

Measurement of the biodegradable  
fraction of dissolved organic matter  
relevant to water reclamation via  
aquifers

Declan Page and Peter Dillon

## Water for a Healthy Country report series

ISSN: 1835-095X

Enquiries should be addressed to:

Declan Page  
Senior Research Scientist  
CSIRO Land and Water  
Private Bag No. 2, Urrbrae SA 5064, Australia  
Ph: +61-8-8303 8748 Fax: +61-8-8303 8750

### **Copyright and Disclaimer**

© 2007 CSIRO To the extent permitted by law, all rights are reserved and no part of this publication covered by copyright may be reproduced or copied in any form or by any means except with the written permission of CSIRO.

### **Important Disclaimer**

CSIRO advises that the information contained in this publication comprises general statements based on scientific research. The reader is advised and needs to be aware that such information may be incomplete or unable to be used in any specific situation. No reliance or actions must therefore be made on that information without seeking prior expert professional, scientific and technical advice. To the extent permitted by law, CSIRO (including its employees and consultants) excludes all liability to any person for any consequences, including but not limited to all losses, damages, costs, expenses and any other compensation, arising directly or indirectly from using this publication (in part or in whole) and any information or material contained in it.

## CONTENTS

<b>EXECUTIVE SUMMARY</b> .....	1
<b>INTRODUCTION</b> .....	2
<b>SOURCES OF ORGANIC MATTER</b> .....	2
<b>QUANTIFICATION OF ORGANIC MATTER</b> .....	3
<b>Chemical oxygen demand and permanganate index</b> .....	3
<b>Biochemical oxygen demand</b> .....	3
<b>Determination of organic carbon</b> .....	4
<b>Determination of organic nitrogen</b> .....	5
<b>CHARACTERISATION OF ORGANIC MATTER</b> .....	5
<b>Fractionation methods</b> .....	5
<b>Fractionation using XAD resins</b> .....	5
Membrane fractionation.....	6
Size exclusion chromatography.....	6
<b>Indirect methods</b> .....	6
UV absorbance.....	7
Fluorescence spectroscopy.....	7
DBP formation potential.....	7
<b>Direct Methods</b> .....	7
Analysis of carbohydrates in water.....	7
Analysis of amino acids in water.....	8
<b>Advanced Techniques for Characterisation of NOM</b> .....	8
<b>CHARACTERISATION OF BOM</b> .....	8
<b>Bioassays based on bacterial growth</b> .....	9
Batch cultures of known bacterial species.....	9
Batch cultures of indigenous bacterial species.....	11
Biofilm colonization monitors.....	12
<b>Bioassays based on measurement of changes in dissolved organic carbon concentrations</b> .....	12
BDOC with batch cultures.....	13
BDOC with batch cultures and sand.....	13
BDOC with bioreactors.....	14
<b>Comparison of techniques for measuring BOM</b> .....	16
<b>BIOSTABILITY OF WATER IN MAR APPLICATIONS TO REDUCE CLOGGING</b> ..	18
<b>Effect of BOM on biological stability</b> .....	18
<b>Defining threshold values to achieve biostability</b> .....	19
<b>PROPOSED BOM MEASUREMENT METHOD FOR PREDICTING CLOGGING IN MAR</b> .....	20
<b>CONCLUSIONS</b> .....	21
<b>REFERENCES</b> .....	22

## List of Tables

Table 1 Modifications to the original AOC method (modified after Prévost <i>et al.</i> 2005) .....	10
Table 2 Modifications to the original BDOC method .....	15
Table 3 Parameters of BOM measurement.....	17
Table 4 Threshold values of BOM for biologically stable waters (modified from Prévost <i>et al.</i> 2005) .....	19

## List of Figures

Figure 1 Overview of the quantification of dissolved organic matter .....	4
---	---

## **EXECUTIVE SUMMARY**

In recent years there has been considerable effort made to characterise biodegradable organic matter (BOM) due to its myriad influences on potable water treatment processes. In managed aquifer recharge, BOM is equally important as it has been considered the limiting nutrient in determining the rates of biologically mediated clogging in injection wells. This becomes especially important for nutrient rich reclaimed waters such as storm water and reclaimed water in unconsolidated aquifers.

BOM in water is defined as the fraction of dissolved organic carbon that can be utilised by bacteria for anabolic or catabolic purposes. The character of BOM is highly complex and is a function of the origin of the water being recharged. The issue of predicting and controlling water biostability has fuelled recent research in the water industry and has been the driver for this review.

To effectively address issues related to BOM, methods are required by which to measure its concentration. Two methods have received most attention, the assimilable organic carbon (AOC) assay and the biodegradable dissolved organic carbon (BDOC) assay. However, to date neither method is able to measure the entire BOM pool. All methods used to measure BOM are bioassays using heterotrophic bacteria. Chemical methods alone are inadequate due to the heterogeneity and complexity of BOM.

A recommendation of this report is that the most suitable method for determining BOM in the context of water reclamation via aquifers is the commonly used BDOC method, which is available in many commercial laboratories. Furthermore direct measurement of clogging in standardised columns of porous media is likely to yield more reliable, simpler and cheaper estimates of clogging than standardised measures of BOM, including BDOC. Understanding microbial communities responsible for clogging will be helpful in understanding how to inhibit biofilm development, and further work has been proposed to address this goal.

## INTRODUCTION

The term natural organic matter (NOM) is generally used to describe a broad group of organic compounds present in all natural waters. Although the original term refers to organic matter from natural sources, NOM may also contain organic matter from domestic, industrial, or agricultural sources. The relevant contribution from these anthropogenic sources is highly specific and may even dominate in some water sources such as industrial process waters. Surface waters generally contain higher organic matter concentrations than groundwaters. The high variability and complexity of the characteristics of NOM play a significant role in its behaviour with respect to water treatment and managed aquifer recharge (MAR). The presence of NOM also influences the content of micropollutants, since it may bind and transport inorganic and organic pollutants (Pelekani *et al.* 1999; Croué *et al.* 2003).

In recent years there has been considerable effort made to characterise NOM, since it plays a major role in many processes involved in drinking water treatment (e.g. Zappia *et al.* 2000, Franzmann *et al.* 2000; 2002; Warton *et al.* 2005; Page 2006). Analogously to the issues NOM causes in water treatment, the character of NOM may influence MAR operational applications: from quality of source water to clogging issues in injection wells (Schippers *et al.* 1995). Understanding which fractions of NOM are removed by various treatment processes allows the optimisation of treatment to improve the removal of physical and chemical processes (e.g. coagulation, adsorption, biofiltration).

A fraction of dissolved organic matter (DOM), which is the water soluble component of NOM, is a complex mixture of aromatic and aliphatic hydrocarbon structures. Given that the organic matter present in treated waters is essentially present in dissolved form, the particulate component can be considered negligible and is not further discussed in this report. As a consequence the term biodegradable organic matter (BOM) is used equivalently to dissolved biodegradable or labile organic matter. The BOM in water is a fraction of NOM that can be consumed, under certain conditions, by microorganisms for catabolic or anabolic purposes. The matrix and properties of BOM are highly complex and are a function of the water's origin and transport pathway. The issue of controlling and predicting the biostability of water draws heavily from research into drinking water quality, treatment and distribution. Better knowledge of the biodegradation rates in biological processes, such as biofiltration and the clogging dynamics in near well injection systems is necessary to quantify the effect of BOM removal on water quality and clogging.

## SOURCES OF ORGANIC MATTER

Many attempts have been made to characterise the main sources of NOM. The NOM found in water is primarily from the decay and leaching processes of organic materials from plants, animals and microorganisms and their transportation in water (Page *et al.* 2002a). The degradation processes may result from microbial activities. Depending upon the source of organic matter, NOM can be characterised as autochthonous or allochthonous, which implies the origin within a given system or outside of the system respectively. When referring to drinking water, allochthonous organic carbon generally originates from terrestrial catchments, while autochthonous organic carbon is derived from the biota (e.g. algae) growing in the water. Although the relative contribution of allochthonous and autochthonous may vary within the system, there are distinct characteristics that may be traced to the source (Page *et al.* 2002a). Others have shown (Biber *et al.* 1996) that the relative proportions of autochthonous organic matter in lake water is maximal in summer when biological activity is high,

while allochthonous organic matter is highest in winter when terrestrial runoff is highest. The relative contributions of the various sources of NOM can thus be highly variable. Factors inducing variability include the position within the catchment; seasonal and climatic changes and major hydrological events; point sources such as industrial or agricultural discharges and non-point sources such as urban or agricultural runoff. Because of this high variability, the source and resulting composition cannot easily be predicted.

## QUANTIFICATION OF ORGANIC MATTER

This following section details some of the most common analytical procedures used to quantify NOM.

### Chemical oxygen demand and permanganate index

The method used to determine the chemical oxygen demand (COD) uses a dichromate reflux and has been used to determine overall organic matter content in water and wastewater. Common values for COD vary from 5 – 15 mg / L for surface waters and are correlated to organic carbon with an approximate ratio of 2 - 3 mg O/ mg C. However the method detection limit (5 mg / L O<sub>2</sub>) restricts the application of this method to wastewaters.

Determination of organic content using the acidic potassium permanganate (permanganate index) has also been used in soils (Skjemstad *et al.* 2006) and waters, but the low oxidizing potential of potassium permanganate and the conditions of oxidation specified in the method (APHA 1998) do not allow complete oxidation of organic carbon. For surface water the permanganate index can vary from 2 – 15 mg O<sub>2</sub>/L. These two oxidation techniques are gradually being replaced by more accurate techniques for measuring organic carbon.

### Biochemical oxygen demand

Biochemical (biological) oxygen demand (BOD) was first introduced in 1908 as a test to infer the general quality of the water and its degree of pollution by biodegradable organic matter. BOD is not an accurate quantitative test and should be considered as providing an indicator of the quality of a water body. Like COD, BOD measures the rate of uptake of oxygen by micro-organisms in the sample of water at a fixed temperature (20°C) and over a given period of time (usually 5 days) in the dark. To ensure that all other conditions are equal, a very small amount of micro-organism seed is added to each sample being tested. This seed is typically generated by diluting activated sludge with de-ionised water. BOD is similar in function to chemical oxygen demand (COD), in that both measure the amount of organic compounds in water. However, COD is less specific since it measures everything that can be chemically oxidised rather than just levels of biology active organic matter. BOD is used as a gauge of the effectiveness of wastewater treatment plants. Various commercial devices are available for its determination.

