INHIBITION OF BIOFILM REGROWTH IN POTABLE WATER SYSTEMS

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Water Research Commission

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Executive Summary

1. BACKGROUND TO THE STUDY AND PROBLEM STATEMENT

In South Africa, as elsewhere, the degradation of the bacteriological quality of water in distribution systems is currently one of the main problems facing suppliers of potable water. The major water quality objectives in water supply consist of the removal of microorganisms which cause waterborne diseases and the prevention of re-contamination of drinking water by these organisms during distribution.

The need to provide adequate water supplies to communities is a major objective of the South Africa Government's Reconstruction and Development Programme. To ensure bacteriologically safe drinking water, drinking water producers try to avoid bacterial growth in a drinking water distribution system. This could be possible by identifying appropriate disinfection processes and pipe materials used for the distribution of potable water.

Bacterial growth in a drinking water distribution system mainly occurs at internal surface of the pipes. Attention has been drawn to various aspects of biofilm formation in potable water systems. Studies have shown that regrowth or biofilm formation in potable water systems is linked to the presence of nutrients (van der Wende and Characklis, 1990), and that bacteria embedded in matrices in biofilms are protected from disinfectants (Fletcher and Marchall, 1982, LeChevallier *et al.*, 1988). Detachment of bacteria from this biofilm may thus affect the water quality. Investigators have shown that bacterial growth occurring at pipe walls depends on different factors: concentrations of disinfectant, water temperature and concentration of BDOC which is the main substrate allowing bacterial growth in the drinking water (Block *et al.*, 1993, LeChevallier *et al.*, 1993; Servais *et al.*, 1995; Servais, 1996). Another factor which has received less attention up to now is the effect of pipe composition on the densities of bacteria fixed on the pipe walls. The characteristics of the material composing pipes may greatly influence the densities of bacteria fixed in a distribution system. It is

well known that the roughness of the material using for the distribution of potable water contribute to bacterial attachment (Pedersen, 1990; Percival *et al.*, 1998). Momba *et al.* (1998) reported a higher yield in viable bacteria on stainless steel coupons than on cement coupons during the first 8 h of their experimental study. Van der Kooij *et al* (1995) also observed that the materials in contact with drinking water could release biodegradable compounds into drinking water, thus enhancing biofilm formation.

A study performed by Momba *et al.* (1998, 1999, Momba 1997) showed that even in the presence of high concentrations of residual disinfectants (13.20 -16.5 mg.ℓ^{-1} hydrogen peroxide, 1-1.5 mg.ℓ^{-1} monochloramine, 0.2-0.5 mg..ℓ^{-1} chlorine) biofilm formation was obvious on stainless steel and cement coupons within the first day of the exposure. However, in laboratory-scale units with a residual disinfectant, biofilm regrowth was limited. Monochloramine and hydrogen peroxide were found to be much more effective in controlling the regrowth of biofilm in laboratory-scale units compared to other disinfectants (ozone, UV, chlorine). In addition, this investigation revealed that ozone was highly effective within the first 2 h as shown by the average kill percentage of 99.999% of heterotrophic bacteria. Nevertheless, the decay of residual ozone occurred earlier than with other disinfectants (chlorine, monochloramine and hydrogen peroxide). This consequently led to faster bacterial regrowth in potable water systems. The water provided by such systems can fail to meet drinking water standards, thus affecting people's heath when used in food production or by direct consumption or through a lack of good hygiene and sanitation when handling water.

To limit the accumulation of bacteria and the formation of biofilm in potable water distribution systems, two parameters must be taken into account: disinfection efficiency and the presence of residual disinfectant in water systems. Until now, most investigation have been concentrated on the efficiency of each disinfectant and its presence as a residual in potable water distribution systems. The need therefore exists to study the combination of two disinfectants: one as a primary disinfectant (because of its immediate efficacy) and the other as a secondary disinfectant which can play the role of residual to prevent and control bacterial or biofilm regrowth in potable water distribution systems.

The initial research proposal stipulated the use of ozone as the primary disinfectant followed by hydrogen peroxide or monochloramine to provide effective treatment which could inhibit bacterial or biofilm regrowth in drinking water distribution systems for as long as residual hydrogen or monochloramine persists. Because of its worldwide use, chlorine could be used as a primary disinfectant, followed by monochloramine or hydrogen peroxide.

The revised research programme aimed at investigating the inhibition of bacterial and biofilm regrowth only in a chlorinated water distribution system. To have a comparative study which allows for an evaluation of the processes, groundwater and surface water were proposed as test water sources while stainless steel and galvanised mild steel were used as pipe materials for the study of biofilm. Moreover, the study with groundwater was found to be especially important for South Africa as most rural communities use water from wells or boreholes (Momba and Notshe, 2000; Momba and Mnqumevu, 2000). Therefore the quality of this water needs to be determined before it is supplied to the communities.

In 1999, the first results on surface water showed that a combined chlorine-monochloramine process provides an effective treatment for the inhibition of planktonic and attached bacteria as long as a concentration of *ca* 0.35 $mg.\ell^{-1}$ monochloramine persists in drinking surface water systems. On the basis of these first results, it has been recommended by the steering committee members to perform a further study which aimed to compare the effect of various pipes materials (used in South Africa, especially in rural communities) on biofilm formation in a combined chlorine-monochloramine potable water system.

To identify the type of pipe materials used in South Africa for the distribution of potable water, the addresses of Water Treatment Authorities (Urban and rural treatment plants) were provided by WRC. A questionnaire was set and sent to 500 Water Treatment Authorities. Data collected from this questionnaire revealed 11 types of pipe materials used in the following percentage: polyvinyl chloride - PVC (25%), asbestos cement -AC (21%), asbestos (19%), unplasticised polyvinyl chloride - uPVC (16%), steels (8%), cement-C (4%), bitumen coated (3%), medium density

polyethylene - MDPE (2%), copper (1%), galvanised mild steel (1%), mortar lined steel (1%), cast iron (1%). Data from the questionnaire also revealed that 84.2% of Water Treatment Authorities use only chlorine (especially chlorine gas) as the main disinfectant and the exact dose used for the disinfection of water remain unknown (65%). This mostly concerns rural Water Treatment Plants. Moreover, the problem of bacterial regrowth and biofilm formation is well known only in urban Water Treatment Plants (15.8%) whereas 84.2 % of the Water Treatment Authorities in rural communities do not understand or ignore it due to the fact that no monitoring of the microbiological quality of water is performed in the Water Treatment Plants. Therefore, there was a need to conduct such a project in order to improve the quality of drinking water, especially in rural communities of South Africa and to train more people, especially black communities who will be able to solve the problem of water quality monitoring .

During the study period, the effect of PVC, uPVC , MDPE, asbestos cement, and cement on biofilm formation was identified and a comparative study was performed using statistical analysis. The DOC concentration for each laboratory-scale water system was determined. From the findings of this study, Water Authorities will be able to establish a regulation that will help drinking water producers in South Africa to choose pipe materials which minimize the possibility of biofilm development in an effort to improve the quality of distributed potable water.

2. PROJECT OBJECTIVES

2.1 Inhibition of bacterial and biofilm regrowth in a chlorinated potable water system - Project No K5/1023/1999 -2000.

The initial research programme aimed to investigate the inhibition of bacterial regrowth and biofilm regrowth in ozonated and chlorinated water distribution systems. To achieve this goal, the following objectives are to be pursued:

1. To use ozone or chlorine as the primary disinfectant followed by monochloramine or hydrogen peroxide as secondary disinfectant in assessing their ability to prevent the deterioration of the microbial quality of ozonated and chlorinated water in distribution systems as long as residual monochloramine or residual hydrogen peroxide persists.

2. To evaluate the influence of chloramination and hydrogen peroxide disinfection on the inhibition of biofilm regrowth in ozonated water systems as well as in chlorinated water systems using a laboratory-scale system.

The revised research programme aimed at investigating the inhibition of bacterial and biofilm regrowth only in a chlorinated water distribution system. No ozone treatment nor hydrogen peroxide post-treatment was performed during the study period. To achieve this goal the following objectives were pursued:

1. To use chlorine as a primary disinfectant followed by monochloramine as a second disinfectant in order to assess their ability to prevent the deterioration of the microbial quality of chlorinated water in distribution system by ensuring that a residual monochloramine persists.

    i. At the first level of extension, the influence of the chloramination process on the inhibition of **bacterial regrowth** (coliform and heterotrophic bacteria) in chlorinated water distribution systems using a laboratory-scale unit must be investigated.

    ii. At the second level of extension, the influence of chloramination on the inhibition of **biofilm. regrowth** in chlorinated water distribution systems using a laboratory-scale unit must be evaluated.

2.2 Comparing the effect of various pipe materials on biofilm formation in a combining chlorine-chloramine potable water system - Project No K8/418/2001

This research programme aimed to :

1. Compare the density of fixed bacteria colonising different pipe materials in a chlorine-chloramine distribution systems.

2. Compare the concentration of dissolved organic carbon (DOC) released by different pipe materials in a chlorine-chloramine distribution systems.

3. Improve the knowledge on the impact of pipe materials on densities of fixed bacteria and help drinking water producers in South Africa to choose pipe materials which minimize the possibility of biofilm development in an effort to improve the quality of distributed potable water.

3 SUMMARY OF MAJOR FINDING AND CONCLUSIONS REACHED

3.1 Project-K5/1023/1999-2000

The ultimate aim of this study was to evaluate a combined chlorine-monochloramine disinfection process for the inhibition of bacterial and biofilm regrowth in a laboratory-scale system. The emphasis was on the maintenance of an effective disinfectant residual throughout the system. To have a comparative study which allowed the evaluation of the process, surface water and groundwater were used as the intake water sources; stainless steel and galvanized mild steel were used for the study of biofilm regrowth. Disinfection was carried out using *ca* 2.5 $mg.\ell^{-1}$ chlorine followed by *ca* 1.5 $mg.\ell^{-1}$ monochloramine. The monitoring of the microbiological quality of treated water or bactericidal effectiveness of the process relied on coliforms, heterotrophic plate count and total bacteria.

Whereas coliforms (total coliforms, *E. coli*, and *Salmonella*) were still visible in chlorinated surface water and groundwater systems 24 h after chlorination, no coliforms were observed 20 min and 24 h after the addition of monochloramine in the combined water system. The effectiveness of a combined chlorine-monochloramine process was also confirmed statistically when using F-ratio and Least Square of Differences tests. This leads to conclude that a combined chlorine-monochloramine process provides an effective treatment which results in the early complete elimination of coliform bacteria in drinking water systems.

The present study confirms the instability of chlorine which led to bacterial regrowth in chlorinated surface and ground water systems between 24 and 504 h while a decrease in bacterial numbers (from 4 log $cfu.ml^{-1}$ to 1 $cfu.ml^{-1}$ or <1 $cfu.cm^{-2}$) was detected in combined chlorine-monochloramine water systems. A combined chlorine-monochloramine process resulted in a longer residual monochloramine that could be detected up to 168 and 678 h d in groundwater and surface water system respectively. Less than 1 bacterial count (viable or total cells) was detected in both combined water systems and on surfaces of both types of piping material exposed to the combined chlorine-monochloramine water systems. The findings revealed that a combination of chlorine and monochloramine provides an effective treatment for the inhibition of bacterial and biofilm regrowth in drinking water as long as the residual monochloramine concentration persists above *ca* 0.35 $mg.\ell^{-1}$ for surface and *ca* 0.2 $mg.\ell^{-1}$ for groundwater.

During the experimental study, the potential for bacterial regrowth was observed between 14 d and 28 d, and between 678 and 720 h in chlorine-monochloramine groundwater system and chlorine-monochloramine surface water systems, respectively. This was mainly due to the presence of a very low concentration of monochloramine residual in treated surface water (0.15 $mg.\ell^{-1}$) and the absence of disinfectant monochloramine residual in ground water . In the absence of the residual monochloramine, the bacterial counts increased up to the maximum counts of 4 $log.cfu.m\ell^{-1}$ and 5 $log.cfu.m\ell^{-1}$ HPC in combined treated surface water and groundwater systems respectively. On the surfaces of stainless steel and galvanised mild steel, bacterial counts reached the maximum counts of 5 $log.cfu.cm^{-2}$ for attached viable bacteria and 6 $log.cells.cm^{-2}$ for total cells.

Although a longer effective treatment was found for the surface water than for the groundwater, no significant difference in bacterial regrowth counts was detected in both types of water source. Neither was any significant difference in bacterial counts noted within stainless steel and galvanised mild steel.

To improve the quality of potable water in water distribution systems and to make significant progress toward compliance with bacteriological quality standard at the point of consumption, it is recommended that chlorine be used as primary disinfectant followed by monochloramine as secondary disinfectant. This study also suggests that water suppliers, especially in rural communities, combine chlorine and monochloramine processes in order to comply with bacteriological standards at the point of consumption as well as in the reservoirs or in household containers during the storage of potable water. It is therefore important to take appropriate actions to correct water quality problems where the health of consumers is at risk.

To be cost effective and sustainable, a suitable education and information programme on a sustained effective disinfection should be urgently implemented in small municipal water treatment plants. The need, therefore, exist to develop capacity amongst Local Water Authorities. This will lead to an understanding of the complexities of water quality and its assessment in rural communities.

3.2 Project K8/418/2001

To have a comparative study which allowed the identification of the effect of various pipe materials on biofilm formation, surface water was used as the intake water sources, plastic-based pipe materials (PVC, UPVC, MDPE) and cement-based pipe materials (cement and asbestos cement) were used as test pipe materials. Disinfection was performed using *ca* 2.5 $mg.\ell^{-1}$ initial chlorine followed by *ca* 1.5 $mg.\ell^{-1}$ monochloramine. The evaluation of the process relied on attached coliforms and heterotrophic plate count bacteria. Scanning electron microscopy (SEM) was used to confirm the presence/absence of biofilm on the surface of pipe materials. From the present study, two important relations were drawn: firstly between the formation of biofilm

and the type of disinfection process, secondly between the density of fixed cells and the generic type of pipe materials.

General data indicated the colonization of all test pipe materials by microorganisms under chlorination process within the first 20 min and over the remainder of the study period. The addition of monochloramine in the chlorinated water system (24 h after chlorination) resulted in the removal of coliforms and heterotrophic bacteria attached to pipe materials. Less than 1 cfu.cm^{-2} viable bacteria (excepted for PVC) was observed on the surface of test pipes between 48 and 168 h. However, the factor time cannot be ignored in determining the effect of pipe materials on biofilm formation in potable water systems. Bacterial regrowth occurred on the surface of all pipe materials between 168 and 672 h. The capability of bacterial regrowth occurring of the surface of test materials during this period was linked to the depletion of the concentration of monochloramine residual. Because the disinfectant decays as water flows through the pipes, chlorinated water may again receive an additional monochloramine disinfection at some critical location every 168 h.

Statistical evidence showed that the generic type of pipe materials did greatly influence the density of bacteria in laboratory-scale systems. Cement-based materials (total means after 672 h: coliform bacteria-5 cfu.cm^{-2}, heterotrophic plate count-2 log cfu.cm^{-2}) significantly (at $p<0.05$ and $p<0.01$) supported less fixed bacteria than plastic-based materials (total means after 672 h: coliforms-8 cfu.cm^{-2} and HPC-3 log.cfu.cm^{-2}). No significant difference in attached bacterial counts was found between the same generic type of pipe materials. This study recommends the use of cement and asbestos cement pipes for the distribution of chlorine-monochloramine treated water.

Statistical evidences also showed that all system parameters (temperature, pH, turbidity, dissolved organic carbon, total nitrogen, sulphate) had no significant effect on bacterial number at $p < 0.05$, implying that the presence of an effective monochloramine residual in the chlorinated water system remains one of the most important factors in controlling the effect of pipe materials on biofilm formation.

4. RECOMMENDATION FOR FUTURE RESEARCH

A future scientific work has to be conducted on the strategies ensuring a sustainable effective disinfection in small municipal water distribution systems. To achieve this goal, a case-study must be conducted in Alice Water Treatment Plant (Eastern Cape Province) and the following objectives must be pursued:

1. Assess the operational constraints of the existing disinfection system in Alice Water Treatment Plant.
2. Assess the occurrence of biofilm formation on the surface of pipe materials which can lead to the deterioration of potable water quality in the Alice distribution system.
3. Assess the level of biodegradable dissolved organic carbon in the Alice water distribution system which can be the cause of bacterial regrowth.
4. Assess the extent to which disinfection of Alice water treatment plant is practised and evaluate the cost of water treatment in small municipal water treatment plants.
5. Apply a sustained effective disinfection in Alice treatment plant for the removal of attached bacteria on the surface of pipe materials and for the inhibition of biofilm formation/bacterial regrowth in the water distribution system.
6. Ensure the maintenance of an effective residual disinfectant throughout the water distribution system (from the treatment point to point of consumption).

As previously stated, the factor time cannot be ignored in determining the microbiological quality of potable water systems. This exposure time always depends on the distance between the treatment point and the end points. It would be therefore interesting in the future works to determine some critical locations between the treatment point and the end points and to quantify the effect of the distance on biofilm regrowth after the addition of monochloramine in the chlorinated water system.

5 LIST OF PRODUCTS

5.1 Degree

BSc Honours Microbiology- S Nadaliso
BSc Honours Microbiology- MA Binda
BSc Honours Microbiology-N Makala

5.2 Chapter in Books

MOMBA MNB (2000) Influence of chloramination on bacterial regrowth in a chlorinated surface water laboratory-scale system. *In: Research Advances In Water Research.* (Ed, Global Net Work. Kerala, India. **1,** 51-62.

5.2 Articles

MOMBA MNB and BINDA MA (2001) Combining chlorination and monochloramination processes for the inhibition of biofilm formation in drinking water systems. *J. Appl. Microbiol.***91,** 1-8.

5.3 Papers in Preparation for publication

MOMBA MNB, NDALISO S and BINDA MA (2002) The effect of a combined chlorine -monochloramine process for the inhibition of bacterial and biofilm regrowth in surface water and groundwater systems: A comparative study. To be submitted for publication in the SA J. Sci.

MOMBA MNB and MAKALA N (2002) Comparing the effect of various pipe materials on biofilm formation in a combined chlorine-chloramine potable water laboratory-scale system. To be submitted for publication in the *J. Water SA.*

5.3 Conference Presentation

MOMBA MNB and TYALI T (2000) Use of monochloramine as secondary disinfectant for the inhibition of bacterial regrowth and accumulation of indicator micro-organisms in a laboratory-scale system model receiving chlorinated water. Poster Presentation at *1st World Congress of the International Water Association (IWA); Paris/France 3-7 July 2000.*

MOMBA MNB and NDALISO S (2001) The disinfection efficacy of a combined chlorine-monochloramine process against bacterial regrowth in a groundwater laboratory-scale systems. Poster presentation at the *IWA 2nd World Congress*. Berlin/Germany 15-19 October 2001.

MOMBA MNB , NDALISO S and binda ma (2002) The effect of a combined chlorine - monochloramine process for the inhibition of bacterial and biofilm regrowth in surface water and groundwater systems. Poster presentation at the *IWA 3rd World Congress*,Melbourne/Australia 7-12 April 2002.

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LIST OF ABBREVIATION

A:	Alice chlorinated water
AC:	Asbestos cement pipe
AF:	Alice water after filtration -before chlorination
AFC_1-AFC_2:	Chlorinated surface water
$AFCM_2$:	Chlori-monochloramine treated water
AOC:	Assimilable organic carbon
ATC:	Attached total cells
AVB:	Attached viable bacteria
BDOC:	Biodegradable dissolved organic carbon
BRC:	Batch reactor with cement -based pipe materials
BRP:	Batch reactor with plastic - based pipe materials
C:	Cement pipe
CB:	Coliform bacteria
DOC:	Dissolved organic carbon
EC:	*Escherichia coli*
GW:	Ground water
GWC1-GWC2:	Chlorinated groundwater
GWC2M:	Chlorine-monochloramine treated water
HPC:	Heterotrophic plate count bacteria
IJ:	Injured bacteria
LSD:	Least Square of Differences
MDPE:	Medium density polyethylene
MS:	Galvanised mild steel
P:	Pipe
PVC:	Polyvinyl chloride
Sal:	*Salmonella*
SEM:	Scanning electron microscopy

SS:	Stainless steel
T:	Time
TC:	Total coliform
TOC:	Total organic carbon
TRT:	Treatment
uPVC:	unplasticised polyvinyl chloride

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CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 GENERAL INTRODUCTION

In South Africa, as elsewhere, the degradation of the bacteriological quality of water in distribution systems is currently one of the main problems facing suppliers of potable water. The major water quality objectives in water supply consist of the removal of microorganisms which cause waterborne diseases and the prevention of re-contamination of drinking water by these organisms during distribution.

The need to provide adequate water supplies to communities is a major objective of the South Africa Government's Reconstruction and Development Programme. To ensure bacteriologically safe drinking water, drinking water producers try to avoid bacterial growth in a drinking water distribution system. This could be possible by identifying appropriate disinfection processes and pipe materials used for the distribution of potable water.

It is well known that any surface in contact with water can be colonized by microorganisms resulting in the formation of biofilms. Microbial growth in biofilms is enhanced over suspended growth due to the flux of substrates to the cells (kjelleberg and Hermansson, 1984). The formation and composition of biofilms is dependent on environmental conditions such as water quality, temperature, hydraulic of the system, age of water in the system (Pirou *et al.*, 1997, Momba *et al.*, 1998) as well the type of substratum present for colonization (Cloete *et al.*, 1992). Nutrition availability on surfaces may be higher due to surface-associated organic materials (Davies and McFeters, 1998; LeChevallier and McFeter, 1990). The aqueous environment and the substratum then influence the selection of microorganisms that will predominate in the forming biofilms

(Lutterbach and de Franca, 1996). If nutrient concentrations are sufficiently low, starvation of suspended cells may occur (Marita, 1998). However, these environments may favour the process of bacterial biofilm formation (Mueller, 1996).

It has been estimated that 99% of all bacteria in natural environments exists in biofilms or at least are attached to surfaces (Costerton *et al.*, 1987). In water distribution systems, the occurrence of biofilms is associated with technical and hygienic problems (Armon *et al.*, 1997). Awareness has grown that potable water leaving the treatment plant may undergo substantial changes in quality during transport through the distribution system to the consumers due to biofilm formation on the pipes (Donlan and Pipes, 1988; LeChevallier *et al.*, 1991). It has been reported that microorganisms growing in biofilms can cause taste and odour (Astier *et al.*, 1995) or are pathogenic. A large variety of pathogenic bacteria isolated from biofilms both in chlorinated and non-disinfected water distribution systems includes the survival of *Legionella pneumophila, Salmonella typhimurium* (Armon *et al,* 1997) and *Campylobacter* ssp (Buswell, *et al.*, 1998).

Studies have shown that biofilm system can also enhance cell survival with respect to the biocide or other chemical stressors (van der Wende *et al.*, 1988; LeChevallier and McFeters, 1990; Chen *et al.*, 1992). An attached form of growth is beneficial to the organisms, especially under low nutrient conditions (Muller, 1996). Although chlorination is a standard method of disinfection in South Africa, the evidence that this process is ineffective against biofilm formation is growing (LeChevallier *et al.*, 1990, Momba *et al.*, 1998, 1999). Experimental studies have shown that biofilms attach to the inner surface of the distribution system even in the presence of free residual chlorine concentration higher than 2.5 $mg.\ell^{-1}$ (LeChevallier *et al,* 1990; de Beer *et al.*, 1994; Momba *et al.*, 1998, 1999). However this concentration is highly recommended by the South African Water Industry for the disinfection of potable water. On one hand, the ineffectiveness of chlorine in inhibiting bacterial biofilm formation is due to the neutralisation of chlorine by biofilms, the inability of chlorine to penetrate the biofilm matrix, and the insensitivity of biofilm members that exhibit a lower growth rate (LeChevallier *et al.*, 1990; de Beer *et al.*, 1994). On the other hand, the stability of residual monochloramine and its efficiency in limiting biofilm formation in water distribution system is a fact greed upon by a number of researchers (LeChevallier *et al.*, 1990; Mathieu *et al.*, 1993, Yu *et al.*, 1993, Momba *et al.*, 1998). Yu and coworkers in 1993 reported that

although monochloramine was a weaker disinfectant, it was more effective in removing attached bacteria from the substratum compared to free chlorine. Momba and coworkers (1999) reported that residual chlorine lasted for 24 h while residual monochloramine persisted up to 48 h. These authors also observed the attachement of *E. coli* in a biofilm formation occurring in the non-disinfected and chlorinated water distribution systems while no *E. coli* were detected on the SS slides exposed to the monochloramine treated water for the whole study period. As a secondary disinfectant monochloramine could be relied upon to achieve the task of inhibiting biofilm formation and bacterial regrowth in distribution systems, thus improving the quality of potable water from the point of treatment to the end consumer.

The control of biofilms within the distribution system involve an integrated approach using a combination of several types of action, for example reducing the amount of organic material in the water (van der Kooij *et al.*, 1995), changing the piping materials to those which have a reduced biofilm forming potential or changing the disinfection regime (LeChevallier *et al.*, 1990).

