FACTORS INFLUENCING BIODEGRADABLE DISSOLVED ORGANIC CARBON MEASUREMENT

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ABSTRACT: The inocula requirements and other experimental conditions for the biodegradable dissolved organic carbon (BDOC) procedure have been investigated and optimized to facilitate its use as a routine parameter for characterizing treatment plant performance. Initial inocula of unfiltered secondary effluent, commercial biochemical oxygen demand seed, mixed-liquor suspended solids (MLSS), and incubation temperatures of 20 and 37°C were examined using standard solutions of known DOC, treatment plant effluents, and ozonated treatment plant effluents. Among all inocula tested, MLSS provides the fastest BDOC exertion rate. It is possible to measure BDOC within five days using a larger volume of MLSS inoculum. The results from this research may be used to support the development of a standard BDOC method for water quality assessment.

INTRODUCTION

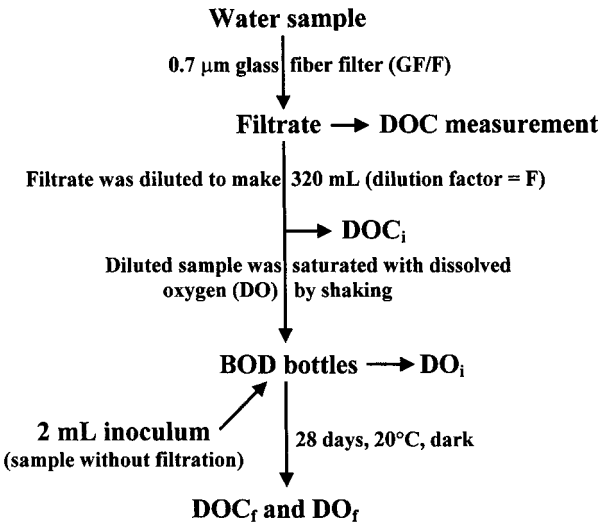
The biodegradability of organic matter in both raw and treated waters continues to be a topic of interest. This is because the residual biodegradable organic matter (BOM) in treated water can serve as a substrate that promotes the regrowth of microorganisms. Many researchers (Rittmann and Snoeyink 1984; Kemmy et al. 1989; Huck 1990; Servais et al. 1993) have suggested that limiting the amount of BOM in treated water is an effective strategy to minimize microbial regrowth, which may reduce the initial disinfectant dose and consequent generation of disinfection by-products.

Biodegradable dissolved organic carbon (BDOC) has been a widely used parameter for quantifying the amount of BOM in drinking water during the past decade. All BDOC procedures rely on the reduction of DOC following the exposure of water samples to either suspended or media-attached microorganisms (Servais et al. 1987, 1989; Joret et al. 1988; Lucena et al. 1990; Mogren et al. 1990; Ribas et al. 1991; Frias et al. 1992; Kaplan and Newbold 1995). The BDOC has been used to indicate the efficiency of water-treatment processes, such as biological activated carbon systems, as well as the quality of raw and treated waters.

In the wastewater-treatment field, Hiser and Busch (1964) introduced a test similar to BDOC for soluble wastes, called total biological oxygen demand. The test measures the reduction of chemical oxygen demand (ΔCOD) as a function of time in a batch system. Gaudy (1972) suggested that the concept of ΔCOD can be extended to continuous-flow systems for biological treatment design and operation and water quality assessment. Gaudy and Gaudy (1988) later stated that not only the ΔCOD but the reduction of total organic carbon (ΔTOC) also can be used as a tool in a bioassay to quantify the amount of the organic matter that can be metabolized by acclimated microorganisms; however, the concept did not receive widespread acceptance.

Khan et al. (1998a) modified a BDOC batch procedure (Servais et al. 1989) used in the drinking water industry for determining BDOC in waters with moderate DOC (4–15 mg/L) such as reclaimed and secondary treated wastewaters. A diagram of the modified BDOC batch procedure is shown in Fig. 1. By following the modified procedure, concurrent determinations of three water quality parameters, DOC, biochemical oxygen demand (BOD), and BDOC, are possible. Khan et al. (1998a) also showed that BDOC is a more sensitive and more precise parameter than BOD and COD.

This paper presents research that attempts to remove the technical barriers associated with the modified BDOC protocol (Khan et al. 1998a) so that BDOC can be used as a routine parameter for wastewater treatment and reclamation plants. The research focuses on the use of different inoculum types and sizes for a more rapid BDOC determination and simultaneous determinations of BDOC and soluble BOD_5 ($SBOD_5$). Three types of samples, specific compounds, secondary effluents, and ozonated secondary effluents were used in this study. The BDOC concentrations and the kinetics of BDOC exertion



- A seed control (sample b) was prepared the same way by adding 2 mL inoculum to 300 mL of dilution water saturated with DO. DOCs and DOs were recorded as DOC_{bi}, DO_{bi}, DOC_{bf}, and DO_{bf}.

$$BDOC\ (mg/L) = [(DOC_i - DOC_f) - (DOC_{bi} - DOC_{bf})]F$$
$$SBOD_u\ (mg/L) = [(DO_i - DO_f) - (DO_{bi} - DO_{bf})]F$$

- For secondary effluent samples, DO_f was determined after 5 days ($SBOD_5$), and the incubation was continued for another 23 days for BDOC determination.

FIG. 1. Modified BDOC Batch Procedure

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resulting from different inoculum types and sizes were compared.

BACKGROUND

Although Khan et al. (1998a,b) have shown that BDOC can indicate successfully the performance of a wastewater reclamation plant and secondary effluent quality, the modified BDOC batch protocol still suffers from the following limitations and disadvantages.

Long Incubation Time

The modified BDOC protocol procedure requires an incubation time of 28 days. This long incubation time was designed to allow the maximum exertion of BDOC while minimizing the size and the concentration of the inoculum served during the incubation. The minimization of the inoculum size and concentration was intended to limit the release of organic carbon (soluble, nonbiodegradable microbial products), which may cause the underestimation of BDOC.

