Use of an enriched inoculum for determination of biodegradable dissolved organic carbon (BDOC) in drinking water

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ABSTRACT

The original BDOC procedure requires the use of indigenous bacteria as a seed. Most of the time in original water samples either bacteria are insufficient in numbers or the diversity is not enough to reflect the biodegradable part of DOC. In this study, instead of using the water sample itself as an inoculum as in the original BDOC test, the bacteria originating from the Ömerli reservoir in Istanbul were acclimated in a suspended growth system to remove readily and slowly biodegradable DOC fractions from the reservoir water. This modified BDOC procedure was first tested on standard acetate solutions and later on raw and ozonated waters of the Ömerli reservoir. Additionally, the results of the modified procedure were compared with the original one by also testing the effectiveness of the indigenous seed from the reservoir. In order to determine the most suitable inoculum amount in the modified BDOC procedure, different seeding ratios like 1:100, 1:250 and 1:500 (v/v) were tested. In both raw water and ozonated waters, higher BDOC readings were achieved at a seeding ratio of 1:100 than the original procedure. The results showed that the modified procedure resulted in more accurate results compared to the original one and that using an acclimated culture can bring an improvement in BDOC measurement.

Key words | biodegradable dissolved organic carbon, biodegradation, drinking water, inoculum

INTRODUCTION

Natural organic matter (NOM) is the all-encompassing term used to describe the non-synthetic organics ubiquitously present in surface waters. NOM can be divided into two fractions: the biodegradable organic matter (BOM) can be used by bacteria as a source of energy and carbon. The second fraction is refractory to biodegradation (e.g., nonbiodegradable) and has little effect on bacterial regrowth (Volk & LeChevallier 2000). BOM is of particular interest because it promotes regrowth of microorganisms in distribution systems. Several biological tests have been developed to assess the level of BOM in water (Huck 1990). All bioassays for the measurement of BOM involve the use of bacteria. These bioassays are mainly based on two concepts: The easily assimilable organic carbon (AOC) measures the growth of a bacterial inoculum in response to the amount of nutrient in water, and biodegradable dissolved organic carbon (BDOC) measures the fraction of DOC that heterotrophic bacteria can utilize as a source of energy and carbon (Volk & LeChevallier 2002). Although AOC and BDOC parameters are useful surrogates for BOM, each suffers from limitations (Huck 1990; Kaplan et al. 1994). There is no reference method that quantifies all of the BOM present in a sample.

The major applications of BDOC measurement are generally in the examination of raw and treated waters in terms of biodegradability and for evaluation of biological treatment system performance. Joret et al. (1991) suggested that BDOC values represent 10–30% of the total dissolved organic carbon content of drinking waters. After the water treatment, the biodegradable organics should be absent in
order to limit bacterial regrowth (Block et al. 1993). Similarly, Servais et al. (1993) have stated that biological stability within the distribution system is associated with a BDOC concentration of 0.16 mg/L or less in the finished water even in the absence of disinfectant.

In the original BDOC measurement procedure developed by Servais et al. (1987), the sample is filter sterilized (by filtering through a 0.45 μm filter) and then reinoculated with some part of the sample that was filtered through a 2 μm pore size for the removal of particle and the protozoa. Incubation at 20°C in the dark is carried out for a period of up to 30 days. BDOC is calculated from the difference between the initial concentration and the plateau at minimum bacteria. There have been a number of modifications to the original BDOC method like increasing the incubation time (McDowell et al. 2006), using attached bacteria instead of suspended inoculum (Park et al. 2004).

A modification in the original BDOC procedure was required within the scope of our study about biological activated carbon (BAC) system when the biodegradation inside the columns was much greater than the influent BDOC concentrations (Yapsakli 2008). Our hypothesis was that the use of an acclimated suspended culture in the BDOC test would result in more correct measurements if the added seed had a high microbial diversity. Therefore, instead of using the water sample itself as an inoculum as in the original BDOC test, bacteria grown in a suspended growth system were used and the performance of such a BDOC seed was tested. The bacteria originated initially from the Ömerli reservoir were acclimated to both readily and slowly biodegradable DOC in this water. This way it was expected that bacteria produced special enzymes to remove organics which were hard to biodegrade.

