Method development for measuring biodegradable organic carbon in reclaimed and treated wastewaters

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ABSTRACT: Analyses that measure oxygen demand, such as biochemical oxygen demand (BOD) and chemical oxygen demand (COD) analyses, have long been used as indicators of contamination and wastewater treatment plant efficiency. They measure the tendency of pollutants to react with oxygen, which is generally a good indicator of the stability or level of treatment. Both parameters include reactions with organic as well as inorganic substances and suffer from a lack of precision and accuracy at low concentrations, which are becoming increasingly more important. Biodegradable dissolved organic carbon (BDOC) analysis is a relatively new procedure that has advantages over both BOD and COD analyses, including insensitivity to inorganic oxidations. A modified BDOC procedure was developed to characterize the performance of advanced treatment methods, such as those used in municipal water reclamation and secondary-treated wastewaters, where moderately low dissolved organic carbon concentrations (4 to 15 mg/L) are routinely encountered. The development of the modified BDOC procedure was based on a combination of the existing batch BDOC protocol and BOD techniques. Various aspects and incubation conditions were investigated to finalize the procedure. Nitrification does not interfere with the procedure. It is possible to simultaneously determine the soluble BOD (SBOD) under certain conditions. The procedure has reduced variability and increased precision as compared to BOD and COD analyses. *Water Environ. Res.*, **70,** 1025 (1998).

KEYWORDS: secondary-treated wastewater, organic carbon, reclaimed wastewater, biodegradation, biodegradable dissolved organic carbon, analyses.

Introduction

Removal of organic contaminants is a primary goal of both water and wastewater treatment. Traditional methods of evaluating treatment efficiency measure the tendency of the contaminants to react with oxygen. Biochemical oxygen demand (BOD), chemical oxygen demand (COD), and total oxygen demand (TOD) have all been used. Organic carbon is frequently one of the main components of the contaminants, and for this reason total organic carbon (TOC) removal is sometimes used as a primary indicator of treatment plant efficiency.

All four measurements have advantages and disadvantages. The BOD measurement provides the best estimate of the reactivity of the contaminants in the natural environment, but is insensitive and imprecise at low concentrations. The COD measurement provides no indication of biodegradability and has limited precision and accuracy at values less than 5 mg/L (APHA *et al.*, 1989). The COD procedure also uses toxic reagents and produces hazardous wastes.

The presence of certain contaminants or conditions interfere with BOD, COD, and TOD analyses. The BOD test measures the presence of both carbonaceous and nitrogenous oxygen demands, which are becoming known as CBOD and NBOD, respectively. Sometimes it is desirable to have one analysis measure both demands, but more often, separate measurement of the demands is preferred (Albertson, 1995). The COD test does not completely oxidize some ring organics such as pyridine and related compounds, and it is subject to interference at high chloride concentrations (APHA *et al.*, 1989). The TOD procedure is an instrumental method that combusts the sample and provides rapid response. Unfortunately, if nitrate is present in the sample, a false reading is produced when some of the nitrate is reduced to nitrite or other nitrous oxides. The indicated TOD is sometimes negative for samples with low oxygen demand and high nitrate concentration. Analysis of TOC is a rapid procedure with low detection limits (0.05 to 0.10 mg/L) and excellent precision, and has fewer of the previously cited disadvantages. Therefore, it is frequently used as an indicator of contaminants in potable waters as well as reclaimed and treated wastewaters. Its primary disadvantage is that it provides no indication of the biodegradability of the contaminants.

This paper presents an adaptation of a previous method (Servais *et al.*, 1989) for quantifying the biodegradable dissolved organic carbon (BDOC) in drinking water (dissolved organic carbon [DOC] < 4 mg/L) for use in evaluating reclaimed and secondary-treated wastewaters (DOC = 4 to 15 mg/L). A modified batch protocol was developed using a combination of the existing batch BDOC protocol and BOD techniques. With the use of this new protocol, it was expected that simultaneous determinations of DOC, BDOC, and soluble BOD (SBOD) of tested waters could be achieved. A companion paper (Khan *et al.*, 1998) shows the applications of the modified BDOC protocol for the analysis of reclaimed and secondary-treated wastewaters. Although it has not been tested with other wastewaters, the protocol, with appropriate modifications, should be applicable to water samples with high DOC, such as primary-treated wastewater.

Background

In 1987, Servais *et al.* introduced the BDOC parameter. Huck (1990) defined BDOC as the portion of organic carbon in water that can be metabolized by heterotrophic microorganisms. Beside causing taste, odor, and color in water, BDOC can react with some disinfectants to form undesirable products. In addition, BDOC can be a problem in drinking water by inducing regrowth in the distribution system; such regrowth inhibits or defeats disinfection.

