CHAPTER 1

1. INTRODUCTION

1.1 Introduction and Overview of the study

Membrane separation systems are widely used to purify water of different qualities including seawater, brackish water and waste water. Dissolved particulate matter in the feed water can deposit on the membrane surface, contributing to the overall resistance of the process. This occurrence is identified as fouling (Abd El Aleem, Sugair & Alahmad 1998).

Pontie, Thekkedath, Duchesne, Jacquement, Luparc and Suty (2005:3) define membrane fouling as “pore plugging and external pore blocking, resulting from deposition of particles and colloids on the membrane surface and precipitation of smaller dissolved materials within the membrane pores and on the membrane surface.”

Reverse osmosis membranes are designed primarily for the retention of monovalent ions and are widely used for water desalination or industrial water production. Most feedwater contaminants are rejected to a variable but generally significant degree (99%). Membrane fouling by the rejected species at the membrane surface is the major cause of long term flux decline in 76% of the RO elements from more than 80 plants in Japan (Schneider, Ferreira, Binder & Ramos 2005).

Fouling mechanisms in membranes include inorganic salt precipitation (contributed by sparingly soluble salts), organic (mostly natural organic matter and effluent organic matter), colloidal (a result of accumulation of a colloidal cake layer on the membrane surface) and microbiological (usually caused by bacterial biofilms formation) (Kamire, Bouguecha & Hamrouni 2008; Herzberg & Elimelech 2007; Abd El Aleem *et al*. 1998).

Different types of fouling can occur simultaneously influencing each other, e.g. biofouling might enhance concentration polarization which stimulates inorganic scaling (Vrouwenvelder, Paassen, Folmer, Hoffman, Nerderlof & Van der Kooj 1998). However, because of the complex nature of fouling, many studies on RO fouling have focused on one foulant type for the sake of simplicity (Tran, Bolto, Gray, Hoang & Ostarcevic 2007). Hence this study's focus is on biofouling.

Biofouling is difficult to control, even by reducing the number of microorganisms in the feed water, they can multiply although their number is greatly reduced, and they will do so if nutrients are available. Prevention methods such as disinfection, Micro-filtration/Ultra-filtration pre-treatment in technical systems leads neither to sterility nor can it be maintained over a long period of time, microorganisms will always invade and colonise the system. Thus, if removed to the extent of 99.99%, there are still enough cells that can grow at the expense of biodegradable substances in the feed stream (Ivnitsky, Katz, Minz, Shimoni, Chen, Tarchitzky, Semiat & Dosoretz 2005; Goosen, Sablani, Al-Hinai, Al-Obeidani & Al-Belushi 2004).

Microbial attachment and growth on the membrane surface leads to the formation of biofilms, which consists of microbial cells embedded in an exopolymeric matrix produced by the microbes, (Schneider *et al*. 2005, Hall-Stoodley & Stoodley 2005).Due to a wide range of contributing factors, like surface type, availability of nutrients and oxygen, microbial species, flow velocity of the surrounding liquid, etc., biofilms are quite diverse, (Meyer 2003).

In membrane systems biofouling represents the Achilles heel of the process because all other fouling components such as inorganic dissolved substances can be removed by pre-treatment, microorganisms on the other hand are particles which can multiply. Thus, even if 99.99% of such organisms are removed, there are still enough cells that can multiply as long as there are enough nutrients available in the water (Flemming, Schaule, Griebe, Schmitt & Tamachkiarowa 1997).

The combined effect of the different membrane fouling mechanism is the formation of a fouling layer, which reduces membrane flux and may also affect salt rejection adversely. Operation costs are increased because of the need for periodical chemical cleaning, which requires additional manpower and chemicals.

To understand the reason for deteriorating membrane performance, operating conditions, feedwater quality, pre-treatment design and the chemicals in use must be considered. Even then, this information may be insufficient to pinpoint the cause of the problem. In a severely fouled plant it may be necessary to analyze the foulant within the membrane itself by a destructive autopsy procedure (Dudley & Darton 1996).

In this study the effect of biofouling was examined through water analysis and autopsy of a fouled spiral wound RO membrane after 10 years of service in a water treatment facility. Water analysis encompassed chemical tests for physical parameters and elemental analysis, while microbiological analysis included microbial enumeration and molecular identification. The membrane autopsy involved visual inspection, microscopy, microbial enumeration and molecular identification. The study highlights the contribution of biofouling to the deterioration of membranes for water treatment.

1.2 Purpose of study

At Sasol, in order for the boilers to run effectively and be protected against scaling that will reduce efficiency, the feedwater to the boilers has to be purified from all total dissolved solids (TDS) and total suspended solids (TSS) to the following specifications:

- Conductivity of 0.1 µS/cm
- Silica of 0.02 ppm
- Total hardness of nul ppm
- Oxygen of 0.005 ppm and
- Iron of 0.005 ppm

Hence, the RO membranes are being used to remove the dissolved salts from the water.

The Sasolburg RO plant experienced difficulties (i.e. fouling) related to RO membranes being cleaned at too high frequencies and eventually having to be replaced. The general objective of this project was to better understand the impact of biofouling on RO membranes.

The decrease in performance of membranes in water re-use and purification systems due to fouling is a major concern. Fouling necessitates frequent chemical cleaning and this ultimately shortens membrane life, thus imposing a large economic burden on membrane plant operation, (Herzberg & Elimelech 2007).

The economics of membrane technology depend on operation and maintenance as well as the permeate flux with operation time. Fouling and flux decline are two of the most important factors affecting the cost of the membrane treatment process. It is therefore necessary to minimise membrane fouling because the deterioration of permeate flux undermines the economics and operational efficiency of the process (Gwon, Yu, Oh, & Ylee 2002).There is, therefore, a genuine need to

improve understanding of how to overcome problems of biofouling and wherever possible to prevent the formation of biofilms.

Biofouling is a complex process affected not only by the mechanical features of the equipment and the conditions of operation, but also by the metabolic requirements of the microorganisms and their presence in the system. A great deal of research has already been published on the effects of different variables but as research equipment becomes more sophisticated, it becomes more and more possible to investigate mechanisms at the microbial level. Such knowledge may assist in a better understanding of potential mitigation techniques (Melo & Bott 1997).

Fouling of RO membranes places a large economic restriction on membrane plant operation, as noted earlier. Hence it is important to determine the dominant mechanism of fouling in an RO plant and put mitigation techniques to limit fouling. Through such this study, an understanding of the RO system would help the plant operators to run the system efficiently for longer periods before they experience fouling problems.

1.3 Study aim and objectives:

Aim: To study the effect of biofouling on the membrane water purification plant at Sasol (Sasolburg) by investigating the quality of water that is purified by the RO system and the extent of fouling that is attributed to biofouling.

Objectives:

- To understand the water purification system and to identify important points (sampling points) in the system which could possibly contribute to the biofouling.
- To conduct the enumeration and molecular identification of microorganisms found in water samples which could foul the RO membrane.
- To determine the chemical constituents of the water samples, that may be contributing to fouling.
- To perform an autopsy on a fouled membrane to determine the water components that are retained on the membrane surface this may contribute to fouling.
- To put forward possible recommendations to alleviate or prevent fouling of the system.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Water treatment

2.1.1 Conventional water treatment

There are various treatment methods for making water safe and appealing to consumers. These methods depend primarily on the characteristics of the raw water. For industrial purposes, the impurities that may be present in water supplies may briefly be grouped as follows: dissolved mineral matter, dissolved gases turbidity and sediment, colour and organic matter as well as microorganisms. Whether or not these impurities are harmful depends on (i) the nature and amounts of the impurities present, (ii) the uses to which the water will be put, and (iii) tolerances of various impurities for each use (American water works Association 1984).

The quality of water required for industrial purposes therefore depends on its end use or uses. As the tolerances for various impurities vary according to these uses, the quality of the water required in each case may differ greatly (Nordell 1961).

Many conventional water treatment plants use a combination of coagulation, sedimentation, filtration and disinfection to provide clean and safe water to the public. Worldwide, a combination of coagulation, sedimentation and filtration is the mostly widely used treatment technology, and has been used since the 20th century.

The coagulation process involves adding iron or aluminium salts, such as aluminium sulphate, ferric sulphate, ferric chloride or polymers to the water. These chemicals are called coagulants, and have a positive charge. The positive charge of the coagulant neutralizes the negative charge of dissolved and suspended particles in water. When this reaction occurs, the particles bind together or coagulate (this process is sometimes also called flocculation). The larger particles

or floc are heavy and quickly set at the bottom of the water supply. This settling process is called sedimentation (Safe Drinking Water Formulation 2006).

The diagram below (Figure2.1) illustrates the process of coagulation, flocculation and sedimentation.

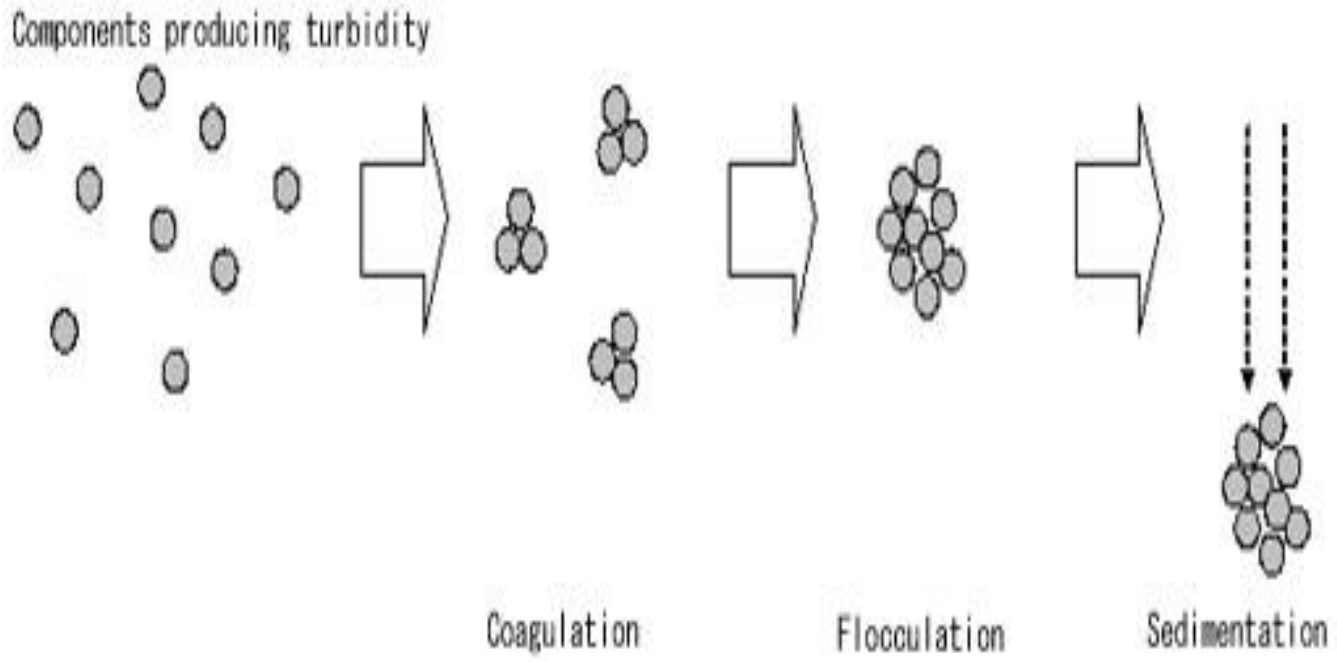

Figure 2. 1 Illustration of coagulation, flocculation and sedimentation
(<www.apec-vc.or.jp/.../images/8000/2-1.jpg>, Retrieved 23/08/2009)

Coagulation can successfully remove a large amount of organic compounds, including some dissolved organic material known as dissolved organic carbon (DOC). Coagulation can also remove suspended particles, including inorganic precipitates, such as iron.

The second stage in conventional water treatment systems is filtration, which removes particulate matter from water by forcing the water to pass through porous media. The filtration system consists of filters with varying sizes of pores and is often made up of sand, gravel and charcoal, as illustrated in Figure 2. 2.

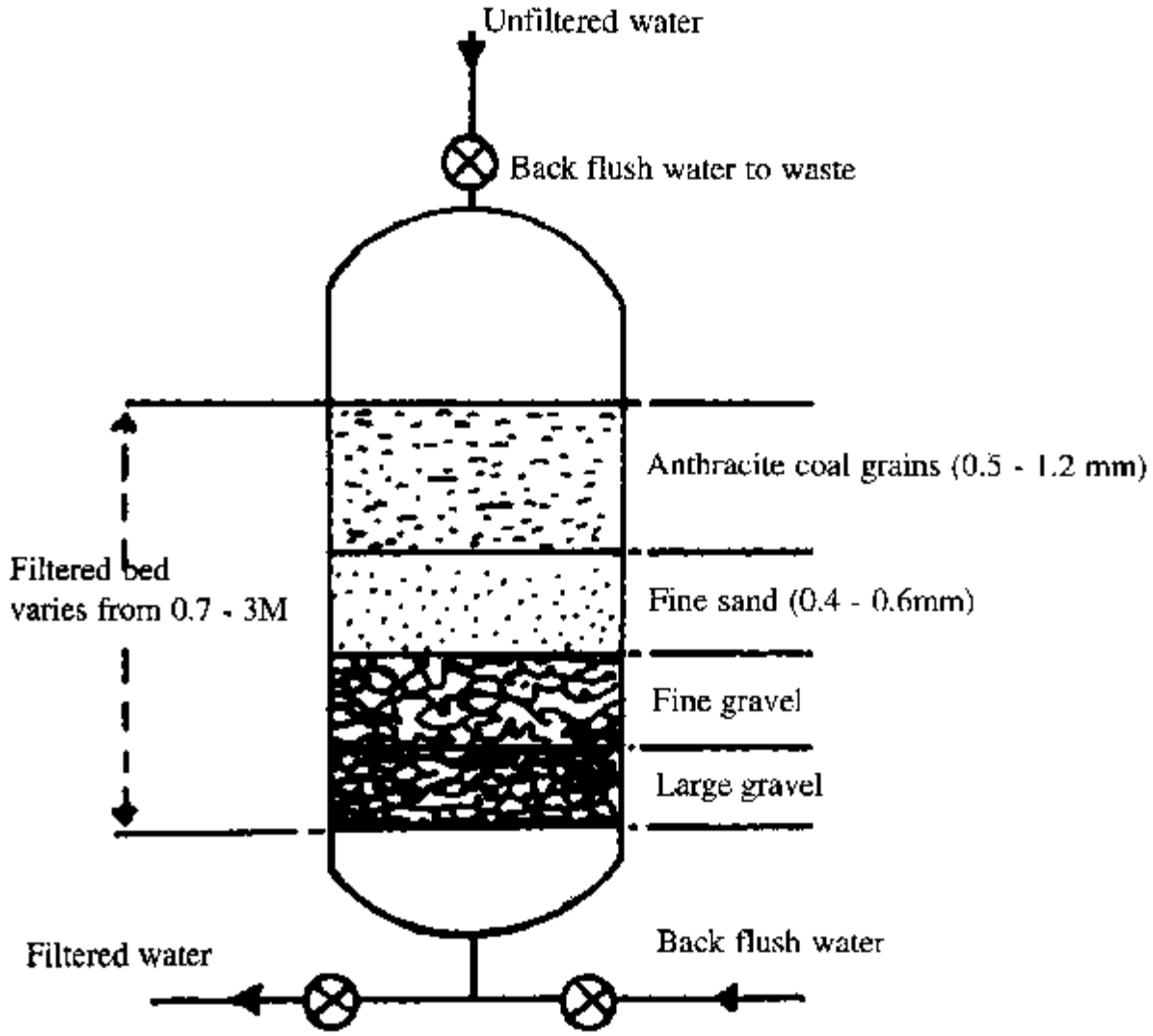

Figure 2. 2 Illustration of sand filtration
(<www.fao.org/docrep/x5624e/x5624e07.gif>, Retrieved: 23/08/2009)

There are two basic types of sand filtration; slow solid filtration and rapid sand filtration. Slow sand filtration is a biological process, because it uses bacteria to treat water. The bacteria establish a community on the top layer of sand and clean water as it passes through by digesting the contaminants in the water. The layer of microbes are called biofilms, and requires cleaning every couple of months, when it gets too thick and flow rate declines. After the biofilms are removed, the bacteria must be allowed several days to re-establish a community before filtering can resume.

A slow sand filtration system requires large areas of land to operate in because the flow rate is between 0.1 and 0.3 metres per hour. Due to the land area that is required and the down-time for cleaning, rapid sand filters, which were developed in the early 20th century, are much more prevalent today.

Rapid sand filtration is a physical process that removes suspended solids from water. Rapid sand filtration is much more common because it has fairly high flow rates and requires relatively little space in which to operate. During rapid sand filtration, the water flows at a rate of up to 20 metres per hour. The filters are generally cleaned twice a day with backwashing and are put back into operation immediately (SDWF 2006).

2.1.2 Membrane technology

Membrane technology has emerged, in the last ten years, as the main contributor to solve water shortage problems. Reverse osmosis (RO), microfiltration (MF) and membrane bioreactors (MBR) are applied today in this field. Compared to conventional water treatment, membrane technology offers the advantage to specifically remove contaminants from the water depending on the pore size and the surface physical properties of the membrane, thus offering a targeted product quality according to needs (Jacquemet, Gaval, Gherman & Schrotter 2006).

The significance need to use membranes in the water treatment process is due to various factors. Lack of freshwater resources first led to the development of membranes for use in desalination. In recent years other factors have helped to extend the use of membrane techniques in all areas of water and wastewater treatment, examples include:

- ever stricter and often contradictory regulations on water quality: a process that physically removes pathogens, membranes make it possible for disinfection to occur without producing any undesirable by-products.

- the growing importance of water re-use: wastewater has now become a resource in cities as well as in arid areas. Membranes are an ideal tool for recycling water due to their extensive moderation range.

- technical advances: membrane technology has been in use in the water treatment processes for less than 50 years. After a long research and development phase, its industrial application has accelerated once the main patents passed into the public domain (Munier 2010; Lopez- Ramirez, Coello & Quiroga 2006).

Despite a fast growing market, membrane technology still has to reach the level of application of many competing water treatment processes and has limitations due to technical and economic issues. For example, the price of membrane systems, which has decreased over the last decade, is still high. Furthermore, cost related to operation and maintenance could be significant. Above all, fouling represents the major constraint to more cost-effective, and therefore expanded, application of membrane technology in water treatment. Membrane fouling has a great impact on process performance such as energy consumption or water production (Jacquemet *et al*. 2006).

In summary, membrane technology is increasingly playing an important role in the purification of wastewater. As a result of growing demand for high quality water, purification of wastewater has become a preferred means of supplementing the water resource. In particular, high quality reclaimed waste water is being used for industrial purposes. For example, it is being used for boiler feed water and process water. RO membranes have proven to successfully treat such water and will continue to play an important role in the future.

2.2 Membrane processes

A membrane may be defined as a material through which one type of substance can pass more readily than other. Membrane materials vary widely in both physical structure and chemical composition. However, the most fundamentally important property, which provides the basis for membrane categorisation, is that of the mechanism by which the separation is achieved. This characteristic separates membranes into one of the two broad groups, either dense or porous (Table 2.1), (Mark, Burgess & Duncan 2004).

Table 2. 1 Dense and porous membranes for water treatment (Mark *et al*. 2004)

Dense	**Porous**
Reverse Osmosis Separation based on different solubility and diffusion rates of water (solvent) and dissolved species (solutes) in water.	Ultra filtration Separation of both large dissolved solute molecules and suspended colloidal particles by size exclusion.
Electro dialysis Separation based on differing ionic size, charge and charge density of solute ions using ion exchange membranes.	Microfiltration Separation of suspended solids from water by size exclusion.
Nano filtration Separation through combination of charge rejection, solubility, diffusion and sieving.	

Table 2. 2 Membrane characteristics (Srikanth 2008**)**

Process	**Membrane type and pore radius**	**Membrane material**	**Process driving force**	**Applications**
Microfiltration	Symmetric microporous, 0.1-1.0 microns	Cellulose nitrate or acetate, polyvinlidene diflouride (PVDF), polyarrides polysulfone, PTFE, metal oxides, etc.	Hydrostatic pressure difference at approx. 10-30 psi.	Bacteria and cyst filtration, potable water treatment, RO pre-treatment.
Ultrafitration	Asymmetric microporous, 0.001-0.1 microns	Polysulfone, polypropylene, nylon 6, acrylic copolymer	Hydrostatic pressure difference at appox. 30-100psi	Separation of macromolecular solutions, RO pretreatment, potable water treatment
Reverse osmosis	Asymetric skin type, 5 to 10 angstroms	Thin film composite, cellulose acetate, aromatic polyamide	Hydrostatic pressure difference at approx. 200-1000 psi	Separation of salts and micro-solutes from solutions
Electrodialysis	Cation anion exchange membrane	Sulfurated cross-linked polystyrene	Electrical potential gradient	Desalting of ionic solutions
Gas separation	Assymmetric homogeneous polymer	Polymers and copolymers	Hydrostatic pressure and concentration gradients	Separation of gas mixtures
Pervaporation	Asymetric homogeneous polymer(A non-porous membrane)	Polyacrylonitrile polymers	Vapour pressure gradient	Separation of aerotropic mixtures
Nanofiltration	Thin –film membranes	TFC^{TM}	50-150 psi	Removal of hardness and desalting

Synthetic membranes are used to remove different solutions and particles in the water treatment process, as shown in Table 2.2. The extent of fouling is strongly dependent on the types of membrane process and type of feed used (Tarazaga, Campderrros & Padilla 2006).

2.3 Reverse Osmosis (RO)

Osmosis is a natural phenomenon in which a solvent (usually water) passes through a semi-permeable barrier from the side with lower solute concentration to the higher solute concentration side (Williams 2003). As shown in Figure 2.3a, water flow continues until chemical potential equilibrium of the solvent is established.

Osmosis is the movement of water (red dots) through a semi-permeable membrane to a higher concentration of solutes (blue dots). Water easily moves through cell membranes by osmosis.

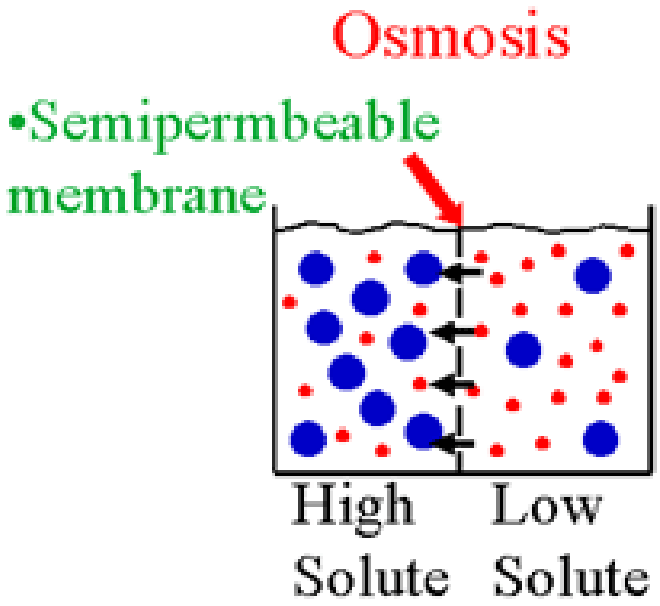

Figure 2. 3a Schematic representation of osmosis (Rico and san'chez 2008)

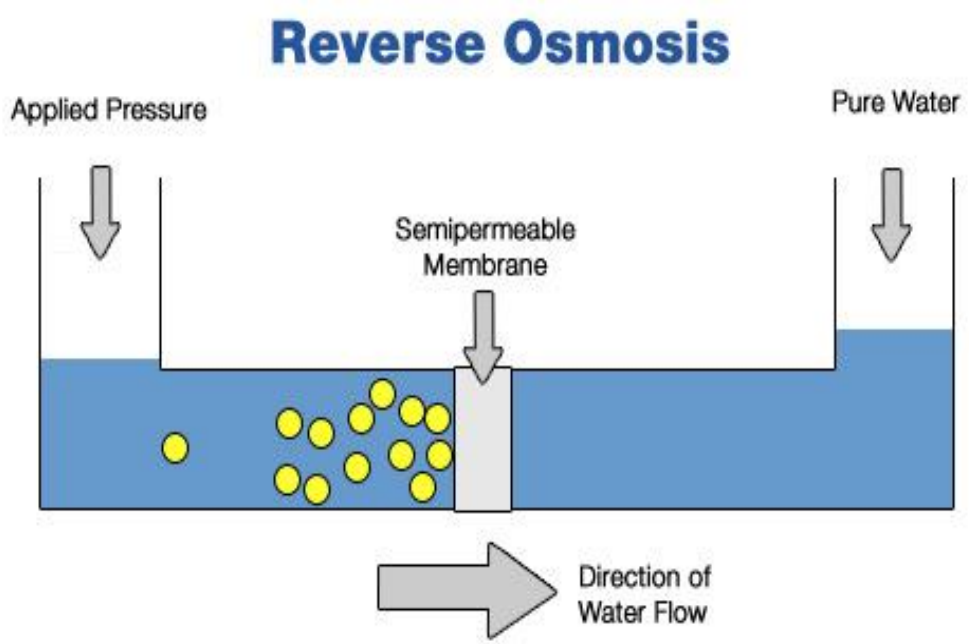

Figure 2.3b 1 Schematic representation of reverse, (Rico & San'chez 2008)

At equilibrium the pressure difference between the two sides of the membrane is equal to the osmotic pressure of the solution. To reverse the flow of water (solvent), a pressure difference greater than the osmotic difference is applied (see Figure 2.3b); as a result, separation of water from the solution occurs as pure water flows from the high concentration side to the low concentration side. This phenomenon is termed reverse osmosis, (it has also been referred to as hyper filtration), (Williams 2003).

A reverse osmosis membrane acts as a semi-permeable barrier to flow in the RO process, allowing the selective passage of a particular species (solvents, usually water), while partially or completely retaining other species (solutes). Chemical potential gradients across the membrane provide the driving forces for solute and solvent transport across the membrane.

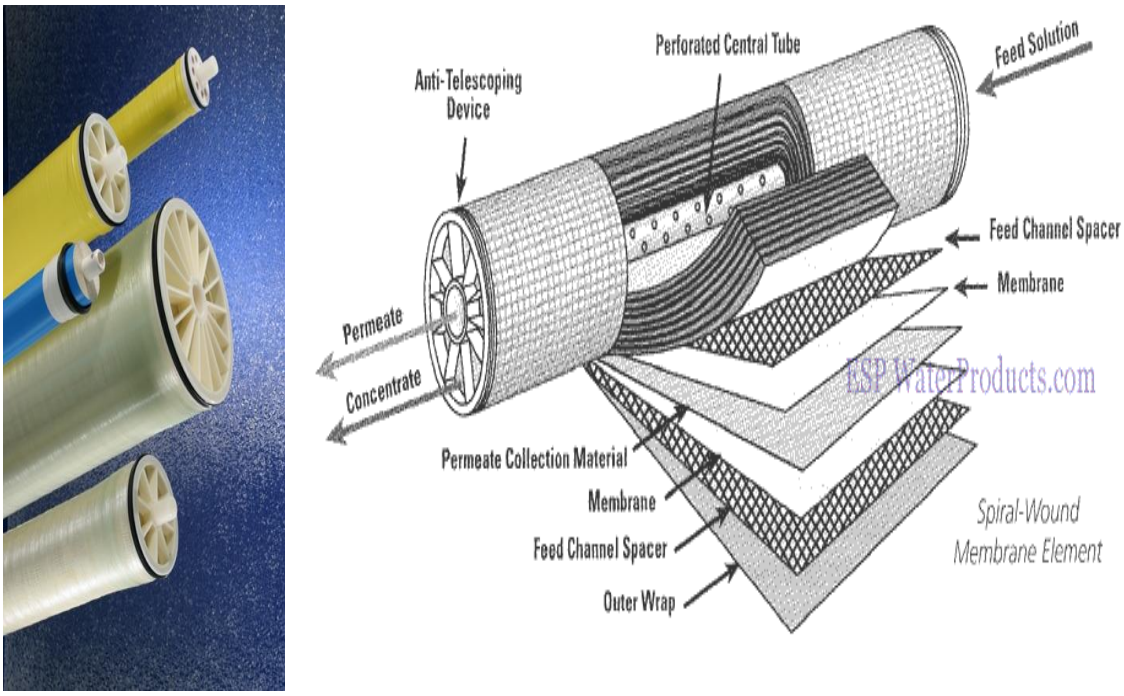

Figure 2. 4 Diagrammatic representation of spirally wound RO membrane, (ESP 2009)

The RO process uses either spiral wound or hollow fibre membranes. Plants consist of pressure vessels containing several membrane elements in series. RO technology is applied to treat surface water, well water and sea water. A spiral wound membrane (Figure 2.4) element consists of membrane material with a mesh spacer wound around a plastic product water carrier, all sealed in a fibre glass casing (Dudley 1996).

