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Impact of a Hydrate-Based Marine Desalination Technology on Marine Microbiota and Water Quality

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14. ABSTRACT Marine Desalination Systems (MDS) is currently developing new technology in gas hydrate formation to supply potable water. To scale this technology to support different country needs there is a requirement to assess the environmental impact of desalination. The NRL MDS collaboration on biological influence of marine desalination focuses on water quality of processed water and environmental impact of the marine desalination application. With this segment of the project accomplished, the subsequent evaluation will focus on the impact of large scale marine desalination on environmental quality. To assess the environmental impact of the marine desalination system there is a need to understand resulting changes to key bacterial and elemental cycles. Seawater vertical transport, alteration of water flow, and filtration have potential to change the microbial community diversity, substrate availability, electron acceptors and key element speciation. Microorganisms surveyed in this analysis control elemental cycles that set environmental quality for the entire aquatic food chain. This study initiates assessment of the effect of the MDS process on water quality and the growth rate of the natural bacterial assemblage. We measured heterotrophic bacterial production, dissolved organic carbon (DOC) concentration and stable isotope values, and fluorescence spectra of the organic matter that partition into the hydrate and waste water streams.					
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IMPACT OF A HYDRATE-BASED MARINE DESALINATION TECHNOLOGY ON MARINE MICROBIOTA AND WATER QUALITY

Lab Report: Marine Desalination
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INTRODUCTION

Shortages of potable water affect 88 developing countries where 80-90% of all diseases and 30% of all deaths result from poor water quality (Leitner 1998, see also review by Miller 2003). Water short fall in many countries is at the level of petroleum availability. With the current predictions of global population expansion this problem will increase the need for a solution. One strategy for generation of high quality potable water involves desalination via clathrate crystal formation. Forty years ago, the US Department of the Interior supported construction of two freeze desalination pilot plants that were based on clathrate formation (CCl_2F_2 , and butane) (Miller 2003). Dendritic hydrate crystal formation made it difficult to separate the freshwater crystals from the aqueous brine (Barduhn 1982). A Bureau of Reclamation study (McCormack and Andersen 1995) was followed by a partially successful pilot test conducted at the Natural Energy Laboratory of Hawaii (McCormack and Niblock 1998, Miller 2003). The primary issues still involved clathrate crystal separation which led to a subsequent program to determine the filterability of crystals, the surveying alternate higher temperature clathrate formers and design and operation of a wash column (McCormack and Niblock 2000, Miller 2003).

Marine Desalination Systems (MDS) is currently developing new technology in gas hydrate formation to supply potable water. To scale this technology to support different country needs there is a requirement to assess the environmental impact of desalination. It is important to determine the MDS technology impact on local water quality and marine microbiota through partitioning of dissolved organic carbon and brine production in wastestreams. High local fluctuations in salinity that would inevitably result from the hydrate formation may be comparable to those seen in sea ice formation. The internal environment of sea ice is known as the brine channel system and is characterized by low temperature, high salinity and elevated concentrations of dissolved nutrients and dissolved organic carbon (Kaartokallio et al. 2005). These changes are known to affect the composition of estuarine and marine bacterial assemblages (Ezura et al. 1974, Rheinheimer 1977) and particularly psychrophilic marine bacteria (Helmke and Weyland 1995, Morita 1976, Morita et al. 1973, Stanley and Morita 1968). The latter class of bacteria may be important in the expected deep water hydrate formation zone for the MDS technology. Bacteria change the composition of their cell membranes in response to local environmental conditions and these alterations can make them more or less susceptible to drastic temperature and salinity gradients (Nichols et al. 2000). These unique environments lead to differences in bacterial community structure between sea ice assemblages, such as those in the hydrate, and those in the open water, such as those that might be produced in the process streams of the MDS technology (Del Giorgio and Bouvier 2002, Kaartokallio et al. 2005). The bacterial community in naturally occurring hydrate formations has been reported to harbor unique eubacteria and archebacteria genotypes not reported elsewhere (Reed et al. 2002, Mills et al. 2005) including methanotrophic *Archaea* (Lanoil et al. 2001). Deep sea methane hydrate assemblages are known to be adapted to the elevated salinity that would be commensurate with hydrate formation coupled with the generation of interstitial brine (Kaye and Baross 2004). These microorganisms are responsible for key elemental cycles in nature that affect the ecosystem health.

This study initiates assessment of the effect of the MDS process on water quality and the growth rate of the natural bacterial assemblage. We measured heterotrophic bacterial production, dissolved organic carbon (DOC) concentration and stable isotope values, and fluorescence spectra of the organic matter that partitions into the hydrate and waste water streams.

MATERIAL AND METHODS

Sampling

Samples were collected in 250 mL Nalgene bottles previously acid-washed (2% HCl) and rinsed with MilliQ grade water. Samples for stable isotope analyses were collected in cleaned (acid-washed and then baked at 450 °C for 6 h) glass bottles using clean techniques (e.g., gloves). Atlantic Ocean seawater was collected from Indian River Inlet, DE on an incoming tide. MDS system source water was collected from Tampa Bay surface water. After operation of the MDS hydrate formation and dissociation system (Figure 1), water samples were collected from above the hydrate diaphragm (upstream) and below the hydrate (downstream). In addition, frozen hydrate crystals were collected and melted at room temperature (hydrate water).

Synchronous fluorescence

Seawater samples (3 mL) were used for Synchronous Fluorescence (SF) measurements on a Shimadzu RF5301 spectrofluorometer operating in the synchronous mode. Excitation wavelength range will be 236 to 600 nm with a 14 nm offset. All spectra acquired were calibrated to the water Raman signal and normalized to instrument corrections.