Lag Period

A kinetic study of the BDOC exertion during the procedure shows that, at the incubation temperature of 20°C, the exertion may lag. The long incubation time also was attributed to the lag. Because the procedure requires an acclimated inoculum, the lag was likely generated by an inadequate number of viable cells provided at the beginning of the incubation.

Inability to Determine BDOC and $SBOD_5$ of Secondary Effluents Simultaneously

Although BOD is not as precise or sensitive as BDOC, it is advantageous to have a BDOC procedure that can provide concurrently BOD information. When the modified BDOC procedure was applied to secondary effluents, the dissolved oxygen consumptions after 5 days of incubation were always less than the criteria of 2.0 mg/L specified by *Standard* (1989). The kinetic study during the incubation also suggested that the amount of BDOC exerted during the first 5 days of the incubation is very small (10.3 ± 9.5% of the final BDOC). This supports the speculation regarding the lag period and insufficient inoculum.

Difference between Final BDOC ($BDOC_{28}$) of Secondary Effluents Incubated at 20 and 37°C

Statistically significant differences (one-tailed t test, $p < 0.05$) in $BDOC_{28}$ of secondary effluents incubated at 20 and 37°C were observed. Similar results were not found for reclaimed wastewaters; the differences in $BDOC_{28}$ incubated at 20 and 37°C were statistically insignificant (one-tailed t test, $p > 0.05$). The $BDOC_{28}$ of the secondary effluents at 20°C was only 75 ± 12% of that exerted at 37°C. This suggests that the rate of reaction is controlling the $BDOC_{28}$ and not the ultimate BDOC. Increasing initial inoculum volume or mass is one way to increase the rate of reaction.

It can be seen that all of these limitations and disadvantages may be attributed to the small-size inoculum (2 mL of unfiltered effluent). Employing a larger inoculum size and/or a more concentrated inoculum should mitigate the limitations and disadvantages; however, separation of microorganisms after the incubation will be required as another step in the procedure.

By increasing the cell mass used during the incubation, Joret et al. (1988) successfully developed a more rapid assay for measuring BDOC in drinking water. In their procedure, a 300-mL water sample was inoculated with 100 g of prewashed biologically active sand collected from a water-treatment plant that does not have prechlorination. The prewashing of the sand is required to reduce the possibility of sample contamination. During incubation at 20°C, the sample was aerated and DOC was measured daily until there was either no change or an increase in DOC, which usually occurred after 3 to 5 days. The difference between initial DOC and minimum DOC was taken as BDOC.

The procedure by Joret et al. (1988) greatly reduces the time of the test because a large amount of biomass is used (biofilm on the surface of the sand). Nevertheless, the procedure is still subject to several weaknesses. First, daily monitoring of DOC is labor and time-consuming. Moreover, although the use of sand as a support medium may not release organic carbon (because of the prewashing), the adsorption of organics (instead of biodegradation) may occur. Finally, the aeration during incubation can strip volatile organic carbon, which may be biodegradable.

EXPERIMENTAL METHODS

In this study, a glass fiber filtration (GF/F, Whatman) step after the incubation was inserted into the modified BDOC batch procedure. Larger volume and/or more concentrated inocula, such as commercial BOD seed (Polyseed, Polybac Corporation, Bethlehem, Pa.) and mixed-liquor suspended solids (MLSS) were explored in addition to the 2-mL unfiltered effluent inoculum. All inocula were used to determine BDOC in standard solutions, secondary effluents, and ozonated secondary effluents. The MLSS inoculum was collected from the same treatment facility as the sample on the last day of the sampling period and used within 24 h without prerinsing. After the inoculum was brought back to the laboratory, it was aerated continuously to maintain aerobic condition. The well-mixed inoculum was introduced to the sample using a wide-tip pipette. For standard solutions, the effluent and MLSS inocula were collected from the Chevron refinery wastewater-treatment facility in El Segundo, Calif. The kinetics of BDOC exertion for all inocula also were observed.

Standard Solution Experiment

Sodium acetate and phenol standard solutions were studied. Each compound was used to prepare three solutions that had approximate DOC concentrations of 2, 5, and 10 mg/L (a total of six standard solutions), respectively. Four inocula, 2 mL of unfiltered effluent, 10 mL of unfiltered effluent, 2 mL (recommended by the manufacturer) of commercial BOD seed, and 2 mL of MLSS were tested. Using each of the four inocula, the BDOC test was performed without SBOD determination at two incubation temperatures: 20 and 37°C (4 × 2 experimental design). To investigate the BDOC exertion kinetics, filtered samples were collected and measured for TOC at 1, 2, 3, 4, 5, 7, 10, 15, 20, and 28 days.

Secondary Effluent Experiment

Unchlorinated secondary effluent grab samples were collected daily for 4 consecutive days from each of the following three low solids retention time (SRT) high-purity oxygen (HPO) activated-sludge municipal wastewater-treatment plants (WWTPs):

- Hyperion WWTP, Playa del Rey, Calif., 8.8 m^3/s, SRT = 1.4 days, operated by the Los Angeles Bureau of Sanitation
- Joint Water Pollution Control Plant, Carson, Calif., 8.8 m^3/s, SRT = 2.4 days, operated by the Sanitation Districts of Los Angeles County

- Sacramento Regional WWTP, ElK Grove, Calif., 7.4 m^3/s, SRT = 2.0 days, operated by the Sacramento Regional County Sanitation District

The foregoing flow rates are nominal flow rates and only the secondary portions of the Hyperion plant and Joint Water Pollution Control Plant are reported. The experimental design for these sets of samples is similar to the design described for the standard solution experiment with two exceptions. The first difference is that the 2-mL MLSS and 2-mL commercial BOD inocula were tested in duplicate at 20°C, and $SBOD_5$ measurement was attempted simultaneously on the duplicate. It was necessary to use a duplicate for the $SBOD_5$ measurement because the bottles for the BDOC measurement were opened daily to take samples. The second difference is that the TOC of filtered samples was measured only at 5, 10, 15, 20, and 28 days when the effluent inocula (2 and 10 mL) were used.