The design of spiral wound membrane elements makes them a perfect environment for the growth of microorganisms that form a biofilm on the membrane surface and on the spacing material in the narrow feed channels. The biofilm acts as a trap for the other particulate matter which quickly builds up as biomass (Al-Ahmad, Abdul Aleem, Mutiri & Ubaisy 2000).

The design of hollow fibre membranes (Figure 2.5) is also susceptible to the accumulation of foulants due to the close proximity of the fibres within the module

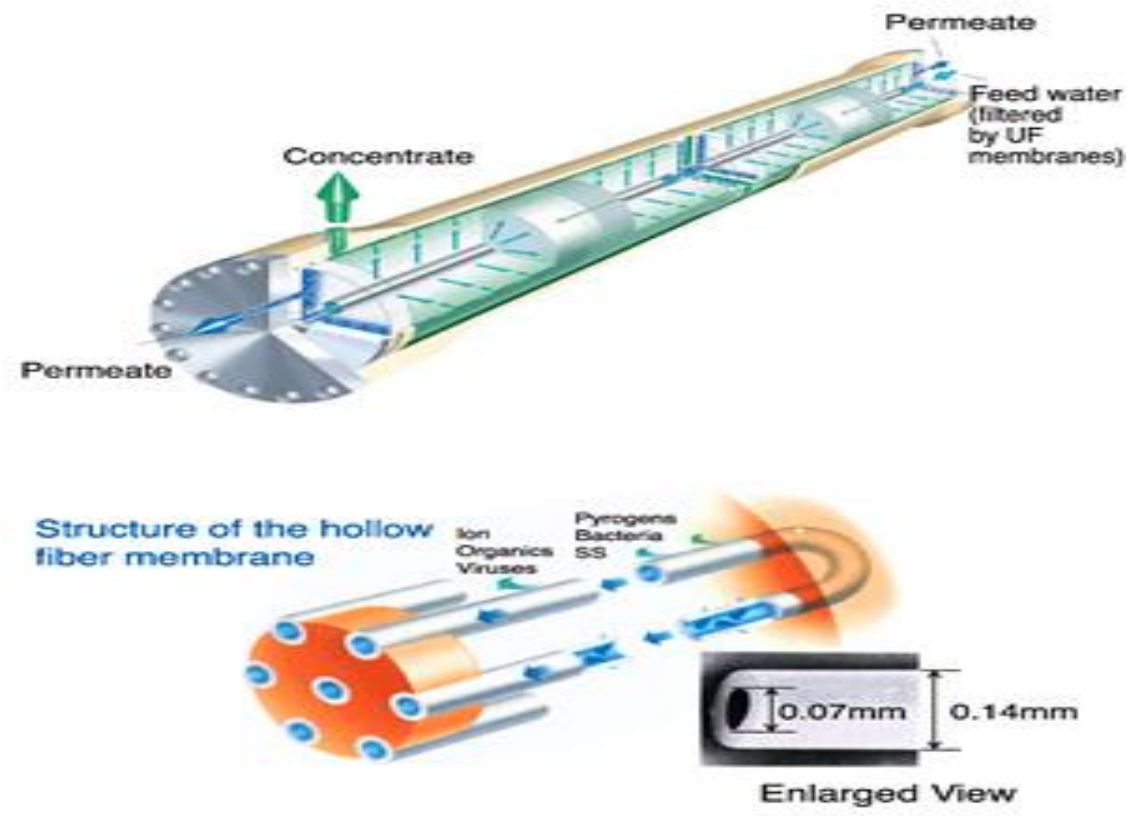

Figure 2. 5 Diagrammatic representation of hollow fibre RO membrane
(<http://www.f-suiki.or.jp/english/seawater/img/img_3.jpg>, Retrieved: 14/05/09)

casing. Reverse osmosis membranes are basically designed for the retention of monovalent ions and are widely employed for water desalination or industrial water production. Most feed water contaminants are rejected to a variable but generally significant degree (>99%). Membrane fouling by the accumulation of rejected species at the membrane surface is the major cause of long-term flux decline in membrane operations. For example, fouling was responsible for flux decline in 76% of the RO elements from more than 80 plants in Japan (Schneider *et al*. 2005).

RO membranes have gained interest due to their wide application in both desalination plants and waste water treatment processes. However, their design

which allows growth of microorganisms, limits their efficient use in the water treatment processes. The biofilm acts as a trap for other particulate matter which quickly builds up as a biomass. Indeed, biofouling of RO membranes is a major source of many problems in water treatment processes.

2.4 Fouling

Fouling in membrane systems can be caused by a number of common foulants. Foulants may be classed into one of four major categories: sparingly soluble inorganic compounds, colloidal or particulate matter, dissolved organic substances and microorganisms (biofouling).

2.4.1 Inorganic fouling/scaling

Scaling means the deposition of particles on a membrane, as illustrated in Figure 2.6, causing it to plug. It is an unwanted effect that can occur during nano filtration and reverse osmosis processes.

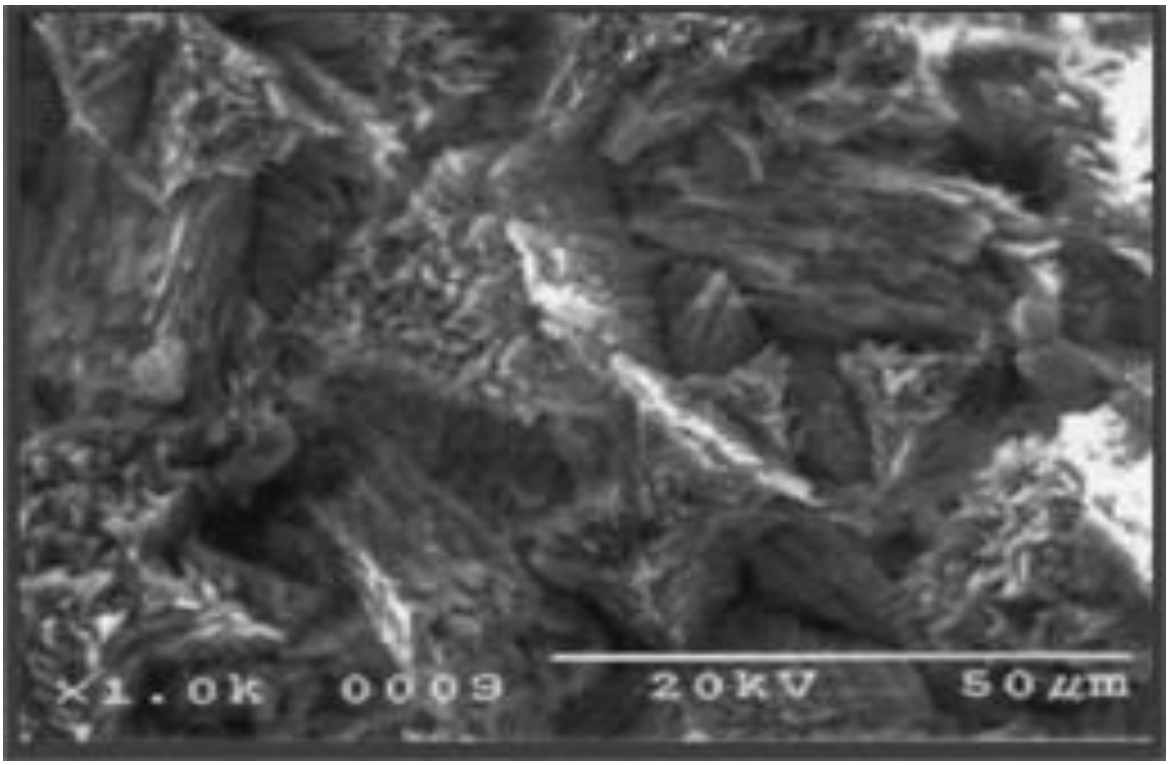

Figure 2. 6 SEM image of scaling, (Pearce 2007)

Scaling occurs when soluble inorganic compounds in feed water concentrate and precipitate onto the membrane surface and form a scale. This scale can be very difficult to remove and treatment often depends on the type of scale that is on the membrane. Scale formation due to exceeding the solubility limits of these compounds is extremely detrimental to RO system effectiveness and membrane life. Common inorganics that lead to scaling are calcium carbonate, calcium sulphate and silica. The feed water is often treated by methods such as acid injection, softening or addition of a scale inhibitor to reduce or eliminate these scaling compounds (Rome, Smith, Moe & Martherne 2000).

Scaling results in higher energy use and a shorter life span of the membranes because these will need cleaning more often. During these processes a high conversion is desired, because this will limit the loss of raw materials and energy. Dependent on conversion, about 75 to 90 percent of the feed water will be converted to the desired product. During the process, the membrane concentrate absorbs salts. Inorganic salts, such as calcium carbonate and barium sulphate, which are water-insoluble, can become over-saturated. This causes them to precipitate. The precipitation of water-insoluble salts on the membrane is more likely to occur when conversion is high.

Scaling causes the nominal flux to decrease. The consequences are, as has been noted before, a higher energy use, an increase of the cleaning frequency and a shorter life span of the membranes. This will cause the membrane water treatment process to become much more expensive. Adding acids or anti-scalants to the system can prevent the precipitation of salts. Acids decrease the over-saturation of calcium carbonate, while anti-scalants decrease precipitation levels. A membrane filtration unit performs optimally at maximum conversion and a minimal dose of acids and anti-scalants, without the occurrence of scaling (Lenntech 2009).

2.4.2 Colloidal fouling

Colloidal particles are ubiquitous in natural waters. Colloids cover a wide range, from a few nanometers, colloidal silica, iron, aluminum, and manganese oxides, organic colloids and suspended matter and calcium carbonate precipitates. In the pH range of natural waters, most colloids carry a negative surface charge. The surface charge of aquatic colloids reflects their surface chemical properties and the chemical composition of natural waters (Yiantsios & Karabelas 1998).

During membrane fouling, colloids accumulate on the membrane surface or within the membrane pores and adversely affect both the quantity (permeate flux) and quality (solute concentration) of the product water. For RO, nanofiltration (NF), and perhaps ultra filtration (UF) membranes, colloidal fouling is caused by the accumulation of particles on the membrane surface in a so-called cake layer, as shown in Figure 2.7. This cake layer provides an additional hydraulic resistance to water flow through the membrane and, thus reduces the product flux.

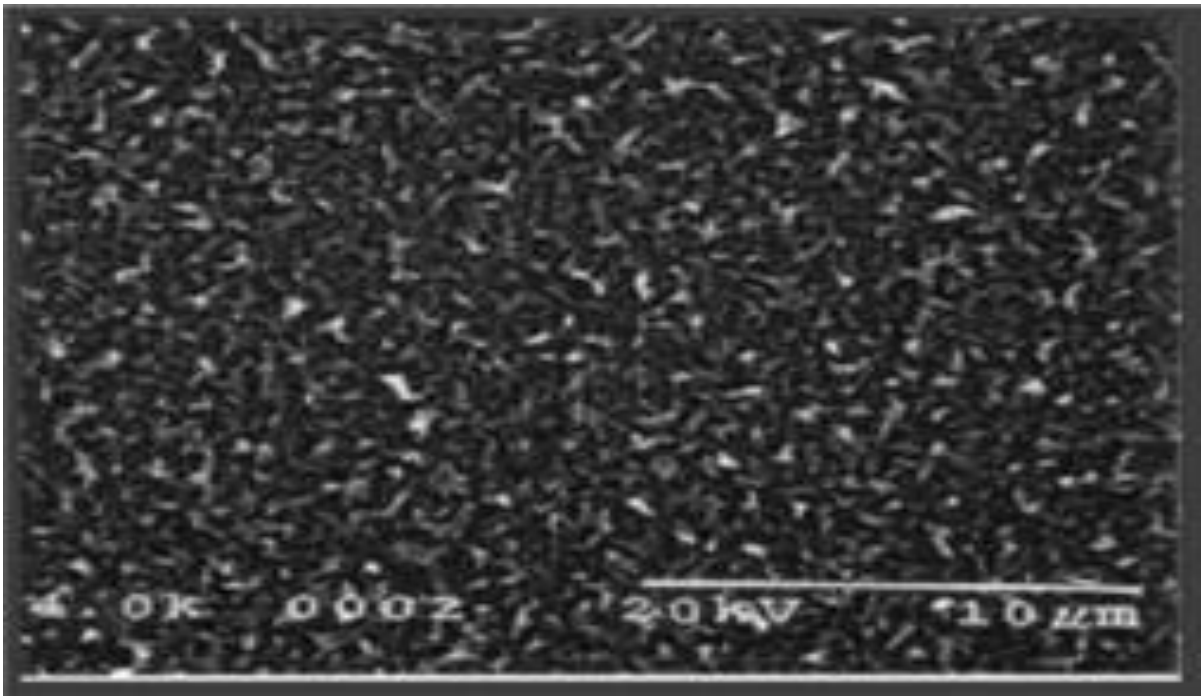

Figure 2. 7 SEM image of particle and colloidal fouling, (Pearce 2007)

For microfiltration (MF) membranes pore plugging can be an important fouling mechanism, in addition to particle accumulation on the membrane surface. The extent of pore plugging and cake layer formation depends on the relative particle size of the particles compared to the membrane pore size. Because RO membranes are considered “non porous”, the sole mechanism of RO colloidal fouling is by cake layer formation (Zhu & Elimelech 1997).

2.4.3 Organic fouling

Organic fouling is the attachment of materials such as oil or grease to the membrane surface. Organic fouling is governed in part by interactions between the membrane surface and the organic foulants, as well as between the organic foulants themselves. Membrane pores may be blocked by adsorption of low molecular weight compounds. The adsorption of organic macromolecules capable of strong intermolecular interactions or the entrapment of crystals by sticky organic polymers may lead to irreversible gel formation on the membrane surface (Schneider *et al.* 2005), as in Figure 2.8.

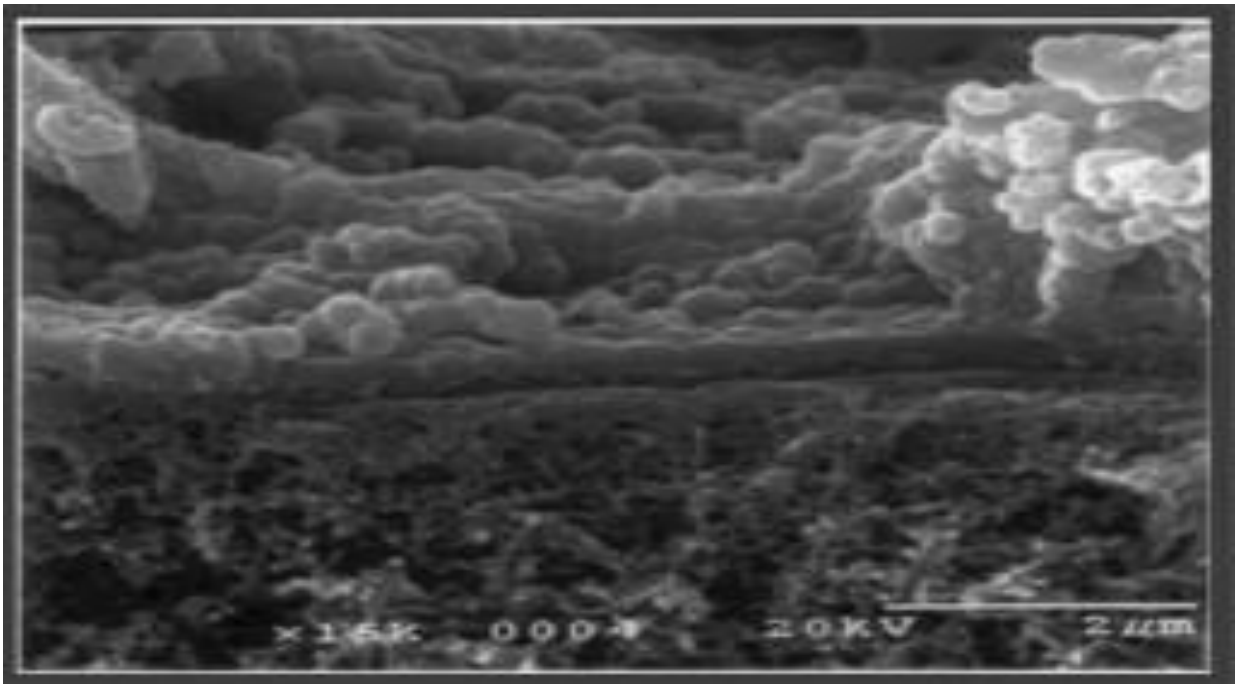

Figure 2. 8 SEM image of organic adsorption, (Pearce 2007)

It is common in RO systems used for treating industrial effluent streams. Filtration or carbon adsorption can be used to remove organic materials from feed water. The presence of organic material influences the formation of the biofilms. In the first phase of biofilm formation process the organic constituents adsorb to the membrane surface followed by the reversible adsorption of the bacteria, (Carnaham, Bolin & Surrat 1995).

2.4.4 Biofouling

2.4.4.1 Membrane biofouling

Membrane biofouling may be defined as accumulation of micro-organisms (biofilms) on a surface of membranes at such a level that it causes operational problems (Belfer, Gilron, Daltrophen & Oren 2005). When a clean membrane surface is exposed to liquid containing microorganisms and dissolved organic molecule, biofilm formation starts to build up (Al- Ahmad *et al*. 2000). Microbial attachment and growth on the membrane surface leads to the formation of biofilms, which consist of microbial cells embedded in an extracellular polymeric substances matrix by the microbes (Tran *et al.* 2007).

Biofouling is usually caused by the accumulation of microorganisms such as bacteria, fungi and algae on the membrane surfaces (as shown below in Figure 2.9) forming the harmful biofilms, through a multi-step and complex formation process (Dudley 1996; Meester, Van Groenestijn & Gerriste 2005).

The first step in biofilm formation involves rapid adsorption of organic molecules to the membrane surface. This organic layer conditions the membrane surface and enhances subsequent microbial adhesion. The next step is the adhesion of microorganisms to the conditioned surface; this step is followed by a phase of continued microbial adhesion, the growth of adhered cells, and the subsequent production of extracellular polymer. Then the microbial cells become attached to

the surface and the accumulated biomass is recognised as a biofilm (Herzberg & Elimelech 2007).

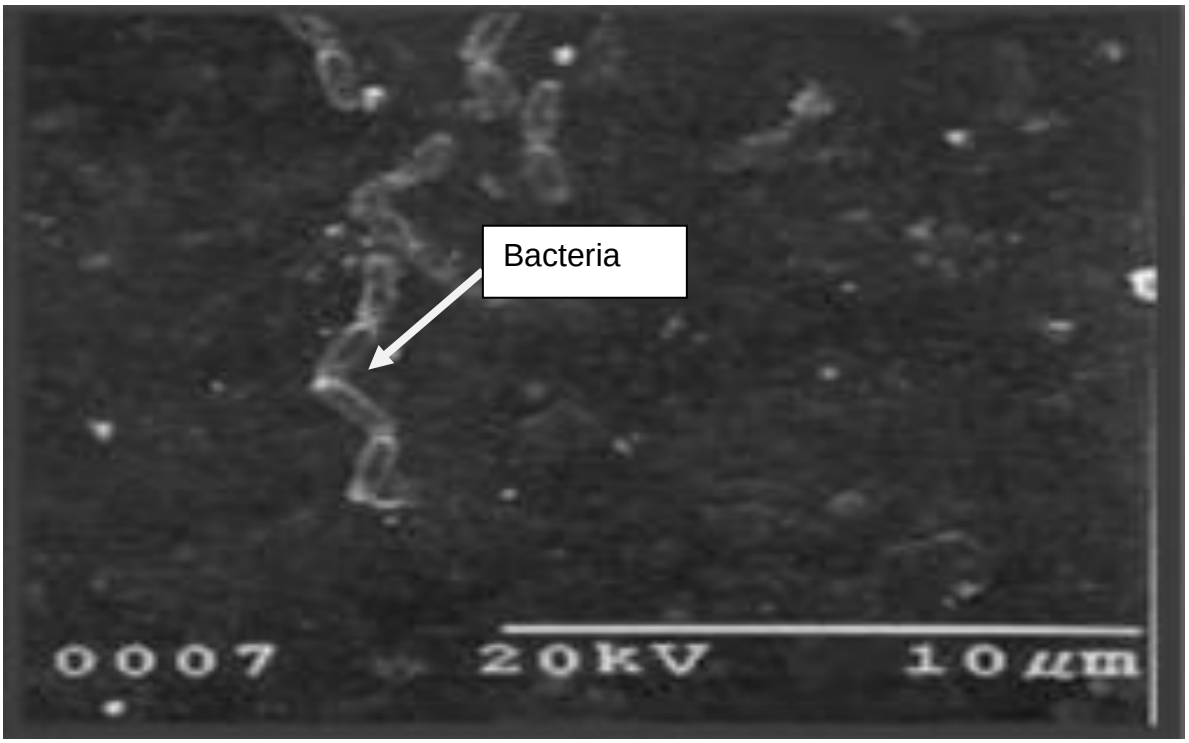

Figure 2. 9 SEM image of Biofouling (Bartels 2004)

In fact, the design of spiral wound membrane elements and the condition at the membrane surface are perfect for the attachment, accumulation and growth of microorganisms. The environment at the separating surface is confined, high in nutrients and the narrow channel space between the membrane leaves is subjected to relatively low cross flow velocity (0.1 m/s) with limited turbulence.

Once the biofilm has become established, it provides an ideal environment for the further growth of microorganisms. These can form as thick biofilms within a polysaccharide and water material, which adheres to the membrane surface, and the plastic spacer material, which separates the membrane leaves. The resulting biofilm once formed can act as a trap for other particular matter, which may quickly build up as a dense biomass (Al-Ahmad *et al.* 2000).

Membrane biofouling is a significant problem for reverse osmosis (RO) systems, in particular for RO membranes. The attachment of bacteria to membrane surfaces and subsequent biofilm growth in the spiral wound RO membrane elements strongly influence RO system performance and RO plant productivity. Problems are due primarily to an increase in the differential pressure of the RO modules, the long term membrane flux reduction of the RO plant, and the deterioration of product water quality as a result of high levels of biomass accumulation on RO membrane surfaces. Once in progress, biofouling regularly and persistently hampers the RO water treatment process (Bereschenko, Heilig, Nederlof, Van Loosdrecht, Stams & Euverink 2000).

Biofouling has adverse effects on the RO membrane systems, e.g. flux decline of the RO plant, significant increase in the pressure drop of the RO modules, increase in salt passage and biodegradation and failure. Biofouling is one of the most serious problems associated with the RO membrane system which has not yet effectively been solved (Al Ahmad *et al.* 2000).

Although distinction between organic, inorganic, colloidal and biofouling is very important, RO membranes in a typical operation are likely to be exposed to all types of fouling. However, due to the complex nature of fouling, many studies on RO membrane fouling have focused on one type for the purpose of simplicity (Tran, *et al*. 2007; Flemming 2002; Klahre & Flemming 2000; Kim, Sangyoung, Seungkwan, Youngsook, Mijin, Jihyang & Taehyun 2009). Hence, in this study the focus is on the biofouling effect on the RO water purification membranes.

2.4.4.2 The biofilm formation process

Biofilms are made up of microbial cells and extra cellular polymer products which often switch from free-living lifestyle to a surface–adapted as well as multi-cellular lifestyle. Depending on environments, their lifestyle becomes highly differentiated, exhibiting a developmental sequence as well as forming complex structures. Biofouling through successive biofilm formation on the membrane surface rapidly deteriorates the membrane performance (Kim *et al.* 2009).

Biofilms are involved in the biogeochemical pathway of Carbon, Nitrogen, Hydrogen, Sulphur, Phosphorus and most metals. Self purification processes in nature are performed by biofilm organisms, and some biotechnics in drinking and wastewater treatment are based on biofilms (Flemming 2002).

Biofilms are involved in the separation process on RO membranes from the very beginning, after a few hours of operation; a biofilm develops and participates in separation (Ivnitsky *et al.* 2005). Once a biofilm has become established, it provides an ideal environment for the further growth of microorganisms. These can form as thick biofilms within a polysaccharide and water material, which adheres to the membrane surface, and the plastic spacer material, which separates the membrane leaves. The resulting biofilm, once formed, can act as a trap for other particulate matter, which may quickly build up as a dense biomass (Al-Ahmad *et al*. 2000).

Biofouling occurs only when biofilm development exceeds a certain ‘threshold of interference’. This is the case when performance parameters like permeate flux fall below given limits (Griebe & Flemming 1998). Biofilm formation involves accumulation of microorganisms on the membrane surface. Bacteria accumulate on membrane by two processes: attachment (adhesion, adsorption) and growth (multiplication), (Belfer *et al*. 2005), as indicated in Figure 2.10.

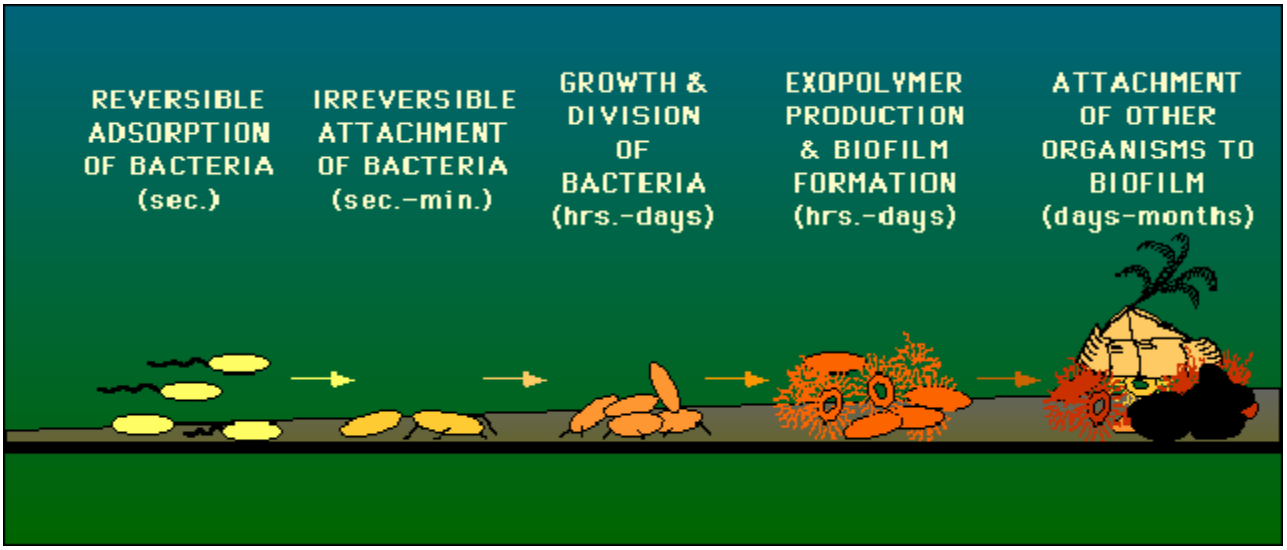

Figure 2. 10 Illustration of biofilm formation process (Todar 2008)

Biofilm bacteria exhibit dynamic adhesion in which bonding to the membrane increases with time due to the biosynthesis of adhesive extracellular biopolymers. EPS enhances the survival and robustness of the biofilm microorganisms by serving as a chemically reactive diffusion transport barrier retarding convective flow and slowing the penetration of antimicrobial agents into the biofilm. In addition the EPS matrix reinforces cellular bonding to membranes and stabilizes the biofilm thereby reducing its susceptibility to sloughing by hydrodynamic shear as well as protecting microorganisms against biocides and bio-dispersants (Ivnitsky *et al*. 2005).

Biofilm microorganisms obtain carbon and energy for growth from dissolved feed water organics although adsorbed nutrients may also be mobilized and scavenged directly from surfaces. The soaked up organics provide biofilm cells with higher nutrient levels than are present in the bulk fluid. Thus, the biofilm lifestyle enables microorganisms to survive and multiply even in extremely low nutrient environments (Kim, Chen & Yuan 2006).

It is therefore important to emphasize that biofilm formation is supported by nutrients available in the RO system. Once the biofilms have established

themselves on the RO membrane they act as a trap for other particulate matter, hence facilitating other types of fouling.

2.4.4.3 The role of EPS in biofilms

In aquatic environments, bacterial EPS exist as part of dissolved organic matter and in particulate matter such as microbial mats, biofilms, etc. EPS generally contains molecular weight compounds with charged functional groups possessing both adhesive and absorptive properties (Bin, Boasheng, Min, Taishi & Zhenghong, 2008).