This study main purpose of the present study was to investigate the inhibition of bacterial regrowth and biofilm formation in potable water distribution systems. To achieve this goal three parameters must be taken into account: disinfection efficiency, the presence of residual disinfectant in water systems and the type of pipe materials. Until now, most investigation have been concentrated on the efficiency of each disinfectant and its presence as a residual in potable water distribution systems. The need therefore exists to study the combination of two disinfectants: one as a primary disinfectant (because of its immediate efficacy) and the other as a secondary disinfectant which can play the role of residual to prevent and control bacterial or biofilm regrowth in potable water distribution systems.

1.2 LITERATURE REVIEW

It is well known that without water and a safe environment, cultural and economic development is impossible. Therefore, it is important to improve levels of human health by meeting the basic environmental health need such clean and safe drinking water and effective sanitation.

Historically potable water has been defined as water that may be consumed in any desired amount without concern for adverse effects on health. Potable water should not contain pathogenic microorganisms which may be harmful to human health. To achieve this goal, disinfection occupies a key position as it is indispensable for the prevention or reduction of waterborne infectious diseases.

The deterioration of the bacteriological quality of water in the distribution system is currently one of the main problems facing potable water suppliers. It is an established fact that the origin (source) of intake, the type of disinfectants, the piping material and the retention periods of water in distribution systems can lead to pronounced changes in microbial quality of potable water distribution systems. This chapter deals with the influence of the above factors on bacterial regrowth or biofilm formation in potable water systems.

1.2.1 Sources of potable water

Drinking water, which is essential for life, social development and economic growth, has become scarce in recent years because of the uneven growth of population and industrial water demands. Because of this tremendous increase in the consumption of water for various purposes, in South Africa water is regulated in terms of the Water Act of 1996 when it became necessary to apportion water more fairly between agriculture, industry, metropolitan, urban and rural users (Bell, 1997).

The raw water sources can be grouped as surface water, groundwater, spring water and rainwater harvesting. Selection of raw water source as drinking water involve a number of factors: 1) adequate quality throughout the year, 2) water quality that is amenable to treatment and 3) some protection of the watershed from domestic, industrial and agricultural pollution (Office of Drinking Water, 1978). This study mainly deals with surface water and groundwater.

1.2.1.1 Surface Water

Surface water can be divided into rivers, streams, lakes, small dams and ponds. In South Africa the flow of river and streams varies widely with seasons and this affects the quality of water. During wet seasons water may have a low dissolved-solid content but high turbidity and in dry seasons the

water flow is low because the dissolved-solids are more concentrated (Heap, 1985). Surface water is often bacteriologically unsafe, contains a high sediment load and requires more complicated treatment than groundwater (Sami and Murray, 1998). Surface water is unsafe because its quality is subjected to frequent and dramatic changes in microbial quality as a result of variety of activities on a watershed. These changes are caused by discharges of municipal raw water or treated effluent at specified point source locations into the receiving water (rivers, lakes) or by storm-water runoff into the drainage basin at non-point locations all over the watershed. The quality is also influenced by occasional re-circulation of organisms in bottom sediments. An approximative 100 to 1000-fold increase in fecal coliform bacteria has been reported in bottom sediments compared to the overlying water (van Donsel and Geldrich, 1971). Prior to consumption, surface water must either be protected or must undergo adequate treatment.

1.2.1.4 Groundwater

Groundwater is the main source of potable water in rural areas. It consists of seepage ditches, infiltration drains, wells and boreholes. Seepage ditches and infiltration drains are known as galleries because they are used to withdraw groundwater at the surface. These sources are usually preferred because their yield and quality are stable. They require the least treatment. They are easily protected and can be located close to the community. Seepage ditches are easy to construct, have a large capacity and a long useful life. However water collected in them is susceptible to contamination which makes them less suitable. Infiltration drains have pores, perforation or open joints which allow the groundwater to enter (Heap, 1985). The wells are suitable when near surface aquifers are found in weathered material and where the population is widely dispersed. The depth of the well depends on the type of the soil and fluctuations of ground water table. Most wells need an inner lining, which provides protection against caving and helps to prevent polluted water from seeping in from the surface (Heap, 1985). Boreholes can be drilled into consolidated or unconsolidated, confined or unconfined aquifers. They are suitable for low volume water supplies.

In South Africa it is estimated that 13% of the total water consumption in 1980 was from groundwater sources of which approximately 10% were used for metropolitan as well as for household consumption in urban areas (Bell, 1997). In 1993 groundwater contributed about 15% to SA's water supply and it is of great importance when one considers that more than 280 towns

and villages use groundwater (Engelbrecht, 1993). SA agricultural sector has been reported to be the biggest user of groundwater and it has been estimated that 78% of all groundwater is used for irrigation, 7% for rural domestic purposes and 6% for stock watering (Conrad *et al.*, 1999).

For many decades groundwater has been considered to be of unquestionable excellence because the soil barrier is often effective in providing the isolation of this high quality source water from surfaces pollutants (Geldreich, 1990). The natural quality of groundwater in the weathered aquifers remained excellent because of its short residence time within this zone and also because leachable minerals have been removed through years of leaching (Conrad *et al.*, 1999). In recent years, however, there has been a growing suspicion that groundwater quality protection is not always assured through soil barriers and that its contamination by different sources occurs world wide (Momba *et al.*, 2000). Natural processes and human activities directly or indirectly cause change in groundwater quality. The most prevalent sources of groundwater contamination are waste disposal, mining operations, agricultural activities such as application of fertilizers and pesticides, urban development and a high concentration of chemicals (Engelbrecht, 1993, Wright, 1999).

The waters from shallow wells are frequently polluted with variety of waste from surface runoff. Periago *et al* (2000) reported that the application of cattle slurry as fertilizer is the major cause of diffuse-source water pollution in many agricultural regions. Previous studies performed by Momba and co-workers (Momba and Notshe, 2000; Momba and Mnqumevu, 2000) reported the poor hygienic quality of Nkonkobe district groundwater in the Eastern Cape Province of South Africa. These studies revealed that the level of heterotrophic bacteria and coliforms in both reservoirs and stand-pipe water from groundwater were above the limits allowed by South African *Water Quality Guideline for Domestic Use* (DWAF, 1996). The deterioration of groundwater quality standards of Nkonkobe district could be linked to agricultural activities as well as to the presence of many pit latrines surrounding the villages in Nkonkobe district. The microbial contamination of any water sources is well known as one of the cause of waterborne disease outbreaks. Because of the transmission of diseases in water, protection of water supplies from microbial contamination has been a major research focus in recent years.

The problem of groundwater contamination lead to the report made by Engelbrecht (1993) that

groundwater protection is important, as surface water supplies will be insufficient to meet all South Africa 's drinking water needs in the near future. Due to the contamination of groundwater, certain principles for the treatment and disinfection of water have been established. Disinfection of this water source is generally very effective as a microbial barrier because of the uniformity of quality and the absence of constituents that interfere with organism inactivation (Geldreich, 1989).

1.2.2 Disinfection of potable water

Disinfection is a very important step in the treatment of drinking water to prevent the risk of waterborne diseases. Disinfection methods were primarily developed for municipal drinking waters and can be classified into three categories: chemical, physical and photochemical. Inadequate disinfection of drinking water can lead to human disease or death. The need to disinfect water is shown by the estimation that each year 500 million people occupying 25 % of the world's hospital beds are victims of water associated diseases through the consumption of unwholesome water (Grabow, 1979). This gives disinfection a high priority in the treatment of potable water. The objectives of disinfection are: to inactivate all harmful organisms during treatment, and to maintain this quality during water distribution.

Different disinfection agents such as chlorine, chlorine dioxide, ozone, chloramine and UV (Ultraviolet radiation) control microorganism's occurrence and growth in drinking water. This study focuses on chlorination and monochloramination.

1.2.3.1 Chlorination

Chlorination of drinking water is the addition of chlorine directly to water. Most cities and tows in South Africa use chlorine as their standards water disinfectant. Chlorine is available in three forms, liquid: NaOCl (sodium hypochlorite), powder: Ca $(OCl)_2$ (calcium hypochlorite) and gas (Cl_2). All these forms of chlorine react with water to produce hypochlorous acid (HOCl) as follows:

$$Cl_2 + H_2O \rightarrow H^+ + Cl^- + HOCl$$

$$NaOCl + H_2O \rightarrow Na^+ + OH^- + HOCl$$

$$Ca(OCl)_2 + 2H_2O \rightarrow Ca^+ + 2OH^- + 2HOCl$$

Hypochlorous acid dissociates to a proton and a hypochlorite ion:

$$HOCl \rightarrow H^{+} + OCl^{-}$$

The hypochlorous and hypochlorite ion are together known as the free available chlorine. This free chlorine reacts with the multitude of inorganic and organic compounds. It acts more rapidly in an acid or neutral water than under alkaline conditions and this indicates that hypochlorous acid is the more powerful bactericide than ionized hypochlorite (Strobel and Diester, 1990). Chlorine is a very efficient oxidizing, bleaching and disinfecting agent. Its dose is related to the chlorine demand of water. The chlorine demand is the amount of chlorine required to react chemically with organic substances as well as other substances, which may occur in the water.

The precise action by which chlorine kills bacteria in water is not well understood. It inactivates microorganisms by the oxidation of proteins and nucleic acids to chloramines and other chloro-compounds. The small size of the HOCl molecules and its electric neutrality enables it to pass readily through the cell wall of bacteria. This brings the powerful oxidizing agent into immediate contact with the cell membrane, which plays vital role in cell metabolism. Chlorine also interferes with certain enzymes in the bacterial cells, since these enzymes are involved in respiratory activities and have sulphydryl group (-SH) which are vulnerable to oxidation (Grabow, 1979). The hypochlorous (OCl^{-}) ion reacts rapidly with cytosine and adenine, slowly with guanine and hardly at all with uracil. In *E. coli* OCl^{-1} terminates adenosine-triphosphate (ATP) production by inhibiting transport of the fermentative substrate, glucose and respiratory succinate, while at the same time inactivating the membrane-localized proton-translocating ATP-synthase, which is important for respiration (Verville and Herson, 1989).

The advantage of chlorination is that it is inexpensive, easy to use, effective in killing bacteria, can remain within the system for 1 to 2 d and as long as the chlorine residual is present, it protects water in storage and in the distribution system (Hua *et al.*, 1999; Momba, 1997). It also decreases the incidence of thyphoid fever, controls intestinal infectious diseases, and increases the effect of water purification. It has played an important role since mid 1970's (Junli *et al.*, 1997). However,

an important disadvantage of chlorination is that the reaction of chlorine with natural organic substances in the water produces trihalomethanes (THM), which are believed to be carcinogenic. It has been reported that the addition of chlorine at a high concentration will kill most bacteria but the chlorine residual will soon disappear in a distribution system with a high chlorine demand, allowing bacterial regrowth (Gibbs, *et al.*, 1990 and Momba *et al.*, 2000). According to Gibbs *et al.* (1990) the chlorine dose is limited by customer complaints due to the ability of chlorine to produce odours and to the formation of trihalomethanes (THM's). According to the Rand Water Board, chlorinated water can be produced by the addition of 1-2 $mg.\ell^{-1}$ chlorine solution (1.1-1.2 $mg.\ell^{-1}$ chlorine during winter) for surface water or 0.6-0.8 $mg.\ell^{-1}$ for groundwater, to maintain a free chlorine residual of 0.5 $mg.\ell^{-1}$ after contact time of 20 min.

In order to achieve a balance between sufficient chlorination to ensure microbiological quality and at the same time provide customers with water which they find pleasant to drink, it is necessary to understand the mechanism of chlorine decay in water distribution systems (Hua *et al.*, 1999). Chlorine concentration decreases continuously with time in any potable water distribution system. This is due to chlorine decay and to reactions with substances of both organic and inorganic nature (Kruger *et al.*, 2000). This decay in water distribution systems is also due to the reaction of chlorine with the pipe wall (Powell *et al.*, 2000). For these reasons most kinetic models describing chlorine decay in natural water have been established empirically or semi-empirically. Therefore, the total decay constant (k) is often expressed as the decay due to the chlorine demand of the pipe, known as the wall decay (k_w) and that due to the quality of the water itself, known as the bulk decay (k_b)

$$k = k_w + k_b$$

It is known that both the wall and the bulk decay are dependent on the temperature (Hua *et al.*, 1999)

1.2.3.2 Chloramination

Chloramination of drinking water has become a popular alternative to chlorine (Watters' *et al.*, 1989). Chloramines were widely used in the 1930's but their usage decreased with the discovery of breakpoint chlorination and its better disinfection ability. When compared to free chlorine and other disinfection agents, they are weak disinfectants for bacteria, protozoa and viruses and increase the risk of pathogens reaching the consumer. Because of this, chloramines are more commonly used

as a secondary disinfectant especially in water with high chlorine demand to provide bactericidal protection, control algae and bacterial aftergrowth (Vogt and Regli, 1981). The use of chloramines as disinfectants is a subject to particular interest to the water treatment field because chloramines do not form chlorinated organics (Norman *et al.*, 1980)

The process of chloramination has been referred to as combined residual chlorination or the ammonia-chlorine process. The process of monochloramine formation is based on the reaction of HOCl with ammonia. Monochloramine is produced rapidly in pH range between 7 – 9 and within the molar ratio $Cl_2 : N \leq 1:1$ or 4:1 by mass (Momba, 1997). The mechanisms in which the monochloramine inactivate bacteria in drinking water has been postulated to be similar to that of free chlorine. Monochloramine enters the cell and attacks its nucleic acid and proteins (Jacangelo *et al.*, 1991).

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 reduction of THM's during the treatment (Symons, 1980). Moreover, as a disinfectant in potable water chloramines offer a number of advantages. They persist in water for a longer time and may be used as an indicator to the cleanliness and freedom from pollution of water distribution system. Chloramine residuals restrain the regrowth or multiplication of bacteria (Twort *et al.*, 1974). They help to prevent the development of unpleasant tastes and are useful in the control of microorganisms in settling basins, filters and distribution systems where heavy chlorine dosages can result in taste problems. Chloramines also provide strong bactericidal effect when organic matter is present, reduce chlorine requirements, assure adequate dosage without fear of overdosing and afford good stability (Norman *et al.*, 1980). The major disadvantage of chloramines in drinking water is their deteriorating effect on taste and odour (Strobel and Diester, 1990). Since this disinfectant functions as an oxidant, most research on the health effect of chloramines has been directed at its oxidative effect on blood. The results of such research have been negative, but for kidney dialysis patients, there is a need for caution since chloramines have been found to induce hemolytic anaemia (Kreft *et al.*, 1985; Daniel *et al.*, 1993).

1.2.3 Effect of disinfection on biological stability of potable water

Drinking water is defined as biologically stable when its assimilable organic carbon (AOC) level is less than 10-20 μg acetate-C.ℓ^{-1} without disinfection or less than 50-100 μg acetate-Cℓ^{-1} after disinfection (Hu *et al.*, 1999).

The biological stability of drinking water has been gaining considerable attention in the field of water treatment and water quality maintainance (Hu *et al.*, 1999). Occurrence of unexplained coliform bacteria in potable water (bacterial regrowth) has been reported over the past 10 years by drinking water utilities. Bacterial regrowth is usually reduced by water treatment followed by the maintenance of disinfectant residual in a microbiologically secure distribution system (Drikas *et al.*, 1993). Hu *et al.* (1999) also reported that biologically stable drinking water is one which would not cause reproduction of coliform and heterotrophic bacteria. Disinfection agents have been found to split organic compounds. This unstable fraction of organic carbon has been suggested to be the main nutrient for microbial regrowth in distribution networks (Miettinen *et al.*, 1997). Studies have shown that bacterial regrowth or biofilm formation in potable water distribution systems occurs mainly due to the presence of nutrients (van der Wende and Characklis, 1990).

The link between biodegradable organics in drinking water and bacterial regrowth in potable water distribution systems has been noted since 1980 (LeChevallier *et al.*, 1987 and 1991, and Hu *et al.*, 1999). Most of the microbes in drinking water are heterotrophic and need organic compounds for their carbon and energy sources (Miettinen *et al.*, 1997). Microbial occurrence and growth in drinking water are controlled by different disinfection agents such as chlorine, monochloramine, etc.

1.2.3.1 Biofilm formation in potable water systems

The term "biofilm" refers to a layer of microorganisms usually between 20-70 μm thick (Pederson, 1982), in an aquatic environment held together in a polymatrix attached to a substratum such as pipes. It has been discovered that the distribution system is often important in the determination of the final quality of potable water (Momba *et al.*, 2000). One of the reasons for the increase of bacterial numbers increase during distribution is the biofilm formation on the material used for

drinking water distribution. Oligotrophic conditions inherent in drinking water preclude extensive planktonic microbial growth because of low levels of organic carbon and nutrient levels (Mueller, 1996).

Attachment of a single bacterial cell is the first step towards biofilm formation (Pederson, 1982). Biofilm development is a result of successful attachment, communication and subsequent growth of microorganisms on substrata. The nature of biofilms that develop in flowing water systems depends on characteristics of the flow system and the flowing water itself (Pederson, 1982). The growth of these bacteria on a surface is accompanied by the production of extracellular polymers (Donlan and Pipe, 1994).

Other factors such as the types of surface material, the microbial occurrence in the water, the concentration and quality of nutrients, the temperature and the hydraulics of the system also play a vital role in the accumulation of microorganisms on the surfaces (Donlan and Pipes, 1994). The formation of biofilms can be considered as a developmental cycle, because once a mature biofilm forms, toxic metabolic wastes could accumulate among the densely packed cells. The solution is therefore to create space between loosely packed clusters of cells so that bacteria migrate slightly from the surface as they excrete polysaccharides that serve as a biofilm matrix giving it a slimy nature. As the biofilm architecture develops, cells cluster in pillar-and-mushroom-like structures, with water channels between them for nutrient transportation and waste product excretion. This water therefore acts as primitive circulatory system. The sloughing-off of individual cells from the biofilm completes the developmental cycle (Kolter and Losick, 1998).

The deterioration of drinking water quality between disinfection time and the time it reaches the consumer is due to bacterial regrowth or bacterial after growth (Momba, 1997). The members of biofilms enjoy three main privileges 1) concentration of trace organics which serve as nutrients, 2) surfaces associated bacteria tend to produce extracellular polymeric substances which further concentrate trace growth factors, 3) protection from antagonistic agents such as disinfectants (Lappin-Scott and Costertan, 1995). Lower sensitivity of biofilm cells to biocides is due to differences in the physiological state associated with lower growth rate . Furthermore, biocides might not be able to penetrate the biofilm matrix (de Beer *et al.*, 1994) or even biocide

neutralization can also occur inside a biofilm.

1.2.3.2 Factors determining regrowth / aftergrowth

The term bacterial regrowth refers to the recovery of injured bacteria during the treatment process and their starting to multiply in water. The term bacterial after growth denotes growth of microorganisms native to the water distribution system (Momba *et al.*, 2000). As previously stated, factors such as the quality of the water, the type of surface material, the concentration and the quality of nutrients, the temperature and the piping material used for distribution of water play a vital role in biofilm formation (Donlan and Pipes, 1994).

1.2.3.2.1. Nature and concentration of biodegradable compounds

Water distribution systems are heterotrophic, thus heterotrophic bacteria growing in the water require the presence of reduced organic compounds to serve as their sources of energy and oxygen as final acceptor of hydrogen. In order for regrowth to occur in drinking water distribution systems, there must be the presence of source of energy (biodegradable compounds). These biodegradable compounds are either present in treated water or leached by the materials in contact with the drinking water (van der Kooij, 1999). Microorganisms use organic or inorganic hydrogen and inorganic or organic carbon as sources of energy and carbon, respectively. They can use the following as hydrogen acceptors: oxygen, nitrate, sulphate, carbon dioxide or carbon. The use of these compounds allows scientists to characterize bacteria according to the compounds they use for particular purposes (Momba *et al.*, 2000).

Biodegradable organic carbon is made up of Biodegradable Dissolved Organic Carbon (BDOC) and assimilable organic carbon (AOC). Biodegradable Dissolved Organic Carbon is organic the fraction of the organic content of a water sample that can potentially be biologically oxidised during water treatment. Its content in water includes both low molecular weight organic compounds and moderately high molecular weight organic compounds (DWAF, 1996). Water quality constituents and properties related to dissolved organic carbon (DOC) content include:

1) Chemical oxygen demand (COD), a measure of oxygen requirement of organic matter.

2) Biological oxygen demand (BOD), a measure of biochemically-mediated oxygen demand of water that is indicative of the portion of organic carbon easily oxidized by microorganisms

3)Total organic carbon (TOC), a measure of the organic carbon found in solution and in suspension (DWAF, 1996). TOC has not been found to be a good predictor of bacterial regrowth because some of it is unavailable for microbial degradation (Rizet *et al.,* 1982).

Joret and collaborators (1991) reported that *E. coli, Klebsiella pneumonia,* and *Enterobacter cloacea* can regrow in river water samples with a 3.2 $mg.\ell^{-1}$ initial DOC and a 1.4 $mg.\ell^{-1}$ initial BDOC. A chemical analysis is not helpful in characterizing dissolved organic matter because of its complexity and variability (Servais *et al.,* 1989). The use of bioassay procedure such as BOD was introduced to determine BDOC, but its lack of sensitivity and specificity prevents its use in drinking water treatment and distribution (Servais *et al.,* 1989). Some other experimental procedures that do not provide direct BDOC measurement but rather indices of bacterial growth on the organic matter present in the water sample were proposed. Most of these procedures are based on the original assimilable organic carbon (AOC) method developed by van der Kooij and coworkers in 1982 (Servais *et al.,* 1989).

Assimilable organic carbon (AOC) represents the fraction of biodegradable organic carbon that can be converted into new cellular material. The concentration of AOC is expressed in µg acetate-C equivalent per litre (van der Kooij, 1999). Van der Kooij, from a study he performed in 1999 concluded that calculations based on AOC uptake in distribution system demonstrate that biofilm formation can be linked with AOC removal data and also that very low concentrations of BDOC can cause a considerable biofilm formation. Bacteria can metabolize dissolved organic carbon and assimilable organic carbon carbon within a period of a few days to a few months. There are methods used to determine the concentration and availability of the biodegradable organic carbon compounds present in water, such a the van der Kooij AOC concentration as µg acetate carbon equivalent per liter and Werner BDOC methods methods.

1.2.3.2.2 Residence time and biocide residuals

Residence time of a disinfectant refers to the time that the disinfected water stayed with disinfectant before its use or consumption. In distribution systems, water quality changes in accordance with the residence time (Momba, 1997) and the longer the distribution distance, the longer the residence time and therefore the lower the biocide residual concentration. The absence of any antagonistic

agents, coupled with the extremely oligotrophic environment associated with purified water systems promotes bacterial adhesion (Lappin-Scott and Costertan, 1995). The role that disinfection play on biofilm formation will be discussed in the section 1.2.4.

1.2.3.2.3. Temperature

Bacteria are able to grow over a wide range of temperatures and this has been shown by many researchers. Temperature changes in distribution systems can come as a result of seasonal changes. Donlan and collaborators in 1994, discovered that biofilm accumulation was greater in warm water (15 to 25°C) than in cold water. All the above mentioned factors are very important in determining the effectivity of disinfectants and thus bacterial biofilm formation.

1.2.3.2.4 pH

The pH of distributed water is very important for the effective disinfection of the water. If the pH of the water being distributed exceeds the working range of the biocide, it will lead to bacterial regrowth or aftergrowth in the distribution system. The procedure of killing micoorganisms by chlorination depends on a pH within the permissible pH ranges of 6.5 – 9.5 with the disinfection effect decreasing as the pH increases (Momba, 1997). It has been shown that hypochlorous acid (HOCl) can react in three different directions:

i) at high pH levels (pH > 8) hypochlorite ions (OCl^-) predominate the reaction;

ii) at moderate pH levels (pH < 8) HOCl predominates the reaction;

iii) at low pH levels (pH< 1) Cl_2 predominates the reaction (Viljoen *et al.*, 1997).

Chloramination on the other hand is limited to the 6 – 9 pH range for disinfection (Momba, 1997).

1.2.4 The role of disinfection processes and piping material on biofilm formation

Microbes growing in biofilms deteriorate the water quality once they are released into the water. Control of biofilms within the distribution system must involve an integrated approach such as changing the disinfection regime, changing the piping material etc (Kerr *et al.*, 1999). To prevent or disrupt biofilm formation biocides are employed. A variety of disinfection processes, such as ozonation, chloramination, monochloramination, hydrogen peroxide, and UV irradiation are used

to prevent biofilm formation on the surface of piping materials in water distribution systems. The efficiency of these biocides in the prevention of biofilm production varies because of their different abilities to penetrate the biofilm (Momba *et al.*, 2000). The piping material also plays a very important role in the maintenance of high water quality because it can supply the bacteria in the water with nutrients through the leaching of organic compounds. This section focuses on disinfection processes namely chlorination and monochloramination.

1.2.4.1 Disinfection processes

In this study, chlorination and chloramination were chosen firstly, because chlorination is the standard disinfection method used in South Africa, and secondly because the combination of these disinfection methods promises to be more effective in biofilm inhibition than chlorination alone.