Servais *et al.* (1989) suggested a batch protocol for determining BDOC in drinking water. They filtered 200 mL of the sample using a 0.22-μm membrane filter that had been previously conditioned by washing with distilled water and the sample. They used a 2-mL inoculum created by filtering a portion of the sample through a 2-μm filter to remove protozoans. This inoculum should be well acclimated to the organic compounds in the sample. They incubated the sample in the dark at 20°C for 28 days and calculated the BDOC from the difference between the initial and final DOC values.

Their BDOC protocol is a bioassay test similar to the BOD test. Biodegradation in the environment is simulated by using an inoculum collected from the same location as the sample. Hence, BDOC could be suitable for monitoring the effectiveness of biological treatment. Servais *et al.* (1987) also proposed bacterial biomass and mortality measurements during the incubation for determination of BDOC (instead of DOC reduction). Although the biomass and mortality approach is more sensitive, it is not widely used, because it is more time consuming and requires more complicated techniques.

An alternative to the BDOC procedure, called assimilable organic carbon (AOC), was invented by van der Kooij *et al.* (1982). The AOC is the portion of the organic carbon that can be synthesized to cellular material by a single bacterial strain. In the AOC determination method, a preheated water sample is seeded with a pure strain of *Pseudomonas fluorescens* P17. The sample is incubated at 15°C, and bacterial growth is monitored daily by colony counts (spread plate techniques) until the maximum growth is reached. By concurrently determining the growth yield of bacteria in solutions of known acetate concentration, the maximum growth can be converted to AOC and expressed as micrograms acetate–carbon equivalent per litre.

Because the AOC test measures cell growth of a single strain, the test does not guarantee that all the assimilable carbon will be measured. The inoculum may not be capable of metabolizing all contaminants, and no acclimation is possible. van der Kooij (1987) and van der Kooij *et al.* (1989) included a *Spirillum* strain, NOX, in the procedure as an alternative seed or a dual strain seed because of the inability of *P. fluorescens* P17 to metabolize oxalic acid, which is one of the products commonly formed during ozonation. For reclaimed and secondary-treated wastewaters, in which a large variety of compounds may be present, an acclimated, mixed-culture inoculum should provide a better indication of the degradable organic carbon than would any single or dual strain inocula.

To shorten the BDOC determination period, Kaplan and Newbold (1995), Lucena *et al.* (1990), and Ribas *et al.* (1991) introduced dynamic reactors for BDOC measurement. A glass-column reactor is filled with an inert support media (filter sand or glass balls) to which high concentrations of microorganisms responsible for DOC consumption are attached. The sample is passed through the column, and the BDOC value can be calculated from the difference between the DOC values of the inlet and outlet samples. This approach has reduced the measurement time from 28 days to approximately 3 hours. However, its main weaknesses are difficulty in standardizing the method and a long start-up period.

The batch BDOC method is relatively unknown in the wastewater treatment field. Its application is limited to water samples with low DOC concentrations (<4 mg/L) because of dissolved oxygen (DO) consumption limitation during incubation (unless samples are diluted). The DO concentration at the end of the incubation period should be sufficiently high (generally $\geq$1.0 mg/L) that it is never rate limiting.

Methodology

Protocol. The water sample was filtered through a 0.7-μm glass-fiber filter (GF/F, Whatman, Whatman International Ltd., Maidstone, England) previously rinsed with 300 mL of deionized (DI) water, containing a TOC content of less than 0.20 mg/L. The filtrate was analyzed for TOC and reported as DOC. A dilution factor, F, was calculated to ensure that adequate DO (DO $\geq$ 1.0 mg/L) remained at the end of the test. Several trials or multiple dilutions may be required for samples with unknown DOC or BOD values. The dilutions were made with DI water having a known TOC of less than 0.20 mg/L to produce at least 320 mL of combined volume, and placed in a washed container with at least 20% gas volume. The mixture was saturated with DO by shaking. After shaking, a 20-mL sample was collected, measured for TOC, and recorded as DOC*i*. The mixture was next placed in a washed BOD bottle. The DO was measured with a washed probe (to prevent sample contamination), and recorded as DO*i*. A 2-mL inoculum of unfiltered water sample was then added. This inoculum should contain either microorganisms present in the environment from which the sample was collected or other acclimated organisms. The bottle was water sealed and incubated in the dark without shaking at 20 $\pm$ 0.5°C for 28 days. At the end of the incubation, the DO was measured and recorded as DO*f*. Then, 20 mL of the supernatant was collected and measured for TOC directly, without any filtration, and recorded as DOC*f*. A seed control (sample *b*) was prepared in the same way except that the 2-mL seed was added to 300 mL of dilution water with no sample, and the values were recorded as DO*bi*, DOC*bi*, DO*bf*, and DOC*bf*. The BDOC and ultimate SBOD (SBOD*u*) were calculated using the following equations:

