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ACCESSION NO: 0225218 **SUBFILE:** CRIS
PROJ NO: ALAW-2011-00361 **AGENCY:** NIFA ALAK
PROJ TYPE: SMALL BUSINESS GRANT **PROJ STATUS:** NEW
CONTRACT/GRANT/AGREEMENT NO: 2011-33610-30480 **PROPOSAL NO:** 2011-00361
START: 01 JUL 2011 **TERM:** 29 FEB 2012 **GRANT YR:** 2011
GRANT AMT: $99,991

INVESTIGATOR: Thoms, R.

PERFORMING INSTITUTION:
CFD Research Corporation
215 Wynn Drive, NW, Flr 5
Huntsville, ALABAMA 35805

NOVEL DEWATERING METHOD FOR COST EFFECTIVE HARVESTING OF MICROALGAE BIOMASS

NON-TECHNICAL SUMMARY: A novel process is proposed which will allow continuous dewatering of algae growth culture in a device which is robust (no moving parts) and readily scalable through process parallelization. The process makes use of innovative passive (low energy input) flow control and does not need any chemical pre-treatment or external heat energy input. Microalgal cultivation affords the promise of renewable production of liquid transportation fuels with dramatically lower net carbon emissions than petroleum-based fuels. Although current production costs for algal biomass are not competitive with petroleum-derived fuel prices, microalgae have a number of compelling characteristics that argue for their development over other biofuel crops. The leading advantage is the fact that algae crops have the potential to yield much more oil from an acre of land (1000-4000 gal/acre/yr) than traditional oil seed crops. A roadblock in the cost-effective production of **algal** based **biofuels** is the high cost incurred to harvest the algal cells from the growth culture for further downstream processing. This project will greatly reduce the energy intensity and cost of algae harvesting, a key process located between the algae growth process and the oil extraction process. Removal of this roadblock is required for domestically sourced carbon-neutral algae based biofuels and bio-products to become commercially viable.

OBJECTIVES: CFDRC has conceptualized and performed preliminary assessment of a novel apparatus which uses fluid dynamics to dewater algae growth culture. The driving factors in the development of this design are to minimize production costs and operating costs while retaining superior dewatering efficiency. This project will enable the research and development needed to build, test, and optimize this technology.

APPROACH: CFDRC will make extensive use of computational fluid dynamics (CFD) tools and techniques to understand the effects and optimize device geometry and operating conditions. Design parameters will be investigated and the effect of operating conditions on performance will be assessed; conditions will include flow rate (or pressure drop), and cell parameters (size and specific gravity). A 1st generation design will be fabricated and experimental results will be used to validate computational models which in turn will be used to optimize the design for maximum separation efficiency and minimum pressure drop. Then a 2nd generation prototype will be designed, fabricated, and tested to verify improved performance. Lastly we will propose a complete system for further development under Phase II.

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ACCESSION NO: 0224484 **SUBFILE:** CRIS
PROJ NO: ARZR-2010-04194 **AGENCY:** NIFA ARZR
PROJ TYPE: AFRI COMPETITIVE GRANT **PROJ STATUS:** EXTENDED

CONTRACT/GRANT/AGREEMENT NO: 2011-67009-30112 **PROPOSAL NO:** 2010-04194
START: 01 FEB 2011 **TERM:** 31 JAN 2016 **GRANT YR:** 2012
GRANT AMT: $797,570

INVESTIGATOR: Hu, Q.

PERFORMING INSTITUTION:
Office for Research & Sponsored Projects Administration
ARIZONA STATE UNIVERSITY
TEMPE, ARIZONA 85287

DEVELOPING BEST MANAGEMENT PRACTICES PLAN FOR PREVENTION AND TREATMENT OF ZOOPLANKTON CONTAMINATION IN ALGAL CROP PRODUCTION

NON-TECHNICAL SUMMARY: The proposed project addresses the Program Area Priority: Crop Protection for Sustainable Feedstock Production Systems. Contamination of cultures and grazing of algae by zooplankton (e.g., rotifers, amoebas and protozoa) represents the most challenging issue for sustainable algal mass culture, preventing algae from being a practical source of oil crops for production of bioenergy and bioproducts. To meet this challenge, we will 1) survey zooplankton contamination in commercial algal production systems; 2) determine biotic and abiotic factors affecting the occurrence, population dynamics and impact of grazing zooplankton on algal crop production; 3) develop a rapid, quantitative diagnostic method and early warning system for the detection of grazing zooplankton using a multiphasic approach; 4) evaluate various chemical and physical means to prevent and treat zooplankton; and 5) develop a comprehensive Best Management Practices Plan (BMPP) for prevention and treatment of zooplankton to ensure sustainable production of algal crops. We will conduct laboratory and outdoor experiments with typical production strains (e.g., Nannochloropsis sp., Chlorella sp. Dunaliella sp. and Cyclotella sp.) grown in both open ponds and closed photobioreactors. We will apply expertise in algology, zoology, cell biology, bio-imaging, genomics and bioinformatics, as well as chemical and physical treatment methods to the study. The proposed project will provide a detailed understanding of the factors influencing the occurrence, population dynamics, impact and control of zooplankton on algal mass culture. The comprehensive BMPP developed will enable sustainable **algal** crop production for **biofuels** and bioproducts.

OBJECTIVES: Goal: To develop a Best Management Practices Plan (BMPP) for sustainable **algal** crop production for **biofuels** and bioproducts. Objectives: 1) survey zooplankton contamination in commercial algal production systems; 2) determine biotic and abiotic factors affecting the occurrence, population dynamics and impact of grazing zooplankton on algal crop production; 3) develop a rapid, quantitative diagnostic method and early warning system for the detection of grazing zooplankton using a multiphasic approach; 4) evaluate various chemical and physical means to prevent and treat zooplankton; and 5) develop a comprehensive Best Management Practices Plan (BMPP) for prevention and treatment of zooplankton to ensure sustainable production of algal crops. Expected outputs: To provide a detailed understanding of the factors influencing the occurrence, population dynamics, impact and control of zooplankton on algal mass culture and develop the BMPP to ensure sustainable **algal** crop production for **biofuels** and bioproducts.

APPROACH: We will conduct laboratory and outdoor experiments with typical production strains (e.g., Nannochloropsis sp., Chlorella sp. Dunaliella sp. and Cyclotella sp.) grown in both open ponds and closed photobioreactors. We will apply expertise in algology, zoology, cell biology, bio-imaging, genomics and bioinformatics, as well as chemical and physical treatment methods to the study.

PROJECT CONTACT:

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ACCESSION NO: 0214672 **SUBFILE:** CRIS
PROJ NO: CALR-2008-03809 **AGENCY:** NIFA CALR
PROJ TYPE: OTHER GRANTS **PROJ STATUS:** TERMINATED
CONTRACT/GRANT/AGREEMENT NO: 2008-38908-19300 **PROPOSAL NO:** 2008-03809
START: 15 AUG 2008 **TERM:** 14 AUG 2011 **GRANT YR:** 2008
GRANT AMT: $690,580

INVESTIGATOR: Bezerra, J. A.

PERFORMING INSTITUTION:
CALIFORNIA STATE UNIV., FRESNO FOUNDATION
FRESNO, CALIFORNIA 93726

CALIFORNIA STATE UNIVERSITY AGRICULTURAL RESEARCH INITIATIVE-FFI 2008

NON-TECHNICAL SUMMARY: 1. Domestication and modern breeding has narrowed the number of species used for food such that only a small fraction of the extant plant biota is utilized. The goal of this sub grant is to examine minor crops and non-domesticated plant species for their potential nutritional or pharmaceutical value by screening plants that have been the focus of the Composite Genome Project (CGP) for their biological activity including a) total anti oxidant activity; b) vitamin A content; c) cell proliferation; d) cell cytotoxicity; e) transcript abundance of apoptotic genes. 2. Most dairy rations use imported corn and soy to satisfy energy and protein needs for the lactating dairy cow. This represents the largest portion of feed expenditures for most dairymen, roughly 50 to 60 percent of total milk production costs. The goal of this sub grant is to establish the economic and production efficiency of alternative feeds common to the western region of the United States as replacements for costly imported grains currently used in the dairy industry. 3. Microalgae will be one of the most desired feedstock for biofuels production in the future. Theoretically, biodiesel produced from algae appears to be the only feasible solution today for replacing petrodiesel completely by a renewable source. The long-term objective of this sub grant is to help convert energy consuming farms to energy generating, locally sustainable rural communities through the use of microalgae in the production of biofuel. 4. The increased utilization of cereal grains, particularly corn, for ethanol production in the U.S. has resulted in a major increase in the cost of livestock production. The objective of this sub grant is to obtain matching funds for the acquisition of a microcapillary flow cytometer for animal health monitoring to determine the effect of various feed additives on animal performance, animal health and feed cost reductions. 5. The current method of determining the amount of rot in wine grapes at harvest, visual inspection by members of the wine grape inspection service, is flawed. This sub grant proposes to develop an objective, rapid, impartial, accurate and precise quantification protocol that requires minimal technical expertise by personnel of fungal (microbial) rot of grapes in the vineyard and at the winery receiving station. 6. About 50% of the US winegrape production is located in the warmer regions of California that are generally considered too hot for making premium wines. Temperature in these regions often exceeds the optimum for fruit color development. The goal of this sub grant is to explore the potential of abscisic acid (ABA) application in enhancing color development and extractability as well as fruit and wine chemistry for major red winegrape varieties that will result in a significant increase in wine grape quality and value. .

OBJECTIVES: 1.The goals and objectives of this research are to: identify, qualify and determine a. grapevine response to ABA application in terms of vine growth, fruitfulness, fruit composition, and yield components to identify its additional benefits and potential limitations b. the best concentration and application time to the winegrape industry for commercial application c. the best concentration and application time to the winegrape industry for commercial application d. the efficacy of ABA application in enhancing color development and extractability by applying ABA to the fruiting zone at various concentrations and development stages 2.The goals and objectives of this research are to: a. Quantify and identify VOCs associated with the rot of wine grapes by specific molds, yeast and bacteria. b. Set of protocols that will quantify rot in wine grapes through VOC measurement c. Calibration for FTIR that can quantify rot in grape juice d. A quick, accurate, precise, affordable and user friendly instrumentation that can be used at the grape-stand to quantify grape rot. 3. The objective of this research is to purchase of a microcapillary flow cytometer to assist in the development of a better understanding of how feed additives can be utilized in poultry diets to help reduce feed costs by monitoring animal health. 4. The goals and objectives of this research are to: investigate the merits and limitations of growing algae as a biofuel feedstock and to determine if it is a viable alternative to petrodiesel, renewable, an environment friendly energy source and presents a sustainable energy conservation practice 5.The goals and objectives of this research is to: a. Replace a portion of the energy requirements of a dairy ration with high performing annual and perennial rye grass pasture blends b. find the most profitable balance of pasture and grain supplementation for dairy rations while utilizing alternative sources of harvested feeds common to the western region of the United States. 6. The goals and objectives of this research are the evaluation of minor crop Composite species for nutritional and phtyochemical potential by quantifying its total antioxidant capacity and determining its vitamin A content using chlorophyll as a proxy, phytochemical effects on cell proliferation and cell death using various assays, phytochemical effects on cell death using a cell proliferation assay, phytochemical effect on cell death using a cell viability assay, and phytochemical effects on cell death using a cell viability assay

APPROACH: All California State University Agricultural Research Initiative sub grants have been submitted for scientific and practical application peer review to at least five qualified academic and or industry representatives with specific expertise in each sub grants subject matter. Research progress and ultimate findings are made widely available in layperson terminology in the public domain through industry and public meetings, presentations, trade publications, news articles and via web based technologies. While refereed professional journal articles and meeting presentation are encouraged, dissemination is directed at farmers, ranchers, processors and consumers who need it most by requirement. Sub grant information dissemination and project benefits extend well beyond farm gate receipts to include improvements in public health and safety, environmental conditions, regional and state economic development and international competitiveness. Sub grant projects build upon and add value to other successful applied research, enhance student experiential learning opportunities and augment, enhance and extend the basic research conducted by the nations land grant universities and USDA. These sub grant projects will provide experiential learning, paid employment and industry exposure opportunities for participating graduate and undergraduate students from at least four CSU campuses with colleges of agriculture geographically distributed throughout

California. The integration of this and other research and academic curricula activities by faculty research scientists further ensures that students not directly participating in these research sub grants also benefit from the knowledge gained from these projects. This is of particular significance for California and the nations agricultural industry because the CSUs colleges of agriculture graduate more than 52 percent of Californias agriculture related majors and more than 90 percent of its specialized majors in areas such as food and nutrition studies, agricultural engineering, and plant and horticultural sciences.

PROGRESS: 2008/08 TO 2011/08
OUTPUTS: 08-09-001 Buckley - Evaluation of Minor Crop Composite Species for Nutritional and Phytochemical Potential - During this project one graduate was trained. Project materials and information have been presented at the annual ARI Showcase at Cal Poly Pomona, Pomona, CA for students, faculty, industry and the public as well as at the American Society of Biologist. These findings will be published in the peer reviewed journal. 08-09-002 Daley - Sustainable Feeding Strategies for Western Dairy Production - Ten industry, university, and professional presentations and extension outreach presentations have been completed to date throughout California and the United States for groups such as, but not limited to, the 2009 Applied Agricultural Economic Association Annual Meeting; Western Organic Dairy Producers Alliance Conference in Twin Falls Idaho; and the Organic Farming Conference, CSU Chico, College of Agriculture. Additionally, the project's student research assistant received a dual major in Agricultural Business and Animal Science related to her work on the project and was subsequently admitted into the University of California Davis Agriculture Economics Department graduate school at. 08-09-003 Gu - Application of Abscisic Acid (ABA) To Enhance Color Development and Extractability in Red Winegrapes - All experiments were conducted as proposed. Results were disseminated through journal publication and professional presentations for scientific audiences as well as ARI reports and outreach-education presentations for the grape and wine industry. Nine professional presentations and seven extension and outreach presentations have been completed to date. 08-09-004 Humphrey/ Peterson - Acquisition of a Microcapillary Flow Cytometer for Animal Health Monitoring - Multidisciplinary experiments have been conducted across departments at California Polytechnic State University. Three senior projects have been associated with and benefited from the project's research activity. Four professional and industry presentations have been completed to date to groups such as the Poultry Science Association and the 2009 California Animal Nutrition Conference. - 08-09-005 Thornton - Quantification of rot in wine grapes - The research team have made twice yearly presentations to the California Wine Industry Advisory Board (CWIAB) during August and December in 2009 and 2010. - 08-09-006 Yildiz - Development and Optimization of Microalgae Cultivation in Photobioreactors - All lab scale photobioreactor models and experiments were completed. Full-size PBR was acquired but never put into full production due to extended technical difficulties with power supply. It was determined that the simplest way to reduce the solar gains is to provide shading with a shading cloth, curtain, or whitening the greenhouse glazing. PARTICIPANTS: 08-09-001 Nancy Buckley, Ph.D. Professor College of Biological Sciences Cal Poly, Pomona - PI project leader planned and supervised experimentation, oversaw research activities, and coordinated activities of the research team; Youngsook You, Ph.D. Senior Research Associate Co-PI grew all the plant materials and performed plant extraction, XTT, BrdU, GeXP, Chlorophyll, FC assays; David Still, Ph.D. Professor, College of Agriculture project coordinator was responsible for experimental design and conceived experiments. 08-09-002 Cynthia A. Daley, Ph.D. College of Agriculture, CSU Chico - project lead, data analysis, reports, project coordination, publications, presentations; Bahoui Song, Ph.D. Agriculture Economist, CSU Chico College of Agriculture - economic analysis, co-author; Darby Holmes, Staff, College of Agriculture, CSU Chico - field data collections. 08-09-003 Sanliang Gu, Ph.D. Ricchiuti Chair of Viticulture Research, Jordan College of Agricultural Sciences and Technology (JCAST), California State University, Fresno - PI initiatied research projects, monitored progress, prepared reports and manuscripts, and presented at professional and outreach meetings; Hemant Gohil. Postdoc Fellow, conducted research projects including setting up and running HPLC system, setting up vineyard trials, sampling, and analyzing fruit and wines. 08-09-004 Daniel Peterson, Ph.D. Associate Professor, Animal Science Dept., College of Agriculture, Food, and Environmental Sciences, Cal Poly State University, San Luis Obispo - PI assumed project directorship, instrumentation oversight and utilization, and faculty researcher and student research assistant training and supervision upon the departure from the University of the original PI, Brooke Humphrey. 08-09-005 Drs. Thornton and Rodriguez are both from the Dept. of Enology and Viticulture, JCAST, California State University, Fresno - Roy Thornton, Ph.D. Professor - PI initiated research projects, monitored progress, prepared reports and manuscripts, and presented at professional and outreach meetings; Susan Rodriguez, Ph.D. Research Fellow/Lecturer and Gary Takeoka, Ph.D. Research Scientist at USDA-ARS Western Regional Research Center, Albany, CA, collected and analyzed field and laboratory data. 08-09-006 Drs. Yildiz, Kaminaka, Hampson and Kelly are faculty research scientists from the Bioresources & Agricultural Engineering Dept., Cal Poly State University, San Luis Obispo. Ilhami Yildiz Ph.D. Associate Professor - PI was responsible for supervising all project activities and ensuring that objectives were completed within the project budget; Stephen Kaminaka, Ph.D. Professor was responsible for coordinating and facilitating activities and aiding in evaluating project progress, meeting agency requirements, and dissemination; Brian Hampson, Ph.D. Professor was responsible for algae microbiology and conducting biochemical analysese; Shaun F. Kelly, Ph.D. Associate was in charge of electronics, controls, and lighting; and Shikha Rahman, Ph.D. was in charge of hydrodynamics and visualization studies. Ten graduate and undergraduate students not listed due to space limitations also contributed to these projects. TARGET AUDIENCES: California State University Agricultural Research Institute (ARI) sub grants are exemplary illustrations of the California State University System (CSU) working for California and the nation through collaborative university-industry applied research partnerships. Its research, outreach-education, and technology transfer activities augment, enhance and extend the basic research conducted by the nation's land grant universities. Its project and program results are made widely available in the public domain in layperson terminology, and technology transfer assistance and information dissemination are provided directly to producers, processors, and consumers who need it most. All ARI program benefits extend well beyond farm gate receipts to include improvements in public health and safety, jobs creation and retention, regional economic development, environmental conditions, and/or international competitiveness. Participating faculty researchers and students benefit from expanded "real world" hands-on applied research that is directly applicable to industry priorities and is of high value to society. The resulting integration of research and academic curricula greatly enhances undergraduate and graduate

student learning opportunities and professional leadership development. And the agricultural industry and consumers are afforded greater direct access to the wealth of CSU faculty and research staff expertise. This is of particular significance to the nation and its agricultural industry because the CSU annually graduates more than 52 percent of California's agriculture-related majors and more than 90 percent of its specialized majors in areas such as food and nutrition studies, agricultural engineering, plant sciences, and horticultural services. The ARI programs and sub grants also enables exploration of solutions for many of the strategic gaps in our knowledge base that otherwise might not be studied by for-profit companies and institutions because there is little or no specific monetary incentive to do so, despite their importance to the long-term sustainability and international competitiveness of our nation's agricultural industry. ARI research, information dissemination, and technology transfer activities will help ensure the continued vitality and long-term sustainability of the nation's agriculture industry well into the future. These six ARI sub grants are specifically targeting the nation's general agricultural community; specialty crop growers and processors; western regional dairy industry; national organic dairy industry; agricultural educators, university extension agents, and consultants; agricultural students interested in sustainable livestock feeding systems; winegrape growers; viticulture and plant growth researchers, teachers, and extension agents; wine grape growers, brokers, buyers, and winery operators; Algal biofuel industry; and state, local, and federal policy makers. PROJECT MODIFICATIONS: 08-09-001 Buckley - Evaluation of Minor Crop Composite Species for Nutritional and Phytochemical Potential - Unanticipated instrumentation frailer and lengthy repaired timelines resulted in the use of total phenol content to assess total antioxidant content. Studies have shown a high correlation between total phenols as assessed by the FC method and total antioxidants as assessed by ORAC for a wide variety of plant species. This indicates that the majority, but not all, of the antioxidant content of a plant is due to phenolic compounds. 08-09-002 Daley - Sustainable Feeding Strategies for Western Dairy Production - No project modifications were required. 08-09-003 Gu - Application of Abscisic Acid (ABA) No project modifications were required. 08-09-004 Humphrey/ Peterson - Acquisition of a Microcapillary Flow Cytometer for Animal Health Monitoring - Daniel Peterson, Ph.D., Associate Professor, Animal Science Department, College of Agriculture, Food, and Environmental Sciences, Cal Poly State University, San Luis Obispo - PI assumed project directorship, instrumentation oversight and utilization, and faculty researcher and student research assistant training and supervision upon the departure from the University of the original PI, Brooke Humphrey. 08-09-005 Thornton - Quantification of rot in wine grapes - The identified project technology was developed and technology transfer information was disseminated. However, after 18 months, the external funding body believed that the wine industry was not ready to use the technology presented. The imponderables such as timing of growth of each mold, and the quantity and speed of growth, all have an impact upon the quantities of end product present in the sample and ultimately in the gondolas during and after harvest. Until such time as these uncertainties are resolved the application may not be useful. 08-09-006 Yildiz - Development and Optimization of Microalgae Cultivation in Photobioreactors - The project was completed early due to the project director unexpectedly leaving the university. Continuation of additional post project research opportunities were not assigned to other faculty researchers.

IMPACT: 2008/08 TO 2011/08
08-09-001 Buckley - Evaluation of Minor Crop Composite Species for Nutritional and Phytochemical Potential - Project experiments demonstrated a specific target of the extract and that the induction of apoptosis was species specific and not a general response despite the close phylogenetic relationship among plants. Thirty species representing seven genera from the Compositae family were evaluated. Extracts with no effect on cell death did not show significant changes in apoptotic gene expression. However, genes involved regulation of the cell cycle was down-regulated by the extract which strongly induced cell death in the HL60 cells. Apoptotic events were consistent with the down-regulation of the key anti-apoptotic gene, Bcl-2. 08-09-002 Daley - Sustainable Feeding Strategies for Western Dairy Production - The objective of this research was to determine the economic impact of reduced grain feeding systems under intensive grazing management. It has shown that higher net profit per cow can be achieved by decreasing the concentrate supplementation levels from 24 to 12 percent of dry matter intake (DMI) with no impact on milk yield and little impact on milk quality in an intensively managed, pastured based dairy with high forage quality. Therefore, forages may be a suitable substitute for grain inputs under intensive grazing management where pastures provide ample high quality vegetation. 08-09-003 Gu - Application of Abscisic Acid (ABA) To Enhance Color Development and Extractability in Red Winegrapes - This project demonstrated the effectiveness and potential of Abscisic Acid (ABA) application to enhance color development and extractability in red winegrapes. ABA application enhanced fruit and wine color up to 85% in the warmer regions for winegrape production, where more than 50% of the tonnage is produced in the US. ABA treatments were effective in enhancing skin anthocyanins in all years. 08-09-005 Thornton - Quantification of rot in wine grapes - An increasing number of VOCs were detected over the time span of the experiment. The type and size of the VOCs also varied with time of incubation. Some VOCs, primarily present in the grapes declined over time, whereas some of those produced by microbial action increased. The data was subjected to detailed analysis by time of elution on the column. The range from 3.75 to 9.5 minutes 2 - and 3-methyl butanal, 2-pentanone declined, ethyl acetate, 2-methyl propanol, propyl acetate, 3-methyl-3-butene -1-ol, 2-and2-methylbutanol all increased. The concentrations of monoterpene hydrocarbons, alpha-pinene, limonene and gamma-terpinene increased. The concentrations of 2-methyl-2-bornene, 1-methylcamphene and 2-methylenebornane increased rapidly during the inoculation time. These compounds have been postulated to be dehydration or degradation products of 2-methylisoborneol but further studies are needed to clarify their origin. 08-09-006 Yildiz - Development and Optimization of Microalgae Cultivation in Photobioreactors - Full scale industry conditions for optimization of temperature control were calculated for horizontal closed-loop photobioreactors under any temperature and weather condition.

PUBLICATIONS (not previously reported): 2008/08 TO 2011/08
1. Daley, Cynthia. 2009. Northeastern Organic Dairy Producers Alliance Newsletter.
2. Daley, Cynthia. 2011. Western Organic Dairy Producers Alliance Newsletter.
3. Gu, S., S. Jacobs, and G. Du. 2011. Efficacy, rate and timing of applications of abscisic acid to enhance fruit anthocyanin contents in Cabernet Sauvignon grapes. Journal of Horticultural Science and Biotechnology. 86:505-510.

4. Gohil, H. and S. Gu. 2011. Effect of temperature and abscisic acid on profile of skin anthocyanins in Cabernet Sauvignon berries. American Society for Horticultural Sciences Annual Conference. Waikoloa, Hawaii.
5. Gu, S., S. Jacobs, E. Mallea, and Y. Fang. 2010. Efficacy of ABA application to enhance fruit and wine color in warm region Syrah and Merlot grapes. National Conference of American Society for Enology and Viticulture. Seattle, Washington.
6. Gu, S., E. Mallea, and Y. Fang. 2010. Effect of temperature and ABA on skin anthocyanin, tannin, and phenolics in Cabernet Sauvignon berries. National Conference of American Society for Enology and Viticulture. Seattle, Washington.
7. Mallea, E. B., Y. Fang, and S. Gu. 2010. Development of tannin and phenolics in skins and seeds of Syrah berries in a warm region. National Conference of American Society for Enology and Viticulture. Seattle, Washington.
8. Mallea, E. B., Y. Fang, and S. Gu. 2010. Effect of ABA on tannin and phenolics in skins and seeds of Syrah berries. National Conference of American Society for Enology and Viticulture. Seattle, Washington.
9. Gu, S., E. Mallea, Y. Fang, S. Jacobs, G. Du, and R. Wample. 2009. Effect of ABA application to enhance fruit color on vine vigor, yield components, and fruit composition in Cabernet Sauvignon grape in a warm region. Annual Meeting of American Society for Enology and Viticulture. Napa, California.
10. Gu, S., S. Jacobs, G. Du, X. Guan, and R. Wample. 2008. Efficacy of ABA application to enhance fruit color of Cabernet Sauvignon grape in a warmer growing region. Annual Meeting of American Society for Enology and Viticulture. Portland, Oregon.
11. Gu, S., K. Kaigas, M. Kaigas, G. Du, S. Jacobs, and R. Wample. 2008. Efficacy of ABA application to enhance wine color of Cabernet Sauvignon in a warmer growing region. Annual Meeting of American Society for Enology and Viticulture. Portland, Oregon.
12. Gu, S. and X. Guan. 2006. Effect of ABA application on berry color development in Cabernet Sauvignon, Syrah, and Merlot grapevines. Annual Meeting of American Society for Enology and Viticulture. Sacramento, California.
13. Mallea, E.B. 2010. Effect of ABA on tannin development in Syrah berries. Department of Viticulture and Enology, California State University, Fresno.
14. D Amato, J. and Humphrey, B.D. (2010) Dietary arginine levels alter markers of arginine utilization in peripheral blood mononuclear cells and thymocytes in young broiler chicks. Poultry Science 89(5):938-947.
15. Mehlitz, Thomas 2009 Temperature Influence and Heat Management Requirements of Microalgae Cultivation inPhotobioreactor. Department of Agricultural Enginering Technology, California Polytechnic State University, San Luis Obispo.

PROJECT CONTACT:

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ACCESSION NO: 0225963 **SUBFILE:** CRIS
PROJ NO: CALW-2010-05126 **AGENCY:** NIFA CALW
PROJ TYPE: AFRI COMPETITIVE GRANT **PROJ STATUS:** NEW
CONTRACT/GRANT/AGREEMENT NO: 2011-67012-30652 **PROPOSAL NO:** 2010-05126
START: 01 SEP 2011 **TERM:** 31 AUG 2013 **GRANT YR:** 2011
GRANT AMT: $130,000

INVESTIGATOR: Terashima, M. T.

PERFORMING INSTITUTION:
CARNEGIE INST.
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STANFORD, CALIFORNIA 94305

DISCOVERING KEY REGULATORS OF ALGAL LIPID SYNTHESIS USING HIGH-THROUGHPUT CHARACTERIZATION OF MUTANTS CARRYING UNIQUE DNA BARCODES

NON-TECHNICAL SUMMARY: Biofuels derived from **algal** lipids are expected to provide substantially for the increased energy demands in the future. Microalgae are capable of photosynthetically producing triacylglycerol, which can act as a biofuel precursor. There are many advantages of **algal** feedstock for **biofuels**, such as high biomass yields per cultivation area and the non-competitive cultivation requirements with conventional agriculture. Although numerous studies have recognized the potential of **algal biofuels**, current **algal** lipid production schemes are expensive and inefficient, calling for continued research and optimization in algal lipid production. Further research of photosynthesis and lipid accumulation is

crucial for the engineering of algae to maximize photosynthetic lipid yields for biofuels purposes. The slow pace of discovery of the genetic components of lipid metabolism using small-scaled genetic approaches was a limiting factor in algal lipid research. I propose to advance our understanding of algal lipid metabolism using a novel high-throughput screening tool for the green alga Chlamydomonas reinhardtii, the leading organism for the study of algal photosynthesis and lipid metabolism. This tool, which is currently being established in the laboratory of Dr. Martin Jonikas (Carnegie Institution for Science), will enable simultaneous measurements of growth rates of hundreds of thousands of individual mutants in a pooled culture. This genetic tool also allows for the identification of the non-functioning gene for each mutant strain. Through this project, I will adapt and optimize this high-throughput genetic screening tool for Chlamydomonas to identify key genes in lipid metabolism. I will generate a list of mutants that have increased or decreased lipid production levels, effectively leading to the identification of genes that are likely to be involved in lipid metabolism. After this, I will isolate and characterize several mutant strains with altered lipid production levels, focusing especially on those mutants defected in genes with possible regulatory roles. This will be important for the engineering of algae with higher lipid accumulation for biofuel production.