Dissolved organic carbon and stable isotope composition

Seawater samples (40 mL) were filtered through 0.2 µm (nominal pore diameter) Teflon filters into cleaned glass vials and sealed with Teflon-lined silicone closures. H₃PO₄ (85%) was added to adjust the pH of samples to ~3 and then samples were sparged for 10 min with UHP Helium. DOC measurement were conducted by heated (98°C) persulfate oxidation on an OI Analytical 1010 TOC analyzer employing an NDIR detector that measures CO₂ evolved from the oxidation. Potassium hydrogen phthalate was used to construct a calibration curve. Stable carbon isotopes of DOC ($\delta^{13}\text{C}$ -DOC) were measured on the evolved CO₂ that vents from the OI instrument. Teflon tubing connected to the 1010 vent carries the CO₂ to a 750°C reactor containing elemental copper and silvered cobaltous reagent to remove halogens. The scrubbed CO₂ was then re-focused using a Porapak GC column and transferred through a ConFlo III interface to the inlet of a ThermoElectron DeltaPlusXP isotope ratio mass spectrometer (IRMS). $\delta^{13}\text{C}$ -DOC values was normalized to the VPDB international stable isotope standard of L-glutamic acid ($\delta^{13}\text{C}$ -DOC = -26.2‰).

Tangential flow filtration

A PALL MiniTan microcassette tangential flow filtration system was used to separate DOC compounds greater than 1000 Daltons. A peristaltic pump was used to recirculate 100 mL of water in the filtration system. SF, DOC, and $\delta^{13}\text{C}$ -DOC was measured on the filtrate and the retentates of the TFF system to estimate the changes to these properties induced by selective removal of higher molecular weight organic C compounds.

Dissolved inorganic carbon

Dissolved inorganic carbon was measured using a Somma coulometer (Johnson et al. 1987).

Heterotrophic bacterial production

The leucine incorporation method (Kirchman et al. 1985, Kirchman 1993, Smith and Azam 1992) was used to measure bacterial production as adapted by Montgomery et al. (1999). A 0.50 μL aliquot of wet surface sediment from each station was added to 2 mL centrifuge tubes (three experimental and one control) which were pre-charged with [^3H -4,5]-L-leucine ($154 \text{ mCi mmol}^{-1}$). The sediment was extracted from the benthic grab sample and added to the 2 mL tube using a 1 mL plastic syringe with the end cut off. One mL of 0.45 μm nom. pore dia. (Acrodisk, Gelman) filtered bottom water (collected <1 m above bottom) was then added to each tube to form a sediment slurry. Samples were incubated for 1-2 h at *in situ* temperatures and subsequently processed by the method of Smith and Azam (1992). A constant isotope dilution factor of 1000 was used for all samples. This was estimated from actual measurements of sediment dissolved free amino acids (Burdige and Martens 1990) and saturation experiment estimates (Tuominen 1995). One mL syringed samples of wet sediment were dried at 50 $^{\circ}\text{C}$ and used to convert production values to dry weight. Leucine incorporation rate was converted to bacterial carbon using factors determined by Simon and Azam (1989).

RESULTS AND DISCUSSION

Water Quality

Salinity

The salinity of the seawater from the source (Tampa Bay) and various process streams (Figure 1) were determined for 12 experiments generating hydrates. The primary goal of the desalination process is to create low salinity hydrate by excluding sea salt ions from the hydrate matrix and partitioning them into the downstream waste water. Depending on the volume of the hydrate produced relative to that of the process water, downstream salinity may measurably increase. Over the course of the 12 experiments, salinity of the melted hydrate water ranged from 0 to 7 ‰ with 10 of the samples ranging from 0 to 3 ‰ (Figure 2). As expected, source water and upstream water ranged from 27 to 34 ppt and covaried. Oddly, salinity in the downstream water actually decreased slightly ranging from 27 to 31 ‰. It was expected that the downstream water would increase in salinity with formation of the low salinity hydrate.

pH

Source water pH ranged from 6.6 to 7.5 among the 12 experiments and was lower in upstream (5.5 to 6.5) and downstream (5.7 to 6.2) samples (Figure 3). In the melted hydrate water, the total pH range was much greater (4.7 to 6.4) with seven of the experiments ranging from 4.7 to 5.3 and four experiments ranging from 6.0 to 6.4 (Figure 3).

Dissolved organic carbon

DOC concentration increases from 2 to 4 mg C L^{-1} in the source water to 2 to 14 mg C L^{-1} in the hydrate (though all but one sample are 2 to 7 mg C L^{-1}) to 3 to 8 mg C L^{-1} in the upstream water and 5 to 12 mg C L^{-1} in the downstream water (Figure 4). Relative to the source water, DOC concentration increases in all media suggesting that the gas involved in hydrate formation (propane) or other compounds associated with the gas that is bubbled into the upstream water is contributing to DOC in the process streams. The $\delta^{13}\text{C}$ -DOC values in the source water range from about -24 to -26 (Figure 5). With the exception of the two anomalous hydrate values of -20, the range of $\delta^{13}\text{C}$ -DOC values for the upstream, downstream and hydrate samples are all lighter (-24 to -30) than that in the source water. When $\delta^{13}\text{C}$ -DOC values are compared with increasing DOC concentration, isotope values become lighter,

changing from -24 at 2 mg L^{-1} to around -30 at 10 to 12 mg L^{-1} DOC (Figure 6). Petroleum hydrocarbons, such as the gas that was used to form the hydrates, have $\delta^{13}\text{C}$ values around -28 to -30 . These data suggest that the dissolved hydrocarbon gas used to form the hydrate was influencing both the DOC concentration and the stable carbon isotope values in the process water and hydrate. Before future experiments are conducted the source of the DOC will be identified and the system will be redesigned to prohibit this contamination. Hydrate formation has recently focused on carbon dioxide as the catalyst gas and this may avoid future experimental problem.