## Determination of organic carbon

The elemental components of NOM are primarily carbon, oxygen, nitrogen, hydrogen and sulphur, all bound together in a wide range of complex configurations. Elemental analysis of carbon, where carbon is completely oxidized can be performed on dried residues of organic matter. Usually NOM concentration is expressed in carbon units, because this is the dominant compound by weight in organic matter.

Organic carbon contained in NOM can be either particulate organic carbon (POC) or dissolved organic carbon (DOC). POC is defined as the fraction of organic matter retained on a 0.45 µm membrane (APHA 1998).

In drinking water applications, organic carbon is commonly used as a surrogate for the total amount of NOM. The carbon content is usually assessed by total organic carbon (TOC) or DOC. Most methods for measuring DOC in water are based on high-temperature catalytic oxidation, ultraviolet/persulphate oxidation or some combination of these processes.

DOC measurement is considered a key parameter in evaluating the efficiency of the treatment processes with respect to NOM removal. It is also used to measure BOM by certain methods (biodegradable dissolved organic carbon, BDOC).

TOC and DOC in raw waters vary considerably depending upon the source. Concentrations of TOC and DOC remain below 2 mg/L in most groundwater. DOC in surface waters varies widely, 1- 20 mg/L, upland streams (1 - 3 mg/L) contain less than rivers (2 - 10 mg/L), while wetlands may contain high DOC concentrations (10 - 60 mg/L).

Figure 1 illustrates the relationship between the different methods described to measure dissolved organic carbon.

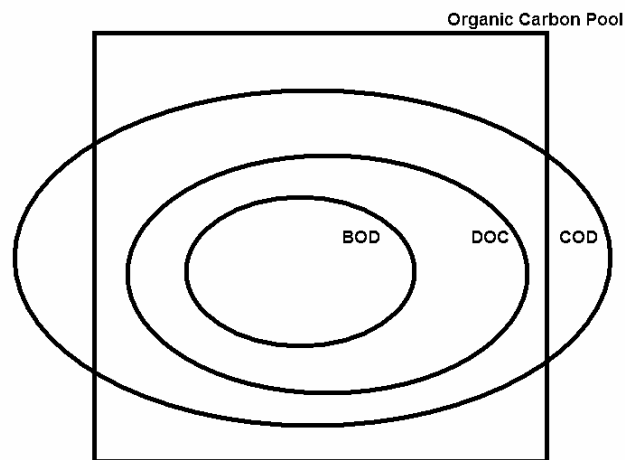


Figure 1 Overview of the quantification of dissolved organic matter

Note that COD measurements include other oxidisable species such as chloride ions and as such encompasses a greater spectrum than the organic carbon pool. The relationships hold that quantification measures of  $COD \geq DOC \geq BOD$ .

## Determination of organic nitrogen

Organic nitrogen compounds are present in natural waters primarily in the form of peptides, polypeptides, proteins, and amino sugars (Aiken 1985; Bruchet *et al.* 1990). Acid hydrolysis is commonly used to breakdown these large amino compounds into their constituent amino acids which are then identified by routine analytical methods (APHA 1998).

Since its introduction in 1883, the Kjeldahl nitrogen determination method has been refined and tested on a wide variety of substances for the determination of total nitrogen. The Kjeldahl method contains three steps; digestion, distillation and titration. This approach measures both organic and inorganic nitrogen and, thus, is used to calculate organic nitrogen in water by also measuring the initial ammonium concentration and subtracting this from the total.

The determination of dissolved nitrogen can be performed using nitrate titration following elevated temperature digestion of organic matter (Egeberg *et al.* 1999). A few attempts to determine dissolved organic nitrogen (DON) are based on its conversion to mineral forms but these techniques suffer from inaccuracy. Emerging analytical tools tend to combine both DOC and DON determination in water with the same multipurpose instrument using thermocatalytic oxidation with infrared detection for carbon analysis and either electrochemical detector or chemiluminescence for nitrogen.

## CHARACTERISATION OF ORGANIC MATTER

The approaches used to characterise NOM can be grouped into specific or general techniques. For the most part, specific techniques address determination based on the chemistry of the compounds, while general approaches commonly refer to fractionation techniques or to the indirect determination using spectral or physical properties of the NOM. The specific techniques apply to the characterisation of target compounds of NOM that have similar chemical properties (e.g. amino acids, carbohydrates). Conversely, the general techniques are aimed at generic groups of NOM that may contain various specific components.

### Fractionation methods

Some techniques used for chemical characterisation require either sample hydrolysis or digestion prior to analysis and therefore are destructive. With these methods, the chemical structures of the polymeric constituents of NOM are not preserved, as intermolecular bonds are broken during hydrolysis. Due to the chemical complexity of such substances, a fractionation step can help to reduce the molecular heterogeneity and achieve better insight into chemical composition (Picolo *et al.* 2002). The techniques based on fractionation address the issue of segregating groups of compounds based on their physical properties.

### Fractionation using XAD resins

Amberlite XAD resins have been extensively used to adsorb organic solutes from water since the early 1970s (Aiken 1988). The use of XAD resins is intended not only to isolate the NOM but to fractionate out the humic substances as well based on the affinity of NOM components to the functional groups of

the resins. Humic substances is a generic term for the fraction of hydrophobic NOM that can be adsorbed on XAD8 resin at pH 2 and then eluted at pH 13. The humic substances (i.e. humic and fulvic acids) can account for 40 - 80% of DOC of surface waters. Fulvic acids represent the main fraction of humic substances in surface waters (up to 90%). A larger fraction of NOM can be isolated and fractionated using two columns in series filled with XAD8 and XAD4 resins (Aiken *et al.* 1992).

Aiken (1988) makes a critical evaluation of the use of macroporous resins for the isolation of humic substances in water. He reports that the fractionation of DOC in a sample varies depending upon many factors including the type of resin, the amount of sample passed through the resin and the eluents used. Another criticism of the fractionation technique is the potential alteration of NOM due to the wide range of pH values involved.

## Membrane fractionation

The use of membranes to fractionate NOM is based on the isolation of different fractions according to molecular cutoff of the membranes. The membrane techniques include reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF) and microfiltration (MF). The fractionation pathways can be considered based on the physical characteristics of the compounds of interest; MF and UF are molecular size driven, while separation by RO and NF is controlled by diffusion factors and ionic charges.

Membrane filtration is an alternative way to concentrate NOM, which minimizes the potential for structural alteration of NOM. Due to their low molecular weight cutoffs, NF and RO can recover up to 90% of the DOC, but concentrate salts which may interfere with subsequent analyses.

## Size exclusion chromatography

Size exclusion chromatography (SEC) is based on the differential separation of molecules of different sizes flowing through a porous matrix. The compounds are primarily separated according to hydrodynamic molecular size which combines hydrophobic and electrostatic interactions between the resin and the analyte compounds, thereby creating an effective size of the compounds (Her *et al.* 2002). SEC is a rapid and reproducible method for characterizing macromolecules according to their molecular weight and size distribution and is now commonly used to characterise the various fractions of NOM.

## Indirect methods

Alternative methods for characterising NOM are not based on the measurement of specific components in the organic matter and are used to complement specific and advanced characterisation techniques.

## UV absorbance

The absorbance of UV light is a general characteristic of the pool of molecules forming DOC. UV absorbance provides overall structural information on NOM. UV absorbance at 254 nm has been used as a simple surrogate for measuring DOC levels (Krasner *et al.* 1996).

The specific UV absorbance (SUVA) is defined as the UV absorbance of a water sample determined at a given wavelength normalised for DOC concentration. SUVA has been used as a surrogate for DOC aromaticity because it correlates well with NMR data for a wide range of DOC isolates. It thus provides an assessment of the general chemical composition of DOC subject to spatial and temporal variations.

## Fluorescence spectroscopy

Because of DOM's complexity, the measurement of fluorescence represents a simple and rapid technique used to characterise DOM. The fluorescent fractions of DOM exhibit intensity peaks at specific wavelengths. Although many advances have been made in the application of fluorescence spectra to characterise DOM, the assignment of structures responsible for the fluorescence behaviour is still largely undetermined and as such finds most promise in fingerprinting-like applications.

## DBP formation potential

The chemical reactivity of NOM can be used as a general characterisation of NOM using the disinfection by product (DBP), and for chlorine disinfection the trihalomethane formation potential (THMFP) (Page *et al.* 2002b). The oxidant demand is an indirect parameter used to assess the ability of NOM to react with oxidants. There has been some success in linking general chemical properties to mechanisms of reactivity with chlorine and subsequent formation of chlorination by-products (Croué *et al.* 1999). Although powerful analytical tools provide needed insight into these mechanisms, a relatively simple measurement such as SUVA appears to be an excellent surrogate parameter to predict THMFP (Krasner *et al.* 1996).

## Direct Methods

A broad range of compounds have been reported to contribute to the organic carbon of natural waters. However due to the heterogeneity and complexity of the DOC pool, only a limited proportion (~20%) can be identified at the molecular level (Gremm and Kaplan 1998). Methods for determining amino acids and carbohydrates are described below because of their dominance and relevance to biodegradability of NOM.

## Analysis of carbohydrates in water

Thurman (1985) identified carbohydrates as a major constituent of the small pool of identifiable DOC molecules. Carbohydrates dissolved in water are present in the form of mono or polysaccharides that are either free or bound to DOM. Gremm and Kaplan (1998) have shown that the concentration in rivers varies with the hydrologic conditions of the catchment. They report increases in the dissolved

total carbohydrates during storm events. Merlet *et al.* (1996) determined that fructose, glucose, rhamnose, mannose and galactose are major compounds found in various river waters. The total carbohydrate concentrations ranged from 150 to 250  $\mu\text{g C / L}$  and represented 4 - 5% of the DOC of these surface waters. They also report that glucose is a major component of carbohydrates either in shallow (60 m) or deep (up to 1,870 m) groundwaters. Lower carbohydrate contents ranging from 7 to 30  $\mu\text{g C / L}$  have been reported in these groundwaters, which represents 1.5 – 20% of the DOC.