**METHODS**

**Enrichment of a culture as an inoculum**

Instead of using the water sample itself as an inoculum, the bacteria grown in a batch suspended growth reactor were used. Bacteria existing in the Ömerli water were enriched by adding readily biodegradable substrates (acetate and glucose) and by supplying air in a 4 L batch reactor. Glucose and acetate were added to the reactor in order to enhance and accelerate the bacterial activity. During this enrichment, three liters of the liquid volume in the reactor was replaced with fresh Ömerli water every day in order to supply the necessary nutrients as well as to enable bacteria to produce specific enzymes for biodegradation of this DOC. Therefore, the suspended growth system contained bacteria that originated from the reservoir itself and were acclimated to both readily and slowly biodegradable DOC by producing special enzymes. At later stages of reactor operation, glucose/acetate supplementation was stopped and the reactor was fed with Ömerli water only. Initial and final DOC values were measured on a daily basis. This culture was used as an inoculum in BDOC experiments.

**The modification of the original BDOC procedure**

BDOC experiments were conducted with standard acetate solutions and Ömerli reservoir water (Figure 1). Acetate was chosen as a standard because of its easy biodegradation. Acetate solutions at concentrations of 1 mg/L, 2.5 mg/L and 5 mg/L were tested. Raw water samples were brought from the Ömerli reservoir, which supplies water to some regions in Istanbul at a rate of 1,000,000 m³/d. It is a big water supply reservoir in Istanbul providing approximately 48% of the city’s drinking water. The year-round measurements indicate that the dissolved organic carbon (DOC) and total organic carbon (TOC) of the source varied between 3.5–5.8 mg/L and 4.1–6.0 mg/L, respectively (Yapsakli 2008).
DOC was analyzed using a Teledyne-Tekmar Apollo 9000 Model TOC analyzer. The method followed is described in Standard Methods: 5310B Total Organic Carbon High Temperature Combustion Method (APHA, AWWA and WEF 1998).

BDOC was measured in duplicate for each sample and inoculum ratio tested which is similar to the method developed by Servais et al. (1987). A brief description of the method is as follows: the raw water and ozonated water samples were filtered through a 0.45 µm glassfiber filter paper (Schleicher & Schuell, Germany), measured for DOC and inoculated with seed at different proportions.

In order to determine the best inoculum amount for the BDOC procedure, different seeding ratios like 1:100, 1:250 and 1:500 (v/v) were tested. In all experiments 400 ml of solution was used. For this purpose, 4 ml, 1.6 ml and 0.8 ml of seed was taken from the batch suspended growth reactor in order to prepare 1:100, 1:250 and 1:500 seeding ratios, respectively. In case of standard acetate solutions, necessary nutrients like MgSO4, CaCl2, FeCl3 and phosphate buffer were added in amounts described in Biochemical Oxygen Demand (BOD) determination (5210B, Standard Methods).

The mixture was incubated at 20°C in an incubator (TS-606-G, WTW) for 28 days. DOC was measured again after incubation. The difference between the first and 28th day DOC reading was BDOC. Also, samples were taken at regular intervals to follow the trend for BDOC exertion with respect to days.

RESULTS AND DISCUSSION

Batch reactor operation for acclimation of microorganisms in Ömerli water

The aim was to acclimate bacteria that could degrade the organic matter in raw water to be used as a seed in the BDOC. The initial and final DOC profiles of the batch reactor are shown in Figure 2.