Synchronous fluorescence (SF) profiles of organic matter in coastal waters can indicate the presence of certain moieties common to either terrestrially derived carbon (allochthonous: fulvic and humic acids) or marine derived (autochthonous: phytoplankton and other primary producers) (Figure 7). SF emission spectra derived from the source water were typical for estuarine systems with both terrestrial and phytoplankton related signals (Figure 8). The upstream process water spectra maintain many of the features of the source water spectra, as expected, but add a fluorescence peak at around 270 nm . This feature is also seen with the downstream water spectra and is the only peak in the hydrate water spectra (Figure 8). This peak is most likely produced from the addition of the dissolved hydrocarbon gas used to form the hydrate. The hydrate also appears to have excluded much of the organic matter signature associated with the source water.

Marine Microbiota

Process water production

The MDS process can affect the natural bacterial assemblage in primarily two ways: metabolic rate (production) and assemblage composition. Changing water quality can impact the microbial assemblage by reducing microbial production which would result from lower organic substrate or through environmental stresses caused by changes in chemical speciation and composition. There was a large range in bacterial production, 26 to $157 \mu\text{g C L}^{-1} \text{ d}^{-1}$, in the source water samples among the five experiments in which this was measured (experiments #4, 8, 10, 11, 12), though this range is typical for estuarine waters (Figure 9). Samples from the process water were much lower in bacterial production ranging from 0.08 to $0.28 \mu\text{g C L}^{-1} \text{ d}^{-1}$ in the upstream water, 0.08 to $0.21 \mu\text{g C L}^{-1} \text{ d}^{-1}$ in the downstream water, and 0.10 to $0.35 \mu\text{g C L}^{-1} \text{ d}^{-1}$ in the melted hydrate water (Figure 9). The MDS process or the sample collection appeared to dramatically affect bacterial growth based on the difference in production between the source water and process water samples.

Toxicity

Subsequent experiments were designed to differentiate between changes in bacterial production related to sample shipment and storage prior to the measurement and those due to changes in some water quality parameter that generally affects bacterial growth. Bacterial production was measured on unamended Atlantic Ocean surface seawater (100% treatment) and compared with samples that had additions of process water (10, 50% treatments) and to the process water alone. If there were no water quality parameters in the process water that were inhibitory to marine bacteria, then there would be a linear relationship among the production values between the Atlantic Ocean water alone (100% treatment) and the process water (0% treatment) (dotted line; Figure 10).

There was little effect of the Tampa Bay source water on Atlantic Ocean water, that is, the production value was the sum of relative contributions of the source waters (Figure 10). Bacterial production of the Atlantic Ocean water was inhibited by addition of all three process water samples (downstream, upstream and hydrate waters) (Figure 10). This suggests that there was some feature or

component of the process water that was inhibitory to bacterial metabolism of natural marine assemblages. Among the possible inhibiting factors is pH, low dissolved oxygen, and dissolved hydrocarbons though the 10% decrease in dissolved oxygen that would result from the 10% process water addition would not be likely to so dramatically decrease bacterial production in the Atlantic Ocean water. Likewise, given the buffering capacity of seawater, the pH change might not be enough to inhibit production to the extent seen in this experiment (Figure 10).

Future experiments will combine measurements of microbial community diversity, production and growth efficiency to assess the impact on the population and key elemental cycles.

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Figure 10. Heterotrophic bacterial production ($\mu\text{C L}^{-1} \text{d}^{-1}$) mixed with different percentages of Tampa Bay source water, upstream and downstream water.

