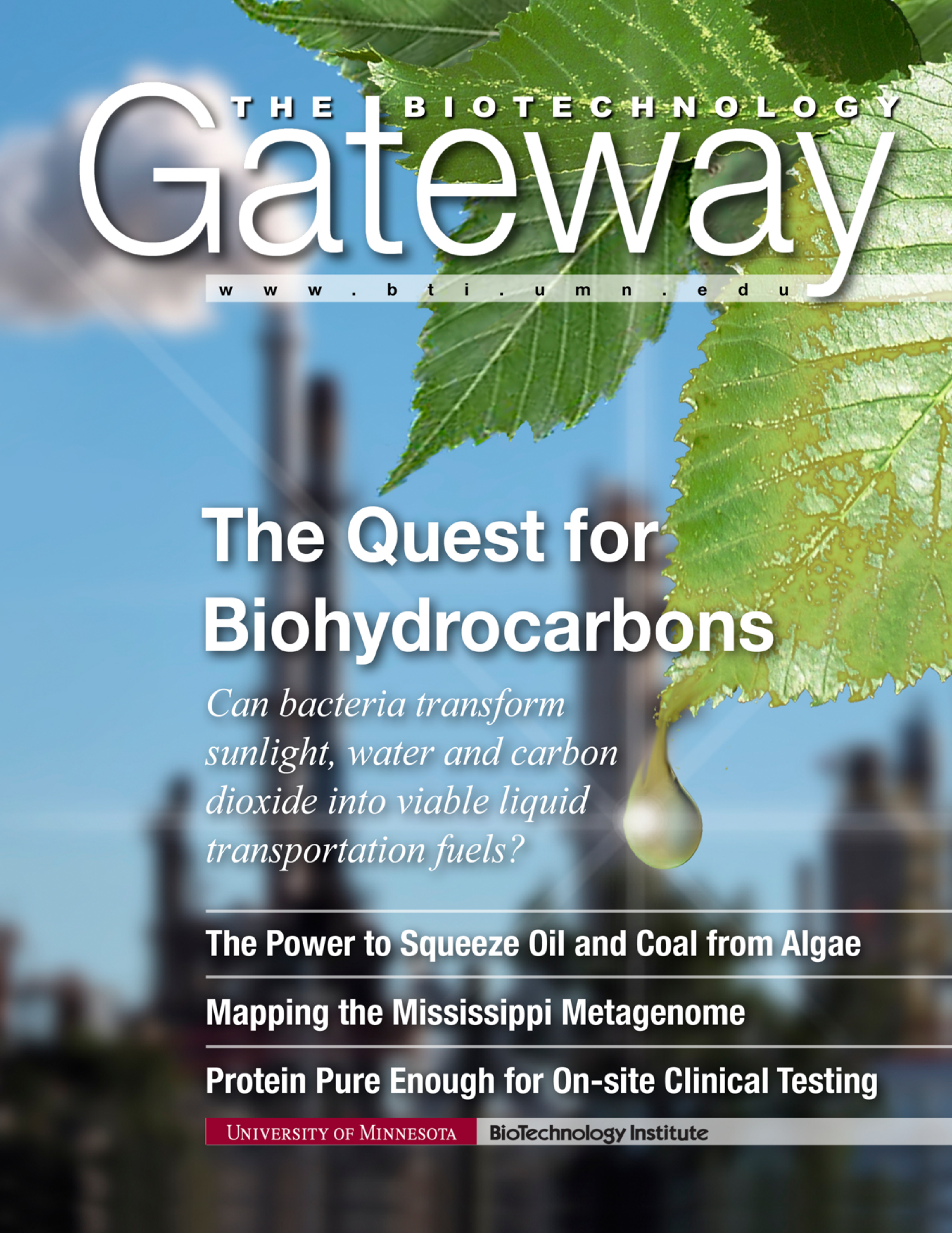

THE BIOTECHNOLOGY
Gateway
www.bti.umn.edu

The Quest for Biohydrocarbons

Can bacteria transform sunlight, water and carbon dioxide into viable liquid transportation fuels?

The Power to Squeeze Oil and Coal from Algae

Mapping the Mississippi Metagenome

Protein Pure Enough for On-site Clinical Testing

UNIVERSITY OF MINNESOTA BioTechnology Institute

Why BTI is an Important Gateway

The University of Minnesota BioTechnology Institute (BTI) has a long tradition and world-class expertise in the biological process of biocatalysis. In 2004, it became home to the University's Biocatalysis Initiative, which was created to focus and fund research in the areas of biocatalysis and biological biotechnology – processes which promise a safer and more sustainable future.

Biocatalysis is the use of biological catalysts, called enzymes, to transform biological materials (plants, algae, bacteria, etc.) into useful products. Plants and algae themselves contain enzymes which convert carbon dioxide in the air to nutritious carbohydrates such as sugar and starch. Selected enzymes are typically used to reduce reaction times to a matter of seconds for biological transformations that might otherwise require several months to reach completion. And because enzymes remain unchanged in the process, one enzyme molecule can be used to catalyze many transformations. Biocatalysis differs from standard industrial chemical reactions in that the biological transformation using enzymes requires less extreme temperatures, requires little or no additional energy input, and does not produce unwanted byproducts.

