THE USE OF STRIPPED GAS LIQUOR (SGL) AS PROCESS COOLING WATER: ANALYSIS OF THE EFFECTS OF OPERATIONAL CONDITIONS ON MICROBIAL COMMUNITY DYNAMICS, FOULING, SCALING AND CORROSION

by

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B. Sc. (Hons.) (University of the Free State)

Dissertation submitted in fulfilment of the requirements for the degree

MAGISTER SCIENTIAE (MICROBIOLOGY)

in the

School for Environmental Sciences and Development: Microbiology

North-West University, Potchefstroom Campus

Potchefstroom, South Africa.

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December 2007

SUMMARY

Sasol plants gasify more than 30 million tons per annum of coal via the Fischer-Tropsch process resulting in the production of synthesis gas and a condensate stream. After gravimetric separation of the tar products, the solvent extraction of phenolics and the steam stripping of ammonia from the condensate stream, a complex effluent referred to as stripped gas liquor (SGL) is generated. SGL has the potential to be used as process cooling water. Microorganisms, scale deposit and corrosion are three major problems associated with the operation of cooling water systems. Parameters that have an influence on the fouling, scaling and corrosion of industrial cooling towers include cycles of concentration (COC), flow velocity (FV) and pH. The aim of this study was to evaluate the effects of COC, pH and FV on the rates of fouling, scaling and corrosion as well as on the microbial community dynamics in cooling towers using SGL as process cooling water.

Stripped gas liquor contains hydrocarbons which can interfere with PLFA analysis. There was, therefore, a need to optimise the selective extraction of hydrocarbons so as to eliminate their interference with PLFA analysis. The method used for the extraction of hydrocarbons before silicic acid fractionation succeeded in removing the hydrocarbons and their interference and was, therefore, determined to be appropriate method to use in subsequent analyses.

Even though the two lab-scale cooling towers were of identical design their similarity or dissimilarity needed investigation. Physico-chemical analysis results confirmed that the two cooling towers were operated under identical operational conditions. The two cooling towers were found to be similar in terms of the fouling, scaling and corrosion rates as well as microbial community structure. The two cooling towers could be operated in parallel using different operational conditions with any differences in results being a reflection of the effects of operational conditions.

The effects of pH, FV and COC on the rates of fouling, scaling and corrosion as well as microbial community dynamics were evaluated with the aid of a 2^3 multi-factorial experimental design. Cycles of concentration was found to have the most profound effect on the fouling, scaling and corrosion rates followed by pH. Cycles of concentration and pH were found to have the most profound effect on microbial community dynamics. The use of the 2^3 multi-factorial experimental design also allowed the determination of optimal conditions for the operation of the cooling

towers using SGL as feed water. These conditions were found to be 5 COC, a pH of 7.5 and a FV of 0.6 m/s. In conclusion, the use of a 2^3 factorial experimental design, physico-chemical, PLFA and DGGE analyses were instrumental in the successful determination of the effects of COC, pH and FV on the rates of fouling, scaling and corrosion as well as on the microbial community dynamics in the cooling towers using SGL as process cooling water.

OPSOMMING

Sasol aanlegte vergas meer as 30 miljoen ton steenkool jaarliks via die Fischer-Tropsch proses wat lei tot die produksie van sintese gas en 'n kondensaat stroom. Na gravimetriese skeiding van die teerprodukte, ekstraksie van die fenoliese produkte en stoomstroping van ammoniak vanuit die kondensaat stroom, bly 'n komplekse afvalstroom oor wat bekend staan as "Stripped gas liquor" of SGL. Die SGL het die potensiaal om as proses verkoelingswater aangewend te word. Mikro-organismes, skaalvorming en korrosie is die drie hoofprobleme wat geassosieer word met die bedryf van verkoelingswaterstelsels. Faktore wat die bevuiling, skaalvorming en korrosie van industriële koeltorings beïnvloed sluit die volgende in: konsentrasiesiklusse (COC), vloeisnelheid (FV) en die pH. Die doel van hierdie studie was om die effekte van konsentrasiesiklusse, vloeisnelheid en pH op die tempo van bevuiling, skaalvorming en korrosie asook die mikrobiese bevolkingsdinamika te bestudeer in koeltorings wat SGL as verkoelingswater gebruik.

SGL bevat koolwaterstowwe wat kan inmeng met PLFA analises. Gevolglik is dit nodig om 'n metode vir selektiewe ekstraksie van die koolwaterstowwe te optimaliseer om die inmenging hiervan op PLFA analises te oorbrug. Die metode wat gebruik is vir die koolwaterstof ekstraksie voor fraksionering van die PLFA was suksesvol in die verwydering van die koolwaterstowwe en die gevolglike inmenging. Die metode is as geskik geag vir gebruik in gevolglike analises.

Alhoewel die twee laboratoriumskaal koeltorings van identiese ontwerp was, moes die eendersheid of verskillendheid bepaal word. Fisies-chemiese analises het inderdaad getoon dat die twee koeltorings onder identiese kondisies funksioneer. Daar is bevind dat die koeltorings eenders reageer in terme van bevuiling-, skaalvorming- en korrosietempo's, sowel as mikrobiese gemeenskapstruktuur. Die twee koeltorings kon dus in parallel bedryf word onder verskillende operasionele toestande met enige verskille in resultate wat 'n effek sou wees van die operasionele toestande.

Die effek van pH, vloeisnelheid en konsentrasiesiklusse op die bevuiling, skaalvorming en korrosie, asook die mikrobiese gemeenskapsamestelling is geëvalueer met behulp van 'n 2^3 multi-faktoriese eksperimentele ontwerp. Konsentrasiesiklusse is getoon om die grootste invloed te hê op die bevuiling,

skaalvorming en korrosie, gevolg deur pH. Konsentrasiesiklusse en pH is ook getoon om die sterkste effekte te hê op die mikrobiese gemeenskap dinamika. Die gebruik van die 2^3 multi-faktoriese eksperimentele ontwerp het dit ook moontlik gemaak om die optimale kondisies te bepaal vir bedryf van die koeltorings met SGL as verkoelingswater. Die kondisies is as volg: 5 konsentrasiesiklusse, pH 7.5 en 'n vloeisnelheid van 0.6 m/s. Samevattend was die toepassing van die 2^3 multi-faktoriese eksperimentele ontwerp, fisies-chemiese, PLFA en DGGE analises instrumenteel in die suksesvolle bepaling van die effek van konsentrasiesiklusse, pH en vloeisnelheid op die bevuiling, skaalvorming en korrosie asook die mikrobiese gemeenskap dinamika in die koeltoring waar SGL as verkoelingswater gebruik is.

This work is dedicated to my loving wife, Phumeza
and my parents Velapi and Vuyelwa

Basic research is what I am doing when I do not know what I am doing.

~Wenher von Braun~

ACKNOWLEDGEMENTS

I wish to convey my most sincere appreciation and gratitude to the following persons and institutions for their contributions to the successful completion of this study:

First and foremost God, almighty, who guided me and gave me the strength to carry on whenever I felt like giving up,

Prof. C.C. Bezuidenhout, School for Environmental Sciences and Development: Microbiology, North-West University, Potchefstroom Campus, for his guidance and support, and for never giving up on me,

Prof. K.J. Riedel, who was the originator of this research project, and Mr. P.J. Jansen van Rensburg, School for Environmental Sciences and Development: Microbiology, North-West University, Potchefstroom Campus,

Dr. L. Tiedt, Electron Microscopy, North-West University, Potchefstroom Campus,

Dr. T. Phillips, Mr. T. Stenden and Ms. J. Swarts, Sasol Technology, Research and Development (Sasol R&D) Sasolburg,

Sasol Technology, Research and Development (Sasol R&D) Sasolburg, for their technical and financial support of this study,

Mohau Booysen, your help and co-operation during the practical part of this study was greatly appreciated,

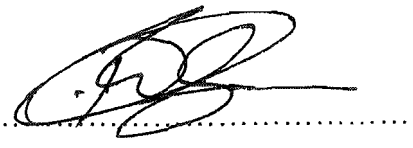
My parents, brothers and sisters for their patience, support and encouragement throughout my university career, and

Most importantly my wife, Phumeza, for her love, patience, encouragement and support. I love you ma' Mkwayi.

DECLARATION

The experimental work conducted and discussed in this dissertation was carried out in the School for Environmental Sciences and Development: Microbiology, North West University, Potchefstroom Campus, Potchefstroom, South Africa. The experimental part of this study was conducted during the period of 2001 to 2003 under the supervision of Prof. K.J. Riedel and the co-supervision of Prof. C.C. Bezuidenhout and Mr. P.J. Jansen van Rensburg.

This study represents original work undertaken by the author and has not been previously submitted for degree purposes to any other university. Appropriate acknowledgements have been made in the text where the use of work conducted by other researchers has been included.



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10/12/2007

Date

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CHAPTER 1: INTRODUCTION

1.1 INTRODUCTION AND PROBLEM STATEMENT

Global freshwater resources are under threat and the threats to freshwater ecosystems result in the continued inability to meet basic human needs for water (Gleick *et al.*, 2001). South Africa is semi-arid with an average rainfall of approximately 450 mm per annum (DWAF, 2004a). The average rainfall in the Upper Vaal Water Management Area (WMA) decreases from 800 mm per annum (mm/a) in the south east to 600 mm/a in the north west (DWAF, 2004b). These values are close to the world average of approximately 860 mm/a. This water, however, has to be distributed to the rest of the country. As a result, South Africa's water resources are, in global terms, scarce and extremely limited and need to be preserved. Sasol is the second biggest industrial water user in the Upper Vaal WMA. In 2000 Sasol I in Sasolburg and Sasol II & III in Secunda required 2 and 3 percent, respectively, of the total volume of water in the Upper Vaal WMA. This is equivalent to 71 200 ML/a and 106 800 ML/a, respectively (DWAF, 2004b).

Sasol plants gasify more than 30 million tons per annum of coal via the Fischer-Tropsch process resulting in the production of synthesis gas and a condensate stream (van Nierop *et al.*, 2000; van Dyk *et al.*, 2006). After gravimetric separation of the tar products, the solvent extraction of phenolics and the steam stripping of ammonia from the condensate stream, a complex effluent referred to as stripped gas liquor (SGL) is generated (van Nierop *et al.*, 2000). Since large volumes (60 ML/d) of SGL are produced by Sasol on a daily basis, the SGL has a potential to be used as process cooling water. The use of SGL as cooling water would enhance the water conservation measures at Sasol by reducing the volume of raw water intake, as well as reducing the final effluent volume after bio-treatment (van Nierop *et al.*, 2000).

There are, however, several problems associated with the operation of open evaporative cooling systems. These problems include microbial growth and the resultant microbially induced corrosion (MIC) (Chourday, 1998). Biofilms and scale formation in cooling water systems interfere with heat transfer across metal surfaces, lower fluid velocity (FV), increase energy utilisation and decrease operation efficiency (Brozel and Cloete, 1991; Cloete *et al.*, 1994; Smith *et al.*, 2003). Microbially induced corrosion can affect all types of metals used in cooling water systems resulting in

metal failure (Chourday, 1998). Microorganisms, scale deposit and corrosion are the major problems in cooling circuit water system (Congmin *et al.*, 2007). Parameters that have an influence on the fouling, scaling and corrosion of industrial cooling towers include pH, cycles of concentration (COC) and FV (IWS, 1994).

Conventional microbiological methodology has been applied in investigating the bacteria in industrial water systems. These methods are, however, not well suited for the detection of microbial community structure *in situ* (White *et al.*, 1996). For a better understanding of microbial diversity, other techniques that are quantitative, more representative and differentiative are required (Muyzer, 1999). PLFA analysis can be used to quantify community structure and biomass without relying upon cultivation of microorganisms (White *et al.*, 1996c; Ibekwe *et al.*, 2007). PLFA analysis provides an estimate of viable microbial biomass and physiological status of the microbial community, since PLFAs reflect the phenotypic response of microorganisms to environmental conditions (Villanueva *et al.*, 2004). Denaturing gradient gel electrophoresis (DGGE) can be used to study the microbial biodiversity and population dynamics of complex ecosystems over space and/or time (Camu *et al.*, 2007). DGGE is a powerful tool to discern changes in microbial community structure in a variety of habitats (Sievert *et al.*, 1999). The technique is capable of differentiating between two DNA molecules that differ only by a single base pair (Sheffield *et al.*, 1989). PLFA and DGGE were used in this study for the analysis of microbial community structure within the cooling towers.

1.2 AIM AND OBJECTIVES

The aim of this study was to evaluate the effects of COC, pH and FV on the rates of fouling, scaling and corrosion as well as on the microbial community dynamics in cooling towers using SGL as process cooling water. The objectives of this study were to:

i. Optimise the selective extraction of hydrocarbons from SGL so as to eliminate the interference of hydrocarbons with PLFA analysis,
ii. Determine the similarity or dissimilarity of two cooling towers in terms of the fouling, scaling and corrosion rates as well as microbial community structure when these were operated under similar operational conditions,
iii. Determine the effects of pH, COC and FV on the rates of fouling, scaling and corrosion of mild steel, as well as the structural diversity of the microbial

communities in both the planktonic and sessile phases using a 2^3 multi-factorial experimental design.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Global freshwater resources have been under threat and the threats are on the rise and are now reaching critical levels. These threats include increasing surface and groundwater pollution, global climatic changes that affect water supply and demand, resurgent water related diseases, and the destruction and degradation of freshwater ecosystems. The threats to freshwater ecosystems result in the continued inability to meet basic human needs for water; the increasing conflicts among urban, agricultural and environmental water interests; growing number of endangered and threatened species; and new outbreaks of water related diseases (Gleick *et al.*, 2001). Population growth and increasing development have increased the demand for better utilization of the limited water supplies (Choi *et al.*, 2002).

South Africa is located in a predominantly semi-arid part of the world. The climate varies from desert and semi-desert in the west to sub-humid along the eastern coastal area. The South African average rainfall was about 450 mm per year (mm/a) in 2004, down from 497 mm/a in 1997 (DWAF, 1997; DWAF, 2004a). These figures are well below the world average of about 860 mm/a. As a result, South Africa's water resources are, in global terms, scarce and extremely limited.

The Upper Vaal Water Management Area (WMA) covers part of four provinces viz. Gauteng, Free State, Mpumalanga, and North West provinces. The average rainfall in the WMA decreases from 800 mm/a in the south east to 600 mm/a in the north west with the potential evaporation increasing from 1300 mm/a in the south east to 1700 mm/a in the north west (DWAF, 2004b). The balance of water (available water less water requirements) in the Upper Vaal WMA is expected to decrease from 19 million m^3/a in 2000 to a shortfall of 44 million m^3/a in 2025. The total gross water use for the Vaal River System for the year 2000 was estimated at 3560 million m^3/a (DWAF, 2004b).

Nearly 20% of the gross domestic product (GDP) of South Africa originates from the Upper Vaal WMA. This is the second largest contribution to the national wealth amongst all the WMAs. The manufacturing sector contributes over 30% to the gross

geographic product (GGP) generated in the WMA, followed by trade at just over 15% and both finance and mining slightly higher than 10% (DWAF, 2004b).

In order for a sustainable future to be secured emphasis should be placed on integrated water resource management (DWAF, 1997; DWAF, 2004b). Gleick *et al.* (2001) suggested that efforts to explore non-structural alternatives to water supply should be widely encouraged, including efficiency improvement, demand management and wastewater reuse.

Sasol plants gasify more than 30 million tons per annum of coal to synthesis gas, which is converted to more than 200 fuel and chemical products via the Fischer-Tropsch process (van Nierop *et al.*, 2000; van Dyk *et al.*, 2006). Sasol produces the equivalent of 150 000 barrels per day of fuels and petrochemicals from coal via its indirect liquefaction process. The process produces more that 40% of South Africa's liquid fuel requirements (van Dyk *et al.*, 2006). During the gasification process coal is converted to crude gas under pressure and at high temperatures in the presence of steam and oxygen (Figure 2.1; van Dyk *et al.*, 2006). The aqueous stream, resulting from the cooling of the crude gas, contains valuable co-products such as tars, phenols and ammonia. After gravimetric separation of the tar products, the solvent extraction of phenolics and the steam stripping of ammonia from the condensate stream, a complex effluent referred to as stripped gas liquor (SGL) is generated (van Nierop *et al.*, 2000). A summary of the Sasol process is illustrated in Figure 2.1.

Coal gasification wastewater characteristics, of which SGL is the main stream, are similar to coke plant wastewater (Zhang *et al.*, 1998). Their contaminants include ammonia, cyanide, thiocyanide, and many toxic organic contaminants such as phenols, mono- and poly-cyclic nitrogen containing aromatics, oxygen and sulphur-containing heterocyclic compounds and poly-aromatic hydrocarbons (Figure 2.1; Zhang *et al.*, 1998). The effluent from the coal-gasification process can contain more than 3 g/L of phenols (Yang *et al.*, 2006). Phenolics account for 80% of the total COD in these waters (Zhang *et al.*, 1998).

Water is the preferred medium for removing this excess heat from process streams in industrial applications because of its low cost, availability in large quantities, ease of handling under process conditions, and it's very good and useful thermal properties (de Almeida *et al.*, 1997; Chourday, 1998). Open evaporative cooling water systems

(Figure 2.2) provide economical heat sinks because they can handle high heat loads with minimal water loss (Chourday, 1998). Water scarcity and environmental considerations have ensured that open recirculating cooling tower systems are the most acceptable and widely adopted method for cooling industrial water (de Almeida *et al.*, 1997).

The water used for the cooling is usually potable water or untreated surface waters. In some cases the water is an industrial effluent that cannot just be released into the river, and even if it could it would be more cost effective to use this effluent as cooling water than to buy millions of litres of water per day. In an attempt to reduce the intake of raw water from the environment, re-use of process effluents as cooling water has been implemented at Sasol, South Africa.

Large volumes (60 ML/d) of SGL are produced by Sasol on a daily basis. SGL has the potential to be used as process cooling water. The use of SGL as cooling water would enhance the water conservation measures at Sasol by reducing the volume of raw water intake to the synfuels complex, as well as reducing the volume of final effluent water that is returned to the Vaal River after bio-treatment (van Nierop *et al.*, 2000). Although an increase in the pollutant concentration will be associated with the use of SGL as cooling water, the total load in the final effluent being released will be decreased. Approximately 9 ML/d of SGL are currently being used by Sasol as cooling water. With the use of SGL instead of potable water as cooling water Sasol has reduced their water cost by at least R15 million per annum based on 2006 – 2007 financial year Rand Water industry tariffs (http://www.randwater.co.za/NewsDetails.asp?NewsID=830, accessed 22/11/2006).

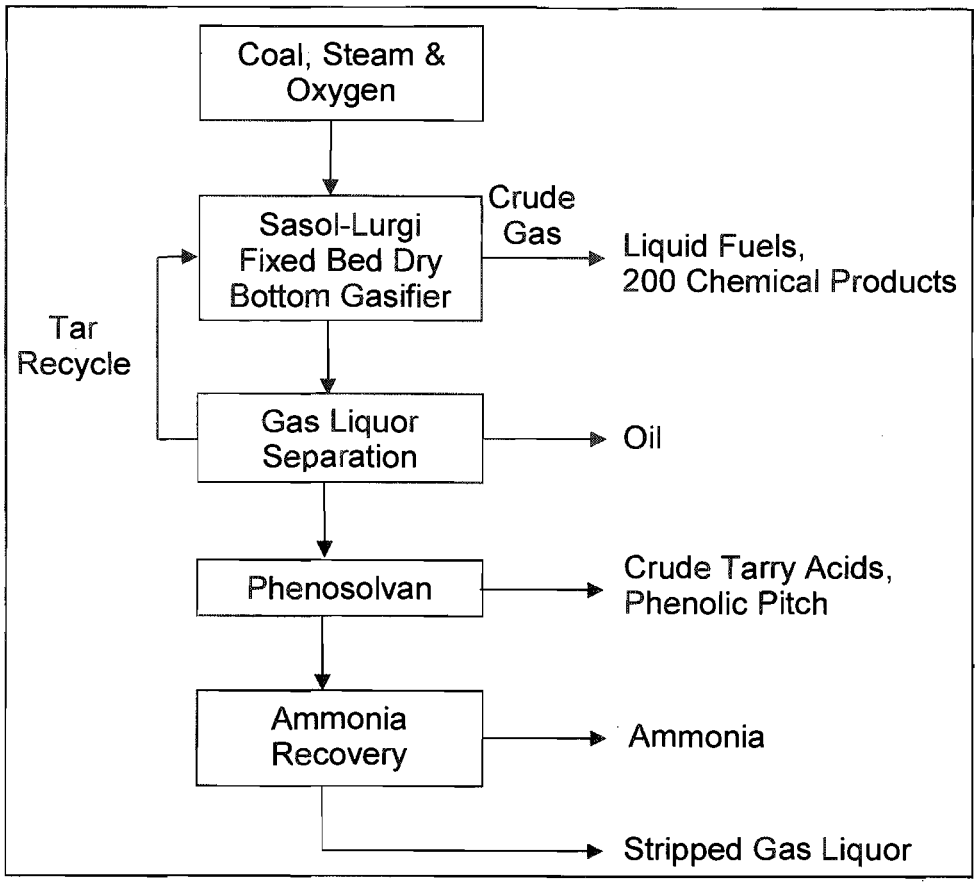


Figure 1. The coal gasification to stripped gas liquor process at Sasol (van Nierop *et al.*, 2000; van de Venter, 2005).

The use of industrial effluents in cooling water systems affects both the quantity and community structure and function of microbial populations in the cooling water systems. This needs to be considered as microbial populations can affect the operation of the cooling water systems.

Due to the presence of microorganisms in most cooling water systems degradation of certain components of the effluent is also possible, allowing the cooling water system to operate as a bioreactor (Weissman *et al.*, 1998; Tanji *et al.*, 2007). This would result in a reduction of the final effluent organic load on the receiving water body. The volume of water released would also be reduced due to large volumes of water being lost through the evaporation that occurs during the cooling process.

There are, however, several problems associated with the operation of open evaporative cooling systems. Microorganisms, scale deposit and corrosion are the major problems in cooling circuit water system (Congmin *et al.*, 2007). Favourable conditions for the development of fouling, scaling and corrosion problems exist in open recirculating cooling water systems with cooling towers (de Almeida *et al.*,

1997). Stripped gas liquor contains nutrients that could possibly encourage the growth of bacteria. Conditions in cooling water systems are favourable for the growth of bacteria (elevated temperatures and high dissolved oxygen levels), leading to fouling. Biofilms and scale formation in cooling water systems interfere with heat transfer across metal surfaces because they act as insulators due to their low conductivity; they also lower FV, increase energy utilisation and decrease operation efficiency (Brozel and Cloete, 1991; Cloete *et al.*, 1994; Smith *et al.*, 2003). Microbially induced corrosion (MIC) is the corrosion of metals, which occurs directly or indirectly as a result of microbial metabolism (von Holy, 1987). Microbially induced corrosion has been shown to correlate with specific biofilm activities and can affect all types of metals used in cooling water systems (Chourday, 1998; White *et al.*, 1999).

Fouling, scaling and MIC cost industry millions of Rands a year. Fouling of heat exchanger has been estimated to cost in the order of 10^{10} Euro per year in Europe (Bott, 1995). Microbially induced corrosion accounts for approximately 10% of all metallic corrosion, resulting in costs of around R400m per annum in South Africa alone (Brözel and Cloete, 1989). This is money that could be effectively used in other parts of the business. Since biofilms consist of a large diversity of microorganisms there is a vast array of interactions that occur in the biofilm that can lead to microbially induced corrosion (MIC). Analysis of the microbial community diversity in these biofilms would lead to a better understanding of these interactions. This understanding would aid in the control and combating of biofouling and MIC.

Conventional microbiological methodology has been applied in investigating the bacteria in industrial water systems. However, these methods are limited in their ability to accurately detect bacterial numbers and microbial community function and structure *in situ* (White *et al.*, 1996b). These methods are not well suited for the assessment of microbial community composition or in situ phenotypic activity of microorganisms. This has necessitated the development of new techniques that are more representative and more quantitative for the study of microbial diversity. Several biochemical assays have been developed for the detection of specific microorganisms associated with biofouling and MIC (Little *et al.*, 1998). Unlike the culturing techniques these techniques are not dependent on microbial culturability but they measure constitutive properties including PLFA, cell bound antibodies, DNA, and adenosine-5'-phosphosulfate reductase and hydrogenase (Little *et al.*, 1998).

2.2 COOLING TOWERS

A cooling water system consists of a cooling tower, a pumping system and a network of heat exchangers or coolers arranged in parallel, in series or in a combination of both (Picon-Nunez *et al.*, 2007). Cooling towers are commonly used in large thermal systems, such as most industrial power generation units, refrigeration and air conditioning plants, chemical and petrochemical industries, to reject waste heat. The towers are designed to cool a warm water stream through evaporation of some of the water into an air stream (Figure 2.2). There are several types of cooling towers. The mechanical draft cooling tower is probably the most common wherein the water enters at the top of the tower as a spray and flows downward through the tower. Ambient air is drawn into the tower using fans, and flows in a counter or crosscurrent direction to the water stream (Figure 2.2). If the fans are at the bottom of the tower and blow the air upward past the water stream, the tower is called a forced draft tower, whereas if the fans are at the top, it is an induced draft tower (Qureshi and Zibair, 2006).

Heat exchangers have tubes with cool water running through them and the hot process stream running over the tubes with cool water. Since the hot and cold water run counter current the process stream becomes cooler but the water in the heat exchanger becomes hotter. The water inside the heat exchanger then needs to be cooled down. This cooling is accomplished with the aid of cooling towers. Open recirculating cooling tower systems take water from the cooling tower sump, circulate it through the process equipment requiring cooling, and returns the water through the evaporation unit, where the water is cooled as some of it evaporates (Figure 2.2; Garrett-Price *et al.*, 1985). In a counter-flow cooling tower, the hot process water to be cooled is sprayed into an upward flowing air stream using a number of nozzles. The nozzles are arranged in such a way that the water is uniformly distributed over the fill material. Due to heat and mass transfer, the water temperature is reduced while the air enthalpy is increased because the air is heated and saturated by the water as it moves up (Qureshi and Zibair, 2006).

The concentration of dissolved and suspended solids, and salts, as well as the natural action of water on system metals and temperature variations within the cooling water system, leads to a number of water related problems (Chourday, 1998). Microorganisms, scale deposit and corrosion are the major problems in cooling circuit water systems (Congmin *et al.*, 2007). Recirculation of the water in cooling towers

results in the concentration of dissolved and suspended substances due to the evaporation, thereby promoting scale formation, biofouling, and subsequent macro fouling of the system and eventually microbially induced corrosion (Cloete *et al.*, 1994). Make up water must be added to replace the water that is lost via evaporation and blow down (bleeding) is required to prevent excessive concentration of dissolved and suspended solids in the cooling water (Garrett-Price *et al.*, 1985).

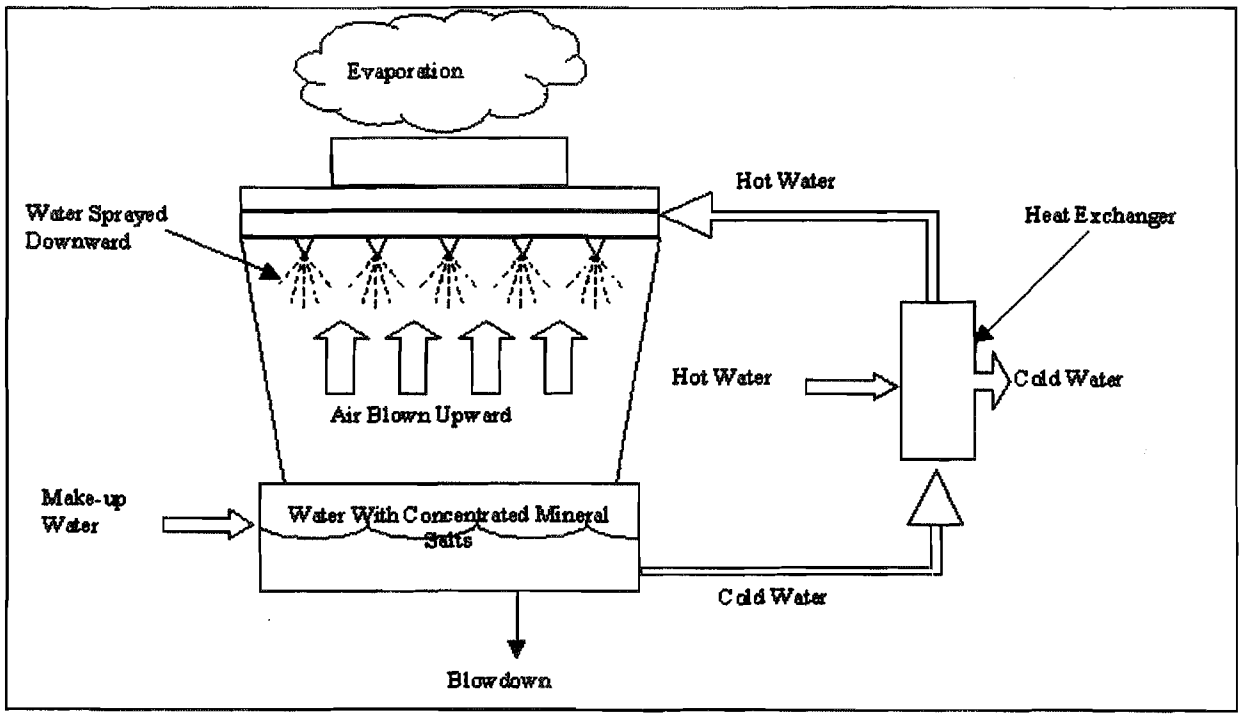


Figure 2. Schematic representation of a typical open re-circulating cooling water tower.

Fouling in cooling water systems can be classified as biological (biofouling), chemical (scaling) or physical (de Almeida *et al.*, 1997). In cooling water systems microbial growth could be very high due to the presence of nutrients, favourable temperatures and high residence time (Ludensky, 2003). Open recirculating cooling water systems also offer plenty of water, are exposed to sunlight, have a pH range of 6 to 9, have good aeration and provide a continuous source of bacteria from makeup water and ambient air (Chourday, 1998). The contamination of cooling water promotes the fouling of heat exchanger surfaces (de Almeida *et al.*, 1997). This contamination may either be from leakages in the process plant, from the makeup water, or from the scrubbing of the air in the cooling towers (de Almeida *et al.*, 1997; Chourday, 1998).

The microorganisms present in the cooling towers can be categorised as either planktonic or sessile. Planktonic microorganisms are dispersed in the cooling water,

whereas sessile microorganisms are attached to surfaces. Sessile microorganisms are known as a biofilm (Tanji *et al.*, 2007). Microbially induced corrosion, which is a result of the formation of biofilms, is a serious problem in cooling systems (Chourday, 1998). Fouling and corrosion in cooling towers are deeply interrelated phenomena (de Almeida *et al.*, 1997). Corrosive processes generate precipitates that result in fouling, which in turn initiate the formation of differential aeration cells in oxygen saturated cooling water, followed by corrosion under the deposits (de Almeida *et al.*, 1997).

2.3 FOULING

Fouling is the accumulation of scale, organic matter, corrosion products, coke, particulates or other deposits on a heat transfer surface (Kukulka and Devgun, 2007). Fouling in cooling water systems can be classified according to its causes. The causes are biofilm formation due to microbial adhesion and proliferation, scale formation by precipitation of salts that have exceeded their solubility limit, due to the concentration effect in cooling water systems, and deposit formation from suspended particles in the circulating water (de Almeida *et al.*, 1997). Fouling is complex, costly, and affects many different industries. Its development is more rapid in flowing systems where adequate nutrients are available, as is found in most cooling tower systems (Kukulka and Devgun, 2007). Increased biofouling can result in increased corrosion rates due to microbial action (microbially induced corrosion).

Biofouling is the undesirable deposition and growth of organisms on surfaces in contact with liquid media, resulting in the formation of biofilms (Flemming *et al.*, 1998; Kumar and Anand, 1998). A biofilm is the functional consortium of microorganisms and extra-cellular polymer substances (EPS) in association with a solid surface (Kumar and Anand, 1998). Bacteria in natural systems seldom appear in pure culture. Biofilms, therefore, consist of multi-species communities (White *et al.*, 1999). The EPS form as much as 50 to 90% of the biofilms' organic carbon (Christensen and Characklis, 1990).