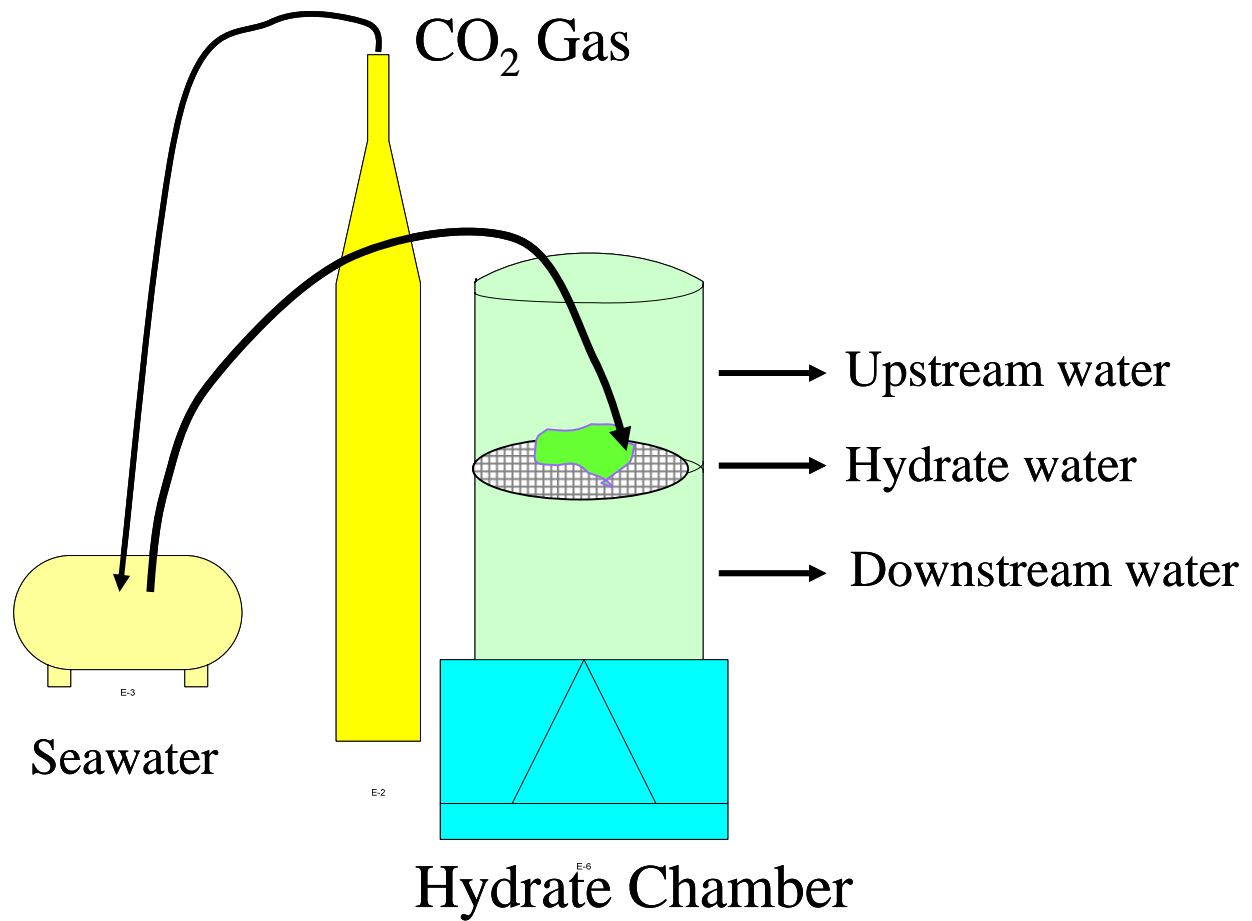


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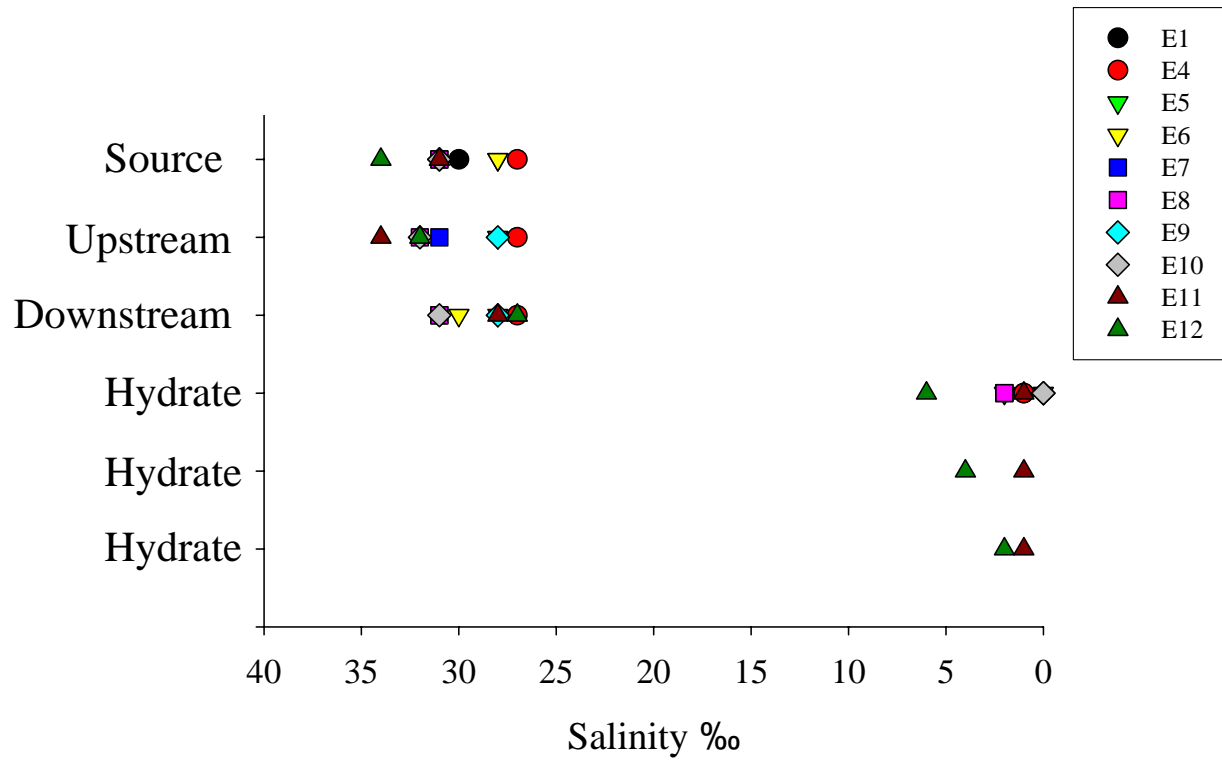


