

## **Biological CO oxidation in the Sargasso Sea and in Vineyard Sound, Massachusetts**

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## **Acknowledgements**

We thank Ollie Zafiriou for serving as Chief Scientist and catalyst for these cruises. Special thanks to the captains and crews of the *R/V Endeavor* (URI) and *R/V Weatherbird II* (BBSR) for their accommodations and assistance. The manuscript was greatly improved by the comments of two diligent reviewers. This research was supported by National Science Foundation grants OCE-98-11208 and OCE-01-36876, and the Reinhard Coastal Research Center and Coastal Ocean Institute grant BI-10918.

## ABSTRACT

In situ dissolved carbon monoxide in oligotrophic waters follows a diel cycle varying from 0.3 - 0.5 nmol L<sup>-1</sup> before dawn to 2.5 – 3 nmol L<sup>-1</sup> in early afternoon when photo-production of CO exceeds biological CO-oxidation and other sinks. Coastal waters may contain up to 15 nmol L<sup>-1</sup> [CO] in the daytime. Assays to measure the rate of CO bio-oxidation typically involve the addition of labeled CO to sealed samples, resulting CO-concentrations above ambient levels during incubation (up to 9 nmol L<sup>-1</sup> CO). We find that biological oxidation of CO obeys first-order kinetics when incubated with up to 4 nmol L<sup>-1</sup> [CO] in coastal water samples, and between 4 and 10.8 nmol L<sup>-1</sup> in oligotrophic waters. At higher [CO], kinetic behavior transitions to zero-order or saturation kinetics. CO oxidation rate coefficients obtained in dark incubations were not representative of the entire diurnal period, as others have assumed. Biological CO oxidation rate coefficients  $k_{co}$  measured in dark incubations of Sargasso Sea surface water in summer were  $0.020 \pm 0.002 \text{ h}^{-1}$  (mean  $\pm$  st.dev) and an order of magnitude greater than those measured in situ during daylight hours ( $0.002 \pm 0.001 \text{ h}^{-1}$ ). Dark and in situ rate coefficients in early spring were  $0.006 \pm 0.004 \text{ h}^{-1}$  and  $0.003 \pm 0.001 \text{ h}^{-1}$ , respectively. In dark incubations of Vineyard Sound water,  $k_{co}$  was  $0.127 \pm 0.038 \text{ h}^{-1}$ . The apparent half-saturation constant  $K_{app}$  for CO ranged from 2.04 to 5.44 nmol L<sup>-1</sup> CO in both environments.

## Introduction

Carbon monoxide (CO) is the third most abundant carbon species in the atmosphere, after CO<sub>2</sub> and methane. Although the dominant sources of atmospheric CO are anthropogenic (Conrad and Seiler 1982a; Müller 1992), the major source of CO in the surface waters of the ocean is the abiotic photo-oxidation of chromophoric dissolved organic material (CDOM) initiated by UV or near-UV light (Mopper et al. 1991; Valentine and Zepp 1993; Pos et al. 1998). Great uncertainty exists regarding the source strength of CO derived from the photodegradation of CO; past open ocean water studies have estimated oceanic CO flux between 13 Tg yr<sup>-1</sup> (Bates et al., 1995) and 200 Tg yr<sup>-1</sup> (Conrad et al. 1982; Kieber et al. 1990), though a more recent study has estimated a global “blue-water” photochemical source of 120 Tg CO yr<sup>-1</sup> (Zafiriou et al. 2003). Because the surface waters of the world’s oceans are saturated with CO with respect to the atmosphere, they are a source of atmospheric CO (Swinnerton et al. 1970).

Daytime vertical profiles of dissolved [CO] show maximum values in the surface water and decreasing [CO] with depth as light in the UV wavelengths is attenuated (Seiler and Schmidt 1974; Xie et al. 2001). The diel variation of dissolved CO occurs in the entire euphotic zone, with decreasing amplitude at greater depths reflecting decreasing light intensity. In contrast with conditions within the euphotic zone, CO concentration varies little with time below the mixed layer. This indicates that the net exchange of CO between the euphotic zone and the underlying deep ocean water is very small and cannot account for the observed diurnal variation within the euphotic zone (Conrad et al. 1982; Jones 1991; Zuo et al. 1998).

The diurnal scale of variability in aquatic CO in the surface waters of the world’s oceans results from production by solar photolysis of CDOM and fast acting sinks that are provided by microbial oxidation (Conrad and Seiler 1980; Jones and Amador 1993) and atmospheric

exchange (Conrad et al. 1982; Zuo and Jones 1995). In oligotrophic Sargasso Sea surface waters, CO concentration increases from dawn to a maximum (2.5 -3.5 nmol L<sup>-1</sup>) in middle afternoon, followed by a rapid decline in the early evening to a minimum (0.3 - 0.5 nmol L<sup>-1</sup>) just before dawn (Xie et al. 2001; O. Zafiriou, pers. comm.). CO in the water column is therefore short-lived and is strongly coupled to the day/night cycle via photo-production and biology. It is likely that most of the CO produced in situ is consumed by microbial activities (Conrad et al. 1982). The estimated microbial CO sink of  $32 \pm 18$  Tg CO-C per year (Zafiriou et al. 2003) suggests that CO processes are a non-trivial component of the oceanic carbon budget, and most photolytically produced CO is cycled internally by the bacterioplankton. Whether the CO is transferred into the atmosphere or oxidized to CO<sub>2</sub>, it represents a direct loss of otherwise refractory DOM carbon in natural waters (Mopper et al. 1990; Zuo and Jones 1997).