Bacteria EPS are a complex mixture of macromolecules including polysaccharides, proteins and nucleic acids. EPS serves to firmly anchor cells to the surfaces and to stimulate additional microbial colonization (Belfer *et al*. 2005). Results from a study by Khor, Sun, Lui and Leckie (2007), suggested that polysaccharides were the main binding agent (structural element of the EPS matrix), which attached onto the membrane surface (adhesive) and hence provided the connection for the further attachment of polysaccharides, proteins and bio-particles.

Life embedded in the EPS matrix offers important advantages for biofilm organisms. They can maintain stable arrangements of synergistic micro consortia of different species and thus facilitate the degradation of complex substances (Wimpenny 2000). The matrix can gather nutrients from the environment and is thus part of the microbial strategy for survival under oligotrophic conditions (Flemming 2002).

In membrane water purification systems, biofilms bacteria exhibit dynamic adhesion in which bonding to the membrane increases with time due to the biosynthesis of adhesive biopolymers. EPS enhances the survival and robustness of the biofilms microorganisms by serving as a chemically reactive diffusion transport barrier retarding convective flow and slowing the penetration of antimicrobial agents into the biofilms. In addition, the EPS matrix reinforces cellular bonding to membranes

and stabilizes the biofilms thereby reducing its susceptibility to sloughing by hydrodynamic shear (Ivnitsky *et al*. 2005).

2.4.4.4 Effects of fouling on RO membrane systems

The poor performance in water treatment membrane plants is primarily due to the accumulation of extracellular polymeric substance (EPS) secreted by microorganisms entering the membranes within the feed water. This adhesive polysaccharide material can act as a trap for other organic debris and as a source for further microbiological growth; this is the problem of biofouling (Karime *et al.* 2007).

The combined effect of different membrane fouling mechanisms is the formation of a fouling layer, which has the following adverse effects on RO systems:

- “Membrane flux decline: This is due to the formation of low permeability on the membrane surface.
- Increasing the differential pressure and feed pressure: This also results from the formation of biofilm resistance with its lower permeability in order to maintain the same production rate. Damage to the membrane element may occur if operating pressure exceeds the manufacturer recommendations.
- Membrane biodegradation: Microorganisms typically produce acidic by-products which are concentrated at the membrane surface where they can cause the most damage.
- Increased salt passage: By reducing turbulent flow, biofilms increase the accumulation of dissolved ions at the membrane surface thus increasing the degree of concentration polarization. This has the effect of increasing the salt passage through the membrane and reducing the quality of the product water.
- Increased energy requirements: Related to higher pressure are requirements to overcome the biofilm resistance and the flux decline” (Abd El Aleem *et al.* 1998: 20; Al-Ahmad *et al.* 2000: 175).

Operation costs are increased because of the need for periodical chemical cleaning, which requires additional manpower and chemicals (Schneider *et al*. 2005).

2.5 Relationship between water quality and biofouling

One of the critical factors affecting the development of the biofilm is feed water quality e.g. temperature, pH, dissolved oxygen content and presence of organic and inorganic nutrients. Once a micro-organism has found an environment to which it is suited, growth will proceed unless conditions in the system become hostile. Aerobic bacteria are present in warm, shallow and sunlit surface water with dissolved oxygen, an optimum pH of 6.5-8.5and an abundance of organic and inorganic nutrients. Anaerobic bacteria, on the other hand, are present in closed water systems with little or no dissolved oxygen and can be activated if sufficient nutrients are introduced. Both types of bacteria can exist at different locations in the same system, (Abd El Aleem *et al.* 1998: 21).

Feed water quality dictates the performance of the membrane system, which in turn limits system recovery, plant output rate and water quality. It is necessary to monitor feed quality and the performance of the individual pre-treatment system components so as to optimise plant economics and efficiency. Feed water quality is normally characterised by a silt density index (SDI).

The plant operator must measure the SDI and the scaling potential of the feed water in order to monitor and control membrane fouling. Microbiological analyses are also needed to monitor biological activity and biological potential throughout the RO plant sections. Other significant tests required for feed water are Dissolved Oxygen Content (DOC), pH measurement, Biological Oxygen Demand (BOD) and Total Oxygen Content (TOC), which can characterise the organic and biological impurities present in the feed water (Al-Ahmad *et al.* 2000).

Accurately monitoring the fouling potential of RO feedwater has many limitations at this time. Most existing measurements of colloidal and biological fouling potential are fairly good qualitative indicators but inaccurate quantitative indicators. Even with their limitations, they provide valuable information and must be used. Understanding their limitations, however, helps us interpret the often confusing and conflicting results (Paul & Abanmy 1990).

2.6 Monitoring techniques for biofouling

It is of utmost importance to monitor biofilm development, in order to optimise the time and extent of countermeasures. Conventional methods rely on sampling defined surface areas or on exposure of test surfaces (coupons) with subsequent analysis in the laboratory. A classic example is the so-called “Robbins device”

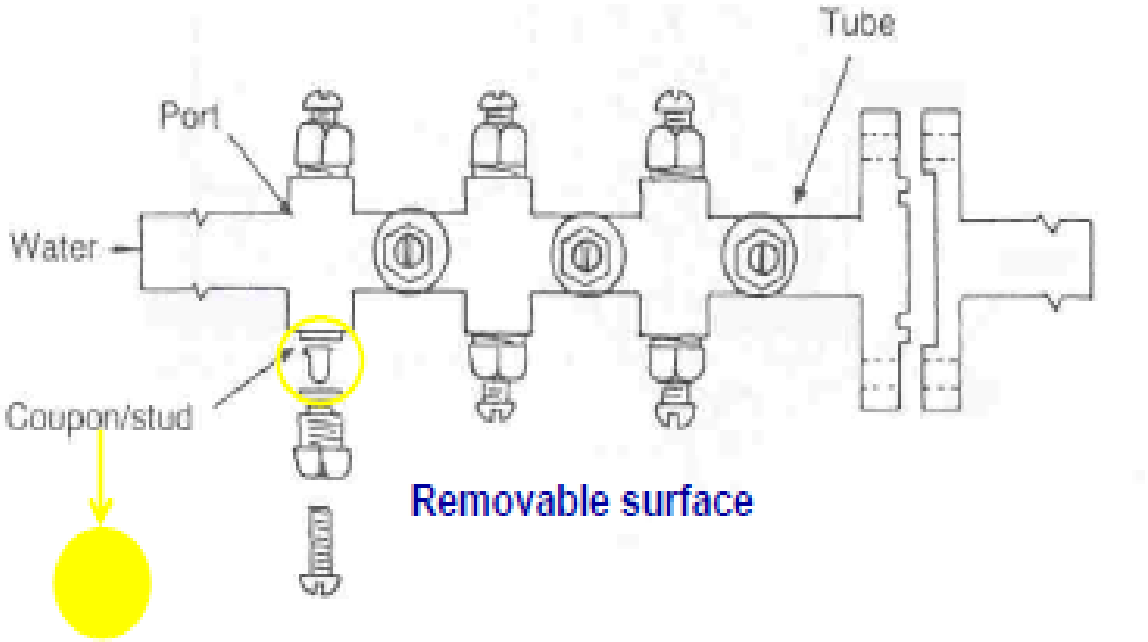

Figure 2. 11 Illustration of Robbins device in the detection of biofilms
(Flemming & Strathmann 2010)

which consists of plugs inserted flush with pipe walls, thereby experiencing the same shear stress as the wall itself. The Robbins device provides quantifiable samples of biofilms growing on submerged surfaces in aqueous systems. The stud surface of the device, as shown in Figure 2.11, which is exposed to the flowing bulk

fluid, can be aseptically removed from the system and sampled for biofilm bacteria (Cloete, Jacobs & Brozel 1998).

The disadvantage of such systems is the time-lag between analysis and result. Other methods which report biofilm growth on–line, non-destructively and in real time have been invented. They are all based on physical methods. One example is a fibre optic device (Figure 2.12), which has an illuminated fibre integrated in the test surface and measures the scattering of light by material deposit on the tip.

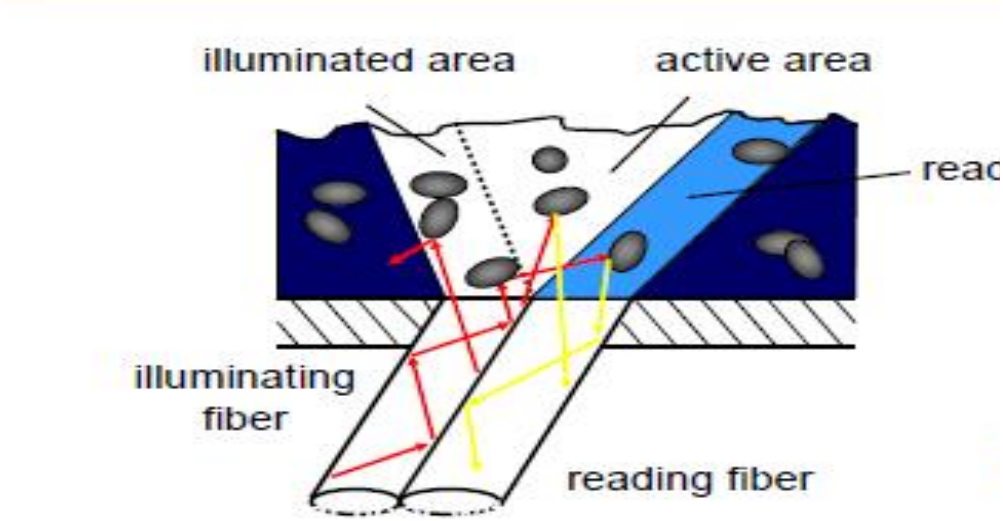

Figure 2. 12 Illustration of the Fibre optical device (Flemming 2002**)**

Another method uses two turbidity-measuring devices, one of which is constantly cleaned. The difference between the signals is proportional to the biomass development on the non-cleaned window, (Flemming 2002).

Al-Ahmad *et al. (*2000:177-178), indicate the following techniques as reliable and applicable for monitoring and detection of biofouling in RO membrane systems:

1. “Physical inspection: regular and thorough inspection of various components such as pre-treatment piping, cartridge and media filters and membrane system manifolds can be helpful in revealing the presence of any biological accumulation. A physical inspection of a spiral wound membrane element may show signs of biofouling in smell and colour. If biofouling has indeed

progressed, physical damage of the membrane element structure may also be observed.

2. System performance analysis: It is normal practice in any RO plant to monitor the following significant parameters precisely:

- The pressure difference along the RO modules.
- The pressure drop across the cartridge filters, the media filters and the pipes of the pre-treatment sections.
- The permeate flow rate and purity of water produced.
- The salt rejection.
- The silt density index (SDI) of feed water entering the membrane section.

3. Water sampling and routine analysis: Collecting reliable water samples at key points throughout the RO plant is an essential part of any meaningful biological- monitoring program. Routine collection of feed, product and brine water samples should be stared as soon as the RO plant goes on stream. The main objective of this sampling and analysis technique is to be able to localize or isolate the source of any bioactivity before it starts to spread and affect the other parts of the system especially the RO membranes.

4. The silt density index (SDI) of the RO feed water, product water and brine rejects are also measured. Dissolved oxygen content, pH, COD, TOC and bacterial counts are thoroughly determined in these routine samples.

5. Culturing techniques: These techniques are used to determine the types and concentration of the microbiological species present in the system in a given period. Two main methods are usually used for this biological analysis, i.e. the direct count method and incubation method."

In conclusion, the RO system needs reliable biofilm monitoring devices, i.e. representing the biofilm accumulation on membrane surface. Sacrificial module

elements are useful for destructive analysis (Autopsy). Membrane autopsy is a technique used to identify the cause of poor membrane performance. This requires a sacrificial membrane element to be removed from the plant for destructive analysis. The poor performance in water treatment membrane plants is primarily due to the accumulation of EPS secreted by microorganisms entering the membranes in the feed water (Karime *et al*. 2008). Fouling causes product quality to deteriorate, the output to decline and energy consumption to increase to the point where it may become necessary to replace the membrane. The content of water and organic carbon is indicative in a fouled membrane. If it is high, there is a high probability that the material is a biofilm. The presence of ATP or respiration activity indicates living organisms (Flemming 1997). Analytical techniques are used to determine the nature of the membrane foulant present on the membrane surface (Karime *et al*. 2008, Dudley 1996).

Membrane autopsy procedure involves dissection of membranes, visual inspection of the surface membrane as well as other components of the module to check for any physical damage. Such damage indicates a high degree of fouling caused by excessive pressure drops across the membrane (Dudley 1996). Furthermore, the foulants and the membrane itself are analysed by various analytical techniques. A wet chemical analysis of the foulants deposited on the membrane is carried out to identify the chemical composition of the foulant. The SEM analysis gives detailed micrographic view of the membrane surface, which shall help in establishing the nature of the deposits. Biological analysis is conducted to study the possibility of biofouling (Al-Amoudi & Farooque 2002).

2.7 Biofouling control techniques

2.7.1 Feed pre-treatment

In the late 1990s, two strategies were strongly proposed to prevent and control membrane biofouling: (i) physical removal of bacteria from the feed water of membrane system (e.g. microfiltration or ultra-filtration pre-treatment) and (ii) metabolic inactivation of bacteria by applying biocide dosage or UV irradiation (Ridgway 1997). At present, the focus is on nutrient removal by biological pre-treatment (e.g. sand filtration) and modification of membranes (disinfectant resistant and low fouling), (Vrouwenvelder *et al.* 2009).

The degree of this pre-treatment usually depends on the nature of the feed, the membrane type and configuration, the recovery level expected and the frequency of membrane cleaning. Minimum pre-treatment is required for a clean well water with a low SDI<2 and a temperature acceptable to the membrane. In this case a 10- micron cartridge filter and antiscalant dosing to prevent the scale formation are sufficient. For larger systems additional flocculation and filtering processes are usually required to remove suspended solids (as illustrated in Figure 2.13). For medium sized plants, the most frequently selected methods for pre-treatment are the use of sand and multimedia filters, granular activated carbon (GAC) and cartridge filters (Al-Ahmad *et al.* 2000).

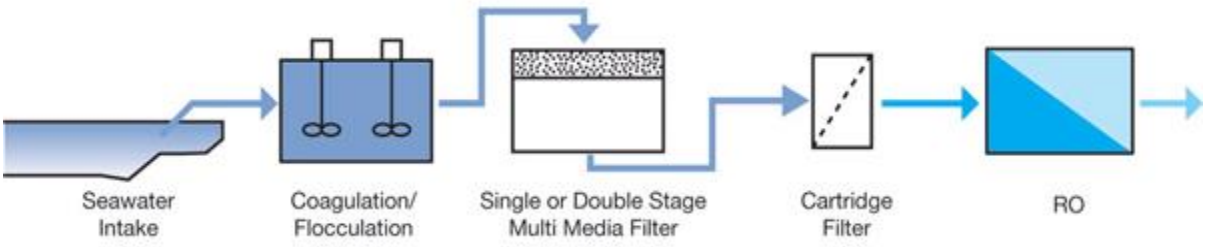

Figure 2. 13 Convectional RO pre-treatment process (Wolf & Siverns 2004)

2.7.2 Membrane cleaning

The need for cleaning is indicated by a reduction of the product water output. As a general guide, cleaning is recommended when any of the following parameters change by 10-15%:

- A decrease in the product water flux
- A decrease in the salt rejection
- An increase in pressure drop
- An increase in the feed

The cleaning chemicals used, and the frequency of cleaning cycles are determined by the biofilm composition, the membrane material and the degree of fouling present. Cleaning procedures are usually given by the membrane manufacturers (Al-Ahmad *et al*. 2000). In general, a cleaning strategy will include the following two steps:

- Weakening of the biofilm matrix, mostly by chemicals such as oxidants, (for example, chlorine, ozone, hydrogen, peroxide, peracetic acid, etc.) by alkaline treatment, tensides, enzymes or complex-forming substances or bio-dispersants. Bio-dispersants are based on polyethylene glycol and are supposed to weaken the interaction between biofilm and support material. A combination of various agents may increase the effectiveness (Flemming 1997).

In essence chemical control generally involves the use of biocides to kill microorganisms or biostats to reduce their activity. Chlorine has been the preferred biocide for many years because it's cheap and available. However, its use is becoming severely restricted due to its detrimental effects on the quality of water discharged back to the natural environment.

The addition of chemicals to the water system may be done in three ways.

1. Continuous. The maintenance of a fixed concentration in the circulating water. The dose depends on the concentration and species of the microorganisms present.
2. Shock. An intermittent dose of relatively high concentration maybe once in 24 hours.
3. Pulse. Involves dosing on a fixed schedule but more frequently than shock dosing, maybe once per hour for example (Bott 2009).

The method of dosing will, in general, depend on the season and the quality of the water involved.

- Removal of the biofilm by mechanical forces such as rinsing with water, air, steam, or combination or by application of sponge balls, brushing or ultrasonic (Flemming 1997).

In summary, biofouling is a major concern for operators of reverse osmosis installations. Proper monitoring of each section of the RO plant is very important to detect any biofilm formation at its early stage so as to eliminate it and prevent its growth. Proper pre-treatment and precise membrane cleaning are efficient techniques to control the biofouling problem.

CHAPTER 3

3. METHODOLOGY

3.1 Area of study

Sasol (originally Suid Afrikaanse Steenkolen en Olie) is a South African company involved in mining, energy, chemicals and synfuels. In particular, they produce petrol and diesel. The Sasolburg complex is located in the Freestate (Figure 3.1), South Africa.

Figure 3. 1 Google map of Sasol, Sasolburg site (Google Earth 2011)

3.2 Sampling site

The RO plant is located at steam station 3, as shown in figure 3.2 below. The purpose of the RO plant is to provide a good quality feed water for the Demineralization plant in order to reduce the purification load on the plant hence reducing the amount of effluent produced.The feedwater to the boilers has to be free from total dissolved solids and total suspended solids. This is very important so as to enable the boilers to run efficiently and be protected from scaling.

Figure 3. 2 Google map of the RO plant site (Google Earth 2011)

3.2 Reverse osmosis plant

This study on the effect of biofouling on RO membranes was done at a full- scale water treatment plant, (Sasol, Sasolburg in the Free State region in South Africa). Operative process steps of the plant are illustrated in Figure 3.3. As indicated in Figure 3.3, pre-treatment processes precede the RO system. This section consists of the following:

- Steam feed heating in order to control the temperature of the feed water at a desired temperature.
- Sand filtration by five sand filters in parallel in order to remove large suspended particles in the feed water.
- SMBS (sodium meta-bisulphate) addition in order to reduce free chlorine in the feed water.
- pH control, by means of hydrochloric acid addition, at between 6.8 and 7.2 in order to reduce the scaling potential of the feed water.
- Anti-scaling addition in order to reduce $CaCO_3$ scaling potential of the water.

The RO system is divided into four smaller units of equal size, operated independently and in parallel for the following reasons:

- The demand for water varies considerably, which means that only one or two of the smaller units could handle the demand at certain times.
- Maintenance and CIP can be performed on one unit without interfering with the other three. This implies that the plant is able to maintain 75% of the maximum production capacity during maintenance of other units.

Each RO unit consists of 13 pressure vessels in a 7:4:2 configuration. Each vessel contains spiral wound polyamide membranes that make a total of 78 membranes per unit. Additional to the membranes, which are the key equipment in the plant the RO unit also consists of three pressure multistage pumps and two filters in series (see Annexure A).

Figure 3. 3 RO plant simplified flow diagram (courtesy of Sasol)

3.2 RO2 unit pressure

Figure 3.2 illustrates the RO pressure representation in the installation. Differential pressure one (DP1) is a difference between second stage pressure and feed pressure while differential pressure two (DP2) is the difference between third stage pressure and second stage pressure. The rise in DP1 and decrease in DP2 may be an indication of fouling.

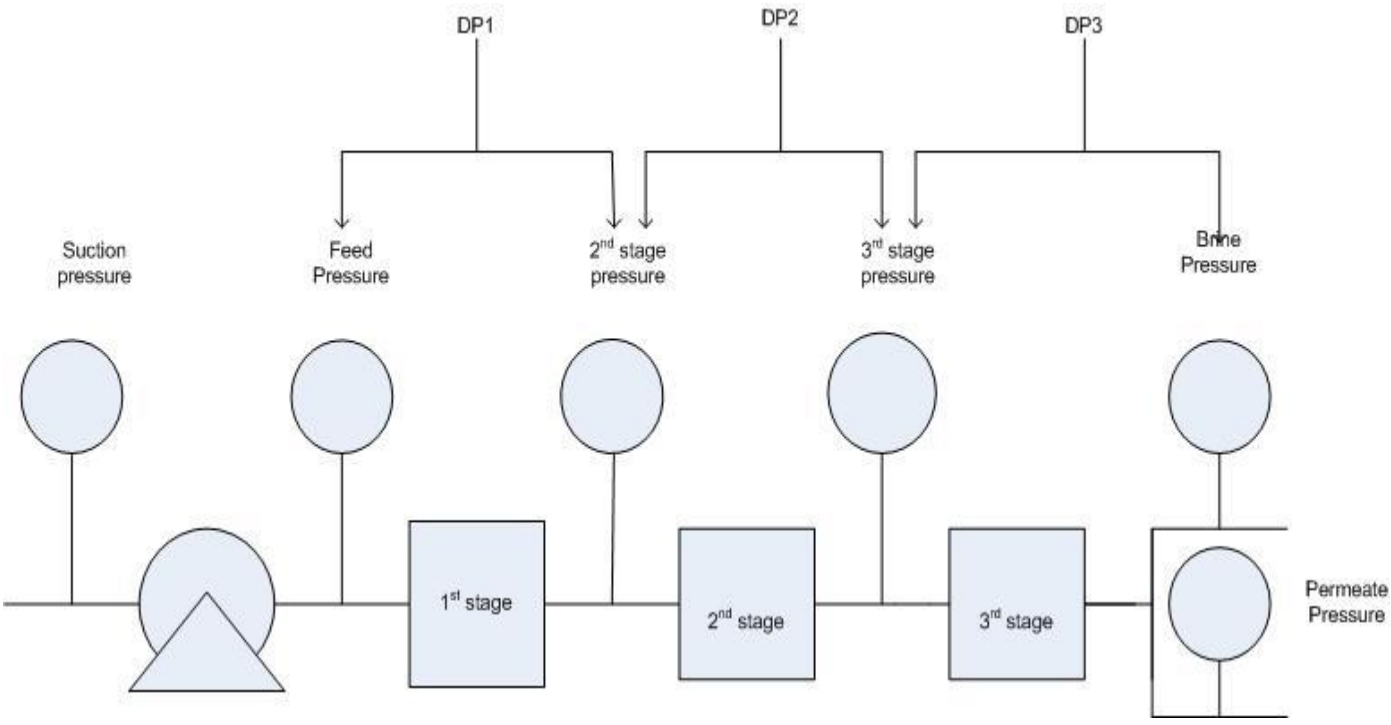

Figure 3. 4 Schematic representation of RO pressure (courtesy of Sasol)

Differential pressure 1 (DP1) = 2nd stage pressure – feed pressure

Differential pressure 2 (DP2) = 3rd stage pressure – 2nd stage pressure

Differential pressure 3 (DP3) = brine pressure – 3rd stage pressure

3.3 Water sampling and analysis

Water quality may be defined by its physical, chemical and biological characteristics. Physical parameters include colour, odour, temperature and turbidity. Solids may be further subdivided into suspended and dissolved solids as well as organic and inorganic fractions. Chemical parameters associated with the organic content of water include biological oxygen demand (BOD) and chemical oxygen demand (COD). Inorganic chemical parameters include salinity, hardness, pH, as well as concentration of ionized metals such as iron and manganese and anionic entities such as chlorides, sulphates, sulphides, nitrates and phosphates. Bacteriological parameters include coliforms and other microorganisms.

Collecting reliable water samples at key points throughout the RO plant is an essential part of biological monitoring programme. The sampling points should be chosen so as to adequately cover the entire system, from the raw water intake to the RO product header line (Al-Ahmad *et al*. 2000).

In this study, samples were collected from six points initially, that is, feed holding tank, before sand filter, after sand filter, after cartridge filter (feed), permeate and brine. After sampling five times , the sampling points were reduced to three (feed, permeate and brine) as the focus was on what goes in and out of the membrane, to help determine the effect of biofouling on the RO membrane. Samples were collected into sterile 2 litre bottles. Water samples were analysed for microbiological components immediately after sampling. The water samples were stored at 4°C until further chemical analysis.

3.3.1 Chemical analysis

3.3.1.1 pH

pH is one of the environmental factors with the greatest relevance to the growth of microorganisms. Microbes prevail in certain ranges of pH that favour their nutrition, reproduction and survival. H1 9025 microcomputer pH meter (HANNA instruments) was used to measure pH.

3.3.1.2 COD and BOD

Together with the chemical oxygen demand (COD), the biological oxygen demand (BOD) is an important parameter for the estimation of biodegradable organic compounds. The COD value was measured using Test 0-26 CSB160 COD (15-160 mg/l) test kit. 2ml of the sample was added to the reaction tube and was digested on a heating block (Nanocolor vario 3) at 48°C for 2hours.After digestion, the sample was shaken, allowed to cool and the reading was taken at 436nm wavelength using Nanocolor 500D photometer.

The Oxitop control/ OxiTop measuring system was used for the determination of BOD. BOD was measured by incubating a sealed water sample over a period of five days at 20°C. The samples were prepared by adding the nitrification inhibitor solution to the samples (20 drops NTT solution/l of sample). A magnetic stirrer rod was placed in each sample bottle, and using tweezers two tablets of sodium hydroxide were added through the stopper. The OxiTop measuring sensor head was tightly screwed onto the measuring bottle. The measurements were started using the OxiTop controller. The samples were then placed on a stirring platform in the thermostat cabinet and the motor drive of the stirring system was switched on. The samples were incubated for five days. After five days the measured values were read.

3.3.1.3 Turbidity

Turbidity is a suspension of fine colloidal particles that do not readily settle out of solution and can result in cloudiness. It is determined by a Nepholometer that measures the relative amount of light able to pass through a solution. Turbidity is reported as NTU (Nepholometric Turbidity Units). Typical RO has a maximum of 1.0 NTU for the feed water. H1 98703 Turbidimeter was used to measure turbidity.

3.3.1.4 Conductivity, Total dissolved solids (TDS) and salinity

Conductivity is a measure of the ability of water to transmit electricity due to the presence of dissolved ions. Absolute pure water with no ions will not conduct an

electric current. Conductivity, TDS and salinity were measured using H1 9828 Multiparameter (HANNA instruments).

3.3.1.5 Elemental analysis

Elemental analysis was done using test kits. The following tests were done, Test 1-48 Silica, Test 0-43 Hardness 20 (Calcium + Magnesium),Test- 45 Potassium, Test 55 Ortho and total phosphate 45,Test 0-37 Iron, Test 0-88 total Nitrogen TNa and b 220, Test 0-66 Nitrate 250 and Test 1-53 Copper. The procedure was followed as per test kit.

3.3.2 Microbiological analysis

3.3.2.1 Microbial Enumeration

Samples were diluted in 0.85% saline solution and subjected to duplicate plate counts. Using the membrane filtering technique, a 100 ml water sample was filtered through a 45 µm filter paper disc and plated on different types of media. The plates were incubated at 28ºC for 48 hours. The number of microorganisms present after 24 hrs were counted and expressed as colony forming units per volume (cfu/ml) of the sample. The techniques used for microbiological analysis are as shown in Table 3.1 below. The detail of each procedure is as indicated in Annexure F.

Table 3. 1Microbiological population techniques

Analysis	**Technique**	**Growth medium**
Aerobes	Membrane filter	R2A
Psuedomonas	Membrane filter	Psuedomonas agar
Moulds	Spread plate	Malt extract
Slime formers	Membrane filter	Sabourad dextrose agar
Anaerobes	Pour plate	Plate count agar
Coliforms	Membrane filter	Coliform agar

3.3.2.2 Statistical analysis

Microbiological data were analysed using the QI Macros 2010 software. It was used to plot the box and whiskers' chart. A box and whisker chart is used to display a set of data so that it can easily be seen where most of the numbers are.

