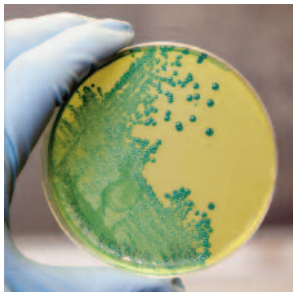
electrofuels

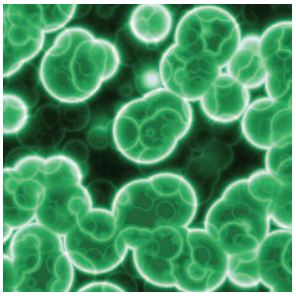
SBE's Conference on Electrofuels Research

Providence, RI • November 6-9, 2011

SBE'S CONFERENCE ON ELECTROFUELS RESEARCH

NOVEMBER 6-9, 2011, PROVIDENCE, RI





Society for Biological Engineering

An AIChE Technological Community

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LETTER FROM ORGANIZING COMMITTEE

In an effort to expand and accelerate the investigation of novel sources of alternative energy, the Society for Biological Engineering (SBE) is sponsoring a new conference series on Electrofuels Research.

The conference brings together key participants in energy innovation--engineers, scientists, venture capital investors, entrepreneurs, large corporations, and government officials--to share ideas and research strategies for developing and deploying new liquid transportation fuels. Instead of using petroleum or biomass, the processes to be discussed use microorganisms to harness chemical and electrical energy from sources such as solar-derived electricity or hydrogen or earth-abundant metal ions to convert carbon dioxide into liquid fuel with remarkably high efficiency.

Keynote speakers include:

- **Dennis Beal,** Vice President of Global Vehicles for FedEx and former Vice President of Physical Assets at FedEx Freight
- **Sharon E. Burke,** Assistant Secretary of Defense for Operational Energy Plans and Programs
- **Daniel Nocera,** a founder of Sun Catalytix and the Henry Dreyfus Professor of Energy and Professor of Chemistry at MIT

In addition to these incredible keynote speakers, each of the ARPA-E grant awardees will be presenting their research.

We thank you for your interest in the advancement of renewable fuels and are sure you will enjoy the incredible program in store!

Sincerely,

Gregory Stephanopoulos
Chair, Conference on Electrofuels Research

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Sunday, November 6	
8:00 am - 2:00 pm	**Registration**
2:00 pm - 2:15 pm	**Opening Remarks** Greg Stephanopoulos; Eric Toone (ARPA-E)
2:15 pm - 3:00 pm	**Keynote Address: Dennis Beal (FEDEX): "Electrification in Action"**
3:00 pm - 3:30 pm	**Break and Networking**
3:30 pm - 5:30 pm	**Session 1: ADVANCES IN ELECTROFUELS I** **Chair: Greg Stephanopoulos**
3:30 pm - 4:15 pm	Greg Stephanopoulos (Massachusetts Institute of Technology): "Bioprocess and Microbe Engineering for Total Carbon Utilization in Biofuel Production"
4:15 pm - 5:00 pm	Michael Lynch (OPX Biotechnologies): "Diesel Production via Fatty Acid Synthesis Utilizing Hydrogen and Carbon Dioxide Feedstocks"
5:00 pm - 5:30 pm	**Break and Networking**
5:30 pm - 7:30 pm	**Panel: VIEW FROM THE INDUSTRIAL WORLD**
5:30 pm - 5:35 pm	**Chair: Jonathan Burbaum (ARPA-E)**
5:35 pm - 5:50 pm	Jennifer Holmgren (CEO LanzaTech): "An Alternative Renewable and Clean Energy Source: Gaseous Carbon"
5:50 pm - 6:05 pm	Thomas Jarvi (CTO Sun Catalytix): "Generation of Renewable Fuel From Sunlight and Water"
6:05 pm - 6:20 pm	Dan Robertson (Joule Unlimited): "Direct Photosynthetic Production of Hydrocarbon Fuels"
6:20 pm - 7:30 pm	Discussion
7:30 pm - 9:30 pm	**Dinner (Grand Ballroom)**

Monday, November 7	
7:30 am – 8:30 am	**Continental Breakfast**
8:30 am - 12:00 pm	**Session 3: ADVANCES IN ELECTROFUELS II** **Co-Chairs: Bob Kelly and Aaron Hawkins**
8:30 am - 9:15 am	Bob Kelly (NC State): "Hydrogen-Dependent Conversion of CO_2 to Liquid Electrofuels by Extremely Thermophilic Archaea"
9:15 am - 10:00 am	Luisa Gronenberg (UCLA): "Reduction of CO_2 to C4 and C5 Higher Alcohols"
10:00 am - 10:30 am	**Break and Networking**
10:30 am - 11:15 am	Christopher Brigham (Massachusetts Institute of Technology): "*Ralstonia Eutropha* and the *De Novo* Biosynthesis of Isobutanol"
11:15 am - 12:00 pm	Mark Worden (Michigan State University): "Mass Transfer Issues in Gas-Intensive Fermentations"
12:00 pm - 12:45 pm	Harold May (Medical University of South Carolina): "Electrosynthesis by Microbial Communities"
12:45 pm – 3:30 pm	**Lunch Break (on your own)**
3:30 pm - 5:45 pm	**Session 4: ALGAE** **Chair: Kim Ogden**
3:30 pm - 4:15 pm	Kim Ogden (Arizona): "Cultivation Strategies for Microalgae to Produce Biofuels"
4:15 pm - 5:00 pm	Zoee Perrine (PhyCal): "Strategies for Improving Photosynthetic Biomass Yield of Microalgal Cultures"
5:00 pm - 5:45 pm	Edward Frank (Argonne National Labs): "Life Cycle Analysis of Algal Fuels with the GREET Model"
5:45 pm - 6:15 pm	**Break and Networking**
6:15 pm - 7:15 pm	**Keynote Address: Daniel Nocera (MIT): "Inexpensive Hydrogen from Solar and Water"**
7:15 pm - 8:00 pm	**Reception and Break for Poster Viewing**
8:00 pm – 9:30 pm	**Dinner (Grand Ballroom)**

Tuesday, November 8	
7:30 am – 8:30 am	**Continental Breakfast**
8:30 am – 12:00 pm	**Session 5: ADVANCES IN ELECTROFUELS III** **Chair: Scott Banta**
8:30 am - 9:15 am	Steve Singer (Lawrence Berkeley National Laboratory): “Microbial-Electrocatalytic Production of Biofuels”
9:15 am - 10:00 am	Derek Lovely (University of Massachusetts, Amherst): “Microbial Electrosynthesis: The Shortest Path from the Sun to Fuel”
10:00 am - 10:30 am	**Break and Networking**
10:30 am - 11:15 am	Jeffrey Way (Harvard Medical School – Wyss Institute): “Engineering Bacterial Electron Uptake and CO_2 Fixation for Electrofuel Synthesis”
11:15 am - 12:00 pm	Scott Banta (Columbia University): “ Electrofuel Production Using Ammonia or Iron as Redox Mediators in Reverse Microbial Fuel Cells”
12:00 pm – 3:00 pm	**Lunch (on your own)**
3:00 pm - 6:30 pm	**Session 6: HYDROGEN and CO_2 SUPPLY** **Chair: Bob Farrauto**
3:30 pm - 3:45 pm	Robert Farrauto (BASF): “Hydrogen Generation for Fuel Cells”
3:45 pm - 4:30 pm	George Bollas (University of Connecticutt): "H_2 Production Options and Their CO_2 Footprint"
4:30 pm - 5:00 pm	**Break and Networking**
5:00 pm - 5:45 pm	Howard Herzog (Massachusetts Institute of Technology): “Production of CO_2 – Sources and Processes”
5:45 pm - 6:30 pm	Edward F. Kiczek (Air Products): “Cost Effective Hydrogen Production: Today and the Near Future”
6:30 pm - 7:00 pm	**Break and Networking**
7:00 pm - 7:30 pm	**Keynote Address: Sharon Burke (Defense for Operational Energy Plans and Programs): “Electrofuels, Energy Use, and National Defense”**
7:30 pm – 9:00 pm	**Dinner (Grand Ballroom)**

Wednesday, November 9	
7:30 am – 8:15 am	**Continental Breakfast**
8:15 am – 10:30 am	**Session 7: ADVANCES IN ELECTROFUELS IV** **Chair: Wayne Curtis**
8:15 am – 9:00 am	Curt Fischer (Ginkgo Bioworks): “Design, Construction, and Testing of Metabolic Modules for *E. coli* based Electrofuels Production”
9:00 am - 9:45 am	Wayne Curtis (Pennsylvania State University): "Development of Rhodobacter as a Versatile Microbial Platform for Fuels Production"
9:45 am - 10:30 am	Robert Tabita (Ohio State University): “Carbon Dioxide to Biofuels by Facultatively Autotrophic Hydrogen Bacteria”
10:30 am - 11:00 am	**Break and Networking**
11:00 am - 12:30 pm	**Panel: The Business Side of Electrofuels** Moderator: William Aulet (Managing Director MIT Entrepreneurship Center) Panelists: Simon Upfill-Brown (Terrabon), Colin South (Novogy), TBD (Agrivida)
12:30 PM	**Conference Ends**

KEYNOTE SPEAKER ABSTRACTS AND BIOS

DENNIS R. BEAL, Vice President, Global Vehicles at FedEx Express, Inc.

Dennis is Vice President of Global Vehicles at FedEx Express, Inc., a subsidiary of FedEx Corp, the world's largest express transportation company. Dennis has been in the transportation industry for over 40 years and joined FedEx in 2001. He served as Vice President, Physical Assets at FedEx Freight and joined FedEx Express in July, 2010 as Vice President, Global Vehicles.
Beal has lived throughout the United States and is a native of west Tennessee. He believes in giving back to the community and has served on numerous Boards for charitable organizations.

Keynote Address:
Dennis will speak on "Electrification in Action" - A discussion about the learning from utilizing a growing number of electric vehicles around the world and a look at the viability of this model.

SHARON E. BURKE, Assistant Secretary of Defense for Operational Energy Plans and Programs

Sharon was sworn in as the Assistant Secretary of Defense for Operational Energy Plans and Programs on June 25, 2010. Ms. Burke is the principal advisor to the Secretary and Deputy Secretary of Defense on operational energy security and reports to the Under Secretary of Defense for Acquisition, Technology, and Logistics. She is the inaugural Assistant Secretary for the office, which was created to strengthen the energy security of U.S. military operations. The mission of the office is to help the military services and combatant commands improve military capabilities, cut costs, and lower operational and strategic risk through better energy accounting, planning, management, and innovation. Operational energy, or the energy required to train, move, and sustain forces, weapons, and equipment for military operations, accounted for 75 percent of all energy used by the Department of Defense in 2009.