Nonozonated versus Ozonated Secondary Effluent Experiment

Unchlorinated secondary effluent grab samples were collected daily for 2 consecutive days from each of the following three high SRT (fully nitrified) activated-sludge municipal WWTPs, which are operated by the Chino Basin Municipal Water District:

- RP1 WWTP, Ontario, Calif., 1.6 m^3/s, SRT = 15 days
- RP2 WWTP, Chino, Calif., 0.21 m^3/s, SRT = 15 days
- Carbon Canyon WWTP, Chino, Calif., 0.3 m^3/s, SRT = 50 days

These effluent samples have low DOC and low BDOC. The first half of each sample was filtered (GF/F, Whatman) and the second half was filtered and ozonated. The ozonation system consists of an Ozone Research and Equipment Corporation Ozone Generator model 03V10, air feed (Ozone Research and Equipment Corporation, Phoenix, Ariz.), and a 2-L ozonation vessel (Pyrex separatory funnel). All components used are resistant to ozone (Teflon tubing, Teflon and stainless steel fittings, stainless steel flow meter, glass gas-sampling tube with Teflon septum and valves, and sintered-glass diffuser).

The sample collected on the 1st day from each plant was ozonated with an ozone dose of 16–20 mg O_3(g)/L at 5.75 L/min for 40 min. Such a high dose of ozone was provided to insure the maximum increase in biodegradability. The UV absorbance at 254 nm (UV_{254}) was measured at different times to indicate relative biodegradability as the samples were ozonated. It should be noted that the purpose of this experiment was not to investigate an optimum ozone dose but to test the BDOC procedure performance with different inocula. The 1st day samples were used to determine ozone contact time for the 2nd day samples. The ozone contact time was selected to minimize UV_{254}. Influent and effluent gas-phase ozone concentrations and liquid-phase ozone concentrations were monitored. The UV_{254} versus ozonation time for the 1st day samples indicates that the destruction of the UV_{254} absorbing compounds (conjugated double bond) occurred largely in the first 5 min. After 15 min of ozonation, an increase of UV_{254} was observed for the effluent samples from the RP2 and Carbon Canyon plants. Thus, the second day samples were ozonated with the same conditions but for 15 min only.

Without concurrent $SBOD_5$ determination, the BDOC test was performed on samples from both days employing four different inocula, 2 mL of unfiltered sample, 2 mL of MLSS, 5 mL of MLSS, and 10 mL of MLSS at two incubation temperatures (20 and 37°C) on ozonated and nonozonated samples ($4 \times 2 \times 2$ experimental design). The kinetics of BDOC exertion were observed for all cases following the same approach described in the foregoing standard solution experiment section except that the 2-mL effluent inoculated samples were measured for TOC only at 5, 10, 15, 20, and 28 days.

Analyses and Measurements

The BDOC and $SBOD_5$ were determined using the modified BDOC batch protocol (Khan et al. 1998a). A Hewlett-Packard diode array spectrophotometer model 8452A (Hewlett-Packard Company, Palo Alto, Calif.) and a 1-cm quartz cell were used to determine UV_{254}. The spectrophotometer was first adjusted to reach zero absorbance with water blank (deionized water containing less than 0.20 mg DOC/L). Each sample then was analyzed three times (three portions) and the mean value was taken as the UV_{254}. Gas-phase ozone was measured by following the procedure described in Collins et al. (1989). Liquid-phase ozone was determined using the Indigo method as specified in *Standard* (1989).

RESULTS AND DISCUSSION

Standard Solutions

Fig. 2(a) shows the BDOC exertion as a function of time when sodium acetate solution with a DOC concentration of 9.97 mg/L was examined. Complete BDOC exertion or DOC depletion occurred within 5 days for all four inocula at both temperatures. Regardless of the inoculum and the incubation temperature, the procedure was able to provide a precise prediction of BDOC at any time from 5 to 28 days. This would not be possible if the procedure did not include the seed control technique. Fig. 2(b) shows the DOC concentrations created by the inocula. The initial DOC of the blank water before adding the inoculum was 0.12 mg/L. The plots suggest no evidence of continuing DOC release due to the endogenous respiration (soluble microbial products) at any incubation time and temperature for all four inocula; the DOC was relatively constant over the 28-day period. For five other standard solutions, the BDOC exertion curves are all similar to the curves shown in Fig. 2(a) except for the standard solutions with lower DOC concentrations (2 and 5 mg DOC/L); the BDOC was fully exerted in a shorter time (1–3 days, data not shown).

The recoveries of acetate and phenol during the BDOC procedure at incubation times of 5 and 28 days are presented in Table 1. The recoveries at 5 days are shown specifically because it is expected to use the same incubation time as used

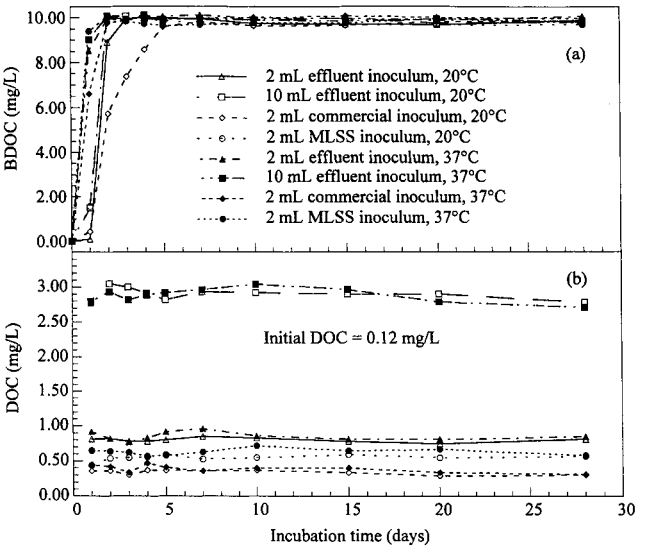

FIG. 2. BDOC Exertion and Inoculum DOC: (a) BDOC of Sodium Acetate with Initial DOC of 9.97 mg/L; (b) DOC Created by Adding Inoculum to Blank (Seed Control)

