

**FLORIDA STATE UNIVERSITY**  
*Establishment of the Center for Marine Bioenergy Research: Systems Approach to BioEnergy Research (SABER)*

**PI:** Joel E. Kostka **Co-PIs:** William Cooper, Michael Wetz  
**Students:** Claire Smith (Ph.D.), Kristina Welch (M.S.)

**Description:** This project is carried out under the auspices of FSU's Institute for Energy Systems, Economics, and Sustainability (IESES) funded by the Florida legislature (<http://www.ieses.fsu.edu/>) since 2008. Within IESES, the Systems Approach to Bioenergy Research (SABER) program was created to blend fundamental and applied research on sustainable, biologically-based fuel alternatives and renewable energy strategies. SABER also focuses on recycling or clean up of environmental pollution (greenhouse gases, excess nutrients) associated with energy production and use. The centerpiece of this project is the development of state-of-the-art technology to tap aquatic algae as a biomass source for fuel production. IESES is particularly focused on coupling algal cultivation to wastewater remediation. IESES has recently partnered with the City of Tallahassee T. P. Smith Waste Water Treatment Plant in order to study the growth of local fresh water algae in waste water for use as biofuel. Presently, one of the drawbacks to algal biofuels is that to obtain significant biomass, the algae must be fertilized with additional nutrients. Our solution to this issue is to grow the algae in secondary treated waste water, which has high levels of essential nutrients. The two main objectives of this project are to: 1) perform both laboratory and field experiments to test for species-specific growth potentials, as well as for the effects of different environmental parameters, including light, CO<sub>2</sub>, and nutrient availability on microalgal growth rates and lipid production, and 2) determine the extent to which microbes (i.e. bacteria), which are exceptionally abundant in waste water, act as either competitors (for nutrients, carbon) or symbiotically with algae. To do this we are examining the bacterial community present in the waste water and detecting community shifts that occur during algae cultivation. We are also examining the nutrient uptake dynamics between bacteria and algae by monitoring the usage and production of nitrogen, phosphorous, and carbon-containing compounds. With better understanding of the microbial and biogeochemical processes occurring in waste water during algae cultivation, engineering approaches may be proposed in order to further optimize algal growth in waste water.

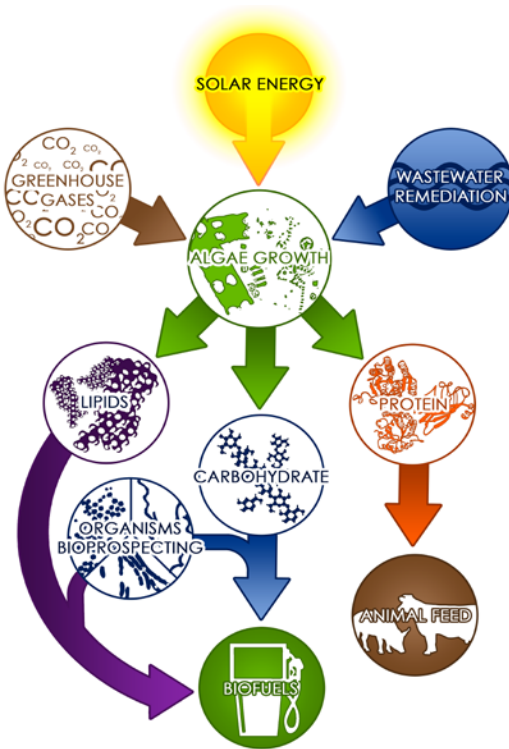
**Budget:** \$300,000

**External Collaborators:** City of Tallahassee

### **Progress Summary**

**Progress to Date:** We are developing a state-of-the-art, off-the-grid algal cultivation facility which will allow us to produce algae with a minimal ecological footprint. Once cultivated, the algae yield lipids, carbohydrates, and protein, which can be processed into biofuels using microorganisms or used as biomass in animal feed. Wastewater from the process (and other sources) can then be reintroduced into the system during cultivation, making this a highly efficient, ecologically friendly alternative for producing fuel from the sun. A multidisciplinary team of faculty and students at FSU has been assembled to cover biofuels R&D across the entire value chain from biomass feedstock production to distribution and greenhouse gas life cycles. Areas of ongoing research include: algal strain selection and growth optimization, biomass analysis and conversion, and the use of green power in sustainable, carbon neutral algal cultivation. SABER is particularly focused on coupling algal cultivation to wastewater remediation.