### Analysis of amino acids in water

Amino acids are another specific group of compounds that can be quantified in water. Similarly to carbohydrates, amino acids in water are present as free or included in peptides, proteins or other organic molecules that may be either free or bound to the NOM. The concentration of amino acids is reflected in the water source; total dissolved amino acid concentrations of 20 to 350  $\mu\text{g/L}$  in groundwater, 50 to 1000  $\mu\text{g/L}$  in river waters and up to 6000  $\mu\text{g/L}$  in lakes (Thurman 1985). The average contribution to DOC is 2 - 3% but can be as high as 13% in some eutrophic lakes dominated by algal blooms.

## Advanced Techniques for Characterisation of NOM

Different sets of advanced techniques have been developed to provide molecular level detail on NOM. These techniques have aimed at providing more detailed information on structural and elemental composition of organic matter.

Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) has been used for structural studies of Australian NOM (Chow *et al.* 1999; Page *et al.* 2002a; Page 2006). The effects of salts are minimal therefore concentration techniques such as reverse osmosis and freeze drying can be used. It involves the rapid heating to high temperature at which all biomacromolecules (e.g. carbohydrates or amino acids) are degraded to lower molecular weight products of thermal decomposition (Page 2006). The natural biomacromolecules which are the precursors of bulk NOM are clearly identified with their specific fragments with little interference among the biomacromolecules.

Although NOM from different sources will produce similar pyrolysis biomarker subunits, the structural variation of the subunits provides a specific fingerprint to each organic material. During pyrolysis the pyrolysate is assumed to represent the total organic matter; however some organic contents are poorly detected by mass spectrometry (Page 2006).

Nuclear Magnetic Resonance (NMR) spectrometry using solid state  $^{13}\text{C}$  or liquid state  $^1\text{H}$  has also been used for NOM characterisation (Skjemstad *et al.* 2005). The  $^{13}\text{C}$  NMR technique is very effective for obtaining average functional group distributions but provides limited information on the individual constituents. Furthermore, NMR is influenced by the salt content, especially paramagnetic ions such as iron (III) which are often present in isolated NOM samples.

## CHARACTERISATION OF BOM

To effectively address the issues related to BOM, methods are required to measure BOM concentrations. Specific applications for BOM assays include water resource assessment, water

treatment decisions, process optimisations, and process monitoring. There is no reference method that quantifies all BOM present in a sample, and an important consideration in understanding what fraction of the total NOM pool is being measured. Conversely, little is known of the relative importance of the most biologically labile BOM constituents such as carbohydrates and amino acids relative to more refractory BOM such as biomacromolecules.

All methods for measuring BOM concentrations involve the use of bacteria. Chemical methods alone are inadequate for BOM estimation because diversity involving thousands of potentially degradable compounds. Some measurement methods focus on the growth of the bacteria, while others measure the activities of the microbes by assaying chemical changes in test water.

## **Bioassays based on bacterial growth**

Bacteria transport organic molecules across their cell walls, degrading or oxidizing some compounds with an accompanying release of chemical energy (catabolism) and incorporating other compounds through biosynthetic reactions into biomass and new cells (anabolism). Bioassays based on bacterial growth focus on the fraction of BOM that is incorporated into biomass and the production of new cells, although the fraction that is respired is considered when estimating growth yields. These BOM measurements can be divided into two major categories: those that involve cultures of known bacteria and those that involve cultures of the indigenous bacterial community.

### **Batch cultures of known bacterial species**

The assimilable organic carbon (AOC) concentration in a water sample is governed by simple Monod type kinetics; it is proportional to the density of organisms that can grow in it. This is measured by the AOC method where a low concentration of bioassay organisms is inoculated into culture vessels containing a sterile test water, the cultures are sealed and incubated at 15°C, and over a period of several days to weeks the density of the cells are enumerated with sufficient frequency to characterise the population growth curve and obtain the maximum cell densities. Cell densities are converted into carbon (usually acetate) concentration equivalents using empirically derived yield factors.

Two bacterial species isolated from drinking water in the Netherlands, *Pseudomonas fluorescens* P-17 and *Spirillum* strain NOX, are the primary bioassay organisms used in the AOC assay. Implicit in their use is the assumption that their metabolic capabilities either correlate with or are representative of a broader range of bacteria found in drinking water supplies and distribution systems.

Because the bioassay organisms are able to grow at low concentrations of organic substrate, the AOC bioassay is extremely sensitive to organic carbon contamination. Theoretically concentrations of 1 µg C/L can be detected with the AOC method; however in practice the detection limit is closer to 10 µg AOC/L (APHA 1998).

There have been a number of modifications to the original AOC method but few have repeated application or uptake. These modifications are listed in Table 1.

Table 1 Modifications to the original AOC method (modified after Prévost *et al.* 2005)

Improvement sought	Modification	Reference
Original method	Standardised AOC method based on stepwise inoculation with <i>Pseudomonas fluorescens</i> P-17 and <i>Spirillum</i> NOX	van der Kooij <i>et al.</i> (1982); APHA (1998)
Increased speed	Incorporation of 4 species in the inoculum	Kemmy <i>et al.</i> (1989)
	A single fast growing <i>Acinetobacter</i> used for the inoculum	Kang <i>et al.</i> (1997)
	Simultaneous inoculation with <i>Pseudomonas fluorescens</i> P-17 and <i>Spirillum</i> NOX	van der Kooij (1990)
	Increased inoculation density	Frias <i>et al.</i> (1994)
	Increased incubation temperature	LeChevallier <i>et al.</i> (1993)
	ATP bioassay of organisms	LeChevallier <i>et al.</i> (1993)
	Rapid macroscopic technique	Bradford <i>et al.</i> (1994)
	Rapid technique based on flow cytometry	Hammes and Egli (2005)
Reduction of contamination potential	40mL vials batched instead of 1 L flask	Kaplan <i>et al.</i> (1993)
Greater sensitivity	Natural microbial consortium used for inoculum	Hammes and Egli (2005)
Greater biostability	Samples heated to 72°C then cooled on ice instead of pasteurization	Escobar and Randall (2000)
	Use of membranes to sterilize the water instead of pasteurization	Yoro <i>et al.</i> (1999)

Regardless of how the assay is performed, the conversion of cell densities to AOC concentrations involves the use of yield coefficients. The bioassay was developed as an index of regrowth potential, not an absolute measure of carbon concentration. When a yield coefficient is used to convert the density of cells into units of concentration, these are expressed as substrate C equivalences. Expressing the AOC concentration in units of carbon extends the method beyond its original purpose, implicitly assumes a yield coefficient, and applies the coefficient to a mixture of substrates in a test water. Yield coefficients differ between P-17 and NOX by a factor of 3 for acetate and yield coefficients of P-17 alone differ by a factor of 4 between organic acids and amino acids. Pure cultures are required for the determination of the yield coefficient and the organic substrate must be the limiting nutrient.

In summary, the particular advantages of the AOC bioassay are that it is sensitive, precise, standardised by bioassay organisms, generally has a response parameter (colony forming units per millilitre) that exceeds the blank by an order of magnitude (high signal to noise ratio) and it gives a measure of the potential to form new biomass. The disadvantages to the AOC method include its sensitivity to organic carbon concentration, a response parameter in units of substrate C equivalencies and the length of time required to perform the test. In addition although the method has been used in The Netherlands as a reference for the potential for clogging in aquifer storage and recovery wells (Olsthoorn 1982; Hijnen and van der Kooij 1992) there is no known link between the reference organisms and clogging in water reclamation applications and as such its utility for estimating BOM with reference to clogging is tenuous.

## Batch cultures of indigenous bacterial species

Testing the growth of indigenous bacteria in water samples forms the second major group of BOM measurement methods based on bacterial growth in batch culture. There are three basic approaches, each based on different techniques for quantifying growth.

The first method is based on the measurement of turbidity, and is termed the bacterial regrowth potential (BRP) assay (Werner 1984; Withers *et al.* 1996; Page *et al.* 2002b). In this method, the water sample is first sterilized, amended with a mineral salt solution to ensure BOM is the limiting nutrient, and placed in a temperature controlled cuvette. The inoculum is prepared by washing the bacteria off the membrane used to sterilize the sample. Cell numbers and densities are adjusted to produce an initial density of  $5 \times 10^4$  cells / mL. The cuvette is constantly stirred except during turbidity measurements and the system is run until the batch cultures reach a stationary phase. This technique results in growth curves over time for the bacterial community. Two parameters are derived from the growth curve: growth rate, calculated as the slope during exponential growth, and the growth factor, calculated as the ratio of final to initial turbidity. Because the bacteria are indigenous, the results should be particularly relevant to the water sampled, though it does measure the growth of suspended rather than attached bacteria. This is of particular importance to clogging as the attached bacteria are generally thought to produce the biofilm causing clogging in MAR applications, similarly to the growth of biofilms in slow sand filters (Page *et al.* 2006). The method is augmented by additional measurements which are often made including the determination of cell numbers by microscopy and change in DOC concentration which provides some measure of BOM concentration and cellular yield. However, with a suspended batch culture grown for 40 - 60 hours at 22°C it is unlikely that all the most biologically labile BOM is metabolised (McDowell *et al.* 2006). Under some circumstances, growth can be detected from turbidity changes, but no change in DOC concentration is detected (Hamsch and Werner 1993).

A second method based on the growth of indigenous bacteria in a batch culture is analogous to the AOC bioassay, except that the inoculum is a mixture of unknown species and the test parameter is AOC (Stanfeld and Jago 1989). The method has many of the same benefits and problems of the AOC method. An important difference however, is that an ATP measurement of a batch culture composed of mixed bacterial species is more difficult to accurately convert into cell numbers because of the differences in species based on ATP content per cell. As an alternative, enumeration by flow cytometry has been used (Stepanauskas *et al.* 2000; Hammes and Egli 2005).