In the first ten days of reactor operation, despite acetate and glucose additions, no biodegradation was observed. Then, the organic carbon was increased on a regular basis from 10 mg C/L to 40 mg C/L by supplementing glucose and acetate to increase bacterial growth. Between days 10 and 25, there was a noticeable increase in biodegradation efficiency which reached a maximum of 83%. Mixed Liquor Volatile Suspended Solids (MLVSS) concentration at Day 25 increased up to a maximum value of 140 mg/L. At this point, organic carbon addition was stopped and the reactor was fed with a mixture of Ömerli raw and ozonated water only.

Because the organic carbon in the Ömerli water was lower than previous days and bacteria concentrations were high with respect to biodegradable organic matter, cell lysis was expected in the system. As a result of the breakage of cell membrane upon cell lysis, organic matter was solubilized in the solution. Therefore, the initial DOC was observed to be lower than the final DOC in the subsequent ten days. At the end of 40 days, the initial and final values did not differ from each other. The initial and final DOC values belonging to 40–140 days are presented in Figure 2b.

Figure 2 | Initial and final DOC profiles in the batch reactor used for enrichment of bacteria from the Ömerli Reservoir at (a) time interval 0–40 days, (b) time interval 40–140 days.
Between days 40–60, the initial DOC decreased due to the change in raw water quality which may occur during a turnover in the lake. The average DOC removal was 20% at this time period.

Between days 100–140, the system was fed with ozonated water only. In this case, the biodegradation efficiency increased up to 40%. Ozonated water contains less refractory NOM compared to raw water since ozonation encourages the formation of low molecular weight substances at the expense of larger compounds and reduces aromaticity and double bonds (reduction of UV absorption) (Meijers 1977; Langlais et al. 1991; Volk et al. 1993). Ozonation was applied to raw water at a rate of 2 mg O\textsubscript{3}/mg DOC in order to increase the BDOC production. This rate represents the optimum ozone dosage which maximizes the BDOC production and at the same time does not result in more than 5% DOC decrease in the Ömerli raw water. The BDOC amount increased as much as 95% with respect to raw water upon ozonation (Yapsaklı et al. 2009).

**BDOC exertions of standard solutions**

The modified BDOC procedure was first tested on standard acetate solutions (1 mg/L, 2.5 mg/L and 5 mg/L). In addition to using the seed from the batch reactor, the indigenous seed from the reservoir (Ömerli water which has been filtered through 2 μm) was also tested. This way, the performance of the modified procedure was compared with the original procedure. BDOC was measured in duplicate for each sample and inoculum tested. In the original procedure, a seeding ratio of 1:100 (v/v, 1 ml seed is added into 100 ml water sample) was used. In the current study, different seeding ratios like 1:100, 1:250 and 1:500 were tested to determine the optimum seed ratio. When the bacterial concentration in the inoculum is high, cell lysis can occur during the BDOC procedure. This occurs because the cell contents become soluble after cell lysis, which in turn increase the DOC concentration in the solution. In this case, the measured BDOC would be lower than the actual value. Since the bacterial concentration in the suspended growth culture is higher compared to the original seed of the BDOC test, different seeding ratios should be tested in order to avoid such situations. The results are presented in Figures 3, 4 and 5.

![Figure 3](https://iwaponline.com/wst/article-pdf/9/2/149/419107/149.pdf)  
**Figure 3** | BDOC exertion at an acetate-C concentration of 1 mg/L.

![Figure 4](https://iwaponline.com/wst/article-pdf/9/2/149/419107/149.pdf)  
**Figure 4** | BDOC exertion for 2.5 mg/L Acetate-C.
more time would be required for complete exertion. The rate of substrate utilization at 2.5 mg C/L was obviously higher compared to 1 mg C/L which is an expected result when working at low substrate concentrations.

Figure 5 shows the BDOC exertion when the acetate-carbon concentration was 5 mg C/L. Similar to previous results, complete BDOC exertion was seen within three days when acclimated seed was used. On the other hand, 10 days was required for complete exertion when indigenous seed was used.