OBJECTIVES: I propose to further our understanding of lipid synthesis in Chlamydomonas reinhardtii, by systematically identifying key genes and pathways required for the photosynthetic production of lipids. I will do this by adapting a high-throughput genomic tool that will allow us to simultaneously measure photosynthetic growth rates and lipid accumulation levels in a large collection of mutants. Using this data, I will be able to identify key genes in which defects cause increased or decreased lipid accumulation. This will provide candidates for genetic engineering efforts to increase lipid production. Aim 1: Adapt and optimize a high-throughput genetic screening tool for C. reinhardtii. A genetic tool is being developed in the laboratory of Dr. Martin Jonikas that enables tracking of growth rates or identification of genes of interest after selective enrichment from a pooled culture with hundreds of thousands of mutants. I am to further establish this method for my project by testing and optimizing the technique to allow for relative quantification of mutant strains. Aim 2: Identify mutations that alter lipid accumulation I will use the tool from Aim 1 to define a set of mutations that cause altered lipid accumulation while taking growth rate differences into account. I expect to generate a list of genes that cause increased or decreased lipid accumulation when mutated. This list is likely to also include genes that have never been characterized previously in addition to genes known to affect lipid accumulation when mutated, such as genes involved in starch accumulation. Aim 3: Identify and characterize key transcriptional regulators controlling lipid accumulation. Candidate transcriptional regulators of lipid accumulation will be identified by searching for genes containing transcription factor domains among the hits from Aim 2. The roles of these regulators will be characterized by analyzing their physiology as well as their transcriptional response to lipid-accumulating conditions. I expect that mutants that lack potential transcription factors involved in lipid accumulation would show altered transcriptional response and altered lipid accumulation. Timeline: 1. Testing quantification using DNA barcode counts: 08/01-09/30/2011. 2. Applying genetic tool to larger pool of mutants (determine growth rates, measure lipid accumulation levels, generate a list of genes important in lipid synthesis): 10/01/2011-03/31/2012. 3. Isolate mutants in putative transcription factors important in lipid synthesis: 04/01-06/30/2012. *Year 1 milestone: Generate a list of genes important in lipid synthesis and isolate a set of mutants lacking important regulatory factors in lipid synthesis. 4. RNA-seq analyses of these mutants vs WT under nitrogen starvation: sample preparation and data analyses: 07/01-11/30/2012. 5. Confirm altered lipid accumulation levels in isolated mutants: 12/01/2012-1/31/2013. 6. Test other parameters in the mutants such as high-light lipid accumulation and growth under HSM conditions: 02/01-03/31/2013. 7. Final data analyses and writing papers: 04/01-07/31/2013. *Project duration: 08/01/2011-07/31/2013.

APPROACH: To monitor growth of mutants under specific conditions, I will utilize a tool that allows for simultaneously tracking of individual Chlamydomonas mutants grown in a pooled culture. Growth rates of strains or relative enrichment of strains can be determined through sequencing and quantifying the mutant-specific "DNA barcodes" consisting of genomic DNA flanking the insertion marker. I will test the quantification of extracted DNA barcodes by mixing known amounts of different mutant strains in varying proportions. I will extract the DNA barcodes from the pools of mutants and quantify the barcodes using Illumina sequencing to compare the experimentally determined ratios with the true values. The optimized genetic tool will be used to identify mutants with altered lipid accumulation. Cells will be subject to random insertional mutagenesis with the marker cassette containing the AphVIII gene. I will pool the mutants and grow them at a constant cell density in media with 10 mM NH_4^+ in a photobioreactor in turbidostat mode. A sample (T0) will be taken from cells growing at the exponential phase and a second sampling (T1) will be taken after continued growth for two days at constant density. The T0 and T1 DNA barcodes will be extracted, sequenced and counted to determine mutant growth rates. Next, lipid accumulation will be induced by transferring to 0mM NH_4^+ media. After two days of growth, half of the culture will be collected for analysis and the remaining cells will be stained with Nile Red, a dye that causes fluorescence proportional in intensity to lipid content. I will enrich for cells with very low and very high fluorescence using a flow cytometry-based cell sorter. DNA barcodes from all samples will be extracted, sequenced and counted. This will allow for the identification of mutants with altered lipid accumulation by comparing the barcode counts between the unsorted and enriched mutants. I will correlate the growth rates with the lipid content of the enriched mutants to check whether the mutants with altered lipid accumulation grow normally under N-replete conditions. Mutants with severe growth defect under normal conditions could be disrupted in genes involved in unrelated cellular aspect and the altered lipid accumulation could be an effect of slowed growth. From the list of mutants showing altered lipid accumulation, I will search for those with DNA binding domains, identifying genes putatively important in the metabolic shift occurring under lipid-accumulating conditions. Once the putative transcription factor genes are identified, mutants in these genes will be isolated using PCR-based screening. I will measure the transcriptional response to N-starvation in the wild-type and the mutants by RNA-seq to characterize whole transcriptome responses to N-starvation. Lipid accumulation in the mutants will also be monitored again to confirm increased or decreased lipid content compared to the wild-type. If the mutants show absence of certain transcriptional responses and the expected changes in lipid accumulation under N-starvation, this will confirm the role of the missing transcription factor in each mutant in regulating lipid accumulation.

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ACCESSION NO: 0226036 **SUBFILE:** CRIS
PROJ NO: IDA01457 **AGENCY:** NIFA IDA
PROJ TYPE: HATCH **PROJ STATUS:** NEW
START: 01 JUL 2011 **TERM:** 30 JUN 2016 **FY:** 2011

INVESTIGATOR: Moller, G.

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NEW APPROACHES FOR SUSTAINABLE WATER AND ENERGY PROCESSES, AND TEACHING SUSTAINABILITY

NON-TECHNICAL SUMMARY: 1-Sustainable Water: Catalytic Oxidation, Reactive Filtration of Wastewater. We will continue testing the catalytic oxidation process at the bench and pilot scale. Two of our patents forward reactive filtration into the arena of catalytic oxidation using HFO as a sacrificial, heterogeneous-homogeneous f-orbital catalyst with ozone, for advanced oxidation water treatment. In full deployment, the technology is designed to fully sterilize degraded water, neutralize odor and color, remove suspended solids and nutrients, and mineralize emerging contaminants of concern. The aim of this technology is well beyond the current wastewater treatment paradigm of nutrient removal and disinfection (not sterilization). The research is necessitated by increasing demands on water and its reuse, as well as the emergence of TrOCs of concern in wastewater discharge, such as pharmaceutical and metabolite residues, and hormonally active substances, in addition to antibiotic resistant microorganisms. 2-Sustainable Energy Processes: Acoustic Cell Disruption, Lipid Extraction and Trans-esterification of Algae Biofuel. For assessment of serial-flow, acoustic algal cell disruption, lipid extraction and trans-esterification efficacy, we will use novel multi-focal, parabolic collimators mounted in individual tubes or in serial multi-tube process apparatus. The multi-focal parabolic collimators operate with a range of transducers at multiple power levels, individually modified for target process frequencies. The collimators have demonstrated the ability to apply a focused beam of megasonic energy (980 kHz) in a tube reactor configuration eliminating energetic loss on reactor boundaries. 3-Teaching Sustainability: Principles of Sustainability. Principles of Sustainability is an online course that uses multiple modes of technology to assist in student learning. Doculectures in each of the learning areas form the basis of this experimental pedagogy. A doculecture is a university level lecture, formalized in content, and supported with active media such as subject themed video, photographs, animations, and text, coupling information intensity with the audiovisual warmth and intensity of a documentary film. The visuals are chosen to help the student experience the subject dimensionally beyond a typical Power Point based lecture. Music and sound are added to assist in this dimensionality. The lectures and background sounds are often filmed in stereo to better the learning experience with the virtually enhanced audio dimension of the production. The doculectures are available in streaming SD and HD (720p) embedded on the Principles of Sustainability course site, and will be available in full 1080p and cell phone format for download.

OBJECTIVES: 1-Sustainable Water: Catalytic Oxidation, Reactive Filtration of Wastewater. The aim of this research is to develop and deploy an aggressive catalytic oxidation process for treatment of wastewater to destroy pathogens and TrOCs, such as highly bioactive pharmaceutical residues. In this research, we are shifting the paradigm of wastewater treatment to cost-effective, high-flow tertiary treatment, water sterilization and mineralization of dissolved organic Compounds of Concern, rather than the current approach of disinfection, often incomplete, and then discharge. The technology addresses the environmental release of organisms with antibiotic resistance genes, capable of insertion into mobile platforms such as plasmids, transposons, or integrons, that have the ability to transform natural microbial ecosystems into a reservoir of resistance genes and platforms. This research will yield important new knowledge about the application of a recently patented, continuously renewable catalytic oxidation process for sustainable water reuse and recycling. 2-Sustainable Energy Processes: Acoustic Cell Disruption, Lipid Extraction and Trans-esterification of Algae Biofuel. Significant challenges remain on industrializing algal bio-fuel processing and these include engineered production and water management, harvest of the lipid fraction, and biomass recovery, among others. Acoustic energy in the ultrasonic range has been used for cell disruption in biology labs for decades. Thus it is an effective way to burst algal cells to initiate oil recovery processes. Principle work on this has been limited to energy density analysis and oil recovery, and regard to oxidative damage to the lipid fraction. In addition, we will use acoustic energy to aid the trans-esterification process used to make plant/vegetable oils (VO) into as form that is readily usable as biodiesel. Using base catalysis driven reactions, acoustic energy can be highly efficient in rapidly converting

VO to biodiesel. For assessment of serial-flow, acoustic algal cell disruption, lipid extraction and trans-esterification efficacy, we will use novel multi-focal, parabolic collimators mounted in individual tubes or in serial multi-tube process apparatus. 3-Teaching Sustainability: Principles of Sustainability. Sustainability is a broad area of inquiry, rapidly changing as we develop new knowledge on human practices that are more sustainable or less sustainable. Our gaps in knowledge are great, but the task of growing a more sustainable global community is greater. It is the mission of the Principles of Sustainability course to provide students with a broad understanding of sustainability in the multiple human dimensions that it is manifested. Upper division and graduate level students from many disciplines will find the courseware of broad interest, intense in some areas and introductory in others, but complete in a desire to present the landscape of a general study in sustainability. The course attempts to synthesize linkages and commonalities of understanding through a presentation of the major elements in the field.

APPROACH: 1-Sustainable Water: Catalytic Oxidation, Reactive Filtration of Wastewater. We will continue testing the catalytic oxidation process at the bench and pilot scale. Two of our patents forward reactive filtration into the arena of catalytic oxidation using HFO as a sacrificial, heterogeneous-homogeneous f-orbital catalyst with ozone, for advanced oxidation water treatment. In full deployment, the technology is designed to fully sterilize degraded water, neutralize odor and color, remove suspended solids and nutrients, and mineralize emerging contaminants of concern. The aim of this technology is well beyond the current wastewater treatment paradigm of nutrient removal and disinfection (not sterilization). The research is necessitated by increasing demands on water and its reuse, as well as the emergence of TrOCs of concern in wastewater discharge, such as pharmaceutical and metabolite residues, and hormonally active substances, in addition to antibiotic resistant microorganisms. 2-Sustainable Energy Processes: Acoustic Cell Disruption, Lipid Extraction and Trans-esterification of Algae Biofuel. For assessment of serial-flow, acoustic algal cell disruption, lipid extraction and trans-esterification efficacy, we will use novel multi-focal, parabolic collimators mounted in individual tubes or in serial multi-tube process apparatus. The multi-focal parabolic collimators operate with a range of transducers at multiple power levels, individually modified for target process frequencies. The collimators have demonstrated the ability to apply a focused beam of megasonic energy (980 kHz) in a tube reactor configuration eliminating energetic loss on reactor boundaries. 3-Teaching Sustainability: Principles of Sustainability. Principles of Sustainability is an online course that uses multiple modes of technology to assist in student learning. Doculectures in each of the learning areas form the basis of this experimental pedagogy. A doculecture is a university level lecture, formalized in content, and supported with active media such as subject themed video, photographs, animations, and text, coupling information intensity with the audiovisual warmth and intensity of a documentary film. The visuals are chosen to help the student experience the subject dimensionally beyond a typical Power Point based lecture. Music and sound are added to assist in this dimensionality. The lectures and background sounds are often filmed in stereo to better the learning experience with the virtually enhanced audio dimension of the production. The doculectures are available in streaming SD and HD (720p) embedded on the Principles of Sustainability course site, and will be available in full 1080p and cell phone format for download.

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ACCESSION NO: 0204620 **SUBFILE:** CRIS
PROJ NO: ILLU-802-389 **AGENCY:** NIFA ILLU
PROJ TYPE: HATCH **PROJ STATUS:** TERMINATED
START: 01 OCT 2005 **TERM:** 30 SEP 2011 **FY:** 2011

INVESTIGATOR: Seufferheld, M. J.

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NEWLY DISCOVERED BACTERIAL ORGANELLE: IMPLICATIONS IN PHOSPHORUS METABOLISM

NON-TECHNICAL SUMMARY: One of the principal sources of water contamination with high levels of phosphorus is domestic and agricultural waste. Livestock production could be a significant source of phosphorus contamination through runoff and soil erosion from manure-amended soil. At the present, little is known about the microbes involved in phosphorus

removal from wastewater and their function there. The microbiology of phosphorus accumulating bacteria is complex and one of their characteristics is the ability to synthesize polyphosphate bodies or volutin granules. During the process of phosphorus removal from wastewater, bacteria are subjected to several types of stresses such as nutritional, osmotic and pH changes and anaerobiosis. It is not clear what functions of polyP bodies in bacteria, but we hypothesize that polyP may have a significant role during stress survival. The discovery of the acidocalcisome in bacteria offers a unique opportunity to have a more comprehensive understanding of the metabolic and biochemical process involved in phosphorus metabolism and cycle in phosphorus accumulating bacteria.

OBJECTIVES: During the process of phosphorus removal from wastewater, bacteria are subjected to several types of stresses such as nutritional, osmotic and pH changes and anaerobiosis. It is not clear what functions of polyP bodies in bacteria, but we hypothesize that polyP may have a significant role during stress survival. The discovery of the acidocalcisome in bacteria offers a unique opportunity to have a more comprehensive understanding of the metabolic and biochemical process involved in phosphorus metabolism and cycle in phosphorus accumulating bacteria. We will approach this goal by first understanding the dynamics of polyphosphate metabolism in our model organisms. The specific objective of this proposal is to investigate the phosphorus-containing compounds and H+-pyrophosphatase present in acidocalcisomes of prokaryotes and eukaryotes.

APPROACH: We have identified the following phosphorus-containing compounds as present in acidocalcisomes of C. reinhardtii and A. tumefaciens: (1) inorganic pyrophosphate and (2) short and long chain polyPs. Our first approach will be to use different methods of extracting phosphorus-containing compounds from whole C. reinhardtii and A. tumefaciens and analyze the extracts by 31P-NMR as it has been done in unicellular eukaryotes. This will allow us to identify the relative amount of each compound (PPi , tri-, tetra-, penta-polyP). The length of the long chain polyP (which is not detectable by 31P NMR) will be established using urea-PAGE electrophoresis. Second we will use fractionation methods to separate acidocalcisomes and then extract the fractions and analyze the phosphorus-containing compounds present in acidocalcisomes by 31P NMR. Third, enzymatic treatment of total extracts or of extracts of subcellular fractions (with inorganic pyrophosphatase, exopolyphosphatase, etc) and analysis of the products will facilitate the quantification of each phosphorus-containing compound.

PROGRESS: 2005/10 TO 2011/09
OUTPUTS: The discovery of a bacterial organelle, the acidocalcisome, that is similar to eukaryotic organelles, has challenged the microbiology dogma that the prokaryotic cells lack sophisticated cytoplasmic organization. Also, the acidocalcisome is the first known organelle that is conserved from bacteria to humans. The acidocalcisome is an acidic and polyphosphate calcium rich organelle that was first discovered in eukaryotes including the algae Chlamydomonas reinhardtii. Although the exact role of acidocalcisomes in cellular function is yet to be understood, these organelles, along with the synthesis of polyphosphate (polyP), have been associated with the capacity of microorganisms to overcome stress. There is mounting evidence that polyphosphates play a central role in microbial adaptation to stress and are associated with virulence in several pathogenic bacteria. This research was focused on testing the hypothesis that acidocalcisomes are important for the stress tolerance and virulence of bacteria pathogens. We worked with two model systems: (1) Helicobacter pylori, the causal agent of human gastric ulcer disease and stomach cancer, and (2) Agrobacterium tumefaciens, which contains acidocalcisomes and is the plant pathogen that causes the proliferation of tumors in plants, known as Crown Gall disease, by transfecting tumor-inducing genes into the host plant. Both plant host and A. tumefaciens stress responses are critical for successful transfer of these genes, and to successfully colonize the human gut, H. pylori must resist the pH, oxidative, and nutritional stresses encountered within the stomach. We used mutants from these two bacterial species that lack the enzyme responsible for the synthesis of polyP, which is the main component of acidocalcisomes. The A. tumefaciens mutant exhibits altered tumorogenesis, while the H. pylori mutant cannot colonize the stomach mucosa. This research has uncovered the presence of acidocalcisomes within the H. pylori cytosol and suggests that the lack of polyP affects the type V H. pylori secretory system. We also investigated the origin of this organelle. Investigation of the evolution of this organelle is constrained by the lack of DNA in it, in contrast to that found in chloroplasts and mitochondria. To overcome this problem, we worked with protein sequences and structural domains of the membrane-bound vacuolar proton translocating pyrophosphatase (V-H+PPase), an enzyme diagnostic of the vacuole and other acidic organelles. Phylogenetic evolutionary analysis of the V-H+PPase functional domains and the universality of volutin granules reveal that the acidocalcisomes may have appeared earlier than the divergence of the superkingdoms. Furthermore, we studied the role of acidocalcisome in algae stress responses in a mutant of Chlamydomonas reinhardtii that lacks a protein involved in calcium movement in the cell. We found that this protein is localized in the acidocalcisome. This mutant has significantly higher photosynthetic efficiency than the WT, survived for long period of time without media replacement and was able to grow and produce more biomass under nutritional deficiencies. PARTICIPANTS: Dr. Manfredo J. Seufferheld, Ph.D. Principal Investigator. He was responsible for supervising all aspects of the research proposed in this project. He was responsible for training graduate students, and, along with the collaborators, oversight of the publication of manuscripts resulting from this work. The Principal Investigator is working specifically in the area of molecular, physiological and ecological mechanisms of microbial stress responses. Dr. Seufferheld supervised 5 graduate students: David Park studied biophysical responses of stress in an algae model. Yi-Chun Chen worked on a Chlamydomonas mutant that was able to survive higher light intensity and overall was more fitted to environmental stress. Yan Zhou was working with Chlamydomonas mutant studying how this mutation affected photosynthesis efficiency by assessing the Photosystem II electron transfer. Peter Rohloff conducted research using a Chlamydomonas ptx2 knockout mutant as model to study Ca cellular influx. Jia-Min Bai was involved in the generation of two mutants of polyphosphatase kinase and exopolyphosphatase in Agrobacterium. Six undergraduate students participated in this research: Eun-Ik Koh worked in the characterization of acidocalcisome-like structures in H. pylori. Eun-Ik was awarded with the prestigious 2009 Francis and

Harlie Clark Undergraduate Research Award and received the 2010 Undergraduate Achievement Award. Umar Batthi was involved in the characterization of an Agrobacterium mutant related to polyphosphate synthesis. Yung Koo Lee investigated changes in polyphosphate species during cadmium exposure in Chlamydomonas. Shiyang Yan was involved in the characterization of Agrobacterium PPK and PPX mutants under stress conditions. Katie Walen was involved in the complementation of Agrobacterium PPK and PPX mutants. Alexandra Barbanova participated in a project involving the generation of the vacuolar pyrophosphatase mutant of Agrobacterium tumefaciens. Two postdoctoral fellows also were involved. Dr. Rodolfo Quintana's research was on the characterization of Helicobacter pylori and Agrobacterium tumefaciens mutants and their responses to stress. Dr. Emma Gachomo was studying the role of polyphophates in bacterial virulence. The PI established several collaborations: Dr. Steve Blanke, an expert in Helicobacter pylori, and Dr. Steve Farrant, an expert in Agrobacterium tumefaciens both from the Department of Microbiology at the U of I. Drs. Kim, Whitfield and Caetano-Anolles G. from the Department of Entomology and Crop Sciences provided expertise in protein evolution, phylogenetic analysis and bioinformatics. The PI collaborated with Dr. Pittendrigh from the Dept. of Entomology at the U of I. Dr. Seufferheld provided expertise in molecular responses of toxins using insect as a model. TARGET AUDIENCES: The target audiences for this project who will benefit from our data include microbiologists, stress physiologists, molecular evolutionists, and cellular biologists. Several manuscripts have been published and several are currently in preparation. The knowledge generated by this reached U.S. and International audiences. PROJECT MODIFICATIONS: Nothing significant to report during this reporting period.

IMPACT: 2005/10 TO 2011/09
The demonstration that polyP and the acidocalcisomes are important in stress tolerance and virulence in plant and human pathogens have important implications in the understanding of microbial physiology and development of new therapeutic alternatives to treat pathogens and design better transformation protocols base on agrobacteria. Also, the confirmation that Helicobacter pylori has an acidocalcisome reinforce the important of this organelle. We found that the absence of polyP and acidocalcisomes in H. pylori uncouple the secretion of the toxin (VacA) needed for H. pylori colonize the stomach. This protein is secreted by a type V system, which is the largest group of secreted proteins in gram-negative bacteria, and several of these proteins play essential roles in the pathogenesis of bacterial infections. This data will be significant to understand virulence and design new therapeutic strategies against pathogenic microbes. The importance of the V-H+PPase function and the evolutionary dynamics of these domains support the early origin of the acidocalcisome organelle. The implications that the acidocalcisome could already have been present in the Last Universal Common Ancestor is remarkable and highlights the possibility that a high degree of cellular compartmentalization could already have been present in the organism from which the tree kingdoms of life evolved. In addition, this new perspective has the potential to shed light on events that drove the evolution of other sophisticated membrane-bound compartments of the eukaryotic cell. These findings form the foundation for continuing investigations in the origin and early evolution of life, as well as the evolution of the eukaryotic endomembrane system. Algae have several key advantages: faster growth rates than other plant species, the ability to grow in marginal environments, the ability to take up excess nutrients in eutrophic waters, and in certain species, high oil composition. However, there are unresolved issues with the process of transforming inputs into algal biomass and producing biofuels. One of the most significant opportunities for improvements lies within the algae themselves. The investigation of the acidocalcisomes in algae offers an excellent model to study acidocalcisomes of algae and their role in stress regarding biofuel production. The findings of this research have been translated into several manuscripts and several of them appeared in journals with high impact factors. In addition, one article received special recognition by being selected for the cover of the Journal of Applied and Environmental Microbiology. The publication about the origin of the acidocalcisome received wide-scale national media attention and a significant amount of international news coverage. In addition, there are several publications in preparation. Moreover, the results of this research were shared in scientific meetings, symposiums and invited talks in U.S., China, Ecuador and Argentina. As extension of this work other work has been produced in collaboration with the Department of Entomology that resulted in high impact publications with national and international distribution.

PUBLICATIONS (not previously reported): 2005/10 TO 2011/09
1. Seufferheld, M.J., Kim, K.M., Whitfield, J., Valerio, A. and Caetano-Anolles, G. 2011. Evolution of vacuolar proton pyrophosphatase domains and volutin granules: Clues into the early evolutionary origin of the acidocalcisome. Biology Direct. 6:50.
2. Seufferheld, M., Whitfield, J. and Caetano-Anolles, G. 2011. Press Release: http://news.illinois.edunews111005LUCA ManfredoSeufferheld JamesWhitf ield Caetano Anolles.html.
3. Sun, L., Li, H.-M., Seufferheld, M.J., Margam, V., Jannasch, A., Diaz, N., Riley, C.P., Sun, W., Li, Y.-F., Muir, W.M., Wu, J.X.J., Zhang, F., Chen J., Barker, E.L., Adamec, J. and Pittendrigh, B. 2011. Systems-scale analysis reveals pathways involved in cellular response to methamphetamine. PloS One, 6(4):e18215.
4. Seufferheld, M.J. and Pittendrigh, B. 2011. Press Release: http://news.illinois.edu/news/11/0420meth BarryPittendrigh.html.
5. Pittendrigh, B.R., Berenbaum, M.R., Seufferheld, M.J., Margam, V.M., Strycharz, J.P., Yoon, K.S., Sun, W., Lee, S.H. and Clark, J. 2011. Simplify, simplify: Lifestyle and compact genome of the body louse provide a unique functional genomics opportunity. Communicative and Integrative Biology. 4(2): 188-91.
6. Zhou, Y., Schideman, L., Govindjee and Seufferheld, M.J. 2011. Improving the photosynthetic productivity and light utilization efficiency in green algae: Metabolic and physiological characterization of an advantageous mutant of Chlamydomonas reinhardtii. (In Preparation).
7. Quintana, R., Gachomo, E., Blanke, S. and Seufferheld, M.J. 2011. Helicobacter pylori possesses a polyphosphate rich acidocalcisome-like organelle that is involved in VacAsecretion. (In Preparation).
8. Quintana, R., Gachomo, E. and Seufferheld, M.J. 2011. Polyphosphates stored in acidocalcisomes are important for growth, motility and virulence of Agrobacterium tumefaciens. (In Preparation).
9. Menes, R.J., Ordonez, O., Estevez, C., Seufferheld, M.J. and Farias, M.E. 2008. Bacterial diversity and novel halophilic

bacteria from an extreme high- altitude andean wetland. 12th International Symposium on Microbial Ecology, Australia. August 17-22, 2008.
10. Zhou, Y., Schideman, L., Govindjee and Seufferheld, M.J. 2010. Improving the photosynthetic productivity and light utilization efficiency in green algae: Metabolic and physiological characterization of an advantageous mutant of Chlamydomonas reinhardtii. 15th International Congress on Photosynthesis and pre-meeting Beijing, China. August 18-27, 2010.
11. Quintana, R., Gachomo, E., Khan, S., Farrand, S.K. and Seufferheld, M.J. 2011. Polyphosphates stored in acidocalcisomes are important for growth, motility and virulence of Agrobacterium tumefaciens. 32nd Annual Crown Gall Conference. Middleton, Wisconsin. October 29-30, 2011.
12. Seufferheld, M.J. 2008. Bacterial acidocalcisomes: Physiological and evolution implications. National University of Rio Cuarto. Cordoba, Argentina. UIUC online seminar presentation. April, 2008.
13. Seufferheld, M.J. 2009. Microbial stress responses: From virulence to biofuels. China Agriculture University, Beijing. September 8, 2009.
14. Seufferheld, M.J. 2009. Microbial stress responses: From virulence to biofuels. Jilin University, China. September 11, 2009.
15. Seufferheld, M.J. 2009. Microbial stress responses: From virulence to biofuels. Zhejiang University, China. September 14, 2009.
16. Seufferheld, M.J. 2010. Systems biology reveals pathways involved in cellular response to the methamphetamine syndrome. Catholic University of Buenos Aires, Argentina. June 18, 2010.
17. Seufferheld, M.J. 2010. The acidocalcisome: Implications in stress physiology, phosphorus metabolism and evolution. Department of Microbiology, College of Agronomy, UBA, Buenos Aires, Argentina. June 23, 2010.
18. Seufferheld, M.J. 2010. The use of microalgae in biofuel research. Catholic University of Ecuador. December 18, 2010.
19. Seufferheld, M.J. 2010. Invited to co-organize a symposium in Sustainable Production of Biofuels Using Algae and Wastewater. Quito, Ecuador. December 15-22, 2010.

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Item No. 7 of 27

ACCESSION NO: 0223849 **SUBFILE:** CRIS
PROJ NO: ME0-2010-03356 **AGENCY:** NIFA ME.
PROJ TYPE: AFRI COMPETITIVE GRANT **PROJ STATUS:** EXTENDED
CONTRACT/GRANT/AGREEMENT NO: 2011-68005-20039 **PROPOSAL NO:** 2010-03356
START: 15 DEC 2010 **TERM:** 14 DEC 2012 **GRANT YR:** 2011
GRANT AMT: $50,000

INVESTIGATOR: Xie, X.; Goodell, B.; Perkins, B.; Jellison, J.; LeBlanc, L.; Rubin, J.; Hunt, G.; Wilson, W.

PERFORMING INSTITUTION:
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GROWTH OF MICROALGAE ON LIGNOCELLULOSIC BIOMASS SUGARS FOR ENHANCED BIO-OIL AND FOOD SUPPLEMENT PRODUCTION - PLANNING

NON-TECHNICAL SUMMARY: This project will support the development of a 2011 Regional Bioenergy Coordinated Agricultural Project focused on enhanced production of bio-oil and human food supplement from algae grown on lignocellulosic sugars. This project will develop alternative feedstocks and novel technologies to support bio-fuel production in regions of the US where sunlight is limited and the traditional wood products industry is in decline. The growth of algal biomass for sustainable bioenergy has fewer ecological and social costs compared to the use of higher plants grown on land. Algal bio-oil has been identified as a key replacement for petroleum-based diesel as it requires no substantial modification to existing infrastructure. Autotrophic growth of algal biomass for bio-oil production has low biomass productivity and geological limitations. The advantages of heterotrophic and mixotrophic growth of algae for bio-oil production include increased lipid productivity and the ease of scale-up. However, scientific and technological supports are lacking and additional research is needed to overcome bottleneck issues. Researchers from the University of Maine and the Bigelow Laboratory for Ocean Sciences have formed a team of scientists with expertise in biomaterials science, biology, food science and nutrition, marine sciences/analytic chemistry, economics and marine algae and phytoplankton. This team seeks to develop and implement a

planning program that links stakeholders from industry, academia, local communities and municipal governments in a synergistic collaboration that can clearly define and effectively solve the critical problems associated with large-scale growth of algal biomass on organic carbon sources for sustainable bio-oil production.

OBJECTIVES: The long-term goal of this project is to develop scalable and commercially viable technologies for the production of **algal** oil based **biofuels** and food supplements using high lipid content microalgae grown on lignocellulosic sugars. This proposal addresses the need to establish the necessary collaborations, initiate research planning, collect preliminary data and conduct necessary economic analysis. The immediate goal of this planning project is to establish an effective collaboration across different disciplines and institutions who can successfully conduct research focused on bio-oil and food supplement production from microalgae grown on low-cost lignocellulosic sugars. A subsequent proposal will be generated and submitted to NIFA in FY 2011 to support continued research.

APPROACH: Our proposed work has two major components. Initially we must seek effective methods of establishing a productive collaboration that facilitates the progress of this initiative. The second component involves building the research capacity to conduct the research and gather preliminary information to establish the feasibility of the research and development plan. Exploration, collaboration and evaluation will be addressed in our initial work through a series of coordinated research exchanges, preliminary studies, a formal seminar series and a series of interactive planning meetings. Planning meetings will include components related to data exchange, identification of core team members and critical functions, identification of consortia structure and core members, appropriate external linkages, economic and environmental analysis and ultimately the evaluation of preliminary results, the identification of potential problems and subsequent grant submission.

