REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
26-08-2021	Final Report	1-Aug-2014 - 31-Jul-2016

4. TITLE AND SUBTITLE
Final Report: Tennessee Photobioreactor Facility for Bioenergy Applications

5a. CONTRACT NUMBER
W911NF-14-1-0429

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611103

6. AUTHORS

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of Tennessee at Knoxville
Office of Sponsored Programs
1534 White Avenue
Knoxville, TN 37996 -1529

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
65203-LS-RIP.1

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Barry Bruce
UU	UU	UU	UU		19b. TELEPHONE NUMBER 865-974-4082

RPPR Final Report
as of 26-Aug-2021

Agency Code: 21XD

Proposal Number: 65203LSRIP **Agreement Number: W911NF-14-1-0429**
INVESTIGATOR(S):

Name: Brad Binder
Email: bbinder@utk.edu
Phone Number: 8659747994
Principal: N

Name: Barry Bruce
Email: bbruce@utk.edu
Phone Number: 8659744082
Principal: Y

Name: Paul Frymier
Email: pdf@utk.edu
Phone Number: 8659742421
Principal: N

Organization: **University of Tennessee at Knoxville**
Address: Office of Sponsored Programs, Knoxville, TN 379961529
Country: USA

DUNS Number: 003387891 EIN: 626001636
Report Date: 31-Oct-2016 Date Received: 26-Aug-2021
Final Report for Period Beginning 01-Aug-2014 and Ending 31-Jul-2016
Title: Tennessee Photobioreactor Facility for Bioenergy Applications
Begin Performance Period: 01-Aug-2014 **End Performance Period:** 31-Jul-2016
Report Term: 0-Other

Submitted By: Barry Bruce Email: bbruce@utk.edu
Phone: (865) 974-4082

STEM Degrees: **STEM Participants:**

Major Goals: 1) Instrumentation was ordered
2) Instrumentation arrived from the Czech Republic
3) Training on-site and online was proved from PSI
4) Systems were put into use
5) Biomass from the bioreactors has been used for multiple experiments

Accomplishments: 1) Instrumentation was ordered
2) Instrumentation arrived from the Czech Republic
3) Training on-site and online was proved from PSI
4) Systems were put into use
5) Biomass from the bioreactors has been used for multiple experiments

RPPR Final Report
as of 26-Aug-2021

Training Opportunities: Instrumentation was used to support graduate work and undergraduate training:

Jon Nguyen was an undergraduate who worked on running these bioreactors. He is now a Ph.D. student at Arizona State University

Richard Simmerman completed his Ph.D. and is now an industrial Post-doc

Kha Nguyen completed his Ph.D. is now a Staff Scientist at the EPA

Meng Li completed his Ph.D. and is now a postdoc at the University of Washingon

Nate Brady completed his Ph.D. and is now a postdoc at Sandia national LAb,

Results Dissemination: 1. Shelaev, I.V., Mamedov, M.D., Gostev, F.E., Aybush, A.V., Li, M., Nguyen, J., *Bruce, B.D. and Nadtochenko, V.A. (2018) Comparisons of electron transfer reactions in a cyanobacterial Tetrameric and Trimeric Photosystem I complex. Photobiology and Photochemistry, 94(3): 564-569. DOI: 10.1111 /php.12886

2. Li., Meng, Calteau, A., Semchonok, D.A., Witt, T.A., Nguyen, J., Sassoon, N., Boekema, E. J., Julian Whitelegge, J., Gugger, M., and *Bruce, B.D. (2019) Tetrameric Photosystem I in Cyanobacteria: Implications in Physiology and Evolution. Nature Plants 5:1309-1319. https://doi.org/10.1038/s41477-019-0566-x

3. Cherepanov, D.A., Brady, N.G., Shelaev, I.V., Nguyen, J., Gostev, F.E., Mamedov, M.D., Nadtochenko, V. A, and *Bruce, B.D. (2020) PSI-SMALP, A Detergent-free Form of Cyanobacteial Photosystem I Reveals Faster Femtosecond Photochemisty. Biophysical Journal, 118:2, 337-351. https://doi.org/10.1016/j.bpj.2019.11.3391