TABLE 1. Recoveries of Sodium Acetate and Phenol during BDOC Procedure

Inoculum and incubation temperature (1)	RECOVERY (%) = [(BDOC/DOC_0) × 100]						Average ± SD (8)
	DOC_0 = 2 mg/L		DOC_0 = 5 mg/L		DOC_0 = 10 mg/L		
	5 days (2)	28 days (3)	5 days (4)	28 days (5)	5 days (6)	28 days (7)	
(*a*) Sodium acetate standard solutions (actual DOC = 1.94, 4.89, and 9.97 mg/L)							
2 mL Effluent, 20°C	93.8	99.5	99.2	99.4	100.2	99.2	98.6 ± 2.4
10 mL Effluent, 20°C	92.8	96.9	96.5	98.2	99.3	98.4	97.0 ± 2.3
2 mL Commercial, 20°C	99.5	98.5	100.0	100.0	96.3	99.1	98.9 ± 1.4
2 mL MLSS, 20°C	97.4	97.9	99.4	98.8	99.1	97.2	98.3 ± 0.9
2 mL Effluent, 37°C	101.5	101.0	102.7	101.4	100.9	101.1	101.4 ± 0.7
10 mL Effluent, 37°C	99.5	98.5	100.0	99.4	99.5	98.9	99.3 ± 0.5
2 mL Commercial, 37°C	93.3	99.0	99.8	100.4	99.6	100.1	98.7 ± 2.7
2 mL MLSS, 37°C	86.6	95.4	93.3	97.8	96.8	98.0	94.7 ± 4.3
Average ± SD	95.6 ± 4.9	98.3 ± 1.7	98.9 ± 2.8	99.4 ± 1.2	99.0 ± 1.6	99.0 ± 1.2	98.4 ± 2.8
(*b*) Phenol standard solutions (actual DOC = 1.87, 4.60, and 9.81 mg/L)							
2 mL Effluent, 20°C	93.6	98.4	101.3	98.7	95.3	97.8	97.5 ± 2.7
10 mL Effluent, 20°C	97.3	100.5	103.7	103.5	101.3	99.3	100.9 ± 2.5
2 mL Commercial, 20°C	96.3	95.7	97.8	98.7	95.2	99.2	97.2 ± 1.7
2 mL MLSS, 20°C	100.0	94.7	99.8	97.2	98.9	98.9	98.3 ± 2.0
2 mL Effluent, 37°C	100.5	102.1	103.0	101.5	98.9	98.7	100.8 ± 1.7
10 mL Effluent, 37°C	111.8	107.0	104.8	102.8	101.3	101.6	104.9 ± 4.0
2 mL Commercial, 37°C	98.4	97.3	100.4	98.7	97.2	99.1	98.5 ± 1.2
2 mL MLSS, 37°C	86.1	91.4	96.1	97.6	95.7	97.5	94.1 ± 2.5
Average ± SD	98.0 ± 7.2	98.4 ± 4.8	100.9 ± 3.0	99.8 ± 2.4	98.0 ± 2.5	99.0 ± 1.2	99.0 ± 4.0

in the BOD procedure. For standard solutions with DOC concentrations of approximately 5 and 10 mg/L, the procedure was able to estimate the BDOC precisely and accurately, assuming that the two compounds are 100% biodegradable. The standard deviations are less than or equal to 3.0% when different inocula and/or different temperatures were used for the same sample.

The BDOC was underestimated when 2 mL of MLSS was employed as an inoculum at 37°C for the approximately 2-mg DOC/L acetate and phenol standard solutions. A case of BDOC overestimation was found when the approximately 2-mg DOC/L phenol standard solution was inoculated with 10 mL of unfiltered effluent and incubated at 37°C. These inaccuracies may be attributed to the unavoidable errors (personnel and method) involved with the BDOC determination and the effect of the errors is more obvious as the initial DOC concentration of the sample decreases. The overall accuracy and precision of the procedure are high (98.4 ± 2.4% for acetate and 99.0 ± 4.0% for phenol).

Fig. 3 shows the effects of the inoculum and incubation temperature on the BDOC exertion for sodium acetate standard solution with a DOC of 9.97 mg/L incubated for 0 to 5 days. A lag period of approximately 1 day was observed for all cases at 20°C whereas the BDOC was exerted either completely or almost completely after 1 day at 37°C. Fig. 3 also indicates that the 2-mL commercial BOD seed was the least effective inoculum (slowest BDOC exertion) at both temperatures. Similar results such as those shown in Fig. 3, were obtained for the other two sodium acetate standard solutions (data not shown).

For phenol standard solutions, longer lag periods of up to 2 days were observed for some cases at 20°C (data not shown). This is likely because acetate is easier to biodegrade than phenol. Even at 37°C, the 2-mL effluent and 2-mL commercial BOD seed inocula also suffered from a lag period for most cases. Quantitative biodegradation kinetics could not be determined because of the limited data; however, it is apparent that the 2-mL commercial BOD seed inoculum provided the slowest degradation. Even though the BOD seed inoculum consists of a broad spectrum of specialized bacteria as specified by the manufacturer, the data seem to indicate that MLSS and secondary effluents are better inocula.

