



First Annual CREATE-IGERT Symposium



**Thursday, October 16, 2008
UC Davis ARC Ballroom**



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CREATE-IGERT Distinguished Lecture and Symposium Schedule

8:00-8:30am	Registration & Coffee
8:30-9:00am	Welcome, Introductions and Overview of the CREATE-IGERT Training Program (Prof. Karen McDonald, CREATE-IGERT Director)
<i>Session 1: Biofuels & Biorefineries</i>	
9:00-9:30pm	Prof. Pamela Ronald (5min) & Dawn Chiniquy (20min+5min questions)
9:30-10:00pm	Prof. Katy Dehesh (5min) & Elenor Castillo (20min+5min questions)
10:00-10:15pm	Coffee Break
10:15-10:45am	Prof. Karen McDonald (5min) & Ben Lindenmuth (20min+5min questions)
10:45-11:10am	Industry Presentation (25min)
<i>Session 2: Environmental Sustainability</i>	
11:10-11:40am	Prof. Clark Lagarias (5min) & Timothy Butterfield (20min+5min questions)
11:40-12:05pm	Prof. C. S. Prakash, Tuskegee University (20 min + 5 min Q/A)
12:05-12:30pm	Industry Presentation or Dalya Lateef, Tuskegee University (25min)
12:30-1:30pm	Lunch
<i>Session 3: Plant-Made Products</i>	
1:30-2:00pm	Prof. Jean VanderGheynst (5min) & Chris Simmons (20min+5min questions)
2:00-2:30pm	Prof. Satya Dandekar (5min) & Tiffany Glavan (20min+5min questions)
2:30-3:00pm	Industry Presentation (30min)
<i>Distinguished Lecture</i>	
3:00pm	Speaker Introduction—Prof. Abhaya Dandekar, Dept of Plant Sciences
3:05-4:00pm	Dr. Chris Somerville, EBI (50min+5min questions)
<i>Poster Session</i>	
4:00-5:00pm	Afternoon Poster Session (Organized by Focus Area) & Snacks
5:00pm	Closing Remarks—Prof. Karen McDonald

**IGERT: Collaborative Research and Education in
Agricultural Technologies and Engineering (CREATE)**
(NSF Award DGE0653984)



UC Davis P.I.s & Co P.I.s

Karen McDonald, Principal Investigator – UC Davis
Abhaya Dandekar, Co-Principal Investigator – UC Davis
Martina Newell-McGloughlin, Co- Principal Investigator – UC Davis
Pamela Ronald, Co-Principal Investigator – UC Davis
Jean VanderGheynst, Co-Principal Investigator – UC Davis
Denneal Jamison-McClung, Program Coordinator – UC Davis

Tuskegee University P.I.s & Co P.I.s

Luther Williams, Principal Investigator – Tuskegee University
Jesse Jaynes, Co-Principal Investigator – Tuskegee University
C.S. Prakash, Co-Principal Investigator – Tuskegee University

IGERT: Collaborative Research and Education in Agricultural Technologies and Engineering (CREATE)

NSF Award DGE0653984

August 15, 2007 – July 31, 2012

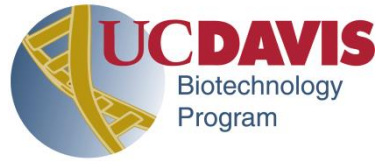
UC Davis has been awarded the multi-institutional IGERT: Collaborative Research and Education in Agricultural Technologies and Engineering (CREATE) grant from The National Science Foundation in the amount of \$599, 824. The grant is under the direction of Karen A. McDonald; Department of Chemical Engineering, with co-PIs: Abhaya M. Dandekar, Department of Plant Sciences; Jean S. VanderGheynst, Department of Biological and Agricultural Engineering; Martina Newell-McGloughlin, UC BREP; and Pamela C. Ronald, Department of Plant Pathology. The lead institution is the University of California at Davis, Davis, CA and collaborating institutions are Tuskegee University, Tuskegee, AL (Luther S. Williams, PI); National University of Ireland, Maynooth, Ireland (Dr. Phil Dix, PI); Teagasc Oak Park Research Centre, Carlow, Ireland (Dr. James Burke, PI).