$$\text{BDOC (mg/L)} = [(\text{DOC}_i - \text{DOC}_f) - (\text{DOC}_{bi} - \text{DOC}_{bf})]F \quad (1)$$

$$\text{SBOD}_u \text{ (mg/L)} = [(\text{DO}_i - \text{DO}_f) - (\text{DO}_{bi} - \text{DO}_{bf})]F \quad (2)$$

where F = (mL of dilution water + mL of sample)/mL of sample, observing the criteria of (DO*i* $-$ DO$_f$) $\geq$ 2 mg/L and DO$_f$ $\geq$ 1 mg/L (APHA *et al.*, 1989).

When the 5-day SBOD (SBOD$_5$) was of more interest than SBOD*u*, such as in secondary-effluent samples, the DO concentrations of both the sample and seed controls were measured after 5 days of incubation and recorded as DO$_5$ and DOb_5. The SBOD$_5$ was calculated by substituting DO$_5$ and DOb_5 (DO*f* and DO*bf*, respectively) in Equation 2. For BDOC determination, after measurement of DO, 100 mL of the mixture was discarded and the mixture was resaturated with DO by shaking. The incubation was continued for 23 days (total incubation period of 28 days). During the second incubation period, the DO in the mixture was recharged by shaking the bottle daily. This ensured adequate DO should nitrification occur. The above procedure for simultaneous SBOD determination is general. Some other techniques, such as dilution water check and preparation, sample storage, and sample dechlorination or deozonation, if re-

quired, should be incorporated and performed as described in *Standard Methods for the Examination of Water and Wastewater* (APHA *et al.,* 1989).

The modified BDOC protocol presented above differs from the original batch protocol in many aspects. It was eventually developed from the original procedure after several series of experiments to identify problems and sources of error. The type of filter used for DOC determination was changed from 0.22-μm cellulose acetate (CA) membrane filter to 0.7-μm glass-fiber filter. It was found that the CA membrane releases a substantial amount of organic carbon. The leaching of organic carbon from the CA membrane was studied in detail, and the results are described and discussed in the next section. A BOD bottle was used for incubation. Both BOD bottles and glass-fiber filters are standard equipment in water quality laboratories. To maintain the similarities in microbial types and activities between the actual environment and the incubation, the seed employed in the modified protocol was not filtered. As a result, the seed should contain both heterotrophic bacteria and protozoans. Dissolved oxygen was measured before and after the incubation to determine SBOD and to confirm its adequacy throughout the incubation period. The dilution and seed control were incorporated to make the protocol workable with moderately high-DOC waters and to produce more accurate results.

Dissolved Organic Carbon and Dissolved Oxygen Measurements. Dissolved organic carbon was measured with a Dohrmann Total Organic Carbon Analyzer, model DC-80 (Xertex Corporation, Santa Clara, California), using ultraviolet promoted persulfate oxidation and infrared spectrometry. The analyzer was calibrated daily using 10 mg TOC/L potassium hydrogen phthalate (KHP) standard solution and the multiple-point calibration procedure recommended by the manufacturer. The analyzer has a usable range of 0.10 to 20.00 mg/L (limit of quantitation to limit of linearity) and a detection limit of 0.04 mg/L for a sample size of 1 mL. The mean value of three DOC measurements was reported. Dissolved oxygen was measured using a YSI 58 DO Meter and a YSI 5720 DO Probe (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio).

Results and Discussion

The original BDOC procedure (Servais *et al.*, 1989) was first used to measure BDOC of the effluent samples from the sand filter, the primary ozonation columns (5 columns), and the biological activated carbon (BAC) filter of the Lake Arrowhead wastewater reclamation pilot plant (Madireddi *et al.*, 1997). Problems and inconsistent BDOC results were encountered using the original procedure. To identify the problems and sources of error, various aspects of the original protocol were evaluated and modified. The main modification was inclusion of the dilution and/or DO recharge (shaking) techniques to avoid oxygen depletion during the incubation. The filter type, inoculum origin and size, and inoculum filtration (after incubation) were all investigated. Control experiments using prepared samples containing known compounds (dextrose and sodium acetate) were also conducted. After the complete development, the modified protocol was employed to determine BDOC of secondary effluents. Reduction of the incubation period was attempted by agitating the sample, increasing the incubation temperature, and studying the protocol kinetics.