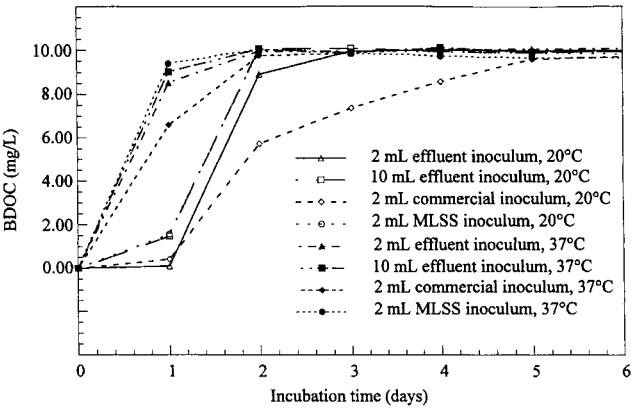

FIG. 3. Effects of Inoculum and Incubation Temperature on Exertion of BDOC for Sodium Acetate Solution with DOC of 9.97 mg/L

Applying any of the four inocula, the modified BDOC protocol responds to the standard solutions with high precision and accuracy after 1–5 days. The 10-mL effluent and 2-mL MLSS inocula react with the substrates (compounds) faster than the other two inocula and are good candidates for use in reducing the incubation time. The reaction of each inoculum to different types of samples (standard solutions versus actual treated wastewater) may not be the same. Therefore, a study on the response of all four inocula to secondary effluent samples also was conducted.

Secondary Effluents

Concurrent determinations of $SBOD_5$ and BDOC were successful only when using the 2-mL MLSS inoculum. When inoculating the undiluted samples with the 2-mL BOD seed inoculum, the reductions of dissolved oxygen were less than the minimum criteria of 2.0 mg/L established by *Standard* (1989). Four additional samples were collected daily for 4 consecutive days from each plant (listed in the methodology section on secondary effluent experiment) for the concurrent determinations. The $SBOD_5$ and $BDOC_5$ (the same bottle) correlate weakly but significantly ($r = 0.61$ and $p < 0.001$, data not shown). A much weaker but significant correlation (r =

0.35 and $p < 0.05$, data not shown) between $SBOD_5$ and $BDOC_{28}$ was found. This is not surprising because there are many factors governing the rate of BOD exertion, and consequently the precision of BOD_5 is very poor. The correlation would be stronger if a longer incubation time for BOD measurement was allowed. This hypothesis is supported by the result presented in Khan et al. (1998b), which shows stronger and significant correlations between ultimate SBOD ($SBOD_{28}$) and $BDOC_{28}$ of reclaimed wastewater samples ($r = 0.70-0.85$ and $p < 0.01-0.0005$).

Fig. 4, which is a plot of normalized BDOC ($BDOC_t/BDOC_{28}$ using 2 mL MLSS at 37°C) versus incubation time (t), shows the effects of inoculum and temperature on BDOC exertion. Each value shown is an average normalized BDOC of 12 samples. Standard deviations of the normalized BDOCs are not shown in Fig. 4 (as error bars) but in Table 2 because they visually interfere with the other information. After testing a few samples, it was discovered that the DOC reductions in the samples inoculated with 2 mL of commercial BOD seed were very small, and the daily DOC monitoring during the first 4 days was later canceled. The rate of BDOC exertion can be ranked visually from the fastest to the slowest as follows: 2 mL MLSS at 37°C, 10 mL effluent at 37°C, 2 mL effluent at 37°C, 2 mL MLSS at 20°C, 10 mL effluent at 20°C, 2 mL commercial BOD seed at 37°C, 2 mL effluent at 20°C, and 2 mL commercial BOD seed at 20°C. When plotting DOC in the seed control against incubation time, the results are similar to Fig. 2(b); DOC remains relatively constant throughout the incubation (data not shown).

The average normalized BDOCs at 28 days shown in Fig. 4 can be divided into two groups: the group with the average normalized BDOC from 0.61 to 0.73 and the group with the average normalized BDOC from 0.90 to 1.00. The first group includes all four inocula at 20°C and the commercial BOD seed inoculum at 37°C; the second group consists of the other three inocula at 37°C. Only the 2-mL commercial BOD seed inoculum does not predict distinctly higher BDOC at 37°C. This suggests an inability of the commercial BOD seed inoculum to utilize some organic compounds that are unable for the other inocula at 37°C. To be able to degrade those compounds, the commercial BOD seed inoculum may need to be acclimated.

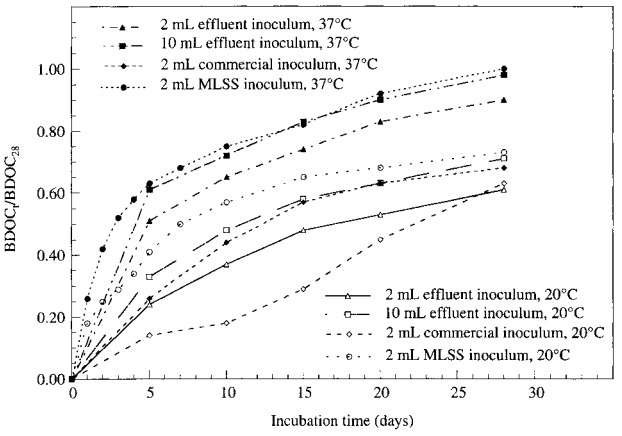

FIG. 4. Effects of Inoculum and Incubation Temperature on Exertion of BDOC for Secondary Effluent Samples from HPO Plants

It can be seen from Fig. 4 that the 2-mL MLSS is the most effective inoculum (fastest rate in the group) and therefore is the main candidate to replace the 2-mL effluent inoculum for shortening the incubation time. The lag period was not observed when inoculating with 2 mL of MLSS at both incubation temperatures. The disadvantages of the commercial BOD inoculum are noted. Although the 10-mL effluent inoculum was able to degrade almost as rapidly as the 2-mL MLSS inoculum, the possible error involved in the use of a large volume of 10 mL (introducing more DOC to the sample) is a potential disadvantage. The use of a larger volume inoculum should be practiced only when a quantitative benefit can be obtained (faster rate or shorter incubation time).

Additional studies were performed to determine if $BDOC_{28}$ could be estimated from $BDOC_5$ (Khan 1997). The $BDOC_5$ using the 2-mL MLSS inoculum at 37°C was not significantly different than the $BDOC_{28}$ using the 2-mL effluent inoculum at 20°C (t test, $p > 0.05$). Strong and significant correlation also existed between the two conditions ($r = 0.89$, $p < 0.0005$). Unfortunately, the $BDOC_5$ using the 2-mL MLSS inoculum at 20°C was significantly lower than the $BDOC_{28}$ using the 2-mL effluent inoculum at 20°C (t test, $p < 0.05$) and the BDOC values between the two conditions correlated weakly ($r = 0.64$, $p < 0.025$). It may be possible to use 5-day BDOC inoculated with 2 mL of MLSS at 37°C to estimate 28-day BDOC at 20°C but further development is required.

