INVESTIGATION OF THE EFFICACY OF BDOC PROTOCOLS USED IN BIOFILM MEASUREMENT AND MONITORING

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A research report submitted to the Faculty of Engineering and the Built Environment, University of the Witwatersrand, Johannesburg, in Partial Fulfilment of the requirements for the degree of Masters of Science in Engineering.

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DECLARATION

I declare that this research report is my own unaided work. It is being submitted for the Master of Science in Engineering by coursework and research to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

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(Signature of Candidate) (Day) (Month) (Year)

ABSTRACT

Access to good quality drinking water is essential for the maintenance of public health. To guarantee a steady supply of good quality water, water treatments plants are designed to provide potable water that meets national and, where necessary, local water quality standards. While the protection of natural water resources against pollution, and proper treatment of water at treatment plants are both crucial to the provision of safe drinking water, the reality is that the quality of treated water can degrade during distribution.

Microbial proliferation within distribution systems can cause problems such as unpleasant tastes and odours as well as the proliferation of pathogenic microorganisms. For most utilities, it is biofilm that grows on pipe surfaces that act as permanent inocula continuously inoculating the bulk water as it flows through the distribution system. Distribution system biofilm growth and the resulting impact on water quality can be minimized by various treatment processes, designed to remove biodegradable organic matter (BOM) from the water. The removal of BOM is of great importance to water utilities because it eliminates bacterial regrowth and the many associated water quality issues. Hence, the spatial and temporal mapping of biodegradable organic carbon (BDOC) offers water utilities an effective strategy in managing the BOM in the distribution system.

This research is aimed at evaluating the applicability of BOM measurement protocols to monitoring biostability and biofilm formation potential within a drinking water distribution system (DWDS). This study specifically investigated the efficacy of a simplified version of the high-density BDOC test as a protocol for monitoring BDOC in finished water. The high-density BDOC protocol was found to be a more streamlined approach in contrast to the assimilable organic carbon (AOC), and provides a suitable monitoring mechanism for lowering biofilm formation potential in DWDSs.

DEDICATION

Firstly, to God Almighty for His steadfastness, love and grace for actualization of my dreams

And

To the cherished memory of my beloved mother, Ezinne Esther Nlemchukwu Olugbuo

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AOC	**Assimilable Organic Carbon**
BAS	**Biologically Active Sand**
BDOC	**Biodegradable Organic Carbon**
BDS	**Blue Drop System**
BOM	**Biodegradable Organic Matter**
CFU	**Colony Forming Units**
DBPs	**Disinfection by-products**
DO	**Dissolved Oxygen**
DOC	**Dissolved Organic Carbon**
DWDSs	**Drinking Water Distribution Systems**
DWAF	**Department of Water Affairs and Forestry**
DWAS	**Department of Water Affairs**
EPS	**Extracellular Polymeric Substances**
HPC	**Heterotrophic Plate Count**
JW	**Johannesburg Water**
MAP	**Microbially Available Phosphorus**
NBDOC	**Non-Biodegradable Dissolved Organic Carbon**
NDIR	**Non-Dispersive Infrared**
NOM	**Natural Organic Matter**
NPOC	**Non-Purgeable Organic Carbon**
pH	**Power of hydrogen**
SANS	**South African National Standards Specification**
SPC	**Standard Plate Count**
THMs	**Trihalomethanes**
TOC	**Total Organic Carbon**
TC	**Total Coliforms**
USEPA	**United States Environmental Protection Agency**
UV	**Ultra-Violet**
WHO	**World Health Organization**
WRF	**Water Research Foundation**

CHAPTER ONE: BACKGROUND TO THE STUDY

1.0. Introduction

The provision of good quality water continues to be a major problem affecting many communities in developing countries. Lack of good quality water does not only have implications for public health but also economic growth. South Africa is a semi-arid country with low rainfall (average of 500 mm per annum) and high evapo-transpiration. The highly variable spatial distribution of rainfall across the country adds to the sparse availability of fresh water (Adewunmi *et al.,* 2010). Given the scarcity of water in South Africa, effective water management practices are essential to preserve the available resources (DWAS, 2013).

Distribution systems (DS) are major part of water utilities. Distribution systems are designed to provide safe portable water from a treatment plant to consumers in the required quantity and quality at a satisfactory pressure at all times (Biyela, 2010; Walski *et al.,* 2003). Studies of drinking water networks have shown that the major source of biomass inside distribution networks is attached to pipe surfaces as biofilms, which affect the water quality and increase the maintenance cost of the distribution network (Farkas *et al.,* 2012; Boe-Hansen *et al.,* 2003; Van Der Wende & Characklis, 1990). Pipe biofilms have been linked to the deterioration of water quality, corrosion of pipes, odour and taste problems and may harbour pathogens (Simoes *et al.,* 2012). It is therefore important to understand how best biofilms can be controlled in DWDS.

The concentration of biodegradable organic matter in drinking water is one of the fundamental elements that determine the extent to which heterotrophic bacterial growth can occur during water distribution (Prevost *et al.,* 1998). Numerous methods have been developed to measure biodegradable organic matter. These methods are discussed by Huck (1990) and Servias *et al,* (1989); however, there are conflicting views, regarding the appropriate method(s) that may be considered best for measuring biodegradable organic matter. A simple method for determining biodegradable organic carbon is proposed. The principle of this method is to sterilize by filtration the water sample containing the organic matter to be tested, to inoculate it with an autochthonous bacterial population, and to measure the decrease of Dissolved Organic Carbon (DOC). Any measurable drop in DOC during the incubation is attributed to oxidation of carbon by bacteria (LeChevallier & Au, 2004; Al Dufour *et al.,* 2003; Servias *et al.,* 1989).

Biofilms are made up of bacteria in a polymeric structure. In order to minimise the risk to public health, high chlorine residual is required to maintain biostability in systems that have biofilms. It is therefore important to understand how best biofilms can be controlled or prevented in drinking water distribution systems (DWDS) to minimize their negative impact.

Understanding the factors that influence microbial activity is also critical in combating biofilm growth. The majority of previous studies have been laboratory or pilot plant-based and few results are available for field conditions.

There are other methods, as have been discussed by Escobar and Randal (2001). Reporting on both Assimilable Organic Carbon (AOC) and Biodegradable Organic Carbon (BDOC), they argued that AOC gives an indication of potential microbial regrowth for a specific strain or defined mixtures of bacteria whereas BDOC gives an indication of microbial growth and chlorine residual demand as well as the disinfection by-product (DBP) potential. Several experimental procedures have also been proposed, most of them are based on the original assimilable organic carbon method developed by van der Kooij *et al*., (1990). In this method, the water sample is sterilized by heating it at 60°C for 30 minutes and inoculated with a pure strain of bacteria (*Pseudomonas Fluorescents or Spirillum* Sp. NOX), and the bacterial growth is monitored by plate count. The maximum bacteria enumerated are usually obtained after 4 to 7 days of incubation. It is taken as an index of assimilable organic carbon; this index is converted to a carbon equivalent by reference index to a calibration obtained by measuring the growth of the same strain on a range of acetate concentrations. This method's results however do not provide a direct measurement of BDOC but rather are indicative of bacterial growth on the organic matter present in the sample.

In contrast to these methods, Servais *et al.,* (1987) proposed a procedure which involved sterilization, reinoculation with a natural assemblage of bacteria, and incubation at 20°C. During the incubation, bacterial biomass and bacterial mortality rate were monitored; BDOC was calculated from the bacterial mortality flux. The values of BDOC found by this method were much higher than those found by the index methods. Huck (1990) listed many methods of BDOC measurement which may be divided into two categories namely biomass-based methods and DOC-based methods. Biomass based methods measure the growth of microorganisms over time and by means of a calibration can be used to approximate the BDOC. The biomass methods are complex and time consuming and they only provide an indirect measure of BDOC. DOC-based methods measure the change in DOC concentration

over time. This decrease in DOC is assumed to be a result of the metabolism of organic compounds by microorganism. Therefore these methods provide a direct measurement of BDOC (Vonkl *et al.,* 2015; NWRI, 2012; Allgeier *et al.*, 1996).

1.1. Problem Statement

With the severely constrained water resources, South Africa is in dire need of effective water management practices to preserve the available resources. As stated previously, while there are many methods that can be used to measure biodegradable organic matter, there are conflicting views, regarding the appropriate method(s) that may be considered best and most appropriate for measuring biodegradable organic matter in the Johannesburg water distribution system. There is a need to describe and validate a protocol of biodegradable organic matter that measures these microorganisms which can be removed in treated water using biological processes. Using the water distribution system of Johannesburg that serve a population of about 3.8 million, this research is centred on the use of BDOC to measure the presence of these microorganisms in the system.

1.2. Research Aim and Objectives

The primary aim of this research is:-

- To test the efficacy of BDOC protocol as a method for measuring and monitoring the potential for bacterial growth and biofilm formation in drinking water distribution system.

The specific objectives of the study are outlined as follows:-

- To assess and standardize the applicability of both BDOC and AOC in measuring and monitoring bacterial growth and biofilm formation in drinking water distribution systems.
- To determine and validate the efficiencies of these protocols (BDOC and AOC) used in measurement and monitoring of the bacterial growth and biofilm formation, and to combine these protocols with other water quality parameters for a comprehensive biostability assessment.
- Then to recommend the most effective protocol to be used for the measuring and monitoring of biofilm formation in the JW's drinking water distribution system.
- To compare BDOC and water quality parameter values under laboratory and field conditions.

1.3. Scope of Work

The focus of this research is on the water quality in the DWDSs, specifically describing and validating a protocol to determine the BDOC.

1.4. Research Limitations

If the samples are not well preserved upon collection it may compromise the laboratory results. Very strict procedures were followed during the collection, transportation, and handling of samples to ensure that they were not compromised. Therefore at the sampling location, specific precautions that were taken to protect the integrity of samples included, but were not limited to, sterilizing all glassware that was to come into contact with the samples; flushing taps for about 5 minutes prior to sample collection, flame sterilization of tap nozzle, sterilization of the sample bottles, collection of the sample in a 1 litre container, transportation in ice-packed storage to the Environmental laboratory. Analysis is usually done within eight hours after collection. These measures ensured not only the integrity of the results of the analysis of each sample but also the consistency of the results across samples.

1.5. Research Assumptions

The period of sampling is considered representative of the overall long term biofilm formation and accumulation within the water distribution of Johannesburg. The sampled points provide a good spatial distribution of biofilm formation and accumulation in the Johannesburg water distribution system.

1.6. Research Question

The research question in this research study is, “How accurate is the BDOC protocol in monitoring and measuring of biofilm growth potential?” Distribution systems are faced with the problem of biofilm growth. The management strategies adopted by water utilities have not been sufficiently robust in dealing with this problem. The use of BDOC protocol will be useful in measuring and monitoring of biofilm growth. The information provided by this protocol will feed into the management strategies of water utilities in limiting bacterial growth in water distribution systems.

1.7. Area of Study: Johannesburg Water

Johannesburg Water (JW) is an independent water utility that serves approximately 3.8 million people within the City of Johannesburg. As it is the case with all utilities, JW reports to the Department of Water and Sanitation. JW buys treated potable water from Rand Water. This water is then transported throughout Johannesburg in a network made up of

approximately 11 000km distribution pipes. The company also operates six wastewater treatment works.

In accordance with South African National Standards Specification (SANS 241), JW has a robust water quality monitoring programme that includes among other things the monitoring of the distribution system water quality. JW has achieved the blue drop status for four consecutive assessments (Johannesburg Water, 2014). The blue drop programme is an initiative created by the Department of Water and Sanitation (DWS) to ensure that water utilities comply with regulation standards of drinking water and that they employ adequate water monitoring programmes. All drinking water quality compliance data are required to be submitted to the Department of Water Affairs on the Blue Drop System (BDS) at a monthly frequency. This data is used to assess drinking water quality compliance. The achievement by Johannesburg Water of blue drop status means that the utility complies with all the regulatory and operational standards that the water is safe for consumption (DWAS, 2008).

For the purpose of this research samples were collected in strategic locations of the Johannesburg water distribution network for tests and analysis.

1.8. Overview Chapters

In this section, a brief outline of the structure of the remaining chapters in this research report is highlighted:

Chapter 2 covers the literature review.

Chapter 3 entails the details of the research design such as sampling protocols and experimental procedures.

Chapter 4 comprises the analysis and the discussions of the results obtained in this study.

In **Chapter 5,** the major contributions of this research are summarized and recommendations for future studies are outlined.

CHAPTER TWO: LITERATURE REVIEW

2.0. Introduction

It is an established fact that the state of the distribution system is vital in determining the quality of drinking water at the point of use (Momba *et al*., 2000; Hammes *et al*., 2010; Lu *et al*., 2014). The growth of pathogenic and toxigenic microorganisms in drinking water are the major risks confronting the water sector, and their occurrence have significant impacts and threats on public health (Momba *et al.,* 2000; WHO, 2001). The degradation of the quality of potable water due to bacterial growth inside distribution systems is a cause for concern. Therefore there is need for water utilities to have robust strategies for monitoring the water quality in the distribution system, managing bacterial growth and biofilm formation, amongst other things.

This chapter reviews the literature on the methods used to measure biodegradable organic matter, and the conflicting views on the effectiveness of BDOC in relation to AOC measurement as the means of evaluating the biostability of distributed water. Since biostability is the balance between the ability of potable water to support microbial growth through the presence of substrates and the ability of the same water to control microbial growth through the presence of disinfectant residuals, this literature review looks at factors that promote bacterial growth and biofilm formation in distribution systems.

2.1. Controlling Microbial Activity in Drinking Water Distribution Systems

Microbial growth in drinking water systems is a very complex process, resulting from a number of inter-relating processes. The hydraulic conditions within the distribution system, the presence of biodegradable substrates and temperature all play a role in determining the extent of microbial activity within distribution systems. Van der Kooij (2003) showed that microbial activity depends on the availability of sources of energy and carbon for the formation and preservation of biomass. The most important energy source in treated water is organic carbon, but ammonia may also fuel growth in some water. Therefore monitoring microbial activity in distribution systems is needed in order to avoid water quality deterioration which may result in non-compliance with regulations (e.g. SANS 241), consumer complaints, disease or engineering problems (WHO 2011). The following approaches can be used for controlling (limiting) microbial activity according to van der Kooij, 2003:

- Maintaining a disinfectant residual in the entire distribution system.
- Optimizing the water distribution system in order to prevent stagnation and sediment accumulation.
- Minimizing the concentration of organic matter entering the distribution system (Simoes, 2013).
- Distributing biologically stable drinking-water in a system with non-reactive biologically stable materials.

2.1.1. Biological Stability

Biological stability (biostability) can be defined as the ability of water or a material in contact with water not to support microbial growth in the absence of a disinfectant (Rittman & Snoeyink, 1984; Woolschlager, 2000). Naturally, biologically stable water does not encourage the growth of microorganisms during its distribution as a result of lack of growth substrates (Rittmann & Snoeyink, 1984).

AOC and BDOC parameters are used to measure the nutritional potential of water directly or indirectly in terms of carbonaceous organic compounds (Kaplan, Reasoner & Rice, 1994); they are not intended for routine monitoring. In the terminology of water safety plans, they are used for "process validation". Depending on the water composition of inorganic, organic carbon could also be a growth limiting factor (Fang *et al.,* 2009; Hammes *et al.,* 2010). Biostability is basically dependent on the concentration of disinfectant residual, assimilable organic carbon (AOC) and biodegradable dissolved organic carbon (BDOC).