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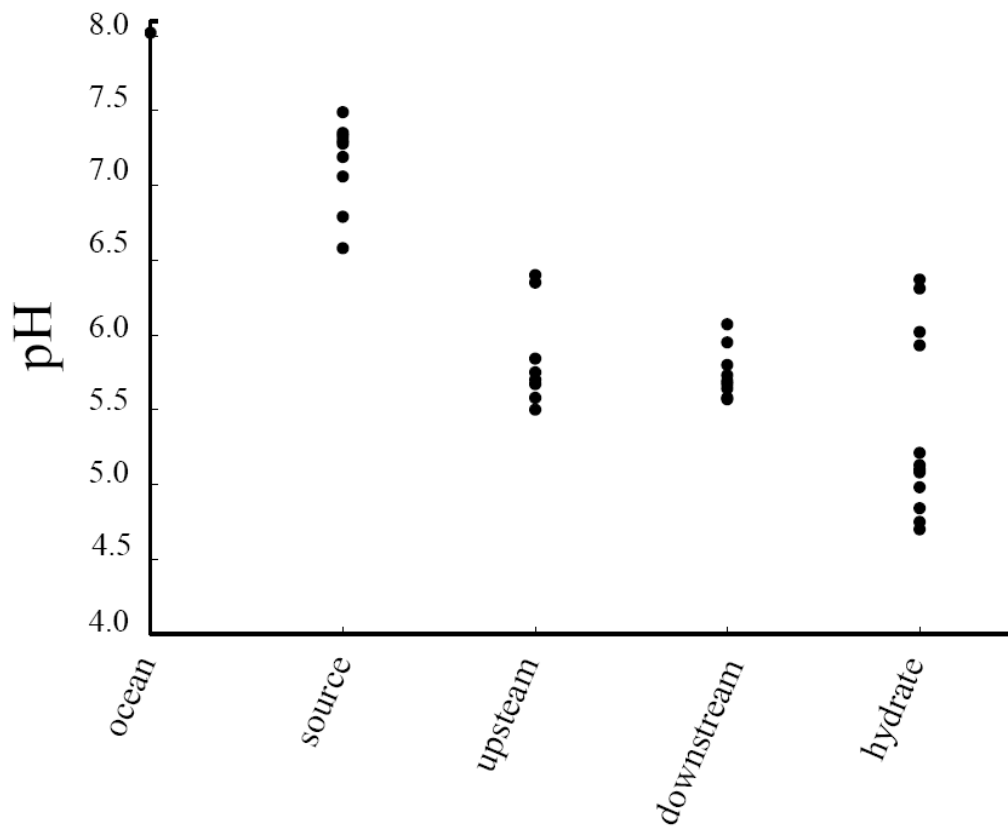


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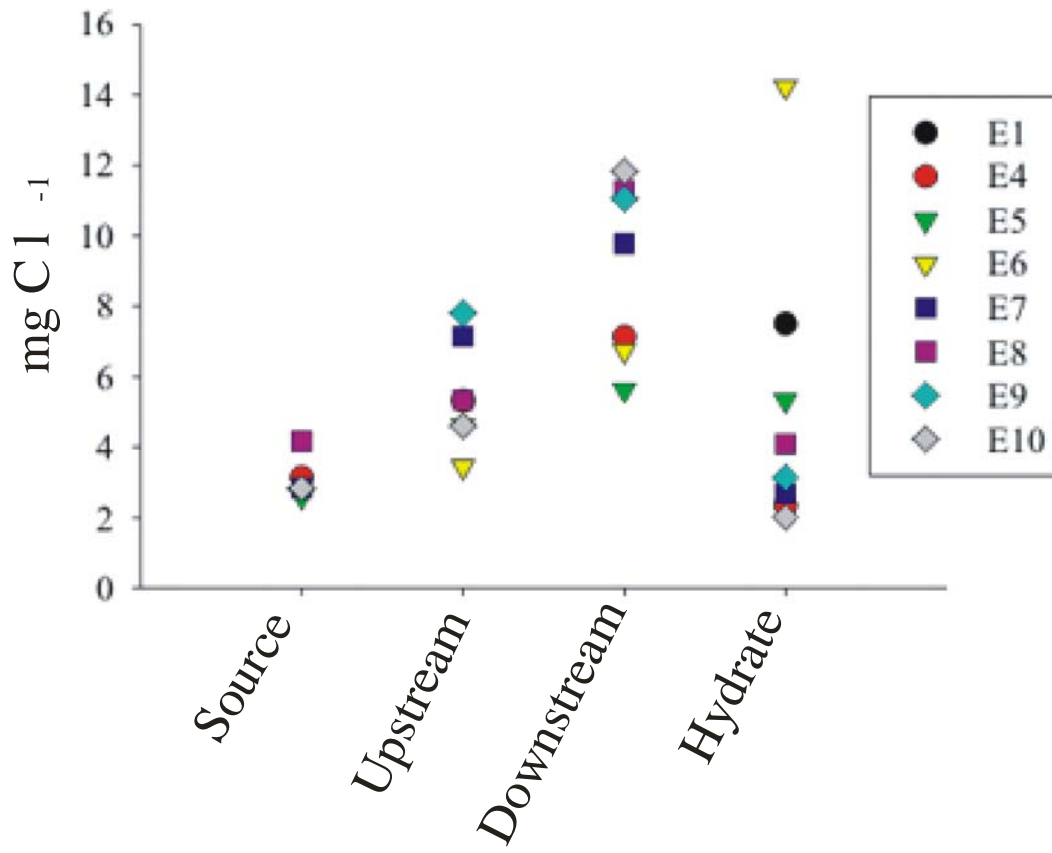


