



Controls on biochemical oxygen demand in the upper Klamath River, Oregon

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ABSTRACT

A series of 30-day biochemical oxygen demand (BOD) experiments were conducted on water column samples from a reach of the upper Klamath River that experiences hypoxia and anoxia in summer. Samples were incubated with added nitrification inhibitor to measure carbonaceous BOD (CBOD), untreated to measure total BOD, which included demand from nitrogenous BOD (NBOD), and coarse-filtered to examine the effect of removing large particulate matter. All BOD data were fit well with a two-group model, so named because it considered contributions from both labile and refractory pools of carbon: $BOD_t = a_1(1 - e^{-a_0 t}) + a_2 t$. Site-average labile first-order decay rates a_0 ranged from 0.15 to 0.22/day for CBOD and 0.11 to 0.29/day for BOD. Site-average values of refractory zero-order decay rates a_2 ranged from 0.13 to 0.25 mg/L/day for CBOD and 0.01 to 0.45 mg/L/day for BOD; the zero-order CBOD decay rate increased from early- to mid-summer. Values of ultimate CBOD for the labile component a_1 ranged from 5.5 to 28.8 mg/L for CBOD, and 7.6 to 30.8 mg/L for BOD. Two upstream sites had higher CBOD compared to those downstream. Maximum measured total BOD₅ and BOD₃₀ during the study were 26.5 and 55.4 mg/L; minimums were 4.2 and 13.6 mg/L. For most samples, the oxygen demand from the three components considered here were: labile CBOD > NBOD > refractory CBOD, though the relative importance of refractory CBOD to oxygen demand increased over time. Coarse-filtering reduced CBOD for samples with high particulate carbon and high biovolumes of *Aphanizomenon flos-aquae*. There was a strong positive correlation between BOD, CBOD, and the labile component of CBOD to particulate C and N, with weaker positive correlation to field pH, field dissolved oxygen, and total N. The refractory component of CBOD was not correlated to particulate matter, instead showing weak but statistically significant correlation to dissolved organic carbon, UV absorbance at 254 nm, and total N. Particulate organic matter, especially the alga *A. flos-aquae*, is an important component of oxygen demand in this reach of the Klamath River, though refractory dissolved organic matter would continue to exert an oxygen demand over longer time periods and as water travels downstream.

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1. Introduction

Dissolved oxygen is essential to fish and other aquatic life. In rivers, the concentration of dissolved oxygen is a sum of processes that include reaeration, transport, photosynthesis, respiration, nitrification, and decay of organic matter (Cox, 2003). The biochemical oxygen demand (BOD) test is one widely applied method to quantify the consumption of oxygen in the water column from the decay of organic matter (carbonaceous BOD, or CBOD) and nitrification of ammonia (nitrogenous BOD, or NBOD). Samples are incubated for a specified number of days (e.g. BOD₅, BOD₂₀, BOD₃₀), and the amount of oxygen consumed over that time period is measured. CBOD can be determined directly by adding a chemical that

inhibits the activity of nitrifying bacteria, thus limiting oxygen consumption to the decay of organic matter alone. The difference between total BOD and CBOD is assumed to be BOD from nitrification (NBOD).

The BOD test can be used to investigate the controls on consumption of dissolved oxygen in the water column and to derive decay rates for water quality models. Historically, and still commonly today, BOD data are fit to a first-order kinetic model (Phelps, 1944; Stamer et al., 1983; American Public Health Association, 2005):

$$BOD_t = BOD_u(1 - e^{-kt}) \quad (1)$$

where BOD_t is the exerted biochemical oxygen demand (mg/L of O₂) at time t , BOD_u is the ultimate BOD (mg/L of O₂), k is a first-order decay rate (1/day), and t is time (days).

This first-order model, however, does not always fit BOD data well, and other BOD model formulations have been put forth. These alternate models include those of other reaction orders such as multiorder or half-order (Hewitt et al., 1979; Borsuk and Stow, 2000; Van Le and Adrian, 2004–2005; Roeder et al., 2008), and two-group

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models, such as double exponential models (Otten et al., 1992; Mason et al., 2006; Esslemont et al., 2007). The two-group models follow the hypothesis that there are both labile and refractory components of carbon with different decay rates. Labile carbon is bioavailable and decays rapidly, while refractory carbon is more resistant to microbial decay. The existence of pools of organic matter with different decay rates is well documented and used in carbon-cycle studies (e.g. Ittekkot, 1988) and water quality models (e.g. Cole and Wells, 2008). Both particulate and dissolved organic matter can have components that decay at different rates; for example phytoplankton, which was abundant during this study, can comprise both labile and refractory fractions (Jewell and McCarty, 1971; Otsuki and Hanya, 1972a; Otten et al., 1992).

The objective of this study was to examine the controls on oxygen demand in a 21-mile (34-km) reach of the upper Klamath River that can experience anoxia and hypoxia in summer. To achieve this objective, long-term (30-day) BOD and CBOD experiments were conducted and decay rates were derived for modeling and analysis.

2. Methods

2.1. Site background

The Klamath River flows about 255 mi (410 km) through southern Oregon and northern California to the Pacific Ocean. The river begins at Link River Dam at the outlet of Upper Klamath Lake (Fig. 1); the

dam regulates lake level, controls downstream flow, and diverts water for hydropower or irrigation. The first mile of the river downstream of Link River Dam is named Link River. The Klamath River proper begins in a wide, shallow portion named Lake Ewauna, near the town of Klamath Falls at an elevation of 4100 ft (1250 m). Twenty mi (32 km) downstream, Keno Dam controls river elevation and downstream flow.

Travel time through this reach in summer is approximately 6–10 days. River velocities measured in May 2007 ranged from 0 to 0.24 m/s (Sullivan et al., 2008). Mean annual discharge in water years 2007 and 2008 was 32.4 and 30.3 m³/s, respectively. The climate of this region is semiarid, with most precipitation occurring in fall and winter from eastward moving Pacific weather systems; average annual rainfall is 35.4 cm. Annual average temperature is 9.2 °C, with average July temperature of 20.4 °C (Oregon Climate Service, 2008).

Sampling sites in this reach have been classified as having “very poor” water quality status by the Oregon Water Quality Index (Mrazik, 2007). A large population of algae, predominantly the blue-green alga *Aphanizomenon flos-aquae* (AFA), enters the river from Upper Klamath Lake in summer (Sullivan et al., 2008, 2009), which causes an increase in particulate organic carbon (Fig. 2). AFA filamentous colonies typically occur as “flakes” that are often described as resembling grass clippings in appearance. Dissolved organic carbon (DOC) also enters the reach in flow from Upper Klamath Lake and other tributaries. Besides elevated summer levels of algal biovolume,