Biodegradable Dissolved Organic Carbon: Biodegradable dissolved organic carbon can be defined as that fraction of the dissolved organic carbon which can be metabolized by bacteria within a period of time (Pierree *et al*., 1989). BDOC values are said to represent 10–30% of the total dissolved organic carbon content of drinking water (Escobar & Randal, 2001). BDOC can be used to quantify the organic carbon that can be removed by biological treatment (e.g. bio-filtration) and serves as a benchmark for process efficiency. Removal of BDOC during treatment can result in lower chlorine demand, lower disinfection by-product (DBP) formation in the distribution system and lower regrowth potential. For most waters there is a difference between BDOC and AOC levels.

A rapid assessment of the BDOC value can be obtained from changes in DOC concentrations following the passage of water through a column containing a support with an adapted

microbial community (Lucena *et al.,* 1990; Ribas *et al.,* 1991). The BDOC method developed by Servais *et al.,* (1989) measures the reduction of DOC due to oxidation by an indigenous microbial community during an incubation period of 30 days. Joret and Levi (1986) modified this method by using the biological filter and an incubation period of 7 days.

The key variables that must be considered when choosing a BDOC method include the duration of the analysis, the nature of the inoculum, and the characteristics of the water to be measured. Test duration is an important factor and BDOC analysis methods vary widely in the time required for analysis, ranging from two hours (Frias *et al.,* 1992) to six weeks (Trulleyova & Rulik, 2004).

Assimilable Organic Carbon: This is that portion of the degradable organic carbon that can be converted to cell mass and expressed as a carbon concentration by means of a conversion factor or calibration. AOC represents the most readily degradable fraction of biodegradable dissolved organic carbon and it typically comprises about 0.1–9.0% of the TOC (Escobar & Randal, 2001). Assessment of the assimilable organic carbon concentration is based on growth measurements with a mixture of two selected pure cultures in a sample of pasteurized water contained in a meticulously clean glass clogged in Erlenmeyer flask (van der Kooij *et al.*, 1982c).

According to work done by van der Kooij (1992), biostability was achieved when AOC reduction concentrations were below 10 μg of C/l, that is at these low levels the heterotrophic plate counts (HPC) remained low. Based on these observations, it was concluded that AOC values below 10 μg of C/l are indicative for drinking water with a limited regrowth potential for bacteria contributing to HPC values (van der Kooij, 1999).

2.2. Techniques for Measurements of Biostability and Biofilm Formation Potential

Several methods have been developed to measure biofilm formation in distribution system. In evaluating and comparing methods, it is essential to define the purpose for which the measurement is being made. There are few microbial measurement methods that can relate to biofilm directly hence the attached bacteria usually have to be removed from the substratum. Removal of intact cells may be a difficult task, since the choice of procedure is a balance between obtaining high removal efficiency and minimising the stress, inactivation or even destruction of the cells. Possible procedures include flushing (LeChevallier *et al*., 1987), sonication (Clark *et al.,* 1994), centrifugation (Camper *et al.,* 1985a), and swapping (Boe-Hansen *et al.,* 2002b) which can be combined with the use of detergents. Different methods

for measuring the biodegradability of the organic matter present in water have been developed. These techniques measure either AOC or BDOC. The principal test conditions of each of the biomass-based methods are summarized in the Table 2.1;

2.2.1. Comparison of Methods

Table 2:1: Conceptual Comparison of BDOC Methodologies (Huck, 1990)

Author	Water Preparation	Inoculation	Incubation Conditions	Measured Parameters	Result Expression
Servais, Billen, and Hascoet; 1987	Filtration	Suspended bacteria from river water	28 days 20°C	DOC	$BDOC = DOC_i - DOC_f$
Joret Levi 1986; Joret, Levi, & Gilbert; 1989	-	Bacteria fixed on sand	1 week 20°C	DOC	$BDOC=DOC_i - DOC_{mini}$
Mogren, Scarpino; 1989	-	Bacteria fixed on sand	5 Days 20°C	DOC	$BDOC=DOC_i - DOC_5$
Frias, Ribas, and Lucena; 1992	Filtration	Column with bacteria fixed on porous glass particles	2.5hr 20°C	DOC	$BDOC=DOC_{inflow} - DOC_{outflow.}$

It is important to note that there have been many variations of these biological assays tests developed by different researchers as seen in Table 2.1, to measure BDOC and each involves the indirect measurement of the biodegradable organic carbon fraction (Huck, 1990). These assays differ in how samples are treated, such that each measures a different fraction of BDOC and no one measures adequately the total amount of BDOC in drinking water (Huck, 1990).

Each of these methods classified above has certain advantages and disadvantages with regards to the complexity of investigations, incubation time and personal choice of

preparation. To be able to generate a standard method measuring the bacterial regrowth potential in drinking water, more details about the degradation of BOM function should be known. Therefore one has to be careful when reviewing the literature to understand the origins of the assay. Most studies have used the BDOC assay by Joret *et al.,* (1986; 1989) with some slight modifications. The purpose of most of the modifications was to shorten considerably the response time needed in the method with the incubation time. Also seen in the table the Servias *et al.*, (1987) measurement performed longer which can delay time needed for the next possible action in line with the treatment efficiency.

2.2.2. Comparative Studies of AOC and BDOC Protocols

According to different authors, assimilable organic carbon and biodegradable dissolved organic carbon have been understood to be the two known parameters that are used for the evaluation of regrowth potential and biological stability of drinking water. Both AOC and BDOC protocols are challenging to measure and both require a high level of proficiency and carefulness when carrying their tests.

The advantages of the BDOC method are that it is both sensitive and precise to perform. Since the BDOC reading corresponds to the difference between two DOC analyses, the minimum detectable concentration is linked to the detection limit of the DOC analyser. For AOC, because the bioassay organisms are able to grow at low concentrations of organic substrate, the AOC bioassay is extremely sensitive to organic carbon contamination. Regardless of how the assay is performed, the conversion of cell densities to AOC concentrations involves the use of yield coefficients. AOC measurement essays are time consuming and require a high level of expertise (Page & Dillon, 2007). AOC and BDOC have repeatedly measured separately as indicators of bacterial regrowth, or together as indicators of bacterial regrowth and disinfection by-product formation potential, respectively. The AOC concentration may be regarded as a measure for the biological stability of the water for heterotrophic growth while the measure BDOC may be regarded as an indication of the relative potential for DBP formation in drinking water (van der Kooij, 1990; Escobar & Randall, 2001).

The use of AOC measurements in water treatment performance monitoring and optimisation provides useful insights for managing distribution systems, but its application remains limited due to the extremely different environments between the inoculation and water distribution systems from which the bioassay organism is based. The BDOC assay is centered on

determining the reduction of dissolved organic carbon as a result of complete biological degradation (Servais *et al.*, 1987; Escobar, 2001). Most comparative studies report low BOM estimates from the AOC method than those from the BDOC methods. This can be explained by the differences in the metabolic capabilities of the bacteria. AOC is based on the metabolic capability of bacteria inoculated while BDOC is based on the metabolic activities of an unknown but larger number of species (Boe-Hansen *et al.*, 2003; NWRI, 2012).

It is important to note that the relationship between AOC, BDOC, and biostability vary from system to system. Van der Kooij (1992) proposed that water containing an AOC level of 10 μgC/l or lower could be considered biologically stable, while LeChevalier *et al.*, (1991) suggested that 50 μgC/l as a limit for coliform regrowth. Servais *et al.*, (1995) suggested that water containing a BDOC level below 0.15 mg C/l could be considered biologically stable. Though these values may be applicable as a rule-of-thumb for practical purposes, the scientific basis for proposing them is poor and considerable biological growth has been observed at AOC concentrations less than 10 μg-ac/L.

The AOC and BDOC methods are theoretically similar when it comes to degradability of bio-available carbon. The fundamental difference in the two methods is that BDOC assay assess the concentration of DOC removed through microbial community (usually biofilm related growth), whereas AOC assays usually assess the amount of cells count produced through consumption of bio-available carbon (Ribas *et al*., 1991; LeChevallier, 2003; WRF, 2012). The relationships between AOC or BDOC to DOC were investigated by different authors and conclusions were made of no overall trend shown since the relationship depends on the type of organic matter present. It was observed that the AOC/DOC ratio in raw water can vary seasonally, while the BDOC/DOC ratio did not show any trends in its variation. These authors concluded that the correlations between AOC/BDOC, between AOC/DOC, and between BDOC/DOC were statistically significant. AOC and BDOC have shown to give different information on the nature of changes in potential biostability of finished water (Huck, 1990; Kaplan *et al.*, 1994).

2.3. Biofilms: Formation and Microbial Diversity

Biofilms are collections of microbial cells that accumulate at solid–liquid interfaces and are entrapped within a gelatinous matrix comprising mostly insoluble extracellular polymeric substances (EPS) that the cells secrete (Fang *et al.*, 2009; Flemming & Wingender, 2010). While other types of microorganisms such as viruses, protozoa, and fungi can all attach to

biofilms and thrive there, biofilm pioneer communities are always bacteria. Even among bacteria, some species are better biofilm formers than other species due to their ability to produce copious amounts of EPS (Davey & O'toole, 2000; USEPA, 2002).

Biofilm can form on pipes, or sediment deposits. Bacteria within these films can negatively affect the quality of the water during distribution for example, biofilm growth:-

- Can be a starting point for a trophic food web leading to the proliferation of organisms visible to the naked eyes such as Asellus (WHO, 2004).
- Can increase turbidity, cause taste, unwanted odours and colouration (WHO, 2008).
- Can enhance corrosion of pipes materials (USEPA, 2002).

Biofilm formation is governed by four main factors:-

- The deposition and adsorption of both living and dead microorganisms from the aqueous to the solid phase;
- The continued erosion of the biomass by the flowing water;
- The growth of the attached microbes at the expense of the available biodegradable organic matter, which can either be nitrogenous or carbonaceous organic matter and;
- The death and/detachment of the attached microorganisms.

Biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings. Biofilm is a fixed system that can be adapted internally to environmental conditions by its inhabitants. The beneficial impact of biofilms is that it can be used for the treatment of water and wastewater. The engineers have been exploring these benefits for the past century. Also biofilms can be used in the bioremediation of hazardous waste sites and polluted aquifers (Centre for Biofilm Engineering, 2008).
It is important to understand the different methods to assess the microbial activity in water. Many researchers have published contrasting findings regarding the factors affecting microbial activity in drinking water systems. Some of the differences in the various findings have been attributed to the assessment methods. Understanding the method that was used in analyzing the microbial activity also helps with contextualizing the findings (Donlan, 2002; Stoodley *et al.*, 2004).

Knowing the type of microorganisms that can persist on pipe surfaces, and analysing their growth requirements will enable us to control them.

Figure 2.1 illustrates the development and growth of biofilm in water distribution systems and treatment plants, and it provides an insight into the strategies that can be adopted in controlling them.

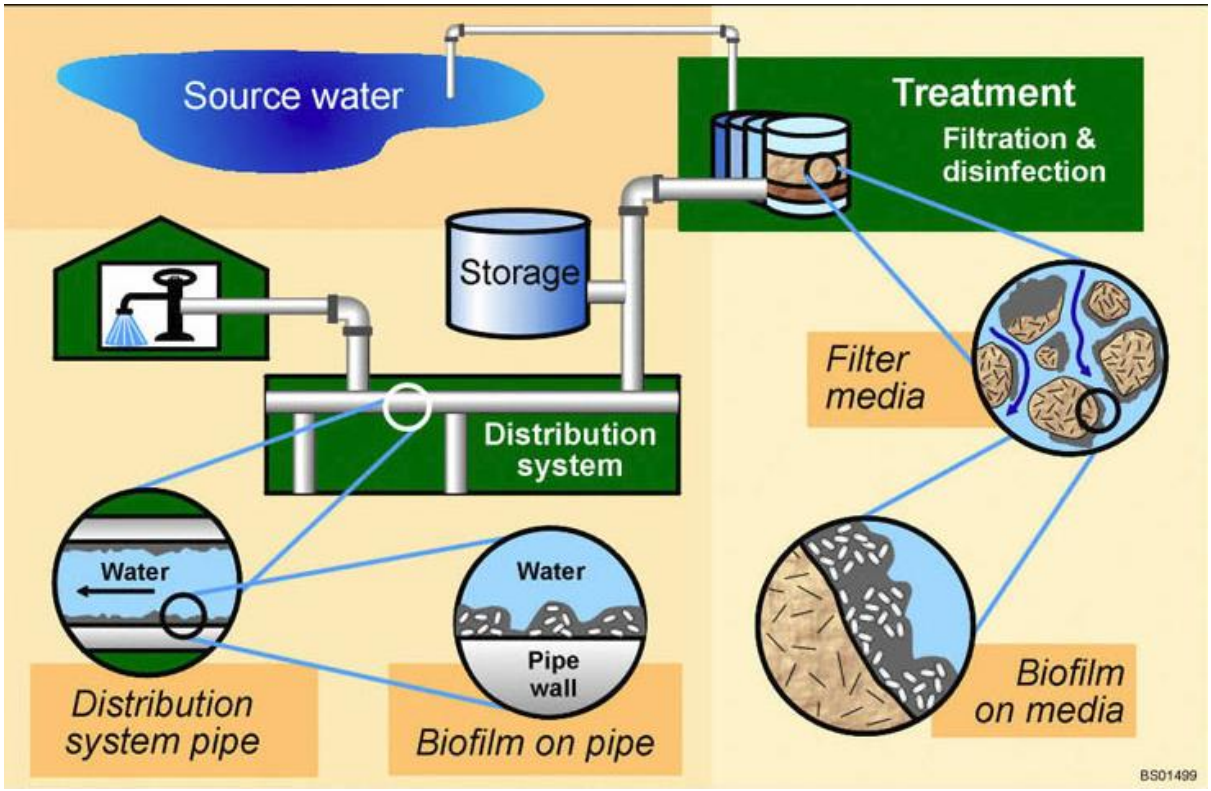

Figure 2.1: The Description of Biofilm Growth (YashodharaKambam, 2013)

2.3.1. Bacteria

Accumulation of substantial quantities of organic materials unto the surfaces of the pipes leads to bacterial growth on such surfaces. Once the bacteria population reaches a critical density they start to turn out to a jelly-like substance that is viscous in nature and this is a characteristic of a biofilm.

2.3.1.1. Heterotrophic Bacteria

These are bacteria that use organic carbon as a source of food and energy. Heterotrophic bacteria are naturally found in water. When heterotrophs survive the disinfection process they can colonize the distribution system. Heightened densities of heterotrophs may be introduced through cross connections, backflow events or repair operations, (Geldreich, 1990a and USEPA, 2002). Heterotrophic Bacteria are often measured by heterotrophic plate count (HPC). The HPC method accounts for the cultivable, heterotrophic bacteria which are said to

form less than 1% of the total population of the microorganisms found in drinking water (Grimes, 2000).