The IGERT program, entitled Collaborative Research and Education in Agricultural Technologies and Engineering (**CREATE**), will provide a structured and well-integrated graduate research and educational training program focused on a **unifying theme of transgenic plants and *in-vitro* plant systems for the production of industrial non-food products and biopharmaceuticals. Research focus areas are 1) Plant-Made Products, 2) Biofuels and Biorefineries, and 3) Environmental Sustainability.** Across the three broad focus areas, specific attention will be paid to **the scientific, engineering, environmental, regulatory, economic, intellectual property, societal and global issues** associated with plant biotechnology.

The **Project Objectives** for IGERT are to:

1. CREATE a **framework for interdisciplinary graduate training** that will foster an environment for revolutionary breakthroughs at the interface of plant science, biotechnology, and engineering.
2. CREATE **new scientific knowledge**, engineering technologies, tools, methods, processes, and global understanding to advance the fields of plant science, biotechnology, engineering and areas at the interface of these disciplines, particularly those related to the underlying theme.
3. CREATE and cultivate the **integrative skill set** in graduate student trainees, faculty trainers, and postdoctoral scholar participants using the underlying theme as the focus.
4. CREATE a training program to **attract, retain, and graduate doctoral students from diverse backgrounds** who are not only top-rated scientists and engineers but also have the variety of skills and understanding to approach problems from integrated perspectives, allowing them to become the academic, industrial, national laboratory, and/or policy leaders in areas related to the unifying theme.
5. CREATE a **Masters to PhD Bridge Program** that strengthens research and graduate training linkages between UC Davis and Tuskegee University in areas related to plant biotechnology and provides a guided transition for MS students at Tuskegee into doctoral programs at UC Davis.

To apply for IGERT support, students **must be a member of the DEB**



Designated Emphasis in Biotechnology Program (DEB)

Goals and Mission of the DEB

The Designated Emphasis in Biotechnology (DEB) is an inter-graduate group program that allows Ph.D. students to receive and be credited for training in the area of biotechnology. The DEB provides a nurturing interactive environment to promote integration of multiple disciplinary approaches to the conduct of research and to promote learning in biotechnology. The mission is to prepare well-educated students to approach problems with creativity and flexibility. The program will provide tools for the students to be leaders, visionaries, entrepreneurs, researchers and teachers in the broad area of biomolecular technology.

DEB Mission:

- To provide well-coordinated, cross-disciplinary training of graduate students in critical areas of biomolecular technology research.
- To promote interdisciplinary research environments that integrate basic biological science, engineering and computational disciplines.
- To allow cross-disciplinary training and trainee experience in a biotechnology company or cross-college laboratory.

Students come from a wide array of disciplines: Participating graduate programs currently include **25 programs**: Agricultural and Environmental Chemistry; Animal Biology; Applied Science; Biochemistry and Molecular Biology; Biological Systems Engineering (formerly Biological & Agricultural Engineering); Biomedical Engineering; Biophysics; Cell & Developmental Biology; Chemical Engineering; Chemistry; Civil and Environmental Engineering; Comparative Pathology; Entomology; Genetics; Immunology; Materials Science and Engineering; Mechanical and Aeronautical Engineering; Food Science; Microbiology ; Molecular, Cellular and Integrative Physiology (formerly Physiology); Nutrition; Pharmacology & Toxicology; Plant Biology; Plant Pathology; and Statistics. The DEB program supplements a student's Ph.D. curriculum and those completing the program will obtain an official designation on their diploma & transcript indicating a qualification in biotechnology. Example: **Doctoral Degree in Microbiology with a Designated Emphasis in Biotechnology**

***CREATE-IGERT Fellows must be enrolled in the DEB**



Session 1: Biofuels & Biorefineries

SPACE RESERVED FOR KATY DEHESH AND ELENOR CASTILLO

Trainee: Elenor Castillo

Faculty Trainer: Katy Dehesh

Blah

Blah

Blah blah

TRANSIENT *in planta* EXPRESSION OF CELLULOSE-DEGRADING ENZYMES: PLANT TISSUES AS BIOREACTORS

Trainee: Ben Lindenmuth

Faculty Trainer: Karen McDonald

Campus: UC Davis

To ferment the large amount of fuel alcohol needed to displace a significant fraction of domestic gasoline consumption, a massive amount of sugar is required. One source of these sugars is cellulosic biomass, and one method of converting cellulose to sugar is enzymatic hydrolysis with cellulase enzymes. To generate enough sugar for fuel alcohol biorefineries, large amounts of enzyme would be required. This research explores the use of living plant tissues as economically feasible bioreactors for the production of cellulase enzymes. Plant tissues are infiltrated with *Agrobacterium tumefaciens*, which transfer into the plant cells a gene for the thermostable β -1,4-endoglucanase from *Acidothermus cellulolyticus*. Significant yields of this enzyme can be obtained after several days while the gene is expressed transiently. Results are presented for optimizing the ratio of *Agrobacterium* to plant biomass, examining the importance of activating the *Agrobacterium*'s *vir* regulon, and evaluating the storage conditions of the infiltrated plant tissues.