Keynote Address:
Ms. Burke will discuss the context in which the Administration and Department of Defense consider our energy use and national defense, including the strategic defense environment, global energy supply and demand trends, and defense energy use. That context is the foundation of the Operational Energy Strategy, released by the Department in June 2011, which Ms. Burke will describe. She will then discuss how electrofuels align with that strategy, and how improvements in this technology may lead to advances on the battlefield.

DANIEL NOCERA, Massachusetts Institute of Technology is a founder of Sun Catalytix and the Henry Dreyfus Professor of Energy and Professor of Chemistry at MIT.

Professor Nocera is also Director of MIT's Solar Revolutions Project and the ENI Solar Frontiers Center. A leading researcher in renewable energy at the molecular level, he studies the basic mechanisms of energy conversion in biology and chemistry, with primary focus on the photogeneration of hydrogen and oxygen from water. Professor Nocera received a B.S. from Rutgers University in 1979 and Ph.D. from California Institute of Technology in 1984.

Keynote Address:
"Inexpensive Hydrogen from Solar and Water"

Electrofuel Production Using Ammonia or Iron As Redox Mediators in Reverse Microbial Fuel Cells

Scott Banta[1], *Kartik Chandran[2] and Alan C West[1]*
[1]Chemical Engineering, Columbia University, New York, NY
[2]Earth and Environmental Engineering, Columbia University

The production of electrofuels requires the efficient transport of electrons from an electrochemical system into a biological system. We have approached this challenge by identifying natural chemical mediators that 1) can be easily reduced electrochemically and 2) are natural substrates for different bacterial strains, thus eliminating the need to engineer this aspect of primary metabolism in the biological hosts. In our first project we have constructed a reverse microbial fuel cell using the ammonia oxidizing bacteria, *N. europaea*. These cells grow planktonically and they efficiently oxidize ammonia to nitrite while fixing carbon dioxide. We have developed an electrochemical reactor to reduce the nitrite back to ammonia so that we are producing biomass from electricity and air. We have recently engineered the *N. europaea* cells to produce isobutanol, which is a transportation infrastructure compatible biofuel. In a second project we are working with A. ferrooxidans, which is an iron oxidizing bacteria used in biomining operations. The oxidized iron can be readily reduced electrochemically, and efforts are underway to engineer these cells to make isobutanol as well. As these processes are developed and optimized, they may be able to produce biofuels and other petroleum derived chemicals from electricity and air.

Hydrogen Production Options and their Carbon Dioxide Footprint

Lu Han and ***George M. Bollas,*** *Department of Chemical, Materials & Biomolecular Engineering, University of Connecticut, 191 Auditorium Road, Unit 3222, Storrs, CT 06269-3222, USA*

Molecular hydrogen is an energy carrier but not an energy resource. Hydrogen use directly as an energy source or indirectly in fuel upgrading processes requires the energy intensive step of producing it. Albeit the high energy requirements for the production of molecular H_2 in most of the existing and in many of the proposed processes for H_2 production, there is a significant CO_2 footprint. Therefore, when considering the upgrading process of environmentally-friendly fuels (electrofuels, biofuels, Fischer-Tropsch Synthesis, etc.) one has to account for the CO_2 footprint of the H_2 used in the upgrading step (typically a hydrotreatment process). This presentation will briefly summarize existing and proposed technologies for H_2 production and analyze them in terms of their thermodynamic efficiency and CO_2 footprint. Focus will be given to novel processes capable of producing CO_2-free H_2 or processes for H_2 production with *in-situ* CO_2 capture

Ralstonia Eutropha and the *De Novo* Biosynthesis of Isobutanol

Christopher Brigham[1], *Jingnan Lu[2], Claudia Gai[1] and Anthony J. Sinskey[3]*
[1]Biology, MIT, Cambridge, MA
[2]Chemistry, MIT, Cambridge, MA
[3]Biology and Health Sciences and Technology, MIT, Cambridge, MA

Isobutanol (IBT) can be used as a 100% replacement for gasoline in existing automobile engines, has >90% of the energy density of gasoline and is compatible with established fuel distribution infrastructure. Using a carefully designed production pathway, we have modified the genetically tractable bacterium *Ralstonia eutropha* to produce IBT using a *de novo* pathway. Production of this product can be achieved by directing the flow of carbon through a synthetic production pathway involving the branched-chain amino acid biosynthesis pathway, a heterologously expressed ketoisovalerate decarboxylase, and a broad-substrate-specificity alcohol dehydrogenase. We have demonstrated that all components of the engineered pathway are functional in an *R. eutropha* IBT production strain. Furthermore, we have demonstrated production of IBT using the engineered strain in fructose- and pyruvate-grown cultures. The motivations and the methods used to engineer *R. eutropha* to produce IBT are expected to culminate in production of the liquid transportation fuel from CO_2, H_2, and O_2.

View from the Industrial World

Jonathan Burbaum, *ARPA-E (Panel Moderator)*

Microbial-Electocatalytic Production of Biofuels

Swapnil Chhabra[1], *Steven Singer[1], Harry R. Beller[1], Jana Mueller[1], Yi-Chun Yeh[1], Christopher Chang[2], Jonah Jurss[2] and Claudio Fillipone[3]*
[1]Lawrence Berkeley National Laboratory
[2]University of California-Berkeley
[3]Logos Technologies

We are developing an integrated Microbial-Electro Catalytic (MEC) system consisting of *Ralstonia eutropha* as a chemolithoautotrophic host for metabolic engineering coupled to a small-molecule electrocatalyst for the production of biofuels from CO_2 and H_2. In this strategy, *R. eutropha* is being engineered to produce hydrocarbons through the fatty-acid and isoprenoid biosynthesis pathways, and to produce butanol by diverting the biosynthetic pathway to produce polyhydroxyalknoates. In parallel, we are developing techniques to display proteins on the *R. eutropha* outer-membrane to bind water-soluble H_2-evolving catalysts for autotrophic growth. In this talk, we will present results demonstrating the overproduction of fatty acids and the production of fatty-acid derived alkanes by introduction of heterologous genes into *R. eutropha*. The production of isoprenoid hydrocarbons was also demonstrated in *R. eutropha* through the expression of heterologous terpene synthases. We have also exported multiple heterologous proteins engineered with reactive functional groups to the surface of *R. eutropha* cells and bound fluorescent small molecules and metal-containing complexes to these proteins. We will also discuss a one-step chemical conversion of butanol to a hydrocarbon mixture closely resembling jet fuel and the construction of a bioelectrochemical reactor for autotrophic growth and biofuel production by *R. eutropha*.

Rhodobacter As a Biofuel Production Platform and Associated Autotrophic Bioreactor Design

Wayne R. Curtis[1], *Joe Chappell[2], Bruce E. Logan[3], Amalie L. Tuerk[1], John A. Myers[1] and Nymul E. Khan[1]*
[1]Chemical Engineering, Penn State University, University Park, PA
[2]Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY
[3]Civil and Environmental Engineering, Pennsylvania State University, University Park, PA

The overall goal of this ARPA-E project is to produce a hydrocarbon fuel feedstock from CO_2 based on microorganism growth on H_2 or direct electron feeding from a cathode. The approach is to genetically engineer C34 branched hydrocarbon biosynthesis from the algae Botryococcus braunii into the purple non-sulfur bacterium *Rhodobacter capsulatus*. An update on progress of alternative approaches to genetic engineering this

pathway will be presented. Efficient transformation has been achieved, stable vectors have been adapted and synthetic operons have been constructed. The importance of integrating organism design and bioreactor design will be discussed in the context of productivity bounds imposed by microbial energetics and gas mass transfer limitations. A scaled down (< 10-mL) autotrophic bioreactor capable of achieving kLa in excess of 1000/hr will be described (by comparison to typical shake flask mass transfer rates of < 50/hr). This system is being multiplexed to provide for screening of performance of transgenic autotrophs. Progress in developing chemostatic autotrophic bioreactors for critical kinetic analysis as well as dead-end fermentation for complete gas conversion will also be presented.

Hydrogen Generation for Fuel Cells

Robert Farrauto, *Research Vice President, BASF Corporation, Iselin, New Jersey USA Adjunct Professor, Earth and Environmental Engineering, Columbia University, New York City*

Structured (monolithic) reactors, with catalyzed washcoats, are now commonplace in pollution abatement technologies. The largest application is automotive emission control for gasoline and diesel engines. The potential for use in chemical applications is now emerging commercially especially as we move towards a hydrogen economy.

At BASF (formerly Engelhard Corporation) we have begun to commercialize catalyzed monolithic reactors for hydrogen generation from a variety of fuels. These structures offer a number of advantages over more traditional base metal oxide packed bed particulate catalysts used in large hydrogen plants, especially for distributed hydrogen,. Precious metal catalyzed washcoated reactors, are compact, free from attrition, have low coke make, enjoy robust performance, and low pressure drop. An important design parameter is the ability to minimize heat transfer resistance for the endothermic steam reforming reaction by depositing washcoat on heat exchangers allowing great throughputs of product.

Today's seminar will discuss various washcoat reactor designs for hydrogen generation for a variety of fuels. Examples for residential combined heat and power, distributed power for cell phone towers and portable power will be discussed.

Design, Construction, and Testing of Metabolic Modules for *E. coli-based* Electrofuels Production

Curt Fischer [1]***,*** *Satoshi Yuzawa* [2]*, Woncheol Kim* [2]*, Leonard Katz* [2]*, Sean Poust* [2]*, Jeffrey Fortman* [2]*, Jay Keasling* [2]*, Justin Siegel* [3]*, Amanda Lee* [3]*, Catherine Louw* [3]*, David Baker* [3]*, Mary Lidstrom* [3]*, Reshma Shetty* [1]*, and Jason Kelly* [1]

[1]Ginkgo Bioworks
[2]University of California, Berkeley
[3]University of Washington

Natural organisms have the capacity many non-tradtional source of energy to power their metabolism, including hydrogen, electricity, or formic acid. Many electrofuels approaches seek to use these natural organisms as an engineering platform for the production of fuels and chemicals from non-traditional feedstocks. We are taking an orthogonal approach -- to reconstitute the pathways for autotrophic energy utilization and for fuels production in an organism that does not naturally possess any of these capabilities. This "bottom-up", synthetic biology approach ensures complete control over all aspects of electrofuels production in a finished organism, opens the possibility for the construction of artificial pathways for the carbon fixation, energy harvesting, and fuels production, and allows *E. coli*, an organism already used in a broad variety of industrial fermentations, to be used for electrofuels production.