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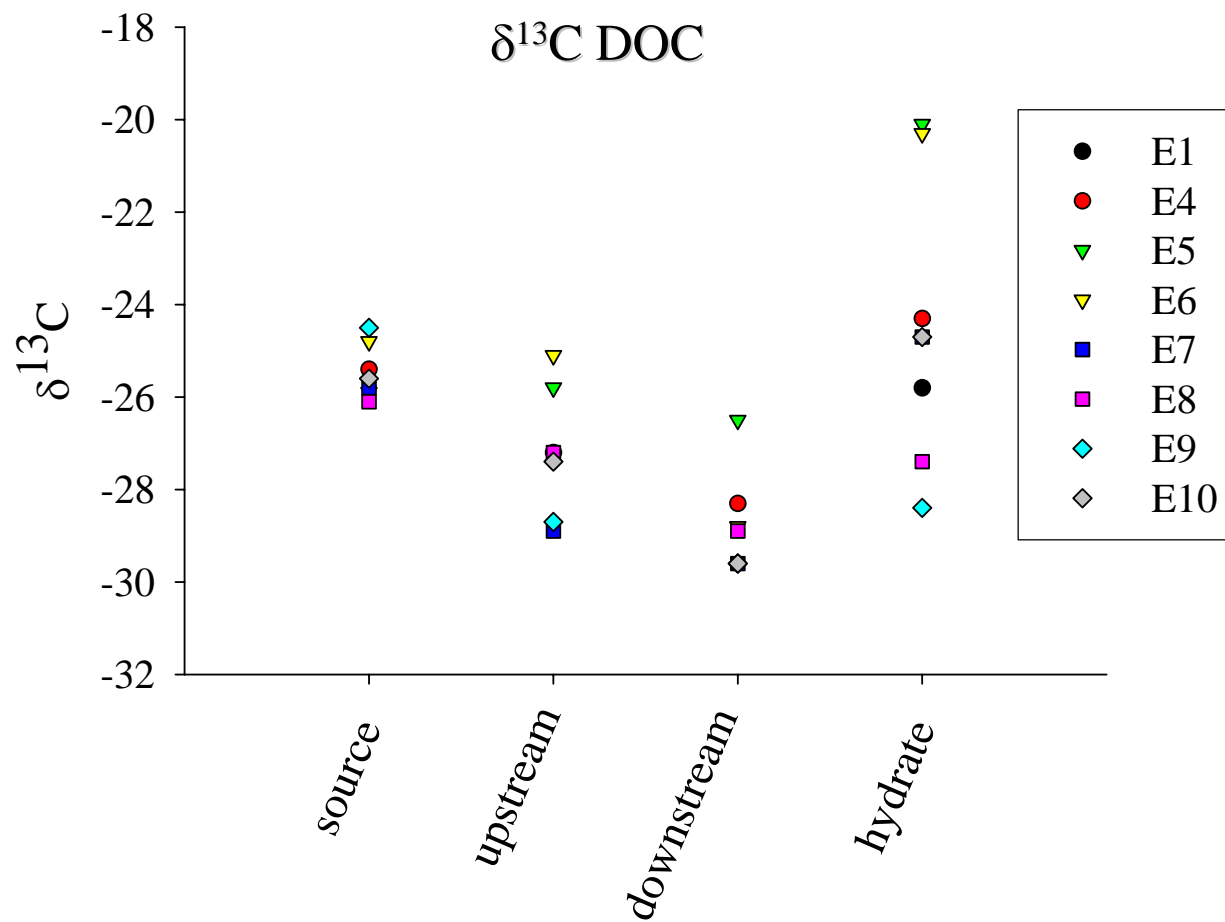


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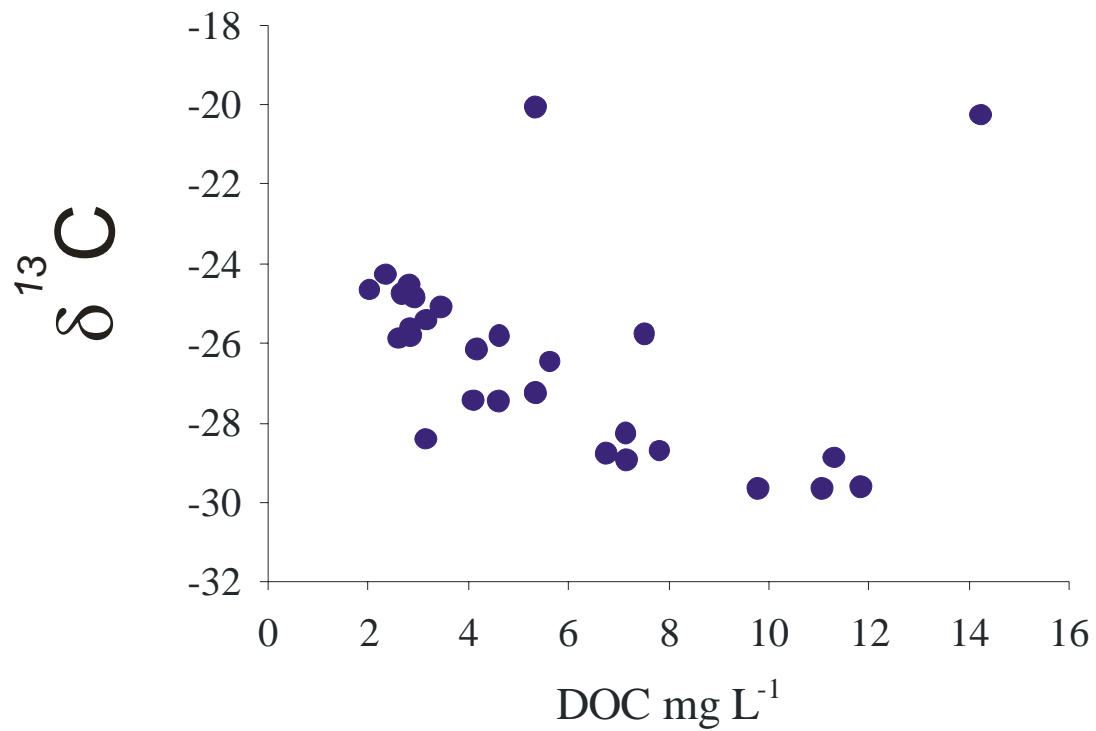