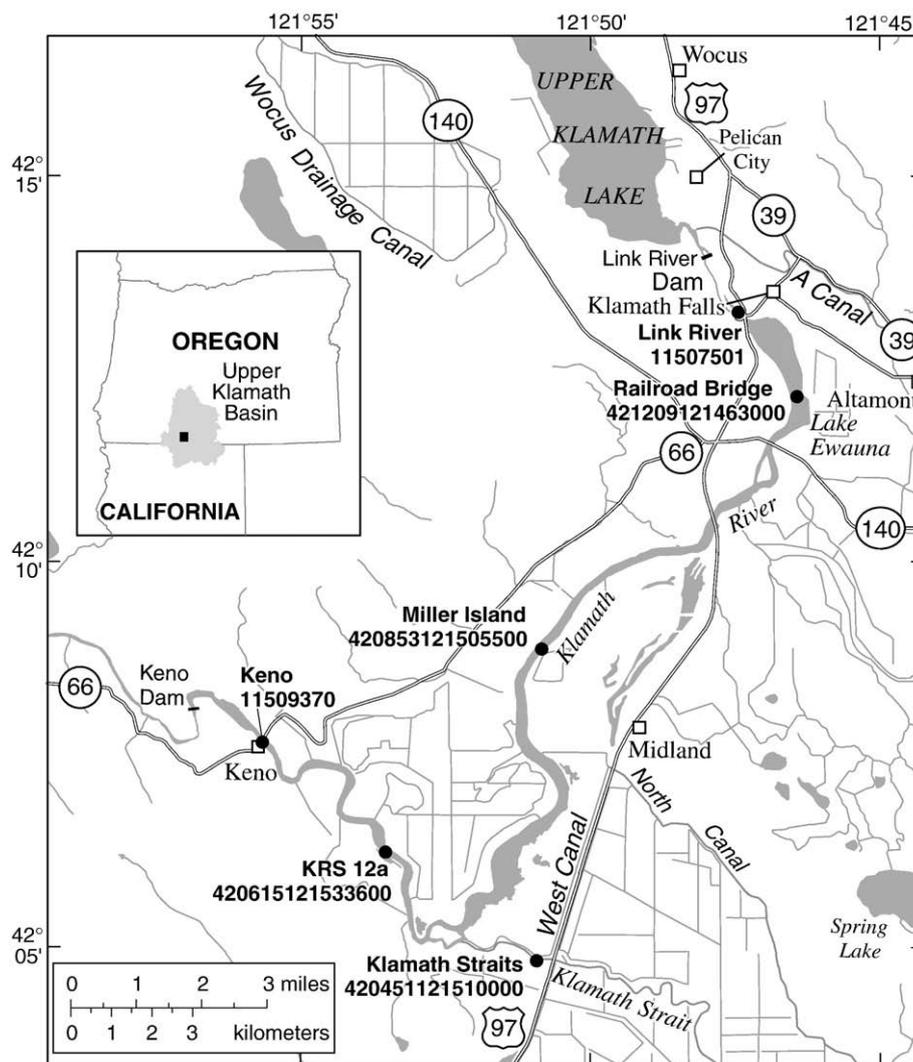


Fig. 1. Map of the study area and sample sites and identification numbers (kilometers and miles).

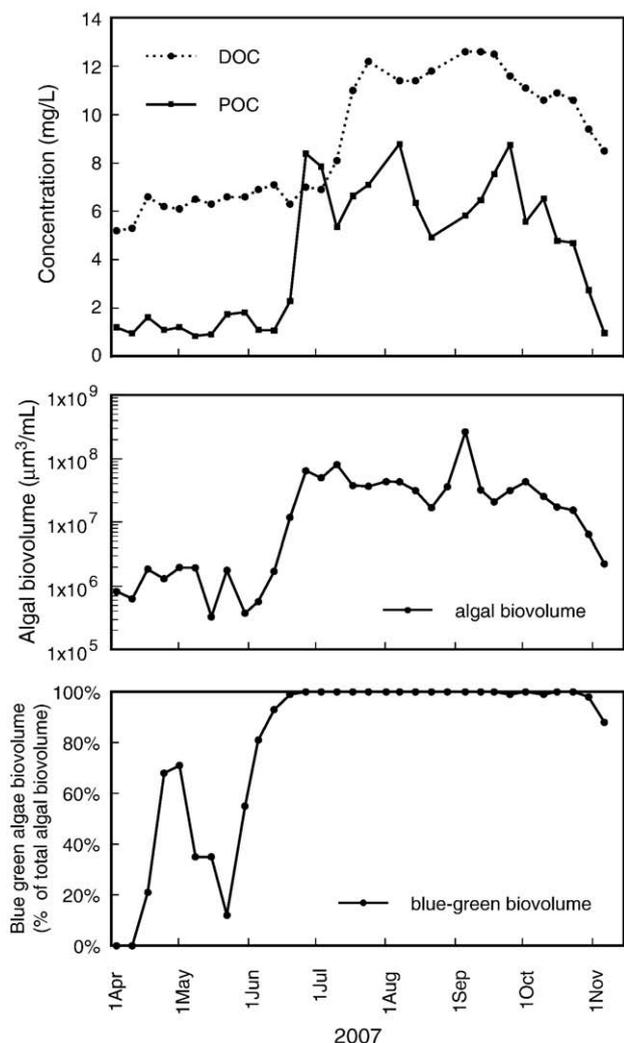


Fig. 2. Concentrations of particulate organic carbon (POC), dissolved organic carbon (DOC), algal biovolume, and blue-green biovolume as a percent of the total. Samples collected at Link River.

Data from Sullivan et al. (2008).

chlorophyll *a*, and organic carbon in the river upstream of Keno Dam, ammonia concentrations and pH are elevated then as well, and dissolved oxygen concentrations vary spatially and temporally, from 200% saturation to anoxia/hypoxia, even at the river surface. Water quality factors, especially low dissolved oxygen, have been implicated as detrimental to fish health in the Klamath River Basin (National Research Council, 2004). Total maximum daily load (TMDL) regulations have been initiated for dissolved oxygen and ammonia year-round, and for chlorophyll *a* and pH in summer, in an attempt to address some of these issues. For water quality models and regulatory decisions, there is a need for more quantitative data on processes and reaction rates that control water quality in this reach of the Klamath River.

2.2. Field sampling

Five mainstem sites in this reach were sampled for BOD; samples were also taken from one tributary, the Klamath Straits Drain (Fig. 1). Samples were collected on 6 days from late August to early September 2006, weekly or every 2 weeks from late June to early September 2007, and approximately every 2 weeks in July and August 2008. Samples were collected with a van Dorn sampler at a noted depth and processed with a churn. At four of the mainstem sites, samples were taken both

near-surface (0.5 m depth) and 1 m from the bottom (between 2 and 4.5 m depth). Unfiltered samples for BOD analysis were churned directly into 300 mL glass BOD bottles, and kept on ice and in the dark. At the same time each BOD sample was collected, field parameters were measured (water temperature, pH, dissolved oxygen concentration, specific conductance), and samples were collected for analysis of total nitrogen and phosphorus; particulate carbon and nitrogen; filtered orthophosphate, nitrite, nitrite + nitrate, ammonia, DOC, alkalinity, UV absorbance at 254 nm, and specific UV absorbance (SUVA). SUVA has been correlated with percent aromaticity of DOC (Weishaar et al., 2003), an indication of DOC's refractory nature. Particulate carbon and nitrogen were analyzed by high-temperature combustion from the material that did not pass through a baked glass fiber filter (0.7 μm nominal pore size); DOC was analyzed from the filtrate. In pilot work, particulate inorganic carbon was never above the reporting level (0.12 mg/L), so particulate carbon is denoted as particulate organic carbon (POC). Selected samplings also included phytoplankton and zooplankton enumeration and species identification, and bacterial abundance and morphological subgroups. Further details on sampling and analysis of constituents other than BOD are provided in Sullivan et al. (2008, 2009).

A subset of samples collected in 2007 were coarse-filtered in the field using fluorocarbon mesh with a 210-μm pore size (Spectrum Laboratories, CA) to remove large AFA flakes from the sample, but let bacteria through. Bacterial counts of unfiltered and coarse-filtered samples were similar.

