1 .	b	b	b	b	a
2. OW	7.59	2 b	.	b	b	b	a
3. UVW	7.65	3 b	b	.	b	b	a
4. CAW	7.66	4 b	b	b	.	b	a
5. HPW	7.68	5 b	b	b	b	.	a
6. RAW	8.06	6 a	a	a	a	a	.

OW = ozonated water

CAW = chlorinated water

UVW = UV irradiated water

CMW = chloraminated water

HPW = water treated with hydrogen peroxide

RAW = raw water

LSMeans = Least Squares Means

LCount = count transformed by taking logarithms 10

a = treatments with significant difference

b = no significant difference

APPENDIX 2

STATISTICAL DATA FOR GROUNDWATER : WATER SAMPLES

2.1 Results of ANOVA for comparing heterotrophic bacteria for different water treatments, source waters 1 & 2, and the interaction of TRT and source waters (using the first 48 h as a co-variate).

Source of variation	DF	P
Source waters 1 & 2	1	0.3347
Treatment (TRT)	5	0.0116
Source waters 1 & 2 * TRT	5	0.9989
First 48 h	1	0.0036

2.2 Multiple comparisons (LSMEANS) for heterotrophic bacteria between treatments based on the ANOVA results of Appendix 2.1.

Treatment	LCount LSMEANS	Exceedance Probability LSMEANS					
		1	2	3	4	5	6
1. CMW	5.02	1 .	b	b	a	a	a
2. HPW	5.15	2 b	.	b	b	b	a
3. OW	6.07	3 b	b	.	b	b	a
4. CAW	6.24	4 a	b	b	.	b	b
5. UVW	6.29	5 a	b	b	b	.	b
6. RAW	6.37	6 a	a	a	b	b	.

OW = ozonated water CMW = chloraminated water

CAW = chlorinated water HPW = water treated with Hydrogen peroxide

UVW = UV irradiated water RAW = raw water

LSMeans = Least Squares Means LCount = count transformed by taking logarithms 10.

a = treatments with significant difference

b = no significant difference.

2.3 Results of ANOVA for comparing heterotrophic bacteria and total bacteria for different water treaments, source waters 1 & 2, and the interaction of TRT and source waters (using 35 d as a co-variate).

Source of variation	DF		P	
	HPC	TB	HPC	TB
Source waters 1&2	1	1	0.4744	0.5490
Treatment (TRT)	5	5	0.0487	0.0037
Source waters 1&2 * TRT	5	5	0.9218	0.8488
35 d	1	1	0.0001	0.0001

HPC = heterotrophic bacteria DF = degree of freedom

TB = total bacteria P = exceedance probability

2.4 Multiple comparisons (LSMEANS) for heterotrophic bacteria between treatments based on the ANOVA results of Appendix 2.3.3

Treatment	LCount LSMEANS	Exceedance Probability LSMEANS					
		1	2	3	4	5	6
1. CMW	4.50	1 .	b	b	a	a	a
2. HPW	5.08	2 b	.	b	b	b	a
3. CAW	5.32	3 b	b	.	b	b	b
4. OW	5.61	4 a	b	b	.	b	b
5. UVW	5.73	5 a	b	b	b	.	b
6. RAW	6.16	6 a	a	b	b	b	.

CMW = chloraminated water HPW = water treated with hydogen peroxide

CAW = chlorinated water UVW = UV irradiated water

OW = Ozonated water RAW = raw water

a = treatments with significant difference

b = no significant difference

LSMeans = Least Squares Means

LCount= count transformed by taking logarithms 10

2.5 Multiple comparisons (LSMEANS) for total bacteria between treatments based on the ANOVA results of Appendix 2.3.

Treatment	LCount LSMeans	Exceedance probability - LSMeans					
		1	2	3	4	5	6
1 CMW	7.37	1 .	b	b	a	a	a
2 CAW	7.41	2 b	.	b	b	a	a
3 UVW	7.52	3 b	b	.	b	b	b
4 HPW	7.54	4 a	b	b	.	b	b
5 OW	7.60	5 a	a	b	b	.	b
6 RAW	7.69	6 a	a	b	b	b	.

CMW = chloraminated water HPW = water treated with hydogen peroxide

CAW = chlorinated water UVW = UV irradiated water

OW = ozonated water RAW = raw water

a = treatments with significant difference

b = no significant difference

LSMeans = Least Squares Means

Lcount = count transformed by taking logarithms 10

APPENDIX 3

STATISTICAL DATA FOR SURFACE WATER: BIOFILMS

3.1 Results of ANOVA for comparing heterotrophic bacteria for different water treatments (TRT), coupons (stainless steel and cement) and the interaction of TRT and coupons (using the study period as a co-variate).