2.3.1.2. Total Coliforms

Total coliform bacteria occur naturally in water, soil, and vegetation. Coliform bacteria are unlikely to cause disease. However, their occurrence in drinking water is an accepted indicator of the possibility of the presence of disease-causing organisms (pathogens) in the water distribution system. Total coliforms belong to the family *Enterobacteriacea*e. Examples of enterobacteria include *Escherichia, Klebsiella, Enterobacter, Citrobacter, Serratia,* and many others (Leclerc *et al.,* 2001). Coliforms are used to verify the efficacy of treatment as well as the reliability of the water distribution systems **(**WHO, 2006). The main reason for using the coliforms as primary microbial indicator of drinking water quality is the fact that even though they do not have an adverse effect they are usually present along with the enteric pathogens.

2.4. Factors That Promotes Biofilm Formation

There are many factors that contribute to the growth of biofilms inside distribution systems including high water temperatures, the presence of oxidisable organic matter, and the absence of disinfectant residuals amongst others (LeChevalier *et al.*, 1990a; Smith *et al.,* 1990).

2.4.1. Environmental Factors

Temperature: Temperature is widely recognized as an important factor in bacterial growth. In climates where water temperatures are warm, bacterial growth may be very rapid (LeChevallier, *et al.,* 2004; WHO, 2003). A study by Momba and colleagues (2000) showed a seasonal distribution of species diversity of the Standard Plate Count (SPC) population present in distribution water. For example, there was greater species diversity in the warmer period than during the cold winter months (Momba *et al.*, 2000).

The efficiency of the treatment plant, the growth rate of the microorganisms, efficiency of disinfection, loss of residuals and the rate of corrosion are all influenced by temperature (LeChevallier *et al.*, 1996).

2.4.2. Nutrient Availability

One of the most important factors in determining the extent of biofilm growth is the availability of nutrients. To grow, organisms must have access to all substances they require

to synthesize cell materials and generate energy. For coliform and heterotrophic bacteria, the principal nutrients sources are organic carbon, nitrogen, and phosphorus (Mains, 2008).

Carbon: Low levels of organic carbon can suppress microbial growth. Organic carbon is utilized by heterotrophic bacteria for production of new cellular material (assimilation) and as an energy source (dissimilation) (USEPA, 1996; Pipe-Martin *et al.*, 2010).

Biodegradable dissolved organic carbon is another test frequently used to define the concentration of nutrients that are available for bacterial growth in water. The variance in carbon levels validates the amount of nutrient readily available for bacterial growth (Joret & Levi, 1986).

2.4.3. Hydraulic Effects

Flow rates and residence times have an impact on microbial activity. The microbial growth on the surface of the pipes may be regulated by the velocity of flow in the distribution systems. An increase in the velocity will increase the flux of nutrients in the system thereby transporting large amounts of disinfectants; this will in turn increase the shearing of biofilms from the pipes surfaces (Melo & Vieira, 1999; Lehtola *et al.*, 2006). Distribution system hydraulics can also affect corrosion, sediment accumulation, and biofilms attachment and detachment (Donlan & Pipes, 1988).

2.4.4. Corrosion

Corrosion of iron pipes can influence the effectiveness of chlorine-based disinfectants for inactivation of biofilm bacteria (LeChevallier *et al.*, 1990; 1993a). Corrosion accounts for heavy economic losses in potable water distribution systems (WRF & USEPA, 2012).

When there is a continuous distribution of corroding metals that are of high concentration in the distributed water, this in turn affects water quality negatively, due to these metals (Lehtola *et al.*, 2002). Corrosion can interfere with the integrity of pipes and may create leaks and bursts. A study by LeChevallier *et al.*, (1990, 1993) established that rusting of iron pipes can control the effectiveness of chlorine-based disinfectants for inactivation of biofilm bacteria.

2.4.5. Sediments Accumulation

Sediment accumulation occurs within storage facilities due to inert conditions which promote particle settling. Potential water quality problems associated with sediment accumulation

include increased disinfectant demand, microbial growth, disinfection by-product formation, and increased turbidity within the bulk water (USEPA, 2002).

The microorganisms are well protected from the disinfectants such as chlorine, ultra-radiation, ozonation, etc. with the help of these sediments and corrosion products (LeChevallier *et al.*, 1990) thus reducing the effectiveness of disinfectants even when applied. The presence of sediments can also cause inaccuracies to the free chlorine residual result which is sometimes used as an indication of the level of microbial activity in water.

2.5. Disinfection Methods and its Impact on Distribution System

Chlorination, ozonation, and chloramination disinfection amongst others have been used as disinfection methods for drinking water.

Chlorination: This is an effective disinfectant against viruses and bacteria, but to a reduced extent against protozoa. Although chlorination is effective in reducing microbial regrowth, its application has been associated with increased concentrations of halogenated disinfection by-products e.g. trihalomethane (THM) which affects the taste and odour of drinking water leading to complaints by consumers (Standfield *et al.,* 2003). The chlorine residual rapidly declines in the distribution system, usually after about a 10hr residence time, the concentration has dropped below 0.1 mg/litre.

Chlorine is a strong oxidant that oxidizes many dissolved inorganic substances and the natural organic matter remaining in the water after treatment. Consequently, a substantial loss of chlorine occurs in these reactions (referred to as chlorine decay), which is most rapid immediately after chlorine is added to the water (Fisher *et al.*, 2011). Chlorine also enhances the corrosion process.

Chloramination: This disinfectant method is used on a large scale for distribution system residual maintenance and has replaced free chlorine residuals (LeChevallier *et al*., 1988b; 1990). Addition of monochloramine in chlorinated water can result in the removal of biofilm from the pipe walls (Momba & Binda, 2002). Its application has a number of advantages over free chlorine (WHO, 2003; USEPA, 2004):

- It has an extensive half-life than chlorine therefore it can retain biostability for a far-reaching time than that of chlorine.
- It yields a lesser amount of THM.

- A significant accomplishment of using monochloramine is the decrease in cases of legionellos is related with chlorinated water supplies (Kool *et al*, 1999a).

And some of the short comings that are associated with chloramination include the following:

- Reaction with elastomers.
- Monochloramine is toxic to humans which limits its full concentration in water.

In order to reduce the amount of chloramine added, Momba and Binda (2002) suggested the combination of chlorination and chloramination processes for the inhibition of biofilm formation.

Ozonation: This is increasingly becoming the most widely used disinfectant. Its advantages over chlorination include:

- It can remove or transform undesired colours tastes, odours, toxins and micro pollutants from the water.
- It leads to smaller concentrations of halogenated disinfection by-products.

Ozonation has however been greatly criticized for its tendency to increase the AOC concentration in water. To remove the AOC after ozonation, treatment with biological filtration (biologically activated carbon and or sand filtration) can be found. Ozonation has some disadvantages such as the instability of ozone which requires on-site production and the generation of mutagenic and carcinogenic agents such as bromide in the treated water (Richardson, 2003).

2.5.1. Impact of Disinfectant Residual on Distribution System

Maintaining a consistent disinfection residual is extremely important for properly operated distribution systems, Friedman *et al.,* (2010).

LeChevallier, Welch, and Smith (1996) recommends that at least 0.2mg/L free chlorine or 0.5mg/L chloramines are present at the far ends of the distribution system to limit coliform regrowth. Elevated water temperature, high AOC/BDOC levels, and low disinfection residuals especially may result in microbial growth problems (Volk & LeChevallier, 2000).

2.6. Summary on Review of Literature

This literature review addresses the causes of water quality deterioration in distribution systems in general and biofilm measurement and monitoring in particular.

The survey revealed that a lot of progress has been made in understanding the dynamics of biofilms and its negative effects on the water quality in distribution system. The most critical factors that affect biofilm growth are also discussed in this section.

One of the main concerns associated with biofilms in distribution systems is the possible presence of pathogenic bacteria that may survive and multiply while protected in the biofilm matrix. As such, further research aiming at characterizing distribution system biofilms would help assess the contribution of biofilms to the presence of pathogens in distribution systems.

Of particular interest to this study is BOM which is generally seen as the key limiting factor that is responsible for bacterial growth in the water distribution system. This has been reviewed in detail. Therefore, one of the most effective methods in controlling the bacterial growth in the distribution system is then to limit the amount of BOM vital for the growth of heterotrophic bacteria in treated water. There are different methods of measurements developed in assessing BOM in water which were investigated; the pros and cons as well as the conflicting views are reviewed thereby providing some pointers to appropriate measures to pursue in the study.

In terms of the key substrate for microbial growth there is a general consensus amongst investigators that AOC and BDOC are the key limiting substrates for microbial activity. The comparative studies of AOC and BDOC are addressed. Both BDOC and AOC require proficiency when carrying out the assays.

This review also showed that there are several biofilm measurement techniques each with advantages and disadvantages but the suitability of these techniques depends on the biofilm parameters to be investigated.

The survey also addresses the global issue of disinfectant residuals and microorganism inactivation in drinking water distribution systems as well as all the numerous factors that affect the presence of disinfectants and microorganisms in distribution systems. It is usually argued that a disinfectant residual may control microorganisms in drinking water distribution systems due to treatment innovation at the plant, biofilm released from the pipe walls, pipe leaks, and other possible sources of contamination. However results from various studies

indicate that microorganisms survive in distribution systems despite the prevailing presence of a disinfectant residual.

In sum the literature review has provided insight into the research question: ‘How accurate and efficient is the BDOC protocol in monitoring and measuring of biofilm growth potential?’ The literature has clarified the tools to measure and monitor biofilm growth in water distribution systems.

If this protocol can be verified and proven to be reliable it would be extremely beneficial to water utilities in South Africa. It would be useful in formulating effective management strategies which will ensure compliance to drinking water regulations.

CHAPTER THREE: METHODOLOGY

3.0. Background

This chapter provides descriptions of the study area, sampling regime, as well as experimental protocols employed in this research. One of the main tasks of this research was to evaluate two protocols widely used in determining BOM in treated water and standardizing them for replicability of their measurements in the Environmental Engineering laboratory in the School of Civil and Environmental Engineering. This proposed strategy seeks to fulfil the goal of assessing the more viable protocol for monitoring of biofilm formation in JW's drinking water distribution system.

The choice of BDOC measurement method was established after the conceptual comparison in the literature survey for assessing BOM in water distribution system. The adopted approach in this study is based on the BAS technique of the BDOC procedure. The geographical origin of the inoculum used may have little or no effect on the measurements, hence the sand samples were collected from the Environmental Engineering laboratory and used for the BAS analyses. These microbial communities established in the BAS are referred to as Reactors A to F in this study. In parallel with the use of BDOC method, AOC protocol was used to measure the BOM in drinking water distribution system. Regardless of how the assay is prepared, the AOC bioassay is extremely sensitive to organic contamination.

Also, other water quality parameters were measured to provide a comprehensive assessment of biostability of drinking water distribution systems, to reflect the second objective of the study. In conjunction with these two protocols, these water quality indices provide insight into the potential for monitoring bacterial growth and biofilm formation in JW's drinking water distribution system. The experimental techniques used for these water quality analyses are specified in Table 3.2.

Furthermore the growing and testing of biofilm in a concrete pipe under controlled conditions in the Environmental Engineering laboratory was another way of achieving the research objectives of the study, thereby obtaining a fundamental knowledge of biofilm development in distribution systems. These results, presented in this study for BIOFILM 1, BIOFILM 2 and BIOFILM 3, were then used to determine the efficacy of the BDOC protocol as stated in the study objectives.

Due to the large size of JW's distribution system as mentioned in chapter 1, only a small section of the distribution system which is made up of AREA 10 and AREA 5 was examined in this study. This area was chosen due to historical water quality problems, particularly the residual disinfectant depletion and extensive bacteria growth. It is important to note that water quality problems in this section of the network did not reflect any failure in meeting the treatment goals at the plant.

Thirteen sampling sites, which are found in these two areas (AREA 5 & AREA 10), were chosen for this analysis. These sites were selected by taking into consideration their proximity to the treatment plant, retention times and the flow conditions (e.g. storage reservoirs, dead ends, main pipes) which are factors that affect water quality.

The sampling plan is premised on providing an overview of the biofilm formation and accumulation in the Johannesburg water distribution system. Having sampling points with wide spatial spread are most likely to present a good spatial distribution of these parameters to both test the precision of the BDOC protocol and assess the biostability of the JW's water distribution system. The number of sampling sites chosen in this study represents a good compromise between in-depth water quality monitoring which is necessary to monitor biostability and the financing that was available for the completion of this research project.

The sampling sites include reservoir tanks as well as distribution pipes. Water samples were collected from these sites fortnightly for a period of 3 months.

Table 3.1 shows the sampling sites data. The codes used in this report are the official codes used by Johannesburg Water.

Table 3.1: The JW Sampled Sites with the Description

Sample Sites Codes	**Descriptions**
RW251	Reservoir inlet (Randburg area)
RW253	Distribution network
RW080	Reservoir Inlet (Northcliff area)
RW081	Reservoir outlet
RW082	Distribution network
RW083	Tower outlet
RW084	Tower inlet
RW104	Reservoir inlet
RW105	Reservoir outlet
RW106	Distribution network
RW107	Reservoir inlet (Fairland area)
RW108	Reservoir outlet
RW109	Distribution network

3.1. Water Quality Testing

All water samples were tested to determine values of water quality parameters from which comprehensive assessments could be made of the state of the water. These include dissolved organic carbon, biodegradable dissolved organic carbon, assimiliable organic carbon, pH, temperature, free chlorine and total chlorine. Experimental techniques provided in Table 3.2 were used for the water quality analysis.

All samples for chemical and microbiological analysis were collected in glass bottles that had been previously autoclaved. Where needed, 5mL 0.1N sodium thiosulfate was added per liter of sample to neutralize chlorine. Prior to sample collection all taps were flushed for approximately 3 minutes. All samples collected were labelled with the sample code,

collection point, date and time of collection, as well as temperature**.** The samples were then stored in cooler boxes packed with ice and transported back to the laboratory for analysis. Sample analysis was always done within eight hours of sample collection.

The choice of BDOC measurement method was established after the conceptual comparison in the literature survey for assessing BOM in water distribution system.

Table 3.2: The Techniques used in measuring the Water Quality Parameters

PARAMETER	**TECHNIQUE USED**
BDOC	A simplified version of the high-density BDOC test (Joret *et al.*, 1991; Allegier *et al*., 1996; Woolschlager, 2000).
AOC	The standard method (van der Kooij, 1989). The method involved growth of Pseudomonas *brenneri* strain P-17 to a maximum density in water samples and controls.
DOC	Combustion-infrared technique on a total organic carbon analyser according to standard method 5310, a combustion-infrared.
pH	A portable pH meter.
TEMPERATURE	A certified alcohol thermometer.
Chlorine (Free & Total)	DPD method according to Standard Methods (Clesceri *et al*,. 1998).

3.1.1. Dissolved Organic Carbon Determination

The DOC analyses were measured using the combustion-infrared technique on a total organic carbon (TOC) analyser (Teledyne Tekmar, TOC). Prior to analysis all samples were filtered through a 0.45 μm filter.

Preparation of Calibration Standards

A concentration of 1000 mg/L of stock solution was obtained from Lab house, South Africa. The stock solution was diluted to 100 mg/L by pipetting 10 mL of the stock solution and diluting with distilled water to a volume of 100 mL. The TOC analyzer auto diluted the stock standard to prepare the calibration standard solutions listed on the table below;

Table 3.3: Preparation of Calibration Standards

Standards	Concentration (mg/L)
1	Blank
2	1
3	5
4	10
5	20
6	25

3.1.2. BDOC Determination

BDOC was measured using a simplified version of the high-density BDOC test (Joret *et al.*, 1991; Allegier *et al*., 1996; Woolschlager, 2000). It involves using Biologically Active Sand (BAS) on which the growth of the inoculum is established, thereby offering the advantage of ease and speed of measurement of organic matter in water. The sampling was done with six batch reactors (Reactors A to F) from which the results give representative biofilm measurements. In order to validate and standardize the method, the adopted BAS was tested at several times until they were proven to be biologically active and ready to be used for the measurement.