PROGRESS: 2010/12 TO 2011/12
OUTPUTS: Effective collaborations among participating organizations have been established. The Bigelow Laboratory for Ocean Sciences has identified one diatom, Cyclotella cryptica, for bio-oil production. The growth conditions of the diatom and Chlorella protothecoides grown on cellulosic sugars have been optimized in the laboratory of University of Maine for maximum biomass production. An analyzing method had been developed for fast test of the lipid content of algae. The preliminary results showed that the algal oil extracted from C. protothecoides grown on glucose contains up to 816mg neutral lipids per gram of the extracted oil. Selected scalable oil extract methods, including mechanical pressing, freeze and thaw, electroporation, osmotic shock, were tested for effective release of the algal oil. None of those methods could effective break the algal cell wall. The traditional organic solvent extraction method was also studied. The results demonstrated that only limited amount of lipids could be extracted out of the dried algae stock without breaking the algal cell wall. It was identified that the extraction process contributed much to the costs of algal oil production. The collaborations have been expanded to additional partners. Scientists from the Bigelow Laboratory for Ocean Sciences and the National Renewable Energy Laboratory were invited to give presentations on campus. Those presentations were incorporated into a graduate student seminar focused on sustainable biomaterials and bioenergy. Several professors of the University of Pennsylvania and Virginia Tech were invited to discuss the technique, economic, and policy aspects related to the proposed bio-oil production technology. Some PIs of this project were invited by other universities to conduct collaborative research on bioconversion of lignocellulosic materials for sustainable bioenergy production. We also successfully established partnership with the industry. The companies interested in collaborative projects included Solazyme, Inc., FMC Corporation, LP building Products, and Technological Innovations, LLC. A general agreement has been reached among all of the organizations that to use the cellulosic residues from wood products industry as a sugars source for heterotrophic growth of algae was a viable approach for sustainable production of boi-oil, value-added structural building products, and food supplements. We also involved the Passamaquoddy Tribe in Maine for potential partnership on development of sustainable bioenergy for the tribal community through implanting the heterotrophic growth technology into their existing algal growing facility. We have managed to provide youth education by developing an individual project for the University of Maine's Upward Bond summer programs on campus. One junior high school student was trained on growth of algae on organic carbon sources in our participating laboratories on campus during the summer of 2011. As a result of the extensive collaboration, we have submitted one Letter of Intent to the Biomass Research and Development Initiative and another one to the National Institute of Food and Agriculture during the lifetime of this project. PARTICIPANTS: Nothing significant to report during this reporting period. TARGET AUDIENCES: Nothing significant to report during this reporting period. PROJECT MODIFICATIONS: The project has been extended to December 14, 2012. The extension will allow additional data collection and analysis needed for preparing publications. It also allows the established collaboration to explore other funding opportunities on bioenergy production.

IMPACT: 2010/12 TO 2011/12
We are preparing two research papers to publish the results from our studies on the heterotrophic growth of algae on cellulosic sugars and on the bioconversion of lignocellulosics into sugars. The education project of this project helped the student to develop an interest in pursuing a Science and Engineering degree in his future college education. The invited presentations on algal biofuels production increased the awareness of current students in sustainable bioenergy. The established network with the universities, the research institutes, the tribal government, and the companies will facilitate future collaborations on sustainable bioenergy production.

PUBLICATIONS (not previously reported): 2010/12 TO 2011/12
Daniel C. Eastwood, Dimitrios Floudas, Manfred Binder, Andrzej Majcherczyk, Patrick Schneider, Andrea Aerts, Fred O. Asiegbu, Scott E. Baker, Kerrie Barry, Mika Bendiksby, Melanie Blumentritt, Pedro M. Coutinho, Dan Cullen, Ronald P. de Vries, Allen Gathman, Barry Goodell, Bernard Henrissat, Katarina Ihrmark, Havard Kauserud, Annegret Kohler, Kurt LaButti,

Alla Lapidus, Jose L. Lavin, Yong-Hwan Lee, Erika Lindquist, Walt Lilly, Susan Lucas, Emmanuelle Morin, Claude Murat, Jose A. Oguiza, Jongsun Park, Antonio G. Pisabarro, Robert Riley, Anna Rosling, Asaf Salamov, Olaf Schmidt, Jeremy Schmutz, Inger Skrede, Jan Stenlid, Ad Wiebenga, Xinfeng Xie, Ursula Kues, David S. Hibbett, Dirk Hoffmeister, Nils Hogberg, Francis Martin, Igor V. Grigoriev, Sarah C. Watkinson. 2011. The plant cell wall-decomposing machinery underlies the functional diversity of forest fungi. Science. 333: 762-765.

PROJECT CONTACT:

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ACCESSION NO: 0183224 **SUBFILE:** CRIS
PROJ NO: MICL01940 **AGENCY:** NIFA MICL
PROJ TYPE: HATCH **PROJ STATUS:** REVISED
START: 01 SEP 2009 **TERM:** 31 AUG 2014 **FY:** 2011

INVESTIGATOR: Benning, C.

PERFORMING INSTITUTION:
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REGULATION OF LIPID METABOLISM IN PLANTS AND ALGAE

NON-TECHNICAL SUMMARY: Seed oils provide food for human and animal consumption but also feedstocks for high energy transportation biofuels such as biodiesel or even jet fuels. However, the oil yield of currently grown seed oil crops such as canola, soybean, corn or sunflower is limited. Therefore, biofuels derived from temperate seed oil crops can only replace a small fraction of current transportation fuel needs without competing with the production of feed and food. Engineered plants that produce oils in tissues other than developing embryos have the potential to increase the oil yield per available land. Moreover engineering oil in vegetative tissues such as leaves, stems or roots enhances the energy density of potential fuel crops. The nature of the portfolio of biofuel crops or photosynthetic organisms that could be utilized for biofuel production is not yet defined. However, it is clear that potentially viable options aside from dedicated biofuel crops with novel properties include microalgal biomass enriched in oils (triacylglycerols). Unicellular microalgae species are known that have the propensity to produce and accumulate oil up to 60% of their cell mass. Knowledge and understanding of biological model systems coupled with recent advances in genetic and genomic technologies have opened a wealth of new possibilities. These new advances promise rapid progress towards the development of algal systems that could potentially produce oil on a scale that might soon supplement and possibly one day replace fossil high energy density liquid fuels. Algae have the potential advantage over conventional crops that they can be grown on marginal land not suitable for conventional agriculture. Thus, microalgal production systems do not compete with the production of food and feed using conventional crops. Moreover, algal culture systems are explored that can take advantage of flue gases emitted by coal-firing plants and waste streams of sewage treatment facilities. Because of the currently uncertain nature of biofuel feedstocks, it seems prudent to explore the potential of land based biofuel crops and microalgae in parallel. Both have the potential to transform agriculture as we currently know it and it seems likely that local solutions will require different feedstocks. The longterm goal of the proposed research is to gain a fundamental understanding of the regulation of oil biosynthesis in plants and algae. It is expected that this basic knowledge can be explored to engineer new biofuel crops and enhanced microalgal production strains in support of building a sustainable biofuel economy.

OBJECTIVES: The long term practical goals of this project are aimed at the development of novel **biofuel** crops and **algal** strains that are suitable for the production of biofuel feedstocks under different conditions of nutrient and light supply. Analysis of WRI1 function in Arabidopsis. The focus will be on the expression of the gene, namely alternative splicing and the study of different gene products and their function during embryo development and seedling establishment. We expect to find an optimal version of WRI1 best suited for the engineering rutabaga and other biofuel crops. In addition, we might uncover a new mechanism for regulation of gene expression by sugars. 1. Changing carbon partitioning in the rutabaga storage organ from starch to oil. A combinatorial synthetic biology approach will be pursued by constructing transgenic lines to block starch accumulation, to inhibit lipid and fatty acid degradation, to enhance glycolysis using the WRI1 transcription factor and to directly increase oil biosynthesis by expression of genes encoding oil biosynthetic enzymes. We expect to generate a series of

transgenic rutabaga with alter metabolism of the storage organ. Analyses of the transgenic plants for changes in composition (foremost oil content) and metabolism, or changes in growth and morphology should provide a realistic basis for the evaluation of engineered rutabaga as a novel biofuel crop. 2. Identification of factors critical to oil accumulation in Chlamydomonas. The focus will be on characterizing the available oil accumulation mutants and the affected genes, and on testing predicted transcription factors upregulated during nutrient deprivation. We expect that identification of the genes disrupted in these mutants will provide insights into the enzymes, regulatory factors and mechanisms involved in oil biosynthesis. The expression of putative transcription factors and other regulators under non-inducing conditions should allow us to test whether any of these genes provide novel targets for the engineering of oil accumulation in green microalgae. 3. Exploring Nannochloropsis as a model for the study of oil accumulation in a marine alga. Different strains available to the academic public will be tested for their growth and for their propensity to take up and insert DNA markers into their genome. The genome for a promising strain will be sequenced and annotated. Transcript profiling will be done under oil inducing and non-inducing conditions. Data and protocols will be rapidly made available to the scientific community through a website to promote the establishment of this alga as a model. We anticipate that genome sequence and transcript profiling will provide an important tool for the analysis of oil biosynthesis in this marine alga. Availability of the genome sequence will enable comparative genomics by including a few other microalgal genomes currently available. Establishing transformation and homologous recombination protocols for this alga will be most rewarding. If stable homologous recombination can be established, possibilities for the use of this alga as a model open up that go beyond that of Chlamydomonas.

APPROACH: 1. Analysis of WRI1 function in Arabidopsis. Public cDNA databases contain at least two different clones that derive from the Arabidopsis WRI1 locus. They differ in the last intron resulting in different C-termini of the respective protein. We plan to construct different cDNA versions and test them for complementation of the wri1-1 mutant and ectopic expression in Arabidopsis wild type using a promoter with broad tissue specificity. Phenotypes observed are morphology of seeds and plants, lipid content in embryos and other tissues, germination of seeds in the presence and absence of sugars, presence and stability of the mRNA and protein in the presence and absence of sugars etc. A second approach is focused on the analysis of a second wri1-2 allele. 2. Changing carbon partitioning in the rutabaga storage organ from starch to oil. Rutabaga transformation has been established in the lab. The type of explant, Agrobacterium strain, duration of infection and concentration of plant selection agent has been optimized. From calli, shoots form on medium containing optimized concentrations of plant growth regulators and selection agents within 3-4 weeks. Regenerated shoots are rooted on medium with optimized concentrations of auxin and selection agent within 3 weeks. Rooted plants are first grown in growth chambers and then acclimatized to greenhouse conditions. Cold treatment is needed to induce flowering. A series of constructs is currently under investigation. 3. Identification of factors critical to oil accumulation in Chlamydomonas. Confirmed high/low TAG mutants will subjected to an analysis of the insertion locus by a combination of established approaches including SiteFinding-PCR and plasmid rescue. Genes surrounding these loci will be tested to probe for larger deletions induced by the gene disruption process. These methods have previously been used in my lab to analyze Chlamydomonas lipid mutants. Once candidate genes have been identified, RNAi strategies will be used targeting candidate genes in the wild type to replicate the phenotype of the mutant. 4. Exploring Nannochloropsis as a model for the study of oil accumulation in a marine alga. Different publically available strains of Nannochloropsis will be tested for growth and their ability to integrate marker genes into the genome. In addition conditions for oil accumulation will be tested. Marker genes and a target sequence vector will be developed for testing of homologous recombination. We will start with selectable marker cassettes used in Chlamydomonas and focus initially on a non essential gene, for example a fatty acid desaturase. We plan to generate ~400Mb Nannochloropsis genomic sequences (~10X coverage), both single and pair-end reads, for assembling the first draft Nannochloropsis genome.

PROGRESS: 2011/01 TO 2011/12
OUTPUTS: Typically triacylglycerol (TAG) accumulates in developing seeds and little is known about the regulatory mechanisms and control factors preventing oil biosynthesis in vegetative tissues in most plants. We obtained proof of concept by engineering Arabidopsis thaliana to ectopically overproduce the transcription factor WRINKLED1 (WRI1) involved in the regulation of seed oil biosynthesis. Furthermore, we reduced the expression of APS1 encoding a major catalytic isoform of the small subunit of ADP-glucose pyrophosphorylase (AGPase) involved in starch biosynthesis using an RNAi approach. The resulting AGPRNAi-WRI1 lines produced 5.8-fold more oil in vegetative tissues than plants with WRI1 or AGPRNAi alone. The same constructs were introduced into rutabaga. We identified a number of transgenic plants (T1 generation) that accumulate oil in leaf tissues. The best lines contained up to 5% dry weight TAG in leaves, but showed reduced growth. Thus carbon partitioning from starch to oil was altered in these lines demonstrating a promising strategy to enhance energy content in vegetative tissues. Carbon partitioning is also relevant to microalgae that are considered as feed stocks for biofuel production. Nitrogen (N)-deprivation was used in the model green alga Chlamydomonas reinhardtii to induce triacylglycerol accumulation and changes in developmental programs such as gametogenesis. Global screening for mutants deficient in oil accumulation or turnover succeeded in the identification of several candidate genes involved in the process. The analysis of one of the low-oil mutants is nearing completion. It is deficient in a galactolipid lipase as determined by in vivo pulse chase label experiments and in vitro enzyme assays. This mutant is also bleached providing clues for the role of TAG formation under nutrient starvation conditions. Noteworthy is also the identification of a novel fatty acid desaturases responsible for 16:4 fatty acids specifically in the thylakoid lipid monogalactosyldiacylglycerol. Going beyond green alga, a publically available strain of the marine microalgae Nannochloropsis oceanica was selected based on growth and antibiotic sensitivity for further analysis. Sequencing of its genome and transcriptome using 454 and Illumina technology has been completed. Annotation and analysis of the data is underway. A transformation protocol for this alga has been developed and the construction of new and improved transformation vectors based on the obtained genomic sequence information is under way. PARTICIPANTS: Sanjaya (Postdoc), Que Kong (Postdoc) Miller, R. (Graduate Student) Wu, G. (Graduate Student), Vieler, A. (Postdoc), Li, X. (Graduate Student), Zauner, S. (Postdoc), Liu, B. (Graduate Student), Jaruswan Warakanont (Graduate Student), Chia-Hong Tsai (Graduate Student), Sears, B.B. (Collaborator at MSU) Kuo, M.H. (Collaborator at MSU) Hegg E.L. (Collaborator at MSU), Eva

Farre (Collaborator at MSU) Shachar-Hill, Y. (Collaborator at MSU) Shiu, S.H. (Collaborator at MSU), John Ohlrogge (Collaborator at MSU) TARGET AUDIENCES: 03/06/11 Keystone Symposium, on Biofuels, Singapore. Regulation of Triacylglycerol Synthesis and Turnover in Microalgae. 04/07/11 Arizona State University, Tempe AZ, Regulation of Triacylglycerol Synthesis and Turnover in Microalgae. 06/22/11 Symposium on Complexity and Systems Biology of Microbial Biofuels, Univ. of Warwick, UK. Regulation of Triacylglycerol Synthesis and Turnover in Microalgae. PROJECT MODIFICATIONS: Not relevant to this project.

IMPACT: 2011/01 TO 2011/12
Understanding the conversion of photosynthetic sugars into triacylglycerols will be essential for the engineering of novel biofuel crops or to enhance vegetable oil production. Triacylglycerols found in plant oils (vegetable oils) are the feed stock for biodiesel, fatty acid methylesters derived from plant oils. Current seed oil crops have not sufficient yields per growth area to be considered a reasonable alternative for replacing a substantial fraction of fossil liquid transportation fuels. However, by developing new biofuel crops which lead to improved oil yield per growth area, biodiesel from plant oils could replace a more significant fraction of renewable fuels. Developing rutabaga as a biofuel crop might provide Michigan's sugar beet farmers with an alternative biofuel crop that could be handled, harvested, and processed using methods established for sugar beets. Our knowledge of the regulation of oil biosynthesis in microalgae is lagging far behind that about this process in model plants like Arabidopsis. However, applying modern genetics and genomics tools to suitable model algae will rapidly provide us with regulatory factors, such as transcription factors that control oil biosynthesis under stress conditions. While it is no longer a challenge to obtain genomic information for any organism, the availability of genetic and molecular tools, e.g. transformability, gene disruption technology, haploid state, ability to conduct sexual crosses, and ease of foreign gene expression will distinguish model alga. At this time, the unicellular green alga Chlamydomonas reinhardtii is closest in fulfilling these requirements because of its genomic and genetic resources. We will use this organism's available tool kit and resources to identify regulatory factors, controlling oil production in this model green alga. In the long term, it will be necessary to move on to other microalgae that are under consideration as biofuel feed stock producers such as Nannochloropsis oceanica. This alga is phylogenetically very different from Chlamydomonas and will require the development of new toolkits. The long term goal is to provide the basic knowledge to develop microalgae into a viable alternative for the production of biofuels that does not compete with food production by agricultural crops.

PUBLICATIONS (not previously reported): 2011/01 TO 2011/12
1. 1. Sanjaya, Durret, TP., Weise, S,E,, and C. Benning. 2011. Increasing the energy density of vegetative tissues by diverting carbon from starch to oil biosynthesis in transgenic Arabidopsis. Plant Biotech, J., 9:874-883.
2. 2. Castruita, M., Casero, D., Karpowicz, S.J., Kropat, J., Vieler, A., Hsieh, S.I., Yan, W., Cokus, S., Loo, J.A., Benning, C., Pellegrini, M., and S.S. Merchant. 2011. Systems biology approach in Chlamydomonas reveals connections between copper nutrition and multiple metabolic steps. Plant Cell, 23:1273-1293

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ACCESSION NO: 0221540 **SUBFILE:** CRIS
PROJ NO: MICW-2010-01534 **AGENCY:** NIFA MICW
PROJ TYPE: OTHER GRANTS **PROJ STATUS:** EXTENDED
CONTRACT/GRANT/AGREEMENT NO: 2010-38889-20726 **PROPOSAL NO:** 2010-01534
START: 15 APR 2010 **TERM:** 14 APR 2012 **GRANT YR:** 2010
GRANT AMT: $468,000

INVESTIGATOR: Ng, K. Y.

PERFORMING INSTITUTION:
WAYNE STATE UNIV
DETROIT, MICHIGAN 48202

CENTER FOR RENEWABLE TRANSPORTATION FUEL, MI

NON-TECHNICAL SUMMARY: Sustainable energy production has become a high priority in addressing the economic and strategic impacts of limited fossil fuel resources. Moreover, fossil fuel consumption has taken a toll on the environment and

global climate. In view of these issues, much attention has been directed at production of biofuels from plant-derived biomass. Since plant-derived biofuels are produced by photosynthetic reduction of atmospheric CO2, a fuel cycle based on such fuels will be near CO2-neutral and will contribute minimally to greenhouse gas emissions. Algae have great potential for production of hydrocarbons for production of **biofuels. Algal** productivity per unit area is superior to land-based crops and, importantly, algal production systems for biomass and biofuels should not utilize land capable of food crop production. However, at present there are no algal production systems that can produce biofuel precursors economically. Limitations include less-than-optimal oil/hydrocarbon content, slow growth rates, and expensive techniques for cultivation and hydrocarbon extraction and conversion to usable fuel. Transformative technologies in genetics, photonics, catalysis, and bioreactor design and control are necessary to overcome these limitations. Our immediate goals are to redirect hydrocarbon production in algae into better biofuel precursors and to investigate the physiologic regulation of this process, to develop highly efficient renewable energy-based illumination strategies to achieve ultrahigh cell densities in novel bioreactor systems, and to determine how production, extraction, and conversion of hydrocarbons can be maximized. Our ultimate goal is to develop a sustainable algal system for photosynthetic production of hydrocarbons and their conversion to biofuels that can be used in existing national infrastructure and transportation systems.

OBJECTIVES: Our specific objectives are: to produce, by genetic engineering, algal stains that synthesize hydrocarbons convertible to biofuels with minimal processing, and to investigate the regulation of hydrocarbon production by these strains to direct future strain improvements; to develop novel photobioreactor designs with improved energy and control systems to investigate the effects of light intensity, distribution, CO2 level, pH, nutrient concentration, hydrodynamics and other culture conditions on the growth kinetics and hydrocarbon production of algae in a photobioreactor system; and to develop novel, environmentally friendly, downstream extraction and conversion processes to convert **algal** biomass to **biofuels**. Our collaborative approach will be integrated, cooperative and synergistic. Its three key components will add a new dimension to the existing scientific knowledge base regarding the use of algae as an efficient non-food crop **biofuel** source, and move **algal** biomass to the forefront as an integral part of nation's emerging bioeconomy. The impact of the proposal is the advance of algal genomic, photobioreactor design, extraction and conversion technologies, resulting in transformative progress towards the development of algal systems producing oil and hydrocarbon on a scale that should soon supplement and eventually replace fossil high energy density liquid fuels. This is consistent with the goal of State-Federal food and agricultural programs to develop sustainable non-food crop based biomass to biofuels. The results of this project would contribute to the required paradigm shift necessary to achieve the goal of 36 billion gallons per year of biofuels production by 2022 mandated by the Energy and Security Independence Act of 2007.

APPROACH: The project will be conducted to develop a fundamental understanding of an algae-based biofuel technology platform utilizing multidisciplinary expertise that will lead to and support an emerging bioeconomy. The proposed work takes advantage of recent advances in: genetics and genomics of the model algae; optical fiber and LED-based lighting systems; biofuel properties and characterization, biofuel catalysis, and novel photobioreactor design. Specifically, we propose to: 1. Genetically engineer algal strains synthesizing improved triacylglycerols or advanced biofuels; 2. Understand the effects of environmental conditions on the growth kinetics and hydrocarbon production and secretion characteristics of algae strains; 3. Design and validate photobioreactor systems through dynamic simulations and novel reactor design; and 4. Develop novel catalytic systems for simultaneous extraction and conversion of **algal** biomass to **biofuels**.

PROGRESS: 2010/04 TO 2011/04
OUTPUTS: Dissemination of results for this project has been through presentations at professional meetings and publication in journals. Specifically, the following presentations were made: 1. Shuli Yan, Craig DiMaggio, Siddharth Mohan, Manhoe Kim, Huali Wang, Steven Salley and Simon Ng, "Simultaneous Esterification and Transesterification for Biodiesel Production," presented at the 10th AIChE Annual Meeting in Salt Lake City, 2010. 2. Shuli Yan, Craig DiMaggio, Siddharth Mohan, Manhoe Kim, Huali Wang, Steven Salley and Simon Ng, "A Novel Class of Solid Base Catalysts in Transesterification of Vegetable Oils with Methanol," presented at the 10th AIChE Annual Meeting in Salt Lake City, 2010. 3. H.L. Wang, M. Kim, C. DiMaggio, S. Yan, S. O. Salley, and K.Y.S. Ng, "Biojet Production from Hydrocracking of vegetable oil," presented at the 10th AIChE Annual Meeting in Salt Lake City, Utah, 2010 4. H.L. Wang, M. Kim, C. DiMaggio, S. Yan, S. O. Salley, and K.Y.S. Ng, "Biojet Production from Catalytic Cracking of Soybean Oil Using HY Zeolite," presented at the 101th AOCS Annual Meeting in Phoenix, AZ, 2010 5. Haiying Tang, Meng Chen, Nadia J. Abunasser, Mario Enrique Danton Garica Perez, Steven O. Salley, and K. Y. Simon Ng. "Potential Resources of Microalgae Oils for Biofuels Feedstock Production," presented at the AIChE's 2010 Annual Meeting in Salt Lake City, UT, Nov. 7 -12. 6. Haiying Tang, Mario Enrique Danton Garica Perez, Meng Chen, Nadia J. Abunasser, Steven O. Salley, and K. Y. Simon Ng, "Culture of Microalgae Chlorella minutissima for Biofuels Feedstock" presented at the AIChE's 2010 Annual Meeting in Salt Lake City, UT, Nov. 7 -12. 7. Haiying Tang, Mario Enrique Danton Garica Perez, Meng Chen, Nadia J. Abunasser, Steven O. Salley, and K. Y. Simon Ng, "The Effects of Illumination Intensity and Period on Growth Rates and Fatty Acid Composition of the Microalgae Dunaliella tertiolecta" at the 101th AOCS 2010 Annual Meeting in Phoenix , AZ, May, 2010. 8. Manhoe Kim, Craig DiMaggio, Shuli Yan, Steven O. Salley and K. Y. Simon Ng, "Sulfur Level Changes in Brown Grease Conversions with Sulfuric Acid and Heterogeneous Zirconia-Supported Metaloxides Catalysts," AIChE annual meeting, Salt Lake City, UT 9. Manhoe Kim, Craig DiMaggio, Shuli Yan, Steven O. Salley and K. Y. Simon Ng, "Esterification of Free Fatty Acids with Methanol Over Metaloxides Supported ZrO2 Catalysts," AIChE annual Meeting, Salt Lake City, UT. 10. M. Kim, C. DiMaggio, S. Yan, S.O. Salley, K.Y. Simon Ng, "Methylester Preparation from Brown Grease by Using Heterogeneous Catalysts," 101st AOCS, May 16-19, 2010, Phoenix Convention Center, Phoenix, AZ. PARTICIPANTS: Nothing significant to report during this reporting period. TARGET AUDIENCES: Nothing significant to report during this reporting period. PROJECT MODIFICATIONS: Nothing significant to report during this reporting period.

IMPACT: 2010/04 TO 2011/04
This project has resulted in advances in several areas related to transportation fuels, notably in the development of new catalysts for conversion of biomass into biodiesel, green diesel, and jet fuel. Additionally, advanced techniques for mass culture of oil-producing algae have been developed, which should contribute to further progress towards realizing the potential of algae as a feedstock for renewable fuel production. Technology developed from this project has led to two new patent applications.

PUBLICATIONS (not previously reported): 2010/04 TO 2011/04
1. Manhoe Kim, Craig DiMaggio, Shuli Yan, Steven O. Salley and K. Y. Simon Ng The effect of support material on the transesterification activity of CaOLa2O3 and CaOCeO2 supported catalysts, Green Chem., 2011, 13, 334 339.
2. Manhoe Kim, Craig DiMaggio, Shuli Yan, Huali Wang, Steven. O. Salley, K.Y. Simon Ng, Performance of heterogeneous ZrO2 supported metaloxide catalysts for brown grease esterification and sulfur removal, Bioresource Technology 102 (2011) 2380 2386.
3. Meng Chen, Haiying Tang, Hongzhi Ma, Thomas C. Holland, K.Y. Simon Ng, and Steven O. Salley, Effect of nutrients on growth and lipid accumulation in the green algae Dunaliella tertiolecta, Bioresource Technology, Volume 102, Issue 2, January 2011, Pages 1649 1655.
4. Haiying Tang, Nadia J. Abunasser, Mario Enrique Danton Garcia Perez, Meng Chen, K. Y. Simon Ng, Steven O. Salley. Potential of Microalgae Oil from Dunaliella tertiolecta as a Feedstock for Biodiesel, Applied Energy, in press, 2010.

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ACCESSION NO: 0212956 **SUBFILE:** CRIS
PROJ NO: MOX-LEE **AGENCY:** NIFA MO.X
PROJ TYPE: EVANS-ALLEN **PROJ STATUS:** TERMINATED
START: 01 OCT 2007 **TERM:** 30 SEP 2011 **FY:** 2010

INVESTIGATOR: Lee, K.

PERFORMING INSTITUTION:
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RESEARCH AND DEVELOPMENT PROGRAM FOR MICRO-ALGAE CULTIVATION, OIL EXTRACTION AND CONVERSION TO BIODIESEL

NON-TECHNICAL SUMMARY: Depleting petroleum reserves and environmental concerns with the greenhouse gases are putting biogenic (plant-derived) oil esters at the forefront as the zero net carbon dioxide contributing alternative for use as fuels (e.g., biodiesel), technical fluids (e.g., lubricating and insulating bio-oils), and resins (e.g., bio-epoxy resin) for plastics. A recent article titled The end of cheap oil in National Geographic magazine highlights a well-known fact that the world is in the twilight of plentiful petroleum oil and alternative sources of energy and raw material must be developed. Biofuel in the form of biodiesel offers one of the most attractive direct replacement of fossil fuel. Significant efforts in this area are already underway as evident from the increasing number of newly installed biodiesel plants in Missouri and around the world. The most common process for producing biodiesel is through the transesterification reaction of vegetable oil or animal fat with an alcohol and a catalyst. Soybeans make up over half of all oilseeds produced worldwide and are currently the main source of biodiesel production. Due to a wide array of applications including nutritional food, animal feed, cooking oil, and recent usage as biodiesel fuel, it is anticipated that the soybean supply will not satisfy the growing demand in the future. There is a great need for the discovery of more productive renewable sources of plant-derived oils. The increasing demand for biodiesel also requires development of more economical and efficient processes for oil extraction and transesterification reaction. The Lincoln University Cooperative Research team in collaboration with the Center for Environmental science and Technology of the University of Missouri Rolla (CEST-UMR) proposes to undertake a comprehensive research and development program aimed at developing an integrated technology for economical production of algae, efficient extraction of oil and conversion to biodiesel. Micro-algae are the fastest growing photosynthesizing unicellular organisms and can complete an entire growing cycle every few days. Some algae species have high oil content (up to 60% oil by weight) and can produce up to 15,000

gallons of oil per acre per year under optimum conditions. The need for an efficient process for the extraction of oil from cultivated algae will be met by developing an economical, safe, and environment-friendly solvent system that can selectively recover triglycerides and efficiently convert them to fatty acid methyl esters for use as the biodiesel fuel and the source of bio-plastic resins. The proposed research and educational program will promote a close collaboration between multi-disciplines (chemistry, biology and mechanical engineering) and multi-institutions (University of Missouri-Rolla, Lincoln University and University of Alabama). By offering exciting research opportunities, and exposing the students to experiential learning curricula, we also seek to recruit, train, and mentor students from underrepresented groups and encourage them to pursue post-baccalaureate degrees in science and engineering disciplines.

OBJECTIVES: Proposed studies are designed to fully develop, evaluate and demonstrate the capabilities of the innovative technology for economical and efficient production of algae-derived oils for use as the source of biofuel. To achieve the overall goal, the proposed work will be performed parallel in two major areas: 1. Micro-algae cultivation and harvest 2. Algae Oil extraction and transesterification to biodiesel The first part of proposed research is directed at the low input, efficient cultivation of micro-algae with optimum oil content: 1. Demonstrate the feasibility to achieve with small-scale laboratory systems the high solar conversion efficiencies and oil productivities required for biodiesel production 2. Isolate and study strains suitable for mass cultures, then apply the productivity enhancement techniques developed under laboratory conditions to these strains. The second part of proposed research is aimed at the direct integration of the economical and improved extraction process and the efficient transesterification process for biodiesel production. The research effort will be directed at development/optimization of each process on a bench scale: 1. Develop and optimize the integrated extraction and transesterification process for economical production of biodiesel from oil-bearing crops such as algae 2. Determine optimal solvent composition for extraction and transesterification 3. Reduce operational costs by eliminating steps such as distillation. The specific objectives will be met through comprehensive experiments in the laboratory designed to optimize solvent and reagent composition and condition for maximizing extraction and transesterification. Comprehensive analysis of methyl soyate and glycerol streams obtained at different stages in the process will be carried out to ascertain the efficiency and establish the need for additional purification. The process should lower the production costs of biodiesel, with the anticipated increase in biodiesel demand the economical impact of the new combined extraction and transesterification process will be very large.