There is great research in biocatalysis being done at the BioTechnology Institute. You can read about some of this research and its applications in this publication. Biological catalysts are being used to develop ways of processing new biofuels, synthesize new drugs and drug delivery systems, process and test foods, break down toxic substances in the environment and develop biodegradable plastics and "biosmart" products with functional coatings. Biocatalysis and pathway engineering are also being used to develop microbial fuel cells, which rely on the organized enzyme reactions in bacteria to generate electric power.

Some biocatalytic processes – such as fermentations – have been employed on an industrial scale for many years, and others of great importance – such as penecillin production and production of amino acids and vitamins – have been devised more recently. However, it is only now that modern collaborative approaches to the biological, chemical, engineering, and information sciences are making possible a broad-ranging understanding and utilization of biocatalysis. Research at the University of Minnesota BioTechnology Institute has facilitated the application of collaborative disciplines necessary to make new advances in biocatalysis – and the Institute remains the gateway to biotechnology.

Michael Sadowsky
Director, BioTechnology Institute

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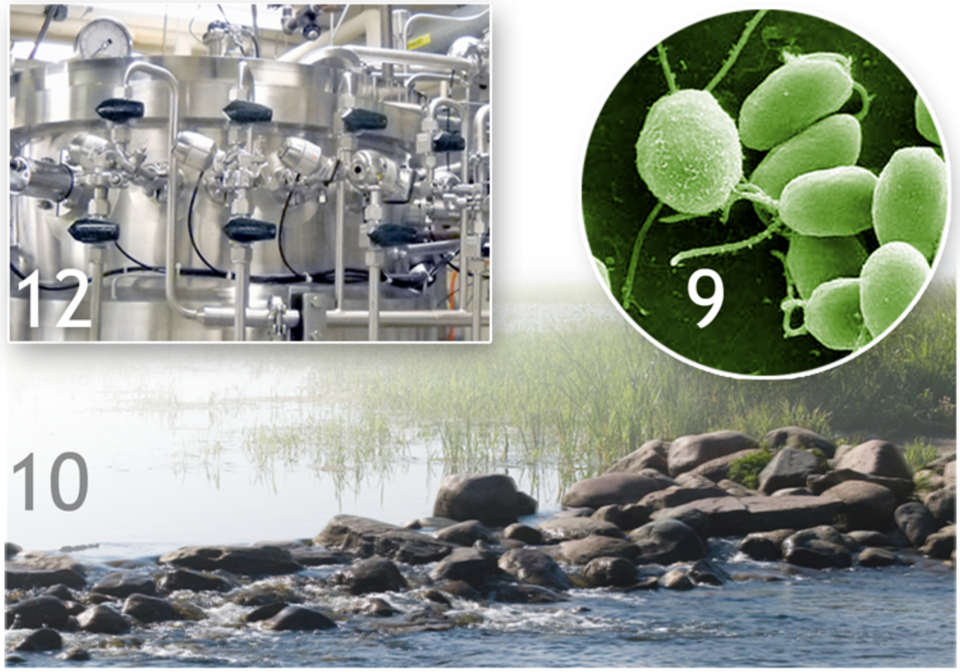


FEATURES

SECTIONS

BioTechnology Institute FACULTY

Alptekin Aksan
Mechanical Engineering

- *Studying the stabilization and transformation of proteins and membranes utilized in tissue engineering*

Brett Barney
Bioproducts, Biosystems Eng.

- *Experimenting with the use of bio-synthetic pathways for creating commodity fuels and high-value products from select bacteria and algae*

Daniel Bond
Microbiology

- *Interested in the physiology of anaerobes & electricity generation by electrode-attached bacteria*

Michael Sadowsky
Director

- *Internationally known and respected for his research work in the area of environmental microbiology*

Robert Brooker
Genetics, Cell Biology & Development

- *Investigating the structure and function of membrane-bound transport proteins*

Antony Dean
Ecology, Evolution & Behavior

- *Making the study of historical adaptation an "experimental science" by examining molecular evolution and enzymology*

BioTechnology Institute faculty have broad expertise in biocatalysis, metabolic engineering/microbial physiology, combinatorial biology, population dynamics, molecular biology, proteomics and focused expertise in defined areas such as bioremediation, biomaterials, biofuels, biosensors, and bioinformatics.