In contrast to the AOC protocol which uses pure strains of bacteria for testing of the organic matter in water, the BDOC protocol provides an easier, quicker and more reliable measurement of DOC concentration since it uses already established bacteria colony. The BDOC procedure involves filtering the water sample containing the organic matter to be tested, inoculating it with an autochthonous bacterial population, and measuring the decrease of DOC concentration due to the carbon oxidization by bacteria.

Materials

i. 1L amber bottles.
ii. Pre-cleaned measuring cylinder.
iii. Distilled water.
iv. Sterile cotton wool, strings, and aluminium foil
v. Syringe filter.
vi. 0.45µm filters.
vii. Sodium acetate.
viii. Waste water.
ix. Shaking incubation.
x. Autoclave.
xi. Pre-washed sand.
xii. 85% of phosphoric acid.

NOTE: All the glassware used during this experiment was washed with 50% nitric acid and rinsed several times with distilled water.

Preparation of Biologically Active Sand

Sterile one litre amber bottles were washed with nitric acid and rinsed with distilled water. These bottles were referred to as Reactors A–F (see Appendix B1) and were used for incubating and growing the microorganisms until they were biologically active and ready to be used for the BDOC determination. Each bottle was filled with 150mL of sand which had earlier been washed with distilled water and oven dried over night at 110^{o}C. The sand was left to cool to room temperature before the commencement of the experiment.

A volume of 550mL of raw waste-water sample was poured into each 1L bottle. The samples were capped with the sterile cotton wool and tied with the threads in order to avoid air entrapment. The samples were then incubated for 5 days on a shaking platform (110 rpm) at 21°C.

After 5 days, the wastewater was decanted and replaced with fresh wastewater. The water was changed every five days, for a total of 28 days.

After 28 days, the wastewater sample was decanted and then replaced with 500mL of sodium acetate solution (200mg/L) which was decanted regularly every 14 days until the testing of water samples could start. The carbon concentration in 200mg/L of sodium acetate could be

calculated as the ratio of mass of C in one mole of sodium acetate multiplied by total concentration of the sodium acetate in one litre solution. The molecular mass of sodium acetate is 82.0343g/mol and the molecular formula of sodium acetate is $C_2H_3NaO_2$, (contains two carbon atoms in sodium acetate). Therefore concentration of carbon in 200 mg/L of sodium acetate solution is :(24.02g/mol)/ (82.0343g/mol) x 200mg/L=58.56mg/L C.

3.1.3. BDOC Measurement

BDOC was estimated by measuring the DOC of water sample, incubating the sample in a batch reactor for 5 days and measuring its DOC again after incubation:

$BDOC=DOC_0 - DOC_5$.

The advantages of the BDOC method are that it is both sensitive and precise to perform. The minimum detectable concentration of the test is 0.1mg C/L or less. Since the BDOC reading corresponds to the difference between two DOC analyses, the minimum detectable concentration is linked to the detection limit.

And the disadvantages are that it takes weeks to complete due to the time of preparing the BAS reactors for the analysis. For work at low concentrations, filtration through membranes is problematic as it is difficult to remove all carbon contamination from the membranes.

3.1.4. AOC Determination

AOC measurement was carried out using Standard Method (van der Kooij, 1989). The AOC protocol was used to measure the BOM in JW's drinking water distribution system. The method involves the growth of Pseudomonas *brenneri* strain P-17, which was imported into South Africa, as it served as the measuring innoculum for the water samples and controls. Assimilable organic carbon protocol is a sensitive method for measuring the growth of heterotrophic bacteria in drinking water due to the bioassy organisms that are able to grow at low concentration of organic substrate. It is also time consuming and requires a high level of expertise.

Materials

i. Pseudomonas *brenneri* P17 culture.

ii. Sodium Acetate.

iii. Potassium Bio phosphate.

iv. Potassium Phosphate Dibasic.

v. Epson Salt.

vi. Ammonium Sulphate.

vii. Sodium Chloride.

viii. Iron (11) Sulphate.

ix. Sodium Thiosulphate.

x. 20% Glycerol.

xi. 2% Peptone.

xii. R2A Agar.

xiii. Nutrient Broth.

xiv. White Light Luminometer.

Procedure

One mL of nutrient broth was used to dissolve the pellet of the P. *brenneri* strain P17. Several drops of this suspension were used to inoculate a second tube of the broth and plate. The tubes were then incubated at 26°C for 24 hours.

After 24 hours, the cultures were retrieved and streaked for purity using R2A agar (prepared as per manufacturer's instructions). The plates were incubated at room temperature for 5 days. The culture was acclimatized to low nutrient by inoculating it in 100mL of dechlorinated sterile tap water and incubated for 7 days at room temperature.

An aliquot of 0.1 mL of the chlorine-neutralized water-adapted culture was used to inoculate 100mL of sodium acetate solution (11.34mg/L) which was incubated at room temperature for

another 7 days.The densities of the cell were then measured at this stage in order to describe the population growth.

Then a 40 mL liquot of water samples which was in adapted culture were inoculated with approximately 10^4 cfu of the strains *Pseudomonas brenneri* strain P-17.

In order to kill vegetative cells in the water, the vials were put into a 70°C water bath for 30 minutes. After cooling, the vials were placed on the sterile table and then were inoculated with 2mL of sodium acetate and were incubated for 5 days. Then the bacterial count was recorded.

3.2. Growing Biofilm in a Set-up Pipe

One way of achieving the fourth research objective of the study, that provides insight into biofilm development in distribution systems, was to conduct a study under controlled (laboratory) conditions in the Environmental Engineering laboratory, and compare the results with those obtained in the field on the JW pipelines.

The set-up in the laboratory provided an environment to observe biofilm growth and its assessment by measuring water quality parameters, such as temperature, biodegradable organic carbon concentration and assimilable organic carbon. It was originally intended to use two pipe materials – a PVC pipe and a concrete one, but the set for the former failed due to leaks that occurred. To allow the biofilm formation, water was made to run continuously thro-ugh the concrete pipe at room temperature for five months. This set-up is shown in Figure 3.1.

Fig 3.1: The Biofilm Set-up in the Laboratory for 5 months.

At the end of five months, three samples were taken from the setup. One sample was 2 litres of the water in the concrete pipe and referred to as Biofilm 1, the second was scrapping of biofilm from the middle part of the pipe wall and referred to as Biofilm 2, and the third was scrapping from the bottom part of the pipe wall and referred to as Biofilm 3. All these three samples were tested for various water quality parameters, including BDOC and AOC, and presented in Table 4.8. It should be noted that samples of Biofilm 2 and Biofilm 3, which are scrapings from the wall of the concrete pipe, could only be analysed for the various water quality tests after being fluidized with distilled water.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.0. Introduction

This chapter presents and discusses the results from this study. The existence of microorganisms in distributed water presents a potential for biofilm formation. As mentioned in Chapters 1 and 2, the presence of biofilm within distribution network can cause operational problems such as bio-corrosion, residual disinfectant depletion, and can protect pathogenic microorganisms from secondary disinfection (Hammes *et al.,* 2006). In this study, water quality parameters were measured to assess biological stability and by extension biofilm formation potential within a section of Johannesburg drinking water distribution network.

4.1. Prepared Biologically Active Sand

The results in Table 4.1 show the extent to which the microbial community established in the BAS reactors could degrade carbon based substrate. It is important to note that the results shown here are for the acetate, a simple carbon based molecule and is readily biodegradable. The efficiencies observed for this compound are expected to be higher than efficiencies most likely to be obtained for the degradation of more complex carbon based substrates in the same BAS reactors and over relatively equal incubation periods.

Table 4.1: Efficiency of BAS Incubators

BATCH	DOC_0 (mg/L)	DOC_5 (mg/L)	BDOC (mg/L)	REACTOR EFFICIENCY
Reactor A	48.91	0.00	48.91	100%
Reactor B	48.21	0.80	47.40	98.3%
Reactor C	49.50	0.16	49.34	99.7%
Reactor D	50.40	0.00	50.40	100%
Reactor E	51.56	0.53	51.03	99%
Reactor F	42.69	0.15	42.54	99.6%

***NOTE**: Detailed results can be found in Appendix B1*

4.2. Water Quality Parameters

Tables 4.2, 4.3, 4.4, 4.5, 4.6 and 4.7 show the results for the water quality analyses carried out between the months of October and December 2015. The data include pH, temperature, DOC, BDOC, free chlorine, and total chlorine. These water quality tests results provided comparative assessments that helped to support the achievement of the objective of the study of measuring and monitoring the potential for bacterial growth and biofilm formation in drinking water distribution systems.

For the month of October (Table 4.2) the average pH was 7.83. It is important to note the narrow range of pH for this month which was between 7.0 to 8.30 pH units. Temperature ranged from 18^{O}C to 23^{O}C, with an average of 20^{O}C. DOC ranged from 3.21mg/L to 3.79mg/L, with an average of 3.49mg/L. The tests for pH, temperature and DOC were carried out fortnightly.

Table 4.2: Water Quality Analysis: October

WEEK ONE	SAMPLING SITES	pH	TEMP	DOC (mg/L)	WEEK TWO	SAMPLING SITES	pH	TEMP	DOC (mg/L)
09/10/2015	RW251	7.0	21°C	3.43	22/10/2015	RW251	8.05	19°C	3.61
09/10/2015	RW253	7.2	22°C	3.21	22/10/2015	RW253	8.08	18°C	3.52
12/10/2015	RW080	7.5	19°C	3.72	26/10/2015	RW080	7.79	21°C	3.38
12/10/2015	RW081	7.7	20°C	3.70	26/10/2015	RW081	7.40	19°C	3.50
12/10/2015	RW082	7.9	22°C	3.45	26/10/2015	RW082	7.86	20°C	3.45
12/10/2015	RW083	7.9	20°C	3.60	26/10/2015	RW083	7.81	20°C	3.49
12/10/2015	RW084	8.1	20°C	3.50	26/10/2015	RW084	8.05	22°C	3.55
12/10/2015	RW104	8.1	19°C	3.45	26/10/2015	RW104	7.93	20°C	3.55
12/10/2015	RW105	8.1	18°C	3.39	26/10/2015	RW105	7.98	20°C	3.53
12/10/2015	RW106	7.9	22°C	3.33	26/10/2015	RW106	7.71	21°C	3.55
12/10/2015	RW107	8.1	23°C	3.36	26/10/2015	RW107	7.55	19°C	3.61
12/10/2015	RW108	8.3	20°C	3.36	26/10/2015	RW108	7.81	19°C	3.79
12/10/2015	RW109	8.1	23°C	3.28	26/10/2015	RW109	7.64	21°C	3.56

***NOTE**: Detailed results can be found in Appendix C1*

Also for the month of October (Table 4.3), BDOC ranged from 0.01mg/L to 1.15 mg/L with an average of 0.36 mg/L. Free chlorine ranged from 0.11mg/L to 1.20mg/L with an average of 0.31mg/L while total chlorine ranged from 1.12 mg/L to 1.84mg/L with an average of 1.53 mg/L. The BDOC, free chlorine and total chlorine were tested once in each monthly sampling routine.

Table 4.3: ***Water Quality Analysis: October***

SAMPLING DATES	SAMPLING SITES	BDOC (mg/L)	FREE CHLORINE (mg/L)	TOTAL CHLORINE (mg/L)
09/10/2015	RW251	0.07	0.11	1.12
09/10/2015	RW253	0.04	1.20	1.20
12/10/2015	RW080	0.01	0.27	1.74
12/10/2015	RW081	1.15	0.17	1.35
12/10/2015	RW082	0.00	0.14	1.35
12/10/2015	RW083	0.73	0.17	1.35
12/10/2015	RW084	0.45	0.40	1.40
12/10/2015	RW104	0.57	0.44	1.70
26/10/2015	RW105	0.00	0.28	1.84
26/10/2015	RW106	0.00	0.14	1.63
26/10/2015	RW107	0.89	0.28	1.81
26/10/2015	RW108	0.00	0.21	1.73
26/10/2015	RW109	0.66	0.19	1.61

NOTE; Detailed result can be found in Appendix C2

For the month of November (Table 4.4), pH ranged between 7.07 to 8.15 pH units and the average was 7.73 pH units. The temperature ranged from 19^{O}C to 25^{O}C, with an average of 21^{O}C. DOC ranged from 3.11mg/L to 6.20mg/L, with an average of 4.73mg/L. There was a slight increase in the DOC results for the samples analysed in the second round of sampling for this month.

Table 4.4: Water Quality Analysis: November

WEEK ONE	**SAMPLING SITES**	**pH**	**TEMP**	**DOC (mg/L)**	**WEEK TWO**	**SAMPLING SITES**	**pH**	**TEMP**	**DOC (mg/L)**
05/11/2015	RW251	7.17	23°C	4.03	16/11/2015	RW251	7.07	21°C	4.50
05/11/2015	RW253	7.40	20°C	4.10	16/11/2015	RW253	7.11	19°C	4.51
12/11/2015	RW080	7.83	22°C	4.90	23/11/2015	RW080	7.46	20°C	6.20
12/11/2015	RW081	7.81	23°C	4.70	23/11/2015	RW081	7.79	19°C	6.10
12/11/2015	RW082	7.73	22°C	4.63	23/11/2015	RW082	7.84	21°C	6.03
12/11/2015	RW083	7.80	21°C	4.60	23/11/2015	RW083	7.95	21°C	6.02
12/11/2015	RW084	7.81	22°C	4.64	23/11/2015	RW084	8.08	21°C	6.11
12/11/2015	RW104	7.82	22°C	4.80	23/11/2015	RW104	8.15	19°C	6.12
12/11/2015	RW105	7.81	22°C	4.82	23/11/2015	RW105	7.98	20°C	3.11
12/11/2015	RW106	7.84	22°C	4.82	23/11/2015	RW106	7.71	21°C	3.11
12/11/2015	RW107	7.86	22°C	4.71	23/11/2015	RW107	7.55	19°C	3.62
12/11/2015	RW108	7.93	23°C	4.71	23/11/2015	RW108	7.81	19°C	3.81
12/11/2015	RW109	7.92	25°C	4.72	23/11/2015	RW109	7.64	21°C	3.61

***NOTE**: Detailed results can be found in APPENDIX D1*

Also for the month of November, (Table 4.5), BDOC ranged from 0.05mg/L to 3.05mg/L with an average of 1.13mg/L. Free chlorine ranged from 0.2 mg/L to 1.19mg/L with an average of 0.34mg/L while total chlorine ranged from 1.20mg/L to 2.03mg/L with an average of 1.58mg/L. The BDOC also has a slight increase in this month of sampling compared to October.