To circumvent the mass-transfer challenges inherent in use of hydrogen or electricity as energy sources, we have chosen formate as an energy carrier for biofuels production. We are constructing modules for formate conversion, carbon fixation, and fuels production that results in isooctanol, a potential drop-in replacement for existing fuels.

Life Cycle Analysis of Algal Fuels with the GREET Model

Edward D. Frank, *Argonne National Labs*

This talk will cover the ability to source the hydrogen from conventional and renewable taking advantage of existing domestic supply. This presentation will cover the recent advancements in hydrogen supply chain systems which are conducive to the role out of new infrastructure at competitive pricing to gasoline today. Also, we touch on sustainable hydrogen pathways that are in operation as well as options for the future.

Microbial Reduction of CO_2 to Higher Alcohols Driven by Electricity

Han Li and ***Luisa Gronenberg,*** *Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, CA*

Microbial reduction of CO_2 to higher alcohols driven by electricity is considered as an effective way to store electricity as liquid fuel. Here we report progress to convert electricity to C4 and C5 higher alcohols, which can be used as liquid fuels. The method, he method links electrochemical generation of hydrogen or formic acid to biological CO_2 fixation and carbon reduction. We genetically engineered a lithoautotrophic organism, *Ralstonia eutropha*, to enable the cell to produce isobutanol and 3-methyl-1-butanol directly from CO_2 using either H_2 or formic acid as the energy source. The liquid fuels generated have energy densities about 100 times higher than current-day batteries.

Production of CO_2 - Sources and Processes

Howard Herzog, *Massachusetts Institute of Technology, Cambridge MA 02139*

The sources of commercial CO_2 today are quite varied. Today's commercial processes prefer sources that have high initial concentrations of CO_2. These include off-gases from ammonia plants, ethanol plants and hydrogen production. Though less concentrated, the flue gas of fossil fuel-fired power plants is a potentially large source of CO_2. One of the cheapest sources is from CO_2 contained in geologic formations. It has also been proposed that CO_2 can be produced from ambient air.

The CO_2 from each of these sources can be characterized by key metrics including quality (concentration and pressure), scale (amount of CO_2), and contaminants. These metrics are critical in deciding the appropriate process required to produce the CO_2 product and their economics. These process options will be reviewed for a variety of sources and their relative costs will be discussed.

An Alternative Renewable and Clean Energy Source: Gaseous Carbon

Dr. Jennifer Holmgren, *LanzaTech Inc., Des Plaines, IL*

The industrialization of emerging markets is drastically increasing the world's energy demand. The International Energy Outlook, in its 2010 reference case, projects that world primary energy consumption will increase by 49 percent (1.4 percent per year) from 495 quadrillion Btu in 2007 to 739 quadrillion Btu in 2035.[1] Crude oil and natural gas resources are currently the main source of energy but are in limited supply and generally are associated with high carbon emissions. Therefore, there is an increasing focus on producing carbon neutral energy and fuels from new feedstocks. In order to meet these challenges, LanzaTech provides a technology solution based on alternative feedstocks while improving process energy efficiency and simultaneously reducing greenhouse gas emissions.

Through gas fermentation, LanzaTech transforms waste into resources. Industrial and chemical processes produce off-gases containing carbon monoxide and/or carbon dioxide that are either vented to the atmosphere, flared, burned as fuel, or converted to power; a sub-optimal use of the carbon and energy contained in these off-gases. The LanzaTech process captures these waste gases with renewable resources and transforms them into energy rich fuels or chemicals such as ethanol, acetic acid, or 2,3-butanediol, among others. Initial estimates indicate that > 30 billion gallons per year of high value products can be produced from steel gases alone; this is a considerable contribution to the worldwide energy and chemical pool. The LanzaTech process offers superior carbon conversion, energy efficiency, and greenhouse gas emission performance compared to conventional and emerging routes to the same products.

The LanzaTech gas fermentation process is described including potential applications in chemical, petrochemical, refinery, gas-to-liquids, and industrial plants (such as coal and steel). Since 2005 LanzaTech has developed its gas fermentation technology from lab-scale batch experiments to a continuous pilot plant which has been in operation since 2008. The technology description includes the scale-up from lab reactors to pilot scale to the first demonstration plant that is currently under construction. The first LanzaTech technology to be commercialized is gas fermentation of raw steel mill waste gases to produce ethanol. This is the first process going to commercial scale for the production of bio-fuels from a non-food alternative feedstock.

[1]"IEA - 2010 International Energy Outlook - World Energy Demand and Economic Outlook." http://www.eia.doe.gov/oiaf/ieo/world.html (accessed February 24, 2011).

Generation of Renewable Fuel From Sunlight and Water

Tom Jarvi, *Sun Catalytix Corporation, Cambridge, MA 02139*

Sun Catalytix has been developing technology for energy storage and the generation of renewable fuels in an ARPA-E sponsored program. The program has been focused on the development and deployment of low-cost earth-abundant catalytic materials and light-absorbing semiconductor systems to capture and convert solar energy into chemical energy. This talk will focus on recent work done to couple cobalt-based water-oxidation and nickel-based hydrogen evolution catalysts with silicon-based solar cells. The results demonstrate direct wireless coupling of solar collection with catalytic materials and operate in relatively benign conditions at reasonable conversion efficiency. These results suggest development pathways for solar hydrogen generation using catalyzed particulate solar absorbing materials. Such pathways will be discussed as they may offer relevant means to integrate solar hydrogen generation with relevant microorganisms that require reducing equivalents to generate liquid fuel starting from CO_2.

Hydrogen-Driven Conversion of Carbon Dioxide to Liquid Electrofuels In Extremely Thermophilic Archaea

Michael W.W. Adams[1], and ***Robert M. Kelly[2]***

[1]Biochemistry and Molecular Biology, University of Georgia, Athens, GA
[2]Partners II Bldg, Room 3309, 840 Main Campus Drive, Chemical and Biomolecular Engineering, Raleigh, NC

The recent discovery of novel CO_2 fixation pathways in extreme thermophiles, coupled with the recent availability of genetics tools for these microorganisms, has given rise to new opportunities for producing electrofuels at elevated temperatures. Not only are there favorable bioenergetic advantages associated with these new pathways, but also the prospect for direct recovery of organic solvents at high temperatures. Preliminary metabolic engineering efforts have uncovered to novel gene expression strategies that can minimize cell maintenance requirements. Discussed here will be our current efforts and progress in integrating the 3-hydroxypropionate/4-hydroxybutyrate pathway from the extreme thermoacidophile, *Metallosphaera sedula*, into the heterotrophic hyperthermophile, *Pyrococcus furious*. The objective of the project is to convert a CO_2:H_2 feed into a biofuel solvent.

Cost Effective Hydrogen Production: Today and the Near Future

Edward F. Kiczek, *Air Products*

This talk will cover the ability to source the hydrogen from conventional and renewable taking advantage of existing domestic supply. This presentation will cover the recent advancements in hydrogen supply chain systems which are conducive to the role out of new infrastructure at competitive pricing to gasoline today. Also, we touch on sustainable hydrogen pathways that are in operation as well as options for the future.

Microbial Electrosynthesis: The Shortest Path From the Sun to Fuel

Derek R. Lovley, *Department of Microbiology, University of Massachusetts, Amherst, Amherst, MA*

Microbial electrosynthesis is the process in which microorganisms use electrons derived from electrodes to reduce carbon dioxide to multi-carbon compounds that are excreted from the cell. With microbial electrosynthesis it is feasible to efficiently produce transportation fuels or other desirable organic compounds from a variety of renewable sources of electricity. When electricity is derived from photovoltatics microbial electrosynthesis is an artificial form of photosynthesis in which solar energy drives the conversion of water and carbon dioxide to organic compounds with oxygen as a byproduct. However, microbial electrosynthesis can be much more effective than processes that rely on biological photosystems because photovoltaics are much more efficient in harvesting solar energy and the microorganisms consuming this energy direct over 90% of the electrons received to desired products, which are respiratory end products that are released directly from the cell into the extracellular medium. The microorganisms catalyzing electrosynthesis grow as a biofilm on the electrode surface, simplifying product separation, reducing the generation of wastes, and allowing for continuous production. This contrasts with the batch processes typical of many biofuel strategies.

Proof of concept studies for microbial electrosynthesis have focused on the production of acetate, as a feedstock for the production of other chemicals, and on the direct production of the transportation fuel butanol. A diversity of acetogenic bacteria, that have proton-dependent ATP synthases were found to be capable of accepting electrons from negatively poised electrodes as the sole electron donor for the reduction of carbon dioxide. The acetogen, *Acetobacterium woodii*, which has a sodium driven ATP synthase, was not capable of electrosynthesis. Wild-type strains of acetogens that are effective in electrosynthesis produce acetate as the primary product with columbic efficiencies of ca. 90% and energetic efficiencies of ca. 70%.

Acetyl-CoA, the central intermediate in the Wood-Ljungdahl pathway of carbon dioxide reduction, can serve as the building block for a wide diversity of microbial products. A metabolically engineered strain of *Clostridium ljungdahalii* produced butanol from carbon dioxide via microbial electrosynthesis. Studies are underway to improve the expression of the enzymes involved in synthesizing butanol from acetyl-CoA in order to increase the rates of butanol production. Production of a diversity of other organic commodities from carbon dioxide and electricity are feasible.

Strategies to promote electrode-microbe electron exchange, based on the recent discovery of metallic-like conductivity along the length of Geobacter pili and through Geobacter biofilms, are underway. A scalable reactor design is under evaluation.

Novel Biological Conversion of Hydrogen and Carbon Dioxide Into Diesel

Michael D. Lynch, *Matthew L. Lipscomb, Tanya Warnecke Lipscomb, Hans Liao, PinChing Maness, OPXBIO, 2425 55th St., Suite 100, Boulder, CO 80301*

There is increasing pressure to reduce dependence on foreign petroleum sources. As such, the development of green chemistry routes to produce fuels from renewable feedstocks has been the focus of significant research. Traditional bio-refining processes rely on microbial fermentation of renewable carbon sources such as sugar into higher value products. More recently, work has focused on the use of non-traditional feedstocks in bio-processing such as cellulosic biomass, pyrolysis of waste biomass, or gasification of organic municipal solid waste, to name a few. OPXBIO is developing a novel, engineered microorganism and process that produces a diesel-equivalent fuel from renewable hydrogen (H_2) and carbon dioxide (CO_2). The proposed process will fix CO_2 utilizing H_2 to generate an infrastructure-compatible, energy-dense fuel. The proposed process is scalable, the initial economics are favorable, and the liquid fuel can be used directly as a blending stock in the existing diesel infrastructure.