Mark Distefano
Chemistry

- *Exploring how proteins accelerate chemical reactions and how proteins recognize other molecules with high specificity*

Gary Dunny
Microbiology

- *Studying regulation of expression of genetic transfer functions and the regulation of virulence in gram positive bacteria*

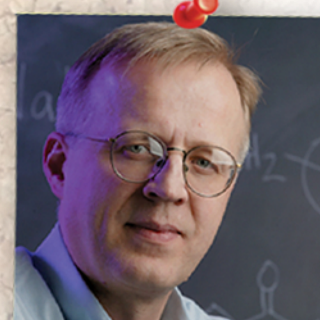
Jeffrey Gralnick
Microbiology

- *Combining research into anaerobic respiration and the physiology of Shewanella bacteria with the study of geomicrobiology*

Raymond Hozalski
Civil Engineering

- *Researching the application of biological processes in the treatment of water, wastewater, and hazardous wastes*

N O T E S



Wei-Shou Hu
Chemical Engineering and Materials Science

- *Working with biochemical engineering, cell engineering and stem cells*

Romas Kazlauskas
Biochemistry Molecular Biology & Biophysics

- *Pursuing Green Chemistry and enzyme modeling, design, and engineering*

George Karypis
Computer Science and Engineering

- *Interests span the areas of data mining, bio-informatics, parallel processing, CAD, and scientific computing*

Yiannis Kaznessis
Chemical Engineering and Materials Science

- Specializes in computer modeling of biological systems, structural bioinformatics, and synthetic bioengineering

Arkady Khodursky
Biochemistry, Molecular Biology & Biophysics

- A focus on functional genomics, analysis of gene expression patterns, microarray applications

Timothy LaPara
Civil Engineering

- Interested in microbial ecology – how organisms interact with each other and with their environment; research explores issues of interest to environmental engineers

Igor Libourel
Plant Biology

- Studying metabollic systems biology, core metabolism in algae

Paige Novak
Civil Engineering

- Specializes in research on the biological transformation of hazardous substances in sediment, groundwater, and wastewater

Patrick Schlievert
Microbiology

- Studies molecular pathogenesis and immunology; streptococci and staphylococci

Christine Salomon
Center for Drug Design

- Recently concentrated her research efforts on finding biological solutions for control of plant disease

Claudia Schmidt-Dannert
Biochemistry Molecular Biology and Biophysics

- *Interested in microorganisms and plants that naturally synthesize a diversity of chemical compounds useful as drugs, new biomaterials and in food applications*

Burckhard Seelig
Biochemistry, Molecular Biology and Biophysics

- *Interests include biocatalysis, Directed Evolution, Protein Engineering, Artificial Enzymes*

Friedrich Srienc
Chemical Engineering and Materials Science

- *Works in the area of biochemical engineering, cell cycle kinetics, biodegradable polymers*

Michael Travisano
Ecology, Evolution, and Behavior

- *Specializes in ecological and evolutionary dynamics, evolutionary genetics, microbial ecology and evolution.*

Kenneth J. Valentas
BioTechnology Institute

- *Past Director of the Institute now working on biofuels-related projects in the areas of process engineering technology and scale-up operations*

Ping Wang
Bioproducts and Biosystems Engineering

- *Studies enzyme engineering and nanotechnology; nanostructured biocatalysts for biotransformations*

Lawrence P. Wackett
Biochemistry, Molecular Biology and Biophysics

- *Research in the areas of biodegradation; dehalogenases; industrial biotransformations; metalloenzymes*

RESEARCH IN ACTION

Prof. Larry Wackett,
UM BioTechnology Institute
College of Biological Sciences

New test kit detects toxic chemical in infant formula and other foods

Melamine is a nitrogen-based industrial chemical used in making plastic countertops, flame-retardant fabrics, and other materials.

In recent years, a few manufacturers – primarily in China – have added melamine as cheap filler to milk and other dairy food products because it boosts the apparent protein content.

Melamine killed six Chinese children and hospitalized 150,000 in 2008 after it was added to milk. It was also linked to deaths of more than 1,000 pets in 2007 after it was added to pet food. Melamine damages the kidneys and can have long-term health consequences for those who survive ingesting it.

Because of these incidents, the World Health Organization put out a call to scientists worldwide to develop a simple, inexpensive way to detect melamine in food. Until now, this required sophisticated laboratory equipment and trained staff.

Two University of Minnesota professors – Larry Wacket and Mike Sadowsky – responded by developing an enzyme, melamine deaminase, which slightly alters the chemical structure of melamine and causes it to release ammonia. Ammonia can be detected in milk by a simple test that turns the milk blue. (Jennifer Seffernick, a research associate in Wackett's lab, discovered the enzyme while conducting research on biodegradation of herbicides.)

BIOO Scientific, a biotechnology company located in Austin, Texas, used the enzyme to create a test kit, which they are distributing in China. The simple, inexpensive kit can be used to detect melamine in milk at regional sites before it is processed. The kit can also be used to detect melamine in processed foods, such as powdered milk, ice cream and chocolate drink.

The new MaxSignal Melamine Enzymatic Assay Kit from BIOO Scientific makes it possile to test inexpensively on site for contamination of milk and other foods.