A third method is based on the integrated flux of bacterial mortality in a water sample (Servais *et al.* 1987). In this method a sample is membrane filtered then re-inoculated with a small sub sample. The sample is then incubated in the dark at 20°C for 10 - 30 days to allow the indigenous bacteria to grow to a maximum and then decline. During the incubation period, cell densities are estimated by microscopic counts and sizes are estimated by microscope and biovolumes are converted to biomass using conversion values from the literature. A bacterial mortality constant is determined from the disappearance of radioactivity in the DNA of bacteria prelabelled with tritiated thymidine. The product of the mortality constant and the change in biomass gives the integrated flux of bacterial mortality (Servais *et al.* 1985). The data obtained by this method agrees well with the BDOC estimated from differences in DOC concentration in batch culture for waters collected before and after biofiltration, but the method is based on methods that are either difficult or laborious to measure, especially bacterial biovolumes, bacterial numbers, and mortality rate estimated by the thymidine method. The method is also dependent on several assumptions, including the conversion of biovolume to biomass estimates which range over an order of magnitude, and all of the assumptions regarding the tritiated thymidine incorporation method (Bell 1993). Because of these numerous assumptions and difficulties this method is rarely used.

## Biofilm colonization monitors

Although methods for measuring the growth of attached bacteria exposed to a continuous flow of water are technically not methods for measuring the concentrations of BOM, they do have many similarities with the BOM methods described above. Most significant is that the objective of both methods is to learn about the growth of bacteria and formation of biofilms. Two very different approaches have been suggested for devices that monitor growth, one using known species of bacteria that are encapsulated under silica gel and connected to the water through an agar film and the other more common approach using the bacterial community present in the test water.

There are several devices that monitor biofilm colonization by the indigenous bacterial community. These devices are placed directly into the flow and include: incorporation sampling devices (Donlan *et al.* 1994), coupon samplers (Schwartz *et al.* 2003), flush mounted coupons (Camper *et al.* 1993), annular reactors (van der Welde *et al.* 1989), rotating disks (Hermanowicz *et al.* 1991) and a column filled with glass cylinders (van der Kooij *et al.* 1995). All of these devices have been utilised in assessing biofilm growth in drinking water distribution systems.

The rotating disk method is relatively rapid (48 hours) and generates growth rates of attached bacteria whereas other methods are longer requiring at least a week to obtain a result. With the rotating disk method, polyvinyl chloride disks are sampled after exposure to test water and the number of one, two and four celled colonies are counted by direct microscopy. Additionally this method assumes that attachment occurs at a constant rate, no detachment occurs and all cells grow at identical rates.

Each of the other biofilm monitoring methods uses a solid substrate of glass, polycarbonate, or metal that is exposed to a continuous flow of the test water for several days to months; the accumulated biomass is then quantified. In the case of the biofilm monitor of van der Kooij *et al.* (1995), glass cylinders are removed periodically from the vertical glass column, extracted with a commercial releasing agent and then analysed for ATP using the luciferin-luciferase reaction.

With annular reactors and flush coupons inserted into pipes there is an attempt to simulate the shear stress experienced by biofilms growing in water distribution systems. The biomass that accumulates in any of these devices can be enumerated using a number of methods, including direct microbial counts, heterotrophic plate counts and ATP.

The advantages of these biofilm monitoring methods are that they integrate a number of variables including organic and inorganic nutrients, temperature, disinfectant, residence time and hydrodynamics into the assessment of biofilm formation. This information is generally not all available from an assay for BOM concentrations. The disadvantage of these methods are that they take a relatively long time to generate results, labour intensive, capital intensive and they do not separate the influence of BOM from the other variables (such as temperature, residence, time, hydraulics, nutrients). Their use in measuring biofilm growth and well clogging are also questionable due to the intrinsic differences of the solid substrates to which the biofilm is attached. While glass substrate-based methods have some similarity to sandy aquifers in terms of the media composition; aquifer sands will also contribute a wider variety of minerals that can facilitate biofilm formation.

## Bioassays based on measurement of changes in dissolved organic carbon concentrations

All bioassays that are based on the measurement of DOC are collectively identified as BDOC assays. Newer instrumentations allows detection limits of DOC as low as 10 µg/L (APHA 1998), but as

BDOC is calculated as the difference between two DOC analyses, the detection limit for BDOC is closer to 20 µg/L. A distinct advantage of using direct measurements of DOC is that the values are in units of carbon, also information is provided on the proportion of DOC that is non-biodegradable.

### BDOC with batch cultures

Testing of batch cultures of indigenous microorganisms to measure concentrations of BOM is conducted with organisms that are either in suspension or attached to a solid media. Such a procedure was originally used to investigate lake water (Krogh and Lange 1932) and has been modified to take advantage of analytical methodology improvements. An example of the assay with suspended bacteria used for drinking water (Servais *et al.* 1987; 1989) filters the test water through a 0.2 µm membrane. This filtered water is then re-inoculated, an initial DOC measurement is made, the sample is incubated, and DOC is measured periodically from the same incubation vessel until no further decrease in DOC is observed. Incubation times are generally 10 - 30 days. However, the kinetics of BDOC metabolism by a suspended inoculum differ for different water qualities (Volk *et al.* 1994). Furthermore, McDowell *et al.* (2006) showed that with more refractory BDOC compounds, 28 day incubation could underestimate BDOC concentrations by as much as 25% compared to an 85 to 120 day incubation. They recommended a more sophisticated method involving: (1) a rapid determination of relatively labile DOC (measurement of DOC removal after 7 days of incubation with added nutrients) and (2) a 42 day incubation with repeated analysis of CO<sub>2</sub> production when determination of decomposition rate constants and a labile and relatively refractory component of DOC is desired.

The advantage of the BDOC method is that it is simple to perform and is both sensitive and precise. The minimum detectable concentration of the test is 0.1 mg 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 of the DOC analyser. Precision of the method has been estimated at 11 - 160 µg C/L for 109 waters in the United States (Kaplan *et al.* 1994) and 3 - 100 µg C/L for 18 source waters in Norway (Charnock and Kjønne 2000). It has also been demonstrated that the source of bacteria is not a significant source of variation for the assay (Prevost *et al.* 1992; Servais *et al.* 1987). The small initial inoculum contains many different species and those capable of degrading the BOM in the test water. Those that consequently grow during the test are selected for over a period of weeks. The disadvantages of this method is that it takes weeks to complete, requires a DOC analyser and the incubation period is selected *a priori*. The release of soluble microbial products of metabolism are not considered BDOC concentrations may be underestimated. For work at low concentrations, filtration through membranes is problematic as it is difficult to remove all carbon contamination from the membranes. The original BDOC method was slightly modified by Kaplan *et al.* (1994) in an attempt to reduce organic carbon contamination and improve the detection limit. Water was filtered through glass microfilters prior to incubation. An additional inoculum was not required because glass fibre filters allow most bacteria in suspension to pass into the filtrate.

### BDOC with batch cultures and sand

A substantial method development of the BDOC assay, developed specifically for drinking waters, involves the use of bacteria attached to sand as the inoculum (Joret and Levi 1986). In this method, sand from a water treatment plant filter is collected and washed with test water until no increases of DOC are measured in the wash. The colonised sand is placed in a 500 mL Erlenmeyer flask (100g sand : 300 mL water) and the batch cultures aerated at 4 L/h with air that is scrubbed by two water washes in series. This procedure again continues until a minimum DOC concentration is obtained usually within 3 – 4 days. The bioassay using bacteria attached to sand was shown to be superior by

Park *et al.* (2004) to the other methods for BDOC determination in terms of its incubation period, recovery, and reproducibility. Assays to estimate the precision of this method generated coefficients of variation ranging from 2.5 - 10% (Joret *et al.* 1988).

The BDOC assay involving sand has most of the advantages of the traditional BDOC technique described earlier and it can be accomplished in shorter time periods because it does not depend upon the growth of the inoculum. Trulleyová and Rulík (2004) and Park *et al.* (2004) both found that the BDOC determination by means of commonly used suspended bacteria as the inoculum made for an underestimation of BDOC between 5% and 25%, compared with attached bacterial community. These findings were ascribed to higher microbial diversity, higher metabolic activity of attached bacteria and abiotic adsorption of organic molecules to inorganic support and biofilm matrix surfaces. As such this method holds the most promise for BOM measurement related to clogging. One disadvantage is that it is difficult to accomplish complete cleaning of the sand, which in turn affects the DOC detection level.

Another type of batch culture with an attached inoculum of indigenous microflora is the recirculating batch reactor. In contrast to the bioassays described previously that use indigenous microorganisms in static batch cultures, the water is passed by assemblages of bacteria attached to inert media. Examples of this method include work with matrices of sand, quartz powder, charcoal (Gimbel and Mälzer 1987) and borosilicate glass (Frias *et al.* 1992). Each of these batch reactors requires an inoculation period and an acclimation period of 80 – 120 days when the source of BDOC is changed. While typical measurements take only 3 - 5 days to complete, the long acclimatisation period would make it problematic to apply to different source waters in practice.

## BDOC with bioreactors

Biological systems similar to the batch reactors just described, but operating in a continuous and flow through mode rather than recirculation, have been used to evaluate biological decomposition of organic compounds following bank filtration of river water (Gimbel and Mälzer 1987). Lucena *et al.* (1990) developed a continuous flow bioreactor as a method of rapidly measuring BDOC in drinking water. The BDOC measurements of a continuous flow bioreactor consist of the difference between the inflow and outflow. The original bioreactor design consisted of a glass column filled with sand and fed with test waters at 4 mL/min in an upflow mode and was modified to include sintered borosilicate beads as a support for bacteria and paired columns (Ribas *et al.* 1991). The bioreactor design was further modified to use commercially available components and has been utilised in several US and European utilities (Ribas *et al.* 1992; Kaplan *et al.* 1996).