Electrosynthesis by Microbial Communities

Harold D. May[1], *Stephen E. Creager[2], J. Michael Henson[2], and R. Sean Norman[3]*

[1]Medical University of South Carolina
[2]Clemson University
[3]University of South Carolina

The goal of this project is to use a carbonless source of electrons, e.g. from wind or solar, to bioelectrochemically reduce CO2 into a liquid fuel that may be integrated into the existing transportation infrastructure. The robust properties of microbial communities, which are very effective at catalyzing operations found in waste treatment and biogas production, will be aimed at the electosynthesis of n-butanol, a compound with chemical characteristics that make it compatible with existing liquid fuel infrastructure. The properties desired in these microbial communities include extracellular electron transfer with metals and electrodes, natural selection of syntrophic interactions, and metabolic redundancy that stabilizes the community of catalysts. Butanol generation will be performed by an acetotrophic, syntrophic bacterial community linked through interspecies hydrogen (or electron) and acetate transfer to an electrode-oxidizing biocathode. An electrochemical approach will be used to drive the evolution of the microbial catalysts and improve biocathode performance, and metagenomics/metatranscriptomics will be used to assess the selection of the community and its genetic adaptation. These results and the engineering of a scalable system will be used to develop an efficient bioelectrochemical reactor, one that will transform our present capabilities into a sustainable one that generates n-butanol from CO_2 without fossil carbon or biomass.

Cultivation Strategies for Microalgae to Produce Biofuels

Kimberly Ogden and Ming Ren, *Department of Chemical and Environmental Engineering, University of Arizona, Tucson, AZ*

There has been quite a debate over the last few years as to the most effective way to cultivate microalgae on the large scale. One group of researchers is convinced that photobioreactors are the best method, however, these have the disadvantage of high capital costs. Others believe that open ponds are the best, but these are more susceptible to variations in weather and contamination. Hybrid systems are one solution for producing large quantities of algae. These systems will be compared and contrasted. Another important cultivation issue is nutrient supplies. Nutrients that are vital for the algal growth include nitrogen, phosphorous, and carbon. Using minimal amounts of the first two is desirable. Hence, how the nitrogen source affects production rates as well as lipid profiles will be presented. Furthermore, a model which predicts yield and productivity will be presented that is applicable for low nitrogen conditions. Strategies for minimizing phosphate use will be briefly highlighted. Finally, use of carbon from non-pure sources such as power plants will be discussed and initial results using flue gas in batch cultivation experiments will be presented.

Strategies for Improving Photosynthetic Biomass Yield of Microalgal Cultures

Zoee Perrine, *Clayton Stroff, Sangeeta Negi and Richard Sayre, Phycal, Inc., Highland Heights, OH 44143*

Rapid growth rates, high solar energy conversion efficiencies, ability to utilize CO_2 from point sources and potentially reduced land requirements, make microalgae ideal feedstock candidates for biofuel production. However, some of the largest losses in photosynthetic efficiency and solar-to-biomass conversion can be attributed to energy dissipation due to non-photochemical quenching under full sunlight intensities when algal light-harvesting and reaction center complexes are light-saturated. Under these conditions, up to 80% of the energy of absorbed photons may be dissipated as heat or fluorescence rather than being utilized for conversion to chemical energy. One strategy for optimizing light utilization particularly at high irradiances, involves reducing the size of the peripheral light-harvesting antenna associated with Photosystem II (PSII). In our study this was achieved by decreasing the levels of the light-harvesting pigment, chlorophyll (Chl) b, which binds to the peripheral antenna of PSII. Targeted suppression of Chl b synthesis was sufficient to yield transgenic algal

strains that assembled smaller PSII antennae. These strains had increased rates of photosynthetic oxygen evolution and high-light growth compared to wild-type (WT). A relative increase in the levels of photoprotective pigments, zeaxanthin and lutein, was also observed in high-light grown transgenic cultures. Ultrastructural microscopic analysis of thylakoid membranes showed looser stacking in the transgenics, which could potentially aid in the turnover and repair of damaged photosynthetic machinery under high light.

Support for using algal strains with smaller PSII antennae for increasing photosynthetic biomass yields was also obtained from growth experiments conducted in the greenhouse in which total biomass and light penetration was measured in ponds of different depths. Small antenna strains allowed for improved light penetration through the culture column relative to WT and had the greatest accumulation of biomass during the brighter growth periods tested presumably due to an improvement in light utilization capacity.

Direct Photosynthetic Production of Hydrocarbon Fuels

Dan Robertson, *Biology, Joule, Cambridge, MA*

Joule Unlimited has used a systems engineering approach to create a biocatalytic, photosynthetic production platform for the synthesis of high combustion quality diesel-range hydrocarbons. The process does not rely on production or processing of biomass. Instead it directly and continuously captures and converts solar energy and CO_2 to drive the synthesis of linear alkanes. The production system uses the highly evolved photosynthetic and carbon fixation machinery of a cyanobacterial organism with inputs of industrial flue gas, brackish or waste water in an inexpensive SolarConverter reactor system.

By obviating biomass production and using direct-to-product synthesis and secretion, the photosynthetic process efficiency can be improved many fold over conventional bio-based photosynthesis. Energy conversion via natural photosynthesis relies upon the absorption of photonic energy to split water and provide electronic charge separation, eg, electromotive force, to drive transduction to a proton motive force and to the chemical currencies of ATP and NADPH used to drive CO_2 fixation and intermediary metabolism. The capture and conversion of the light energy via this energy transduction system is widely accepted to be highly efficient.

The energetic losses in conventional photosynthetic processes are due to channeling of chemical energy to biomass and cell maintenance, and to competing respiratory metabolism, eg, mitochondrial respiration and photorespiration. Photorespiration can dissipate up to 40% of free energy but is obviated by use of inherent cyanobacterial carbon concentrating machinery and processing at high [CO_2]. Engineering the removal of competing pathways, dedication of the majority of carbon to product and continuous synthesis combine to reduce energy loss to a level that supports high process productivity. A comparison of solar capture and conversion efficiencies for an algal biomass process and the direct, continuous process. When applied to the alkane synthesis, the efficiency converts to 15,000 gallons of alkane/acre/yr, far surpassing fermentative or biomass-dependent fuels processes. This treatment of energy conversion efficiency has been validated in practice by measuring the rate of production of fuel product or cell mass.

A metabolic pathway for alkane synthesis and a membrane complex catalyzing secretion of alkane product has been constructed in the organism to efficiently partition fixed CO_2 into product and to secrete alkane, creating a process operating at steady-state without production of biomass. Genetic regulation systems have been engineered in the production organism to control synthesis in process phases by switching carbon between biomass and product and to optimize production within a diurnal cycling regime. Competing carbon utilization pathways are removed.

Processing takes place in a transparent, arrayed SolarConverter system designed to promote efficient utilization of CO_2, to utilize all incoming solar radiation via photosynthesis, to dissipate thermal energy and to provide a mechanism for capture and separation of product. SolarConverters are connected in circulation units where motive force is applied via gas sparging. The circulation units are in turn supplied by a central plant and product recovery occurs in a downstream processing unit operation. The process has been scaled in an outdoor pilot facility in Leander, TX.

Bioprocess and Microbe Engineering for Total Carbon Utilization in Biofuel Production

Greg Stephanopoulos, *Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA*

A novel bioprocessing concept is proposed for the production of biodiesel from CO_2 and hydrogen, CO, or electricity. A core component of the scheme is an oil-hyper-producing microbe that is capable of converting a variety of carbohydrate feedstocks and organic compounds to oil (triacylglycerides) that can be used for biodiesel (FAME) production. Here it is proposed to combine aerobic fermentations of this microbe with anaerobic CO_2 fixing bacteria operating in separate anaerobic fermentors with hydrogen, CO or current (via electrodes) providing electrons for reducing potential. The product of the anaerobic CO_2 fixation is acetate, a compound that can be readily utilized by the aerobic microbe for growth and oil production at close-to-theoretical yields. Preliminary calculations suggest that the economic feasibility of the proposed concept depends critically on the rate of carbon dioxide fixation and acetate production. While typical reported volumetric rates are low, specific rates of CO_2 fixation by acetogens are reasonable and can be significantly enhanced by applying technologies of metabolic engineering and synthetic biology. Hence, a central goal of this research is the engineering and or isolation of organisms capable of rapid fixation of CO_2 and acetate production. Additionally, we will engineer the metabolism of the oleaginous microbe for oil production from acetate at high yield. We will be reporting results on both fronts at this conference. The oversell concept of this research is both novel and cutting-edge, with a high probability of commercial success, and relies on technology proven to varying degrees in different contexts. If successful, it has the potential of unlimited biodiesel production from CO_2 and competitively priced hydrogen or CO in a process that does not impose demands on or changes of land use and is not confined by the need of access to carbohydrate feedstocks.

Bioconversion of Carbon Dioxide to Biofuels by Facultatively Autotrophic Hydrogen Bacteria

F. Robert Tabita[1], *Stephanie A. Smith[2], and S. T. Yang[1]*

[1]The Ohio State University
[2]Battelle Memorial Institute, Columbus, Ohio

Our goal is to use hydrogen bacteria to convert carbon dioxide to infrastructure-compatible liquid biofuels, such as n-butanol, without the requirements of photosynthesis. For these studies, we will use organisms that can grow at the expense of hydrogen, oxygen, and carbon dioxide. We will accomplish our

goals through three major innovations supported by ARPA-E: genetic modifications of bacteria that assimilate carbon dioxide, oxygen, and hydrogen in the dark; development of an industrially scalable bioreactor system for sustainable production of biofuels with these organisms; and a novel approach to recovery of the biofuel (e.g., butanol) from the bioreactor. There will be three levels of advancement taken to accomplish these goal including innovations at the cellular level, systems level, and subsytems level. Hydrogen bacteria normally use carbon dioxide, oxygen, and hydrogen for growth and produce all their cellular constituents from these gases. However, we will short-circuit some of the normal cellular pathways so that the organisms will convert these gaseous inputs into desirable biofuels. While the synthesis of butanol serves as a useful model process, the various genetic modifications will enhance the efficiency of carbon dioxide bioconversion, so that the modified organisms could potentially support an industrial-grade process for many desired biofuels.

This process will potentially result in a significant and economically feasible process to produce biofuels so that the product will eventually out-compete ethanol with respect to price, and be comparable to gasoline. Furthermore, an innovative approach will be taken for generating the gaseous substrates, using a proprietary technology that converts waste biomass into carbon dioxide and hydrogen.