The limited penetration of chlorine into a biofilm was proved not to be simply due to transient diffusion but also to neutralization of the chlorine in the biofilm matrix (de Beer *et al.*, 1994). This neutralization together with microbial conversion of glucose decrease the pH value of the water. Even though it has been reported that glucose from bulk liquid and biofilm material are substrates of chlorine reduction, it has been proven that a 200-fold increase in the glucose concentration did not reduce chlorine penetration. This leaves the biofilm matrix material as a prime substrate for chlorine neutralization (de Beer *et al.*, 1994). The statistical analysis of the sampling data from LeChevallier and coworkers in 1990 showed that coliform densities were not influenced by the maintenance of a free chlorine residual of 0.75 mg/l. The concentration of chlorine in the biofilm is typically 20% of that in the bulk water (Smith *et al.*, 2000).

Chlorine has been shown to react with various components of the system such as construction materials, biomass and other particulate or dissolved components (van de Wende *et al.*, 1989). The use of iron pipes leads to journalistic chlorine consumption due to corrosion (this will be discussed in detail in section 2.4.2). Chlorine consumption also depends on hydraulic conditions (Momba *et al.*, 2000). A chlorine microelectrode sensor is impractical for use in detecting the effect of chlorine contact within the biofilm due to its fragility and noise sensitivity so a lipid analysis of the biofilm was introduced and has been reported to work very well (Smith *et al.*, 2000).

Because monochloramine does not react with capsular material, the biocide is available for inactivation of biofilm organisms (LeChevallier *et al.*, 1990). Comparing chlorine and monochloramine, studies performed by Momba and collaborators in 1998 showed that biofilm formation was related to the depletion of residual disinfectant concentrations. Monochloramine had a longer residual effect in controlling the growth of biofilm cells in the system for a longer period before regrowth occurred. Once no residual disinfectant concentration could be detected there was no significant difference between the viable bacterial counts on any of the stainless steel and cement coupons in chlorinated and monochloraminated water systems. The effectiveness of monochloramine in preventing the attachment of *E. coli* in a developing biofim was also reported by Momba and coworkers (1999). It has been stated that monochloramine does not react with the piping material and at a dose of 4 $mg.\ell^{-1}$ biofilm counts are reduced by more than 100-fold; whereas free chlorine at either at a dose of 1 or 4 $mg\ell^{-1}$ dose remained ineffective (LeChevallier *et al.*, 1990).

1.2.4.2. Piping materials

There are several types of piping materials (ranging from plastics to concrete) employed for water distribution. The examples are polybutylene, copper, polyvinyl chloride (PVC), asbestos, concrete etc. All of these have different constituents that affect the water quality one-way or the other.

A comparative study on the effect of plumbing material on biofilm formation was performed by Rogers and coworkers in 1994. The plumbing materials they used in their study were copper, PVC, and polybutylene. They concluded that copper reduced the number of bacteria in water due to less biofilm forming on the copper at all temperatures examined. The authors also witnessed that copper ions could possibly account for reductions in the pathogenic population. Biofilms in iron, galvanized steel, copper, and PVC pipes treated with 12 $mg.\ell^{-1}$ initial concentration of chlorine and 12 $mg\ell^{-1}$ monochloramine showed the following disinfectant demand in $mg.\ell^{-1}$: for chlorine, iron, galvanized, copper, and PVC, showed 40.0, 7.5, 3.9, and 3.2 respectively; and for monochloramine, they respectively showed a demand of 32.0, 1.8, 0.8, 0.6 (LeChevallier, *et al.*, 1990). Kerr and collaborators performed a study in 1999 in which they compared biofilm attachment and heterotrophic bacterial diversity on cast iron, medium density polyethylene (MDPE), and unplasticised polyvinyl chloride (uPVC) using a New Modified Robbins Device (nMRD) run over

a short period (21 d) and a longer period (7 months). They found that the number of bacteria on each material increased exponentially between 0 and 11 d. The mean doubling time of heterotrophic population on the materials during the exponential phase was 13.3 h for cast iron, and 15.6 h for MDPE and uPVC (Kerr *et al.*, 1999). The piping material can increase the concentration of biodegradable compounds, which can be determined by total organic carbon (TOC) analysis of water, also known to enhance biofilm formation in water distribution systems (van der Kooij, 1999).

Corrosion of the piping material has been proven to result in less concentration of biocide such as chlorine due to ferrous ions production thus rendering chlorine less efficient as a biocide:

$$2\ Fe\ (HCO_3)_2 + Cl_2 \rightarrow Ca\ (HCO_3)_2 \rightarrow 2\ Fe\ (OH)_3 \rightarrow CaCl_2 + 6\ CO_2$$

Corrosion produces ferrous ions, which also consume the chlorine residual. Some of the growth promoting factors of biofilm are carbon from microbial by-products and trace elements and salts from piping extractables (Miltelman, 1995; van de Wende *et al.*, 1989). Many methods have been developed to combat the corrosion of piping material, the most cost effective of which is to apply a protective surface to the internal pipe surface (Ramotlhola *et al.*, 1999). Selection of piping materials and the conditions under which the pipe must operate are very important. The coatings normally used are cement-mortar, unplasticised polyvinyl chloride (uPVC), high -density polyethelene (HDPE), asbestos, cement, mild steel, and galvanized steel. Biofilm may develop on the piping material only if that material supplies the biofilm with nutrients (Rogers *et al.*, 1994). Plastic surfaces are known to leach metal ions at a sufficiently low level to prevent a toxic effect but could possibly contribute to cations essential for enzyme function. The plasticizers may also contribute to this biofilm formation (Rogers *et al.*, 1994). The biofilms forming on piping material as a result of regrowth and aftergrowth could be characterized according to the damage they cause in the pipelines.

1.2.5 Population dynamic of bacterial biofilm in water distribution systems

Numerous studies have been performed to determine the composition of the communities present in various environments such as activated sludge and oligotrophic environments such as drinking

water. It was repeatedly shown that less than 1% of the bacterial population from oligotrophic environments could be cultured (Macdonald and Brozel, 2000).

Bacterial biofilm can consist of only one bacterial species or can be formed by a consortium of different bacterial species. The unison and coordination of their behaviour is achieved through their communication, known as "quorum-sensing". The bacterial population in a biofilm depends on the environment in which the biofilm develops. Bacteria from biofilms show different physiological properties in their response to environmental influences compared with bacteria growing planktonically (Buhler *et al.*, 1985). In a study done by Rogers and coworkers in 1994, it was found that the biofilm that developed on polybutylene contained a diverse mixture of gram negative bacteria, actinomycetes, fungi and protozoa including amoeba. Pseudomonads were the principal pioneers composing 72% of the total bacterial flora (Rogers *et al.*, 1994). Ample microscopic evidence is available to show that most pipes in distribution systems are colonised by microorganisms. Diatoms, algae, protozoans, and bacteria were commonly encountered (LeChevallier *et al.*, 1987). A biofilm, therefore, serves as a focal point where bacterial and protozoal populations interact. This study deals especially with bacterial biofilms.

Pathogens such as *E. coli*, *Legionella bozenmanii*, *Mycobacterium smegmatus*, *etc* readily colonise preformed biofilm developed with *Pseudomonas aeruginosa*, *Acidovorax* sp. and *Bacillus* sp. isolated from drinking water biofilms (Smith *et al.*, 2000). In all the different bacterial biofilms forming in various environments, the Pseudomonads are always present in large number. The reason being that they are the initiators of the biofilm structures and this they do by signaling to surrounding bacteria that it is time to come together and form the biofilm structure (Kolter and Losick, 1998). For example, Corneal infection is a severe sight-threatening complication caused by the *P. aeruginosa* found in biofilms that inhabit contact lenses (Cowell *et al.*, 1999).

In a study performed by Jones and Bradshaw on biofilm in water pipes in 1997, they showed that established biofilms of *Klebsiella pneumoniae* can act as foci for attachment and subsequently release into water a column of *Salmonella enteritidis*. The evidence in the literature suggests that there is a considerable level of interdependence among biofilm organisms, including eucaryotes as well as prokaryotes. This interdependence also involves the formation of complex metabolic

consortia. *E.coli* and *Aeromonas hydrophila* have been proven to be present (Mossa *et al.*, 1999). *P. aeruginosa* has been shown to provide a signaling molecule that is used for bacterial biofilm formation (Kolter and Losick, 1998). The most alarming findings are the presence of opportunistic and pathogenic bacteria such as *Pseudomonas, Mycobacter, Campylobacter, Klebsiella, Aeromonas, Legionella* ssp, *Salmonella typhimurium* and enterotoxigenic *E. coli* (Momba *et al., 2000)*

1.2.6 Health risks associated with the bacterial contamination of drinking water

Water has always been suspected of carrying diseases. It has been reported that waterborne diseases infect around 250 million people in the world, resulting in 10 to 20 million deaths each year (Toze, 1999). Although this problem is usually seen in developing countries without adequate facilities for the treatment of wastewater and drinking water, an increasing trend in waterborne illnesses has also been reported within industrialized nations (McFeters and Singh, 1991).

At present, microbial contamination of water causes over half of waterborne diseases in US (Deborde *et al.*, 1999) and disease outbreak has increased between 1971 and 1985 with more recorded outbreaks than in any previous 15 years interval since 1920. In many rural and growing urban areas of South Africa there is a widespread scarcity, gradual destruction and increased pollution of freshwater resources. The growing populations and their economic needs, as well as the poor progress in sanitation facilities in most black townships constitutes a threat to the water resource and to drinking water. For a long time it has been known that pathogenic and toxigenic microbial agents in drinking water cause disease and death to consumers.

The presence of bacterial pathogens in the drinking water is of great concern because some of them can cause serious harm to the consumer of infected water. Some of the serious pathogens include *Campylobacter jejuni,* which is known to cause gastroenteritis, *Legionella pneumophila,* known to cause Legionnaries' disease and Pontiac fever, *Mycobacterium tuberculosis*, which is responsible for tuberculosis, and *Pseudomonas aeruginosa* which causes urinary and respiratory tract infections (Atlas, 1995). *Salmonella typhi* is known to cause typhoid fever, a disease of the gastrointestinal tract which frequently gives rise to systemic infections. A major breakout of typhoid fever in Great

Britain, investigated by Suckling, killed 43 people in Croydon in 1937 (Twort *et al.*, 1974).

Vibrio Cholerae is a causative agent of cholera which the most devastating of the waterborne diseases. Cholera is transmitted by ingestion of bacteria from polluted water or other material contaminated with faecal matter from infected individual. The patient suffers server diarrhoea, massive dehydration of the body and serious salt imbalance, which impairs the normal function of many organs (Craun, 1984). Recently in the Kwa-Zulu Natal Province of South Africa, the Department of Health reported an outbreak of cholera. In October 2000 this disease caused 31 deaths while about 4000 cases required medical attention.

The genus *Shigella* (*Sh. dysenteriae1, Sh. flexreri, Sh. boydii* and *Sh. sonnei*) is responsible for the bacterial dysentery which can be contracted via water contaminated by human faeces, but is more commonly due to the ingestion of food contaminated by flies or by unhygienic food handlers who are carriers (Towrt et al., 1974). The cause of bacterial dysentery was first recognized in Japan at the end of nineteenth century during an epidemic involving 90 000 cases. In the USA in 1978, data from Centre for Disease Control indicated nearly 25 cases per 100 000 among infants (Craun, 1984). In South Africa the statistics of this disease remain unknown, but rural communities commonly suffer from this disease.

The above outbreaks could also be linked to groundwater contamination. From 1946 to 1977 there were 264 outbreaks and 62 273 reported cases of waterborne diseases due to groundwater contamination in the United States. Groundwater accounts for about 50% of all illnesses (Engelbrecht, 1993). Therefore it is important that water treatment and purification methods for groundwater must include preventive actions against all of the above mentioned diseases.

Increased awareness of the breadth and magnitude of water microbial diseases suggests that new and improved strategies are needed to reduce the health risk posed by microorganisms in drinking water. A combined chlorine-monochloramine process is likely to reduce such health hazards.

CHAPTER 2

INHIBITION OF BACTERIAL AND BIOFILM REGROWTH IN A CHLORINATED POTABLE WATER SYSTEM

2. 1 INTRODUCTION

Drinking water is traditionally disinfected at a treatment plant. To monitor the microbiological quality of such water and to ensure a bactericidal or bacteriostatic activity throughout distribution systems, an effective disinfectant residual must be maintained. Chlorine is the most commonly used disinfectant for the chemical treatment of water. The microbiological and physico-chemical quality of distributed waters coupled with the state of the piping material used determine chlorine levels (Fratteur *et al.,* 1999).

In South Africa, the emphasis of the effective Safe Drinking Water Act (Act No 66 of 1998) and its Amendments is the attainment of water quality standards at the point of consumption. However, the compliance with water quality standards at the outlet from the treatment plant does not prevent the degradation of water quality during transport. Any drinking water distribution system, therefore, will experience an increase in bacterial numbers with distance away from the point of treatment. This increase has been termed regrowth and is recognized as a major problem within many water systems (Clark *et al.,* 1982, Edge and Finch, 1987)

The relation between chlorine residual and bacterial regrowth within any water distribution system remains an important concern to water authorities for a number of reasons. Firstly, the occurrence of high numbers of coliform undermine the validity of routine sampling programs and often lower annual water quality results in the non-compliance categories (Kaye *et al.,* 1999). Secondly, many microorganisms found in distribution systems also have the capacity to behave as opportunistic pathogenic micro-organisms. Kühn *et al.,* (1997) reported the persistence of some potentially pathogenic *Aeromonas* strains within the Swedish drinking water distribution system. *Klebsiella oxytoca* and a high number of *Aeromonas hydrophilla* were also isolated

within the drinking water distribution system of Sydney (Kaye *et al,* 1999). A previous study performed by Momba and Dumani (1999, unpublished) on the microbiological quality of chlorinated water in Alice, Eastern Cape Province of South Africa, indicated not only the regrowth of heterotrophic plate count bacteria and coliforms (Total coliform, *E. coli*, *Salmonella*) but also the presence of Somatic and F-RNA coliphages in chlorinated water at the point of consumption (public stand-pipes). In terms of the South Africa Water quality Guidelines, the indicator levels posed increased risk of infectious disease transmission (DWAF, 1996). The above findings clearly show that poorly managed drinking water distribution systems constitute a possible health risk.

It has also been shown that the maintenance of chlorine residual does not eliminate all bacteria in a water distribution system (Lechevallier *et al.,* 1990, Momba *et al.*, 1998, 2000). However, a study performed by Momba,(1997) showed that the greater persistence of monochloramine residuals was found to control the phenomenon of bacterial regrowth in potable surface water and ground water through a laboratory-scale system. This study also revealed the efficacy of monochloramine in the complete elimination of coliform bacteria after disinfection, leading to a greater understanding of the processes which can inhibit the phenomenon of bacterial regrowth in drinking water distribution systems.

Not only planktonic bacteria, but attached bacteria can be apparent in finished drinking water. The formation of biofilms is a common phenomenon in aquatic environment (Block 1992, LeChevallier *et al.,* 1993; Holden *et al.,* 1995). Drinking water distribution systems provide an oligotrophic environment in which the biofilms growing on the pipe surfaces are thought to adversely affect water quality. Awareness has grown that potable water leaving the treatment plant may undergo substantial changes in quality during transportation through the distribution system to the customer due to biofilm formation on pipes (Donlan and Pipes 1988, LeChevallier *et al.,* 1991).

The occurrence of biofilms is associated with technical and hygienic problems in drinking water distribution systems. Microbes growing in biofilms reduce the quality of water when released into the water flow. Some microbes occurring in biofilms can affect taste and cause odour in water (Astier *et al.*,1995) or be pathogenic. A large variety of pathogenic bacteria have been

isolated from biofilm both in chlorinated and non-disinfected water distribution system. The survival of *Legionella pneumophila*, *Salmonella typhimurium* (Armon *et al.*, 1997), and *Campylobacter* spp (Buswell *et al*, 1998) has been reported.

It is well known that biofilms protect microbes against disinfectants (Block *et al.*, 1993a, Bois *et al.*, 1997; Momba *et al.*, 1998, 1999). The presence of complex biofilms results in the increase of planktonic cells in the drinking water distribution system (van der Wende *et al.*, 1989) as well as in the increase of corrosion of piping materials (LeChevallier *et al.* 1993; Beck, 1995; Holden *et al.*, 1995). The accumulation of micro-organisms on the surface of pipe materials and the formation of biofilms depend on many factors prevailing in the water system. These include the types of surface materials, the disinfectant, the concentration and quality of nutrients, the microbial quality of intake water, the temperature, the hydraulics of the system, the exposure time of water in the distribution system and above all the presence of a disinfectant residual (Roger *et al.*, 1994; Piriou *et al.*, 1997; Momba *et al.*, 1998).

Control of biofilm within the distribution system must involve an integrated approach using a combination of several types of action, for example reducing the amount of organic material in the water (van der Kooij *et al.*, 1995), changing the piping materials to those which have a reduced biofilm forming potential or changing the disinfection regime (LeChevallier *et al.* 1990). Although biofilm formation has been detected on the surfaces of stainless steel and cement coupons exposed to treated (chlorine, monochloramine, ozone, UV irradiation and hydrogen peroxide treated waters) and non disinfected waters (surface water and groundwater), biofilm regrowth was limited in laboratory-scale units with a disinfectant residual (Momba *et al*, 1998). This study also showed that biofilm formation was related to the depletion of residual disinfectant concentration. Monochloramine and hydrogen peroxide had a longer residual effect in controlling growth of biofilm cells in the systems for a longer period before regrowth occurred. Moreover, the susceptibility of bacteria in chloramine treated water was linked to effective disinfectant residual concentrations which could be detected for up to 8 d.

Therefore, the major aim of this work was to investigate the inhibition of bacterial regrowth and biofilm formation in a laboratory-scale system. To achieve this goal chlorine was used as primary disinfectant followed by monochloramine as secondary disinfectant. To have a comparative study

which allowed the evaluation of the process, surface water (partially treated water - water after sedimentation and sand filtration and chlorinated water - reservoir water - from Alice purification system) and groundwater from two villages in Alice were used as test water sources. Stainless steel and galvanized mild steel were used as piping materials for the study of biofilm in a laboratory-scale system.

2.2 MATERIALS AND METHODS

2.2.1 Study site and water sources

The study covered Alice Town and 2 rural communities of Alice namely Dyamala and Kwa-Nkobonkobo locations. Alice is situated in a semi-rural area in the Eastern Cape Province of South Africa. Dyamala and Kwa-Nkobonkobo locations are situated in the northern -east and northern-west of Alice respectively. Alice has its own water purification system which only supplies water to town dwellers. Because of the small capacity of the treatment plant, Alice purification plant cannot supply all the rural communities around Alice with potable water. Consequently, due to the widespread scarcity of freshwater resources in these communities, there was a need for the communities to ask for the development of groundwater supply for drinking water.

The Alice purification system receives its water from a catchment dam that draws water from the Tyume River. The water from the dam then undergoes purification in the treatment plant. The purification involves the following steps: flocculation, sedimentation, sand slow filtration and chlorination, using chlorine gas. Flocculation and sedimentation are enhanced by the addition of alum [$(Al_2(SO_4)_3$] and lime for coagulation of the solids. The chlorinated water is stored in the main storage tank until required. Partially treated water (AF) was used as a control during the experimental study. Chlorinated water from the main storage tank (A) was used to assess the quality of water during its circulation in a laboratory-scale system model and to establish a comparison between water treated at the Alice plant and water treated with a combined chlorine-monochloramine process in the laboratory-scale system.

Kwa-Nkobonkobo and Dyamala locations receive their drinking water from the boreholes. In

both locations, the boreholes are located on the outskirts of the residential area. At Kwa-Nkobonkobo, the water is drawn from the borehole using a windmill and is pumped into a storage tank made of cement. During the study period, the storage tank was regularly left open. In Dyamala, water is drawn using a rotatory hand pump. In both villages, water is taken in drums from a communal standpipe without any prior treatment.

2.2.2 Sampling of test waters

Test waters were collected in 50 ℓ sterile polyethylene drums. A total of 4 drums was used for surface water and 3 for groundwater. Before use, the drums were washed with detergent solution, rinsed with hot water and distilled water and then disinfected with 5 $mg.\ell^{-1}$. free chlorine for 24 h. Sodium thiosulphate (ca 17.5 $mg.\ell^{-1}$) was used to dechlorinate the drums and neutralise any disinfectant residual. Sterile distilled water was then passed through the drums for 24 h after which a total microbiological count was done to check the sterility of the drums.

2.2.3 Laboratory-scale system

The present study was performed using the laboratory-scale system described for the first time by Jacob *et al.*, (1996) for the study of biofouling and used by Momba and coworkers (1998,1999, 2000) for the study of bacterial regrowth and biofilm formation in potable surface water and ground water systems. The laboratory-scale system model illustrated in Fig. 1 was used as a batch reactor. For each batch reactor, two modified Pedersen devices were used to allow biofilm formation to be studied on stainless steel (20 slides) and galvanized mild steel (20 slides). For this purpose, stainless steel (SS) and galvanized mild steel (MS) slides, the size of the microscope slides (75x25x1mm), were installed vertically into the Pedersen device. Three systems were used for disinfected surface waters (A, AFC_1, AFC_2) and one for the control (AF). As to groundwater, two systems were used for disinfected waters (GWC1 and GWC2) and one for the control (GW). Before use the systems were treated in the same way as the drums.

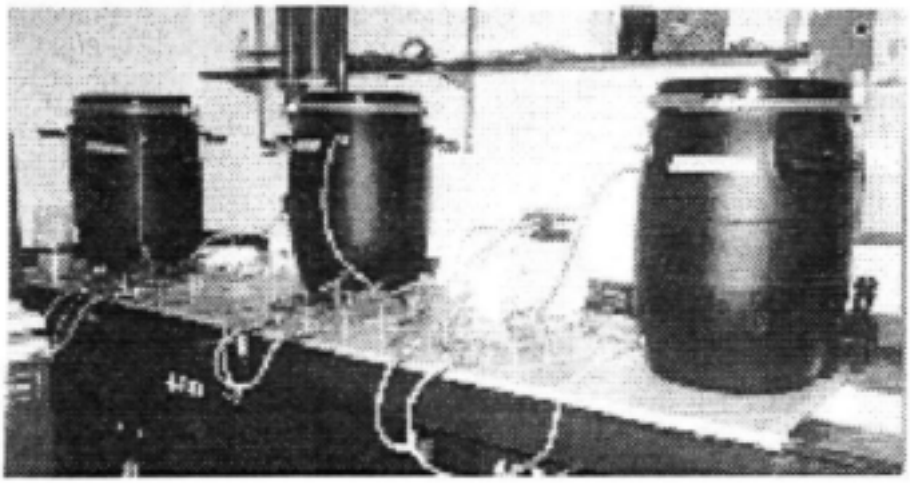

Fig. 1 Photograph showing the laboratory -scale system.

2.2.4 Treatment of test waters

2.2.4.1 Preparation of disinfectant stock solutions

Chlorine stock solution was prepared from sodium hypochlorite (commercial bleach). To obtain a concentration of 100 mg.ℓ^{-1} free chlorine, 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 performed using the N,N-diethyl-p-phenylenediamine (DPD, Sigma) ferrous titrimetric method (APHA-AWWA-WEF , 1998).

The monochloramine solution was prepared by reacting the chlorine with the ammonium sulphate at a NH_4:Cl mass ration of 4:1. When preparing a monochloramine solution, the ammonium sulphate [$(NH_4)SO_4$] (Protea Laboratory) was added first and then the sodium hypochlorite (commercial bleach). The concentration of monochloramine was determined using the DPD (Sigma) ferrous titrimetric method (APHA-AWWA-WEF , 1998).

2.2.4.2 Disinfection of test waters

Chlorinated water was produced by addition of stock solution to give an initial 2.5 mg.l^{-1} free chlorine into the partially treated surface water sample (AF) and groundwater water samples, prior to being transferred to the separate laboratory-scale systems (AFC_1 and AFC_2 for surface water; GWC1 and GWC2 for groundwater). No disinfection was performed for the control

water (AF, GW) and Alice chlorinated water(A). Control and disinfected water samples circulated in separate laboratory-scale systems at a flow rate of 2.8 $\ell.h^{-1}$. Twenty four hours after the circulation of test waters in different systems, 1.5 mg $.\ell^{-1}$ monochloramine was added to AFC_2 (AFC_2M) and GWC2 (GWC2M). No neutralization of any the disinfectants used for the source waters, with the exception of samples on which the microbiological analysis were conducted, was done during the study period.

2.2.5 Physico-chemical analyses

Sample frequency is indicated in the results and was the same for all experiments. Concentrations of residual free chlorine and residual monochloramine were measured using N,N-diethyl-p-phenylenediamine (DPD, Sigma) ferrous titrimetric method (APHA-AWWA-WEF 1998). Temperature, pH, turbidity were determined according to standard methods (APHA, AWWA-WEF 1998). Calcium(Ca^{2+}), Magnesium,(Mg^{2+}) chemical oxygen demand (COD), total nitrogen (N), phosphate (PO_4^{3-}), sulfate (SO_4^{2-}) concentrations were determined according to Spectroquant NOVA 60 manual (1998), using photometric test kits (Merck).