Two commonly used rate measurement methods, the rate of disappearance of dissolved CO in dark incubations and time-series biological oxidation of <sup>14</sup>C-CO to <sup>14</sup>C-CO<sub>2</sub>, quantify different aspects of biological CO transformation. CO loss kinetics have long been assumed to be first-order at environmental concentrations (Jones and Morita 1984; Jones and Amador 1993; Johnson and Bates 1996), such that at low [CO], the rate of CO oxidation accelerates linearly with increasing [CO]. At higher [CO], the concentration of the active enzyme becomes limiting and additional CO cannot accelerate the reaction; the reaction reaches a maximum velocity ( $V_{\max}$ ) and obeys zero-order or saturation kinetics. When first-order kinetics are obeyed, exponential decays may be fitted to time-series data to obtain CO loss rate coefficients  $k_{co}$  expressed as either reciprocal hours (h<sup>-1</sup>) or days (d<sup>-1</sup>). Dark syringe incubation spanning 12 - 24 hours is therefore commonly used to measure microbial CO loss rates (Jones 1991; Jones and Amador 1993; Johnson and Bates 1996), and typically yields net CO loss rate coefficients at

[CO] slightly below the sample's initial [CO]. In contrast, the  $^{14}\text{C}$ -CO oxidation method (Jones 1991; Jones and Amador 1993), directly yields net CO oxidation rates ( $R_{\text{CO}_2}$ ,  $\text{nmol L}^{-1} \text{h}^{-1}$ ) at [CO] levels above most oceanic [CO] values. The [CO] within the incubation mixture is typically two- to ten-fold above oceanic values because  $^{14}\text{C}$ -CO must be added.  $R_{\text{CO}_2}$  is determined by time-series measurements of  $^{14}\text{C}$ -CO<sub>2</sub> accumulation in a sealed headspace, and  $k_{co}$  is determined by normalizing by the total dissolved [CO] within the sample ( $R_{\text{CO}_2} / [\text{CO}]_{\text{inc}}$ ). Accuracy of determined  $k_{co}$  depends upon the degree to which the reaction is first-order. Carbon monoxide oxidizing enzymes may become saturated at higher concentrations of CO and will result in under-estimating  $k_{co}$  determined in samples containing elevated CO levels if first-order kinetics is assumed.

In past efforts to measure microbial CO-oxidation in natural waters, rate determinations in dark incubations were assumed to be representative of the entire photic period (Conrad and Seiler 1982b; Jones and Amador 1993; Johnson and Bates 1996). The present study and few others (Jones and Morita 1984; Jones 1991) suggest that light may inhibit this process in surface waters, though the previous studies of light effects on CO oxidation were done on pure-cultures of CO-oxidizing organisms rather than in situ measurements of CO-oxidation in natural samples. If daytime CO-oxidation rates in surface waters are depressed compared with CO-oxidation occurring overnight, this may have a substantial effect on modeling studies that attempt to balance in situ CO production and sinks if the estimated diel CO consumption rates are based solely on dark incubations. In the present study we conducted CO-oxidation measurements both in the dark and situ during daylight hours, and confirm photo-inhibition of microbial CO oxidation.

## Methods and Materials

Study areas. Fieldwork was conducted during *R/V Endeavor* (URI) cruises EN327 and EN335 in waters near the Bermuda Atlantic Time-series Station (BATS) in August 1999 and March 2000, respectively. The ship was intended to remain approximately in the same water masses by following a free-drifting drogue and optical buoy. EN327 was occupied from Year Day (YD) 218 at 31.37°N, 64.00°W to YD 229 at 31.50°N, 64.03°W, and EN335 started on YD 77 at 32.01°N, 64.02°W and ended on YD 88 at 32.03°N, 64.01°W. Front passage or deviation from the Lagrangian mode was monitored by continually profiling sea surface temperature, salinity, and chlorophyll fluorescence and by recording the current vector versus depth and time via shipboard acoustic doppler current profiler. Surface insolation was recorded at 15 minute intervals by the ship's radiometer. The timing of the two cruises (summer versus early spring) was intended to maximize expected differences in CDOM concentration and the range of key variables, such as wind speed, biology, mixed layer and euphotic zone depths (Xie et al. 2001). High resolution measurements of dissolved [CO] in the surface waters (1m, 4m) were made with an automated continuous-flow-equilibration method and a surface-following outboard water sampler (Xie et al. 2001). Gas analysis was performed with an analytical system modified from an RGA3 Reduction Gas Analyzer (Trace Analytical Inc., Menlo Park, CA). The coastal sampling site was the WHOI Shore Lab pier, 1 km east of Nobska Light, Woods Hole, on a southeast-facing beach on Vineyard Sound, Cape Cod, in Massachusetts.

Sampling, incubation, and analysis. Pelagic samples were collected in 10-L Niskin bottles fitted with silicon O-rings and Teflon<sup>®</sup> coated springs or in custom-built titanium bottles (Doherty et al. 2003) mounted on a CTD rosette. Bulk water was collected from 8 m (summer) or 20 m (spring) within the surface mixed layer early in the morning (0100 – 0300 h) for in situ <sup>14</sup>C-CO

incubations and other assays (Xie et al., unpubl.). Water was drawn from the bottles without contact with the atmosphere into 100 ml ground-glass syringes after three complete flushes with sample water. 25.0 ml sub-samples were dispensed into 130 ml quartz vials for the in situ incubations and into acid cleaned 75 ml borosilicate glass serum vials for dark incubations in the laboratory. The bottles were fitted with crimp-sealed rubber septa. Coastal water samples were collected at the WHOI Shore Lab pier in acid-cleaned bottles or all-glass syringes at 0.5 m depth, in water depths of approximately 2 m.