Filter Type. The initial BDOC analysis using 0.22-μm CA membrane filters provided inconsistent results. The membrane released organic carbon even after being rinsed with 300 mL of DI water and 300 mL of sample. A soak test was performed to determine the amount of organic carbon released from the CA membrane filters (Khan, 1997). The results show that without prerinsing, one 0.22-μm CA membrane filter can release approximately 0.40 to 0.50 mg of TOC (1.40 to 1.70 mg TOC/L in a 300-mL BOD bottle) in the first 24 hours of the soak test. Filters still leached organic carbon in a second 24-hour soak test (0.03 mg of TOC/filter).

The quantity of DOC leached was sometimes more than the DOC reacting during the BDOC analysis. The leached organic carbon was not analyzed, but its biodegradability was evaluated. During the 28-day period, the leached organic carbon degraded, further complicating the use of CA membrane filters. It is possible that the filters were hydrolyzing and releasing TOC. The 0.22-μm CA membrane filters were abandoned. No leaching or adsorption problems were detected with glass fiber-filters, which were used for all subsequent analyses.

Detection Limit, Precision, and Accuracy of the Modified Biodegradable Dissolved Organic Carbon Protocol. The modified BDOC protocol was evaluated following the procedure for determining method detection limit (MDL) in *Standard Methods* (APHA *et al.*, 1989) using five blank samples spiked with sodium acetate (0.40 to 0.50 mg DOC/L). The results indicated an MDL of 0.10 to 0.15 mg/L, which was lower than the MDLs of 5 mg/L and 2.0 mg/L for the COD and BOD_5 tests, respectively. Though the detection limit of the BDOC test was much lower than those for the COD and BOD_5 tests, it alone was not sufficient to conclude that the BDOC test is better than the other two tests. As a consequence, the modified BDOC protocol was further investigated for precision.

To determine precision, the modified BDOC protocol was used on 29 reclaimed-wastewater samples and 43 secondary-effluent samples. The DOC concentrations of these 72 samples ranged from 4.50 to 15.50 mg/L. Each sample was run in triplicate. The mean BDOC concentration and standard deviation (SD) of each sample were calculated. Assuming a linear relationship between BDOC concentration and the precision of the method, a linear regression between 72 mean BDOC concentrations and their SDs yields the following statistically significant relationship (Pearson, $p < 0.000\ 5$):

$$\text{SD (mg/L)} = 0.03(\text{BDOC}) + 0.03 \qquad \text{correlation coefficient, } r = 0.55 \quad (3)$$

Or

$$\text{Coefficient of variation (\%)} = 100\left(\frac{\text{SD}}{\text{BDOC}}\right) = 3.0 + \left(\frac{3.0}{\text{BDOC}}\right) \quad (4)$$

Concentrations of BDOC were measured in 72 samples and ranged from 0.50 to 5.00 mg/L. The SD was 0.05 to 0.18 mg/L, which corresponds to a range in the coefficient of variation (CV) of 3.6 to 9.0%. According to a series of interlaboratory studies on BOD_5 measurements using synthetic water samples (1:1 mixture of glucose and glutamic acid) as described in *Stan-*

Table 1—Accuracy of the modified BDOC protocol.

Standard solution	Actual DOC, mg/L	BDOC, mg/L	Recovery, %
Dextrose	1.41	1.39	97.9
		1.33	94.3
		1.33	94.3
		1.29	101.4
Sodium acetate	0.83	0.81	97.6
		0.81	97.6
		0.80	96.4
		0.84	101.2

dard Methods (APHA *et al.*, 1989), the predicted BOD_5 CV for samples with a BOD_5 of 2 to 30 mg/L (a typical range of BOD_5 for reclaimed and secondary-treated wastewaters) is 16.9 to 40.4%. The CV of the COD procedure is not defined for concentrations in this low range; however, the CV of samples with a COD concentration of 200 mg/L and chloride concentration of 0 to 100 mg/L ranges from 4.8 to 10.8%. At the lower COD concentrations found in reclaimed and secondary-treated wastewaters (5 mg/L $\leq$ COD $\leq$ 80 mg/L) and greater chloride concentrations, the CV should be higher. It can be concluded that the BDOC procedure is more precise than the BOD_5 and COD procedures.

The variability of the BDOC procedure described above can arise from factors related to three sources: instrumentation, personnel/operation, and method. The last two sources of variability are difficult to identify. To estimate the variability resulting from the instrument, a review of the long-term performance of the TOC analyzer was made. During a span of 2 years preceding this study, the same TOC analyzer was used for DOC measurement of various samples, including reclaimed and secondary-treated wastewaters. A review of approximately 2 500 triplicate analyses (DOC $\leq$ 15.00 mg/L) showed a CV of 1.0%. If DOC concentrations of the dilution water used for seed control are low ($\leq$ 0.20 mg/L), the pooled SD of the initial and final DOC measurements of a sample can be used to estimate the variability caused by the instrument. Based on the CV of 1.0% and the actual DOC data before and after incubation of 72 samples used in the precision study, the range of pooled SD caused by the DOC measurements is 0.05 to 0.19 mg/L, which agrees with the range yielded by Equation 3. Accordingly, it seems that the variability in BDOC analysis caused by personnel and method are small, and the precision of the modified BDOC protocol is primarily dependent on the precision of the DOC measurements.

