Comparison of methods for the determination of biodegradable dissolved organic carbon in potable water supply: use of a novel biofilm


ABSTRACT

Potable water treatment in the state of Western Australia is challenged in many instances by the presence of high and variable levels of dissolved organic carbon (DOC), particularly in surface water supplies. In recent years the effects of climate change on local sources, in combination with the ever-present requirement for on-going disinfection effectiveness and disinfection by-product regulation has driven the need for the development of innovative, sustainable processes for the removal of DOC. Extensive pilot plant studies over a number of years have demonstrated the effectiveness of biological filtration for cost-effectively managing a variety of high DOC source waters, consequently biofiltration is seen as a process of choice for organics removal in drinking water in Western Australia. Although there are a number of indicators for measuring the efficacy of organic carbon reduction across biological treatment processes (e.g., DOC concentration, SUVA, etc), none currently are able to reliably inform on the change in the “non-refractory” organic carbon component of DOC (i.e. biodegradable dissolved organic carbon [BDOC]) which arises from microbiological metabolic activity in the biological treatment process. Knowledge of this parameter is of critical importance to the understanding of biological stability of the finished water entering a distribution system. We have therefore investigated a number of analytical procedures in order to develop a robust test method to accurately quantify this important aspect of biofilter performance. The paper reports a comparison between conventional and rapid methods for the determination of BDOC using immobilised biofilms enriched on deep aquifer water. Effective sample collection, preparation and method of analytical analysis, method validation using natural source water with and without addition of standard amounts of assimilable organic carbon (e.g. acetate), and reproducibility of test results are discussed.

Key words | biodegradable dissolved organic carbon (BDOC), biofilm, biofiltration, dissolved organic carbon (DOC), non-purgeable organic carbon (NPOC), reactors

INTRODUCTION

Accurate analytical methods have long been available for the determination of the concentration of non-purgeable organic carbon (NPOC) in water. Of increasing interest are the fractions of the NPOC called “non-refractory” organic carbon, which persist beyond the treatment step, and promote biofilm growth in distribution systems. These “non-refractory” organic carbon components of the NPOC are commonly referred to as the biodegradable
dissolved organic carbon (BDOC) and the assimilable organic carbon (AOC).

Biological assays are required in order to determine the concentration of BDOC and AOC in water. These data can then be used to assess the “biological stability” of water under investigation. However, there is no clear consensus on what constitutes biologically stable water, but some measures that are considered to indicate biological stability in water are given in Table 1.

Various bioassay methods exist (van der Kooij et al. 1982; Servaise et al. 1987; Volk & LeChevallier 2000), however, methods such as these are time consuming and expensive to perform. The method proposed by Joret & Levy (1986), is presently in widespread use by various water testing and research laboratories. However, implementation of a consistent and reliable BDOC method is challenged by the lack of a suitable microbial inoculum, which has the ability to utilise a wide range of naturally occurring organic compounds that are represented in all waters across Australia. A clear approach taken by many researchers and water testing laboratories to resolve this issue has been the implementation of a “proprietary” based bioassay method, involving aspects of established methods but utilising indigenous bacteria typical to the water type to be tested.

New rapid methods for the determination of BDOC in water are available (Lucena et al. 1990; Ribas et al. 1991; Kaplan & Newbold 1995; Kahn et al. 2003). Comparisons have been made between the method developed by Servaise (1987) and the rapid method developed by Lucena et al. (1990) and Kaplan & Newbold (1995). Outcomes from this research indicate there is the potential for the use of the rapid method by the Australian water industry.

Briefly, the rapid BDOC (R-BDOC) methods employ the use of a single glass column which contains a known amount of inert support medium (typically sand or porous glass beads) on which biofilm has been established. Water is continuously pumped through the reactor and the amount of BDOC corresponds to a difference in DOC concentration between the influent and effluent water entering and exiting the reactor. The determination of BDOC by this method can usually be performed within 3 hours, as opposed to 14 days typically required to complete the Joret & Levy (1986) method (conventional method [C-BDOC]).

This study aims to compare the R-BDOC (i.e. Lucena et al. 1990) and C-BDOC (i.e. Joret & Levy 1986) methods in terms of their ability to determine the concentration of BDOC in various water types, taking into account analysis time, reliability, reproducibility, robustness and other practical considerations.

### MATERIALS AND METHODS

#### DOC analysis

DOC analysis was carried out at Curtin University using a Shimadzu Total Organic Analyser (TOC-Vws).

