Bioproducts, Sciences & Engineering Laboratory
Major achievements from August 2008-August 2009.

1. We have built important facilities such as pretreatment, fermentation and analytical facilities. The pretreatment system consists of a custom-designed 10 liter reactor/pretreatment tank and 100 liter flash tank that uses wet oxidation. The pretreatment reactions occur in pressurized, superheated water at a temperature above the normal boiling point of water (100 °C), but below the critical point (374 °C). The equipment is constructed of 316L grade stainless steel, and incorporates state-of-the-art technology licensed by WSU from a leading international cellulosic bioethanol technology company. This equipment allows WSU and PNNL researchers to study methods to convert lignocellulosic biomass such as agricultural crop residues, forest residues and other wastes into feedstocks for subsequent biochemical and thermochemical processing. The pretreated biomass allows WSU and PNNL researchers to effectively and efficiently separate the biomass into the component cellulose, hemicellulose and lignin fractions. These individual components are the feedstocks for subsequent research into enzymatic hydrolysis, fermentation and distillation of bioethanol fuel and bioproducts, and are also used as feedstocks by BSEL and Pacific Northwest National Laboratories (PNNL) researchers for research into thermochemical conversion of biomass into biofuels and bioproducts.

We have also made significant advances in developing the advanced fermentation capabilities at BSEL. We have acquired a variety of fermentation units (bioreactors) ranging in size from shaker flask (less than 1 liter), up to 100 liters. These bioreactors are ideal tools for researching lab and bench-scale fermentation experiments as well as providing outstanding platforms for fermentation systems modeling. Each fermenter system is fully equipped to measure and control pH, temperature, dissolved oxygen (DO), level/foam and agitation. Most of the BSEL fermenters also use removable autoclavable pump heads mounted on head plates to make preparations for autoclaving and subsequent set up much simpler. In addition, WSU has acquired fully integrated computerized monitoring and control software that is modular, easy to operate, and provides essential parallel replication to help ensure reproducible results. The largest of our fermenters is a 100 liter Allegheny Bradford bioreactor that includes controller and user computers, a control panel and manuals. The reactor system is made from 316L grade stainless steel, and is designed to operate at 302° F at pressures of up to 50 PSI.

The heat jacket allows WSU and PNNL researchers to conduct fermentation studies at a range of temperature conditions, and to precisely control those temperatures. This 100 liter bioreactor is an essential tool to research and solve issues related to scaling up research from lab scale, to pilot scale to commercial scale.

We have also, in conjunction with PNNL, developed a world-class analytical laboratory at WSU/BSEL. We have acquired a wide range of analytical equipment which includes:

- Dionex ICS-3000 Reagent-Free™ Ion Chromatography (RFIC™) system. The ICS-3000 is a unique, high resolution chromatograph that will increase the productivity of our research at BSEL by combining high performance, intelligent automation, and integrated data management along with state-of-the-art control.

- UltiMate 3000 Rapid Separation LC (RSLC) system accelerates high performance liquid chromatography (HPLC) for unrivaled performance and flexibility. Precision-engineered instrumentation, advanced data processing and highly optimized chemistries meet all chromatographic performance challenges. Industry-leading RSLC technology will allow researchers and technicians to work over an extensive flow-pressure footprint, covering the full range of HPLC, including conventional and ultrafast LC, on one system.

- Leica AM6000 inverted microscope with micromanipulators. This microscope combines the functions of a fully automated inverted research microscope with those of electronic micromanipulators. The Leica AM6000 with micromanipulation system will allow the BSEL team to observe and manipulate specimens simultaneously. Using the contrast or illumination manager, BSEL researchers will be able to use the Leica AM6000 and the built-in high resolution digital camera to capture razor-sharp, brilliant images required to support research publications.

- Gerhardt THO500 Orbital Incubating Shaker. Proper culturing of the microbes needed for research at BSEL requires specialized incubating equipment. The Gerhardt THO500 Orbital Incubating Shaker offers a unique, patented state-of-the-art incubation shaker with a large, programmable orbital motion. The sophisticated construction guarantees controlled and steady conditions for continuous operation as well as homogenous mixture

of cultures thanks to constant speed and temperature. A large transparent and internally lit incubator chamber allows easy observation of cultures from all sides.