$^{14}\text{C}$ -CO oxidation assay.  $^{14}\text{C}$ -labeled CO was generated by dehydration of 3.7 MBq  $^{14}\text{C}$ -labeled formate (2.07 GBq/mmol, 7:3 ethanol:water, ICN Biomedical) with 1.0 ml 36N anhydrous sulfuric acid in a sealed 120 ml serum vial kept at room temperature for 24 hours. The mixture was made alkaline with the gradual addition of 5.0 ml 10 mol L<sup>-1</sup> NaOH to sequester incidentally produced  $^{14}\text{C}$ -labelled CO<sub>2</sub>. Labeled product was transferred to an evacuated Tedlar<sup>®</sup> gas sample bag by displacement with CO-free distilled water.  $^{14}\text{C}$ -CO activity (DPM ml<sup>-1</sup> gas) in the stock bag was determined by injecting a 1 ml aliquot with a gas-tight syringe into a >400°C heated copper column (0.6 cm inner diameter x 20 cm) packed with copper oxide chaff as catalyst for oxidation of  $^{14}\text{C}$ -CO to  $^{14}\text{C}$ -CO<sub>2</sub>. The carrier gas was zero-grade compressed air at a flow-rate of 60 ml min<sup>-1</sup>. The  $^{14}\text{C}$ -CO<sub>2</sub> product was captured quantitatively when bubbled through a paired series of scintillation vials containing a mixture of 10.0 ml scintillation cocktail (Scintiverse II) and 0.2 ml hyamine hydroxide (ICN Biomedical, Inc.) downstream of the catalyst.  $^{14}\text{C}$  activity was measured via liquid scintillation spectroscopy in the laboratory.

For dark incubations, 1.0 ml aliquots of  $^{14}\text{C}$ -CO stock were injected into replicate pairs of septum-sealed vials containing 25 ml sample. Ambient room-air CO was determined with a reduced gas analyzer (RGA3, Trace Analytical). Dark incubations were performed in the



laboratory and maintained at in situ temperature. Incubations were terminated with 1.0 ml of 10 mol L<sup>-1</sup> NaOH injected into the reaction mixture to raise the pH of the sample to >9. The killed samples were shaken vigorously to sequester CO<sub>2</sub> product. “Zero-time” (t<sub>0</sub>) bottles were killed by injecting NaOH within 5 minutes of introducing <sup>14</sup>C-CO. Duplicate dark-incubated time-series samples were sacrificed with NaOH at three-hour intervals. <sup>14</sup>C-activity was assessed in the NaOH-stabilized samples at a shore-based laboratory, where <sup>14</sup>C-CO<sub>2</sub> was released, trapped, and assayed using the methods described by Griffiths et al. (1982). Total moles of CO within each incubation vessel included the CO contained in the 25 ml aqueous phase (derived from the in situ CO concentration at the depth of sample collection), the CO contained in the headspace of the bottle (derived from CO content of the lab atmosphere when the vial was sealed and the total volume of the bottle less 25 ml), and the moles CO injected at time-zero (t<sub>0</sub>). Total dissolved [CO] required the Bunsen solubility coefficient (β) for CO (Weisenburg and Guinasso 1979), which varies as a function of temperature and salinity.

BATS substrate-series. Water was collected by Niskin flask on a Kevlar wire and drawn into an acid-cleaned 2 liter Erlenmeyer flask. The sample water was sparged for 30 min with zero-grade air passed through a heated CuO catalyst that oxidized all environmental CO, allowing a tightly constrained specific activity of the label to be used in the assay. CO-free sample water (25.0 ml) was dispensed into 70 ml serum vials that had been flushed with CO-free air for 30 seconds at a flow rate of 5.0 ml s<sup>-1</sup>. Headspaces were continuously flushed with CO-free air until each vial was sealed. Labeled CO was then injected with a gas-tight syringe into each vial set. Multiple parallel time-series incubations under defined aqueous CO concentrations ranging between 0.01 and 10 nmol L<sup>-1</sup> were performed (0.1 nmol L<sup>-1</sup> -intervals between 0 – 1.0 nmol L<sup>-1</sup>, and approximately 1 nmol L<sup>-1</sup>-intervals between 1.0 and 10 nmol L<sup>-1</sup>) to establish the [CO] where

$V_{\max}$  of CO oxidation occurs. The time-series incubations consisted of paired samples incubated in the dark at in situ temperature and sacrificed approximately every 2 hours in 8-hour incubations.

Daytime in situ measurements. Six dawn-dusk in situ optical buoy incubations were performed in collaboration with O. Zafiriou (WHOI Dept. Chemistry) over an eight-day period in August 1999 (EN327), and seven incubations over an eight-day period in March 2000 (EN335). The in situ  $^{14}\text{C}$ -CO incubations were performed using water collected from a common depth within the mixed layer (8 m in summer, 20 m in spring) in order to isolate depth-dependent light-field as the variable affecting CO oxidation. Sample pairs were prepared as previously described in 130-ml quartz vials and incubated at discrete depths using an optical buoy developed for this purpose. The buoy was a cross-braced tetrahedron of 2.54 cm aluminum pipe 3 m on a side. The triangular base was floated at each corner by small, paired 35.5 cm Norwegian floats so that the apex, which bears a radio transmitter beacon, strobe light, and pennant, was maintained above the sea surface. A central sample-line attachment point was ~0.7 m above the water surface and 2 m from the corner floats, minimizing shadowing. Samples were attached neck-down in light, strong stainless steel cages at adjustable depths to a weighted wire rope. Light-shadowing was estimated to average <5%. The buoy was deployed at dawn and recovered at dusk, resulting in end-point measurements of CO oxidation over the integrated photic period. The quartz bottles for in situ incubations possessed a relatively large (>100 ml) headspace that provided a large reservoir of CO that was in equilibrium with the CO in the aqueous phase. A potential doubling of aqueous [CO] due to photo-production, when equilibrated into the headspace, resulted in <5% change in  $^{14}\text{C}$ -CO specific activity and hence, measured rates.