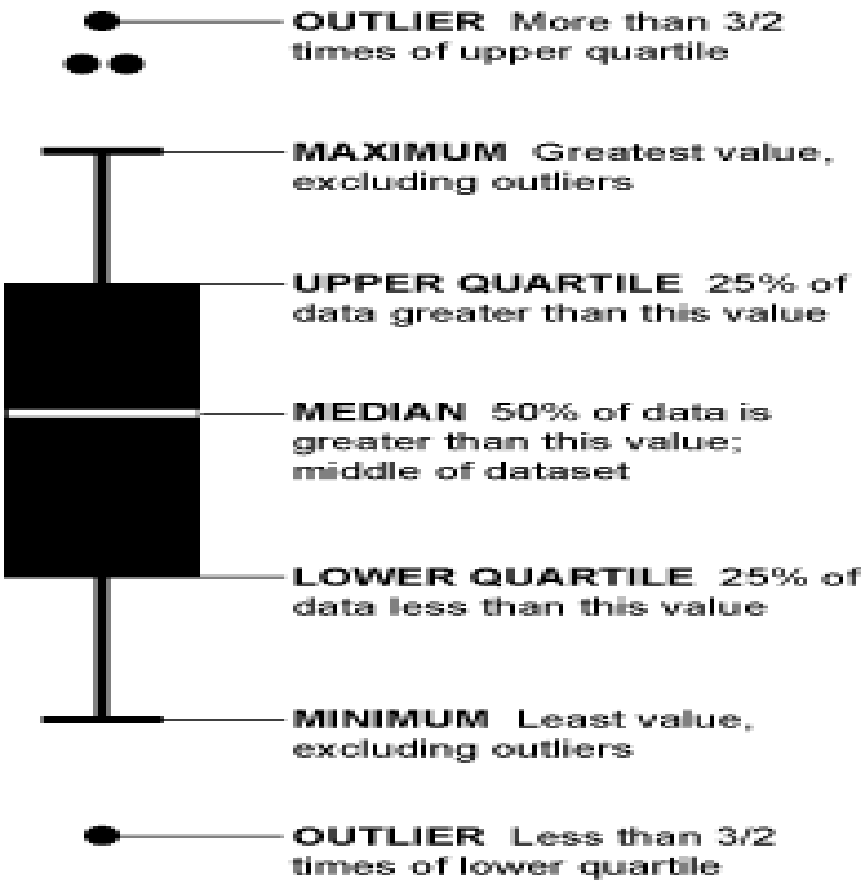

Figure 3. 5 Illustration of the box and whisker chart (Flowing data 2011)

3.3.2.3 Microbial Identification

3.3.2.3.1 Bacteria Identification using the API20E

Different types of colonies from the media plates were picked and purified. Pure samples were used to inoculate strips of the API20E for *Enterobacteriacea* identification.

3.3.2.3.2 DNA extraction, PCR and Sequencing

Cultures from water samples were subjected to DNA extraction using the Zymo Research Fungal/Bacterial soil DNA extraction kit. The DNA was stored at -20 ° C until further processed. PCR amplifications for sequencing were performed with the standard bacterial primer pair 27F and 1492R.

3.4 Membrane autopsy

A fouled RO membrane was collected from the full-scale water treatment plant, (Sasol, Sasolburg). The fouled membrane was wrapped around in plastic and kept intact and in a wet condition until arrival in the laboratory. The outer casing and the ends of the membrane were inspected closely before dissection. After dissection the membrane was analysed by various techniques as follows:

3.4.1 Visual inspection

The fouled membrane was inspected for physical damage along the glue lines and feed spacer material.

3.4.2 Microscopy

Samples of fouled membrane were examined and images taken. A zeiss stemi “2000” stereo microscope and zeiss axioskop phase contrast microscope with axiovision 4 software were used.

3.4.3 Elemental analysis

The sample was prepared for elemental analysis as in microbial enumeration. The sample was then tested for presence of calcium, silica and magnesium using test kits as in water analysis.

3.4.4 Alcian Blue stain

Alcian blue staining was used to confirm the presence of biofilm matrix. Alcian blue stains acidic polysaccharides are often present in the biofilm matrix (exopolymeric substance—EPS), (Rayner, Veeh and Flood, 2004). Staining was carried out by the direct addition of an aqueous solution of 0.1% Alcian blue 8GX (Sigma) to the

surface of the sample. After 20 min, samples were gently flushed with 0.45 µm filter-sterilized water to remove excess dye. The stained samples were then mounted in two to three drops of sterile water, and a cover slip was applied and then examined under the microscope.

3.4.5 Microbiological enumeration

An area of 100 cm^2 of fouled membrane was scraped off the fouling layer. The fouling material was placed in a beaker with 100 ml of 0.85% NaCl in distilled water. A homogenous mixture was obtained through shaking. The sample was then plated on different types of media (as in Table 3.1) for determination of total microbial counts.

3.4.6 ATP analysis

The biomass parameter used to determine the concentration of biomass on the membrane is the adenosine triphoshate (ATP). ATP (ATP/cm^2) gives an indication of the total amount of the active biomass. The quantification of ATP can be used for a variety of different purposes. Because ATP is the “coin” for energy transfer for almost all living organisms yet rapidly degrades in the absence of viable organisms, its existence can be used to identify the presence of viable organisms. Measurement of ATP has been used for the detection of bacteria on surfaces and quantification of bacteria in water or somatic cells in culture.ATP analysis was done using a test kit. The procedure followed is as indicated in the Total control BWWT (TCB) - Protocol (see Annexure B).

3.4.7 Microbial Identification

3.4.7.1 Bacteria Identification using the API20E

Different types of colonies from the media plates were picked and purified. Pure samples were used to inoculate strips of the API20E for *Enterobacteriacea* identification.

3.4.7.2 DNA extraction, PCR and sequencing

The scrapings from a fouled membrane were subjected to DNA extraction using the Zymo soil DNA extraction kit. The DNA was stored at -20 ◦ C until further processed. PCR amplifications for sequencing were performed with the standard bacterial primer pair 27F and 1492R.

3.4.7.3 PCR set up

Primers:

27F: GAGTTTGATCCTGGCTCAG

1492R: GGTTACCTTGTTACGACT

PCR Mixture: 12.5 µl 2X econotaq mastermix , 9.5 µl ddWater , 1 µl of each primer (10 µM) , 1 µl genomic DNA (or some of the colony from the mixture)

Run Mixture: 95°C for 5 min, Then 10 cycles: 95°C for 30 sec, 50°C for 30 sec, 72°C for 2 min

Then 15 cycles:

- 95°C for 30 sec
- 52°C for 30 sec
- 72°C for 2 min

Another 20 cycles:

- 95°C for 30 sec
- 55°C for 30 sec
- 72°C for 2 min

Final extension: 10 min

The PCR product was sequenced at Inqaba Biotech. The sequences obtained were analysed using the CLC main workbench 5 software.

4. RESULTS AND DISCUSSION

Biofouling, which is the accumulation of biomass on a surface by growth or deposition to such a level that causes operational problems, is difficult to quantify. However, diagnosis can be justified if a relation is found between the encountered operational problems and biomass accumulation as determined within adequate parameters (Vrowenvelder & Van der Kooj 2001).

The Sasolburg RO plant experienced fouling problems related to RO membranes being cleaned at too high frequencies and eventually being replaced. Although the main cause of these problems was unknown, biological growth was suspected as being the primary contributor. To assess the level at which microorganisms impacted on the fouling, an analysis of the operating conditions, pre-treatment system and the water quality was done.

The water analysis was done by analysing water samples from the feed holding tank through the pre-treatment system, the feed, permeate and brine. An autopsy of a fouled membrane was also carried out to determine the extent at which biofouling had contributed to the deterioration of the membranes. It was also important to do an autopsy so as to give a comparison to the results of water analysis.

4.1 Analysis of the operating conditions of the plant

The RO plant installation at Sasol, Sasolburg is composed of four independent units (RO1, RO2, RO3 and RO4) of equal size. For the purpose of this study focus was on the unit 2 (RO2). An analysis of the operating conditions of RO2 indicated a progressive increase of pressure drop as presented by DP1 (Figure 4.1). Until the end of April the value of the pressure drop remained in the limit of 10 kPa, and then it underwent a rapid increase until it reached 22 kPa. On the other hand DP 2 indicated a decrease in the permeate pressure, which implies a decrease in the product output. This phenomenon can be explained as a sign of membrane fouling.

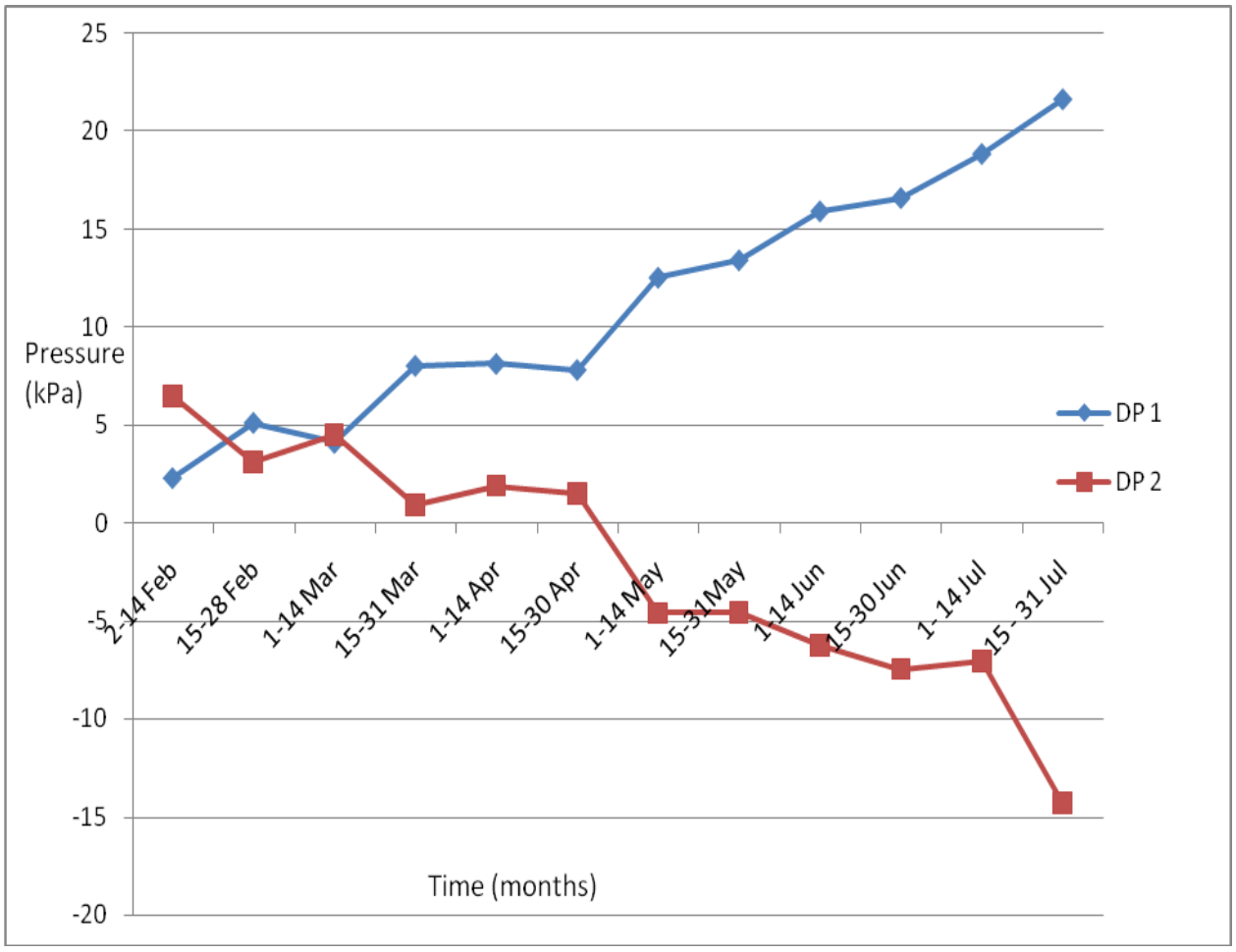

Figure 4. 1 Reverse osmosis pressure drop for RO2

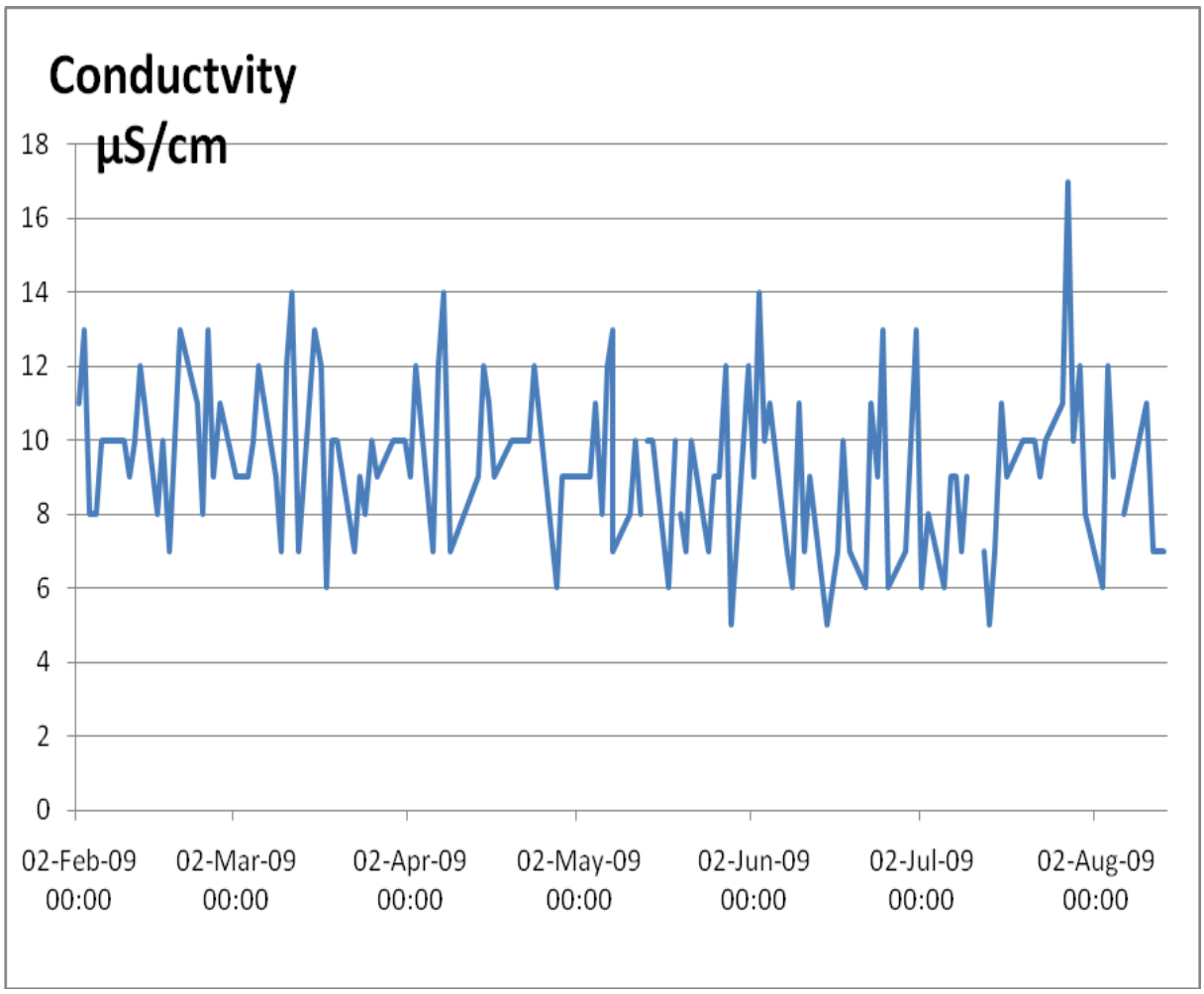

Figure 4. 2 Permeate conductivity for RO2

Figure 4.2 indicates a conductivity of permeate over a period of six months. Conductivity in the permeate increased to beyond 13 mS/m which is the limit in this RO membrane specification. An increase in conductivity indicates decrease in the quality of permeate, which is also a sign of fouling.

The decreasing of performances in the RO was therefore due to problems of which their level, nature and origin could be understood through sampling and analysis of water in the operating plant as well as a membrane autopsy of a fouled membrane.

4.2 Water analysis

According to Characklis (1990), water quality is a critical factor in assessing or controlling biofouling in virtually any system. Hence, six sampling points were analysed. These sampling points included the feed holding tank (FHT), before sand filter (BSF), after sand filter (ASF), feed, permeate and brine. Sampling was done fortnightly for a period of eight months.

4.2.1 Analysis of the feed holding tank, before sand filter and after sand filter samples

The analysis of the first three sampling points, (FHT, BSF and ASF), indicated an improvement in the water quality from one sampling point to the next. The figure below shows that pH is stable at around 8.2.

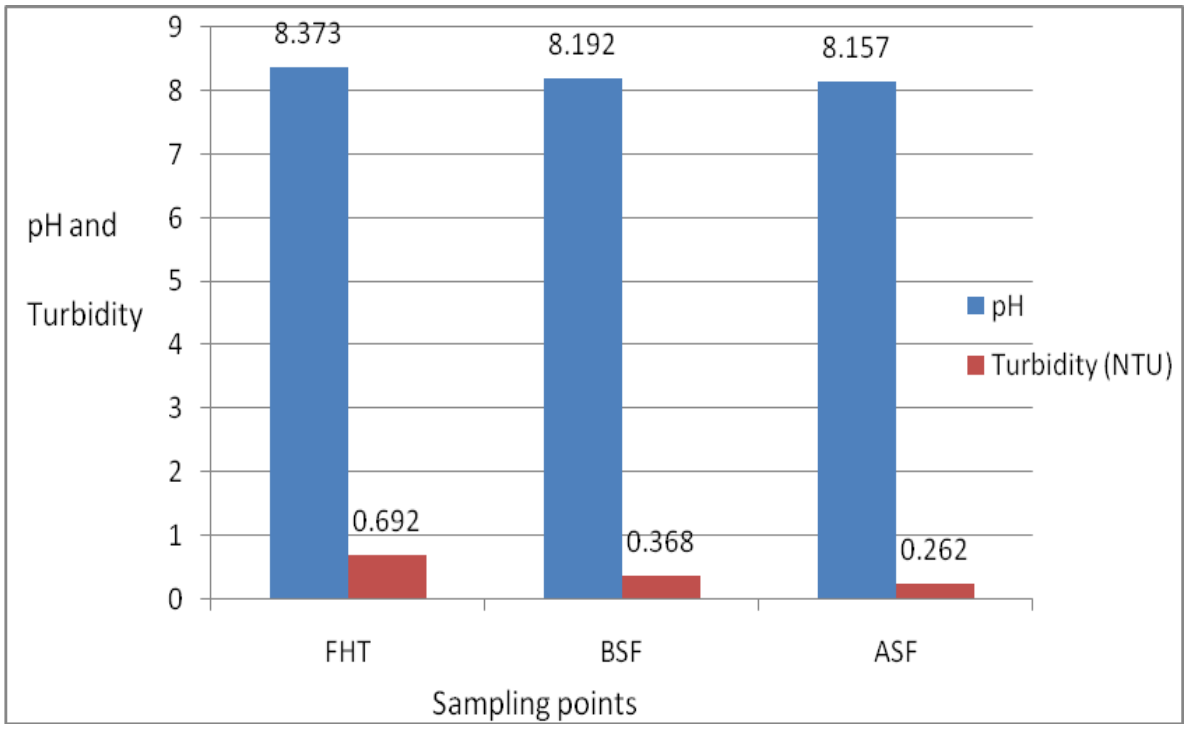

Figure 4. 3 Average pH and Turbidity for FHT, BSF and ASF sampling points

Figure 4.3 also indicates a decrease in turbidity of water by 53% from FHT to BSF and further decrease from BSF to ASF. The decrease in turbidity shows that the sand filters play an important (pre-treatment) part in improving the quality of water

before it gets to the RO membranes. This is supported by a decrease in the number of colony forming units at each stage of the sampling points as shown in Figure 4.4.

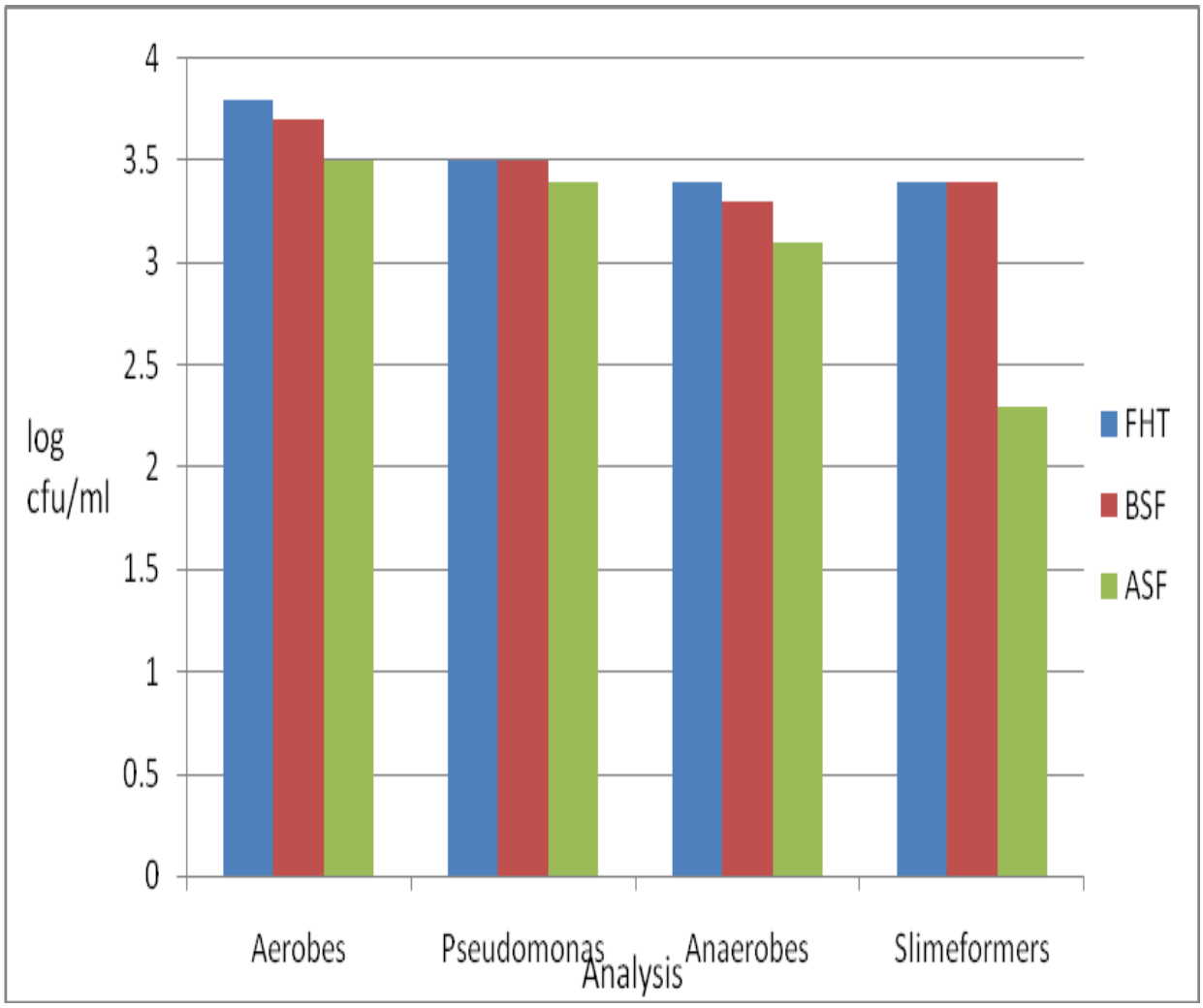

Figure 4. 4 Bacterial counts for FHT, BSF and ASF sampling points

Although what happens between the first three sampling points is important in indicating the effectiveness of the pre-treatment system, ultimately the quality of feedwater is the one that determines the extent and the type of fouling. Hence the sampling points were reduced to feedwater, permeate and brine.The first three sampling points were eliminated after three months of sampling.

4.2.2 Analysis of feed, permeate and brine samples

The table below (Table 4.1) indicates the averages for analysis of water samples from the RO plant. pH values of the feedwater were found to be stable at around 8.

Table 4. 1 Average chemical analyses of water samples from RO plant

	Feed water	Permeate	Brine
pH	8	7.26	8.15
Turbidity(NTU)	0.25	0.16	0.43
Conductivity(µS/cm)	121	6	607
TDS (ppm)	81	3.8	405
Salinity	0.09	0	0.435
Chemical Oxygen demand (COD) (mg/l)	13	0	40
Biological Oxygen Demand (BOD) (mg/l)	4.6	4.8	-

This is due to that pH of the feed was being stabilized by addition of hydrochloric acid. On the other hand permeate pH was found to be almost neutral as this is cleaned water while brine pH was high at 8.15 due concentration of the impurities rejected by the membrane.

Turbidity values were found to be low in all samples, (feed-0.25 NTU, permeate-0.16 NTU and brine-0.43).This was expected as the water samples had gone through pre-treatment (sand filtration), and had no cloudiness.

The organic pollution in terms of COD and BOD in the feedwater was 13mg/l and 4.6mg/l respectively. In the permeate COD and BOD were found to be 0 and 4.8mg/l.The absence of COD in the permeate indicates that almost all chemical components were retained on the membrane surface. On the other hand, BOD seems to have increased indicating that there is still food or organic carbon that bacteria can oxidize.

The RO feed-water contained approximately 20-fold more total dissolved solids (TDS) than the permeate water. The TDS correlates with the conductivity, In

particular, the high values of conductivity in the feed and brine samples are supported by high values of TDS whereas for permeate both values are low.

Dissolved matter may consist of highly soluble salts, such as carbonates, sulphates and silica. Suspended solids, on the other hand, may consist of inorganic particles, colloids and biological debris such as bacteria and algae. Organic carbon and other nutrients then provide energy and building blocks for cell synthesis by microorganisms present in the water system. Organic and inorganic material may also add to the accumulation by collecting in the adsorptive biofilm.

The high level of TDS in the feedwater is problematic, since during the RO process, the volume of water decreases and the concentration of dissolved ions and suspended particles increase. Suspended particles may settle on the membrane surface, thus blocking feed channels and increasing friction losses (pressure drop) across the system. Sparingly soluble salts may precipitate from the concentration stream, create a scale on the membrane surface and resulting in lower water permeability through the RO (flux decline), (Hydranautics 2001).

Therefore, one of the critical factors affecting the development of the biofilm is the water quality, e.g., pH, BOD, presence of organic and inorganic nutrients. Once microorganisms have found an environment to which they are suited, growth will proceed unless conditions in the system become hostile.

In conclusion, the chemical analysis (Table 4.1) values are in a range that can allow the growth and survival of microorganisms. For example, pH ranges between 7 and 8. Total Dissolved solids (81 ppm in feedwater) indicate that there are nutrients available to support the growth of microorganisms.

4.2.3 Elemental analysis

Elemental analysis indicated the presence of chlorine, magnesium, calcium, silica and potassium. Nitrates, zinc and copper were also present in small amounts (Figure 4.5). From the analysis of the feed it can be seen that especially magnesium, calcium, silica, chloride and potassium are retained on the membrane surface. These were also later found to be some of the constituents of the foulants on the membrane surface. Silica was deposited on the membrane surface, most probably as a result of sand particles in the feed. These results correlate with what has been found in other studies (Butt, Rahman & Baduruthamal 1997; Schneider *et al.* 2005 and Tran *et al*. 2007).