Numerous biological, chemical, and physical parameters influence the BDOC measurement, including bioreactor colonization, empty bed contact time, temperature and influent DOC concentration. Organisms present in the bioreactor feedwater provide the inoculum for colonization. Colonization is generally facilitated by higher inoculum density and viability, warmer temperatures and higher BOM concentration but can take 6 months or more. Once colonized, replicate bioreactors provide a high degree of precision, with a coefficient of variation reported at 6.3% for replicate bioreactors (Kaplan and Newbold 1995). Experiments with azide treatment have demonstrated that the removal of DOC is primarily a biological phenomenon (Kaplan and Newbold 1995) and that a large microbial biomass within the bioreactor provides a reservoir of potential metabolic activity that buffers the bioreactors from changes in chemical and physical parameters. Experiments with bioreactors that were continuously fed vs. those fed from reservoirs shows that the prefilter used to protect the bioreactor from particles contained in the test water function as pre-bioreactors as well, reducing the quantity of BDOC fed to the bioreactor (Dubreuil *et al.* 1997). Additionally, bioreactors fed with either 75 µm or 1 µm filtered test water did not show any appreciable difference (Dubreuil *et al.* 1997).

The bioreactor has a plug flow of carbon and energy supplies in a single direction. Hence the observed biomass gradients within the bioreactor and information on biofilm models (Rittmann and McCarty 1980) suggest that metabolic gradients are also likely and are important in establishing ecological niches that permit the many species of bacteria to inhabit the bioreactor.

The plug flow bioreactor has been used to characterise the composition of DOM in a surface water (Volk *et al.* 1997). Data collected on five separate occasions revealed that humic substances comprised 72 - 78% of the DOM, and on average 22% of the humic substances were degraded in the bioreactors. These humic substances represented 68 - 85% of the total BDOC pool. Total carbohydrates comprised 9 - 16% of the DOC and accounted for 22 - 43% of the BDOC, with more than half of them bound to the humic substances. Amino acids accounted for 2 - 6% of the BDOC and similarly were bound to the humic substances. Without a better understanding of the degradation kinetics of the BOM constituents, it is difficult to know their actual importance to microbial processes contributing to clogging. However, work with model compounds in annular reactors have revealed higher specific growth rates for bacteria fed with carbohydrates and amino acids than with humic substances (Ellis *et al.* 2000).

The most striking advantage of the continuous plug flow bioreactor is that a measurement is possible within the space of a few hours, the time it takes the water to travel through the reactor. This allows the monitoring of BDOC concentrations in nearly real-time and hence repeat sampling may be accomplished if required.

The major disadvantage of a plug flow bioreactor is that it requires considerable time (~100 days) for colonization. Bioreactor effluent also includes DOC excreted by biofilm organisms and thus the measured BDOC may be an underestimate of the actual concentrations, possibly by as much as 32% (Kaplan and Newbold 1995). In addition, the bioreactor, like all BDOC type assays requires carbon limitation. Hence the addition of inorganic nutrients and oxygen may be required in some instances where carbon is not limiting.

There have been a number of modifications to the original BDOC method but few have repeated application or uptake. These modifications are summarised in Table 2.

Table 2 Modifications to the original BDOC method

Improvement sought	Modification	Reference
Original method	Standardised BDOC method based on measurement of DOC	Servais <i>et al.</i> (1987); APHA (1998)
Increase size of measured BDOC pool	Use of attached bacteria	Joret and Levi 1986; Park <i>et al.</i> (2004)
	Increase incubation time	McDowell <i>et al.</i> (2006)
Increased speed	Use of attached bacteria	Trulleyová and Rulík (2004); Park <i>et al.</i> (2004)
	Use of recirculating batch reactor	Gimbel and Mälzer (1987); Lucena <i>et al.</i> (1990)
	Use of a plug flow bioreactor	Volk <i>et al.</i> (1997)
Reduction of contamination potential	Pre-filtering of test water	Kaplan <i>et al.</i> (1994)

## Comparison of techniques for measuring BOM

At present there are no absolute measures of BOM that can be used as a standard for all other assays, and the different methods all differ in their minimum detection limits and applicability. The comparison of BOM methods described above are compared in Table 3.

It should be noted that not all methods are in common use. Some methods have been standardised such as the AOC and BDOC methods (APHA 1998).

Attempts to identify the entire pool of BOM are complicated by the fact that biodegradation is a function of both bacterial enzymes and the character of DOM. An equally important issue is to determine what BOM is important to clogging in MAR. Some assays such as AOC are designed as indices rather than try to identify the entire BOM pool. To be useful these indices must be good predictors of clogging. In contrast BDOC assays attempt to quantify as much of the BOM as possible. Some authors have concluded that AOC and BDOC assays are complimentary and both should be performed (Escobar and Randall 2000; Charnock and Kjønno 2000).

With such a great variety of methods for measuring BOM concentrations, it is of interest to determine how each method compares to the others. Several studies have done this for AOC and BDOC (Kaplan *et al.* 1994; Volk *et al.* 1994; Charnock and Kjønno 2000).

Two general conclusions from these comparisons are that:

- estimates of BOM from the AOC method are lower than those from the BDOC methods; and
- estimates of BDOC based on suspended bacteria are less than using attached bacteria.

In addition, correlations between methods of the same type (i.e. two ways of measuring BDOC) tend to be strong, but correlations between different types (i.e. AOC and BDOC) tend to be variable – strong in some studies and weak in others. The observation that AOC estimates of BOM are less than BDOC is universal. This can be explained by the differences in the metabolic capabilities of the bacteria. AOC is based on the metabolic capability of two bacteria while BDOC is based on the metabolic activities of an unknown but larger number of species. In addition the bacteria for the BDOC assay often comes from the test water which implies the selection for particular metabolic pathways has already occurred. Comparisons between the AOC assay and the BDOC assays are not necessarily valid because the yield coefficient for the AOC bioassay organisms must be assumed and these vary depending upon the character of the BOM tested.

The reason for the differences between the BDOC measurements using suspended and attached bacteria is not always apparent, but may result from differences in the diversity of the metabolic pathways by the bacterial communities or the bacterial density. It is generally acknowledged that sessile growth of bacteria involves significantly different metabolic pathways to planktonic growth. Biofilms are a matrix of cellular and extracellular material; the extracellular material is mostly produced by the bacteria in the biofilm, but much of this process of carbon cycling within a biofilm is poorly understood. Suffice to say there are many reasons why suspended bacteria might give a different BDOC measure to attached bacteria. Both techniques use the indigenous bacterial communities; however, the bacterial species collected in a single grab sample may differ from those present in a continuous flow bioreactor. This refers to not only the initial strains of bacteria that colonize the media but also to the seasonally changing populations within the bacterial community that have the potential to colonize the bioreactor. Additionally the presence of the media for the attached bacteria should establish a greater variety of niches for species colonization.

Table 3 Parameters of BOM measurement

BOM measurement method	Test water pre-treatment	Inoculum	Temperature (°C)	Culture Type	Duration	Test parameter	Reference
AOC / cell growth	Pasteurization	<i>Pseudomonas fluorescens</i> P-17 / <i>spirillum</i> NOX	15	batch	5-2.5 days	colony counts or ATP	van der Kooji <i>et al.</i> (1982a; 1982b); Le Chevallier <i>et al.</i> (1993)
AOC / microscopy	membrane filtration	<i>Pseudomonas fluorescens</i> P-17 / <i>spirillum</i> NOX	20	batch	24 hours	viable cells	Bradford <i>et al.</i> (1994)
Coliform growth response	membrane filtration	<i>Enterobacter cloacae</i>	20	batch	5 days	colony counts	Rice <i>et al.</i> (1990)
bacterial growth	membrane filtration	Indigenous bacteria	20	batch	1-2 days	turbidity	Werner (1984)
bacterial growth	membrane filtration	Indigenous bacteria	22	batch	2-5 days	ATP	Stanfield and Jago (1989)
bacterial mortality	membrane filtration	Indigenous bacteria	20	batch	10-30 days	bacterial abundance and mortality	Servais <i>et al.</i> (1987)
BDOC / suspended bacteria	membrane filtration	Indigenous bacteria	15 - 25	batch	10-30 days	DOC	Servais <i>et al.</i> (1987)
BDOC / sand bacteria	unfiltered	bacteria on sand filter	20	batch	2-7 days	DOC	Joret and Levi (1986)
BDOC / batch reactor	unfiltered	Indigenous bacteria	20	recirculating batch	3-5 days	DOC	Gimbel and Mälzer (1987); Frias <i>et al.</i> (1992)
BDOC / bioreactor	Glass fibre filtered	indigenous bacteria	10 - 25	continuous	2 hours	DOC	Ribas <i>et al.</i> (1991).

## **BIOSTABILITY OF WATER IN MAR APPLICATIONS TO REDUCE CLOGGING**

When bacteria utilize BOM, part of it is catabolised into carbon dioxide through respiration and the rest is used for biomass production. In unconsolidated aquifers and ASR applications, an increase in bacterial density is usually observed as a decrease in hydraulic conductivity of the aquifer material or decrease in flow rate for injection wells related to clogging. This is typically caused by not only the increase in bacterial density but also the production of exopolymers such as polysaccharides and slimes present in biofilms.

This increase in bacterial numbers due to the utilization of BOM in the injection well and the associated clogging phenomenon can be defined in terms of biological instability of the water. The importance of this instability results from the effects of several parameters and their interactions: BOM flux, temperature, hydraulic conditions, mineralogy and geochemistry and influent bacterial cells.

### **Effect of BOM on biological stability**

Bacteria proliferate in the ASR injection well of unconsolidated aquifers as a result of the consumption of BOM in the recharge waters. In most cases, BOM is assumed to be the sole limiting nutrient for bacterial and biofilm growth (Schippers *et al.* 1995).

The amount of BOM in recharge water varies over a broad concentration range as a function of the recharge water quality (e.g. stormwater versus treated wastewater). For traditional treatment trains, the BDOC values are generally in the 0.2 to 1 mg C/L range (Kaplan *et al.* 1994). In the case of high performance treatment trains such as nanofiltration or reverse osmosis, BDOC concentrations < 0.2 mg C/L (detection threshold of the method) have been reported (Agbekodo *et al.* 1994; Laurent *et al.* 1999).

However, even relatively low concentrations of BOM are sufficient to produce a large number of bacteria. Thus when considering bacterial yield (the part of the BOM consumed that is used for biomass production) of 30% (Barillier and Garnier 1993), the consumption of 0.1 mg C/L enables the synthesis of 0.03 mg C/L of bacterial biomass, i.e.  $\sim 10^9$  bacteria / L.