The adhesion of microorganisms onto surfaces is a function of the surface roughness with microorganisms attaching better to the rougher surfaces (Garrett-Price *et al.*, 1985). *Pseudomonas* spp. are normally the first to adhere to the surfaces because they posses polar flagella, therefore they attach very well (Cloete *et al.*, 1994). The types of microorganisms that are mainly responsible for the formation of biofilms are

the heavily encapsulated, fast growing bacteria such as species of *Arthrobacter*, *Aerobacter*, *Proteus*, *Bacillus* and *Pseudomonas* (Chourday, 1998). Bacterial detachment from and absorption into the biofilm provides a means of interaction between the planktonic bacteria and the biofilm. Low local fluid velocities promote the attachment of microorganisms to surfaces. The growth of these biofilms is limited by the shearing that occurs at high fluid FV (Garrett-Price *et al.*, 1985). Water carries the detached cells and they then attach on another surface resulting in biofilm formation downstream (Characklis and Cooksey, 1983).

The formation of biofilms offers bacteria certain advantages. Biofilms support a very wide range of microorganisms, as can be seen in Figure 3 and Figure 2.4 (Nielsen *et al.*, 1997; Little *et al.*, 1998). This allows for the establishment of biological niches, allowing organisms that could not compete or survive in a completely mixed suspended growth system to thrive successfully in biofilms (Bishop, 1997). Bacteria in biofilms are in close proximity with other bacteria with which they are in mutualistic relationships and, therefore, have greater access to nutrients (Cloete *et al.*, 1994). Sulphate reducing bacteria, chemo-organotrophic bacteria, and bacteria involved in iron-manganese cycles are the most commonly found microorganisms in biofilms (Figure 2.4; von Rege and Sand, 1998). Biofilms also account for microbially induced corrosion (MIC) of metal surfaces since the microbial species within biofilms can give rise to MIC (Poulton *et al.*, 1995). The processes that can lead to MIC are illustrated in Figure 2.4.

Biofilms consist of complex microbial communities that interrelate between the aerobic, facultative and anaerobic layers of the biofilm in terms of substrate utilisation and waste removal (Figure 2.4). One should therefore not isolate individual organisms or groups of organisms but look at the microbial community as whole in order to gain some insight into the dynamics that exist in biofilms.

Biofouling is a complex process that is hard to measure and requires potentially hazardous products to control (McCoy *et al.*, 1998). The formation of biofilms interferes with heat transfer across metal surfaces, increasing heat transfer resistance, since biofilms act as insulators due to their low conductivity (Cloete *et al.*, 1994). A biofilm will most probably form on the cooling tower's fill when the circulating water has a high biological oxygen demand (Beyer, 1993). The cleaning of the heat transfer surfaces sometimes requires long shutdown periods (2-3 days),

resulting in high cleaning, repair and lost production costs and results in the reduction of operational hours (de Almeida *et al.*, 1997; van Agtmaal *et al.*, 2007).

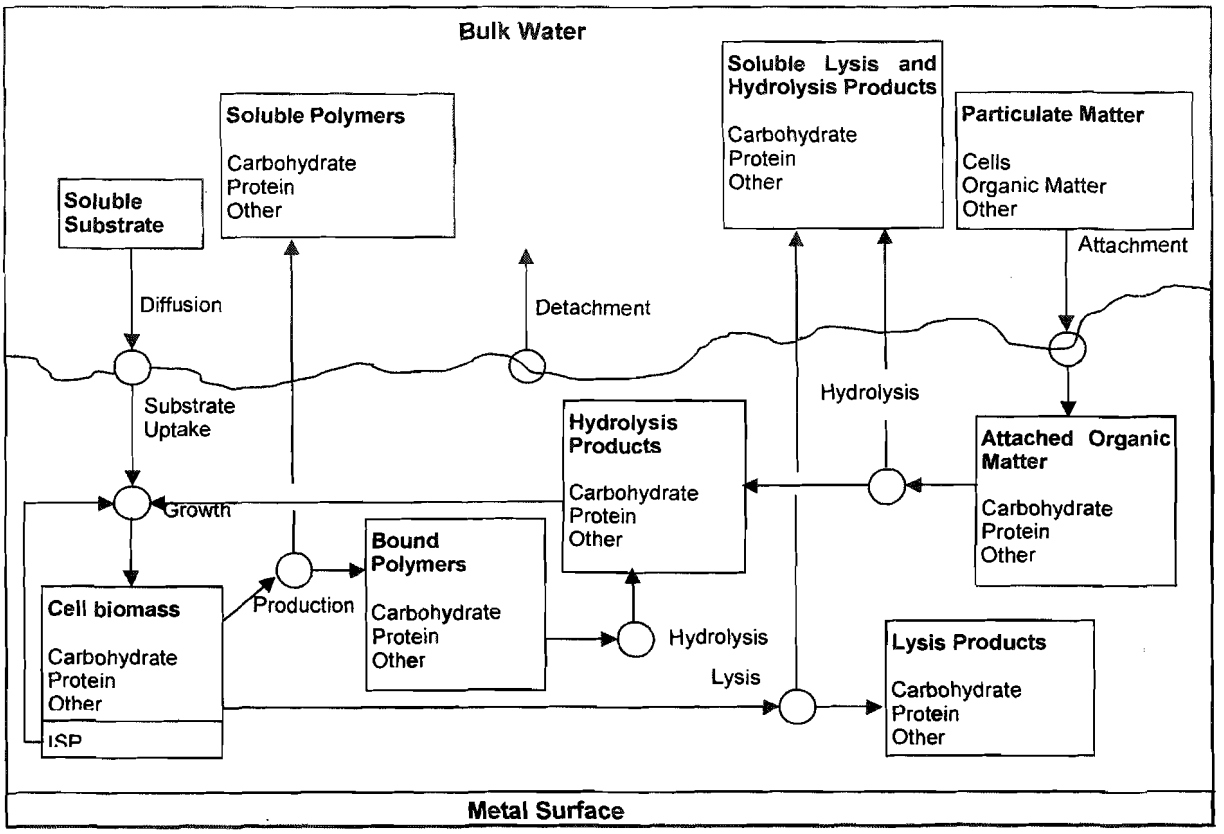


Figure 3. Suggested model of the composition of a biofilm and the main processes that occur within it (adopted from Nielsen *et al.*, 1997)

Low FV may provide stagnant areas that enhance biofilm formation and the precipitation of organic and inorganic contaminants from the circulating water (IWS, 1994). It is, therefore, generally expected that an increase in flow rates would prevent bacterial attachment and growth on metal surfaces. Soini *et al.* (2002) reported that FV of 1.5–5.2 m/s had an insignificant effect on biofilm formation. They did, however, also find that an increase in the FV decreased bacterial densities indicating preferable biofilm formation on areas with low FV. Lehtola *et al.* (2006), however, reported a 13 to 15 times increase in biofilm formation with increased FV of water from 0.03 to 0.28 m/s. Chen *et al.* (2005) reported that biofilm adhesive strength increased as the fluid velocity increased from 0.6 to 1.6 m/s. They also found that the biofilms generally grew as a more compact pattern at the higher fluid velocity. Kobrin (1994) reported fouling and subsequent stainless steel failure, due to microbially induced corrosion, at flow rates of 2.4 to 3 m/s.

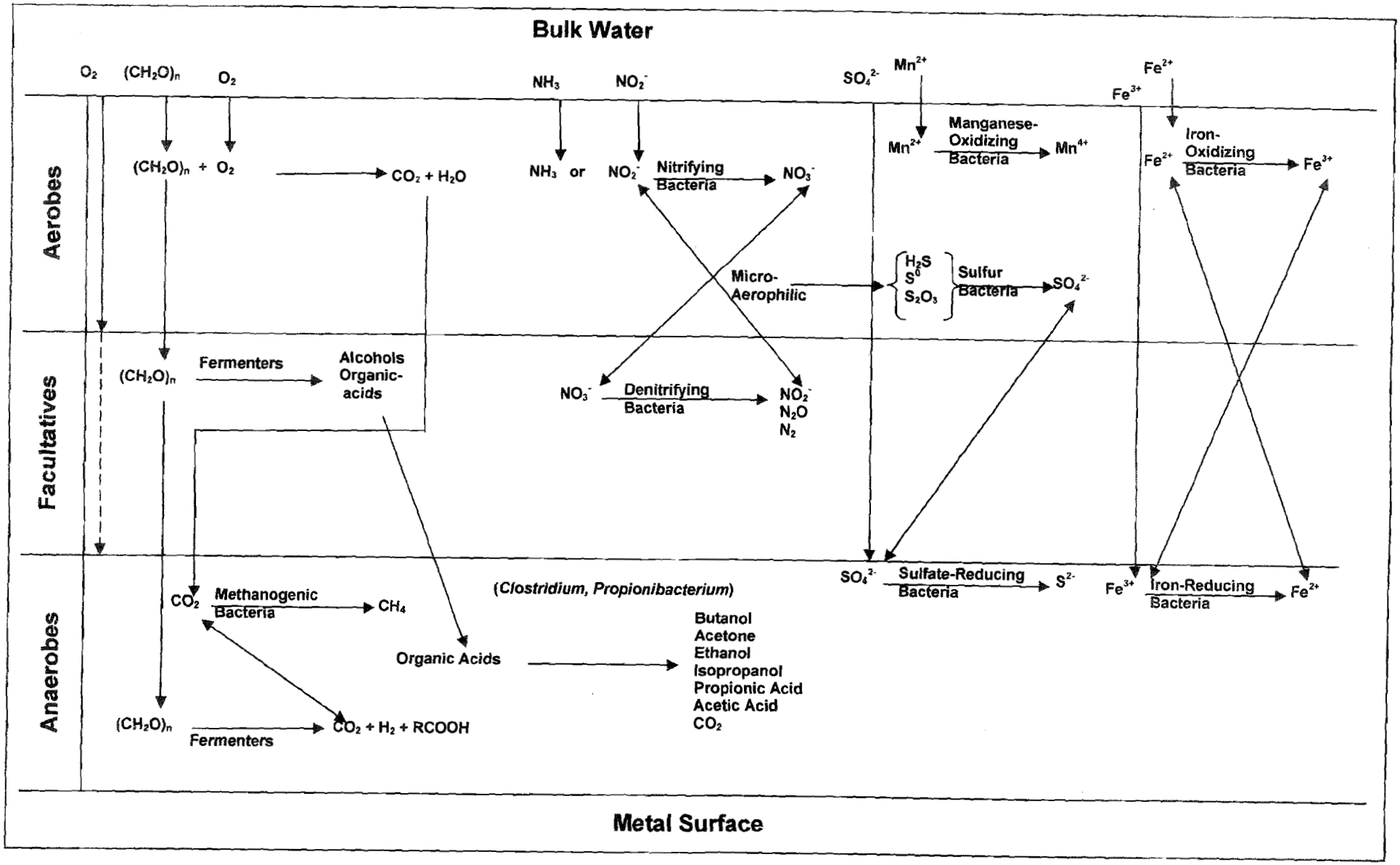


Figure 4. Strata within a typical biofilm and possible reactions within it (Little *et al.*, 1998)

Increasing FV helps to transport the nutrients of bulk water into the biofilm, causing the bacteria within the biofilms to multiply more quickly (Tsai, 2006). Brito and Melo (1999) found that a transient variation in the liquid velocity from 1.5 up to 13.2 m/h, imposed after the biofilm had reached steady-state conditions, increased the internal mass transport by 20% on average. Conversely, increasing FV is also helpful to the sloughing of biofilms and the penetration of disinfectants into biofilms, thus suppressing biofilms formation (Tsai, 2006). Kukulka and Devgun (2007) suggest that stronger shear forces will not entirely prevent fouling, but will lead to thinner and firmer deposits. Cloete *et al.* (2003), however, found that biofilms were detached at flow rates of 3 – 4 m/s. The IWS (1994) stated that increased FV result in reduced fouling rates but warned that the increased flow rates can result in increased erosion/corrosion and impingement attack problems, especially if suspended matter is carried in the cooling water.

2.4 SCALING

A number of mineral salts cause scaling in cooling water systems. Most of these mineral salts are present in the make-up water supply (IWS, 1994). Even if the make-up water is not hard, the circulating water in a cooling-tower system is generally hard due to the evaporation of water leaving mineral ions behind in the circulating water (Lee and Cho, 2002). Calcium carbonate ($CaCO_3$) is the most common constituent of scale found in cooling water systems in hard water areas (IWS, 1994). Calcium carbonate can be formed when a sharp rise in pH favours the decomposition of bicarbonate: $Ca^{2+} + HCO_3^- + OH^- \rightarrow CaCO_3 + H2O$ (Rakanta *et al.*, 2007). Scale often observed in industry includes calcium carbonate, calcium sulphate, barium sulphate, silica, and others (Kim *et al.*, 2001).

Scale formation is affected by factors such as pH, ion concentration, temperature, heat transfer, water composition, chemical treatment, plant design, FV and retention time (IWS, 1994). A lower pH generally leads to increased solubility of salts and therefore a reduced scaling potential (IWS, 1994). The concentration effect in cooling water systems may result in scale formation due to the precipitation of salts that have exceeded their solubility limits (Cloete *et al.*, 1994; de Almeida *et al.*, 1997). As hard water is heated, its solubility changes resulting in scale formation on heat exchangers. This happens in the case of calcium and magnesium carbonates that exhibit inverse solubility behaviour with temperature (Kim *et al.*, 2001; Picon-Nunez *et al.*, 2007).

Theoretical Langelier Saturation Index (LSI) or/and the Ryznar Stability Index (RSI) can be used as indicators of the scaling or corrosion potential of water (Al-Rawajfeh, and Al-Shamaileh, 2007; Melidis *et al.*, 2007). These indices are used under the assumption that if water is scale forming, corrosion rates will be negligible. As a consequence waters that are not scale forming are considered corrosive when interpreting the LSI. The latter index is the most widely used indicator of cooling water scaling potential but provides no indication of the amount of scale or actual precipitation of $CaCO_3$. The index indicates the driving force for scale formation and growth in terms of pH (as a master variable), alkalinity (reported as $CaCO_3$ in mg/L), calcium concentration (as Ca^{2+} in mg/L), total dissolved solids (TDS in mg/L) and water temperature. The RSI combines empirical data with theory of calcium carbonate saturation to predict the scale forming tendency and how corrosive the water is (Rakanta *et al.*, 2007).

A scaling layer can offer some protection against corrosion, implying that higher scaling rates should generally result in lower corrosion rates (IWS, 1994). Marin-Cruz *et al.* (2006), however, established that corrosion and scale processes are closely related and occur simultaneously at different sites of the metallic surface. Rakanta *et al.* (2007) also found that the presence of chloride ions, associated with scale formation, increased corrosion as a result of the formation of galvanic cells caused by the difference of oxygen concentration in the water and in areas where there was a salt cover or entrapment of water. Scaling causes loss of capacity for thermal exchange and in severe cases restricts water flow (Marin-Cruz *et al.*, 2006; Rakanta *et al.*, 2007).

2.5 CORROSION

Conditions that influence corrosion rates in cooling water systems are conditions of metal surfaces (deposits, pitting corrosion under deposits), nature of the environment (low pH leads to increased corrosion rates), oxygen content, flow rate (increased flow rate increases the oxygen access to the surface), and water quality (hard waters are scale forming and less corrosive) (Rakanta *et al.*, 2007). The corrosion rate of metals in cooling towers can be increased by dissolved oxygen and dissolved carbon dioxide, the latter lowering the pH. This means that even if the water is alkaline the system can be affected by oxygen corrosion (IWS, 1994).

The following are the elementary reactions of the corrosion process, $Fe^0 \rightarrow Fe^{2+} + 2e^-$ (1) and $O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$ (2). The reaction of carbon steel dissolution (Eq. (1)) will generate corrosion products, whereas the oxygen reduction reaction (Eq. (2)) will produce OH^- ions, giving rise to a local modification of pH. Both reactions promote the formation of Fe(II), and Fe(III) compounds on the surface including iron oxides (Fe_3O_4, Fe_2O_3), hydroxides ($Fe(OH)_3$), sulphates ($FeSO_4$) and chlorides ($FeCl_3$). The oxygen reduction reaction additionally gives rise to scaling due to the presence of Ca(II) and HCO_3^- ions in the medium, according to the reaction: $Ca^{2+} + HCO_3^- + OH^- \rightarrow CaCO_3 + H_2O$ (3). Given that reactions (1) and (2) occur simultaneously in different zones of the same surface, the scale process will be coupled to the corrosion process (Marin-Cruz *et al.*, 2006).

Weight loss measurement of metal coupons is one of the methods that is used to determine corrosion rates. Rao *et al.* (2000) used carbon steel coupons to determine the corrosion rate in a cooling water circuit. Choi *et al.* (2002) also used carbon steel coupons to determine the effects of an inhibitor on carbon steel dissolution in synthetic cooling water through weight loss measurements. The advantage of using corrosion coupons is that their use is relatively simple and inexpensive. Disadvantages include the assumption that corrosion is uniform and that the corrosion rate is integrated over the exposure time. Extreme variations in corrosion rate cannot be detected (de Bruyn, 1996).

2.5.1 Microbially Induced Corrosion (MIC)

MIC can be defined as the corrosion of metals, which occurs directly or indirectly as a result of microbial metabolism and has been shown to correlate with specific biofilm activities (von Holy, 1985; White *et al.*, 1999). Microbiologically induced corrosion (MIC) occurs when a unique combination of biological factors interact with the chemistry of the water, temperature, FV, component metallurgy and the presence of organic and inorganic fouling materials (Martinez *et al.*, 2004). Microorganisms can modify the near-surface environmental chemistry by microbial metabolism and may interfere with electrochemical processes occurring at the metal-environment interface, promoting localised corrosion and affecting all types of metals (Chourday, 1998; Angell, 1999; Chen *et al.*, 2007). In 1995 Poulton *et al.* estimated that microbially induced corrosion accounted for approximately 10% of all metallic corrosion in South Africa, resulting in costs of approximately R400m per annum. This figure could have substantially increased since 1995.

Biofilms result in reduced oxygen concentrations near the metal surface and many bacteria that are active fermenters under such conditions result in the production of aggressive acids that corrode metal surfaces (Figure 2.4; Gaylarde and Morton, 1997). de França and Lutterbach (1996) and Lewandowski *et al.* (1997) stated that microbial activity in biofilms accelerates fouling processes and may result in the corrosion of industrial appliances. The presence and metabolic activities of bacteria on metal surfaces produce environments that can alter rates of partial reactions in corrosion processes and shift corrosion mechanisms (Figure 2.4; Congmin *et al.*, 2007). The most severe microbiologically induced corrosion takes place in aquatic solution where physiological groups of aerobic and anaerobic microorganisms interact (Congmin *et al.*, 2007). Although both the aerobic and anaerobic bacteria cause MIC, the corrosion reactions are different. Microbially induced corrosion does not produce a unique form of localised corrosion. Instead, it can result in pitting, crevice corrosion, under deposit corrosion, enhanced galvanic corrosion and erosion corrosion (Little *et al.*, 1998, White *et al.*, 1999).

2.5.1.1 Organisms Involved in Microbially Induced Corrosion

Several groups of microorganisms are responsible for MIC. The bacteria causing corrosion of iron and steel include sulphate-reducing bacteria (SRB), acidophilic *Thiobacillus* spp., iron-reducing bacteria, iron-oxidizing bacteria, etc. (Chen *et al.*, 2007). Other organisms involved in MIC include acid producing microorganisms, methanogenic bacteria and algae. The most devastating MIC occurs in the presence of microbial consortia in which many types of bacteria interact in complex ways within the biofilm structure, as can be seen in Figure 2.4 (Little *et al.*, 1998). One must therefore not be misled by the discussion of the groups of microorganisms individually. It would be very difficult, if not impossible, to discuss the biofilm as a whole mainly because the interactions that exist in a mature biofilm are highly complex and not fully understood.

Sulphate reducing bacteria cause corrosion by acting as cathodic depolarisers and by producing corrosive hydrogen sulphide (Figure 2.4; Odom, 1990; von Holy, 1987). Even though not commonly known sulphate reduction also occurs under aerobic conditions (Santegoeds *et al.*, 1998). This implies that SRB can cause corrosion under both aerobic and anaerobic conditions. Anaerobic SRB are, however, the

most significant contributors to the MIC of iron and ferrous alloys in aquatic and terrestrial habitats (Beech *et al.*, 1998). Corrosion due to SRB occurs mostly where metal structures are covered by biofilm and, as a result, the metal surface is devoid of oxygen (Cloete *et al.*, 1994; Chourday, 1998). Under anaerobic conditions SRB corrode steel and other alloys four times faster than when corrosion is oxygen-promoted (Cloete *et al.*, 1994).

Chen *et* al. (2007) showed that iron oxidising bacteria (IOB) are able to adhere to steel surfaces and cause serious corrosion damage. Iron-oxidising bacteria are recognised for their ability to deposit iron hydroxide in structures outside their cells, creating environments that are conducive to corrosion, especially on metals that are prone to crevice corrosion (Chourday, 1998). The IOB, *Gallionella* spp., oxidises ferrous to ferric iron ($Fe^{2+} \rightarrow Fe^{3+} + e^-$), thereby catalysing tubercle deposition, especially on stainless steel welding seams, where bacterial deposits are rich in both iron and manganese (Iverson, 1987; Brozel and Cloete, 1989). Cells and metal ions form dense deposits that effectively exclude oxygen from the metal surface and initiate a series of events that are individually or collectively extremely corrosive (Chourday, 1998).

Ferric iron is insoluble, except at low pH, thereby protecting the metal surface from further corrosion due to chemical activity (Cloete *et al.*, 1994). Iron-reducing bacteria reduce ferric iron (Fe^{3+}) to soluble ferrous iron (Fe^{2+}), removing layers of oxide from the metal surface thereby forcing mineral replacement reactions that lead to further corrosion of the metal (Iverson, 1987; Angell, 1999). *Clostridium* spp., *Desulfovibrio* spp., *Vibrio* spp., *Bacillus* spp. and *Micrococcus* spp. are capable of anoxically reducing Fe^{3+} (Cloete *et al.*, 1994). This group of bacteria therefore promotes corrosion indirectly.

Both the organic and inorganic acids produced by microorganisms can be highly corrosive to metals. A number of organic acids have been associated with metal corrosion; these include acetic acid, butyric acid, carbonic acid, glycolic acid, tricarboxylic amino acids and fatty acids (Figure 2.4). Organic acids produced by a number of bacteria, including *Lactobacillus delbrueckii*, have been found to cause extensive corrosion of iron (Iverson, 1987). *Propionibacterium* spp. produce propionic and acetic acids and *Clostridium* spp. produce primarily acetic acid, which have been implicated in the corrosion of metals (Montville *et al.*, 1985; Holt *et al.*,

1994). Organic acids of fungal origin have reportedly corroded aluminium, steel and copper (Iverson, 1987).

Acidophilic sulphur-oxidising bacteria *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* produce sulphuric acid (H_2SO_4) from the oxidation of sulphur, sulphides or sulphates. This results in localised pH depression to as low as 1 (Iverson, 1987; Chourday, 1998). The drop in pH results in severe thinning of metals where these organisms are in contact with the metal (Chourday, 1998). Methanogenic bacteria induce corrosion and oxidation of elemental iron and mild steel via cathodic depolarisation. In the process methane gas is produced (Figure 2.4) as a result of the chemical production of low levels of hydrogen gas ($8H^+ + 4Fe^0 + CO_2 \rightarrow CH_4 + 4Fe^{2+} + 2H_2O$) (Boopathy and Daniels, 1991). Algae produce molecular oxygen, corrosive organic acids, slime, and nutrients for other organisms involved in MIC (Cloete *et al.*, 1994). They grow in the presence of light and will, therefore, mainly proliferate in the light exposed areas of cooling water systems (IWS, 1994). The blue-green algae *Nostoc* spp. and *Anabaena* spp. have been implicated in mild and stainless steel weld-seam corrosion and hydrogenase-positive *Chlorophyta* spp. and *Cyanophyta* spp. aggravate corrosion through cathodic depolarisation (Cloete *et al.*, 1994).

2.6 CULTURE DEPENDENT BACTERIA MONITORING METHODS

Two methods, based on the determination of viable counts, are frequently used to monitor microbial presence and activity in water systems. These techniques are the plate count technique and the most probable number (MPN) technique (Cloete *et al.*, 1996). The Biolog system has also been used to determine the functional diversity of microbial communities (Zhang *et al.*, 2008).

2.6.1 The Plate Count Method

The plate count method is based on the culturability of the microorganisms in the sample under observation. This technique assumes that a visible colony will develop from each organism in the sample (Bott *et al.*, 1983). The advantage of culturing techniques is that they are extremely sensitive in that low numbers of bacteria grow to easily detectable higher numbers in the proper growth medium (Little *et al.*, 1998). Identification of microorganisms based solely on this method yields valuable information (Kawai *et al.*, 2002). The method, however, only accounts for 0.1% to

10% of the total community detected by direct counting methods (Macnaughton *et al.*, 1997).

2.6.2 The Most Probable Number (MPN) Method

The MPN method is based on a statistical estimation of the viable biomass. It is assumed that even if a test tube with growth medium were inoculated with one cell, growth would occur (Hopton *et al.*, 1972). Although the MPN method gives higher bacterial counts than the plate count method; this method also has significant disadvantages. Probabilistic estimations are used, which results in a higher frequency of error (Melchiorri-Santolini, 1972). All MPN methods tend to underestimate the number of bacteria in environmental samples due to clumping of cells and attachment to particulate matter (Vester and Ingvorsen, 1998). The MPN method, therefore, represents only a fraction of the total count observed microscopically (Hopton *et al.*, 1972).

2.6.3 Community Level Physiological Profiles (Biolog)

The functional diversity of a microbial community can be determined using the community level physiological profile (CLPP) technique. The technique is easily applicable and is feasible for use in large-scale industrial or environmental studies (Palojarvi *et al.*, 1997). The CLPP technique is capable of differentiating bacterial communities from the same (or different) environment under different conditions (Kelly and Robert, 1998). Cookson *et al.* (2007) obtained similar results when they used CLPP, DGGE and PLFA. The technique is, however, limited by the differential growth of micro-organisms that occurs in the individual Biolog wells (Garland and Mills, 1991), the need for the standardisation of the inoculum, and the systems' dependence on the growth of the micro-organisms in the Biolog wells.

Culture dependent methods have considerable limitations for the analysis of industrial and environmental samples (White *et al.*, 1996c). Conventional microbiological methods provide little insight into the *in situ* microbial community structure, nutritional or environmental status, the evidence of toxicity, or the evidence of phenotypic expression of specific metabolic activities (White *et al.*, 1996c). These methods are limited because only a small percentage of all microorganisms can be cultured (Ibekwe *et al.*, 2007). Between 500 and 50 000 attached bacterial cells have been reported for each planktonic cell in suspension (Costerton and Lashen, 1984;

Jacobs *et al.*, 1996). The common practice for monitoring industrial water systems still, however, frequently involves the determination of planktonic bacterial numbers resulting in an underestimation of bacterial numbers and types of bacteria present in the biofilm (Cloete *et al.*, 1998).

2.7 CULTURE INDEPENDENT METHODS FOR MICROBIAL COMMUNITY DYNAMICS

Traditional microbiological techniques, such as light microscopy and culturing, have a limited use for the classification and identification of microorganisms (Muyzer, 1999). For a better understanding of microbial diversity other techniques that are quantitative, more representative and differentiative are required (Muyzer, 1999). Several molecular techniques have been developed in order to study natural samples (Kawai *et al.*, 2002) These techniques measure constitutive properties including PLFA, cell bound antibodies, DNA, adenosine-5'-phosphosulfate reductase and hydrogenase (Little *et al.*, 1998, Muyzer, 1999). PLFA and nucleic acids have been shown to provide a qualitative and quantitative measure of microbial diversity and community composition (White *et al.*, 1998). With the use of these techniques it has become evident that microbial diversity is much greater that previously anticipated (Muyzer, 1999).

2.7.1 Electron Microscopy

Chemical analysis can indicate the cause of fouling, scaling by hardness or amorphous silica, microbial contamination, clay particles etc., but scanning electron microscopy (SEM) can provide more in-depth information on the fouling layer (de Roever and Huisman, 2007). Rao *et al.*, (2000) used SEM to study MIC and observed the presence of ensheathed filamentous iron bacteria encrusted with corrosion products as well as significant pitting and SRB induced corrosion in the form of concentric rings beneath the tubercles.

SEM can be combined with an energy-dispersive X-ray spectrometer (EDS), which enables one to obtain the elemental/chemical composition of the area or particle being imaged by SEM (de Roever and Huisman, 2007). The benefit of SEM is that the study matter does not have to be removed from the surface concerned and can, therefore, be studied *in situ*.

2.7.2 Microbial Community Structure (PLFA Analysis)

Phospholipid fatty acids (PLFA) are stable components of the cell walls of most microorganisms and occur only in the membranes of living cells (Winding *et al.*, 2005; Palojarvi *et al.*, 1997). When bacteria are cultured under standardised conditions, they maintain a constant fatty acid composition, which is specific for a genus or even a species (Keweloh and Heipieper, 1996). It is possible to quantify different groups of microorganisms by this method, and individual PLFAs can be related to microbial community structure (Winding *et al.*, 2005). Several unique classes of PLFAs can be used to characterise microbial communities as they are specific for specific subgroups of microorganisms e.g., Gram negative or Gram positive bacteria, methanotrophic bacteria, fungi mychorrhiza and actinomycetes (Zelles, 1999). PLFA analysis produces descriptions of microbial communities based on the functional groupings of fatty acid profiles. PLFA analysis can, therefore, be used to quantify community structure and biomass without relying upon cultivation of microorganisms (White *et al.*, 1996c; Ibekwe *et al.*, 2007).

Fatty acids are designated by the total number of carbon atoms as well as the number of double bonds with the position of the double bond closest to the methyl end (ω) of the molecule. Configuration of the double bonds is indicated as *cis* (c) or *trans* (t). For example, 16:1ω7c is a PLFA with 16 total carbons with one double bond seven carbons from the methyl end in the *cis* configuration. Fatty acids that are branched are designated as *iso* (i) or *anentio* (a) if the methyl branch is one or two carbons from the ω end (i15:0), respectively. Cyclopropyl (cy) fatty acids are designated by the total number of carbons (cy17:0) (Jack *et al.*, 1992).

External factors such as temperature, pH, nitrogen source and salinity affect PLFA composition (Dowling *et al.*, 1986). Specific PLFA patterns can indicate physiological stress (White *et al.*, 1996). Factors such as the metabolic state, environmental conditions and toxins have an effect on the PLFA composition of the cell membrane (Frostegard *et al.*, 1993). Exposure to stressful or toxic environments results in an increase in specific trans monoenoic PLFA relative to their *cis* isomers and also an increase in the cyclopropane to monoenoic PLFA ratio (Jack *et al.*, 1992; White *et al.*, 1996). Bacteria can quickly adapt to toxic conditions by using trans/cis isomerisation resulting in their membranes being more stable thus retaining their intracellular physiological balance (Guckert *et al.*, 1991). An increase in trans/cis ratios of these isomers greater than 0.1 indicate a possibility of toxicity or starvation, whereas

healthy organisms have a ratio of <0.05. Within minutes or hours after death the cell's PLFAs are hydrolysed to produce diglycerides (DG) (White *et al.*, 1996). The difference (and ratio) between viable and non-viable cells can therefore be easily determined by comparing the PLFA and DG content of a sample. Since the lipid composition reflects defined responses to the environment during growth, the analysis of PLFA patterns can, therefore, be used for the determination of nutritional status, structural diversity and the effects of certain disturbances upon the microbial community (Frostegard and Baath, 1996; Smith *et al.*, 2000; Temina *et al.*, 2007). The determination of the total PLFA has also been shown to give an accurate quantitative measure of the viable microbial biomass. However, problems of extraction efficiency exist (Jack *et al.*, 1992; Winding *et al.*, 2005).

In comparison to conventional microbiological techniques, the analysis of the PLFAs analyses provides an indication of biodiversity and the phenotypic activities of each community (White *et al.*, 1996). Despite the fact that the determination of the structural diversity of microbial communities, using PLFAs, is more complex than the conventional plate counting assay, the entire microbial community can be monitored, *in situ*. Although internal metabolic cycles can be hidden they play a crucial role in the biofilm and will be reflected in the structure of the microbial community (Santegoeds *et al.*, 1998).