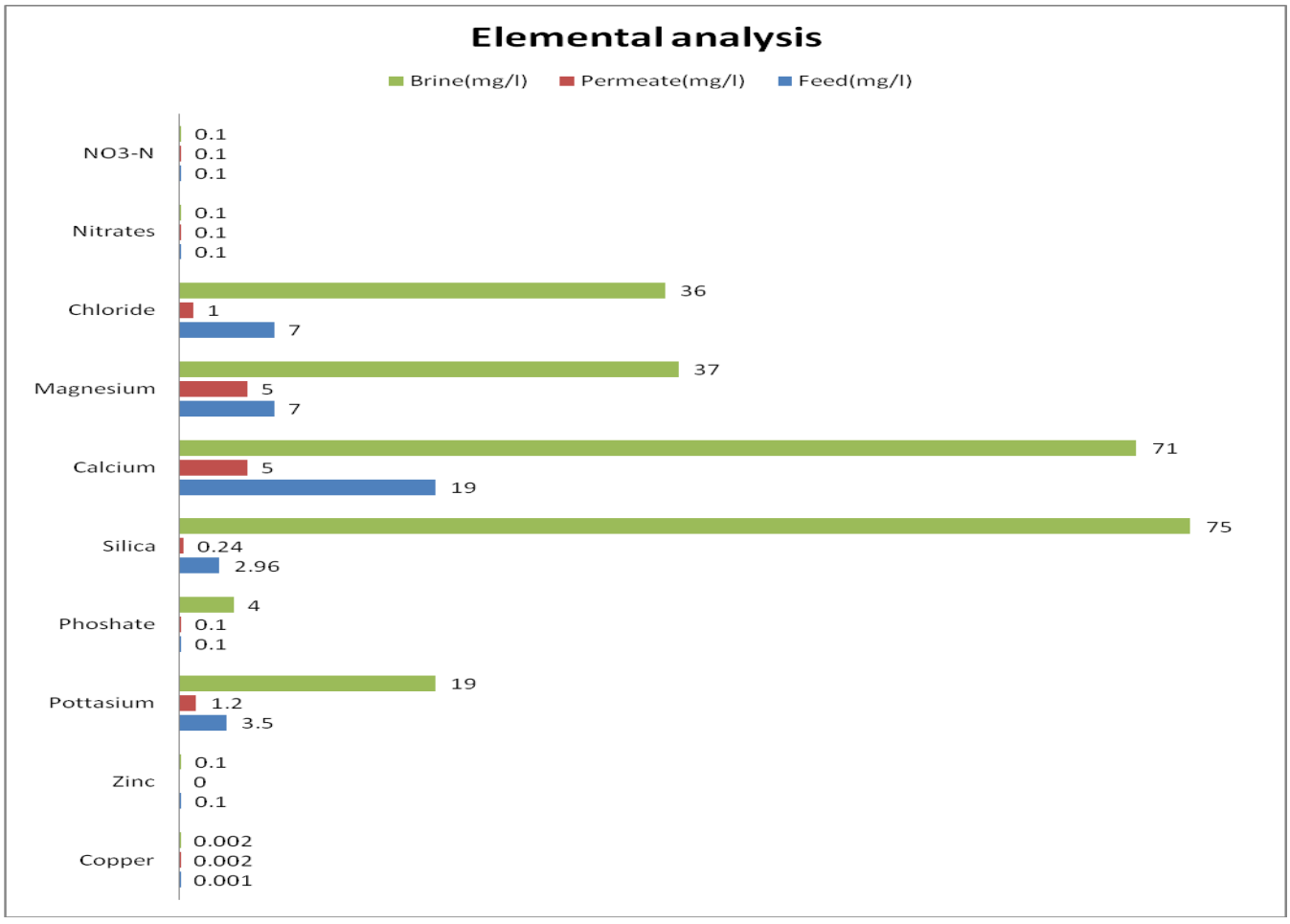

Figure 4. 5 Elemental analysis of water samples

In addition, generally, the presence of negative ions, including bicarbonate, silicate and sulphate, in the RO feed is important for the precipitation of various compounds. Common deposits found on fouled RO membranes include aluminium silicates, carbonate compounds of calcium and magnesium, and sulphate compounds of calcium, strontium and barium. Metal ions, most notably calcium ions, may also form complexes with natural organic matter, giving rise to the subsequent formation of intermolecular bridges among organic foulant molecules and enhanced membrane fouling. Also, when a fouling layer has developed on the membrane surface, the layer may entrap and hinder back diffusion of dissolved salt ions, resulting in an increase in concentrations of salt ions near the membrane surface (Tran *et al*. 2007).

4.2.4 Microbiological analysis

Microbiological analysis was done for water samples, that is, feed water, permeate and brine to enumerate and identify the microorganisms present. Bacteria are theoretically physically incapable of passage from the feed-water surface of the RO membrane to the permeate surface. In actual practice, microorganisms can gain access to the permeate water collection system via small leaks in the rubber O-ring seals which connect adjacent membrane modules or via microscopic holes or other imperfections in the membranes (Ridgway *et al*. 1983).

However, there is no correlation between the cell numbers in the water phase and the site and extent of biofilms. Cells can be eroded by shear forces, they can leave the biofilms as a bunch of cells and eventually parts of the biofilms can be sloughed off, giving rise to substantial increase of cell numbers in bulk. All these events occur randomly, (Flemming 1997).

4.2.4.1 Microbiological enumeration

The feedwater sample accounted for high bacterial counts between log 1.5 cfu/ml and log log 3.8 cfu/ml, (figure 4.6).On the other hand, the permeate sample showed reduced bacterial counts between log 1.4 cfu/ml and log 2.5 cfu/ml, (figure 4.7).

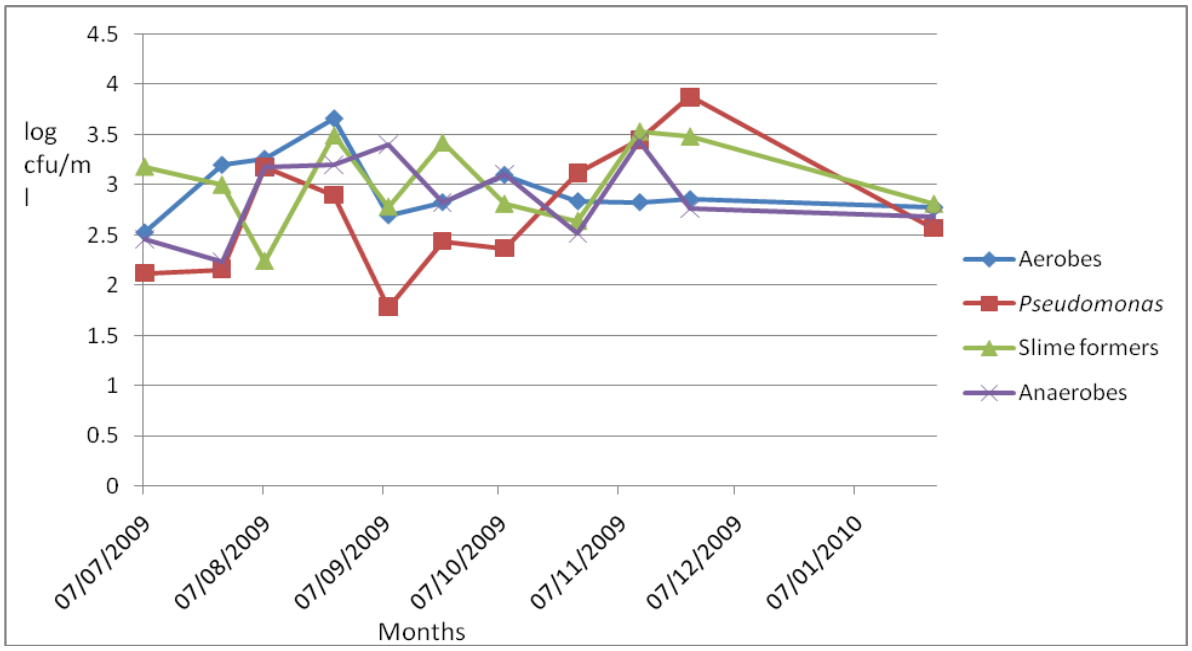

Figure 4. 6 Feedwater counts

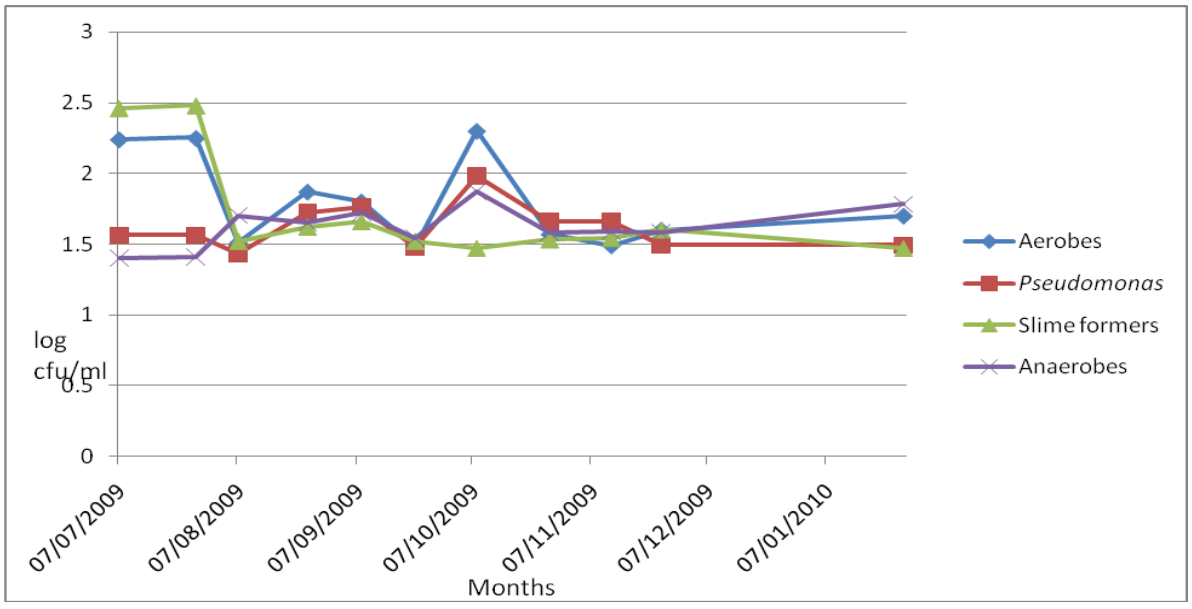

Figure 4. 7 Permeate counts

It was aslo noted that in the feedwater the counts differed randomly with the sampling dates while the permeate sample show a decrease in counts after approximately four weeks which could be atributed to cleaning in place(CIP treatment).The permeate counts aslo remained between the range log 1.5 cfu/ml and log 2cfu/ml from mid October due to the replacement of the membrane.

The brine sample had the highest counts which ranged between log 2.5 cfu/ml and log 4 cfu/ml.This could be attributed to high concentration of nutrients in the brine water.

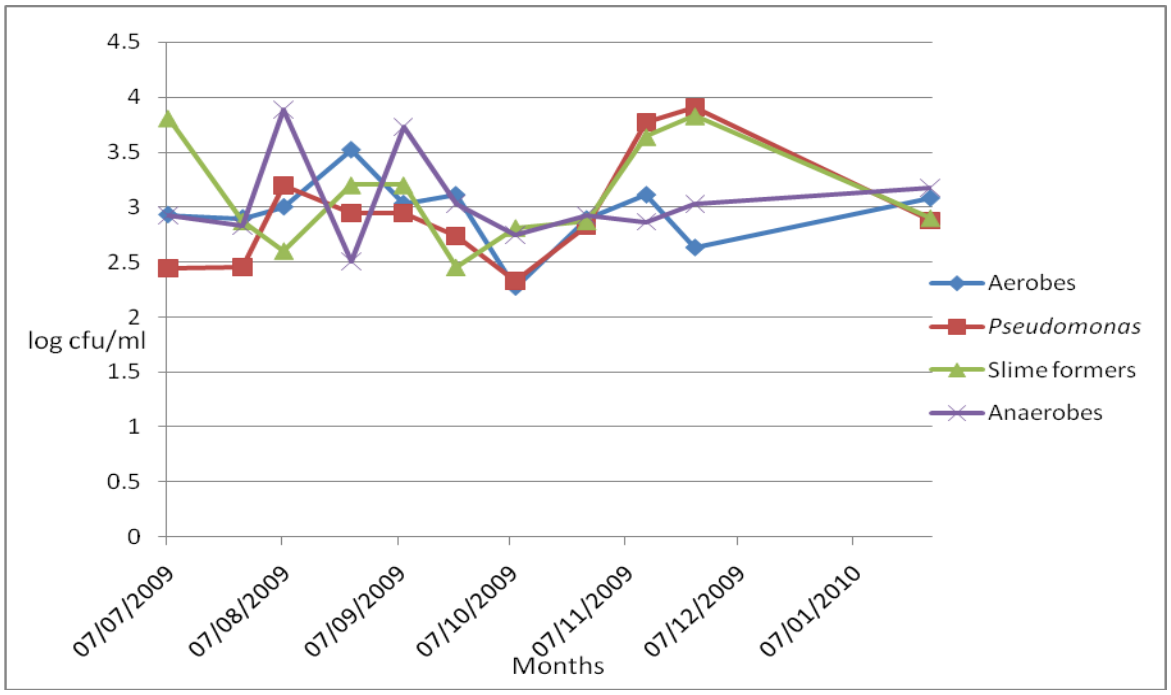

Figure 4. 8 Brine counts

It has been concluded from other studies (Flemming *et al*., 1997; Baker and Dudley, 1998; Herzberg and Elimelech, 2007) that bacteria base load of $10^{2\ to}\ 10^{4}$ cfu/ml present on every membrane surface without causing operational problems, therefore if the bacterial load is more than 10^{4} cfu/ml, the biofilm will cause operational problems.

4.2.4.2 Statistical analysis of water microbial counts

Microbiological analysis data was analysed statistically using the QI Macros software.

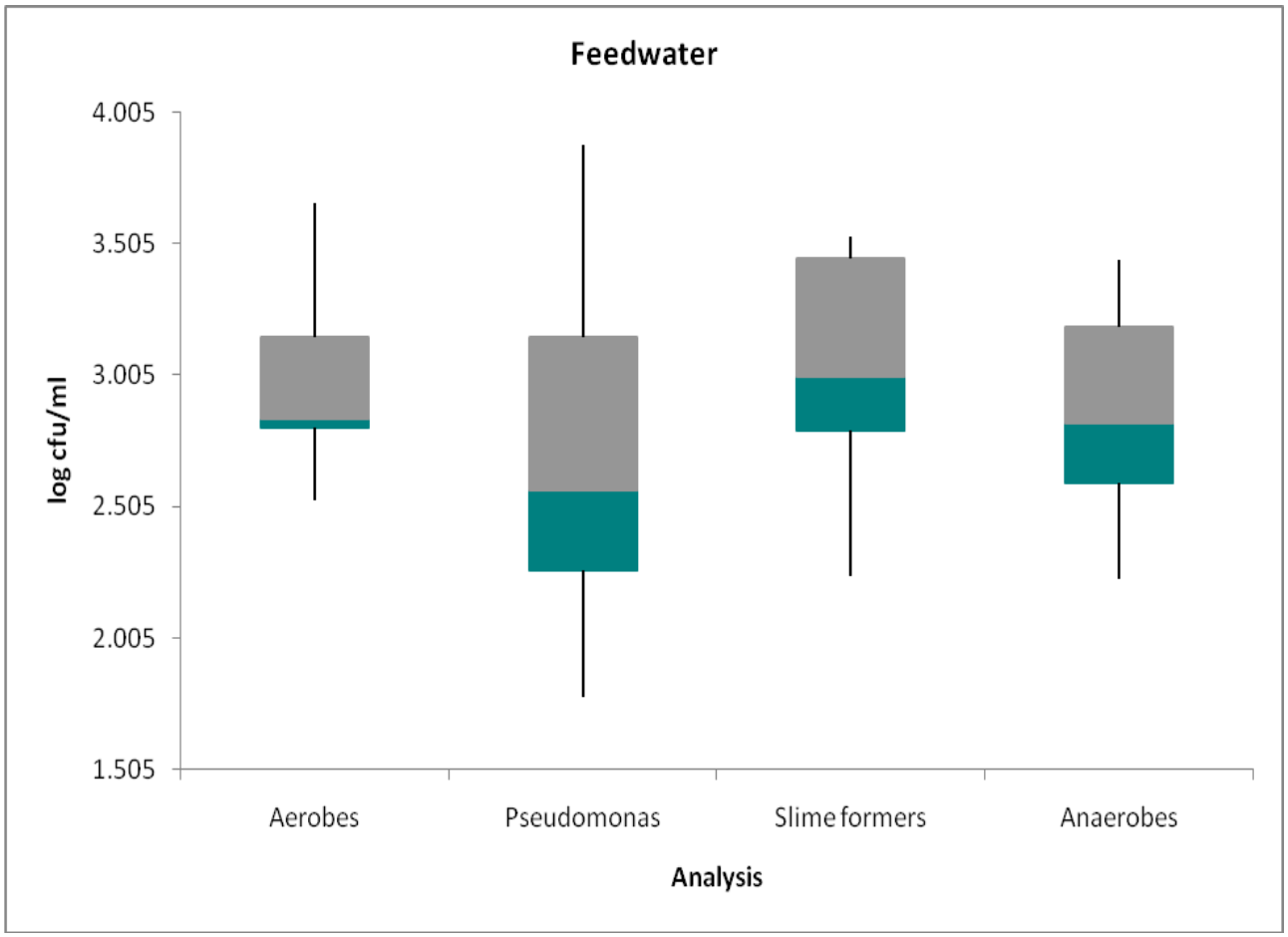

Figure 4. 9 Feedwater counts

Microbial analysis of the feed water indicated that there were a high number of microorganisms in all samples as shown by most values being above average in all the samples. The analysis also indicated wide count variability in the *Pseudomonas* compared to aerobes, slime formers and anaerobes. Feed water analysis also indicated high numbers of *pseudomonas* and slime formers and low values of aerobes and anaerobes, (figure 4.9).

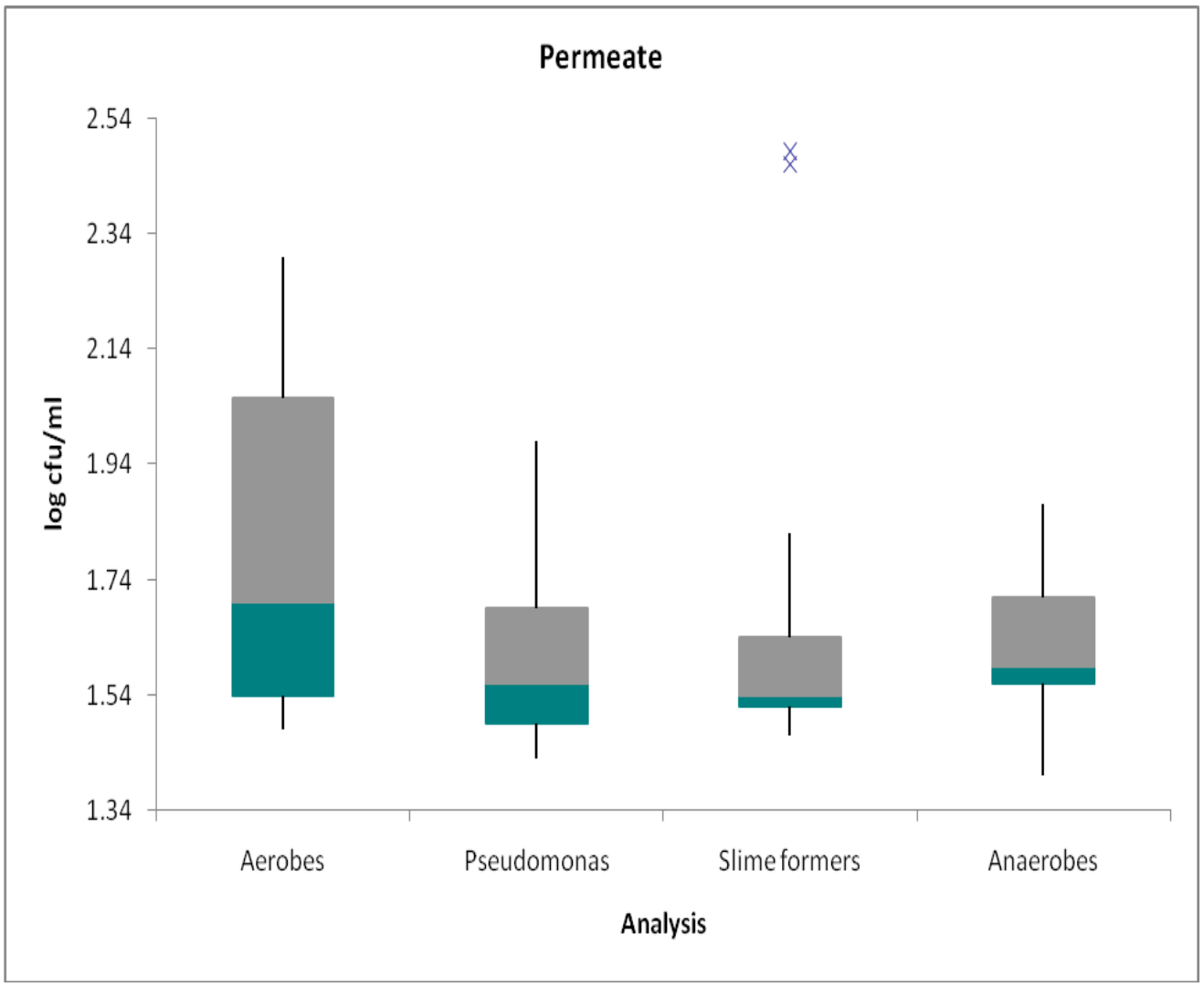

Figure 4. 10 Permeate water counts

On the other hand the permeate water analysis indicated high values of aerobes and low values of pseudomonas and slime formers, (figure 4.10). Aerobes also indicated wide count variability.

In the brine samples, slime formers and *Pseudomonas* were found to have high counts whereas aerobes and anaerobes had low values. Brine samples were also found to have a high number of outliers compared to feed and permeate samples, as indicated in figure 4.11.

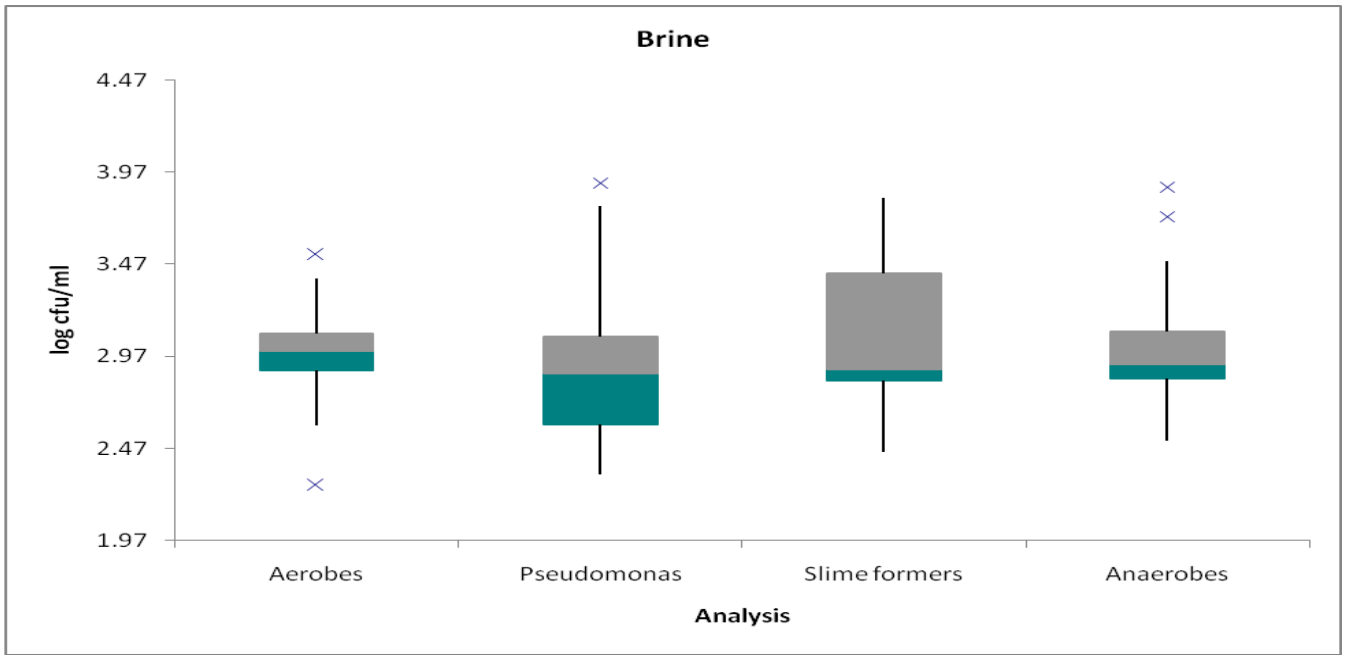

Figure 4. 11 Brine counts

A biofilm can be composed of aerobic (requiring oxygen) and anaerobic (not requiring oxygen) bacteria. Oxygen-requiring bacteria actually form a layer over the non-oxygen-requiring bacteria. Thus, the anaerobic bacteria exist in a low-oxygen microclimate while the aerobic bacteria have a neighbour that's not competing for the oxygen they require. Meanwhile, they both benefit from the protective nature of a biofilm. Hence the significant counts of anaerobes were found to be present in the samples, (Barnes and Caskey, 2002).

In summary, the statistical analysis indicates that from all water samples the dominant group of microorganisms are the *Pseudomonas*. It is also clear that the feed and the permeate samples had few outlier values indicating a uniformity in the feed and permeate water samples as well as low sampling errors. On the other hand, the brine samples had a high number of outliers most probably due to the high concentration of the rejected (retentate) species which can contribute to a sharp increase of microorganisms at some given point.

4.3 Membrane autopsy

In the Sasol RO plant, the need to replace the RO membrane element was necessitated by the conditions that were prevailing in the plant, as indicated by Figures 4.1 and 4.2. A fouled RO membrane element (X-20, Foulgard Technology, Figure 4.12) was retrieved from RO2 unit, wrapped in a plastic and taken to the laboratory for analysis.

Figure 4. 12 Fouled membrane sample

4.3.1 Visual inspection of the fouled membrane

In the laboratory the fouled membrane was cut open and visual inspection conducted. Visual inspection of the surface of the membrane showed no signs of physical damage. The O-rings and the glue lines were still intact even though there were some brown deposits on the surfaces. Light–brown slimy deposits were also

observed on all sections of the surfaces of all membrane leaves, Figure 4.12. The support fibre showed no indication or evidence of fouling, through visual inspection.

Figure 4. 13 Section of fouled membrane

4.3.2 Macroscopic analysis of membrane surface

Optical microscopy

Optical microscope (OM) was used to produce images of the membrane sample at magnification levels much higher than possible with naked eye but lower than the levels achieved with SEM. A zeiss stemi “2000” stereo microscope and zeiss axioskop phase contrast microscope with axiovision 4 software were used.

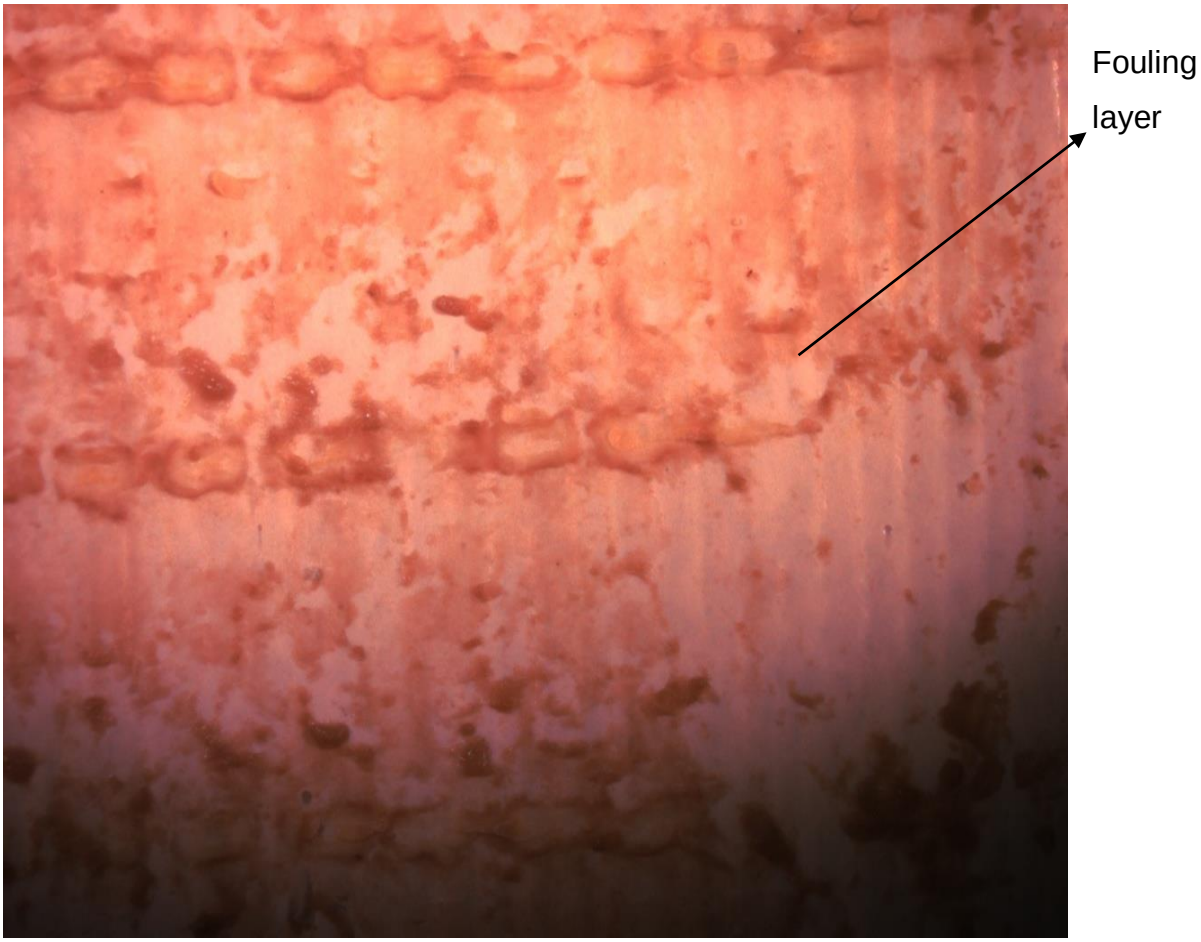

Figure 4. 14 Fouled membrane surface at X100 magnification

The fouling layers on the membrane appeared similar on all surfaces each having a pattern superimposed by the feed spacers that separate adjacent envelopes in an intact RO membrane element. The light brown layer could be easily scraped off the surface with a scalpel. The loose structure of the biomass present on the membrane surface suggests that it had been produced rapidly and recently.