Incubating at 37°C prohibits the simultaneous determinations of $SBOD_5$ and BDOC. To preserve the simultaneous determinations, it was decided to abandon the 10-mL effluent and 2-mL commercial BOD seed inocula and to focus on the utilization of larger sizes of MLSS inoculum to increase the exertion at 20°C. As a consequence, the use of 5 and 10 mL MLSS inocula for determining BDOC in nonozonated and ozonated secondary effluents was later studied along with the use of the 2-mL effluent and 2-mL MLSS inocula.

Nonozonated versus Ozonated Secondary Effluents

The effluent samples from the high SRT plants (listed in the experimental methods section on nonozonated versus ozonated

TABLE 2. Average Normalized BDOC and Standard Deviation for Secondary Effluent Samples from HPO Plants

Incubation time (days) (1)	AVERAGE NORMALIZED BDOC ± SD							
	20°C				37°C			
	2 mL effluent inoculum (2)	10 mL effluent inoculum (3)	2 mL commercial inoculum (4)	2 mL MLSS inoculum (5)	2 mL effluent inoculum (6)	10 mL effluent inoculum (7)	2 mL commercial inoculum (8)	2 mL MLSS inoculum (9)
1	—	—	—	0.18 ± 0.05	—	—	—	0.26 ± 0.04
2	—	—	—	0.25 ± 0.04	—	—	—	0.42 ± 0.04
3	—	—	—	0.29 ± 0.07	—	—	—	0.52 ± 0.06
4	—	—	—	0.34 ± 0.08	—	—	—	0.58 ± 0.05
5	0.24 ± 0.05	0.33 ± 0.05	0.14 ± 0.04	0.41 ± 0.08	0.51 ± 0.08	0.61 ± 0.06	0.26 ± 0.06	0.63 ± 0.05
7	—	—	—	0.50 ± 0.07	—	—	—	0.68 ± 0.05
10	0.37 ± 0.07	0.48 ± 0.06	0.18 ± 0.06	0.57 ± 0.07	0.65 ± 0.10	0.72 ± 0.09	0.44 ± 0.06	0.75 ± 0.05
15	0.48 ± 0.07	0.58 ± 0.05	0.29 ± 0.07	0.65 ± 0.03	0.74 ± 0.08	0.83 ± 0.06	0.57 ± 0.07	0.82 ± 0.04
20	0.53 ± 0.07	0.63 ± 0.05	0.45 ± 0.06	0.68 ± 0.05	0.83 ± 0.08	0.90 ± 0.09	0.63 ± 0.08	0.92 ± 0.02
28	0.61 ± 0.06	0.71 ± 0.06	0.63 ± 0.04	0.73 ± 0.07	0.90 ± 0.07	0.98 ± 0.07	0.68 ± 0.07	1.00

secondary effluent experiment), which are biorefractory (low BDOC), were used for several reasons. If a larger volume of MLSS inoculum can reduce the BDOC incubation time for these samples, it should be able to do that for other secondary effluents as well. When comparing the BDOC exertion rate between nonozonated and ozonated samples of the same effluent, using the recalcitrant secondary effluents should offer the most distinctive results. Finally, in the reclamation facilities with an ozone/granular activated carbon system, the influent of the system usually is recalcitrant (ozonated secondary effluents represent reclaimed wastewaters).

The DOC concentrations in the seed controls for the samples from the RP1 plant for four inocula at both temperatures used in this experiment were plotted against incubation time as shown in Fig. 5. Tremendous release of DOC caused by cell decay was observed in the seed control using the 10-mL MLSS inoculum at both temperatures. This release occurred as early as 10 days. The seed control data for the samples from the other two plants also are similar; significant release of DOC was encountered only when using the 10-mL MLSS inoculum (data not shown). These seed control data produced inconsistent and unreliable BDOC results. The degree of cell decay (endogenous respiration) in the sample may not be the same as in the seed control because the substrate concentrations are different. At the time of DOC release, the seed control is in the death phase (either accelerating or decelerating autodigestion) of the growth curve whereas the sample may be in other growth phases. It is important to stress that the purpose of having a seed control in the procedure is to account for the additional DOC in the sample caused by the properties (impurities) of the inoculum and not endogenous respiration. Accounting for the release of DOC caused by endogenous respiration in the sample may be possible but will be too complicated for the procedure.

It was decided to normalize $BDOC_t$ for all four inocula at both temperatures with the $BDOC_{28}$ exerted at 37°C using the 5-mL MLSS inoculum and not to report the normalized $BDOC_{t>10}$ for the samples inoculated with 10 mL of MLSS. Figs. 6(a and b) (for nonozonated and ozonated effluents, respectively) show the average normalized BDOC (six samples) versus incubation time. Standard deviations of the normalized BDOCs are presented separately in Table 3 because they visually impair the other information. Fig. 6 clearly illustrates the effects of inoculum and incubation temperature on BDOC exertion rate for both nonozonated and ozonated samples. Furthermore, a comparison between Figs. 6(a and b) indicates that the exertion rate of the nonozonated samples is much slower than that of the ozonated samples of the same effluent using the same inoculum at the same temperature.

Using the 2-mL effluent inoculum on nonozonated samples at 20°C yields very low $BDOC_{28}$ values (barely above the detection limit of 0.15 mg/L). This indicates that the 2-mL effluent inoculum from high SRT plants sometimes may not provide an adequate number of cells and may underestimate the $BDOC_{28}$. As can be seen in Fig. 6(b), there is a difference in the average normalized $BDOC_{28}$ using the 2-mL effluent inoculum at different temperatures. At a significance level of 0.05 (t test), the $BDOC_{28}$ differences between the two temperatures using the 2-mL effluent inoculum are not significant (Khan 1997). This verifies that the difference between the final BDOC ($BDOC_{28}$) of secondary effluents incubated at 20 and 37°C is because of both inadequate inoculum and the recalcitrant nature of secondary effluents, especially those from high SRT plants.