Session 2: Environmental Sustainability

BILIPROTEIN PHOTORECEPTORS AND PHOTORECEPTION IN PLANTS

Trainee: Timothy Butterfield

Faculty Trainer: J. Clark Lagarias

Campus: UC Davis

J. Clark Lagarias*, Timothy Butterfield, Lixia Shang, Wei Hu, Nathan C. Rockwell, Sindy Liao Chan, Alexander King, Keenan C. Taylor, Shelley Martin and Gaganjoat Sidhu

Department of Molecular and Cellular Biology. The University of California, Davis, CA 95616

Research in my lab focuses on the phytochromes, a family of light sensing biliproteins found in plants and cyanobacteria, which mediate responses mainly to red and far-red light in the environment. Our investigations are biochemical in nature focusing on structure-function and evolutionary relationships of the extended superfamily of phytochromes and the enzymes responsible for the synthesis of its linear tetrapyrrole (bilin) prosthetic group. A long-term goal of these investigations is to rationally alter the natural responses of plants to their light environment through modification of the biosynthesis, structure and function of phytochromes. One avenue of investigation seeks to define how tetrapyrrole and light signals are perceived by the phytochrome molecule and transduced to downstream target molecules, addressing the hypothesis that prokaryotic and eukaryotic phytochromes transduce both signals by protein phosphorylation via distinct biochemical mechanisms. These studies are presently exploiting the exciting discovery of a class of constitutively active mutants that confer dominant gain-of-function signaling activity in transgenic plants. A second line of investigation focuses on the family of ferredoxin-dependent bilin reductases, enzymes responsible for the biosynthesis of the bilin precursors of phytochrome and phycobiliprotein chromophores. Utilizing recombinant enzymes, biochemical and biophysical approaches have been used to determine the cofactor composition, substrate/inhibitor specificity and kinetic parameters for individual members of this family. Chemical modification studies, in combination with site-directed mutagenesis, domain swapping and x-ray crystallographic analyses, seek to elucidate the structural basis for the catalytic specificity of each family member. A third line of investigation exploits our ability to reconstitute holophytochrome biosynthesis in bacteria and yeast in order to identify mutants in phytochromes and the bilin reductases. Using high throughput optical screens, these directed evolution studies are expected to provide insight into the structural basis of phytochrome signaling as well as to generate mutant phytochromes with novel spectroscopic properties. Specifically, we hope to develop phytochrome-based fluorescent tag that emit in the near infrared.



Session 3: Plant Made Products

A KINETIC MODEL OF T-STRAND TRANSFER DURING AGROINFILTRATION

Trainee: Chris Simmons

Faculty Trainer: Jean VanderGheynst

Campus: UC Davis

Chris Simmons* and Jean VanderGheynst*

Biological and Agricultural Engineering, UC Davis

Agrobacterium-mediated gene transfer (agroinfiltration) is a common plant transformation technique. However, the efficacy of agroinfiltration varies widely between plant species. We are interested in studying the kinetics of gene transfer from *A. tumefaciens* to plant cells. In the process, we plan to create a methodology for identifying the rate limiting steps in the gene secretion pathway that act to reduce transformation efficiency in various plants. During agroinfiltration, genetic material from Agrobacterium, the T-strand, is exported from the bacteria into plant cells. Ultimately, genes housed on the T-strand may be expressed by the infected plant cells via transient expression. A variety of virulence (Vir) proteins encoded by Agrobacterium facilitate this DNA secretion process. Unique Vir proteins form complexes with the T-strand at distinct steps in the pathway. Immunoprecipitation will be used to isolate T-strands at key pathway steps from extracts of agroinfiltrated plant tissue based on their associations with certain Vir proteins. Once isolated, quantitative PCR will be used to determine the concentration of T-strands at each pathway step. We will collect T-strand concentration data over time for each pathway step. This data will be used to fit parameters in a power-law mass balance describing the flux of T-strands through each step of the agroinfiltration pathway. This model will be used to identify rate-limiting steps of the pathway. This model will be a powerful tool for optimizing in planta transient expression of agroinfiltrated genes and will have implications for the biopharmaceutical and biofuel industries, where plant-based transient expression may be desirable



Distinguished Lecture

**Distinguished Lecturer
Dr. Chris Somerville
Director, Energy Biosciences Institute (EBI)**

**Presents On:
“Cellulosic Biofuels”**



UC Davis Activities & Recreation Center (ARC) Ballroom
October 16, 2008
3:00 – 4:00 pm

As Director of the Energy Biosciences Institute, Dr. Somerville oversees all open activities at the institute, including research, communications, education and outreach.