Results suggest that the acclimated seed could completely biodegrade acetate in a reasonable time at both low and high concentrations. Even in the case of a 1:500 seed ratio enough microorganisms were present in the test solution to degrade acetate as at higher ratios. On the other hand, the bacteria concentration in the Ömerli water was limited which had a strong influence on biodegradation rates. The indigenous seed of Ömerli reservoir failed to completely biodegrade acetate at a concentration of 1 mg C/L in 28 days. Increase in acetate concentration resulted in relatively faster biodegradation rates when indigenous seed was used. Biodegradation rate obviously decreased when substrate became limiting.

**BDOC exertions of Ömerli raw and ozonated water samples**

BDOC test was repeated for the same seeding ratios for both raw water and the ozonated waters of Ömerli reservoir. For raw water samples at an initial DOC of 5.18 mg/L, the average BDOC concentrations at different inoculum ratios are presented in Figure 6 with respect to incubation time.

A seeding ratio of 1:100 resulted in higher BDOC exertion than others. Obviously, at lower ratios the bacteria were not enough in numbers to achieve the same ultimate BDOC values. Moreover, 51% higher BDOC readings were achieved by using a seeding ratio of 1:100 with respect to the original procedure. This indicates that there was a portion of DOC that could be degraded by acclimated bacteria but not by indigenous inocula. This was an expected result since the indigenous seed was not as acclimated as the other three inocula. The average ultimate BDOC exertion for the acclimated seed was 15.0%, 10.0% and 9.8% when a seeding ratio of 1:100, 1:250 and 1:500 were used, respectively. BDOC results at different seeding ratios were found to be statistically significant according to ANOVA analysis and 95% confidence interval (P < 0.05). In the original procedure this percentage of biodegradable substrate was as low as 8.3%. Ultimate BDOC value of 0.66 mg/L in raw water was reached within 14 days of incubation time when the seeding ratio was 1:100. When the original procedure was followed or when a seeding ratio other than 1:100 was applied, ultimate BDOC values cannot be reached at the end of 28 days.

For ozonated water samples with an initial DOC of 5.05 mg/L, the average BDOC concentrations are presented in Figure 7. Ozonated water was obtained by applying an ozone dose of 2 mg O₃/mg DOC to raw water. In ozonated samples, all seeding ratios resulted in similar BDOC exertions which were found to be statistically insignificant. On the other hand, conducting the test at a seeding ratio of 1:100 resulted in 72% higher BDOC readings than the original procedure. The average ultimate BDOC exertion for acclimated seed was 36.0%, 35.7% and 35.4% when seeding ratios of 1:100, 1:250 and 1:500 were used,
respectively. In the original procedure this percentage of biodegradable substrate was as low as 21%. In ozonated water samples, BDOC exertion was completed at the end of 23 days of incubation time. The highest BDOC exertion was observed as 2.03 mg/L at a seeding ratio of 1:100. In this case, the rate of BDOC exertion in ozonated water samples was lower compared to raw water, indicating that completion of BDOC exertion was retarded even if the biodegradability of raw water was increased by ozonation. This observation may be attributed to formation of slowly biodegradable organics upon ozonation.

The BDOC exertion in raw and ozonated water samples can be fitted into first order kinetic model assuming that ultimate BDOC (BDOCu) values are reached when a seeding ratio of 1:100 is used both in raw and ozonated water samples. The first order kinetic model given below is also used for examining BOD exertion kinetics.

\[
\text{BDOC}_t = \text{BDOC}_u \times (1 - e^{-kt})
\]

where:

- BDOC\(_t\) = BDOC at time \(t\) (mg/L), BDOC\(_u\) = Ultimate BDOC (mg/L), \(k\) = First order rate constant (day\(^{-1}\)), \(t\) = Incubation time (day)

\(k\) constants obtained by regression analysis are shown in Table 1 together with their correlation coefficients. BDOC exertions for all samples can be fitted well to the first order model. BDOC\(_u\) concentrations were taken as 0.66 mg/L and 2.03 mg/L for raw and ozonated water, respectively, assuming that BDOC concentrations at a seeding ratio of 1:100 reflect the ultimate BDOC.