Engineering Bacteria to Absorb Electrons from an Electrode, Fix CO_2, and Synthesize a Biofuel

***Jeffrey Way*[1], *and Pamela Silver*[2]**
[1]Wyss Institute, Harvard University, Boston, MA
[2]Systems Biology, Harvard Medical School, Boston, MA

The premise of the electrofuels concept is similar to that of photosynthetically derived biofuels: electrons are used as chemical reducing equivalents to drive the reduction of carbon dioxide into a fuel molecule. However, the synthesizing organism must be a microbe that can pick up that can pick up electrons from an electrode, rather than generating them from a light-driven water-splitting reaction. We are taking a synthetic-biological approach to the electrofuel concept, with the expectation that the final organism must be significantly engineered and that it might be necessary to re-engineer every aspect of any electrofuel synthesis genetic elements.

One goal of our project is to develop genetic modules corresponding to electron uptake, CO_2 fixation, and biofuel synthesis. The essential elements of an electron uptake system have been modularized and transported into *E. coli* (Jensen et al., PNAS 2010), and modules for synthesis of biofuel genetic modules have been created by numerous groups. However, to the best of our knowledge no one has functionally introduced a complete carbon fixation pathway into a heterotrophic organism, so we will describe in some detail our strategy and progress toward this goal.

PRESENTATION INSTRUCTIONS

SPEAKERS

Speakers should plan to meet the session chair at least 15 minutes prior to the session. Please sit in the front of the room during your session.

Your presentation must be uploaded to the conference computer at least 30 minutes before the session. The presentation needs to be in either Power Point or PDF format. It is the job of the speakers and session chairs to ensure that all talks are ready for presentation. Speakers will have 15 minutes for their talk, including questions. Please help us remain on time.

POSTER PRESENTERS

Please set up your poster in the designated room on Monday afternoon, November 7th. Posters may be left up until Wednesday morning, November 9th.

IMPORTANT ADDRESSES

SBE staff will be available at the Registration Booth in the Prefunction Area outside of the Grand Ballroom at the following times:

Sunday, November 6: 12 pm - 2 pm
Monday, November 7: 7 am - 12 pm
Tuesday, November 8: 7 am - 11 am
Wednesday, November 9: 7 am - 3 pm

Following the conference, you may reach SBE's Technology Associate, Derek Lapiska, by email at derel@aiche.org or by phone at (646) 495-1381.

Optimizing Immobilized Enzyme Performance in Cell-Free Environments to Produce Liquid Fuels

Joseph Grimaldi, *Cynthia Collins and Georges Belfort, Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY*

Active research to produce energy via biofuel cells or through the bioconversion of sugars to liquid fuels offers exciting new alternates to fossil fuel. We focus here on the bioconversion route that uses enzymes to convert sugars to butanol, for example. While these enzymatic routes offer great promise and excellent selectivity for the production of biofuels, enzymes exhibit slow kinetics, display low volume capacity in solution and exhibit product feed back inhibition. These limitations have to be overcome so that biofuels can be produced in an economically viable fashion.

Our approach is quite different to most others in that we produce the requisite enzymes via rDNA technology with *E. coli*, and then use these enzymes *in vitro* coupled with pervaporation to produce and continuously remove the desired butanol, respectively. Initially, we are interested in immobilizing a model enzyme, beta-galactosidase, and determining its reaction rates adsorbed on or tethered to flat convex or concave surfaces (AIM 1). These experiments are currently in progress. We plan to next study the separate reactions of immobilized keto acid decarboxylase (KDC) and alcohol dehdrogenase (ADH) (i.e. the product of the first enzyme is the substrate for the second one) on the best two surfaces (Aims 1 & 2) to produce long-chain alcohols. In collaboration with a protein engineer, Dr. Cynthia Collins, Assistant Professor, Chemical and Biological Engineering, RPI, we have ordered the strain and DNA from NIZO of KDC, and plan to clone it into an *E. coli* expression vector with a His tag. ADH is available commercially.

Hydrogen-Driven Conversion of Carbon Dioxide to Liquid Electrofuels In Extremely Thermophilic Archaea: Metabolic Engineering of Pyrococcus Furiosus

Angeli Menon[1], *Ifeyinwa J. Iwuchukwu[1], Matthew Keller[1], Therese Leuko[1], Aaron S. Hawkins[2], Yejun Han[2], Hong Lian[2], Andrew J. Loder[2], Robert M. Kelly[2] and Michael W.W. Adams[1]*

[1]Biochemistry and Molecular Biology, University of Georgia, Athens, GA
[2]Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC

The goal of this project is to metabolically engineer an extremely thermophilic archaeon to directly use hydrogen for the conversion of carbon dioxide into C2, C3 and C4 compounds that can be used to generate biofuels. The selected host, *Pyrococcus furiosus*, is a genetically tractable, anaerobic hyperthermophile that produces hydrogen and captures low potential electrons to generate reducing power in the form of NADPH which can be used to drive CO_2 fixation. The CO_2 fixation pathway is the recently discovered novel 3-hydroxypropionate/4-hydroxybutyrate (3-HP/4-HB) pathway that uses NADPH as the reductant and is unique to thermophilic and hyperthermophilic archaea. The autotrophic CO_2 fixation pathway from the thermophilic archaeon, Metallospheara sedula has been selected for integration into the host genome. The CO_2 fixation pathway genes will be integrated into the chromosome of P. furiosus in three steps, each comprising the sequential enzymes required to make key intermediates within the pathway: sub-pathway 1 (SP1, 3-HP), sub-pathway 2 (SP2, 4-HB) and sub-pathway 3 (SP3, acetylCoA). To date we have engineered auxotrophic P. furiosus strains that contain the M. sedula 3-HP/4-HB SP1 gene module in their genomes. The strains differ in that transcription of SP1 is driven either by a highly expressed native constitutive promoter or an inducible promoter. We will discuss the characteristics of these strains, as well as the methodology that has been developed to date to measure and optimize the expression of the M. sedula 3-HP/4-HB SP1 enzymes and the production of 3-HP in *P. furiosus*.

Functional Analysis of the 3-Hydroxypropionate/4-Hydroxybutyrate CO_2 Fixation Cycle in the Extremely Thermoacidophilic Archaeon Metallosphaera Sedula

Yejun Han[1], *Aaron S. Hawkins[1], Hong Lian[1], Andrew J. Loder[1], Ifeyinwa J. Iwuchukwu[2], Matthew Keller[2], Therese Leuko[2], Angeli Menon[2], Michael W.W. Adams[2] and Robert M. Kelly[1]*

[1]Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC
[2]Biochemistry and Molecular Biology, University of Georgia, Athens, GA

The 3-hydroxypropionate/4-hydroxybutyrate (3HP/4HB) cycle is central to autotrophic carbon fixation by the extremely thermoacidophilic archaeon *Metallosphaera sedula*. Thirteen enzymes and three sub-pathways comprise this cycle, in which acetyl-CoA incorporates two molecules of inorganic carbon two yield two acetyl-CoA molecules. This cycle represents a potentially efficient route for biological conversion of CO_2 into liquid electrofuels and chemical intermediates important to the chemicals industry.

Most genes encoding the enzymes of 3HP/4HB cycle have been identified in the genome of M. sedula, except for 4-Hydroxybutyryl-CoA synthetase, although several candidates for this enzyme have been proposed through homology alignment and transcriptomic analysis of H_2/CO_2 autotrophy. Most genes of 3HP/4HB cycle were cloned from M. sedula and expressed in *E. coli* through a variety of strategies. The biochemical properties of the recombinant enzymes were further characterized at 37ºC and 75ºC. In addition, the sub-pathway 1 (SP1), sub-pathway 2 (SP2), and other parts of 3HP/4HB cycle were re-constituted *in vitro* with recombinant enzymes. For example, using two previously uncharacterized enzymes, methylmalonyl-CoA epimerase and methylmalonyl-CoA mutase, (R)-Methylmalonyl-CoA could be converted to succinyl-CoA by two successive catalytic reactions. A key intermediate, 3-hydroxypropionate, was formed from malonyl-CoA by malonyl-CoA reductase and malonate semialdehyde reductase. The *in vitro* pathway for producing 3-hydroxypropionate (3-HP) from CO_2 and acetyl-CoA, and producing 4-hydroxybutyrate (4-HB) from 3-HP using recombinant enzymes of SP1 and SP2 are being re-constituted *in vivo* and *in vitro*. Furthermore, the complete pathway will be optimized *in vitro*, based on kinetic parameters and biochemical properties of recombinant enzymes.

Tethering Hydrogen-Producing Catalysts to the Outer Membrane of Biofuel-Producing *Ralstonia Eutropha* H16

Yi-Chun Yeh[1], *Jonah Jurss[2], Jana Mueller[1], Harry R. Beller[1], Christopher Chang[2], Steven Singer[1] and Swapnil Chhabra[1]*

[1]Lawrence Berkeley National Laboratory
[2]University of California-Berkeley

We are developing an integrated Microbial-Electro Catalytic (MEC) system consisting of *Ralstonia eutropha* for metabolic engineering coupled to a small-molecule electrocatalyst for the production of biofuels from CO_2 and electrogenic H_2. Wild-type *R. eutropha* can grow heterotrophically on organic substrates or chemolithoautotrophically on CO_2/H_2 under aerobic conditions. Upon nutrient limitation, *R. eutropha* directs a large portion of its reduced carbon flux toward the synthesis of polyhydroxybutyrate (PHB). Using synthetic biology approaches, we are developing heterologous metabolic pathways in *R. eutropha* for the production

of biofuels. Additionally, we aim to employ a Co-polypyridine complex that, with electrical input, converts water to H_2 at high rates for microbial growth and biofuel production. To enable site-specific labeling of the cell surface, we engineered target proteins with a genetically encoded short peptide with a reactive functional group that serves as an efficient chemical handle for labeling applications. We were able to demonstrate the translocation of the target protein to the *R. eutropha* cell surface via an autotransporter protein and are now optimizing the conditions for surface tethering of the catalyst.

Metabolic Engineering of *Ralstonia Eutropha* H16 for Hydrocarbon Production From CO_2

Jana Mueller, *Yi-Chun Yeh, Swapnil Chhabra, Steven W. Singer and Harry R. Beller, Lawrence Berkeley National Laboratory*

R. eutropha H16 is a model for facultative chemolithoautotrophic bacteria able to grow with organic substrates or H_2/CO_2 under aerobic conditions. When experiencing nutrient limitation, *R. eutropha* H16 directs most of the reduced carbon flux to synthesis of polyhydroxybutyrate (PHB), a biopolymeric compound stored in granules. Diverting this substantial metabolic flux may be a promising method to produce biofuels at high titers from biomass substrates (sugars, organic acids) or H_2/CO_2. Therefore, we deleted genes required for PHB synthesis in *R. eutropha* and inserted heterologous pathways to produce hydrocarbon biofuels. We constructed defined *R. eutropha* mutants that produced no PHB when grown with organic substrates under nutrient-limited conditions, but produced large amounts of pyruvate, which was excreted into the medium. We also constructed expression vectors containing heterologous genes for hydrocarbon production and transformed these vectors into *R. eutropha* wild-type and PHB- strains. Target hydrocarbons were detected by GC/MS analysis when these strains were grown under heterotrophic conditions. Current efforts are directed to increase the titer of hydrocarbon production by manipulating *R. eutropha* fatty acid biosynthesis and by improving heterologous protein expression. Optimized conditions are also being developed for autotrophic production of hydrocarbons by engineered *R. eutropha* strains.