Figure 4. 15 Fouled membrane surface at X1000 magnification

Figure 4.15 shows a heavy fouling layer covering the membrane. This layer may consist of viable bacteria, fungi and organic debris, likely to include polysaccharides excreted by some of the forms of bacteria present. Inorganic components may also be present.

The feed spacer was not as heavily fouled as the membrane surface; patches of the foulant however could be clearly seen at X100 magnification (figure 4.16).

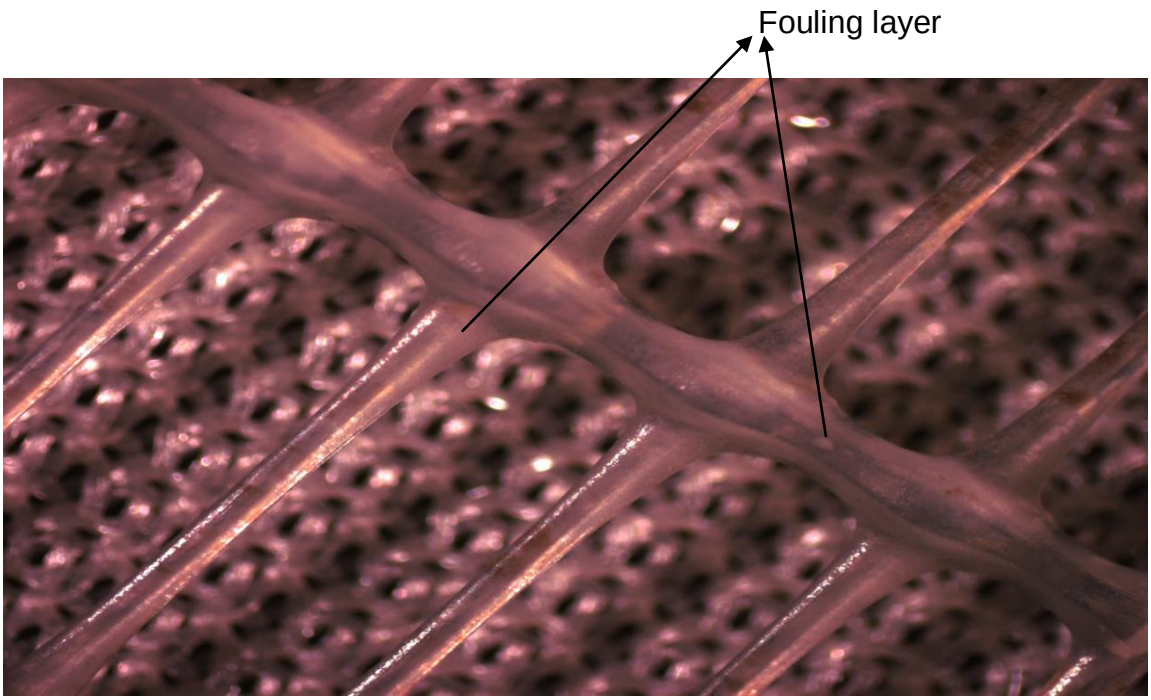

Figure 4. 16 Feed spacer at X1000 magnification

4.3.3 Alcian Blue Stain

A section of the membrane leaf was stained with Alcian Blue stain to confirm the presence of the biofilms. As shown below, the blue stain was retained after destaining indicating the presence of biofilms.

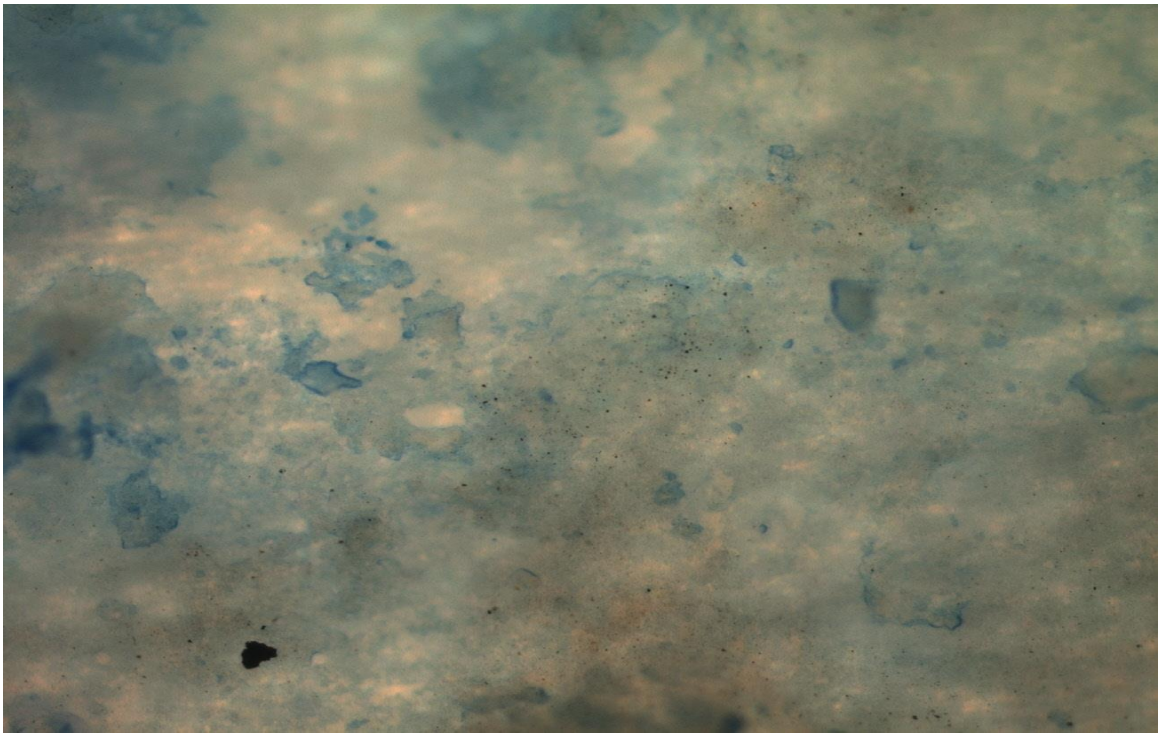

Figure 4. 17 Alcian Blue stain at X1000 magnification

In a study on adhesion of microorganisms to polymer membranes (Kochkodan *et al*. 2008) it was shown that the fluxes of membranes with deposited native *Psuedomonas putida* microorganisms decreased with time of membrane exposure to natural surface water due to the growth of bacteria on the membrane surface.
Therefore presence of biofilm on the fouled membrane sample indicates that there was accumulation of microorganisms on the surface of the membrane, hence a potential of biofouling.

4.3.4 Bacterial enumeration

The total numbers of colony forming units (cfu) detected in the fouling material scraped from the membrane surface area of 100 cm^2 is shown below on Table 4.2. The membrane sample consisted of sixteen leaves and three leaf samples were used. The samples were taken from the first leaf (top layer), eighth leaf (middle layer) and finally on the sixteenth leaf (Bottom layer).

Table 4. 2 Bacterial counts (cfu/cm^2): membrane sample

Membrane sample	*Pseudomonas*	Aerobes	Slime formers	Anaerobes
Top layer	2.9 X 10^4	1.6 X 10^4	8.1 X 10^3	1.6 X 10^4
Middle layer	2.4 X 10^4	2.9 X 10^4	3X 10^4	4.9 X 10^4
Bottom layer	2.7 X 10^4	2.4 X 10^4	8 X 10^3	3.2 X 10^4

The total number of cfu on this membrane surface is higher than the cfu in the water samples but low compared to the amount of cfu detected in other membrane autopsy studies.

Plate count values ranging from 2X 10^5 to 6X10^7 Cfu/cm^2 have been reported for fouled RO membrane (Ridgway *et al*., 1983; Flemming *et al*., 1997; Baker and Dudley, 1998; Hu etal., 2006). The large variability of viable counts in membrane fouling studies is probably caused by the difficulties associated with analysis of the microbiota of environmental biofilms. Viable counting on laboratory media requires separation of cells from the matrix without damaging the organisms. The relatively mild sonication or other separation methods employed may not result in the complete separation of all cells. Cell clump deposited on semisolid agar media will result in a single colony count for that clump and may , therefore, cause a considerable underestimation of viable cell populations in membrane biofilms, (Schniider *et al*.,2005).

An important factor contributing to low counts is that cells in bulk water are adapted to life in low nutrient environments. Bio-available carbon values in such waters are typically below 1-2 mg/l, as opposed to the relatively high carbon concentrations employed in laboratory media such as R2A.The high carbon concentrations select for organisms that are capable of growing in high nutrient environments, which may represent only a small fraction of bacterial population in the bulk water. Typically less than 1% of microbial cells in membrane fouling layers are recoverable as viable counts. The viability of cells not recovered on laboratory media is unknown (Schneider *et al.* 2005).

4.3.5 ATP analysis

Adenosine Triphosphate is a microbial "energy'' storing compound, which is indicative of viable organisms. Relatively low concentrations of ATP were detected in the fouling material indicating the presence of small number of viable microorganisms, as shown in Table 4.3.

Table 4. 3 ATP analysis

Sample	cATP	BSI%	ABR
Top layer	37	31	2
Middle layer	31	12	2
Bottom layer	31	41	1

cATP (cellular ATP) is the concentration (quantity) of living organisms.
BSI (Biomass stress index) is the stress level (quality) of the living organisms.
ABR (Active Biomass Ratio) is the percentage of solids that are alive.
(Calculations done as in Appendix D).
The low counts and the small number of viable microorganisms on the membrane sample may be also attributed to the cleaning in place (CIP treatment) that was done on the plant two weeks before the fouled membrane was taken out. However, the mere presence of the microorganisms and an average of 33% concentration of

cellular ATP is an indication that an important biological activity was present on the fouled membrane sample.

4.3.6 Microbial identification

Morphology of isolated microorganisms

The slide images below show microorganisms that were found to be present in the fouled membrane sample. Microscopic investigation of the biofilm obtained from the membrane showed that most of the microorganisms present on the membrane surface were the rod shaped microorganisms.

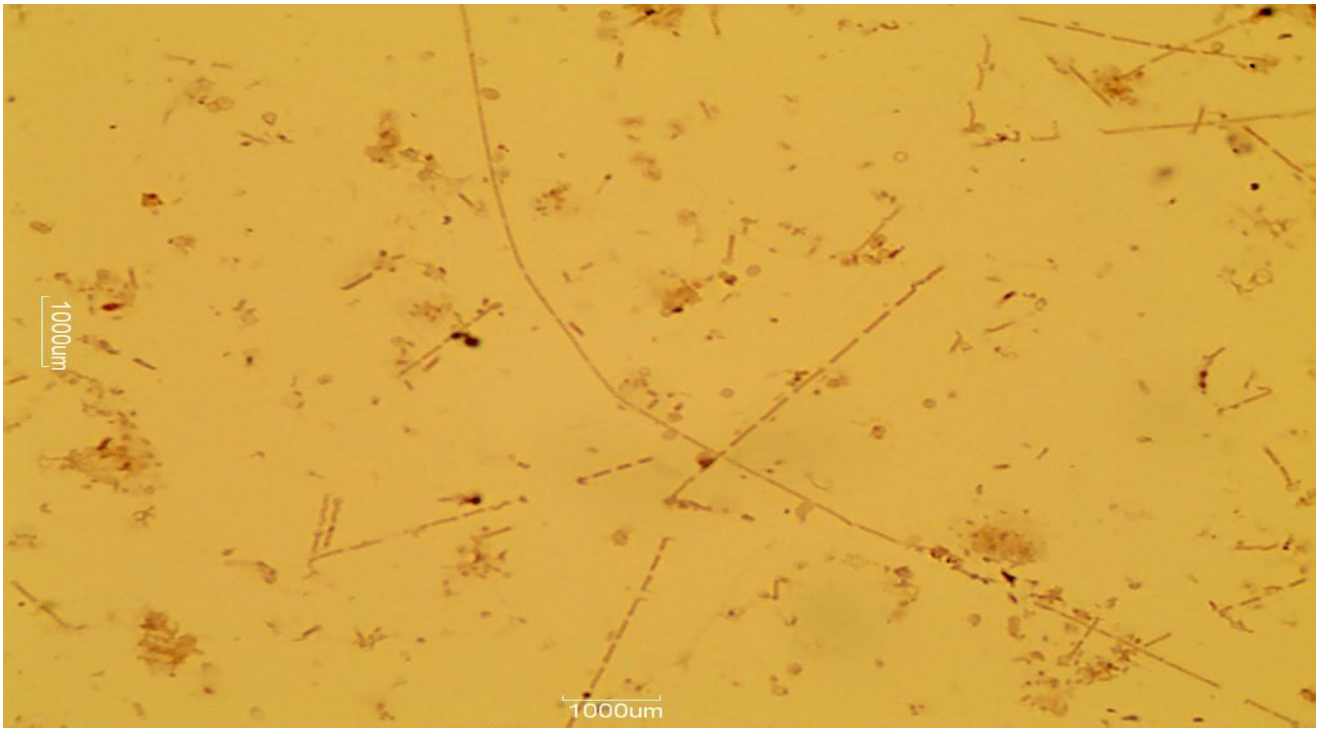

Figure 4. 18 Gram stain: rod and cocci shaped bacteria

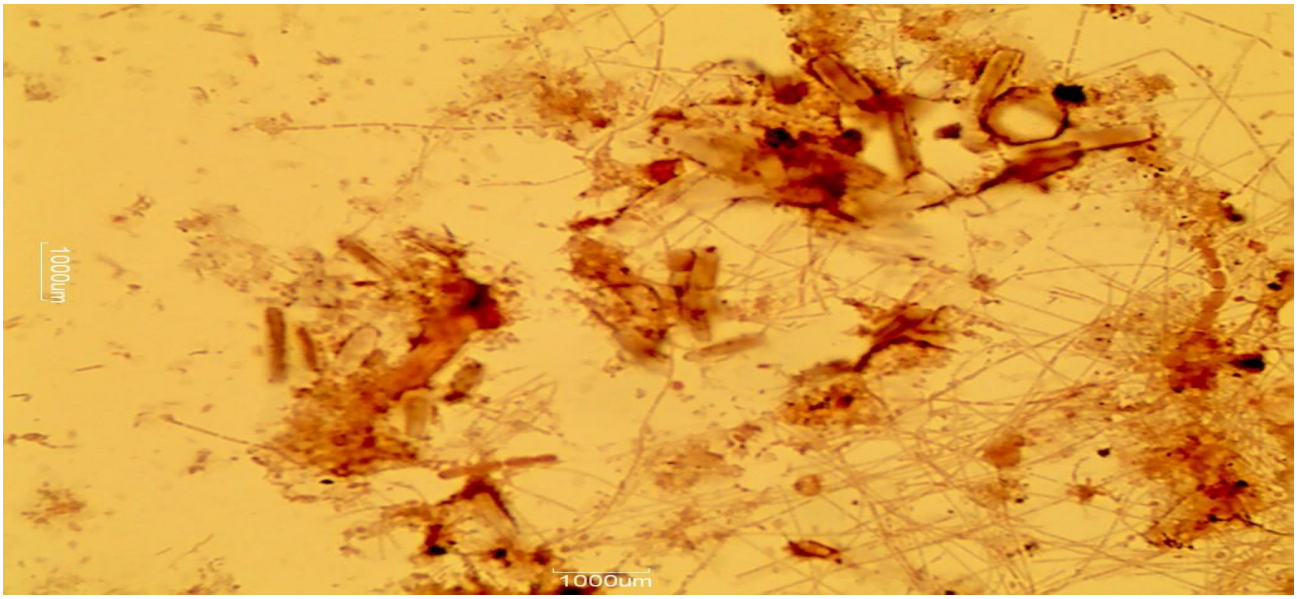

Figure 4. 19 Gram stain: rod, cocci shaped bacteria and diatoms

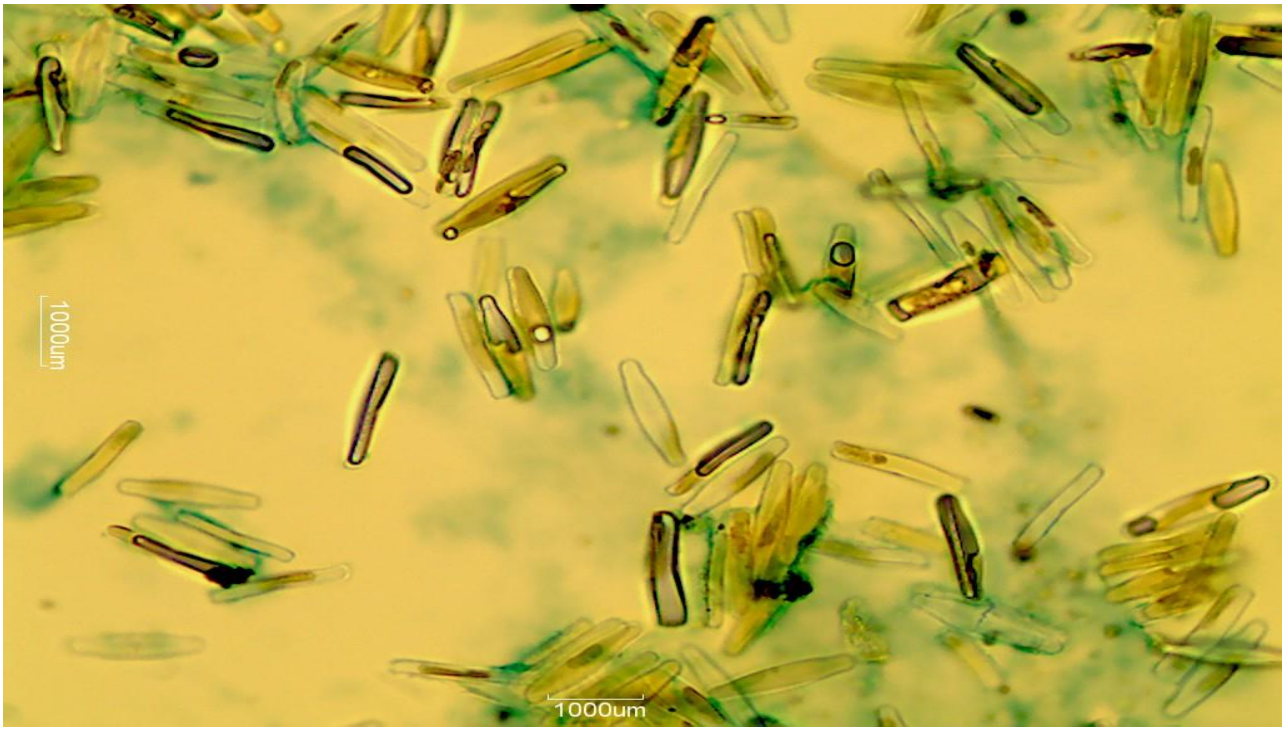

Figure 4. 20 Alcian blue stain: Diatoms

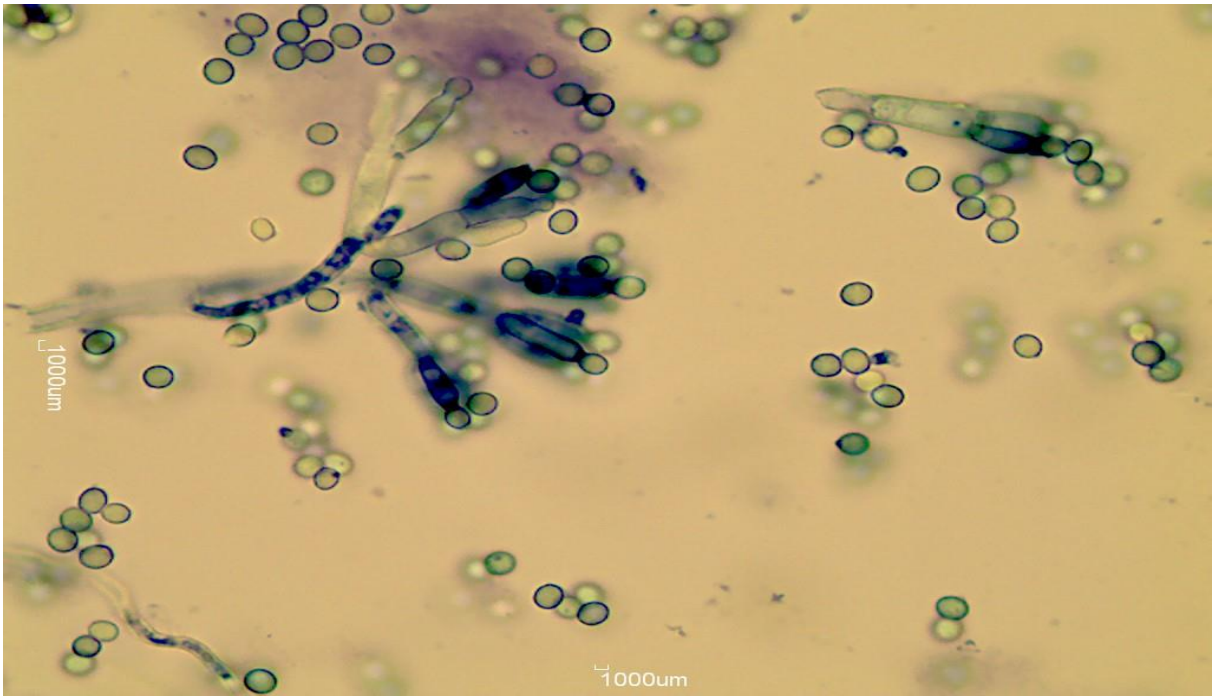

Figure 4. 21 Methylene blue stain: Fungi

In addition high numbers of diatoms and fungi were present on the membrane surface as shown in figure 4.20 and 4.21 respectively.

Identification of bacteria using API20E

Pure bacterial isolates were recovered from PCA and R-2A media. These were identified using the API20E test kit. A total of five generic groups were identified. These included the *Serratia, Enterobacter, Klebsiella, Chromobacterium and Psuedomonas*. The major bacteria genera associated with the membrane surface were the *Psuedomonas.*

4.3.7 Chemical analysis

Analysis of the membrane foulant indicated the presence of nitrates, silica and calcium. The elemental components were also found to be present in the feed water. This shows that these elements were retained on the membrane surface.

The presence of silica in membrane autopsy has been recorded in other studies. Silica is the second most common foulant in autopsy.

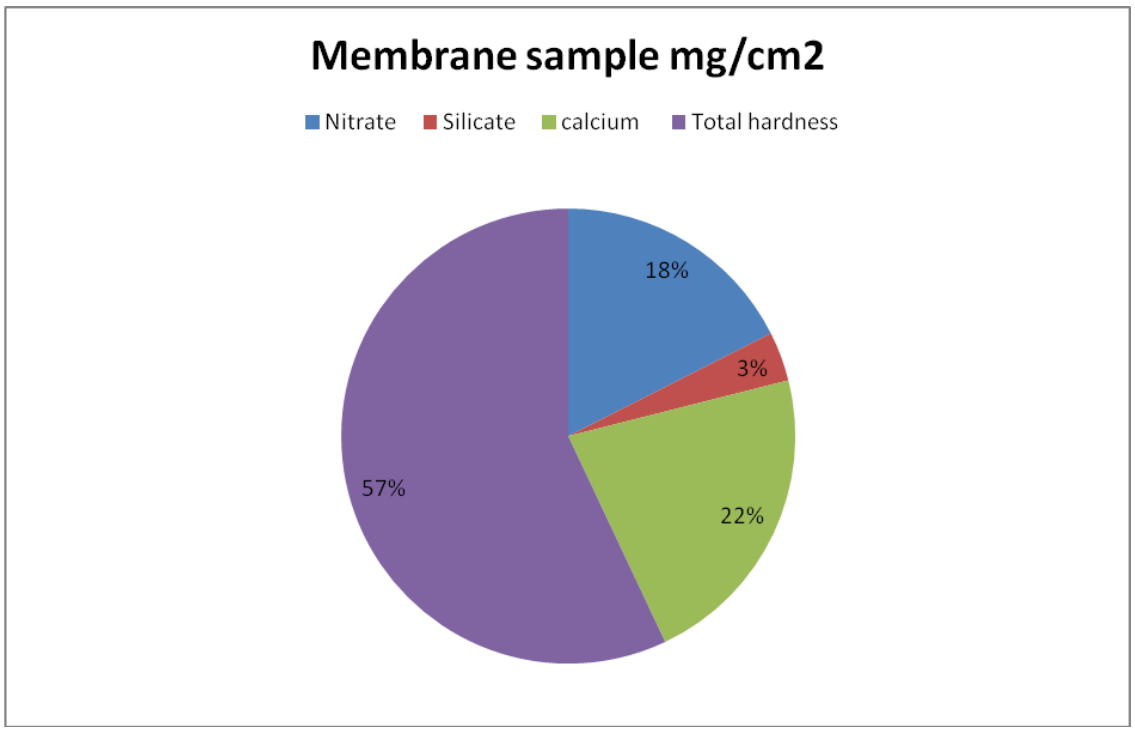

Figure 4. 22 Chemical analysis of the membrane sample

Presence of trivalent cations such as Al^{3+} and Fe^{3+} encourages the precipitation of silica. In a study by Graham *et al. (*1989), a significant amount of silica (along with Al, Ca, Mg and Fe) in the membrane deposit was observed, even though precipitation of silica was not expected. They concluded that at least part of the deposit could be complex silicates of these cations.

Comparison of the feedwater and permeate samples indicate that some of the elements are retained on the membrane surface (see appendix B). For example total hardness for the feedwater was at 26% and permeate at 16%, indicating that 62% of the salts were retained on the membrane surface. Figure 4.17 shows a total hardness of 57% hence supporting the fact that there is retention of elements on the membrane surface.

4.3.8 DNA extraction, PCR and Sequencing

DNA samples were extracted from water samples and from the membrane biofilm. Extracted DNA was confirmed by running a 1% agarose gel electrophoresis. The amplified product was also run on a 1% agarose and the picture was taken using a Gel documentation system (Figure 4.23).

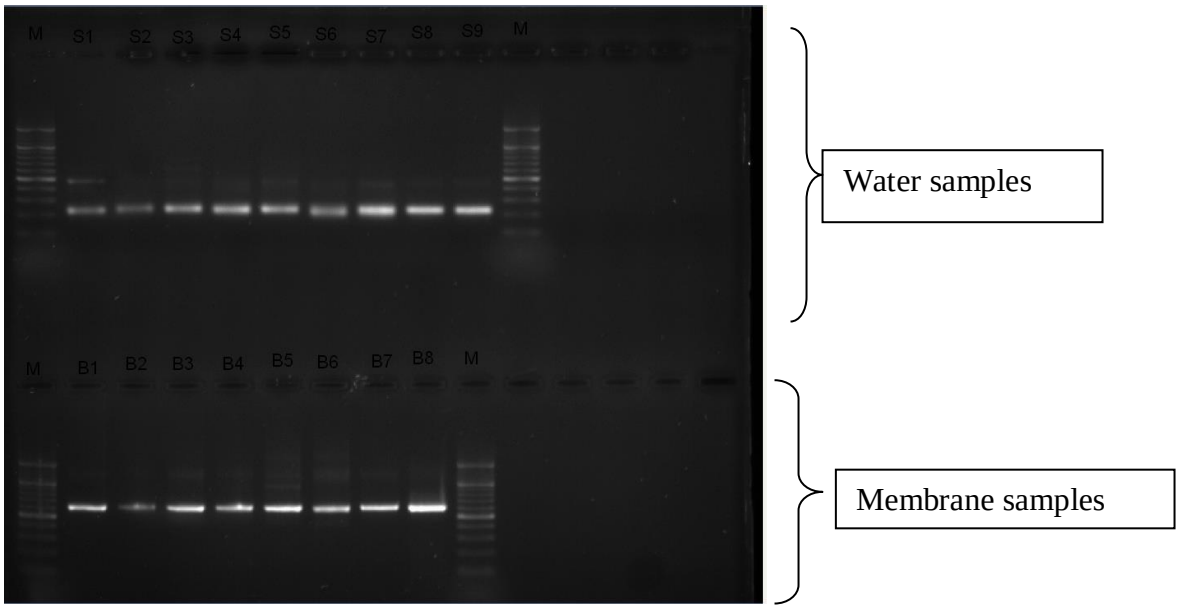

Figure 4. 23 Amplified DNA from water and membrane samples

The PCR product was then sent for sequencing. The results from sequencing indicated that *Pseudomonas species* was the dominant organism present in the water and biofilm samples.