APPROACH: Collaborative experiments outlined below will be conducted by the interdisciplinary research team consisting of microbiologist (Dr. Keesoo Lee), chemist (Dr. Paul Nam) and phycologist (Dr. Fabio Rindi). Part 1. Experimental Approach for Micro-Algae Cultivation and Harvest: Micro-algae strain: Of many algal strains available for investigation of growth and oil production properties, the best candidates recommended are from two classes, the Chlorophyceae (green algae) and the Bacilliarophyceae (diatoms). However, the ideal algae stain(s) for oil production will likely be different for each location, particularly for growth in outdoor ponds. Algal culture technique: We will initially cultivate them by the indoor culture method utilizing the greenhouse facility at Lincoln University. For mass cultivation of algae we will also explore the continuous culture method in which a supply of fertilizer enriched water is continuously pumped into a growth chamber and the excess culture is simultaneously removed, maintaining the cultures close to the maximum growth rate. Quantification of algal biomass: The quantity of algal biomass present in cultures will be determined by counting the number of cells or through measurement of volume, optical density or weight. Cellular volume will be measured by centrifuging samples and measuring the volume of the concentrated paste. Dry weight of the algal biomass will be determined by weighing after the lyophillization. Harvesting micro-algae: High-density algal cultures will be concentrated by chemical flocculation followed by centrifugation to remove excess water. Part 2. Experimental Approach for Algal Oil Extraction and Conversion to Biodiesel: Research efforts will be directed at the optimization of three areas; extraction parameters, conditions for solvent/oil separation, and direct transesterification. Comprehensive analysis of methyl soyate and glycerol streams obtained from varied processing conditions will be carried out to ascertain the efficiency and quality. Oil Extraction: We will explore solvent system that can selectively extract triglycerides and efficiently convert them to fatty acid methyl esters for use as the biodiesel fuel. All experiments will be carried out with micro-algae obtained from small-scale production. Extraction solvents investigated will be mixtures of HFC/hexanes in varied ratios. Transesterification: Fatty acid methyl esters (biodiesel) will be synthesized through base catalyzed transesterification of extracted algae oil with methanol. Gas Chromatograph/Mass Spectrometer Analysis of Biodiesel: Comprehensive analysis of biodiesel and glycerol streams obtained at different stages in the process will be carried out to ascertain the efficiency and establish the quality of the product. Cost Analysis for Algae Oil Extraction and Conversion to Biodiesel: For the process model to estimate micro-algae derived biodiesel production costs, we will use a commercial software available. The cost estimation of the proposed methods will be compared with the conventional biodiesel production from soybean feedstock.

PROGRESS: 2007/10 TO 2011/09
OUTPUTS: The flue gas carbon dioxide from a 60 MW coal-fired power plant was used to cultivate the native microalgae in five deep circular pools that can hold total 10,000 gallons. The effect of flue gas without any desulfurization treatment on microalgae growth and biomass production was evaluated under the Midwest weather conditions. Utilization of the wastewater nutrients for microalgae cultivation was investigated. The results showed the potential of using microalgae for bioremediation of wastewater to reduce nitrogen and phosphorus. Since the prevalence of herbicides such as atrazine in agricultural runoff can present a problem, the maximum tolerance level of atrazine in wastewater for growing algae was determined. The ability of microalgae to use the soluble carbonate salts for their growth was examined as a way to capture and utilize carbon dioxide from power plants if the algae cultivation ponds are located in the distance. Harvesting microalgae from the cultivation ponds has been a major hurdle. The conventional harvesting methods such as centrifugation and filtration are time and labor intensive processes. Gravimetric settlement with the aid of cationic flocculants is the most widely employed technique, but suffers from the difficulty in recovering microalgae flocs. Furthermore, recycling of these flocculants after the harvesting process is not possible. A novel and efficient process is developed that involves the flocculation of microalgae with specially

synthesized particles to efficiently settle the microalgae, easily remove flocs using magnet, and recycle the recovered flocculant particles. Catalyst-free transesterification reaction in the supercritical alcohol is investigated for the production of biodiesel fuel and epoxy resin. High temperature and pressure conditions help to accelerate the transesterification reaction since the supercritical alcohol has an enhanced contact with the oil. The supercritical transesterification reaction is conducted without the traditional acid/base catalyst and allows easier recovery of pure biodiesel product. The supercritical alcohol method is also employed with small-volume reactors that are designed for the fast analysis of oleaginous biomass samples. PARTICIPANTS: Nothing significant to report during this reporting period. TARGET AUDIENCES: Nothing significant to report during this reporting period. PROJECT MODIFICATIONS: Nothing significant to report during this reporting period.

IMPACT: 2007/10 TO 2011/09
The proposed microalgae-based bioenergy research program will support two high priority areas: sustainable, renewable bio-energy/fuel and global climate change. The interest toward biomass as alternative sources of energy and raw material is on the rise due to concerns about depleting petroleum reserves and greenhouse gas problem. Biomass is considered a renewable energy resource with net zero carbon emissions due to the fact that CO2 in the atmosphere is fixed through photosynthesis. Increasing demand for biofuel has exposed a great need for the discovery of more productive, non-food sources of oils and readily-fermentable biomass to produce biodiesel and ethanol, respectively. Successful demonstration of the algal biomass and biofuel production using the flue gas CO2 from coal-fired power plant emission will ultimately contribute to the reduction of greenhouse gases and make a positive impact in the area of global climate change. Multidisciplinary research activities addressing these critical issues will also help advance the body of knowledge in basic and applied sciences involving food and agriculture. The proposed research and educational capacity building project will involve multidisciplinary approaches in the areas of biology, chemistry, and engineering at multiple locations (Lincoln University of Missouri, Missouri University of Science & Technology, and the Chamois Electric Power Plant) for the production of microalgal biomass and conversion to the usable forms in the area of energy and agriculture. By working together with a major research university as well as the energy producing industry, Lincoln University will offer exciting research opportunities and expose students to experiential learning curricula. These opportunities will help to recruit, train, and mentor quality students from underrepresented groups and strengthen the Nation's scientific and professional workforce.

PUBLICATIONS (not previously reported): 2007/10 TO 2011/09
Modi, D., Nam, P., and Lee, K. (2010) Transesterification of Bio-Renewable Oils with Supercritical Alcohols. Abstracts of Papers, 2010 AIChE Annual Meeting, Salt Lake City, UT, Nov. 7, 2010 Dudenhoeffer, N., Viswanathan, T., Lee, K., and Nam, P. (2011) Algae for bio-fixation of flue gas carbon dioxide and sustainable biomass production. Abstracts of Papers, 16th Biennial Research Symposium, Association of Research Directors, Atlanta, GA. April 9-13, 2011 Dudenhoeffer, N., Nam, P., Kandasamy, G., and Lee, K. (2011) Microalgal Biomass Production Coupled with Bio-Fixation of Flue Gas Carbon Dioxide. Abstracts of Papers, 47th Annual Meeting of Missouri Academy of Science, Jefferson City, MO, April 15-16, 2011 Webpage Article. Oct. 29, 2010. KBIA, Columbia, MO, "Fueling hope in algae", http://www.kbia.org/news/fueling-hope-in-algae Webpage Article. Oct. 27, 2010. Harvest Public Media, "Fueling hope in algae", http://www.harvestpublicmedia.org/article/fueling-hope-algae News Blog. Oct. 6, 2010. Harvest P

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ACCESSION NO: 0223012 **SUBFILE:** CRIS
PROJ NO: MOX-LEE10 **AGENCY:** NIFA MO.X
PROJ TYPE: OTHER GRANTS **PROJ STATUS:** NEW
CONTRACT/GRANT/AGREEMENT NO: 2010-38821-21444 **PROPOSAL NO:** 2010-02521
START: 15 AUG 2010 **TERM:** 14 AUG 2013 **GRANT YR:** 2010
GRANT AMT: $499,984

INVESTIGATOR: Lee, K.; Nam, P.

PERFORMING INSTITUTION:
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MICROALGAE-BASED BIOFUELS AND BIOPRODUCTS

NON-TECHNICAL SUMMARY: Although the R&D on the use of microalgae has been carried out for many decades, the microalgae resources for biofuel production are still in the early stages. The production of microalgae for high value products, i.e., nutritional supplements, is well established. Species like Spirulina, Haematococcus, Chlorella and Dunaliella are cultivated in open ponds for the production of nutritional products, specialty animal feed, etc. Constant increases in the price of fossil fuels and concerns about greenhouse gases over the past few years have placed microalgae back into the R&D limelight as an alternative biodiesel source. Nevertheless, low value algal products for biodiesel need a massive boost in R&D to overcome technical difficulties and the large cost advantage of other biodiesel feedstocks. Microalgae strains already isolated from Missouri and surrounding areas of the Midwest through our ongoing research activities with a goal to find strains that are resistant to invasion and adapt well to local environments, will be evaluated for mass cultivation and conversion. A pilot open-pond algae cultivation system that utilizes flue gas CO2 from a coal-fired power plant has been constructed this year and will be available for the proposed R&D to facilitate the commercialization of microalgae-based bioremediation and biofuel technologies. The research will specifically focus on the selection of algal strains suitable for the region and the improvement of technologies involving harvesting/dewatering processes, conversion to biodiesel and bioethanol, and examination of the residual algal biomass as a bio-fertilizer. Success of the project will enable us to generate highvalue/high quality agricultural products from sustainable sources and ultimately benefit Midwestern farmers. In addition, this project seeks to produce many graduates with training in alternative energy and material areas that are in great demand.

OBJECTIVES: Overall goals of this multidisciplinary (phycology, microbiology, chemistry, and engineering), multi-institutional (Lincoln University of Missouri, Missouri University of Science & Technology, regional electric power cooperatives, and USDA-ARS laboratory) joint research and educational project are: (1 to find solutions to the difficulties encountered in the R&D of microalgae biomass production and conversion to biofuels and other biomaterials, and (2 to train students to integrate knowledge across disciplines through experiential learning opportunities for underrepresented students in bioenergy-related science and engineering. Outputs expected are: (1) successful demonstration of the **algal** biomass and **biofuel**/material production using the flue gas CO2 from coal-fired power plant emission, (2) reduction of green house gases and positive impact on the global climate change, and (3) increased number of students trained in bioenergy and agricultural science and engineering.

APPROACH: The research will specifically focus on the selection of algal strains suitable for mass cultivation in the Midwestern region and the improvement of technologies involving harvesting/dewatering processes, conversion to biodiesel and ethanol, and examination of the residual algal biomass as sources for bio-fertilizer and plastics. A pilot open-pond algae cultivation system that utilizes flue gas CO2 from a coal-fired power plant will be tested for the integration of microalgae-based bioremediation and biofuel technologies. We envision a system that integrates the microalgae cultivation facility with the biorefinery that is dedicated to algae-based biofuels and bioproducts. This integrated system will bypass a number of traditional refining processes and allow self-supported, economical production of microalgae biomass and conversion to biofuels and biomaterials. Final product of the proposed research work will be proof-of-concept for such a system.

PROGRESS: 2010/08 TO 2011/08
OUTPUTS: Various algal samples collected from Midwest USA were screened for prospective algal strains for their possible application in bioremediation of flue gas carbon dioxide from coal fired power plants and biofuel production. Initially, 38 microalgae strains were screened by their 18S rRNA and rbcL genes sequence analysis. All these strains were established in liquid and solid media. Total lipids and their respective fatty acids profiles were analyzed by GCMS using authentic standards and also by comparing the mass spectra. Scenedesmus sp. LU1 was isolated from a freshwater body near Lincoln University and was characterized based on its morphological features and 18S rRNA sequence analysis. Scenedesmus sp. LU1 was cultured in different autotrophic media BBM, BG11 & F2 along with their respective trace mix enriched commercial fertilizers HJ and MG media. The microalgae Scenedesmus sp. LU1 was also analyzed for its single-cell protein (SCP) contents. Heterotrophic cultivation of algae is also gaining importance in utilizing waste waters rich in organic compounds and hence initial screening for the potential algal strains for their application in heterotrophic and mixotrophic culture conditions experiments are underway. Excessive use of synthetic fertilizers leads to soil degradation over time and runoff contamination of water systems. Bio-fertilizers composed of a consortium of live formulates of microorganisms are a possible solution which will promote long-term soil health and the quality of crop products, as well as mitigating the environmental hazards associated with synthetic fertilizer. Wet slurries comprising of microalgae and natural micro-flora, were evaluated for the bio-fertilizer. From the nutrient compositional analysis of corn plants grown with this microalgae-based bio-fertilizer, total plant nitrogen increased greatly. Addition of algal bio-fertilizers to farm soil was as effective as a commercial organic fertilizer in improving the overall height and collar length of corn plant. Although the algal biomass is less recalcitrant to ethanol fermentation due to the absence of lignin, an effective pretreatment method is required for the starch and cellulosic components that need to be hydrolyzed into fermentable sugars. The current research is directed at the technology innovation to advance the algal biomass pretreatment and bioethanol conversion processes in order to improve the yield and reduce the cost. The hydrothermal pretreatments in addition to dilute acid in sealed vessels are being evaluated for the efficient conversion of algal biomass to monomeric sugars suitable for ethanol fermentation. The pretreated and hydrolyzed algae will be fermented using industrial yeast, Saccharomyces cerevisiae, and analyzed for ethanol and byproducts. PARTICIPANTS: Nothing significant to report during this reporting period. TARGET AUDIENCES: Nothing significant to report during this reporting period. PROJECT MODIFICATIONS: Nothing significant to report during this reporting period.

IMPACT: 2010/08 TO 2011/08

The research and education activities continue to help to achieve the ultimate goal of producing algae-based alternative fuels and materials that are economical and sustainable. The microalgae research are showing their potential as higher-yielding, new bio-based feedstocks that do not compete with the current food and feed production, and can relieve strains on the price and natural resources from increased utilization of food crops for producing biodiesel and ethanol. Potential environmental benefit from algal biomass production seems to be the recycling of carbon dioxide generated by coal-fired power plants that will contribute in reducing green house gases and making a positive impact on the global climate change. Utilization of wastewater or saline water for the algae cultivation and of farm runoff as a source of nutrients seems to be also feasible. The project continues to provide the opportunity for graduate and undergraduate students to engage in hands-on laboratory and field experiments. Participating in the collaborative and summer research activities, students had direct access to multi-faceted research equipment and facilities and became exposed to a diverse array of research projects involving algae cultivation and conversion processes for biofuels and biomaterials. Students developed problem-solving skills and learned broad scientific knowledge. This also created opportunities for students from a number of different disciplines to collaborate and learn. Multi-institutional collaboration between Lincoln University and Missouri University of Science & Technology provided a mechanism to extend the outreach to an institution without a graduate program and adequate research facilities/equipments via making available summer research collaboration and student research opportunities. Another component of the outreach activity was the development and transfer of new technology to the agricultural community and bioenergy/biomaterial industry.

PUBLICATIONS (not previously reported): 2010/08 TO 2011/08
1. Kandasamy, G., Nam, P., and Lee, K. (2011) Microalgae as Bio-Fertilizer. Abstracts of Papers, 16th Biennial Research Symposium, Association of Research Directors, Atlanta, GA. April 9-13, 2011
2. Kandasamy, G., Dumbach, D., Nam, P., and Lee, K. (2011) Microalgae Based Bio-Fertilizers Evaluated for Corn Plant. Abstracts of Papers, 47th Annual Meeting of Missouri Academy of Science, Jefferson City, MO, April 15-16, 2011

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ACCESSION NO: 0222121 **SUBFILE:** CRIS
PROJ NO: NEB-30-119 **AGENCY:** NIFA NEB
PROJ TYPE: HATCH **PROJ STATUS:** NEW
START: 01 APR 2010 **TERM:** 31 MAR 2015 **FY:** 2010

INVESTIGATOR: Bailey, C.

PERFORMING INSTITUTION:
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IDENTITY AND FUNCTION OF SUMO IN CHLAMYDOMONAS REINHARDTII

NON-TECHNICAL SUMMARY: This will contribute to knowledge important to solve three agricultural issues: (1) Chlamydomonas reinhardtii is an algae that can be genetically modified to optimize production of **algal**-based **biofuels**. (2) Cilia are important in clearing airways of the throat and lungs. Bovine upper respiratory infections are a leading cause of loss of production on farms and ranches. Understanding how cilia work is important in helping develop robust systems to prevent and treat bovine upper respiratory infections. (3) Cilia and tails of sperm have very similar structures and underlying biological mechanisms. Studying cilia advances the knowledge of healthy moving sperm, which are important to animal husbandry and production.

OBJECTIVES: The major focus of this application is to functionally characterize SUMO proteins and their target proteins in Chlamydomonas reinhardtii, with the focus on flagellar development, stress response and the carbon concentrating mechanism. The proposed study will be guided by three specific aims: 1) identify SUMO protein(s) involved in flagellar and cilia development in algae (Chlamydomonas reinhardtii), invertebrates (Tetrahymena pyriformis), and animal cells (spermatozoa and bovine airway epithelial cells); 2) functionally characterize newly identified target proteins involving flagellar and cilia development, stress response and carbon concentrating mechanisms; 3) analyze SUMO-like fusion proteins and their function in Chlamydomonas reinhardtii. We propose to address the role of SUMO protein in plant biology and animal health by

characterizing functions of SUMO we identified in algae and those previously identified in other species to understand critical questions of organelle development, stress response, and carbon concentrating mechanisms. The long-term potential impact of my work is an increase in mobility and sustenance of algae to produce biofuels, upper-respiratory health of bovine production, and improvement in sperm motility in breeding programs. The three specific aims of this application are as follows: Specific Aim 1: Identify SUMO protein(s) and their target proteins involved in flagellar and cilia development in algae, invertebrate protozoa, and mammalian cells. Specific Aim 2: Functionally characterize newly identified target proteins involving flagellar and cilia development, stress response and carbon concentrating mechanisms. Specific Aim 3: Analyze SUMO-like fusion proteins and their function in Chlamydomonas reinhardtii.

APPROACH: Specific Aim 1: Identify SUMO protein(s) and their target proteins involved in flagellar and cilia development in algae, invertebrate protozoa, and mammalian cells. Our working hypothesis, based on strong preliminary data, is that flagellar protein(s) are SUMOylated during flagellar development. Flagella and cilia share many similarities. This leads to investigating the putative role of SUMO in cilia formation in invertebrate protozoa and animal cells. Others have shown in axons that a dynein binding protein is SUMOylated, while the non SUMOylated protein binds to kinesin. We will pursue whether dynein, kinesin or a protein that binds flagellar proteins is SUMOylated in algal flagellar development and in cilia of other organisms. Specific Aim 2: Functionally characterize newly identified target proteins involving flagellar and cilia development, stress response and carbon concentrating mechanisms. We have published and non-published data that target proteins are SUMOylated during flagellar development and stress response. We have preliminary data that SUMO96 transcripts are elevated at very low CO2 concentrations, suggesting that SUMOylation is involved in the carbon concentrating mechanisms of algae. We will pursue identifying target proteins, and investigating the temporal and spatial SUMOylation of the target proteins in Chlamydomonas reinhardtii. In addition, bioinformatics of the Chlamydomonas genome, analysis of specific sequences, and genome to genome comparisons will identify putative target proteins. Specific Aim 3: Analyze SUMO-like fusion proteins and their function in Chlamydomonas reinhardtii. We have identified three SUMO-like proteins through bioinformatics. These putative gene products are SUMO fusion proteins, and present a novel finding. We will pursue investigating the temporal and spatial expression of these genes at both the transcript and protein level. We propose that these proteins are expressed as SUMO fusion proteins due to the critical nature of SUMOylation for the correct function of the protein. We will test this hypothesis using a combination of RNAi, overexpression, and mutagenesis.

PROGRESS: 2009/10 TO 2010/09
OUTPUTS: This project was disseminated at national and local meetings: The American Society of Biochemistry and Molecular Biology national meeting and at the University of Nebraska, Lincoln research fair poster session. PARTICIPANTS: Cheryl Bailey is an Assistant Professor, Department of Biochemistry, University of Nebraska, Lincoln. Bailey trained on a specialized separation procedure at Applied Biosystems Instruments. Stephanie Matejka was a graduate student, Department of Biochemistry, University of Nebraska, Lincoln. Stephanie took courses in biochemistry, presented her work to the Department of Biochemistry and nationally at the American Society of Biochemistry and Molecular Biology, and learned new techniques in the laboratory. Amy Knobbe is a graduate student in the Department of Biochemistry, University of Nebraska, Lincoln. Amy learned managerial skills by training undergraduates and working with Stephanie Matejka. Jacob Johnson was an undergraduate in the Department of Biochemistry, University of Nebraska, Lincoln. He trained in both computer and laboratory techniques related to this project. TARGET AUDIENCES: Target audiences are students, who include first generation to go to college. Efforts are providing training and mentoring of students to develop as scientists and as future employees. PROJECT MODIFICATIONS: Nothing significant to report during this reporting period.

IMPACT: 2009/10 TO 2010/09
A protein in the isolated cilia of Tetrahymena thermophila and Chlamydomonas reinhardii was detected with an anti-SUMO antibody. This is significant because it indicates the possibility of a modified protein in the same sub-cellular organelle in plant and protist. Cilia are implicated in many cellular processes. The resources of personnel and laboratory supplies helped produce these data.

PUBLICATIONS (not previously reported): 2009/10 TO 2010/09
Stephanie Lynn Matejka, Amy Knobbe, Jacob Johnson, Donald P. Weeks and Cheryl Bailey. SUMO (small-ubiquitin-like modifier) in Tetrahymena thermophila. FASEB J.April 2010. 24 (Meeting Abstract Supplement) 843.2

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ACCESSION NO: 0223502 **SUBFILE:** CRIS
PROJ NO: NEB-35-116 **AGENCY:** NIFA NEB

PROJ TYPE: HATCH **PROJ STATUS:** NEW
START: 01 SEP 2010 **TERM:** 31 AUG 2015 **FY:** 2010

INVESTIGATOR: Van Etten, J. L.

PERFORMING INSTITUTION:
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CHARACTERIZATION OF LARGE ALGAL VIRUSES AND THEIR GENE PRODUCTS

NON-TECHNICAL SUMMARY: There is an increasing interest in using algae as an efficient renewable source of high quality oils for biofuels. However, it is obvious that if algae are grown in large-scale raceways that pathogens, including viruses, will become a major issue. Our laboratory has as much experience as anyone in the world on algal viruses and we plan to continue studying these fascinating viruses. About 30 years ago we discovered and began to characterize the first member of what is now a rapidly increasing family of viruses (Phycodnaviridae) that infect eukaryotic algae. Phycodnaviruses and putative phycodnaviruses are huge (150 to 220 nm in diameter) icosahedral, dsDNA-containing (genomes up to 560 kb) viruses that are ubiquitous in aqueous environments throughout the world. The development of environmentally sustainable, economically viable sources of renewable biofuels is a major goal for the United States and the world. Exacerbation of global climate change associated with the use of fossil fuels is a global challenge requiring new sources of cleaner carbon-neutral fuels. Lipid-rich algae, which require CO2 for growth, are potentially an efficient renewable source of high quality oils that can serve as fuel feedstocks. However, if algae are grown on a large scale then pathogens, including viruses, will become a major issue. For the most part, the threat of viruses has been ignored by people promoting algae. Algal viruses are potentially a greater problem for algae than higher plant viruses are to higher plants because higher plant viruses are usually vectored by insects. Thus, one needs two components for a virus disease outbreak on higher plants, while algal viruses only require one component because they are not vectored. The phycodnaviruses have many additional properties that justify their continued study including: i) Some phycodnaviruses are predicted to have more than 600 protein encoding genes, which are more genes than some bacteria. ii) The viruses are sources of new and unexpected genes. Some of the chlorella virus genes encode commercially important enzymes such as DNA restriction endonucleases, whereas others encode enzymes that are the smallest in their class and may represent the minimal catalytic unit. Consequently, these small proteins often serve as models for mechanistic and structural studies. iii) The viruses are an important source of genetic elements, e.g., promoters and enhancers, as well as enzymes, for genetically engineering plants and algae. iv) The phycodnaviruses probably have a common ancestor with the poxviruses (e.g. smallpox virus) and African swine fever virus (lethal to swine and is quarantined in the USA). Thus chlorella virus properties may be relevant to these other important viruses. v) Phycodnaviruses play a dynamic, albeit largely unknown, role in regulating phytoplankton communities in aqueous environments, such as termination of massive algal blooms commonly referred to as red tides and brown tides. We are well prepared to conduct the proposed research because of our 30 years of work with the chlorella viruses, which were discovered at the University of Nebraska-Lincoln (UNL).

OBJECTIVES: 1. Continue studies on virus PBCV-1 structure and the infection process using 5-fold symmetry averaging reconstruction and tomography procedures. 2. Continue to investigate glycosylation of the chlorella virus major capsid proteins. 3. Evaluate the role of SUMOylation in PBCV-1 replication, specifically cytoplasmic/nuclear transport. 4. Evaluate the role of a chlorella host metacaspase in virus PBCV-1 replication, specifically DNA packaging. 5. Explore chlorella resistance to virus infection. 6. Continue to characterize interesting PBCV-1 gene products.

APPROACH: 1. The virus PBCV-1 structure experiments will be done in collaboration with the Rossmann's group at Purdue University. This is one of the best virus structure groups in the world and they have the appropriate equipment for the studies. We provide the highly purified virus. 2. Some of the virus structure work will be conducted in collaboration with the mass spectroscopy center at the University of Nebraska. A new faculty member is arriving in Sept, 2010 who has extensive expertise in analyzing post translationally modified proteins. We will provide him with the appropriate material for analysis. We will be identifying and cloning the virus-encoded genes involved in the process. 3. Objectives 3, 4, and 6 will require the growing of the virus and standard cloning and biochemical assays. 4. Objective 5 will dependent our extensive experience in growing algae and purifying the viruses that infect them.

PROGRESS: 2010/10 TO 2011/09
OUTPUTS: This project is to characterize large dsDNA viruses, commonly referred to as chlorella viruses that infect freshwater algae. These viruses and their evolutionary relatives, which include the poxviruses (e.g. smallpox and the gigantic amoeba virus called megavirus), contain more protein encoding genes (i.e. 350 to 1100) than any other virus. The chlorella viruses are ubiquitous in freshwater from around the world and they can reach titers of >100,000 infectious particles per ml of native water. We study many aspects of the chlorella viruses and their gene products, often with collaborators at other institutions. Some of our current projects include: i) Continue to attempt to produce an atomic structure of the prototype chlorella virus PBCV-1. Currently, we are at about 8 angstron resolution. PBCV-1 attachment to its host and the initial infection process differs from all other viruses that infect eukaryotic organisms. Unexpectedly, we discovered that chlorella virus PBCV-1 has a unique vertex with a spike structure; this was published this year. ii) Unlike all other glycoprotein-containing viruses,

the chlorella viruses encode most, if not all, of the machinery used to glycosylate their major capsid protein. Futhermore, the glycosylation process occurs in the cytoplasm rather than in the traditional endoplasmic reticulum - Golgi pathway used by eukaryotes. During the past year we have identified several virus mutants that contribute to the glycosylation process. iii) We are determining the DNA structural organization in PBCV-1 virions. The DNA is neutralized by basic proteins rather than cations and polyamines that is typical for bacterial viruses. iv) We have conducted an intensive transcriptome analysis during the first hour of PBCV-1 infection. The sequences are currently being analyzed by bioinformatics collaborators at the University of Marseille. v) A functional virus-encoded potassium transporter was expressed and biochemically characterized (manuscript in press). vi) We led the effort to have the green alga Coccomyxa subellipsoidea sequenced by JGI. This is the first eukaryotic microorganism from a polar environment to be sequenced and a manuscript describing its annotation is about ready for submission. PARTICIPANTS: Mr. Jim Gurnon, technologist at UNL, Dr. Dave Dungian, research associate professor at UNL, Dr. Irina Agarkova, research assistant professor at UNL. Dr. Janet Rowe, Postdoctoral Researcher at UNL who left in March of this year. Cristian Quispe, graduate student at UNL. As indicated above we also collaborate with several laboratories around the world. TARGET AUDIENCES: An international group of scientists who study the dynamic role of viruses in regulating phytoplankton communities in aqueous environments such as the termination of massive algal blooms commonly referred to as red tides and brown tides. People involved in the emerging algal biofuels industry will also benefit from our work. PROJECT MODIFICATIONS: Nothing significant to report during this reporting period.

IMPACT: 2010/10 TO 2011/09
The 46.2 Mb genome of one of the chlorella virus hosts, Chlorella variabilis, was sequenced and annotated. Chlorella species are considered to be excellent candidates for large-scale algal biofuels production and this is the first Chlorella isolate to have its genome sequenced and annotated. This paper generated quite a bit of interested in the scientific community. We are completing the sequence and annotation of another 48.8 Mb green alga genome, Coccomyxa subellipsoidea, which represents the first eukaryotic microorganism from a polar (Antarctica) environment to have its genome sequenced.

PUBLICATIONS (not previously reported): 2010/10 TO 2011/09
1. Greiner, T., Ramos, J., Alvarez, M., Gurnon, J.R., Kang, M. Van Etten, J.L., Moroni, A., and Thiel, G. (2011). A functional HAK/KUP/KT-like potassium transporter encoded by chlorella viruses. Plant Journal (in press).
2. Van Etten, J.L. (2011). Giant viruses. Am. Scientist. 99, 304-311.
3. Van Etten, J.L. and D. Dunigan(2012). Chloroviruses: not your everyday plant virus. Trends Plant Sci. (in press) (Cover photo).
4. Wulfmeyer, T., C. Polzer, G. Hiepler, K. Hamacher, R. Shoeman, D.D. Dunigan, J.L. Van Etten, M. Lolicato, A. Moroni, G. Thiel, and T. Meckel. 2012. Structural organization of DNA in chlorella viruses. PloS One (in press).
5. Wilson, W. H., J.L. Van Etten, D.S. Schroeder, K. Nagasaki, C. Brussaard, G. Bratbak, and C. Suttle. (2012). Phycodnaviridae. In: Virus Taxonomy, IXth Report of the ICTV (A.M.Q. King, M.J. Adams, E.B. Carstens, E.J. Lefkowitz, eds), pp. 219-262. Elsevier/Academic Press, Amsterdam.
6. Van Etten, J.L. (2012). Genus Chloroviruses (Phycodnaviridae). In: The Springer Index of Viruses, 2nd edition. C. A. Tidona and G. Darai (eds). pp. 1242-1252. Springer-Verlag, Berlin.
7. Smith, D.R., F. Burki, T. Yamada, I.V. Grigoriev, J.L. Van Etten, and P.J. Keeling. (2011). The GC-rich mitochondrial and plastid genomes of the green alga Coccomyxa give insight into the evolution of organelle DNA nucleotide landscape. PloS One 6, e23624.
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9. Van Etten, J.L. (2011). Another really, really big virus - Commentary. Viruses 3, 32-46.

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ACCESSION NO: 0212419 **SUBFILE:** CRIS
PROJ NO: NEV003GN **AGENCY:** SAES NEV
PROJ TYPE: STATE **PROJ STATUS:** EXTENDED
CONTRACT/GRANT/AGREEMENT NO: NOT APPLICABLE
START: 15 JUL 2007 **TERM:** 30 SEP 2012 **FY:** 2011

INVESTIGATOR: Cushman, J.; Shintani, D.; Harper, J.

