Report as of FY2007 for 2006NV104B: "Microbial and Phytoplankton Impacts on Endocrine Disrupting Contaminants: Las Vegas Wash and Lake Mead, NV"

Publications

Project 2006NV104B has resulted in no reported publications as of FY2007.

Report Follows

Title: Microbial and Phytoplankton Impacts on Endocrine Disrupting Contaminants: Las Vegas Wash and Lake Mead, NV

Synopsis: Only recently has it become known that microorganisms metabolize endocrine degrading compounds (EDCs), mediating processes including biodegradation, bioaccumulation, and deconjugative activation. Organisms at the bottom of the food web may thus the control the fate, persistence, and activity of agents whose greatest impacts are at the highest trophic levels. This award has been used to develop a preliminary dataset and facilitate program development at DRI focused on the role of natural microorganisms in controlling EDC ecology in the Las Vegas Wash. Highlights to date include the securing of roughly matching funding through USGS/SNPLMA for the funding a graduate student at UNLV and the development of preliminary results and techniques through the contributions of several undergraduate researchers. Tools developed in association with this project to date have revealed that EDC-degrading microorganisms are present in Las Vegas Wash and can be obtained in pure culture. Numerical abundance of EDC-degraders in the Wash appears to increase from about 10^2 in the upper Wash to 10^4 below the major wastewater discharges, representing about $1/100^{th}$ of the total microbial bioburden. The availability of techniques and collaborations developed to date will enable very quick progress with the enrollment of a graduate student dedicated solely to this project in September of 2007.

Annual Report

Problem and research objectives: Lake Mead serves as the principal water supply for much of the southwestern US. Extending 15 miles from Las Vegas proper to Las Vegas Bay of Boulder Basin, the Wash contributes in excess of 100 million gallons per day of treated wastewater to Lake Mead. Among the contaminants inadvertently delivered to the Wash via wastewater, endocrine disrupting chemicals (EDCs) represent a class of growing concern. Most notably, EDCs alter the structure of the vertebrate endocrine system, leading to reproductive impairment of wildlife. The most potent are medical EDCs such as 17β-estradiol (E2), and ethinylestradiol (EE2, the main ingredient in birth control pills). These compounds have been detected in basin water samples (Snyder, 1999) and fish blood plasma (Goodbred, 1999). Evidence, including tissue abnormalities and reproductive effects (Goodbred, 1999, Snyder, 1999), that these contaminants are bioactive is mounting. For example, male carp (*Cyprinus carpio*) in the Wash and Las Vegas Bay display low gonadosomatic index values, low sperm counts, gonadal macrophage aggregates, and elevated concentrations of egg yolk protein, vitellogenin (Goodbred, 1999, Snyder, 1999).

Certain microorganisms, through the activity of 17β-hydroxysteroid dehydrogenase (Lanisnik, 1992) have the ability to deconjugate EE2 to its bioactive intermediate E2 (Weber, 2005), although very little is known concerning the abundance and diversity of ECD-degraders. Thus, microbial communities may play a role in determining the environmental fate and persistence of these compounds and research into microbial mechanisms underlying EDC-degradation is timely. We propose that the fortuitous experiment embodied by Las Vegas Wash, a river composed mostly of treated wastewater, represents an outstanding resource for the elucidation of mechanisms underpinning the environmental processing of EDCs. The work described here details initial methods development towards these ends and provides some of the first information concerning the abundance, activity, and diversity of EDC-degrading microorganisms from the Southwest U.S.

Methodology: Water samples were collected from five sites along the Las Vegas Wash: including an upstream site (East Desert Inn road) lacking exposure to any wastewater source, the outfalls for the Las Vegas Pollution Control Facility and the Henderson Wastewater Treatment Plant, and a downstream site at Pabco Road. A sample of opportunity was also collected from the Tronox Inc. discharge (perchorate bioremediation).