2.7.3 Microbial Community Structure (DGGE Analysis)

Denaturing gradient gel electrophoresis (DGGE) has been developed to assist in the study of microbial biodiversity and population dynamics of complex ecosystems over space and/or time (Camu *et al.*, 2007). DGGE analysis of PCR amplified 16S and 18S rDNA fragments has frequently been used in the fingerprinting of natural bacterial and fungal populations, respectively (Watanabe *et al.*, 2001). The technique is capable of differentiating between two DNA molecules that differ only by a single base pair (Sheffield *et al.*, 1989). Kawai *et al.* (2002) found that the method can detect bacteria that cannot be detected on culture media. With DGGE one can obtain a qualitative and a semi-quantitative picture of a microbial community without the need to isolate and culture its single components (Camu *et al.*, 2007).

The general strategy for genetic fingerprinting of microbial communities using the DGGE technique consists of the extraction of nucleic acids (DNA and RNA), amplification by PCR and the analysis of the PCR products (Muyzer, 1999). The

incorporation of a 40 base-pair GC-rich clamp in the 5' end of the forward primer is necessary for the optimal resolution of the fragments in the denaturing gradient of the DGGE gel (Muyzer *et al.*, 1993). Specific primers can be designed for specific groups of microorganisms, for example, eubacteria, fungi, archae, sulphate-reducing bacteria and methanotrophic bacteria. The design of primers targeting exact groups has, however, proved difficult as there are deficiencies in most primer sets (Winding *et al.*, 2005).

The separation of the different DGGE bands depends on the melting behaviour of the PCR products and not on the size of the nucleotide fragment (Muller *et al.*, 2001). Separation of DNA fragments in DGGE is based on the decreased electrophoretic mobility of partially melted double stranded DNA molecules in polyacrylamide gels (Muyzer *et al.*, 1993). The polyacrylamide gels contain a linear gradient of DNA denaturants (a mix of urea and formamide) (Muyzer, 1999). The denaturation of the DNA proceeds in discrete melting domains, which are stretches of base pairs with an identical melting temperature (Muyzer *et al.*, 1993). Upon the domain with the lowest melting temperature reaching its melting temperature, a transition from a helical to partially melted DNA molecule occurs, and the migration of the molecule stops (Muyzer *et al.*, 1993). Differences in the sequences of these domains cause the melting temperatures to differ (Muyzer *et al.*, 1993). Dar *et al* (2007) found that DNA samples that had the same sequences had a similar melting behaviour. The limitation of this method concerns species co-migrations because despite sequence differences, the melting temperatures of co-migrated V3 fragments of 16S rRNA genes are similar and they, therefore, migrate at the same position in denaturing gels (El-Baradei *et al.*, 2007).

The number of DNA derived bands relates to the presence of bacterial populations that are above the detection limit of DGGE, while the RNA derived bands reflect the predominantly active populations (Dar *et al.*, 2007). Camu *et al.* (2007) states that the detection limit of PCR-DGGE is generally between 4 and 6 log colony forming units per gram or higher depending on the bacteria investigated. This technique is able to detect more than 90% of the most numerous species of a community without discriminating living cells from cells in a viable but not cultivable state (Camu *et al.*, 2007). The technique is capable of differentiating between two DNA fragments of the same length that differ by base pair sequences or even by a single base pair (Sheffield *et al.*, 1989; Kawai *et al.*, 2002). It is reliable, reproducible, rapid and

relatively inexpensive (Muyzer, 1999). The technique also has the added advantage of the possibility of identifying community members by the sequencing of excised bands or by hybridisation analysis with specific probes, which is not possible with other fingerprinting techniques, except temperature gradient gel electrophoresis (Muyzer, 1999).

Denaturing gradient gel electrophoresis is a powerful tool to discern changes in microbial community structure in a variety of habitats (Sievert *et al.*, 1999). The method can be used in the simultaneous analysis of multiple samples, allowing the monitoring of complex microbial community dynamics due to seasonal fluctuations or other environmental perturbations (Muyzer, 1999). This method has been used to study microbial community diversity in soil (Kozdroj and van Elsas, 2001), drinking water (Fonseca *et al.*, 2001), sediments (Spring *et al.*, 2000), hydrothermal vents (Brinkhoff *et al.*, 1999), saline and hypersaline lakes (Foti *et al.*, 2007), bioreactors (Ahn *et al.*, 2007; Dar *et al.*, 2007), fermentation processes and products (Camu *et al.*, 2007; El-Baradei *et al.*, 2007), to assess the impact of pollutants (Kozdroj and van Elsas, 2001; Muller *et al.*, 2001), and other diverse environments.

The preceding paragraph has indicated the successful and widespread use of this technique in determining the effects of various conditions on microbial community dynamics. This procedure has made it possible to identify the presence and relative abundance of different species and thus, to profile microbial populations in both a qualitative and a semi-quantitative manner (Muyzer *et al.*, 1993).

2.8 CONCLUSION

The global and South African future outlook on water is bleak if efforts to explore non-structural alternatives to water supply are not widely encouraged. These alternatives include efficiency improvement, demand management and wastewater reuse. Several South African companies, including Sasol, have started reusing their effluents and waste water in a bid to achieve lower or zero effluent discharge scenarios. The use of industrial effluents as cooling water would enhance the water conservation measures by reducing the volume of raw water intake, as well as reducing the volume of final effluent water that is released. The disadvantages for the use of industrial effluents as cooling water include microbial and abiotic fouling, scaling and corrosion, which cost industry millions to control. These are affected by pH, COC and FV.

Conventional microbiological methodology has been applied in investigating the bacteria in industrial water systems. These methods are, however, limited in their ability accurately detect bacterial numbers, function and structure *in situ*. Due to the limitations of conventional microbiological techniques, several biochemical assays have been developed for the detection of specific microorganisms associated with biofouling and MIC (Little *et al.*, 1998). These techniques include the analysis of the microbial community structure using PLFA and DGGE analysis. Industry would benefit significantly from the use of these techniques, instead of the traditional techniques due to the advantages these techniques offer over the conventional techniques.

The aim of this study was, therefore, to evaluate the effects of COC, pH and FV on the rates of fouling, scaling and corrosion as well as on the microbial community dynamics in cooling towers using SGL as process cooling water. The objectives of this study were to:

i. Optimise the selective extraction of hydrocarbons from SGL so as to eliminate the interference of hydrocarbons with PLFA analysis,
ii. Determine the similarity or dissimilarity of two cooling towers in terms of the fouling, scaling and corrosion rates as well as microbial community structure when these were operated under similar operational conditions,
iii. Determine the effects of pH, COC and FV on the rates of fouling, scaling and corrosion of mild steel, as well as the structural diversity of the microbial communities in both the planktonic and sessile phases using a 2^3 multi-factorial experimental design.

CHAPTER 3: OPTIMISATION OF THE SELECTIVE EXTRACTION OF HYDROCARBONS IN SGL DURING LIPID EXTRACTION

3.1 INTRODUCTION

The phospholipid fatty acids of microorganisms occur only in the membranes of living cells and have a short turnover time (Palojarvi *et al.*, 1997). When bacteria are cultured under standardised conditions, they maintain a constant fatty acid composition, which is specific for a genus or even a species (Keweloh and Heipieper, 1996). Phospholipid fatty acid (PLFA) analysis has an advantage over other procedures because specific fatty acids are specific to bacteria (Gillian and Hogg, 1984; Pennanen *et al.*, 1998). Phospholipid fatty acids can therefore be considered as a fingerprint for microbial communities (Peterson and Klug, 1994). Temina *et al.* (2007) found that the fatty acid compositions of *Nostoc* spp. were affected by environmental conditions. This technique is, therefore, capable of discerning changes in microbial communities due to environmental conditions. Details of this aspect have been discussed in Section 2.7.2.

The effluent from the coal-gasification process, as used by Sasol, can contain more than 3000 mg/L of organic chemicals (Yang *et al.*, 2006). Phenolics account for 80% of the total COD in these waters (Zhang *et al.*, 1998). Stripped gas liquor (SGL) is a waste water stream from the coal gasification process obtained after gravimetric separation of tar products, solvent extraction of phenolics and steam stripping of ammonia from the condensate stream (van Nierop *et al.*, 2000). Even after the solvent extraction of phenols a significant amount still remains in SGL. Since large volumes (60 ML/d) of SGL are produced by Sasol on a daily basis, the SGL has a potential to be used as process cooling water.

The method for PLFA analysis requires the extraction of lipids, their fractionation, methylation and quantitative gas chromatography-mass spectrometry (GC-MS) analysis (White *et al.*, 1996b). The use of SGL as cooling water poses problems for the analysis of the structural diversity of microbial communities using PLFAs because it contains hydrocarbons. Hydrocarbons and cholesterol have been found to interfere with GC-MS analysis of PLFA prepared from complex biological samples (Nightingale *et al.*, 1999; Ali and Cole, 2001). For the abundant fatty acids, including 16:0, 18:0, 18:1*n*-9, 18:2*n*-6 and 20:4*n*-6, standard GC methods are fully adequate, but for fatty

acids present at 1% or less of total cellular fatty acids, or for late-eluting fatty acids, alternative methods may be needed (Nightingale *et al.*, 1999). It was, therefore, important to be able to selectively remove these hydrocarbons from SGL since this is the medium in which the micro-organisms that were to be characterised were present in.

The aim of this part of the study was to optimise the lipid extraction procedure to minimise the co-extraction of unwanted hydrocarbons. The objectives of this chapter were to:

i. Determine the effects of selective extraction of hydrocarbons before and after silicic acid fractionation on GC-MS analysis,
ii. Statistically analyse the PLFA data from the two selective extraction procedures to select the appropriate one.

3.2 MATERIALS AND METHODS

3.2.1 Glassware Preparation

All glassware and Teflon-lined caps were washed with phosphate free detergent and rinsed with distilled water. Clean glassware was wrapped in foil and baked at 450°C for at least 4 hours. Caps were rinsed with chloroform and methanol and allowed to dry at room temperature before use.

3.2.2 Sample Preparation

The experiment was conducted as illustrated in Figure 3.1. A freeze-dried culture of *Pseudomonas aeruginosa* (ATCC 15442) was cultured in 100 ml nutrient broth and incubated at 35°C and 200 rpm. After 18 hours 25 ml of the bacterial suspension was sub-cultured into 250 ml nutrient broth and incubated as above.

The following steps were performed in triplicate (Figure 3.1). Twenty five millilitres of the bacterial suspension was aseptically transferred into three sterile 50 ml glass centrifuge tubes. Then 25 ml of SGL was added to the triplicates marked (A) and (B) and 25 ml saline solution (0.85% m/v NaCl) was added to the triplicates marked (C), respectively. These samples were then immediately subjected to PLFA analysis. Triplicates marked (A) were used for the selective extraction of hydrocarbons from the lipid sample after silicic acid fractionation. Triplicates marked (B) were used for the selective extraction of hydrocarbons from the lipid sample before silicic acid

fractionation. Triplicates marked (C) were used as the control and no selective extraction of hydrocarbons was conducted on it (Figure 3.1).

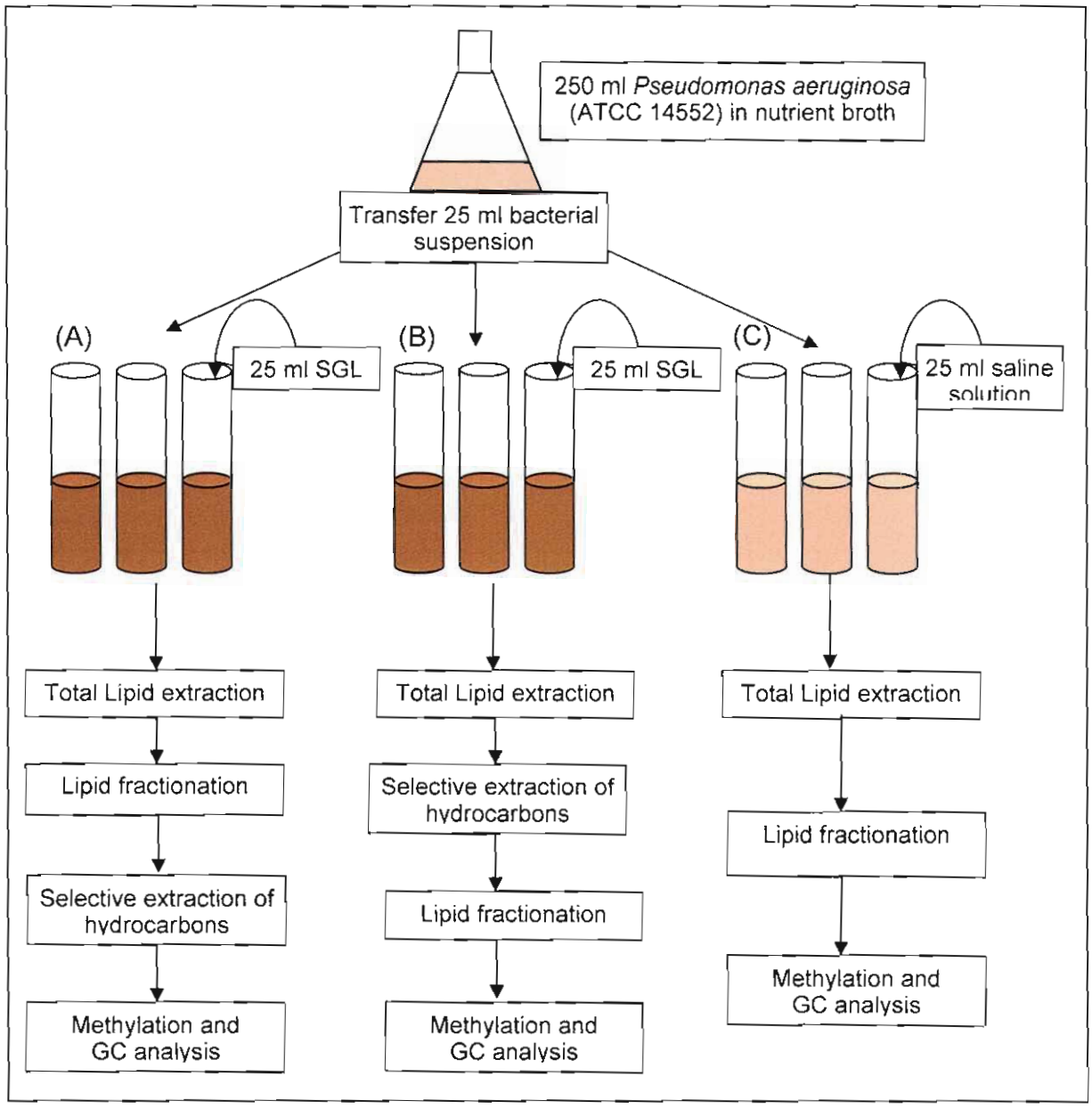


Figure 5. Schematic representation of the experimental set-up for the evaluation of the selective extraction of hydrocarbons (A) after silicic acid fractionation, (B) before silicic acid fractionation and (C) the control lipid samples that were not selectively extracted for hydrocarbons.

3.2.3 Microbial Community Structure (PLFA Analysis)

3.2.3.1 Lipid Extraction, Fractionation and Methylation

Total lipid extraction was performed using a Bligh and Dyer (1959) method as modified by White and Ringelberg (1998). Samples were centrifuged at 2000 rpm for

30 minutes (Harrier 18/80 refrigerated centrifuge, Sanyo) and the supernatant discarded. Four millilitres of phosphate buffer (50mM, pH 7.5), 5 ml of chloroform and 10 ml of methanol were added to the pellets in the centrifuge tubes. The samples were then sonicated for 2 minutes and vortexed for 30 sec. These were again centrifuged at 2000 rpm for 30 min. The liquid phase was decanted into clean red-capped 50 ml centrifuge tubes. Additional 5 ml of chloroform and 5 ml of nano-pure water were added to the decanted liquid phase to split the phases. These were then shaken vigorously, burped and allowed to separate overnight (approximately 18 hours) or until aqueous (upper) phase was no longer cloudy. The upper phase was then discarded.

Five millilitres of chloroform was added to the bottom phase, shaken, burped, vortexed for 30 seconds and centrifuged at 2000 rpm for 15 min. The upper layer was discarded. Five millilitres of nano-pure H_2O was added to the bottom phase, shaken and allowed to stand for 2 hours for phases to separate. Samples were then centrifuged at 2000 rpm for 20 minutes and bottom phases were transferred into glass screw-cap tubes. Lipid samples were then dried under a gentle stream of nitrogen and stored under nitrogen at –20°C.

Hydrocarbons were selectively extracted from the total lipid extracts. The (B) triplicates were used for this step (Figure 3.1). Two millilitres of hexane:chloroform (4:1) and 2 ml of nano-pure H_2O were added to the glass tubes with the dried lipid samples. The samples were then vortexed for 5 seconds and centrifuged at 2000 rpm for 20 minutes. The organic upper phase was recovered and transferred to other glass tubes. This step was repeated twice for a total recovered volume of 6 ml. Lipid samples were then dried under a gentle stream of nitrogen and stored under nitrogen at –20°C until silicic acid fractionation.

The extracted lipid samples were fractionated into neutral lipids, glycolipids, and polar lipids by silicic acid column chromatography (Guckert *et al.*, 1985). Pasteur pipettes were packed with glass wool and baked at 450°C. Pre-weighed silicic acid was activated by dehydration at 100°C for at least 1 hour. Five hundred milligrams of the silicic acid was suspended in chloroform and packed into Pasteur pipettes plugged with glass wool. Silicic acid columns were then flushed with 5 ml of acetone and 5 ml of chloroform, respectively. The lipid sample was then re-dissolved in 100 µl of chloroform and transferred to the silicic acid column. This step was repeated three

times to make sure that all the lipid sample was transferred onto the silicic acid column. The neutral fatty acid fraction, the glycolipids and the phospholipids were eluted into 15 ml glass screw-cap tubes with 5 ml of chloroform, 5 ml of acetone and 10 ml of methanol, respectively. Lipid fractions were then dried under a gentle stream of nitrogen and stored under nitrogen at –20°C.

Phospholipids from the (A) triplicates were re-extracted with a selective extraction step to remove any hydrocarbons from the extract (Figure 3.1). The samples were dissolved in 2 ml of 1M KOH and heated for an hour at 100°C to form salts of the fatty acids. Samples were then extracted three times with 2 ml of hexane to extract the contaminants and leave the PLFAs behind. pH was decreased to approximately 2.0 by the addition of 0.24 ml of concentrated HCl thereby hydrolyzing the salts. These were then heated for 6 hours at 100°C. Phospholipids were then extracted in 2 ml chloroform and dried under a gentle stream of nitrogen and stored under nitrogen at –20°C.

Phospholipids from the (A), (B) and (C) triplicates were trans-esterified to fatty acid methyl esters by a mild alkaline methanolysis procedure (Figure 3.1) according to Guckert *et al.* (1985). Five hundred microliters of chloroform, 0.5 ml of methanol and 1 ml of methanolic KOH (0.28 g KOH in 25 ml methanol), respectively, were added to the glass screw-cap tubes containing the phospholipid fractions. The sample was then vortexed for 30 seconds, incubated at 60°C for 30 minutes and then allowed to cool. Two millilitres of hexane, 200 µl of glacial acetic acid (1N) and 2 ml of H_2O (nano-pure) were added to the sample, respectively. The sample was then again vortexed, centrifuged at 2000 rpm for 5 minutes and the upper organic phase transferred into another glass screw-cap tube. The bottom phase was further extracted, three times, by adding 2 ml of hexane, vortexing for 30 seconds and centrifuging at 2000 rpm for 5 minutes. The top phase was always transferred into the glass screw-cap tube with the organic phase. The organic phase was then dried under a gentle stream of nitrogen, re-dissolved in an internal standard, transferred into a GC vial before GC analysis.

3.2.3.2 GC Conditions

The methylated samples were analysed using a Hewlett Packard 6890 II Plus gas chromatograph equipped with a Hewlett Packard 7683 injector, a Hewlett Packard 7683 auto sampler, a flame ionisation detector and a J&W Scientific DB-1 column (30

m x 0,25 mm x 25 µm). The initial temperature of 60°C, increased at a rate of 20°C per minute to 150°C where it remained constant for 4 minutes. The temperature was then ramped up to 230°C at 7°C per minute and kept constant at 230°C for 2 minutes. Then the temperature was again ramped up to 300°C at 10°C per minute and kept constant at 300°C for 3 minutes. Samples were injected in split-less mode. The inlet and detector temperatures were 230°C and 300°C, respectively. Helium was used as the carrier gas at a flow rate of 1 ml min^{-1}. Data was integrated using the HP Chem Station (Rev. A.07.02 682) software. Methyl nonadecanoate (C19:0) was used as internal standard and the concentration of PLFAs expressed as equivalent peak area response to the internal standard.

3.2.3.3 Statistical analysis

Data obtained from GC-MS analysis was separated (A), (B), and (C) triplicates according to the preceding analysis (Figure 3.1) and entered into an Excel spreadsheet. The arcsine square root transformation was applied to mole percent PLFA data. Lipid profiles of the different experiments, indicating microbial community structure, were represented as bar graphs. Different samples were compared to each other, based on lipid classes using the Tukey Test in Statistica 6.0 (Statsoft, Inc., Tulsa, OK) and box and whisker plots of the results were generated with a 95% confidence limit. Additionally hierarchical cluster analysis was performed from the transformed PLFA data using Ward's method with Euclidean distances.

3.3 RESULTS AND DISCUSSION

After GC analysis of the lipid samples, chromatograms were obtained (Figure 3.2). Upon examination of these chromatograms it was evident that PLFA profiles of the (B) samples (Figure 3.2 (B)) were similar to those of the (C) samples (Figure 3.2 (C)). Figure 3.2 clearly illustrates that chromatograms from (B) and (C) were similar, while (A) was different from the other two. The major peaks (retention times 9 to 25 minutes) in (C) and (B) are also found in (A). However in the latter case they are very small. The (A) samples still contained a considerable amount of hydrocarbons, as can be seen in the retention times above 27 minutes (Figure 3.2). Poerschmann *et al.* (2007) found that nC_{22}–nC_{32} alkanes were indicative of petroleum hydrocarbon contamination.

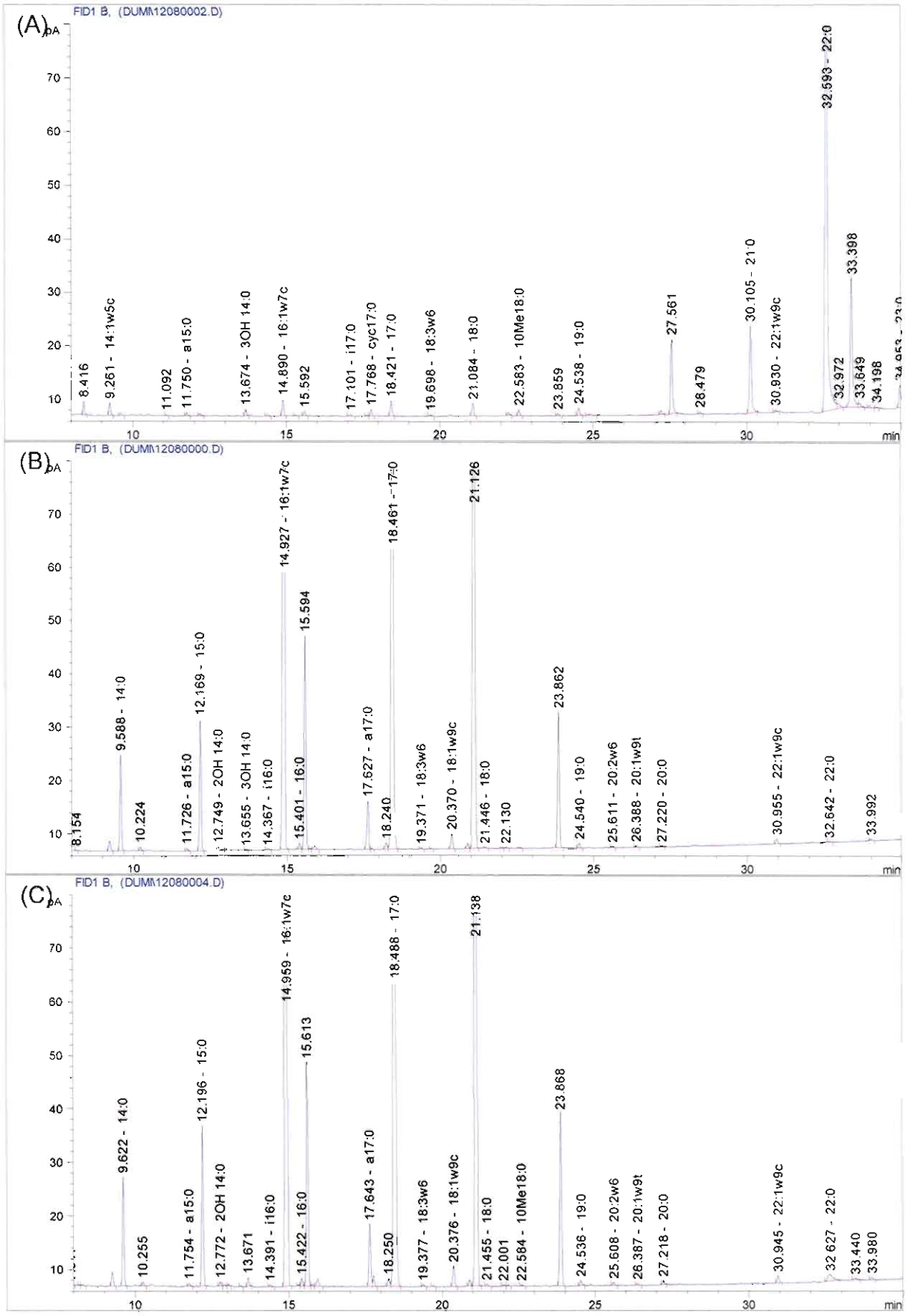


Figure 6. Chromatograms of the lipid samples that were selectively extracted for hydrocarbons (A) after silicic acid fractionation, (B) before silicic acid fractionation and (C) the control lipid sample that were not selectively extracted for hydrocarbons.

Figure 3.3 also clearly illustrates that the lipid group structures from (B) and (C) were similar, while (A) was different from the other two. Terminally branched saturated fatty acids, indicative of Gram positive bacteria (Zelles, 1999), and poly-unsaturated (polyenoic) fatty acids, indicative of microeukaryotes (Villanueva *et al.*, 2004), were present in high concentrations in the (A) samples. Their concentrations were greatly reduced in the (B) and (C) samples. The high concentration of these fatty acids in the (A) samples can be attributed to the hydrocarbons found in SGL that were not selectively extracted and therefore methylated with the sample. This could lead to the results being improperly construed to imply an increase in Gram positive bacteria and fungi in a microbial community due to the presence of hydrocarbons.

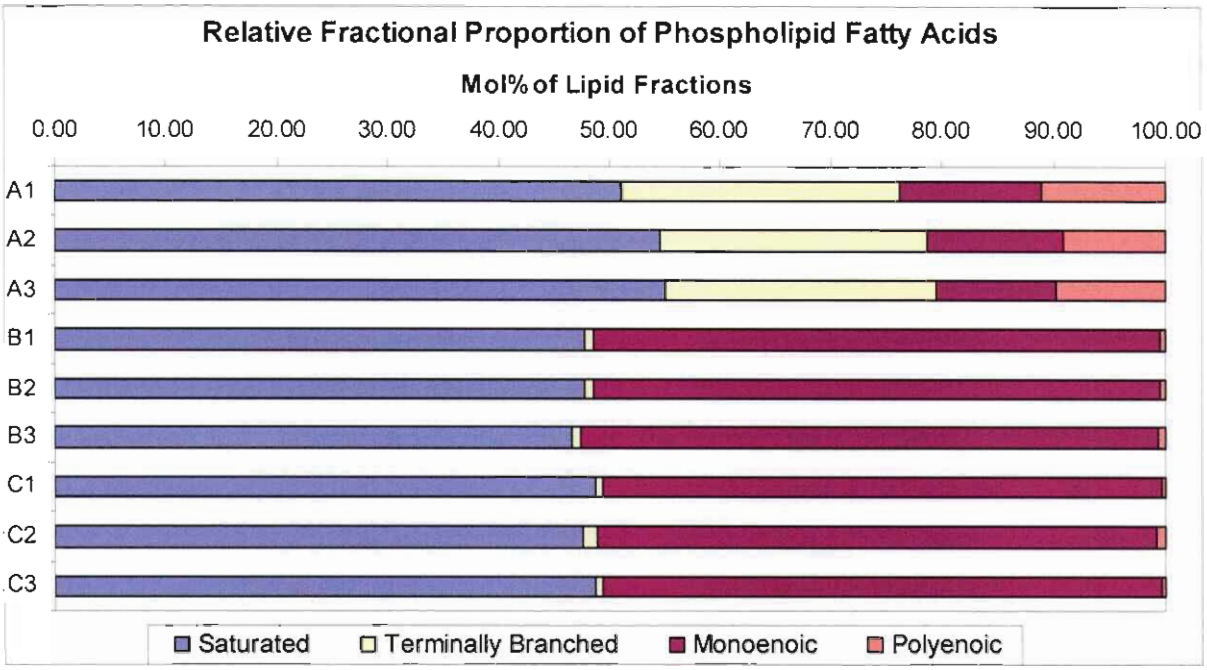


Figure 7. Proportions of the major lipid groups obtained from the (A), (B) and (C) samples.

To statistically verify the similarities and dissimilarities the lipid data was subjected to the Tukey Test and box and whisker plots ($p<0.05$) of the results were plotted (Figure 8). Any overlap between the box and whiskers means that there is no significant statistical difference between the particular data points. Upon examination of the box and whisker plots it is evident that (B) and (C) were similar, while (A) was different from the other two in terms of their normal saturated, monoenoic, polyenoic and terminally branched saturated fatty acids.

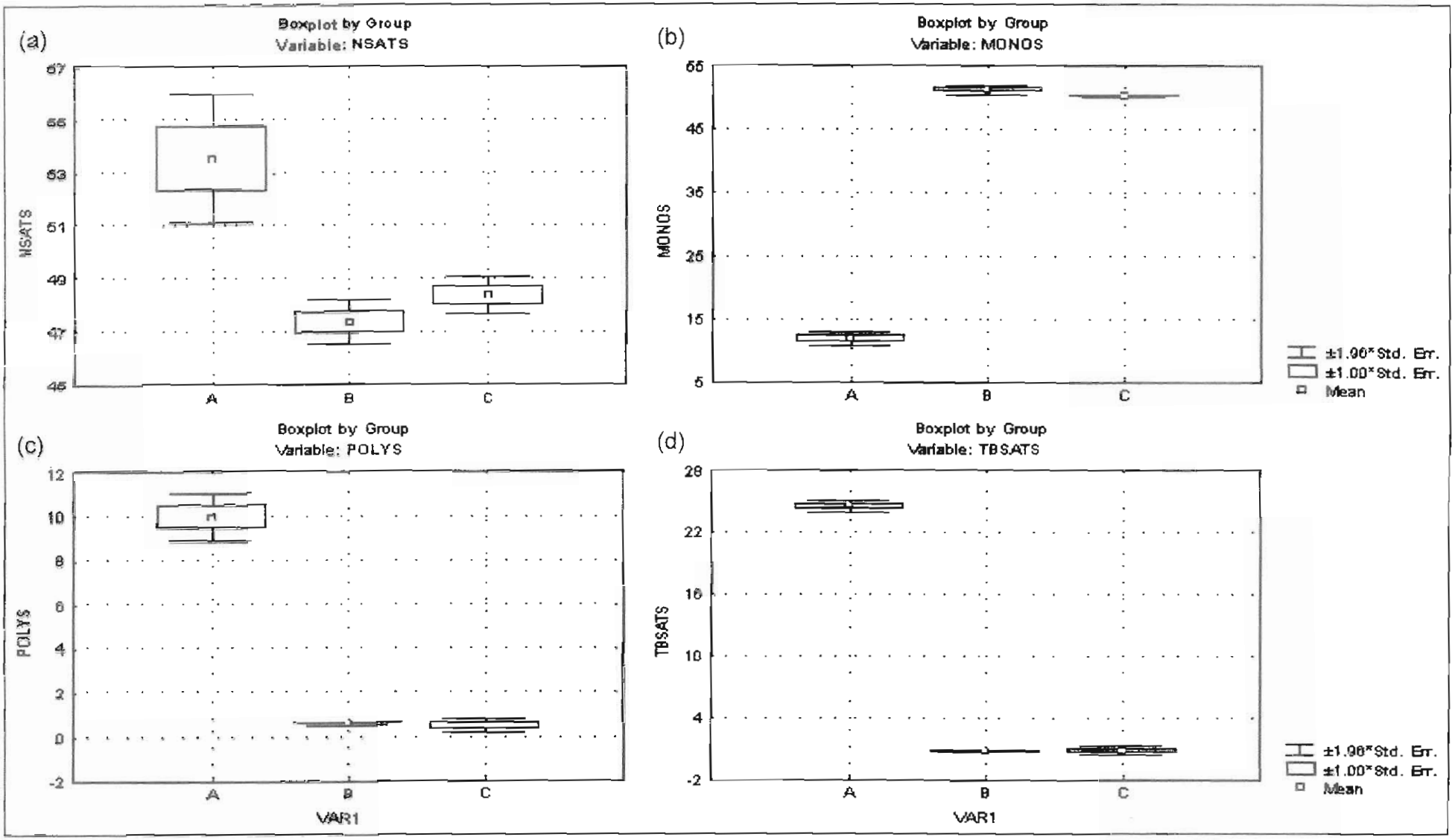


Figure 8. Box and whisker plots illustrating the statistical differences ($p>0.05$) or lack thereof between the (A), (B) and (C) samples in terms of the normal saturated (NSats) (a), monoenoic (Monos) (b), polyenoic (Polys) (c) and terminally branched saturated fatty acids (TBSats) (d).