The EBI is a new research and development organization that harnesses advanced knowledge in biology, the physical sciences, engineering and environmental and social sciences to devise viable solutions to global energy challenges and reduce the impact of fossil fuels to global warming.

Dr. Somerville is a Professor in the Department of Plant and Microbial Biology at the University of California, Berkeley. His research focuses on the characterization of proteins implicated in plant cell-wall synthesis and modification. He has published more than 200 scientific papers in plant and microbial genetics, genomics, biochemistry and biotechnology.





Poster Session

A KINETIC MODEL OF T-STRAND TRANSFER DURING AGROINFILTRATION

Trainee: Chris Simmons

Faculty Trainer: Jean VanderGheynst

Campus: UC Davis

Chris Simmons* and Jean VanderGheynst*

Biological and Agricultural Engineering, UC Davis

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CRYOPRESERVATION AND VIABILITY ANALYSIS OF TRANSGENIC RICE ORYZA SATIVA CELLS

Lucas Arzola*, Corey Dodge, and Karen McDonald

Department of Chemical Engineering and Materials Science, University of California, Davis

Cryopreservation has been used successfully to preserve plant cells. This technique eliminates the need of constant subculturing, reduces the risk of contamination, and offsets the possibility of genetic drift. A cryopreservation protocol was developed for a rice cell culture that produces 100kDa recombinant gelatin. Cells were first subjected to an osmotic pretreatment to reduce intracellular water content to below 10%. They were then placed in a cryoprotective solution of glycerol, sucrose, and DMSO. This was followed by a two-step freezing method, first to -80 °C, then to -196 °C in liquid nitrogen. Procedure efficiency was determined by measuring cell viability using the Evans Blue Dye uptake method, and the triphenyl tetrazolium chloride cellular respiration indicator. Cells were thawed after periods of 1, 3, 7, 15, and 30 days of liquid nitrogen exposure. Initial cryopreservation studies indicated an approximated 50% viability for all time points. Optimization of this technique will provide the opportunity to create master cell banks, important in the preservation of valuable plant cell lines commercialized in industrial bioreactors.

Effect of Antimicrobial Synthetic Peptide D4E1 on Infestation of Cotton Seedling Disease and on Soil Microbial Diversity

Trainee: LaKisha Odom

Faculty Trainer: Jessie Jaynes

Campus: Tuskegee University

LaKisha Odom*, Conrad Bonsi, Ramble Ankumah, Jessie Jaynes, Marceline Egnin, Lanell Ogden, and Desmond Mortley

Department of Agriculture and Environmental Sciences. Tuskegee University, AL, 36088

Cotton seedling disease is a fungal disease complex comprised of several fungal pathogens such as *Pythium*, *Rhizoctonia*, *Fusarium*, and *Thielaviopsis*. In Alabama, the two main fungal pathogens associated with Cotton Seedling Disease are *Rhizoctonia Solani* and *Pythium ultimum* which belong to the orders of Basidiomycete and Oomycete respectively. Cotton Seedling Disease, which results in loss of cotton production revenues totaling over 10 million dollars per year, in Alabama alone, has no known disease resistant cultivars. Recently, a synthetic antimicrobial peptide *D4E1* was designed which has been demonstrated in greenhouse studies to confer tolerance to the fungal pathogens *Aspergillus* and *Fusarium*. *D4E1* was also found to have broad-spectrum antimicrobial action, and to be active against fungi belonging to the orders Ascomycete, Basidiomycete, Deuteromycete and Oomycetes, as well as bacterial pathogens *Psuedomonas* and *Xanthomonas*. In view of *D4E1*'s antifungal activity and broad spectrum antimicrobial action, this study is being conducted to examine both the efficacy of this peptide on the control of Cotton Seedling Disease Complex in transformed cotton plants in a field setting and on the effects of this peptide on soil microbial community. Two 150 x150 ft test plots, which had been identified as having the pathogens of interest, were arranged in a completely randomized design and were assigned either one of 3 cultivars of cotton transformed with *D4E1* or a control cultivar. Each cultivar and control was then examined and evaluated to determine the level of infestation. Preliminary results obtained show that all three cultivars were more resistant to cotton seedling infestation than the non-transformed variety. Microbial diversity is being evaluated by extracting whole community DNA, followed by Denaturant Gradient Gel Electrophoresis (DGGE) to determine which communities, if any, are altered as a result of the introduction of an antimicrobial synthetic peptide *D4E1*. Enzymatic activity will also be evaluated in order to determine whether or not introduction of transgenic plants will have any effect on soil enzymatic activity.

