COVER PAGE

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Principal Investigator Information: Harvey Hou	**Recipient Organization:** Alabama State University 915 S Jackson St Montgomery, AL 36104-5615 Country: USA **UEI:** DLJWLMSNK627

ACCOMPLISHMENTS

The project aims to overcome barriers to recruitment and retention of underrepresented minorities with comprehensive training activities in microbiology and energy science. The research activities will serve as training and mentoring opportunities for underrepresented students (undergraduates and graduates) and postdoctoral researchers, led by scientists from the National Renewable Energy Laboratory (NREL), Washington University in St. Louis (WUSTL), and ASU.

Objective 1: Isolate microalgae and cyanobacteria strains in Alabama and the Gulf Coast region. This will involve collecting microalgae and cyanobacteria strains in selected locations in Alabama and the Gulf Coast regions, enriching them in liquid culture, isolating the strains using plates, screening them by microscopy, and identifying them using 16S/18S rDNA PCR and Sanger sequencing. We do not expect major issues in this objective. The microalgae and cyanobacteria isolates will be deposited in the UTEX collection, and a new ASU Cyanobacteria Collection Center will be established as part of this project.
Objective 2: Study carbon uptake and metabolite overflow, and the ability to degrade plastics/oil polymers in these strains, under various culture conditions. As the tri-institutional collaborative team, we plan to investigate energy regulation and management in algal strains by monitoring metabolic end-products in the growth media, as well as in whole cells, using bioanalytical techniques such as high-performance liquid chromatography (HPLC) and liquid chromatography with mass spectrometry (LC-MS). Understanding the dynamic energy flows in cyanobacteria and microalgae will inform new strategies for producing biofuels and chemicals by controlling the energy and carbon sinks.
Objective 3: Teaching innovation by integrating research activities into the curricula at ASU. We will integrate research into the classroom and laboratory modules in a variety of ASU classes, including general chemistry, forensic science survey, forensic chemistry, forensic toxicology, capstone senior seminar, and graduate seminar. Specifically, we will implement innovations, including new lectures, experiments, and ultimately new courses, based on the research activities of CREATE. In addition, we will organize two conferences: one broad STEM Annual Research Frontier Symposium in the spring semester and one focused energy science workshop, entitled "cyanobacteria and algae research and education (CARE) workshop in the fall semester.

1.1 Research Objective 1: Isolation of microalgae and cyanobacteria strains in Alabama and the Gulf Coast region.

The tri-institutional (NREL-WUSTL-ASU) cyanobacteria team has established the local cyanobacteria collection center at Alabama State University in the spring of 2025. The name of the center is “ASU RENEW Cyanobacteria Collection Center,” which is led by PI Harvey Hou. The Center is housed in Hatch Hall at Alabama State University. A Scientific Committee, including the Co-PIs, is formed, and an Advising Committee consisting of the world-leading experts in the field of photosynthesis and the cyanobacteria community, including the Curator of UTEX Culture Collection of Algae at UT-Austin (UTEX) and the Director of Bigelow National Center for Marine Algae and Microbiota (NCMA). The members of the Advising Committee are invited and to be confirmed.

We began the experiment by collecting soil samples from Forest Park in St. Louis, Missouri. After collection, the samples were enriched for nitrogen-fixing strains by adding nitrogen-deficient media (BG11–N) to the soil. Within a few days, the soil turned green as cyanobacteria began to grow. Cyanobacterial cells were then collected and cultured in liquid and solid media for further characterization. Microscopic analysis revealed the presence of multiple cyanobacterial strains in most of the samples collected (SLFP #1-10). Spectroscopic analysis of the samples showed significant differences in the nitrogen-rich light-harvesting antenna, suggesting variability in the nitrogen-fixing efficiencies of the strains. Further experiments on the N-fixing and chemical analysis of overflow metabolism in the strains under different light intensities are in progress at Alabama State University and Washington University in St. Louis.

We isolated nitrogen-fixing cyanobacteria from water and soil samples collected on the NREL campus. The growth of nitrogen-fixing cyanobacteria, as well as the isolation and purification process of the algae, was explored and identified. DNA sequencing was used to identify the isolated strains. The measurements of the N-fixing and chemical analysis of overflow metabolism in the strains will be further investigated.

At ASU, we have collected cyanobacteria samples from freshwater and soil in local areas of Montgomery, Alabama, as well as from the ocean at Dolphin Island in Mobile, Alabama. The total number of cyanobacteria samples is 211. The samples were isolated by plates with a common algal medium (BBM) under moderate light (50-100 mmol m-2 s-1) and screened by microscopy. We found 24 positive strains and will further purify them by plating. Characterization of the samples involves physiological assays to evaluate growth and development under various light and nutritional conditions. The cellular protein and pigment abundance in the samples was assessed using UV-vis spectroscopy and the Fourier transform infrared (FTIR) method. Future work will be placed on 16S rRNA sequencing to verify taxonomic identity. The microalgae and cyanobacteria isolates will be deposited in the UTEX strain collection, and a new ARMCCC will be established within this project.

1.2 Research Objective 2: Study carbon uptake and metabolite overflow, and the ability to degrade plastics/oil polymers in these strains, under various culture conditions

Alpha-ketoglutarate (AKG) plays a crucial role in cellular metabolism, primarily as an intermediate in the citric acid (or Krebs) cycle to produce ATP. We have observed previously that glycogen synthesis mutant DglgC is able to secrete alpha-ketoglutarate (AKG) as a major overflow end-product (Cano et al, 2018). There is no detectable overflow of AKG in the wild-type cyanobacteria. Our chemical analysis revealed that AKG at approximately 200 µM of metabolite overflow was present in the growth medium of the cyanobacterium Synechocystis sp. PCC 6803 was observed at high light conditions. The LC-MS and FTIR support the overflow of AKG in cyanobacteria. This line of evidence supports that there is a lower quantity of metabolite overflow of AKG in WT cyanobacteria, as an alternative mechanism for carbon sink and energy management. Further investigation will focus on the AKG transporters and the regulation of AKG overflow in cyanobacteria.