Power to Squeeze Oil & Char from Algae

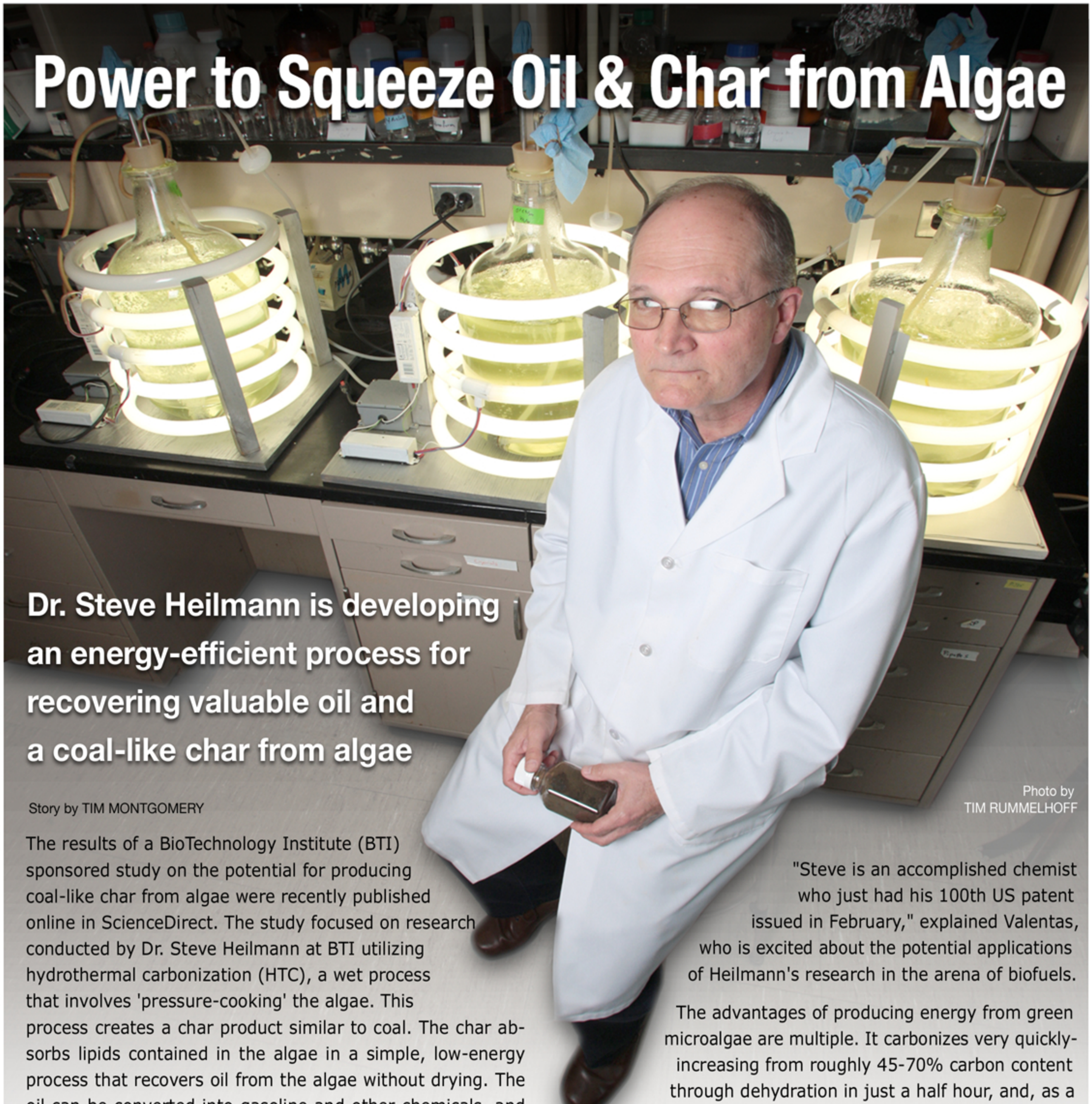


Photo by TIM RUMMELHOFF

Dr. Steve Heilmann is developing an energy-efficient process for recovering valuable oil and a coal-like char from algae

Story by TIM MONTGOMERY

The results of a BioTechnology Institute (BTI) sponsored study on the potential for producing coal-like char from algae were recently published online in ScienceDirect. The study focused on research conducted by Dr. Steve Heilmann at BTI utilizing hydrothermal carbonization (HTC), a wet process that involves 'pressure-cooking' the algae. This process creates a char product similar to coal. The char absorbs lipids contained in the algae in a simple, low-energy process that recovers oil from the algae without drying. The oil can be converted into gasoline and other chemicals, and the char can be burned to generate heat and electricity.

"The advantage of the HTC process is in its simplicity," says Heilmann. "Through basic heating under moderate pressure, we're able to make chars of bituminous coal quality and accomplish in a half hour what takes nature millions of years."

Dr. Heilmann, a University of Iowa graduate, retired after 34 years as a scientist with the 3M Corporation. He is an organic polymer chemist who began working on the algal coal project 2 years ago, utilizing lab space at the Biotechnology Institute at the invitation of former BTI Director Ken Valentas, who initiated the project.

"Steve is an accomplished chemist who just had his 100th US patent issued in February," explained Valentas, who is excited about the potential applications of Heilmann's research in the arena of biofuels.