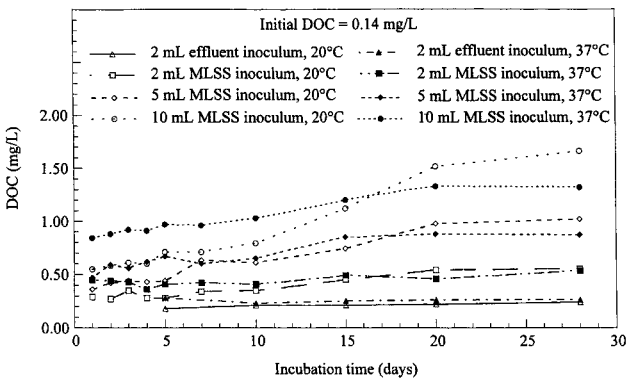

FIG. 5. DOC Produced by Inoculum in Blank Water (Seed Control) for Samples from RP1 WWTP

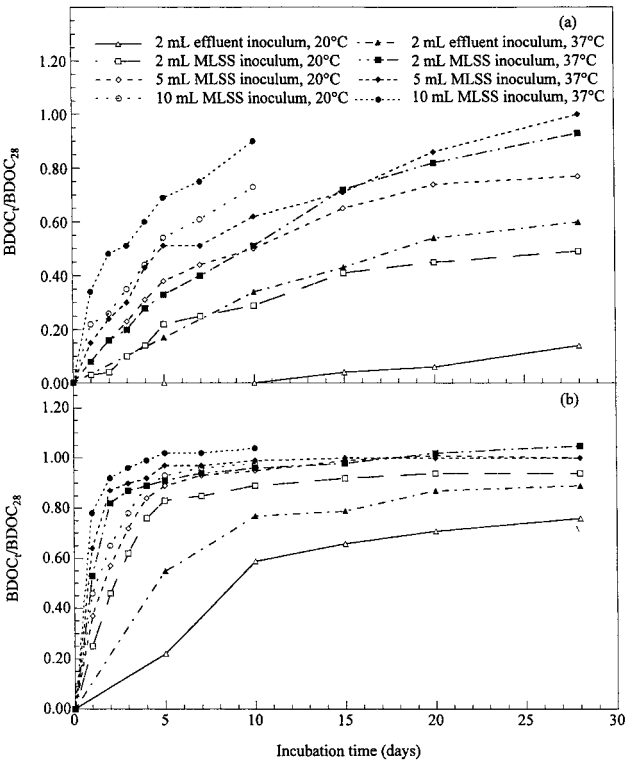

FIG. 6. Effects of Inoculum and Incubation Temperature on Exertion of BDOC for Secondary Effluents from High SRT Plants: (a) Nonozonated Samples; (b) Ozonated Samples

The use of the 10-mL MLSS inoculum for determining BDOC in nonozonated secondary effluent provided the fastest exertion among inocula tested. To retain the ability to perform simultaneous determinations of BDOC and $SBOD_5$, the BDOC procedure has to be performed at 20°C. According to Fig. 6(a), the average normalized $BDOC_5$ using the 10-mL MLSS inoculum at 20°C is about the same as the average normalized $BDOC_{28}$ using the 2-mL MLSS inoculum at 20°C. Khan (1997) showed that the $BDOC_5$ using the 10-mL MLSS inoculum at 20°C and the $BDOC_{28}$ using the 2-mL MLSS inoculum at 20°C are not significantly different (t test, $p > 0.05$). This indicates that the $BDOC_{28}$ using the 2-mL MLSS inoculum at 20°C can be reached in 5 days using the 10-mL MLSS inoculum at 20°C. Because of a small number of samples (six samples), a weak and insignificant correlation ($r = 0.64$, $p < 0.10$) was observed between the $BDOC_5$ using the 10-mL MLSS inoculum at 20°C and the $BDOC_{28}$ using the 2-mL MLSS inoculum at 20°C (Khan 1997).

Even though it has not been tested the 10-mL MLSS inoculum should be able to provide the simultaneous determinations of $SBOD_5$ and BDOC of other secondary effluents, such as those from low SRT and/or HPO plants, as the 2-mL MLSS inoculum because more BDOC is exerted. As an example, adding 10 mL of 1,000 mg/L MLSS in a BOD bottle

TABLE 3. Average Normalized BDOC and Standard Deviation for Nonozonated Secondary Effluent Samples from High SRT Plants