Table 4.5: ***Water Quality Analysis: November***

SAMPLING DATES	SAMPLING SITES	BDOC (mg/L)	FREE CHLORINE (mg/L)	TOTAL CHLORINE (mg/L)
05/11/2015	RW251	0.00	0.27	1.70
05/11/2015	RW253	0.00	1.19	1.20
12/11/2015	RW080	0.11	0.35	1.82
12/11/2015	RW081	0.10	0.25	1.46
12/11/2015	RW082	0.00	0.20	1.42
12/11/2015	RW083	0.05	0.23	1.42
12/11/2015	RW084	0.00	0.30	1.46
23/11/2015	RW104	2.74	0.33	2.03
23/11/2015	RW105	2.96	0.27	1.85
23/11/2015	RW106	2.78	0.20	1.51
23/11/2015	RW107	3.05	0.31	1.81
23/11/2015	RW108	2.94	0.24	1.62
12/11/2015	RW109	0.00	0.24	1.30

NOTE; Detailed result can be found in Appendix D2

For the month of December (Table 4.6), the pH ranged between 7.71 and 8.05 pH units and the average was 7.89 pH units. Temperature ranged from 21^{O}C to 25^{O}C, with an average of 23^{O}C. DOC ranged from 4.57mg/L to 5.51mg/L, with an average of 4.99mg/L. There was a slight increase in pH for this month.

Table 4.6: Water Quality Analysis: December

WEEK ONE	SAMPLING SITES	pH	TEMP	DOC (mg/L)	WEEK TWO	SAMPLING SITES	pH	TEMP	DOC (mg/L)
02/12/2015	RW251	7.84	21°C	5.51	14/12/2015	RW251	7.84	21°C	5.47
02/12/2015	RW253	7.86	22°C	4.81	14/12/2015	RW253	7.86	22°C	4.71
07/12/2015	RW080	7.84	22°C	5.21	21/12/2015	RW080	7.84	22°C	5.21
07/12/2015	RW081	7.97	23°C	4.91	21/12/2015	RW081	7.98	22°C	4.91
07/12/2015	RW082	7.82	25°C	5.13	21/12/2015	RW082	7.82	25°C	5.13
07/12/2015	RW083	7.85	22°C	4.93	21/12/2015	RW083	7.85	22°C	4.93
07/12/2015	RW084	7.84	22°C	4.97	21/12/2015	RW084	7.84	22°C	4.97
07/12/2015	RW104	7.93	25°C	4.92	21/12/2015	RW104	7.94	25°C	4.92
07/12/2015	RW105	8.04	23°C	4.60	21/12/2015	RW105	8.05	23°C	4.57
07/12/2015	RW106	8.05	25°C	4.92	21/12/2015	RW106	8.05	24°C	4.92
07/12/2015	RW107	7.71	24°C	4.99	21/12/2015	RW107	7.72	24°C	4.99
07/12/2015	RW108	7.90	23°C	5.10	21/12/2015	RW108	7.90	22.5°C	5.08
07/12/2015	RW109	7.88	24°C	4.99	21/12/2015	RW109	7.88	24°C	5.00

***NOTE**: Detailed result can be found in Appendix E1*

The BDOC for the month of December (Table 4.7) was not carried for about half of the sampling sites due to the close of academic session and the non-availability of the drivers of the School who had gone for the holidays. For the sampled sites, BDOC ranged from 0.43mg/L to 2.01mg/L with an average of 1.36mg/L. Free chlorine ranged from 0.12mg/L to 1.01mg/L with an average of 0.25mg/L while total chlorine ranged from 0.38mg/L to 2.02mg/L with an average of 1.22mg/L.

Table 4.7 ***Water Quality Analysis: December***

SAMPLING DATES	SAMPLING SITES	BDOC (mg/L)	FREE CHLORINE (mg/L)	TOTAL CHLORINE (mg/L)
02/12/2015	RW251	1.20	0.19	1.27
02/12/2015	RW253	0.43	1.01	1.01
07/12/2015	RW080	2.01	0.15	1.90
07/12/2015	RW081	1.85	0.15	0.44
21/12/2015	RW082	-	0.13	0.54
07/12/2015	RW083	1.02	0.12	0.54
07/12/2015	RW084	1.51	0.13	0.38
07/12/2015	RW104	1.95	0.31	2.02
07/12/2015	RW105	0.91	0.17	1.87
21/12/2015	RW106	-	0.19	1.57
21/12/2015	RW107	-	0.28	1.78
21/12/2015	RW108	-	0.19	1.46
21/12/2015	RW109	-	0.19	1.14

NOTE; Detailed result can be found in Appendix E2

The Figure 4.1 presents the average DOC of the sampled three months. Except for the month of November when there was elevated DOC values at sampling sites RW80, RW081, RW 082, RW083, RW084 and RW104, the DOC remain fairly uniform at all the sampling sites for the months of October and December. The DOC values varied from the month of October to December.

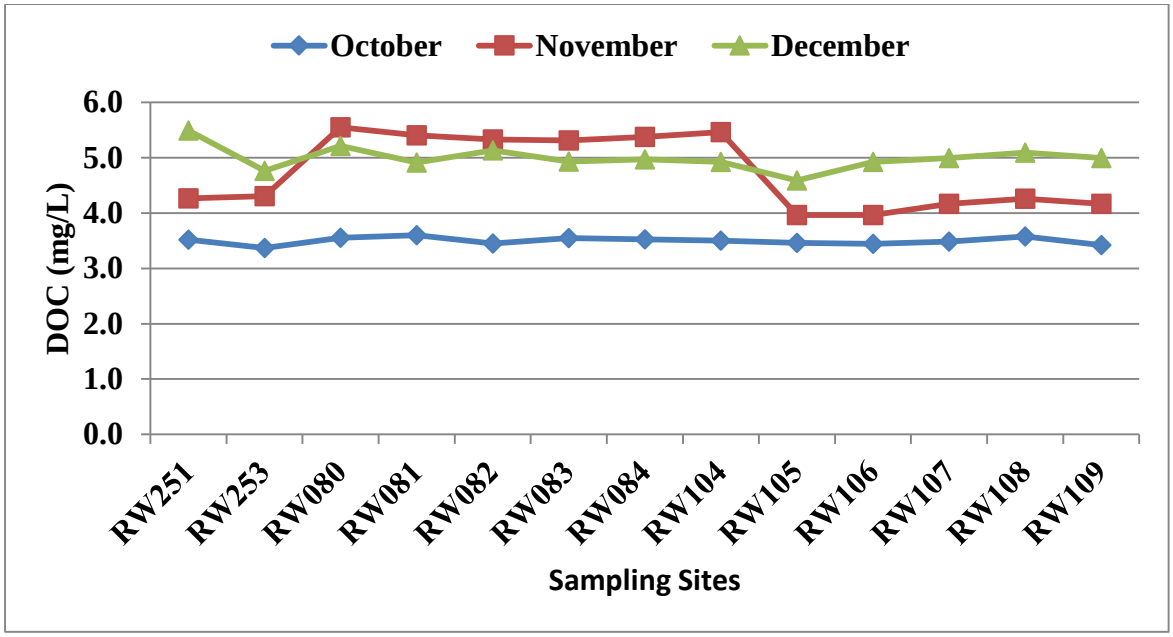

Figure 4.1: The Average DOC results at various Sampling Sites

Figure 4.2 presents the combined results of BDOC for the sampled months of October and November and part of December. The results indicate higher values of BDOC in November than other months for half of the sampling sites, and conversely for the other sites where BDOC values were insignificant.

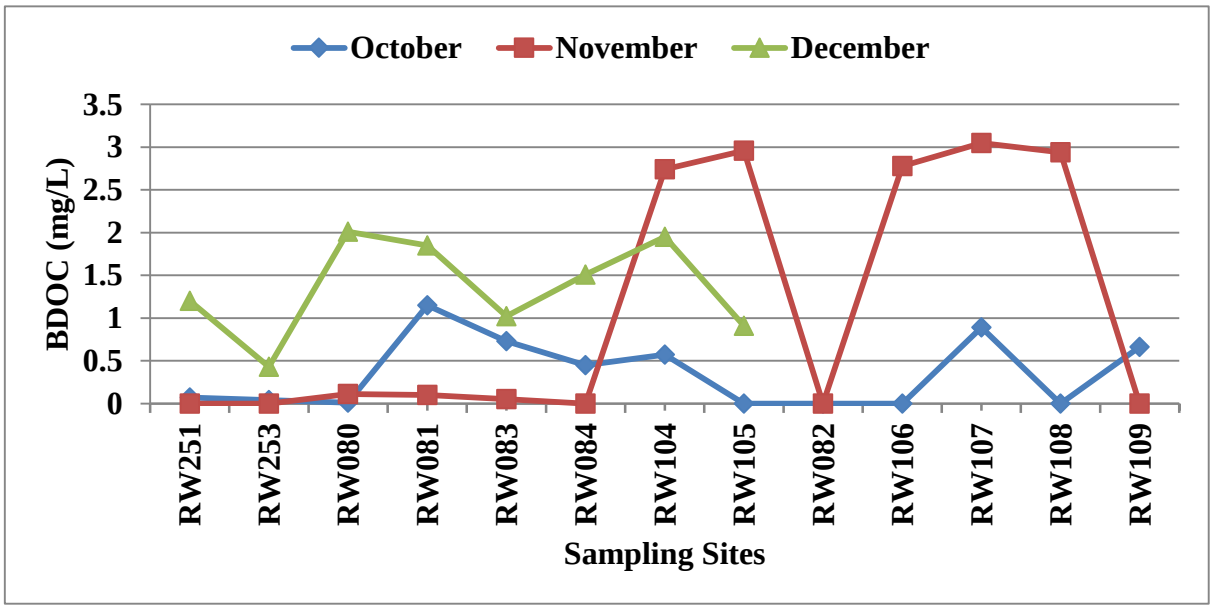

Figure 4.2: The Average BDOC results at various Sampling Sites

Figure 4.3 presents the average results for the free chlorine throughout the round of sampling. It indicates a significantly high values of free chlorine at site RW253 which is an outlet point

from the reservoir. At all sites there was little variation in free chlorine over the three month period.

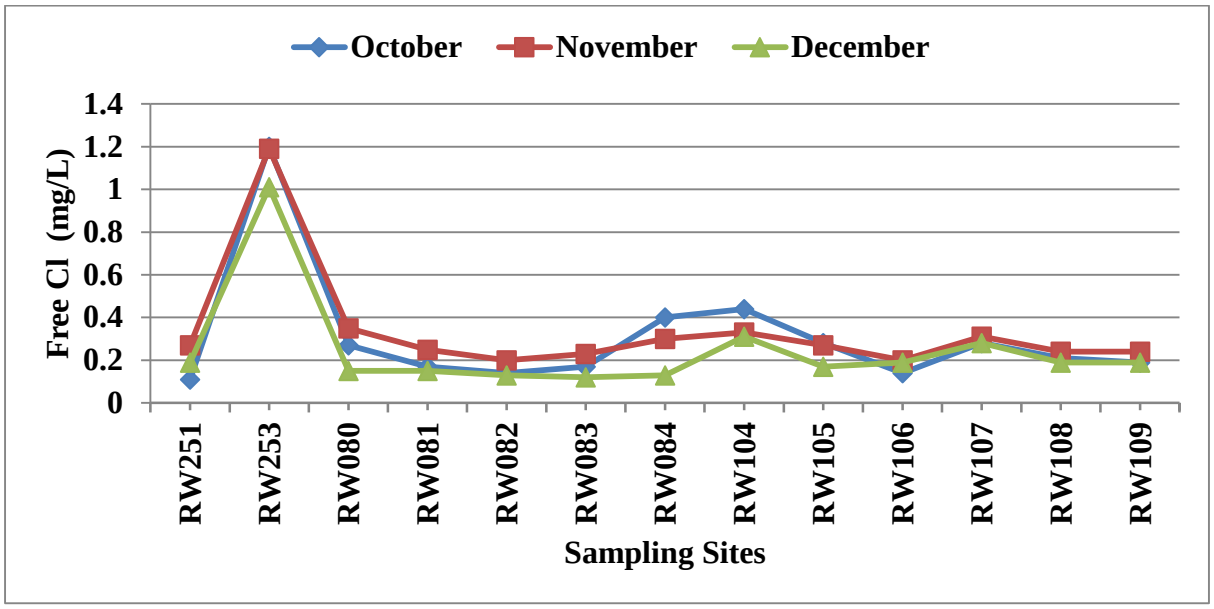

Figure 4.3: The Average results of Free Chlorine at various Sampling Sites

Figure 4.4 summarises the results of the total chlorine for the three months period of sampling. The total chlorine variation shown in the Figure 4.4 is small except for the month of December when reduced values were observed at sites RW081, RW082, RW083, and RW084 which are sites that are close to each other.

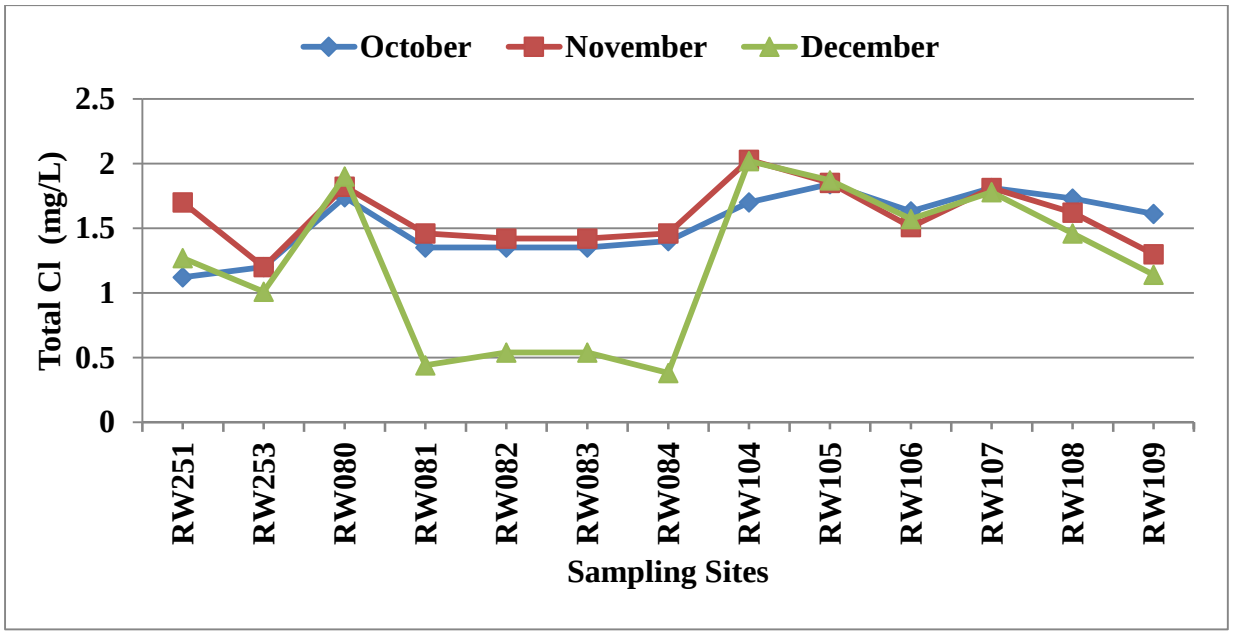

Figure 4.4: The Average results of Total Chlorine at various Sampling Sites

Figure 4.5 presents the combined result of pH for all the three months period. At all sampling sites, the pH values belong to the alkaline phase.