It is difficult to determine the accuracy of the modified BDOC protocol because it is a bioassay method and the true value can never be known. Two biodegradable compounds, dextrose and sodium acetate, were used to prepare standard solutions having DOC concentrations of 1.40 mg/L and 0.80 mg/L, respectively. The BDOC results in Table 1 show that the predictions were very accurate. Recovery ranged from 94.3 to 101.4% for dextrose standard solution and from 96.4 to 101.2% for sodium acetate standard solution. The protocol should provide even more accuracy with standards that have higher DOC concentrations. Response of the modified BDOC protocol to samples containing standard compounds with higher DOC concentrations is being studied.

Inoculum Origin and Size. Table 2 shows a comparison of the BDOC values determined using inoculum from two different sources: BAC filter effluent and sand filter effluent. The results for this case show that the BDOC measurement is not a function of the inoculum origin. Most of the difference between the two inocula are within the method's precision range, and their significance levels (Student's *t*-test) are $\geq$ 0.05. For a later procedure, it was decided to inoculate the sample with the unfiltered sample. For samples that might not contain sufficient microorganisms to serve as an inoculum, such as ozonated samples, the BAC filter inoculum was used.

Three inoculum sizes were investigated, and the results are shown in Table 3. The inoculum size does not significantly affect the BDOC determination for these conditions. The differences between the BDOC values using different inoculum sizes (2 mL versus 4 mL and 2 mL versus 1 mL) fall within the method's precision and are insignificant. An inoculum size of 2 mL was chosen for the remainder of the study.

Filtration after Incubation. Microbial growth occurs during the BDOC procedure. To determine whether the cells interfere with the procedure or there is a significant TOC associated with cell mass, a series of experiments was performed with and without glass-fiber filtration before final TOC analysis. Table 4 shows the results, which indicate that filtration had no significant effect. For simplicity and convenience, the modified BDOC procedure therefore does not require filtration of the sample after the incubation.

Agitation of Samples, Temperature of Incubation, and Kinetics of the Protocol. It was expected that agitating samples or incubating at a higher temperature would reduce the incubation period. Fourteen reclaimed-wastewater samples and 39 secondary-effluent samples were used to study the effects of agitation and incubation temperature on protocol kinetics. Agitation was provided by leaving 30% headspace in the incubation bottle and shaking at 100 r/min throughout the incubation. Simultaneous determination of SBOD could not be performed with this procedure. A temperature of 37°C was chosen because it is the upper limit of the mesophilic range. Four different incubation conditions (agitation at 20°C, no agitation at 20°C, agitation at 37°C, and no agitation at 37°C) were studied. Duplicates were run for all conditions. Kinetics were investigated by collecting samples for TOC analysis at 5, 10, 15, and 20 days in addition to the final determination at 28 days. The BDOC results (mean values of duplicates) for different conditions were compared.

Significance levels (*t*-test) of the differences in BDOC concentrations measured for agitated and nonagitated reclaimed-wastewater samples were computed. At each temperature, only 1 of 70 observations had a significance level below 0.05. The significance levels of the differences were all above 0.05 at 15, 20, and 28 days of incubation. Thus, at both incubation temperatures, agitation had no effect on the protocol kinetics (data not shown).

Figure 1 shows the significance levels of the differences in BDOC concentrations of reclaimed-wastewater samples when incubated at 20°C and 37°C (regardless of agitation condition because it has no effect on BDOC determination). Points falling below the horizontal line at 0.05 are significant at a significance level of 0.05 using the one-tailed *t*-test. All 28 observations had

Table 2—Inoculum origin effect on BDOC determination.