The advantages of producing energy from green microalgae are multiple. It carbonizes very quickly-increasing from roughly 45-70% carbon content through dehydration in just a half hour, and, as a growing plant, can double its biomass in 2-3 hours. Burning the resulting char is also carbon neutral, because the process isn't based on any fossil fuel and doesn't add any new carbon to the atmosphere. Greater environmental benefits also exist since the green microalgae can be used to capture carbon dioxide from the burning of fossil fuels to fuel its own growth.

According to Heilmann, large-scale success of the project will be determined by whether or not microalgae production can be ramped up and the hydrothermal carbonization process made continuous. "We'll know within five years if algae can be farmed and whether it can capture carbon dioxide from coal-burning factories."

Mapping the MISSISSIPPI Metagenome

Story by MARY HOFF

What's the Mississippi River like as it starts its 2,300-mile journey from the forests of Minnesota to the Gulf of Mexico? And how does it change along the way? Michael Sadowsky, director of the BioTechnology Institute (BTI), aims to answer at least part of that big question with help from some very little friends.

Sadowsky is leading a project to gather genetic information from the microbes that teem in the river's headwaters at the Itasca Biological Station and Laboratories and, eventually, at various spots downstream. The study will yield valuable insights into how human activity alters the river along its path. Perhaps more important, it will provide CBS students an appetite-whetting taste of metagenomics – an emerging discipline that involves studying the combined genetic material of microorganisms harvested from a natural setting rather than examining single species of organisms grown in the lab.

Known as the Minnesota Mississippi Metagenome Project – M3P for short – the initiative started as an inspiration CBS Dean Robert Elde had after following the forays of genomics guru Craig Venter, who extracted microbial genes from ocean water as a way to explore a new dimension of marine biodiversity and search for unknown organisms with traits beneficial to humans.

Minnesota may not boast an ocean, but we do host the first 600 miles of America's most-storied river. Put that together with the BTI's high-throughput genomic screening facilities, and you have a one-of-a-kind education and research experience just waiting to happen. Elde applied for and received $400,000 in federal stimulus money to set up a program in which students learn the logistics of metagenomics while improving understanding of the river's micro-ecosystem.

"The purpose of the whole project is to start looking at what's in the Mississippi River," says CBS education specialist Jane Phillips, who is heading up the academic aspects of the project. "We are so connected to the Mississippi here on campus. It kind of runs through our blood."

Photo courtesy JANE PHILLIPS

COLLEGE OF BIOLOGICAL SCIENCES

With Sadowsky at the helm, students will create two types of genomic libraries from Mississippi microbes. One, a collection of ribosomal DNA, can be used to assess the biodiversity within the sample. The other, made up of stretches of Mississippi microbe DNA that have been inserted into E. coli, provides a massive collection of genes that can be evaluated for their function. Using these libraries, students will assess biodiversity and look for traits such as antibiotic and heavy metal resistance and toxin-degrading enzymes that tell them something about the environment in which the organisms evolved.

"It's not those experiments in science class when you already know the answer," Sadowsky says. "Everything the students find will be new."

Sadowsky's hope is to use the two-year project to leverage additional funding that would bring in other CBS researchers and extend sampling to other stretches of the Mississippi River. By comparing results of samples from different places, he says, we can get a better understanding of human influences on the river and how they vary with time and location.

"The possibilities are endless," he says.

Chris Stenland, left, will supervise and coordinate the protein purification process in a facility jointly directed by Dr. Marc von Keitz of the BRC and Dr. Vadim Gurvich of ITDD, pictured below.

PURE PROTEIN

The basis for a Partnership in New Drug Development

Story and photos by TIM MONTGOMERY

The Biotechnology Resource Center (BRC) has partnered with the Institute for Therapeutics, Discovery and Development (ITDD) to upgrade its protein purification capabilities. Thanks to the Minnesota Partnership for Biotechnology and Medical Genomics (established by the University of Minnesota and the Mayo Clinic in Rochester), the BRC/ITDD partnership now offers on-site scaled up production and purification of proteins to meet standards set by the U.S. Food and Drug Administration (FDA) for Phase I and Phase II clinical trials. The partnership between the BRC and ITDD gives the University a unique opportunity to make significant advances in the area of therapeutic biologics - therapeutic proteins created by biological processes.

Dr. Vadim Gurvich

Dr. Marc von Keitz

Few academic institutions have the infrastructure and facilities necessary to comply with the rigorous good manufacturing practices (GMP) required by the FDA and necessary for development of therapeutic proteins and natural products compounds in a cost-effective manner. The BRC/ITDD partnership is one of the few in academia who offer FDA-compliant GMP capabilities for drug substances.

Established in 1986 as the central research facility of the University of Minnesota BioTechnology Institute, the BRC has become a state-of-the-art laboratory for scaling up new technologies for bioprocessing. Through its fermentation facilities, the BRC is producing protein entities for industrial use and as diagnostic reagents. The partnership with ITDD introduces a protein purification capability that raises output from the level of crude industrial product to a clinical grade protein.