On each occasion when sub-samples of water from experimental flasks or reactors were collected for determination of the concentration of BDOC, prepared standards using 2.0 and 8.0 mg L$^{-1}$ phthalate (as carbon) and a bore water (W257, NPOC = 1.80 ± 0.03 mg L$^{-1}$) were included for analytical quality assurance and validation purposes.

The instrumental method for NPOC was set up so that multiple analyses were performed for each sample. The maximal standard deviation that was acceptable was ± 0.015 mg/L, and the maximum acceptable coefficient of variation was 0.2%, ensuring an accuracy of ± 0.05 mg/L for the NPOC analysis component in each BDOC determination.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Value</th>
<th>Comment</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOC</td>
<td>10 µg L$^{-1}$</td>
<td>AOC uptake by biofilm limited at &lt; 10 µg L$^{-1}$</td>
<td>van der Kooij et al. (1999)</td>
</tr>
<tr>
<td>AOC</td>
<td>&lt; 50 µg L$^{-1}$</td>
<td>For AOC in a system with chlorine residual</td>
<td>LeChevallier et al. (1991)</td>
</tr>
<tr>
<td>BDOC</td>
<td>200 µg L$^{-1}$</td>
<td>In water with no disinfectant residual</td>
<td>Piriou et al. (1998)</td>
</tr>
<tr>
<td>BDOC</td>
<td>500 µg L$^{-1}$</td>
<td>In water &lt; 15°C with no disinfectant residual</td>
<td>Piriou et al. (1998)</td>
</tr>
<tr>
<td>BDOC</td>
<td>≤ 150 µg L$^{-1}$</td>
<td>In water without a disinfectant residual</td>
<td>Servaise et al. (1995)</td>
</tr>
</tbody>
</table>
Determination of the concentration of NPOC in any one sample requires three separate analyses from which the mean representing the overall result is obtained. In addition, the corresponding standard deviation concentration (SD conc.) and coefficient of variation (i.e. CV%) are also obtained. If the SD conc. and CV% are greater than the accepted method SD conc. and CV Max, a fourth and possibly a fifth analysis maybe performed. The only change that was made to manufacturer's methods was that the accepted SD conc. was lowered from $0.05 \text{ mg L}^{-1}$ to $0.01 \text{ mg L}^{-1}$ and the accepted CV% was lowered from 1.0% to 0.20%. This effectively ensures that for each sample, five separate analyses are always performed. The mean is then obtained from the three individual analyses which give the lowest SD and CV%.

**Glassware**

All glassware was prepared in accordance with Australian Standard Methods for the determination of dissolved organic carbon in water (ASM method 6040b).

**Filtration**

To mitigate preparative contamination (Karanfil *et al.* 2003), all test waters were filtered through 0.45 $\mu$m Supor [Life Sciences] (PES) membrane and the filtrate concentration of NPOC was determined within 2 hours after collection.

**Bioreactors**

Where possible all material used in the construction of the bioreactors was glass. All other materials such as tubing and aeration tanks were made from either high grade marine stainless steel or low carbon leaching polymers such as Norprene and PFA.

Siran® glass beads were used as support media used to immobilise and grow indigenous biofilms. Siran® is an open-pored sintered glass sphere of 1–5 mm diameter with 60–300 $\mu$m pore diameters that provide a 90,000:1 surface to volume ratio.

The influent water to the reactors was sourced from bore W257 located in compound 257 at the Wanneroo Ground Water Treatment Plant (WGWTP) north of Perth, Western Australia. As W257 water is sourced from deep below the surface (ca 800 m below) and contains little or no oxygen, its oxygen content was raised from $<1.0 \text{ mg L}^{-1}$ to ca. 6.0 $\text{ mg L}^{-1}$ via a dual aeration pre-treatment step. A schematic diagram of the setup at the WGWTP is given in Figure 1.

**Establishing a biofilm**

Most (ca. 75%) of Perth's metropolitan water supply is derived from confined deep (800 m) and unconfined shallow aquifers (ca. 50 m). Generally in Western Australia water quality improves with depth. The concept of

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**Figure 1** | Schematic diagram describing how the inoculum is developed at Wanneroo Ground Water Treatment Plant.

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immobilising bacteria from relatively organically impoverished environments is novel.

Hence a novel approach to grow indigenous biofilms on an inert support media (Siran®) in aerated ground water from WGWTP was undertaken.

