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RENEWABLE BIO-SOLAR HYDROGEN PRODUCTION FROM ROBUST OXYGENIC PHOTOTROPHS

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RUTGERS THE STATE UNIVERSITY OF NEW JERSEY

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Final Report

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14. ABSTRACT This project produced advances in two areas: 1) biological hydrogen production, and 2) electrocatalysts. Topic (1) is highlighted by successful genetic engineering of acyanobacterium to redirect the anaerobic catabolism of glycogen, stored from a prior photosynthetic stage, from the normal glycolytic pathway to an alternative pathway that produces significantly more hydrogen. Topic (2) is highlighted by the synthesis of two new electrocatalysts made from earth-abundant materials that catalyze the oxidation of water to O₂ and the reduction of water to H₂, respectively. These were patented and were licensed commercially where they are in development for applications in commercial electrolyzers for renewable hydrogen production.					
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2013-2015 AFOSR Funded Publications:

Archival
Publications:

- 1) Robinson, D.M., Y.B. Go, M. Mui, G. Gardner, Z. Zhang, D. Mastrogiovanni, E. Garfunkel, J. Li, M. Greenblatt and G.C. Dismukes, *Photochemical water oxidation by crystalline polymorphs of manganese oxides: structural requirements for catalysis*. J Am Chem Soc, 2013. **135**(9): p. 3494-501.
- 2) Xu, Y., L.T. Guerra, Z. Li, M. Ludwig, G.C. Dismukes and D.A. Bryant, *Altered carbohydrate metabolism in glycogen synthase mutants of Synechococcus sp. strain PCC 7002: Cell factories for soluble sugars*. Metab Eng, 2013. **16**: p. 56-67.
- 3) Guerra, L.T., Y. Xu, N. Bennette, K. McNeely, D.A. Bryant and G.C. Dismukes, *Natural osmolytes are much less effective substrates than glycogen for catabolic energy production in the marine cyanobacterium*

- Synechococcus sp. strain PCC 7002*. *Journal of Biotechnology*, 2013. **166**: p. 65– 75.
- 4) McNeely K., Kumaraswamy G.K., et al. (2014). Metabolic Switching of Central Carbon Metabolism in Response to Nitrate: Application to Autofermentative Hydrogen Production in Cyanobacteria. *Journal of Biotechnology*, 182-183, 83-91.
 - 5) Smith, PF, Kaplan C, Sheats JE, Robinson DM, McCool NS, Mezle N, Dismukes CG. 2014. What determines catalyst functionality in molecular water oxidation? Dependence on ligands and metal nuclearity in cobalt clusters. *Inorganic chemistry*. 53(4):2113-21.
 - 6) Kumaraswamy, G.K., Tiago Guerra, et al. (2013). Reprogramming the Glycolytic Pathway for Increased Hydrogen Production in Cyanobacteria: Metabolic Engineering of NAD⁺-dependent GAPDH. *Energy & Environmental Science*, 6, 3722-3731.

Changes in

Research objectives: none

Change in AFOSR

Program Manager, none if any:

Extensions granted

or milestones slipped, if any: none

100%

Survey Software powered by SurveyGizmo

1. **Title of Project:** Renewable Bio-Solar Hydrogen Production from Robust Oxygenic Phototrophs: The Second Generation
2. **Grant Number:** FA9550-11-1-0231
3. **Period of this report:** 9/1/13 – 1/1/15
4. **Institution:** The Waksman Institute of Microbiology and the Dept of Chemistry & Chem. Biology, Rutgers The State University of New Jersey, Piscataway, NJ
5. **Telephone & Email:** 732-445-6786; dismukes@rci.rutgers.edu)
6. **Contributing Personnel (20 in total):**
 - G. Charles Dismukes, PhD, Principal Investigator, Distinguished Professor
 - Gennady Ananyev, PhD, Research Associate Professor
 - Kumaraswamy G. Kenchappa, PhD, Postdoctorate Researcher

- Anagha Krishnan, Busch Scholar graduate fellow, 5th year continuing PhD candidate
- Graeme Gardner, NSF IGERT trainee fellowship, 5th year continuing PhD candidate
- Paul Smith, NSF IGERT trainee fellowship, 5th year continuing PhD candidate
- 6 PhD Rotation Students:
- 10 Undergraduate researchers: Kinan Tadmori, Shannon, Artun Hoscan, Chris Kaplan, Kelly Patraju, Yifan Wang, Gram Townsend, Donald Chawla, Jessica Gonzalez, and Nicholas Mezle.