The unique protein production and protein purification capabilities provided by the combined resources of the BRC and ITDD allows scientists to efficiently evaluate new therapeutic strategies in the development of new drugs and drug treatments.

Mic.E.

A Proven Path to Rewarding Careers in Biotechnology

Story and photos by TIM MONTGOMERY

The Microbial Engineering Master of Science (Mic.E.) program offered by the BioTechnology Institute cross-trains students in engineering and the biological sciences. The program is a pathway for industrial careers in biotechnology or for further studies leading to the Ph.D. in related biotechnology disciplines. With an increasing need to engineer renewable sources of energy, the program and its graduates are in demand.

"Mic.E. coursework in microbiology, genetics and biochemical engineering is helping me in my work," said Indraneel Shikhare, a recent graduate of the Mic.E. program who was hired as a Research Associate in Organism Development by Mascoma Corporation, a biofuels company in New Hampshire working to develop advanced cellulosic ethanol technologies. "It (the program) gives experience with biological research to students with an engineering background and an engineering dimension to biology/biochemistry majors - and such candidates are valued by industry."

The biotechnology industry views the Mic.E. degree as an engineering degree. And while 36 percent of students graduating from the Mic.E. program have continued on to Ph.D. programs, 64 percent have found industrial employment with companies such as Cargill, 3M, Merck, Pfizer, Dow, General Mills, Exxon and others. MicE graduates have very good career opportunities at competitive engineering salaries.

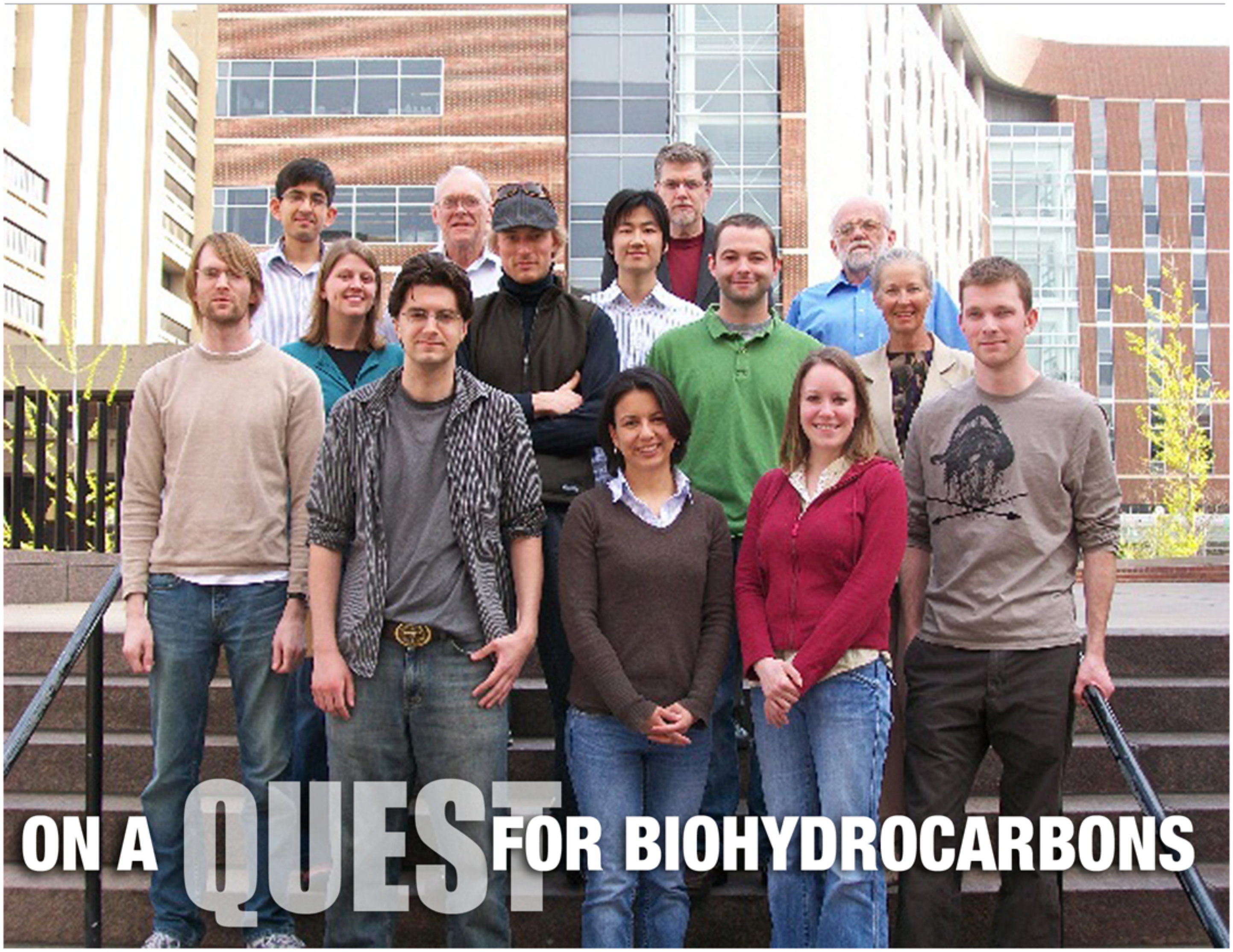
"The Mic.E. program is doing something to build up the midwest biotechnology workforce," explained former BTI director Ken Valentas, "and it's also doing something to build up students' marketability."