**Rapid BDOC method**

The rapid BDOC method was setup based on the method described by Lucena et al. (1990). However in this instance ground water was used to immobilise indigenous bacteria on the support media instead of surface water. The concentration of BDOC using the method was obtained by subtraction of the concentration of NPOC in effluent water from the reactor from the concentration of NPOC in the influent water to the reactor. In order to determine an optimum flow rate for the removal of BDOC, W257 water was passed through each reactor at rates of 100, 50, 10, 5 and 2 mL per minute. After 14 bed volume exchanges of water at the respective flow rate, the concentration of dissolved oxygen (DO) in waters influent to and effluent from the reactors was determined.

**Conventional method**

A fourth reactor filled with approximately 2.0 L of Siran® glass bead was used to immobilise and grow biofilms in W257 water which then could be used as a source of inoculum in the modified conventional Joret & Levy (1986) BDOC method. Briefly this involved;

Approximately 200 grams of prepared inoculum was added to 600 mL of dechlorinated test water giving a typical ratio of 1:3. To ensure that oxygen was not limiting for biological growth, filtered medical grade air was delivered to each flask via a sintered glass bubbler at a rate of approximately 68 mL min⁻¹. The DOC concentration for all test waters was determined prior to dosing with the inoculum and recorded as the DOC concentration at time zero. After the inoculum was added to the test water the concentration of DOC was determined over several days at specified times (typical 1, 5 and 24 hrs and every 24 hours thereafter until a minimum DOC concentration had been observed). As in the rapid method, BDOC concentrations are derived from the difference between the initial and minimum DOC concentrations. On each sampling occasion, 60 mL of sample water was taken and 20 mL of this water was used to rinse the filter membrane and the remainder used for the DOC analysis.

For purposes of completeness, a number of water types (e.g. surface and ground water), with and with standard additions of easily (acetate-C) and slowly (i.e. Oxalate-C) biodegradable organic carbon, were used during method comparison tests and they are as follows:

1. Raw aerated W257 bore water, with and without organic carbon (as Acetate-C) standard additions of 0.2 mg L⁻¹ and 0.8 mg L⁻¹.
2. WGWTP enhanced (i.e. Alum 80 mg L⁻¹) conventionally clarified water.
3. Raw surface water from Salmon Gums, a regional town ca. 950 km from Perth, with and without standard additions of 0.5 mg L⁻¹ Acetate-C and 1.0 mg L⁻¹ Oxalate-C.

**Inoculum preservation study**

To determine the impacts of preservation and storage on W257 inoculum species composition and organic carbon degradation potential, W257 biofilm was preserved according to methods as described by Joret & Levy (1986). Briefly, siran® glass beads, laden with a mature biofilm enriched on water from bore W257, was washed several times with milliQ water until the concentration of NPOC in the washate was ≤0.1 mg L⁻¹ and stored at 4°C in sterile dechlorinated tap for approximately 1 month. After this time, 16S rDNA PCR-DGGE analysis was performed on “fresh” (n = 2) and “preserved” (n = 2) W257 inoculum. The “old” inoculum had been preserved for a period of ca. 7 months.

Comparison of “fresh” v “old” biofilm degradation potential involved determination of the concentration of BDOC in Denmark River raw surface water, which was known to contain relatively large amounts of NPOC (ca. 12 mg L⁻¹), Little Darkin River water (NPOC ~ 3.0 mg L⁻¹) and W257 aerated raw water (ca. 1.8 mg L⁻¹).

**16S rDNA PCR-DGGE analysis of W257 biofilm samples**

Samples (10 ml) were centrifuged (10,000 × g, 10 minutes) to collect any biofilm that was present in the water.
The supernatant was removed, and the pelleted biomass was resuspended in 60 μl of sodium dodecyl sulfate (SDS) and other cell disrupting agents before being processed using a Mo Bio Powersoil DNA isolation kit (MO BIO Laboratories Inc.) to extract genomic DNA from the biofilm samples. The extracted DNA was run on a 1% agarose gel to ensure the quality of the DNA.

PCR of 16S rRNA genes was performed using the 27F primer (bacteria) and the 1492R primer (universal) as previously described (Plumb et al. 2002). Amplification of ~500 bp fragments of the 16S rRNA gene, and subsequent analysis of fragments using DGGE was performed as described previously (Hawkes et al. 2006) except that the denaturing gradient of 50 to 80% was used. Nucleic acid sequence data was obtained from excised bands from the DGGE profiles using an automated cycle sequencing service provided by Royal Perth Hospital. Partial 16S rRNA sequences were compared with sequences in the publicly accessible GenBank database using the Basic Local Alignment Search Tool (BLAST; Altschul et al. 1997).

RESULTS AND DISCUSSION

The concentration of NPOC and BDOC in WGWTP enhanced clarified and MIEX clarified waters, Salmon Gums and aerated W257 water, with and with standard additions of acetate and/or oxalate, are given in Table 2.