The following bacteria were found to be dominant in water samples:

Feedwater: uncultured *Psuedomonas sp*,

Permeate: uncultured *Pseudomonas*, *Dugella sp* and *Oxalobacteria*,

Brine: *Acinobacter tandoii, Acinobacter sp* and *Bacillus sp*.

From the membrane biofilm sample the dominant bacteria was found to be *Pseudomonas putida* and *Serratia marcescens*.

The dominant organism, that is, *Psuedomonas species*, found in this study and other reports show a wide metabolic diversity and fitness to the environment. *Psuedomonas species* which are ubiquitous bacteria in soil and wastewater

treatment plants are related to the degradation of a broad range of synthetic and natural organic compounds (Ivnitsky *et al.* 2007). Hence their abundance in this RO system.

It has been demonstrated that many related slime–producing bacteria, including *Psuedomonas* species and *Aerobacter* species, are associated with biofilms which develop on the surfaces of other kinds of RO membranes, (Ridgway *et al.* 1983). *Pseudomonas* species has also been identified as a fast adhering species out of all tap water microfloras (Goosen *et al*. 2004).

Serratia marcescens is a species of Gram-negative, rod-shaped bacterium in the family *Enterobacteriaceae*. Due to its ubiquitous presence in the environment, and its preference for damp conditions, *S. marcescens* is commonly found growing in natural environment, including soil, water and surfaces of plant parts. The potential of *S.marcescens* to utilize a wide range of nutrients is expressed clearly by its ability to survive and grow under extreme conditions including disinfectant, antiseptics and double distilled water. *S. marcescens* is a motile organism and can grow in temperatures ranging from 5–40°C and in pH levels ranging from 5 to 9 (Hejazi & Falkiner 1997).

CLC main workbench 5 software was used for sequence alignment and phylogenetic tree construction (Figure 4.24). The phylogenetic tree scale indicates 80 nucleotide substitutions per 100 nucleotides. The tree shows two main clades indicating close evolutionary relationship. A close relation between bacteria from different sampling points was observed (for example, S1_1492-R_D05 from the membrane biofilm and P2_1492-R_D05 from the permeate water). The divergence present in these species could be due to the high chance of mutation because of need to adapt to different conditions at different sample points along the RO system.

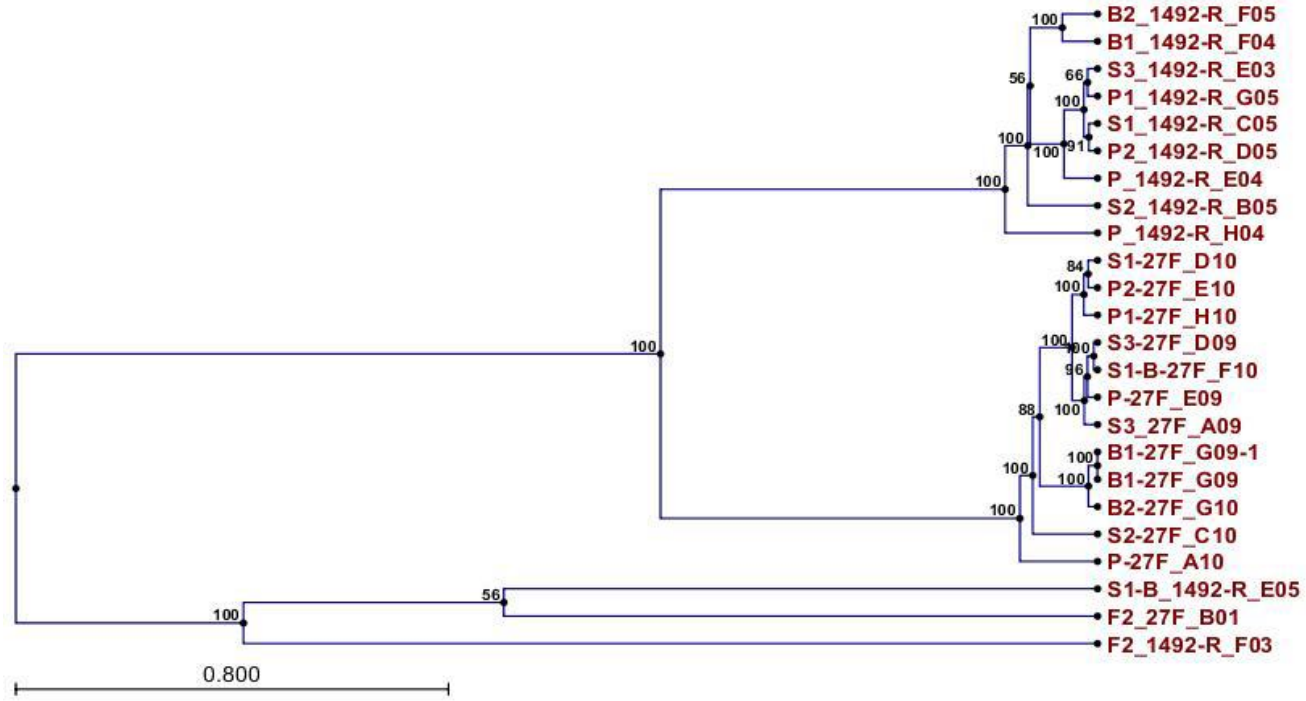

Figure 4. 24 Phylogenetic tree

Phylogenetic relationship between the 16S rDNA of bacterial samples from bulk water and membrane samples.S, membrane sample; F, feedwater; P, permeate; B, brine. The tree was constructed using the UPGMA algorithm.Numbers before the branch points represent percentages of bootstrap resampling based on 100 trees. Bootstrap values below 50% are not represented. The scale bar represents the expected number of substitutions averaged over all sites analysed.

In summary, there is a relation between organisms from different sampling points. The close relationship supports the fact that microorganisms in the feed water are passed on to the permeate and brine while others remain on the membrane. As microorganisms are exposed to different environments, they adapt to the particular environment in order to survive. Microorganisms retained on the membrane surface have the potential to multiply and produce biofilms that results in biofouling problems in the RO system.

CHAPTER 5

5.1 Conclusion and recommendations

The deterioration in performance of the RO system was found to be related to biofouling. Analysis of the operating conditions of the RO plant indicated a rise in pressure drop and a decline in the permeate quality. This indicated that the plant was experiencing fouling problems. It has been noted in Vrouwenvelder, Loordrecht and Kruithof (2010) that in pre-treated water the pressure drop increase over the membrane modules in the installation is directly related to membrane fouling.

An analysis of the RO system indicated that the pre-treatment system reduces the bacterial load in the feed but does not render it sterile as some of these microorganisms pass through to the permeate surface. Total colony forming units in each analysis indicated high numbers in the brine (fig. 4.8) and feed (fig.4.6) while permeate samples had relatively low counts (fig. 4.7). A reduced bacterial load in the permeate shows that some of them are retained in the residual (brine). The retained microorganisms would remain in the brine while some are deposited on the membrane surface, hence contributing to fouling.

Microbiological analyses indicated that the dominant group of bacteria present in the water samples were the *Pseudomonas* (fig. 4.9). Molecular Identification of the bacteria pointed out *Pseudomonas* to be the dominant genera in the water sample. *Psuedomonas* is a slime producer; hence its presence may contribute to biofilm formation which is the problem of biofouling.

Elemental analysis of water samples indicated the presence of chlorine, calcium, silica, potassium, phosphates and nitrates. Comparison of the feedwater and the permeate indicated that some of these elements are retained on the membrane surface (see appendix D. The high values for conductivity and presence of chlorine

in the feedwater can be attributed to hydrochloric acid which is dosed to maintain pH.

The membrane autopsy confirmed the possibility of biofouling being responsible for the poor performance of the membrane system. The cfu/cm^2 on the fouled membrane ranged between 8 X 10^3 and 4.9 X 10^4.

Molecular identification of bacteria indicated *Pseudomonas putida* to be the dominant species on biofilm scrapings from the membrane surface. The presence of ATP in the fouling layer (Table 4.3) suggested that the fouling bacteria are metabolically active on the membrane surface. These metabolically active cells have the potential to grow and multiply rapidly under *in situ* temperature and pressure conditions. These bacteria can also use other dead bacterial cells associated with the fouling layers as nutrients or soluble organic nutrients concentrated at the membrane surface, hence forming biofilms. The Alcian blue stain confirmed the presence of biofilm on the fouled membrane.

As water passes through the RO membrane a wide variety of soluble inorganic compounds normally present in feedwater become highly concentrated at the membrane surface where precipitation may occur. The elements like nitrate, silica and calcium found to be present on the membrane sample were also detected in the feedwater sample. This indicates that these elements were retained on the membrane surface during the water purification process. When elements concentrate and precipitate on the membrane surface, they impede water flux and provide suitable microenvironment for the adhesion and proliferation of microorganisms.

Finally, it can be concluded that although biofouling might not be the major contributor to fouling it definitely played an important role in the process.The membrane autopsy has also shown that it is a useful tool in proving the presence of biofilm on fouled membranes. It gives a clear indication of the cause of problems in

RO systems. Hence, effective control of fouling requires good monitoring techniques of the operating conditions, microbiological and chemical components in the RO system. The autopsy, on the other hand, is able to confirm the fouling present in the system and this would help indicate possible improvements in the pre-treatment of water before reverse osmosis.

The following recommendations can be proposed:

Prevent biofouling by:

(i) efficient pre-treatment to reduce concentration of nutrients,
(ii) performing preventive or curative cleaning, and
(iii) combining pre-treatment and cleaning.

Since biofouling potential is composed by the ubiquitous microorganisms and the availability of nutrients, biofilm accumulation could therefore be reduced by decreasing the nutrient concentration. The filter systems are useful tools in reducing the nutrient concentration and thus reducing fouling.

- Regular maintenance of sand filters to avoid sand particles in the feed would help reduce flight particles from entering the RO system and contributing to fouling.
- Use antiscalant to prevent precipitation of salts by ensuring it is dosed correctly at appropriate intervals.
- Clean membranes immediately when the performance of the plant declines.

- Chemicals dosed to water feeding the membrane installation should not contain easily biodegradable components. Even well defined –chemical pure grade-acids need close attention and monitoring, since handling and transport may introduce nutrients contributing to biofouling.

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ANNEXURE A RO system mechanical equipment

1. Plant feed

RWB, condensate water is fed into a 50 m^3 concrete buffer tank, which is equipped with a slow agitator. Steam is fed into the tank in order to control the temperature of the RO feed at a optimum A 30 kW centrifugal pump (with backup) transfers water from this tank into the sand filters.

2. Sand filters *(*FK 2001 to 2004 and FK 2012)

Five multi-media sand filters, each with a diameter of 3m and designed to handle a flow rate of between 58 and 68 m^3/hr, are installed in parallel to remove most of the larger colloidal material suspended in the water.

3. Booster pumps

From here a 37 kW centrifugal booster pump (with backup) transfers the filtered water form the booster pumps into the RO Units.

4. Backwash pump

A single 7.5kW centrifugal pump serves as a common backwash pump for all four sand filters.

5. RO units

The RO units (RO1, RO2, RO3 and RO 4) are located next to each.

Each unit comprises the following:

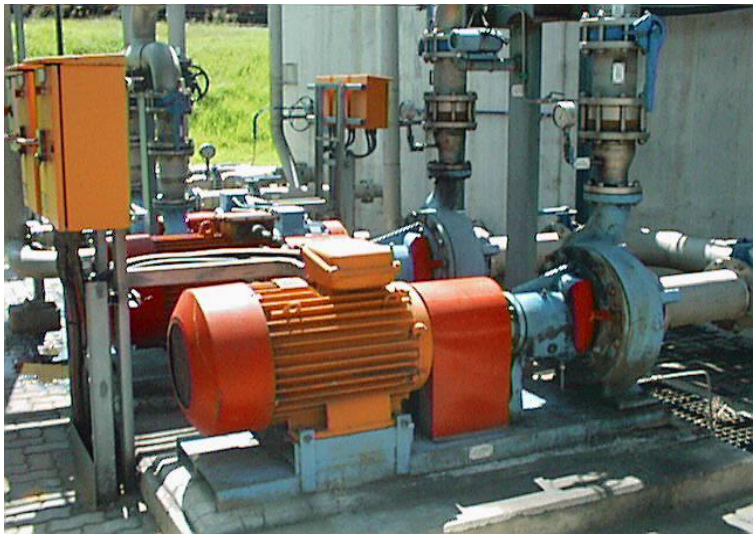
Two sequential cartridge filter pressure vessels (20 μm and 5 μm), each vessel containing 27 polypropylene spun cartridge filter elements. (FK2005 to 2010). RO 4 only has a single cartridge filter (FK2011) with a single 90 μm absolute polypropylene spun cartridge filter element.

6. CIP tank

A single 4 m^3 stainless steel tank and a 7.5 kW centrifugal pump is available for in-situ cleaning of the membranes, e.g. all three units are serviced by a common CIP tank. It is estimated that RO units would require a CIP once a week.

7. Permeate System

A 130 m^3 concrete tank receives permeate from the RO section and provides ^{buffer} storage capacity between the RO plant and the desalination plant. A 55 kW centrifugal pump (with backup) transfers the water from the permeate tank to the desalination plant.



8. Concentrate system

The brine product from the RO section flows into a 50 m^3 concrete tank. RWB can be added to dilute the brine to acceptable concentrations. The diluted brine is finally pumped to a either the Demin plant from where it is transferred to Chlorine Coalplex or into the Polifin's effluent system, by means of a 37 kW centrifugal pump (with backup). A sump pump transfer water from the plants trenches to the concentrate tank

9. HCl Dosing system

Diluted hydrochloric acid (5% by mass) is stored in a polypropylene tank with a capacity of 3.2 m^3. This tank is located in the bunded area at the south-eastern end of the RO complex from where it is pumped into the RO feed in order to control the pH of the RO feed water

10. SMBS Dosing

SMBS (Sodium Meta-Bisulphate) solution is manually prepared in a polypropylene make-up tank with a capacity of 400 liters. An 0.55 kW centrifugal pump transfers the solution to a polypropylene storage tank that has a capacity of 500 liters

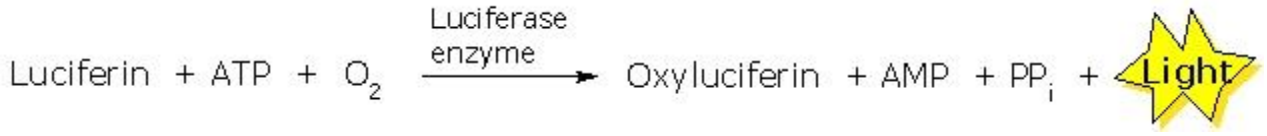
11. Anti-scalent Dosing

FN217 is stored in a 1 m^3 stainless steel tank, which is also located next to the flocculant tanks. Three dosing pumps supply the anti-scalent to each RO unit individually

ANNEXURE B ATP analysis protocol

ATP is easily and accurately measured by treating an ATP-containing sample with a reagent that reacts with ATP and produces light that can be measured in a luminometer. The amount of light produced is proportional to the amount of ATP in the sample. This reaction occurs naturally in fireflies to produce their characteristic light.

In this reaction, a compound, luciferin, reacts with ATP and a molecule of oxygen with the help of an enzyme catalyst, luciferase to yield an oxidized form of luciferin, adenosine monophosphate (AMP), and pyrophosphate (PP_i). Energy is also evolved in the form of light. The chemical equation is:



ATP measurement method:

A measured amount of water sample containing bacteria is collected for analysis.

A reagent, known as a “lysing agent,” whose function is to break apart bacterial cell walls and membranes to release ATP into the bulk water, is added to the sample.

ATP reacts with the luciferin-luciferase test reagents, producing light in quantities which can be measured by a sensitive luminescence detector.

The sample containing the reagents is placed in the detector, and light is measured in Relative Light Units (RLU). The RLU reading from the instrument is directly proportional to the amount of ATP in the sample, which, in turn, is a function of the number of bacteria in the sample and their

metabolic activity. The analysis can be conducted in a cuvette, with sample, lysing reagent and luciferin-luciferase reagents pipetted into the cuvette; the cuvette is then placed in the detector, where light measurement occurs. Alternatively, an ATP pen can be used. Sample (water or deposit) is collected on the swab, and the swab is returned to the pen, where pre-loaded reagents are released; the pen is then placed in the detector, where light measurement occurs.

PRODUCT FACT SHEET

TCB™ — Total Control for Biological Wastewater Treatment

aqua-tools.com

LUMINULTRA™

How and Where is this Test Kit Used?

Total Control for Biological Wastewater Treatment (TCB) test kits are based on the measurement of ATP and are designed for high-solids, high-biomass concentration applications, such as biological wastewater treatment plants. Through two separate analyses on a single sample, you can accurately determine ***biomass concentration, health, and solids activity*** in:

- ✓ Aerobic Reactors
- ✓ Anaerobic Reactors
- ✓ Activated Sludge
- ✓ UASB Reactors
- ✓ Collection Systems
- ✓ Raw Wastewater
- ✓ Biosolids or Soil
- ✓ Treated Effluent

What do the Results Mean?

The results obtained using TCB are used as an indicator of total biomass concentration and health. With two simple measurements, you will be able to determine the quantity and quality of biomass in your sample as well as the fraction of total solids that are living within **minutes**. This information can be used to quickly troubleshoot and optimize the biological process and over time lead to continuous improvement.

How Does the Test Kit Work?

TCB allows the user to perform two analyses, Total ATP (**tATP™**) and Dissolved ATP (**dATP™**). For the detailed procedure, consult the TCB Quick-Reference Guide. Results are used to calculate:

Parameter	Description	Calculation & Units	Range
cATP™ Cellular ATP	A measure of total biological concentration	**cATP = tATP – dATP** (ng cATP/mL)	1 to 20,000
BSI™ Biomass Stress Index	A measure of biomass health	**BSI = dATP / tATP** (% dead biomass)	0 to 100
ABR™ Active Biomass Ratio	The fraction of total solids that are living	**ABR = (cATP* 0.5) / TSS** (% living solids)	0 to 100

Test Kit Sizes

Cat. #	Number of Analyses
TCB-50	50 tATP, 50 dATP, 25 UltraCheck 1

** UltraCheck 1 analyses are used to convert luminometer output (Relative Light Units, RLU) to ATP concentrations.*

Test Kit Contents

Included in TCB Kits	Storage Temp.	Shelf Life
Luminase *Luciferase Enzyme Reagent*	4°C	3 mo.
UltraCheck 1 *1 ng/mL ATP Standard*	20°C	6 mo.
UltraLyse 30 *ATP Extraction Reagent*	20°C	12 mo.
UltraLute *tATP Dilution Buffer*	20°C	12 mo.
LumiSolve *dATP Stabilizing Buffer*	20°C	12 mo.

Required for Test Kit Use

Item	Cat. #
Luminometer + Calculation and Interpretation Software	Kikkoman C-100
100-1000µL Micropipettor 500-5000µL Micropipettor 4-face Test Tube Rack 1000µL Micropipette Tips 5mL Micropipette Tips 15mL Test Tubes and Caps	Starter kit Aqua-tools.com
2.5mL Culture Tubes	Luminometer Tubes

Document v2.0
Visit www.aqua-tools.com to check for updates

ISO 9001:2000

LuminUltra™ Technologies Ltd.
Innovative Biological Monitoring Technologies
4 40 Regent Street, Regent Tower, Suite 630, Fredericton, New Brunswick, Canada E3B 3Z8
Tel: (506) 459-4777 Fax: (506) 453-4980 www.luminultra.com

Authorized Distributor
Marc RAYMOND
aqua-tools.com
Domaine de la Brisette
36 rue de la Falaise
78126 Allainville-sur-Mauldre
France
contact@aqua-tools.com
aqua-tools.com

ISO 9001:2000
LuminUltra™ Technologies Ltd.
Innovative Biological Monitoring Technologies
440 King Street, King Towers, Suite 636, Fredericton, New Brunswick, Canada E3B 5H8
Tel: (506) 459-8777 Fax: (506) 453-9860 www.luminultra.com

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Marc RAYMOND
aqua-tools.com
Domaine de la Brissette
36 rue de la falaise
78126 Aulnay-sur Mauldre.
France
contact@aqua-tools.com
aqua-tools.com

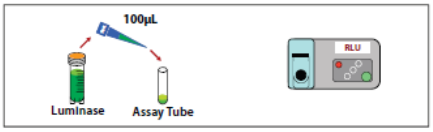
Total Control BWWT (TCB) - PROTOCOL

UltraCheck™ 1 Calibration

Perform one calibration for each set of analyses!

1. PREPARATION

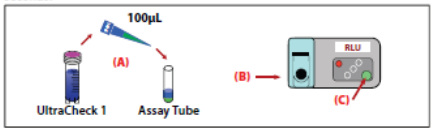
Prepare the following: 100µL of Luminase into a 12x55mm tube.



NOTE: Remove Luminase from fridge – 1 hour prior to measurement.

2. ASSAY

Add 100µL of UltraCheck 1 to the Assay Tube (A). **Immediatly** place the Assay Tube into the Luminometer (B), hit Enter (C), and record the RLU_{UC1} after 10 seconds.



NOTE: If the assay produced less than 5,000 RLU, LuminUltra recommends that new Luminase be obtained.

ATP: The Basics

Total Control technology from LuminUltra™ is based on the measurement of Adenosine Triphosphate (ATP). ATP is a direct and interference–free indicator of total biomass. ATP is measured using the **firefly luciferase assay**, where a sample containing ATP is introduced to a solution containing the enzyme Luciferase, which naturally occurs in the tails of fire flies, to produce light. The light is detected in a Luminometer.

$$ATP + luciferin + O_2 \xrightarrow[Mg^{++}]{Luciferase} AMP + PP_i + oxyluciferin + Light$$

Calculations

The following formulae are used to calculate Total Control parameters. For easy calculation, make use of LuminUltra's easy-to-use **LumiCapture™** software!

$$tATP\ (ng/mL) = RLU_{tATP} / RLU_{UC1} \times 306$$

$$dATP\ (ng/mL) = RLU_{dATP} / RLU_{UC1} \times 101$$

cATP (Cellular ATP) is the concentration (quantity) of living organisms.

$$cATP\ (ng/mL) = tATP - dATP$$

BSI (Biomass Stress Index) is the stress level (quality) of the living organisms.

$$BSI\ (\%) = dATP / tATP$$

ABR (Active Biomass Ratio) is the percentage of solids that are alive.

$$ABR\ (\%) = (cATP \times 0.5) / TSS$$

Kit Use and Interpretation

TCB is designed for measurement of biomass in biological wastewater treatment. **Measure** tATP and dATP on each sample, **convert** using UltraCheck results, and then **calculate** cATP, BSI and ABR.

Although each process or application is different, use these guidelines to establish your control program.

Process	Parameter	Good Control	Preventive Action Required	Corrective Action Required
Process Influent	BSI (%)	<50	50 to 75	>75
Bioreactor	cATP (ng/mL)	*Process Specific		
	BSI (%)	<30	30 to 50	>50
	ABR (%)	>25	10 to 25	<10
Process Effluent	cATP (ng/mL)	<50	50 to 250	>250

** The optimum cATP control guidelines for your process will depend on reactor type and influent qualities. In general, deviation from previous values by ±25% should be considered a preventive guideline and ±50% should be considered corrective.*

- Avoid analysis contamination by always using a new pipette tip for each pipetting step!
- Because ATP and bacteria are present on skin, it is important not to touch the surface of pipette tips.
- Because QGA is a test for biological characteristics, and biological characteristics will begin to change immediately upon collection, **analyze samples within 2 hours of collection**.
- Consult reagent labels for storage conditions.
- Download MSDS at **www.aqua-tools.com**.

Document v3.0
Visit www.aqua-tools.com to check for updates

ANNEXURE C Protocol for protein and sugar determination of membranes

Protein determenation

Reaction solutions

Solution A:

1g Na (Sodium tartrate) plus 50 g NaCO3 (Sodium carbonate) dissolve in 225ml of 1N NaOH dilute to final volume of 500ml with 1N NaOH.

Solution B:

2g Na (Sodium tartrate) plus 1 g CuSo4.5H2O (Copper sulphate) disolve in 90ml H2Oand dilute to final volume of 100ml with 1N NaOH.

Solution C:

Dilute 50ml Folin-Ciocalteu reagent with 50ml distilled water (Solution should be prepared fresh daily)

Solution D:

Dissolve 50g SDS (Sodium dodecyl sulphate) in 900 ml of a 0.5N NaOH solution. Heat solution to dissolve SDS. Dilute solution to 1000ml with distilled water. IMPORTANT HEAT SOLUTION BEFORE USE TO TEMPERATURE ABOVE 28 °C.

KEEP ALL SOLUTIONS IN DARK AT 4 °C.

Standard curve

- Make a 1mg/ml standard BSA solution
    - Weigh of 50mg (0.05g) BSA transfer to a 50ml volumetric flask. Dilute BSA with distilled water to final volume of 50-ml (Write down the exact amount of BSA that was weighed) IMPORTANT DON'T SHAKE SEVERELY AS PROTEIN WILL DENATURATE.
- Make dilution range in triplicate as shown in table .

Test Tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
µl of standard BSA solution	0	25	50	75	100	150	200	250
µl distilled water	250	225	200	175	150	100	50	0
µg Proteins in the tube	0	25	50	75	100	150	200	250

- To each of the test tubes add:
  - 500µl of SolutionD, incubate at 30°C for a period of 2 hours. Vortex solution every 30 minutes
  - Add 500µl of a 2N NaOH solution, Vortex
  - Immediately add 900µl of solution A , Vortex and incubate for 30min at room temperature.
  - After 30 minutes add 100µl of solution B, Vortex, incubate for 20 minutes at room temperature.
  - After 20minutes of incubation add 3ml of solution C, vortex, incubate at room temperature for 30 minutes.
  - Read the absorbance at 650nm after standardisation with test tube 1. Write down the absorbance readings in table.

Test Tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
First range	0							
Second range	0							
Third range	0							
Average range	0							

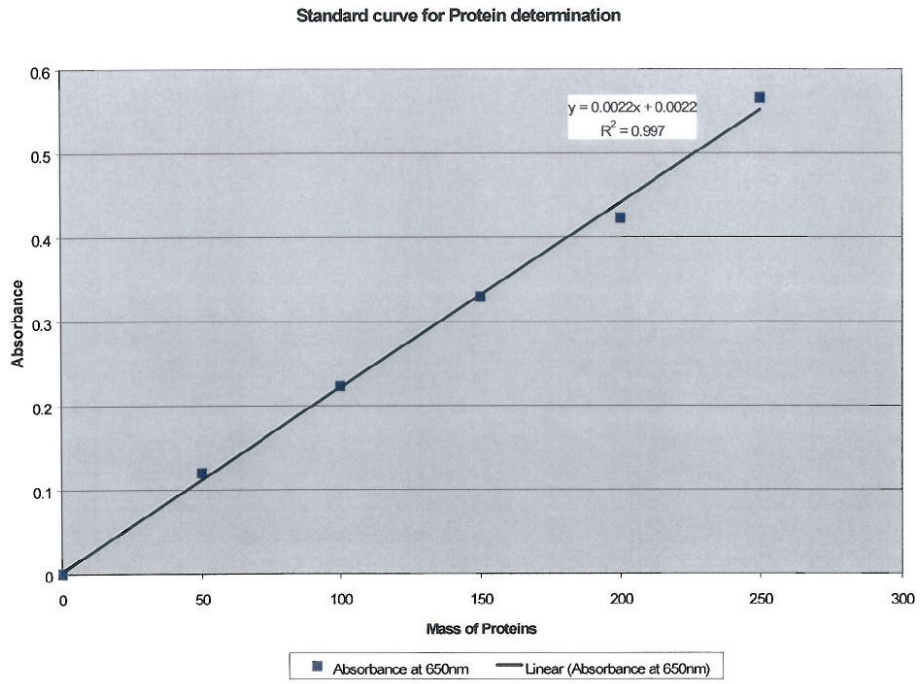
Protein Determination of membranes:

Take 25cm^2 (or any specific size available) piece of membrane. Write down specific size membrane. Cut membrane into smaller pieces to fit into test tubes, use an unfouled membrane as a blank.