Source of variation	DF	P
TRT	5	0.7568
Coupons	1	0.8032
Coupons & TRT	5	0.9687
Days	1	0.0001

3. 2 Results of ANOVA for comparing total bacteria for different water treatments (TRT), coupons (stainless steel and cement) and the interaction of TRT and coupons (using the study period as a co-variate).

Source of variation	DF	P
TRT	5	0.0031
Coupons	1	0.0012
Coupons & TRT	5	0.6255
Days	1	0.0001

DF = degree of freedom P = exceedance probability

3. 3. Multiple comparisons (LSMEANS) for attached total bacteria between treatments based on the ANOVA results of Appendix 2.2

Treatment	LCount LSMEANS	Exceedance Probability LSMEANS
		1 2 3 4 5
1. CMW	5.23	6
2. CAW	5.46	1 . b b b a
3. HPW	5.47	a
4. OW	5.56	2 b . b b b
5. UVW	5.63	a
6 RAW	5.99	3 b b . b b
		a
		4 b b b . b
		a 5 a b b b .
		a
		6 a a a a a
		.

CMW = chloraminated water

CAW = chlorinated water

HPW = hydrogen peroxide treated water

a = treatment with significant difference

b = treament with no significant difference

OW = ozonated water

UVW = UV irradiated water

RAW = control (raw water)

LSMEANS = Least Square Means

LCount = count transformed by taking logarithms 10

3. 4. Multiple comparisons (LSMEANS) for attached total bacteria between coupons based on the ANOVA results of Appendix 2.2 .

Coupons	Treatments	Lcount LSMEANS
cement	chloramine	5.33
cement	hydrogen peroxide	5.65
cement	ozone	5.70
cement	UV irradiation	5.72
cement	chlorine	5.74
cement	control	6.34
stainless steel	chloramine	5.12
stainless steel	hydrogen peroxide	5.30
stainless steel	ozone	5.42
stainless steel	UV irradiation	5.53
stainless steel	chlorine	5.18
stainless steel	control	5.63

3. 5 Results of ANOVA for comparing total bacteria for different water treatments (TRT), source waters (1 and 2) and the interaction of TRT and coupons (using the study period as a co-variate).

Source of variation	DF	P
Source waters	1	0.2547
TRT	5	0.3532
Source waters & TRT	5	0.5658
Days	1	0.0001

3. 6 Results of ANOVA for comparing total bacteria for different water treatments (TRT), stainless steel coupons (removal of the DAPI on coupons) and the interaction of TRT and coupons (using the study period as a co-variate).

Source of variation	DF	P
TRT	5	0.2305
Wash	1	0.0001
Wash & TRT	5	0.1981
Days	1	0.0001

DF = degree of freedom P = exceedance probability

Wash = washed coupons (the removal of DAPI stain for epifluorescence microscopy) and non-washed coupons

3. 7 Multiple comparisons (LSMEANS) for total bacteria between treatments, washed & non-washed coupons based on the ANOVA results of appendix 2.6.

TRT	Wash	Lcount LSMEANS	Average cells.cm^{-2}
HPW	N	5.79	3.52 X 10^5
UVW	N	5.94	7.33 X 10^5
OW	N	6.15	9.24 X 10^5
CMW	N	6.22	1.10 X 10^6
CAW	N	6.23	1.65 X 10^6
RAW	N	6.29	1.10 X 10^6
CMW	Y	5.13	6.25 X 10^5
CAW	Y	5.18	3.82 X 10^5
HPW	Y	5.30	4.74 X 10^5
OW	Y	5.42	8.73 X 10^5
UVW	Y	5.53	8.57 X 10^5
RAW	Y	5.63	9.74 X 10^5

CMW = chloraminated water
CAW = chlorinated water
HPW = hydrogen peroxide treated water
OW = ozonated water
UVW = UV irradiated water
RAW = raw water
a = treatment with significant difference
b = treatment with no significant difference

Y = washed coupons

N = non-washed coupons

LSMEANS = Least Square Means

LCount = count transformed by taking logarithms 10

APPENDIX 4

STATISTICAL DATA FOR GROUNDWATER: BIOFILMS

4.1 Results of ANOVA for comparing heterotrophic bacteria attached to the stainless steel and cement coupons, for different water treatments (TRT), using the study period as a co-variate.

SOURCE WATER 1				
Source of variation	DF Stain.steel	DF Cement	P Stain.steel	P Cement
Treatments	5	5	0.7880	0.8589
35 days	1	1	0.0002	0.0001
SOURCE WATER 2				
Source of variation	DF Stain.steel	DF Cement	P Stain.steel	P Cement
Treatments	5	5	0.9271	0.9751
35 days	1	1	0.0003	0.0001

4. 2 Results of ANOVA for comparing heterotrophic bacteria attached to the stainless steel and cement coupons, for different water treatments (TRT), source waters (1 and 2) and the interaction of TRT and source waters, using the study period as a co-variate.