2.3. Biochemical oxygen demand experiments

BOD samples were transported to the laboratory and incubation began the same day as sample collection. Incubation procedures were based on those in Standard Methods 5210 C. Ultimate BOD test (American Public Health Association, 2005). Samples were incubated for at least 30 days in the dark at 20 °C. Some samples were incubated for 60 days. Dissolved oxygen concentrations were measured at intervals, and samples were reaerated (and remeasured) if concentrations were projected to drop below 2 mg/L before the next measurement. Natural bacteria populations were used in this study; no bacterial seed was added to samples. One set of samples was monitored for chlorophyll *a* concentration several times over a 2-week period. The amount of oxygen consumed per unit of chlorophyll *a* was calculated at each time that chlorophyll *a* was measured.

Some samples were incubated without nitrification inhibitor, and thus measured total BOD. Others were treated with a nitrification inhibitor, Hach formula 2533 TCMP (2% 2-chloro-6 (trichloromethyl) pyridine on sodium sulfate, 0.16 g per 300 mL) to measure CBOD. To examine the effect of the nitrification inhibitor, some bottles were treated with higher concentrations. Doubling the concentration of inhibitor reduced CBOD₃₀ by an average of 3.7 mg/L O₂. This effect is probably due to the fact that it can be difficult to target an inhibitor to affect solely one type of bacteria; it is likely that populations of carbonaceous bacteria were affected by higher concentrations of inhibitor. Alternatively, it could be that only the higher concentration was completely effective at inhibiting nitrification. In any case, these tests emphasized that BOD is an operationally defined test and that the nitrification inhibitor, as well as other factors such as incubation temperature, can affect results.

Replicates, blanks, and standards were also incubated for 30 days. The mean relative percent difference (RPD) of 12 sets of CBOD₃₀ replicates was 5.4% with a range of 0.1–15.0%. The mean oxygen consumption by eight blanks was 0.1 mg/L; the maximum oxygen consumed by any blank was 0.5 mg/L over 30 days. Standards were run by spiking duplicate river water samples with BOD standard, a mixture of glutamic acid and glucose (Wibby Environmental, Ricca Chemical). Samples for standard spikes were coarse-filtered in the

field with the fluorocarbon mesh. A second set of duplicates was run unspiked to determine background BOD from the river water. Nitrification inhibitor was added to standards.

CBOD and BOD data were fit with models to extract decay rates and the standard 5-day and 30-day oxygen demands. Each dataset was graphed and curve-fit with Grace (Grace Development Team); curve-fit results were double checked with SAS (SAS Institute, Inc.). Using SAS, a Tukey's Studentized range test was applied to CBOD model parameters and $CBOD_5$ and $CBOD_{30}$ to test for significant differences between sites and sampling periods. Three sampling periods were defined for this test: June 15–July 14, July 15–August 14, and August 15–September 15. CBOD and BOD results were tested for correlation, using Pearson correlation coefficients, to field parameters, particulate carbon and nitrogen, total nitrogen and phosphorus, DOC, UV absorbance at 254 nm and SUVA.

3. Results

3.1. Carbonaceous biochemical oxygen demand (CBOD)

3.1.1. CBOD model and rates

The shape of the CBOD oxygen demand versus time curves was similar for most samples. The rate of oxygen consumption was highest and most nonlinear at the start of the incubation, then slowed to a longer period of slower and more linear oxygen uptake (Fig. 3). There were a few samples, especially from the Klamath Straits Drain, that did not show fast initial consumption of oxygen. Demand from those samples was generally low, and oxygen consumption followed a more linear form. Because initial nonlinear oxygen consumption was typically followed by slower linear consumption, CBOD data from all sites were fit well by a two-group model:

$$BOD_t = a(1 - e^{-a_0 t}) + a_2 t \quad (2)$$

where $a_1(1 - e^{-a_0 t})$ represents the first-order demand from the more labile pool of carbon, and $a_2 t$ represents the zero-order demand from the more refractory group of carbon. BOD_t is the exerted oxygen demand (mg/L) at time t , a_1 is the ultimate BOD (mg/L) for the labile group, a_0 is the first-order decay rate (1/day), a_2 is the zero-order decay rate for the refractory group, and t is time (days). Mean error (ME) and mean absolute error (MAE) were calculated between each dataset and its corresponding two-group model. CBOD ME averaged 0.03 mg/L O_2 and MAE averaged 0.24 mg/L O_2 .

Applying the first-order-only model (Eq. (1)) to these data resulted in curves that did not capture the true temporal pattern in the data (Fig. 3). For the 1-group model, average CBOD ME was -0.05 mg/L O_2 and MAE 0.47 mg/L O_2 . Despite the nonideal fit using the first-order-only method, values of $CBOD_5$ and $CBOD_{30}$ calculated by the two methods were similar, and in order to facilitate comparison with rates determined from other studies, decay rates from the first-order-only model are reported here also. Caution should be used, however, when using the first-order model to predict long-term BOD from short period datasets. Unless noted, discussion hereafter refers to results fit with the two-group model (Eq. (2)).

Site-average values of the first-order decay rate a_0 ranged from 0.15 to 0.22/day (Table 1). The Tukey's test did not show any conclusive differences between sites or time periods for this parameter. Site-average values of the zero-order decay rate a_2 ranged from 0.13 to 0.25 mg/L/day. The Tukey's test showed significant temporal differences in this decay rate. Values of a_2 from samples taken between June 15 and July 14 averaged 0.12 mg/L/day (0.07 mg/L/day standard deviation), and were statistically lower than samples taken later in the season, between July 15 and September 14, which averaged 0.22 mg/L/day (0.09 mg/L/day standard deviation).

Average site $CBOD_5$ ranged from 4.1 to 15.5 mg/L and average site $CBOD_{30}$ ranged from 11.7 to 32.3 mg/L (Table 2). Site-average values

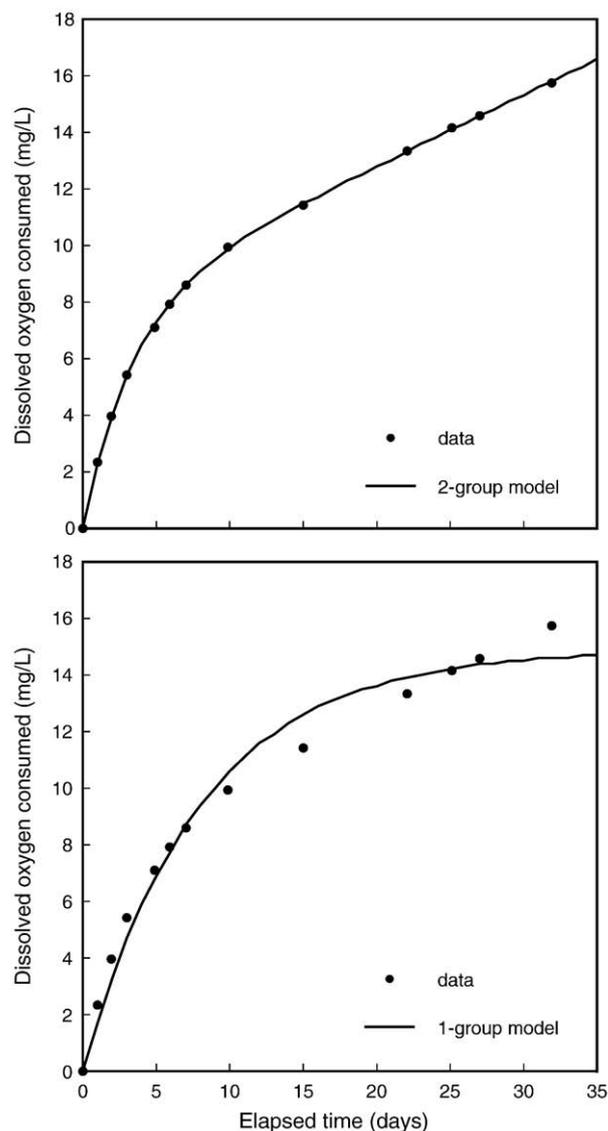


Fig. 3. Comparison of one set of CBOD data fit with the 2-group model (top) and the first-order-only model (bottom). The better fit with the 2-group model was typical. Sample collected from site KRS 12a on July 24, 2007.

