Nitrification kinetics

Causes and Characterization of the San Joaquin River Hypoxia

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Project Description

Dissolved oxygen (DO) concentrations in the Stockton Deep Water Ship Channel (DWSC) respond rapidly to changes in flow under conditions that are poorly understood. For example, the DO declined from 8 mg/L on January 1, 2003 to 0 mg/L in early February. The DWSC at Rough and Ready Island remained anoxic throughout most of February. More recently in May, 2004, the DO fell from 8 mg/L to 3 mg/L immediately after VAMP inputs ceased, causing the net flow to fall from 2500 to 400 cfs. Other extreme events are also well documented, but poorly understood and problematic to model accurately.

Preliminary data suggest that high ammonia concentrations, exacerbated by low net flows in the San Joaquin River, are the primary cause of the acute hypoxia observed in the DWSC during the winter months. Research is continuing to verify this hypothesis by monitoring the recovery of this river reach and contributing factors. Kinetic rate studies of oxygen demanding substances are being performed in the laboratory and will be compared with analyses of field observations. Early kinetic rates investigation results suggested that ammonia and nitrite oxidizing bacteria population dynamics play an important role. As such, identification and enumeration of these microorganisms was added to the investigation.

This study investigates the causes for extreme dissolved oxygen deficits that were common in 2003 and 2004. Some of the mechanisms responsible for these severe episodes have been quantified. Quantification of important parameters necessary to describe and predict the DO response in the DWSC have also been performed.

Objectives

To determine the causes for the hypoxic conditions in the San Joaquin River several approaches are underway or proposed:

- Determination of the kinetic rates of decay of biochemical oxygen demand (BOD), carbonaceous BOD (CBOD), nitrogenous BOD (NBOD), and ammonia for water collected from the San Joaquin River with laboratory experiments.

- Determination of the kinetic rates of decay of BOD, CBOD, NBOD, and ammonia from longitudinal field measurements performed during critical low DO events.

- Establish mechanistic relationships between nitrification kinetics and bacteria populations to enhance modeling accuracy of ammonia utilization and associated DO depletion. Ammonia and nitrite oxidizing bacteria will be enumerated with standard MPN techniques and real-time polymerase chain reaction (PCR) methods. Identification and relative enumeration of these bacteria and the total bacterial community will also be attempted with DNA sequencing and

terminal restriction fragment length polymorphism (TRFLP). Perhaps of more value, the latter technique provides a characteristic "fingerprint" of the bacteria species present in a water sample, which could be used to trace organisms back to their source.

- Extend the DNA sequencing and TRFLP method to algal species. This rapid identification technique may permit tracking algae back to a source based on their DNA fingerprint. This will provide a means of determining the oxygen depletion associated with particular algal species entering in the DWSC. The TRFLP technique could also yield another method for assigning load allocations to responsible upstream parties if the monitoring were extended above Mossdale.

Scope of work

Field and laboratory investigation include the following components.

- Field monitoring above and within the critical reach of the DWSC before and during periods of low flows. Monitoring will be performed as appropriate during extreme events.
    - Longitudinal water quality surveys with *in situ* water column profiles of temperature, DO, pH, turbidity, fluorescence (chlorophyll *a*) at the following navigational lights (Lt) or stations: Lt 24, Lt 28, Lt 34, Lt 38, Lt 48, Rough and Ready Island (RRI) at the Department of Water Resources (DWR) continuous monitoring station, below the City of Stockton wastewater effluent outfall (near City Station R2A), in French Camp Slough, and above the influence of the City of Stockton effluent discharge (approximately 2 miles above the outfall). Two stations in the Turning Basin will also be added to assess the contribution of algae transported to the DWSC.
    - Collection of water samples near the surface or at mid-depth to be analyzed in the laboratory for BOD, CBOD, ammonia, nitrate, chlorophyll *a*, and pheophytin *a*.
- Laboratory kinetic rate studies of BOD, CBOD and nitrification.
    - BOD and CBOD decay rates are determined with long-term monitoring of DO in closed reactors maintained at river temperature and 20°C. The CBOD test is performed with a nitrification inhibitor. Duplicate trials are established for selected sampling locations.
    - Nitrification rates are quantified by measuring the long-term monitoring of ammonia and nitrate in closed reactors maintained at river temperature and 20°C. Duplicate or triplicate trials are established for selected sampling locations and times. Important nitrification mechanisms associated with ammonia and nitrite oxidizing bacteria populations will be performed.
- Development of molecular methods for bacterial and algal species identification and relative enumeration.
    - The presence and abundance of ammonia-oxidizing bacteria (AOB) will be determined for water samples taken from three locations in the San Joaquin River: upstream at the wastewater outfall, downstream near Rough and Ready Island, and a midstream site. Each site will be sampled at three different times. At each site on each sampling trip, 2 replicate samples will be taken, generating approximately 18 samples overall. Samples