A dendogram (UPGA with Euclidian distances) of the PLFA data was constructed (**Error! Reference source not found.**). The dendogram also clearly illustrates that (B) and (C) were similar, while (A) was different from the other two. This confirms what was seen in Figures 3.2, 3.3 and 3.4.

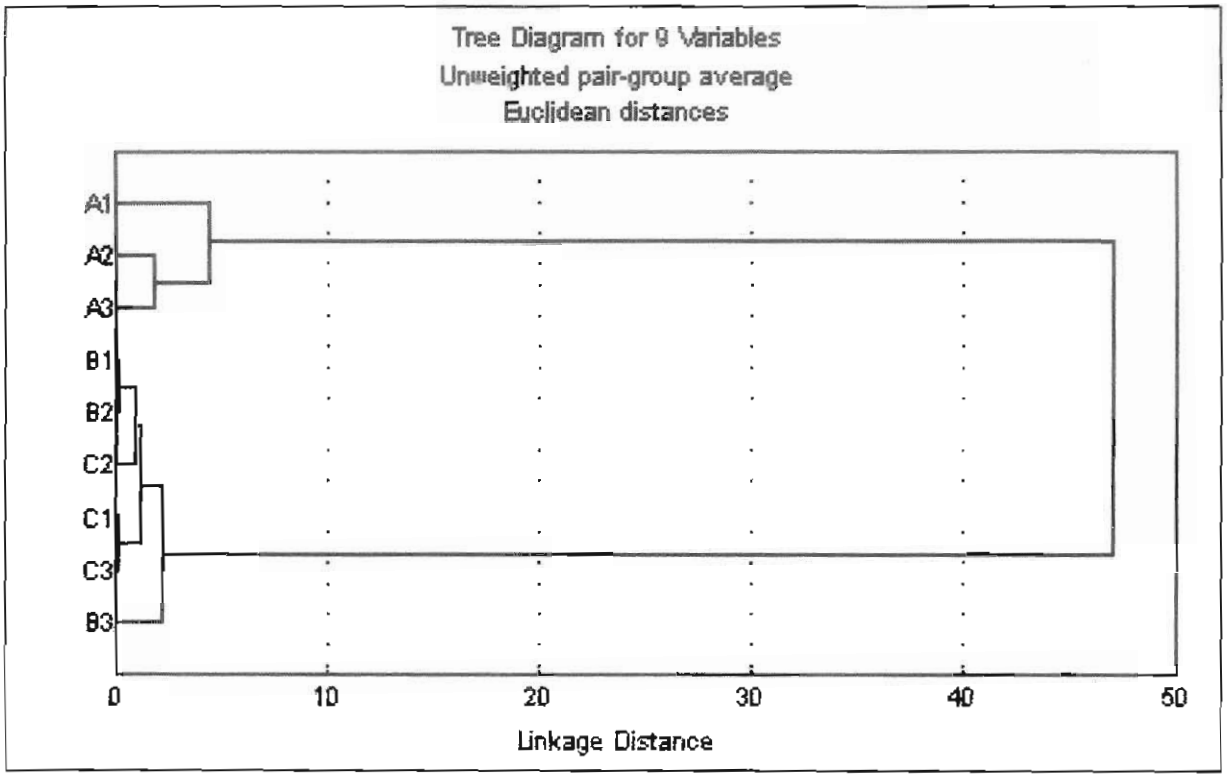


Figure 9. Dendogram of the lipid data illustrating the grouping of the (A), (B) and (C) samples.

Fang and Findlay (1996) used hexane to elute polycyclic aromatic hydrocarbons from the silicic acid column, but the phospholipid fraction still required further purification. The method used in this study for the selective extraction of the (B) samples did not require any further purification of the PLFA samples. Ringelberg *et al.* (2007) used methylene chloride to elute petroleum hydrocarbons from the silicic acid column. They found a significant positive correlation between microbial biomass and petroleum hydrocarbon concentrations. They attributed this to the petroleum hydrocarbons resulting in increased microbial growth. Aries *et al.* (2001) conducted an experiment, similar to the one in this study, where they compared the PLFA composition of a bacterial community grown in an ammonium acetate medium and in a Blend Arabian Light petroleum medium. They also noted differences in PLFA composition in response to petroleum hydrocarbons. They attributed the change in PLFA composition to the effects of hydrocarbons on the microbial community

structure. The bacterial culture in this experiment was, however, subjected to lipid extraction immediately after the addition of SGL and not allowed to grow in the SGL medium. It is, therefore, clear that the change in PLFA composition seen in this experiment was due to the inadequate selective extraction of the hydrocarbons present in SGL.

3.4 CONCLUSION

In this chapter the effects of selective extraction of hydrocarbons before and after silicic acid fractionation on GC-MS analysis were determined. The PLFA data from the two selective extraction procedures was statistically analysed. The chromatograms, community structures, box and whisker plots and dendograms clearly indicated the impact of contaminant hydrocarbons on the analysis of microbial community structure by PLFA analysis. The method used to selectively extract hydrocarbons before silicic acid fractionation was determined to be appropriate method to use going forward.

The aim of this part of the study, to optimise the lipid extraction procedure to minimise the co-extraction of unwanted hydrocarbons, was therefore, achieved.

CHAPTER 4: DETERMINATION OF THE SIMILARITY OR DISSIMILARITY OF TWO COOLING TOWERS IN TERMS OF PHYSICO-CHEMICAL PROPERTIES AND MICROBIAL COMMUNITY STRUCTURE

4.1 INTRODUCTION

A cooling water system consists of a cooling tower, a pumping system and a network of heat exchangers (Figure 2.2; Picon-Nunez *et al.*, 2007). Cooling towers are used to reject waste heat. The towers are designed to cool a warm water stream through evaporation of some of the water into an air stream (Qureshi and Zibair, 2006). There are, however, inherent problems associated with the operation of cooling towers using SGL. These problems include fouling, both biological (biofouling) and non-biological (sedimentation), scaling and microbially and chemically induced corrosion of the cooling tower sump, packing material and heat exchanger tubing.

The factors that affect the degree of fouling, scaling and corrosion include make-up water composition, ion concentration, temperature, cycles of concentration (COC), fluid velocity (FV) and pH (IWS, 1994; de Almeida *et al.*, 1997). The composition of the make-up water contributes to the fouling and scaling of cooling water systems due the amount of chemical compounds it contains. Hard waters, for example, are scale forming and less corrosive (de Almeida *et al.*, 1997; Rakanta *et al.*, 2007). A lower pH generally leads to increased solubility of salts and metals and therefore a reduced scaling potential but also an increased corrosion potential (IWS, 1994; Rakanta *et al.*, 2007). Recirculation of water in cooling towers results in the concentration of dissolved and suspended substances due to evaporation. Cycles of concentration is the ratio of dissolved salt concentration in the circulating water relative to dissolved salt concentration in make-up water. An increase in COC promotes scale formation, biofouling, macro fouling of the system and eventually microbially induced corrosion (MIC) (Cloete *et al.*, 1994; de Almeida *et al.*, 1997). Make up water must be added to replace the water that is lost via evaporation and bleeding (blow down) is required to prevent excessive concentration of dissolved and suspended solids in the cooling water (Garrett-Price *et al.*, 1985). As hard water is heated, its solubility changes resulting in scale formation on heat exchangers. This happens in the case of calcium and magnesium carbonates that exhibit inverse solubility behaviour with temperature (Kim *et al.*, 2001; Picon-Nunez *et al.*, 2007). Low FV may provide stagnant areas that enhance biofilm formation and the

precipitation of organic and inorganic contaminants from the circulating water (IWS, 1994). The corrosion rate of metals in cooling towers can be increased by dissolved oxygen. An increased flow rate increases the oxygen access to the surface, thereby, promoting corrosion (IWS, 1994; Rakanta *et al.*, 2007).

In cooling water systems microbial growth could be very high due to the presence of nutrients, exposure to sunlight, favourable temperatures ranging from 30°C to 60°C, a pH range of 6 to 9, good aeration and a continuous source of bacteria from makeup water and ambient air (Chourday, 1998; Ludensky, 2003). Microorganisms in the cooling water systems attach to the surfaces of water systems and this leads to the formation of biofilms that are responsible for biofouling and MIC (Santegoeds *et al.*, 1998; Wolfaardt and Cloete, 1992). Microorganisms can modify the near-surface environmental chemistry by microbial metabolism and may interfere with electrochemical processes occurring at the metal-environment interface, promoting localised corrosion and affecting all types of metals (Chourday, 1998; Angell, 1999; Chen *et al.*, 2007). It is important to monitor biofouling in relation to scaling and corrosion as inter-relationships exist. The products of scale and corrosion may provide an ideal habitat for the growth of bacteria, and bacteria could be the major cause for corrosion (IWS, 1994).

Each of the experimental runs to be conducted using the cooling towers were envisaged to last at least 6 weeks and 8 experiments had to be carried out in the next chapter. Due to time constraints it was necessary to determine whether the operation of multiple cooling towers under similar operational conditions would yield similar results. This would allow the use of two cooling towers in parallel, testing different operational conditions with any differences in results reflecting the effects of operational conditions and not differences in the cooling towers themselves.

The aim of this chapter was to determine the similarity or dissimilarity of two cooling towers in terms of the fouling, scaling and corrosion rates as well as microbial community structure when these were operated under similar operational conditions. The objectives of this chapter were to:

i. Determine the rates of fouling, scaling and corrosion of two cooling towers operated under identical conditions,

ii. Determine microbial community structure by phospholipid fatty acid (PLFA) analysis and scanning electron microscopy (SEM) of two cooling towers operated under identical conditions,

iii. Statistically analyse the data to determine similarity or dissimilarity of the two cooling towers.

4.2 MATERIALS AND METHODS

4.2.1 Cooling Tower Design and Operation

Two cooling towers were used in the experiment (**Error! Reference source not found.**). The cooling towers were made of perspex and the tubing was PVC with an internal diameter of 18mm. Packing material was polypropylene 25mm Pall V-rings with a surface area of $1m^3$ each (Mass and Heat Transfer Technology (Pty) Ltd, Roodeport, South Africa). Cooling air was supplied by a 115 kw fan on the side of the cooling tower sump. The cooling towers were fed with stripped gas liquor (SGL), whose volume in the cooling towers was controlled by two volume level probes. Total cooling tower volumes were maintained between 8L and 10.44L. Sump volumes were maintained between 5.34L and 7.66L. The cooling towers were fitted with three mild steel corrosion coupons and four microscope slides each.

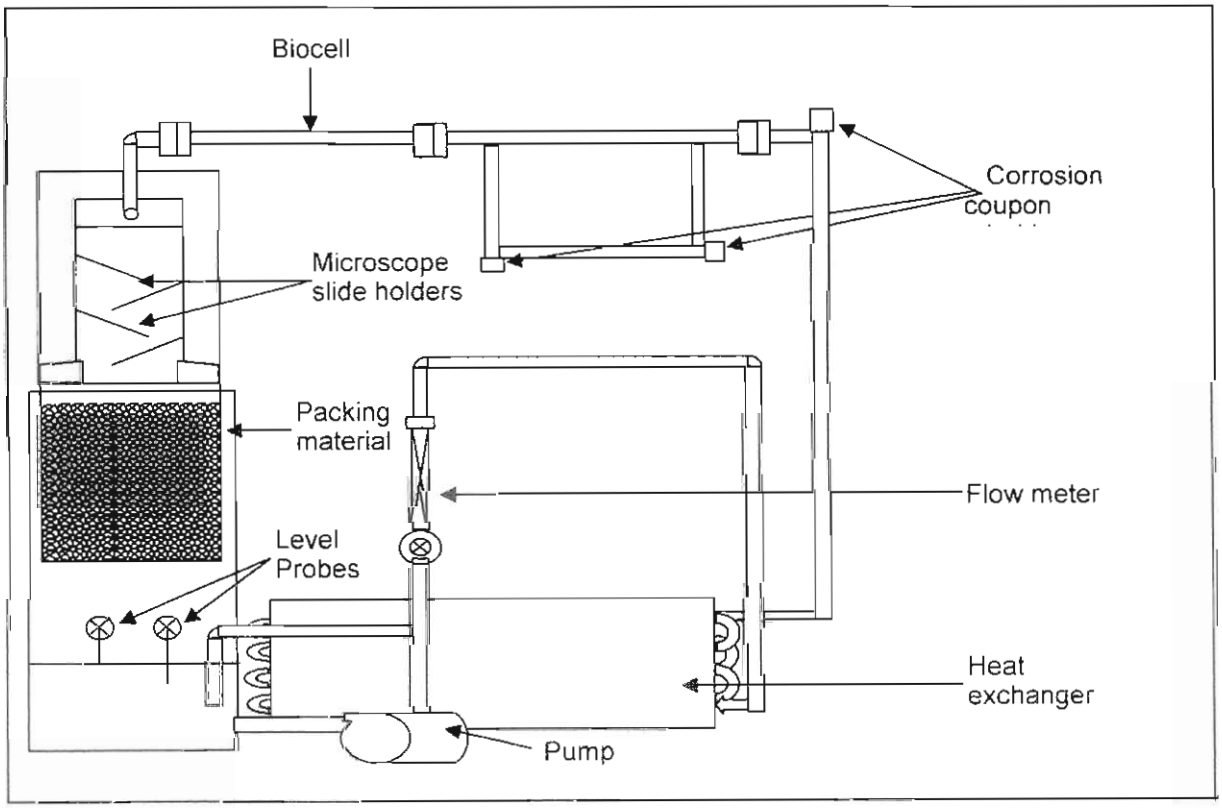


Figure 10. Schematic representation of the cooling towers.

Two lab-scale cooling towers were operated under similar conditions with SGL as make-up water. The two lab-scale cooling towers were operated with a FV of 1,5 m/s, a ΔT of 10°C (temperature difference between water exiting the heat exchanger and water in the sump), a pH of 9 in the feed (maintained with 25% NH_4OH), a sump temperature not exceeding 35°C and 13 COC (using fluoride as an indicator). Microscope slides and mild steel AquaCorr™ probes were used for scanning electron microscopy (Section 4.2.3). The experimental period was a total of 59 days. The first 21 days were for acclimatisation, after which biocells (Mechanical Engineering Department, North West University, South Africa), mild steel corrosion coupons and mild steel heat exchanger tubing were inserted. After a further of 21 days of operation the mild steel corrosion coupons were replaced with a second set of mild steel corrosion coupons. Seventeen days later the corrosion coupons, heat exchanger tubing and biocells were removed and analysed accordingly. The rates of fouling, scaling and corrosion were determined from the corrosion coupons and heat exchanger tubing (Section 4.2.4). The biofilm obtained from the biocells was used for microbial community structure analysis using PLFA (Section 4.2.5).

4.2.2 Physico-Chemical Analyses

Several physico-chemical parameters were measured daily during the study. These parameters were fluoride concentration, conductivity, pH, sump temperature, Δ T, corrosion rate (mm/year), chemical oxygen demand (COD) and total suspended solids (TSS). Fluoride, conductivity, pH, sump temperature and the ΔT were all determined with the use of specific probes (WTW, Germany). Chemical oxygen demand was determined spectrophotometrically using Spectroquant, Nova 60 (APHA *et al.*, 1985; Merck, Darmstadt, Germany). Corrosion rate was determined with the AquaCorr™ system (Rohrback Cosasco Systems, USA). Total suspended solids were determined gravimetrically with a Whatman GF/A filter paper (110 mm diameter) (APHA *et al.*, 1985). Cycles of concentration were determined by conductivity and fluoride concentration. The COC were determined according to the equation:

$$\textit{Cycles of concentration} = \frac{\textit{Conductivity or Fluoride in sump water}}{\textit{Conductivity or Fluoride in make up water}}$$

Other parameters were determined three times a week. These parameters were ammonia, calcium, iron, phenol and volatile fatty acids. Ammonia was determined spectrophotometrically using Spectroquant, Nova 60 (APHA *et al.*, 1985; Merck,

Darmstadt, Germany). Calcium and total iron concentrations in the bulk liquid were determined by atomic absorption. Volatile fatty acids were determined by steam distillation and subsequent titration. Phenol was determined by Sasol R&D, Sasolburg.

4.2.3 Electron Microscopy

The microscope slides from the cooling towers were subjected to electron microscopy according to Tiedt *et al.* (1999). Biofilms on the microscope slides were fixed in 70% ethanol, processed through an acetone series (70%, 80%, 90%, 100% and 100%) and critical point dried with liquid CO_2. The samples were then carbon coated and analysed using an FEI Quanta 200 ESEM.

4.2.4 Fouling, Scaling and Corrosion

Fouling, scaling and corrosion rates were determined from the corrosion coupons and the heat exchanger tubing using a modified ASTM method as optimised by Sasol Research and Development, Sasolburg (ASTM, 1999). Corrosion coupons, heat exchanger tubing and U-bends were rinsed with acetone to remove any oil and grease from the surface and then weighed before being used in the cooling towers. Heat exchanger tubing and corrosion coupons were dried at 105°C, for 2 hours and 24 hours respectively, and weighed to determine fouling. The corrosion coupons and heat exchanger tubing were then washed in 10% NaOH for 3 minutes, dried at 105°C and weighed to determine scaling. They were then washed in inhibited hydrochloric acid for a maximum of 25 minutes, dried at 105°C and weighed to determine corrosion. The rates of fouling, scaling and corrosion were determined by weight loss measurement. Fouling and scaling rates were expressed as weight per unit area per unit time ($mg/dm^2/d$). The corrosion rate was expressed as depth of penetration per unit time (mm/y).

Results obtained were subjected to parametric and non-parametric statistical analyses using Statistica 6.0 (Statsoft, Inc.). Parametric and non-parametric data were subjected to Tukey's honest significant difference test (HSD), breakdown analysis of variance (ANOVA) and one way ANOVA. Results of these were plotted in box and whisker plots.

4.2.5 Microbial Community Structure (PLFA Analysis)

The biofilm samples obtained from the biocells in the cooling towers were subjected to total lipid extraction, fractionation and methylation as described in Section 3.2.3.1. The optimised selective extraction method was used. Gas chromatography analysis was performed as in Section 3.2.3.2.

4.3 RESULTS AND DISCUSSION

4.3.1 Physico-Chemical Analyses

The COC were calculated from fluoride concentration and conductivity. From the results (Figure 4.2) it is evident that the COC determined in terms of fluoride are always higher than when determined using conductivity. The two parameters were on average parallel to each other. Once the cooling towers were stable the COC remained relatively constant at approximately 13 and 9 for fluoride and conductivity, respectively. Conductivity is normally used for the determination of the COC but the disadvantage is that it is can be affected by pH. An increase in pH results in the precipitation of dissolved mineral ions whereas a reduction in pH would result in the re-suspension of precipitated mineral ions (IWS, 1994). Kim *et al.* (2001) also found a difference between the COC values determined by conductivity and obtained from mass balance determination. They attributed the difference to the precipitation of dissolved mineral ions in water. Lee and Cho (2002) determined the cycle of concentration using the chloride concentration as it was not affected by their process.

The pH in the feed was maintained at approximately 9.5 and this resulted in the pH in the cooling towers stabilising at approximately 7 (Figure 4.3). The drop in pH could possibly due to the volatilisation of the ammonia used to adjust the pH as it is cycled through the cooling towers (APHA, 1985). The cooling towers were operated at 31-35°C and large amounts of air were blown through the SGL. Another possibility for the drop in pH would be the nitrification of the NH_4OH by the microbial community in the cooling towers resulting in the formation of NO_3^- and NO_2^-. Nitrates and nitrites were not monitored during this experiment and would have to be determined in future studies so as to determine to what extent each of these phenomena affected the drop in pH.

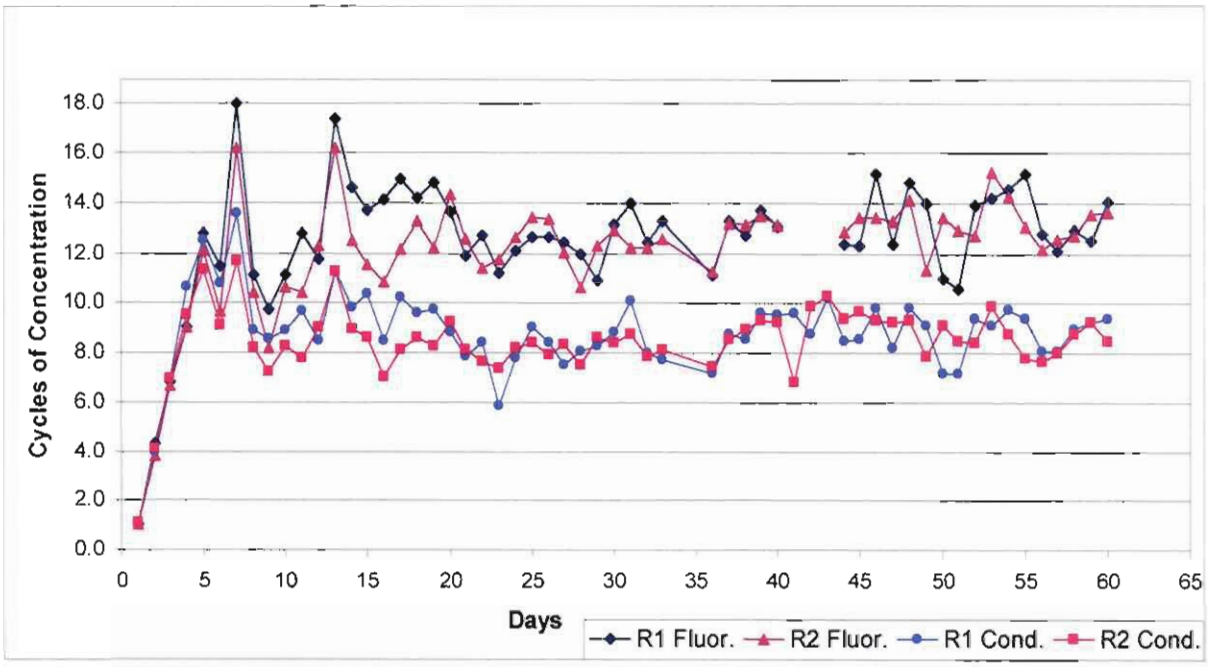


Figure 11. Cycles of concentration based on fluoride concentration and conductivity.

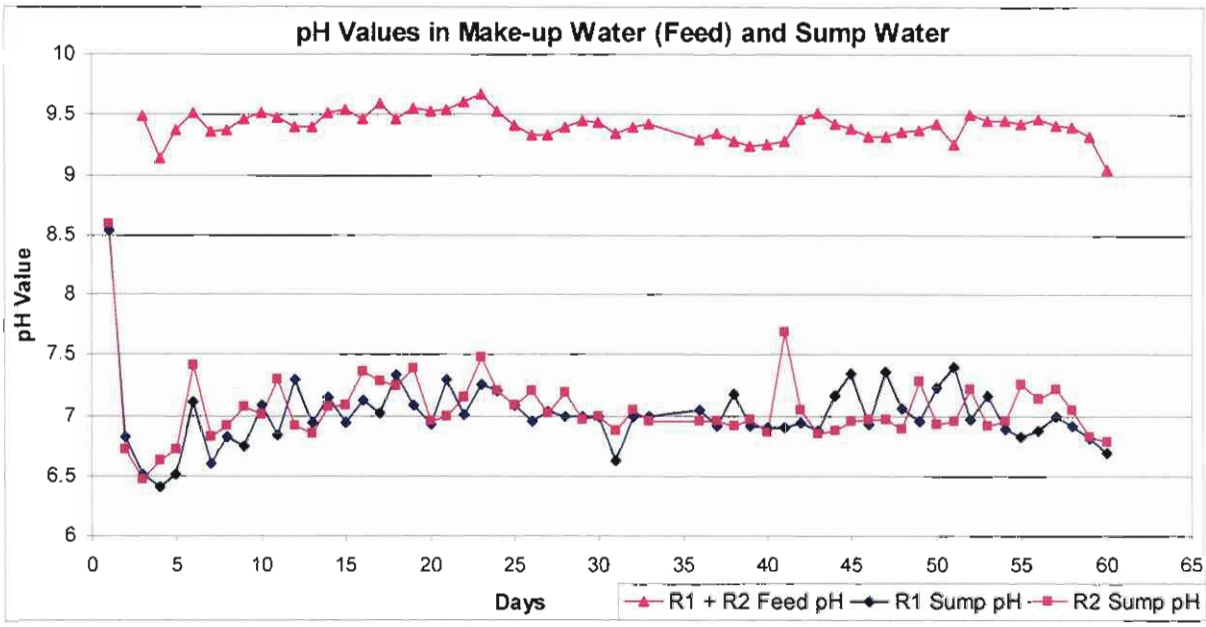


Figure 12. pH values in make-up water and sump water.

The sump temperature remained below 35°C at a ΔT of 10 for the first 28 days after which an increase was observed (Figure 4.4). This increase in the sump temperature was more profound in cooling tower 1. On day 30 the skin temperature (the temperature of the aluminium heating block in the heat exchanger) was decreased so as to lower the sump water temperature. This resulted in a drop not only in the sump temperature but also in the ΔT. The packing material was then rinsed in tap water and that resulted in a decrease in the sump temperature and at the same time maintained a ΔT of 10. Qureshi and Zibair (2006) also observed that the fouling of

the packing material interferes with air and water flow through the tower resulting in a reduction in the overall thermal effectiveness of the tower.

The ΔT of the cooling towers was maintained at 10°C and it remained relatively stable for the first 42 days after which fluctuations were observed. The fluctuations were mainly due to the fact that the flow in the cooling towers kept dropping and did not remain stable. Unstable flow can be attributed to fouled heat exchanger tubing since fouling results in increased fluid frictional resistance (Cloete *et al.*, 1996). Rosmaninho *et al.* (2008) found that when the flow rate is kept constant, the presence of a deposit on the surface of a channel tends to increase the pressure drop due to the deposit roughness. Kim *et al.* (2001) also found that the flow rate of the circulating water decreased by up to 50% due to scale formation in the heat exchanger surfaces in their cooling tower test unit after as little as 100 to 200 hours.

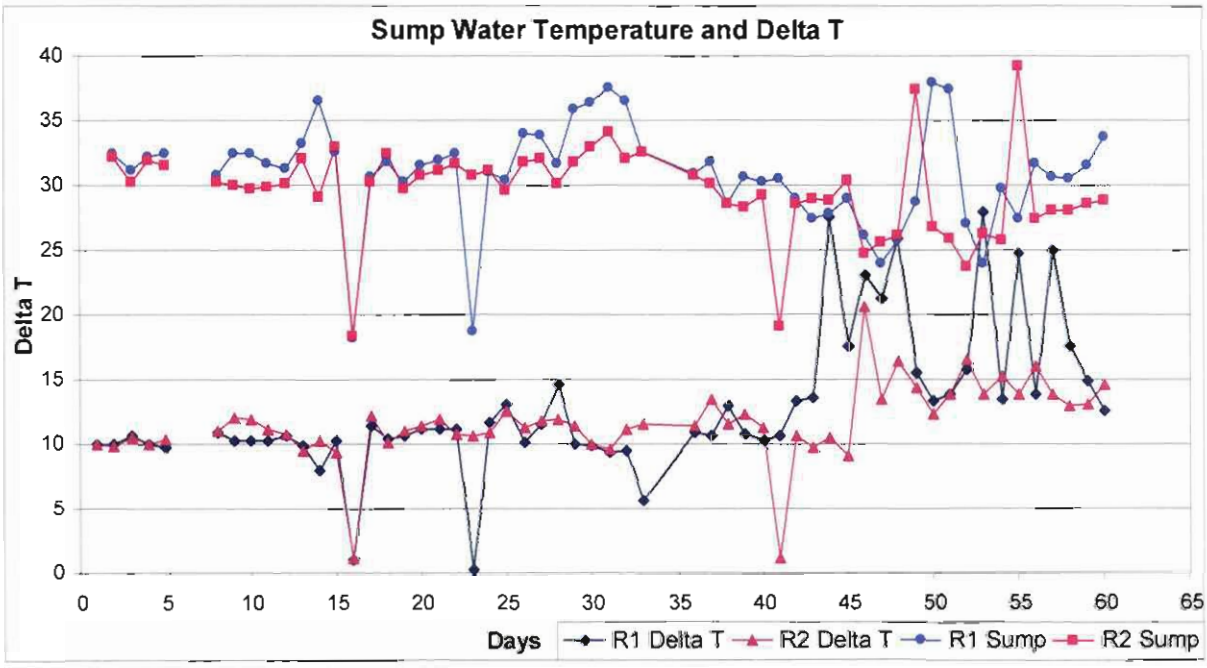


Figure 13. Sump water temperature and delta T (ΔT).

Within the first 5 days there was a sudden 7-fold increase and drop in iron concentration and corrosion rate as measured using the AquaCorr™ system (Figure 4.5). Though initially high, the corrosion rate dropped due to an accumulation of a protective fouling layer on the metal surface. A fouling layer may have protected the heat exchanger tubes from corrosion by the SGL flowing through them in the heat exchangers (Iverson, 1987). The fouling layer also probably absorbed the iron, not allowing it to go into the bulk liquid and, therefore, could not be measured. The decreasing corrosion rate and iron concentration could, therefore, be attributed to an

increasing fouling layer. This fouling layer may have resulted in the formation of anaerobic conditions next to the metal surface (Gaylarde and Morton, 1997). These anaerobic conditions favour the proliferation of anaerobic microorganisms, including sulphate reducing bacteria, which have been implicated in the corrosion of metals (Hamilton, 1985; Beech *et al.*, 1998; Choudhary, 1998).

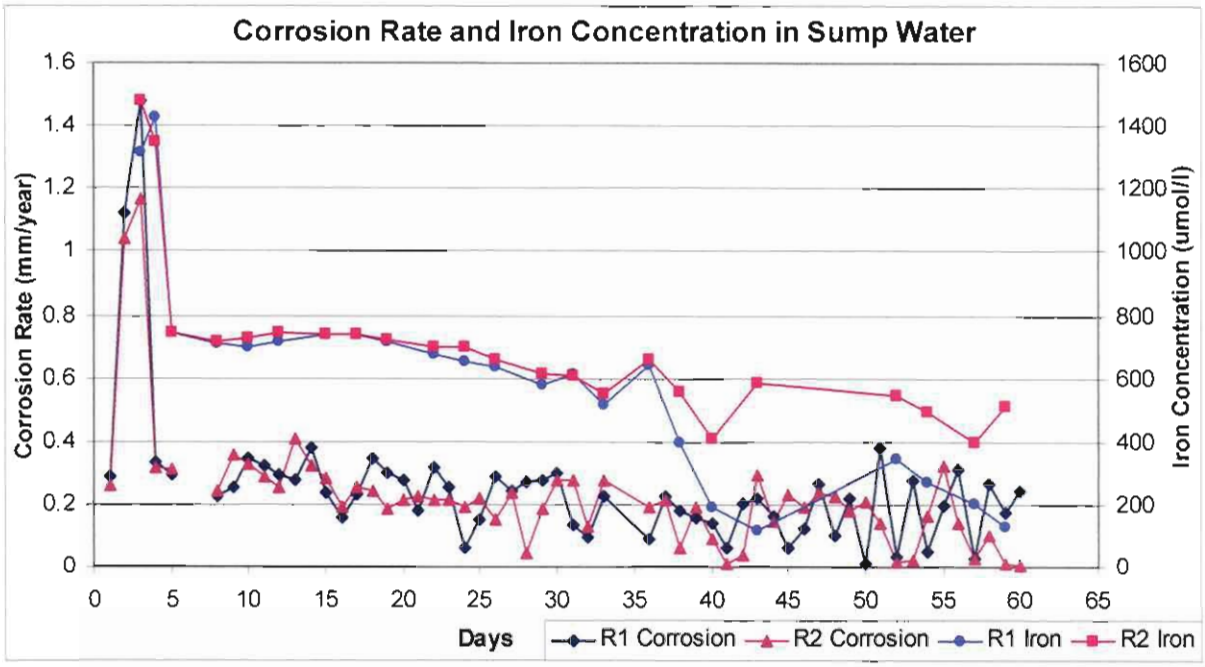


Figure 14. Corrosion rates as measured using the AquaCorr system and iron concentration in sump water.