of the ultimate CBOD for the labile group a_1 ranged from 5.5 to 28.8 mg/L (Table 1). Results from the Tukey's test showed that $CBOD_5$, $CBOD_{30}$, and a_1 were significantly higher at the two upstream sites, Link River and Railroad Bridge, compared to mainstem sites further downstream and the tributary Klamath Straits Drain. The maximum measured 5-day ($CBOD_5$) and 30-day ($CBOD_{30}$) oxygen demands for any sample were 24.5 and 50.5 mg/L, respectively, for a surface sample from Railroad Bridge in mid-August of 2007. That sample also had the second highest algal biovolume, $(99.2 \times 10^6 \mu m^3/mL)$; 99.7% AFA) of any sample in this study. No statistically significant temporal difference in $CBOD_5$, $CBOD_{30}$, or a_1 from June 15 to September 15 was found for this sample set.

Considering the labile and refractory groups of organic matter that are modeled by the two-group model, the labile group comprised most of the CBOD: an average of 84% of $CBOD_5$ and 63% of $CBOD_{30}$. The oxygen demand from the refractory group was a smaller fraction, and that demand was more similar between sites. For instance, site-average refractory $CBOD_5$ ranged only between 0.7 to 1.3 mg/L. Over the same time period, the labile carbon site-average oxygen demand ranged from 2.9 to 14.8 mg/L.

Table 1

Mean (and standard deviation) of carbonaceous BOD (CBOD) model rate coefficients and parameters for sites sampled in this study.

Site	Sample depth mean, m	Count	One-group model		Two-group model		
			k 1/day	$CBOD_u$ mg/L O ₂	a_0 1/day	a_1 mg/L O ₂	a_2 mg/L/day
Link River	0.5	19	0.12 (0.02)	25.8 (6.9)	0.17 (0.04)	19.4 (7.5)	0.20 (0.10)
Railroad Bridge (all)	1.4	12	0.11 (0.02)	27.2 (11.9)	0.16 (0.06)	21.9 (14.3)	0.16 (0.11)
Surface	0.5	6	0.12 (0.02)	33.1 (13.3)	0.15 (0.03)	28.8 (15.9)	0.13 (0.12)
Bottom	2.3	6	0.10 (0.02)	21.3 (7.3)	0.17 (0.08)	14.9 (9.1)	0.20 (0.09)
Miller Island (all)	2.0	17	0.11 (0.03)	15.7 (5.2)	0.21 (0.06)	9.6 (4.5)	0.18 (0.08)
Surface	0.5	9	0.11 (0.03)	16.7 (5.1)	0.20 (0.06)	10.8 (4.3)	0.18 (0.09)
Bottom	3.8	8	0.10 (0.03)	14.5 (5.3)	0.21 (0.07)	8.4 (4.7)	0.18 (0.08)
KRS 12a (all)	2.0	12	0.10 (0.04)	17.0 (5.4)	0.20 (0.06)	10.4 (7.5)	0.19 (0.08)
Surface	0.5	6	0.11 (0.03)	19.9 (6.0)	0.20 (0.07)	13.9 (8.9)	0.19 (0.10)
Bottom	3.4	6	0.08 (0.04)	14.2 (2.8)	0.20 (0.05)	6.9 (4.0)	0.19 (0.05)
Keno (all)	1.9	20	0.10 (0.04)	15.3 (5.7)	0.21 (0.07)	9.1 (5.9)	0.19 (0.10)
Surface	0.5	12	0.11 (0.03)	16.4 (5.9)	0.22 (0.07)	10.4 (5.9)	0.20 (0.11)
Bottom	4.1	8	0.07 (0.04)	13.6 (5.2)	0.20 (0.08)	7.2 (5.9)	0.16 (0.10)
Klamath Straits	0.5	10	0.04 (0.02)	18.5 (5.3)	0.17 (0.06)	5.5 (4.4)	0.25 (0.05)

Results from both the one-group (Eq. (1)) and two-group models (Eq. (2)) are included. [Count = number of samples].

3.1.2. Change in chlorophyll *a* during incubation

In a 2-week test, concentrations of chlorophyll *a* decreased in the CBOD sample bottles (Fig. 4). Loss of chlorophyll *a* in the sample taken at Link River was particularly large, and after 14.5 days, only 2% of the original chlorophyll *a* remained. For all data in this experiment, the average amount of oxygen consumed per unit of chlorophyll *a* was 0.31 mg/μg, from 0.12 mg/μg for a Link River sample to 0.71 mg/μg for a Klamath Straits Drain sample. Cohen (1990) found carbonaceous BOD to consume 0.20 mg of oxygen per μg of chlorophyll *a* for experiments with additions of the green algae *Chlamydomonas reinhardtii*.

3.1.3. Effect of coarse-filtration

Removing large particulate matter with a 210 μm screen filter (which would include most AFA flakes and large zooplankton) reduced the measured CBOD (Fig. 5). Surface samples at Keno and Link River, which had high POC concentrations (10.6 and 5.8 mg/L POC), had especially large decreases in CBOD: 89% and 78% reduction in CBOD₅ compared to unfiltered samples. The shape of the CBOD curve also changed at those sites. The fast initial consumption of oxygen, modeled by the first-order part of the equation, generally decreased upon coarse-filtration, suggesting that coarse organic matter made up much of the labile component of organic matter. The linear portion (zero-order) of the curve remained largely unchanged by filtration, suggesting that smaller particulate matter

and dissolved organic matter made up this more refractory portion of organic matter.

The samples at Miller Island were less changed by coarse-filtration. It is unknown why this was the case, but there were differences in algal biovolumes and species between Miller Island and the other two sites. Miller Island had the lowest algal biovolume of the three sites on the day sampled. AFA, whose large size makes it more likely to be screened out by a coarse filter, made up only 70% of algal biovolume at Miller Island, while comprising 97% and 100% of algal biovolume at Keno and Link River, respectively. Cryptophytes made up 26% of algal biovolume at Miller Island on that date, whereas they were virtually absent (<1%) at Keno and Link River).

3.2. Total BOD and NBOD

Total BOD data were also fit with the two-group model (Fig. 6). Data were fit by the model well, though total BOD data sometimes were more variable in the incubations after day 20. Site-average BOD values for a_0 ranged from 0.11 to 0.29/day; values of a_1 ranged from 7.6 to 30.8 mg/L; values of a_2 ranged from 0.01 to 0.45 mg/L/day (Table 3). Average site BOD₅ ranged from 5.6 mg/L to 15.9 mg/L and average site BOD₃₀ ranged from 18.4 mg/L to 35.3 mg/L (Table 4). The maximum BOD₅ and BOD₃₀ demands for any sample were 26.5 mg/L and 55.4 mg/L, respectively, for the surface sample from Railroad Bridge in mid-August of 2007. As with CBOD data, the two-group model produced a better fit (ME 0.05 mg/L O₂,

Table 2

Mean (and standard deviation) of carbonaceous BOD (CBOD) at 5 and 30 days.