Sampling date	Treatment unit	DOC, mg/L	BDOC, mg/L		BDOC difference, mg/L	Significance level of the difference (*t*-test)
			2 mL BAC filter inoculum	2 mL sand filter inoculum		
10/13/94	Sand filter	8.49	1.61	1.28	0.33	0.07
	Ozonation column 5	5.89	1.85	1.91	0.06	0.31
	BAC filter	4.71	0.67	0.40	0.27	0.05
10/25/94	Sand filter	8.67	2.26	2.02	0.24	0.12
	Ozonation column 5	8.36	2.95	3.34	0.39	0.10
	BAC filter	6.40	1.56	1.56	0.00	0.50
11/01/94	Sand filter	7.45	0.98	1.23	0.25	0.08
	Ozonation column 5	6.99	2.18	2.10	0.08	0.28
	BAC filter	5.05	0.85	0.80	0.05	0.26

significance levels below 0.05 at 5 and 10 days (14 observations for each incubation time). Only two and four observations, respectively, had significance levels above 0.05 at 15 and 20 days. At 28 days, all 14 observations had significance levels above 0.05, indicating that incubating reclaimed-wastewater samples at the two temperatures resulted in significantly different BDOC concentrations at 5, 10, 15, and 20 days of incubation. However, the final BDOCs or BDOCs at 28 days of incubation at the two temperatures were not significantly different.

The effect of incubation temperature on protocol kinetics is further shown in Figure 2, which is a plot of normalized mean BDOC (mean of BDOCs exerted at time t, regardless of agitation condition, divided by the mean of BDOCs exerted after 28 days of incubation at the same temperature, regardless of agitation condition) versus incubation time. The error bars represent SDs generated from normalized mean BDOC values of 14 samples. Figure 2 relies on the assumption that the BDOC exerted after 28 days of incubation ($BDOC_{28}$) was approximately equal to ultimate BDOC ($BDOCu$). It was speculated that BDOC exertion or DOC decrease during the incubation would follow first-order kinetics. Relying on the same assumption ($BDOC_{28} \approx BDOCu$), logarithmic transformations of the remaining fractions of BDOC (1 - mean value in Figure 2) were performed. The slope of a linear regression between the transformed values and incubation time, multiplied by -1, is an initial estimate of the first-order rate constant (k). This initial value was used to calculate $BDOC_{28}/BDOCu$. Then, $BDOCt/BDOCu$ values were estimated as the mean value in Figure 2 multiplied by $BDOC_{28}/BDOCu$. A new k value was obtained from a new linear regression between $\ln[1 - BDOCt/BDOCu]$ and incubation time. The iteration was performed until there was no change in the k value.

Figure 3 shows the final results of the iterations; the k values were 0.024 and 0.095 d^{-1} for 20°C and 37°C, respectively. These rate constants were used to calculate $BDOCt/BDOCu$ values, and the results were compared to the actual values (mean value in Figure 2 multiplied by $BDOC_{28}/BDOCu$). The $BDOC_{28}/BDOCu$ values were 0.49 and 0.93 for 20°C and 37°C, respectively. According to the comparisons (residuals) and $BDOC_{28}/BDOCu$ values, only the BDOC exertion at 37°C followed first-order kinetics. This may be because the inoculum size was small; therefore, the incubation at 20°C had a lag period.

Similar results were obtained when secondary-effluent samples were used to study the agitation effect on the BDOC exertion rate. Agitation did not accelerate the exertion of BDOC (data not shown). Incubation temperature had a pronounced effect on the BDOCs of secondary-effluent samples during the incubation. Unlike the results shown in Figure 2, the differences were significant (<0.05, t-test) even at the end of the incubation. Figure 4, which is a plot of normalized mean BDOC (mean of

Table 3—Inocolumn size effect on BDOC determination.

Sampling date	Treatment unit	DOC, mg/L	BDOC, mg/L			BDOC difference, mg/L	Significance level of the difference (*t*-test)
			1 mL BAC filter inoculum	2 mL BAC filter inoculum	4 mL BAC filter inoculum		
08/13/94	Sand filter	9.66	—	2.87	2.98	0.11	0.26
	Ozonation column 5	8.04	—	2.57	2.68	0.11	0.25
	BAC filter	6.06	—	1.88	1.85	0.03	0.41
09/09/94	Sand filter	7.12	—	1.71	1.98	0.27	0.10
	Ozonation column 5	6.84	—	2.04	2.04	0.00	0.50
	BAC filter	5.32	—	0.90	0.83	0.07	0.26
10/06/94	Sand filter	7.26	1.12	1.16	—	0.04	0.31
	Ozonation column 5	9.44	4.57	4.58	—	0.01	0.48
	BAC filter	5.29	1.51	1.61	—	0.10	0.21

Table 4—Effect of inoculum filtration on BDOC determination.