PERFORMING INSTITUTION:
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BIOFUELS FROM SALT BASIN ALGAE: A RENEWABLE ENERGY CROP FOR CARBON SEQUESTRATION

NON-TECHNICAL SUMMARY: The goal of this research is to develop salt-loving microalgae as alternative biofuel feedstocks. Existing oilseed feedstocks derived from terrestrial plants cannot satisfy the current or projected transportation fuel needs of the U.S. Biofuel producing algae are one of the only avenues available for high-volume capture and reuse of CO2 generated in biomass-, coal- or natural gas-fired power plants and can provide the intermountain west with a major new "cash crop" without putting additional demands on freshwater supplies needed for residential, industrial and agricultural use. Nevada researchers and producers are uniquely enabled to leverage the geothermal, high solar radiation, ample land area, and salt basins to produce microalgea in a scalable and economically viable manner. The research rationale is to identify the algal strains that have the greatest economic potential for biofuel production. The purpose is to identify key components of the endogenous TAG biosynthetic pathway to learn how to improve oil production and alter desirable oil characteristics with immediate and significant impact on the emerging **algal** feedstock **biofuels** industry. In addition, a room-size "demonstration" scale production facility will be developed to educate scientists, investors, and the lay public about the potential feasibility of the algae-to-biodiesel conversion process and related technologies. Systems will be readily scalable with a minimum of capital investment in contrast to closed bioreactor systems that are capital intensive and not readily scalable.

OBJECTIVES: The long-term goal of the proposed research is to optimize and implement the use of halophytic microalgae as a biofuel crop. Halophytic green algae are ideally suited as a non-seasonal, renewable energy resource for the arid western U.S. because they are 30 times more productive than terrestrial feedstocks (e.g., soy, canola), can be grown on marginal lands with brackish or saline water unsuitable for traditional agriculture, and provide unlimited potential for sequestration of CO2 from biomass, coal, and gas-fired power plants. Research goals are 1) to determine the oil production potential for 20 strains of Dunaliella that can grow in the Great Basin and Intermountain West; 2) to conduct mutant screens to identify optimal stains of Dunaliella with enhanced production of triacylglycerols (TAGs) suitable for conversion to biodiesel and 3) to identify genes that control oil production by microarray expression profiling. Education and extension aims of the proposal are: 1) to develop a room-size "demonstration" scale production facility whose purpose will be to educate scientists, investors, and the lay public about the potential feasibility of the algae-to-biodiesel conversion process and related technologies and 2) to develop pilot-scale "proof-of-concept" production facilities in Wabuska and Lovelock, NV with private sector collaborators to optimize biodiesel production from algal feedstocks that leverages geothermal and/or biomass cogeneration resources and to assess the economic feasibility and impact of algae-to-biodiesel production systems on local communities.

APPROACH: The approach is to grow Dunaliella spp. under a set of defined conditions to ascertain which conditions promote optimal oil production. Conditions tested will include salt concentration, pH, light intensity, and nutrient availability. A variety of approaches will be used to select for and isolate mutants that have improved oil content, including buoyant density gradients, lipid staining, and gas chromatography. Fatty acid profiles of all strains will be monitored to ensure their suitability for biodiesel production. We will leverage the availability in late 2007 of the complete genome sequence of Dunaliella salina to fabricate an oligonucleotide-based microarray and to monitor changes in mRNA profiles during nutrient starvation-induced increases in TAG production. We will implement algae-to-biodiesel production systems by creating working test-bed facilities at room-scale and pilot-scales to optimize biodiesel production from algal feedstocks that leverages geothermal and/or biomass cogeneration resources and to assess the economic feasibility and impact of algae-to-biodiesel production systems on local communities.

PROGRESS: 2010/01 TO 2010/12
OUTPUTS: During the project reporting period, PI Cushman presented several invited lectures including: Nevada Renewable Energy Consortium Lecture Series at the University of Nevada, Las Vegas, NV on September 7, 2010 and the 2010 Pacific Rim Summit of the Biotechnology Industry Organization (BIO) in Honolulu, HI on December 12, 2010. In addition, graduate student Hathwaik presented a poster at the 2nd Annual Meeting of the Nevada Renewable Energy Consortium (NVREC) at the University of Nevada, Las Vegas, NV on August 20, 2010. Postdoctoral scholar Hiibel presented three posters at the 2nd Annual Meeting of the Nevada Renewable Energy Consortium (NVREC) at the University of Nevada, Las Vegas, NV on August 20, 2010; at the UNR College of Engineering in Reno, NV on October 14, 2010; and at the Annual Meeting of the American Institute of Chemical Engineers (AIChE), November 7-12, 2010, Salt Lake City, UT. Reports on the Dunaliella salina organelle genomes (Smith et al., 2010), EST collections from salinity shocked cells (Alkayal et al., 2010), and a review on Dunalliella (Ramos et al., 2011) have now been published or submitted. PARTICIPANTS: To date, two graduate students (Mark Lemos and Leyla Hernandez-Gomez) and one postdoctoral research associate (Dr. Sage Hiibel) are being trained as independent scientists and participate in our regular meetings and conference calls. Each is receiving advising and mentoring from the project director about their various research activities and their career development goals. Four undergraduate students (Kim Rafter, Samantha Kertson, Alexander Lewis, and Brian Lelly) have also been trained on the project. One lab manager has been trained on the project in a wide range of preparative and analytical techniques related to the specific requirement of the project: Rebecca Albion, Staff Research Associate II, University of Nevada, Reno (UNR). Research on the use of wastewater is being conducted in collaboration with Dr. Eric Marchand in the Department of Civil and Environmental Engineering at UNR. Research on algal paste extraction is being conducted in collaboration with Dr. Charles Coronella in the Department of Chemical and Metallurgical Engineering at UNR. Dr. Juergen Polle of Brooklyn College, City University of New York collaborates with us on all aspects of Dunaliella genetics and molecular biology. Private sector partners include Dr. John

Bebout, Bebout & Associates (http://bebout-and-associates.com/), Mr. Jeffrey Eppink, Enegis LLC, (http://www.enegis.com/), and Alton Reich, Streamline Automation (http://streamlineautomation.biz/site/). TARGET AUDIENCES: The project results and outcomes were targeted to the scientific community at national and international scientific meetings, specifically to research scientists and undergraduate and graduate students and post-doctoral researchers conducting research on the development of algae as feedstocks for biofuel production. Outreach projects are also being targeted to the lay public through poster presentations at an annual meeting of the Nevada Renewable Energy Consortium (NVREC) at the University of Nevada, Las Vegas, NV and research outcomes are being presented to scientific audiences through oral and poster presentations at regional, national and international meetings. PROJECT MODIFICATIONS: Nothing significant to report during this reporting period.

IMPACT: 2010/01 TO 2010/12
Analysis of growth rates, triacylglycerol, free fatty acid content, and insoluble starch content for nineteen (19) strains of halophytic microalgae (Dunaliella salina) has been completed and analysis of results is being finalized for publication. Dunaliella cells contain a relatively low triacylglycerol content averaging only 0.56% on a dry weight basis (range = 0.18-1.91%) with the following fatty acid compositions: linolenic C18:3 > linoleic C18:2 > palmitic C16:0 > oleic C18:3, > palmitoleic C16:1 = hexadecadienoic C16:2 > C16:4. In addition, we have explored the use of centrate, the liquid fraction created when anaerobically digested wastewater sludge is dewatered for disposal purposes, as a low cost, nutrient sources for growing algae. Interestingly, eleven (of 18) salt-water Dunaliella species were shown to grow in up to 50% v/v centrate. The 18S rRNA gene and adjacent internal transcribed spacer (ITS) regions were used to provide a facile method to unambigouously identify and classify the Dunaliella strains. The sequencing of 18s rDNA gene and adjacent ITS region has been completed for eighteen and nineteen strains, respectively. We have also begun the sequencing of ~30 different freshwater green algae strains including Chlorella, Neochloris, and Nannochloropsis species. Transgressive selection trials of wildtype and ethyl methyl sulfonate (EMS) mutagenized strains of Dunaliella salina CCAP 1918 have continued through 53 rounds of selection of wildtype and mutant cells by buoyant density gradient centrifugation. Flow cytometry with Nile Red dye staining has been performed successfully to assess the effects of reiterative selection and associated lipid content changes. Both buoyant density gradient centrifugation and flow cytometry followed by fluorescence activated cell sorting can be effectively used to select for significant increases in lipid and/or starch content from within wildtype or mutagenized algal cell populations without resorting to genetic engineering. Currently, a ~4 X assembly of the genome has been completed by JGI resulting in complete mitochondrial (28.8 kb) and plastid (269 kb) genome sequences which have now been published (Smith et al., 2010). The nuclear genome contains 60% repetitive DNA consisting mainly of simple sequence repeats and this is making contig assembly especially challenging with only 191 MB of 350 Mb currently being assembled. EST sequencing of mixed cDNA libraries derived from various environmental stress conditions by Sanger (37,391 ESTs) and Roche 454 (2,252,211 ESTs) sequencing produced over 1.5 M cleansed reads and about 21,000 unigenes. Analysis of a small collection of 2831 ESTs from salt-shocked D. salina cells has also been completed (Alkayal et al., 2010). Such information, along with additional rounds of paired end reads, will be needed to improve the genome assembly and annotation. In order to identify genes that control oil production, an experiment using a custom microarray containing probes for 21,000 unigenes derived subjecting D. salina cells to 0, 12, 24, 48, and 72 h nutrient deprivation has been completed with the largest change in gene expression patterns occurring after 72 h.

PUBLICATIONS (not previously reported): 2010/01 TO 2010/12
1. Smith DR, Lee RW, Cushman JC, Magnuson JK, Tran D, Polle JEW. (2010) The Dunaliella salina organelle genomes: large sequences, inflated with intronic and intergenic DNA. BMC Plant Biology. 10:83.
2. Alkayal MF, Albion RL, Tillett RL, Mark S. Lemos, Hernandez-Gomez L, Cushman JC. (2010) Expressed Sequence Tag (EST) profiling in salinity shocked Dunaliella salina reveals high expression of protein synthetic apparatus components. Plant Science. 179: 437-449.
3. Ramos AA, Polle J, Tran D, Cushman JC, Jin ES, Varela JC. (2011) The unicellular green alga Dunaliella salina Teod. As a model for abiotic stress tolerance: Genetic advances and future prospects. Algae. Submitted.

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ACCESSION NO: 0227974 **SUBFILE:** CRIS
PROJ NO: NJ12116 **AGENCY:** NIFA NJ.
PROJ TYPE: HATCH **PROJ STATUS:** NEW
START: 01 OCT 2011 **TERM:** 30 SEP 2016

INVESTIGATOR: Lam, E.

PERFORMING INSTITUTION:
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SUSTAINABLE ENERGY PRODUCTION USING DUCKWEED BIOMASS THROUGH OPTIMIZED WASTE-TO-FUEL TECHNOLOGIES

NON-TECHNICAL SUMMARY: The quest for renewable energy alternatives to fossil fuels that have low carbon footprints has become a global priority. In response to the urgent call for significant decreases in Greenhouse Gas (GHG) emission, the Renewable Fuel Standard provision of the Federal Energy Independence and Security Act of 2007 requires 36 billion gallons of biofuels to be used in our nation's transportation fuel supply by the year 2022. Of these, 21 billion gallons are expected to derive from cellulosic and other "second generation (i.e. non-corn starch-based)" biofuels. Two major alternative biofuel strategies are being pursued worldwide. In the so-called second generation biofuels, technologies are being optimized for conversion of cellulosic feedstock materials into sugars for subsequent fermentation. However, cellulose is heavily fortified in plant-based feedstocks and requires significant energy input and enzyme pretreatments to aide its transformation into fermentable sugars with current technologies. With current estimated production cost of cellulosic ethanol at about 3 times that of corn-starch ethanol, it is unclear if and when cellulosic bioethanol will become economically viable. The situation with algal biodiesel, also called third generation biofuel, is perhaps even a bit worse since the scale-up of this approach has been particularly problematic. One of the major issues, for example, is the economical separation of algal biomass from the aqueous medium in which it has been growing. A recent life cycle analyses of these different biofuel feedstocks have raised significant concerns over their true environmental impact, especially for **algal biofuels**. In our consideration of alternative sources of renewable biomass that can be "domesticated" for energy production, we believe the Lemnaceae family of aquatic plants, commonly called duckweeds, holds great potential for the development of a commercially viable feedstock as a micro-crop for fuel production. The chief characteristics that make duckweeds ideal for waste-to-energy conversion are their rapid growth rate, easy harvesting potential, and ability to grow directly on existing wastewater sites. To realize these advantages of this micro-crop system, my laboratory will carry out research to develop new aquatic agronomic methods for deploying selected duckweed strains as a waste-to-fuel platform on local sites in New Jersey. In the next five years, we endeavor to 1) create a functional and sustainable pilot pipeline in which bioethanol can be produced from duckweed harvested from wastewater sites, 2) optimization of the harvesting and processing methods to improve the economic output of the system, 3) carry out a full Life-Cycle-Analysis of the completed demonstration pipleline by the end of the 5 year project, and 4) educating the public and relevant agencies on the potential of this novel micro-crop and facilitating research on this aquatic plant model as well as its applications.

OBJECTIVES: 1. Identification of optimal strains of duckweed for biomass production from municipal wastewater. Systematic comparison of growth rate, starch and protein content will be performed with selected strains from our collection. Using both synthetic rich media as well as wastewater samples obtained from two different sites in New Jersey, this work will aim to identify high growth rate duckweed strains that also can accumulate high concentrations of target components such as starch (for bioethanol production) or protein (for animal feed). 2. Creating a sustainable wastewater-to-fuel pipeline with the duckweed platform. By harvesting indigenous duckweed that currently thrives on the fertilizer run-off containment ponds of the Pinelands Nursery in South Jersey and producing fuel-grade ethanol from this biomass, we seek to create a complete demonstration pilot for this technology. In addition to using this pilot to attract investors, this pipeline will also allow us to carry out important LCA studies to determine the economics and environmental impact of this platform. 3. Outreach activities using the duckweed platform. In addition to continuing our ongoing outreach activities with the 4-H and Waksman High School Scholar programs on campus, we will seek out international and national opportunities for implementing the duckweed biofuel platform in disadvantaged communities through the involvement of philanthropic groups such as em[Power] and Engineers Without Borders. At the present time, we are orchestrating activities in Mexico, Brazil and Pakistan through local contacts in these countries. Expected Impact: Through our activities described here, we endeavor to develop duckweeds into an economically sustainable source for renewable fuel production worldwide. A key focus for our research work will be to couple wastewater treatment to fuel production that is driven by solar energy through duckweed. In this way, we believe duckweed can be the "greenest" fuel possible with minimal GHG emission compared to corn ethanol or algal biodiesel. We expect our work will play a major role in getting this new crop system adapted and perhaps spark a new source of renewable fuel in the near future of 2 to 5 years. The success of this project will have local impact in New Jersey and elsewhere in the U.S. as well as internationally by creating a new industry for the agronomic deployment of duckweed micro-crops. This will translate into jobs and societal benefits in the new Green Economy that is rapidly growing worldwide.

APPROACH: 1). Growth Rate Comparison on Wastewater from Ponds/Lagoons. Selected strains of duckweed will be grown on sterile MS liquid medium and then transferred to open containers containing one or the other wastewater sample at 100 mL each. These will then be grown in a roof-top greenhouse at the PI's laboratory and growth rate compared as for aseptic samples. Comparison of data obtained from this study to those with aseptic plant growth media should provide clear information on the most appropriate strains with the fastest biomass production rates on these two types of wastewater source. 2). Sugar and Starch Content Quantification. We will use the YSI 2700 Select Biochemistry Analyzer (YSI incorported, Yellow Springs, OH) for fast measurements (< 1 min per sample). Briefly, the homogenized mixtures will be mixed with 4 ml of

Starch Assay Buffer and autoclaved for 1 hour to solubilize starch. After the autoclaved samples are cooled, 1 ml of 1 mg/ml amyloglucosidase (Sigma # A7420) is added to each. The samples will then be incubated for 30 minutes. 25 microliter sample from each of the before and after amyloglucosidase treatment samples will then be used to measure sucrose and dextrose content on the YSI 2700 dual channel analyzer. 3). Protein Content Quantification. Total protein content of the duckweed samples after drying will be determined as described before using the standard Lowry protein determination procedure. Total protein produced per dry weight will be quantified for the selected strains under different growth conditions and media. 4). Duckweed Harvesting Technology Development. In the first year of this project, we will experiment with and optimize the use of the Pond-HippoTM, a commercially available duckweed harvester (EcoPond Rescue LLC, Fl.) that we have just purchased this year. In the second and third years of our project, we will systematically harvest the endemic duckweed biomass from the Pinelands site over a six-month period (from late April to mid-October). 5). Fermentation and Fuel Grade Ethanol Production. Pilot studies will be performed to demonstrate the feasibility of the bifunctional duckweed platform by producing ethanol from harvested duckweed. For bioethanol production capability, standard fermentation assays will be used to quantify the amount of ethanol that can be produced from the selected duckweed strains grown on standard plant growth media and the two wastewater streams, following the established protocol for the saccharification of corn starch. Dry duckweed with high starch content will be mixed with water and hydrolysis of the duckweed starch will be conducted at the temperature of 50-90 degree Celcius (deg C) with the addition of hydrolases. The hydrolysis will take about 4-5 hours. The hydrolysate will then be fermented with baker's yeast to produce ethanol. After fermentation, the ethanol concentration in the fermentation broth will be determined with the YSI Select Biochemistry Analyzer. To produce fuel-grade ethanol from the fermented "beer", we will utilize the recently developed MicroFueler that is being developed by the start-up company E-Fuel (Paso Robles, CA).

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ACCESSION NO: 0226839 **SUBFILE:** CRIS
PROJ NO: NYC-127477 **AGENCY:** NIFA NY.C
PROJ TYPE: HATCH **PROJ STATUS:** NEW
START: 01 OCT 2011 **TERM:** 30 SEP 2014

INVESTIGATOR: Lei, X. G.; Austic, R. E.; Miller, D. D.

PERFORMING INSTITUTION:
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DEVELOPING A NEW GENERATION OF FEED PROTEIN SUPPLEMENTS FROM MARINE BIOFUEL PRODUCTION

NON-TECHNICAL SUMMARY: The long-term goal of this project is to develop novel feed protein supplements from the biofuel production of algae. We will conduct six feeding experiments to test to test effects of various levels of defatted **algal** meal from **biofuel** production on growth performance, biochemical response, nutritional and health values of meats and eggs, and potential toxicity in corn-soybean meal based diets for broiler chickens, laying hens, and weanling pigs. This project helps address two major real-world issues/problems. The first is about the steady supply and now sources of feed protein for the animal production. Feed accounts for 75% of the total expense of animal production and protein represents the second most expensive feed ingredient. The most commonly-used feed protein supplement is soybean meal that is added at 20 to 30% in diets for swine and poultry. In the US, 6 million metric tons of soybean meal is used for only pig feeding per year. With an increasing demand for human consumption due to the population expansion, the continuous use of soybean meal as the major feed protein will become more expensive and less steady. Many algal species contain 45 to 55% of protein. Although the amino acid profile and the nutritional quality of the algal proteins are only slightly inferior to the high-quality proteins, there has been no systematic nutritional assessment of algal proteins or any attempt to improve their quality as a feed supplement. The second issue is that the biofuel production is still less affordable than fossil sources of energy in terms of efficiency. Using co-products as high-quality animal feed protein will add cash values to make this industry competitive. Because this project will create opportunities for many segments of the society including biofuel and feed industry, animal producers, and general public and next generations, federal funds are essential for the initial research and development to generate concrete scientific evidence to attract private funds.

OBJECTIVES: The long-term goal of this project is to develop novel feed protein supplements from the biofuel production of algae. Success of this project will add significant values to the biofuel production and spare high-quality soybean protein used in animal feeding for human consumption. There are three specific objectives: 1. To determine nutritional values of graded levels of defatted **algal** meal from **biofuel** production in replacing soybean meal and(or) a mixture of corn and soybean meal in diets for broiler chicks and laying hens; 2. To determine nutritional values of graded levels of defatted **algal** meal from **biofuel** production in replacing soybean meal and(or) a mixture of corn and soybean meal in diets for pigs; 3. To optimize nutritional effects and to determine health values of the selected defatted algal co-product source and level in diets for poultry and pigs. Our research will help create a completely new source of high-quality feed protein supplements from the **biofuel** fermentation of **algal** biomass for swine and poultry feeding. This intended outcome will have numerous significant impacts on many segments of the society. First, the feed protein supplements out of biofuel fermentation will add a great cash value to the new industry and make the operation affordable. This will in turn make the US biofuel industry highly competitive in the global market. Second, the new feed protein supplements will allow feed companies in the US to launch new commodities. Third, livestock producers will have a new type of feed protein supplement as an alternative source to the expensive soybean or fishmeal. Fourth, the general public will have high-quality edible proteins such as soybean spared from animal feeding for consumption, and will benefit from a new source of bioenergy and feed as well as less use of land and fresh water for energy and food production. Overall, findings from this project will help in developing new sources of energy and feed protein, in preserving natural resource, in protecting our environment, and in exploring new opportunities. Altogether, it will help make animal agriculture and biofuel production in the US more competitive and sustainable. New York State is a highly-industrialized and populated State. Taking a leadership in new technology such as biofuel and alternative agriculture that is addressed in this project will enable the State to compete well with other more traditional-agriculture-oriented States or countries.

APPROACH: Three feeding experiments will be conducted first to test effects of various levels of defatted **algal** meal from **biofuel** production on growth performance, nutritional biochemical response, and potential toxicity in corn-soybean meal based diets for broiler chickens, laying hens, and weanling pigs. Their response to graded dietary levels of defatted algal meal will be tested at the following conditions: 1. A corn-soybean meal based diet (BD) as the control (0% algal product) 2. The BD will be supplemented with 7.5% defatted algal meal to replace 7.5% soybean meal. Synthetic amino acids and corn oil will be added to make this diet isoenergetic and similar profiles of the most limiting amino acids to the BD 3. The BD will be supplemented with 7.5% defatted algal meal to replace 2.5% soybean meal + 5.5% corn. No extra amino acid or oil will be supplemented. 4. The BD will be supplemented with 10% defatted algal meal with 2.2% soybean meal + 7.5% corn. No extra amino acid or oil will be supplemented. Based on results from the first three Experiments, we will conduct three additional experiments to determine effects of the selected microalgal protein supplements and their appropriate inclusion rate in diets for swine and poultry on health values and eating quality of their products (meats and eggs). We will also attempt to optimize the nutritional and health values of the selected defatted algal product by a combined supplementation of digestive enzymes. The basic design of these three experiments for the respective types of animals (Broilers, laying hens, and pigs) will be as follows: Treatment 1: Control as the commercial, complete corn-soybean meal basal diet (BD) Treatment 2: BD + the selected optimal type/dose of defatted algal meal Treatment 3: The same as Treatment with inclusions of phytase, protease, and xylanase. The sources and amounts of the three hydrolytic enzymes will be decided in year 3 with our industrial collaborators based on practical recommendations and product availability. In addition to the growth performance, biochemical, and toxicological responses, we will determine yield, chemical composition, health value, and eating quality of eggs and meats produced by animals fed the selected defatted algal meals. The taste panel evaluation will be assisted by Dr. Dennis D. Miller and other experts in the Department of Food Science.

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ACCESSION NO: 0216392 **SUBFILE:** CRIS
PROJ NO: NC02274 **AGENCY:** NIFA NC.
PROJ TYPE: HATCH **PROJ STATUS:** TERMINATED
START: 01 OCT 2008 **TERM:** 01 OCT 2011 **FY:** 2010

INVESTIGATOR: Liehr, S. K.

PERFORMING INSTITUTION:
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RALEIGH, NORTH CAROLINA 27695

IMPACT OF SWINE LAGOON COVERS FOR ENERGY RECOVERY AND GAS EMISSION REDUCTION ON NUTRIENT MANAGEMENT

NON-TECHNICAL SUMMARY: Environmental and economical issues associated with conventional anaerobic swine waste treatment lagoons continue to be difficult to resolve. One option that is rapidly gaining attention in North Carolina is the installation of non-permeable covers over existing lagoons. Such covers have the potential to provide multiple environmental benefits, including reduced odor, ammonia, particulate, and greenhouse gas emissions, as well as rainwater exclusion for volume reduction. The recent emergence of a voluntary market for carbon offset credits, which could be generated from capture of escaped methane, offers a possible revenue source to offset the capital cost and to increase farm income as a result of installing lagoon covers. A possible mandatory greenhouse gas reduction program in the future could expand this possibility. In addition to revenue from sale of carbon credits, capture of methane and utilization for generation of electricity could provide further income. An additional impact of these covers is the conservation and concentration of nitrogen in the lagoon liquid. Experiences with other types of covered waste management structures indicate that nitrogen dynamics are likely to change substantially. The conserved nitrogen could be considered a benefit if there is a beneficial use for additional nitrogen on the farm, or if technologies are developed to recover the nitrogen in a marketable product. Whether or not the additional nitrogen is beneficial, this aspect of covered lagoons must be considered in management of the lagoon system. Current programs in North Carolina to test lagoon covers offer an opportunity to document some of these effects. Activities of this research will include determining changes in nitrogen concentrations due to lagoon covers and making recommendations regarding changes to nutrient management plans that will be required. We will also explore options for additional nitrogen management strategies, including nitrogen removal technologies and nitrogen recovery.

OBJECTIVES: Although covering of waste lagoons offers opportunity for an affordable technology for making lagoon spray-field technology more environmentally acceptable, the complete environmental effect of these covers is not yet known. The goal of this research is to evaluate the changes in nitrogen dynamics that occur as a result of covering swine lagoons with a non-permeable cover. This project will include the following specific objectives: (1) determine the impact of covering existing lagoons for the purpose of capturing methane on nitrogen accumulation in the lagoon and (2) make any warranted recommendations for nitrogen management modifications and explore options for effective nitrogen management strategies for covered lagoons, including nitrogen recovery.

APPROACH: Environmental Credit Corp. (ECC) has received funding to demonstrate the lagoon cover technology by installation on approximately 8 swine farms in North Carolina. To date (fall, 2008), two sites have been identified, and covers were installed on a total of four lagoons at these sites in late spring of 2008. ECC has agreed to cooperate with us on monitoring lagoon quality and to share with us their monitoring data of methane from the lagoons. Other groups in North Carolina are also planning to demonstrate the lagoon cover technology, and we will try to gain permission to monitor them as well. We plan to monitor at least 8 to 10 covered lagoons over the project period. Various parameters of lagoon quality will be monitored, although nitrogen is the primary emphasis of this study. Parameters to be measured are total Kjeldahl nitrogen, total ammonia nitrogen, nitrate, total phosphorus, ortho-phosphate, chemical oxygen demand, total solids, volatile solids, suspended solids, and alkalinity. Analysis will be conducted at the NCSU Environmental Analysis Laboratory (BAE Dept.). Samples will be taken monthly from sample ports in the lagoon cover. Three methods will be used to evaluate changes that occur as a result of the cover installation. (1) Lagoon quality will be measured before (when possible) and for at least one year after cover installation. (2) Previous NCDA farm data will also be examined as part of the data available prior to cover installation. (3) We will attempt to find a similar lagoon (proximity, production type, company / farmer / integrator) as a control for each covered lagoon, and samples will be taken at same time. Comparison to a control lagoon will allow us to evaluate how season and/or weather events affect changes. The combination of these three approaches will allow us to develop a reasonable determination of the effects on nitrogen dynamics. This project involves making use of the information collected to make recommendations for nitrogen management for covered lagoons. Three approaches will be taken. (1) Analysis of the nitrogen levels will allow us to predict a range of nitrogen concentration factors likely to result from lagoon covers. This information will be used directly with existing nutrient management tools to project the effect on nutrient management plans. (2) Preliminary exploration of options for nitrogen management other than land application will be done. Modifications of nitrogen removal technologies will be proposed based on the extensive performance and economic data collected during studies at NCSU. (3) Recovery of nitrogen as a fertilizer product will be explored. Nitrogen (mostly as ammonia) is difficult to separate from water, and historically this has not been considered economically feasible. Our preliminary tests of ammonia stripping indicate that this might become a feasible step in nitrogen recovery, considering recent substantial increases in the price of commercially available nitrogen fertilizer. Additional analysis through modeling of ammonia stripping and adsorption will continue in order to explore the likelihood of feasibility.

PROGRESS: 2008/10 TO 2011/10
OUTPUTS: The covered lagoon monitoring data indicated that nitrogen concentrations will increase enough to require modifications for nitrogen management. This result has been consistent throughout the project, and has been disseminated to the public at intervals during the project period. Results were presented at an EPA-sponsored workshop and field tour on September 18, 2008 held in Clinton, NC. The workshop was entitled: Environmental and Economic Benefits of Capturing Swine Manure Methane, and was well attended by researchers, policy makers, regulators, and farmers. (See agenda and presentations at http://www.epa.gov/agstar/workshop08.html) An update of monitoring data was provided to an interagency meeting of NRCS and North Carolina Division of Soil and Water Conservation staff on April 29, 2009 in Raleigh, NC. A report containing nitrogen monitoring data was submitted to the North Carolina Pork Council. The extent of increase in nitrogen concentration in the one year following cover installation was explained. This report was made available to pork producers in

North Carolina through the NC Pork Council. A final project report containing nitrogen monitoring data was submitted to the North Carolina Pork Council. The extent of increase in nitrogen concentration during the 2.3 years following cover installation was explained. This report will be made available to pork producers in North Carolina through the NC Pork Council. PARTICIPANTS: Mark Rice, Extension Specialist, Biological & Agricultural Engineering Dept., North Carolina State University C. Mike Williams, Director, Animal Waste Management Center, North Carolina State University TARGET AUDIENCES: Pork producers Environmental regulators Policy makers PROJECT MODIFICATIONS: Nothing significant to report during this reporting period.

IMPACT: 2008/10 TO 2011/10
Environmental Credit Corp. (ECC) received funding from the USDA NRCS Conservation Innovation Grant (CIG) program for the purpose of demonstrating covered lagoons in an innovative carbon credit incentive program. Four swine lagoons on two separate farms in North Carolina were covered. Covers were installed on all four in April, 2008, and monitoring was initiated in May, 2008. Nitrogen concentration data prior to lagoon cover installation are not available. Nitrogen concentrations increased substantially in all four lagoons since cover installation. The amount of increase was determined using the difference in estimated concentrations at the beginning and end of the study. Initial concentrations were estimated using a linear trendline through the first six months of data points. The final concentrations were estimated as the average over the last four months, when concentrations appeared to have stabilized. By this method, the nitrogen concentrations at the Farm 1 increased by 72% and 115% in Lagoons 1 and 2, respectively. At Farm 2, nitrogen concentrations increased by 107% and 102% in Lagoons 3 and 4, respectively. We were not able to collect data to calculate a complete water balance for the system, so we do not know how much of the nitrogen concentration increase is due to exclusion of rainwater from the lagoons and how much is due to prevention of volatilization of ammonia gas. Much of the rainwater was excluded, but evaporation losses were also greatly reduced. We can compare concentrations of other elements to get an indication of the effect of rainwater exclusion. Total phosphorus (TP) is a relatively conservative element in wastewater lagoons and does not have a volatile form. Phosphorus concentrations in the lagoon liquid did not increase during this study, and actually decreased. It does not appear that rainwater exclusion is the main reason for the nitrogen concentration increases. The rapid increase in nitrogen concentrations in covered lagoons that was observed during the monitoring phase of this project has allowed us to alert producers to the likelihood that additional nitrogen management will be required if covers are installed on existing lagoons. These results have also stimulated interest in nitrogen management techniques and, especially, in potential nitrogen recovery technologies. Three approaches to nitrogen recovery were investigated: 1) ammonia stripping, 2) membrane separation, and 3) algal biofuel production. All have problems in terms of immediate application to swine farms in North Carolina. These technologies may be expensive and management intensive, and have not yet been tested enough for swine waste treatment. The technology of lagoon covers can have a beneficial effect on reducing carbon footprint of swine farms in North Carolina, primarily by methane collection and by subsequent energy production from the methane. Nitrogen recovery from the lagoon would have a lesser effect.