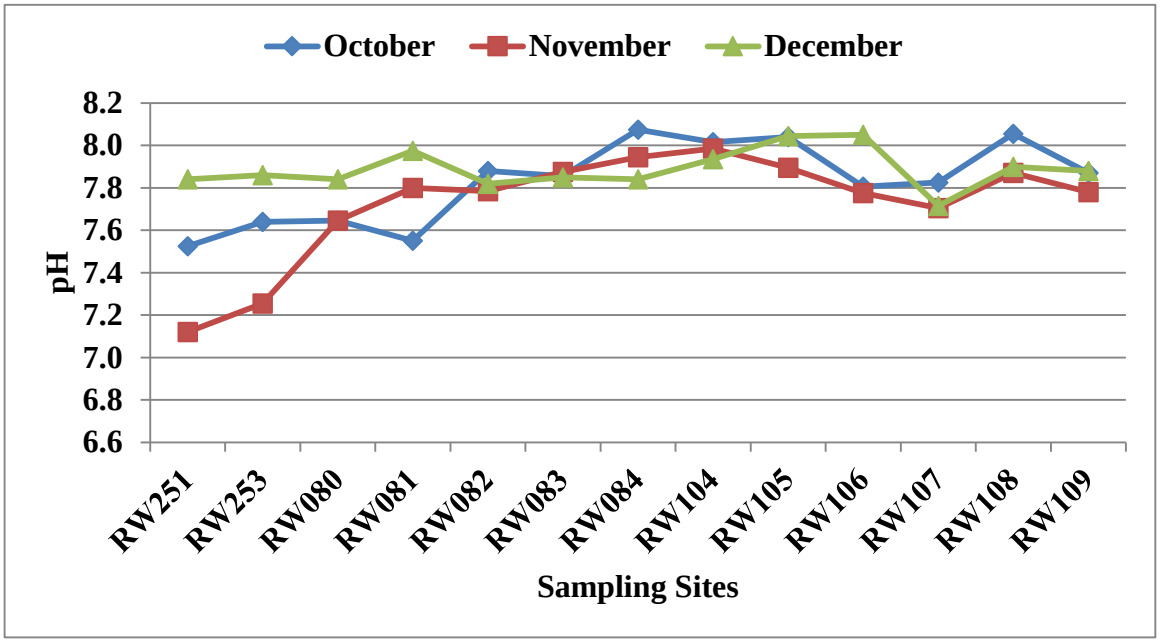

Figure 4.5: The Average results of pH at various Sampling Sites

Figure 4.6 presents the average result of the temperature. In general the values of temperature in the month of December in this figure are relatively higher than other months.

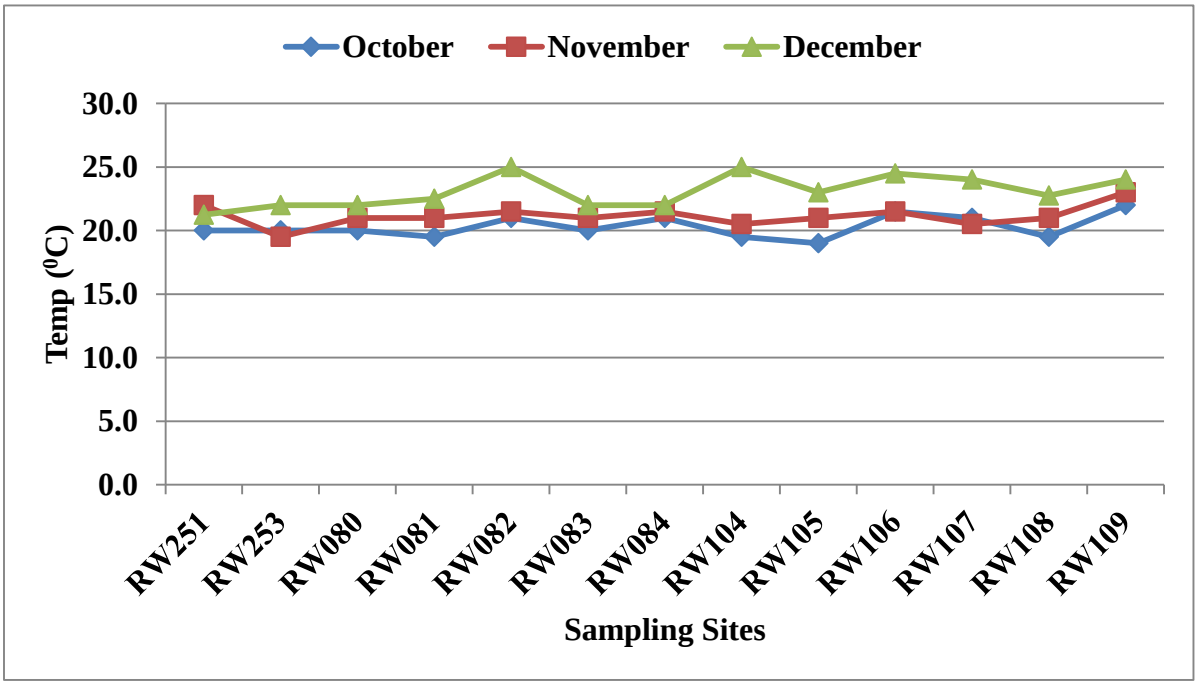

Figure 4.6: The Average results of Temperature at various Sampling Sites

4.3. Biofilm Analysis in the Set-up Pipe

Table 4.8 shows the results of the biofilm analysis from the concrete pipe that was set up in the Environmental Engineering laboratory. The average BDOC concentration is 0.37mg/L, the average DOC concentration is 2.71mg/L, and the average pH is 6.67pH units. The concentrations of free and total Chlorine are found to be insignificant.

Table: 4.8: Biofilm Analyses of the Set-up Pipe

PARAMETERS TESTED	BIOFILM 1	BIOFILM 2	BIOFILM 3
BDOC	0.57 mg/L	0.20 mg/L	0.53 mg/L
DOC	5.47 mg/L	1.08 mg/L	1.59 mg/L
pH	7.67	6.63	5.71
FREE CHLORINE	<0.03mg/L	<0.11mg/L	<0.11 (mg/L)
TOTAL CHLORINE	<0.03mg/L,	<0.22mg/L	<0.22 (mg/L)

***NOTE**: Detailed results can be found in Appendix F1&F2*

The results in Table 4.9 show the correlation between BDOC and other water quality parameters. There were no strong correlations, positive or negative between BDOC concentrations and the water quality parameters monitored in this study.

Table 4.9: Correlations between BDOC and other Water Quality Parameters

Month	pH/BDOC	Temp/BDOC	DOC/BDOC	Free Cl/BDOC	Total Cl/BDOC
October	0.03	0.00	0.22	0.11	0.11
November	0.55	0.15	0.38	0.21	0.62

4.4. Discussions

The results obtained from all the analysis carried out in this study are discussed below according to each water quality test.

4.4.1. BAS

The data shown in Table 4.1 proves that the sand was significantly active biologically. This is confirmed by the results which, with the introduction of 200mg acetate-C/L into the sand, showed DOC concentrations after 5 days to be less than 2.0mgC/L, as can be seen in Table

4.1 that had average DOC_5 of 0.27mgC/L (Joret *et al* 1991; Najm *et al*., 2000; Escobar & Randall, 2001; Park *et al*., 2004).

The BDOC values from the BAS were obtained after 5 days of the incubation, and this is in contrast to other methods that take between 21 and 28 days of incubation (Servais *et al*., 1989; Huck, 1990; Ribas *et al.,* 1991; Snider, 2011). The faster incubation period is an advantage that can be exploited in biostability monitoring.

Furthermore, the use of BAS in relation to the AOC protocol offers savings in cost, time for preparation and handling, as the protocol requires standardized inoculum of pure bacteria strains, pre-treatment of the samples which have to undergo pasteurization, sterilization, filtration and other aseptic laboratory techniques, and careful handling to avoid contamination of the samples.

The geographical origin of the sand which is used as the inoculum may have little or no effect on the measurement; the sand can be collected from any environment without any specific preparations. This is an advantage of the BAS in that its standardization and the reliability of its results do not depend on the source of the sand.

4.4.2. DOC

The target water quality range (SANS 241) for DOC in potable waters is 0 to 5mg/L. This range is regarded to have no effect on health, aesthetic (colour, taste and odour) or THM formation during chlorination. Ranges from 5 to 10 mg/L of DOC pose a slight health risk and depending on DOC composition may also pose a higher risk for THM formation during secondary chlorination.

The DOC results presented in Tables 4.2, 4.4 and 4.6, and Figure 4.1 show a range from 3.11mg/L to 6.20mg/L. The average DOC result for December was noted to be slightly high (4.99mg/L) which suggests a higher potential for bacterial growth during that month.

4.4.3. BDOC

The high density sand bioassay is a very reliable technique for the measurement of the biodegradable fraction of dissolved organic matter relevant to water distribution systems. This technique is better than other methods for BDOC measurement due to its relatively short incubation period, high reproducibility amongst other things. This method was chosen based on its direct measurement on DOC which is considered as a universal indicator of organic matter presence in water.

On careful observation, there are spatial and temporal variations of the BDOC data presented Tables 4.2, 4.4 and 4.6, and Figure 4.2. There was no discernible spatial trend in the BDOC values at the various sampling points in the JW's water distribution system. It is of concern when BDOC exceeds 0.15mg/L which indicates high potential for biofilm formation and proliferation of bacteria and micro-organisms within the pipeline. Such concerns present themselves at some sampling sites in the months of November and December (Figure 4.2).

Many factors influence the observed BDOC values at the various sampled sites. The presence of residual chlorine in drinking water provides protection against disease-causing organisms as discussed in the literature review. The SANS 241 code champions the maintenance of the residual chlorine of <0.5mg/L at all points in the distribution system in order to prevent biofilm formation. Another factor relates to the hydraulics of the flow in the pipes, reservoirs and other appurtenances in the distribution systems. High turbulent intensity of the flow in the pipes tends to prevent the growth of biofilm. Poor integrity of joints at valves, which promote ingress of contaminants into the distribution system, promotes biofilm formation. Regular flushing and maintenance of the reservoirs should help in distributing the disinfectant residual to all portions of the distribution system and scour existing biofilms.

The average temperature during the sampling period at all sites steadily increased from October to December, while the BDOC values were highest in December for the sites sampled and in November for the other sites. This suggests that the temperature influences the biological reactions in the pipe, and can potentially promote biofilm formation.

In summary, this study places emphasis on the monitoring of the BDOC over space and time in the water distribution system so that appropriate treatment and management protocols can be designed and used to assess the system's biological stability and to trigger remedial actions when high BDOC values are encountered.

4.4.4. AOC

Although AOC is also an important protocol for monitoring BOM in water distribution systems, it is susceptible to errors and unreliable results. This was observed in the analysis carried out in the study. The density and bacteria count on the plate counts exceeded the reading limit of the equipment. This is seen in all the tests carried out for all the sampled points of the JW sites (see Table 3.1) of this study.

The same results for AOC were observed for the biofilm samples – Biofilm 1, Biofilm 2 and Biofilm 3 – that were taken from the concrete pipe of the laboratory investigation. Therefore the bacteria counts could not be directly correlated to the biofilm formation thereby making the AOC protocol result inconclusive in this report, and this can be attributed to the principle on which this method is based thereby underrating the BOM present in the drinking water distribution system. Thus the accuracy and relevance of this protocol have proven to be debatable.

4.4.5. Biofilm Formation in Set-up Pipe

Although no comprehensive investigation was carried on the influence of pipe materials on biofilm growth and development, the results from the concrete pipe used for the laboratory investigation compared to those from the pipes of JW provides some indicative insight into the influence of pipe material on biofilm growth. The BDOC values in the concrete pipe varied between 0.20mg/L and 0.57mg/L, and these were within the ranges observed in the JW water distribution system. However, the maximum observed BDOC values in the water distribution system were higher for the three months than those observed in the concrete pipe laboratory set-up.

Based on the literature survey, it was shown that the type of pipe materials has great influence on the density of bacteria (Kerr *et al.*, 1999). Pipe materials may also influence the quality of the water and biofilm formation potential. The literature survey specified that pipe material influences biofilm accumulation through release of compounds which supports biological growth, corrosion and degree of roughness. Whereas these observations have been made about pipe material influence on biofilm accumulation, it was not possible to replicate these due to the limited types of pipes used.

4.4.6. The Relationship between AOC and BDOC and the Implications for Water Quality Monitoring and Management

Some reports on the Johannesburg Water distribution system have indicated that all the elements necessary for the growth of microorganisms are present within their distribution network. Of all nutrients present, carbon is the one that is available in largest quantities. Because of this one can assume that carbon is the main limiting nutrient for bacterial growth and regrowth within JW's drinking water distribution network.

In principle, if carbon is the limiting nutrient there is supposed to be a strong positive correlation between the concentration of biodegradable organic matter and bacterial densities in the distributed water. Because of their reliance on carbon (for growth) one can expect

heterotrophic bacteria to dominate microbial communities within JW's distribution network. Therefore nutrient monitoring in general and specifically the monitoring of biologically available carbon is necessary in order to maintain biological stability throughout the network. Of all methods currently used for the measurement of biologically available organic carbon the measurement of AOC and BDOC seem to be the most popular.

While BDOC and AOC are sometimes used together, to characterise the biostability of drinking water, this is neither possible nor affordable for some utilities. For utilities that can only monitor one indicator it becomes crucial for utility managers to understand the distinction between AOC and BDOC so that they can pick one that will be the most reliable as a tool for monitoring biological stability in their network. In general distribution systems that frequently report high heterotrophic densities are better served by AOC monitoring and distribution systems that report rapid and or/or severe residual depletion as well as heightened disinfection by-product formation are better served by BDOC monitoring.

An evaluation of JW's historical water quality data in general and in the area monitored in this study in particular showed the loss of residual to be the biggest source of biological stability. Results from this study highlight the loss of disinfectant residuals within the network. Therefore JW will be better served by BDOC monitoring.

CHAPTER FIVE: CONCLUSION & RECOMMENDATION

5.1. CONCLUSION

The focus of this research is specifically on the water quality in the DWDSs, with particular emphasis on describing and validating a protocol for BDOC determination. The unique aspect of this research is that it is the first time that biofilm growth potential monitoring and measuring using high-density BDOC method has been carried out in JW's distribution system, and the overall conclusions of the study are outlined below.

With the achievement of a biologically active sand, the BAS technique has been found from this study to be effective in determining BDOC protocol within a five day period, showing it to be a reliable, effective and speedy technique. Therefore, the BDOC protocol has proven to be a reliable and efficient protocol for routine monitoring and measurement of biofilm formation in Johannesburg water distribution system. With the use of the BAS technique for BDOC determination, lower cost and ease of sample preparation and handling are attained when compared with the AOC protocol.

The choice of an appropriate assessment method for the determination of the biodegradable organic matter present in water is at the heart of decision-making of those who manage water distribution systems. AOC and BDOC have been understood to be the two key parameters used for the assessment of regrowth potential and biological stability of drinking water. This study has demonstrated that BDOC is proven to perform better than AOC protocol when quantifying BOM in water. In all measurements, the density of the bacteria in the plate counts exceeded the reading limit, which made the AOC results inconclusive. On the other hand BDOC measurements quantify a fraction of the BOM pool and can be used to identify hotspots that have high potential for biofilm formation in the distribution system. This is of huge significance to the water utility when monitoring its distribution to be able to speedily identify any health risks to consumers.

The conducted research involved various phases, from testing the reliability of biologically active sand for BDOC determination, to field investigations at various sampling points on Johannesburg water distribution network, and to growing of biofilm in concrete pipe in the Environmental Engineering laboratory setting. In the first phase, the information gathered

from the literature provided the basis for the experimental procedures. The data collected in this phase indicated that the sand used was significantly active biologically and considered valid for the BDOC determination.