Sampling date	Treatment unit	DOC, mg/L	BDOC, mg/L		BDOC difference, mg/L	Significance level of the difference (*t*-test)
			With filtration	Without filtration		
08/13/94	Sand filter	9.66	2.87	3.03	0.16	0.20
	Ozonation column 1	8.63	2.44	2.20	0.24	0.13
	Ozonation column 2	8.66	2.59	2.79	0.20	0.16
	Ozonation column 3	8.35	2.84	2.68	0.16	0.20
	Ozonation column 4	8.24	2.77	2.53	0.24	0.14
	Ozonation column 5	8.04	2.57	2.73	0.16	0.19
	BAC filter	6.06	1.88	1.85	0.03	0.39

BDOCs at time t regardless of agitation condition divided by mean of $BDOC_{28}$ exerted at 37°C regardless of agitation condition), illustrates the differences. At 28 days, the BDOC exerted at 20°C was only 75% (± 12%) of the BDOC exerted at 37°C. The assumption that $BDOC_{28}$ is approximately equal to BDOCu was not true for this case. This difference may have occurred because secondary-effluent samples are more recalcitrant than ozonated reclaimed-wastewater samples. The modified BDOC protocol is being investigated to detect the biodegradability of ozonated secondary effluents. The results will be reported in a future publication.

Investigation of the BDOC exertion rate constants for secondary-effluent samples followed the same process described above for reclaimed-wastewater samples, and the results were similar. Only the BDOC exertion at 37°C agrees with the first-order model, and the $BDOC_{28}$/BDOCu value is 0.85. The k value of 0.068 d^{-1} for 37°C (data not shown) indicates that the BDOC exertion of secondary-effluent samples is much slower than the BDOC exertion of the ozonated, reclaimed-wastewater samples (k value of 0.095 d^{-1}). The incubation temperature of 20°C was still chosen for the modified protocol because it is the standard temperature that has been used in most of the analytical procedures, including the BOD test. In addition, considering BOD_5, which is only 68% of BODu, it is believed that BDOC exerted after 28 days of incubation (approximately 64% of BDOCu) is sufficient to indicate secondary-effluent quality (Khan *et al.*, 1998).

Simultaneous Determinations of Biodegradable Dissolved Organic Carbon and Soluble Biochemical Oxygen Demand. The protocol was used successfully for simultaneous determinations of BDOC and SBODu of reclaimed-wastewater samples. Nitrification does not affect BDOC determination, but nitrification inhibitors such as 2-chloro-6-(trichloromethyl) pyridine and allylthiourea will interfere with the BDOC exerted during the procedure. The BDOC and $SBOD_5$ of secondary-effluent samples could not be determined simultaneously because oxygen consumption in these samples after 5 days of incubation did not meet the depletion criteria (≥2 mg/L). Inoculating the sample with 2 mL of the unfiltered sample might not have provided an adequate seed for $SBOD_5$ measurement. This problem can be solved by increasing the inoculum size and/or using a more concentrated inoculum; however, for simultaneous determinations of BDOC and $SBOD_5$, separation of microorganisms after the incubation will be required. Inclusion of cell-separation techniques, such as filtration or centrifugation, in the modified BDOC procedure is being studied. It is also expected that increasing the inoculum size or using a more concentrated inoculum will eliminate the lag phase and reduce the incubation period.

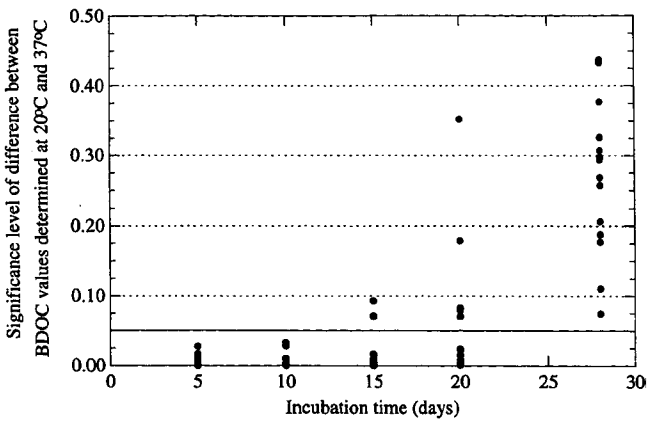

Figure 1—Incubation temperature effect on determination of BDOC in reclaimed wastewater samples.

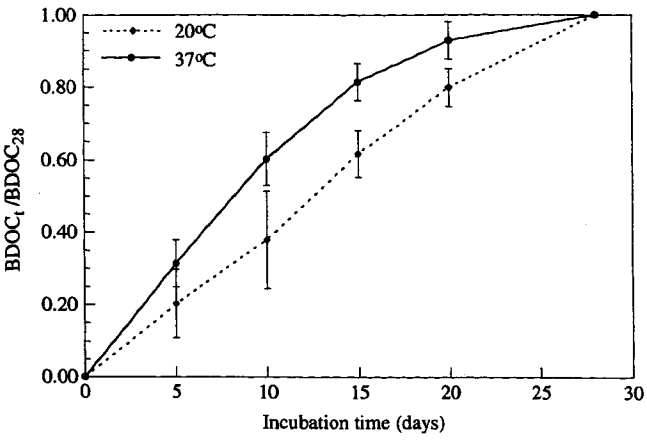

Figure 2—Incubation temperature effect on the protocol kinetics of reclaimed wastewater samples.