Figure 1. The centerpiece of the SABER project is the development of state-of-the-art technology to tap aquatic algae as a biomass source for fuel production.



Summary of Research Progress: 15 strains of microalgae (representing 4 genera of Chlorophytes) were isolated from Lake's Bradford & Munson (near Tallahassee, Florida) and established as monospecific cultures. Batch culture growth experiments were conducted to identify those with highest growth rates and lipid production in an idealized media (Chu 10) and in wastewater. Two strains were selected for more detailed growth studies in municipal wastewater. Algal growth was limited by low phosphorous levels and high pH levels in the waste water, indicating that pH buffering is needed. Evidence from microcosm experiments indicates that cyanobacteria may be outcompeting algae for CO<sub>2</sub>, suggesting that these organisms should be considered for further considered as a biomass feedstock source grown in wastewater. Experiments are being run in summer, fall and winter to take advantage of seasonal variability in municipal wastewater nutrient concentrations and speciation.

In the first month, PI Kostka and co-PI Wetz visited companies engaged in algal biofuels research throughout the state. Contact was made with the following four different biofuels companies: the Midwest Research Institute (MRI) of Palm Bay, Petroalgae in Melbourne, Aurora Biofuels of Vero Beach, and Algenol of

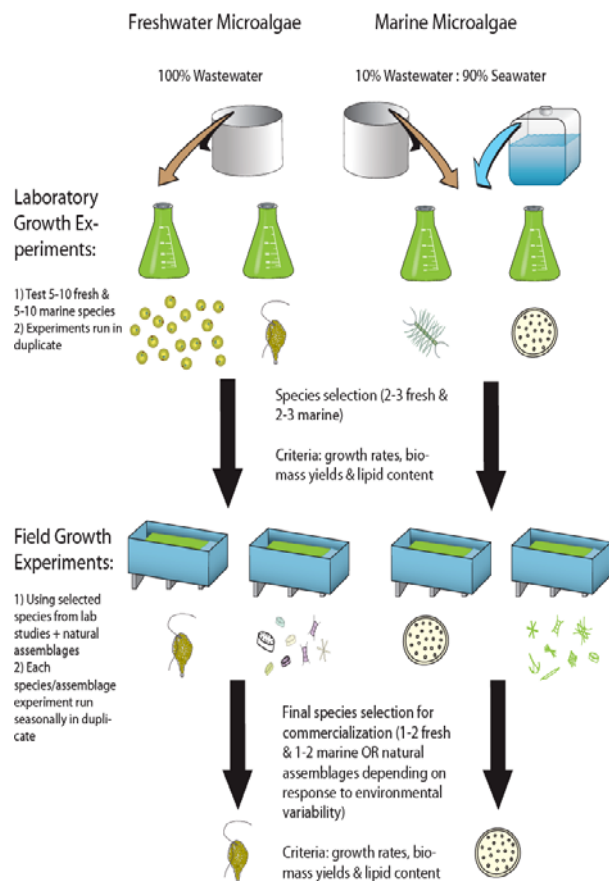
Bonita Springs. Most of the companies provided us with a tour of their facilities and we discussed potential research collaborations. Aurora Biofuels informed us that they already have a university partner from Florida Institute of Technology. Algenol was the only company that was not interested in further collaboration. Thus, we pursued further contacts with Petroalgae and MRI. Contact with MRI resulted in the submission of two research proposals and we remain in communication on further opportunities (please see Deliverables section). Petroalgae contacted us at the beginning of the summer about leasing land for the construction of algal cultivation facilities and they asked us if we would be interested in teaching a workshop to their personnel on algal cultivation. The partnership with Petroalage requires that we build an marine algal cultivation facility close to the ocean. Thus, we are currently in discussions with the Director of the FSU Coastal and Marine Laboratory, Dr. Felicia Coleman, to move forward on this partnership with Petroalgae.