The second phase of the research was a field investigation which evaluated BDOC, AOC and other water quality parameters on samples obtained from the 13 strategic locations along Johannesburg water distribution network. This phase provided insight into the spatial and temporal distribution of water quality parameters linked to biostability and biofilm formation potential. However, we found that there was no significant correlation between BDOC and other water quality indices such pH, DOC, free and total Chlorine. This does not detract from the usefulness of this study which would entail the use of the BDOC protocol by the water utility as a stand-alone protocol to identify hotspots when high values (>0.15mg/L) are encountered and to put in place remedial actions to mitigate the potential for biofilm formation.

The final phase was the cultivation of biofilm in a concrete pipe in the laboratory. This was intended to complement the field investigations of phase two. Although the investigation was limited on the pipe materials and flow hydraulics, the results obtained after analysing biofilm in the concrete pipe in the laboratory set up provided preliminary understanding on how biofilm develops in such pipe materials.

5.2. Recommendation

Maintenance of chlorine residual and regular disinfection and flushing of the distribution systems is highly recommended to minimize bacterial growth and biofilm formation. A residual disinfectant can control regrowth of microorganisms that remain after primary disinfection and minimize the microbial interactions with pipe wall biofilms. Also secondary disinfection protects against reinoculation of the flowing water by microbes trapped in the biofilm which can occur through sloughing or erosion of the biofilm. Disinfectant residuals can also reduce the amount of viable organisms available to become adsorbed to the biofilm.

Johannesburg Water should consider measuring BDOC as part of their regular monitoring programme. The AOC test method is very meticulous, costly, requires high expertise, and error prone, and as such it is not considered an ideal protocol in monitoring biofilm in water

distribution system for JW. The BDOC method which utilises natural bacterial inocula obtained from BAS is a more streamlined method of biofilm measurement than the AOC measurement. The use of BDOC protocol seems to be more suitable based on its theoretical and practical consideration which leads to easier standardization. Therefore with JW having achieved the Blue Drop status for four consecutive years and with all the relevant equipment and expertise available, the BDOC protocol, that is affordable, can be used routinely. The cost of this additional monitoring routine will far outweigh the benefits to residents of Johannesburg in terms of improved health through potable water, improved productivity and thereby having less reduced medical expenses.

Finally this research will greatly benefit the water treatment facilities and public health because it will indicate how biologically stable the water in Johannesburg is. If potable water produced by the JW has high regrowth potential, containing high BDOC, primarily it has to be known and then studied in more detail on strategies to minimize BDOC which will help to protect water consumers more from potential microbial contact that may cause adverse health effects.

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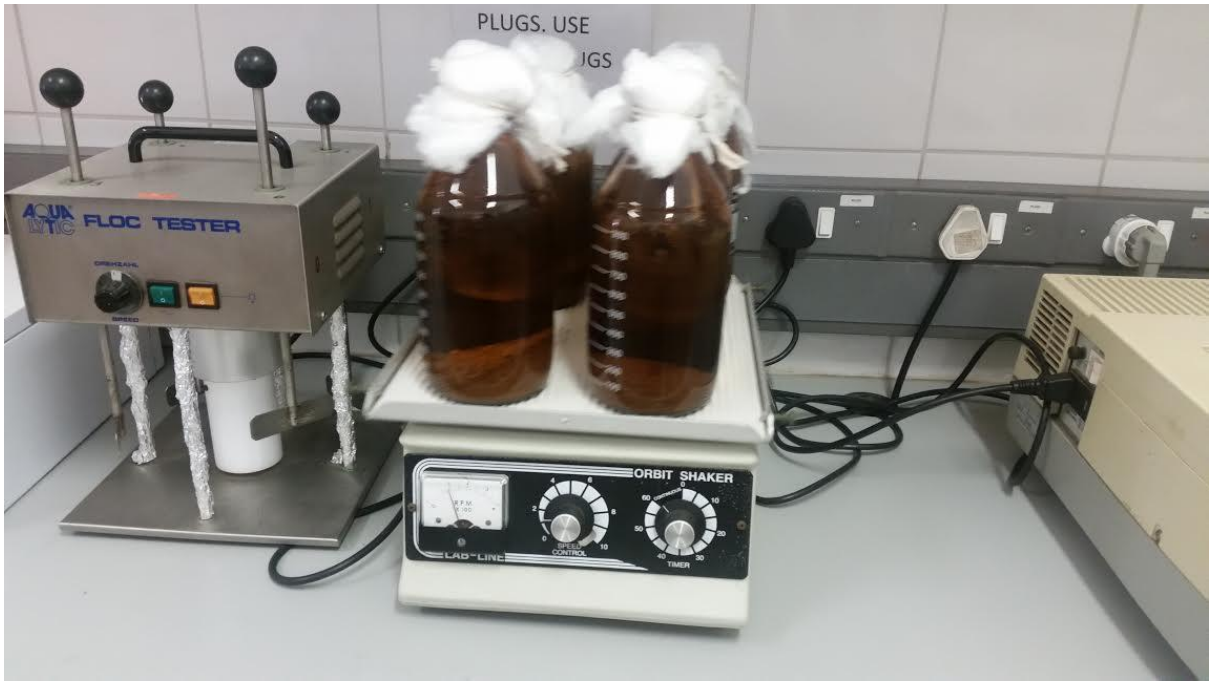
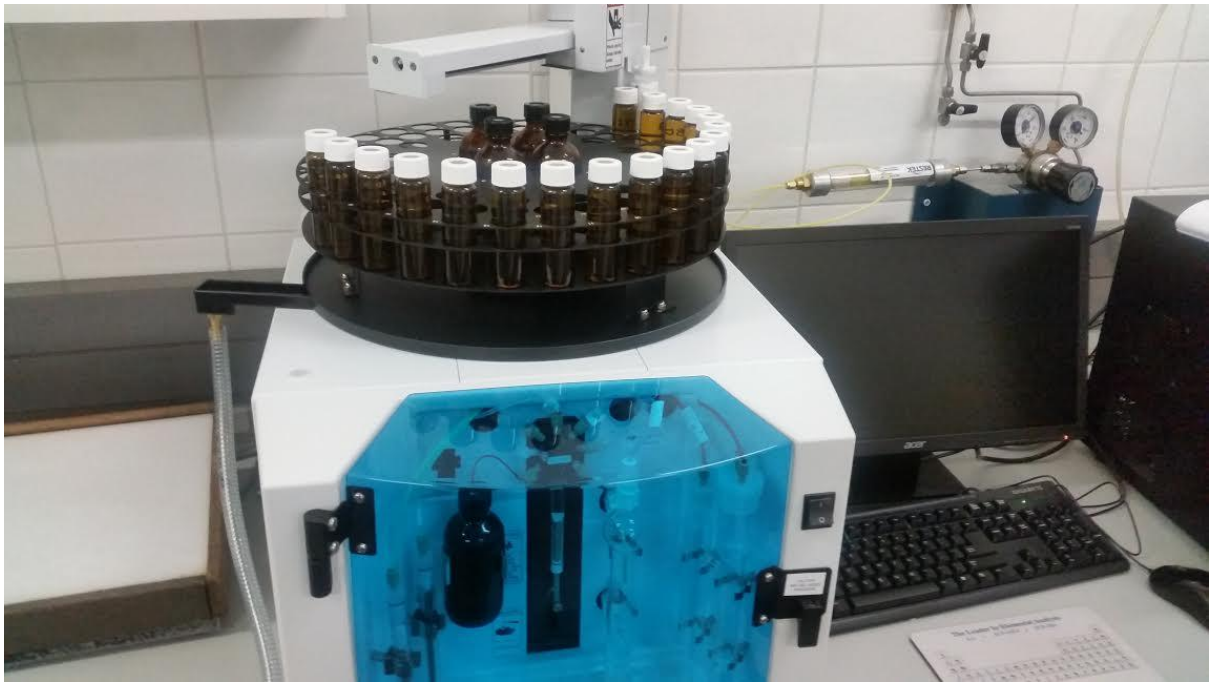
APPENDIXES

APPENDIX A



A1: The BAS Batch Reactors on Orbital Shaker



A2 Teledyne Tekmar Total Carbon Analyser Torch with DOC and BDOC Sample

A 3: Teledyne Tekmar Total Carbon Analyser Torch with DOC and BDOC Samples

A 4: The Biofilm Set-up in the Laboratory for 5 months

APPENDIX B

TABLE B1: TRIPLICATE RESULTS OF BAS

Reactor A				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/05	1	48.9910	0.0000	48.9910
	2	50.0944	0.0000	50.0944
	3	47.6392	0.0000	47.6392
Mean		48.91	0.00	48.91
SD		1.23	0.00	1.23
%RSD		2.51	#DIV/0!	2.51
Reactor B				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/05	1	47.4564	0.7850	46.6714
	2	48.0745	0.8812	47.1933
	3	49.0862	0.7452	48.3410
Mean		48.21	0.80	47.40
SD		0.82	0.07	0.85
%RSD		1.71	8.70	1.80
Reactor C				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/05	1	48.4440	0.1450	48.2990
	2	51.4362	0.1697	51.2665
	3	48.6258	0.1724	48.4534
Mean		49.50	0.16	49.34
SD		1.68	0.02	1.67
%RSD		3.39	9.30	3.39
Reactor D				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/05	1	47.5397	0.0000	47.5397
	2	53.5663	0.0000	53.5663
	3	50.1083	0.0000	50.1083
Mean		50.40	0.00	50.40
SD		3.02	0.00	3.02
%RSD		6.00	#DIV/0!	6.00

Reactor E				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/05	1	49.0783	0.5849	48.4934
	2	53.4372	0.6343	52.8029
	3	52.1683	0.3752	51.7931
Mean		51.56	0.53	51.03
SD		2.24	0.14	2.25
%RSD		4.35	25.88	4.42
Reactor F				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/05	1	42.7047	0.2496	42.4551
	2	41.7829	0.0326	41.7503
	3	43.5905	0.1685	43.4220
Mean		42.69	0.15	42.54
SD		0.90	0.11	0.84
%RSD		2.12	72.98	1.97

APPENDIX C

Table C1: THE TRIPLICATE RESULTS OF DOC: OCTOBER

RW 251			RW 251		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/10/09	1	3.5285	2015/10/22	1	3.6867
	2	3.4204		2	3.5551
	3	3.3481		3	3.6111
Mean		3.43	Mean		3.62
SD		0.09	SD		0.07
%RSD		2.65	%RSD		1.83
RW 253			RW 253		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/10/09	1	3.3018	2015/10/22	1	3.6078
	2	3.1105		2	3.4459
	3	3.2338		3	3.5231
Mean		3.22	Mean		3.53
SD		0.10	SD		0.08
%RSD		3.02	%RSD		2.30
RW 080			RW 080		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/10/12	1	3.7755	2015/10/26	1	3.2658
	2	3.6745		2	3.5895
	3	3.7226		3	3.2836
Mean		3.72	Mean		3.38
SD		0.05	SD		0.18
%RSD		1.36	%RSD		5.38
RW 081			RW 081		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/10/12	1	3.5827	2015/10/26	1	3.4584
	2	3.7242		2	3.5476
	3	3.6820		3	3.5215
Mean		3.66	Mean		3.51
SD		0.07	SD		0.05
%RSD		1.98	%RSD		1.31

RW 082			RW 082		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/10/12	1	3.4088	2015/10/26	1	3.4019
	2	3.3939		2	3.4426
	3	3.5512		3	3.5169
Mean		3.45	Mean		3.45
SD		0.09	SD		0.06
%RSD		2.52	%RSD		1.69
RW 083			RW 083		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/10/12	1	3.4301	2015/10/26	1	3.4027
	2	3.7128		2	3.5368
	3	3.6119		3	3.5223
Mean		3.58	Mean		3.49
SD		0.14	SD		0.07
%RSD		4.00	%RSD		2.11
RW 084			RW 084		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/10/12	1	3.4763	2015/10/26	1	3.5655
	2	3.6682		2	3.5729
	3	3.3473		3	3.5244
Mean		3.50	Mean		3.55
SD		0.16	SD		0.03
%RSD		4.62	%RSD		0.74
RW 104			RW 104		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/10/12	1	3.3671	2015/10/26	1	3.5381
	2	3.5425		2	3.5887
	3	3.4424		3	3.5277
Mean		3.45	Mean		3.55
SD		0.09	SD		0.03
%RSD		2.55	%RSD		0.92

RW 105			RW 105		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/10/12	1	3.2823	2015/10/26	1	3.5302
	2	3.5149		2	3.6464
	3	3.3604		3	3.4160
Mean		3.39	Mean		3.53
SD		0.12	SD		0.12
%RSD		3.50	%RSD		3.26
RW 106			RW 106		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/10/12	1	3.4104	2015/10/26	1	3.4123
	2	3.3548		2	3.6464
	3	3.2358		3	3.6049
Mean		3.33	Mean		3.55
SD		0.09	SD		0.12
%RSD		2.68	%RSD		3.51
RW 107			RW 107		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/10/12	1	3.1293	2015/10/26	1	3.6746
	2	3.4372		2	3.5912
	3	3.5046		3	3.5933
Mean		3.36	Mean		3.62
SD		0.20	SD		0.05
%RSD		5.96	%RSD		1.31
RW 108			RW 108		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/10/12	1	3.1786	2015/10/26	1	3.5306
	2	3.4017		2	4.1226
	3	3.4916		3	3.7112
Mean		3.36	Mean		3.79
SD		0.16	SD		0.30
%RSD		4.80	%RSD		8.01
RW 109			RW 109		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/10/12	1	3.1857	2015/10/26	1	3.5331
	2	3.3627		2	3.6057
	3	3.2953		3	3.5294
Mean		3.28	Mean		3.56
SD		0.09	SD		0.04
%RSD		2.72	%RSD		1.21

Table C2: THE TRIPLICATE RESULTS OF BDOC: OCTOBER

RW 251				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/09	1	2.8557	2.7799	0.0758
	2	3.0190	2.9692	0.0498
	3	2.8927	2.9624	0.0697
Mean		2.92	2.90	0.07
SD		0.09	0.11	0.01
%RSD		2.93	3.70	20.89
RW 253				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/09	1	2.5293	2.5094	0.0199
	2	2.5876	2.4592	0.1284
	3	2.6204	2.3456	0.2748
Mean		2.58	2.44	0.14
SD		0.05	0.08	0.13
%RSD		1.79	3.44	90.70
RW 080				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/12	1	3.0198	3.3328	0.0313
	2	3.3098	3.5608	0.0000
	3	3.2116	3.5004	0.0000
Mean		3.18	3.46	0.01
SD		0.15	0.12	0.02
%RSD		4.64	3.41	173.21
RW 081				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/12	1	3.2767	2.0534	1.2233
	2	3.4523	2.2997	1.1526
	3	3.3787	2.2959	1.0828
Mean		3.37	2.22	1.15
SD		0.09	0.14	0.07
%RSD		2.62	6.37	6.09