4.3.2 Electron Microscopy

Scanning electron microscopy micrographs of the AquaCorr probes and microscope slides revealed similarities in microbial community structure between the two cooling towers (Figure 4.6). The accumulated fouling layer can be seen in the Figure 4.6(a) micrographs, with 2(a) having a smoother fouling layer. The fouling layer allowed the formation of crevices in which bacteria accumulated (Figure 4.6b). Figure 4.6(2b) showed some crystal formation which, possibly, would have resulted in scaling. These slight differences between the two sets of micrographs could be attributed to the irregular flow rates experienced towards the end of the experiment possibly due to fouling. Fouling results in a reduction in the overall effectiveness of the tower by interfering with air and water flow through the tower resulting in a pressure drop (Kim *et al.*, 2001; Qureshi and Zibair, 2006).

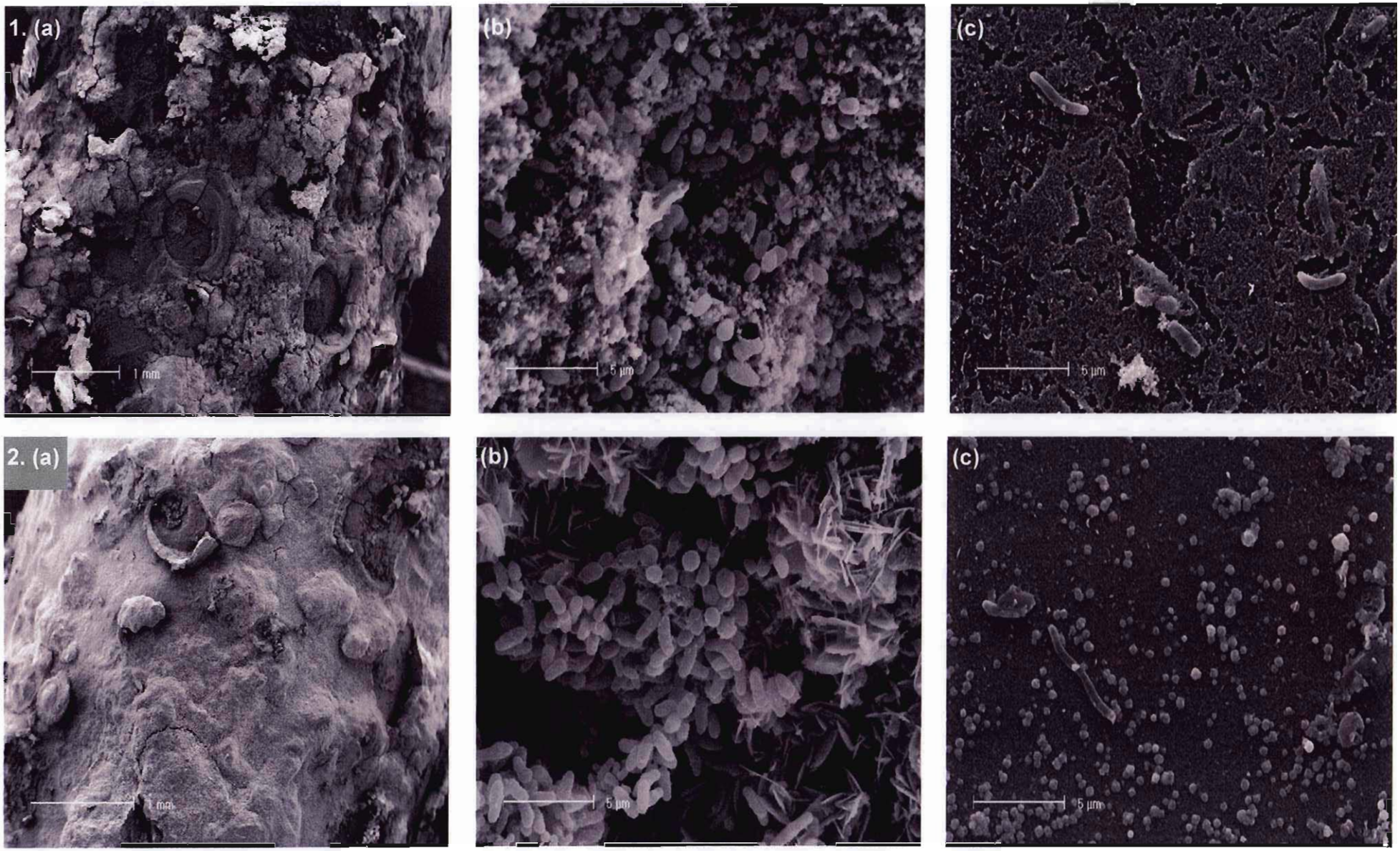


Figure 15. Scanning electron micrographs of AquaCorr probes at low magnification (bar scale = 1mm) (a), AquaCorr probes at high magnification (bar scale = 5µm) (b) and the microscope slides at high magnification (bar scale = 5µm) in reactor 1 and 2, respectively.

4.3.3 Fouling, Scaling and Corrosion

The results obtained from the analysis of the fouling, scaling and corrosion rates of the corrosion coupons and heat exchanger tubing from cooling towers 1 and 2 are presented in Appendix A (Table A1). The fouling, scaling and corrosion rate results were subjected to parametric and non-parametric statistical analyses using Statistica 6.0 (Statsoft, Inc.). Tukey's honest significant difference test (HSD) was used on the parametric and non-parametric data to determine the statistical significance of variance between various samples (Appendix A, Table A2). From the results of Tukey's HSD analysis for both cooling towers it is evident that there was no significant statistical difference between the corrosion coupons from the two corrosion coupon phases in terms of fouling, scaling and corrosion. The results from the Tukey's HSD analysis were plotted in box and whisker plots. Any overlap between the box and whiskers means that there is no significant statistical difference between the particular data points. The box and whisker plots confirmed that there was no significant statistical difference between the corrosion coupons from the two corrosion coupon phases in terms of fouling, scaling and corrosion for both cooling towers (Appendix A, Figure A1).

The data for both the 1st and 2nd corrosion coupon phases was combined and analysed as one for each cooling tower. The data for both the corrosion coupons and the heat exchanger tubing was then subjected to Tukey's HSD analysis. The results were again subjected to breakdown and one way ANOVA and plotted in box and whisker plots (Figure 4.7). There was also no significant statistical difference between the corrosion coupons from cooling towers 1 and 2 in terms of the fouling (Figure 4.7a), scaling (Figure 4.7b) and corrosion (Figure 4.7c) rates. There was, however, a significant statistical difference between the fouling, scaling and corrosion data for heat exchanger tubing from cooling towers 1 and 2 possibly due to the instability towards of the experiment, which would have resulted high temperature variations at the heat exchanger surfaces. Furthermore a significant statistical difference existed between the heat exchanger tubing and the corrosion coupons in terms of the fouling and corrosion rates (Figure 4.7). This could be attributed to the corrosion coupon phases being 21 and 17 days, respectively, while the heat exchanger tubing were in the system for 38 days.

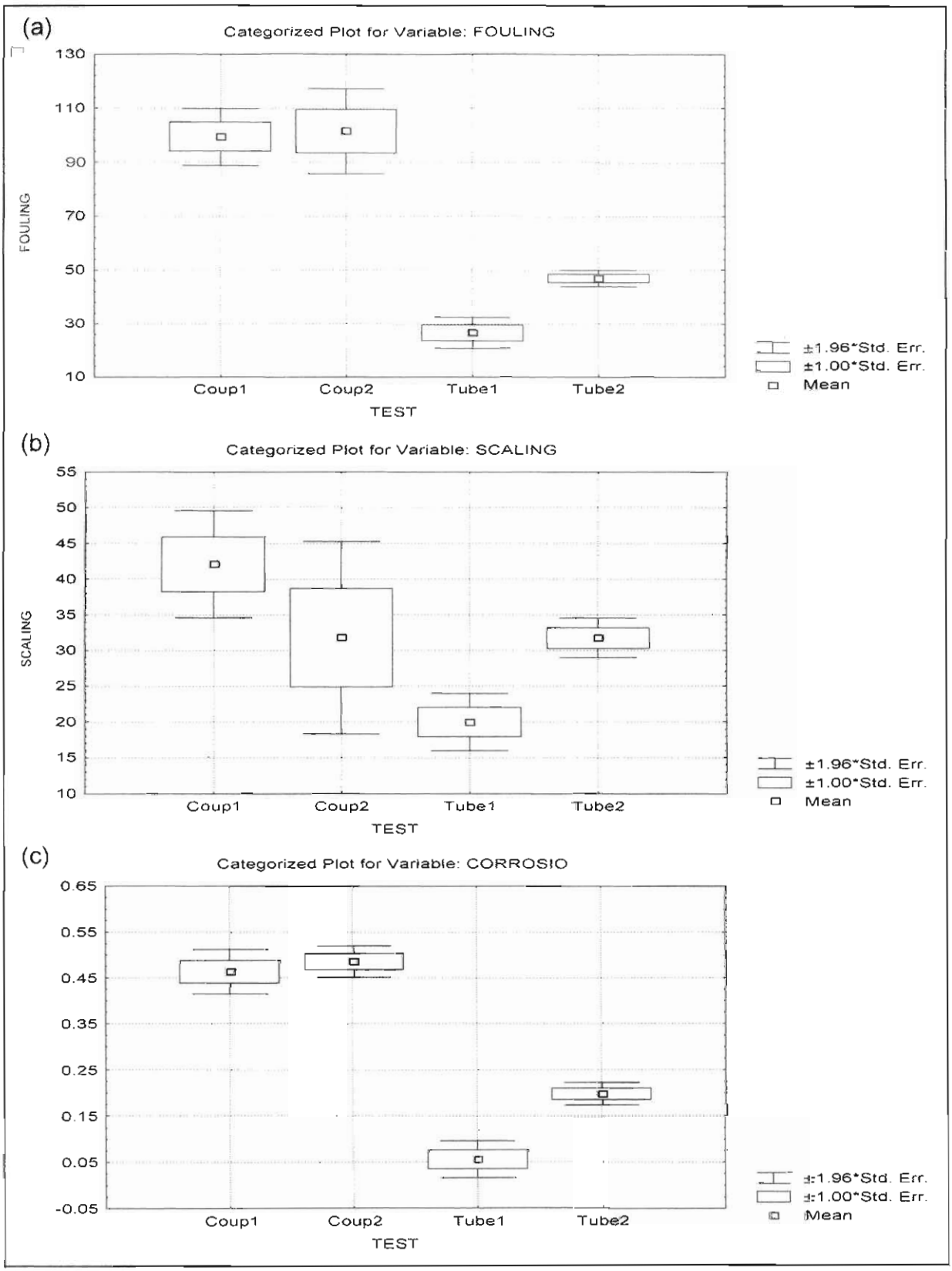


Figure 16. Box and whisker plot of the fouling (a), scaling (b) and corrosion (c) rates in cooling towers 1 and 2, respectively. Coup1 and Coup2 denote the corrosion coupons from cooling tower 1 and 2, respectively. Tube1 and Tube2 denote the heat exchanger tubing from cooling tower 1 and 2, respectively.

4.3.4 Microbial Community Structure (PLFA Analysis)

The detection of unique lipids can infer the presence of certain groups of microorganisms. Terminally branched saturated PLFAs are generally considered to be indicative of Gram positive bacteria (Zelles, 1999). On the other hand, monounsaturated/monoenoic PLFAs are generally considered to be indicative of Gram negative bacteria (Wilkinson, 1988). Saturated PLFAs are generally considered to be indicative of all genera, whereas polyenoic PLFAs are generally considered to be indicative of microeukaryotes (Villanueva *et al.*, 2004).

Figure 4.8 was plotted from the PLFA analysis of the microbial community structure of the biofilm samples from the biocells. Gram negative bacteria were the most prominent group of bacteria. Microeukaryotes/fungi were found in concentrations similar if not slightly higher than Gram positive bacteria. From the figure (Figure 4.8) it is evident that there was high reproducibility between results of the triplicate samples obtained from each cooling tower. A similarity between the microbial community structures present in the two cooling towers was also evident.

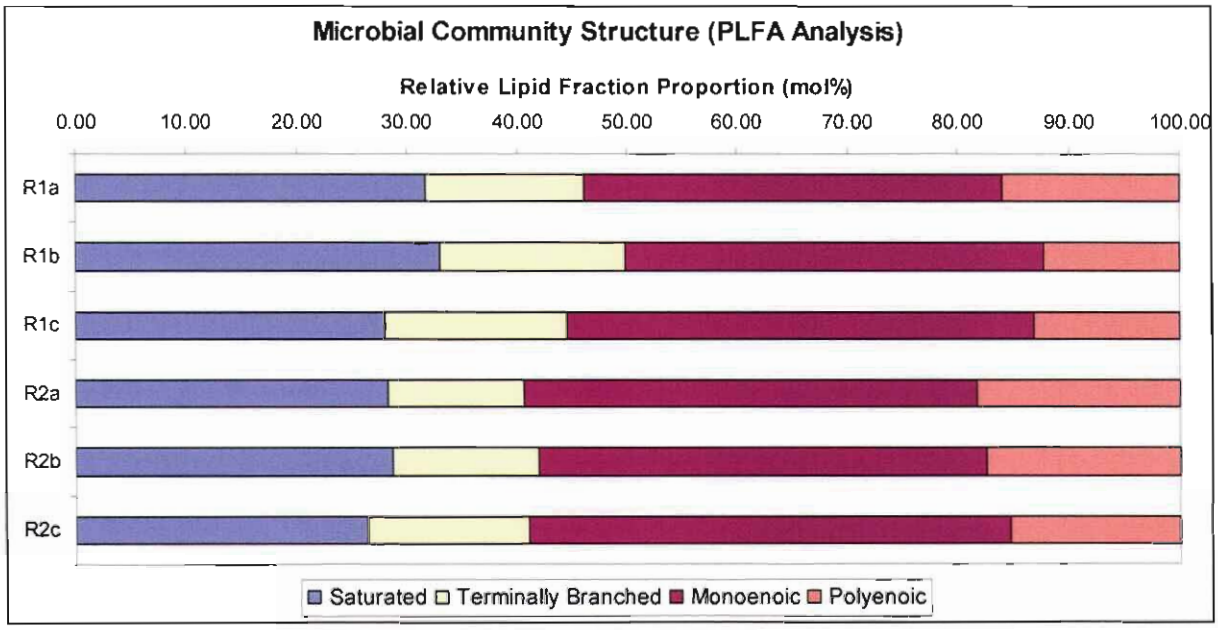


Figure 17. Proportions of the major lipid groups obtained from triplicate biofim samples obtained from the biocells in cooling towers 1 and 2, respectively with a, b and c denoting the triplicates from each cooling tower.

4.4 CONCLUSIONS

Physico-chemical analysis results confirmed that the two cooling towers were operated under identical operational conditions. The physico-chemical analysis results indicate that 14 days are required to reach steady state conditions. Furthermore the cooling towers cannot be operated continuously for longer than a total of 42 days, otherwise they become unstable. The rates of fouling, scaling and corrosion were determined and statistically analysed. There was no significant statistical difference between cooling towers 1 and 2 within a 95% confidence limit in terms of the fouling, scaling and corrosion rates of the corrosion coupons. Microbial community structure was determined by phospholipid fatty acid (PLFA) analysis and scanning electron microscopy (SEM). The results also showed that the structure of the microbial communities in the two cooling towers were similar.

The work carried out in this chapter clearly indicates that the two cooling towers were similar in terms of the fouling, scaling and corrosion rates as well as microbial community structure when they were operated under similar operational conditions. This will, therefore, allow the operation of the two cooling towers in parallel under different operational conditions. Any differences in results will be reflection of the effects of operational conditions and not differences in the cooling towers themselves.

CHAPTER 5: FOULING, SCALING, CORROSION AND STRUCTURAL DIVERSITY OF THE MICROBIAL COMMUNITIES IN SGL FED COOLING TOWERS

5.1 INTRODUCTION

Since large volumes (60 ML/d) of stripped gas liquor (SGL) are produced by Sasol on a daily basis, SGL has a potential to be used as process cooling water. Use of SGL as cooling water would enhance the water conservation measures at Sasol by reducing the volume of raw water intake, as well as reducing the volume of final effluent water that is returned to the Vaal River after bio-treatment (van Nierop *et al.*, 2000). There are, however, several problems associated with the operation of open evaporative cooling systems. Microorganisms, scale deposit and corrosion are the major problems in cooling circuit water system (Congmin *et al.*, 2007). Parameters that have an influence on the fouling, scaling and corrosion of industrial cooling towers include pH, COC and FV (IWS, 1994).

Scale formation is affected by pH (IWS, 1994). A lower pH generally leads to increased solubility of salts and, therefore, a reduced scaling potential but a low pH also leads to increased corrosion rates (IWS, 1994; Rakanta *et al.*, 2007). Iron oxide containing corrosion products on metal surfaces can adsorb humic substances thereby stimulating and/or supporting biofilm development (Butterfield *et al.*, 2002).

Recirculation and evaporation of the water in cooling towers results in increased COC due to the concentration of dissolved and suspended substances thereby promoting scale formation, biofouling, and subsequent macro fouling of the system and eventually microbially induced corrosion (Cloete *et al.*, 1994). Increased COC may result in scale formation due to the precipitation of salts that have exceeded their solubility limits (Cloete *et al.*, 1994; de Almeida *et al.*, 1997). Hard waters are less corrosive but scale forming (Rakanta *et al.*, 2007).

Low FV may provide stagnant areas that enhance biofilm formation and the precipitation of organic and inorganic contaminants from the circulating water (IWS, 1994). Increasing FV is, therefore, helpful to the sloughing of biofilms and the penetration of disinfectants into biofilms, thus suppressing biofilms formation (Tsai, 2006). On the other hand, an increased flow rate increases the oxygen access to the

metal surface, thereby increasing the corrosion rate of metals in cooling towers (IWS, 1994; Rakanta *et al.*, 2007).

Microbial communities have an effect on cooling water systems and, therefore, need to be analysed. Conventional microbiological methods have been applied in investigating bacteria in industrial water systems (Tanji *et al.*, 2007). These methods are, however, not well suited for the detection of microbial community structure *in situ* (White *et al.*, 1996). For a better understanding of microbial diversity, other techniques that are quantitative, more representative and differentiative are required (Muyzer, 1999). The analysis of PLFA patterns is an important tool for the determination of nutritional status, structural diversity, biomass and the effects of certain disturbances upon the microbial community without relying upon cultivation of microorganisms (Frostegard and Baath, 1996; White *et al.*, 1996c; Smith *et al.*, 2000; Ibekwe *et al.*, 2007; Temina *et al.*, 2007). Phospholipid fatty acid provides descriptions of microbial communities based on functional groupings of fatty acid profiles; however, it does not have the capability to identify microorganisms at the species or strain levels (Ibekwe *et al.*, 2007). Denaturing gradient gel electrophoresis can also determine microbial biodiversity and population dynamics of complex ecosystems over space and/or time and can detect and identify community members with high specificity to the species and strain levels (Camu *et al.*, 2007; Ibekwe *et al.*, 2007). However, DGGE cannot be used to determine bacterial numbers, nutritional status and stress levels experienced by microbial communities like PLFA can. Combining the two methods would, therefore, be very powerful in determining microbial community dynamics and has been successfully used previously. Harnesmaa *et al.* (2005) found that the PLFA and DGGE data were in agreement when they evaluated changes occurring in Scots pine rhizosphere microbial communities. Ibekwe *et al.* (2007) also found strong agreement between PLFA and DGGE data in their study of microbial communities in a developing soil ecosystem. The combined use of these methods would, therefore, give a better understanding of microbial community dynamics.

Phospholipid fatty acid analysis can be used to quantify biomass. Several conversion factors have been used in the quantification of biomass using PLFA. Balkwill *et al.* (1988) used a conversion factor of 5.9×10^{12} cells per gram which was derived from a value of 1×10^{8} pmol PLFA/gram of bacterial cells. Kieft *et al.* (1994) used a conversion factor of 5.9×10^{4} cells/pmol of PLFA which was derived from an

average value of 1 x 10^4 mol of PLFA/gram (dry weight) of cells and 5.9 x 10^{12} cells per gram (dry weight). Jiang *et al.* (2006) used a conversion factor of 2 x 10^4 cells/pmol of PLFA. White *et al.* (1996d) used 1.4-4 x 10^4 cells/pmol of PLFA, whereas Frostegard and Baath (1996) calculated an average value of 1.4 x 10^{-17} mol PLFA/cell. Kieft *et al.* (1994) found that the numbers they determined by PLFA were in approximate agreement with direct microscopic counts, but slightly higher. Jiang *et al.* (2006), however, found that the numbers they determined by PLFA analysis were consistently lower than the numbers they determined by acridine orange direct counting. Conversion factors used in the calculation of bacterial numbers are based on mean PLFA concentrations for wide ranges of bacteria (Frostegard and Baath, 1996). In the present study it was important to determine the effect of operational conditions on microbial community dynamics and the choice of conversion factor to be used was, therefore, insignificant. The same profile would be obtained even though the bacterial numbers obtained would differ. It is, however, crucial that the same conversion factor be used throughout this specific study.

An increase in the ratio of cyclopropanoic fatty acids (cyclo/ω7c ratio) represents a shift to conditions that slow down the growth rate of bacteria. This ratio ranges from 0.05 (exponential phase) to 2.5 (stationery phase) in Gram negative bacteria (Villanueva *et al.*, 2004). The ratios of the trans and cis isomers of the monounsaturated PLFA 18:1ω7 can be used to indicate possible environmental stress or starvation in microorganisms. A trans/cis ratio greater than 0.1 indicates a possibility of toxicity or starvation, whereas non-stressed microbial populations have a ratio of <0.05 (Villanueva *et al.*, 2004). Bacteria can quickly adapt to toxic conditions by using trans/cis isomerisation resulting in their membranes being more stable thus retaining their intracellular physiological balance (Guckert *et al.*, 1991).

It is possible to quantify different groups of microorganisms by this method, and individual PLFAs can be related to microbial community structure (Winding *et al.*, 2005). Terminally branched saturated PLFAs are generally considered to be indicative of Gram positive bacteria (Zelles, 1999). On the other hand, monounsaturated/monoenoic PLFAs are generally considered to be indicative of Gram negative bacteria (Wilkinson, 1988). Saturated PLFAs are generally considered to be indicative of all genera, whereas polyenoic PLFAs are generally considered to be indicative of microeukaryotes (Villanueva *et al.*, 2004).

The general strategy for genetic fingerprinting of microbial communities using DGGE consists of firstly, the extraction of nucleic acids (DNA and RNA), secondly, the amplification of genes encoding the rRNA, and, thirdly, the analysis of the PCR products using the DGGE technique (Muyzer, 1999). Separation of DNA fragments in DGGE is based on the decreased electrophoretic mobility of partially melted double stranded DNA molecules in polyacrylamide gels, which is decreased compared to that of the completely helical form of the molecule (Muyzer *et al.*, 1993). Polyacrylamide gels contain a linear gradient of DNA denaturants (a mix of urea and formamide) (Muyzer, 1999). The number of DNA derived bands relates to the presence of individual species that are above the detection limit of DGGE (Dar *et al.*, 2007). In the fingerprinting of natural bacterial populations 16S rDNA fragments are used and for fungal populations 18S rDNA fragments are used (Watanabe *et al.*, 2001). It can be assumed that the individual distinguishable bands represent distinct species, however, these results should be interpreted with some caution because the possible occurrence of multiple copies of 16S and 18S rDNA genes in individual species may result in an increased number of phylotypes and therefore an overestimation of the microbial diversity (Rasmussen and Sorensen, 2001; Jonkers *et al.*, 2003). Specific primers can be designed for specific groups of microorganisms, e.g., eubacteria, fungi, archae, sulphate-reducing bacteria, methanotrophic bacteria, etc.

Species diversity is often used as a measure of environmental health. There are two parts to diversity: the number of species and the evenness of the distribution. The assumption is that where there are more species the habitat is more diverse. Furthermore, an evenly distributed community is more diverse than an unevenly distributed community with the same number of species (Hedrick *et al.*, 2000). Microbial communities within contaminated ecosystems are typically less diverse than those in non-stressed systems (Macnaughton *et al.*, 1999). Diversity can be determined by using the Shannon-Weaver diversity index. The advantage of this index is that it takes into account the number of species and the evenness of the species. The index is increased either by having more unique species, or by having greater species evenness. Ampe and Miambi (2000) used the Shannon-Weaver diversity index to determine the structural biodiversity of DGGE bands found in foods of different origins. Gafan *et al.* (2005) used the Shannon-Weaver diversity index to investigate differences in the microbiota of plaque.

Due to SGL being a complex medium containing hydrocarbons it would also be necessary to optimise DNA extraction and DGGE analysis. In Chapter 3 the selective extraction of hydrocarbons from SGL was successfully optimised. In Chapter 4 it was determined that the cooling towers had to be operated for approximately 14 days to reach steady state conditions, but could not be operated continuously for longer than 42 days, otherwise they become unstable. It was also determined that the two cooling towers were similar. Results of experiments conducted on the two cooling towers could, therefore, be compared to each other with any differences in results being a reflection of only the effects of operational conditions.

Experimental factorial design aims at limiting the number of experiments normally required to study the influence of the most important factors involved in a given reaction (Rafiqul *et al.*, 2000). An X-level factorial design for k factors is denoted as X^k. This means that for a 2^3 factorial design is used to test three factors (operational parameters) at two different levels (high and low) resulting in 8 experiments. Casas *et al.* (1997) achieved the optimization of a synthetic medium for *Candida bombicola* growth by a 2^4 factorial design. Rafiqul *et al.* (2000) used a 2^3 factorial experimental design for the optimization of the co-liquefaction process of coal and a sugar cane waste product with respect to oil yield. Perkiomaki *et al.* (2003) used a 2^2 factorial experimental design to assess the effects of moderate amounts of continuous acid and metal deposition on humus microbial activity and community structure. Aust *et al.* (2006) also used a 2^4 factorial experimental design to determine the effects of start temperature for crystallization, cooling rate, heating rate, and elution speed on the separation quality of temperature-rising elution fractionation using a heterophasic ethylene/propylene copolymer. The use of factorial experimental design, therefore, has great potential for the optimization of operational conditions in cooling towers.

The aim of this chapter was, therefore, to investigate the influence of varying pH, COC and FV on the rates of fouling, scaling and corrosion of mild steel, as well as the structural diversity of the microbial communities in both the planktonic and sessile phases using a 2^3 multi-factorial experimental design. The objectives of this chapter were to:

i. Determine the effects pH, COC and FV on fouling, scaling and corrosion rates using a 2^3 multi-factorial experimental design,

ii. Determine the effects of pH, COC and FV on microbial community structure as determined by PLFA and DGGE analysis using this 2^3 multi-factorial experimental design.

5.2 MATERIAL AND METHODS

5.2.1 2^3 Multi Factorial Experimental Design

Statistica 6.0 (Statsoft, Inc., Tulsa, OK) was used to generate a 2^3 multi-factorial design of the experiments to enable comprehensive statistical analysis of the results that would be obtained. A randomised 2^3 factorial design matrix (Appendix B, Table B1), was obtained from Statistica 6.0 and used to define the experimental setup as illustrated in Figure 5.1 below. Experiments were conducted under such conditions. The 2^3 factorial experimental design required 8 experiments to be performed to optimise the operational conditions of cooling towers operated using SGL. The two cooling towers were operated in parallel for a total of four runs. Experiments 1&2, 3&4, 5&6, and 7&8 were run in parallel using cooling towers 1 and 2, respectively.

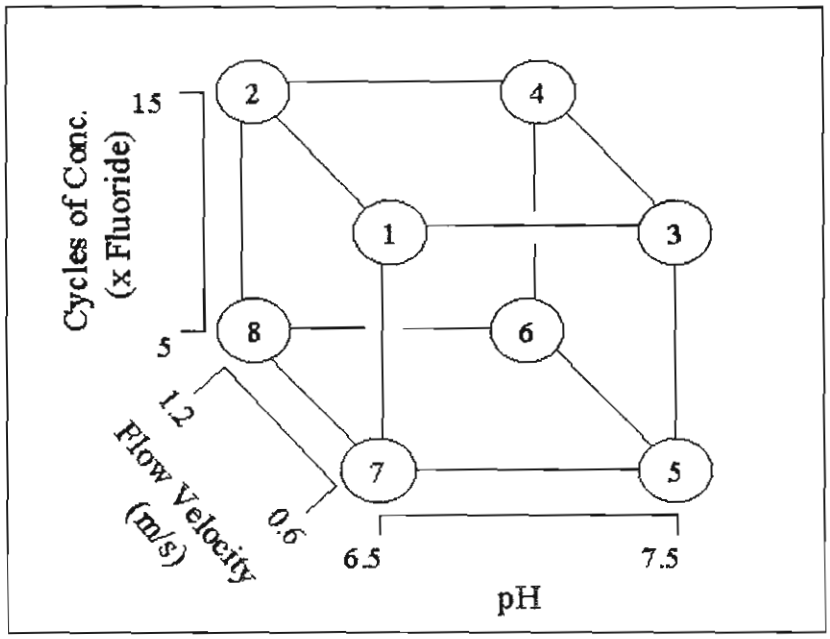


Figure 18. Experimental layout as determined by the randomised 2^3 factorial design matrix.

5.2.2 Experimental Setup

The cooling towers used are as described in Section 4.2.1. The cooling towers were operated with pH values of 6.5 and 7.5 (maintained with concentrated H_2SO_4 and

25% NH_4OH, respectively), FV of 0.6 and 1.2 m/s in the heat exchanger tubing (8mm ID, mild steel), 5 and 15 COC, a ΔT of 10°C and a sump temperature not exceeding 35°C.

For each experiment the cooling towers were operated for approximately two weeks to reach steady conditions, after which PVC biocells, mild steel corrosion coupons and mild steel heat exchanger tubing were inserted. After 14 days the corrosion coupons were removed and analysed. These were immediately replaced with another set. There were thus two corrosion coupon phases per experiment. Heat exchanger tubing remained in the heat exchangers for 28 days.

After completion of each experiment the corrosion coupons, heat exchanger tubing and biocells were removed and analysed accordingly. Based on the results obtained from the corrosion coupons and the heat exchanger tubing the rates of fouling, scaling and corrosion were determined. The sump liquid sample and the biofilm obtained from the biocells were analysed by PLFA and DGGE to determine the structural diversity of the microbial communities in the cooling towers.

5.2.3 Fouling, Scaling and Corrosion

The rates of fouling, scaling and corrosion were determined as described in Section 4.2.4.

5.2.4 Microbial Community Structure (PLFA Analysis)

The total lipid extraction, selective extraction of hydrocarbons, fractionation, methylation and gas chromatography mass spectrophotometry analyses were performed as described in Section 4.2.5.

5.2.4.1 PLFA Data Analysis

Phospholipid fatty acid (PLFA) data was analysed according to Section 3.3.3.3. Fouling, scaling and corrosion rate data was combined with the PLFA data and redundancy analysis (RDA) ordination diagrams were plotted using Canoco for Windows 4.0 (GLW-CPRO).

5.2.5 Microbial Community Structure (DGGE Analysis)

5.2.5.1 DNA Extraction

Biofilm samples were scraped off biocell sections using a sterile spatula and frozen in 1.5 ml microfuge tubes. These were stored at –80°C until DNA extraction. Fifty millilitres of the planktonic phase was centrifuged at 4000 rpm in sterile falcon tubes. The supernatant was discarded and the pellet was frozen and stored at –80°C until DNA extraction.