- Agilent Gas chromatography-mass spectrometry (GC/MS), model 5975C, bundled with a CTC Analytics' CombiPAL and GC PAL automated sample injectors. BSEL researchers will use the GC/MS technology for the analysis of biofuels and other biologically derived hydrocarbon compounds. A high performance GC/MS is essential to allow BSEL researchers to comply with ASTM Method D-6584 for free and total glycerin, as well as a GC-headspace sampling method to comply with the European Method EN 14110 for the determination of residual methanol (or ethanol) in biofuels. Additionally, other GC testing methods are described for Simulated Distillation to comply with ASTM Method D-2887, as well as methanol-purity testing. This will also allow BSEL researchers to sample highly volatile hydrocarbon samples very rapidly and with high precision. The Agilent 5975C GC/MS ChemStation software seamlessly integrates the CTC sample injectors which improve the speed, efficiency and flexibility of the GC/MS. The autosampler is unique in that it will allow BSEL researchers to perform liquid, headspace and Solid Phase Micro-extraction (SPME). SPME is a patented sample preparation technique for GC based on the adsorption of analytes directly from an aqueous sample onto a coated, fused-silica fiber and desorp the analyte. This sampling technique is fast, easy to use and eliminates the use of organic solvents. The SPME fiber can be inserted into the liquid sample or suspended above the sample for headspace sampling. In headspace sampling, the SPME fiber acts as a "chemical pump," forcing compounds out of the liquid phase into the headspace and then into the fiber.

- Thermo Scientific Trace GC Ultra delivers superior performance, while ensuring simple and reliable performance. The Thermo Scientific TRACE GC UltraTM is built around the recognized quality, reliability and ruggedness of the established trace GC technology, but adds speed, reliability and automation features that will provide researchers at BSEL with a very significant increase in sample throughput, with analyses performed up to 30 times faster than with a standard trace GC, without compromises in precision.

- MiniOpticon real-time PCR detection system with a compact two-color real-time detector built on a MJ Mini cycler. Research at the BSEL facility includes identification, isolation and purification of enzymes such as cellulases, hemicellulases, and other glycosyl hydrolases synthesized by fungi and bacteria work together in a synergistic fashion to degrade the structural polysaccharides in biomass. In addition, the equipment will allow BSEL and PNNL researchers to rapidly identify non-cultivatable or slow-growing microorganisms such as mycobacteria, anaerobic bacteria, archaeal organisms from tissue culture assays and reactors.

- Purifier Logic Class II Biosafety Cabinet. This equipment provides BSEL researchers with protection from accidental exposure to potentially hazardous particulates, chemicals or organisms that require Biosafety Level II or III containment. During operation, room air is drawn into the inlet grille at the work access opening and is filtered through a supply HEPA filter. Approximately 70% of the HEPA-filtered air is circulated through the cabinet, while 30% passes through an exhaust HEPA filter and is discharged. These biohazard cabinets are designed to discharge HEPA-filtered exhaust air directly and safely into the laboratory. If required, BSEL personnel can also directly duct the canopy into the laboratory air handler system to allow researchers to also work on applications involving minute quantities of volatile toxic chemicals and tracer amounts of radionuclides as an adjunct to microbiological research.

In addition to the major systems already listed, WSU/BSEL has also acquired a broad range of other laboratory equipment, including:

- LF Horizontal Flow Clean Bench (ENVIRCO, New Mexico, USA)

- Incubator (Forced convection incubator, BINDER, Tuttlingen, Germany

- Sanyo top loading portable autoclave (MLS-3781L, Wood Dale, IL, USA)

- UV/VIS Spectrophotometer (JENWAY, Essex, UK)

2. WSU researchers have embarked on innovative new lines of research designed to develop new, infrastructure ready advanced biofuels designed to help the US reduce our dependence

on imported oil and improve our energy security. Toward this end, WSU hired three new post-doctoral researchers who have each started new lines of research under the tutelage of Dr. Ahring, including:

- Research into fungus that can break down plants into simple sugars, the basic components of ethanol. Several species of fungus contain a number of enzymes, cellulases, with potent catalytic properties that break down plants. After fermentation, simple sugars can easily be transformed into biofuels such as ethanol. First generation biofuels, made from grain or beet fermentation into ethanol, have certain limitations including competition with food uses of the feedstocks. Second generation biofuels made cellulose contained in forest and agricultural wastes (tree cuttings, corn cobs, straw, etc.) do not have these limitations, as they complement pre-established agricultural activity, have a better CO_2 balance, and aren't used for food applications.