Genome-Scale Modeling of Microbial Electrosynthesis for Electrofuel Production

Harish Nagarajan [1], *Juan Nogales* [2], *Merve Sahin* [2], *Ali Ebrahim* [2], *Derek R. Lovley* [3], *Bernhard O. Palsson* [4], *Adam M. Feist* [5] *and Karsten Zengler* [5]

[1] Bioinformatics and Systems Biology, University of California - San Diego, La Jolla, CA
[2] Bioengineering, University of California - San Diego
[3] Department of Microbiology, University of Massachusetts, Amherst, Amherst, MA
[4] Bioengineering, UCSD, La Jolla, CA
[5] Bioengineering, University of California - San Diego, La Jolla, CA

A novel mechanism, known as microbial electrosynthesis, in which microorganisms directly use electric current to reduce carbon dioxide to multi-carbon organic compounds that are excreted from the cells into extracellular medium, has recently been discovered. Microbial electrosynthesis differs significantly from photosynthesis in that carbon and electron flow is primarily directed to the formation of extracellular products, rather than biomass. However, extensive knowledge about the metabolism of the organism as well as its extracellular electron transfer pathways is critical to realize the potential of this technology for the production of the desired fuel compound. So far, only a few acetogenic microorganisms have been shown to be capable of accepting electrons from the cathode to reduce carbon dioxide to limited organic compounds such as acetate and 2-oxobutyrate. Constraint-based metabolic modeling and analysis has been useful for discovering and understanding new capabilities and content in bacteria, as well as in guiding metabolic engineering efforts for targeted production.

In this study, we present the application of this constraint-based modeling technique on an electrosynthetic bacterium, *Clostridium ljungdahlii*, to characterize the process of electrosynthesis for autotrophic synthesis of multi-carbon organic compounds such as butanol. Following the established protocol, we have reconstructed the genome-scale metabolic network of an electrosynthetic organism that comprises of 687 metabolic reactions encoded by 604 genes. This reconstruction captures all the major central metabolic, amino acid, nucleotide and lipid biosynthetic pathways as well as the pathways for the synthesis of major cofactors and vitamins. More importantly, the key carbon dioxide fixation pathway in *C. ljungdahlii* (Wood-Ljungdahl pathway) and energy conservation pathways have been reconstructed in detail. Importantly, this reconstruction represents one of the first detailed descriptions of key electrosynthesis pathways. We have identified key components that are potentially involved in extracellular electron transfer in *C. ljungdahlii*. The genome-scale model is interrogated using established computational approaches and will be validated based on physiological data under different growth conditions. We will further employ *in silico* strain-design tools on the validated metabolic model in order to optimize butanol production under electrosynthetic conditions.

In summary, this study presents the genome-scale metabolic network and extensive metabolic characterization of the various growth phenotypes of an electrosynthetic organism, *C. ljungdahlii*. This also represents the first metabolic network of a homoacetogen. We discuss the potential of this network to serve as a strain-design platform for optimizing microbial electrosynthesis.

Microbial Electrosynthesis: Metabolic Engineering, Adaptive Evolution, and System Optimization

Kelly P. Nevin, *Ching Leang, Toshiyuki Ueki, Pier-Luc Tremblay and Derek R. Lovley, Microbiology; University of Massachusetts Amherst*

Microbial electrosynthesis is the process in which microorganisms are provided with electrons at an electrode surface to promote the reduction of carbon dioxide to multi-carbon compounds, such as fuels and other organic commodities. Previous studies in our laboratory demonstrated that a diversity of acetogenic microorganisms were capable of producing acetate from carbon dioxide with electrons derived from electrodes at high columbic and energetic efficiencies. The purpose of the studies summarized here was to enhance the rate of microbial electrosynthesis and to metabolically engineer strains that could produce the transportation fuel butanol.

Of all the strains evaluated, *Sporomusa ovata* was the acetogen most effective in electrically driven acetate production. In order to enhance this process, selective pressure for more rapid acetate production was placed on this organism. To date, an 18-fold increase in acetate production rates has been achieved. Genome resequencing is underway to determine which mutations are associated with enhanced acetate production rates. Furthermore, modifying reactor conditions led to further increases in acetate production rates.

In order to evaluate the possibility of directly producing transportation fuels with microbial electrosynthesis, studies have been conducted with two other genera for which it was considered

that genetic systems could readily be established. *Clostridium ljungdahlii* is naturally effective in electrosynthesis and heterologous expression of genes for butanol production in *C. ljungdahlii* was previously reported. We significantly increased transformation rates *00* and produced the first gene knockouts in this organism. In order to produce butanol at high rates, genes for the enzymes necessary to convert acetyl-CoA, the key intermediate in the Wood-Ljungdahl pathway, are being incorporated into the chromosome with a gene knock-in method that we developed. Furthermore, we have deleted the genes that catalyze alternative pathways for acetyl-CoA metabolism to further direct electrons toward butanol production.

A surprising finding was that Geobacter metallireducens, which is not an acetogen, was also capable of electrosynthesis. The pathway for carbon dioxide reduction is different than that for *C. ljungdahlii* and appears to proceed via the reverse TCA cycle. Genes for butanol production were detected in *G. metallireducens* and slow rates of butanol production were possible with wild-type cells. In order to enhance rates of butanol production a genetic system for markerless gene deletion was developed. This method is now being employed to block pathways that compete with butanol synthesis. For example, formate was a significant electrosynthesis product in wild-type cells and thus the formate dehydrogenase is being deleted to prevent formate production.

To date microbial electrosynthesis has only been attempted in laboratory-scale reactors with volumes of no more than 1 liter. Evaluation of several novel reactor designs that should be amenable to scale up to industrial scale is underway.

Enhancement of H_2 and CO_2 Uptake for the Production of Hydrocarbon Fuels in Cupriavidus Necator

Pin-Ching Maness, *Carrie Eckert, Ryan Sullivan, Grant Balzer and Jianping Yu, Biosciences Center, National Renewable Energy Laboratory, Golden, CO*

The facultative chemolithotrophic bacterium *Cupriavidus necator* (also known as *Ralstonia eutropha*) is able to grow autotrophically on a mixture of hydrogen, carbon dioxide, and oxygen, as well as heterotrophically on various organic carbon sources. Metabolic engineering in this microbe to produce a hydrocarbon-based electrofuel from H_2 and CO_2 has immense potential in decreasing the country's demand for oil. The hydrocarbon produced can be used directly as a blending stock in the existing petroleum-based infrastructure or as feedstock in a catalysis process leading to jet fuels. *Cupriavidus necator* recruits three hydrogenases to accomplish H_2 metabolism, namely the membrane-bound (MBH), soluble (SH) and regulatory hydrogenase (RH). Collectively these enzymes oxidize H_2 to generate NAD(P)H and ATP, both of which are required to carry out CO_2 fixation through the Calvin-Benson-Bassham (CBB) cycle. As such, optimization of efficient H_2 and CO_2 uptake is the main goal of this research, supported by the DOE ARPA-E Electrofuels Program. Directed approaches to improve H_2 and CO_2 uptake and their utilization focused on two strategies: (1) hydrogenase over-expression by introducing additional copies of genes encoding MBH, SH, and their maturation machinery; and (2) optimizing transcriptional regulations to afford over-expression of Ribulose-1, 5-bisphosphate carboxylase/oxygenase (RubisCO) protein involved in the CBB pathway. In the first effort, we discovered that the RH is naturally mutated in the *C. necator* parental strain to afford constitutive expression of both MBH and SH under carbon-limited growth conditions. Coupling this with the transformation of additional copies of these hydrogenases has resulted in over-expression of MBH and SH, leading to a three-fold improvement in H_2-uptake activity, *in vitro*. The enhanced hydrogenase activity is corroborated by higher protein expression based on Western blots. To enhance RubisCO activity, initial efforts aimed at manipulating the promoter and terminator regions in the cbb operon, leading to a six-fold improvement in RubisCO activity over wild-type, *in vitro*. Integration of both H_2 and CO_2 uptake enhancements into a single production strain has been completed. Work is underway to determine both *in vitro* and in situ H_2 and CO_2 uptake enhancement in the engineered strains, with the overarching goal of providing abundant energy and reducing equivalents towards the economic production of hydrocarbon fuels in *C. necator*, with the latter carried out by OPXBIO (Boulder, CO).

Marine Sediment Seeded Biocathodes for Gas Phase CO_2 Removal

John M. Pisciotta[1], *Wayne Curtis[2], and Bruce E. Logan[1]*

[1]Civil and Environmental Engineering, Pennsylvania State University, University Park, PA
[2]Chemical Engineering, The Pennsylvania State University, University Park, PA

Electron accepting biocathodes were produced by establishing Microbial Fuel Cell (MFC) bioanodes in marine sediments, then electrically inverting the carbon brush electrodes into cathode working electrodes of dual chamber, Microbial Electrolysis Cells (MECs). Linear sweep voltammetry (LSV) and long term potentiostatic measurements documented patterns of electron uptake at negative applied potentials below -550 mV (versus Ag/AgCl) in 50 mM PBS lacking organic sources of energy or carbon. Sediment electrode biofilms established under unpoised MFC electrode potentials were readily inverted into electron-accepting MEC biocathodes. Conversely, sediment electrode biofilms established under negatively poised conditions (-200 mV versus Ag/AgCl), like sterile control electrodes, demonstrated minimal uptake of electrons when transferred as MEC cathodes. Gas chromatography (GC) analysis revealed a significant, time dependent reduction in cathode chamber headspace CO_2 only with biocathodes originally established under unpoised MFC conditions. Similarly, abundant cathode chamber growth, as revealed by an increase in turbidity and presence of bacteria upon microscopic examination, was evident for the groups established under the unpoised MFC conditions. Bacterial colonies were successfully isolated from biocathodes using a salt amended PBS agar incubated in an atmosphere containing CO_2 + H_2. Bacterial colonies did not grow on this substrate in the presence of H_2 alone. Periodic GC measurements of cathode chamber headspace detected changing concentrations of hydrogen and methane in the unpoised groups which may be indicative of microbial metabolism under the electrotrophic culture conditions administered. Sterile transfer and inoculation of 1:5 diluted catholyte into sterile control MEC reactors reproducibly induced electron uptake on graphite, carbon brush or carbon rod cathode substrates following a roughly 4 day lag period. Ongoing efforts, including analysis of 16s clone libraries, aim to better characterizing the microorganisms thus enriched and isolated. This study provides a simple and effective method for enriching electrotrophic bacteria on electrodes for the purpose of gas phase CO_2 removal with possible conversion to useful product.