Dissolved organic carbon (DOC)- Samples were prepared according to Mathieu *et al.*, (1993). This method was slightly modified during this study. Instead of glass syringes, sterile plastic syringes were used for the filtration of samples. For each water sample, 10 ml were sampled with a plastic syringe (pre-treated with nitric acid) and were filtered through 0.2µm Durapore membrane (Millipore, GVWP 02500). Before filtration of the samples, each membrane was rinsed 10 times with 10 ml volume of MilliQ water. The sample filtrates were collected in glass bottles (pretreatment: 200°C for 8 h), 30 µl of nitric acid were added and the glass bottles were hermetically sealed and sent to Council for Scientific and Industrial Research (Pretoria/South Africa) for the measurement of DOC concentrations.

2.2.6 Microbiological analyses

Sample frequency is indicated in the results and was the same for all experiments. The experimental data are based on two replicates of the study.

2.2.6.1 Water samples

Chlorinated and chloraminated samples were collected in sterile bottles previously rinsed with MilliQ water , which contained sodium thiosulphate (ca 17.5 mg.ℓ^{-1}).

Coliform bacteria - Coliform bacteria were enumerated by membrane filter procedure using a filter with a 0.45µm pore size (APHA- AWWA - WEF, 1998). Membranes (Whatman) were placed on chromocult coliform agar (Merck) for the enumeration of total coliforms (TC), presumptive *E coli* (Ec) and presumptive *Salmonella* (Sal). Characteristic colony colours were used for the identification of coliform bacteria (Merck). A confirmation test for faecal coliform in groundwater was conducted using m-FC agar. Injured coliforms (IJ) were recovered by placing the membranes on mT7 agar (Difco) (LeChevallier *et al.*, 1983). Each determination was performed in triplicate.

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

2.2.6.2 Biofilm formation

The colonized stainless steel (SS)and galvanized mild steel (MS) (75 x 25 x1 mm) slides were aseptically removed from the Pedersen devices 24 h after the circulation of the A, AF, AFC_1, AFC_2/ AFC_2M , GW, GWC1 and GWC2/GWC2M waters in laboratory-scale systems and later once a week with the exception of the last week where the slides were taken twice.

Attached viable bacteria - The removed slides (two for each type of pipe materials) were put into sterile plastic bottles with 20 ml sterile MilliQ water. To detach biofilms from the slide surfaces, the bottles were votexered for 2 min using a vortex mixer (VM-300). For each water system, two slides (50 x 25 x 1 mm) were removed and heterotrophic plate count bacteria were enumerated by the standard spread plate procedure using 2RA agar (Difco), incubated at 28°C for 7 d (Reasoner and Geldreich, 1985). Analyses were carried out in duplicate. The following equation was used to calculate the number of HPC bacteria (cfu.cm^{-2}):

HPC (cfu.cm^{-2}) = N x D/Surface area of slides

where N= average number of colonies and D=dilution factor

Total cell densities- The removed slides were placed in sterile petri dishes. Total cell counts were measured directly on SS and MS slides, 0.2 ml DAPI (1µg.ml^{-1} working concentration) (Boehringer Mannheim GmbH) was added to the slides. The staining was carried out in a dark room for 7 min. The slides were rinsed with sterile diluent consisting of MilliQ water and 50% gluteraldehyde (Electron Microscopy Sciences). The cells on the surfaces were counted with epifluorescence microscope. A minimum of 20 microscope fields for each slide were counted for total count. Total cells were expressed in cell.cm^{-2} and calculated using the following equation :

Bacterial density (cell.cm^{-2}) = N*f*/a
where N*f* = average number of cells per microscope field and a = Area of microscope field (49 x 10^{-8}cm^{2})

Visualization of biofilm formation - The removed slides (25 x 25 x 1mm) were put into sterile plastic bottles containing 2.5 % gluteraldehyde in a phososphate buffer (GTA). The biofilm formation was visualized using the scanning electron microscope (SEM).

2.2.7 Statistical analyses

To compare variation in treatment, Least Square of Differences (LSD) were applied to the bacteria counts with the latter as the dependant variable. The bacteria counts were transformed by taking the logarithm base 10 in order to stabilize the variance. The number of days from the outset of the experimental were used as a co-variate. A correlation was established between bacteria counts and disinfectant residual concentrations and between bacteria count and physico-chemical values of waters

2.3 RESULTS

2.3.1 Surface water

2.3.1.1 Physico-chemical quality of test waters

With the exception of DOC in AF and AFC_1, organic and inorganic compounds in water systems complied with the *South Africa Water Quality Guidelines*. The same temperature values (average) of 21.18 °C was found in all water systems. The Turbidity in disinfected waters was approximately the same but was higher when compared to the control water. Some variations in pH values were recorded in the test waters (Table 2.1) although the pH was between 7.2 and 7.8 and complied with the limits allowed by *South African Water Quality Guideline for Domestic Use* (et water quality range: 6.0-9.0; DWAF, 1996).

Table 2.1 Physico-chemical characteristics of different surface water systems during the experimental study.

Test Water	Parameter (Means)									
	T° (°C)	pH	NTU	Ca^{2+} $mg.l^{-1}$	Mg^{2+} $mg.l^{-1}$	COD $mg.l^{-1}$	DOC $mg.l^{-1}$	N $mg.l^{-1}$	PO_4^{3-} $mg.l^{-1}$	SO_4^{2-} mgl^{-1}
A	21.18	7.20	3.24	11.50	14.00	10.60	5.08	1.26	0.50	10.80
AF	21.18	7.20	2.85	11.20	12.80	15-95	9.95	2.14	0.58	11.40
AFC_1	21.18	7.80	3.25	10.40	11.20	11.85	7.10	1.24	0.56	14.90
AFC_2/AFC_2M	21.18	7.30	3.15	12.17	9.20	11.35	5.55	2.70	0.54	21.40

A: Alice chlorinated water from the tank, AF: After filtration water, AFC: After chlorination water, AFCM: Chlorine-monochloramine treated water.

2.3.1.2 Disinfectant residuals

No disinfectant residual was found in A water even on the day of sampling. A gradual depletion of free chlorine concentration from 2.5 to 0.5 $mg.\ell^{-1}$ occurred in AFC_1 and AFC_2 24 h after disinfection, after which no residual was noted in AFC_1 for the remainder of the experimental

study (Figs.2.3-2.4). A combination of free chlorine and monochloramine in AFC_2 led to the long persistence of the disinfectant residual that could be detected up to 672 h. The monochloramine concentration decreased from 1.5 to 0.15mg.l^{-1} between 24 and 672 h. Complete disappearance was noted after 720 h (Fig. 2.4).

2.3.1.3 Microbiological features in test waters

Coliform bacteria

Characteristic colony colours on Chromocult coliform agar made easy identification of coliform where total coliforms (salmon to red colonies) were differentiated from presumptive *E. coli* (dark blue to violet colonies) and presumptive *Salmonella* (light blue to turquoise colonies) during the study period. As shown in this Table 2.2, a similar initial number of 4 log cfu $.10^{-2}$ ml total coliforms was isolated from A and AF water. Low counts were noted for *E. coli* and *Samonella* compared to total coliforms. The study also revealed the presence of injured bacteria in A water when using mT7 agar. A gradual decrease of coliform bacteria counts was noted in all systems. However, complete elimination of bacteria occurred earlier in AFC_2M (24 h after chlorine-monochloramine disinfection) system than in AFC_1 (72 h after chlorination), A and AF (168 h) systems. Although the count for presumptive *E.coli* and *Salmonella* were low, their persistence was noted in A (168 h for both organisms) and AF (168 h for *E. coli)* systems (Table 2.2).

Heterotrophic plate count bacteria

Figs. 2.2 - 2.4 illustrate the microbiological features related to the average numbers of HPC in all systems. An initial heterotrophic bacterial count of 4 log cfu.ml^{-1} was found in AF and A water systems (Fig. 2.2). This number increased to 6 log cfu.ml^{-1} in A system between 0 and 72 h, after which the bacterial count returned to that present within the system 24 h after the circulation of water. For the remainder of the study period, bacterial count was 5 log. cfu.ml^{-1}. As to AF system, bacterial counts ranged between 5 and 6 log cfu $.ml^{-1}$ although some fluctuations took place from time to time.

Table 2.2 Coliform bacterial counts(averages) in test surface water systems during the experimental study

Time	Test Water/Coliform bacteria (cfu.10^{-2}ml)															
	A				*AF*			*AFC$_1$*				*AFC$_2$/AFC$_2$M*				
	TC	Ec	Sal	IJ	TC	Ec	Sal	TC	Ec	Sal	IJ	TC	Ec	Sal	IJ	
0 h	$1.51x10^4$	4	0	40	$1.54x10^4$	3	9	$1.54x10^4$	3	9	-	$1.54x10^4$	3	9	-	
20 min AC	N/A				N/A			17	3	4	5	17	0	3	2	
24 h	$2.07x10^4$	4	5	25	$3.00x10^3$	7	5	1	1	1	1	1	1	1	1	
20min AM	N/A				N/A			N/A				0	0	0	0	
48 h	21	3	1	10	26	6	0	1	0	0	1	0	0	0	0	
72 h	29	2	2	9	18	3	0	0	0	0	0	0	0	0	0	
168 h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

A: Alice chlorinated water from the tank, AF: After filtration water, AFC: After chlorination water, AFCM: Chlorine-monochloramine treated water, AC: After chlorination, AM: After monochloramination, N/A: not applicable. TC: total coliform, Ec: *Escherichia coli*, Sal: Salmonella, IJ: Injured bacteria.

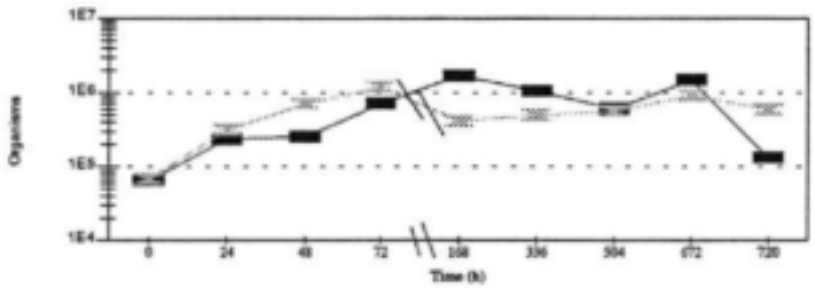

Fig.2.2 Growth of heterotrophic plate count bacteria in AF and A water systems during the experimental study.

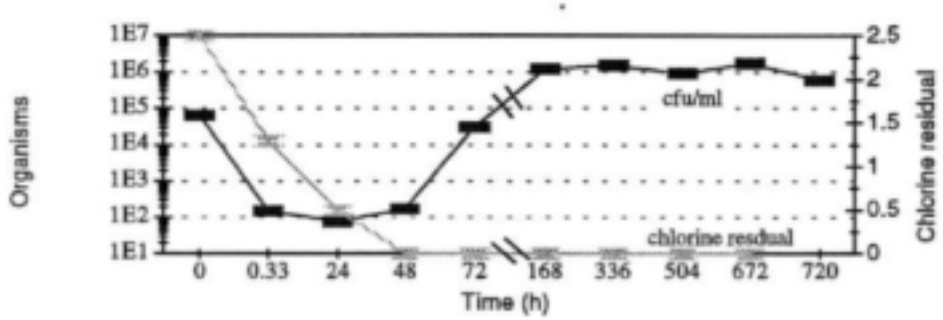

Fig. 2.3 Regrowth of heterotrophic bacteria during the depletion of chlorine residual in the chlorinated surface water system (AFC_1).

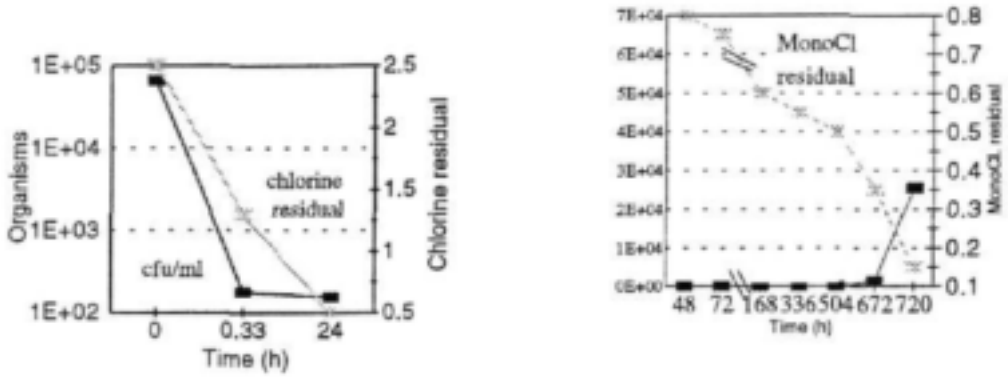

Fig. 2. 4 The effect of a combined chlorine-monochloramine disinfection process on the inhibition of heterotrophic plate count bacteria in the AFC_2M water system.

Chlorination of AF water resulted in a decline in HPC bacterial numbers in AFC_1 (Fig. 2.3) and AFC_2 (Fig.2. 4). Within the first 24 h, bacterial count in AFC_1 was 1 log cfu.ml^{-1}, after which this number returned to that originally present within AF water and numbers of HPC bacteria increased to a level greater than that originally present in AF. Although some fluctuations were observed, bacterial counts ranged between 5 and 6 log. cfu.ml^{-1} in AFC_1. The addition of monochloramine in the chlorinated water system (AFC_2) resulted in a total decline of HPC numbers. Less than 1 cfu.ml^{-1} was noted between 48 h and 504 h in the AFC_2M water system, after which bacterial number increased and returned to that originally found within AF water system for the rest of the study period (Fig.2.4).

2.3.1.4 Microbiological features on slides

Attached viable bacteria.

Average plate count values on slides surface are given in Figs. 2.5 -2.12. Within the first 24 h after the circulation of untreated and disinfected waters in separate systems, 4 log cfu.cm^{-2} HPC colonized the slides exposed to the control water (AF) and Alice chlorinated (A) water systems whereas 2 log cfu.cm^{-2} HPC colonized those exposed to chlorinated surface water (AFC_1 and AFC_2) systems. In the first three systems, a maximum plate count of 5 log cfu.cm^{-2} HPC covered SS and MS, but at different times (Figs. 2.5-2.7). In A system, this maximum HPC number was detected on SS within 72 h, after which bacterial count returned to that initially present on the slides and remained constant for the rest of the study period. On MS, the maximum plate count

was reached within 336 h and remained constant over the remainder of the study period (Fig.2.5). As to the AF water system, this number was observed on SS and MS slide surfaces within 46 h, after which some fluctuations took place from time to time (Fig. 2.6). In AFC_1 water system , the maximum plate of 5 log. $cfu.cm^{-2}$ was reached on SS and MS in 72 and 48 h respectively. The viable bacterial count remained constant on SS between 72 and 504 h whereas no specific change occurred on MS (Fig.2. 7). In the combined water system (AFC_2M), less than 1 $cfu.cm^{-2}$ was detected between 48 and 504 h, after which the viable counts of heterotrophic bacteria reached 3 log $cfu.cm^{-2}$ on SS and varied from 3 to 4 log $cfu.cm^{-2}$ on MS for the rest of the study period (Fig. 2.8).

Attached total cells (Epifluorescence)

As shown in Figs.2. 9-2.11, the average cell densities were higher in A (8 log $cells.cm^{-2}$) and AF(7 log $cells.cm^{-2}$) laboratory scale systems than in AFC_1 (6 log $cells.cm^{-2}$) (and AFC_2 water systems within the first day. For the rest of the experimental period, the average cell counts attached to the SS and MS surfaces varied between 6 and 7 log $cells.cm^{-2}$ in A water systems (Figs. 2.9). Stainless steel and MS slides exposed to AF system reached maximum cell densities of 8 log $cells.cm^{-2}$ and 7 $cells.cm^{-2}$ respectively. Similar observations were also noted on the surface of slides exposed to chlorinated water (Fig.2.11). No specific change in total cell counts occurred 20 min after the addition of monochloramine. Less than 1.$cell.cm^{-2}$ was counted between 48 h and 504 h after which a total bacteria count of 6 log $cells.cm^{-2}$ occurred on SS and MS within 678 h (Fig.2.12).

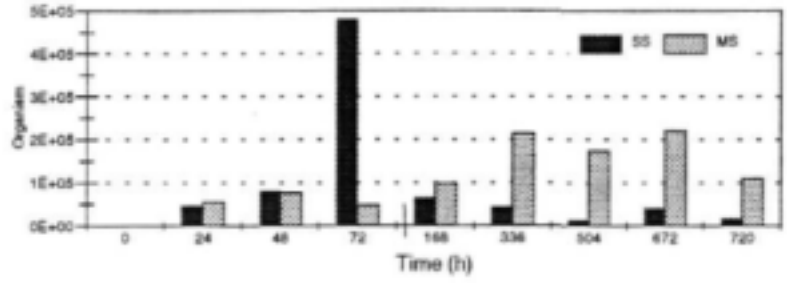

Fig.2.5 The average count of viable bacteria attached to the surface of stainless steel and galvanized mild steel slides exposed to the Alice chlorinated water system (A).

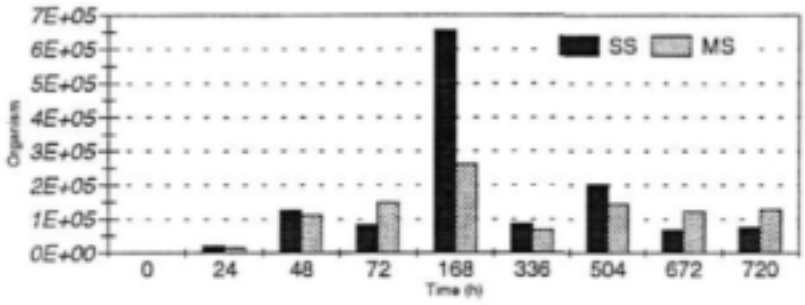

Fig. 2.6 The average count of viable bacteria attached to the surface of stainless steel and galvanized mild steel slides exposed to the AF water system.

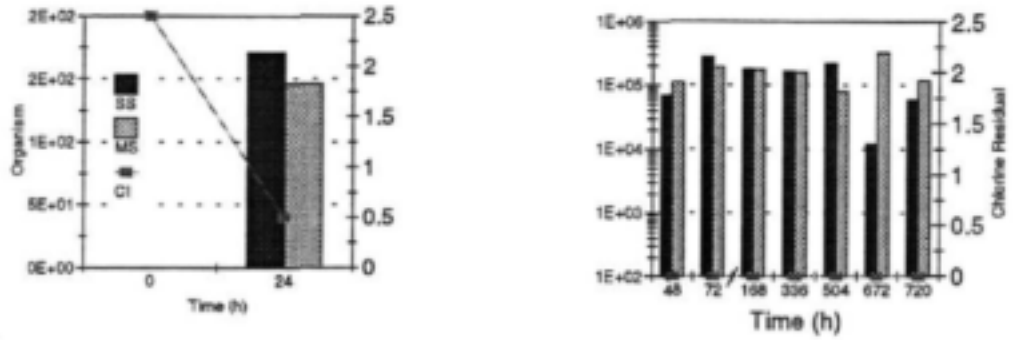

Fig. 2.7 The average count of viable bacteria attached to the surface of stainless steel and galvanized mild steel slides exposed to the chlorinated (AFC_1) water system between 0 and 24 h, and between 48 and 720 h.

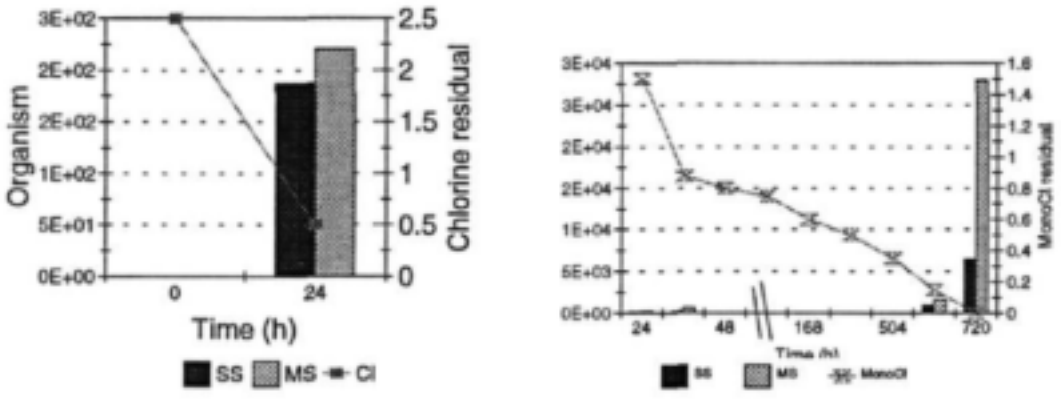

Fig.2.8 The average count of viable bacteria attached to the surface of stainless steel and galvanized mild steel slides exposed to the chlorinated surface water system (AFC_2) before and after the addition of monochloramine (AFC_2M).

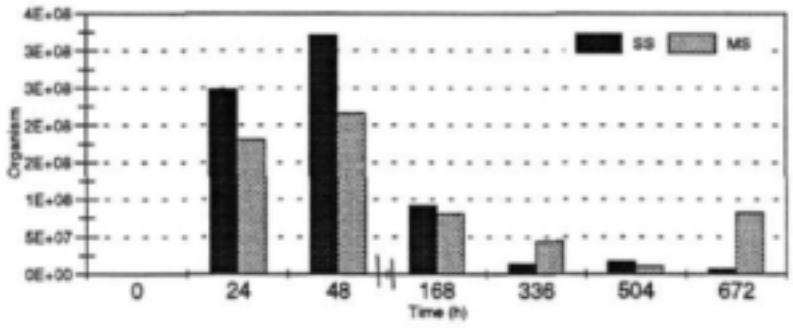

Fig. 2.9 The average counts of Total cells (epifluorescence) attached to the surface of stainless steel and galvanized mild steel slides exposed to the Alice chlorinated water system (A).

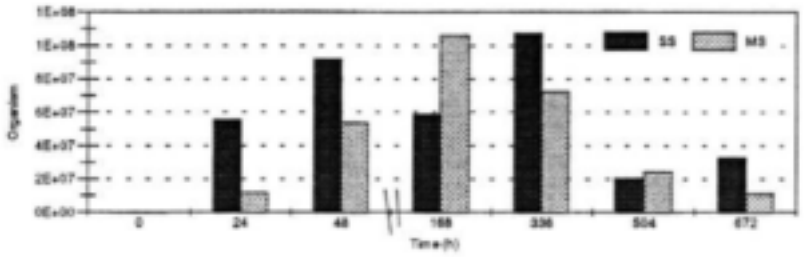

Fig.2.10 The average of total cells (epifluorescence) attached to the surface of stainless steel and galvanized mild steel slides exposed to AF water system.

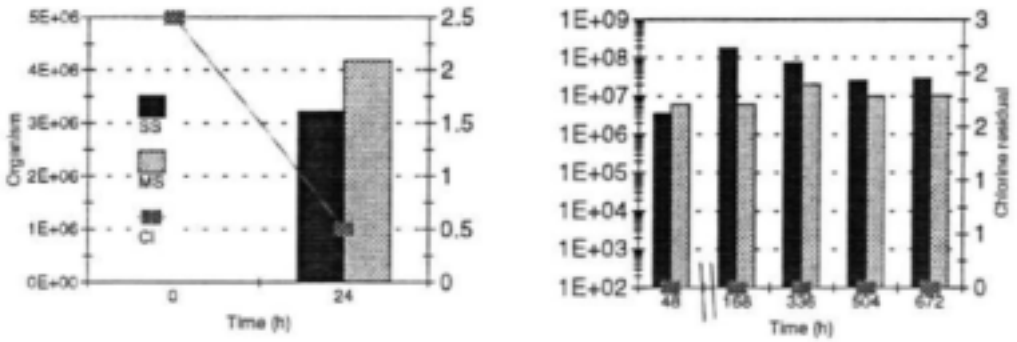

Fig. 2.11. The average counts of total cells (epifluorescence) attached to the surface of stainless steel and galvanized mild steel slides exposed to chlorinated water system (AFC_1) between 0 and 24 h, and between 48 and 672 h.