- Add 2.5ml distilled water and 5ml of solution D to membrane in the test tube incubate at 30°C for a period of 3hrs. Vortex solution every 30min.
- After 2 hours incubation withdraw 750µl of this solution containing proteins removed from the membrane by Solution D
- Add 500µL of a 2 N NaOH solution , vortex

- Immediately add 900µl of solution A , Vortex and incubate for 30min at room temperature.
- After 30 minutes add 100µl of solution B, Vortex, incubate for 20 minutes at room temperature.
- After 20minutes of incubation add 3ml of solution C, vortex, incubate at room temperature for 30 minutes.
- Read the absorbency at 650nm after standardisation with tube that contained unfouled membrane. Write down the absorbency readings in table.

Sample number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
ABS reading (650nm)								
µg protein from standard curve								
µg protein/Cm^2 membrane								



Sugar determenation

Reaction solutions

Sulfuric acid reagent:

95.5% Sulfuric acid.

Phenol reagent:

Phenol 5% (5g in 100ml) solution in water.

KEEP ALL SOLUTIONS IN DARK AT 4 °C.

Standard curve

- Make a 0.5mg/ml standard D-glukcose solution (0.03g)
    - Weigh of 25mg (0.03g) D-Glucose transfer to a 50ml volumetric flask. Dilute D-Glucose with distilled water to final volume of 50-ml (Write down the exact amount of D glucose that was weighed).
- Make dilution range in triplicate as shown in table .

Test Tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
µl of standard D-Glucose solution	0	200	400	600	800	1000	1200	1600
µl distilled water	2000	1800	1600	1400	1200	1000	800	600
µg D-Glucose in the tube	0	50	100	150	200	250	300	350

- To each of the test tubes add:
    - 1ml of the 5% Phenol reagent.
    - 5ml Sulfuric acid reagent. This reagent should be injected directly to the liquid in the test tube and not against the side of the test tube.
    - Allow the test tubes to stand for 20 minutes, vortex.
    - Incubate at 30°C for 20 minutes.
    - Read absorbance at 490nm after standardisation with test tube 1. Write down the absorbance readings in table.

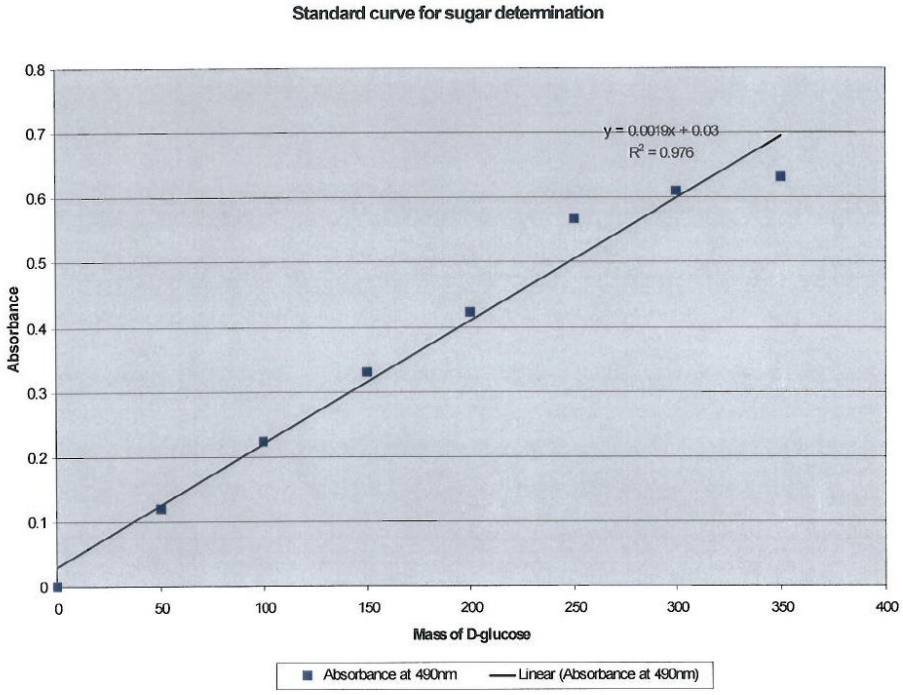
Test Tube	1	2	3	4	5	6	7	8
First range	0							
Second range	0							
Third range	0							
Average range	0							

Sugar Determination of membranes:

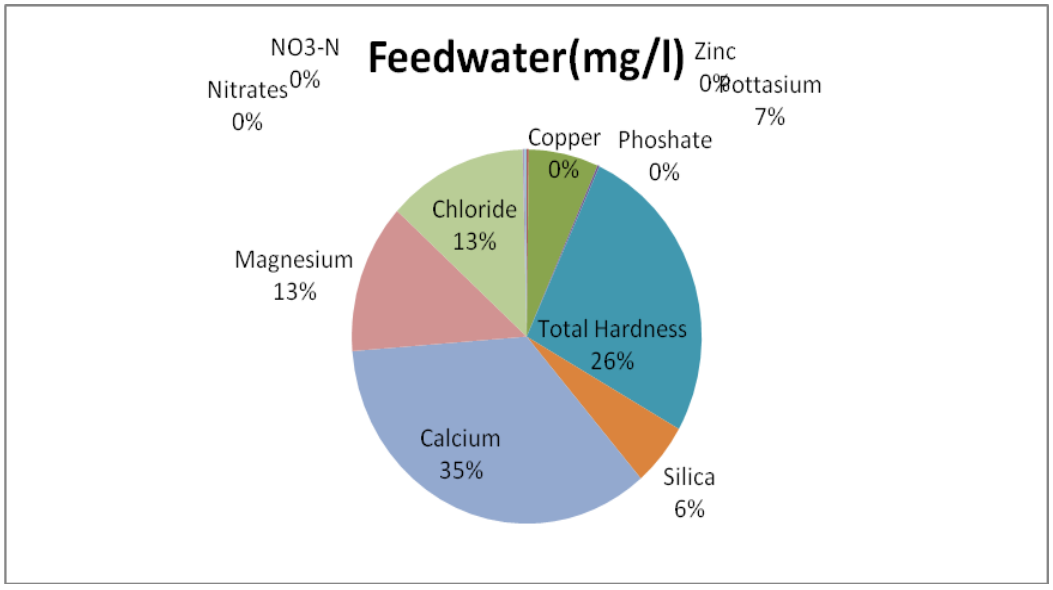
Take 25cm^2 (or any specific size available) piece of membrane. Write down specific size membrane. Cut membrane into smaller pieces to fit into test tubes, use an unfouled membrane as a blank.

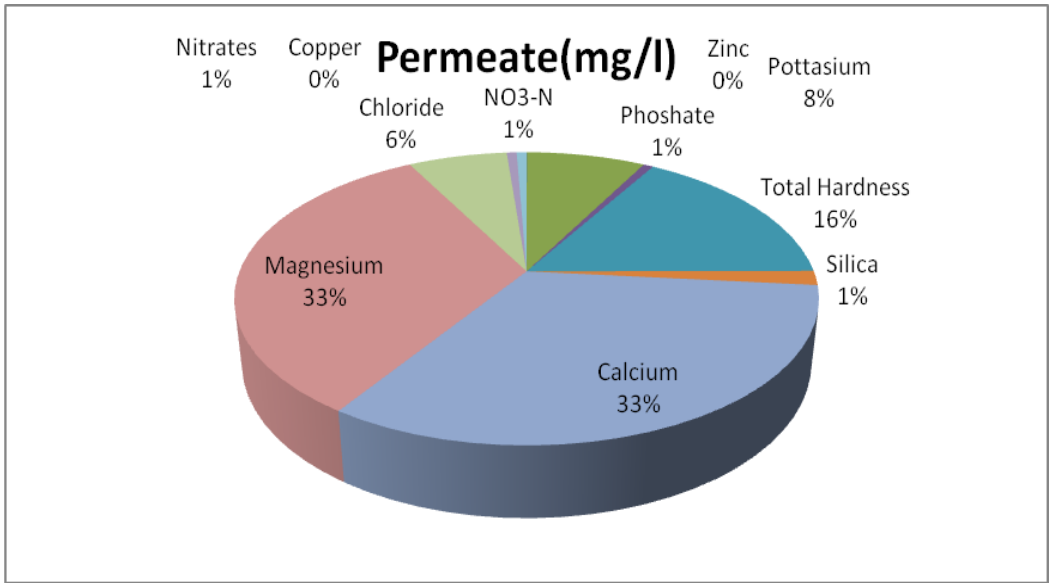
- Add 2ml distilled water to membrane in the test tube.
- Add 1ml Phenol reagent.
- Add 5ml Sulfuric reagent.
- Allow to stand at room temperature for 20 minutes, vortex.
- Incubate at 30°C for a period of 30 minutes.
- Read the absorbance at 490nm after standardisation with tube that contained unfouled membrane. Write down the absorbance readings in table.

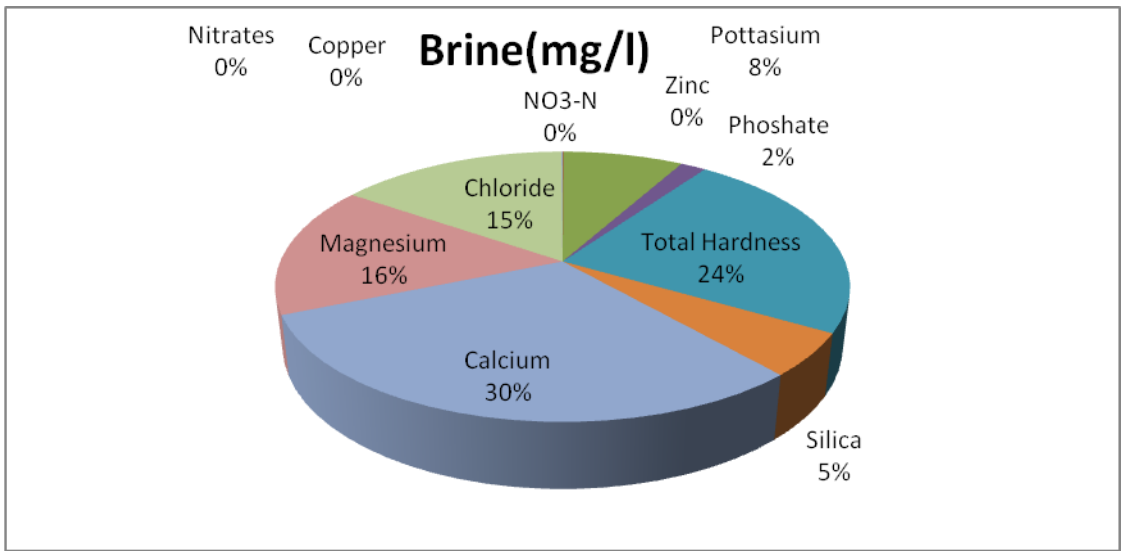
Sample number	1	2	3	4	5	6	7	8
ABS reading (490nm)								
µg D-glucose from standard curve								
µg D-glucose/cm^2 membrane								


Standard curve for sugar determination
y = 0.0019x + 0.03
R² = 0.976
Absorbance
Mass of D-glucose
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
50
100
150
200
250
300
350
400
Absorbance at 490nm
Linear (Absorbance at 490nm)

ANNEXURE D Results for elemental analysis of feed, permeate and brine samples






Brine(mg/l)
Nitrates 0%
Copper 0%
NO3-N 0%
Zinc 0%
Pottasium 8%
Phoshate 2%
Chloride 15%
Magnesium 16%
Total Hardness 24%
Calcium 30%
Silica 5%

ANNEXURE E BLAST search results

R2A-S1-27F_D10 (1177 letters)

```
>  emb|AM989284.1|  Pseudomonas sp. AKB-2008-HE68 partial 16S rRNA gene,
strain AKB-2008-HE68
Length=848

 Score = 1166 bits (631),  Expect = 0.0
 Identities = 631/631 (100%), Gaps = 0/631 (0%)
 Strand=Plus/Plus

Query  160  TCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATG
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Sbjct  181  TCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATG
240

Query  220  GCTCACCAAGGCTACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGA
279
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  GCTCACCAAGGCTACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGA
300

Query  280  GACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGC
339
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGC
360

Query  340  CTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTG
399
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Query  400  GGAGGAAGGGTACTTACCTAATACGTGAGTATTTTGACGTTACCGACAGAATAAGCACCG
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Query  460  GCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACT
519
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540

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579
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Sbjct  541  GGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCT
600
```

```
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639
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660

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720

Query  700  GATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTC
759
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780

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Sbjct  781  CACGCCGTAAACGATGTCAACTAGCCGTTGG  811


Sbjct  655  TAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACT
714

Query  700  GATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTC
759
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  715  GATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTC
774

Query  760  CACGCCGTAAACGATGTCAACTAGCCGTTGG  790
            ||||||||||||||||||||||||||||||
Sbjct  775  CACGCCGTAAACGATGTCAACTAGCCGTTGG  805
```

PCA_S2_27F_H08 (1164 letters)

```
>  gb|GU354317.1|  Pseudomonas putida strain L1-5 16S ribosomal RNA
gene, partial
sequence
Length=1498

 Score = 1240 bits (671),  Expect = 0.0
 Identities = 671/671 (100%), Gaps = 0/671 (0%)
 Strand=Plus/Plus

Query  120  AATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATG
179
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Sbjct  158  AATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATG
217

Query  180  AGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAAC
239
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct  218  AGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAAC
277

Query  240  TGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
299
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  278  TGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
337

Query  300  GCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTG
359
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Sbjct  338  GCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTG
397

Query  360  AAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACC
419
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457

Query  420  TTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGG
479
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Sbjct  458  TTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGG
517

Query  480  TAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTT
539
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  518  TAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTT
577

Query  540  CGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCGAG
599
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  578  CGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCGAG
637

Query  600  CTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGG
659
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Sbjct  638  CTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGG
697

Query  660  AAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGC
719
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Sbjct  698  AAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGC
757

Query  720  GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGC
779
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  758  GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGC
817
```

```
Query  780  CGTTGGAATCC  790
            ||||||||||| 
Sbjct  818  CGTTGGAATCC  828
```

PCA-S3-27F_D09 (1161 letters)

```
>  gb|GU325691.1|  Pseudomonas sp. DQ-02 16S ribosomal RNA gene, partial
sequence
Length=1178

 Score = 1166 bits (631),  Expect = 0.0
 Identities = 631/631 (100%), Gaps = 0/631 (0%)
 Strand=Plus/Plus

Query  160  TCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCT
219
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227

Query  220  CACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGAC
279
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Sbjct  228  CACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGAC
287

Query  280  ACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTG
339
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Sbjct  288  ACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTG
347

Query  340  ATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGA
399
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Sbjct  348  ATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGA
407

Query  400  GGAAGGGCAGTAAGCTAATACCTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCT
459
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  408  GGAAGGGCAGTAAGCTAATACCTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCT
467

Query  460  AACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGG
519
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Sbjct  468  AACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGG
527

Query  520  CGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGG
579
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Sbjct  528  CGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGG
587
```

```
Query  580  AACTGCATCCAAAACTGGCAAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAG
639
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  588  AACTGCATCCAAAACTGGCAAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAG
647

Query  640  CGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGAT
699
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  648  CGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGAT
707

Query  700  ACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCAC
759
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  708  ACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCAC
767

Query  760  GCCGTAAACGATGTCAACTAGCCGTTGGAAT  790
            |||||||||||||||||||||||||||||||
Sbjct  768  GCCGTAAACGATGTCAACTAGCCGTTGGAAT  798
```

PCA-Feed-_1492-R_E04 (1114 letters)

```
>  gb|HM012038.1|  Uncultured Pseudomonas sp. clone S2-346 16S ribosomal
RNA gene,
partial sequence
Length=1190

 Score =  520 bits (281),  Expect = 2e-144
 Identities = 281/281 (100%), Gaps = 0/281 (0%)
 Strand=Plus/Minus

Query  160   CTGCGATCCGGACTACGATCGGTTTTGTGAGATTAGCTCCACCTCGCGGCTTGGCAACCC
219
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1024  CTGCGATCCGGACTACGATCGGTTTTGTGAGATTAGCTCCACCTCGCGGCTTGGCAACCC
965

Query  220   TCTGTACCGACCATTGTAGCACGTGTGTAGCCCAGGCCGTAAGGGCCATGATGACTTGAC
279
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  964   TCTGTACCGACCATTGTAGCACGTGTGTAGCCCAGGCCGTAAGGGCCATGATGACTTGAC
905

Query  280   GTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCCTTAGAGTGCCCACCATAACG
339
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  904   GTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCCTTAGAGTGCCCACCATAACG
845

Query  340   TGCTGGTAACTAAGGACAAGGGTTGCGCTCGTTACGGGACTTAACCCAACATCTCACGAC
399
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct  844   TGCTGGTAACTAAGGACAAGGGTTGCGCTCGTTACGGGACTTAACCCAACATCTCACGAC
785

Query  400   ACGAGCTGACGACAGCCATGCAGCACCTGTGTCAGAGTTCC  440
              ||||||||||||||||||||||||||||||||||||||||
Sbjct  784   ACGAGCTGACGACAGCCATGCAGCACCTGTGTCAGAGTTCC  744
```

R2A-Permeate 1492-R_H04 (1145 letters)

```
>  gb|EU057876.1|  Oxalobacteraceae bacterium CH29-4 16S ribosomal RNA
gene, partial
sequence
Length=1399

 Score =  857 bits (464),  Expect = 0.0
 Identities = 475/481 (98%), Gaps = 2/481 (0%)
 Strand=Plus/Minus

Query  150   AGTTGCAGACTACAATCCGGACTACGATACACTTTCTGGGATTAGCTCCCCCTCGCGGGT
209
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1248  AGTTGCAGACTACAATCCGGACTACGATACACTTTCTGGGATTAGCTCCCCCTCGCGGGT
1189

Query  210   TGGCGGCCCTCTGTATGTACCATTGTATGACGTGTGAAGCCCTACCCATAAGGGCCATGA
269
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1188  TGGCGGCCCTCTGTATGTACCATTGTATGACGTGTGAAGCCCTACCCATAAGGGCCATGA
1129

Query  270   GGACTTGWSRCSTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCATTAGAGTGC
329
              |||||||   | ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1128  GGACTTG--ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCATTAGAGTGC
1071

Query  330   TCTTTCGTAGCAACTAATGACAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCA
389
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1070  TCTTTCGTAGCAACTAATGACAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCA
1011

Query  390   CGACACGAGCTGACGACAGCCATGCAGCACCTGTGTAATGGTTCCCTTTCGGGCACTCCC
449
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  1010  CGACACGAGCTGACGACAGCCATGCAGCACCTGTGTAATGGTTCCCTTTCGGGCACTCTC
951

Query  450   AAATCTCTCCGGGATTCCATCCATGTCAAGGGTAGGTAAGGTTTTTCGCGTTGCATCGAA
509
              |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  950   AAATCTCTCCGAGATTCCATCCATGTCAAGGGTAGGTAAGGTTTTTCGCGTTGCATCGAA
891
```

```
Query  510   TTAATCCACATCATCCACCGCTTGTGCGGGTCCCCGTCAATTCCTTTGAGTTTTAATCTT
569
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  890   TTAATCCACATCATCCACCGCTTGTGCGGGTCCCCGTCAATTCCTTTGAGTTTTAATCTT
831

Query  570   GCGACCGTACTCCCCAGGCGGTCTACTTCACGCGTTAGCTGCGTTACCAAGTCAATTAAG
629
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  830   GCGACCGTACTCCCCAGGCGGTCTACTTCACGCGTTAGCTGCGTTACCAAGTCAATTAAG
771

Query  630   A  630
             |
Sbjct  770   A  770
```

R2A-Permeate-27F_A10 (1175 letters)

```
>  gb|EU194880.1|  Duganella sp. A22 16S ribosomal RNA gene, partial
sequence
Length=1367

 Score = 1088 bits (589),  Expect = 0.0
 Identities = 590/591 (99%), Gaps = 0/591 (0%)
 Strand=Plus/Plus

Query  160   CAAGACCTCATGCTCATGGAGCGGCCGATATCTGATTAGCTAGTTGGTGGGGTAAAGGCC
219
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  150   CAAGACCTCATGCTCATGGAGCGGCCGATATCTGATTAGCTAGTTGGTGGGGTAAAGGCC
209

Query  220   TACCAAGGCAACGATCAGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGAACTGAGAC
279
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  210   TACCAAGGCAACGATCAGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGAACTGAGAC
269

Query  280   ACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGCAACCCTG
339
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  270   ACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGCAACCCTG
329

Query  340   ATCCAGCAATGCCGCGTGAGTGAAGAAGGCCTTCGGGTTGTAAAGCTCTTTTGTCAGGGA
399
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  330   ATCCAGCAATGCCGCGTGAGTGAAGAAGGCCTTCGGGTTGTAAAGCTCTTTTGTCAGGGA
389

Query  400   AGAAAAGGGAYTGGCTAATATCTGGTCCTCATGACGGTACCTGAAGAATAAGCACCGGCT
459
             |||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  390   AGAAAAGGGACTGGCTAATATCTGGTCCTCATGACGGTACCTGAAGAATAAGCACCGGCT
449
```

```
Query  460  AACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGG
519
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  450  AACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGG
509

Query  520  CGTAAAGCGTGCGCAGGCGGTTTTGTAAGACTGTCGTGAAATCCCCGGGCTCAACCTGGG
579
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  510  CGTAAAGCGTGCGCAGGCGGTTTTGTAAGACTGTCGTGAAATCCCCGGGCTCAACCTGGG
569

Query  580  AATGGCGATGGTGACTGCAAGGCTAGAGTTTGGCAGAGGGGGGTAGAATTCCACGTGTAG
639
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  570  AATGGCGATGGTGACTGCAAGGCTAGAGTTTGGCAGAGGGGGGTAGAATTCCACGTGTAG
629

Query  640  CAGTGAAATGCGTAGATATGTGGAGGAACACCGATGGCGAAGGCAGCCCCCTGGGTCAAA
699
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  630  CAGTGAAATGCGTAGATATGTGGAGGAACACCGATGGCGAAGGCAGCCCCCTGGGTCAAA
689

Query  700  ACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG  750
            ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  690  ACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG  740
```

PCA-Brine-27F_G09 (1158 letters)

```
>  emb|FM177776.1|  Acinetobacter sp. TDB-2008b partial 16S rRNA gene,
strain A23
Length=1407

 Score = 1166 bits (631),  Expect = 0.0
 Identities = 631/631 (100%), Gaps = 0/631 (0%)
 Strand=Plus/Plus

Query  160  ACTTGTGACCTTGCGTTAATAGATGAGCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAG
219
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  147  ACTTGTGACCTTGCGTTAATAGATGAGCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAG
206

Query  220  GCCTACCAAGGCGACGATCTGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGA
279
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  207  GCCTACCAAGGCGACGATCTGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGA
266

Query  280  GACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGGAACC
339
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct  267  GACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGGAACC
326

Query  340  CTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCTTATGGTTGTAAAGCACTTTAAGCGA
399
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  327  CTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCTTATGGTTGTAAAGCACTTTAAGCGA
386

Query  400  GGAGGAGGCTCTCTTGGTTAATACCCAAGATGAGTGGACGTTACTCGCAGAATAAGCACC
459
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  387  GGAGGAGGCTCTCTTGGTTAATACCCAAGATGAGTGGACGTTACTCGCAGAATAAGCACC
446

Query  460  GGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCGAGCGTTAATCGGATTTAC
519
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  447  GGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCGAGCGTTAATCGGATTTAC
506

Query  520  TGGGCGTAAAGCGTGCGTAGGCGGCTTTTTAAGTCGGATGTGAAATCCCCGAGCTTAACT
579
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  507  TGGGCGTAAAGCGTGCGTAGGCGGCTTTTTAAGTCGGATGTGAAATCCCCGAGCTTAACT
566

Query  580  TGGGAATTGCATTCGATACTGGGAAGCTAGAGTATGGGAGAGGATGGTAGAATTCCAGGT
639
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  567  TGGGAATTGCATTCGATACTGGGAAGCTAGAGTATGGGAGAGGATGGTAGAATTCCAGGT
626

Query  640  GTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGCGAAGGCAGCCATCTGGCC
699
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  627  GTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGCGAAGGCAGCCATCTGGCC
686

Query  700  TAATACTGACGCTGAGGTACGAAAGCATGGGGAGCAAACAGGATTAGATACCCTGGTAGT
759
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  687  TAATACTGACGCTGAGGTACGAAAGCATGGGGAGCAAACAGGATTAGATACCCTGGTAGT
746

Query  760  CCATGCCGTAAACGATGTCTACTAGCCGTTG  790
             ||||||||||||||||||||||||||||||
Sbjct  747  CCATGCCGTAAACGATGTCTACTAGCCGTTG  777


>
```

R2A-Brine-27F_H09 (871 letters)

```
>  gb|HM028663.1|  Bacillus sp. III_B33 16S ribosomal RNA gene, partial
sequence
Length=1414

 Score =  150 bits (81),  Expect = 7e-34
 Identities = 81/81 (100%), Gaps = 0/81 (0%)
 Strand=Plus/Plus

Query  240  GCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCC
299
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  226  GCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCC
285
Query  300  AGACTCCTACGGGAGGCAGCA  320
            |||||||||||||||||||||
Sbjct  286  AGACTCCTACGGGAGGCAGCA  306
```

PCA-Brine-_1492-R_F05 (1084 letters)

```
>  gb|GU570444.1|  Uncultured bacterium clone 3351 16S ribosomal RNA
gene, partial
sequence
Length=1079
Score =  852 bits (461),  Expect = 0.0
 Identities = 461/461 (100%), Gaps = 0/461 (0%)
 Strand=Plus/Minus

Query  150  GAGTTGCAGACTCCAATCCGGACTACGATCGGCTTTTTGAGATTAGCATCCTATCGCTAG
209
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  982  GAGTTGCAGACTCCAATCCGGACTACGATCGGCTTTTTGAGATTAGCATCCTATCGCTAG
923

Query  210  GTAGCAACCCTTTGTACCGACCATTGTAGCACGTGTGTAGCCCTGGTCGTAAGGGCCATG
269
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  922  GTAGCAACCCTTTGTACCGACCATTGTAGCACGTGTGTAGCCCTGGTCGTAAGGGCCATG
863

Query  270  ATGACTTGACGTCGTCCCCGCCTTCCTCCAGTTTGTCACTGGCAGTATCCTTAAAGTTCC
329
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  862  ATGACTTGACGTCGTCCCCGCCTTCCTCCAGTTTGTCACTGGCAGTATCCTTAAAGTTCC
803

Query  330  CACCCGAAGTGCTGGCAAATAAGGAAAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACA
389
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  802  CACCCGAAGTGCTGGCAAATAAGGAAAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACA
743
```

```
Query  390  TCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTATGTAAGTTCCCGAAGGCACC
449
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  742  TCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTATGTAAGTTCCCGAAGGCACC
683

Query  450  AATCCATCTCTGGAAAGTTCTTACTATGTCAAGACCAGGTAAGGTTCTTCGCGTTGCATC
509
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  682  AATCCATCTCTGGAAAGTTCTTACTATGTCAAGACCAGGTAAGGTTCTTCGCGTTGCATC
623

Query  510  GAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAGT
569
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  622  GAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAGT
563

Query  570  CTTGCGACCGTACTCCCCAGGCGGTCTACTTATCGCGTTAG  610
            ||||||||||||||||||||||||||||||||||||||||
Sbjct  562  CTTGCGACCGTACTCCCCAGGCGGTCTACTTATCGCGTTAG  522
```

ANNEXURE F Microbiological population techniques

Membrane filter Technique

1. The filtering unit was assembled for use.
2. The membrane filter (45µm) was placed into the funnel assembly
3. 100ml of the water sample was pored into the funnel
4. The vacuum was turned on and the sample was allowed to draw completely through the filter.
5. The membrane filter was then place on a prepared petri dish
6. The plates were incubated at 28°C for 48 hours.

Spread plate

0.1 ml of serially diluted samples were placed in the middle of agar plates. The sample was spread over the surface with the help of the L-rod. After 24 hours of incubation at 37°C the colonies were counted.

Pour plate

1. Water samples were serially diluted
2. 1ml of diluted samples was place in sterile Petri dishes
3. 20 ml of molten agar which was cooled to 45°C was added, swirling to mix.
4. The agar was allowed to solidify on a flat table top.
5. The petri dishes were then incubated at the appropriate temperature.