Source of variation	Degree of freedom (DF)		Exceedance probability (P)	
	Stain.steel	Cement	Stain.steel	Cement
Source waters	1	1	0.7059	0.7076
Treatments (TRT)	5	5	0.7762	0.9421
Source waters & TRT	5	5	0.9233	0.9026
35 days	1	1	0.0001	0.0001

4.3 Results of ANOVA for comparing attached heterotrophic bacteria for different water treatments (TRT), coupons (stainless steel and cement) and the interaction of TRT and coupons, using the study period as a co-variate.

Source of variation	Degree of freedom (DF)		Exceedance probability (P)	
	S. water 1	S. water 2	S.water 1	S.water 2
coupons	1	1	0.0018	0.0061
Treatments (TRT)	5	5	0.6922	0.9671
Coupons & TRT	5	5	0.9632	0.9392
35 days	1	1	0.0001	0.0001

4.4 Multiple comparisons (LSMEANS) for attached heterotrophic bacteria between coupons based on the ANOVA results of Appendix 4.3 - Source water 1.

Coupons	Treatments	LCount LSMEANS
cement	hydrogen peroxide	6.19
cement	chloramine	6.58
cement	ozone	6.76
cement	UV irradiation	6.79
cement	control (raw water)	6.82
cement	chlorine	6.84
stainless steel	hydrogen peroxide	5.77
stainless steel	chloramine	6.14
stainless steel	ozone	6.12
stainless steel	UV irradiation	6.10
stainless steel	control (raw water)	6.18
stainless steel	chlorine	5.82

LSMEANS = Least Square Means

LCount = count transformed by taking logarithms 10

4.5 Multiple comparisons (LSMEANS) for attached heterotrophic bacteria between coupons based on the ANOVA results of Appendix 4.3 - Source water 2.

Coupons	Treatments	Lcount LSMEANS
cement	control	6.37
cement	hydrogen peroxide	6.50
cement	chlorine	6.52
cement	UV irradiation	6.57
cement	ozone	6.62
cement	chloramine	7.01
stainless steel	control	6.04
stainless steel	hydrogen peroxide	5.97
stainless steel	chlorine	5.88
stainless steel	UV irradiation	6.19
stainless steel	ozone	5.85
stainless steel	chloramine	5.96

4.6. Results of ANOVA for comparing total bacteria attached to the stainless steel and cement coupons, for different water treatments (TRT), using the study period as a co-variate.

SOURCE WATER 1				
Source of variation	DF Stain.Steel	DF Cement	P Stain.Steel	P Cement
Treatments	5	5	0.6522	0.9104
35 days	1	1	0.0001	0.0001
SOURCE WATER 2				
Source of variation	DF Stain.steel	DF Cement	P Stain.steel	P Cement
Treatments	5	5	0.5722	0.8493
35 days	1	1	0.0001	0.0001

4.7 Results of ANOVA for comparing toatal bacteria attached to the stainless steel and cement coupons, for different water treatments (TRT), source waters (1 and 2) and the interaction of TRT and source waters, using the study period as a co-variate.

Source of variation	Degree of freedom (DF)		Exceedance probability (P)	
	Stain.steel	Cement		
Source waters	1	1	0.1242	0.2903
Treatments (TRT)	5	5	0.2507	0.9221
Source waters & TRT	5	5	0.9927	0.7120
35 days	1	1	0.0001	0.0001

4.8 Results of ANOVA for comparing attached total bacteria for different water treatments

(TRT), coupons (stainless steel and cement) and the interaction of TRT and coupons, using the study period as a co-variate.

Source of variation	Degree of freedom (DF)		Exceedance probability (P)	
	S. water 1	S. water 2	S.water 1	S.water 2
coupons	1	1	0.2282	0.0001
Treatments (TRT)	5	5	0.6600	0.7044
Coupons & TRT	5	5	0.9889	0.4794
35 days	1	1	0.0001	0.0001

4.9. Multiple comparisons (LSMEANS) for attached total bacteria between coupons based on the ANOVA results of Appendix 4.8 - Source water 2.

Coupons	Treatments	Lcount LSMEANS
cement	control	7.86
cement	hydrogen peroxide	7.93
cement	chloramine	7.98
cement	chlorine	7.98
cement	UV irradiation	8.05
cement	ozone	8.08
stainless steel	control	7.84
stainless steel	hydrogen peroxide	7.76
stainless steel	chloramine	7.74
stainless steel	chlorine	7.64
stainless steel	UV irradiation	7.78
stainless steel	ozone	7.78

LSMEANS = Least Square Means

LCount = count transformed by taking logarithms 10