In drinking water distribution systems, where most work on biostability of waters has been performed, it has been possible to establish relationships between the concentration of organic matter and the bacterial biomass in a distribution system. Mathieu *et al.* (1992) have observed a log linear relationship between the density of attached or suspended bacteria and the amount of DOC degraded in a pipe loop system. To study the effect of BDOC on bacterial dynamics, Servais *et al.* (1992; 1993; 1995) have analysed data from situations found in several full-scale distribution systems. The results collected covered a wide range of situations in terms of BDOC in effluent waters from treatment plants (0.1 - 0.7 mg C/L). The mean abundance of fixed bacteria in the distribution systems studied varied from 0.3 -  $2.6 \times 10^7$  cells / cm<sup>2</sup>. When these values were plotted as a function of BDOC in the finished water, a correlation was obtained which suggested the amount of BDOC entering a drinking water distribution system is a major factor controlling the fixed bacterial biomass that colonized the internal surface of the pipes. In the same distribution systems a correlation has been observed between the abundance of fixed and suspended bacteria (Servais *et al.* 1995). This correlation is explained by

the observation that in areas without chlorine, the bacteria in suspension come primarily from the detachment of fixed bacteria.

These results are in accordance with those obtained by Volk and LeChevallier (1999) on annular reactors installed in two drinking water utility systems. In both cases, two reactors were initially fed with treatment plant effluent water. When biofilm densities reached a plateau, biofiltration was installed ahead of the second reactor, reducing the nutrient levels entering this second reactor (by around 50% measured in terms of AOC and BDOC for both treatment plants). This study showed that biofilm densities were related to the amount of biodegradable material entering the system. After a period of 6 months, biofilm densities were reduced by 0.5 to 1.0 log unit. Volk and LeChevallier (1999) mention that a period of several months following implementation of biofiltration was required before an effect on bacterial quality (as measured by total plate counts) in the system was observed.

## Defining threshold values to achieve biostability

Several attempts have been made to define the threshold concentration of BOM below which water can be considered biologically stable. This work has primarily occurred in the context of drinking water research. These threshold values call on different methodologies for the measurement of BOM concentration (AOC, BDOC) and different criteria for the measurement of biological stability (Table 4).

Table 4 Threshold values of BOM for biologically stable waters (modified from Prévost *et al.* 2005)

Parameter followed	Threshold value	Measurement technique	References
No coliform growth	< 0.05 mg C/L	AOC	LeChevallier <i>et al.</i> (1991)
	≤ 0.15 mg C/L	BDOC	Volk and LeChevallier (2000)
No HPC growth	< 0.01 mg C/L	AOC	van der Kooij (1992)
No <i>E. coli</i> growth	< 0.1 mg C/L	AOC	LeChevallier <i>et al.</i> (1996)
No <i>V. cholerae</i> growth	< 0.05	AOC	Vital <i>et al.</i> (2007)
No BDOC increase	≤ 0.15 mg C/L @ 20°C	BDOC	Volk (1994)
	≤ 0.30 mg C/L @ 15°C	BDOC	Volk (1994)
	≤ 0.15 mg C/L	BDOC	Servais <i>et al.</i> (1995)
	≤ 0.25 mg C/L	BDOC	Niquette <i>et al.</i> (2001)
	≤ 0.15 mg C/L	BDOC	Laurent <i>et al.</i> (1997)
No increase in resistance	< 0.01 mg C/L	AOC	Olsthoorn (1982); Hijnen and van der Kooij (1992)

Van der Kooij (1992) proposed an AOC level ≤ 10 µg/L to maintain the stability in terms of colony counts of heterotrophic bacteria. Values proposed by LeChevallier *et al.* (1991; 1996) and Volk and LeChevallier (2000) (Table 4) are defined as AOC concentrations that must not be exceeded if the appearance of coliforms in the distribution systems under study are to be prevented. Similarly, Volk

and Joret (1994) defined BDOC 0.15 mg C/L as a critical threshold if coliform prevention is a prime objective.

The threshold value of BDOC defined by Servais *et al.* (1995) has been based on the relationship between the consumption of BDOC in the system and the BDOC content leaving the treatment plant. The correlation indicated that the consumption of BDOC is proportional to BDOC entering the system and there exists a threshold value below which there is no longer any consumption of BDOC in the system. This implies that when BDOC output from the plant is below this threshold, the bacterial activity is so limited it can no longer be measured by BDOC consumption. This threshold can therefore be considered the maximum admissible value for water to be considered biologically stable. Similar results were found by Volk (1994), Niquette *et al.* (2001) and Laurent *et al.* (1997) for other distribution systems (Table 4). Olsthoorn (1982) related clogging in recharge wells and BOM and derived a threshold criteria of  $\leq 10 \mu\text{g/L}$ . This threshold value is likely to be dependent on other characteristics of the recharge water such as total suspended solids concentrations and the aquifer characteristics such as transmissivity.

As mentioned previously, these thresholds all use different methodologies for the measurement of BOM and different criteria exist for the measurement of biological stability. Nevertheless, only small discrepancies exist between them indicating that although BOM is only one of the factors affecting biostability it is probably one of the most important.

## **PROPOSED BOM MEASUREMENT METHOD FOR PREDICTING CLOGGING IN MAR**

Previous work on defining the biostability of water and associated critical threshold values (Table 4) have been primarily in the drinking water distribution system context. A new measurement method for BOM and definition of biostability in the well clogging context are required for MAR applications. To this end a new definition of biostability of recharge waters is proposed: that there be less than a defined decline in the intrinsic permeability of a media for the duration of the test. Durations of the test may require experimentation to confirm an appropriate time in which biostability can be measured.

Of the BOM measurement methods described above, all methods have their limitations, but the modified BDOC method employing sand appears to be the best candidate for determining the largest portion of the BOM pool. Alternatively the bioreactor is a rapid method for BOM quantification which also utilises attached bacteria. Appropriate environmental conditions are required in the test bioreactor that better simulate the near injection well environment.

As such the development of an in-house method for BOM determination specific to water reclamation via aquifers and predicting clogging appears difficult. It seems unlikely that an improved method for BOM determination can be found given the unknown microbiology of clogging in injection wells and the limitation of current methods. In addition, all current methods are considered to be indices, development of a modified BOM determination method would be unlikely to improve on this situation. Further, given their great cost in terms of capital setup, method development and quality control there seems little value in deriving another test that is unlikely at this stage to provide further specific information.

## CONCLUSIONS

BOM measurement techniques have been used since 1982 and a significant number of techniques are currently available. Numerous studies in the 1990s provided ample information on their application when studying treatment methods such as biofiltration and evaluating the results in model and full scale systems but there have been limited applications with respect to well clogging and water reclamation via aquifers. When comparing these methods and discussing the results with respect to water reclamation in aquifers a few general conclusions stand out:

There are no absolute measures of BOM that can be used as a standard for all other assays. Two BOM methods (AOC and BDOC) have been standardised, but they were developed as tools for management of biofilm growth in drinking water distribution systems and their value for management of clogging in MAR is unknown.

Some assays such as AOC are designed as indices rather than to quantify the entire BOM pool. Their application in water quality, treatment performance and optimisation have provided useful insights to managing distribution systems, but their application in the management of clogging in MAR is unlikely to be successful due to the extremely different environments between the near injection well and water distribution systems from which the bioassay organism is sourced.

BDOC methods attempt to quantify as much as the BOM pool as possible. Batch techniques are well established and on-line bioreactors give promise of rapid results. It can be argued that the results using fixed bacteria such as in bioreactors is more representative for biofilm processes and hence MAR biostability determinations.

## REFERENCES

- Agbekodo, M.K., Legube, B., Coté, P. and Bourbigot, M.M. (1994) Performance de la nanofiltration pour l'élimination de la matière organique naturelle: Essais sur l'usine de Mèry-sur-Oise, *Revue des Sciences de l'Eau*, 7 183-200.
- Aiken, G.R. (1985) Isolation and concentration techniques for aquatic humic substances. In *Humic substances in Soil, Sediment, and Water – Geochemistry, Isolation and Characterization*. Edited by G.R. Aiken, D.M. McKnight, R.L. Wershaw and P. MacCarthy, Chapter 14, 363-385.
- Aiken, G.R. (1988) A critical evaluation of the use of macroporous resins for the isolation of aquatic humic substances. In *Humic Substances and their role in the Environment*, Edited by F.H. Frimmel and R.F. Christman, Proceedings of the 1987 Dahlem Conference, 15-28.
- Aiken, G.R., McKnight, D.M., Thorn, K.A. and Thurman, E.M. (1992) Isolation of hydrophilic organic acids from water using nonionic macroporous resins, *Organic Geochemistry*, 18, 567-573.
- APHA (American Public Health Association) (1998) Standard Methods for Examination of Water and Wastewater. 20<sup>th</sup> ed, Washington DC, USA.
- Barillier, A. and Garnier, J. (1993) Influences of temperature and substrate concentration on bacterial growth yield in Seine River water batch cultures, *Applied Environmental Microbiology*, 59, 1678-1682.
- Bell, R.T. (1993) Estimating the production of heterotrophic bacterioplankton via incorporation of tritiated thymidine, In *Handbook of Methods in Aquatic Microbiology*, Edited by Kemp, P.F., Sherr, B.F., Sherr, E.B. and Cole, J.J., Lewis Publishers, Boca Raton, USA, 495-508.
- Biber, M.V., Gülaçar, F.O. and Buffle, J. (1996) Seasonal variations in principal groups of organic matter in a eutrophic lake using Pyrolysis/GC/MS, *Environmental Science and Technology*, 30, 3501-3507.
- Bradford, S.M., Palmer, C.J., Olson, B.H. (1994) Assimilable organic carbon concentrations in Southern California surface and groundwaters, *Water Research*, 28, 427-435.
- Bruchet, A., Rousseau, C. and Mallevalle, J. (1990) Pyrolysis-GC-MS for investigating high-molecular weight THM precursors and other refractory organics, *Journal of the American Water Works Association*, 82, 66-74.
- Camper, A.K., Hayes, J.T., Jones, W.L., and Zilver, N. (1993) Persistence of coliforms in mixed population biofilms, In *Proceedings of the AWWA Water Quality Technology Conference*, Denver, USA, 1653-1661.
- Charnock, C. and Kjønne, O. (2000) Assimilable organic carbon and biodegradable dissolved organic carbon in Norwegian raw and drinking waters, *Water Research*, 34, 2629-2642.
- Chow, C.W.K., van Leeuwen, J.A., Drikas, M., Fabris, R., Spark, K.M. and Page, D.W. (1999) The impact of the character of natural organic matter in conventional treatment with alum, *Water Science and Technology*, 40, 97-104.