Based on these outcomes, there was no significant difference between methods for the determination of the concentration of BDOC in W257 raw, W257 dosed with 0.8 mg L⁻¹ C-acetate, Salmon Gums Raw and Wanneroo GWTP enhanced waters. It also represents the first occasion where use of groundwater derived microbial inoculum was used to determine the concentration of BDOC in surface water.

However, less than expected concentrations of R-BDOC in W257 + 0.2 mg L⁻¹ C-acetate, Salmon Gums + 1.0 mg L⁻¹ and C-oxalate were measured compared to C-BDOC values for the same waters. In addition, the concentration of R-BDOC in Salmon Gums + 0.5 mg L⁻¹ C-acetate was 0.642 mg L⁻¹ which was approximately 40% less than the concentration of C-BDOC (i.e. 0.944 mg L⁻¹).
In all cases determination of the concentration of BDOC using the conventional method yielded BDOC values closer in concentration (up to 98%) to those expected in background and spiked ground and surface test waters, compared to values achieved using the rapid method.

There are several factors which may have caused the observed reduction in R-BDOC performance (most notably observed for the Salmon Gums + 1.0 mg L$^{-1}$ C-oxalate and the Salmon Gums + 0.5 mg L$^{-1}$ C-acetate test waters) compared to the C-BDOC method:

- Biofilm conditioning prior to conducting the rapid BDOC experiment
- Oxygen limitation
- BDOC character type
- Contribution of soluble microbial products

To obtain improved R-BDOC accuracy it maybe required that all of these factors should be considered and optimised when analysing any one particular test water. Nevertheless, the C-BDOC method was independent of some of these factors resulting in improved performance and reliability. Based on these outcomes the C-BDOC method was chosen as the preferred bioassay tool to proceed with further optimisation.

**Preserved v fresh W257 inoculum**

DGGE analysis of the “fresh” and “old” biofilm samples yielded a moderate number of different bands in the profiles for each sample (see Figure 2). The more prominent bands were detected in all samples. Bands 1–6, 7, 9 and 10 appeared to be present in all samples, while bands 8 and 11 only appeared in “fresh” biofilm samples. All of the bands detected in “old” biofilm appeared present in “fresh” biofilm samples. “Fresh” biofilm contained a number of faint bands that were not present in “old” biofilm samples. Eleven bands were cut from the DGGE gel, and sequenced. Results of BLAST analysis of sequence data obtained from each of the excised bands is shown in Table 3.

Most of the sequence data obtained from excised DGGE bands were not highly similar (≤ 98% similarity) to previously described species. It is therefore likely that these sequences represent novel species. A number of the sequences were less than 90% similar to the closest matching database sequences, suggesting that these sequences possibly represent novel genera. Despite this, identification to the level of phylum suggested that the W257 biomass contains a moderate level of diversity with representatives of three different phyla within the domain Bacteria. Three different subclasses of the Proteobacteria (alpha, beta and gamma) were represented in the biomass sample. The relative dissimilarity of most of the band sequence data to that from previously described species makes drawing conclusions about the microbial physiology of these population members difficult. Table 1 provides brief information on the source and physiology of the closest matching strains. For the small number of DGGE sequences that were highly similar to the closest matches in the database (e.g. Bands 4, 5 and 10), some insight into