Site	Sample depth mean, m	Count	CBOD ₅ mg/L O ₂	Labile CBOD ₅ mg/L O ₂	Refractory CBOD ₅ mg/L O ₂	CBOD ₃₀ mg/L O ₂	Labile CBOD ₃₀ mg/L O ₂	Refractory CBOD ₃₀ mg/L O ₂
Link River	0.5	19	11.9 (3.8)	10.9 (3.8)	1.0 (0.5)	25.3 (7.0)	19.2 (7.4)	6.1 (3.0)
Railroad Bridge (all)	1.4	12	12.1 (6.5)	11.3 (6.8)	0.8 (0.5)	26.4 (12.0)	21.5 (14.1)	4.9 (3.2)
Surface	0.5	6	15.5 (7.3)	14.8 (7.6)	0.7 (0.6)	32.3 (13.5)	28.4 (15.6)	3.9 (3.6)
Bottom	2.3	6	8.7 (3.6)	7.7 (3.9)	1.0 (0.5)	20.5 (7.2)	14.6 (8.8)	5.9 (2.7)
Miller Island (all)	2.0	17	6.6 (2.0)	5.7 (2.0)	0.9 (0.4)	14.9 (4.4)	9.5 (4.4)	5.4 (2.5)
Surface	0.5	9	7.3 (2.0)	6.4 (2.0)	0.9 (0.4)	16.1 (4.4)	10.7 (4.3)	5.4 (2.6)
Bottom	3.8	8	5.9 (1.8)	5.0 (1.8)	0.9 (0.4)	13.6 (4.3)	8.3 (4.5)	5.4 (2.5)
KRS12 a (all)	2.0	12	6.9 (3.1)	6.0 (3.3)	0.9 (0.4)	15.9 (5.9)	10.3 (7.3)	5.6 (2.3)
Surface	0.5	6	8.6 (2.9)	7.7 (3.4)	0.9 (0.5)	19.3 (5.8)	13.7 (8.6)	5.6 (3.1)
Bottom	3.4	6	5.2 (2.4)	4.2 (2.5)	0.9 (0.3)	12.5 (3.8)	6.9 (4.0)	5.6 (1.5)
Keno (all)	1.9	20	6.4 (3.6)	5.4 (3.5)	0.9 (0.5)	14.5 (6.6)	8.9 (5.9)	5.6 (3.1)
Surface	0.5	12	7.5 (3.5)	6.5 (3.3)	1.0 (0.6)	16.4 (6.6)	10.3 (5.8)	6.1 (3.3)
Bottom	4.1	8	4.7 (3.5)	3.9 (3.4)	0.8 (0.5)	11.7 (6.0)	6.8 (5.8)	4.9 (2.9)
Klamath Straits	0.5	10	4.1 (2.3)	2.9 (2.3)	1.3 (0.2)	12.8 (4.4)	5.3 (4.2)	7.5 (1.4)

All results are from the two-group model (Eq. (2)), and CBOD components from the labile (group 1) and refractory (group 2) fractions are also shown. [Count = number of samples].

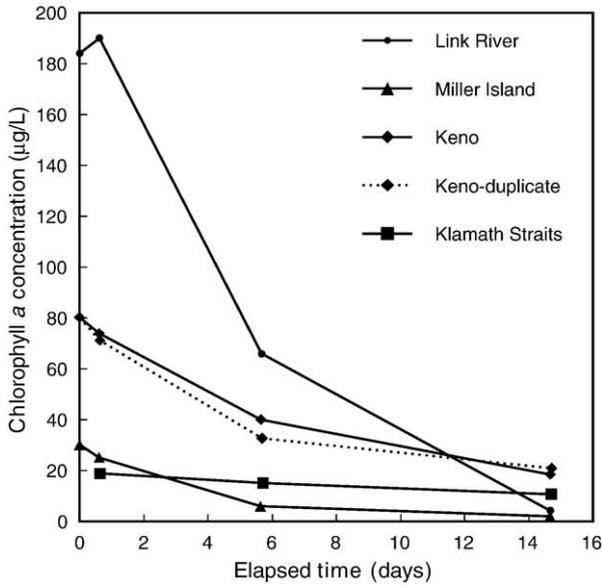


Fig. 4. Change in chlorophyll a concentration in CBOD bottles over the first two weeks of incubation. Samples were collected on August 12, 2008.

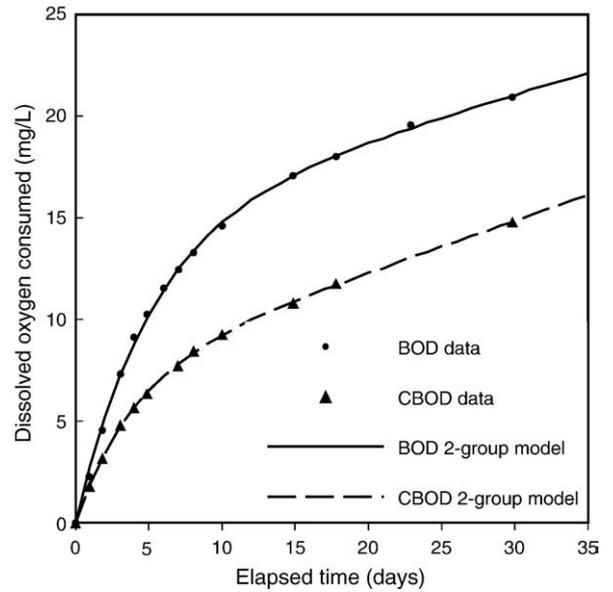


Fig. 6. CBOD and BOD data and 2-group model curve fits. Samples were collected at Keno on August 8, 2007.

MAE 0.39 mg/L O₂) than the first-order-only model (ME -0.05 mg/L O₂, MAE 0.63 mg/L O₂).

The difference between CBOD and BOD is expected to be nitrogenous BOD (NBOD) from the activity of nitrifying bacteria which consume oxygen in the conversion of ammonia to nitrite and eventually nitrate. Site-average NBOD₅ ranged from 1.4 mg/L to 3.2 mg/L and site-averaged NBOD₃₀ ranged from 5.3 mg/L to 8.3 mg/L.

With NBOD and the labile and refractory components of CBOD, there were three components of water column BOD calculated from the data in this study. At 5 days, averaging data from all sites, labile organic matter made up 61% of oxygen demand, nitrogenous demand made up 27% of oxygen demand, and refractory organic matter 12%. At thirty days, 44% of accumulated demand was from labile organic matter, 30% from nitrogenous demand, and 26% from refractory organic matter.