We found a significant amount of glycerol overflow in the growth medium of cyanobacteria, as indicated by an HPLC peak at a retention time of 13.6 min in the late phase of the growth period, which was assigned to glycerol using an external standard solution. FTIR data supported the identification of glycerol overflow in cyanobacteria. The glycerol overflow is different from the AKG/pyruvate overflow in time, in which the AKG/pyruvate overflow occurs first, followed by the overflow of glycerol. This idea provides specific new insights into metabolite overflow in cyanobacteria for energy management and may offer a novel approach to glycerol production from CO2. The transporters of glycerol are aquaglyceroporins and caveolins, which are submicroscopic integral membrane proteins that are particularly abundant in many mammalian cells. Future effort will be on the overflow mechanism and regulation of metabolite glycerol, including transporters in cyanobacteria.

We observed the mM-scale formate overflow in cyanobacteria at high light conditions (500 µE). The HPLC peak at retention time 14.6 min is assigned to formate by using the standard. The formate peak increases during the growth period, suggesting a possible role in energy management, similar to that of AKG and pyruvate. The enzymatic assay confirmed the identification of formate in metabolite overflow in cyanobacteria. FTIR data supported the notion that formate overflow in cyanobacteria occurs under high light conditions. This idea provides specific new insights into metabolite overflow in cyanobacteria and offers a novel approach for CO2 to formate production via cyanobacterial metabolite overflow. In future work, we intend to investigate the mechanisms of formate overflow and optimize experimental conditions for formate production under various growth conditions.

1.3 Research Objective 3: Teaching innovation by integrating research activities into the curricula at ASU

In the spring semester of 2025 and fall semester of 2205, PI has implemented the cyanobacteria research in the five classes, general chemistry (CHE 141, CHE 141L, CHE 142, and CHE 142L), forensic science survey (FRS 220), forensic chemistry (FRS 320 and 664), forensic toxicology (FRS 432 and 642) and senior capstone seminar (FRS 499). A total of 200 undergraduates is exposed to the CREATE project with opportunities for internships at NREL and WUSTL.

The undergraduate internship positions at NREL, WUSTL, and ASU in the summer of 2025 have a huge impact on the ASU community. Over 50 undergraduates have applied for the positions. To maintain student enthusiasm, we established the ASU cyanobacteria community, “ASU Cyano Academy.”

In 2025, we initiated a new event, “Annual Research Literature Competition.” The purpose of the event is to focus on the critical reading, interpretation, analysis, and evaluation of literature in the STEM fields by participants supervised by their research advisors, with a focus on the limitations of previous work and potential future directions in research. The Competition is expected to promote the preparation in critical assessment and review of research literature, and encourage the participation of the next generation of leaders and the workforce in STEM fields, especially among underrepresented student populations.

The undergraduate students who worked in the laboratory are given an opportunity to present at the following event, organized by the tri-institutional team, “Annual Cyanobacteria and Algae Research and Education (CARE) Workshop.” The event is open to members of the ASU Cyano Academy who do not have the opportunity to work in the laboratory on cyanobacteria through the CREATE project.

To increase the visibility of the undergraduate students’ achievements in cyanobacteria, the PI has organized the 2025 Annual Research Frontier Symposium at ASU. The theme of this year's Symposium is "Publication in STEM Research." The 2025 Annual Research Frontier Symposium has received 58 abstracts from 14 institutions in 12 states.

In FY25, NREL provided hands-on research training to one undergraduate student (Lydia Davies-Balogun) in summer 2025 and supported their research and conference presentations throughout the year. NREL provided research data for multiple other ASU students to analyze and present at conferences. NREL presented at an ASU research seminar, helped recruit

and evaluate ASU postdoc candidates, and helped organize and present at student-centered events hosted by ASU, including the CARE workshop, Research Frontiers Symposium, and Research Literature Competition.

PI Harvey Hou and Co-PI Jianping Yu are working on the book project, “Photosynthesis: Solar Energy Conversion and Management,” to be published by Springer. The book proposal has been approved. We are now inviting potential authors to the book project and expect to publish the book in 2028.

PRODUCTS

The products shown below include only Publications with a 'Published' status and Intellectual Properties with a 'Granted' status. Products with other statuses are not included in this report. The Revision Type indicates whether a product is New (newly added), Updated (existing product modified), or No Change (existing product reported without modifications) during the current budget period. Note that 'Updated' statuses may appear more frequently as products progress through the publishing process. All products listed have been reported for the current project period of this award.

PUBLICATIONS

There are no publications to report.

INTELLECTUAL PROPERTIES

There are no intellectual properties to report.

PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

The table below only contains participants who have identified an affiliation with the Awardee Institution. Participants from any associated subawards may not be included in this count.

PARTICIPANTS DETAIL

Project Role	Number of People	Total Person Months Worked
Co-Project Director	4	4
Graduate Student (Research Assistant)	1	6
Other Professional	2	13
Postdoctoral (scholar, fellow or other postdoctoral position)	1	4
Principal Investigator/Project Director	1	1
Staff Scientist (doctoral level)	2	2
Undergraduate Student	1	2
Total Responses	**12**	**32**

PARTNERS DETAIL

There are no partners to report.

IMPACT

This project has a significant impact on research infrastructure, fundamental research, and mentoring students. In particular, this project enables the isolation and characterization of algae and cyanobacteria strains, providing new resources for research on energy production, oil spill mitigation, and the degradation of microplastics.