![Figure 2](https://iwaponline.com/ws/article-pdf/8/6/633/419011/633.pdf)
<table>
<thead>
<tr>
<th>Band</th>
<th>Closest Match (% similarity)</th>
<th>Phylogeny</th>
<th>Source/Physiology</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Flavobacterium succinicans</em> (88)</td>
<td><em>Bacteroidetes</em></td>
<td>Isolated from a salmon fin, facultatively anaerobic glucose fermenter</td>
<td>DSMZ catalogue entry</td>
</tr>
<tr>
<td>2</td>
<td><em>Pseudomonas mendocina</em> strain JY4 (81)</td>
<td><em>γ-Proteobacteria</em></td>
<td>Isolated from soil via enrichment with ethanol, facultative anaerobe, denitrifier, opportunistic pathogen</td>
<td>Genbank database entry, <em>Aragone et al. 1992</em></td>
</tr>
<tr>
<td>3 *</td>
<td><em>Pseudomonas mendocina</em> strain JY4 (76)</td>
<td><em>γ-Proteobacteria</em></td>
<td>See above</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Pseudomonas mendocina</em> strain JY4 (99)</td>
<td><em>γ-Proteobacteria</em></td>
<td>See above</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>Sphingomonas</em> sp. F1-1 (98)</td>
<td><em>α-Proteobacteria</em></td>
<td>Isolated from petroleum contaminated soil</td>
<td>Genbank database entry</td>
</tr>
<tr>
<td>6</td>
<td><em>Hydrogenophaga bisanensis</em> strain K102 (88)</td>
<td><em>β-Proteobacteria</em></td>
<td>Isolated from textile dyeworks wastewater, facultative anaerobe</td>
<td><em>Yoon et al. 2008</em></td>
</tr>
<tr>
<td>7</td>
<td><em>Leptothrix</em> sp. MBIC3364 (87)</td>
<td><em>β-Proteobacteria</em></td>
<td>Isolated from a hot spring, possible iron and manganese oxidiser</td>
<td>Genbank database entry</td>
</tr>
<tr>
<td>8</td>
<td><em>Leptothrix ginsengisoli</em> (94)</td>
<td><em>β-Proteobacteria</em></td>
<td>Isolated from ginseng field soil</td>
<td>Genbank database entry</td>
</tr>
<tr>
<td>9 *</td>
<td><em>Flectobacillus</em> sp. EPACd7 (87)</td>
<td><em>Bacteroidetes</em></td>
<td>Isolated from aquatic environments</td>
<td>Genbank database entry</td>
</tr>
<tr>
<td>10</td>
<td><em>Nitrospira moscoviensis</em> (99)</td>
<td><em>Nitrospirae</em></td>
<td>Isolated from a corroding iron pipe, autotrophic aerobic nitrite oxidiser, or anaerobic hydrogen oxidiser and nitrate reducer</td>
<td><em>Ehrich et al. 1995</em></td>
</tr>
<tr>
<td>11</td>
<td>“<em>Candidatus Nitrospira defluvii</em>” (91)</td>
<td><em>Nitrospirae</em></td>
<td>Enriched from nitrifying bioreactor, autotrophic aerobic nitrite oxidiser</td>
<td><em>Spieck et al. 2006</em></td>
</tr>
</tbody>
</table>

*Sequence match due to poor quality sequence data, despite repeated efforts to generate better data.
the possible role of these population members can be gained. The closest matching bacterial species are all found in either soil or aquatic environments. *Nitrospira moscoviensis* is capable of both aerobic growth via nitrite oxidation or anaerobic growth via nitrate reduction using hydrogen as an energy source (Ehrich 1995). *Pseudomonas mendocina* is a facultative anaerobe, capable of organotrophic growth using oxygen or nitrate as an electron acceptor. This species is also regarded as an opportunistic pathogen (Aragone 1992). Additional research is required in order to determine more precisely the physiology of the W257 biomass population members.

“Old” and “fresh” biofilm NPOC degradation profiles in Quinninup raw surface water, Little Darkin River water and W257 aerated raw water are shown in Figure 3. Clearly, preservation had a marked effect on biofilm performance for the degradation of NPOC in untreated surface and raw aerated ground waters. This outcome was not expected and was contrary to those observations reported by Joret & Levy (1986) BDOC method, which demonstrated inoculum as old as 8 months has been used for the determination of BDOC in test waters. Nevertheless, biofilm preservation should be avoided and only fresh biofilm used as required.

The concentration of dissolved oxygen (DO) in effluent water from each rapid reactor at 2, 5, 10, 50 and 100 mL per minute flow rates are shown in Figure 4. The reduction in DO (measured as % DO consumed) achieved by reactors at the various flow rates tested showed that the optimum flow rate was in the range 2–5 ml per minute. With this, a flow rate of 2 mL per minute was incorporated into the overall rapid BDOC method. This compared well with Lucena et al. (1990) in which a flow rate of 3 mL per minute was used.

**CONCLUSIONS**

In all cases determination of the concentration of BDOC using the conventional method yielded BDOC values closer in concentration (up to 98%) to those expected in background and spiked ground and surface test waters, compared to values achieved using the rapid method.

W257 biofilm contains a moderate level of microbial diversity with representatives of three different phyla within the *Bacteria* domain. Comparison of sequenced data using BLAST revealed low similarities to known species and genera.

The outcomes presented here possibly represent the first instance where indigenous bacteria, immobilised and grown on inert glass media (i.e. Siran glass beads) in deep aquifer water, was used for the determination of the concentration of BDOC in ground and surface water, with and without standard additions, of easily biodegradable and slowly biodegradable organic carbon.

Biofilm preservation should be avoided and only fresh biofilm used as required.
REFERENCES