PUBLICATIONS (not previously reported): 2008/10 TO 2011/10
No publications reported this period

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ACCESSION NO: 0024187 **SUBFILE:** CRIS
PROJ NO: NC05159 **AGENCY:** SAES NC.
PROJ TYPE: STATE **PROJ STATUS:** EXTENDED
CONTRACT/GRANT/AGREEMENT NO: NOT APPLICABLE
START: 30 JUN 1964 **TERM:** 30 SEP 2020 **FY:** 2011

INVESTIGATOR: Robarge, W. P.

PERFORMING INSTITUTION:
SOIL SCIENCE
NORTH CAROLINA STATE UNIV
RALEIGH, NORTH CAROLINA 27695

ANALYTICAL LAB FOR SOILS

OBJECTIVES: Provide analytical services needed by the soil scientists in their research including technicians to perform most standard analyses and tests.

APPROACH: Not Provided

PROGRESS: 2010/10 TO 2011/09
OUTPUTS: The Analytical Service Laboratory provides direct support for various research projects (for a fee) that require analytical assistance in terms of sampling protocols, sample handling, analytical methods development and data interpretation from analysis of various solid and liquid sample matrices. Clientele including project leaders, staff and students from 11 different departments at North Carolina State University, and 7 different departments from other universities in the region and elsewhere. Assistance was also provided to a North Carolina-based entity needing assistance with a full chemical analysis of pool-water with algae growth, a Colorado based entity needing low-level metals analysis during start-up of a water-treatment plant in Virginia, and one criminal case. Types of sample matrices processed were varied: mine-drainage stream water, digests of bacteria, metal pellets, metal polymers, metals in carbon structures, nanoparticles and catalysts used in drug delivery, lignin oil, calcium soaps, algae plant material, algal biofuels and catalysts, glycerol samples, fatty acids/oils, liver cells, fly ash from coal-fired power plants, plant-derived food colorings, silver content in cell media, gold nanoparticles for tissue inflammation studies, media from bioreactors, fresh water invertebrates, fish food, soils, fertilizers, and leachate from soil lysimeter studies. Primary analytical request was total elemental analysis of the sample matrix, although metal speciation analysis for As and Se was also conducted for a number of toxicology studies. State-of-the-art analytical instrumentation used in these analyses included a dual-view ICP-OES, and LC-ICP-MS. Various digestions techniques employed included high-pressure microwave digestion, muffle furnaces and standard hot plate or block digestion systems. All analyses are carried out using high-purity grade reagents, multipoint calibration curves, replicate analyses (when sample matrices allow), and NIST CRMs when available. PARTICIPANTS: Not relevant to this project. TARGET AUDIENCES: Not relevant to this project. PROJECT MODIFICATIONS: Not relevant to this project.

IMPACT: 2010/10 TO 2011/09
The Analytical Service Laboratory is one of a number of service centers at North Carolina State University. The laboratory employs a variety of state-of-the-art analytical instrumentation whose cost, maintenance, and successful operation prohibits duplication within the university. The laboratory is staffed with experienced personnel who can provide analytical services on unique sample matrices with a high probability of success. Analytical costs are averaged over the university community resulting in a stable, affordable fee structure for project leaders for consistency in costs for grant-supported research.

PUBLICATIONS (not previously reported): 2010/10 TO 2011/09
No publications reported this period

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ACCESSION NO: 0204414 **SUBFILE:** CRIS
PROJ NO: NC06823 **AGENCY:** NIFA NC.
PROJ TYPE: HATCH **PROJ STATUS:** EXTENDED
START: 01 OCT 2005 **TERM:** 30 SEP 2011 **FY:** 2011

INVESTIGATOR: Burkholder, J. M.

PERFORMING INSTITUTION:
PLANT BIOLOGY
NORTH CAROLINA STATE UNIV
RALEIGH, NORTH CAROLINA 27695

EUTROPHICATION OF SELECTED AQUATIC ECOSYSTEMS IN NORTH CAROLINA

NON-TECHNICAL SUMMARY: A. Nutrient over-enrichment has been identified as one of the most important water pollution problems affecting U.S. freshwaters and estuarine/marine waters. B. An experimental approach is needed, in combination with long-term datasets of environmental conditions, to quantify and understand impacts of nutrient pollution on aquatic ecosystems. Such datasets are valuable in evaluating influences of changing land use in watersheds, and of the efficacy of management strategies such as targeted total maximum daily loads to reduce pollutant loadings. This project will strengthen understanding about nutrient inputs and impacts in major reservoirs used for recreation and potable water supplies in North Carolina. It will also provide continuity for the most detailed, long-term dataset on the Neuse Estuary over the past decade. In addition, the research will advance understanding about impacts of a major new nonpoint pollution source, industrialized animal agriculture, as well as influences of climate and human impacts on estuarine water quality and ecosystem response in the hurricane-prone North Carolina area. Finally, this project will integrate new technology, real-time remote monitoring, and modeling to advance scientific understanding about estuarine ecosystems.

OBJECTIVES: The objectives of this research are (i) to examine chronic impacts of nutrient over-enrichment and high suspended sediment inputs on phytoplankton and benthic microalgae in selected freshwater and estuarine systems; (ii) to improve quantification of nutrient sources and loadings to the Neuse and Cape Fear estuaries; and (iii) to extend a long-term monitoring and research effort in the Neuse Estuary, to strengthen trend analyses of changes in nutrients and SS concentrations and loadings over time.

APPROACH: For the freshwater component of this research, the nutrient regimes; suspended solids and light regimes; composition, abundance, and productivity of phytoplankton; and prevalence of cyanobacteria (known to be stimulated by nutrient over-enrichment) and their toxins will be compared in newer versus older major reservoirs (20-30 years post-fill and 60-85 years post-fill respectively) used for recreation and potable supplies. Second, the growth response and toxin production of major toxigenic bloom-forming taxa from these reservoirs to P, N, and N+P enrichments across a gradient of suspended solids concentrations will be examined in a series of laboratory experiments. For the estuarine component of this research, first, longitudinal statistical analyses will be conducted using ~decadal databases produced by State-certified laboratories, to assess trends in nutrient concentrations over time and the influence of a major new (since the late 1980s) source of nonpoint pollution in the Neuse and Cape Fear watersheds, concentrated animal feed operations (CAFOs). This effort will include a comparison of (i) streams draining CAFO-dense watersheds, (ii) streams in non-CAFO-dense watersheds but impacted by point source discharges, and (iii) streams that are little affected by CAFOs or point sources. Second, the growth response and toxin production of major toxigenic bloom-forming taxa from the Cape Fear and Neuse estuaries to P, N, and N+P enrichments across a suspended solids concentration gradient will be experimentally examined. Third, six real-time remote monitoring stations will be maintained in the mainstem Neuse Estuary, sited off- channel in known problem areas for hypoxia, algal blooms, and fish kills and disease. The meteorological and hydrological data will be augmented with continued (12 years ongoing) biweekly sampling for nutrients, phytoplankton assemblages and biomass, and suspended solids concentrations. The dataset, in combination with twelve years of previously collected data, will be used to improve assessment of trends in nutrient concentrations and loadings, and response variables(dissolved oxygen, phytoplankton biomass) in the Neuse River Estuary.

PROGRESS: 2009/10 TO 2010/09
OUTPUTS: ACTIVITIES - I directed the Center for Applied Aquatic Ecology (CAAE). The CAAE assisted the Cities of Raleigh and High Point in safeguarding the drinking water supplies depended upon by more than 700,000 North Carolinians in the Piedmont and Triad. Our automated platform stations near the intakes of these water supplies provided real-time data 24/7 to track sudden pollution spikes and harmful algal blooms, so that the water treatment plant managers could take immediate actions when needed to protect the health safety of all of these citizens. The CAAE continued to characterize environmental and water quality conditions, algal blooms, and fish kills on the Neuse Estuary, including real-time data at several automated stations. I taught Phycology (PB774), Environmental Issues in Aquatic Ecology (PB595W), and a STEM Education Seminar, Aquatic Ecology and Pedagogical Applications (EMS 496/622/822 or TDE 490/610). I received consistently outstanding teaching evaluations. I mentored 3 undergraduate students and 4 graduate students in research projects, and 1 gifted high school teacher who is a Kenan Fellow. In outreach education, the CAAE's Floating Classroom Program continued to provide hands-on experience in aquatic science to hundreds of 8th grade and high school students and their teachers. EVENTS - I co-organized and conducted a workshop about harmful algae for water treatment plant operators that was sponsored by the North American Lake Management Society. I served on the organizing board for an international conference on harmful algae. SERVICE - I served as a consultant to the U.S. EPA on ballast water treatment. The CAAE provided water quality analyses, algal identifications, and flow cytometric analyses for faculty at three UNC system universities. We also provided real-time water quality data and graphics to secondary and high school teachers as requested. These data were used by the teachers to develop class lessons and laboratory exercises. PRODUCTS - I secured more than $1 million in funding for the CAAE. We provided lipid-rich mass-algal culture for sustainable biofuel production as part of a major NSF grant funding NCSU and partners from two small businesses. The CAAE website (http://www.ncsu.edu/wq/) was strengthened for use by teachers, resource managers, legislators, and scientists in providing real-time information on major NC waters, and updates on aquatic technology, methods, and equipment. The CAAE continued to build high-frequency databases for major water supply reservoirs (7 years ongoing) and the Neuse Estuary (17 years ongoing). Two graduate students completed their degrees on CAAE research. DISSEMINATION - I engaged in outreach activities to advance knowledge about harmful algal blooms for potable water treatment plant personnel statewide, and explained emerging water quality issues for K-12 teachers. I presented our research findings at various scientific conferences. The CAAE's Floating Classroom Program was recently highlighted as a central feature of an educational video produced by the Kenan Fellows Program, called Teaching Students to Think Outside the Book, which is being used by K-12 teachers across the state. PARTICIPANTS: INDIVIDUALS WHO WORKED ON THIS PROJECT - Robert Reed, Researcher, CAAE; Elle Allen, Research Associate, Center for Applied Aquatic Ecology (CAAE); Carol A. Kinder, Data Manager, CAAE; Jenny James and Linda MacKenzie, water quality analytical specialists, CAAE; Eric Morris, field technician, CAAE; David DeMaster, MEAS-NCSU, Reide Corbett, ECU; Michael Mallin, UNCW; Patricia Glibert, U MD; Kim Null, U CA; Allasanne Ouattara, University of Abobo-Adjame, Ivory Coast; Meghan Rothenberger, Lafayette College, PA; Parke Rublee, UNC Greensboro; Tom Wentworth, NCSU; Paul Zimba, Texas A&M; and Peter Moeller, NOAA-NOS, Charleston. OPPORTUNITIES FOR TRAINING AND DEVELOPMENT - I taught several courses to 35 students; I served as mentor for 3 undergraduates in research projects about aquatic science, as well as 4 graduate students on thesis projects and 1 Kenan Fellow. I also provided outreach education to ~50 secondary school and high school teachers, and to several hundred of their students. The CAAE's website provided materials for virtual training in water quality analysis and aquatic science to many K-12 teachers across the state. TARGET AUDIENCES: More than 700,000 people in Raleigh and surrounding municipalities, and in the city of High Point in the Triad region, were served by this project in the CAAE's efforts to safeguard major drinking water supplies. Undergraduate and graduate students from NCSU were served by this project in classroom and laboratory training for young scientists. Nearly 650 secondary school students (about one-third of whom were

economically disadvantaged) and their teachers received experiential training in aquatic science through our outreach education program, The Floating Classroom, including development of new educational materials. Through this program, the families of these children were also affected by dissemination of information designed to make citizens of all ages better stewards of our state's public trust water resources. PROJECT MODIFICATIONS: Not relevant to this project.

IMPACT: 2009/10 TO 2010/09
I contributed the following significant outcomes and impacts as changes in knowledge, actions or conditions: Strengthened protection of our state's drinking water supplies is essential for continued, sustainable economic development, a fact recognized by Business North Carolina. The advanced technology that we recently patented is being used to provide early-warning detection of pollution spills and toxic algal blooms to help safeguard the water supplies depended upon by 700,000 North Carolinians. In 2010 our data on water quality in Falls Lake translated into major policy actions: The CAAE's data demonstrated impairment and violation of the state's water quality standard for algal biomass (as chlorophyll) throughout the lake. These data were also an important influence on the North Carolina General Assembly in passing the Falls Lake Rules, new legislation to improve protection of this important drinking water supply reservoir. Other CAAE data on nutrient stimulation of potentially toxic cyanobacteria in major drinking water supply reservoirs are being used by resource managers as support for additional measures to restrict development in the watersheds of some of the state's most important water supply reservoirs, in order to reduce nutrient loading. Our research on the complex nutrition of estuarine and marine coastal harmful algae helped to lead to a major published scientific consensus on the importance of nutrient pollution in stimulating harmful algal species that previously were not linked to nutrient over-enrichment. This consensus is important in altering management strategies in efforts to mitigate the economic impacts of these organisms. Our long-term study of the Neuse Estuary is demonstrating the comparative importance of climatic events (major storms, extended droughts) versus management actions in affecting nutrients and other pollutant loads to the estuary. The long-term Neuse watershed land use/water quality analysis is helping to guide resource managers and policy makers about land use practices needed for improved water quality. I first-authored syntheses to guide the science of protecting water quality in major drinking water reservoirs, and to guide research on the pervasive impacts of nutrient pollution on commercially important shellfish species. I co-authored a third synthesis describing on-line, state-of-the-art, real-time equipment for monitoring water quality. Our outreach education efforts aboard the CAAE's U.S. Coast Guard-certified research ship, RV Humphries, advanced understanding of hundreds of middle school students and their teachers about emerging issues affecting North Carolina estuaries. Other education outreach advanced knowledge of potable water treatment plant personnel about harmful algal blooms.

PUBLICATIONS (not previously reported): 2009/10 TO 2010/09
1. Burkholder, J.M., Frazier, W., and Rothenberger, M.B. 2010. Source water assessment for harmful and noxious algae. Chapter 19 in Algae Manual, by the American Water Works Association, Denver, CO.
2. Burkholder, J.M. and Shumway, S.E. 2011. Bivalve shellfish aquaculture and eutrophication, in: Shumway, S.E. (editor), Shellfish and the Environment. Wiley, New York (in press).
3. Pate, S.E., Burkholder, J.M., Shumway, S.E., Hegaret, H., Wikfors, G.H., and Frank, D. 2010. Effects of the toxic dinoflagellate Alexandrium monilatum on survival, grazing and behavioral response of three ecologically important bivalve molluscs. Harmful Algae 9: 281-293.
4. Reed, R.E., J.M. Burkholder and E.H. Allen. 2010. Current online monitoring technology for surveillance of algal blooms, potential toxicity and physical-chemical structure in rivers, reservoirs and lakes, Chapter 1 in Algae Manual, by the American Water Works Association, Denver, CO.
5. Burkholder, J.M., Reed, R.E., Allen, E.H., and Kinder, C.A. 2010. Climate Change and Harmful Algal Blooms in the Southeast. North American Lake Management Society (NALMS), Winston-Salem, NC (abstract)
6. Burkholder, J.M., Reed, R.E., Kinder, C.A., Allen, E.H., James, J., and Mackenzie, L. 2010. Water Quality in Upper and Lower Falls Lake, a Major Potable Water Supply Reservoir in the Upper Neuse Watershed. UNC Water Resources Research Institute, Raleigh, NC (abstract)

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ACCESSION NO: 0216906 **SUBFILE:** CRIS
PROJ NO: SC-1700391 **AGENCY:** NIFA SC.
PROJ TYPE: HATCH **PROJ STATUS:** NEW **MULTISTATE PROJ NO:** S-1041
START: 01 OCT 2008 **TERM:** 30 SEP 2013 **FY:** 2011

INVESTIGATOR: Walker, T. H.

PERFORMING INSTITUTION:
Environmental Engineering & Earth Science
CLEMSON UNIVERSITY
CLEMSON, SOUTH CAROLINA 29634

THE SCIENCE AND ENGINEERING FOR A BIOBASED INDUSTRY AND ECONOMY

NON-TECHNICAL SUMMARY: Advantages offered by the proposed marine algal biomass production process include: 1) Elimination of the need for vast areas of high quality farm, forest and/or pasture land, 2) Elimination of the need for intensive inputs of fertilizers, pesticides and other energy intensive inputs, 3) Avoidance of the need to produce large quantities of bulky lignocellulosic biomass suitable mainly as low-value solid fuels or requiring expensive conversion processes not yet commercially available, 4) Elimination of the need for extended crop growing periods, with seasonal harvests requiring large investments in underutilized harvesting, transport, pretreatment and storage facilities, 5) Elimination of negative environmental impacts resulting from nutrient losses to local surface and groundwater and increased global greenhouse gas (GHG) emissions resulting from production and degradation of required synthetic fertilizers. Furthermore, the use of brine shrimp cultures as an algal harvest and conversion process offers additional advantages; including;1) Capacity of Artemia to grow on a wide variety of algal genera eliminating the need for expensive algal species control techniques in mass algal culture operations, 2) Avoidance of the difficult and costly need to directly harvest, concentrate and dry microalgae biomass and, 3) Significant cost reduction resulting from the ease of extraction of Artemia-oil as compared to solvent boiling used in conventional algal-oil extraction. A preliminary techno-economic analysis will be provided to evaluate the potential for producing biodiesel from CEP algal/brine shrimp production systems. Important drivers for this analysis will be algal and lipid productivity in outdoor, open-air systems, as well as algal harvestability using the described techniques. Costs will be projected for extracting oil efficiently from the biomass, including the quantity of oil extracted per mass of biomass harvested and projected costs to perform the extraction at various scales. The resulting analysis in intended to provide preliminary estimates of the quantity of oil that could be produced per unit CEP area, along with estimates of the costs for labor, energy, and supplies needed to operate the system and the cost of the biofuel and biofertilizers produced by the system. We plan to address the impact of potential future advances in this technology, in particular projected higher productivities resulting from genetic improvements in algal strains, as well as potential improved economic and regulatory price supports.

OBJECTIVES: (1) Reduce costs of harvesting, handling and transporting biomass to increase the competitiveness of biomass as a feedstock for biofuels, biomaterials and biochemicals. (2) Improve biofuel production processes. (3) Identify, develop and evaluate sustainable processes to convert biomass resources into biochemicals, biocatalysts and biomaterials. 4) Demonstration of continuous marine algal production and coupled brine shrimp biomass production with biomass quality maintained within existing greenhouse-covered marine aquaculture production system located at Clemson University, 5) Optimization of two-phase solvent composition/volume requirements with wet brine shrimp biomass grind and elution sequences for maximum oil recovery at minimum energy input, 6) Optimization of high value oil, and biodiesel yield from the combined algal-brine shrimp biomass production process and, 7) Preliminary mass, energy and economic analysis of algal-brine shrimp oil and biodiesel production process.

APPROACH: 1) Demonstration of algal brine shrimp biomass production and quality; Marine (wild type) green algal biomass and cyanobacterial biomass (bluegreen algae) will be produced in pre-existing 0.0625 acre greenhouse covered partitioned aquaculture system (PAS units) located at Clemson University. The algal biomass will be used to feed 1000 liter field and 20 liter laboratory cultures of high density brine shrimp cultures using previously developed (and patented) brine shrimp culture techniques. Algal productivity, algal standing crop and algal population composition will be stabilized and maintained using populations of filter-feeding tilapia and shellfish, comprising a "designed aquaculture ecosystem," a technique perfected at Clemson University over the last 15 years. 2) Optimization of two-phase solvent composition/volume requirements; Preliminary experiments showed that a 3/2 hexane to isopropanol (v/v) mixture could be used to rapidly separate the oil and water fraction from raw wet brine shrimp biomass (90% water content) at room temperature. Additional trials will be conducted to examine the possibility to reduce solvent volume by animal grinding and centrifugation to enhance oil/protein fractionation prior to solvent separation and enrichment. Harvested brine shrimp biomass is separated from brine shrimp water and oil content by grinding the wet animal tissue directly using a Kinematica Polytron homogenizer in the suspensions of organic solvents at room temperature. The resulting oil/solvent and water/solvent mixture will be further separated using a centrifuge at 3400 rpm for 3 minutes. 3)Optimization of high value oil, and biodiesel yield; Algal biomass to brine shrimp biomass conversion efficiencies can range from 25 to 50% (algal dry wt to brine shrimp dry) depending primarily on algal feed concentration. Brine shrimp oil content typically ranges from 10 to 20% of total weight, depending on the algal diet oil content. Algal oil composition and content varies with net algal productivity, with higher net algal productivity typically yielding lower total algal oil content. Therefore, optimum combined algal/brine shrimp system lipid productivity and lipid composition will depend on the combination of these three variable. A matrix of combined growth trials will be conducted consisting of algal productivities of 3, 6 and 12 gmC/m2-day controlled by algal cell harvesting rate, and algal population compositions of bluegreen vs green algal populations controlled using filter feeding fish and shellfish, with brine shrimp reactor steady state algal cell concentration controlled by reactor loading rate and animal density.4) Mass, energy and economic analysis; Using the data obtained from tasks 1, 2 and 3, total yield (as biodiesel) vs. required solvent volume and solvent evaporation energy requirements will be calculated. A preliminary model of net energy yield and cost of algal/brine shrimp production and brine shrimp oil extraction and concentration will be determined for the range of critical production and oil extraction parameters.

PROGRESS: 2010/01 TO 2010/12

OUTPUTS: Biodiesel is produced from the transesterification of lipids and is defined as simple monoalkyl esters of long chain fatty acids that meet the requirements of biodiesel fuel standards such as ASTM D6751 or EN 14214. Advantages over petroleum diesel fuel (petrodiesel) include positive energy balance, superior lubricity and biodegradability, domestic and renewable origin, low or no sulfur content, superior flash point and lower overall exhaust emissions. The alcohol employed commercially in the transesterification of triacylglycerols (TAG) is methanol, which results in the production of fatty acid methyl esters (FAME). Other alcohols including ethanol and butanol may also be used to prepare fatty acid ethyl (FAEE) and butyl (FABE) esters, respectively. Transesterification of refined cottonseed oil was carried out with methanol, ethanol, 1-butanol and various mixtures of these alcohols at a constant volume ratio of alcohol to oil of 1:2 using KOH (1 wt%) as catalyst to produce biodiesel. Microalgal biodiesel is considered as second-generation biofuel, which have advantage in avoiding threat to food supplies and biodiversity. Moreover, microalgal lipid has been considered as a very good candidate for biodiesel production because of its higher lipid content, shorter time growth cycle, and need for less land compared to other energy crops. Chlorella protothecoides have been studied to achieve both high biomass and lipid production in heterotrophic condition by using different carbon sources. The cost of feedstock accounts for 60-70% of the total cost of the biodiesel. For realizing commercial production of biodiesel from heterotrophic C. protothecoides lipid, lower cost and effective alternatives to glucose is desirable. In this study, we aim (1) to evaluate the growth of Chlorella protothecoides on three carbon substrates, glucose, pure glycerol, and crude glycerol from biodiesel production (2) to perform fed-batch fermentations to improve biomass and lipid production by using a lower cost carbon substrate, crude glycerol. This is also the first study to investigate the C. protothecoides on the crude glycerol. Lignocellulosic material such as switchgrass comprises of three major components including lignin, hemicellulose and cellulose. For the conversion of lignocellulosics into ethanol, cellulolyticbiological conversion (cellulolytic) method or thermochemical conversion (gasification) is generally employed. Ammonia soaking pretreatment (ASP) is the one of the recently developed pretreatment processes for herbaceous feedstocks like switchgrass and is operated at moderate temperatures (60 C) with less formation of inhibitory compounds taking place compared to other pretreatments. The main aim of the present ethanol-based research was to evaluate the effect of ammonia soaking pretreatment on different switchgrass particle sizes for the production of cellulolytic enzymes using Trichoderma reesei Rut C-30 compared with other substrates such as non-pretreated switchgrass and Solka- Floc. PARTICIPANTS: USDA-ARS Southwestern Cotton Ginning Research Laboratory (SWCGRL) in Cooperative 269 R&D Agreement with Eco-Sol, LLC for their support and supply of cottonseed oil. Cotton, Inc. for funding a portion of this project. Bryan Moser, USDA/ARS, National Center for Agricultural Utilization Research, 1815 N. University St, Peoria, Illinois. David Thornton and Rachel Burton of Piedmont Biofuels, Inc. Pittsboro, NC TARGET AUDIENCES: Biodiesel manufacturers and scientists working with bioconversion for ethanol and biodiesel production. PROJECT MODIFICATIONS: Not relevant to this project.

IMPACT: 2010/01 TO 2010/12
In the mixed alcohol transesterifications for cottonseed oil, the formation of methyl esters was faster than ethyl and butyl esters. Cottonseed oil-based biodiesel prepared from methanol to ethanol and butanol volume ratios of 1:1 or greater exhibited enhanced cold flow properties versus neat methyl esters and were within the prescribed limits contained in the ASTM D6751 and EN 14214 biodiesel standards with respect to kinematic viscosity and acid value. Also examined was the influence of blending alkyl esters with ULSD. All blends exhibited improved cold flow properties (CP, PP, and CFPP) versus unblended alkyl esters and significantly enhanced lubricity versus unblended petrodiesel as well as properties within the specified ranges of the petrodiesel standards ASTM D975 and ASTM D7467. These results indicated that the fuel properties of cottonseed oil-based biodiesel can be improved by substituting a portion of the methanol reagent with ethanol and/or butanol during transesterification, albeit at a higher production cost due to the higher price of ethanol and 1-butanol in comparison to methanol. The algal biofuels study showed (1) Chlorella protothecoides could use crude glycerol as carbon substrate; (2) fed-batch mode is a better culture strategy than batch for improving biomass concentration, lipid production and crude glycerol consumption; (3) In fed-batch mode, crude glycerol from the biodiesel production processes could be use directly of C. protothecoides with results similar to those obtained with pure glycerol. Consider the high biomass and lipid production, the crude glycerol-to-lipid fermentation model will provide additional feedstock for biodiesel purpose while offering lower cost carbon substrate and eliminating the problem of crude glycerol disposal, which are important criteria for the bioconversion of industrial byproducts into valuable products. Milled switchgrass was sieved to three different particle size ranges i.e. 0.5-1.0, 1.0-2.0 and 2.0-10.0 mm and the effect of ammonia soaking pretreatment (ASP) were determined. Cellulase production from Trichoderma reesei Rut C-30 using 2 % ASP pretreated and non-pretreated switchgrass in the shake flasks were tested. Enzyme activity of 1.30, 0.92 and 1.42 FPU/ml were observed for fungi grown on 0.5-1.0, 1.0-2.0 and 2.0-10.0 mm pretreated switchgrass, respectively, which were compared to 0.37, 0.39, 0.29 and 2.99 FPU/ml for non-pretreated 0.5-1.0, 1.0-2.0 and 2.0-10.0 mm switchgrass and Solka-Floc, respectively. Enzyme production maintaining pH (pH 5) using 2 % ASP pretreated (particle-size of 0.5-1.0 mm) increased enzyme activity to 2.46 FPU/ml. Enzymatic digestibility tests of ASP pretreated switchgrass resulted in 44 % with 49.2 FPU of cellulase/2 g-BM (Biomass) in 72 hours.

PUBLICATIONS (not previously reported): 2010/01 TO 2010/12
1. Chen YH, TH Walker, 2011. Biomass and lipid production of heterotrophic microalgae Chlorella protothecoides by using biodiesel derived crude glycerol, Biotechnol Letters (in review)
2. Joshi H, BR Moser, TH Walker, 2011. Mixed alkyl esters from cottonseed oil: Improved biodiesel properties and blends with diesel fuel, JAOCS (in review)
3. Joshi H, BR Moser, A Mandalika, TH Walker, 2011. Evaluation of Cold Flow Improver and Antioxidant Additives in Cottonseed Oil Methyl Esters, Bioresource Technol (in review)
4. Jain A, J Toler, TH Kim, TH Walker, 2011. Effect of ammonia soaking pretreatment on switchgrass for production of cellulase using Trichoderma reesei Rut C-30, Biotechnol Biofuels (in review)
5. Joshi H, Moser BR, Shah SN, Smith WF, Walker TH, 2010, Physical properties of biodiesel from several sources blended with ethyl levulinate, Fuel, (in press).

6. Joshi HC, BR Mosur, TH Walker*, 2010, Preparation and fuel properties of biodiesel prepared from soybean oil using mixtures of methanol and ethanol, Biomass and Bioenergy, 34(1):14-20.

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ACCESSION NO: 0215295 **SUBFILE:** CRIS
PROJ NO: SD00H269-08 **AGENCY:** NIFA SD.
PROJ TYPE: HATCH **PROJ STATUS:** NEW
START: 01 OCT 2008 **TERM:** 30 SEP 2013 **FY:** 2010

INVESTIGATOR: Anderson, G. A.; Browning, L.; Butler, E.; Halaweish, F.; Rosentrater, K.; Twedt, M.; Cuello, J.

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ENHANCED BIOFUEL PRODUCTION THROUGH A NEW BIOMASS SOURCE AND IMPROVED DRYING EFFICIENCY IN PRODUCTION PLANTS

NON-TECHNICAL SUMMARY: Energy security of the United States can be advanced with the development of biofuels. The proposed project will enhance energy security by developing a sustainable nonfood/non-feed biomass for biodiesel. The proposed photobioreactor (PBR) system may enhance conventional biomass to ethanol production by increasing profitability. Profitability will be enhanced by using the carbon dioxide produced during ethanol production to create a new source of biomass, namely algae. The economics of ethanol production will also be enhanced by reducing production costs via decreased energy demand in the process. A significant portion of the energy used to produce ethanol is used to dry wet distillers grain. The amount of free water in wet distillers grain may range from 40-60% of the total water in the wet distillers grain (depending on moisture content). A non-thermal method to remove the free water will reduce the energy needed. The system will suspend the suspend the free water and distillers grain in an air stream, break the water droplets into small droplets, separate the water drops from the distillers grain, and use traditional thermal drying to remove water that is not free from the distillers grain. The PBR will require a concentrated source of carbon dioxide. Carbon dioxide sources can be ethanol plants, coal fired power plants, sewage treatment plants, methane digesters, livestock confinement units, and so forth. The PBR will not only add a new source of biomass for energy, it will help keep carbon in the energy chain not releasing it permanently to the atmosphere. Algae are one of the most efficient photosynthetic organisms on earth with a very rapid growth rate making them ideal for concentrated (requiring a small amount of land) biomass production.