- Croué, J.P., Debroux, J.F., Amy, G.L., Aiken, G.R. and Leenheer, J.A. (1999) Natural organic matter: Structural characteristics and reactive properties, In *Formation and Control of Disinfection By-Products in Drinking Water*, Edited by P.C. Singer, Denver, USA.
- Croué, J.P., Benedetti, M.F., Violeau, D. and Leenheer, J.A. (2003) Characterisation of copper binding of humic and nonhumic matter isolated from the South Plate River: Evidence for the presence of nitrogenous binding site, *Environmental Science and Technology*, 37, 328-336.
- Dillon, P. and Toze, S. (2005) Water quality improvements during aquifer storage and recovery. Volume 1: Water quality improvements processes, *American Water Works Association Research Foundation (AwwaRF)*, Report number 91056F, Denver, USA.
- Donlan, R.M., Pipes, W.O. and Yohe, T.L. (1994) Biofilm formation on cast iron substrata in water distribution systems, *Water Research*, 28, 1497-1503.
- Dubreuil, G.M., Prévost, R., Desjardins, R. and MacLean, R.G. (1997) Bioreactors for the rapid determination of biodegradable dissolved organic carbon (BDOC) in drinking water: feed mode impact, *Environmental Technology*, 18, 363-374.
- Egeberg, P.K., Eikenes, M. and Gjessing, E.T. (1999) Organic nitrogen distribution in NOM size classes, *Environment International*, 25, 225-236.
- Ellis, B.D., Butterfield, P., Jones, W.L., McFeters, G.A. and Camper, A.K. (2000) Effects of carbon source, concentration and chlorination on growth related parameters of heterotrophic biofilm bacteria, *Microbiology and Ecology*, 38, 330-347.
- Escobar, I.C. and Randall, A.A. (2000) Sample storage impact on the assimilable organic carbon (AOC) bioassay, *Water Research*, 34, 1680-1686.
- Franzmann, P.D., Zappia, L.R., Masters, D., Heitz, A., O'Leary, B. (2000) An evaluation of biologically activated carbon filters and sand biofilters at the Wanneroo treatment Plant in July 2000. *CSIRO Land and Water Consultancy Report*.
- Franzmann, P.D., Zappia, L.R., Allpike, B., Heitz, A., Masters, D. and O'Leary, B. (2002) Effects of chlorine treatment on Assimilable Organic Carbon, Biodegradable Organic Carbon and the molecular weight distribution of DOC in pilot biofilter effluents at Wanneroo. *CSIRO Land and Water Consultancy Report*.
- Frias, J., Ribas, F. and Lucena, F. (1992) A method for the measurement of biodegradable organic carbon in waters, *Water Research*, 35, 4444-4454.
- Frias, J., Ribas, F. and Lucena, F. (1994) Substrate affinity from bacterial strains and distribution water biofilms, *Journal of Applied Bacteriology*, 76, 182-189.
- Gibbs, R.A., Scutt, J.E., Carroll, B.T. (1993). Assimilable organic carbon concentration and bacterial numbers in a water distribution system, *Water Science and Technology*, 27, 159-166.
- Gimbel, R. and Mälzer, H.J. (1987) Testfilter zur Beurteilung der Trinkwasserrelevanz organischer Inhaltsstoffe von Fließgewässern, *Vom Wasser*, 69, 139-153.

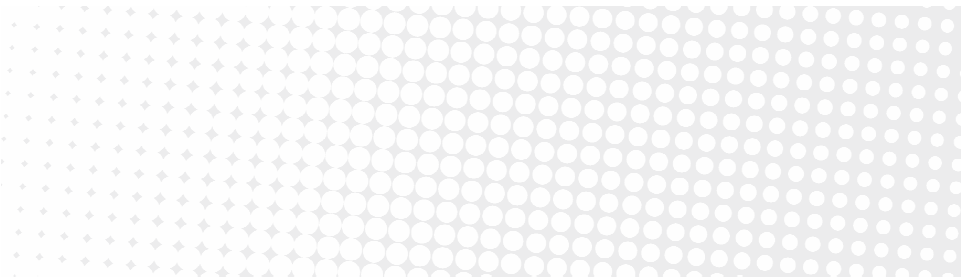
- Gremm, T.L. and Kaplan, L.A. (1998) Dissolved carbohydrate concentration, composition and bioavailability to microbial heterotrophs in stream water, *Acta Hydrochimica et Hydrobiologica*, 26, 167-171.
- Hamsch, B. and Werner, P. (1993) Control of bacterial regrowth in drinking-water treatment plants and distribution systems, *Water Supply – International Water Supply Association*, 11, 299-308.
- Hammes F.A. and Egli, T. (2005) New method for assimilable organic carbon determination using flow-cytometric enumeration and a natural microbial consortium as inoculum, *Environmental Science and Technology*, 39, 3289–3294.
- Her, N., Amy, G., Foss, D., Cho, J., Yoon, Y. and Kosenka, P. (2002) Optimization of method for detecting and characterizing NOM by HPLC-size exclusion chromatography with UV and on-line DOC detection, *Environmental Science and Technology*, 36, 1069-1676.
- Hermanowicz, S.W., Price, M. and Rohan, J. (1991) Growth of attached bacteria in drinking water: A method of assessment of water biostability, In *Proceedings of the AWWA Water Quality Technology Conference*, Denver, USA, 497-508.
- Hijnen, W. A.M. and van der Kooij, D. (1992) The effect of low concentrations of assimilable organic carbon (AOC) in water on biological clogging of sand beds, *Water Research*, 26, pp. 963–972.
- Huck, P.M. (1990). Measurement of biodegradable organic matter and bacterial growth potential in drinking water, *Journal of the American Water Works Association*, 82, 78-86.
- Joret, J.C. and Lévi, Y. (1986) Méthode rapide d'évaluation du carbone éliminable des eaux par voie biologique, *La Tribune du Cebedeau*, 39, 3-9.
- Joret, J.C. Lévi, Y., Dupin, T. and Gibert, M. (1988) Rapid method for estimating bioeliminable organic carbon in water In *Proceedings of the AWWA Annual Conference and Exposition*, Denver, USA, 1715-1725.
- Joret, J.-C., Lévi, Y., Dupin, T., Gibert, M. (1989). The measurement of bioeliminable dissolved organic carbon (BDOC): a tool in water treatment, *Water Supply*, 7, 41-45.
- Kang, J.W., Kim, J.B., and Koga, M. (1997) Determination of assimilable organic carbon (AOC) in ozonated water with *Acinetobacter calcoaceticus*, *Ozone Science and Engineering*, 18, 521-534.
- Kaplan, L.A., Bott, T.L. and Reasoner, D.J. (1993) Evaluation and simplification of the assimilable organic carbon nutrient assay for bacterial growth in drinking water, *Applied Environmental Microbiology*, 59, 1532-1539.
- Kaplan, L.A., Reasoner, D.J. and Rice, E.W. (1994) A survey of BOM in US drinking waters, *Journal of the American Water Works Association*, 86, 121-132.
- Kaplan, L.A. and Newbold, J.D. (1995) Measurement of streamwater biodegradable dissolved organic carbon with a plug flow bioreactor, *Water Research*, 29, 2696-2706.
- Kemmy, F.A., Fry, J.C. and Breach, R.A. (1989) Development and operational implementation of a modified and simplified method for determination of assimilable organic carbon (AOC) in drinking water, *Water Science and Technology*, 21, 155-161.

- Krasner, S.W., Croué, J.P., Buffle, J., Perdue, E.M. (1996) Three approaches for characterizing NOM, *Journal of the American Water Works Association*, 88, 66-79.
- Krogh, A. and Lange, E. (1932) Quantitative untersuchung uber Plankton, Kolloide, und geloste organische und anorganische substanzen in dem Furesee, *Internationaler Review der Gesamten Hydrobiologie*, 26, 20-53.
- Laurent, P., Pévost, M., Cigana, J., Niquette, P. and Servais, P. (1999) Biodegradable organic matter removal in biological filters: Evaluation of the CHABROL model, *Water Research*, 33, 1387-1398.
- LeChevallier, M.W., Schulz, W. and Lee, R.G. (1991) Bacterial nutrients in drinking water, *Applied Environmental Microbiology*, 57, 857-862.
- LeChevallier, M.W., Shaw, N.E., Kaplan, L.A. and Bott, T.L. (1993) Development of a rapid assimilable organic carbon method for water, *Applied Environmental Microbiology*, 59, 1526-1532.
- LeChevallier, M.W., Welch, N.J. and Smith, D.B. (1996) Full-scale studies of factors related to coliform regrowth in drinking water, *Applied Environmental Microbiology*, 62, 2201-2211.
- Lucena, F., Frias, J. and Ribas, F. (1990) A new dynamic approach to the determination of biodegradable dissolved organic carbon in water, *Environmental Technology*, 12, 343-347.
- Mathieu, L., Paquin, J.L., Block, J.C., Randon, G., Mailliard, J. and Reasoner, D.J. (1992) Parameters governing bacterial growth in water distribution systems, *Revue des Sciences de l'Eau*, 5, 91-112.
- Merlet, N., Legube, B., Rouillier, L., Lacoste, P. and Croué, J.P. (1996) Characterization of some specific components of biodegradable organic matter in various raw waters and their removal in treatment plants, 4<sup>th</sup> International BOM conference, Waterloo, Canada, June 20-21.
- McDowell, W.H., Zsolnay, A., Aitkenhead-Peterson, J.A., Gregorich, E.G., Jones, D.L., Jödemann, D., Kalbitz, K., Marschner, B., Schwesig, D. (2006) A comparison of methods to determine the biodegradable dissolved organic carbon from different terrestrial sources, *Soil Biology and Biochemistry*, 38, 1933-1942.
- Niquette, P., Servais, P. and Savoie, R. (2001) Bacterial dynamics in a drinking water distribution system in Brussels, *Water Research*, 35, 675-682.
- Olsthoorn T.N. (1982) The Clogging of Recharge Wells, Main Subjects, *Kiwa research communication* 72, Rijswijk, The Netherlands.
- Page, D.W., van Leeuwen, J.A., Spark, K.M. and Mulcahy, D.E. (2002a) Pyrolysis characterisation of plant, humus and soil extracts from Australian catchments, *Journal of Analytical and Applied Pyrolysis*, 65, 269-285.
- Page, D.W., van Leeuwen, J.A., Spark, K.M., Drikas, M., Withers, N., Mulcahy, D.E. (2002b) Effect of alum treatment on the trihalomethane formation and bacterial regrowth potential of natural and synthetic water, *Water Research*, 36, 4884-4892.
- Page, D.W. (2006) Characterisation of organic matter from air, water, soils and waste material by analytical pyrolysis, In *Chromatographic Analysis of the Environment 3<sup>rd</sup> Ed. Chromatographic Science Series Vol 93*, Edited by Nollet, L.M.L., Taylor and Francis Group, Florida, USA.