Characterization and Modification of Enzymes in the 2-Ketoisovalerate Biosynthesis Pathway of *Ralstonia Eutropha* H16

***Jingnan Lu*[1], *Christopher Brigham*[2], *Claudia Gai*[2] and *Anthony J. Sinskey*[2,3,4]**

[1]Chemistry, MIT, Cambridge, MA
[2]Biology, MIT, Cambridge, MA
[3]Biology and Health Sciences and Technology, MIT, Cambridge, MA
[4]4Engineering Systems Division, MIT, Cambridge, MA

2-Ketoisovalerate is an important cellular intermediate for the synthesis of branched-chain amino acids, pantothenate, and various other cellular building blocks and cofactors. This ketoacid not only has a crucial role for the microorganism that produces it, but it can also be used as a precursor molecule for isobutanol or 3-methyl-1-butanol biofuel, pharmaceuticals, and flavor agents. In the metabolically versatile betaproteobacterium *Ralstonia eutropha*, the synthesis of 2-ketoisovalerate from pyruvate is carried out by acetohydroxyacid synthase, acetohydroxyacid isomeroreductase, and dihydroxyacid dehydratase. All three enzymes are regulated though their substrate specificity and also by feedback inhibition. Among them, acetohydroxyacid synthase serves as a gate-keeper for 2-ketoisvalerate biosynthesis, thus demonstrated the tightest regulation. Here we characterized the kinetic parameters and inhibition concentration of each enzyme. We also modified the active site and regulatory subunit of the acetohydroxyacid synthase enzyme in order to bias substrate specificity towards pyruvate and diminish feedback inhibition by valine, both of which increased 2-ketoisovalerate production.

Carbonic Anhydrases: Key Enzymes in CO_2 Metabolism in *Ralstonia Eutropha*

Christopher Brigham[1], *Jingnan Lu*[2], *Anthony J. Sinskey*[1,3,4] and *Claudia S. Gai*[1]

[1]Biology, MIT, Cambridge, MA
[2]Chemistry, MIT, Cambridge, MA
[3]Biology and Health Sciences and Technology, MIT, Cambridge, MA
[4]4Engineering Systems Division, MIT, Cambridge, MA

Ralstonia eutropha is a betaproteobacterium capable of lithoautotrophic growth using H_2 as energy source and CO_2 as the sole carbon source through the Calvin Benson Bassham (CBB) cycle. Carbonic Anhydrases (CAs) are important enzymes in CO_2 metabolism as they catalyze the rapid interconversion between CO_2 and bicarbonate. The understanding of the role of these enzymes on CO_2 assimilation by the bacteria could help in the development of industrial processes, such as biofuel or bioplastic production from carbon dioxide. Based on the genome sequence, four CAs were identified in *R. eutropha* strain H16: H16_A1192, can, can2 and caa. To study these enzymes, each CA was separately overexpressed in *R. eutropha* and activity in the cell extracts was tested using $KHCO_3$ and CO_2 as substrates. Furthermore, recombinant CAs were produced in *E. coli*, purified and biochemically characterized. The high activity measured using CO_2 as a substrate indicate that the Caa enzyme is a suitable target for further study on the potential biotechnological application of *R. eutropha*.

Role of the Photosynthetic Electron Transfer Chain in Electrogenic Activity of Cyanobacteria

John Pisciotta, *Yongjin Zou and Ilia Baskakov, Center for Biomedical Engineering and Technology, University of Maryland, Baltimore, MD*

Certain anaerobic bacteria, termed electrogens, produce an electric current when electrons from oxidized organic molecules are deposited to extracellular metal oxide acceptors. In these heterotrophic "metal breathers", the respiratory electron transport chain (R-ETC) works in concert with membrane-bound cytochrome oxidases to transfer electrons to the extracellular acceptors. The diversity of bacteria able to generate an electric current appears more widespread than previously thought, and aerobic phototrophs, including cyanobacteria, possess electrogenic activity. However, unlike heterotrophs, cyanobacteria electrogenic activity is light dependent, which suggests that a novel pathway could exist. To elucidate the electrogenic mechanism of cyanobacteria, the current studies used site-specific inhibitors to target components of the photosynthetic electron transport chain (P-ETC) and cytochrome oxidases. Here, we show that (1) P-ETC and, particularly, water photolysed by photosystem II (PSII) is the source of electrons discharged to the environment by illuminated cyanobacteria, and (2) water-derived electrons are transmitted from PSII to extracellular electron acceptors via plastoquinone and cytochrome bd quinol oxidase. Two cyanobacterial genera (Lyngbya and Nostoc) displayed very similar electrogenic responses when treated with P-ETC site-specific inhibitors, suggesting a conserved electrogenic pathway. We propose that in cyanobacteria, electrogenic activity may represent a form of overflow metabolism to protect cells under high-intensity light. This study offers insight into electron transfer between phototrophic microorganisms and the environment and expands our knowledge into biologically based mechanisms for harnessing solar energy.

Biocatalytic Coating Enabled Advanced Reactor Designs for Direct Solar Fuels and Electrofuels

Jimmy Gosse, Stefan Thust, Thomas Evans, Thomas Harwood and ***Marc von Keitz,*** *BioCee, Inc., Saint Paul, MN*

For biological direct solar fuels and electrofuels to make a relevant contribution to the world's supply of renewable fuels and chemicals, it will be important to engineer highly productive microorganisms. Advanced microbial strains alone, however, will not be sufficient to ensure economically viable production of these fuels. These organisms also have to be deployed in a cost-effective, and scalable reactor system.

Since all direct solar and electrofuel processes utilize carbon dioxide as a carbon source, a suitable reactor design needs to maximize CO_2 mass transfer to the microbial catalyst. To address this issue, BioCee has developed several multi-phase reactor designs based on its proprietary biocatalytic coating technology. Biocatalytic coatings are biocomposite materials, in which living microorganisms are immobilized in thin, nano-structured polymeric matrices. In these reactor designs, we position biocatalysts at the phase interphase of continuous flow, multi-phase processes, specifically gas-liquid processes (e.g. phototrophic CO_2 sequestration and syngas fermentation) and liquid-liquid processes (e.g. biological desulfurization of petroleum). In this way, substrates from both phases can be supplied to the immobilized cells at high rates of mass transfer, while keeping the bulk phases completely separated, thus simplifying the overall process flow diagram.

In this presentation, we will show several different recent configurations and performance data of biocatalytic coating reactors for some of our current applications.

The Electrocatalytic Oxidization and Reduction of Biomass-derived Oxygenates in a PEM Reactor

Sara K. Green[1], *Geoffrey A. Tompsett[1], Hyung Ju Kim[2], Won Bae Kim[2], George W. Huber[1,]*

[1]Department of Chemical Engineering, University of Massachusetts, 686 N. Pleasant St., Amherst, MA 01003 (USA), Fax: (+1) 413-545-1647, E-mail: huber@ecs.umass.edu

[2]Department of Materials Science and Engineering, Gwangju Institute of Science and Technology (GIST), 261 Cheodan-gwagiro, Buf-gu, Gwangju 500-712, (South Korea), Fax: +82 62 970 2304, E-mail: wbkim@gist.ac.kr

Proton electrolyte membrane (PEM) fuel cells have been developed in the last 20 years to be highly efficient methods for producing electricity from oxygenated compounds. In this presentation, we will demonstrate how PEM technology can be used to produce different fuels and chemicals from biomass-derived oxygenates. Electrochemical oxidation at the anode can be used to selectively oxidize polyols into aldehydes and ketones in the aqueous phase. Glycerol can selectively be oxidized to glyceraldehyde in selectivies above 90%. Biomass-derived molecules can also be reduced in an electrocatalytic PEM reactor, by feeding the oxygenated molecules to the cathode. Protons needed for the reduction can be produced from the electrolysis of water or from hydrogen gas dissociation on the anode side of the reactor. Acetone can be electrocatalytically reduced to isopropyl alcohol (IPA) using both of these proton sources. We will demonstrate how the fuel cell performance changes with acetone concentration, temperature, applied voltage, and pressure.

Nature-Inspired Gas Distributor Design with Application to Low-Temperature PEM Fuel Cells

Jeffrey A. Marquis, *Marc-Olivier Coppens*

This work investigates a novel design for a low-temperature proton exchange membrane fuel cell (PEMFC) flow field[1]. Typical, existing flow field designs suffer from the same fundamental deficiency, namely the non-uniform distribution of reactant gases over the surface of the catalytically active area. This leads to inefficient usage of the expensive, platinum-based catalyst material within the fuel cell.

The proposed, new design is inspired by the architecture of the human lung, which has been shown to be an extremely efficient gas transport network as a result of displaying equipartition of entropy production[2, 3]. Furthermore, the fractal, self-similar geometry of the human lung is capable of performing two other key tasks: evenly distributing oxygen throughout the lung, and slowing down the gas velocity, so that, when the oxygen reaches the alveoli, the oxygen's convective flux is equal to its diffusive flux. These two properties are highly desirable in a fuel cell gas distributor as well.

By evenly distributing reactant gases over the surface of the catalytically active material all the platinum catalyst can be efficiently used. As a result, less platinum can be used to achieve the same power output. The novel gas distributor design is guided by the geometry of the bronchial tree. It has one inlet and 4^n outlets, where n is the number of branching generations. Similar fractal geometries have been used in applications ranging from fluidization and irrigation to electronics cooling[4-6]. A variety of product (liquid water, excess air) removal flow paths are also being studied, including a second fractal network, as well as more conventional serpentine and parallel flow fields.

Simulations of this gas distributor geometry have been conducted using COMSOL Multiphysics 3.5a. The results of these simulations have shown that 8 branching generations are needed to achieve an equality of convective and diffusive fluxes at the outlet of the gas distributor when operating at standard conditions. However, as few as 4 generations, in conjunction with a typical gas diffusion layer will provide a nearly uniform distribution of reactant gases over the catalytically active surface.