Calculations. First order disappearance of CO measured in dark incubated syringes follows the equation  $[\text{CO}]_t = [\text{CO}]_{t=0} \times e^{-k_{co}t}$ , from which the first order rate coefficient,  $k_{co}$  ( $\text{h}^{-1}$ ), was obtained. The CO disappearance rate  $R_{\text{CO}}$  ( $\text{nmol L}^{-1} \text{h}^{-1}$ ) was obtained from  $k_{co}$  and the aqueous concentration of CO using the equation  $R_{\text{CO}} = k_{co} \times [\text{CO}]$ . Using the  $^{14}\text{C}$ -CO oxidation method, first-order rate coefficients were calculated by normalizing the time-series rate of CO oxidation to  $\text{CO}_2$  ( $\text{nmol L}^{-1} \text{h}^{-1}$ ) by the total dissolved  $[\text{CO}]$  ( $\text{nmol L}^{-1}$ ) within each incubation vessel to yield the rate coefficient ( $k_{co}$ ) expressed in reciprocal hours ( $\text{h}^{-1}$ ). Normalization allows comparison with previous work. Nonlinear curve fitting was performed with SAAM II software for kinetic analysis v1.2 (SAAM Institute, University of Washington) and Microsoft Excel, using the Michaelis-Menton kinetic model  $V = (V_{\text{max}} \times [\text{CO}]_{\text{inc}}) \times (K_{\text{app}} + [\text{CO}]_{\text{inc}})^{-1}$ . The kinetic parameters  $V_{\text{max}}$  and  $K_{\text{app}}$  were determined by Lineweaver-Burke analyses of the dataset from each cruise.

## Results

If CO oxidation is the predominant sink for dissolved CO, then CO disappears at a rate consistent with the rate of CO<sub>2</sub> production in closed samples. We tested this hypothesis by simultaneously applying two common methods for measuring rates of biological CO transformation, the dark syringe incubation and <sup>14</sup>C-CO oxidation method, on water collected from Vineyard Sound in March, April, and June 2001. CO<sub>2</sub> production rates calculated from R<sub>CO</sub> (syringe) agreed with <sup>14</sup>C-CO<sub>2</sub> production rates (R<sup>14</sup>C-CO<sub>2</sub>) within 6% in March, 2% in April, and 5% in June (Fig. 1). To further confirm that dissolved CO was being oxidized to CO<sub>2</sub>, the fate of labeled carbon from <sup>14</sup>C-CO was investigated by time-series measurements of <sup>14</sup>C activity appearing in acid-unstable products (<sup>14</sup>C-CO<sub>2</sub> in headspace gas), acid-stable <sup>14</sup>C-POM and <sup>14</sup>C-DOM on filters and in filtrate, respectively. Neither the filter nor the filtrate retained any labeled carbon fixed as stable organic material; the filters and liquid filtrate from these incubations contained only background levels of activity (<50 decays per minute (DPM)). All CO-C was captured quantitatively as CO<sub>2</sub>. Rates measured by either method agree, and there are no unidentified sinks of CO. Dissolved CO consumed by microorganisms is oxidized to CO<sub>2</sub> rather than incorporated into particulate material over the course of 12 – 24 hour incubations.

Diel variations of in situ [CO] and rates of CO oxidation at the coastal sampling location were consistent with other coastal [CO] and CO oxidation measurements in similar environments (Jones and Amador 1993; Jones 1991). CO production is typically greater near coastal waters than open-ocean sites, probably due to larger CDOM content. The mid-day peak of [CO] at the Vineyard Sound location (15 nmol L<sup>-1</sup>), for example, is nearly 5 fold higher than the maximum [CO] measured in the Sargasso Sea (3.13 nmol L<sup>-1</sup>)(Jones 1991; Xie et al. 2002). Similarly, CO-oxidation rate coefficients in coastal waters, while variable on a seasonal or annual scale (Fig. 2),

are 5- to 10-fold greater than those measured in pelagic surface environments, suggesting a very active CO-oxidizing microbial community near-shore. In experiments to estimate kinetic parameters at the coastal site, CO oxidation rates were plotted against corresponding  $[CO]_{inc}$ .  $V_{max}$  ( $0.024 \text{ nmol L}^{-1} \text{ h}^{-1}$ ) and  $K_{app}$  ( $2.04 \text{ nmol L}^{-1}$ ) determined by Lineweaver-Burke analysis were used to construct the Michaelis-Menton model curve (Fig. 3). While in situ  $[CO]$ ,  $V_{max}$ , and  $k_{co}$  greatly differ between the pelagic and coastal waters, apparent half-saturation concentrations are remarkably similar (Table 1).

Sargasso Sea CO bio-oxidation studies. The microbial assemblage in the surface Sargasso Sea is typically subjected to low CO concentrations with maximum exposures of  $\sim 3 - 5 \text{ nmol L}^{-1}$  CO for brief periods in the mid-afternoon during peak photo-production. It is likely that CO-oxidizing enzymes of the active microorganisms become saturated just over peak environmental  $[CO]$ , and rate normalization assuming first-order kinetics underestimates the rate coefficients in incubations containing CO levels greater than that at  $V_{max}$ . Measured rates of CO oxidation in the Sargasso Sea are highly variable depending on depth of sample origin and time of day sampled, and hence, in situ  $[CO]$ . When dark rates were plotted versus  $[CO]_{inc}$ , nearly all of the incubations contain  $[CO]_{inc}$  below the concentration where microbial CO-oxidation was proceeding at  $V_{max}$  ( $10.88 \text{ nmol L}^{-1}$  in summer,  $7.3 \text{ nmol L}^{-1}$  in spring) (Fig. 4). Two spring incubations containing greater than  $7.3 \text{ nmol L}^{-1}$  CO were excluded as outliers in all other analyses. Normalizing each rate by the corresponding  $[CO]_{inc}$  resulted in the first-order rate coefficient,  $k_{co}$ .