- Park, S.-K., Pak, K.-R., Choi, S.-C. and Kim, Y.-K. (2004) Evaluation of Bioassays for Analyzing Biodegradable Dissolved Organic Carbon in Drinking Water, *Journal of Environmental Science and Health Part A—Toxic/Hazardous Substances and Environmental Engineering*, A39, 103–112.
- Pelekani, C., Newcombe, G., Snoeyink, V.L., Hepplewhite, C., Assemi, S. and Beckett, R. (1999) Characterization of natural organic matter using high performance size exclusion chromatography, *Environmental Science and Technology*, 33, 2807-2813.
- Piccolo, A., Conte, P., Trivellone, E., van Lagen, B. and Buurman, P. (2002) Reduced heterogeneity of a lignite humic acid by preparative HPSEC following interaction with an organic acid. Characterization of size-separates by Pyr-GC-MS and <sup>1</sup>H-NMR, *Environmental Science and Technology*, 36, 76-84.
- Prévost, M., Coallier, J., Mailly, J., Desjardins, R. and Duchesne, D., (1992) Comparison of biodegradable organic carbon techniques for process control, *Journal of Water Supply, Research and Technology – Aqua*, 41, 141-150.
- Prévost, M., Laurent, P., Servais, P. and Joret, J.-C. (2005) Biodegradable organic matter in drinking water treatment and distribution, American Water Works Association, Denver, USA.
- Ribas, F., Frias, J. and Lucena, F. (1991) A new dynamic method for rapid determination of the biodegradable dissolved organic carbon in drinking water, *Journal of Applied Bacteriology*, 71, 371-378.
- Rice, E.W., Scarpino, P.V., Logsdon, G.S. Reasoner, D.J., Mason, P.J. and Blannon, J.C. (1990) Bioassay procedure for predicting coliform bacterial growth in drinking water, *Environmental Technology*, 11, 821-828.
- Rittmann, B.E. and McCarty, P.L. (1980) Model of steady state biofilm kinetics, *Biotechnology and Bioengineering*, 22, 2243-2357.
- Schwartz, T., Hoffmann, S. and Obst, U. (2003) Formation of natural biofilms during chlorine dioxide and UV disinfection in a public drinking water distribution system, *Journal of Applied Microbiology*, 95, 591-691.
- Schippers, J.C., Verdouw, J. and Zweere, G.J. (1995) Predicting the clogging rate of artificial recharge wells, *Journal of Water Science Research and Technology – Aqua*, 44, 18-28.
- Servais, P., Billen, G. and Vives-Rego, J. (1985) Rate of bacterial mortality in aquatic environments, *Applied Environmental Microbiology*, 49, 1448-1454.
- Servais, P., Billen, G. and Hascoet, M.C. (1987) Determination of the biodegradable fraction of dissolved organic matter in waters, *Water Research*, 21, 445-450.
- Servais, P., Anzil, A. and Ventresque, C. (1989) Simple method for the determination of biodegradable dissolved organic carbon in water, *Applied Environmental Microbiology*, 55, 2732-2734.
- Servais, P., Billen, G., Bouillot, P. and Benezet, M. (1992) A pilot study of biological GAC filtration in drinking water treatment, *Journal of Water Supply, Research and Technology – Aqua*, 41, 163-168.

- Servais, P., Laurent, P. and Randon, G. (1993) Impact of biodegradable dissolved organic carbon (BDOC) on bacterial dynamics in distribution systems, *Proceedings of the AWWA Water Quality Technology Conference*, Denver, USA, 963-980.
- Servais, P. Laurent, P., Billen, G. and Gatel, D. (1995) Characterisation of dissolved organic matter biodegradability in waters: Impact of water treatment and bacterial regrowth in distribution systems, In *Proceedings of the AWWA Water Quality Technology Conference*, Denver, USA.
- Skjemstad, J.O., Swift, R. and Hayes, M. (2005) Evaluation of changes in NOM during ASR at the Bolivar site, Volume I: Water quality improvements processes, *American Water Works Association Research Foundation (AwwaRF)*, Report number 91056F, Denver, USA.
- Skjemstad, J.O., Swift, R.S. and McGowan, J.A. (2006) Comparison of the particulate organic carbon and permanganate oxidation methods for estimating labile soil organic carbon, *Australian Journal of Soil Research*, 44, 255 – 263.
- Stanfield, G. and Jago, P.H. (1989) Application of ATP determinations to measure the concentration of assimilable organic carbon in water, In *ATP Luminescence*, Edited by Stanley P.E., McCarthy, B.J. and Smither, R. Blackwell Scientific, Oxford, England, 99-108.
- Stepanuskas, R.Farjalla, V.F., Tranvik, L.J., Svensson J.M., Esteves, F.A. and Graneli, W. (2000) Bioavailability and sources of DOC and DON in macrophyte stands of a tropical coastal lake, *Hydrobiologia*, 436, 241-248.
- Thurman, E.M. (1985) *Developments in Biogeochemistry: Organic Geochemistry of Natural Waters*, M. Njihff and W. Junk Publishers, Dordrecht, The Netherlands.
- Trulleyová, S. and Rulík, M. (2004) Determination of biodegradable dissolved organic carbon in waters: comparison of batch methods, *Science of the Total Environment*, 332, 253– 260.
- van der Kooj, D., Visser, A. and Hijnen, W.A.M. (1982a) Determining the concentration of easily assimilable organic carbon in drinking water, *Journal of the American Water Works Association*, 74, 540-545.
- van der Kooj, D., Visser, A. and Oranje, J.P. (1982b) Multiplication of fluorescent pseudomonads at low substrate concentrations in tap water, *Antonie van Leeuwenhoek*, 48, 229-243.
- van der Kooj, D. (1990) Assimilable organic carbon (AOC) in drinking water, In *Drinking Water Microbiology*, Edited by McFeters, G.A., Springer-Verlag, New York, USA, 57-87.
- van der Kooji, D. Veenendaal, H.R. and Baars-Lorist, C., van der Klift, D.W. and Drost, Y.C. (1995) Biofilm formation on surfaces of glass and teflon exposed to treated water, *Water Research*, 29, 1655-1662.
- van der Welde, E., Characklis, W.G. and Smith, D.B. (1989) Biofilms and bacterial drinking water quality, *Water Research*, 23, 1313-1322.
- Vital, M., Fuchslin, H., Hammes, F.A., Egli, T., (2007) Growth of *Vibrio cholerae* O1 Ogawa Eltor in freshwater. *Microbiology*, accepted for publication.
- Volk, C., Renner, C. and Joret, J.C. (1994) Comparison of two techniques for measuring the biodegradable organic carbon in water, *Environmental Technology*, 15, 545-556.

- Volk, C. and Joret, J.C. (1994) Paramètres prédictifs de l'apparition des coliformes dans les réseaux de distribution d'eau d'alimentation, *Revue des Sciences de l'Eau*, 7, 131-152.
- Volk, C.J., Volk, C.B. and Kaplan, L.A. (1997) The chemical composition of biodegradable organic matter in stream water, *Limnology and Oceanography*, 42, 39-45.
- Volk, C.J. and LeChevallier, M.W. (1999) Impacts of the reduction of nutrient levels on bacterial water quality in distribution systems, *Applied Environmental Microbiology*, 65, 4957-4966.
- Volk, C.J. and LeChevallier, M.W. (2000) Assessing biodegradable organic matter, *Journal of the American Water Works Association*, 92, 64-76.
- Warton, B., Zappia, L., Franzmann, P.D., Alessandrino, M., Allpike, B, Masters, D., Raine, A., Heitz, A. (2005) A report on laboratory testing of biofiltration and enhanced coagulation for the removal of THM precursors from Harris River water. Curtin University, Consultancy Report.
- Werner, P. (1984) Untersuchung zur substrateigenschaft organischer wasserinhaltsstoffe bei der trinkwasseraufbereitung, *Zentralblatt fuer Bakteriologie, Mikrobiologie und Hygiene, Abt. 1, Originale B*, 180, 46-61.
- Withers, N; Drikas, M; Hamsch, B (1996). Bacterial regrowth potential in German and Australian waters, *Water*, 24, 59-62.
- Yoro, S.C., Panagiotopoulos, C. and Sempere, R. (1999) Dissolved organic carbon contamination induced by filters and storage bottles, *Water Research*, 33, 1956-1959.
- Zappia, L.R., Masters, D., Franzmann, P.D. (2000) A preliminary evaluation of biologically activated carbon filters and sand biofilters at the Wanneroo treatment plant, *CSIRO Land and Water Consultancy Report*.



### Contact Us

Phone: 1300 363 400

+61 3 9545 2176

Email: [enquiries@csiro.au](mailto:enquiries@csiro.au)

Web: [www.csiro.au](http://www.csiro.au)

### Your CSIRO

Australia is founding its future on science and innovation. Its national science agency, CSIRO, is a powerhouse of ideas, technologies and skills for building prosperity, growth, health and sustainability. It serves governments, industries, business and communities across the nation.