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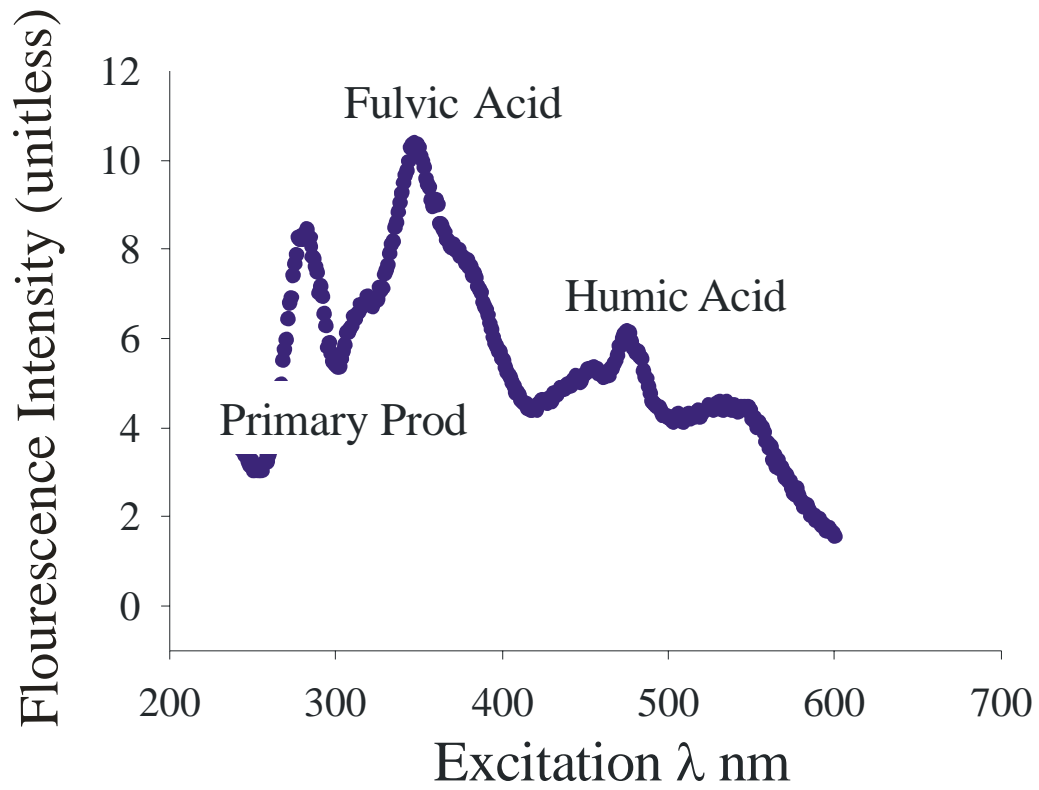


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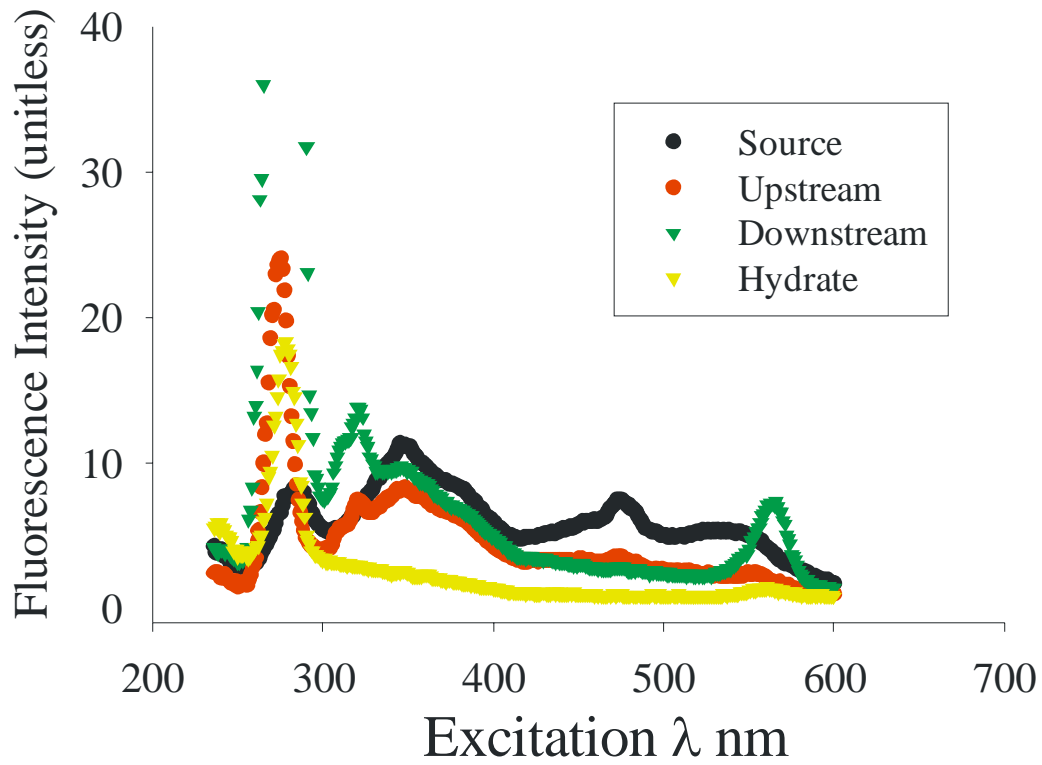