Within the first two months of the project, two excellent Ph.D. students, Claire Smith and Kristina Welch were recruited to work on the project. Claire and Kristina were admitted to the Oceanography Department, and both of them began their work in August. Kristina's research is squarely focused on the growth of algae for biofuels applications. Claire will pursue research on the environmental impacts of biofuels production and is currently carrying out an experiment on carbon biosequestration for a proposal to be submitted to the U.S. Department of Energy in this area.

The algae-to-biofuels research community is growing and changing rapidly. Most investigators have been involved with this research area for less than 2 two years. To develop a unique bioenergy research portfolio for IESES at FSU, we are investigating a number of opportunities. The main focus is on optimizing the growth of marine and freshwater algae to be used as a biomass feedstock for the production of transportation fuels such as ethanol or biodiesel. Industry reports indicate that in order

to develop this technology in a cost effective manner, the growth of algal biomass should be coupled to some other industrial process such as wastewater remediation or the mitigation of carbon dioxide emissions from the flue gas of coal-fired power plants. The growth of marine algae was discussed above. For the growth of freshwater algae, we have developed a number of partnerships and projects are farther along. These projects are focused on using the growth of algal biomass as an inexpensive alternative for the remediation of municipal and rural wastewater. Contact was initiated with Greenpointe, LLC, of Jacksonville, Florida in early August. Greenpointe has initiated a project on the cleanup of wastewater that enters the St. Johns River. We have offered to help them with the analysis of their waste stream and they will contact us when samples are available.

In September, discussions were initiated with the City of Tallahassee. The city's wastewater treatment plant is near capacity. To address future needs in capacity, the city requires alternative means to dispose of reclaimed water as well as methods to reduce operational costs. SABER has entered into a partnership with the city to optimize the growth of algal biomass for fuel from the city's nutrient-rich wastestream. See Figure 2 for details on the research plan. The city has offered the use of land at the plant to build a pilot scale algal cultivation facility. SABER will build the pilot scale facility and the city will offer some analytical services as a match. Design and construction are underway. In the meantime, Ph.D. student, Kristina Welch, will obtain wastewater samples and begin cultivation in October. During our meetings with the city, two other possibilities of collaboration were discussed. The city has an operating biorefinery for transforming vegetable grease into biodiesel through a transesterification process. We will explore the possibility of using this refinery to produce biodiesel from algal biomass. Secondly, we discussed the establishment of a recycling program on campus for oils from food waste to be used as a feedstock for the



production of biofuels. Co-PI Michael Wetz is an accomplished algal physiologist and is leading the algal growth experiments. The growth of algae is a key component that drives the other components of our research plan. Wetz arrived as an assistant professor at FSU in December of 2008. SABER research on algal growth has been hampered by delays in the renovation of the Wetz laboratory. The laboratory was recently finished and algal cultivation has commenced.

Professor Juergen Wiegel, Distinguished Professor of Microbiology at the University of Georgia was recruited by the PI Kostka to be a visiting scientist for IESSES and he has accepted in principle, pending scheduling. Professor Wiegel will advise the project on the conversion of algal biomass to biofuels using microbial enzymes. He will assist with the development of research proposals, and he will also teach a short course in biomass conversion focused on hydrolysis and fermentation of biomass to ethanol.

Professor Wiegel pioneered the study of microorganisms that grow at temperatures above 55 degrees in the absence of oxygen. He has established one of the premier laboratories in the world for the isolation and characterization of such "thermophilic anaerobes." As a post-doc at UGA,

Wiegel isolated *Thermoaerobacter ethanolicus*, which represents a novel thermophilic genus, species, and family and was the first wild-type organism patented in the United States for ethanol production. Recently, his laboratory extended the known limits of life when it isolated new genera of bacteria that thrive in alkaline hot springs and salt flats. Some of his novel isolates are a rich source of industrial-relevant enzymes. His laboratory developed a genetic system for thermophilic anaerobes, which is now used in industry. Wiegel's work has resulted in more than 190 original scientific publications, three patents, and \$5.7 million in extramural funding. In 2007 he received the Bergey's Award, the highest honor in systematic bacteriology, for his contributions to the systematics of thermophilic and alkaliphilic microorganisms in extreme environments. Professor Wiegel is scheduled to visit FSU during spring/summer of 2010.