4. Teodor, A.H., Ooi, E., Medina, J., Alarcon, M.I, Vaughn, M.D.,*Bruce, B.D., and Bergkamp, J.J.* (2021) Functional Characterization of Bipyridine Cobalt (II/III) Redox Mediator Complexes as Photosystem I Electron Donors for Biophotovoltaic Applications. RCS Advances, 11, 10434-10450. DOI: 10.1039/D0RA10221K

5. Brady, N.G., Workman, C., Cawthon, B.J., *Bruce, B.D., and *Long, B. (2021) Protein Extraction Efficiency and Selectivity of Esterified Styrene–Maleic Acid Copolymers in Thylakoid Membranes. ACS Biomacromolecules, 22:6, 2544-2253. https://doi.org/10.1021/acs.biomac.1c00274

6. Teodor, A.H., Thal, L.B., Vijayakumar, S., Chan, M., Little, G., and *Bruce, B.D. (2021) Encapsulation and Stabilization of Cyanobacterial Photosystem I into Aqueous Mesoporous Microspheres using Calcium Carbonate Templates Materials Today BIO 11, 100122. https://doi.org/10.1016/j.mtbio.2021.100122

Honors and Awards: Distinguished Professorship, Charles Postell Chair in Biotechnology, University of Tennessee 2021
Member, Advising Committee, Annual Research Frontier Symposium, Alabama State Univ. (Virtual) 2021
Founder and President, NAPCO (North American Photosynthesis Conference (501 (3)(c) Corporation) 2020
Mentor, Khorana Scholar Exchange Program (COVID delayed) 2020
Chair, Satellite Meeting, International Congress on Photosynthesis, Rotorua, New Zealand (COVID delayed) 2020
Instructor, XXI Congreso Nacional de Ciencia Technologia, y Sociedad, Limon, Costa Rica 2019
Outstanding Senior Research Award, BCMB Dept. UTK 2020
Chancellor's Award for Outstanding Undergraduate Mentor 2019
Outstanding Academic Outreach Award, College of Arts and Sciences Award, UTK 2019
GIAN Faculty, GIAN: Global Initiative for Academic Network, 2019
Ministry of Human Resource Development, Government of India
The University of Hyderabad, 2019 Course Date (TBD)
Co-Chair, 29th Western Regional Photosynthesis Meeting, Bodega Bay, CA 2020
Co-Chair, 28th Western Regional Photosynthesis Meeting, Friday Harbor, WA 2019
International Organizing Committee, Joint ASPB/ISPR Conference, Montreal, CA 2019
International Organizing Committee, "Photosynthesis Research for Sustainability," Hyderabad, IN 2018
Outstanding Senior Research Award, BCMB Dept. UTK 2018
Co-Chair, 27th Western Regional Photosynthesis Meeting, Oracle, AZ 2018
Chair, 26th Western Regional Photosynthesis Meeting, Marconi Conference Center, Marshall, CA2017

Protocol Activity Status:

Technology Transfer: Nothing to Report

RPPR Final Report
as of 26-Aug-2021

PARTICIPANTS:

Participant Type: Graduate Student (research assistant)
Participant: Khoa Nguyen
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Meng Li
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Undergraduate Student
Participant: Jon Nguyen
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: nate Brady
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Partners

,

RPPR Final Report

as of 26-Aug-2021

I certify that the information in the report is complete and accurate:
Signature: Barry D. Bruce
Signature Date: 8/26/21 2:29PM

Final Report:
Submitted by Barry D. Bruce (PI and BCMB Professor)

ARO DURIP grant W911NF-14-1-0429 (UNCLASSIFIED)

This DURIP funded the Acquisition of two Photobioreactors and a continuous flow centrifuge. The following equipment was purchased and installed.

1.0 Description of Purchased Instrumentation: One of the leading suppliers of advanced instrumentations is Photon Systems Instruments (PSI). This company has over fifteen years of experience building specialized instrumentation for growing and characterizing photosynthetic organisms, including higher plants and microorganisms. The PSI team of skilled employees allows seamless integration of optics, electronics, and software expertise to develop an innovative, ever-broadening range of high-end scientific instruments. Although based in the Czech Republic, PSI has a system of distributors that provide close contact and technical support to customers in many countries worldwide. Their outstanding products have been included in many national and international projects supported by the Czech government, other European governments, and European Economic Space (EES) Organization.