will be collected by pumping river water into 1 liter glass bottles, which will be held on ice in the dark for a short period before lab analysis.

Two approaches will be used to test for the presence and abundance of AOB. First, the Most-Probable-Number analysis (MPN) using microplates will be employed (Rowe et al., 1977), with modifications for culturing nitrifying bacteria (Lipponen et al., 2002). This technique is widely used and allows for a crude quantification of AOB, however it is believed that this analysis underestimates the actual AOB quantities (Belser 1979). To provide another, possibly more accurate estimate of AOB quantity, the real-time PCR method will be tested for feasibility (Hermansson and Lindgren 2001). This recent technique detects the amount of AOB-specific DNA in the water sample, rather than the actual living cells. The advantage of real-time PCR is high sensitivity and short analysis time, however the disadvantages are that environmental samples contain PCR-inhibiting chemicals that reduce sensitivity, and exact quantification requires primers that are well-matched to the river bacteria and that appropriate standard curves can be prepared. Considering the present lack of information on the bacterial community in the river, the preparation of such primers and standard curves is problematic. The results of the two analyses will provide two independent sources of data, and allow for a comparison of detection efficiency, which may direct future research efforts.

A third AOB detection method, immunofluorescence, is known to be highly accurate for nitrite-reducing bacteria (NOB) quantification (Feray 1999), however specific DNA sequence data is required for the target organisms. Although this method is not being attempted in this work, the baseline sequence data will be generated in the AOB sequencing analysis study. Future work, pending additional funding, may explore this method.

Furthermore, for the understanding of nitrifying-bacteria diversity in the river, a sequencing study will be conducted to identify the major species in the river. Then, a TRFLP analysis will be generated, with the sequence data allowing for the correlation between terminal restriction fragments (TRF) peaks and specific bacterial species. The fingerprint patterns will permit an analysis of community diversity in several areas of the river. As mentioned above, these data could form the basis for future work on immunofluorescent detection and quantitation of AOB and NOB in the river.

- Molecular-based algal species identification and community fingerprinting will involve the design and testing of DNA primers that are specific for each of the several major phylogenetic groups of algae, including greens, diatoms, blue-greens, golden, etc. Initially, a sequencing study will be conducted that will identify the major species of algae in each taxonomic group. This work involves comparison of river sequences with known sequences in public databases. This information will allow for specific identification of TRFLP markers, correlating each marker with a species or species group. Then, TRFs will be generated, producing fingerprints representing the algal community present in each water sample. With such information it may be possible to correlate particular species with DO depletion, and to track water movement by tracking algal community movement. In addition, the analysis will provide a means of determining the relative abundance of each species.

- Compilation and analyses of complementary data collected by the City of Stockton, Department of Water Resources, Central Valley Regional Water Quality Control Board, and the U.S. Geological Survey. This data will be used with the longitudinal field data to obtain apparent BOD and nitrification rates using a simple finite-section model of the critical reach of the DWSC.

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