Incubation time (days) (1)	AVERAGE NORMALIZED BDOC ± SD							
	20°C				37°C			
	2 mL effluent inoculum (2)	2 mL MLSS inoculum (3)	5 mL MLSS inoculum (4)	10 mL MLSS inoculum (5)	2 mL effluent inoculum (6)	2 mL MLSS inoculum (7)	5 mL MLSS inoculum (8)	10 mL MLSS inoculum (9)
(*a*) Nonozonated samples								
1	—	0.03 ± 0.05	0.08 ± 0.08	0.22 ± 0.07	—	0.08 ± 0.05	0.15 ± 0.08	0.34 ± 0.11
2	—	0.04 ± 0.06	0.16 ± 0.07	0.26 ± 0.06	—	0.16 ± 0.04	0.24 ± 0.07	0.48 ± 0.03
3	—	0.10 ± 0.06	0.23 ± 0.08	0.35 ± 0.05	—	0.20 ± 0.04	0.30 ± 0.07	0.51 ± 0.09
4	—	0.14 ± 0.06	0.31 ± 0.07	0.44 ± 0.05	—	0.28 ± 0.09	0.43 ± 0.05	0.60 ± 0.07
5	0.00 ± 0.00	0.22 ± 0.06	0.38 ± 0.04	0.54 ± 0.04	0.17 ± 0.11	0.33 ± 0.08	0.51 ± 0.06	0.69 ± 0.06
7	—	0.25 ± 0.02	0.44 ± 0.06	0.61 ± 0.03	—	0.40 ± 0.07	0.51 ± 0.08	0.75 ± 0.10
10	0.00 ± 0.01	0.29 ± 0.04	0.50 ± 0.09	0.73 ± 0.11	0.34 ± 0.16	0.51 ± 0.08	0.62 ± 0.05	0.90 ± 0.11
15	0.04 ± 0.03	0.41 ± 0.07	0.65 ± 0.09	—	0.43 ± 0.16	0.72 ± 0.12	0.71 ± 0.06	—
20	0.06 ± 0.03	0.45 ± 0.05	0.74 ± 0.10	—	0.54 ± 0.18	0.82 ± 0.14	0.86 ± 0.06	—
28	0.14 ± 0.04	0.49 ± 0.06	0.77 ± 0.14	—	0.60 ± 0.21	0.93 ± 0.11	1.00	—
(*b*) Ozonated samples								
1	—	0.25 ± 0.09	0.37 ± 0.10	0.46 ± 0.10	—	0.53 ± 0.10	0.64 ± 0.08	0.78 ± 0.05
2	—	0.46 ± 0.06	0.57 ± 0.09	0.65 ± 0.10	—	0.82 ± 0.05	0.87 ± 0.04	0.92 ± 0.05
3	—	0.62 ± 0.06	0.72 ± 0.08	0.78 ± 0.10	—	0.87 ± 0.03	0.90 ± 0.05	0.96 ± 0.03
4	—	0.76 ± 0.04	0.84 ± 0.06	0.89 ± 0.06	—	0.89 ± 0.04	0.92 ± 0.04	0.99 ± 0.04
5	0.22 ± 0.07	0.83 ± 0.04	0.89 ± 0.05	0.93 ± 0.06	0.55 ± 0.16	0.91 ± 0.02	0.97 ± 0.04	1.02 ± 0.04
7	—	0.85 ± 0.04	0.93 ± 0.04	0.96 ± 0.05	—	0.94 ± 0.03	0.97 ± 0.04	1.02 ± 0.03
10	0.59 ± 0.03	0.89 ± 0.03	0.95 ± 0.03	0.98 ± 0.04	0.77 ± 0.06	0.96 ± 0.03	0.99 ± 0.03	1.04 ± 0.05
15	0.66 ± 0.04	0.92 ± 0.05	0.99 ± 0.04	—	0.79 ± 0.07	0.98 ± 0.03	1.00 ± 0.03	—
20	0.71 ± 0.05	0.94 ± 0.05	1.01 ± 0.04	—	0.87 ± 0.06	1.02 ± 0.06	1.00 ± 0.03	—
28	0.76 ± 0.04	0.94 ± 0.03	1.00 ± 0.03	—	0.89 ± 0.06	1.05 ± 0.06	1.00	—

filled with 290 mL filtered sample would produce an SS concentration of approximately 33 mg/L, which is close to the limit for secondary effluents. This is roughly equivalent to performing the BOD test on some secondary effluents without seeding.

Determination of BDOC in ozonated secondary effluents can be completed in 5 days using any of the MLSS inocula tested at both temperatures. However, employing the 10-mL MLSS inoculum would insure the adequacy of viable cells. Based on the data shown earlier (Fig. 5), using the 10-mL MLSS inoculum, significant release of DOC in the seed control should not appear before 10 days. An MLSS inoculum larger than 10 mL is not recommended because a tremendous release of DOC due to the endogenous respiration in the seed control may occur during the first few days of incubation.

CONCLUSIONS

This paper describes the usage of larger size and/or more concentrated inocula in the modified BDOC procedure for reclaimed and secondary treated wastewaters mainly to obtain a shorter incubation time and to accomplish concurrent determinations of $SBOD_5$ and BDOC. These two features are very important for promoting BDOC as a routinely adopted water quality parameter. The modified BDOC protocol using different inocula was tested with standard solutions, secondary effluents from three HPO plants, and nonozonated and ozonated secondary effluents from three high SRT plants. The kinetics of BDOC exertion were monitored and the results provided by different inocula were compared.

Four inocula, 2 mL of unfiltered effluent, 10 mL of unfiltered effluent, 2 mL of commercial BOD seed, and 2 mL of MLSS, were first employed. At incubation time of 5 and 28 days, all four inocula were able to provide accurate and precise BDOC results when tested with sodium acetate and phenol standard solutions. For secondary effluents from three HPO plants, BDOC and $SBOD_5$ could be determined simultaneously only when 2 mL of MLSS was used as an inoculum. The results show that the incubation time may be reduced to 5 days by inoculating the sample with 2 mL of MLSS and incubating at 37°C. It was decided to explore a larger size of MLSS inoculum at 20°C in order to be able to perform BDOC and $SBOD_5$ determinations concurrently.

Nonozonated and ozonated secondary effluent samples from high SRT plants were inoculated with 2 mL of effluent, 2 mL of MLSS, 5 mL of MLSS, and 10 mL of MLSS. The 10-mL MLSS inoculum provided the fastest exertion rate among all inocula used for determining BDOCs of nonozonated and ozonated secondary effluent samples. It is possible to reduce the incubation time to 5 days by employing the 10-mL MLSS inoculum. It is believed that the 10-mL MLSS inoculum can be used to achieve the simultaneous determinations of BDOC and $SBOD_5$ as well as the 2-mL MLSS inoculum. The 10-mL MLSS inoculum demonstrates the ability and potential to remove the disadvantages associated with the use of the 2-mL unfiltered effluent inoculum. The modified BDOC procedure equipped with this new inoculum is a promising alternative method for characterizing reclaimed and treated wastewaters. The procedure with the new inoculum may be promoted as a routine method; however, more applications and more studies on the performance (precision and accuracy) of the procedure are recommended.

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