OBJECTIVES: The objective is to develop the information necessary to design a photobioreactor (PBR) and a biomass drying technology that removes free water mechanically. Specific areas addressed for the PBR are determine the light extinction coefficient for dense algal cultures in a bubbly flow, the effects different light sources (LED, grow lights, fluorescent lights), and gas transfer coefficients for bubbly flows (carbon dioxide, oxygen). The information can be used by engineers to size PBR systems of different sizes. The drying unit objectives are to determine water droplet size relative to biomass particle size that will allow the two to be separated and then develop methods of breaking the water droplets and biomass into the appropriate sizes for separation.

APPROACH: Algae will tend to grow exponentially until either nutrient, light or carbon dioxide limited. Tests will be conducted to determine the grow curves of three or four algal species under different light intensities and types of lights (LED, fluorescent, grow lights, etc.). These tests will be carried out in 200ml flasks with the light source under the flask. Nutrients and carbon dioxide will be supplied in excess of needed for growth. The light intensity, light wavelength spectrum, and algal concentration (mass and cell count per unit volume) will be measured. Light extinction coefficients will also be determined for algal cultures of varying density in bubbly flows for a light source selected from the first testing. Tests will be conducted in flat plate photobioreactors (PBR) varying in width (light path) from 50.8mm to 304.8mm in 50.8mm increments. The PBR will be inoculated with algae and allowed to grow for 14-21d. At 3-4d intervals the air flow to the PBR will be varied from 0-9ml/min and light intensity measurements will be taken at each flow rate. The algal concentration will be determined for each days tests and the width of the bubbly flow will be estimated so the bubbly flow effect can be isolated from the growth medium-algae

areas. Difficulties are anticipated since the bubbly flow changes in width fairly randomly and the bubble concentration likely changes with flow pattern. The light extinction coefficients coupled with carbon dioxide transfer coefficients will help establish the three dimensions of the PBR along with the PBR volume needed to produce the desired amount of biomass (derived from the growth curves). The PBR pH will also impact algal growth. Bubbling large amounts of air laden with carbon dioxide can change the pH. Tests will be conducted with one size PBR selected from the light extinction coefficient tests. Carbon dioxide concentration will be varied in the air bubbling through the PBR. The growth medium pH will be measured and algal growth will be evaluated by cell count and mass per unit volume. The second part of the program deals with drying system. The essence of the system is that it can separate particles (water drops and distillers grain) from each other after it has broken them apart. Test to determine the ability of cyclonic devices and abrupt expansion and contraction devices to suspend plastic beads (1000kg/m3, water) in the size range from 80-2500microns will be conducted to determine what sizes will travel in the air stream. Next the beads representing water will be mixed with beads with a density of 1400-1600kg/m3 representing distillers grain particles. Sizes for testing are to be selected after the uniform bead size tests are evaluated. The tests will verify what size ratios of water drops relative to distillers grain particle sizes can be separated. The next battery of tests will use the plastic beads representing the distillers grain with water. The tests will determine which devices can effectively break the water in the mixture into small enough droplets to be separated from the distillers grain. Quantities measured are the temperature, air water content, air velocity, and static pressure.

PROGRESS: 2010/01 TO 2010/12
OUTPUTS: Three species of algae were grown with three different wavelengths of light at two intensities. The algae species are Chlorella kessleri (UTEX 398), Botryococcus braunii (UTEX 572), and Synechococcus leopoliensis (UTEX 625B). The wavelengths utilized were blue (467nm), red (659nm), and fluorescent (white) light. The first light intensity the current to the blue and red lights was kept at 2.6A with a voltage of 6.5V for the blue light and 11.0V for the red light. The light intensity 1cm from the source was measured at 557, 272, and 163 microwatts/square cm per nanometer for the blue, red, and fluorescent lights. These intensities correspond to 3019, 1389, and 2936 lux for the blue, red, and fluorescent lights respectively. The second light intensity was set at 6030 lux for all three wavelengths. The intensity was achieved by varying the distance the light source was from the surface of the photobioreactor (PBR). UTEX 398 was grown in a N-8 growth medium while UTEX 572 and 625B were grown in AC-H2O medium with Scully's microelements. Room air was used to mix the PBR growth medium and to supply carbon dioxide. The algae were grown for 7 days monitoring cell count, biomass concentration, pH, ORP, and temperature. Two runs were made with UTEX 398. The initial cell counts for UTEX 398 first run were 1,250,000, 1,350,000, and 1,240,000 cells/ml for the blue, red, and fluorescent lights and first light intensity respectively. The cell counts were 100,000 cells/ml for the second light intensity. The cell concentration for the first light intensity increased to 20,000,000, 13,500,000, and 5,000,000 cells/ml for the blue, red, and fluorescent lights while the cell mass went from 0.15-0.45, 0.12-0.4, and 0.06-0.36g/l for the light sources. The second light intensity saw the cell concentrations increase to 18,000,000, 14,000,000, and 14,000,000 cells/ml while the biomass concentration went from 0.125-0.22, 0.2-0.2, and 0.12-0.24 g/l. The red lit algae showed a significant drop in biomass for the first three days. The second time UTEX 398 was cultivated for 7 days. The initial cell concentration was 1,100,000 cells/ml for all lights first light intensity. The blue lit PBR increased cell count to 1,220,000 cells/ml with a biomass increase of 1.6-1.7g/l. the red light cell count increased to 2,850,000 cells/ml with a biomass range of 1.5-2.7g/l. The fluorescent light cell count increased to 35,000,000 cells/ml with a biomass range of 1.6-2.7g/l. UTEX 625B had an initial cell count of 10,400,000 cell/ml for all light sources. The blue lit PBR increased the cell count to 14,000,000 cells/ml with a biomass concentration range of 0.4-0.8 g/l. The red light cell count reached 64,000,000 cells/ml with biomass concentration of 0.2-1.0 g/l. The fluorescent lit PBR reached a maximum cell concentration of 53,000,000 cells/ml with a biomass concentration range of 0.4-1 g/l. UTEX 572 in blue light obtained a cell concentration of 490,000 cells/ml and the biomass ranged from 1-1.7 g/l. The red lit PBR 1,270,000 cells/ml with a biomass range of 1-2.3 g/l while the fluorescent light maximum cell concentration was 1,220,000 cells/ml and the biomass range was 1.1-2.1 g/l. PARTICIPANTS: Dr. Caner Koc was a visiting professor working on the project. He is on leave from Ankara University. His goal is to establish a lab facility modeled after the facilities that he worked in at SDSU. Dr Koc has expressed interest in developing a joint project involving the University of Ankara, South Dakota State University, and the University of Missouri-Columbia. The project would involve algal production systems and methods of converting algal biomass to biofuel. Mr. Eric Hodnefield and Mr. Eric Lanoue are undergraduate students working with the PBR development. They are gaining experience in algal cultivation, cell counting, biomass determination, and the measurement of pH, temperature, dissolved oxygen, and ORP. TARGET AUDIENCES: The target audience is the biofuel industry and those interested in producing algae, cynobacteria, and other photosynthetic organisms that produce biomaterial and biofuels that can be used directly or are building blocks for other industrial products. PROJECT MODIFICATIONS: Nothing significant to report during this reporting period.

IMPACT: 2010/01 TO 2010/12
The testing conducted showed that light wavelength does affect the growth of algae in terms of biomass concentration and cell concentration. UTEX 398 in blue light increased cell concentration by 11% and biomass concentration by 6% while the red light increased cell concentration by 2.5 times and biomass concentration by 1.8 times. The fluorescent light saw similar increases with the cell concentration increasing 3.2 times and the biomass concentration increasing 1.7 times. Blue light is not a good light source for UTEX 398. UTEX 398 grows better with red light with a mixture of other wavelengths. UTEX 625B showed better results with blue light. Biomass concentration increased 2 fold while cell concentration increased 1.27 times. Blue light still did not compete with red and fluorescent light. Cell concentration increased 4 fold for red light and 5.1 times for fluorescent light. The red light biomass concentration increased 4 fold while it increased 2.5 times for the fluorescent light. UTEX 625B grows bet in red light followed by fluorescent light with blue light being the least effect growing light. UTEX 572 had the highest cell concentration increases for all lights compared to UTEX 398 and 625B. Blue light cell concentrations increased 4.8 times while it increased 12.5 times for red light and 12 fold for fluorescent light. The biomass concentration increases were 1.7, 2.3, and 1.9 for blue, red, and fluorescent light. The biomass concentration increases are similar as found

for UTEX 398 and 625B. UTEX 572 cell size must have decreased in the PBR environment provided. The results suggest that blue light is not a good light source for growing the three species of algae. Also, UTEX 572 changes cell size in a PBR. In general red light gave the best growth results. Red light is the least expensive light source to produce on a photon basis. The cost of a red photon coupled with growth of algae make red light the best candidate for growing algae.

PUBLICATIONS (not previously reported): 2010/01 TO 2010/12
Koc, C, G.A. Anderson, and A. Kommareddy. 2010. Use of RGB LEDs and fluorescent lamps as light sources to grow microalgae in a photobioraector (PBR). ASABE Paper No. MBSK 10-105.

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Item No. 22 of 27

ACCESSION NO: 0220456 **SUBFILE:** CRIS
PROJ NO: SD00H348-09 **AGENCY:** NIFA SD.
PROJ TYPE: HATCH **PROJ STATUS:** NEW
START: 01 NOV 2009 **TERM:** 30 SEP 2014 **FY:** 2010

INVESTIGATOR: Zhou, R.

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ENGINEERING CYANOBACTERIA AS A FACTORY TO PRODUCE BIOFUELS AND HIGH VALUE CHEMICALS

NON-TECHNICAL SUMMARY: Our goal is to develop photosynthetic cyanobacteria that are capable of converting either CO2 or sugar into an energy-dense biofuel methylbutenol (MBO) or a high value industrial product isoprene. This will improve resource-use efficiency of biorefineries by recovering CO2 lost from fermentation and improve economic vitality by providing a fungible fuel MBO or a bioproduct isoprene used in the polymers industry. This proposal is based on our successful genetic engineering work in E. coli and the greater potential photosynthetic efficiency of cyanobacteria, which provides a greater MEP pathway flux for making the precursor for isoprene/MBO production. Three objectives of this project are: 1) genetically shunt the native isoprenoid biosynthesis pathway of cyanobacteria to separately produce isoprene/ MBO, by transferring-in the corresponding gene respectively; 2) maximize production and excretion of isoprene/MBO by blocking or reducing the carbon flow to competing pathways; 3) analyze isoprene/MBO production to assess the effectiveness of the genetic engineering work. By redirecting cyanobacterial carbon flow from producing stored bioenergy precursors (i.e. lipids, polysaccharides) to direct production of excreted products (isoprene/MBO), we will create a cellular "factory" that could be exploited in a recirculating photobioreactor system. The recirculating photobioreactor that grows isoprene/MBO producing cyanobacteria will be coupled with membrane units being developed by our industrial partner (Separation Kinetics, Inc) to recover the end products, while recycling cell mass and water. Due to cyanobacterial unique metabolism, isoprene/MBO can be directly produced from CO2 and sunlight using its photoautrophic metabolism; alternatively, isoprene/MBO can be produced from lignocellulosic sugars via its heterotrophic metabolic pathway. Broader impacts of this project are: 1) directly converting CO2 or sugar in to energy-dense fuels through MEP pathway is innovative and if successful will revolutionize the biofuel industry; 2) Developing an efficient process to produce isoprene/MBO from both CO2 and sugars will provide a technology and expertise platform that can be subsequently used for other high value fuels and chemicals; 3) significant reduction of CO2 emission because sugar-based methylbutenol production (let alone CO2-based) via MEP pathway would reduce 50% of CO2 emission compared to fuel ethanol production via fermentation process (e.g. fermentation releases 2 mol CO2/per mol glucose while MEP pathway releases only one mol CO2/per mol glucose); 4) the project serves as an excellent example of research that combines synthetic biology with technology for solar energy utilization; 5) the PI has expertise in molecular genetics and biochemistry, and has extensive experience working with cyanobacteria, thus providing an excellent hands-on training opportunity for postdocs, graduates and undergraduates; 6) broaden the participation of Native American youth and instructors in this project; and 7) the results will be disseminated through international publications and participation at scientific meetings.

OBJECTIVES: Biorefineries that use the biochemical conversion route release one third of the carbohydrate carbon as CO2

during fermentation, as well as significant amounts of low grade heat. For example, a 100 MMGY corn ethanol plant releases over 23 tons/hr of CO2 and 350 million BTU/hr of heat. We propose to dramatically improve biorefinery profitability by photosynthetically converting CO2 and low grade heat into high-value industry products isoprene and methylbutenol (MBO), in photobioreactors located in greenhouses adjacent to biorefineries. Methylbutenol (a C5 long-chain alcohol) has lower vapor pressure, lower hygroscopicity and higher energy density, also making it more suitable as liquid fuels compared to ethanol. Isoprene is a valuable chemical for industrial rubber and elastimer production. Three objectives of this project are: 1) genetically shunt the native isoprenoid biosynthesis pathway of cyanobacteria to separately produce isoprene/ MBO, by transferring-in the corresponding gene respectively; 2) maximize production and excretion of isoprene/MBO by blocking or reducing the carbon flow to competing pathways; 3) analyze isoprene/MBO production to assess the effectiveness of the genetic engineering work. By redirecting cyanobacterial carbon flow from producing stored bioenergy precursors (i.e. lipids, polysaccharides) to direct production of excreted products (isoprene/MBO), we will create a cellular factory that could be exploited in a recirculating photobioreactor system. The recirculating photobioreactor that grows isoprene/MBO producing cyanobacteria will be coupled with membrane units being developed by our industrial partner (Separation Kinetics, Inc) to recover the end products, while recycling cell mass and water. Due to cyanobacterial unique metabolism, isoprene/MBO can be directly produced from CO2 and sunlight using its photoautrophic metabolism; alternatively, isoprene/MBO can be produced from lignocellulosic sugars via its heterotrophic metabolic pathway. Our process will convert fermentation-derived CO2 into MBO for superior fuels or isoprene for biorubber, increasing the profitability of biorefineries, with no additional feedstock cost. This CO2 could be considered a negative value feedstock, reducing CO2 emissions by 2,000 tons for every million gallons of ethanol biomass ethanol produced. We estimate capital costs at $0.75/gal, primarily from greenhouse space and photobioreactor components. Operating costs (estimated at $0.35/gal) will largely be for labor and electrical requirements (pumping), since sunlight will be used for illumination. Assuming only half of the waste heat from a 100 MMGY corn ethanol plant can be captured, it would heat 1 million square ft (23 acres) of greenhouse space. This would be sufficient to produce up to 40,000 tons of MBO using our system, assuming only 50% CO2 fixation. This system will recycle water in the process, not use/generate hazardous/toxic substances, and reduce CO2 emissions. The low-impact environmental footprint will foster rural economic development, and provide an excellent ROI opportunity for corn and biomass-based ethanol facilities.

APPROACH: This work will use standard molecular biology techniques to modify synthetic genes to function in cyanobacteria. The three objectives are below. Objective 1. Engineering cyanobacteria to produce and excrete isoprene into culture fluide; Objective 2. Block or reduce carbon flow to competing pathways to maximize isoprene production; Objective 3. Assessing isoprene production in benchtop bioreactor under both photosynthetic and heterotrophic conditions. To achieve these three objectives, we have detailed 8 tasks to be done below: Task 1 Shunt the native MEP pathway of cyanobacteria to produce isoprene Task 2 Increase MEP pathway flux to DMAPP synthesis Task 3 Substitute genes (dxs,hdr,hds) for the reduction steps Task 4 Block synthesis of glycogen Task 5 Knock out major pyruvate-consuming pathway Task 6 Conditionally knock down the DMAPP flux to terpenoids Task 7. Testing culture for isoprene production Task 8 Isoprene/methylbutenol separation and purification

PROGRESS: 2010/01 TO 2010/12
OUTPUTS: Our goal is to develop photosynthetic cyanobacteria that are capable of converting CO2 into isoprene (C5H8) as rubber building blocks and linalool (C10H18O) as drop-in biofuels. This will improve resource-use efficiency of biorefineries by recovering CO2 lost from fermentation and improve economic vitality by converting this unused CO2 back to a drop-in fuel or chemicals. This proposal is to take advantage of using the highest photosynthetic efficiency of cyanobacteria, which provides a greater MEP pathway flux to make the precursor for production of both isoprene and linalool. Three objectives include: 1) genetically shunt the native isoprenoid biosynthesis pathway (MEP pathway) of cyanobacteria to produce isoprene and linalool by transferring-in the corresponding genes; 2) maximize production and excretion of linalool by blocking or reducing the carbon flow to competing pathways; 3) Identify long-chain hydrocarbons (alkenes/alkanes) innately produced by nitrogen-fixing cyanobacteria; 4) Identify the genes required for biosynthesis of such long-chain alkens/alkanes. Dr. Zhou and one postdoc have been working on this project.This project has also provided hands-on training for four undergraduate students. PARTICIPANTS: Dr. Ruanbao Zhou from Department of Bio-Microbiology has been working on engineering cyanobacteria to produce isoprene and linalool. The postdoc Yusheng Wu (01/2010- 08/2010) was also working on this project. Four undergraduate students received hands-on exoerience on metabolic engineering of cyanobacteria. TARGET AUDIENCES: Companies such as ICM, Inc, SDIP,VeraSun Energy, Separation Kinetics, and KL Process Design are developing biomass to ethanol processes. These companies form the base of our private sector partnerships and will provide the most direct route to commercialization. PROJECT MODIFICATIONS: Because we did not obtain the MBO synthesis gene from our collaborators, we had to modify our project by replacing our model product methylbutenol (C5H10O) with linalool (C10H18O). We also added two new objectives: 3) Identify long-chain hydrocarbons (alkenes/alkanes) innately produced by nitrogen-fixing cyanobacteria; 4) Identify the genes required for biosynthesis of such long-chain alkens/alkanes. Since we made a significant project modification, we want to change the title of our project to be ENGINEERING CYANOBACTERIA AS A FACTORY TO PRODUCE BIOFUELS AND HIGH VALUE CHEMICALS.

IMPACT: 2010/01 TO 2010/12
Outcomes: In the past year, we made substantial progress on objective 1: genetically shunt the native isoprenoid biosynthesis pathway of cyanobacteria to produce isoprene and linalool by transferring-in the corresponding genes. Because we did not obtain the MBO synthesis gene from our collaborators, we had to change our model product to be linalool (C10H18O). Luckily, we have succeeded in engineering Anabaena to produce and secrete linalool by transferring a plant linalool synthesis gene to Anabaena. The linalool production by transgenic Anabaena was confirmed by GC/MS analysis. However, as expected, the yield of linalool from the 1st generation of genetically engineered strain is quite low. Now we are focusing on improving the

linalool productivity. For another model product isoprene, we had a bad luck. Although we successfully transferred an IspS gene (isoprene synthase gene) into Anabaena, the transgenic Anabaena produced no detectable isoprene. Next, we will optimize the IspS codon for Anabaena because the codon modified IspS gene worked in a cyanobacterium Synechocystis 6803 (Lindberg et al., 2010). Impacts: Our process will convert fermentation-derived CO2 into linalool or isoprene, increasing the profitability of biorefineries, with no additional feedstock cost. Engineering a cyanobacterium to directly convert CO2 to excreted end products, which bypasses the expensive, multiple unit operated biomass pathway currently used in cellulosic biofuels or algal oil production. Our model product linalool (C10H18O), a long-chain alcohol with an energy density of 40 mj/kg, heat of vaporization of 0.19 mj/kg, and octane of 102. These features also make linalool suitable for a drop-in biofuel. Linalool is a naturally-occurring terpene alcohol with many commercial applications, such as most frequently used as perfumed hygiene products and cleaning agents. Recently, it has been reported that 2 micro mole (2uM) of linalool is able to completely kill cancer cells (Usta etal., 2009).This will also make linalool as a potential anti-cancer drug. Assuming only half of the waste heat from a 100 MMGY corn ethanol plant can be captured, it would heat 1 million square ft (23 acres) of greenhouse space. This would be sufficient to produce up to 20,000 tons of linalool using our system, assuming only 50% CO2 fixation. This system will recycle water in the process, not use/generate hazardous/toxic substances, and reduce CO2 emissions by 2,000 tons for every million gallons of ethanol biomass ethanol produced. The low-impact environmental footprint will foster rural economic development, and provide an excellent ROI opportunity for corn and biomass-based ethanol facilities.

PUBLICATIONS (not previously reported): 2010/01 TO 2010/12
No publications reported this period

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ACCESSION NO: 0221263 **SUBFILE:** CRIS
PROJ NO: TEX09409 **AGENCY:** NIFA TEX
PROJ TYPE: SPECIAL GRANT **PROJ STATUS:** EXTENDED
CONTRACT/GRANT/AGREEMENT NO: 2010-34228-20689 **PROPOSAL NO:** 2010-01466
START: 01 JUN 2010 **TERM:** 31 MAY 2012 **GRANT YR:** 2010
GRANT AMT: $332,249

INVESTIGATOR: Richardson, J. W.; Outlaw, J. L.

PERFORMING INSTITUTION:
Agri Economics
TEXAS A&M UNIV
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REGIONALIZED IMPLICATIONS OF FARM PROGRAMS ON CROP FARMS

NON-TECHNICAL SUMMARY: The probable economic impacts of changes in the farm bill on the viability of representative crop farms in major production regions of the US will be estimated. Purpose of the project is to provide information to Congress, producers, consumers, and commodity groups as to the consequences of alternative options for the 2008 farm bill and increased production of biofuels.

OBJECTIVES: The primary objective for FY10 is to use the representative farm data base to analyze questions from the House and Senate Agricultural Committees related to alternative ways of implementing proposed options for the 2008 farm bill. A secondary objective is to analyze alternative policies and farm management/marketing strategies being considered for dealing with the increased price and income risk. Specific objectives are: - Use the representative farm data sets, with aggregate policy analyses by FAPRI, to analyze the economic effects of implementing the 2008 Farm Bill. - Use the FLIPSIM model and the representative farm data base to analyze the farm level impacts of alternative risk management programs and management/marketing strategies proposed for dealing with the increased price risk. - Continue updating the representative farms so they remain useful for farm policy analyses. - Host a conference to present policy analysis results to different interest groups.

APPROACH: Representative crop farms data base maintained at Texas A&M will be used with the farm level and policy

simulation model (FLIMSIM) to analyze the farm level effects of policy changes. The FLIPSIM model developed at A&M is the recognized model for conducting Monte Carlo simulation analyses of policy impacts on farms. The policy analyses will be done in a risk context so probabilities of economic viability can be estimated.

PROGRESS: 2010/06 TO 2011/05
OUTPUTS: We updated 20 of the 64 representative crop farms and updated the FLIPSIM model to simulate options in the 2012 farm bill. Research on the economics of micro algae for diesel was expanded on several fronts by developing an economic model and applying it to analyze alternative production scenarios. Expanded the world CGE model to include food crisis identification capabilities and linkages for analyzing the algal biofuel sector. PARTICIPANTS: Individuals Dr. James W. Richardson Co-Project Director - Directed research, prepared reports to Congress, wrote articles, presented papers at professional meetings, and made presentations to commodity groups, Congressional Ag Committees and USDA. Dr. Joe L. Outlaw Co-Project Director - Directed research, prepared reports to Congress, wrote articles, presented papers at professional meetings, and made presentations to commodity groups, Congressional Ag Committees and USDA. Dr. David Anderson Associate Professor and Extension Economist - Conducted research and developed extension programs on impacts of farm programs on dairy, beef, sheep and crop producers. Dr. Henry Bryant Research Assistant Professor - Developed and applied econometric models to analyze impacts of increased demand for biofuel on grain and oilseed prices. Developing a world CGE model to further analyze biofuel impacts. Mr. George Knapek Program Director for Representative Farms - Managed the updating and use of representative farms for policy analysis. Mr. Marc Raulston Research Associate - Updated representative crop, beef, and dairy farms by meeting with panels of producers. Used the farms for farm policy analysis. Mr. Brian Herbst Research Associate - Updated representative crop, beef, and dairy farms by meeting with panels of producers. Used the farms for farm policy analysis. Partner Organization(s) Food and Agricultural Policy Research Institute (FAPRI) at the University of Missouri, Columbia. - They share funds from CSREES on the project. - Dr. Scott Brown and Pat Westhoff develop sector forecasts of crop and livestock prices that are used in AFPC's representative farm analyses. - Mr. Peter Zimmel collaborates with George Knapek in updating representative farms. Training or Professional Development AFPC trains graduate students in the science and art of quantitative policy analysis. The graduate students involved in the project are: - George Knapek - Ph.D. - Jiamin Lu - Ph.D. - Jose Juan Monge - Ph.D. - Myriah Johnson - Ph.D. TARGET AUDIENCES: Target Audiences: - Economists and staffers attached to the U.S. Congressional Agriculture Committees. - Leadership of national, regional, and state farm commodity organizations such as: wheat, corn, cotton, rice, soybeans, milk, beef, sheep and goats. - Farmers and ranchers. - Other stakeholders, such as: agricultural input suppliers, processors, and exporters. Efforts: - Through the AFPC website www.afpc.tamu.edu we provide free copies of all AFPC reports on farm outlook and policy analyses. - Adult education programs for commodity organizations and farmers/ranchers/and other stakeholders were held to present policy analysis results. - Briefings for different policy analyses and the Baseline outlook were provided to the House and Senate Ag Committees and to the USDA World Board. - Papers were presented at professional meetings and journal articles were published from the research. PROJECT MODIFICATIONS: None - research is on schedule, report preparation is on schedule.

IMPACT: 2010/06 TO 2011/05
Under the January 2011 Baseline, 37 of the 64 crop farms are considered in good liquidity condition (less than a 25 percent chance of negative ending cash by 2015). Twelve crop farms have between a 25 percent and a 50 percent likelihood of negative ending cash, and the remaining 15 crop farms have greater than a 50 percent chance of negative ending cash. Furthermore, 44 of the 64 crop farms are considered in good equity position (less than a 25 percent chance of decreasing real net worth during the study period). Ten crop farms have between a 25 percent and 50 percent likelihood of losing real net worth, and ten crop farms have greater than a 50 percent probability of decreasing real net worth. The following discussion provides an overall evaluation by commodity considering both liquidity and equity measures. - FEEDGRAIN FARMS: Nineteen of the 23 feedgrain farms are in good overall financial condition. Three are classified in marginal condition, and one is in poor condition. - WHEAT FARMS: Eight of the 11 wheat farms are classified in good financial condition and three are in marginal condition; no farms are in poor condition. - COTTON FARMS: Eight of the 16 cotton farms are classified in good condition, four are in marginal condition, and four are in poor condition. - RICE FARMS: Two of the 14 rice farms are projected to be in good financial condition, four are in marginal condition, and eight are in poor condition. - DAIRY FARMS: Eleven of the 21 dairy farms are in good overall financial condition. Five are considered to be in marginal condition, and five are in poor condition. - BEEF CATTLE RANCHES: Seven of the 12 cattle ranches are classified in good financial condition, four are in marginal condition, and only one is projected to be in poor condition. Economic analyses of algae farming for biodiesel production showed that the cost per gallon of algae oil is greatly dependent upon the capital investment expense. If the capital costs are large it increase the interest and dividends cost to the point where more than 50% of the cost per gallon is debt servicing costs. As a result algae farms will have to reduce capital costs 5%-60% from engineering cost estimates to gain a reasonable probability of economic success. Costs per gallon of algae oil can be reduced from the $7-$10 range to the $2-$3/gallon range by reducing capital costs. There of course is a trade-off in that smaller reductions in capital costs coupled with 75%-100% increases in biomass production can yield favorable probabilities of economic success. Over the last 12 months, we have completed substantial improvements to our computable general equilibrium model of the world economy. This facilitates analyses that are now under way of many interesting big-picture questions relating to bioenergy technology development, renewable energy policy, climate change, and their effects on ag and energy markets.

PUBLICATIONS (not previously reported): 2010/06 TO 2011/05
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ACCESSION NO: 0208664 **SUBFILE:** CRIS
PROJ NO: VA-135778 **AGENCY:** NIFA VA.
PROJ TYPE: HATCH **PROJ STATUS:** TERMINATED
START: 01 OCT 2006 **TERM:** 31 JUL 2010 **FY:** 2010

INVESTIGATOR: Wen, Z.

PERFORMING INSTITUTION:
BIOLOGICAL SYSTEMS ENGINEERING
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ENHANCING ANIMAL MANURE ANAEROBIC DIGESTION PERFORMANCE: MANURE PRETREATMENT, MICROBIAL COMMUNITY ANALYSIS, AND CO-PRODUCTS DEVELOPMENT

NON-TECHNICAL SUMMARY: The disposal of the animal manure is a challenge faced by animal industries in both Virginia and the United States. Anaerobic digestion (AD) provides an alternative to traditional animal manure management. However, animal manure AD is limited by high fiber content of the manure, and lack of information of the microbial community inside the reactor. This project will address above problems through the practices of manure pretreatment, characterizing microbial communities at different AD stages, and developing co-products.

OBJECTIVES: The goal of this study is to improve the overall efficiency of the animal-manure anaerobic digestion through manure pretreatment, microbial communities analysis, and co-product development. The specific objectives are to (1) enhance biogas production from animal-manure anaerobic digestion by using microorganisms to accelerate the degradation of the fibrous components; (2) characterize, both qualitatively and quantitatively, the microbial communities at different stages of the AD processes by using 16S rDNA-based molecular techniques; and (3) produce high quality fiber products from animal-manure crude fiber to be used in the nursery industry.

APPROACH: Dairy manure slurry will be pretreated by rumen microorganisms through four tasks: (1) isolation of cellulolytic microorganisms from ruminants gastro-intestinal tract; (2) establishment of in-vitro culture of rumen organisms, and optimization of the growth conditions; (3) investigation of the attachment of rumen organisms on the fiber surface; and (4) integration of the rumen organism pretreatment into anaerobic digesters. The experiment will be replicated in triplicate, and the data will be statistically analyzed by one-way ANOVA to compare the significance of the differences observed among the different treatments. After the integration of the pretreatment process with the anaerobic digestion, the microbial community involved in the different stages of anaerobic digestion process, will be monitored qualitatively and quantitatively. The samples to be investigated include rumen fluid, influent after pretreatment, effluent from the acidification reactor, and effluent from the methanogenesis reactor. First, nucleic acids (DNA and RNA) of the microbial consortia will be extracted from anaerobic sludge samples. The partial 16S rDNA fragment from the community DNA will be amplified by polymerase chain reaction (PCR) for

denaturing gradient gel electrophoresis (DGGE) fingerprint. At the same time, a full-length 16S rDNA will be PCR-amplified for cloning and sequencing. Second, to quantify the microbes, total RNA will be hybridized with 16S rRNA-targeted oilgonucleotide probes to quantify the relative abundance of microbial groups by both fluorescence in-situ hybridization (FISH) and membrane hybridization. The probe design will be based on the clone library derived from the sequence analysis by DGGE and cloning. Lastly, the microbial community information will be used to the implementation of anaerobic digestion model (ADM 1). Only the active species (determined by membrane hybridization) and the abundance species (determined by FISH) will be implemented into the kinetic rate equations of ADM1. By omitting the minor bacteria/archaea species and their corresponding kinetic rate equations, the implementation of ADM 1 can be significantly simplified. In addition to above pretreatment and microbial community analysis research, the manure fiber will be processed to produce high quality materials, with the aim of replacing commercial peat moss in the nursery industry. First, different chemical and physical parameters to evaluate the fiber quality for various nursery applications will be established. These parameters include water-holding capacity, air space, porosity, conductivity, ammonium-N, nitrate-N, phosphorus, potassium, sulfur, calcium, magnesium, sodium, iron, aluminum, and trace element (manganese, copper, and zinc). Second, two treatment processes will be used to modify and improve the quality of raw manure fiber, including hot water washing of manure fiber, and treatment with white rot fungi. Third, the effectiveness of prepared manure fiber products used as a soil amendment will be evaluated by germination tests and growth trials of several plants commonly used in the nursery industry.