"Through this program, I'm able to research solutions to real-life problems like helping to develop renewable and biodegradable polymers."

- DAN ROUSE,
B.S. in Biotechnology and Microbiology,
North Dakota State University

"I've enjoyed researching bacterial production of petroleum-like hydrocarbons and exploring the biofuels field in general"

- STEPHAN CAMERON,
B.A. Biology, St. Olaf College,
Northfield, MN



Above, members of the University group working to make biohydrocarbons. In front (l to r) are Brian Michael, Michael Skinner, Neissa Pinzon, Janice Frias, Chris Flynn. Second row (l to r), Erin Surdo, Stefan Thust, Hsu Chiang, Dave Sukovich, and Carol Gross. And in back (l to r) are Aditya Bhan, Jack Richman, Larry Wackett, and Lanny Schmidt.

ON A QUEST FOR BIOHYDROCARBONS

Can Bacteria Transform Sunlight, Water and CO2 into Viable Transportation Fuels?

Story by TIM MONTGOMERY

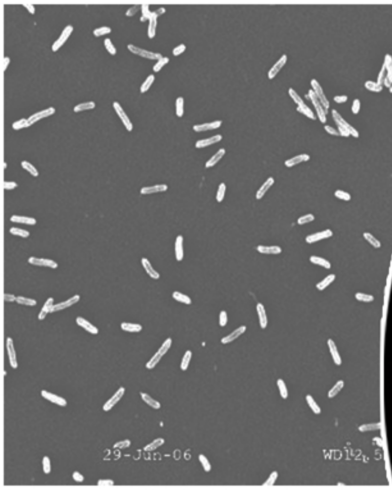
BioTechnology Institute faculty member Larry Wackett is lead investigator for a University of Minnesota team which was selected to receive major stimulus funding through the U.S. Department of Energy's Advanced Research Projects Agency - Energy (ARPA-E) venue for breakthrough energy research. The $2.2 million award finances the team's quest to produce liquid hydrocarbon transportation fuels directly from sunlight, water and carbon dioxide using bacteria.

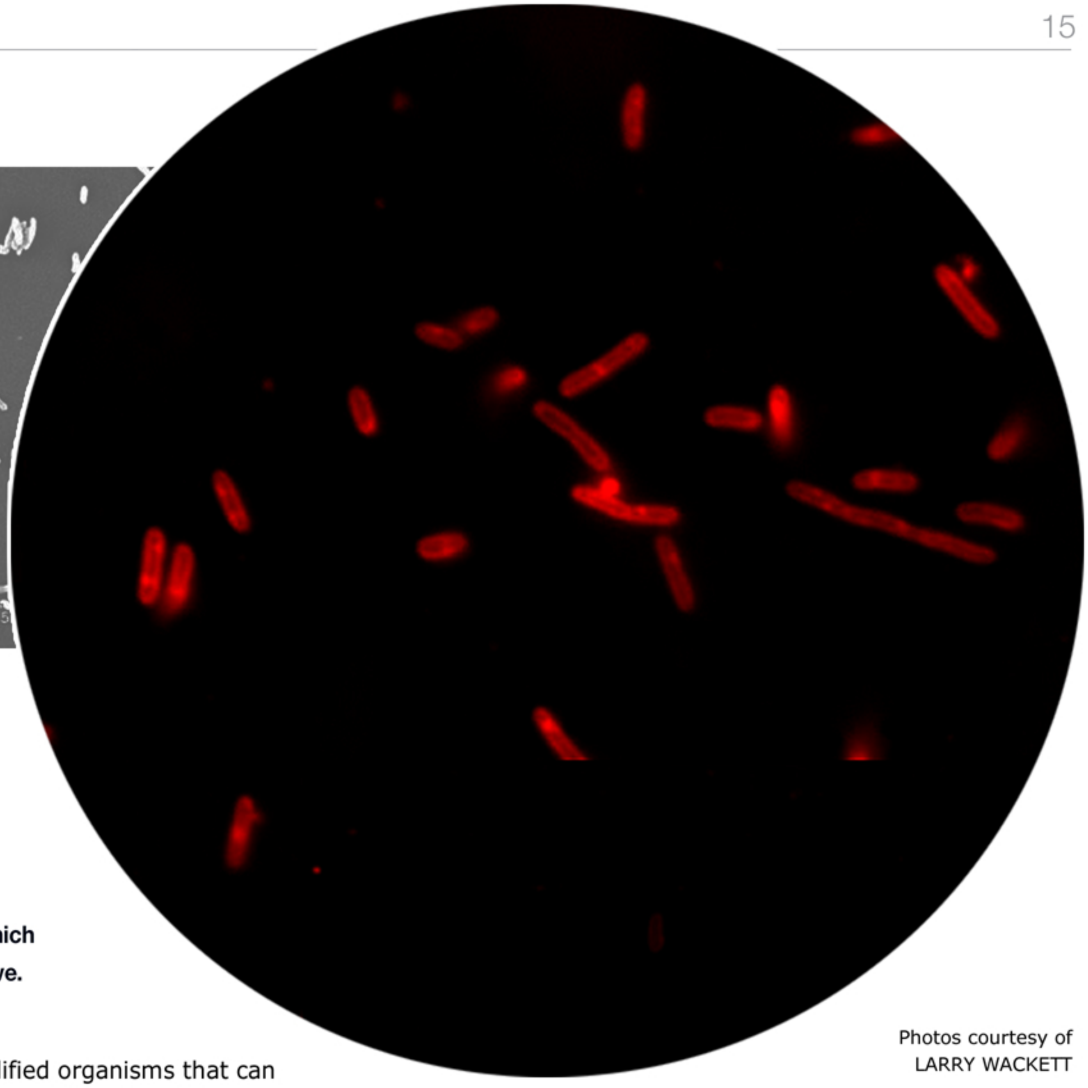
Liquid hydrocarbons extracted from sedimentary rock are the basis of current petroleum fuels. They are a principal source of energy after refining and combustion, and society is heavily invested in the infrastructure necessary for their production, transport and use. But fossil hydrocarbon fuels like coal and petroleum add carbon to the atmosphere when burned, contributing to global climate warming through increased greenhouse gases.