Several modifications of the DNA extraction method used by Sambrook *et al.* (1989) were evaluated for its ability to provide DNA of a quality and quantity that would be sufficient for PCR amplification. SGL contains several hydrocarbon substances that could inhibit PCR. Eventually a method that was adopted from Hillis *et al.* (1990) was used for the DNA extraction. Five hundred microliters of hot 2 x cetyl trimethylammonium bromide (CTAB) isolation buffer (60°C) was added to approximately 250 mg of sessile and planktonic samples (wet weight) in a sterile 1.5 ml microfuge tube. Planktonic samples were obtained by centrifugation of 50 ml of cooling water at 2000 rpm from the cooling tower sump. This was then incubated in a 60°C water bath for 45 minutes, centrifuged at 12000 rpm for 5 minutes (MiniSpin Plus, Eppendorf) and the supernatant transferred to a new sterile 1.5 ml microfuge tube. An equal volume of hot TE buffered Phenol:Chloroform:Isoamyl (PCI) was added and the DNA in the sample extracted for 10 minutes at 60°C, centrifuged at 12000 rpm for 5 minutes and the aqueous phase transferred to a new sterile 1.5 ml microfuge tube. The sample was then re-extracted with an equal volume of TE Buffered Chloroform:Isoamyl (24:1) for 10 minutes at room temperature, centrifuged at 12000 rpm for 5 minutes and the aqueous phase transferred to a new sterile 1.5 ml microfuge tube. One hundred microliters of 5 M NaCl and 1 ml of ice-cold 95 % molecular grade ethanol were added and the DNA was precipitated at -80°C for 1 hour. After the samples were centrifuged at 12000 rpm at 4°C for 5 minutes, the supernatant was discarded. The pellet was washed with 1 ml ice-cold 70% ethanol to remove NaCl, centrifuged at 12000 rpm at 4°C for 5 minutes and the supernatant discarded. This pellet was dried under vacuum (Dura-Dry™ μP, FTS Systems, New York), re-suspended in 25 µl TE buffer, incubated at 37°C for 1 hour to reconstitute the DNA and stored at 4°C.

DNA concentrations were determined spectrophotometrically according to the equation:

$$DNA\ Concentration = A_{260\ nm}\ obtained \times 1\ A_{260\ nm} \times dilution\ factor$$

Where 1 $A_{260\ nm}$ = 50ng/µl DNA (Maniatis *et al.*, 1989; Kozdroj and van Elsas, 2001).

DNA purity was determined according to the equation:

$$DNA\ Purity = A_{260nm} / A_{280nm}$$

A ratio of 1.8 means that the DNA is of good quality, whereas a ratio less than 1.8 means that there is protein contamination and a ratio greater than 1.8 means that there is RNA contamination (Maniatis *et al.*, 1989; Kozdroj and van Elsas, 2001).

5.2.5.2 Polymerase Chain Reaction

The PCR reaction was performed with an Omni-E PCR thermocycler (Hybaid). A number of optimisation reactions were performed to determine the effects of the addition of BSA, glycerol, additional *Taq* and $MgCl_2$ (Appendix B, Table B). Then a second set of optimisation reactions was done to determine the optimum $MgCl_2$ concentration to be added (Appendix B, Table B). The optimised reaction volumes were a total of 25 µl containing 12.5 µl of PCR Master Mix (Roche Diagnostics), 1U of Super-Therm *Taq* (JMR Holdings, UK), 4mM of $MgCl_2$, 50 ng of BSA, 50 pmol of primer pair and 10 ng of DNA preparation.

The primer pair GM5F (5'-CCT ACG GGA GGC AGC AG-3') - 907R (5'-CCG TCA ATT CCT TTG AGT TT-3') was used to amplify the 16S rDNA of members of the domain *Bacteria* (Brinkhof *et al.*, 1998; Moesender *et al.*, 1999). Nested PCR was used to amplify of 18S rDNA fragments. The samples were first amplified with the forward primer NS1-GC (5'-CC AGT AGT CAT ATG CTT GTC-3'; Kowalchuk *et al.*, 1997) and the reverse primer nu-SSU-1196R (5'-TCT GGA CCT GGT GAG TTT CC-3'; Borneman and Hartin, 2000) to obtain a 1179-bp fragment. One µl of the 1179-bp fragment was used for the second PCR step using the forward primer NS1-GC and the reverse primer NS2+10 (5'-GAA TTA CCG CGG CTG CTG GC-3') to obtain a 569-bp fragment of the 18S rDNA (Kowalchuk *et al.*, 1997). A 40-bp GC clamp (5'-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC G-3'; Muyzer *et al.*, 1993) was attached to the 5' ends of the GM5F and NS1-GC forward primers.

The cycling program for the PCR of the 16S rDNA consisted of an initial denaturing step of 95°C for 5 minutes, annealing at 65°C for 30 seconds and elongation at 72°C for 1 minute. This was followed by 34 cycles of denaturing at 95°C for 30 seconds, annealing at 65°C for 30 seconds and elongation at 72°C for 1 minute. Each run was ended with a final elongation step at 72°C for 5 minutes.

The PCR products were electrophoresed on a 1% agarose gel containing 1 μg/ml ethidium bromide at 100 V for 45 minutes in 1X TAE (40mM Tris, 20mM Acetic acid, 1mM EDTA; pH 8.3). The gels were visualised using a GeneGenius Image system (Syngene, UK) and the images were captured and documented using GeneSnap software (Version 3.00.22, Syngene, UK).

5.2.5.3 DGGE

Fifteen microliters of the 16S rDNA PCR products from each sample were analysed on a 6% bis/acrylamide gel containing a gradient of 30 to 60% of the denaturants urea and formamide. The DGGE gels were electrophoresed using a DCode™ Universal Mutation Detection System (Bio-Rad, UK) according to the manufacturer's instructions. The gels were electrophoresed at a constant voltage of 100 V for 16 hours at 60°C in 1X TAE buffer (40mM Tris, 20mM Acetic acid, 1mM EDTA; pH 8.3).

Fifteen microliters of the 18S rDNA PCR products from each sample were electrophoresed on a 6% bis/acrylamide gel containing a gradient of 20 to 50% of the denaturants urea and formamide. The gels were electrophoresed at a constant voltage of 80V for 13 hours at 60°C in 1XTAE buffer (40mM Tris, 20mM Acetic acid, 1mM EDTA; pH 8.3).

Following electrophoresis, the gels were stained for 25 minutes in a 1 μg/ml ethidium bromide solution in 1X TAE then rinsed for 5 minutes in 1X TAE. The gels were visualised using a GeneGenius Image system (Syngene, UK) and the images were captured and documented using GeneSnap (Version 3.00.22, Syngene, UK) software and analysed using Gene Tools (Version 3.00.22, Syngene, UK).

5.2.5.4 DGGE Data Analysis

The DGGE fingerprints obtained were analysed according to the quantity of each diagnostic band using Gene Tools (Syngene, UK). For the determination of the community structure a single band in the gel was selected as the reference band and given an arbitrary intensity value. The relative intensities of the other bands were normalised in relation to the quantity of the band chosen as the reference. The microbial community structure was plotted based on the intensities of the specific bands in relation to their Rf distances along the DGGE gel. The relative band intensity data was also used to plot dendograms of the DGGE data using the unweighted pair group average (UPGA) algorithm and euclidean distances (Statistica 6.0, Statsoft Inc.).

The structural diversity of the bacterial and fungal communities was calculated according to the Shannon-Weaver index of general diversity (Shannon and Weaver, 1963; Ibekwe *et al.*, 2007):

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

H was calculated on the basis of the bands on the gel lane by using the relative intensities of the bands. P_i is the relative probability of the bands in a lane. The relative probability, P_i was calculated as:

$$Pi = n_i / N$$

Where n_i is the relative intensity of a band and N is the sum of all the relative intensities in a lane.

5.3 RESULTS AND DISCUSSION

5.3.1 Fouling, Scaling and Corrosion

The results obtained for the analysis of the fouling, scaling and corrosion rates of the first and second corrosion coupon phases as well as the heat exchanger tubing are illustrated in Figure 5.2. The combination of conditions (FV, pH and COC) that existed at each experiment are shown in Table 5.1 and graphically in Figure 5.1.

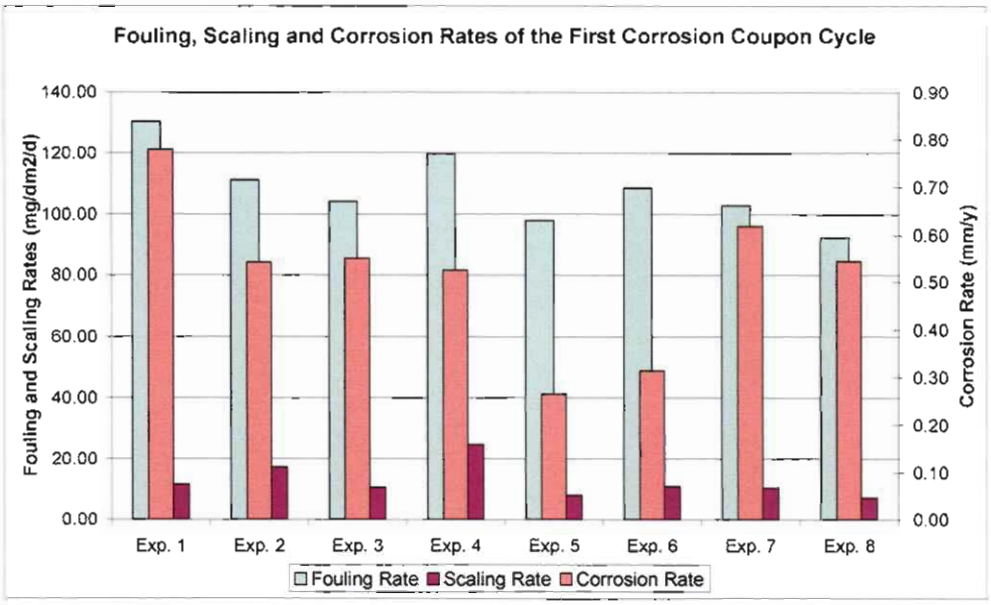
Table 5.1. Experiments conducted and their operational conditions.

Experiment	pH	Flow Velocity	Cycles of Concentration
1	6.5	0.6 m/s	15
2	6.5	1.2 m/s	15
3	7.5	0.6 m/s	15
4	7.5	1.2 m/s	15
5	7.5	0.6 m/s	5
6	7.5	1.2 m/s	5
7	6.5	0.6 m/s	5
8	6.5	1.2 m/s	5

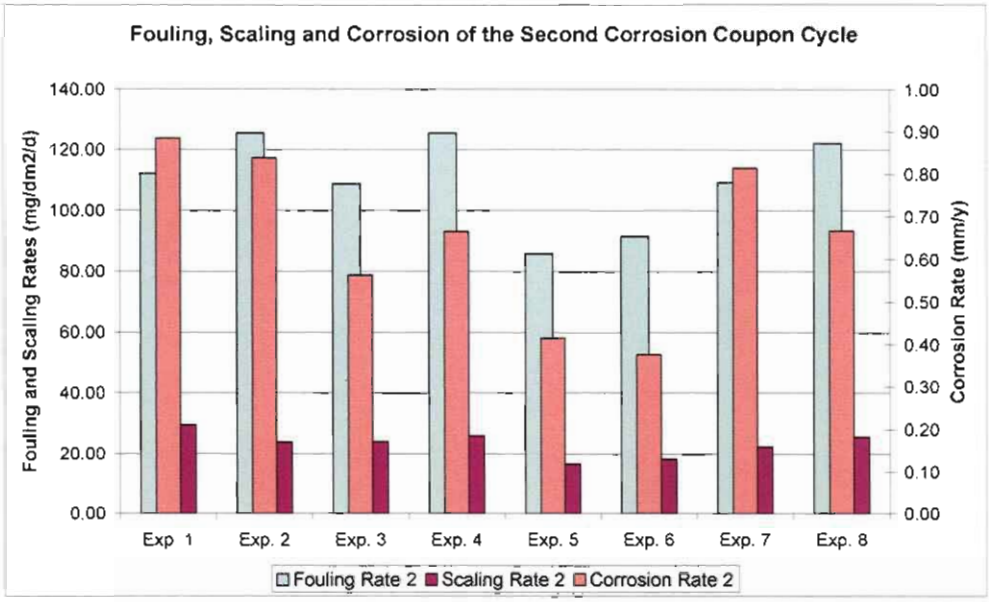
Upon examination of the results obtained from the first corrosion coupon phase (Figure 5.2a) it is evident that the highest fouling and corrosion rates were obtained at experiment 1. The highest scaling rate was obtained at experiment 4. The lowest fouling and scaling rates were obtained at experiment 8. The lowest corrosion rate was obtained at experiment 5. Results obtained from the second corrosion coupon phase (Figure 5.2b) indicate that the highest fouling rate was obtained at experiment 4. Highest scaling and corrosion rates were obtained at experiment 1 and the lowest fouling and scaling rates at experiment 5. The lowest corrosion rate was obtained at experiment 6. The highest fouling, scaling and corrosion rates in the heat exchanger tubing were obtained from experiment 2 (Figure 5.2c). In experiment 8 the lowest fouling rate was observed. The lowest scaling rate was obtained at experiment 7 and the lowest corrosion rate at experiment 5.

Some similarities between the results obtained from the first and second corrosion coupon phase, and the heat exchanger tubing existed. The results obtained from the second corrosion coupon phase and the heat exchanger tubing (Figure 5.2) indicate that a FV of 1.2 m/s (experiments 2, 4, 6 and 8), relative to 0.6 m/s (experiments 1, 3, 5 and 7), generally resulted in higher scaling and corrosion rates. Fifteen COC (experiment 1 to 4) resulted in generally higher fouling, scaling and corrosion rates than 5 COC (experiments 5 to 8) in the first and second corrosion coupon phases, as well as the heat exchanger tubing. Even though the corrosion coupons were in the cooling towers for 14 days per session and the heat exchanger tubing for 28 days the heat exchanger tubing exhibited lower fouling and corrosion rates relative to the corrosion coupons. The scaling rates in the heat exchanger tubing were fairly similar to the scaling rates in the corrosion coupons.

(a)



(b)



(c)

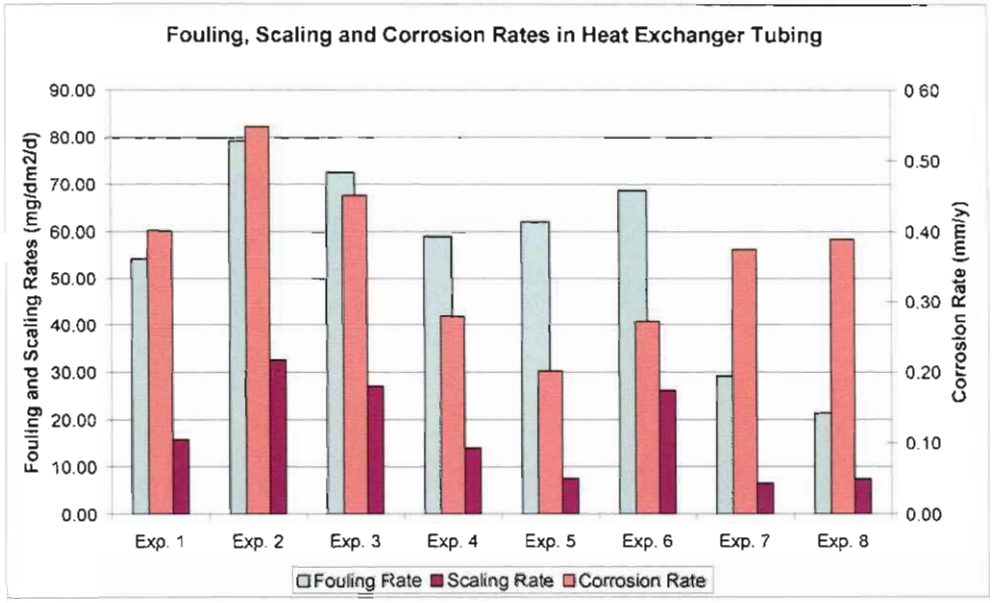


Figure 19. Fouling, scaling and corrosion rates obtained from the analysis of the 1st (a) and 2nd (b) corrosion coupon phases as well as the heat exchanger tubing (c).

Differences in the fouling, scaling and corrosion rates between the two corrosion coupon phases could possibly be attributed to the development of a fouling layer on the pipes and fill of the cooling tower. The fouling of cooling tower fills is one of the most important factors affecting its thermal performance. Fouling reduces cooling tower effectiveness and tower capability with time (Qureshi and Zibair, 2006). This would then result in the two corrosion coupon phases being subjected different thermal conditions. The rate of fouling of heat exchangers due to scaling increases as the temperature of the water rises. This happens in the case of calcium and magnesium carbonates that exhibit inverse solubility behaviour with temperature (Picon-Nunez *et al.*, 2007). It, therefore, stands to reason that with increased fouling over time, the scaling rate would also increase due to the decreased thermal performance of the cooling tower. This is clearly evident in the results illustrated in Figure 5.2.

The differences between the corrosion coupons and the heat exchanger tubing could be attributed to the FV being affected by pipe diameters. Flow velocities were calculated using the diameters of the heat exchanger tubing (8 mm ID) and since the pipe diameter around the corrosion coupons was wider (18 mm ID), the FV around them was much lower. Reduced water velocities can lead to a deposition of suspended matter (fouling) and the creation of differential aeration cells that result in pitting corrosion (IWS, 1994). According to the IWS (1994) a balanced water velocity with a preferred range of 1.5 to 3 m/s is required to prevent corrosion effects. Kobrin (1994), however, reported fouling and subsequent stainless steel failure, due to microbially induced corrosion, at flow rates of 2.4 to 3 m/s.

In order to determine the optimal operating conditions for the operation of cooling towers the experiments that gave the lowest fouling, scaling and corrosion rates from the 1st and 2nd corrosion coupon phases, as well as the heat exchanger tubing were determined (Table 5.2). The results in Table 5.2 clearly illustrate that, overall, the lowest fouling, scaling and corrosion rates in cooling towers operated with SGL as make-up water were achieved experiment 5 since it occurred the most times in this table. Conditions at experiment 5 were a pH of 7.5, a FV of 0.6 m/s and 5 COC.

Table 5.2. Experiments that gave the lowest fouling, scaling and corrosion rates from the 1st and 2nd corrosion coupon phases, as well as the heat exchanger tubing. The conditions at each experiment are shown in parenthesis (pH; FV; COC).

Lowest fouling, scaling and corrosion rates			
	1st Coupon Phase	2nd Coupon Phase	Heat Exchanger Tubing
Fouling	8 (6.5; 1.2; 5)	5 (7.5; 0.6; 5)	8 (6.5; 1.2; 5)
Scaling	8 (6.5; 1.2; 5)	5 (7.5; 0.6; 5)	7 (6.5; 0.6; 5)
Corrosion	5 (7.5; 0.6; 5)	6 (7.5; 1.2; 5)	5 (7.5; 0.6; 5)

From the results in Table 5.2 it is evident that the operational parameter with the greatest overall influence on fouling, scaling and corrosion rates was COC followed by pH. Furthermore they also indicate the importance of COC control. Increased COC may result in scale formation due to the precipitation of salts that have exceeded their solubility limits (Cloete *et al.*, 1994; de Almeida *et al.*, 1997). Cloete *et al.* (1994) also stated that increased COC results in the concentration of dissolved and suspended substances thereby promoting scale formation, biofouling, and subsequent macro fouling of a system. This eventually leads to microbially induced corrosion. The results presented here are thus in agreement with published literature where it is indicated how important the role of COC is in controlling fouling, scaling and corrosion.

A high pH generally leads to decreased solubility of salts and therefore an increased scaling potential but a high pH also leads to decreased corrosion rates (IWS, 1994; Rakanta *et al.*, 2007). The IWS (1994) recommends the use of controlled scaling for the prevention and control of corrosion. A pH of 7.5 is high enough to allow a scaling layer to form. This layer will be sufficient to protect the system against corrosion.

The results obtained in this study also indicate that a lower FV (0.6 m/s) resulted in lower fouling, scaling and corrosion rates. This observation correlates with the findings of Lehtola *et al.* (2006) who reported a 13 to 15 times increase in biofilm formation with increased FV of water from 0.03 to 0.28 m/s. Chen *et al.* (2005) also reported increased biofilm adhesive strength as they increased fluid velocity from 0.6 to 1.6 m/s. Soini *et al.* (2002), however, reported that an increase in the FV decreased bacterial densities indicating preferable biofilm formation on areas with low FV. According to the IWS (1994), low FV may provide stagnant areas that enhance biofilm formation and the precipitation of organic and inorganic contaminants from the circulating water.

The 2^3 factorial experimental design was critical in the determination of the effects COC, pH and FV on fouling, scaling and corrosion rates. Cycles of concentration was determined to have the most considerable effect on the fouling, scaling and corrosion rates in the cooling towers, followed by pH. From the results thus far optimal operational conditions for the reduction of fouling, scaling and corrosion in cooling towers using SGL as make-up water were determined to be 5 COC, a pH of 7.5 and a FV of 0.6 m/s.

5.3.2 Microbial Community Structure (PLFA Analysis)

The number of cells per ml or cm^2 in the planktonic and sessile phases, respectively, was determined according to Balkwill *et al.* (1988), derived from a value of 1×10^8 pmol PLFA/gram of bacterial cells and 5.9×10^{12} cells per gram (Figure 5.3). The number of cells per ml was higher in the planktonic phases of experiments 3 to 6 (pH 7.5) relative to the planktonic phases at experiments 1, 2, 7 and 8 (pH 6.5) (Figure 5.3). Estimated numbers of cells were higher in the planktonic phases of experiments 1 to 4 (15 COC) relative to the sessile phases of each experiment. Estimated numbers of cells were higher in the sessile phases of experiments 5 to 8 (5 COC) relative to the planktonic phases of each experiment (Figure 5.3). A FV of 1.2 m/s resulted in higher estimated cell numbers in the planktonic phase, relative to 0.6 m/s. This could be attributed to the shearing effect of higher fluid velocities of the biofilm layer resulting in more microorganisms staying in suspension (Tsai, 2006). Perkiomaki *et al.* (2003) stated that phospholipid fatty acid analysis mainly targets bacteria. This could imply that total microbial community numbers at the lower pH could possibly have been similar or higher due to the presence of higher fungal numbers, but the PLFA method could have underestimated this due to it targeting mainly bacteria. This aspect would have to be investigated further in future studies.

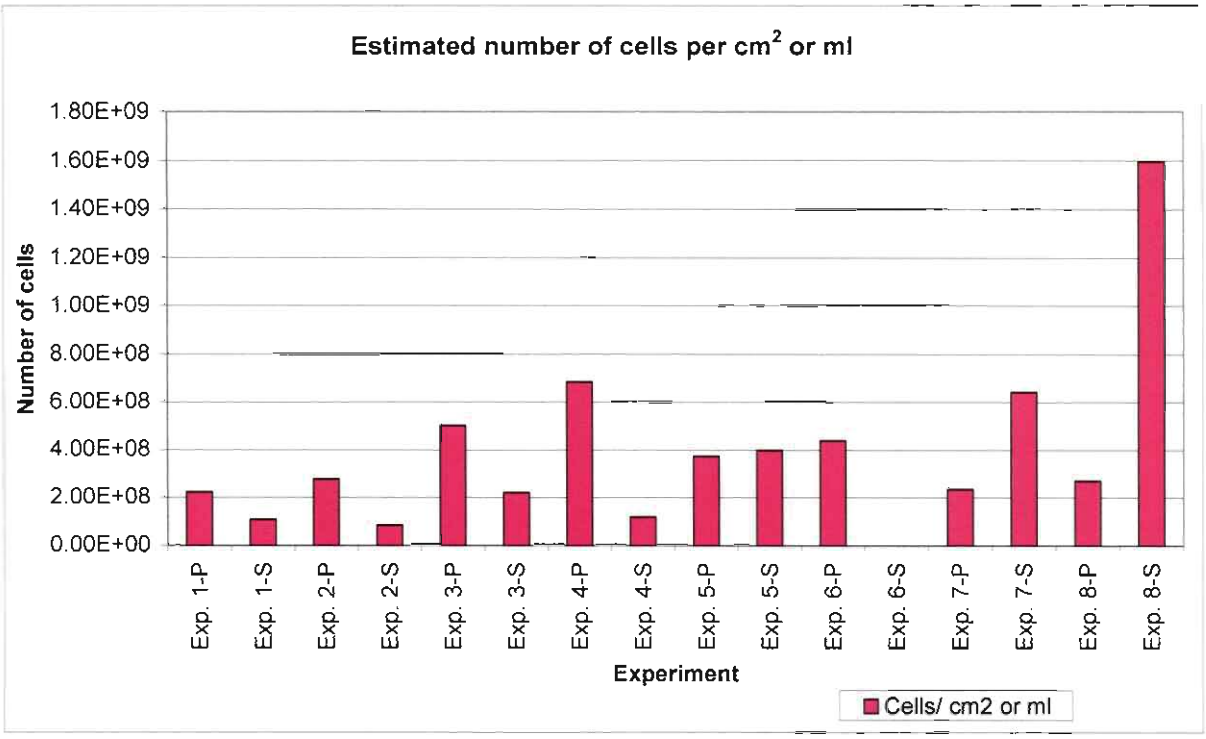


Figure 20. Estimated bacterial numbers per ml of planktonic phase liquid or cm^2 of biofilm as determined according to Balkwill *et al.* (1988).

The microbial communities in the planktonic phase generally had a lower cyclo/ω7c ratio relative to the respective sessile phase microbial communities (Figure 5.4a). This suggests that the planktonic phase microbial communities were more in the exponential growth phase and the sessile phase microbial communities more in the late exponential to stationery phases of growth (Villanueva *et al.*, 2004). The planktonic phase microbial communities in experiments 1, 2, 7 and 8 (pH 6.5) had cyclo/ω7c ratios ranging from 0.02 to 0.04. The cyclo/ω7c ratios in experiments 3 to 6 (pH 7.5) ranged from 0.09 to 0.14 (Figure 5.4a). These figures suggest that the planktonic microbial communities grew more actively at a pH of 6.5. The highest cyclo/ω7c ratios among the planktonic phase microbial communities were found at experiments 3 and 4 (pH 7.5 and 15 COC). The highest cyclo/ω7c ratios among the sessile phase microbial communities were found at experiments 7 and 8 (pH 6.5 and 5 COC). The results illustrated in Figure 5.4 (a) again demonstrate the important role played by COC.

Upon examination of the results of the trans/cis ratio of the PLFA (Figure 5.4b) it became evident that the microbial communities in the sessile phases only experienced metabolic stress at experiments 7 and 8 (5 COC and pH 6.5). The microbial community in the sessile phase in experiment 7 experienced more stress

than the microbial community in experiment 8 and there were, therefore, higher bacterial numbers from experiment 8. The planktonic phases experienced a higher degree of metabolic stress at experiments 3 and 4 (15 COC and pH 7.5).

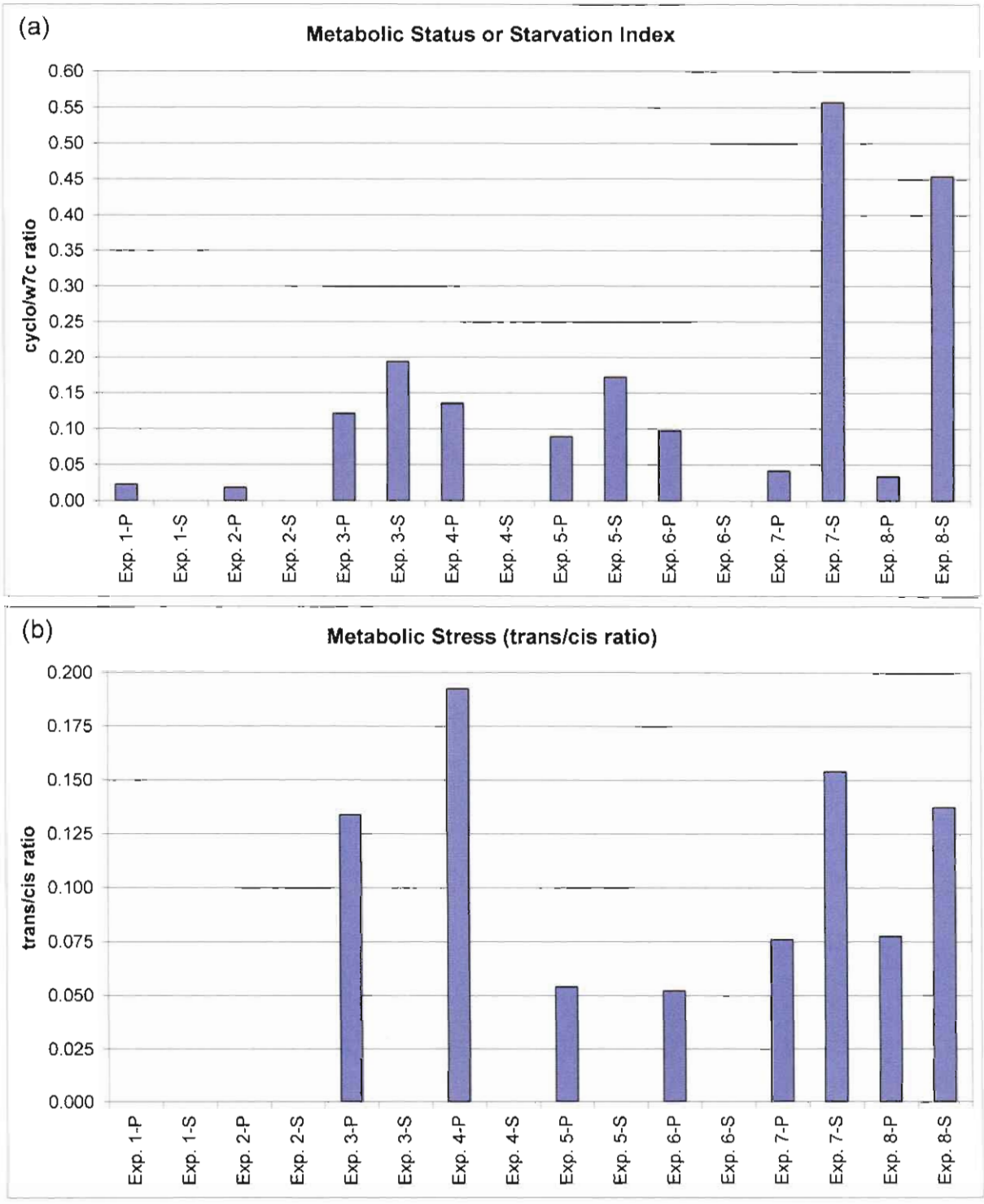


Figure 21. (a) Metabolic status or starvation index as the ratio of cyclopropyl fatty acids to monoenoic PLFAs (cyclo/ω7c ratio). (b) Metabolic stress expressed as the trans/cis ratio of monoenoic PLFAs.

The planktonic microbial communities had the highest estimated bacterial numbers (Figure 5.3), highest cyclo/ω7c ratio (Figure 5.4a) and the highest trans/cis ratio (Figure 5.4b) at experiments 3 and 4 (15 COC and a pH of 7.5). The sessile microbial communities had the highest estimated bacterial numbers (Figure 5.3), highest cyclo/ω7c ratio (Figure 5.4a) and the highest trans/cis ratio (Figure 5.4b) at experiments 7 and 8 (5 COC and a pH of 6.5). This, therefore, implies that an increase in the metabolic stress and the resultant decrease in the growth rate experienced by microbial communities cannot be taken to imply that there will be a decrease in microbial biomass. It also highlights the role of COC and pH on microbial community dynamics.

Upon examination of Figure 5.5 it is evident that there are definite differences in terms of the concentration of Gram positive and Gram negative bacteria between the planktonic and the sessile phases. The concentration of sessile phase Gram negative bacteria was lower at experiments 1 to 4 (15 COC), whereas the concentration of sessile phase Gram positive bacteria was lower at experiments 5 to 8 (5 COC). Cycles of concentration is the major differentiating parameter between Gram positive and Gram negative bacteria.