A substrate-series experiment was conducted in July 2002 aboard the *R/V Weatherbird* (Bermuda Biological Station for Research, Inc.) in the vicinity of the Bermuda Atlantic Time-Series (BATS) station and the previous EN cruises. The substrate series experiment showed

$V_{\max}$  for the CO oxidation reaction of  $0.026 \text{ nmol L}^{-1} \text{ CO}$  oxidized  $\text{h}^{-1}$  and  $K_{\text{app}}$  of  $2.31 \text{ nmol L}^{-1} \text{ CO}$  (Fig. 5). The CO concentration at  $V_{\max}$  was  $4.6 \text{ nmol L}^{-1}$ , and is consistent with that observed in the coastal substrate series ( $4.1 \text{ nmol L}^{-1}$ ). The  $K_{\text{app}}$  for CO measured previously in the Sargasso Sea were  $5.44 \text{ nmol L}^{-1}$  in the summer and  $3.65 \text{ nmol L}^{-1}$  in early spring.

The EN327 (summer) and EN335 (spring) dark and in situ  $k_{\text{co}}$  values were plotted versus depth of origin or depth of incubation. Dark  $k_{\text{co}}$  was  $0.020 \pm 0.002 \text{ h}^{-1}$  (mean  $\pm$  st.dev) in the summer and  $0.006 \pm 0.004 \text{ h}^{-1}$  in the early spring (Fig. 6). The highest  $k_{\text{co}}$  were observed during the summer 1999 cruise where the dark rate coefficients ranged from  $0.015$  to  $0.025 \text{ h}^{-1}$ , with the maximum occurring at  $15 \text{ m} - 30 \text{ m}$ . These dark  $k_{\text{co}}$  were 10-fold higher than the in situ daytime  $k_{\text{co}}$  values for that season, and several-fold higher than both the dark and in situ rate coefficients in the spring. We did not observe increasing CO oxidation rates or  $k_{\text{co}}$  with increasing depth in any in situ incubation in either season, but all the in situ  $k_{\text{co}}$  values were significantly depressed relative to corresponding dark incubations in both seasons.

## Discussion

We found agreement between the two most commonly used methods to estimate CO disappearance rates in natural marine samples, which measure different sides of the assumed mass-balance of CO oxidation. CO is quantitatively converted to CO<sub>2</sub> only in living samples, thus the dominant sink of dissolved CO is in situ microbial oxidation. CO is not incorporated as biomass, though it is possible that autotrophic bacteria may incorporate CO-C after it has oxidized, whether or not they played a role in CO oxidation, but this was not observed in our 12 – 20 h incubations.

The <sup>14</sup>C-CO oxidation method has an advantage over the syringe method in that it can be performed in the presence of photo-production and in situ. The syringe method, while easiest to perform, is limited to dark incubations to prevent interference from photochemical CO production and cannot be used to assess light effects, i.e., potential inhibitory effects of ultra violet radiation on bacterial activity. Application of the <sup>14</sup>C-CO oxidation method in situ shows that daytime CO consumption is negatively affected by daytime light regimes, and rates (and rate coefficients) determined solely from dark incubations can not accurately estimate diel CO turnover. When applying the <sup>14</sup>C-CO oxidation method in waters containing only nanomolar levels of dissolved CO, it is important to consider that accuracy of determined  $k_{co}$  depends upon the assumption that the reaction is first-order. Large additions of <sup>14</sup>C-CO as tracer cause enzyme-saturation and deviation from first-order kinetics which causes under-estimation of  $k_{co}$ . In the Sargasso Sea cruises, where  $K_{app}$  was 5.44 nmol L<sup>-1</sup> in summer and 3.65 nmol L<sup>-1</sup> in spring, the range of [CO]<sub>inc</sub> spanned up to 9.6 nmol L<sup>-1</sup>, suggesting that a few incubations having high [CO]<sub>inc</sub> were in the higher transitional region of the Michaelis-Menton model. Therefore,  $k_{co}$  in incubations containing greater than 7.3 nmol L<sup>-1</sup> CO, calculated under strict first-order

assumptions, may be under-estimated by as much as 50%. This is not perceived as a crucial error in our data since the vast majority of our incubations contained between 3 and 7 nmol L<sup>-1</sup> CO, and deviation from first-order kinetics is negligible in this range.

There are instances of possible under-estimation of  $k_{co}$  due to saturation kinetics in previous studies. To illustrate, Jones (1991), using the <sup>14</sup>C-CO oxidation method in dark incubations, measured rate coefficients for CO consumption in the Sargasso Sea surface waters that were 0.012 – 0.018 h<sup>-1</sup> in July, and 0.0045 – 0.011 h<sup>-1</sup> in September. Jones and Amador (1993), in a study to determine the impact of the Orinoco River on the consumption of CO and CH<sub>4</sub> and the photo-production of CO in the southeastern Caribbean Sea, reported CO oxidation rate coefficients were minimum at a station in Mona Passage (0.028 h<sup>-1</sup>), increased with proximity to the Orinoco River outflow, and were highest near the mouth of the river (0.199 h<sup>-1</sup>). However, Jones (1991) and Jones and Amador (1993) added large amounts of CO (178 nmol L<sup>-1</sup> <sup>14</sup>C-CO as a tracer substrate) to their samples. This added [CO] was far above the ambient [CO] in the areas they investigated (<4 nmol L<sup>-1</sup> in the S.W. Sargasso Sea and 0.9 – 31.6 nmol L<sup>-1</sup> in the E. Caribbean). If the half-saturation concentrations for these areas are similar to those reported here, the  $k_{co}$  values of Jones (1991) and Jones and Amador (1987) are probably underestimated.

In an incubation containing extremely high [CO]<sub>inc</sub> in the Sargasso Sea substrate series, CO oxidation activity was nearly zero when [CO]<sub>inc</sub> = 19 nmol L<sup>-1</sup> CO, or six-fold greater [CO] than peak ambient levels (data not shown). Therefore, saturation kinetics gives way to inhibition kinetics at [CO] between 10 nmol L<sup>-1</sup> and 19 nmol L<sup>-1</sup>. There are no data to interpolate between these two [CO] values as our objective was to determine the transition point between first- and



zero-order kinetics at low [CO], therefore the transition between CO saturation and inhibition kinetics was poorly constrained.