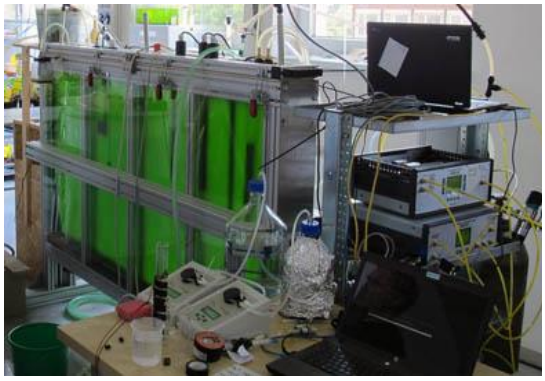
Figure 1: The PSI 120 liter Photobioreactor with controllers.

1.1 Photobioreactors: Photobioreactors are used for the precise phototrophic cultivation of algae and cyanobacteria. They feature a unique combination of the cultivator and monitoring device. Light power and spectral composition and the temperature and aeration gas composition can be set with high accuracy. In addition, cultivation conditions can be dynamically varied according to a user-defined protocol. Light, temperature, and gas composition can oscillate with various amplitudes and frequencies. The growth of the cultures is monitored by the integrated densitometer (OD 720) that measures light scattering at 720 nm. The chlorophyll content of the culture can also be monitored continuously by the differences in optical densities at 680 and 720 nm. The instantaneous physiological state of the culture is measured by the Photosystem II quantum yield (Fm'-Fs)/Fm. All PSI Photobioreactors have a flat-vessel design that brings uniform illumination over the whole volume of cultivated culture. The photobioreactors we purchased were the 25 L and 120 L versions. Large-Scale Photobioreactors are systems that create an artificial environment for the large-scale growing and harvesting of photosynthetic organisms, including cyanobacteria and algae. The 25 L or 120 L size bioreactors may then serve for industrial or semi-industrial production of biomass. They are manufactured as modular systems that enable the customers to build their cultivating apparatus according to their individual experimental needs**.**


Figure 2: The Sharples T1P Tubular Bowl Centrifuge.

1.2 High-speed Harvesting of Algal/Cyanobacterial Biomass using Continuous Flow Centrifuge:

Although these large bioreactors will allow a much more uniform and large culture of cyanobacteria to be collected, they also present a new problem in how the cells must be collected. We currently use traditional centrifugation bottles that hold between 250-1000 ml each. These are then spun in a floor centrifuge (Sorvall RC-6), which typically takes 12-15 minutes to reach full speed, allow cell pelleting by the high G-forces, and then slow down to a stop. This traditional approach would take hours of continuous use to harvest the 25-liter bioreactor and nearly two full days to harvest the 120-liter reactor. Our solution is to purchase a lab-scale continuous flow centrifuge. This centrifuge works by continuously flowing the cell culture through a spinning shaft-style centrifuge that will rapidly pellet the cells in a single step. We will use a reconditioned Sharples Model T1P with a top g-value of 62,000 and a flow capacity of 49 liters per hr. This centrifuge will allow even the larger bioreactor to be harvested in about two hours. An additional feature is essentially a hands-free operation that does not require a dedicated person to harvest the cells repeatedly via typical centrifugation. A photo of this reconditioned model is shown in Figure 2.

2.0 Equipment Purchased, Price and Date:

Name	Model	Vendor	Price	Date	Serial Number
Continuous Flow Centrifuge	Model T1-P	Sharples	$19,000.00	1/14/2016	#15609225
Airlift Photobioreactor 120 liters	120-liters	Photon Systems Instruments	$36,860.55	6/5/2015	# 047037
Airlift Photobioreactor 120 liters	25 liters	Photon Systems Instruments	$32,715.45	6/5/2015	#047068

3.0 Research Projects Related to DURIP Instrumentation:

Both of these bioreactors were used for the growth of large quantities of cyanobacteria cell pellets (~ gm fresh weight/liter).. This has included the growth of the two thermophilic cyanobacteria (*Thermosynechoccocus elongatus* and *Chroococcidiopsis* TS-821) and the mesophilic cyanobacteria, Synechocystis PCC 6803. These bioreactors have supported the work of Dr. Brad Binder (BCMB), Dr. Paul Frymier (BCMB) and Dr. Barry D. Bruce (BCMB).

Specific Projects:

3.1 Influence of light and ethylene on the Motility in Cyanobacteria. This work has addressed how the cyanobacteria Synechocystis PCC6803 has its motility affected by light and exposed to the hormone ethylene. Unfortunately, the use of an airlift bioreactor is not ideal for culturing these strains of cyanobacteria since the shear forces of the airlift bubbles damage the pili associed with motility.