As expected, when a seeding ratio of 1:100 was used in tests, higher kinetic constants were found compared to other seeding ratios as well to the original procedure. On the other hand, the \(k\) value was found to be higher in raw water samples than the ozonated water samples (Table 1). This was also evidenced in Figures 6 and 7 indicating that higher incubation times were required for ozonated water samples compared to raw water samples to reach the ultimate BDOC values. As explained earlier, ozonation might have converted nonbiodegradable organics mostly into slowly biodegradable organics, which then may result in decreases in biodegradation and BDOC exertion rates. It should be noted that the kinetic constants belonging to raw and ozonated water shown in Table 1, were based on different ultimate BDOC values. A lower kinetic constant does not indicate lower ultimate BDOC, but rather reflects how fast the ultimate BDOC value is reached with a specific seed.

In the modified procedure, the rate of BDOC exertion decreased with a decrease in the amount of seed. It is obvious that the initial bacterial amount in the BDOC test played an important role in the rate of exertion. On the other hand, even the 1:500 seeding ratio provided a faster BDOC exertion compared to the original procedure. This study showed that, this BDOC modification resulted in improved BDOC readings with respect to original procedure. There are also a number of other modifications in literature. For example, Khan et al. (2005) reported that using the unfiltered sample seed tended to result in higher BDOC\(_{28}\) than inoculating with the commercial seeds.

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 a 85 to 120 day incubation. They recommended a more sophisticated method involving:

<table>
<thead>
<tr>
<th>Sample</th>
<th>(k) (\text{day}^{-1})</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw water</td>
<td>Original procedure</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>1:100 seed</td>
<td>0.230</td>
</tr>
<tr>
<td></td>
<td>1:250 seed</td>
<td>0.072</td>
</tr>
<tr>
<td></td>
<td>1:500 seed</td>
<td>0.070</td>
</tr>
<tr>
<td>Ozonated water</td>
<td>Original procedure</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>1:100 seed</td>
<td>0.117</td>
</tr>
<tr>
<td></td>
<td>1:250 seed</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>1:500 seed</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Table 1: First-order kinetic constants and correlation coefficients in BDOC exertion of raw and ozonated water samples

![Figure 7](https://iwaponline.com/ies/article-pdf/9/2/149/419107/149.pdf)
(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 CO₂ production when determination of decomposition rate constants and a labile and relatively refractory component of DOC is desired.

Usage of bacteria attached to inert media was tested by several researchers like Joret & Levei 1986; Ribas et al. 1991; Frias et al. 1992; Escobar & Randall 2001 etc. This inert media contains a native biomass that is well adapted to the water under investigation. Obviously high microbial diversity, higher metabolic activity of attached bacteria and reduced testing time (5 to 7 days) made these modifications suitable for application. However, use of attached bacteria may cause some drawbacks. Firstly, it is difficult to accomplish complete cleaning of the sand, which in turn affects the DOC detection level. Second, the bacterial products which are excreted as a result of cell lysis can cause problems in measurement, especially when low BDOC samples are tested. Lastly, abiotic adsorption of organic molecules to inorganic support and biofilm matrix surfaces can overestimate the BDOC readings. In a study conducted by Trulleyova & Rulik (2004), it was stated that 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 (biofilm). They stated that the reason for these findings could be the higher microbial diversity, higher metabolic activity of attached bacteria and abiotic adsorption of organic molecules to inorganic support and biofilm matrix surfaces.