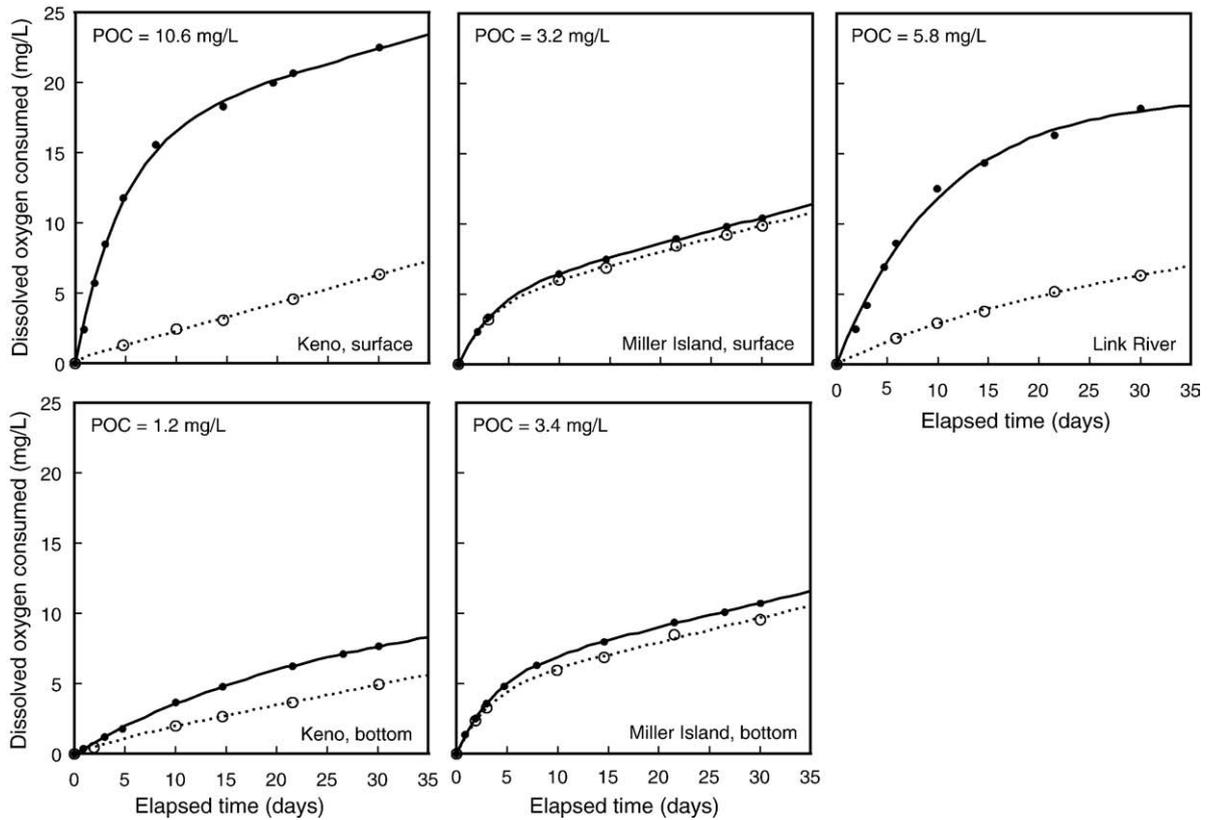


Fig. 5. Comparison of CBOD curves for unfiltered (dark circles, line) and coarse-filtered (210 µm) samples (open circles, dashed line). POC varied between sites, as noted on the figures. DOC was less variable, between 11.2 and 12.6 mg/L. Samples collected on September 5, 2007.

Table 3
Mean (and standard deviation) of total BOD model rate coefficients and parameters for sites sampled in this study.

Site	Sample depth mean, m	Count	One-group model		Two-group model		
			k 1/day	BOD_u mg/L O ₂	a_0 1/day	a_1 mg/L O ₂	a_2 mg/L/day
Link River	0.5	6	0.11 (0.02)	30.1 (4.1)	0.11 (0.02)	29.8 (4.4)	0.01 (0.02)
Railroad Bridge (all)	1.4	8	0.09 (0.04)	33.7 (10.4)	0.16 (0.09)	26.1 (16.7)	0.17 (0.27)
Surface	0.5	4	0.11 (0.04)	37.3 (13.5)	0.16 (0.07)	30.8 (21.0)	0.17 (0.27)
Bottom	2.4	4	0.07 (0.03)	30.1 (6.1)	0.15 (0.12)	21.4 (12.4)	0.18 (0.32)
Miller Island (all)	2.1	9	0.09 (0.02)	24.6 (3.5)	0.27 (0.19)	12.2 (6.8)	0.37 (0.21)
Surface	0.5	5	0.09 (0.03)	24.6 (2.1)	0.29 (0.26)	14.1 (8.2)	0.31 (0.25)
Bottom	4.1	4	0.08 (0.02)	24.7 (5.2)	0.24 (0.09)	10.0 (4.9)	0.45 (0.16)
KRS12 a (all)	1.9	8	0.08 (0.02)	23.9 (5.6)	0.20 (0.10)	11.5 (6.6)	0.33 (0.20)
Surface	0.5	4	0.09 (0.02)	24.8 (5.2)	0.23 (0.13)	14.0 (7.7)	0.33 (0.23)
Bottom	3.3	4	0.06 (0.02)	22.9 (6.5)	0.16 (0.06)	9.0 (5.1)	0.33 (0.20)
Keno (all)	1.9	10	0.08 (0.04)	24.4 (7.3)	0.28 (0.15)	9.8 (4.3)	0.38 (0.21)
Surface	0.5	6	0.11 (0.03)	23.4 (7.0)	0.27 (0.08)	11.3 (2.6)	0.39 (0.23)
Bottom	4.0	4	0.05 (0.02)	25.8 (8.6)	0.28 (0.24)	7.6 (5.8)	0.37 (0.22)
Klamath Straits	0.5	5	0.08 (0.03)	24.2 (8.0)	0.12 (0.05)	17.5 (9.5)	0.19 (0.12)

Results from both the one-group (Eq. (1)) and two-group models (Eq. (2)) are included. [Count = number of samples].

Some sites or sampling dates did not follow this pattern. For instance, by day 30 for the Klamath Straits Drain, which had especially high concentrations of DOC (16–37 mg/L DOC compared to 6–13 mg/L for mainstem sites), accumulated oxygen demand from the refractory component of CBOD was often greater than the labile component (Table 2).

3.3. Correlation to other constituents

CBOD₅, CBOD₃₀, the labile component of the two-group CBOD model, BOD₅ and BOD₃₀ had similar patterns of correlation to other constituents and showed especially high positive correlation to particulate C (Pearson's R , 0.85–0.88) and particulate N (Pearson's R , 0.85–0.87) (Table 5, Fig. 7). They also showed statistically significant positive correlation to field pH and dissolved oxygen, both affected by algal photosynthesis, and total N. Most had significant negative correlation to specific conductance and depth. There was no significant correlation to temperature, total P, DOC, UV absorbance, or SUVA.

The refractory component of the two-group model had different correlation patterns. There were weak but statistically significant positive correlations to DOC, UV absorbance at 254 nm, and total N.

Table 4
Mean (and standard deviation) of total BOD and nitrogenous BOD (NBOD) at 5 and 30 days.

Site	Sample depth mean, m	Count	BOD ₅ mg/L O ₂	BOD ₃₀ mg/L O ₂	NBOD ₅ mg/L O ₂	NBOD ₃₀ mg/L O ₂
Link River	0.5	6	12.6 (3.5)	28.6 (4.9)	2.5 (1.2)	5.8 (1.6)
Railroad Bridge (all)	1.4	8	12.5 (6.8)	30.1 (11.4)	1.7 (0.9)	5.5 (2.9)
Surface	0.5	4	15.9 (8.1)	35.3 (14.4)	1.9 (1.1)	5.3 (3.0)
Bottom	2.3	4	9.1 (3.3)	24.8 (4.6)	1.4 (0.7)	5.8 (3.2)
Miller Island (all)	2.0	9	9.0 (1.8)	23.2 (3.3)	2.3 (0.6)	7.1 (6.3)
Surface	0.5	5	9.1 (1.8)	23.0 (2.3)	2.0 (0.6)	6.3 (3.1)
Bottom	3.8	4	8.7 (2.1)	23.4 (4.7)	2.6 (0.6)	8.1 (2.3)
KRS12 a (all)	2.0	8	7.8 (2.3)	21.1 (4.3)	2.3 (0.2)	7.4 (2.8)
Surface	0.5	4	9.6 (1.6)	23.6 (3.9)	2.4 (0.1)	6.8 (2.9)
Bottom	3.4	4	5.9 (1.1)	18.5 (3.3)	2.2 (0.3)	8.1 (2.8)
Keno (all)	1.9	10	8.3 (3.0)	21.2 (5.9)	2.5 (0.6)	7.6 (3.2)
Surface	0.5	6	10.1 (2.5)	23.0 (7.0)	2.7 (0.6)	7.2 (3.5)
Bottom	4.1	4	5.6 (1.1)	18.4 (2.4)	2.1 (0.5)	8.3 (3.2)
Klamath Straits	0.5	5	8.1 (4.5)	21.5 (7.7)	3.2 (1.2)	7.3 (2.9)

All results are from the two-group model (Eq. (2)). NBOD is calculated as the difference between total BOD and carbonaceous BOD (CBOD). [Count = number of samples].