(Brad Binder, NSF support) **Completed**

3.2 Detergent free isolation of membrane protein complexes using Styrene Maleic Acid Copolymers. This work has generated >8 papers from my group. All of the work using thylakoids from cyanobacteria has use the instruments purchased from the DURIP.

(Barry Bruce, on-going submitted to NSF and NIH- in preparation), **on-going**

3.3 Isolation of different Photosystem I oligomers for Cryo-EM analysis. This work is now in revision at Molecular Plants. It will be a high impact publication that is an extension from our 2019 Nature Plants paper https://doi.org/10.1038/s41477-019-0566-x.

(Barry Bruce, submitted to DOE, will resubmit in 2022), **on-going**

3.4 Isolation of Photosystem complexes for Applied Photosynthesis. This work is an ongoing activite of my lab and we the foundation of the Directors Strategic Initiative funded by ARL with Dr. Cynthia Lundgren at ARL. My lab is still active in this research area and are currently using these bioreactors for this work. We would like to submit a second DURIP to maintain and improve these bioreactors.

(Barry Bruce, funded by ARL and will submit to NSF and DOE in 2022), **on-going**

4.0 Publications Derived from DURIP Instrumentation:

1. Shelaev, I.V., Mamedov, M.D., Gostev, F.E., Aybush, A.V., Li, M., Nguyen, J., *Bruce, B.D. and Nadtochenko, V.A. (2018) Comparisons of electron transfer reactions in a cyanobacterial Tetrameric and Trimeric Photosystem I complex. Photobiology and Photochemistry, 94(3): 564-569. DOI: 10.1111/php.12886
2. Li., Meng, Calteau, A., Semchonok, D.A., Witt, T.A., Nguyen, J., Sassoon, N., Boekema, E. J., Julian Whitelegge, J., Gugger, M., and *Bruce, B.D. (2019) Tetrameric Photosystem I in Cyanobacteria: Implications in Physiology and Evolution. Nature Plants 5:1309-1319. https://doi.org/10.1038/s41477-019-0566-x
3. Cherepanov, D.A., Brady, N.G., Shelaev, I.V., Nguyen, J., Gostev, F.E., Mamedov, M.D., Nadtochenko, V.A, and *Bruce, B.D. (2020) PSI-SMALP, A Detergent-free Form of Cyanobacteial Photosystem I Reveals Faster Femtosecond Photochemisty. Biophysical Journal, 118:2, 337-351. https://doi.org/10.1016/j.bpj.2019.11.3391
4. Teodor, A.H., Ooi, E., Medina, J., Alarcon, M.l, Vaughn, M.D.,***Bruce, B.D.**, and Bergkamp, J.J.* (2021) Functional Characterization of Bipyridine Cobalt (II/III) Redox Mediator Complexes as Photosystem I Electron Donors for Biophotovoltaic Applications. RCS Advances, **11,** 10434-10450. DOI: 10.1039/D0RA10221K
5. Brady, N.G., Workman, C., Cawthon, B.J**., *Bruce, B.D.,** and *Long, B. (2021) Protein Extraction Efficiency and Selectivity of Esterified Styrene–Maleic Acid Copolymers in Thylakoid Membranes. ACS Biomacromolecules, **22:6,** 2544-2253. https://doi.org/10.1021/acs.biomac.1c00274
6. Teodor, A.H., Thal, L.B., Vijayakumar, S., Chan, M., Little, G., and *Bruce, B.D. (2021) Encapsulation and Stabilization of Cyanobacterial Photosystem I into Aqueous Mesoporous Microspheres using Calcium Carbonate Templates Materials Today BIO **11,** 100122. https://doi.org/10.1016/j.mtbio.2021.100122

5.0 Current status and use of instrumentation:

Currently both bioreactors are in operation. However, one of the heating probes has suffered corrosion and will need to be replaced. Also, both of the submersible spectrophotometers need to be replaced. This is very disappointing since it is the major data monitoring component of these two bioreactors that allow the controlled and reproducible growth of the algae or cyanobacteria. One possible solution is to submit a second DURIP to update, improve and replace these damaged/broken components. This should permit another 5-10 year operation of these critical instruments. Fortunately, this DURIP would be considerably less costly than the original proposal.