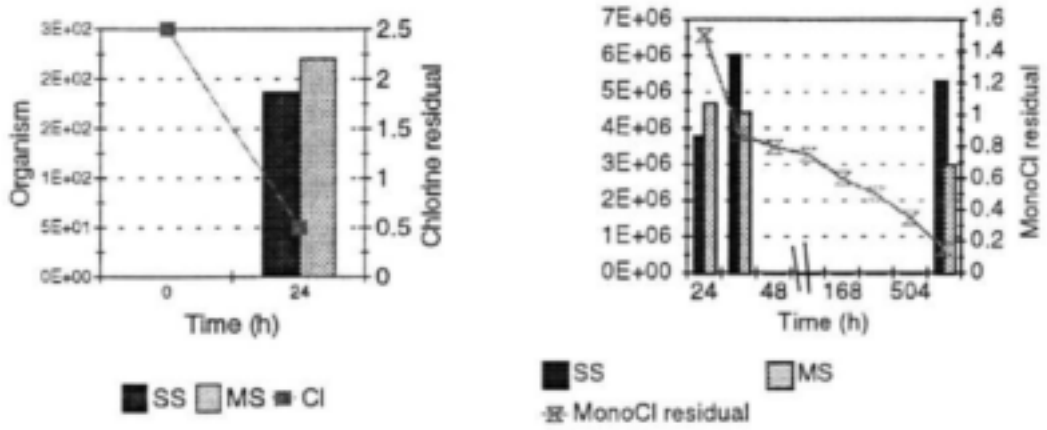

Fig.2.12 The average of total cells (epifluorescence) attached to the surface of SS and MS exposed to the chlorinated water system (AFC_2) before and after the addition of monochloramine (AFC_2M).

Visualization of cells on slides (SEM)

SEM observations indicated the presence of biofilms cells on the slide surfaces exposed to A, AF, and AFC_1 during the study period (Plates.3 to 5, 7 to 9, 11 to 13, 15 to 17). No biofilm cells were detected on the slides exposed to AFC_2M for the first 504 h of the experimental study (Plates 6, 10, 14). The formation of biofilm could be observed on the slides exposed to AFC_2M 678 h after disinfection (Plate 18).

2.3.1.5 Statistical analyses

Analysis of the variance

During the study period, significant differences were found between bacterial counts as well as between treatments at $p > 0.05$.

A significant difference was noted between the four categories of coliform bacteria (TC, Ec, Sal and IJ) with F-ratio test value of 8.83 and $p < 0.0001$. The LSD test gave the following results for the mean counts at $p < 0001$: TC: 3.2 cfu. 10^{-2}ml, Ec: 0.0563 cfu. 10^{-2}ml, Sal: 0.0243 cfu.10^{-2} ml, IJ: 0.0998 cfu 10^{-2} ml. The summary F ratio test for the treatment effect was 11.5 with $p<0001$. The LSD test showed the following mean bacterial counts: A: 1.6 2cfu.10^{-2} ml, AF: 0.932 cfu.10^{-2} ml, AFC: 0.0722 cfu.10^{-2} ml, AFC_2M: 0.0078 cfu.10^{-2} ml .

The summary F-ratio test for the treatment effect on heterotrophic plate count bacteria was 15.04 with $p < 0001$. The LDS test showed AFC_2M being significantly different from A, AF, and AFC with $p < 0001$ and a mean count value of 2.77 cfu. ml^{-1} ml. No significant difference ($p > 0$) in mean bacterial counts was noted between the A, AF, AFC_1 (A: 4.75 x 10^5 cfu. ml^{-1}, AF: 4.50 x 10^5 cfu.ml^{-1}, AFC_1: 7.13 x 10^4 cfu. ml^{-1}).

As regards the attached total cells, the summary F-ratio test for the treatment effect was 43.8 at $p < 00001$. The LSD test showed significant difference between the slides exposed to AFC_2M water systems and those exposed to other systems ($p < 0001$). The LSD means for bacterial counts

Plate 1

Plate 2

Plate 3

Plate4

Plate 5

Plates 1-5 SEM photographs showing the microbiological features of the stainless steels slides before (Plate 1) and 24h after exposure to the A (Plate 2), AF (Plate 3), AFC_1 (Plate 4), AFC_2M (Plate 5) water systems.

Plate 6

Plate 7

Plate 8

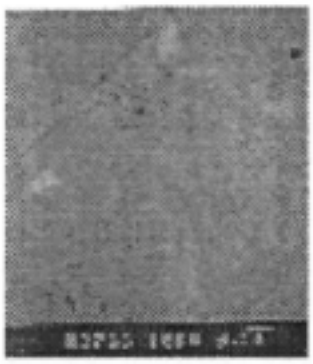

Plate 9

Plates 6-9 SEM photographs showing the microbiological features of the stainless steels slides 336 h after exposure to the A (Plate 6), AF (Plate 7), AFC_1 (Plate 8), AFC_2M (Plate 9) water systems.

Plate 10

Plate 11

Plate 12

Plate 13

Plates 10-13 SEM photographs showing the microbiological features of the stainless steels slides after 720 h exposure to the A (Plate 10), AF (Plate 11), AFC_1 (Plate 12), AFC_2M (Plate 13) water systems.

Plate 14

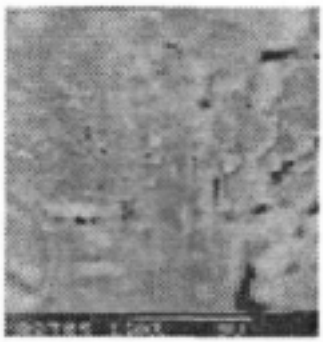

Plate 15

Plate 16

Plate 17

Plate 18

Plates 14-18 SEM photographs showing the microbiological features of galvanized mild steel slides before (Plate 14) and after 24 h exposure to the A (Plate 15), AF (Plate 16), AFC_1 (Plate 17), AFC_2M (Plate 18) water systems.

Plate 19

Plate 20

Plate 21

Plate 22

Plates 19-22 SEM photographs showing the microbiological features of the galvanized mild steel slides after 336 h exposure to the A (Plate 19), AF (Plate 20), AFC_1 (Plate 21), AFC_2M (Plate 22) water systems.

Plate 23

Plate 24

Plate 25

Plate 26

Plates 23-26 SEM photographs showing the microbiological features of the galvanised mild steel slides after 720 h exposure to the A (Plate 23), AF (Plate 24), AFC_1 (Plate 25), AFC_2M (Plate 26) water systems.

were significantly lower on the slides exposed to AFC_2M (SS : 0.18 cfu.cm^{-2}, MS: 0.24 cfu.cm^{-2}) than on those exposed to A (SS: 1.73 x10^4 cfu.cm^{-2}, MS: 1.10 x 10^5 cfu.cm^{-2}), AF (SS: 1.0 x 10^5 cfu.cm^{-2}, MS: 9.8 x 10^4 cfu.cm^{-2}), AFC_1 (SS: 4.5 x 10^4 cfu.cm^{-2}, MS: 6.3 x 10^4 cfu.cm^{-2}). However, no significant differences in bacterial counts were revealed among slides exposed to A, AF and AFC_1 ($P > 0.5$). Within treatments, the LSD test also indicated no significant difference between slides ($p > 0.9$ for AFC_2M, $p > 05$ for A, AF, AFC_1).

The summary F-ratio test for the treatment effect on attached total cells was 24.3 with $p < 0001$. The LDS test showed that cell densities attached to the slides exposed to AFC_2M were significantly different from those attached to the slides exposed to A, AF and AFC with $p <$ 0001. The mean bacterial counts for SS and MS slides exposed to AFC_2M were 1.65 and 1.54 cfu.cm^{-2} respectively, which were significantly different from the mean counts for the slides exposed to A (SS: 7.10 x 10^7 cells.cm^{-2}, MS: 5.08 x 10^7.cells.cm^{-2}), AF (SS: 5.29 x 10^7 cells.cm^{-2}, MS: 3.30 x 10^7 cells.cm-2) and AFC_2 (SS: 1.50 x 10^7 cells.cm^{-2}, MS: 8.29 x 10^6 cells.cm^{-2}). No significant difference were noted between slides within treatments ($p > 0.98$ for AFC_2M. and $p > 0.5$ for A, AF, AFC_1).

Correlation analysis

Correlation was established between the concentrations of bacterial counts and those of various physicochemical parameters in the combined water systems.

Correlation values for bacterial counts with calcium, magnesium, chemical oxygen demand , dissolved organic carbon, total nitrogen, phosphate and sulphate are shown in Table 3. A weak negative correlation was observed between calcium and different group of bacterial counts (CB, HPC, AVB, ATC). Chemical oxygen demand, dissolved organic carbon and phosphate showed a weak positive correlation with all the bacterial counts. Strong positive correlations were registered for magnesium, while a high negative correlation for sulphate and nitrogen with all the bacterial counts (CB, HPC, AVB, and ATC).

A box plot for the physicochemical parameters revealed little variation in the temperature, pH, turbidity and phosphate on the one hand; and large variations in calcium, magnesium, chemical oxygen demand , dissolved organic carbon and sulphate on the other hand (Fig. 2.13).

Table 2.3. Correlations between bacterial counts and concentrations inorganic and organic compounds within surface water systems.

Parameters	Group of bacteria			
	CB r	HPC r	AVB r	ATC r
Calcium	0.07	-0.05	-0.32	-0.30
Magnesium	0.97	0.92	0.88	0.90
COD	0.39	0.51	0.34	0.33
DOC	0.40	0.54	0.45	0.42
Phosphate	0.01	0.16	0.13	0.09
Sulphate	-0.99	-0.96	-0.90	-0.92
Total Nitrogen	-0.61	-0.55	-0.74	-0.74

r = correlation value, CB = coliform bacteria, HPC= heterotrophic bacteria, AVB= attached viable bacteria, ATC= attached total cells, COD= chemical oxygen demand

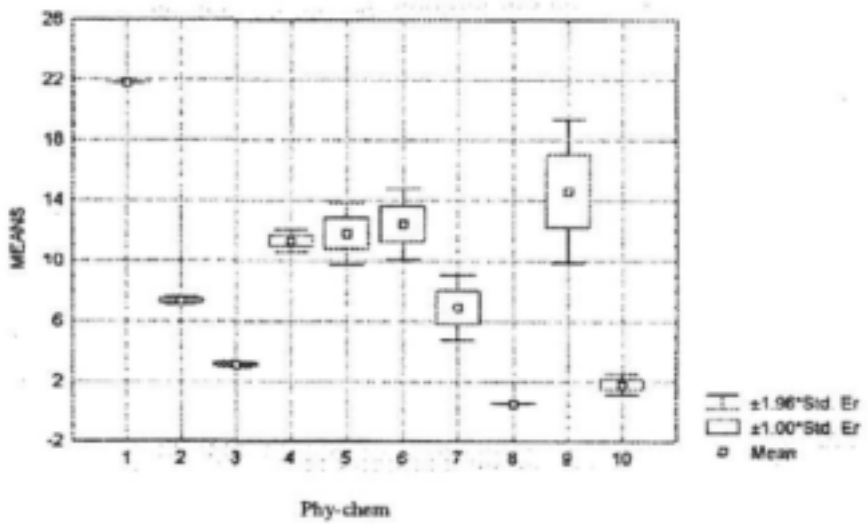

Fig. 2.13 Categorized plot for variables : Mean values for the physico chemical parameters 1: Temperature, 2: pH, 3: Turbidity, 4 Calcium, 5: Magnesium, 6:COD, 7: DOC, 8: Phosphate, 9: Sulphate, 10: Nitrogen.

2.3.2 Groundwater

2.3.2.1 Physico-chemical quality of test waters

With the exception of pH, nitrate, PO_4^{3-} and SO_4^{2-}, organic and inorganic compounds in all laboratory scale systems were above the limit allowed by the *South African Water Quality Standards*. Although an increase in the DOC concentration was observed after the disinfection of groundwater, a higher DOC concentration was recorded in the combined water system (GWC_2M) than in the chlorinated water system (GWC_1). High COD, nitrate, SO_4^{2-} concentrations and high temperatures were also recorded in the combined system during the study period. The turbidity and pH were found to be approximately similar in all the water systems (Table 2.4).

Table 2. 4: The physico-chemical characteristics (average values) of test groundwater water

Water source	Parameter (Means)									
	T° (°C)	pH	NTU	Ca^{2+} $mg.\ell^{-1}$	Mg^{2+} $mg.\ell^{-1}$	COD $mg.\ell^{-1}$	DOC $mg.\ell^{-1}$	N $mg.\ell^{-1}$	PO_4^{3-} $mg.\ell^{-1}$	SO_4^{2-} $mg.\ell^{-1}$
GW	19.88	7.77	2.21	63.75	79.83	5.42	6.27	4.76	0.35	58.67
GWC_1	22.01	7.89	2.34	64.5	77.15	5.43	8.32	3.69	0.49	56.83
GWC_2/ GWC_2M	24.43	7.67	2.18	57.13	77.00	7.14	12.60	9.02	0.4 0	69.25

GW: Alice groundwater from the borehole, GWC_1: After chlorination, GWC_2M: After monochloramination

2.3.2.2 Disinfectant residuals

Over a period of 24 h after disinfection free chlorine concentration decreased from 2.53 to 0.45 $mg.l^{-1}$ in GWC_1 and GWC_2 water systems, after which complete disappearance of free chlorine residual occurred in GWC_1 between 48 h and 72 h. The addition of 1.5 $mg.l^{-1}$ monochloramine in GWC2 water resulted in the persistence of the disinfectant residual that could be detected up

to 7d (Table 4). Between 7 d and 28 d no disinfectant residual was noted in the combined water system (GWC2M).

2.3.2.3 Microbiological features in test waters

Coliform bacteria

Table 2.5 illustrates the average number of coliform bacteria detected in control (GW) and disinfected (GWC_1 and GWC_2/GWC_2M) water systems. Groundwater was characterized by higher counts of total and fecal coliforms than the limit allowed by *South African Water Quality Standards*. The detection of presumed *E.coli* and *Salmonella* in the initial water confirmed the presence of fecal coliform. Although a gradual decrease in coliform bacterial counts was recorded in the control (GW) water system the persistence of coliforms was observed during the entire period of the experimental study.

A higher number of total and faecal coliform was removed in GWC_2 (TC: 99.20%, FC: 0) than in GWC_1 (TC: 98.71%, FC: 86.02%) within 20 minutes, although a similar concentration of 2.53 $mg.\ell^{-1}$ chlorine was used to disinfect groundwater in GWC_1 and GWC_2 systems (Table 2.5). While the presence of coliform was observed between 24 and 672 h in GWC_1, the addition of 1.5 $mg.\ell^{-1}$ monochloramine in GWC_2 led to the complete removal of coliform within 48 h.

Heterotrophic plate count bacteria

An initial heterotrophic bacterial count of 4 log $cfu.ml^{-1}$ was recorded in groundwater before the transfer of water into different systems. In GW, heterotrophic plate count increased from 4 log to 6 log $cfu.ml^{-1}$ between 0 h and 338 h, after which a decrease was noted and the bacterial count was 5 log $cfu.ml^{-1}$ over the remainder of the study period (Fig.2.14).

Twenty minutes after chlorination, a decline in the HPC number (from 4 log to 3 log $cfu.ml^{-1}$) was noted in both GWC1 and GWC2 water systems (Figs.2.15 to 2.16). Over the remainder of the study period bacterial numbers in GWC_1 increased and reached a level greater than that originally present in the initial water; the maximum bacterial number was 6 log $cfu.ml^{-1}$ (Fig. 2.15)

Table 2.5 Groundwater: Coliform bacterial counts (average) in different test water systems during the experimental study

Time	Test Water/Coliform bacteria (cfu.10^{-2}ml)														
	GW					*GWC1*					*GWC2/GWC2M*				
	TC	Ec	Sal	FC	IJ	TC	Ec	Sal	FC	IJ	TC	Ec	Sal	FC	IJ
0 h	2.01x1034	$1.01x10^2$	$1.03x10^2$	93	-	$2.01x10^3$	$1.01x10^2$	$1.03x10^2$	93	-	$2.01x10^3$	$1.01x10^2$	$1.03x10^2$	93	-
20 min AC	N/A			N/A		26	0	27	13	8	16	1	16	0	
						19									
24 h	380	10	34	67	-	10	0	19	0	0	4	1	3	0	0
20min AM	N/A		N/A			N/A					3	1	3	0	0
48 h	185	9	10	35	-	6	0	6	0	0	0	0	0	0	0
72 h	260	12	9	12	-	7	2	4	0	0	0	0	0	0	0
168 h	29	5	10	4	-	18	0	5	0	0	0	0	0	0	0
336 h	74	1	2	0	-	20	1	2	0	0	0	0	0	0	0
504 h	17	6	3	0		7	0	2	0	0	0	0	0	0	0
678 h	16	2	3	4		8	0	1	0	0	0	0	0	0	0

GW: Alice groundwater from the borehole, GWC: After chlorination, GWCM: chlorine-monochloramine treated water, WM: After monochloramine, WC: after chlorination, N/A: not applicable, TC: Total coliform, IJ: Injured coliform, FC: Faecal coliform, Sal: Salmonella, Ec: *Escherichia coli*

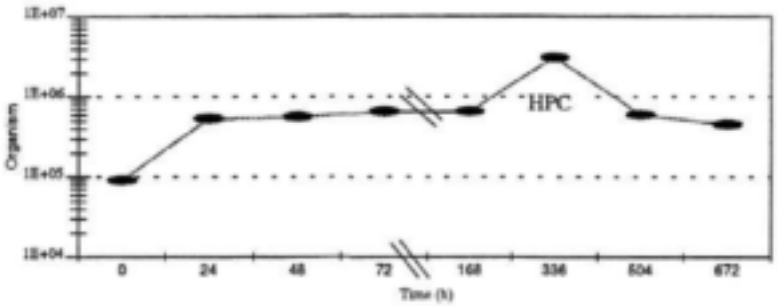

Fig.2.14 Growth of heterotrophic plate count bacteria in the GW water system during the experimental study.

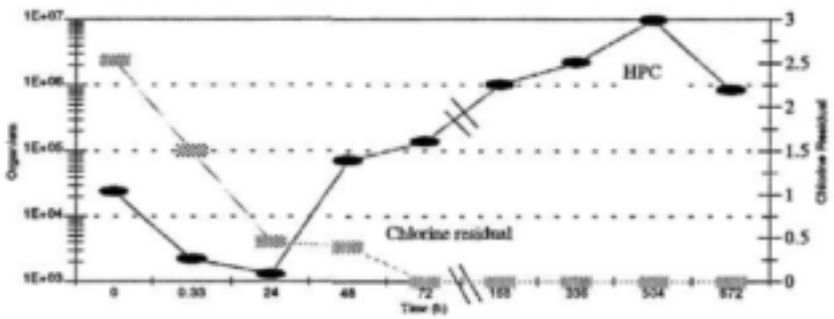

Fig. 2.15 Regrowth of heterotrophic plate count bacteria during the depletion of chlorine residual in a chlorinated groundwater system (GWC1).

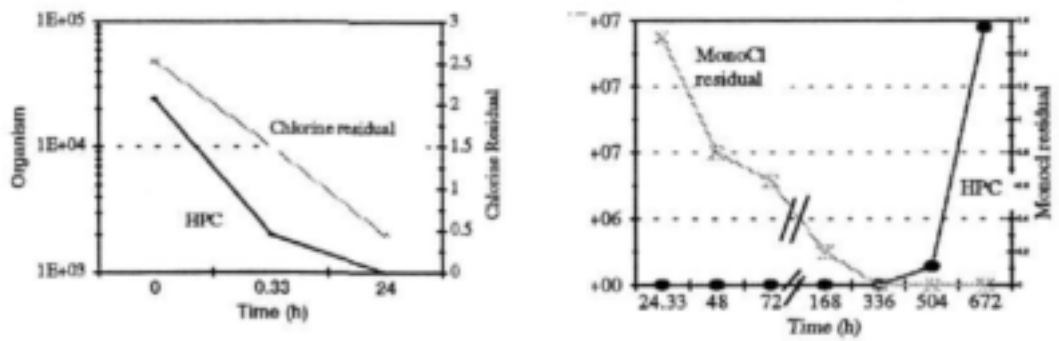

Fig. 2.16 The effect of a combined chlorine-monochloramine disinfection process on the inhibition of heterotrophic plate count bacteria in the GWC2M water system.

In GWC_2, the addition of monochloramine resulted in a total decline of heterotrophic bacteria. Less than 1 cfu.ml^{-1} was recorded in GWC_2M between 24.33 h and 168 h, after which bacterial number returned to that originally present in the initial water within 336 h. Between 336 h and 672 h a maximum count of 7 log cfu.ml^{-1} was recorded in GWC_2M (Fig.2.16).

2.3.2.4 Microbiological features on slides

Attached viable bacteria

Twenty four hours after the exposure of the slides to the untreated water (GW) system, 2 log cfu.cm^{-2} HPC and 3 log cfu.cm^{-2} HPC colonized SS and MS slides respectively. Although fluctuations took place from time to time, no specific increase in attached viable counts was observed on the surface of either SS or MS slides(Fig 2.17).

In the chlorinated water system, viable bacteria attached to the piping material within the first day (Figs.2.18-2.19). In GWC1 2 log cfu.cm^{-2} HPC colonized both SS and MS slides. Between 24 and 672 h, attached viable bacteria on the surface of SS slides reached a maximum plate count of 4 log cfu.cm^{-2} HPC. As to the MS, a decrease in attached viable bacterial counts was observed between 24 and 48 h, after which this number returned to that initially observed within 24 h. Attached viable bacterial count on MS reached a maximum plate of 4 log cfu.cm^{-2} HPC within 504 h and this number remained constant for the rest of the study period (Fig. 2.18).

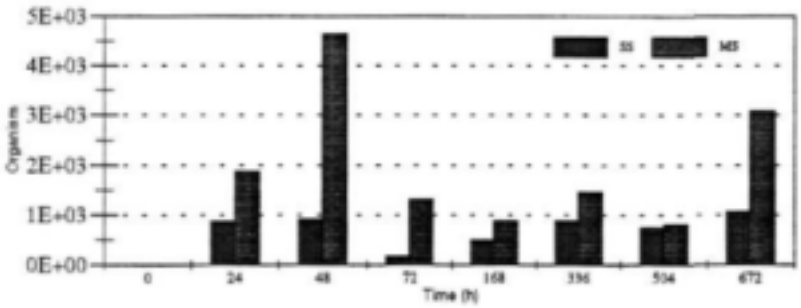

Fig.2.17 The average count of viable bacteria attached to the surface of SS and MS slides exposed to the GW water system.

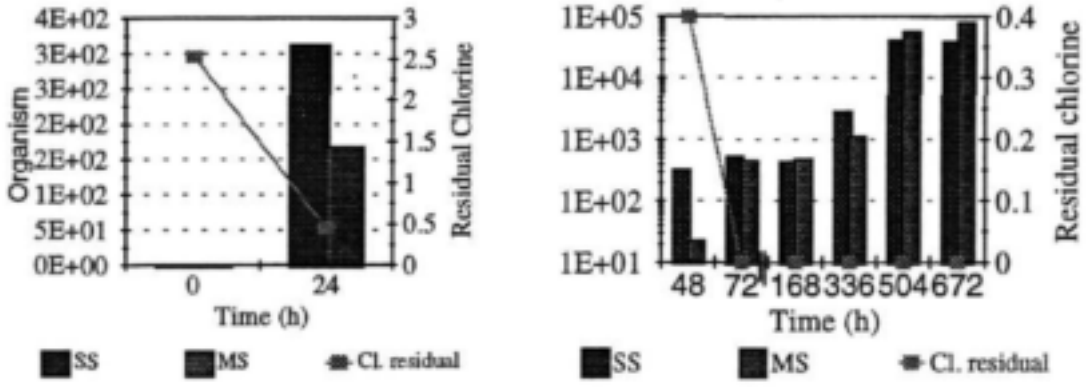

Fig. 2.18 The average count of viable bacteria attached to the surface of SS and MS slides exposed to the GWC1 water system.

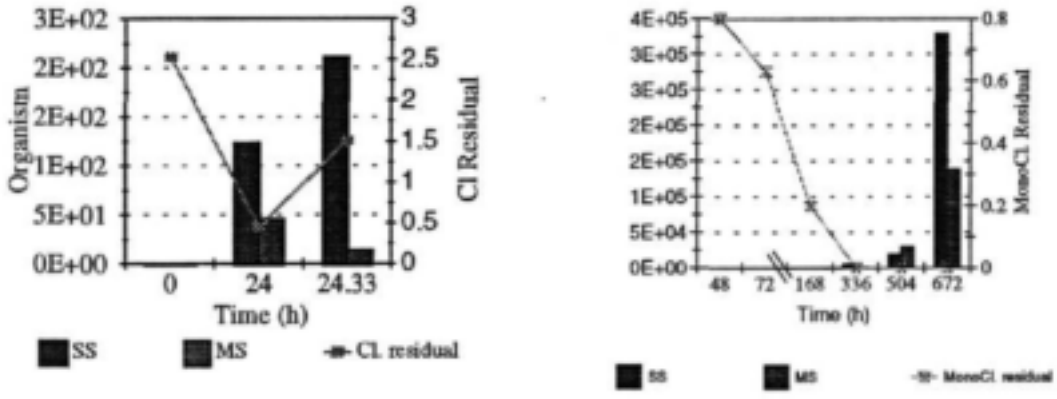

Fig. 2.19 The average count of viable bacteria attached to the surface of SS and MS slides exposed to GWC2/GWC2M water systems.