Our estimates of oceanic and coastal dark CO consumption rates and rate coefficients in the surface water are consistent with many prior studies that used syringe assays (Conrad and Seiler 1982b),  $^{14}\text{C}$ -CO oxidation methods (Jones and Amador 1993; Jones 1991) or calculation using numerical models (Johnson and Bates 1996) to estimate these parameters. Conrad et al. (1982) and Conrad and Seiler (1982) measured the decay of CO in surface samples from the equatorial Atlantic Ocean, and reported rate coefficients on the order of  $0.03 - 0.05 \text{ h}^{-1}$  in dark incubations, and  $K_{\text{app}}$  of  $7 \text{ nmol L}^{-1} \text{ CO}$ . Johnson and Bates (1996) estimated CO loss rate coefficients in the tropical South Pacific by an exponential fit method, and reported mean  $k_{\text{co}}$  values as  $0.032 \pm 0.0071 \text{ h}^{-1}$  in April, and  $0.009 \pm 0.0052 \text{ h}^{-1}$  in December.

An implicit assumption common to the aforementioned studies, however, is that the CO oxidation rates and rate coefficients measured in dark incubations of natural samples is representative of the entire diurnal period. Vanzella et al. (1989) suggested that high light intensity in laboratory assays negatively affects the rate of ammonium and nitrite oxidation by chemolithotrophic bacteria that are potential contributors to in situ CO-oxidation in oceanic waters (Jones and Morita 1983). These experiments were performed with pure-cultures rather than natural water samples. Our daytime in situ  $^{14}\text{C}$ -CO-oxidation incubations with natural water samples clearly demonstrate light-related inhibition of CO oxidation compared to dark incubations. CO-oxidation rates and rate coefficients were significantly depressed during daylight hours at all in situ depths tested (0-35 m) relative to dark incubations.

CO turnover times, often reported in “days” (the reciprocal of the rate coefficient  $\text{d}^{-1}$ ), may be inaccurate if in situ CO oxidation is depressed by daytime light intensities. Slower rates

of CO oxidation during daylight hours translate to over-estimates of diel rate coefficients based on dark incubations and, hence, under-estimates of CO turnover times. For example, the turnover time calculated using only summertime dark incubation values integrated over 24 hours is approximately 2.1 days. When this value is corrected to account for photo-inhibition during the daytime, the integrated turnover time (12 hours daytime, 12 hours dark) is 3.8 days.

There is some discrepancy between estimates of  $k_{co}$  by different groups utilizing diverse methods at the same oceanic site. Najjar et al. (pers. comm.) employed a one-dimensional physical-chemical coupled model to simulate the diurnal cycles of surface-water [CO] and CO-inventory from the same field investigation as this study (EN327 near BATS). The rate coefficient constrained from this model is  $0.091 \text{ h}^{-1}$ , or over four-fold higher than the night-time or dark  $k_{co}$  value derived in the current study. The work of Xie et al. (unpubl.), which was conducted during the same field investigation, employed a nighttime inventory method to determine  $k_{co}$  of  $0.061 \text{ h}^{-1}$ , or 3-fold greater than our value of  $k_{co}$ . Further study is required to resolve the contradictory results from these three groups.

Daytime dissolved [CO] in surface waters increases rapidly because of the combined effects of 1) CO photo-production, 2) light-induced inhibition of the microorganisms that oxidize CO, and 3) saturation of CO-oxidizing enzymes at or near peak daytime dissolved [CO]. Conversely, nighttime dissolved [CO] decreases rapidly because photo-production of CO ceases, the activities of CO metabolizing microorganisms are no longer inhibited, and CO-oxidizing enzymes are no longer saturated with high levels of dissolved CO and resume first-order kinetic behavior.

The  $k_{co}$  determinations in the Sargasso Sea and in Vineyard Sound show an enormous difference in CO-oxidation activity between oligotrophic and coastal waters. This is probably due to the greater abundance of CDOM and, hence, dissolved CO, in combination with greater bacterial cell density in coastal waters. In contrast, the kinetic behavior of CO-oxidation is similar in both environments, reflected by nanomolar affinities for CO at each study site. This may imply that the CO-oxidizing enzymes, or perhaps the phylogenetic or functional microbial group(s) responsible for CO-oxidation, are similar in pelagic and coastal systems.

Microbiological studies to determine the identity, abundance, and phylogenetic relatedness of microorganisms responsible for environmental CO-oxidation have the potential to improve our understanding of this component of carbon cycling in natural waters.

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**Table 1:** Summary of sampling and kinetic parameters for assays conducted during this study.

| Cruise                     | Incubation type            | Temp. (°C) | Mixed layer depth (m) | Surface [CO] diel range (nmol L <sup>-1</sup> ) | Depth sampled (m)              | Depth incubated (m)          | $k_{co}$ (h <sup>-1</sup> ) mean $\pm$ st.dev. n | $V_{max}$ (nmol L <sup>-1</sup> h <sup>-1</sup> ) | $K_{app}$ (nmol L <sup>-1</sup> ) |
|----------------------------|----------------------------|------------|-----------------------|---|--------------------------------|------------------------------|--|---|-----------------------------------|
| EN327 (summer 1999)        | Dark <sup>14</sup> C-CO    | 27         | 32                    | 0.44 - 2.4                                      | 1, 4, 8, 14, 22, 32            | --                           | 0.020 $\pm$ 0.002 n=34                           | 0.186   | 5.44                              |
|                            | In situ <sup>14</sup> C-CO | 27         | 32                    | 0.44 - 2.4                                      | 8                              | 0.3, 1.0, 2.5, 5, 9, 15, 20  | 0.002 $\pm$ 0.001 n=27                           | --  | --                                |
| EN335 (spring 2000)        | Dark <sup>14</sup> C-CO    | 19         | 123                   | 0.3 - 3.13                                      | 1, 4, 5, 9, 10, 15, 20, 22, 33 | --                           | 0.006 $\pm$ 0.004 n=25                           | 0.048   | 3.65                              |
|                            | In situ <sup>14</sup> C-CO | 19         | 123                   | 0.3 - 3.13                                      | 20                             | 0.3, 1.0, 2.0, 5, 10, 20, 30 | 0.003 $\pm$ 0.001 n=39                           | --  | --                                |
| BATS 166 (summer 2002)     | Dark <sup>14</sup> C-CO    | 26         | 30                    | --  | 5                              | --                           | 0.007 $\pm$ 0.003 n=14                           | 0.026   | 2.31                              |
| Vineyard Sound (1999-2000) | Dark syringe               | 5 - 20     | --                    | 1.5 - 12.1                                      | 0.5                            | --                           | 0.127 $\pm$ 0.038 n=8                            | --  | --                                |
|                            | Dark <sup>14</sup> C-CO    | 5 - 20     | --                    | 1.5 - 12.1                                      | 0.5                            | --                           | --   | 0.024   | 2.04                              |