BIOSYNTHESIS OF COLORANTS WITH ANTIBACTERIAL PROPERTIES FROM BACTERIA

Farzaneh Alihosseini and Gang Sun

Division of Textiles and Clothing, University of California, Davis

The use of microorganisms in production of colorants is an alternative to non-renewable resources. We found that a new isolated marine bacterium similar to category of bacteria *Vibrio gazogenes* produces a bright red colorant in a good yield rapidly. The preliminary tests show that this colorant could dye polymers and fibers such as acrylic, wool and nylon and in addition provide antibacterial properties. Due to its low molecular mass, non-polar character and heat stability, it can be used as a disperse dye as well. The UV-Visible spectra of methanol solution has absorption wavelength at λ_{\max} 530nm. The Electro Spray Ionization mass spectroscopy (ESI-MS) and Nuclear Magnetic Resonance (NMR) are used to identify the structure of this compound. The accurate mass measurements show that the colorant has molecular mass of 323.1997 Da. Further analyzing by mass fragmentation, H-NMR and COSY NMR are done and the elementary composition of the colorant has been determined with a formula of $C_{20}H_{25}N_3O$. The light and heat stability of the compound under different conditions proves it as a good colorant for cosmetic, food and textile industries.

CREATE-IGERT SUMMER LABORATORY SHORT COURSES

Larry Joh
Chemical Engineering and Materials Science, UC Davis

Two summer laboratory short courses will be offered to CREATE-IGERT students and their faculty advisors beginning in 2009. Each one-week course will emphasize participation in teams, communication, and proper handling of recombinant materials. The Plant Transformation Methods course will be taught in the UC Davis Plant Transformation Facility with guidance from Professor Pam Ronald and David Tricoli, Manager of the Plant Transformation Facility. The goal is to introduce students to the equipment and methods involved with stable and transient gene expression in representative monocot, dicot, and cell suspension systems. The Recovery and Purification of Plant-Derived Products course will be taught in a new IGERT-funded lab with guidance from Professor Jean VanderGheynst. The goal is to introduce students to the equipment and methods involved with extraction, concentration, purification, and characterization of protein produced in transgenic plants and *in vitro* plant systems. The courses will provide a strong foundation in the principles and practical methods used in protein production from plants as well as the regulatory and environmental considerations.

ASSESSMENT OF TOTAL SYSTEM SUSTAINABILITY IN SMALL FARMS PRODUCTION USING AN AGROECOLOGICAL MODEL

Raymon S. Shange*, Ramble Ankumah, and Robert Zabawa

Dept. of Agricultural and Environmental Sciences, Tuskegee University, Tuskegee, AL, 36088

In light of the effects of environmental and ecological degradation, recent national economic trends, as well as a history of political marginalization, small farmers in the Black Belt of Alabama continue to face debilitating issues with regard to the viability of their farms. Goals of scholarly work have not formulated models that are sensitive to the unique traits of small-scale farms economically, environmentally, and socially. This research proposes a conceptual model that looks at total system sustainability (environmental, economic, and social over the short- and long-term). The model proposes a trinity of subsystems be used to evaluate human influenced ecosystems with case specific indicators. To assess the utility the model, two poultry production systems in the Alabama Black Belt were chosen as case studies to evaluate the impact of production intensity on system sustainability. Using spatial statistics, geographic information systems, and dynamic systems modeling, the project also attempts to model short- to long-term sustainability of the agroecosystem and its components. Data collection is integrative in approach as methodologies were used from environmental, social, and economic disciplines. In utilizing this model for these case studies, environmental indicators include enzyme activity, nitrogen mineralization, organic matter content, pH, and microbial diversity of the soil systems. Indicators of economic viability and social sustainability are included in the models final assessment. It is anticipated that the results will provide an understanding of the integrative nature of human-influenced ecosystems, and that the research method undertaken will prove to be a strong integrative approach to modern issues regarding human and natural systems. In preliminary data collected from the poultry systems, it was shown that the intensively managed system shows evidence of nitrogen accumulation in the soils, as well as narrow C/N ratios as compared to the less intense system. Economic data also suggests that the intensely managed system requires high capital investment that disallows system diversification that results in difficulty to sustain profits, while the low intensity system demonstrates the converse.