For microbial cultivations, the basal freshwater medium, M1 (Myers and Nealson, 1988) was used with estrogens added as the sole carbon and energy source. To this, 10 ppm of EE2, E2, or a combination of both was added from a 5 mg/mL stock solution in HPLC grade methanol. Alternative carbon source controls included a mixture of formate, acetate, lactate, and glucose, each at 5 mM. Enumerations of aerobic heterotrophs were obtained by serial ten-fold dilution and plate counting on solid R2A medium (Reasoner 1985). Densities of aerobic estrogen-degrading microorganisms were estimated by serial ten-fold dilution in liquid M1. Growth was assessed by optical density (OD_{600}, Spectronic 20 spectrophotometer, Bausch & Lomb). Enrichment cultivations were performed using shake flask technique with weekly transfers. Growth curves were performed over a 4-day period in shake flasks at 25°C and sampled every 6 hours for optical density and estrogen concentrations. Microscopic examinations were performed using a Zeiss Axioskop 2 Plus fitted with phase contrast and an

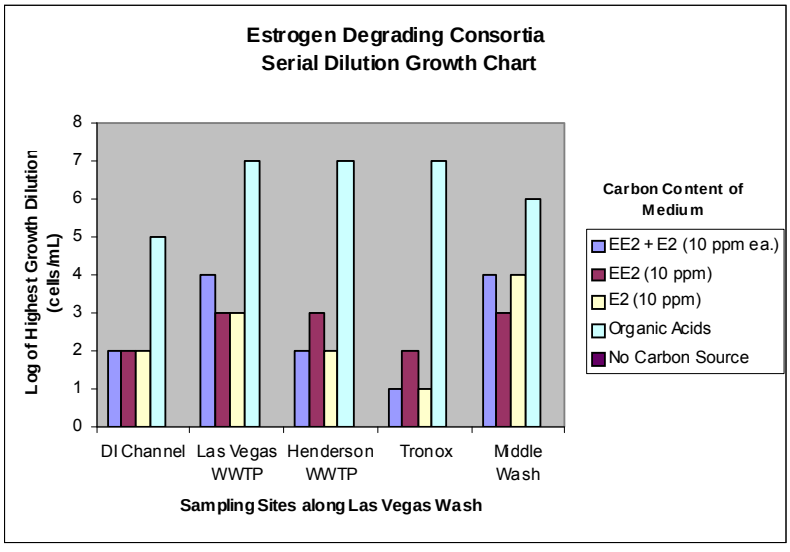

FIG. 2. Media containing multiple types of carbon sources were used to assess the trends in EE2 and E2 degradation at each site. The Middle Wash by far produced the most growth on both all media types. The negative control, which lacked a carbon source, failed to produce growth at any of the sites.

Axiocam MRC digital camera.

Quantitative analysis of EE2 and E2 was performed after filtration (0.2 μm, Millipore Acrodisc) using high performance liquid chromatography (HPLC). (HPLC-UV, Agilent Technologies, 1200 series). Separation was achieved on a Zorbax ODS 4.6 mm ID x 250 mm C-18 reverse phase column with a 70Å pore size and a mobile phase of 40% acetonitrile and 60% water with a flow rate of 1 mL/min. The injection volume was 150 μL, and signals were detected at 220 nm. Retention times for EE2 varied between 13 and 14 min, and that for E2 was steady at 9 min.

Principal findings and significance: A collaboration was established with the USGS SNPLMA-funded endocrine disruptors project under the management of Drs. Steven Goodbred and Michael Rosen of USGS (Objective 1). The 1:1 match and the collaborative infrastructure provided by this team will facilitate the support of a Masters's student at UNLV. A strong candidate from Denver Colorado, Susanna Blunt was recruited and will commence her program in September of 2007. In addition, we were able to recruit one of UNLV's top biology students (Karen Levy, 4.0 GPA) to apply for an receive an NSF EPSCoR undergraduate fellowship, which was used during the academic 2006/2007 year to supplement these funds and initiate methods development that will be required to complete the tasks outlined here. Her success in this project, was instrumental in her receiving a prestigious AMGEN fellowship at Columbia University this summer. Karen will be rejoining the project in the fall of 2007 where this research will be the basis for her undergraduate thesis work in the UNLV Honors College. Thus, the infrastructure and educational goals from this proposal have already exceeded expectations. Research-wise, we have obtained significant preliminary results and these are detailed under Objects 2 and 3 below.