There were weak significant negative correlations to field pH, field dissolved oxygen, and SUVA. There were no significant correlations between refractory BOD and particulate C, particulate N, depth, temperature, specific conductance, or total P.

4. Discussion

The decay of organic matter in the Link River to Keno reach of the Klamath River was consistent with the conceptual model of the existence of at least two pools of organic matter with different rates of decay. Labile organic matter followed first-order decay, and refractory organic matter followed zero-order decay; the use of a model that considered both groups fit the data well. The labile group decayed rapidly, with 80% of its demand expressed in roughly 8 days (for $a_0 = 0.2/\text{day}$). The refractory component decayed at a much slower rate, but consumed oxygen for a longer time than the labile phase. The 60-day experiments demonstrated that oxygen consumption from the refractory group continued for at least that period of time.

Table 5
Correlation coefficients (Pearson) of parameters that may relate to carbonaceous biochemical oxygen demand (CBOD).

	CBOD ₅	CBOD ₃₀	Labile CBOD ₃₀	Refractory CBOD ₃₀	BOD ₅	BOD ₃₀
CBOD ₅	1.00					
CBOD ₃₀	0.98	1.00				
Labile CBOD ₃₀	0.96	0.96	1.00			
Refractory CBOD ₃₀	−0.13	−0.06	−0.36	1.00		
BOD ₅	0.98	0.97	0.96	−0.40	1.00	
BOD ₃₀	0.94	0.94	0.92	−0.34	0.94	1.00
Sample depth	−0.33	−0.34	−0.29	−0.08	−0.38	−0.28
Temperature	−0.01	−0.01	−0.04	0.13	0.15	0.06
Specific conductance	−0.38	−0.33	−0.36	0.17	−0.29	−0.29
pH	0.63	0.58	0.64	−0.30	0.58	0.51
Dissolved oxygen	0.45	0.39	0.48	−0.38	0.56	0.47
Particulate C	0.88	0.88	0.87	−0.17	0.86	0.85
Particulate N	0.87	0.87	0.87	−0.18	0.85	0.85
Total N	0.47	0.56	0.41	0.34	0.59	0.62
Total P	−0.16	−0.07	−0.18	0.39	0.06	0.08
DOC	−0.33	−0.25	−0.33	0.33	−0.21	−0.17
SUVA	0.13	0.11	0.18	−0.27	0.25	0.20
UV absorbance, 254 nm	−0.27	−0.20	−0.26	0.24	−0.06	−0.05

Values in bold are significant at $p < 0.01$ (number of samples = 44, 74, 77, or 88).

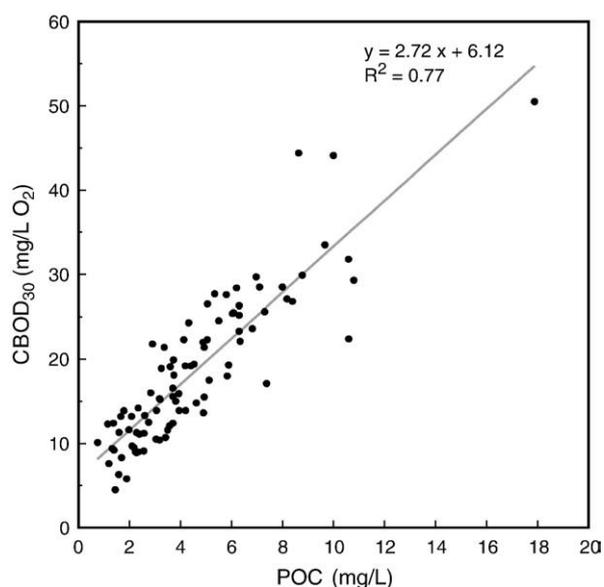


Fig. 7. $CBOD_{30}$ plotted as a function of particulate carbon.

The labile component of oxygen demand could theoretically come from decay of either particulate or dissolved organic matter, but here multiple lines of evidence suggest that it comes from the decay of particulate matter, which in this reach is mainly composed of phytoplankton, especially AFA. This corroborates results from an initial study on this reach which found lower oxygen demand in filtered samples (Deas and Vaughn, 2006). Studies in other rivers have also found close association between oxygen consumption and particulate matter, algae, and chlorophyll *a* (Heiskary and Markus, 2001; Sand-Jensen and Pedersen, 2005; Volkmar and Dahlgren, 2006; MacPherson et al., 2007). This is not always the case, as Fallon and Brock (1979) found that filtration that removed up to 90% of algal biomass did not correspondingly reduce oxygen demand; however, they also found that 99% AFA volume was lost by day 8 of the incubations. Some species of blue-green algae, including AFA, are thought to be the least resistant algal type to decomposition (Hansen et al., 1986). Though these algae die and decompose rapidly in dark incubations, it is probable that some portion of the initial oxygen demand comes from algal respiration in addition to algal decomposition.

The pool of refractory organic matter could also have some contribution from both particulate and dissolved organic matter, but results suggest that it was largely made up of dissolved organic matter in this system. Studies on other systems have also found DOC to be largely refractory, with only a small amount of labile carbon (Coffin et al., 1993; Sondergaard et al., 1995). The pool of refractory matter degrades more slowly and thus consumes less dissolved oxygen over the travel time in this reach. However, refractory matter would contribute to oxygen demand downstream, and it may be important to consider in management scenarios that increase the residence time of water in this reach.

Decay rates of refractory organic matter (a_2) showed statistically significant variation over the summer season, with lower values in early summer and higher values in mid- to late summer. The timing of this cycle had a similar temporal pattern to DOC concentrations at most mainstem sites, and a pattern opposite the temporal pattern of SUVA, which was lowest in mid- and late summer (Fig. 8). Early summer DOC may have had a larger component of allochthonous aromatic and refractory DOC from wetlands in the upper reaches of the watershed, and mid- to late summer DOC a larger contribution of autochthonous labile DOC related to the summer algal bloom (Fig. 2), from, for example, algal cell lysis, or algal photosynthetic or zooplankton grazing excretions (Park et al., 1997).

Also contributing to total oxygen demand in the system was oxygen consumption from nitrification (Table 4). Usually, this demand was less than that from labile organic matter but greater than that of refractory organic matter over the 30-day time period. This demand comes from oxidation of dissolved ammonia as well as oxidation of reduced nitrogen species from algal decomposition. Ammonia concentrations as high as 1.5 mg/L as N and particulate nitrogen concentrations up to 3.9 mg/L were found in field samples for this study.