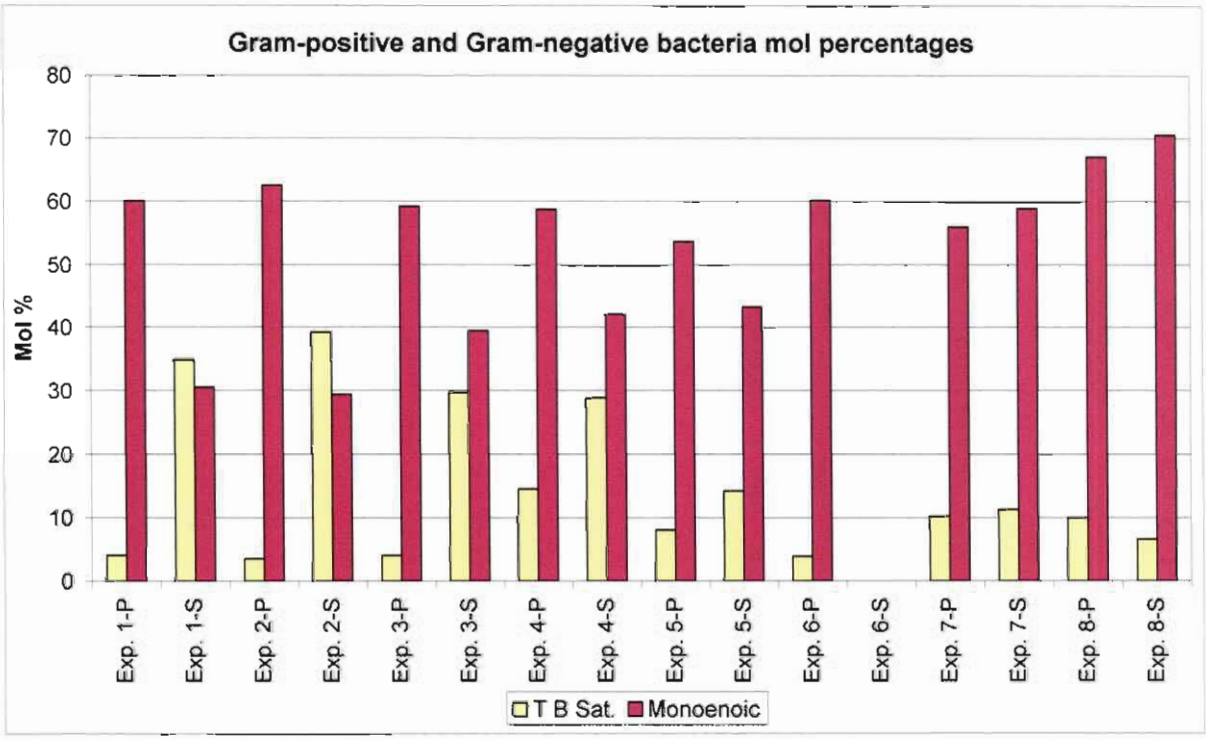


Figure 22. Illustration of the Gram positive and Gram negative mol. percentages (TB Sat. (terminally branched saturated PLFAs) are indicative of Gram positive bacteria, and Monoenoic PLFAs are indicative of Gram negative bacteria).

Upon examination of the total microbial community structures found in the different experimental samples, similarities between the experimental samples were observed (Figure 5.6). The structure of the microbial communities in the planktonic phases of experiments 1, 2, 5 and 6 were similar. pH seems to be the major differentiating factor in the planktonic phase. The structure of the microbial communities in the sessile phases of experiments 1, 2, 3 and 4 (15 COC) were similar. The major differentiating parameter in the sessile phase is COC.

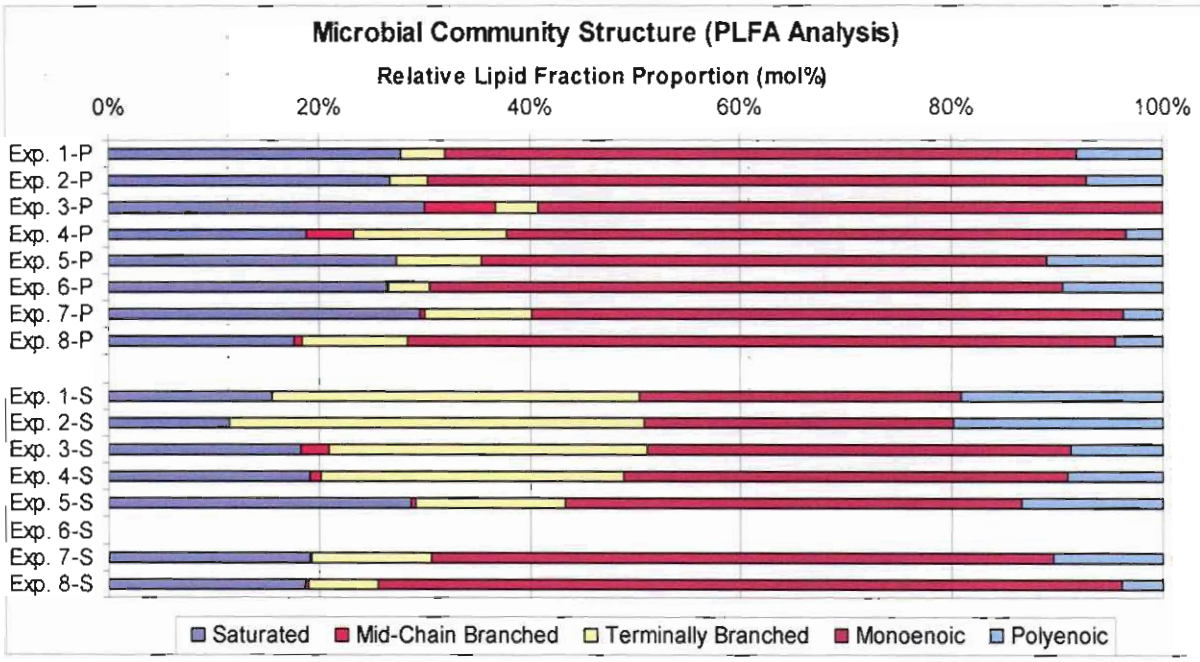


Figure 23. Microbial community structure based on the proportions of the major PLFAs.

Upon examination of the dendograms (Figure 5.7) illustrating the spatial differences between the lipid compositions of the microbial communities in the different experiments, it became evident that the main differentiating factor between the lipid composition of the microbial communities in the planktonic phases was pH, whereas the main differentiating factor in the sessile phase was the COC.

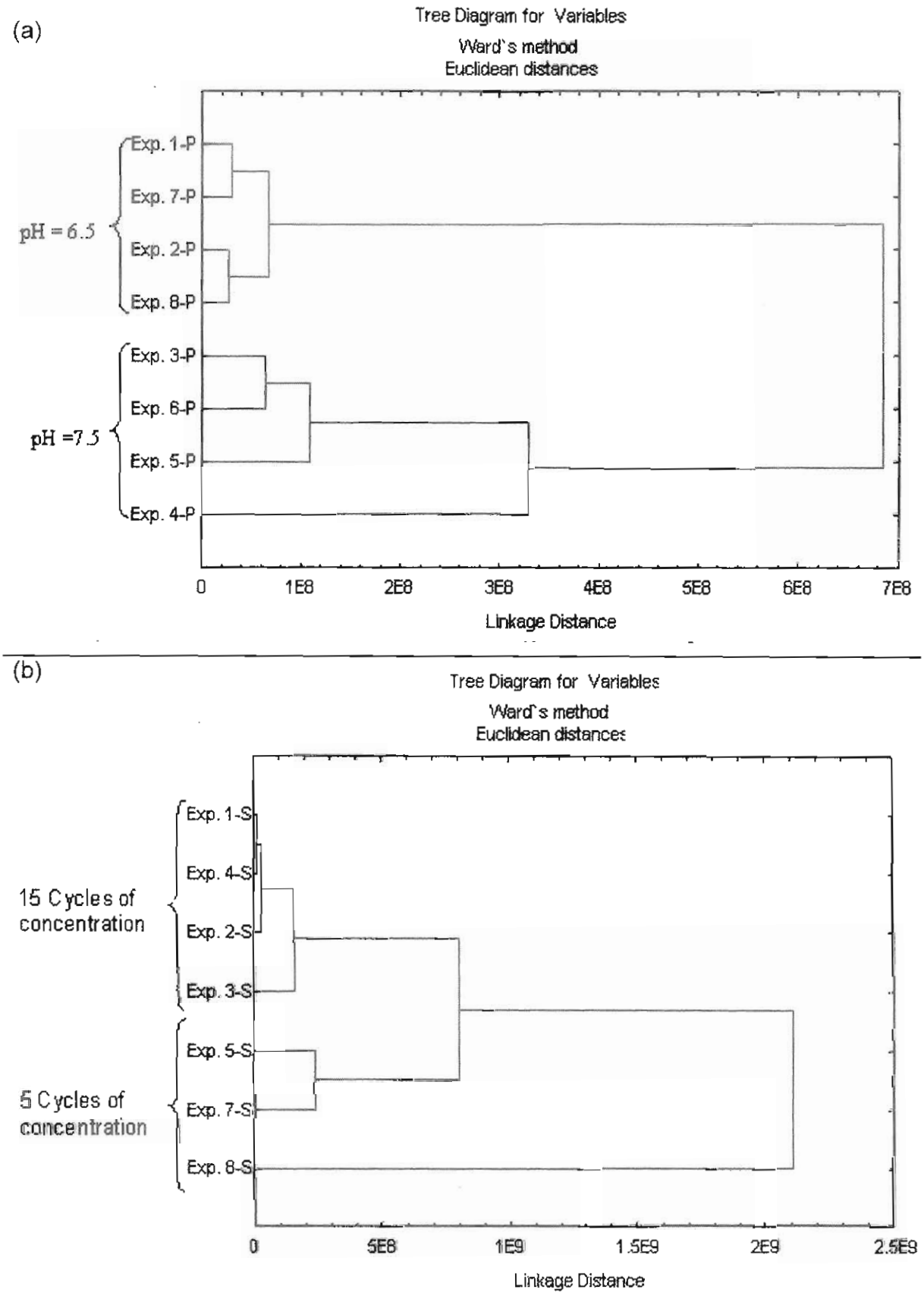


Figure 24. Dendograms illustrating the clustering of samples based on phospholipid fatty acid profiles obtained from the planktonic (a) and sessile (b) phase samples of the different experiments.

5.3.3 Fouling, Scaling, Corrosion and PLFA Analysis

A redundancy analysis (RDA) graph (Figure 5.8) indicates the similarity between samples, variation in data as well as the correlation between different variables. The clustering effect or distance between the samples indicates similarity. Variables or species are represented by the arrows. Shorter arrows indicate smaller variation. Long arrows tend to matter most since the length of the arrow also indicates the slope of response. Direction of the arrow indicates the direction of maximum change. Angles between arrows indicate correlation. Acute angles indicate high positive correlation. An angle of ±180° is indicative of a negative correlation and an angle ≥90° is indicative of no correlation. An angle ≤90° indicates a positive correlation between variables. Relation between samples and variables shows the relative ranking of each sample to each variable. From the RDA correlations between environmental variables and samples can be made. Environmental variables were COC, pH and FV. Samples were fouling, scaling and corrosion rates as well as PLFA analysis data obtained at each experiment.

From the results in Figure 5.8(a) it is evident that an increase in COC resulted in increased fouling, scaling and corrosion rates of both the corrosion coupon phases and heat exchanger tubing. This is in agreement with Cloete *et al.* (1994) who stated that increased COC results in the concentration of dissolved and suspended substances thereby promoting scale formation, biofouling, macrofouling and eventually microbially induced corrosion. Because pH and corrosion rates of the corrosion coupons and heat exchanger tubing are in opposite quadrants it indicates that there was a negative correlation between these two parameters. This implies that a decrease in pH would result in an increase in corrosion rates. This is in agreement with Rakanta *et al.* (2007) who stated that low pH leads to increased corrosion rates. It is also evident, based on arrow length, that FV did not have a profound impact in sample variation. Experiment 5 had a negative correlation with the rates of fouling, scaling and corrosion (Figure 5.8a). This confirms the results obtained in Section 5.3.1 which showed experiment 5 to have the ideal conditions for the operation of the cooling towers using SGL.

Increases in Gram negative (Mono-Unsaturated) and anaerobic bacteria (Mid Chain Branched Saturates) also resulted in increased fouling, scaling and corrosion rates. Fungi (Poly Unsaturated) had a positive correlation with experiment 5, indicating that fungi did not play a considerable role in the fouling, scaling and corrosion of the heat

exchanger tubing and corrosion coupons. A positive association is also apparent between the Gram positive bacteria and biomass, meaning that the biomass consists mostly of Gram positive bacteria. Biomass in the planktonic phase had a negative correlation to the fouling rate implying that an increase in biomass might be due to the microbial community not forming a fouling layer. The reverse would also be true with increased attachment resulting in less biomass in the planktonic phase.

Upon examination of Figure 5.8(b) it is also evident that an increase in COC resulted in an increase in all the fouling, scaling and corrosion rates in the sessile phase. There was a negative correlation between pH and all the corrosion rates, meaning that a decrease in pH would result in an increase in the rates of corrosion. Experiment 5 also had a negative correlation with the rates of fouling, scaling and corrosion in the sessile phase. Gram positive bacteria and fungi also have a positive correlation with all the fouling, scaling and corrosion rates. This implies that the Gram positive bacteria and the fungi are responsible for or enhance the fouling, scaling and corrosion rates in the sessile phase. A positive association is also apparent between the Gram negative bacteria and biomass, implying that the biofilm consists mostly of Gram negative bacteria.

These results indicate that the effect on the rates of fouling, scaling and corrosion, as well as on the microbial community structure (PLFA analysis) was in the order COC >pH>FV. These results also confirm that the ideal conditions for the operation of the cooling towers were obtained in experiment 5 (5 COC, pH 7.5 and a FV of 0.6 m/s).

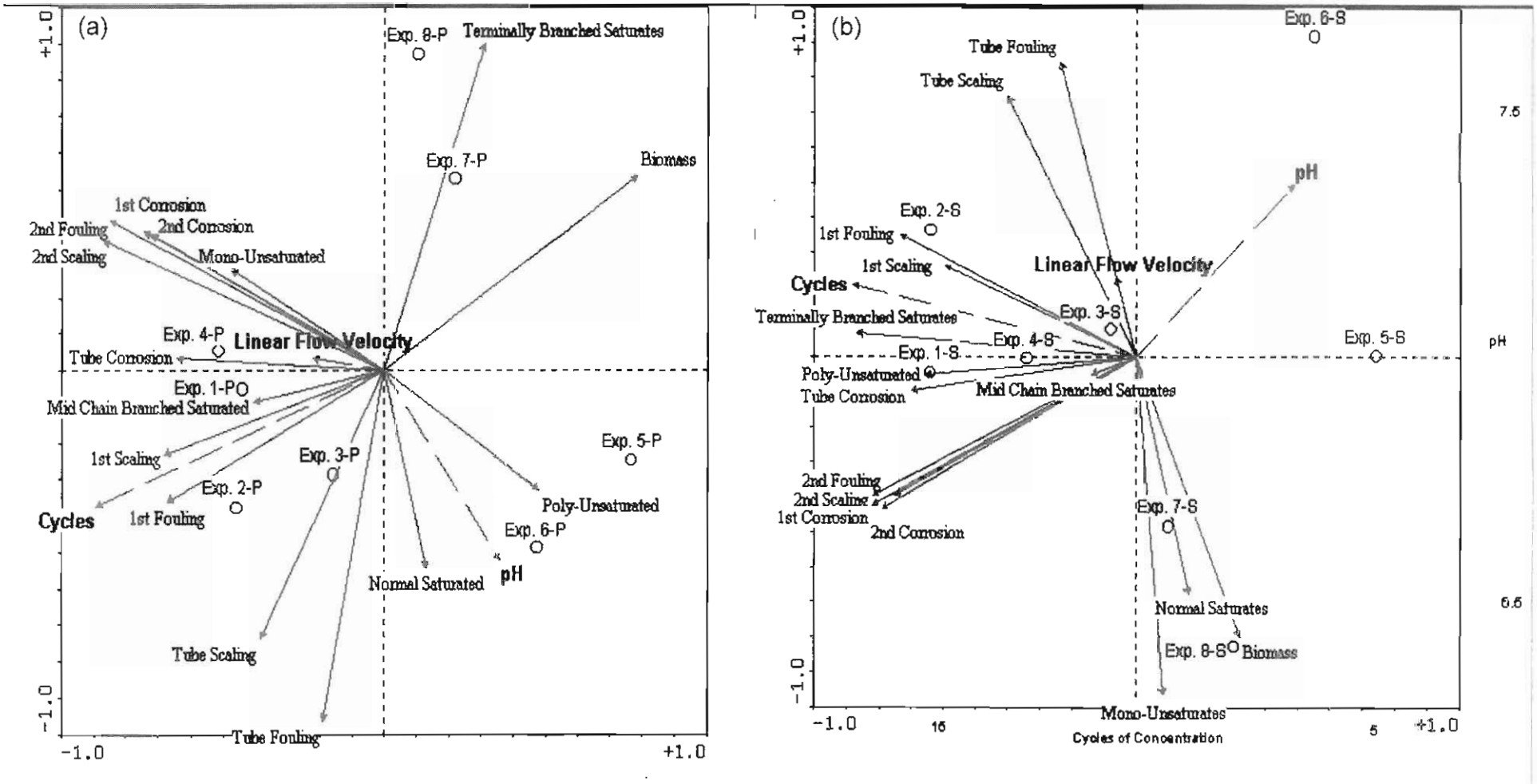


Figure 25. RDA illustration of the correlations between environmental variables (COC, pH, FV) and samples (PLFA groups, fouling, scaling and corrosion rates) in the (a) planktonic and (b) sessile phases of experiments 1 to 8.

5.3.4 Microbial Community Structure (DGGE Analysis)

5.3.4.1 DNA Concentrations and Purity

The DNA concentrations and purity co-efficients obtained from the planktonic phase samples are tabled in Appendix B (Table B3). Upon examination of the DNA concentration data obtained from the respective samples (Appendix B, Table B) it is evident that the DNA concentrations obtained from the planktonic phases were considerably higher at 11.63 ng/µl (standard deviation (SD) = 9.74) than those obtained from the sessile phases 6.87 ng/µl (SD = 5.79). The 260nm/280nm ratios for these phases also differed. The average 260nm/280nm ratio in the planktonic phase was 1.72 (SD = 0.33) and the average 260nm/280nm ratio in the sessile phase was 1.55 (SD = 0.44). These differences in concentrations and purities could possibly be attributed to the DNA extraction and purification protocols that were not optimised sufficiently due to insufficient sample volumes. This could also be attributed to the presence of inhibiting impurities which would be present in higher concentrations in the sessile phase. Published DNA purification procedures may be inadequate for complex environmental samples containing inhibiting impurities (Pennanen *et al.*, 2001) and the fact that no work was done previously on the extraction of DNA from stripped gas liquor means that there was no available method that had been optimised for this kind of work. The difference in DNA concentrations may also be an indication of actual bacterial levels. In the preceding section it was illustrated that bacterial levels based on PLFA data were generally lower in the sessile phase relative to the planktonic phase. The method described in Section 5.2.4.1, although not optimised, worked consistently, providing DNA of a sufficient quantity and quality for PCR and DGGE analysis.

5.3.4.2 PCR Optimisation

PCR protocol was optimised to determine the effects of the addition of bovine serum albumin (BSA), glycerol and $MgCl_2$. It was found that the addition of 1U *Taq* DNA polymerase, 50 ng BSA and 4mM $MgCl_2$ to the PCR mix gave the most consistent results.

Several problems were encountered during the study. The optimised PCR protocol worked for the amplification of the 18S planktonic and sessile as well as the 16S planktonic samples but did not work well with the 16S sessile samples. The main

limiting factor for our optimisation attempts was insufficient sessile sample quantities. Because of this, no studies were conducted on the efficacy of primers in the presence of co-extractants. Different annealing temperatures were also attempted but the best we got for the problematic samples was a smear if it worked at all. At the time when the experiments were conducted we also did not have touchdown PCR facilities. The conditions finally used in this study were the most informative, given the mentioned limitations.

5.3.4.3 PCR and DGGE analyses

The DNA extracted from the planktonic and sessile phases was subjected to 16S and 18S rDNA PCR and electrophoresed on a 1% (w/v) agarose gel. All the planktonic samples that were subjected to 16S rDNA PCR amplified successfully, while only three of the sessile phase samples were amplified. The other sessile samples did not amplify most probably due to inhibition by the presence of co-extracted humic substances. All the planktonic and sessile samples that were subjected to 18S rDNA PCR amplified successfully, except for the planktonic sample from experiment 1.

The successfully amplified DNA samples from both the planktonic and sessile were analysed by DGGE. Figure 5.9 is an illustration of the planktonic 16S and 18S DGGE profiles that were obtained. The presence of bands can be associated with the presence of bacterial (Figure 5.9a) or fungal (Figure 5.9b) strains. The relative intensities of the bands illustrate the increase and decrease of the particular strain concentrations between the different samples. This appearance or disappearance of bands illustrates the effects of varying experimental conditions on microbial community structure in both planktonic and sessile samples.

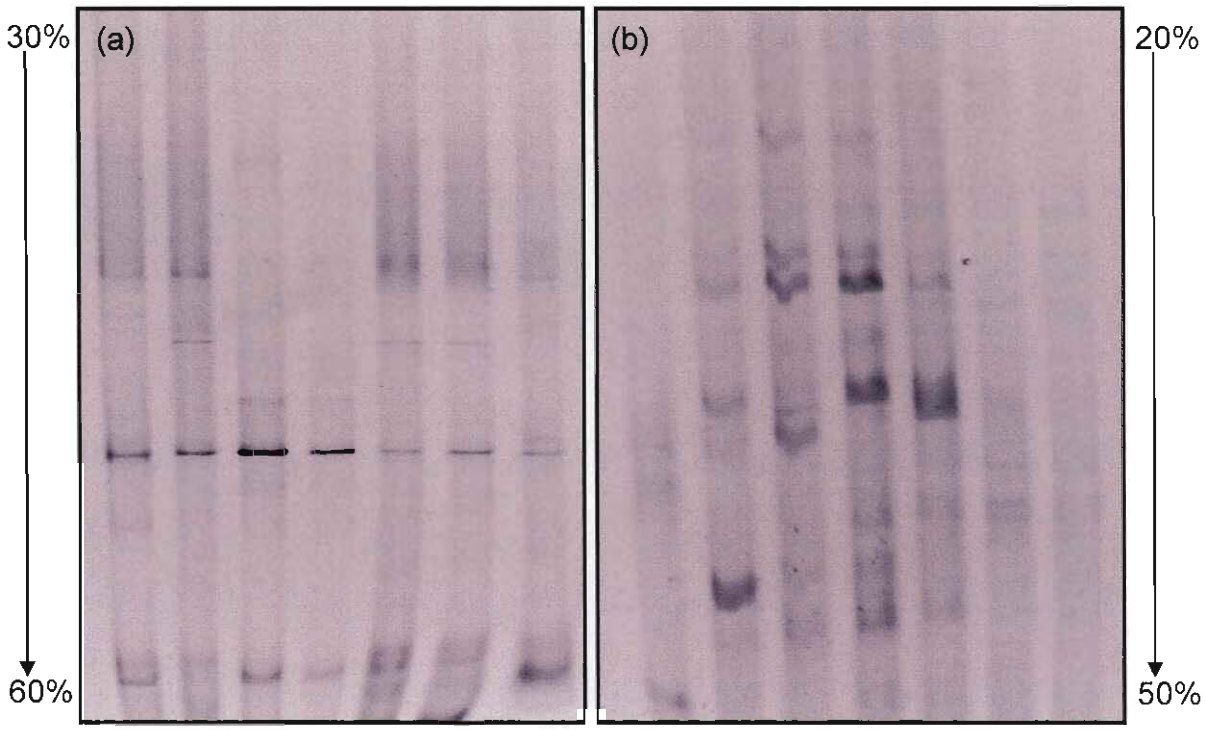


Figure 26. 16S (a) and 18S (b) DGGE profiles as well as denaturing gradients illustrating the effects of operational conditions on the planktonic microbial community structure. Lanes 1 to 7 in both (a) and (b) indicate samples from experiments 2, 3, 4, 5, 6, 7 and 8.

The quantities of the diagnostic bands of each sample were determined relative to the quantity of the reference band and against their Rf distances. This data was used to determine the relative strain abundances in each microbial community. Figure 5.10 illustrates the relative strain abundances in the experimental samples as determined by 16S rDNA (Figure 5.10a) and 18S rDNA (Figure 5.10b) DGGE analysis. When presented this way the data is complex and very difficult to draw conclusions from and hence the need for statistical analysis and the use of indices such as the Shannon-Weaver diversity index (Shannon and Weaver, 1963). The use of the Shannon-Weaver diversity index would greatly simplify the data and make it easier to explain the effects of environmental conditions on microbial community structure diversity. A summary of the Shannon-Weaver diversity index data for 16S and 18S planktonic and sessile phases of the various experiments is presented in Figure 5.11.

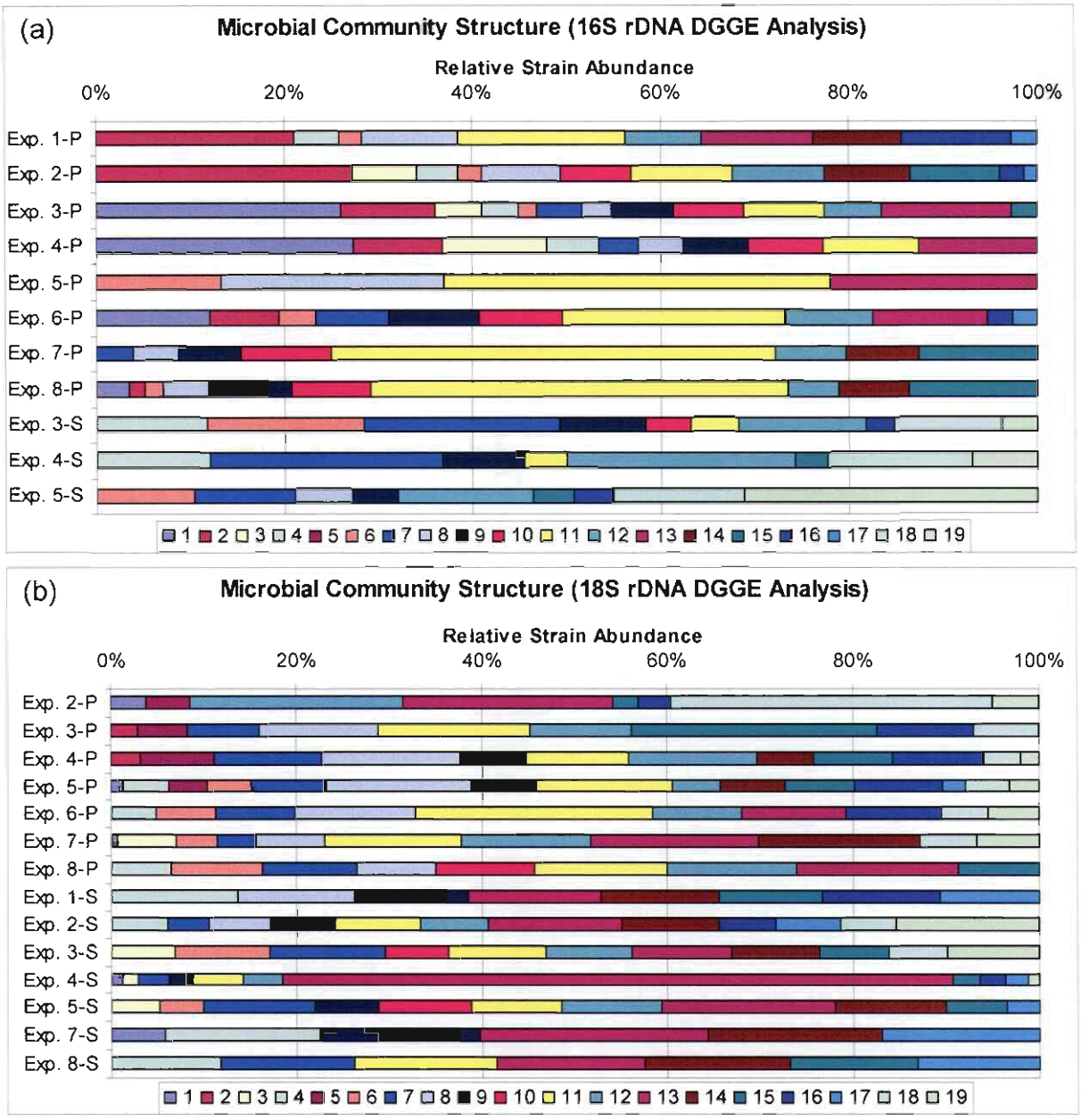


Figure 27. Relative strain abundances of the experimental samples as analysed by (a) 16S rDNA DGGE and (b) 18S rDNA DGGE electrophoresis. Analyses were condensed as described in Section 5.2.5.4.

Upon examination of Figure 5.11 it is evident that 16S rDNA (bacterial) planktonic and sessile microbial communities had the highest diversity in experiment 3. Experiment 3 had a pH of 7.5, a FV of 0.6 and 15 COC and also had the second highest fouling, scaling and corrosion rates in the heat exchanger tubing. This would therefore mean that the optimum operational conditions to minimise bacterial species diversity would be operation at a lower pH, low COC and a high FV. This correlates with the results obtained from the analysis of fouling, scaling and corrosion rates.

Shannon-Weaver indices of the 18S rDNA were highest in experiment 2 and 5 for the sessile and planktonic fungal communities, respectively. Experiment 2 had the highest fouling, scaling and corrosion rates in the heat exchanger tubing, highlighting the important role that fungal communities within biofilms play in the fouling, scaling and corrosion of industrial systems. Experiment 2 had a pH of 6.5, a FV of 1.2 and 15 COC. Experiment 5 which also had the lowest 16S rDNA Shannon-Weaver index was always among the lowest fouling, scaling and corrosion rates for the corrosion coupons and heat exchanger tubing. Experiment 5 had a pH of 7.5, a FV of 0.6 and 5 COC. Cycles of concentration and pH seem to have had the greatest effect on species diversity and evenness.

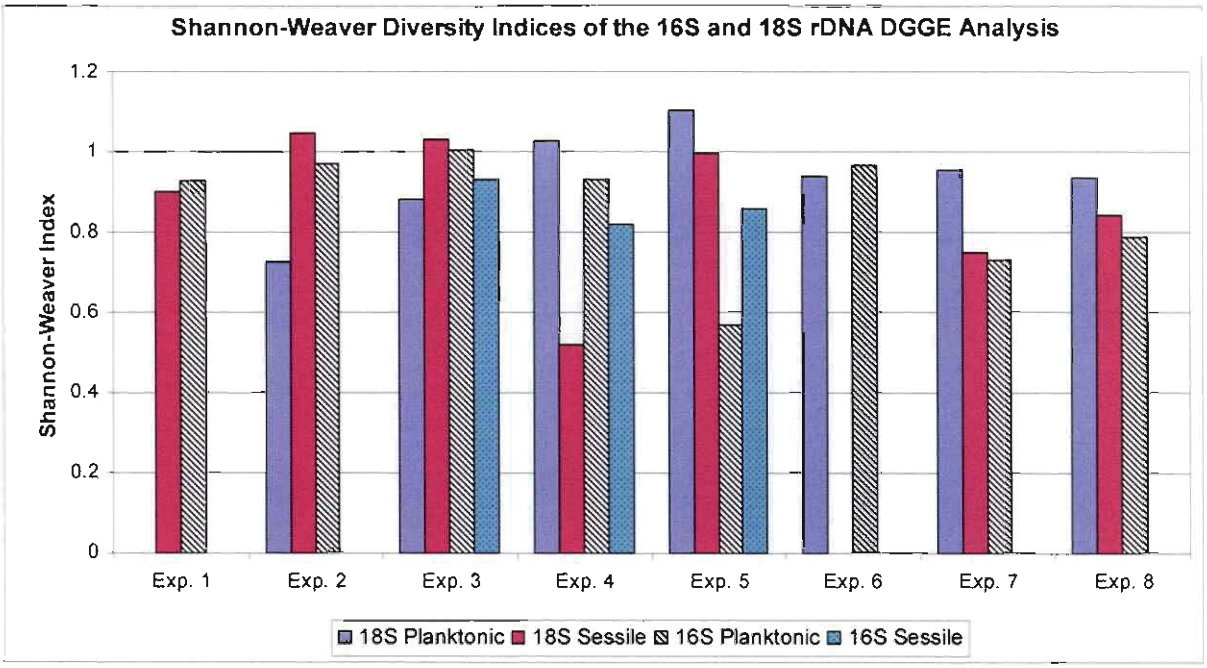


Figure 28. Shannon Weaver Diversity indices of the experimental samples as determined from 16S and 18S rDNA PCR DGGE band presence/absence and intensity.

Absence or presence as well as intensity data was subjected to cluster analysis using Statistica 6.0 software. The resultant dendograms are presented in Figures 5.12 and 5.13. pH was the major differentiating parameter followed by COC in the clustering of the 16S rDNA planktonic samples based DGGE analysis (Figure 5.12). Clusters B and D were both at a pH of 6.5 but cluster B was at 15 COC whereas cluster D was at 5 COC. Cluster C was at a pH of 7.5 with experiments 3 and 4 both being at 15 COC, therefore clustering closer together, and experiments 5 and 6 both being at 5 COC, therefore also clustering closer together.

