EMSL Research and Capability Development Proposals

Development of Live and LC-NMR Microbial Metabolomics Methods for Systems Biology Studies: A Test Case Relevant to Biofuels Production

Project start date: Spring 2009

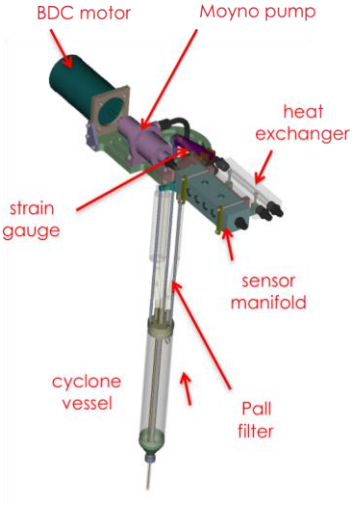
EMSL Lead Investigator:
Nancy Isern, *NMR Spectroscopy and Magnetic Resonance Group, EMSL, PNNL*

Co-investigators:
Paul Majors and John Cort, *Biological Sciences Division, FCSD, PNNL*
Haluk Resat, *Computational Sciences Division, FCSD, PNNL*
Jesse Sears, *NMR Spectroscopy and Magnetic Resonance Group, EMSL, PNNL*
Birgitte Ahring, *Bioproducts, Sciences and Engineering Laboratory (BSEL), Washington State University, Tri-Cities, Richland, Washington*

Consultants:
David Hoyt, *NMR Spectroscopy and Magnetic Resonance Group, EMSL, PNNL*
Kathleen McAteer, *College of Sciences, Washington State University, Tri-Cities, Richland, Washington*

The overall goal of this project is to enhance EMSL's state-of-the-art *in vitro* metabolomics nuclear magnetic resonance (NMR) with advanced *in vivo* NMR bioreactor capabilities for enhanced systems biology studies. There are two specific objectives: 1) develop and cross-validate unique EMSL high-resolution and bioreactor NMR instrumentation and methods, including computational flux analysis, and 2) apply this improved metabolomics capability to study microbial processes relevant to bioenergy development.

Final NMR bioreactor design improvements were implemented using commercial and (as required) homebuilt components. Special considerations were provided for high-magnetic-field compatibility and low electromagnetic noise generation. The brushless direct-current (BDC) motor is detachable—everything else will go into the autoclave for sterilization. All sensor and fluid lines will interface with a new Bioflow 310™ (New Brunswick Scientific) bioreactor controller that recently was purchased for this project. Pall tangential filtration cartridges will provide the higher cell concentrations required for measuring *intracellular metabolite profiles* (ICMPs). A strain gauge weighing system will be employed in an attempt to use the total reactor weight to control the fluid levels. Two cyclone vessels were constructed. The second copy will be used for emergency spare parts as needed, as an offline reactor to prepare for subsequent NMR runs, or as a proposed bioreactor for the new EMSL 750-MHz MRI system due to be delivered by the end of this fiscal year. Currently, the system is being assembled for testing.



NMR bioreactor design

We have refined the design of the triple-resonance NMR probe to coordinate with a planned American Recovery and Reinvestment Act

(ARRA)-funded upgrade of EMSL's Bruker 500 imaging spectrometer. Specifically, this probe will take advantage of a new, second radio-frequency receiver to allow for the development of a novel, *two-NMR-experiments-at-once* capability. Also, it is designed to fit into and be implemented with new (stronger) Bruker imaging/diffusion gradients required for ICMP measurements.

Concurrently, test cultures of *C. saccharolyticus* have been examined using EMSL's high-resolution metabolomics NMR system, and optimal cell extraction procedures have been selected based on the spectroscopic results. Labeling studies are underway using ^{13}C-labeled glucose and xylose in the BSEL bioreactor feedstock, and NMR spectra are being collected on aliquot samples and extracts of cell samples collected from the bioreactor. Data are being analyzed using Chenomx and VNMRJ spectral deconvolution. The *in vitro* high-resolution NMR results will be compared to *in vivo* analysis once the bioreactor has been tested and pressed into service.

Products and Output

New Capability for EMSL Users

NMR bioreactor for *in vivo* studies of microbial communities. The development project is expected to be available for EMSL users in 2011.

Presentations

Isern N.G., D.W. Hoyt, S.D. Burton, H.S. Mehta, and S.A. West. 2010. "Technologies for Tomorrow: Expanded Capabilities at the EMSL User Facility Supporting Innovative Solid- and Liquid-State Research in Biosystems and Materials." presented at the *ENC 51st Annual Conference*, April 18-23, 2010, Daytona Beach, Florida.

Baer, D.R. 2010. "Advanced Capabilities in EMSL: Current and coming attractions." presented at the *5th Annual Department of Energy-Subsurface Biogeochemical Research (DOE-SBR) Principal Investigator Meeting*, March 29-31, 2010, Washington, D.C.