PROGRESS: 2006/10 TO 2010/07
OUTPUTS: Animal manure is typically disposed of through land application. Anaerobic digestion for biogas production and recovering nutrients as slow release fertilizer provide alternative for traditional manure management practices. During the project lifetime, we developed several technologies with the goal of achieving the high biogas production, nutrient recovery, and pathogen reduction; the feasibility of using microalgae for treating animal wastewater for producing renewable biofuel is also explored. First, we developed several pretreatment methods for degrading the lignocellulosic fiber contained in dairy manure. We applied the microwave-treatment technology to dairy manure slurry to enhance the degradation of manure fiber. In addition to microwave pretreatment, we also attempted to use rumen organisms as a pretreatment to enhance the fiber degradation. However, the rumen microorganisms-base pretreatment did not enhance biogas production significantly. Second, the nutrient (nitrogen and phosphorus) recovery from dairy manure was investigated. Still using the microwave treatment technology, it was found that the pretreatment facilitates the conversion of organic and poly-phosphorus (P) into inorganic P which can be used for nutrient recovery through struvite precipitation. We then used the chemical reaction to form the struvite, which was visualized by the Scanning Electronic Microscopy. Further analysis of the P content in the liquid phase (after struvite removal) confirmed the effectiveness of P recovery/removal by microwave pretreatment. Third, we studied the effects of different manure treatment processes on the fate of pathogens contained in the manure by using a rigorous quantitative PCR-based method for accurately assessing pathogens. A protocol to extract DNA from manure samples has been established, with a control mutant E. coil strain being constructed to monitor DNA extraction efficiency. During the project period, we used three different anaerobic digestion processes, i.e., mesophilic anaerobic digestion (MAD), thermophilic anaerobic digestion (TAD), and temperature phased anaerobic digestion (TPAD) for treating dairy manure slurry and then quantified the pathogens presented in the treated manure. The PCR-based method was used for numerating total E. coli contained in the manure effluent. It was found that almost all E. coli cells were killed in the TAD process and the TPAD also resulted in almost 90% E. coli reduction. While the manure treated by MAD still contained a significant amount of E. coli. Finally, we initiated an algae-to-biofuel area during the project period. Algal biofuel production has gained renewed interest in recent years but is still not economically feasible due to several limitations related to algal culture. Our study is to explore a explore the possibility of growing the microalgae as biodiesel feedstock using dairy manure wastewater as growth medium. It was found that the microalgal cells can be successfully grown in manure-based wastewater with 61-79% of total nitrogen removed and 62-93% of total phosphorus removal from the wastewater. PARTICIPANTS: Zhiyou Wen; Ying Jin; Zhenhu Hu; Michael B Johnson TARGET AUDIENCES: Nothing significant to report during this reporting period. PROJECT MODIFICATIONS: Not relevant to this project.

IMPACT: 2006/10 TO 2010/07
Currently, most manure produced in animal operation is disposed through land application. There has been increased pressure from government agencies and the general public on the animal industry to tighten its manure management practices to reduce potential environmental problems. Anaerobic digestion (AD) provides an alternative to traditional animal manure management with a number of benefits including odor control, and reduction in greenhouse gas emissions, and yields beneficial products such as biogas and fertilizer. The microwave-based pretreatment technology developed in this project proved to be an efficient method to break down the recalcitrant manure fiber and thus enhance the biogas production. In addition, the microwave pretreatment increased the nutrient (particularly phosphorus) recovery by changing the form of P from organic status to inorganic status. In addition to biogas production and nutrient recovery, development of a robust method to accurately assess pathogens in manure matrices is needed. The outcome of this project will be a protocol to rapidly and accurately identify and quantify the pathogen organisms in animal manure samples and evaluate the effectiveness of different manure treatment process on the pathogen reduction. The results will impact the U.S. agriculture industry by providing methods for better assessing the fate of pathogenic microbes in dairy waste, and understanding the source, fate and the transport of pathogens in soil, surface and ground water. Algal biomass is a potential feedstock for biofuel production if the right kind of species is cultivated to produce significant quantities of lipids. Using dairy manure wastewater to grow algae can significantly reduce the culture cost while alleviating the environmental problems caused by manure disposal. The outcome of this project will be development of a system to easily grow and harvest microalgal biomass with high lipid content, which can be used as biofuel feedstock. The results will provide a method for better utilization of animal manure wastewater, and will benefit U.S. by potentially reducing the U.S. demand for foreign fossil oil.

PUBLICATIONS (not previously reported): 2006/10 TO 2010/07
No publications reported this period

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ACCESSION NO: 0216562 **SUBFILE:** CRIS
PROJ NO: VA-136228 **AGENCY:** NIFA VA.
PROJ TYPE: HATCH **PROJ STATUS:** NEW **MULTISTATE PROJ NO:** S-1041
START: 01 OCT 2008 **TERM:** 30 SEP 2013 **FY:** 2010

INVESTIGATOR: Zhao, B.

PERFORMING INSTITUTION:
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THE SCIENCE AND ENGINEERING FOR A BIOBASED INDUSTRY AND ECONOMY

NON-TECHNICAL SUMMARY: Biodiesel production is a fast-growing industry in the United States but is limited by the feedstock supply. The feedstock for commercial biodiesel production in the United States is predominately from pure vegetable oils such as soybean or canola oil. Currently, the biodiesel manufacturers are facing changelings of high prices of these feedstock. It is well-known that microalgae accumulate oils that are suitable for producing biodiesel. However, current **algal biofuel** production is facing several major limitations; i.e., the high cost of harvesting algal biomass from diluted culture solution, the contamination of undesired species, and the low oil yield from the algal cells. As a result, the technology is still not commercially viable. The algal culture process developed here is particularly designed to reduce the algal harvesting cost. This sytem allows for easy, high-density collection of algae, and thus reduces incurring the usual costs associated with traditional growth methods. The culture system can be run on a continuous basis, with biomass harvested periodically. After each time of harvest, the system does not need an extra step for algae inoculation again. It can be potentially used to produce large quantities of algal biomass for use as a feedstock, such as in the production of biodiesel.

OBJECTIVES: 1. Improve biofuel production processes 2. Identify, develop and evaluate sustainable processes to convert biomass resources into biochemicals, biocatalysts and biomaterials 3. Identify and develop needed educational resources, develop distance based delivery methods, and develop a trained work force for the biobased economy

APPROACH: In this project, we will develop a novel attached growth system to cultivate microalgae. The algal cells were incubated in a container with an attaching material located in the bottoms, the contianer was placed on a rocker shaker which provided a smooth, gentle rocking motion. The surface of the supporting material was alternatively submerged into the culture solution that provided nutrients for algal growth and then illuminated with light, which provided energy for algal photosynthesis. The algal biomass can be harvested by simply scraping the algal biomass mat from the surface of the materials; the residual biomass attached on the surface serves as the inoculum for the next batch algal culture, and so the additional inoculum steps in the traditional algal culture systems can be avoided. The supporting material, polystyrene foam, is resilient to damage, withstands culture conditions, and is reusable for the foreseeable duration of the algal culture. This sytem allows for easy, high-density collection of microalgae, and thus reduces incurring the usual costs associated with traditional growth methods, such as filtering large quantities of water from the algae. It can potentially be used to produce large quantities of algal biomass for use as a feedstock, such as for biodiesel production.

PROGRESS: 2010/08 TO 2010/11
OUTPUTS: The goal of this project is to establish the culture and transformation system for several microalgae (Botryococcus braunii , Schizochytrium limacinum, and Pythium irregulare) that can be used for biodiesel production. In the last few month, we made progress on several objectives: (1). Optimized the growth condition of Botryococcus braunii to prepare competent cells for electro transformation. At the beginning of the project, we tried to grow B. braunii in autoclaved soil-containing liquid medium. However, the algal cells tend to be contaminated by unknown microbes and aggregated when we tried to prepare competent cells for electro-transformation. We filtered the soil-containing medium through 0.2 um filter, this simple step

completely prevents the contamination problem during the liquid culture process. After collecting algal cells, we tried to re-suspend the pellet in 1.0 M sorbitol instead of 10% glycerol. This method prevents the aggregation of algal cells, which could facilitate the electro-transformation process. The electro-transformation will allow us to transient express targeted genes. (2). Identified antibiotics that could inhibit the growth of B. braunii in liquid medium We tried to grow Botryococcus braunii in liquid medium that was supplemented with Hygromycin, biolophos, Kanamycin, Augmentin at various concentrations. Our results suggest Hygromycin (50mg/L) and biolophos (8 mg/L) can significantly inhibit the growth of B. braunii. However, Augmentin, even at the highest concentration (375mg/L), has no effect on the growth of B. brauni. The result of Kanamycin is not very reproducible. It is possible that Kanamycin is not stable in liquid medium. Based on this result, we designed a binary vector as described in (3) that would allow us to select transformed algal cells using both Hygromycin and biolophos. We will also be able to suppress the growth of Agrobacterium tumefaciens with Augmentin but not affect the growth of B. braunii. PARTICIPANTS: Bingyu Zhao, PI, oversees the whole project. Bing Xu, Graduate student, performed the microalgae culture and transformation. TARGET AUDIENCES: Not relevant to this project. PROJECT MODIFICATIONS: The original PI, Dr. Zhiyou Wen, relocated to Iowa State University in August, 2010. Dr. Bingyu Zhao of Department of Horticulture at VT was changed to the PI of this project.

IMPACT: 2010/08 TO 2010/11
Microalgae has great potential for producing diesel and jet fuel (JP-8) quality hydrocarbons with designer chain lengths and unsaturation content at commercially viable scales and costs. Microalgae species like Botryococcus braunii has the potential to be the highest producer of hydrocarbons for biofuels, using just 3% of the U.S. cropping area to meet U.S. liquid fuel needs without competition with food crops for land. But this has not yet been realized primarily because of the high production costs using natural strains, which are not tailored to meet production requirements. There has been a lack of knowledge on the biochemical pathways and enzymatic properties of hydrocarbon syntheses, and an absence of molecular genetic technologies for improving the organism~{!/~}s production capabilities. In this project, we tried to establish the culture method for Botryococcus braunii, and establish a genetic transformation protocol that allows us to genetically engineer Botryococcus braunii. We found new the growth medium can eliminate the microbial contamination problem during the microalgae culturing process. Several antibiotics have been identified that can be used to select potential transgenic Botryococcus braunii strains. The results will help us establish and select genetic-improved microalgae strains for efficient biodiesel production.

PUBLICATIONS (not previously reported): 2010/08 TO 2010/11
No publications reported this period

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ACCESSION NO: 0214431 **SUBFILE:** CRIS
PROJ NO: VA-428581 **AGENCY:** NIFA VA.
PROJ TYPE: SPECIAL GRANT **PROJ STATUS:** TERMINATED
CONTRACT/GRANT/AGREEMENT NO: 2008-34602-19321 **PROPOSAL NO:** 2008-03482
START: 01 AUG 2008 **TERM:** 31 JUL 2011 **FY:** 2009 **GRANT YR:** 2008
GRANT AMT: $208,348

INVESTIGATOR: Mostaghimi, S.; Quisenberry, S.

PERFORMING INSTITUTION:
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BIODESIGN AND BIOPROCESSING RESEARCH CENTER AT VIRGINIA TECH

NON-TECHNICAL SUMMARY: The goal of this proposal is to support the activities of the Biodesign and Bioprocessing Research Center (BBRC) at Virginia Tech. The mission of BBRC is to enhance the capabilities and economic viability of farmers by conducting cutting edge basic and applied research for the design, production, and recovery of industrial enzymes, biofuels and pharmaceuticals from transgenic and alternative specialty crops, and for conversion of agricultural materials and residues to value-added products. This would include process scale-up of new scientific discoveries to promote entrepreneurship. The final impact will be revitalization and sustaining economies by adding value to crops and agricultural by-

products through biotechnology and Bioprocessing.

OBJECTIVES: The following two objectives will be accomplished:1.Enhance dairy manure anaerobic digestion through fiber treatment, mixing enhancement and microbial community characterization. 2.Develop a novel algal attachment-based culture system for producing oil-rich microalgae from dairy wastewater.

APPROACH: Objective 1. Enhance fiber degradation through aerobic treatment by microorganisms. The scientists at Novozymes have developed a unique aerobic fermentation process to degrade lignocellulosic materials. We will apply this technology to treat the manure fiber that has gone through the anaerobic digestion process. The performance evaluation of this technology will be based on the soluble COD that results from this treatment, the biogas increase in terms of biogas volume and CO2/CH4 composition. Characterize the microbial community in anaerobic digesters. We will investigate the microbial community of the digester qualitatively and quantitatively. The DNA of the microbial consortia from different manure samples will be extracted. We will use DGGE to identify different microorganisms. The partial 16S rDNA fragment from the community DNA will be amplified by polymerase chain reaction (PCR) for DGGE fingerprint. DNA from the resolved band will be recovered for sequencing to identify the microbes of interest. Investigate the mixing behavior of the anaerobic digester. We will use two 20-L laboratory-scale anaerobic digesters for this objective. The mixing of the reactors will be based on the gas-circulation method. Animal manure wastewater at different solid contents (2%, 5% and 7.5%) will be used for digestion. We will evaluate the flow characteristics inside the digester. Mixing efficiency is determined from the distribution defined by the variation in tracer concentration with time by calculating the statistical properties of the distribution. Objective 2. Enhance lipid production of selected algal species. Selected algal species including Chlorella sp. Botryococcus braunii and Pleurochrysis carterae will be evaluated for their lipid/oil production capability. The medium compositions for the algal culture are a combination of manure waste water and Bristol nutrient solution. We will investigate the effects of nutritional (nitrogen and carbon sources) and environmental factors (biomass density, pH, and salinity) on algal lipid production. The production of lipids is dependent on carbon availability. We will study the effect of CO2, bicarbonate and acetate on lipid content (% of dry weight) and productivity (g l-1 d-1). The optimal incubation time will be determined for each alga. Attach the selected algal species on different supporting materials. Once the optimal growth conditions for the algae are determined, the algal species will be grown on the surface of different supporting materials. The attachment of algae to the supporting materials will be studied. The supporting materials, the shaking speed, and the shaking frequency that result in the highest biomass and lipid production will be used in both the bench and pilot-scale ATS systems. Implement the algal culture to an Algae Turf Scrubber system. A bench-scale Algal Turf Scrubber (ATS) algal culture system will be used. The algae will be grown on the screen surface of the ATS system until a thick layer of biomass is formed. The selection of algal species, culture conditions, and supporting material will be based on the results obtained from previous objectives.

PROGRESS: 2008/08 TO 2011/07
OUTPUTS: We conducted both laboratory and field scale studies to test use of alum and iron based salts in combination with polymers to remove phosphorus from dairy manure. Based on the quantity used, one can remove as up to 100% of P from the manure. Our study on chemical phosphorus removal showed that use of chemical + polymers in large volumes of batch manure types is feasible. We successfully demonstrated our designer manure concept at the Virginia Tech dairy farm by chemically treating 2,000 cubic meters of manure in batch using a combination of aluminum chloride and a polymer with a trade name Superfloc 4512. These results provides a viable alternative to manure phosphorus management, to producers who have liquid manure handling systems and are in areas where soils may be saturated with phosphorus, have manure storage for 6 months and may be limited by land area to apply manure. Using chemicals to remove phosphorus can work well with dairies that use flush systems to handle manure. The recovered phosphorus, being small in volume but high in phosphorus content can then be transported out of the Shenandoah Valley, if the objective is to remove the excess phosphorus in the region. In another investigation we successfully developed a novel algal culture system to reduce the biomass harvest cost. When algae cells are grown on the surface, they can be harvested by scraping, and thus, the settling ponds and subsequent centrifugation in traditional microalgal suspended culture systems is avoided. Among various supporting materials tested for algal attachment, polystyrene foam led to a firm attachment, high biomass yield and high fatty acid yield. The biomass attached on the supporting material surface was harvested by scraping; the residual colonies left on the surface served as inoculum for re-growth. Also, dairy wastewater effluent from a local dairy farm was used as a growth medium in order to further reduce algae production costs while providing the valuable service of removing excess nutrients from the wastewater. The attached algal culture removed 61-79% total nitrogen and 62-93% total phosphorus from dairy manure wastewater, depending on different culture conditions. The biomass harvested from the attached growth system (through scraping) had a water content of 93.75%, similar to that harvested from suspended culture system (through centrifugation). Collectively, the attached algal culture system with polystyrene foam as a supporting material demonstrated a good performance in terms of biomass yield, biodiesel production potential, ease to harvest biomass and physical robustness for re-use. PARTICIPANTS: Dr Zhiyou Wen and Jactone Arogo were the principal scientists working on the objectives reported here. TARGET AUDIENCES: Managers of agricultural and municipal waste materials as well as industries involved in development of value-added products and conservation of natural resources would benefit from the results of this study. PROJECT MODIFICATIONS: Nothing significant to report during this reporting period.

IMPACT: 2008/08 TO 2011/07
Our research focused on producing designer manures with the appropriate balance of nitrogen (N) and phosphorus (P) fertilizer values required by different crops. Removing the excess P allows the continued use of manure as a fertilizer in areas such as the Chesapeake Bay that may be restricted by the P-based manure application regulations. Also, removing P from manures prevent over application of P, which is a typical result of applying manure to meet crop N needs. A second benefit of

the designer manure concept is that it recovers and recycles P in an environmentally sustainable manner. This is critically important because rock phosphate reserves, the major source of P for agriculture, are projected for depletion within 50 to 100 years, making recovery and reuse of P necessary for long-term food security. The results of our efforts on Producing oil-rich microalgae from dairy wastewater for algal fuel production can be used for producing algal biomass which can be used as feedstock for producing biofuel as well as high value food products. Currently, algal biofuel production is still far from being economical due to several challenges including high cost of the harvesting biomass and biofuel preparation from biomass. This project aimed to develop new technologies to solve these challenges.

PUBLICATIONS (not previously reported): 2008/08 TO 2011/07
Shen, Y., J.A. Ogejo, and K.E. Bowers. 2011. Abating the effects of calcium on struvite precipitation in liquid dairy manure. Transactions of the ASABE 54(1):325-336 Johnson M, Wen Z. 2010. Development of an attached microalgal growth system for biofuel production. Applied Microbiology and Biotechnology. 85 (3): 525-534. Johnson M, Wen Z. 2009. Production of biodiesel fuel from the microalga Schizochytrium limacinum by direct transesterification of algal biomass. Energy and Fuels. 23 (10): 5179-5183. Ethier S, Woisard K, Vaughan D, Wen Z. 2011. Continuous culture of the microalgae Schizochytrium limacinum on biodiesel-derived crude glycerol for producing docosahexaenoic acid. Bioresource Technology. 102 (1): 88-93.

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ACCESSION NO: 0207953 **SUBFILE:** CRIS
PROJ NO: TEST-00588 **AGENCY:** NIFA WYO
PROJ TYPE: NRI COMPETITIVE GRANT **PROJ STATUS:** TERMINATED
CONTRACT/GRANT/AGREEMENT NO: 2006-35318-17445 **PROPOSAL NO:** 2006-03270
START: 01 SEP 2006 **TERM:** 31 AUG 2010 **FY:** 2009 **GRANT YR:** 2006
GRANT AMT: $242,000

INVESTIGATOR: Herbert, S. K.; Basile, F.; Gomelsky, M.

PERFORMING INSTITUTION:
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BIOLOGICAL FUNCTIONS OF ANTIOXIDANTS ASSOCIATED WITH OXYGENIC PHOTOSYNTHESIS

NON-TECHNICAL SUMMARY: Antioxidants are present in all aerobic cells. They have generated much scientific interest because of their potential value as remedies for human diseases and as tools for improving the stress tolerance of agricultural plants. Attempts to improve stress tolerance of plants by genetic modification of antioxidants have had mixed success, however. It has become clear that a more detailed, mechanistic knowledge of how antioxidants work in living plant cells is needed to understand their adaptive value in nature and to make effective use of them in medicine and agriculture. Our project addresses this need. This project will begin a detailed analysis of how the most common antioxidant enzymes protect specific components of photosynthetic cells as the cells experience oxidative stress, which occurs in crops during periods of drought and cold. This knowledge of what they do in live cells will allow genes for the antioxidants to be used more effectively in genetic modification for greater stress tolerance in agricultural plants.

OBJECTIVES: The goal of this project is to resolve the biological functions of antioxidant enzymes associated with the oxygen-evolving photosynthetic system. The chemical functions of these enzymes are well known but their biological functions, how they work in living plant cells, are not. Past efforts to improve stress tolerance of plants by genetic modifications of antioxidants have yielded mixed success. Basic knowledge of how antioxidants function in living cells is essential to using them effectively for engineering of increased stress tolerance in agricultural plants.

APPROACH: We will take two approaches. Our first approach is make rigorous physiological analyses of mutants in the model cyanobacterium Synechococcus sp PCC 7942 that lack one member of their complement of antioxidant enzymes. These analyses are intended to determine the primary sites of oxidative damage protected by the missing antioxidant enzyme.

We define primary sites of oxidative damage as specific molecules where early oxidative modifications cause loss of normal cell function, often by promoting a cascade of secondary oxidative damage throughout the cell. Identifying the primary sites of oxidative damage that are protected by specific antioxidant enzymes will provide useful knowledge of their biological function, specifically, what cell components they protect and under what conditions of oxidative stress they do so. Our second approach is to determine the patterns of amino acid oxidation that occur in photosynthetic complexes of the thylakoid membrane in the Synechococcus mutants. They will be exposed to mild oxidative stresses and the proteins of their Photosystem I complexes will be analyzed by mass spectroscopy to locate and identify amino acids that have been oxidized. By comparing the temporal and spatial patterns of amino acid oxidations between mutants and wild type, we hope to generate a novel, spatial understanding of how antioxidants protect the oxygenic photosynthetic system in cyanobacteria, and ultimately in plants.

PROGRESS: 2006/09 TO 2010/08
OUTPUTS: The original goal of the project was to understand antioxidant gene function in cyanobacteria so as to inform breeding of crop plants for greater stress tolerance. During the project, rising oil prices generated renewed interest in large-scale growth of algae for biodiesel production. Our project became refocused on improvement of oxidative stress tolerance in algae, both cyanobacteria and the green alga Chlamydomonas. Oxidative stress can strongly limit algal growth in large-scale cultures. Improvement of oxidative stress tolerance is a near term goal of algal domestication for biodiesel production (Wijffels & Barbosa, 2010, Science 329:796). Refocusing the long-term goals of the project on algae greatly shortened the distance between our fundamental study and application of its results in agriculture and industry. 1. Activities - Experiments were performed on antioxidant null mutants of the cyanobacterium Synechococcus elongatus PCC7942. Experiments included assays to quantify loss of enzyme function in the mutants, dose response experiments to quantify growth under defined oxidative stresses, Photosystem II photoinhibition experiments, Photosystem I inhibition experiments, and metabolite profiling. Genetic modifications of the green alga Chlamydomonas were also performed. Grad and undergrad students conducted most experiments so teaching and mentoring were inherent. 2. Services - Free consulting was provided to the Mt. Cement Corporation when they were asked to purchase an ill-conceived algal biodiesel facility for their Laramie, WY cement plant. As a result, they chose to initiate small experiments on algal carbon sequestration rather than purchase a large-scale facility. 3. Products - Multiple plasmid vectors for genetic modification of Synechococcus and Chlamydomonas were made and multiple strains of Chlamydomonas expressing glycoprotein genes from the green alga Volvox were produced. These activities tested methods for genetic modification of Chlamydomonas to improve its oxidative stress tolerance. The transformed strains were also valuable in their own right because they exhibited "auto-flocculation," which is clumping of cells for easier harvest from large-scale cultures. Two patent applications based on this work were submitted to the University of Wyoming (UW) technology transfer office for review. One is formally a patent pending. A 750 liter outdoor photobioreactor was also constructed and tested for mid-scale production of algal biomass and field trials of genetically modified strains. 4. Dissemination - Multiple presentations of the project were given through May 2011. These included 14 seminars and 7 posters. Venues included the DOE National Renewable Energy Lab, Colorado State University, USDA project directors meetings, a Western Photosynthesis Conference, Western Society of Crop Science meetings, a AAAS western section meeting, UW Undergraduate Research Day meetings, UW McNair Scholars meetings, and department seminars at UW. Findings from the project were also incorporated into lectures for biology and agronomy classes at UW, multiple grant proposals, and were discussed with citizens interested in growing algae. PARTICIPANTS: 1. Project director - Stephen K. Herbert directed the project but received no salary. 2. Co-PI - Franco Basile provided analytical chemistry expertise and access to a GC-MS system for analysis of carbohydrate and amino acid profiles but received no salary. 3. Co-PI - Mark Gomelsky supervised construction of Synechococcus mutagenesis vectors but received no salary. 4. Research associate and graduate student - Wendy K. Cecil conducted many of the experiments described under Outputs. She received 50% of her salary from the project for approximately 24 months. 5. Graduate student - Dimitri Ryenkov constructed plasmid transformation vectors for mutagenesis of 2 additional antioxidant genes in Synechococcus elongatus PCC 7942. He received approximately 3 months of salary from the project. 6. Graduate student - Levi G. Lowder conducted physiological experiments, genotyped the Synechococcus strains used to assure they were all Synechococcus, and constructed plasmid vectors for heterologous protein expression in Chlamydomonas. He received approximately 2 months of salary from the project. 7. Undergraduate student - Matthew Link performed dose response experiments and enzyme assays. He received approximately 4 months of salary from the project. 8. Undergraduate student - Bethany Borbely served the project as general lab assistant and performed many physiological experiments. She received approximately 10 months of salary from the project. 9. Undergraduate student - Vanessa Taulo-Millar performed enzyme assays and dose response curve experiments. She received approximately 2 months of salary from the project. 11. Undergraduate student - Connor Thompson performed PS I inhibition assays. He received approximately 4 months of salary from the project. 12. Undergraduate student - Denise Pierre performed dose response experiments and recieved approximately 4 months of salary from the project. 13. Undergraduate student - Lianne Przygocki performed chlorophyll analyses and received approximately 3 months of salary from the project. All of the individuals designated as graduate or undergraduate students above received training and professional development proportional to their participation. TARGET AUDIENCES: The target audience for this project consisted primarily of academic scientists, corporate scientists, science students, and a few members of the general public. The efforts made to deliver science-based knowledge to this target audience are described in the dissemination section of Outputs above and in Publications above. PROJECT MODIFICATIONS: As noted in Outcomes above, the project has been refocused on near term application of knowledge about antioxidants to the domestication of algae for production of petroleum substitutes, including biofuels. The major impact of this modification on actual work performed was addition of Chlamydomonas as a subject organism and the acquisition of methods and materials for heterologous protein expression in Chlamydomonas and other algae. Events outside the project drove this change, primarily rising oil prices and a remarkably sudden new interest and investment in the development of algal biofuels. The overall effect of the project modification was to turn relatively esoteric and fundamental research into experiments and results much closer to direct application. This modification is consistent with recent directives from the USDA and other science funding agencies.

IMPACT: 2006/09 TO 2010/08
As noted in Outputs, the national context of the project changed during its execution. This and changes in knowledge generated by the project itself have led to changes in action, specifically a redirection of the project toward domestication of algae for farm-scale production of petroleum substitutes by genetic modification for improved oxidative stress tolerance. Changes in conditions caused by the project have not yet occurred but can be reasonably expected in the next 5 years. 1. Change in knowledge - To date, the most striking new information generated by the project was that the sodB mutant was inhibited in its rate of Photosystem II (PS II) repair after strong light exposure while the katG and tplA mutants were not. This result defines one of the functions of cyanobacterial and chloroplastic FeSODs as protection of the protein turnover process required for PS II repair. This is consistent with recent studies demonstrating that inhibition of PS II repair is one of the major impacts of oxidative stress on photosynthesis in both plants and algae (Takahashi & Badger, 2011, Trends Plant Sci 16:53). Contrary to current thinking, however, our results are consistent with an inhibition of amino acid biosynthesis rather than protein synthesis generally as the mechanism by which oxidative stress inhibits PS II repair. Current proposals being submitted for the project include genetic modification strategies for maintaining amino acid biosynthesis under oxidative stress. Publications that describe this new perspective are also in preparation. 2. Change in actions - Changes in actions have been limited to project participants, specifically the PI and students working under his supervision. The changes in action included establishment of a small recombinant DNA work area in which plasmid vectors are constructed for genetic modification of algae that are plausible candidates for domestication, beginning with the green alga Chlamydomonas. Changes in action also included efforts to formulate an algal bioenergy group at UW that was integrated with the UW School of Energy Resources (SER). One result of this effort was funding of a small proposal to test the growth of algae under exposure to cement plant flue gas, which includes beneficial carbon dioxide but also oxidants such as sulfur dioxide and nitrous oxides. As part of this project, a 750 liter polyethylene photobioreactor was designed and tested for field trials of genetically modified algae. 3. Changes in conditions - No clear changes in conditions have resulted from the project to date but several local growers and ranchers have expressed interest in producing algae on their land for biodiesel or using algal biomass for soil amendment. One of the latter is a cooperator in a graduate student proposal to the Western SARE program that is now pending. It is hoped that algal production by a few local growers or ranchers, promoted by this project, can occur within 5 years. To this end, the 750 liter polyethylene photobioreactor described in section 2. above will be demonstrated at the Laramie Research and Extension Center greenhouse complex field day in August 2011.

PUBLICATIONS (not previously reported): 2006/09 TO 2010/08
1. Cecil, W.K., Borbely, B., Taulo-Millar, V., Link, M., Fomina, I.R., Herbert, S.K. 2011. Photoinhibition repair in antioxidant null mutants of the cyanobacterium Synechococcus elongatus PCC 7942. In preparation.
2. Lowder, L.G., Herbert, S.K. 2011. Heterologous expression of a Volvox cellular adhesion molecule induces autoflocculation in Chlamydomonas reinhardtii. J. Applied Phycol. In revision.
3. Kreslavski, V.D., Fomina, I.R., Tatarinzev, N.P., Ivanov, A.A., Kosobryukhov, A.A., Biel, K.Y., Herbert, S.K. 2010. NaCl-induced photoinhibition and recovery of photosynthetic activity by the KatG- mutant of the cyanobacterium Synechocystis sp. PCC 6803. Biofizika (Russian Journal of Biophysics) 55(2):252.

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