It is evident, upon examination of the dendograms (Figure 5.13), that pH was the main differentiating parameter in the clustering of the 18S rDNA planktonic and sessile samples based DGGE analysis. Cluster A1 in the planktonic phase had a pH of 6.5 and smaller cluster A2 was at 5 COC. The B cluster was at a pH of 7.5 with the difference between B1 and B2 being that B1 was at 15 COC whereas B2 was at 5 COC (Figure 5.13a). This indicated that COC was also an important differentiating parameter in the planktonic phase. Cluster B in the sessile phase (Figure 5.13b) was at a pH of 6.5. Experiments 1 and 7 were both at a FV of 0.6 and therefore grouped together to form cluster B2, whereas experiments 2 and 8 were at a FV of 1.2. Both clusters C and D were at a pH of 7.5 with cluster C being at a FV of 0.6 and D at a FV of 1.2. This indicates that FV was also an important differentiating parameter in the sessile phase.

The analysis of DGGE data revealed that pH and COC had the most profound effect on microbial community structure. This is similar to the data obtained from the analysis of the fouling, scaling and corrosion rates in relation to PLFA analysis data.

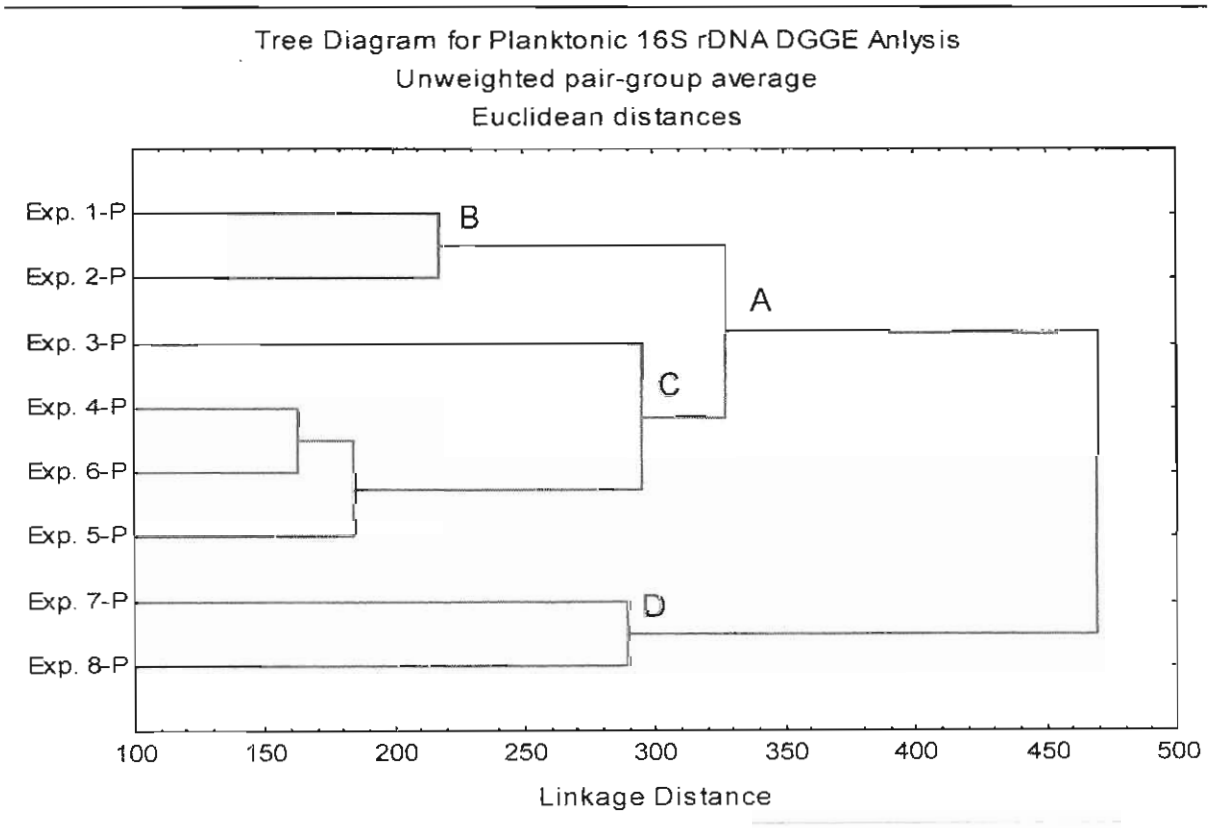


Figure 29. Dendogram illustrating the clustering of samples based on DGGE profiles obtained from the planktonic samples subjected to 16S rDNA DGGE analysis.

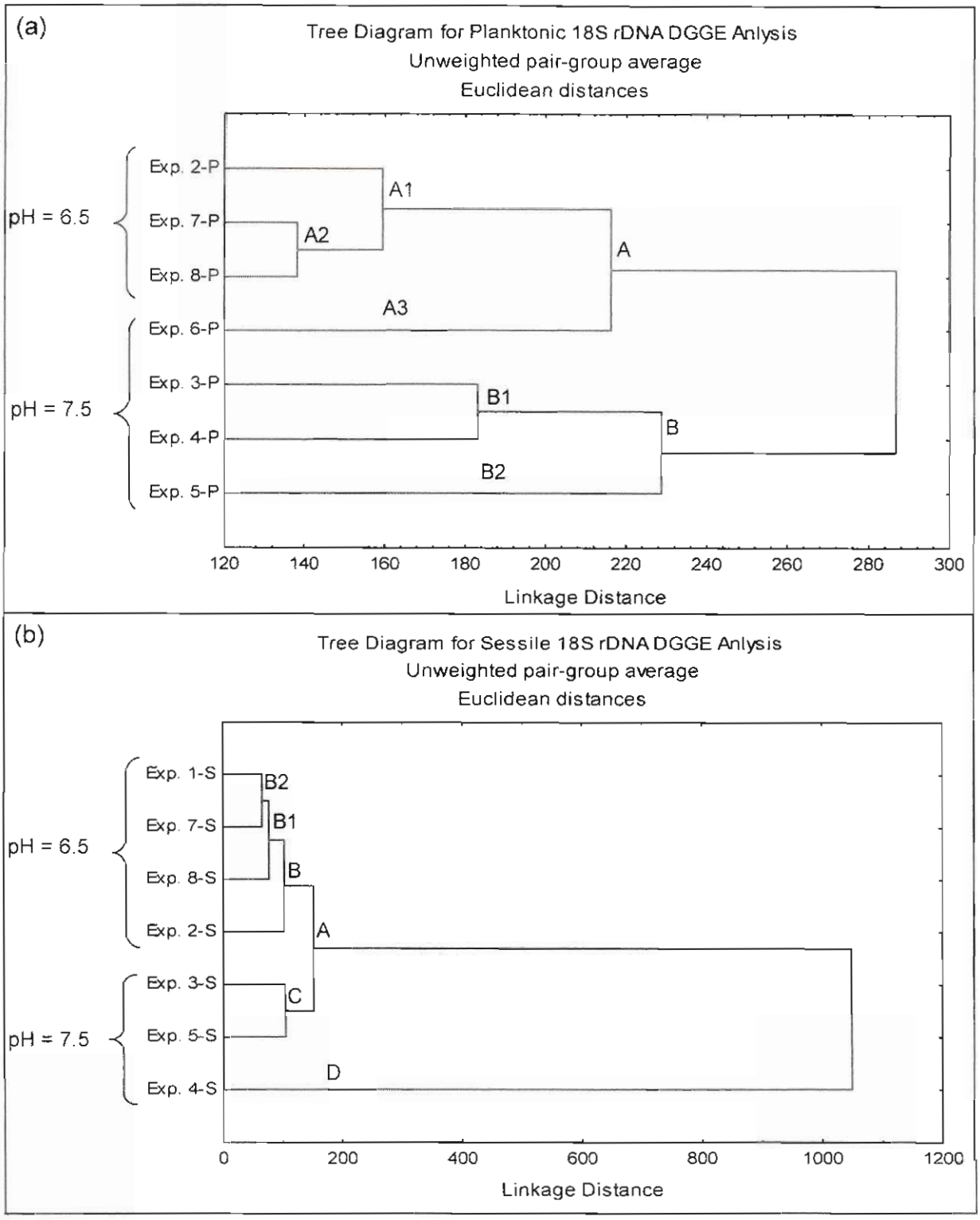


Figure 30. Dendograms illustrating the clustering of samples based on DGGE profiles obtained from (a) the planktonic and (b) sessile samples subjected to 18S rDNA DGGE analysis.

5.4 CONCLUSIONS

A 2^3 multi-factorial experimental design was successfully implemented in determining the effects pH, COC and FV on fouling, scaling and corrosion rates. The operational

parameter with the greatest overall influence on fouling, scaling and corrosion rates was found to be COC followed by pH. From the results obtained from the analysis of fouling, scaling and corrosion rate data optimal operational conditions for the reduction of fouling, scaling and corrosion in cooling towers using SGL as make-up water were determined to be 5 COC, a pH of 7.5 and a FV of 0.6 m/s.

Upon examination of the PLFA analysis data it was evident that the planktonic microbial communities had the highest estimated bacterial numbers, highest cyclo/ω7c ratios and the highest trans/cis ratios at experiments 3 and 4 (15 COC and a pH of 7.5). The sessile microbial communities had the highest estimated bacterial numbers, highest cyclo/ω7c ratio and the highest trans/cis ratio at experiments 7 and 8 (5 COC and a pH of 6.5). Cycles of concentration were also found to be the major differentiating parameter between Gram positive and Gram negative bacteria. The main differentiating factor between microbial communities in the planktonic phases was pH, whereas COC was the main differentiating factor in the sessile phase. These results highlight the role of COC and pH on microbial community dynamics.

Redundancy analysis of fouling, scaling corrosion and PLFA data relative to COC, pH and FV at the different experiments was also conducted. From the results it was evident that an increase in COC resulted in increased fouling, scaling and corrosion rates of both the corrosion coupon phases and heat exchanger tubing. A decrease in pH was also found to result in an increase in corrosion rates. These results indicate that the effect on fouling, scaling and corrosion rates, as well as on microbial community structure (PLFA analysis) was in the order COC >pH>FV. These results also confirmed that the ideal conditions for the operation of the cooling towers were 5 COC, pH 7.5 and a FV of 0.6 m/s.

DNA concentrations and purity obtained from the planktonic phases were considerably higher at than those obtained from the sessile phases. The difference in DNA concentrations may also be an indication of actual bacterial levels. Bacterial levels based on PLFA data were also generally lower in the sessile phase relative to the planktonic phase. Illustration of relative strain abundances in experimental samples as determined by 16S rDNA and 18S rDNA DGGE analysis was found to be complex and very difficult to draw conclusions from. Use of the Shannon-Weaver diversity index greatly simplified the data and made it easier to explain the effects of environmental conditions on microbial community structure diversity. Cycles of

concentration and pH seemed to have had the greatest effect on species diversity and evenness. Experiment 5 (5 COC, pH 7.5 and a FV of 0.6 m/s) had the lowest 16S rDNA Shannon-Weaver index. Dendograms the DGGE data also revealed that pH was the main differentiating parameter, followed by COC, in the clustering of 16S and 18S rDNA planktonic and sessile samples. These results also confirmed that operation at 5 COC, a pH of 7.5 and a FV of 0.6 m/s would not only results in reduced fouling scaling and corrosion but also reduced microbial species diversity.

In conclusion, the 2^3 multi-factorial experimental design implemented in this part of the study was instrumental in determining the effects pH, COC and FV on fouling, scaling and corrosion rates as well as microbial community structure (PLFA and DGGE). Cycles of concentration was found to have the most considerable effect on the fouling, scaling and corrosion rates followed by pH. Analysis of both the PLFA and DGGE data agreed that COC and pH had the most considerable effect on the microbial community structure. The use of the 2^3 multi-factorial experimental design also allowed the determination of the optimal conditions for the operation of the cooling towers using SGL as feed water. These conditions were found to be 5 COC, a pH of 7.5 and a FV of 0.6 m/s.

CHAPTER 6: GENERAL DISCUSSION AND CONCLUSIONS

6.1 INTRODUCTION

The aim of this study was to evaluate the effects of COC, pH and FV on the rates of fouling, scaling and corrosion as well as on the microbial community dynamics in cooling towers using stripped gas liquor (SGL) as process cooling water. The objectives of this study were to:

i. Optimise the selective extraction of hydrocarbons from SGL so as to eliminate the interference of hydrocarbons with PLFA analysis,
ii. Determine the similarity or dissimilarity of two cooling towers in terms of the fouling, scaling and corrosion rates as well as microbial community structure when these were operated under similar operational conditions,
iii. Determine the effects of pH, COC and FV on the rates of fouling, scaling and corrosion of mild steel, as well as the structural diversity of microbial communities in both planktonic and sessile phases using a 2^3 multi-factorial experimental design.

6.1.1 Optimisation and Processing of Fatty Acids for PLFA Analysis

From literature it is evident that SGL contains hydrocarbons (Zhang *et al.,* 1998; van Nierop *et al.,* 2000; Yang *et al.*, 2006). Hydrocarbons and cholesterol have been found to interfere with gas chromatography analysis of PLFA prepared from complex biological samples (Nightingale *et al.*, 1999; Ali and Cole, 2001). It was, therefore, important to first optimise the selective extraction of hydrocarbons from SGL so as to eliminate the interference of hydrocarbons with PLFA analysis. Two methods for the selective extraction of hydrocarbons were evaluated. Effects of selective extraction of hydrocarbons before and after silicic acid fractionation on GC-MS analysis were thus determined.

Chromatograms, community structures, box and whisker plots and the dendograms obtained from the analyses of the PLFA analysis data clearly indicate the impact of the contaminant hydrocarbons on the analysis of the microbial community structure by PLFA analysis. These results also demonstrated that the hydrocarbons in SGL interfered with the analysis of PLFA samples.

The method used for the extraction of hydrocarbons before silicic acid fractionation succeeded in removing the hydrocarbons and their interference. This method was, therefore, determined to be the appropriate to use in subsequent experiments. The objective of this part of the study was, therefore, achieved.

6.1.2 Comparison of Two Lab-Scale Cooling Towers Operated under Identical Conditions

Even though the two available lab-scale cooling towers were of identical design their similarity or dissimilarity was also a concern that needed investigation. The similarity or dissimilarity of two cooling towers in terms of the fouling, scaling and corrosion rates as well as microbial community structure when these were operated under similar operational conditions was, therefore, determined. If similar results are obtained, this would allow two experiments to be run at the same time while ensuring the integrity of the results that would be used for the analysis of the effects of changes in operational parameters on the rates of fouling, scaling and corrosion as well as on microbial community dynamics.

The two lab-scale cooling towers were operated with a FV of 1.5 m/s through the heat exchanger tubing at a pH of 7 and 13 COC. Physico-chemical analysis results confirmed that the two cooling towers were operated under identical operational conditions. Scanning electron microscopy results illustrated that both reactors had similar microbial populations based on morphology and only slightly differed in terms of the thickness of the biofilm. The difference in biofilm thickness was attributed to the unstable FV experienced towards the end of the experimental run. Rates of fouling, scaling and corrosion were determined and statistically analysed. There was no significant statistical difference between cooling towers 1 and 2 within a 95% confidence limit in terms of their fouling, scaling and corrosion rates. Microbial community structure was determined by phospholipid fatty acid (PLFA) analysis and scanning electron microscopy (SEM). The results also showed that the structure of the microbial communities in the two cooling towers were similar.

The work carried out in this part of the study clearly indicated that the two cooling towers were similar in terms of the fouling, scaling and corrosion rates as well as microbial community structure when they were operated under similar operational conditions. This, therefore, allowed the operation of the two cooling towers in parallel under different operational conditions. Any differences in results will be reflection of

the effects of operational conditions and not differences in the cooling towers themselves. Another outcome of this part of the study was that for consistent results to be obtained the cooling towers should not be operated for longer than 42 days, based on the results obtained from the lab-scale cooling towers.

6.1.3 Effects of Changes in Operational Conditions on Fouling, Scaling and Corrosion as well as on Microbial Community Dynamics

The objective of this part of the study was to evaluate the effects of COC, pH and FV on the rates of fouling, scaling and corrosion as well as on the microbial community dynamics in cooling towers using SGL as process cooling water. For this purpose a 2^3 multi-factorial experimental design was used to minimise the number of experiments required. The 2^3 multi-factorial experimental design allowed the evaluation of the effects of pH, FV and COC on the rates of fouling, scaling and corrosion as well as microbial community dynamics. The operational parameters were 5 and 15 COC, pH of 6.5 and 7.5, and FV of 0.6 and 1.2 m/s.

For each experiment the cooling towers were operated for a total of 42 days. In Chapter 4 it was demonstrated that after 42 days the lab-scale cooling towers became unstable. This was probably due to fouling, scaling and / or corrosion affecting FV through the heat exchanger tubing. The cooling efficiency of the packing material was also affected by the formation of biofouling layer due to the high organic load of the stripped gas liquor (Beyer, 1993).

The 2^3 multi-factorial experimental design was instrumental in determining the effects pH, COC and FV on fouling, scaling and corrosion rates. Cycles of concentration was found to have the most considerable effect on the fouling, scaling and corrosion rates followed by pH. Cloete *et al* 1994 stated that increased COC promotes biofouling, and subsequent macrofouling of the system, scale formation, and eventually microbially induced corrosion. FV did not have a significant effect on the rates of fouling, scaling and corrosion probably due to the variation being insufficient. This is despite Lehtola *et al.* (2006) reporting a 13 to 15 times increase in biofilm formation with increased FV of water from 0.03 to 0.28 m/s. Chen *et al.* (2005) also reported that biofilm adhesive strength increased as the fluid velocity increased within the range of 0.6–1.6 m/s.

The effects pH, COC and FV on microbial community structure as determined by PLFA and DGGE analysis were demonstrated. Cycles of concentration affects the concentration of dissolved and suspended substances, thereby promoting biofouling (Cloete *et al.*, 1994). According to Little *et al.* (1998) the sessile microbial communities are capable of maintaining a pH that is radically different from the bulk liquid and are therefore less prone to be affected by minor changes in pH. The impact of changes in operational conditions on microbial communities could thus be explained in terms of the observations made by Cloete *et al.* (1994) and Little *et al.* (1998). During the duration of each experiment the pH, COC and FV were kept constant. The changes in operational conditions between the experiments could, therefore, have an effect on microbial community structure and dynamics. Analysis of the PLFA data revealed that COC had a considerable effect on the microbial community structure followed by pH. Results obtained from the RDA ordination analysis of the PLFA analysis data relative to the fouling, scaling and corrosion rates indicated that COC followed by pH had the most profound effect. Analysis of DGGE data indicated that pH had the most profound effect on the microbial community structure followed by COC.

The 2^3 multi-factorial experimental design also allowed the determination of the optimal operational conditions for the reduction of fouling, scaling and corrosion in cooling towers using SGL as feed water. These conditions were found in experiment 5 and were 5 COC, a pH of 7.5 and a FV of 0.6 m/s.

6.2 CONCLUSION

The presence of hydrocarbons in SGL can interfere with PLFA analysis. The lipid extraction procedure was, therefore, successfully optimised to minimise the co-extraction of unwanted hydrocarbons. The two cooling towers were found to be similar in terms of the fouling, scaling and corrosion rates as well as microbial community structure when they were operated under similar operational conditions. This allowed the operation of the two cooling towers in parallel under different operational conditions with any differences in results reflecting the effects of operational conditions. The 2^3 multi-factorial experimental design was instrumental in determining the effects pH, COC and FV on rates of fouling, scaling and corrosion, as well as on the microbial community dynamics as determined by PLFA and DGGE analysis. Cycles of concentration had the most profound effect on the fouling, scaling and corrosion rates as well as microbial community structure. This was

demonstrated by physico-chemical as well as microbial dynamics data. Using the 2^3 multi-factorial experimental design the optimal conditions for the operation of the cooling towers using SGL as feed water were determined to be 5 COC, a pH of 7.5 and a FV of 0.6 m/s.

6.3 RECOMMENDATIONS

i. The effect of pH on conductivity and ion (fluoride, sodium, etc) concentration and, therefore, COC should be determined.

ii. The effect of nitrification/denitrification on the drop in pH adjusted using NH_4OH should be determined.

iii. The fouling rate of the packing material and effect thereof on the thermal efficiency of a cooling tower should be determined.

iv. Energy dispersive spectrometry (EDS) should be used to analyse fouling, scaling and corrosion layers to obtain structural, compositional and chemical information about the layers. This would allow the determination of, for example, the corrosion products in the fouling layer and the effects of fouling on the electrical and chemical determination of corrosion rates by AquaCorr™ and iron concentration, respectively.

v. The efficacy of primers in the presence of co-extractants should be determined and the selective extraction of hydrocarbons from DNA samples should be optimised.

vi. The sensitivity of PLFA in determining fungal biomass relative to bacterial biomass and the effect thereof in microbial community structure analysis should be determined (Perkiomaki *et al.*, 2003).

vii. The statistical manipulation of DGGE data so as to allow RDA analysis in relation to PLFA, fouling, scaling and corrosion rates would be of great value in bringing all the information together.

In conclusion, this research approach can be used for the optimisation of operational parameters for the re-use of industrial effluents as cooling water to minimise fouling, scaling and corrosion. The operating conditions optimised in this study have been successfully applied to the Sasol (Sasolburg) cooling towers operating with SGL as cooling water.

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APPENDIX

Table A1. Fouling, scaling and corrosion rates of the corrosion coupons and heat exchanger tubing with standard deviations shown in parenthesis. R1 1st Coupon denotes the corrosion coupons in the first corrosion coupon phase in cooling tower 1. R1 2nd Coupon denotes the corrosion coupons in the second corrosion coupon phase in cooling tower 1. R1 Tube denotes the heat exchanger tubes in cooling tower 1. Substitute R1 with R2 for cooling tower 2.

	Fouling Rate mg/dm2/d	Scaling Rate mg/dm2/d	Corrosion Rate mm/y
R1 1st Coupon	107.17 (14.43)	44.26 (13.45)	0.51 (0.05)
R2 1st Coupon	114.85 (19.31)	23.50 (17.09)	0.46 (0.04)
R1 2nd Coupon	91.54 (5.97)	39.88 (4.74)	0.42 (0.04)
R2 2nd Coupon	88.08 (7.11)	40.10 (14.51)	0.52 (0.02)
R1 Tube	26.55 (8.43)	19.99 (5.75)	0.06 (0.06)
R2 Tube	47.01 (4.36)	31.73 (4.07)	0.20 (0.04)

Table A2. Cooling tower 1 and 2 Tukey's HSD analysis results ($p<0.05$ = statistical difference). Statistical Difference shown in red.

FOULING			
Sample	**R1 Tubes**	**R1 Coup1**	**R1 Coup2**
R1 Tubes		0.000198	0.000202
R1 Coup1	0.000198		0.152448
R1 Coup2	0.000202	0.152448	

FOULING			
Sample	**R2 Tubes**	**R2 Coup1**	**R2 Coup2**
R2 Tubes		0.0002	0.000808
R2 Coup1	0.0002		0.013368
R2 Coup2	0.000808	0.013368	

SCALING			
Sample	**R1 Tubes**	**R1 Coup1**	**R1 Coup2**
R1 Tubes		0.006543	0.021395
R1 Coup1	0.006543		0.766484
R1 Coup2	0.021395	0.766484	

SCALING			
Sample	**R2 Tubes**	**R2 Coup1**	**R2 Coup2**
R2 Tubes		0.592938	0.583402
R2 Coup1	0.592938		0.155114
R2 Coup2	0.583402	0.155114	

CORROSION			
Sample	**R1 Tubes**	**R1 Coup1**	**R1 Coup2**
R1 Tubes		0.000198	0.000203
R1 Coup1	0.000198		0.182169
R1 Coup2	0.000203	0.182169	

CORROSION			
Sample	**R2 Tubes**	**R2 Coup1**	**R2 Coup2**
R2 Tubes		0.000199	0.000198
R2 Coup1	0.000199		0.130007
R2 Coup2	0.000198	0.130007	

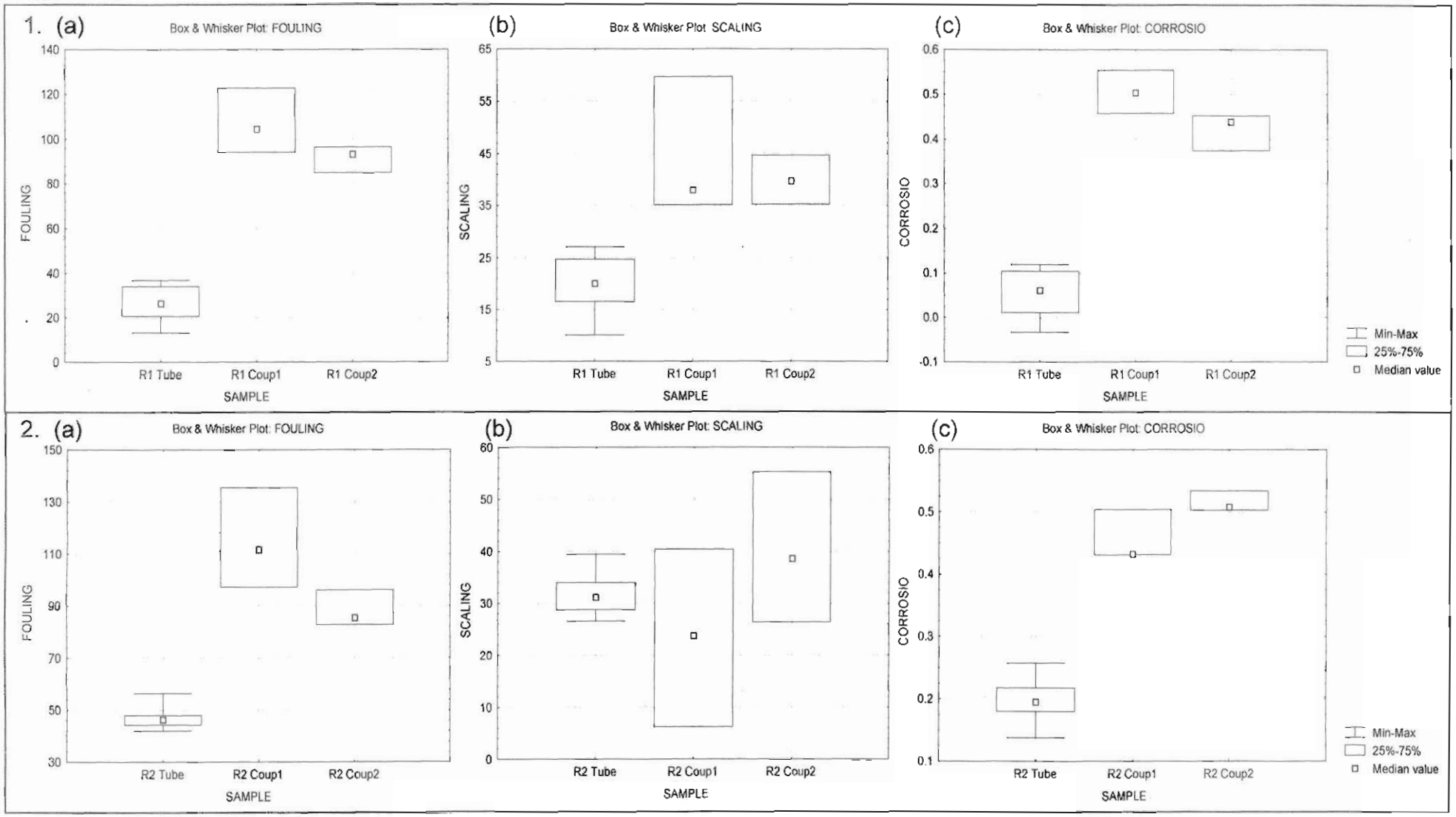

Figure A1. Box and whisker plot of the fouling (a), scaling (b) and corrosion (c) rates in cooling towers 1 and 2, respectively. Coup1 and Coup2 denote the corrosion coupons from cooling tower 1 and 2, respectively. Tube1 and Tube2 denote the heat exchanger tubing from cooling tower 1 and 2, respectively.

APPENDIX B

Table B1. Randomised factorial experimental design matrix.

Experiment	pH	FLOW VELOCITY	Cycles of Concentration
1	-1 (6.5)	-1 (0.6 m/s)	1 (15)
2	-1 (6.5)	1 (1.2 m/s)	1 (15)
3	1 (7.5)	-1 (0.6 m/s)	1 (15)
4	1 (7.5)	1 (1.2 m/s)	1 (15)
5	1 (7.5)	-1 (0.6 m/s)	-1 (5)
6	1 (7.5)	1 (1.2 m/s)	-1 (5)
7	-1 (6.5)	-1 (0.6 m/s)	-1 (5)
8	-1 (6.5)	1 (1.2 m/s)	-1 (5)

Table B2. Fouling, scaling and corrosion rates of the first corrosion coupon phase, second corrosion coupon phase and heat exchanger tubing.

Experiment	First Corrosion Coupon Phase		
	Fouling (mg/dm^2/d)	Scaling (mg/dm^2/d)	Corrosion (mm/y)
Experiment 1	130.358	11.717	0.779
Experiment 2	111.242	17.315	0.541
Experiment 3	104.189	10.702	0.550
Experiment 4	119.848	24.749	0.525
Experiment 5	97.952	8.106	0.265
Experiment 6	108.617	11.046	0.315
Experiment 7	102.922	10.530	0.618
Experiment 8	92.663	7.307	0.543

Experiment	Second Corrosion Coupon Phase		
	Fouling (mg/dm^2/d)	Scaling (mg/dm^2/d)	Corrosion (mm/y)
Experiment 1	112.122	29.481	0.884
Experiment 2	125.475	24.004	0.837
Experiment 3	108.740	24.085	0.563
Experiment 4	125.543	25.967	0.665
Experiment 5	85.910	16.643	0.415
Experiment 6	91.531	18.291	0.376
Experiment 7	109.306	22.388	0.814
Experiment 8	122.197	25.586	0.667

Experiment	Heat Exchanger Tubing		
	Fouling (mg/dm^2/d)	Scaling (mg/dm^2/d)	Corrosion (mm/y)
Experiment 1	54.338	15.743	0.402
Experiment 2	79.243	32.644	0.548
Experiment 3	72.631	27.059	0.451
Experiment 4	59.087	13.998	0.280
Experiment 5	62.170	7.545	0.202
Experiment 6	68.804	26.150	0.272
Experiment 7	29.245	6.527	0.375
Experiment 8	21.402	7.474	0.389

Table B3. DNA concentrations obtained from the respective samples.

Sample	DNA Concentration (ng / µl)	DNA Purity (260nm / 280nm)
Experiment 1 P	27.19	1.271
Experiment 1 S	16.095	1.312
Experiment 2 P	5.315	1.572
Experiment 2 S	4.995	1.401
Experiment 3 P	9.03	1.6
Experiment 3 S	1.33	2.440
Experiment 4 P	10.695	1.548
Experiment 4 S	0.25	
Experiment 5 P	25.735	1.746
Experiment 5 S	4.135	1.52
Experiment 6 P	10.365	1.965
Experiment 7 P	3.485	1.648
Experiment 7 S	9.71	1.306
Experiment 8 P	1.26	2.377
Experiment 8 S	11.58	1.318

Table B4. PCR reaction mixtures for the determination of the effects of the addition of additional *Taq*, BSA, glycerol and $MgCl_2$.

Reagent	1	2	3	4	5	6	7	8
Master Mix (µl)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Additional *Taq* (U)	1	0	1	0	1	0	0	0
H_2O (µl)	3.5	4.5	4.5	5.5	4.5	5.5	6.5	10.5
Primer Mix (pmol)	50	50	50	50	50	50	50	50
BSA (ng)	50	50	50	50	0	0	0	0
Glycerol (5%)	1µl	1µl	0	0	1µl	1µ	0	0
$MgCl_2$ (mM)	4	4	4	4	4	4	4	0
DNA	1µl	1µl	1µl	1µl	1µl	1µl	1µl	1µl

Table B5. Optimisation of the concentration of $MgCl_2$ to be added to the PCR mixture.

Reagent	1	2	3	4	5
Master Mix (µl)	12.5	12.5	12.5	12.5	12.5
Additional *Taq* (U)	1u	1u	1u	1u	1u
H_2O (µl)	5.5	6.5	7.5	8.5	9.5
Primer Mix (pmol)	1	1	1	1	1
BSA (ng)	1	1	1	1	1
$MgCl_2$ (mM)	4	3	2	1	0
DNA (µl)	1	1	1	1	1