In the GWC2 water system, 2 log cfu.cm^{-2} HPC attached to SS and 1 log cfu.cm^{-2} HPC colonized MS within 24 h. Between 24 and 24.33 h no remarkable change in viable count occurred on MS. With the addition of monochloramine in the system (GWC2), less than 1 log cfu.cm^{-2} HPC was detected on MS between 24.33 and 168 h after which bacterial count returned to a level greater than that initially detected on the first 24 h. 1 log cfu.cm^{-2} HPC gradually increased to a maximum count of 5 log cfu.cm^{-2} HPC between 336 and 672 h (Fig.2.19).

Attached total bacteria

An average cell density of 5 log cells.cm^{-2} was noted on SS and MS slides exposed to GW system. This number progressively increased to 6 log cells.cm^{-2} between 24 h and 504 h, after

which no remarkable increase was noted over the remainder of the study period for both MS and SS slides (Fig. 2.20).

In the GWC1 water system, an average count of 4 log cells.cm^{-2} colonised SS and MS slides, after which an increase in bacterial number occurred within 72 h for SS and 168 h for Ms, and bacteria reached an average count of 5 log cells.cm^{-2}. Although some fluctuations occurred on SS slides, the maximum bacterial count was 5 log cells.cm^{-2} for both SS and MS during the study period (Fig. 2.21).

In GWC2, an initial average cell density of 4 log cells.cm^{-2} colonised both types of piping material. This number remained almost unchanged within the first hours of monochloramine addition to GWC2. As shown in Fig. 6, less than 1 cells.cm^{-2} was observed on the slides 24 h after the addition of monochloramine. Between 48 and 168 h no remarkable change occurred in cell density colonising both types of slides, after which bacterial counts returned to a number greater than that initially colonising the slides. An average count of 5 log cells.cm^{-2} was observed on the slide exposed to GWC2M between 168 and 336 h. A maximum average count of 6 log cells.cm^{-2} was observed over the remainder of the study period (Fig. 2.22).

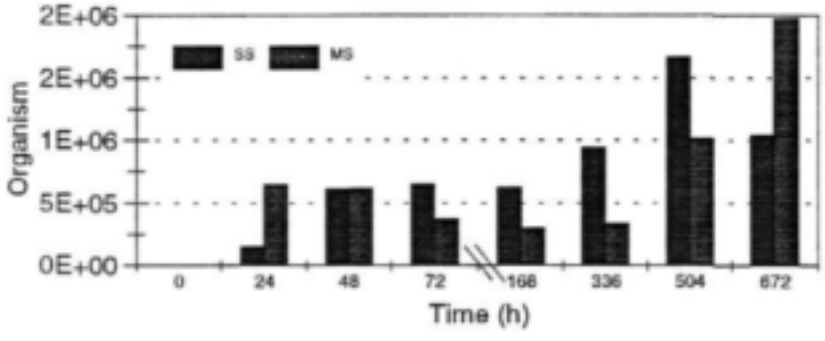

Fig.2.20. The average count of total cells (epifluorescence) attached to the surface of SS and MS slides exposed to the GW water system.

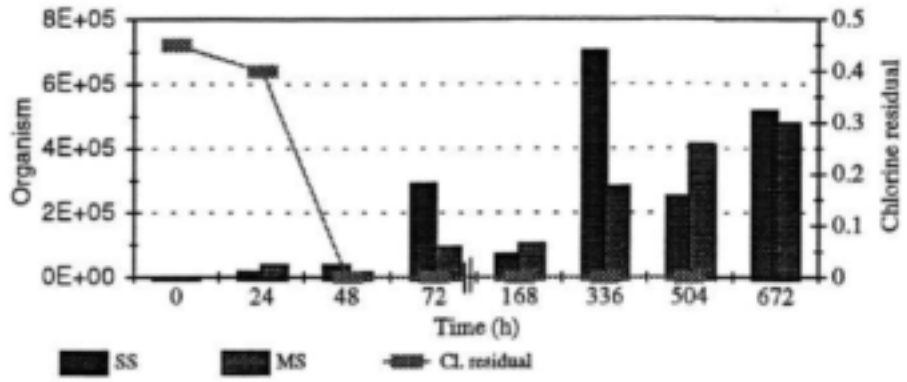

Fig.2.21 The average counts of total cells (epifluorescence) attached to the surface of SS and MS slides exposed to the GWC1 water system.

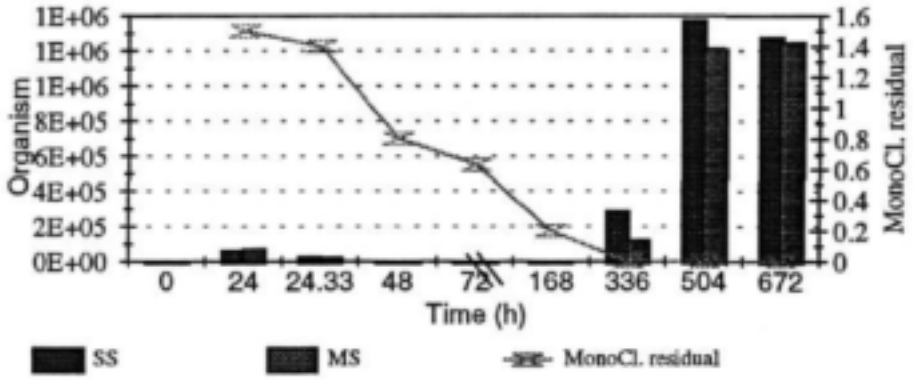

Fig.2.22 The average of total cells (epifluorescence) attached to the surface of SS and MS exposed to the GWC2M water system.

Visualization of cells on slides (SEM)

SEM observations revealed the presence of biofilm cells on the surface of slides exposed to GW, GWC1 and GWC2 during the entire study period (Plates 28 to 30, 31 to 32, 34 to 35,38,39,40 to 42,44,45). No biofilm cells were detected on the slides exposed to GWC2M between 24 and 168 h of the experimental study (Plates 33,43,) after which biofilm cells could be observed on the slides exposed to GWC2M over the remainder of the study period (Plates 36,46).

2.3.2.5 Statistical Analyses

Analysis of variances

When considering the entire experimental study, the F-ratio test as well as the LSD test showed no significant difference in mean counts for HPC (GW = 7.1 x 10^5, GWC_1= 1.4 x10^6, GWC_2M = 2.1 x 10^6) between the three water systems at $p > 0.05$. The summary F-ratio test for equality

of bacterial means between the three systems was 0.3 (p= 0.74). However, taking into account the study period ranging between 24 and 168 h, the F-ratio test and the LSD test showed GWC_2M being significantly different from GW and GWC_1 at $p > 0.05$. The summary F- ratio count for HPC during this period was 6.69 ($p = 0.011$). The LSD test gave the following results for the means counts: GW: 5.86×10^5 , GWC1 :2.5×10^5, GWC2M: 2.0×10^2.

The summary F- ratio test for the three treatment effects of attached viable bacteria (AVB) during the entire period of the experimental study was as follows: 0.92 ($p = 0.42$) and 3.07 ($p = 0.068$) for SS and MS respectively. Between the three systems, no significant difference for the equality of bacterial means at $p > 0.05$ was noted when considering this period. The following means were recorded when using the LSD test: GW= 4.42×10^4, $GWC_1 = 1.05 \times 10^4$, $GWC_2M = 7.54 \times 10^2$). Between 24 and 168 h the summary of the F- ratio test was 11.44 ($p = 0.0017$) for SS slides and 9.07 ($p = 0.004$) for MS slides. The LSD test showed significant difference in AVB counts between the slides exposed to GWC_2M and those exposed to other water systems at $p > 0.05$. The LSD means for attached viable bacteria on SS were 6.66×10^2, 3.78×10^2, and 66 $cfu.cm^{-2}$ in GW, GWC1 and GWC2 respectively, and on MS they were 2.12×10^3, 2.55×10^2, and 11 $cfu.cm^{-2}$ in GW, GWC1 and GWC2 respectively. No significant difference in AVB counts was found among the slides at $p > 0.05$.

As to the attached total cells (ATC), the F- ratio test and the LSD test indicated no significant difference in ATC counts between the slides exposed to disinfectant waters and those exposed to untreated water when considering the entire study period. The summary F- ratio test for the effect of three water systems on ATC counts was as the follows: SS = 2.32 ($p = 0.12$) and MS = 0.64 ($p = 0.54$). The LSD test gave the following mean bacterial counts: GW: 2.11×10^4 , GWC_1: 1.10×10^4, GWC_2M: 2.9 x 103. Between 24 and 168 h the LSD test showed significant difference in total cells attached to the slides exposed to the combining system and those exposed to chlorinated and untreated waters. The summary F- ratio test for the three treatment effects on ATC between 24 and 168 h was 9.05 ($p=0.0017$) for SS and 9.07 ($p=0.004$) for MS. The LSD test means for LSD counts were as follows: GW: 4.36 x 105 $cfu.cm^{-2}$ GWC_1: 8.74×10^4 $cfu.cm^{-2}$, GWC_2M: 1.64×10^4 $cfu.cm^{-2}$ for SS and GW: 5.15×10^5 $cfu.cm^{-2}$, GWC1: 5.72×10^4 $cfu.cm^{-2}$, GWC2: 1.82×10^4 $cfu.cm^{-2}$. No significant difference was reported among the slides.

Plate 27

Plate 28

Plate 29

Plate 30

Plates 27-30 SEM photographs showing the microbiological features of stainless steel slides before (Plate 27) and after 24 h exposure to the GW (Plate 28), GWC1 (Plate 29), GWC2 (Plate 30) water systems.

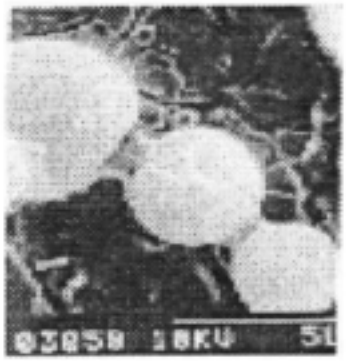

Plate 31

Plate 32

Plate 33

Plates 31-33 SEM photographs showing the microbiological features of stainless steel slides after 168 h exposure to the GW (Plate 31), GW1 (Plate 32), GWC2M (Plate 33) water systems.

Plate 34

Plate 35

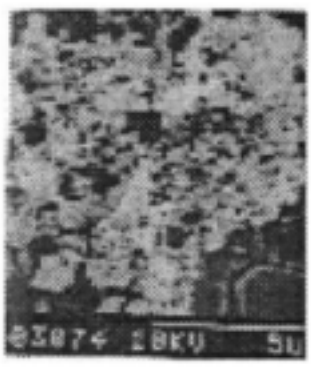

Plate 36

Plates 34-36 SEM photographs showing the microbiological features of stainless steel slides after 720 h exposure to the GW (Plate 34), GW1 (Plate 35), GWC2M (Plate 36) water systems.

Plate 3 7

Plate 38

Plate 39

Plate 40

Plates 37-40 SEM photographs showing the microbiological features of the galvanized mild steel slides before (Plate 37) and after 24 h exposure to the GW (Plate 38), GWC1 (Plate 39),GWC2 (Plate 40) water systems.

Plate 41

Plate 42

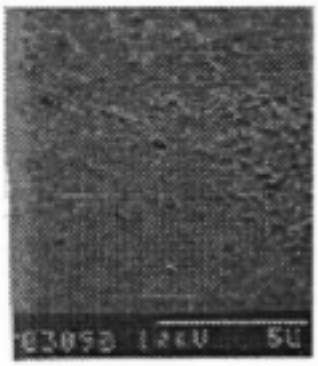

Plate 43

Plates 41-43 SEM photographs showing the microbiological features of galvanized mild steel slides after 168 h exposure to the GW (Plate 41), GWC1 (Plate 42), GWC2M (Plate 43) water systems.

Plate 44

Plate 45

Plate 46

Plates 44-46 SEM photographs showing the microbiological features of stainless steel slides after 720 h exposure to the GW (Plate 44), GW1 (Plate 45), GWC2M (Plate 46) water systems.

Correlation Analysis

Correlation values for bacterial counts with calcium, magnesium, chemical oxygen demand, dissolved organic carbon, nitrate, phosphate and sulfate are illustrated in Table 2.6. A weak negative correlation was observed between calcium and HPC and AVB, COD and AVB, nitrate and ATB, sulfate and CB. A strong negative correlation was observed between calcium and ATB, magnesium, dissolved organic carbon and phosphate also showed a strong negative correlation with CB, while chemical oxygen demand showed a strong negative correlation with HPC and CB. On the other hand chemical oxygen demand and sulfate showed a weak positive correlation with ATB, while magnesium showed a weak correlation with HPC and AVB. Nitrate showed weak positive correlation with HPC, AVB and CB. Dissolved organic carbon and phosphate showed a weak positive correlation with HPC, AVB and ATC. Strong positive correlation was registered between calcium and CB, magnesium and ATB, and sulfate and HPC.

A box plot for the physico-chemical parameters indicated very little variation for temperature, turbidity, pH, phosphate and COD on one hand, and little variation was noted for calcium, magnesium, DOC, nitrate and sulphate (Fig.2.23) .

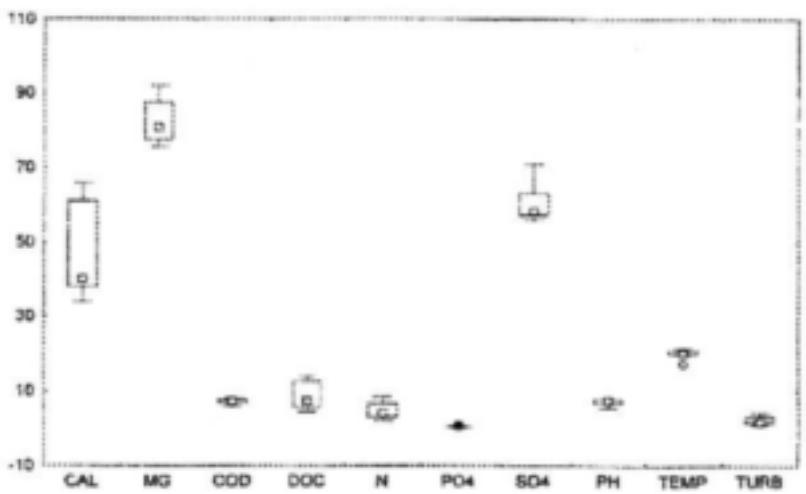

Fig. 2.23. Categorized plot for variables : Means values for the physico chemical parameters.

Table 2.6: Correlation between bacterial counts and concentrations (average values) of inorganic and organic compounds in water systems

	Group of bacteria			
Parameters	CB	HPC	AVB	ATC
Calcium	0.827	-0.279	-0.308	-0.614
Magnesium	-0.534	0.241	0.335	0.817
COD	-0.819	-0.614	-0.046	0.336
DOC	-0.643	0.433	0.555	0.294
Nitrate	0.205	0.240	0.203	-0.086
Phosphate	-0.6101	0.002	0.035	0.036
Sulfate	-0.266	0.749	0.681	0.329

r = correlation value, CB = coliform bacteria, HPC= heterotrophic bacteria, AVB= attached viable bacteria, ATC= attached total cells (epifluorescence), COD= chemical oxygen demand

2.3.3 Comparison of statistical data between surface and ground water combined systems

As indicated in Table 2.7, the F-ratio test as well as the LSD test showed no significance difference in mean counts for all bacterial groups between AFC2M and GWC2M when considering the study period of 504 h.

2.4 DISCUSSION

The aim of this study was to evaluate a combined chlorine-monochloramine disinfection process for the inhibition of bacterial and biofilm regrowth in a laboratory-scale system. The emphasis was placed on the maintenance of an effective disinfectant residual throughout the system. The monitoring of the microbiological quality of treated water or bactericidal effectiveness of the process relied on enumerating the coliforms, heterotrophic plate count and total bacteria.

Table2.7 Comparison of AFC2M and GWC2M for different bacterial groups.

Bacterial group	F- ratio value	P	LSD Means	
			AFC2M	GWC2M
TC	0.74	0.40	1.71×10^3	2.24×10^2
Ec	0.96	0.34	11.44	0.44
Sal	0.93	0.35	12.11	1.11
IJ	1.00	0.33	0.13	0.00
HPC	1.02	0.33	2.12×10^6	7.4×10^3
AVB SS	1.16	0.29	1.55×10^2	3.92×10^4
AVB MS	1.45	0.25	2.79×10^2	1.88×10^4
ATC SS	2.32	0.15	1.68×10^6	3.35×10^5
ATC MS	2.17	0.16	1.35×10^6	2.96×10^5

Disinfectant effectiveness

Results of this study revealed that the intake water from the Alice purification system as well as that of Dyamala and Kwa-Nkobonkobo groundwater (as used by the local community) were of poor quality. Whereas the average counts of 3 log cfu.100ml^{-1} TC and 4 log cfu.l^{-1} HPC were detected in the initial groundwater samples, the averages of 4 log cfu.100ml^{-1} TC and 4 log cfu.1ml^{-1} HPC were recorded in the initial surface water samples. There was no significant difference between Alice chlorinated water (A) and Alice partially treated water (AF). Initial bacterial counts of 4 log cfu.10^{-2}ml total coliforms and 4 log cfu.ml^{-1} HPC were detected in both A and AF laboratory scale systems. In terms of the South Africa Water Quality Standards (DWAF, 1996), such indicator count levels indicate an increased risks of infectious disease transmission.

Our findings confirm previous studies done on the microbiological quality of Alice drinking water (Muyima and Ngcakani, 1998; Momba and Dumani, 1999). High levels of indicator bacteria in Alice chlorinated water could be related to the malfunctioning of the Alice water purification system. Although potable water purification in Alice involves flocculation, sedimentation, sand filtration and chlorination, Alice treatment plant does not have any

laboratory facilities for assessing the dosage of chemicals used for the treatment of water or for routine monitoring of water quality in order to comply with the Standards. Moreover, this chlorinated water is stored in the main tank until required. Even before its distribution to the communities there was no residual disinfectant in the stored water (A). It has been shown that low levels or absence of disinfectant residual in drinking water result in the increase of bacterial counts in water distribution systems (LeChevallier *et al.*, 1987, Clark *et al*., 1994; Momba *et al.*, 2000). Kaye *et al.* (1999) reported that total coliform, *K. oxytoca* and *A. hydrophila* numbers increased after treatment and as the distance from the treatment point increased. This study showed that the presence of a chlorine residual had an negative effect on the final numbers detected. In the presence of a chlorine residual, organism numbers did not return to the original density present within raw water. In the absence of a chlorine residual, total coliforms and *A.hydrophila* increased to levels originally present in raw water and the number of *K. oxytoca* increased above levels originally present in raw water.

The occurrence and survival of high numbers of coliforms and HPC bacteria in Dyamala and Kwa-Nkobonkobo groundwater confirms our previous findings (Momba and Notshe, 2000; Momba and Mnumevu, 2000) which reported the poor quality of groundwater in the Nkonkobe district of the Eastern Cape Province. The deteriorating standard of groundwater quality in this district could be linked to the presence of many pit toilets surrounding Alice locations as well as to farmyards. According to Chilton *et al.* (1995), farmyards constitute a source of groundwater pollution. Periago and coworkers (2000) reported that the application of cattle slurry as fertiliser is the major cause of diffuse-source water pollution in many agricultural regions.

In general, the chlorination process was found to be more effective for surface water (Table 2, Figs.3-4) than for groundwater (Table 5, Figs. 15-16). Chlorination of surface water (AF) was effective in the initial removal of total coliforms (average killing rate: 3 log cfu.10^{-2} ml for surface water) and initial reduction of HPC bacteria (average killing rate: 2 log cfu.ml^{-1} for surface water), within the first 20 min. This effectiveness was greater for total coliforms than for HPC bacteria, presumptive *E coli* and presumptive Salmonella (Table 2.2, Figs.2.3-2.4). Complete disappearance of coliforms in A, AF and AFC_1 occurred within 168 and 72 h respectively (Table 2.2). Although, the chlorination process also resulted in the reduction of coliforms (2 log cfu.10^{-2} ml) and HPC bacteria (1 log cfu. ml^{-1}) in GW1 within the first 20 min,

the persistence of coliforms in the chlorinated water was observed during the entire period of the experimental study (Table 2.5). Dissolved organic carbon concentrations could lead to the persistence of coliform bacteria in a drinking water distribution system. According to Bernhard and Wilhems (1985), treated water containing a DOC concentration below 1 $mg.\ell^{-1}$ is not prone to regrowth. Joret *et al.* (1991) reported that *Escherichia coli, Klebsiella,* and *Enterobacter cloacae* are unable to multiply in finished water samples with initial DOCs of 0.4 and 0.8 $mg.\ell^{-1}$. Comparing these DOC concentrations with those found in the present study as shown in Tables 2.1 and 2.4, it is clear that the levels of DOC concentrations in surface water and groundwater samples were high and could lead to the persistence of coliform and HPC in high numbers in treated waters.

It is also important to note that the decline or elimination of coliforms in untreated and chlorinated surface water and groundwater systems coincided with the increase of HPC bacteria. This could be related to the competition between HPC and coliform bacteria for limited nutrients. Since coliforms have higher growth requirements than HPC bacteria, they are particularly susceptible to competition for nutrients in oligotrophic environments such as drinking water (LeChevallier and McFeters, 1985). The concentrations of organic and inorganic compounds in test waters could also explain the competition between coliforms and HPC bacteria for limiting nutrients as well as the suppression of the proliferation of coliform by high numbers of HPC bacteria. Although, a positive or a negative correlation was observed between organic or inorganic elements presents in surface water samples and bacterial groups, strong positive correlation was revealed with HPC than with coliform bacteria (except for calcium). As to the groundwater samples, with the exception of calcium and nitrate, a negative correlation was found between coliform and organic or inorganic elements while a positive correlation was noted between HPC and all the compounds (except for calcium and COD). The findings of this study confirms those of previous investigators who found a significant correlation between the level of HPC and the rate of coliform decline (LeChevallier and McFeters, 1985). Moreover, it has been reported that coliform populations in drinking water samples held at 5°C and 22°C declined significantly after 24, 30 and 48 h but not after 2, 6, or 12 h (McDaniel and Bardner, 1983). As shown in Tables 2.2 and 2.3, an average temperature of 21.8°C in surface water samples and average temperatures ranging between 19.88 and 24 °C in groundwater samples could also explain the decline of coliform bacteria during our study. Similar observations were also reported

by Momba and Notshe (2000), when a study was performed on the microbiological quality of drinking ground water.

Whereas coliforms (total coliforms, *E. coli*, and *Salmonella*) were still visible in chlorinated surface water (AFC_1 and AFC_2) and groundwater systems (GW1 and GW2) 24 h after chlorination (Tables 2.2 and 2.5) , no coliforms were observed in the combined chlorine-monochloramine treated water system (AFC_2M and GW2M) 20 min and 24 h after the addition of monochloramine in the AFC_2 and GW2 respectively (Figs. 2.3, 2.15). The effectiveness of a combined chlorine and monochloramine process was also confirmed statistically when using F-ratio and LSD tests. This leads to conclude that a combined chlorine-monochloramine process provides an effective treatment which results in the early complete elimination of coliform bacteria in drinking water systems.

Effect of disinfectant residuals on bacterial regrowth

Chlorine was relatively unstable and did not provide a sustainable residual. A concentration of 0.45 ± 0.05 $mg.l^{-1}$ free chlorine in AFC_1, AFC_2 and GWC1, GWC2 systems could be detected only up to 24 h after which decay occurred and no free chlorine residual could be observed in AFC_1 during the remainder of the experimental period and in AGWC1 afer 48 h. The instability of free chlorine in water distribution systems has been reported by previous investigators (Clark, *et al.*,1994, Momba *et al.*, 1998, 1999,2000). It is interesting to note that the absence of the free chlorine residual in the AFC_1 system resulted in an increase of viable bacterial counts from 1 log to 6 $log.cfu.ml^{-1}$. As to the groundwater, even in the presence of 0.4 $mg.\ell^{-1}$ in GWC1 water system, HPC number returned to that initially present within the system (Fig. 2.7). A comparison between chlorinated water (A, AFC_1, GWC1) and untreated water (AF, GW) showed that AF and GW water did not significantly differ from A, AFC_1 and GWC1 water as a result of regrowth in the chlorinated water (Figs.2.2, 2.3, 2.7). Because chlorine residual decays as water flows through the distribution systems, the water may receive additional disinfectant at some critical time. In the present study, a monochloramine concentration of 1.5 $mg.\ell^{-1}$ was added in AFC_2 and GWC2 laboratory systems 24 h after chlorination. This additional disinfectant not only resulted in complete disappearance of coliforms but also in the reduction of HPC bacteria from 2 log to 1 log cfu ml^{-1} in AFC_2M and from 4 log to 3 log $cfu.ml^{-1}$ GW2M. While the phenomenon of bacterial regrowth took place in AFC_1 and GWC1 between 24 and 504 h

(Figs.2.3, 2.15), it is interesting to note a sensible decrease in bacterial numbers in AFC_2M (Fig. 2.4) and GWCM2 (Fig. 2.16). This demonstrates that, firstly bacterial regrowth in the laboratory-scale system was linked to the absence of disinfectant residual, secondly and the microbial efficacy of a combined chlorine-monochloramine treatment which provided a longer monochloramine residual and inhibited bacterial regrowth in AFC_2M and GWC2M water systems. Moreover the findings of this study reveal that a combination of chlorine and monochloramine provides an effective treatment for the inhibition of bacterial regrowth in drinking water as long as the residual monochloramine concentration persists to 0.35 $mg\ell^{-1}$ for surface and to 0.2 $mg.\ell^{-1}$ for groundwater.