Conclusions

A modified bioassay protocol for measuring BDOC in water samples with moderately low DOC, such as reclaimed and secondary-treated wastewaters (4 to 15 mg of DOC/L), has been developed from existing batch BDOC protocol and BOD techniques. The development of the modified procedure was focused on the adequacy of DO throughout the incubation period and simultaneous determinations of DOC, BDOC, and SBOD. The primary advantages of the modified BDOC protocol when compared to BOD and COD tests are higher precision and higher sensitivity.

Glass-fiber filters were used in the modified protocol instead of 0.22-μm CA membrane filters because the membrane filters release a substantial amount of organic carbon, which interferes with the procedure. The modified protocol provides good reproducibility. The precision of the protocol ranges from 0.05 to 0.18 mg/L, while the MDL is approximately 0.15 mg/L. The precision of the new BDOC method is much better than that of BOD and COD methods. The BDOC measurement is not sensitive to inoculum origin and size. Glass-fiber filtration of sample at the end of the incubation has an insignificant effect on the BDOC determination.

Shaking the incubation bottle with 30% gas volume at 100 r/min does not accelerate the kinetics of the exertion. The final BDOC concentrations ($BDOC_{28}$) of reclaimed-wastewater samples provided by incubation temperatures of 20°C and 37°C are not different. For secondary-effluent samples, $BDOC_{28}$ exerted at 20°C is only 75% of $BDOC_{28}$ exerted at 37°C and 64% of BDOC*u*. These values may result from the nature of secondary effluents, which are more biorefractory than reclaimed wastewaters investigated in this research. It was decided to adopt the incubation temperature of 20°C for the modified BDOC procedure because it is the laboratory reference temperature used for the BOD test and most of the water quality analyses. The first-order model can be used to describe the BDOC exertion kinetics only for the incubation at 37°C.

Using the modified BDOC protocol, simultaneous determinations of DOC, BDOC, and $SBOD_u$ of reclaimed wastewaters

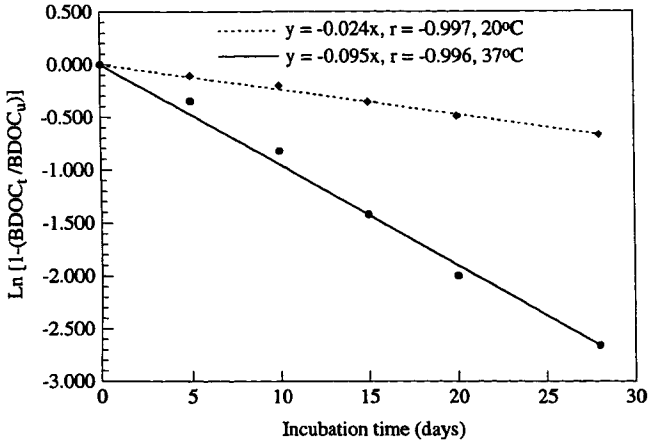

Figure 3—Determination of BDOC exertion rate constants of reclaimed wastewater samples by the first-order model.

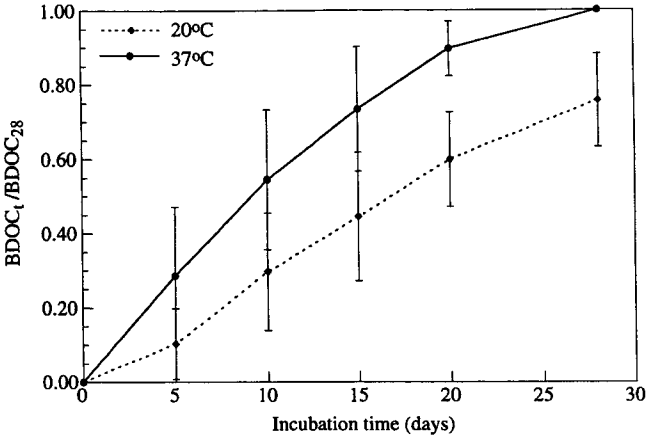

Figure 4—Incubation temperature effect on the protocol kinetics of secondary effluent samples.

can be achieved. Unfortunately, $SBOD_5$ cannot be determined simultaneously with DOC and BDOC of secondary effluents because of inadequate inoculum. The protocol is currently being refined by increasing the inoculum size or using a more concentrated inoculum and adding a cell-separation step. A shorter incubation period is also anticipated.

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