Objective 1: Founding of a joint DRI/USGS/USFWS relationship focused on Lake Mead. This project was designed to dovetail with SNPLMA-funded water quality research being performed by the USGS and USFWS. In this regard, we were able to use this award to leverage a roughly 1:1 match from the USGS SNPLMA project for the support of our graduate student. Final contracting for the supplemental USGS funding is now underway. Delays in the delivery of the SNPLMA funding and the availability of a graduate student have necessitated pushing the start-date for major project activities back to September of 2007 (when Susanna Blunt commences her program).

Objective 2: Identification of major EDC-degrading microoganisms and algae. The first necessary steps were the development of culture media, sampling approaches, and analytical techniques to enable the cultivation of and measurement of E2 and EE2 degradation in microbial cultures. Enrichment cultures and quantitative dilutions using EE2, E2, or combination of the two as the sole carbon source were prepared using M1 medium. Whereas, typically an initial enrichment took about 5 days to reveal significant growth and estrogen

degradation, subsequent transfers grew up in as little 12 hrs, suggesting a conditioning of the microbial community. Individual isolates were obtained from the flask or tube enrichments by streaking for isolation onto solid estrogen-containing medium in purified agar and have been archived at -80^{o}C.

These experiments confirmed that significant bacterial growth was possible with EE2 and/or E2 and that from 10^2 and 10^4 culturable degraders were present in Las Vegas Wash, increasing in abundance moving down stream. These numbers were ca. 3 orders of magnitude lower than total cell counts, suggesting that about 1/1000th of environmental microorganisms in the Wash are estrogen degraders (a significant proportion). Several locations (Tronox and the Henderson plant sites) produced a conspicuous pink colonial estrogen-degrading culture type, visible as clumps to the naked eye and nearly clonal microscopically. We have also observed what appeared to be the same colonial organism free-floating in Las Vegas Wash. The identification of these and isolates yet to be recovered will be a future objective of the project.

Objective 3: Measurement of potential EDC degradation and metabolite generation rates. Using high performance liquid chromatography (HPLC) to quantitatively analyze the consumption of EE2 an E2 and a spectrophotometer to measure bacterial growth, we were able to track estrogen degradation and coincident growth. Growth curves from isolates and aquatic enrichments revealed significant, although sometimes incomplete, degradation of 10 ppm estrogen after an initial lag phase of ca. 48 hrs. Although future experiments must be performed at lower (e.g. more environmentally relevant) concentrations, the results of this work indicate that per-cell degradation rates will be achievable using adaptations of the techniques developed here.

Objective 4: Determination of the possible impacts of proposed deep wastewater delivery to mid Boulder Basin. Not addressed at this time.

Information Transfer Activities : The preliminary methods and approaches tested to date should provide the basis for publication-quality work in the near future. For this to occur, more samples will be required and additional characterization of the isolates will be required, which will become the main focus of our graduate student. Undergraduate, Karen Levy presented her results at the NSF EPSCoR student conference, held at DRI in Las Vegas in April of 2007.

References:

Goodbred, S.L., *et al.* 1999. in *20th Annual meeting, Society of Environmental Toxicology and Chemistry* (Philadelphia, Pennsylvania).

Lanisnik, T., *et al.* 1992. Fungal 17ß-hydroxysteroid dehydrogenase. FEMS Microbiol. Lett. 78:49.

Myers, C.R., and K.H. Nealson. 1988. Bacterial manganese reduction and growth with manganese oxide as the sole electron-acceptor. Science 240:1319-1321.

Reasoner, D.J., and E.E. Geldreich. 1985. A new medium for the enumeration and subculture of bacteria from potable water. Appl. Environ. Microbiol. 49**:**1–7.

Snyder, S.A. *et al.*, 1999. *Environ. Sci. Technol.* 33:2814.

Tuttle, P.L., and Orsak, E.L. 2002. U.S. Fish and Wildlife Service, Southern Nevada Field Office. FFS Numbers: 1F27 and 1F31.

Weber, S., *et al.* 2005. Degradation of estradiol and ethinyl estradiol by activated sludge and by a defined mixed culture. Appl. Microb. Cell Phys. 67:106-112.