RW 083				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/12	1	3.1010	2.2963	0.8047
	2	3.2482	2.4630	0.7852
	3	3.2890	2.5945	0.6945
Mean		3.21	2.45	0.76
SD		0.10	0.15	0.06
%RSD		3.08	6.10	7.72
RW 084				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/12	1	2.9649	2.3975	0.5674
	2	3.2316	2.8207	0.4109
	3	3.2150	2.8565	0.3585
Mean		3.14	2.69	0.45
SD		0.15	0.26	0.11
%RSD		4.76	9.49	24.39
RW 104				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/12	1	3.2660	2.3809	0.8851
	2	3.4072	2.9139	0.4933
	3	3.2682	2.9275	0.3407
Mean		3.31	2.74	0.57
SD		0.08	0.31	0.28
%RSD		2.44	11.37	49.01
RW 105				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/12	1	2.8199	2.4549	0.3650
	2	3.0742	2.5005	0.5737
	3	2.9930	2.3873	0.6057
Mean		2.96	2.45	0.51
SD		0.13	0.06	0.13
%RSD		4.38	2.33	25.39
RW 082				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/26	1	4.4920	4.9432	0.000
	2	4.5954	4.8283	0.000
	3	4.5771	4.9342	0.000
Mean		4.55	4.90	0.00
SD		0.06	0.06	0.11
%RSD		1.21	1.30	0.000

RW 105				
Date	Replicate	Day0 Results (ppm)	Day5 Results (ppm)	BDOC (ppm)
2015/10/26	1	3.8984	5.0262	0.000
	2	4.0478	5.1807	0.000
	3	3.7950	5.2779	0..000
Mean		3.91	5.16	0.000
SD		0.13	0.13	0.00
%RSD		3.25	2.46	0.00
RW 106				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/26	1	4.2479	4.6837	0.0000
	2	4.6564	4.9475	0.0000
	3	4.5721	4.9449	0.0000
Mean		4.49	4.86	0.00
SD		0.22	0.15	0.00
%RSD		4.80	3.12	00.00
RW 107				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/26	1	5.6261	4.4553	1.1708
	2	5.0915	4.3899	0.7016
	3	5.5369	4.7375	0.7994
Mean		5.42	4.53	0.89
SD		0.29	0.18	0.25
%RSD		5.29	4.08	27.79
RW 108				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/26	1	4.3850	5.2474	0.0000
	2	4.6410	5.3752	0.0000
	3	4.6672	5.2358	0.0000
Mean		4.56	5.29	0.00
SD		0.16	0.08	0.00
%RSD		3.42	1.46	00.00
RW 109				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/26	1	5.0682	4.2828	0.7854
	2	5.2185	4.6110	0.6075
	3	4.8939	4.3056	0.5883
Mean		5.06	4.40	0.66
SD		0.16	0.18	0.11
%RSD		3.21	4.17	16.46

APPENDIX D

Table D1: THE TRIPLICATE RESULTS OF DOC: NOVEMBER

RW 251			RW 251		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/11/05	1	4.0988	2015/11/16	1	4.5150
	2	3.9589		2	4.5038
	3	4.0436		3	4.4940
Mean		4.03	Mean		4.50
SD		0.07	SD		0.01
%RSD		1.75	%RSD		0.23
RW 253			RW 253		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/11/05	1	4.0177	2015/11/16	1	4.4427
	2	4.0620		2	4.5834
	3	3.8317		3	4.4256
Mean		3.97	Mean		4.48
SD		0.12	SD		0.09
%RSD		3.08	%RSD		1.93
RW 080			RW 080		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/11/12	1	4.6922	2015/11/23	1	6.2038
	2	4.8328		2	6.0629
	3	4.9456		3	6.3174
Mean		4.82	Mean		6.19
SD		0.13	SD		0.13
%RSD		2.63	%RSD		2.06
RW 081			RW 081		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/11/12	1	4.6137	2015/11/23	1	5.9748
	2	4.9320		2	6.0231
	3	4.5544		3	6.1572
Mean		4.70	Mean		6.05
SD		0.20	SD		0.09
%RSD		4.32	%RSD		1.56

RW 082			RW 082		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/11/12	1	4.5206	2015/11/23	1	6.0884
	2	4.7627		2	5.9686
	3	4.6198		3	6.0214
Mean		4.63	Mean		6.03
SD		0.12	SD		0.06
%RSD		2.63	%RSD		1.00
RW 083			RW 083		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/11/12	1	4.4919	2015/11/23	1	6.0248
	2	4.5464		2	6.1129
	3	4.7693		3	6.1396
Mean		4.60	Mean		6.09
SD		0.15	SD		0.06
%RSD		3.19	%RSD		0.99
RW 084			RW 084		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/11/12	1	4.6710	2015/11/23	1	6.1435
	2	4.6945		2	6.3708
	3	4.5526		3	6.0646
Mean		4.64	Mean		6.19
SD		0.08	SD		0.16
%RSD		1.64	%RSD		2.57
RW 104			RW 104		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/11/12	1	4.9762	2015/11/23	1	5.8970
	2	5.1083		2	6.1435
	3	4.6362		3	6.1379
Mean		4.91	Mean		6.06
SD		0.24	SD		0.14
%RSD		4.96	%RSD		2.32

RW 105			RW 105		
Date	Replicate	DOC Results	Date	Replicate	DOC Results
2015/11/12	1	4.7246	2015/11/23	1	5.9441
	2	5.0683		2	6.1714
	3	4.5897		3	6.2253
Mean		4.79	Mean		6.11
SD		0.25	SD		0.15
%RSD		5.15	%RSD		2.44
RW 106			RW 106		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/11/12	1	4.6043	2015/11/23	1	5.9186
	2	4.7566		2	5.9180
	3	5.0222		3	6.0311
Mean		4.79	Mean		5.96
SD		0.21	SD		0.07
%RSD		4.41	%RSD		1.09
RW 107			RW 107		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/11/12	1	4.8361	2015/11/23	1	6.0992
	2	4.5747		2	6.1617
	3	4.7129		3	5.8567
Mean		4.71	Mean		6.04
SD		0.13	SD		0.16
%RSD		2.78	%RSD		2.67
RW 108			RW 108		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/11/12	1	4.8412	2015/11/23	1	6.1140
	2	4.7768		2	6.0413
	3	4.7077		3	5.9680
Mean		4.78	Mean		6.04
SD		0.07	SD		0.07
%RSD		1.40	%RSD		1.21
RW 109			RW 109		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/11/12	1	4.7039	2015/11/23	1	5.8561
	2	4.6541		2	6.0776
	3	4.7307		3	6.0453
Mean		4.70	Mean		5.99
SD		0.04	SD		0.12
%RSD		0.83	%RSD		2.00

Table D2: THE TRIPLICATE RESULTS OF BDOC: NOVEMBER

RW 251				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/11/05	1	4.5800	6.0355	0.0000
	2	4.6625	5.8377	0.0000
	3	4.6326	5.9851	0.0000
Mean		4.63	5.95	0.00
SD		0.04	0.10	0.00
%RSD		0.90	1.73	00.00
RW 253				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/11/05	1	4.5278	6.8568	0.0000
	2	4.6769	6.7608	0.0000
	3	4.6046	6.7955	0.0000
Mean		4.60	6.80	0.00
SD		0.07	0.05	0.00
%RSD		1.62	0.71	0.00
RW 080				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/11/12	1	5.5723	5.8534	0.11
	2	5.8417	5.7423	0.000
	3	5.5620	6.1323	0.000
Mean		5.66	5.91	0.11
SD		0.16	0.20	0.11
%RSD		2.80	3.40	00.11
RW 081				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/11/12	1	5.6767	5.6317	0.0450
	2	5.7815	5.7801	0.0014
	3	5.6983	5.6038	0.0945
Mean		5.72	5.67	0.05
SD		0.06	0.09	0.05
%RSD		0.97	1.67	99.18

RW 082				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/11/12	1	5.5324	5.9450	0.0000
	2	5.7110	6.0098	0.0000
	3	5.6310	5.7712	0.0000
Mean		5.62	5.91	0.00
SD		0.09	0.12	0.00
%RSD		1.59	2.09	00.00
RW 083				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/11/12	1	5.8554	5.7233	0.1321
	2	5.7289	5.5689	0.1600
	3	5.6005	5.5644	0.0361
Mean		5.73	5.62	0.11
SD		0.13	0.09	0.06
%RSD		2.22	1.61	59.41
RW 084				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/12	1	5.4811	5.8833	0.0000
	2	5.8417	6.0297	0.0000
	3	5.6833	5.7672	0.0000
Mean		5.67	5.89	0.00
SD		0.18	0.13	0.00
%RSD		3.19	2.23	00.00.
RW 109				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/11/12	1	5.7703	6.2713	0.0000
	2	5.7844	6.2474	0.0000
	3	5.9198	6.2423	0.0000
Mean		5.82	6.25	0.00
SD		0.08	0.02	0.00
%RSD		1.42	0.25	00.00

RW 104				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/10/23	1	7.0831	4.5200	2.5631
	2	7.3768	4.5755	2.8013
	3	7.3081	4.4402	2.8679
Mean		7.26	4.51	2.74
SD		0.15	0.07	0.16
%RSD		2.12	1.51	5.84
RW 105				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/11/23	1	7.2161	4.0552	3.1609
	2	7.2161	4.3502	2.8659
	3	7.2240	4.3741	2.8499
Mean		7.22	4.26	2.96
SD		0.00	0.18	0.18
%RSD		0.06	4.17	5.92
RW 106				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/11/23	1	7.1615	4.2974	2.8641
	2	7.2922	4.5067	2.7855
	3	7.2371	4.5355	2.7016
Mean		7.23	4.45	2.78
SD		0.07	0.13	0.08
%RSD		0.91	2.92	2.92
RW 107				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/11/23	1	7.6364	4.1315	3.5049
	2	7.3047	4.3120	2.9927
	3	7.0206	4.3794	2.6412
Mean		7.32	4.27	3.05
SD		0.31	0.13	0.43
%RSD		4.21	3.00	14.26

RW 108				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/11/23	1	7.1189	4.1053	3.0136
	2	7.3285	4.2761	3.0524
	3	7.0740	4.3227	2.7513
Mean		7.17	4.23	2.94
SD		0.14	0.11	0.16
%RSD		1.89	2.70	5.57
RW 080				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2015/11/23	1	7.3240	4.4637	2.8603
	2	7.2501	4.5559	2.6942
	3	7.2166	4.3173	2.8993
Mean		7.26	4.45	2.82
SD		0.05	0.12	0.11
%RSD		0.76	2.71	3.87

APPENDIX E

Table E1: THE TRIPLICATE RESULTS OF DOC: DECEMBER

RW 251			RW251		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/12/02	1	5.5441	2015/12/14	1	5.5441
	2	5.3453		2	5.3453
	3	5.5272		3	5.5272
Mean		5.47	Mean		5.47
SD		0.11	SD		0.11
%RSD		2.01	%RSD		2.01

RW 253			RW 253		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/12/02	1	4.6607	2015/12/14	1	4.6607
	2	4.6565		2	4.6565
	3	4.8150		3	4.8150
Mean		4.71	Mean		4.71
SD		0.09	SD		0.09
%RSD		1.92	%RSD		1.92

RW 080			RW 104		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/12/07	1	6.2038	2015/12/07	1	5.8970
	2	6.0629		2	6.1435
	3	6.3174		3	6.1379
Mean		6.19	Mean		6.06
SD		0.13	SD		0.14
%RSD		2.06	%RSD		2.32
RW 081			RW 105		
Date	Replicate	DOC Results (ppm)	Date	Replicate	DOC Results (ppm)
2015/12/07	1	5.9748	2015/12/07	1	5.9441
	2	6.0231		2	6.1714
	3	6.1572		3	6.2253
Mean		6.05	Mean		6.11
SD		0.09	SD		0.15
%RSD		1.56	%RSD		2.44

RW 082		
Date	Replicate	DOC Results (ppm)
2015/12/07	1	6.0884
	2	5.9686
	3	6.0214
Mean		6.03
SD		0.06
%RSD		1.00

RW 083		
Date	Replicate	DOC Results (ppm)
2015/12/07	1	6.0248
	2	6.1129
	3	6.1396
Mean		6.09
SD		0.06
%RSD		0.99

RW 084		
Date	Replicate	DOC Results (ppm)
2015/12/07	1	6.1435
	2	6.3708
	3	6.0646
Mean		6.19
SD		0.16
%RSD		2.57

RW 106		
Date	Replicate	DOC Results (ppm)
2015/12/07	1	5.9186
	2	5.9180
	3	6.0311
Mean		5.96
SD		0.07
%RSD		1.09

RW 107		
Date	Replicate	DOC Results (ppm)
2015/12/07	1	6.0992
	2	6.1617
	3	5.8567
Mean		6.04
SD		0.16
%RSD		2.67

RW 108		
Date	Replicate	DOC Results (ppm)
2015/12/07	1	6.1140
	2	6.0413
	3	5.9680
Mean		6.04
SD		0.07
%RSD		1.21

RW 109		
Date	Replicate	DOC Results (ppm)
2015/12/07	1	5.8561
	2	6.0776
	3	6.0453
Mean		5.99
SD		0.12
%RSD		2.00

APPENDIX E

Table E2: THE TRIPLICATE RESULTS OF BDOC: DECEMBER

RW 251				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
12/2/2015	1	5.6235	4.6650	0.9585
	2	5.9702	4.7650	1.2052
	3	5.9920	4.7752	1.2168
Mean		5.86	4.74	1.20
SD		0.21	0.06	0.15
%RSD		3.53	1.29	12.16

RW 253				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
12/2/2015	1	6.2926	5.8599	0.4327
	2	6.2889	5.8449	0.4440
	3	6.2977	5.8598	0.4379
Mean		6.29	5.85	0.43
SD		0.00	0.01	0.01
%RSD		0.07	0.15	1.32

APPENDIX F

Table F1: THE TRIPLICATE RESULTS OF DOC: BIOFILM IN A SET-UP PIPE

BIOFILM 1		
Date	Replicate	DOC Results (ppm)
2016/11/05	1	5.3370
	2	5.4954
	3	5.5733
Mean		5.47
SD		0.12
%RSD		2.20

BIOFILM 2		
Date	Replicate	DOC Results (ppm)
2016/11/05	1	1.2268
	2	1.0403
	3	0.9700
Mean		1.08
SD		0.13
%RSD		12.30

BIOFILM 3		
Date	Replicate	DOC Results (ppm)
2015/10/26	1	2.7526
	2	1.0403
	3	0.9700
Mean		1.59
SD		1.01
%RSD		63.59

Table F2: THE TRIPLICATE RSULTS OF BDOC: BIOFILM IN A SET-UP PIPE

BIOFILM 1				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2016/05/11	1	6.4676	5.7732	0.6944
	2	6.0351	5.7743	0.2608
	3	6.0867	5.3300	0.7567
Mean		6.20	5.63	0.57
SD		0.24	0.26	0.27
%RSD		3.81	4.55	47.34
BIOFILM 2				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2016/05/12	1	1.6289	1.9432	0.0000
	2	1.6741	2.0054	0.0000
	3	1.6915	1.9253	0.0000
Mean		1.66	1.96	0.00
SD		0.03	0.04	0.00
%RSD		1.94	2.15	0.00
BIOFILM 3				
Date	Replicate	Day 0 Results (ppm)	Day 5 Results (ppm)	BDOC (ppm)
2016/05/12	1	4.3549	3.7300	0.6249
	2	4.7427	3.8452	0.8975
	3	3.9348	3.8561	0.0787
Mean		4.34	3.81	0.53
SD		0.40	0.07	0.42
%RSD		9.30	1.83	78.12