Tolli, Table 1



**Figure 1:** Comparison of CO assays. (A, C, and E) dark syringe incubations to determine first-order rate coefficients ( $k_{co}$ ) of CO disappearance. (B, D, and F) CO<sub>2</sub> production rate calculated from dark syringe  $k_{co}$  ( $R_{CO} = k_{co} \times [CO]$ ), and CO oxidation rate ( $R_{14CO_2}$ ) directly measured by <sup>14</sup>C-CO oxidation.

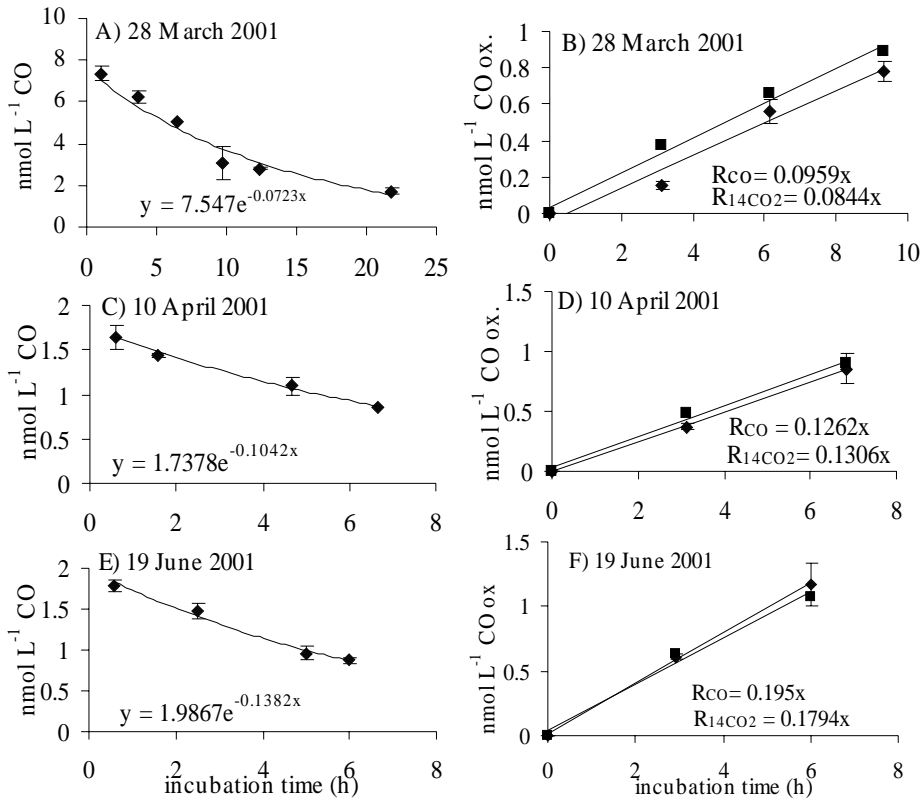
**Figure 2:** Seasonal variability in rate coefficient ( $k_{co}$ ) of CO disappearance in Vineyard Sound water, determined in dark syringe incubations conducted in 2000 and 2001. Mean  $k_{co} = 0.127 \pm 0.038 \text{ h}^{-1}$  (mean  $\pm$  st.dev, n=8)

**Figure 3:** Substrate series to estimate kinetic parameters of coastal microbial assemblage for CO oxidation (Vineyard Sound, 05-Dec-2000).  $[CO]_{inc}$  results from injected tracer only. CO oxidation obeys first-order kinetic behavior up to  $4 \text{ nmol L}^{-1}$  dissolved CO. The Michaelis-Menton kinetic model is shown, constructed from parameters derived from the Lineweaver-Burke double-reciprocal plot (inset).  $V_{max} = 0.024 \text{ nmol L}^{-1} \text{ h}^{-1}$ , and  $K_{app} = 2.04 \text{ nmol L}^{-1}$ .

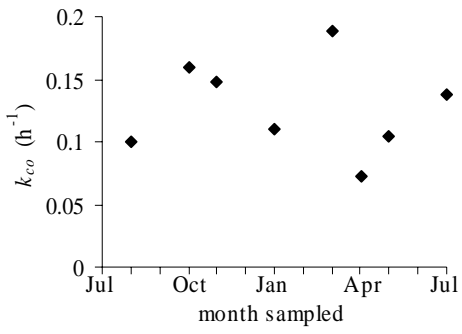
**Figure 4:** Dark rates of CO oxidation determined at various  $[CO]_{inc}$  during EN327 (summer) and EN335 (spring). The Michaelis-Menton model for each dataset is shown, derived from the kinetic parameters that result from Lineweaver-Burke analyses (insets).  $V_{max}$  and  $K_{app}$  in summer were  $0.186 \text{ nmol L}^{-1} \text{ h}^{-1}$  and  $5.44 \text{ nmol L}^{-1}$ , and  $0.048 \text{ nmol L}^{-1} \text{ h}^{-1}$  and  $3.65 \text{ nmol L}^{-1}$  in spring.