The modeled oxygen consumption rates from this study are of the same order of magnitude as those from other studies. Despite the less-good fit of the first-order-only model for this dataset, those results can be compared to other studies that used a first-order-only model. The site-average first-order-only decay rates for total BOD ranged from 0.05 to 0.11/day for this study (Table 3). Volkmar and Dahlgren (2006) found total BOD decay rates of 0.03–0.09/day for the San Joaquin River in California. Otten et al. (1992) found decay rates of 0.06–0.19/day for blue-green algae (*Oscillatoria limnetica*) and

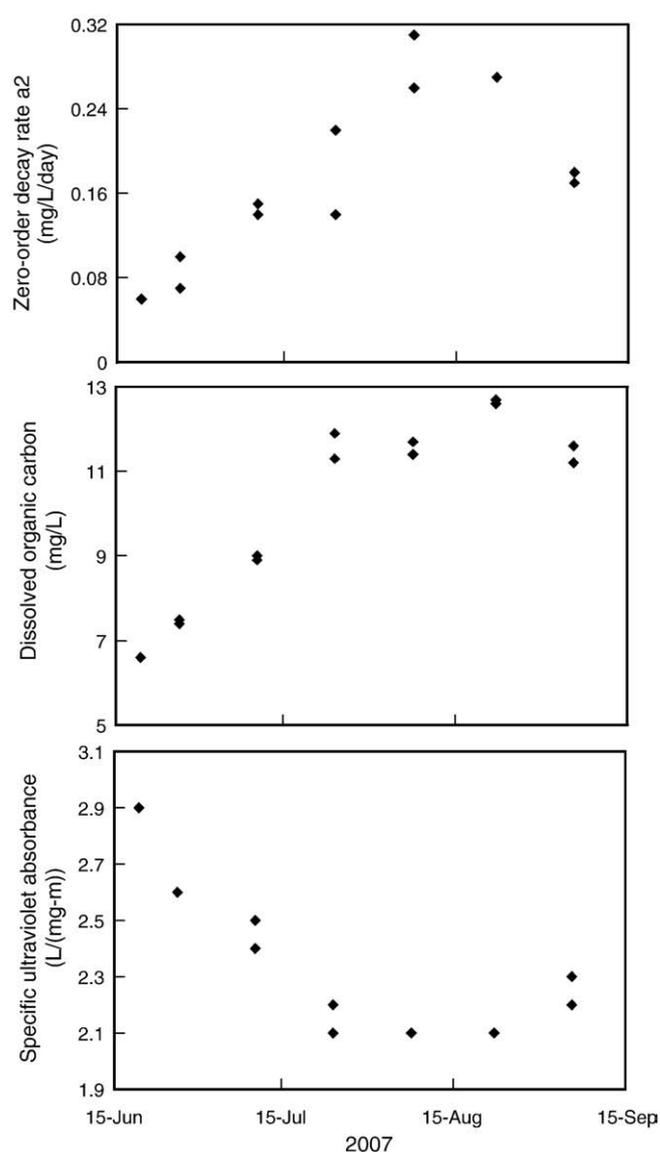


Fig. 8. The zero-order CBOD decay rate (a_2), dissolved organic carbon concentrations (DOC), and specific ultraviolet absorbance (SUVA), a measure of the aromaticity of DOC, at Miller Island in 2007.

0.005/day for refractory detritus. Ostapenia et al. (2009) measured rates of 0.044–0.14/day for six lakes of different trophic status.

The highest oxygen demand occurred at the two upstream sites closest to Upper Klamath Lake. A large amount of algal material enters the river from the lake, and degradation of any non-living algae could occur quickly, within the first days (and first sites) of its travel time through the reach. However, the conclusions of this study are based on incubation experiments with continuously oxic conditions. At some times of the year, portions of this reach of the Klamath River can be hypoxic or anoxic. Decay of chlorophyll has shown to be slower under anoxic conditions (Fallon and Brock, 1979), and Otsuki and Hanya (1972b) found the decomposition rate of algal C and N to be less than half under anoxic conditions. So, degradation rates could change under anoxic conditions, and labile organic matter could be transported downstream.

While this work focused on oxygen demand in water samples, sediments can also exert an oxygen demand on the water column. Doyle and Lynch (2005) measured sediment oxygen demand (SOD) in this reach of the Klamath River in early June of 2003. These measurements were made with specialized in situ chambers over several hours. SOD rates can be compared to BOD rates by considering their effect on oxygen concentrations in the overlying water; dividing the SOD rate ($\text{g}/\text{m}^2/\text{day}$) by the depth of water at the measurement site (m) produces an oxygen loss rate due to SOD in $\text{mg}/\text{L}/\text{day}$. For mainstem river sites, the SOD rate measured in 2003 averaged 0.75 $\text{mg}/\text{L}/\text{day}$ at 20 °C, with a range of 0.12–1.87 $\text{mg}/\text{L}/\text{day}$. Studies performed upstream in Upper Klamath Lake had similar SOD values, but there at least one measurement in late summer was higher: 3.43 $\text{mg}/\text{L}/\text{day}$ (Wood, 2001). Doyle and Lynch (2005) also conducted control experiments in the water column, akin to an in situ BOD measurement. There, they found an average water column oxygen consumption of 1.31 $\text{mg}/\text{L}/\text{day}$, with a minimum of 0.40 and a maximum of 2.00. Results from the present work, extracted as total BOD_1 (oxygen consumption in the first day) to match the same timeframe as SOD measurements, had an average demand of 2.5 $\text{mg}/\text{L}/\text{day}$ with a range of 1.0–6.7 $\text{mg}/\text{L}/\text{day}$. The lower rates of water column oxygen demand in the Doyle and Lynch (2005) study compared to this study are due in part to the fact that their measurements were made in early June, before the main algal bloom, in order to ensure that near-sediment oxygen levels were high enough to measure oxygen loss. This general comparison confirms that oxygen demand in the water column can be an important control on oxygen concentrations in this reach of the Klamath River, and perhaps more important than the SOD in some reaches, though additional data and research may be required to further define these roles.

5. Conclusions

Compared to a 1-group model, a 2-group model that considered both labile and refractory components of organic matter produced a better fit to 30-day biochemical oxygen demand incubation data for the Link River Dam to Keno Dam reach of the upper Klamath River. For mainstem sites, the labile component of BOD, associated with decomposition of particulate algal material, made up most of the oxygen demand in these experiments, especially over the shorter 5-day period. Over longer periods, the refractory component of BOD, associated with dissolved organic matter, made up an increasingly larger portion of BOD. So, while labile BOD could contribute strongly to oxygen demand for the travel time of this reach, refractory BOD would continue to contribute to oxygen demand as water travels downstream. NBOD, from the oxidation of ammonia, also contributed to oxygen demand.

Early summer values of the decay rate a_2 , for refractory material, were lower than mid- to late summer values. Temporal cycles were similar to those for DOC concentrations, and opposite to those for SUVA for most mainstem sites. The nature of this pool of carbon is not

static, and its concentration, composition, and rate of decay can change through the course of an algal bloom.

The two upstream sites, closest to the inflow from Upper Klamath Lake, had significantly higher CBOD_5 , CBOD_{30} , and values of a_1 , the ultimate CBOD for the labile group, compared to sites further downstream and a tributary inflow. The oxygen demand in the water column was of the same magnitude or greater than oxygen demand from sediments, suggesting that a reduction of the upstream load of particulate algal material that enters this reach in summer could limit anoxia and hypoxia in the water column and produce expanded habitat for fish and other aquatic life.

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