Khan et al. (2003) developed a simple and rapid continuous bioreactor procedure using immobilized cells for determining biodegradable dissolved organic carbon (BDOC) in water. Results showed that the feed aerated (FA) bioreactor is a better tool for BDOC determination especially for waters with low initial organic concentrations because of less background DOC released by the immobilized cell systems. Using the FA bioreactor, the accurate and reproducible measurement of BDOC can be achieved within a hydraulic retention time of 3h, and no start-up period is required.

Similar to the modification in this study, Khan et al. (2005) suggested using mixed liquor suspended solids (MLSS) from wastewater treatment plants as inocula for the BDOC test. Although this application is more practical and faster compared to the current study, it has some drawbacks. For example, the bacteria taken from the wastewater treatment plant have some organic matter adsorption ability. This would lead to the erroneous BDOC readings since the adsorbed organic matter will not be necessarily degraded. Conducting a blank sample cannot monitor the adsorption since blank sample does not contain any organic matter. Moreover, the microbial cultures in the wastewater treatment plant are quite different from those found in water systems and change with operational factors such as sludge retention time (SRT).

**Comparison of DOC removal in the batch reactor with BDOC exertion in tests**

In order to evaluate the BDOC reading performance of this modified procedure, BDOC values measured in bioassays were compared with the DOC removal in the aerated suspended growth batch reactor. The difference between the initial and final DOC in the batch reactor reflected the amount of biodegraded DOC. Therefore, measured BDOC values should be close to biodegraded DOC in the reactor since measured BDOC monitors the biodegradable DOC (BDOC). Table 2 shows an example comparison of DOC removal in the batch reactor and BDOC readings.

It was previously shown in Figure 2b that the batch suspended culture could biodegrade up to 40% of initial DOC when the reactor was fed with ozonated water. For example, difference between the initial and final DOC values in the reactors was 1.5 mg/L (average influent DOC: 3.5 mg/L, average effluent DOC: 2.0 mg/L). BDOC of this water was monitored to be 0.76 mg/L by the original BDOC procedure and 1.2 mg/L by the modified procedure.

| Table 2 | An example comparison of BDOC readings with different methods and biodegradation in batch reactor |
|-----------------|---------------------------------|-----------------|
| Raw water       | Original BDOC procedure (mg/L)  | Modified BDOC procedure with 1:100 seed (mg/L) | Biodegraded DOC in the batch reactor (mg/L) |
|                 | 0.43 ± 0.08                     | 0.71 ± 0.17                  | 0.95 ± 0.18 |
| Ozonated water  | 0.76 ± 0.10                     | 1.20 ± 0.12                  | 1.50 ± 0.15 |

Raw water 0.43 ± 0.08 0.71 ± 0.17 0.95 ± 0.18
Ozonated water 0.76 ± 0.10 1.20 ± 0.12 1.50 ± 0.15
In this case, the modified BDOC procedure gave more realistic results compared to the original procedure. Still, the modified BDOC readings were lower than the actual biodegradation observed in the reactor. This can be derived from the fact that BDOC measurement is done using a suspended culture with limited bacteria concentration under poor mixing conditions. Therefore, our assumption is that in general the actual biodegradable part of DOC (BDOC) is underestimated in the BDOC test procedure. Also, our findings indicate that much more organic carbon was biodegraded in the lab-scale BAC filters than the measured BDOC values (Yapsalı 2008).

CONCLUSION

This research revealed the possibility of using an acclimated culture in the BDOC test. The usage of an acclimated seed resulted in more accurate BDOC readings compared to the original method. Despite the efficacy of this modification, the only disadvantage of such system is the requirement of long acclimation periods for the enrichment and operation of a culture. On the other hand, the most striking advantage of using an acclimated culture as a BDOC seed is that the culture contains bacteria which are well acclimated to the organic matter from a specific source. This enrichment culture is not considered to be site-specific. For the cases in which the NOM character in raw water does not vary much between water supply sources the same acclimated culture can be used for examining the BDOC.

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