- Research into a novel fungus that can directly breakdown cellulose and synthesize a variety of long-chain hydrocarbon compounds. WSU researchers are working with the endophytic fungus Gliocladium roseum which has the unique capability of converting cellulose into an extensive series of the acetic acid esters of straight-chained alkanes including those of pentyl, hexyl, heptyl, octyl, sec-octyl and decyl alcohols. Other hydrocarbons were also produced by this organism, including undecane, 2,6-dimethyl; decane, 3,3,5-trimethyl; cyclohexene, 4-methyl; decane, 3,3,6-trimethyl; and undecane, 4,4-dimethyl, and under some growth conditions it is capable of producing other volatile hydrocarbons including heptane, octane, benzene, and some branched hydrocarbons. This fungus could potentially be a completely new source of green energy which posses the metabolic machinery to produce high-energy-related hydrocarbons depending upon the substrate and environmental conditions. However, G. roseum is the only organism that has been shown to produce such an important combination of fuel substances and WSU researchers are working to see if it can be used to produce the diesel compounds from cellulose which would make it a better source of biofuel than anything we use at the moment.

- Research into using various species of thermophilic bacteria for both enzymatic hydrolysis and fermentation. Some species of thermophilic bacteria produce specialized enzymes that may be potentially harnessed to improve ethanol production by allowing the hydrolysis and fermentation steps to be merged together. For a start, some thermophilic bacteria are known to be able to break down cellulose and the enzymes they use to do this may be better able to withstand the heat and acids used in the pre-treatment step than standard cellulases. In addition, under certain conditions, some thermophilic bacteria possess the ability to produce ethanol from sugars via fermentation and some can do so with better yields than many strains of commercial yeasts. WSU researchers are also performing research on microorganisms capable of producing other hydrocarbon compounds, such as isoprene, which are easier to separate from feedstocks than is ethanol. Such work holds the promise of contributing to the significant advances being made in the development of advanced biofuels.

3. We have attracted new 3 Faculty to work in the center who all will take advantage of the facilities made during the first year. These faculty include:

1. Dr. Hanwu Lei – an expert in the use of pyrolysis, which is a thermochemical method for converting biomass into biofuels. Although synthetic diesel fuel cannot yet be produced directly by pyrolysis of organic materials, researchers have found ways to produce similar liquid ("bio-oil") that can be used as a fuel, after the removal of valuable bio-chemicals that can be used as food additives or pharmaceuticals. Fuel bio-oil resembling light crude oil can also be produced by hydrous pyrolysis from many kinds of feedstock, including waste from pig and turkey farming, by a process called thermal depolymerization (which may however include other reactions besides pyrolysis).

2. Dr. Bin Yang – an expert in biomass pretreatment and hydrolysis. Dr. Yang's research will focus on optimization of thermochemical and biochemical pretreatment of biomass, hydrolysis reaction processes and chemistry, process optimization to allow for scale-up from laboratory → bench → pilot →commercial operations.

3. Dr. Xiao Zhang – an expert in the development and extraction of new polymers from lignin made during the biofuels production process. Dr. Zhang's research will focus on

development new, high value materials and products that can be extracted and recovered in a cost-effective, efficient manger from lignin.

4. Dr. Ahring and WSU researchers have established a number of industrial collaborations with important industries in the State of Washington, including:

- EverGreen Renewable Energy,
- Imperium Renewables,
- Energy Northwest
- Battelle
- Weyerhaeuser, and
- Pacific Ethanol, Inc.

5. Dr. Ahring in a partner in the new energy initiative coordinated by Tridec together with other energy entities and industries. A proposal for a local Energy Park is currently under development where BSEL will deliver technology for a biorefinery to be implemented as part of the Energy Park.

Focus on the funding initiatives has been on larger grants, which have demanded collaborations with a number of companies, municipalities, state and federal agencies. A number of these proposal are currently under review while a small SUN grant has been received for testing new energy crops.