During the present study , the potential for bacterial regrowth was observed between 336 and 672 h and between 672 and 720 h in GWC2M and AFC_2M , respectively (Figs.2.4, 2,16). This was mainly due to the presence of a low concentration of monochloramine residual in water (0.15 $mg.\ell^{-1}$ for surface water and less than 1 $mg.\ell^{-1}$ for groundwater). In the absence of the residual monochloramine (336 and 720 h after monochloramination of groundwater and surface water respectively), the bacterial counts increased up to 4 $log.cfu.ml^{-1}$ in both AFC_2M and GWC2M water systems. Previous investigators also reported the increase of micro-organisms in water systems when the residual disinfectant disappeared (Gibbs *el al.*, 1990; Clark *et al.*, 1994; Momba *et al.*, 1998). A comparison between surface water and groundwater showed that the phenomenon of bacterial regrowth occurred earlier in the groundwater system than in the surface water system. This fact could be related to high concentrations of certain inorganic and organic elements in groundwater such as calcium, magnesium, total nitrogen and sulphate (Table 2.4). A positive correlation, therefore, was observed between HPC bacteria and all of the above compounds with the exception of calcium (Table 2.6). Based on the results of this present study, it is clear that the effectiveness of a combined chlorine-monochloramine process was achieved for a longer period in the surface water than in the groundwater. High concentrations of organic and inorganic compounds in the intake water had a negative impact on the duration of the effectiveness of this process.

To improve the quality of potable water in water distribution systems and to make significant progress toward compliance with bacteriological quality standards at the point of consumption, it is recommended that water suppliers reduce the control the concentration of organic and

inorganic elements in the intake water as well as use chlorine as primary disinfectant followed by monochloramine as second disinfectant. In South Africa as elsewhere, many rural communities receive drinking water from public stand-pipes. Household containers or tanks, therefore, are commonly used for the storage of water for domestic use. The typically long residence times of drinking water in these containers cause excessive loss of disinfectant residuals, as the residual reacts with the oxidizable materials in waters. Low levels or absence of disinfectant concentrations promote bacterial regrowth in household containers leading to an increased survivability of humans pathogens such as *E.coli*, *Salmonella* and somatic coliphages (Momba and Dumani, 1999). Based on the results of the present study, it is recommended that water suppliers in rural communities combine chlorination and monochloramine disinfection processes in order to comply with bacteriological standards at the point of consumption as well as during storage of potable water in municipality tanks or household containers.

Biofilm formation and biofilm inhibition

The formation of biofilm was very rapid, especially in slides exposed to A, AF, AFC_1, GW and GWC1 laboratory-scale systems. In surface water systems, the viable bacterial numbers on SS and MS surfaces followed the bacterial number in waters (Fig. 2.5-2.8). In other words, higher viable bacterial counts were noted on slides exposed to untreated (4 log.cfu.cm^{-2}) than on those exposed to disinfected water (2 log.cfu.cm^{-2}) 24 h after chlorination. Similar observations were also noted by Momba *et al.*, (1998). The authors reported that stainless steel and cement surfaces exposed to raw water exhibited higher viable bacterial counts than those exposed to disinfected waters (chlorine, monochloramine, ozone, UV and hydrogen peroxide waters) within the first 24 h. Although 4 log and 3 log.cfu.cm^{-2} viable bacteria were detected in untreated and disinfected groundwater samples respectively, almost a yield of 2 log cfu. cm^{-2} attached viable bacteria was recorded on the surface of slides exposed to untreated and chlorinated waters 24 h after chlorination (with the exception of MS exposed to GWC2) (Figs.2.14-2.15).

Whereas the yields for attached viable bacteria yields were initially similar within each type of water source 24 h after chlorination, a significant difference in bacterial numbers occurred between the two chlorinated water systems with the addition of monochloramine in AFC_2 and GWC2M (Figs.2.7- 2.8 for surface water and Figs. 2.17 and 2.18 for groundwater). Between 48 h and 336 h, the number of viable heterotrophic bacteria on the slides exposed to AFC_1 remained

constant with a level of 5 log cfu.cm^{-2}, whilst <1 cfu.cm^{-2} was noted on the surface exposed to AFC_2M (Fig. 8). With the exception of SS and MS slides exposed to AFC_2M system, viable bacterial counts fluctuated between 4 and 5 log.cfu.cm^{-2} in the chlorinated water surface over the reminder of the study period. The heterogeneity of biofilms could be one possible explanation for the fluctuations in attached viable bacterial counts. Biofilms consist of several groups of micro-organisms with different nutrient requirements, and the presence of microbial groups with different physiology can vary in the biofilm with time (van der Kooij *et al.*, 1995, Lutterbach and de Franca, 1996; Percival *et al.*, 1998). As to the groundwater systems, attached viable bacteria on both types of the slide reached a maximum yield of 3 log and 4 log in the untreated and chlorinated water respectively between 2 and 28 d whilst < 1 cfu.cm^{-2} colonized the slides exposed to the combined system between 2 and 7 d (Fig. 18). Between 168 and 672 h the number of attached viable bacteria on the slides exposed to the combined water system reached 5 log.cfu.cm^{-2} for the rest of the study period. The above data confirm the effectiveness of combined chlorine-monochloramine process not only in the inhibition of bacterial regrowth but also in the inhibition of biofilm regrowth. This inhibition was longer in the surface water system than in the groundwater water system.

In their study, Momba and coworkers (1998) showed that 1 mg.ℓ^{-1} monochloramine and 0.2 mg.ℓ^{-1} free chlorine residuals did not prevent the formation of biofilms. Although the presence of monochloramine residual was found to control the phenomenon of biofilm regrowth on the stainless steel and cement surfaces, biofilm formation was obvious during the whole study period. This means that chlorine or monochloramine alone cannot inhibit total colonization of pipe surfaces by bacteria. The present study also confirms that chlorination alone had no effect on biofilm formation. However , a combination of chlorine and monochloramine led to the decrease of viable biofilm counts from 2 log cfu.cm^{-2} to <1cfu.cm^{-2}. This biofilm plate count could be detected up to 504 hon both SS and MS surfaces for surface water and up to 168 h on the slides exposed to groundwater. The present study demonstrated the inability of HPC bacteria to colonize surfaces of SS and MS in a combined chlorine-monochloramine system. In such a treatment system, the susceptibility of viable bacteria was observed only when the concentration of monochloramine persisted up to 0.35 and 0.2 mg.ℓ^{-1} in surface water and groundwater respectively . A lower monochloramine residual of ±0.15 mg.ℓ^{-1} led to the phenomenon of biofilm regrowth (Fig.2.12). The decay of monochloramine residual in water systems resulted

in the increase of viable fixed bacteria levels (Figs. 2.12 and 2.18). A comparison between SS and MS slides exposed to AFC_2M and GWC2M showed no significant difference between attached viable bacterial counts at $p > 0.05$.

Previous studies have shown that temperatures above than 5°C (Lund and Ormerod, 1995) and 15°C (leChevallier *et al.*,1996, Kaye *et al.*,1999; Zacheus *et al.*,2000) have been associated with bacterial regrowth and biofilm formation in drinking water distribution systems. Although, in the present study, the temperature (above 19°C in all systems) and the high concentration of organic and inorganic compounds could influence the bacterial or biofilm regrowth in water systems, statistical data support that a combined chlorine-monochloramine process and the persistence of monochloramine in the combined system constitute of the most important factors linked to the inhibition of bacterial or biofilm regrowth in a drinking water system.

In both surface water and groundwater, higher numbers of total cells were noted on slides exposed to untreated water (including Alice chlorinated water) than those exposed to chlorinated water (Figs. 2.9-2.11 and 2.19-2.20). High cell counts on slides exposed to untreated water systems could also be visualized when using SEM techniques (Plates 28, 31, 34, 38,41,44). Although a higher number of cells was observed on SS slides than on MS slides exposed to surface water (the colonization of slides by bacteria was equally observed in groundwater), statistically ANOVA showed no significant difference in cell densities at $p > 0.05$. Cells counts measured directly on MS slides were undoubtedly underestimated because of the structure of MS surface. SEM techniques revealed the presence of crystals which rendered difficult the visualization of fixed cells on MS. The present experiment with total cells also confirmed the inability of bacteria to colonize surfaces of MS and SS slides in a combined chlorine-monochloramine with a significant monochloramination residual (from 0.8 to 0.35 $mg.\ell^{-1}$ for surface water and from 0.8 to 0.2 $mg.\ell^{-1}$). Comparing the two water sources, ANOVA showed no significant difference in all bacterial counts when chlorine was used as primary disinfectant and monochloramine as a second disinfectants for the disinfection of surface water and groundwater.

CHAPTER 3

COMPARING THE EFFECT OF VARIOUS PIPE MATERIALS ON BIOFILM FORMATION IN A COMBINED CHLORINE-CHLORAMINE WATER SYSTEM

3.1 INTRODUCTION

The need to provide adequate water supplies to communities is a major objective of the South Africa Government's Reconstruction and Development programme. To ensure bacteriologically safe drinking water, drinking water producers try to avoid bacterial growth in a drinking water distribution system. This could be possible by identifying appropriate disinfection processes and pipe materials used for the distribution of potable water.

Bacterial growth in a drinking water distribution system mainly occurs at internal surface of the pipes. Detachment of bacteria from this biofilm may thus affect the water quality. Investigators have shown that bacterial growth occurring at pipe walls depends on different factors: concentrations of disinfectant, water temperature and concentration of BDOC which is the main substrate allowing bacterial growth in the drinking water (Block *et al.*, 1993, LeChevallier *et al.*, 1993; Servais *et al.*, 1995; Servais, 1996). In developing countries, the effect of pipe composition on the densities of bacteria fixed on the pipe walls remains one of the factors which has received less attention.

The characteristics of the material composing pipes may greatly influence the densities of bacteria fixed in a distribution system. It is well known that the roughness of the material using for the distribution of potable water contributes to bacterial attachment (Pedersen, 1990; Percival *et al.*, 1998). Momba *et al.* (1998) reported a higher yield in viable bacteria on stainless steel coupons than on cement coupons during the first 8 h of their experimental study. Van der Kooij *et al* (1995) also observed that the materials in contact with drinking water could release biodegradable compounds into drinking water, thus enhancing biofilm formation.

A study performed by Momba and his co-workers showed that a combined chlorine-monochloramine process provides an effective treatment for the inhibition of planktonic and attached bacteria as long as a concentration of *ca* 0.35 $mg.\ell^{-1}$ monochloramine persists in drinking surface water systems (Project No K5/1023/0/1, funded by WRC for the year 1999-2000). On the basis of the first results on surface water, it has been recommended by the steering committee members to perform a further study which aimed to compare the effect of various pipes materials (used in South Africa, especially in rural communities) on biofilm formation in a combined chlorine-monochloramine water .

To identify the type of pipe materials used in South Africa for the distribution of potable water, the addresses of Water Treatment Authorities (Urban and rural treatment plants) were provided by WRC. A questionnaire was set and sent to 500 Water Treatment Authorities. Data collected from this questionnaire revealed 11 types of pipe materials used in the following percentage: polyvinyl chloride-PVC (25%), asbestos cement -AC (21%), asbestos (19%), unplasticised polyvinyl chloride- uPVC (16%), steels (8%), cement-C (4%), bitumen coated (3%), high density polyethylene-MDPE (2%), copper (1%), galvanised mild steel (1%), motar lined steel (1%), cast iron (1%). Data from the questionnaire also revealed that 84.2% of Water Treatment Authorities use only chlorine (especially chlorine gas) as the main disinfectant. The initial chlorine dose used to disinfect the intake water remains unknown (65%). This mostly concerns rural Water Treatment Plants. Moreover, the problem of bacterial regrowth and biofilm formation is well known only in urban Water Treatment Plants (15.8%) whereas 84.2 % of the Water Treatment Authorities in rural communities do not understand or ignore it due to the fact that no monitoring of the microbiological quality of water is performed in the Water Treatment Plants. Therefore, there was a need to conduct such a project in order to improve the quality of drinking water, especially in rural communities of South Africa and to form more people, especially black communities who will be able to solve the problem of water quality monitoring.

During the project period, plastic-based -(PVC, UPVC, MDPE) and cement-based (C and AC) materials were used as test pipes. The effect of each pipe material on biofilm formation was identified. Moreover the concentration of DOC in treated water systems was determined. Using statistical analysis, a comparative study was performed.

3.2 MATERIALS AND METHODS

3.2.1 Study site

The experiment was performed using surface water from the Alice purification system. The purification system gets its water from the catchment dam that draws the water from the Tyume River. The water from the dam undergoes purification, which involves sedimentation, sand filtration and chlorination. The test water was collected after filtration just before reaching the chlorination tank. The experimental study of each test water was conducted on the basis on the basis of two replicates and 672 h for each repeat.

3.2.2 Sampling of test waters

Water samples were collected in four 50ℓ sterile polyethylene drums and 1 sterile bottle from the filtration tank. Water sample in the sterile bottle was used to check the initial bacterial counts in water. Before use, the drums were washed with the detergent, rinsed with hot tap water and distilled water, and then disinfected with 5mg.ℓ^{-1} of chlorine for 24h. Twenty four hours after disinfection, sodium thiosulphate (ca 17.5mg.ℓ^{-1}) was added to the drums to stop further chlorination and neutralize any disinfectant residual. The drums were then rinsed with sterile distilled water. Sample waters were then transported to the laboratory and analysis proceeded immediately.

3.2.3 Treatment of test waters

The experimental study was performed using the laboratory-scale system described in section 23. The partially treated surface water was disinfected with 2.5 mg.ℓ^{-1} free chlorine prior to being transferred to four separate laboratory-scale systems. The chlorinated water circulated in laboratory-scale systems at a flow rate of 2.8ℓ.h^{-1}. Twenty four hours after the circulation of test waters in different systems, 1.5 mg.ℓ^{-1} monochloramine was separately added to two chlorinated water systems to form two chlorine-monochloramine treated water systems.

3.2.4 Biofilm analyses

Biofilm experiments were conducted for the chlorinated water and the combined chlorine-monochloramine treated water. For each type of treated water, two series of experiments were

performed: the first series involved the analysis of biofilms on plastic-based materials (PVC, UPVC, MDPE), whereas the second series involved the analysis of biofilms on cement-based materials (cement and asbestos cement). Ten modified Pedersen devices were used to allow the formation of biofilm to be studied. For this purpose, 20 microscope slides made up of each plastic-based materials (25x25x1mm) and cement-based materials (25x25x10mm) were installed vertically into each Pedersen device. Prior to use, the slides were treated in the same manner as the drums and the laboratory-scale system. The colonized pipe slides were removed aseptically from the Pedersen devices. The removed slides (two for each type of pipe material) were put into sterile plastic bottles with 20 ml sterile MilliQ water. To detach biofilms from the slide surfaces, the bottles were vortexed for 2 min using a vortex mixer. Sample frequency is indicated in the results and was the same for all experiments.

Coliform bacteria were enumerated by the membrane filtration procedure using the filter membrane with 0.45um pore size and 47 mm diameter (Millipore). Membranes were placed on Chromocult agar (Merk), and all plates were incubated at 37h. Analyses were carried out in triplicates. The following equation was used to calculate the number of coliform bacteria ($cfu.cm^{-2}$):

$cfu.cm^{-2} = N \times D$ /surface area of slide

Where N =average number of colonies and D =dilution factor

Heterotrophic plate count bacteria were enumerated by the standard plate spread method using R2A agar (Oxoid), incubated at 28°C for 72 hours (APHA, 1998). Analyses were carried out in duplicates. The number of heterotrophic plate count bacteria were also expressed in $cfu.cm^{-2}$, and calculated using the same equation as above. For the visualization of biofilm formation, the removed slides were put into sterile plastic bottles and 2.5% gluteraldehyde (GTA) was added. The biofilm formation was visualized using a scanning electron microscope (SEM).

3.2.5 Physico-chemical analyses

Concentrations of residual free chlorine and residual monochloramine were measured using N,N-diethyl-p-phenylenediamine (DPD, Sigma) ferrous titrimetric method (APHA-AWWA-WEF 1998). Temperature, pH and turbidity were determined according to standard methods (APHA,

AWWA-WEF 1998). Calcium(Ca^{2+}), Magnesium,(Mg^{2+}) chemical oxygen demand (COD), total nitrogen (N), phosphate (PO_4^{3-}), sulfate (SO_4^{2-}) concentrations were determined according to spectroquant NOVA 60 manual (1998), using photometric test kits (Merck).Samples for dissolved organic carbon (DOC) were prepared according to Mathieu *et al.,* (1993) and sent to Council for Scientific and Industrial Research (C.S.I.R- Pretoria/South Africa) for the measurement of DOC concentrations.

3.2.6 Statistical analyses

To compare variations in treatments, ANOVA ($\propto = 0.05$) was applied to the bacterial counts with the latter as the dependant variable. The number for heterotrophic plate count bacteria was transformed by taking the logarithm base 10 in order to stabilize the variance. The number of the days as well as the pipe materials were used to identify their effect of biofilm formation. A correlation was established between attached bacterial counts and disinfectant residual concentrations and between bacterial counts and the concentrations of organic and inorganic compounds in waters.

3.3 RESULTS

3.3.1 Microbiological features on slides

3.3.1.1 Coliform bacteria

Tables 3.1 summarizes the results of a two-way ANOVA for the mean coliform bacterial counts when considering factors time and pipe materials (polyvinyl chloride-PVC, unplasticised polyvinyl chloride-UPVC, medium density polyethylene-MDPE, cement and asbestos cement-AC) exposed to chlorine and chlorine-monochloramine treated waters. The effect of the time, pipe materials and the treatment on biofilm formation is shown in Table.3.4 for both chlorine and monochloramine.

Chlorinated water - The attachment of coliform bacteria on test pipes exposed to chlorinated water was apparent within 20 min (Table 3.1). Twenty hours after chlorination, more coliform bacteria (with a total average count of 6.5 cfu.cm^{-2}) were recorded on the cement pipe than on other pipe materials (Table 3.1). Although pipes materials did not show statistical significant

difference in bacterial counts between 0 h and 24 h, the interaction with factor time showed significant difference in coliform bacterial counts at $p < 0.05$ (Table 3.2). The coliform counts progressively increased between 336 and 672 h. Mean bacterial counts between 48 and 168 h were comparatively lower than those recorded between 336 and 672 h (Table 3.1). Across the pipes, the mean counts on AC and C pipes were significantly lower than those on PVC, UPVC and MDPE pipes at $P < 0.01$(Table 3.1). Both main (pipes, times) and interaction effects showed statistical significant difference in coliform counts at $p < 0.05$ (Table 3.2).

Combined chlorine-monochloraminated water system – The addition of 1.5 mg.ℓ^{-1} mochloramine in the chlorinated system resulted in the removal of coliform bacteria on the surface of all pipe materials between 24 and 48 h (Table 3.1) after which bacterial counts progressively increased to a total average count of 8 cfu.cm^{-2} on PVC, UPVC and HDPE between 168 and 672 h (Table. 3.1). Coliform regrowth on C and AC pipes exposed to the combined system occurred within 336 h. The numbers of bacteria increased from <1 cfu.cm^{-2} to 3 and 1.8 cfu.cm^{-2} respectively, and both pipe materials reached a similar total average count of 5 cfu.cm^{-2} between 336 and 672 h (Table 3.1). The mean bacterial counts were significantly lower on the C and AC pipes than on the PVC, uPVC and MDPE pipes at $p < 0.05$ (Table 3.1). Taking into account the factors time and pipes, ANOVA showed that both the main and interaction effects were all statistically significant at $p < 0.01$. However, under the consideration of all three factors (treatment: chlorine-monochloramine, time and pipe materials), all main effects and the interaction effect between the pipes and time statistically showed significant difference in coliform bacterial counts excepted for pipe materials (Table 3.2).

3.3.1.2 Heterotrophic plate count bacteria

The bacterial counts for HPC were transformed into log values in order to stabilize variances due to the extremely large count values. Tables 3.3 and 3.4 summarize the results of a 2-way ANOVA for the mean heterotrophic bacteria counts when considering factors time and pipe materials (polyvinyl chloride-PVC, unplasticised polyvinyl chloride-uPVC, high density polyethylene-MDPE, cement-C and asbestos cement-AC) exposed to chlorine and chlorine-monochloramine treated waters.

Tables 3.1 Mean counts of coliform bacteria by time and pipe materials in chlorine and chlorine-monochloramine treated water systems ($p < 0.05$*, $p < 0.01$**)

Chlorinated water system

Pipe	0 h	20 min	24 h	Total
PVC	0	1.217	3.217	4.343
uPVC	0	2.867	1.050	3.917
MDPE	0	2.417	1.792	4.209
C	0	3.200	3.308	6.508
AC	0	2.083	1.633	3.716

Pipe	48 h	168 h	336 h	504 h	672 h	Total
PVC	1.750	3.833	3.350	5.883	12.300	24.716
uPVC	1.000	2.017	3.950	7.483	19.483	33.933
MDPE	1.267	1.700	5.150	7.350	8.430	23.897
C	1.100	0.817	5.450	4.067	10.200	21.634
AC	2.017	1.400	1.733	1.867	5.783	12.800**

Chlorine-monochloraminated water system

Pipe	48 h	168 h	336 h	504 h	672 h	Total
PVC	0	0.133	1.433	2.317	4.433	8.316
uPVC	0	0.267	1.850	1.700	4.583	8.400
MDPE	0	0.267	2.450	2.150	3.100	7.967
C	0	0.000	1.567	0.700	3.100	5.367*
AC	0	0.000	0.833	2.617	1.867	5.317*

Table 3.2 The effect of time and pipe materials on coliform bacteria in chlorine and combined chlorine-monochloramine treated waters ($p < 0.05$*, $p < 0.01$**)

Effect	df Effect	MS Effect	df Errors	MS Error	F	P
Chlorinated water system						
Between 0 and 24 h						
P	2	3.796	105	1.603	2.368	0.0573
T	4	62.657	105	1.603	39.087	0.0000*
T x P	8	6.596	105	1.603	4.115	0.0002*
Between 48 and 672 h						
P	4	2.733	125	0.679	4.020	0.0040*
T	4	59.045	125	0.679	86.855	0.0000*
T x P	16	2.269	125	0.679	3.337	0.0001*
Chlorine-monochloraminated water system						
Between 48 and 672 h						
P	4	71.808	125	4.668	15.382	0.0000**
T	4	464.978	125	4.668	99.605	0.0000**
T x P	16	36.555	125	4.668	7.831	0.0000**
TRT	1	1080.721	250	32.555	33.196	0.0000**
P	4	677.795	250	32.555	20.819	0.0000**
T	4	18.172	250	32.555	0.558	0.693
TRT x P	4	231.932	250	32.555	7.124	0.0001**

P: pipe, T: time, TRT: Treatment

Chlorinated water - An initial average density of 2 log cfu.cm^{-2} HPC was recorded on PVC, uPVC and MDPE 20 min after chlorination of surface water, after which this number decreased to 1 log.cm^{-2} for the first two pipe materials and to < 1 log.cm^{-2} for MDPE. Within 24 h the total average density of HPC bacteria on plastic-based materials was 2 log.cm^{-2} after which this

bacterial increased to a maximum total count of 5 log cfu.cm^{-2}. A comparison between test pipes showed no significant difference in bacterial counts at $p < 0.01$. On cement and asbestos cement, an initial average density of 1 log.cm^{-2} HPC was detected. Although a slight decrease in bacterial counts was observed within 24 h on AC, the total count remained the same as that initially observed. Between 20 min and 672 h, total bacterial counts of 4 log and 3 log cfu.cm^{-2} were recorded on C and AC respectively. At $p < 0.01$, no significant difference was observed between the two pipe materials. However, a comparison between different pipe materials showed a lower significant HPC number on cement and asbestos cement than on PVC, uPVC and HDPE at $p < 0.05$ and $p < 0.01$ (Table 3.3).

Chlorine-monochloramine treated water - With the exception of MDPE and AC, a similar number of attached HPC bacteria (1 log.cfu.cm^{-2}) was observed on the surface of all types of pipes 24 h after chlorination. With the addition of 1.5 mg.ℓ^{-1} monochloramine in the chlorinated water system, bacterial counts decreased to less than 1 cfu.cm^{-2} on the surface of test pipes between 48 and 72 h after which the number of HPC bacteria progressively increased and reached maximum total numbers of 3 log cfu.cm^{-2} and 2 log HPC.cm^{-2} on plastic-based pipes and cement-based pipes respectively. Across the pipes (PVC, uPVC, MDPE, C and AC), pipes C and AC showed statistical significant difference in mean counts of HPC bacteria compared to pipes PVC, uPVC and MDPE at $p < 0.05$ and $p < 0.01$ (Table 3.3). While the factor time showed significance difference in bacterial counts at $p < 0.0001$ and $p < 0.0005$ during the entire period of the study in treated waters, no significant difference in HPC numbers was observed when considering the factor pipes and the interaction between both factors after 24 h in chlorinated water (Table 3.4) . However, between 48 and 672 h, the difference in HPC bacterial numbers were found highly significant when taking into account the factor pipes and the interaction of both time and pipe materials in chlorine and chlorine-monochloramine treated water (Table 3.4).