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The centerpiece of this project is the partnership between SABER and the City of Tallahassee. A pilot scale facility is being installed at the Southeast Farm Waste Water Reuse Facility in Tallahassee, which is fed with secondary treated effluent from the waste water treatment plant. At this facility there will be three raceway ponds for continuous cultivation of algae, which will be used for large scale experiments and ultimately converted to biofuel.



Figure 2. Raceway ponds at the Southeast Farm Facility (Left panel) and Secondary treated waste water at the T. P. Smith Waste Water Treatment Plant (Right panel).



Figure 3. Accumulated microalgal biomass in wastewater (Left panel) and an example of a lab-based bioreactor that we are using for larger-scale microalgal biomass production (Right panel).

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Laboratory results show that algal species 10 and 11 are the most efficient growers and best lipid producers (Figures 4 and 5). Thus, these species are currently being further tested under other lab conditions and eventually in the pilot-scale outdoor facility.

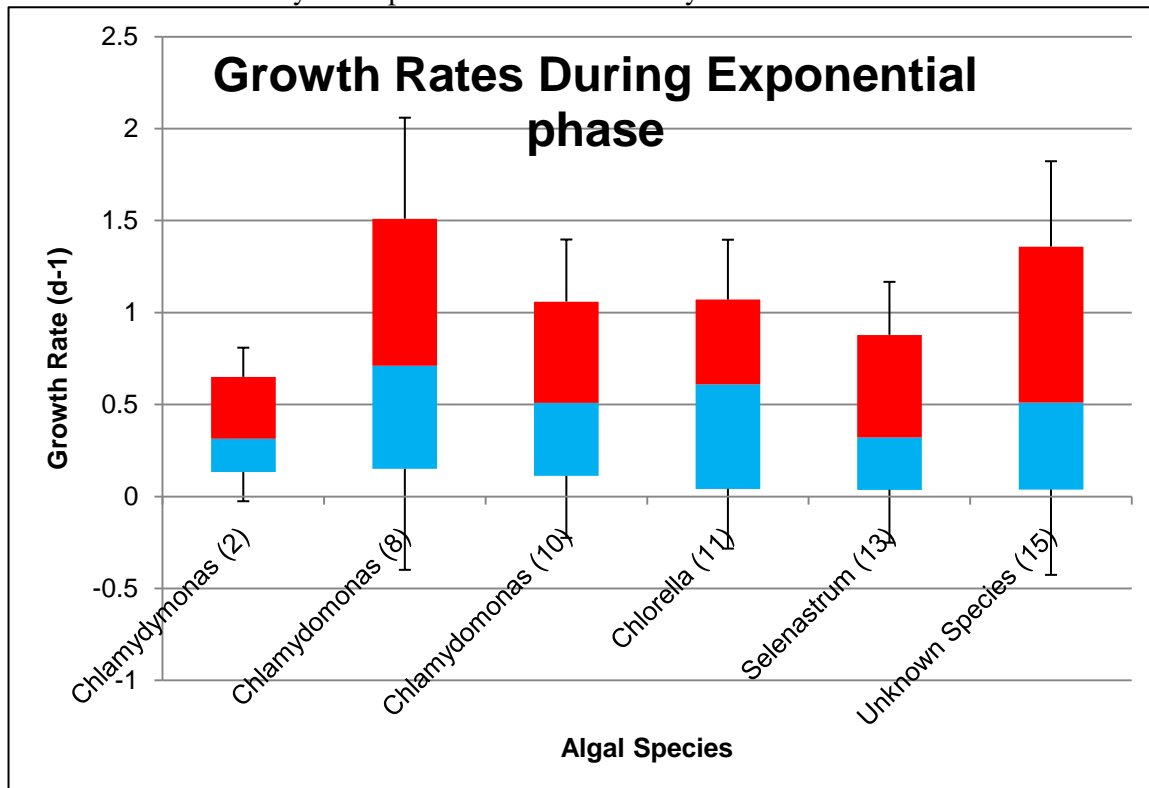


Figure 4. Growth rate of algal strains under ideal conditions in the laboratory.