As a result of these simulations a prototype design of the fractal gas distributor has been generated using the computer-aided-design package NX 7.5. This design has been successfully prototyped by using a high resolution sterolithography machine. These gas distributors are assembled along with other necessary fuel cell components to form a test cell. The performance of this gas distributor is tested using the fuel cell test equipments available within the Center for Automation Technologies & Systems (CATS) at Rensselaer. Key performance characteristics, including average current density, average power output, and platinum usage, will be discussed, and compared to systems that utilize more conventional flow field geometries (serpentine, parallel, and parallel-serpentine).

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Solar Fuels and Fertilizer and Their Link to Sustainable Biomass Production and Conversion

Ronald Michalsky, Peter H. Pfromm, Department of Chemical Engineering, Kansas State University, Manhattan, Kansas, USA

Over 100 million metric tons ammonia produced worldwide per year mainly for fertilizers ensure the food supply for a growing world population and may allow for increased biomass production for biofuels in the near future.

Industrially the Haber-Bosch process synthesizes NH3 catalytically at high pressure and elevated temperature from its elements consuming 5% of all natural gas produced annually, with significant fossil-based CO_2 emissions.

Alternative solar thermochemical NH_3 synthesis from steam and nitrogen produces a sustainable solar fuel and avoids the storage problem for H_2. Production of metallic nitrides by reduction of their metal oxides is a high-temperature and energy-intensive process that may take advantage of concentrated solar radiation as inexpensive and sustainable source for process heat. This work presents a solar thermochemical NH_3 synthesis process sequence of nitride hydrolysis splitting H_2O and absorbing protons released in the formation of NH_3 at ambient pressure, and endothermic metal oxide reduction and nitridation driven by concentrated solar energy.

Experimental data focusing protonation of the nitrogen ions in the solid state to liberate NH_3 via steam hydrolysis of various ionic, covalent, intermediate and interstitial nitrides are presented. A characteristic kinetic parameter such as the solid state diffusion constant will be correlated with the nitride ionicity to discuss the relation between ionic size at the atomic scale to the reaction kinetics in the bulk material.

Analogous synthesis of acetylene by protonation of carbon ions will be contemplated.

Genome-wide Screen of Gene Over-expression Targets for the Development of Acetate and Ethanol Resistance in *Saccharomyces cerevisiae*

Pedro V. Peña[1], Steven Glasker[2], John Hundertmark[2], Jared Cohen[2], and Friedrich Srienc[1,2]

[1]Biotechnology Institute, University of Minnesota, St. Paul, MN 55108
[2]Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN 55455

Bioethanol production from cellulosic biomass is projected to significantly reduce the environmental impact over traditional methods of producing this biofuel. However, microbial growth inhibition by compounds in the biomass hydrolysate and by the desired product itself severely compromises efficiency. Acetate in the biomass hydrolysate, for instance, is present at growth inhibitory concentrations. Moreover, inhibition of growth is intensified by the presence of increasing concentrations of the produced bioethanol. Thus, engineering of resistance to growth inhibitors is essential to improve bioethanol yields, while decreasing the need to detoxify the cellulosic feedstock. Here, we apply the Cytostat cell culture technique, developed in our lab, to screen for acetate and ethanol resistance phenotypes in *S. cerevisiae* cells transformed with a genome-wide, gene over-expression library. The Cytostat selects for inhibitor specific resistance because the continuous culture is maintained at very low cell densities, and thus the selective pressure remains constant. Further, the Cytostat rapidly selects for the fittest, most resistant clone. Using this approach, an acetic acid resistant clone, with nearly double the growth rate of the WT strain in minimal medium supplemented with 40 mM acetic acid, was rapidly enriched from a pool of cells transformed with the library. Similarly, ethanol resistant transformants were isolated, with 40% and 300% improved growth rates over the WT in minimal medium supplemented with either 2.5% or 5% v/v ethanol, respectively. The identification of the over-expressed genes and their roles in the induction of the resistance phenotypes will be discussed.

Revised Molecular Basis of the Promiscuous Carboxylic Acid Perhydrolase Activity in Serine Hydrolases

Tyler Yin, *University of Minnesota, Department of Biochemistry, Molecular Biology & Biophysics and The BioTechnology Institute, 1479 Gortner Avenue, Saint Paul, MN 55108, USA, Fax: (+1)-612-625-5780*

Carboxylic acid perhydrolases catalyze a substitution in carboxylic acids with hydrogen peroxide to form peroxycarboxylic acids. Peroxycarboxylic acids have been shown to degrade lignocellulose to for biofuels production (Yin *et* al. *Bioresour. Technol.* **2011,** *102,* 5183-92). The five x-ray crystal structures of carboxylic acid perhydrolases all show a proline residue in the oxyanion loop, which moves a main chain carbonyl oxygen closer to the active site. Previously, we hypothesized that the closer carbonyl group increases the selectivity of the enzyme for hydrogen peroxide over water (Yin *et* al. *Biochemistry* **2010**, *49,* 1931-42). In this paper, we test this hypothesis, focusing on L29P PFE, which is a 43-fold faster perhydrolase for acetic acid that wild type PFE, and show that this hypothesis is incorrect. First, L29P PFE catalyzes hydrolysis of methyl acetate faster (k_{cat}/K_m = 200 s^{-1} M^{-1}) than perhydrolysis of methyl acetate ((k_{cat}/K_m = 20 s^{-1} M^{-1}), suggesting lower selectivity for hydrogen peroxide. Second, wild type PFE is already highly selective for hydrogen peroxide over water (β_0 = 430 M^{-1}), but selectivity decreases slightly for L29P PFE (β_0 = 220 M^{-1}). This decrease is opposite to the prediction from the hypothesis. Third, the rate of acetyl enzyme formation measured by ^{18}O-water exchange into acetic acid was 26-fold faster in L29P PFE (62 U/mg) than in wild type PFE (2.4 U/mg), which is similar to the 43-fold faster perhydrolysis in L29P PFE. Thus, carboxylic acid perhydrolases increase the formation of the acetyl-enzyme intermediate. Molecular modeling of the first tetrahedral intermediate (T_d1) supports a role for the main chain carbonyl which forms a hydrogen bond with a water molecule.

In vitro Reconstitution and Steady-state Analysis of the Fatty Acid Synthase from *Escherichia coli*

Xingye Yua[1], Tiangang Liu[2,3], Fayin Zhu[3], and Chaitan Khosla[1,2]

[1]Department of Chemical Engineering, Stanford University, Stanford CA 94305
[2]Department of Chemistry, Stanford University, Stanford CA 94305
[3]Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (Wuhan University), Ministry of Education, and Wuhan University School of Pharmaceutical Sciences, Wuhan 430071, P. R. China

Microbial fatty acid derivatives are emerging as promising alternatives to fossil fuel derived transportation fuels. Among bacterial fatty acid synthases (FAS), the *E. coli* FAS is perhaps the most well studied, but little is known about its steady-state kinetic behavior. Here we describe the reconstitution of *E. coli* FAS using purified protein components and report detailed kinetic analysis of the reconstituted system. When all ketosynthases are present at 1 mM, the maximum rate of free fatty acid synthesis of the FAS exceeded 100 μM/min. The steady-state turnover frequency was not significantly inhibited at high concentrations of any substrate or cofactor. FAS activity was saturated with respect to most individual protein components, when their concentrations exceeded 1 mM. The exceptions were FabI and FabZ, which increased FAS activity up to concentrations of 10 mM; FabH and FabF, which decreased FAS activity at concentrations higher than 1 mM; and *holo*-ACP and TesA, which gave maximum FAS activity at 30 mM concentrations. Analysis of the S36T mutant of the ACP revealed that the unusual dependence of FAS activity on holo-ACP concentration was due, at least in part, to the acyl-phosphopantetheine moiety. MALDI-TOF mass spectrometry analysis of the reaction mixture further revealed medium and long chain fatty acyl-ACP intermediates as predominant ACP species. We speculate that one or more of such intermediates are key allosteric regulators of FAS turnover. Our findings provide a new basis for assessing the scope and limitations of using *E. coli* as a biocatalyst for the production of diesel-like fuels.

Bio-based Redox Capacitor to Transfer Biochemical Energy

Eunkyoung Kim, *Yi Liu, William E. Bentley and Gregory F. Payne, Institute for Bioscience and Biotechnology Research and Fischell Department of Bioengineering, 5115 Plant Sciences Building, University of Maryland, College Park, MD 20742, USA*

Electronic devices process information and transduce energy with electrons, while biology performs such operations with ions and chemicals. To establish the connectivity between biology and electronics, we biofabricate a redox-capacitor by modifying a polysaccharide (i.e., chitosan) with a redox-active catechol. We report that these films are rapidly and repeatedly charged and discharged electrochemically via a redox-cycling mechanism in which mediators shuttle electrons between the electrode and film (capacitance ≈ 40 F/g or 2.9 F/cm^2). Further, charging and discharging can be executed under bio-relevant conditions. Enzymatic-charging is achieved by electron-transfer from glucose to the film via an NADPH-mediated redox-cycling mechanism. Discharging occurs by electron-donation to O_2 to generate H_2O_2 that serves as substrate for peroxidase-mediated biochemical reactions. Thus, these films offer the capability of inter-converting electrochemical and biochemical inputs/outputs. Among potential applications, we

anticipate that catechol-chitosan redox-capacitor films could serve as circuit elements for molecular logic operations or for transducing bio-based chemical energy into electricity.

Lumped Hybrid Cybernetic Model (L-HCM): A Potential Tool for Electrofuels Research

Hyun-Seob Song *and Doraiswami Ramkrishna,*
School of Chemical Engineering, Purdue University, West Lafayette, IN 47907, USA

Current biomass-based technologies for producing biofuels rely on carbohydrates derived from plants as the source of reducing power. A new paradigm offered from electrofuels research employs a much more efficient way to produce liquid fuels directly from sunlight and carbon dioxide by harnessing the energy of low-potential electrons. The key to success is to develop microorganisms which are able to extract energy from hydrogen, electricity or other energy-carrier materials and produce liquid fuels in an efficient way. Metabolic models will serve as a useful tool for achieving this purpose. In this poster, we present a dynamic metabolic modeling framework referred to as Lumped Hybrid Cybernetic Model (L-HCM) and seek a possible link to electrofuels research as a potential tool. In general, dynamic metabolic models are burdened with excessive number of parameters most of which are difficult to identify from experiments. This over-parameterization is avoided in L-HCM introducing a concept of rational lumping, which notably reduces the number of parameters without loss of critical elements of metabolism (Song and Ramkrishna, 2010, 2011a). With this feature, the current approach enables incorporation of sufficiently large size networks (such as genome scale networks) into dynamic metabolic modeling framework without extensive data. L-HCMs are shown to accurately predict dynamic behaviors of both mutants (Song and Ramkrishna, 2011b) as well as wild-type strains, consequently offering unprecedented promise for model-driven metabolic engineering.

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