Table 3.3 Mean counts of heterotrophic plate count bacteria by time and pipes after chlorination (* $p < 0.05$ and ** $p < 0.01$)

Chlorinated water system

Pipe	0 h		20 min		24 h		Total
PVC	0		2.63E2		3.98E1		3.03E2
uPVC	0		2.63E2		1.71E1		2.08E2
MDPE	0		7.59E2		6.45		7.66E2
C	0		3.69E1		4.19E1		7.88E1**
AC	0		5.63E1		7.83		6.41E1**
Pipe	48 h	72 h	168h	336 h	504 h	672 h	Total
PVC	1.26E1	0.29	1.06	9.04E2	2.01E5	8.49E5	1.05E5
uPVC	1.06E1	1.63	2.81E1	5.93E2	3.60E5	6.82E5	1.04E5
MDPE	2.66E1	0.05	1.97E1	2.21E2	9.45E5	8.91E5	1.83E5
C	3.08E1	0.06	1.95E1	2.31E2	7.11E3	3.66E3	1.11E4*
AC	2.29	0.00	1.26E1	6.21E1	1.26E2	8.49E3	8.63E3**

Chlorine-monochloramine water system

Pipe	48 h	72 h	168 h	336 h	504 h	672 h	Total
PVC	0.298	0.000	1.629	6.208E1	3.614E2	1.750E3	2.175E3
uPVC	1.629	0.000	0.000	4.864E1	3.811E2	9.183E3	9.614E3
MDPE	0.051	0.000	0.055	4.529E1	4.305E2	1.367E3	1.842E3
C	0.467	0.000	0.000	2.547	3.767E1	3.698E2	4.104E2*
AC	0.298	0.000	0.297	3.076E1	3.251E1	3.243E2	3.881E2**

Table 3.4 The effect of time and pipe materials on heterotrophic plate count bacteria in treated water systems (** chlorine-monochloramine, * significant at given p-levels)

Effect	df Effect	MS Effect	df Errors	MS Error	F	P
Chlorinated water system						
			Between 0 and 24 h			
P	4	0.3304	55	0.4400	0.8100	0.0526
T	2	58.1440	55	0.4400	141.8120	0.0000*
T x P	8	0.5139	55	0.4400	1.2530	0.2867
			Between 48 and 672 h			
P	4	8.8590	80	0.4948	17.9030	0.0000**
T	5	83.6600	80	0.4948	169.0570	0.0000**
T x P	20	1.4700	80	0.4948	2.9710	0.0002**
Chlorine-monochloramine treated water system						
			Between 48 and 672 h			
P	4	25.6438	80	1.6189	15.8398	0.0000**
T	5	154.5582	80	1.6189	95.4711	0.0000**
T x P	20	6.3210 80		1.6189	3.9045	0.0001**
TRT	1	147.904	160	0.70455	209.925	0.0000*
P	4	8.236	160	0.70455	11.689	0.0000*
T	5	146.622	160	0.70455	208.107	0.0000*
TRT x P	4	2.736	160	0.70455	3.8829	0.0049*
TRT x T	5	3.246	160	0.70455	4.6638	0.0005*
P x T	20	1.6110	160	0.70455	2.2866	0.0024*

3.3.2 Physico-chemical quality of test waters

With the exception of DOC levels, the concentrations of inorganic compounds and the level of turbidity in all water systems reflected the limits allowed by *South African Water Quality Guideline for Domestic used* (DWAF, 1996). Compared to the initial water, an increased temperature value was noted in treated waters and this temperature was similar in all treated water systems (Table 3.5).

Although an initial concentration of chlorine (2.5 mg.ℓ^{-1})was added to all four batch reactors to produce chlorinated water systems, a quick decrease of the concentration of free chlorine residual was noted in chlorinated water systems with plastic-based materials than in those with cement-based materials within the first 24 h. Similar observation was also noted with the addition of monochloramine in the chlorinated water systems despite a longer persistence of the residual monochloramine in both combined water systems (Table 3.6).

Table 3.5 Physico-chemical values(average) of the test waters in different batch reactors

	Parameters							
Test water	T (°C)	pH	NTU	COD (mg.ℓ^{-1})	DOC (mg.ℓ^{-1})	N (mg.ℓ^{-1})	PO_4^{3-} (mg.ℓ^{-1})	SO_3^{2-} (mg.ℓ^{-1})
WIo	19.0	7.4	1.4	4.8	17.0	0.6	<0.5	36.0
BRPC	20.5	7.4	0.8	<4.0	16.3	0.6	<0.5	36.5
BRCC	20.5	7.5	0.9	<4.0	11.0	2.1	<0.5	44.0
BRPM	20.5	7.8	0.8	<4.0	4.1	2.2	<0.5	38.0
BRCM	20.5	7.8	0.8	<4.0	7.9	1.3	<0.5	37.5

Wio - Initial water; BRPC- Batch reactor with plastic slides in the chlorinated water system; BRPM- Batch reactor with plastic slides in the chlorine-monochloramine treated water system; BRCC- Batch reactor with cement slides in the chlorine water system

BRCM- Batch reactor with cement slides in the chlorine-monochloramine treated water system

Table 3.6 Decay of disinfectant residuals (average values) in treated water systems

Time	Residual disinfectant			
	Chlorine(mg.ℓ^{-1})		Chlorine(mg.ℓ^{-1})	
	BRP	BRC	BRP	BRC
0 h	2.5	2.5	2.5	2.5
20 min	2.2	2.2	2.2	2.2
24 h	1.15	1.3	1.15	1.3
			Monochloramine (mg.ℓ^{-1})	
	0.75	0.8	1.5	1.5
72 h	0	0	0.9	0.95
168 h	0	0	0.55	0.6
336 h	0	0	0.2	0.3
504 h	0	0	0	0.1
672 h	0	0	0	0

BRP - Batch reactor with plastic-based pipe materials, BRC - Batch reactor with cement-based pipe materials.

3.3.3 Correlation between mean bacterial counts and the concentration of inorganic and organic compounds in test waters

In chlorinated water systems, the temperature, DOC and total nitrogen showed weak negative correlations with coliform bacteria attached to plastic-based pipe materials and cement-based pipe materials, while the pH, turbidity and sulphate showed weak positive correlations. In both cases, these correlations were not significant at $p < 0.05$. Similar statistical observations were also noted in chlorine-monochloramine water systems although positive correlations were registered between mean counts of coliform bacteria and the values of temperature, pH, turbidity and sulphate, and negative correlations were recorded for DOC and total nitrogen (Table .3.7)

Table 3.7 Correlations between attached coliform bacterial counts (mean) and Physico-chemical values (means) in treated water systems (between 48 h and 672 h. at $p < 0.05$)

	Test water							
	Chlorinated water				Chlorine-Monochloraminated water			
Parameter	BRP		BRC		BRP		BRC	
	r	p	r	p	r	p	r	p
Temp(°C)	-0.460	0.430	-0.405	0.499	0.641	0.244	0.691	0.191
pH	0.231	0.708	0.500	0.390	0.269	0.336	0.181	0.771
NTU	0.266	0.667	0.269	0.661	0.115	0.853	0.152	0.804
COD(mg/l)	-	-	-	-	-	-	-	-
DOC(mg/l)	-0.408	0.496	-0.179	0.773	-0.441	-0.457	-0.540	0.347
N (mg/l)	-0.367	0.542	-0.462	0.433	-0.490	0.402	-0.563	0.323
PO_4^{3-} (mg/l)	-	-	-	-	-	-	-	-
SO_4^{2-} (mg/l)	0.281	0.646	0.457	0.900	0.281	0.684	0.079	0.440

BRP: Batch reactor with plastic-based pipe materials; BRC: Batch reactor with cement-based pipe materials; r: correlation, $p<0.05$, -: not considered.

With the exception of the turbidity and the sulphate, negative correlations were registered between the number of heterotrophic plate count bacteria attached to plastic- and cement-based pipe materials exposed to both chlorine and chlorine-monochloramine treated water. Statistical evidence showed no significant difference between bacterial counts and physico-chemical parameters at $p < 0.05$ (Table 3.8).

Table 3. 8 Correlations between attached heterotrophic plate count bacteria (mean log) and the physico-chemical values (mean) in treated water systems (between 48h and 672 h. at $p < 0.05$).

Parameter	Test water: Chlorinated water				Test water: Chlorine-monochloraminated water			
	BRP		BRC		BRP		BRC	
	r	p	r	p	r	p	r	p
Temp(°C)	-0.06	0.93	-0.07	0.91	-0.39	0.51	-0.42	0.48
pH	-0.42	0.49	-0.44	0.46	-0.04	0.95	-0.02	0.98
NTU	0.41	0.50	0.39	0.52	0.43	0.48	0.40	0.51
COD($mg.\ell^{-1}$)	-	-	-	-	-	-	-	-
DOC($mg.\ell^{-1}$)	-0.08	0.90	-0.08	0.90	-0.47	0.42	-0.47	0.42
N($mg.\ell^{-1}$)	-0.27	0.66	-0.28	0.65	-0.41	0.50	-0.43	0.47
PO_4^{3-}($mg.\ell^{-1}$)	-	-	-	-	-	-	-	-
SO_4^{2-}($mg.\ell^{-1}$)	0.04	0.45	0.02	0.97	0.51	0.38	0.48	0.42

BRP: Batch reactor with plastic-based pipe materials; BRC: Batch reactor with cement-based pipe materials; r: correlation, $p<0.05$, -: no considered.

3.3.4 Correlation between mean bacterial counts and the concentrations of disinfectant residuals in test waters

Although negative correlations were registered between the concentrations of disinfectant residuals and the numbers of bacteria on the surface of pipe materials, the water treated with a combined

chlorine-monochloramine disinfectants showed high correlation values. The highest values were then found with coliform bacteria under the combined the chlorine-monochloramine treatment and in this system, the correlations were significant at $p < 0.05$ (Table 3.9).

Table 3.9 Correlations between attached bacterial counts (mean counts for coliform bacteria, mean log-counts for heterotrophic plate count bacteria) and the concentrations of disinfectant residuals in treated water systems (between 48 and 672 h).

	Disinfectant residuals							
	Chlorine				Monochloramine			
Organism	BRP		BRC		BRP		BRC	
	r	p	r	p	r	p	r	p
CB	-0.50	0.39	-0.39	0.52	-0.87	0.05	-0.93	0.02
HPC	-0.39	0.52	-0.41	0.49	-0.54	0.35	-0.63	0.25

CB: coliform bacteria, HPC: heterotrophic plate counts, BRP: batch reactor with plastic based-pipe materials, BRC: batch reactor with cement-based pipe materials, r: correlation, $p<0.05$

3.3.5 SEM - Visualization of bacterial cells on the surface of pipe materials

With the exception of cement and asbestos cement pipes, (Plates 55-56), SEM observation revealed the presence of biofilm cells on the surface of pipe materials exposed to chlorine treated water within the first 24 h. As shown in Plates 52-54, 57-61, 67-71 and Plates 77-81, the adhesion and survival of bacteria dramatically increased on the surface of all test pipes between 24 and 672h. No biofilm cells were detected on the surface of all pipes exposed to the chlorine-monochloramine treated water for the first 168 h of the experimental study (Plates 62-66). Although the formation of biofilm could be observed on these pipes between 336 and 672 h, lower numbers of attached cells were visible on cement and asbestos cement than on PVC, UPVC and HDPE (Plates 72-76, 82-86).

Plate 47-PVC

Plate 48-uPVC

Plate 49-MDPE

Plate 50-C

Plate 51-AC

Plate 52-PVC

Plate 53-uPVC

Plate 54-MDPE

Plate 55-C

Plate 56-AC

Plates 47-56 SEM photographs showing the microbiological feature of plastic-based materials and cement-based materials before (Plates 47-51) and after 24 h exposure (Plates 52-56) to chlorinated water systems

Plate 57-PVC Plate 58-uPVC Plate 59-MDPE Plate 60-C Plate 61-AC

Plate 62-PVC Plate 63-uPVC Plate 64-MDPE Plate 65-C Plate 66-AC

Plates 57-66 SEM photographs showing the microbiological feature of plastic-based materials and cement-based materials after 168 h of exposure to chlorine (Plates 57-61) and chlorine-monochloramine (Plates 62-66) treated water systems.

Plate 67-PVC

Plate 68-uPVC

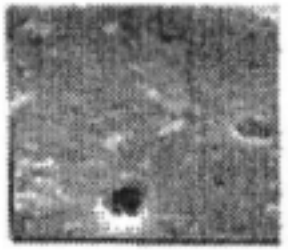

Plate 69-MDPE

Plate 70-C

Plate 71-AC

Plate 72-PVC

Plate 73-uPVC

Plate 74-MDPE

Plate 75-C

Plate 76-AC

Plates 67-76 SEM photographs showing the microbiological feature of plastic-based materials and cement-based materials after 336 h of exposure to chlorine (Plates 67-71) and chlorine-monochloramine (Plates 72-76) treated water systems.

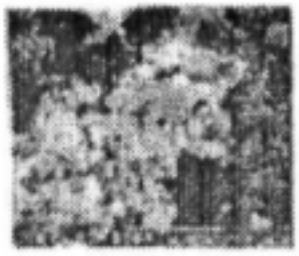

Plate 77-PVC

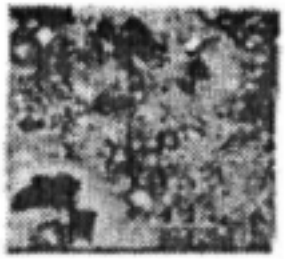

Plate 78-uPVC

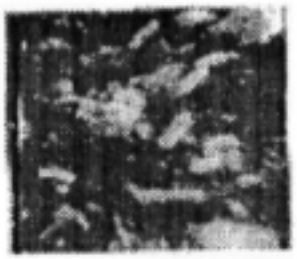

Plate 79-MDPE

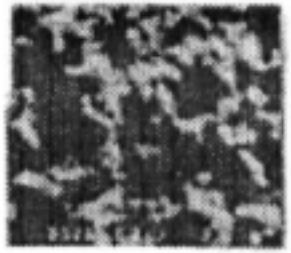

Plate 80-C

Plate 81-AC

Plate 82-PVC

Plate 83-uPVC

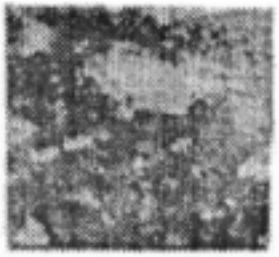

Plate 84-MDPE

Plate 85-C

Plate 86-AC

Plates 77-86 SEM photographs showing the microbiological feature of plastic-based materials and cement-based materials after 672 h of exposure to chlorine (Plates 77-61) and chlorine-monochloramine (Plates 82-86) treated water systems.

3.4 DISCUSSION

From the present study, important relations can be drawn firstly between the formation of biofilm and the type of disinfection process, secondly between the density of fixed cells and the generic type of pipe materials.

General data indicated the colonization of all test pipe materials by microorganisms under chlorination (Tables 3.1 and 3.3, Plates 52-34, 57-61, 67-71, 77-81). This biofilm included both coliforms and herotrophic plate count bacteria. The possibility of the cell attachment (especially within 20 min) on the surface of test pipe materials exposed to chlorinated water is supported by weak correlations of free chlorine residual concentrations with bacterial numbers (Tables 3.6, 3.9). Statistically none of these correlations were significant at $p < 0.05$, which means, although there were inverse relations, these relations were not as significant as it might be expected. Bacterial biofilms developing in distribution systems receiving chlorinated water have been repeatedly reported by previous investigators (LeChevallier *et al.*, 1987, 1988a, 1990; Wende and Characklis, 1990; Camper *et al.*, 1999; Momba *et al.*, 1998,1999, 2000, 2001). Although the present investigation did not deal with the identification of various pathogenic and opportunistic pathogens, the survival and multiplication of heterotrophic bacteria such as *Escherichia coli, Pseudomonas, Aeromonas, Klebsiella, Legionella spp, Mycobacter, Campylobacter, Salmonella typhimurium* and *Helicobacter pylori* have been observed within biofilms occurring in potable water systems with standard chlorine residual concentrations (Engel *et al.*, 1980; Widowsky *et al.*, 1982; Burke *et al.*, 1984; Armon *et al.*, 1997; Mackey *et al.*, 1998; Camper *et al.*,1999; Momba *et al.*, 1999). The capability of *E. coli* to survive a high free chlorine residual dose of 0.7 $mg.\ell^{-1}$ and to attach on pipe materials such as stainless steels has been reported by Momba and co-workers in 1999. The authors pointed out that there were no significant differences in attached *E. coli* counts between the non-disinfected water and chlorinated water. The above findings clearly showed the ineffectiveness of free chlorine in inhibiting the formation of biofilm in potable water systems. Both coliforms and HPC bacterial groups may pose a significant public health risk. Promoting biofilm inhibition/removal directly or developing strategies for adequate control measure would therefore appear to be an attractive alternative approach for water industries.

During the experimental study, monochloramine was used as secondary disinfectant for the inhibition of biofilm formation in the chlorinated water system. Negative correlations were also found between monochloramine residual concentrations and attached bacterial numbers under the chlorine-monochloramine treatment. Compared to the chlorine treatment, these negative correlations were found t much more stronger and significant (at $p < 0.05$) in the combined chlorine- monochloramien water system (Table 3.9). The strength of these negative correlations adds to the evidence of the effectiveness of monochloramine in the chlorinated water system, which was found prominent during the first 168 h of the treatment (with less than 1 $cfu.cm^{-2}$ coliforms and HPC bacteria). Bacterial regrowth between 168 and 672 h was also visualized using SEM techniques. The capability of bacterial regrowth occurring on the surface of test pipe materials during this period was linked to the depletion of the monochloramine residual concentration. At the decreased monochloramine level, biofilm cells tended to increase at higher counts affecting the microbiological quality of treated water within the distribution systems (Momba *et al.* 1998, 2001).

Although both treatments led to the formation of biofilms on the surface of test pipe materials, statistical evidence showed that a combined chlorine-monochloramine remains an attractive approach for the removal and inhibition of bacterial biofilm in potable water systems. The study therefore suggests the implementation of the combined chlorine-monochloramine process in developing countries in order to ensure adequate control measure for the formation of biofilm in potable water distribution system. Because the disinfectant decays as water flows through the pipes, chlorinated water may again receive additional monochloramine disinfection at some critical locations.

It is important to note that the generic type of pipe materials as well as the factor time did greatly influence the density of bacteria in treated water systems (Tables 3.1-3.4). Cement-based materials (C and AC) support less fixed bacteria than plastic –based materials (PVC, uPVC and MDPE). This observation was also confirmed when using SEM techniques. Similar bacterial densities were then recorded on each generic type of test pipes. Statistical evidence showed no significant difference in fixed bacterial densities between cement and asbestos cement. Similar statistical trends were also observed when comparing PVC, uPVC and MDPE. This implies that pipe

materials with similar porosity and roughness seem to support similar densities of fixed bacteria as also observed by Niquette and co-workers (2000). Compared to the stainless steel, the lower densities of bacteria fixed on cement exposed to chlorine, monochloramine, ozone, UV and hydrogen peroxide treated water have been previously recorded within 8 d of the experiment by Momba *et al.* (1998). Although the densities of bacteria fixed on cement-based materials increased with the exposure time, the highest bacterial counts were noted on the surface of plastic-based materials. Even if test pipes alone could not show statistical significant difference in the main effect, their interaction with time resulted in significant difference in bacterial counts. This means that the factor time cannot be ignored in determining the effect of pipe materials on biofilm formation and also in devising a solution for the inhibition of biofilm in potable water distribution systems. Considering factor time, the addition of monochloramine residual in the chlorinated water system at some critical locations might be recommended every 168 h after the initial chlorine-monochloramine treatment.

The exposure time always depends on the distance between the treatment point and the end points. Power and Nagy (1999) reported that the Warragamba zone, a fully treated water system with short distances between the treatment point and the end points, experienced the least problems with bacterial regrowth when compared to other zones which contained dead end points between 20 and 41 km. It would be therefore interesting in the future works to determine some critical locations between the treatment point and the end points and to quantify the effect of the distance on biofilm regrowth after the addition of monochloramine in the chlorinated water system.

Other studies have shown a decrease in nutrients available for growth of microorganisms in water distribution systems, implying utilization of substrates by microorganisms (LeChevallier *et al.*, 1987). During the present experimental study, the levels of organic and inorganic compounds were measured before and after the treatment of test waters in all four batch reactors (Table 3.5). Although a decrease in the levels of certain physico-chemical parameters (pH, COD, DOC) was recorded, statistical evidences showed that all system parameters had no significant effect on bacterial numbers at $p < 0.05$ (Tables 3.7-3.8).

From the findings of the present study, one can conclude that the presence of an effective disinfectant residual in water distributions remains one of the most important factors that contribute to the control of bacterial biofilms. The generic type of pipe materials also plays a second role in devising a solution to biofilm formation in potable water distribution system.

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

Inhibition of bacterial and biofilm regrowth in a chlorinated potable water system

* Results of this study revealed that the intake water from Alice purification system was of poor quality. No significant difference in bacterial counts (CB and HPC) was noted between Alice chlorinated water and non-disinfectant water from Alice purification treatment plant.
* This study confirms the instability of chlorine in terms of providing a sustainable residual. Chlorine alone could not provide effective treatment. This also confirms that chlorination as water treatment process cannot be a guaranteed safeguard against bacterial infection.
* A combined chlorine-monochloramine process resulted in a longer monochloramine residual that could be detected up to 168 h and 672 h in groundwater and surface water respectively.
* A combination of chlorine and monochloramine provided effective treatment for the inhibition of bacterial regrowth and biofilm formation in the laboratory-scale unit as long as monochloramine concentrations persist to *ca* 0.2 and *ca* 0.35 $mg.\ell^{-1}$ in groundwater and surface water respectively.
* Chloramination as a second disinfectant for inhibiting bacterial growth in drinking water systems was statistically proved to be the safest water purification process.
*The phenomenon of bacterial and biofilm regrowth was observed earlier in groundwater system than in surface water surface. Although a longer effective treatment was found in surface water than in groundwater, no significant difference in bacterial regrowth counts was detected in either type of source water.
*To improve the quality of potable water in water distribution systems and to make significant progress toward compliance with bacteriological quality standards at the point of consumption, it is recommended that chlorine be used as primary disinfectant followed by monochloramine as secondary disinfectant.

Comparing the effect of various pipe materials on biofilm formation in a combined chlorine-monochloramine water system.

* This investigation indicated the colonization of all test pipe materials (PVC, uPVC, MDPE, cement and asbestos cement) by coliforms and heterotrophic plate count bacteria within 20 min under chlorination treatment.
* The addition of monochloramine in the chlorinated water system resulted in the removal of coliforms and HPC attached to the pipe materials and less than 1 cfu.cm^{-2} coliforms and heterotrophic plate count bacteria (excepted for PVC) was observed on the surface of test pipes between 48 and 168 h.
* Statistical evidence showed that the generic type of pipe materials did greatly influence the density of bacteria in the water system. Cement-based materials (cement and asbestos) support less fixed bacteria than plastic-based materials (PVC, uPVC and MDPE). No significant difference in attached bacterial counts was found between the generic type of pipe materials.
* The factor time cannot be ignored in determining the effect of pipe materials on biofilm formation in potable water distribution systems.
* Statistical evidence also showed that all system parameters (temperature, pH, turbidity, dissolved organic carbon, total nitrogen, sulphate) had no significant effect on bacterial biofilms at $p < 0.05$, implying that the presence of an effective monochloramine residual in a chlorinated water system remains one of the most important factors in controlling the effect of pipe materials on biofilm formation.
* This study, therefore, recommends the use of cement and asbestos cement pipe for the distribution of chlorine-monochloramine treated water. Because the disinfectant decays as water flows through the pipes, chlorinated water may again receive an additional monochloramine disinfection at some critical locations every 168 h.

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The guidelines can be used by policy-makers and planners when considering alternatives for providing or improving the water supply to small users and rural communities. It will provide them with the necessary information in a concise form to show that upgrading of existing treatment plants offers a cost-effective way of improving the water supply to the communities. Local authorities, hospitals, forestry stations and communities can use the practical guidelines to upgrade their plants effectively. The guidelines specifically point out the advantages, disadvantages and possible pitfalls of the various treatment options. Key guidelines for selection and operation of the treatment options are given. Available databases on water treatment plants in the country as well as information gathering during the study show that of a total number of 880 treatment plants in the country, 587 (or 67%) have capacities of less than 2,5 M□/d (2 500 m^3/d) and, therefore, fall in the category of small water treatment plants.

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