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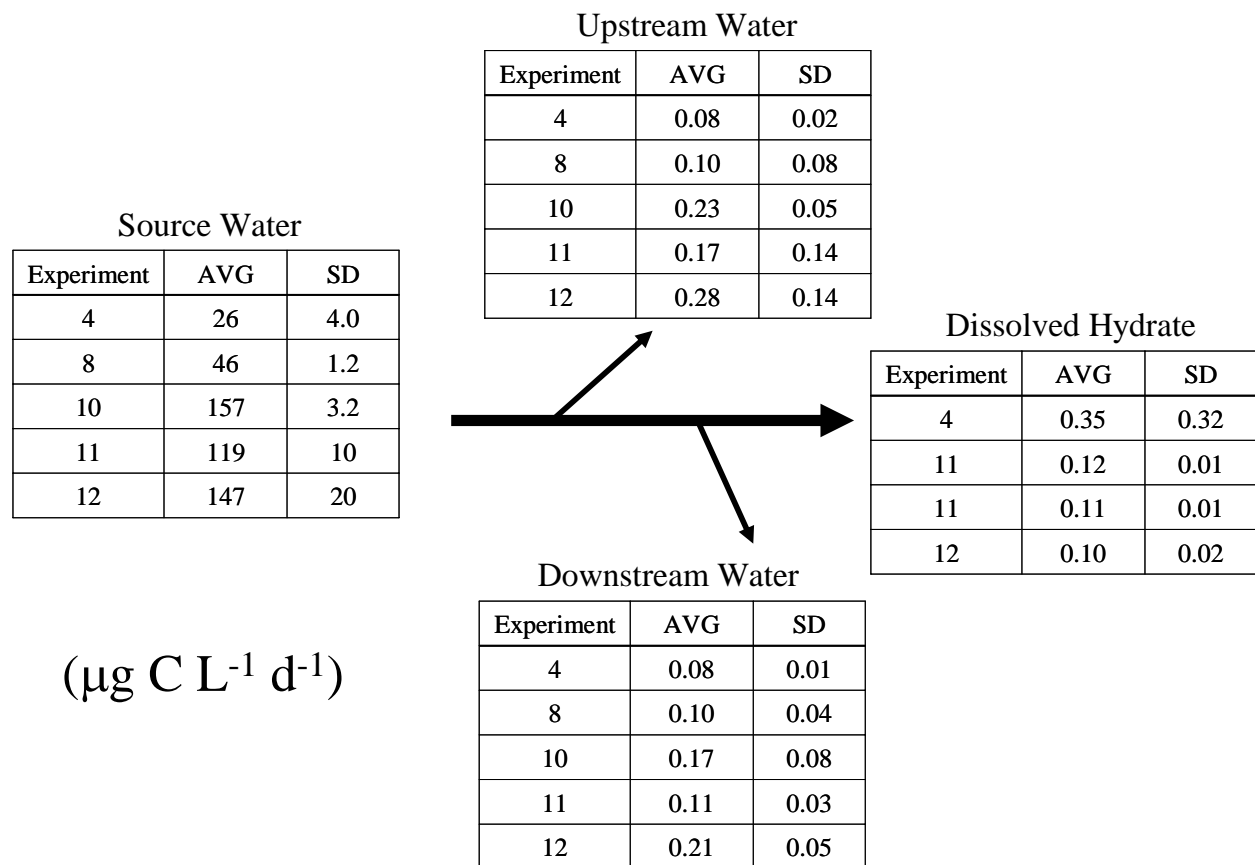


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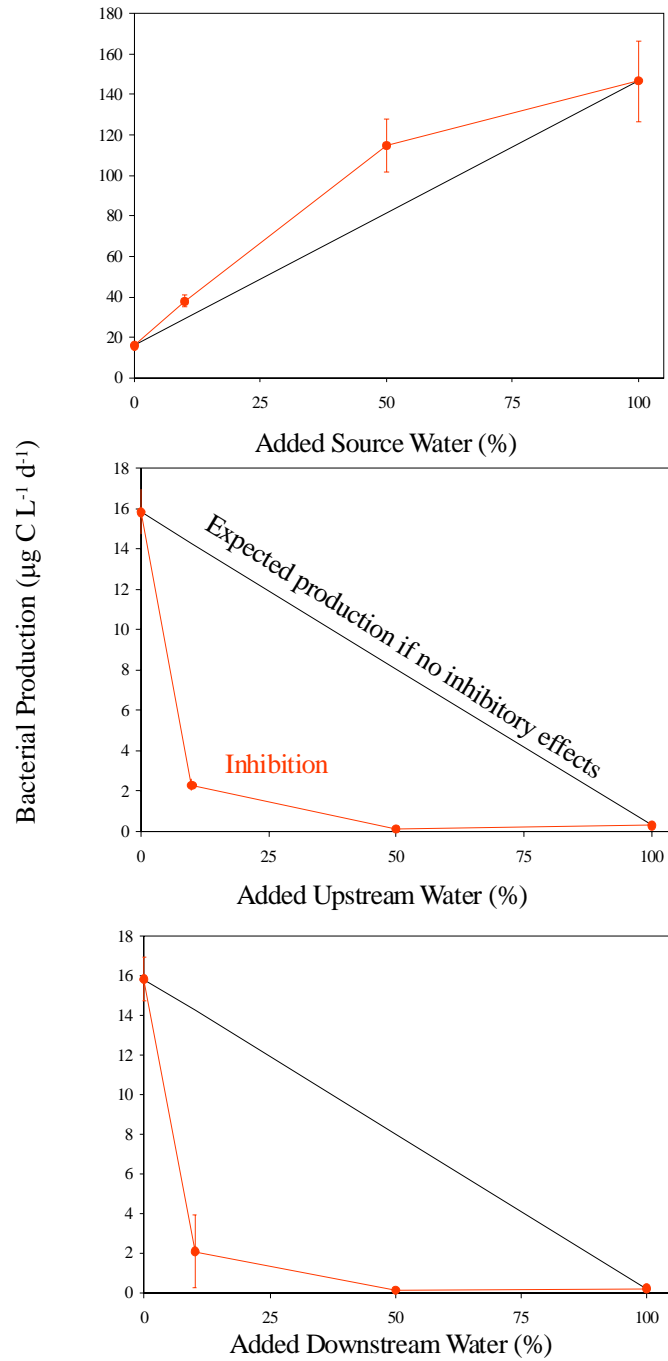


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