**Figure 5:** Sargasso Sea substrate-series, July 2002 near the Bermuda Atlantic Time-series Station (BATS).  $[CO]_{inc}$  results from injected tracer only. The open circle is a killed control ( $5 \text{ mg L}^{-1} \text{ NaCN}$ ). The Michaelis-Menton model is shown, constructed with kinetic parameters that result from Lineweaver-Burke analysis.  $V_{max} = 0.026 \text{ nmol L}^{-1} \text{ h}^{-1}$  and  $K_{app} = 2.31 \text{ nmol L}^{-1}$ .

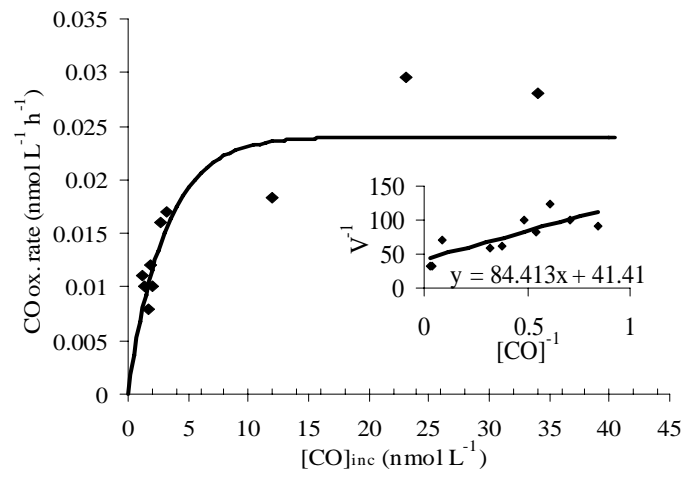
**Figure 6:** Summer (top) and spring (bottom) in situ and dark incubation rate coefficients (mean  $\pm$  SEM) at depths of incubation or depth of sample origin.



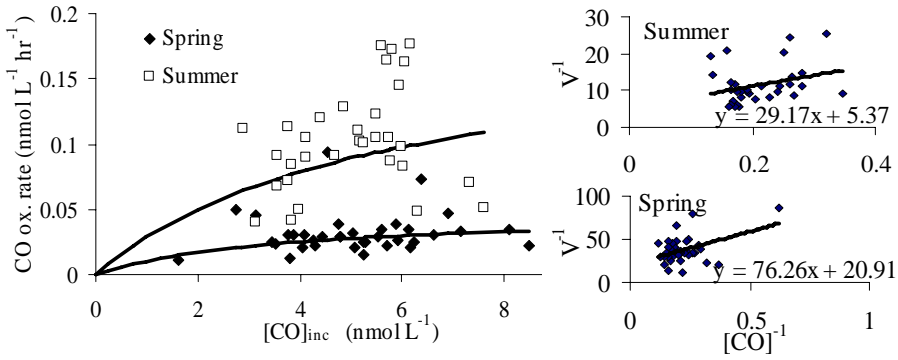
Tolli, Figure 1



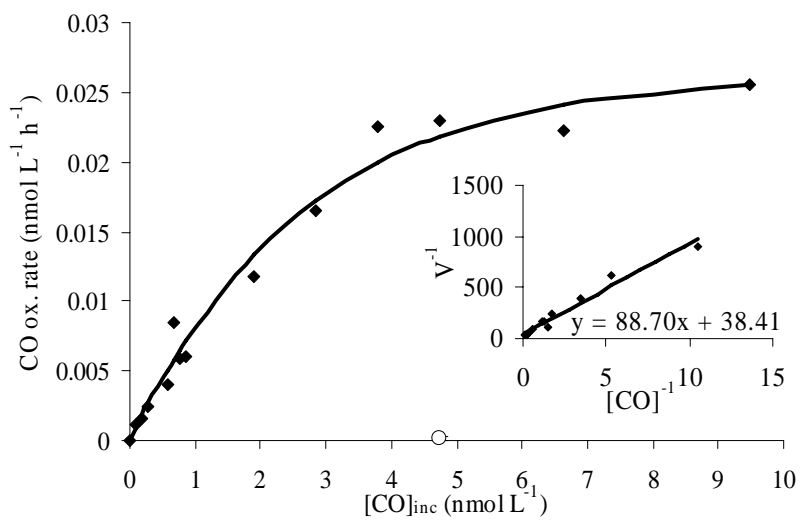
Tolli, Figure 2



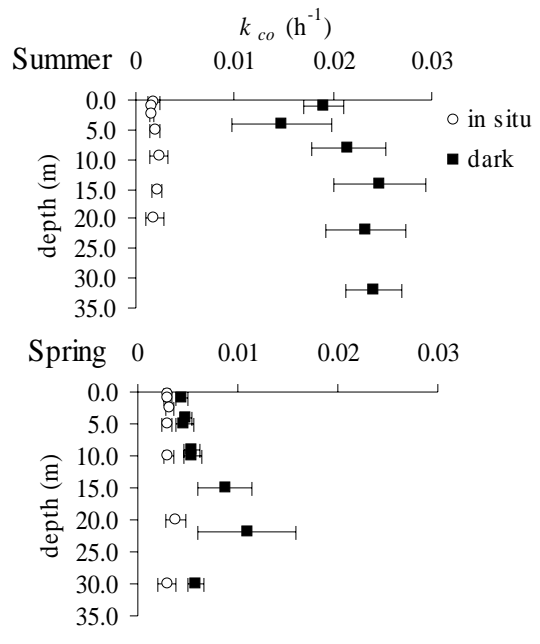
Tolli, Figure 3



Tolli, Figure 4



Tolli, Figure 5



Tolli, Figure 6