The team of University researchers proposes to create clean-burning liquid hydrocarbon fuels from renewable biological sources - in this case, two different types of bacteria cultured together.

"The process we are looking to develop involves keeping biological catalysts producing hydrocarbon fuels continuously for weeks or months," explained Wackett. "This contrasts with the current discontinuous and rather inefficient process for making fuel ethanol."

To accomplish this, a multidisciplinary group of top chemists and engineers are teaming up with a Minnesota start-up company





Shewanella bacteria producing hydrocarbons flouresce in a dye test developed by post-doctoral student Neissa Pinzon as a screening method to determine which bacteria strains are most productive.

Photos courtesy of
LARRY WACKETT

BioCee, to culture together modified organisms that can produce hydrocarbons with those that will feed them in a thin latex biofilm that will provide engineers with a means of structuring a continuous process for producing fuel.

“There are organisms living together and feeding off one another in natural biologic films such as the algae and bacteria that form colored coatings in deserts,” says Wackett. “We’ll see what works best for catalytic applications and feed the organisms to increase production.”

The idea for a "biohydrocarbon" project came about from a collaborative effort between the Wackett Lab and the lab of Jeffrey Gralnick which identified genes involved in the production of a very large hydrocarbon. Working with the Shewanella bacterium, Dave Sukovich, a Ph.D. student in the Wackett lab, discovered a way to significantly broaden the products of this pathway - going from one specific long-chain hydrocarbon to a diverse range of hydrocarbons, similar to an actual fuel profile.

"Shewanella bacteria is the platform that we are developing into a biohydrocarbon production system," commented Gralnick, who was particularly excited about the novel way in which 'food' will be provided for Shewanella to use in making hydrocarbons."

Working in partnership with the Department of Energy's Pacific Northwest National Laboratory (PNNL), University researchers are using a photosynthetic bacteria developed by PNNL that can convert light and carbon dioxide to "feed" the hydrocarbon-producing Shewanella bacteria being altered at the BioTechnology Institute for scaled-up production. The latex biofilm, developed by former BioTechnology Institute faculty member Michael Flickinger and the late L. E. (Skip) Scriven, an Institute of Technology professor, and produced by university start-up BioCee Inc. will provide the environment for growth of the bacteria. University specialists in Chemical Engineering and Materials Science, Aditya Bhan and Lanny Schmidt, will work on "cracking" the thick hydrocarbon output to produce fuel. The availability and contribution of specialists and materials located in close proximity at the University was one of the factors that enhanced the proposal, according to Wackett.

"The view of the people at ARPA-E was that instead of using different yeasts in fermentation, they wanted to hear more proposals that had potential to change the industry," concluded Wackett. "This is a high-risk, high-reward venture."

Biofuels Believe It or Not!

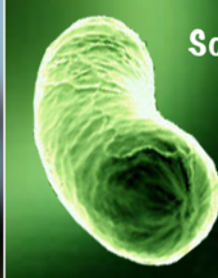
PRAIRIE GRASSES

GROW ABUNDANTLY IN POOR SOIL WITH LITTLE OR NO FERTILIZER

. . . growing prairie grasses on marginal land for use as biofuels can also help reverse global warming, purify water, restore soil for food crops, and provide natural habitats for wildlife

ALGAE grown on wastewater can remove pollutants and make oil that can be turned into fuel

Ethanol is now made from corn and soybeans, but technology will soon be available to convert cellulose (stalks and leaves) from all kinds of plants into ethanol



Some bacteria have the ability to convert organic materials into electricity

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