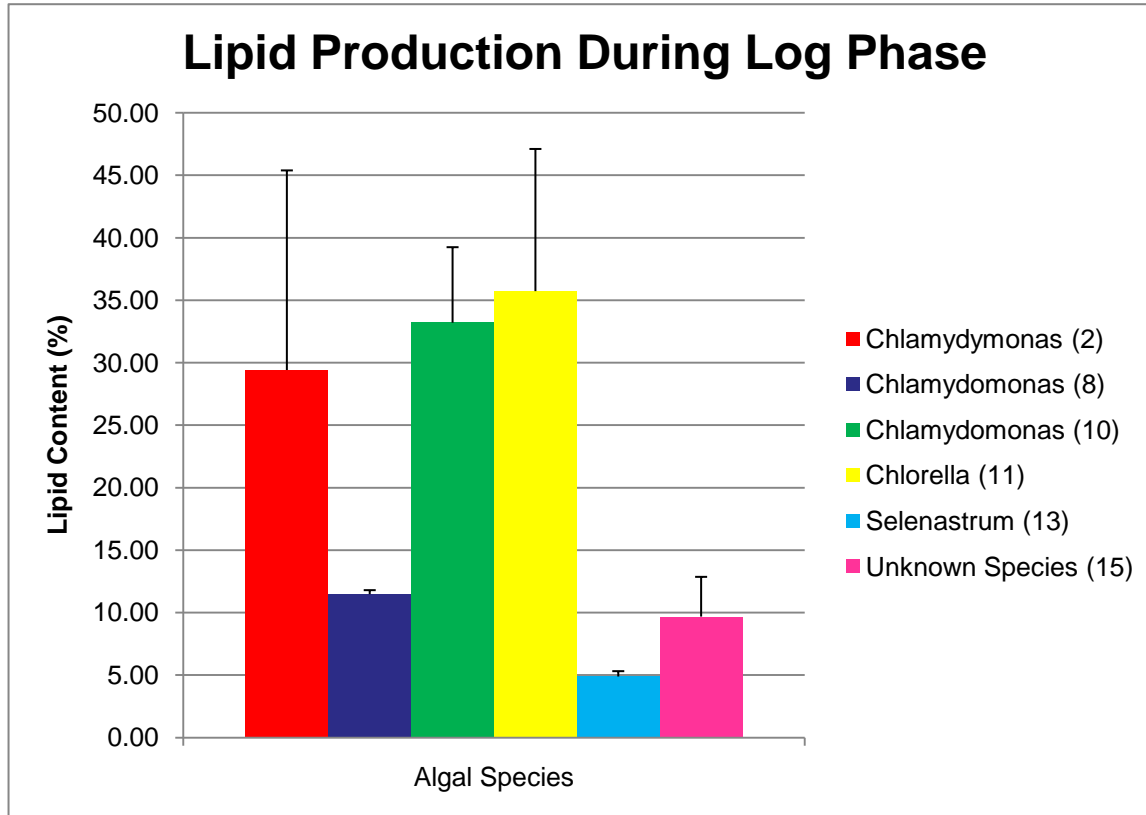


Figure 5. Lipid content of algal species represented in Figure 4.

These species also greatly reduced the nutrient loads of the water they are grown in, particularly the orthophosphate content, suggesting that they will be good candidates for remediation purposes. The next round of experiments will test the impacts of light, CO<sub>2</sub>, and nutrient composition of the wastewater on growth and lipid production.

Deliverables for the period of 10/2009-10/2010:

A total of 13 proposals have been submitted to federal agencies for funding of SABER research. Three projects are funded, 5 were denied, and 5 are pending (see below). A number of biofuels meetings were attended by the PIs. The students presented their research at regional and national meetings. A principal investigator meeting for the SABER program was convened by the PI in March of 2010. The goal of the meeting was to report on research progress and discuss further collaborations within IESES at FSU. This meeting was a resounding success, and the presentations are available upon request. Numerous other meetings were attended by the PI with state and local officials that are not listed.