PLANT CELL SUSPENSION CULTURES AS A BIOPRODUCTION PLATFORM OF RECOMBINANT HUMAN THERAPEUTIC PROTEINS

Ting-Kuo Huang^{*1}, Michael A. Plesha¹, Bryce Falk², Abhaya M. Dandekar³, and Karen A. McDonald¹,

(1) Department of Chemical Engineering and Materials Science, University of California, Davis

(2) Department of Plant Pathology, University of California, Davis

(3) Department of Plant Sciences, University of California, Davis

Plant cell cultures have been investigated for developing as a potential bioproduction platform of recombinant protein especially for human therapeutics due to their intrinsic safety, cost-effective bioprocessing that leads to lower production and downstream costs, and the capacity for post-translation modifications. To realize using plant cell suspension cultures as alternative to traditional prokaryotic and eukaryotic systems for producing biopharmaceuticals in industry, in this study, we investigated various factors which may affect the expression yield and functionality of a recombinant human blood protein, alpha-1-antitrypsin (AAT), in transgenic tobacco cell suspension cultures.

First, we demonstrated that the productivity and functionality of rAAT could be enhanced by using our novel Cucumber mosaic virus (CMV) inducible viral amplicon (CMViva) expression system while comparing with a Cauliflower mosaic virus (CaMV) 35S constitutive promoter expression system or a chemically inducible promoter expression system.

Second, to decrease the proteolytic degradation effects and increase the stability of rAAT during plant cell cultures, a rational induction strategy combining medium exchange and pH control was proposed in bioreactor.

Third, the timing of induction (TOI) is a critical parameter for chemically inducible plant cell cultures in bioreactor. We applied OUR (oxygen uptake rate) of plant cell cultures as physiology indicator for determining the optimal TOI.

Last but not least, amino acids supplied in cultivation medium have been proven that could affect mammalian cells growth and recombinant product yield and quality. We studied the effects of amino acid supplementation on the rAAT production in plant cell cultures using design of experiments (DOE). All of our results have been investigated and further play a foundation for developing plant cell cultures as a platform of biopharmaceuticals production in industry.

DEVELOPMENT OF A NEW ENZYME-BASED BIODIESEL PRODUCTION PROCESS

Weihua Wu*, Zhiliang Fan

Department of Biological and Agricultural Engineering, University of California, Davis, CA,

Biodiesel produced from plant oils and animal fat is advantageous in terms of sustainable resource supply, less emissions, and biodegradability. However, the production cost of biodiesel is substantially higher than petroleum-based diesel due to high feedstock costs, high processing costs and the need for byproduct glycerol disposal. Specific manufacturing problems that lead to the high processing cost of biodiesel production using strong base as the catalyst, which is the process widely used in industry, include high energy cost, difficulty of glycerol recovery, catalyst removal, and the need for waste water treatment. There is a need for lowering the biodiesel production cost by developing alternative approaches for both biodiesel production and byproduct utilization. Enzymatic transesterification by lipase can result in the same products as that catalyzed by strong base and do not have the problems mentioned above. Lipase catalyzed biodiesel production is not utilized in industry, however, because of the high cost of lipase. In the project, we investigate a novel process for biodiesel production. The new process features production of biodiesel in a two-step process. The first-step takes place in an aqueous-organic biphasic system. The byproduct from biodiesel production, glycerol, will be utilized as the carbon source for culturing lipase-producing microorganisms to produce lipase in the aqueous phase. Lipases produced in the aqueous phase will catalyze the hydrolysis reaction to produce free fatty acids in the organic phase. Fatty acid methyl ester (FAME) is produced by esterification of free fatty acids (FFA) and methanol in a second reactor using lipase from the first step. This new process needs no exogenous lipase addition and utilizes the byproduct glycerol. It will lower feedstock costs and manufacturing costs of biodiesel, reduce waste formation, and eliminate the challenge of disposal of low-purity glycerol.