7. Project Accomplishments & Translational Advances:

- **Trained/Mentored 20 Rutgers Students in Research:** 3 predoctoral trainees in progress, 6 predoctoral rotation students trained, 1 honors thesis (BS) in progress, 10 undergraduate researchers trained.
- **Fundamental Scientific Advances:** 8 publications or submitted manuscripts in peer reviewed journals; 5 of these were done in collaboration with other BioSolarH₂ PIs: Drs. D. Bryant and M. Posewitz. See publication list.
- **Intellectual Property Generated:** A patent disclosure was filed at Rutgers and national patent application was filed on December 31, 2013. NICKEL PHOSPHIDES ELECTROCATALYSTS FOR HYDROGEN EVOLUTION AND OXIDATION REACTIONS. USPTO. PCT/US13/78486.
- **Intellectual Property Generated:** A patent was issued to Rutgers by the USPTO: 13/643,439. SPINEL CATALYSTS FOR WATER AND HYDROCARBON OXIDATION This described the synthesis of a nanoparticulate form of cubic spinel phase of Lithium Cobalt Oxide and demonstration of its function as a highly efficient water oxidation catalyst in electrolyzers.

8. Awards:

- Kumaraswamy G. Kenchappa, Busch postdoc fellowship Waksman Institute

9. Industrial and Public Service Related to AFOSR Sponsored Research

- **Translation of Research to Industry, Licensing Stage:** Licensing of AFOSR funded OER SPINEL CATALYSTS USPTO 13/643,439 to NATCO Corporation.
- **Translation of Research to Industry, Commercial Testing Stage:** Patented OER Spinel catalyst USPTO 13/643,439 has been sent to a commercial electrolyzer company (Proton OnSite, Wallingford, CT) for testing and development of a new form electrolyzer.

10. Public Presentations of AFOSR funded research, 2012-2013

1. Talk: Kumaraswamy G.K, **Metabolic engineering of cyanobacteria for hydrogen production**, *Waksman Annual Retreat, September 9, 2013*
2. Talk: Anagha Krishnan, Metabolic and photosynthetic consequence of mutation of starch biosynthesis in *Chlamydomonas reinhardtii*. *Waksman Annual Retreat, September 2014*
3. Poster presentation: Title: Metabolic and photosynthetic consequence of

mutation of starch biosynthesis in *Chlamydomonas reinhardtii*.

4. GC Dismukes, invited speaker, **Gordon Research Conference: Renewable Energy: Solar Fuels**, Ventura, CA Jan, 19-24 2014.
5. GC Dismukes, 2013 **APRA-E Energy Innovation Summit**, Washington D.C. Feb 25, 2013. Rutgers bioinspired electrocatalysts for solar hydrogen and electrical energy storage.
6. GC Dismukes, **SOFI-Creation of the Solar Fuels Institute**, Telluride, CO, convenor: Dr. M. Wasielewski. Demo Project coPI (2014).
7. Advisory: 2014 DOE-H2A Technoeconomic Review Panel; 2013 DOE-Hydrogen Program;

11. 2013-2014 AFOSR Funded Publications:

1. Robinson, D.M., Y.B. Go, M. Mui, G. Gardner, Z. Zhang, D. Mastrogiovanni, E. Garfunkel, J. Li, M. Greenblatt and G.C. Dismukes, *Photochemical water oxidation by crystalline polymorphs of manganese oxides: structural requirements for catalysis*. J Am Chem Soc, 2013. **135**(9): p. 3494-501.
2. Xu, Y., L.T. Guerra, Z. Li, M. Ludwig, G.C. Dismukes and D.A. Bryant, *Altered carbohydrate metabolism in glycogen synthase mutants of Synechococcus sp. strain PCC 7002: Cell factories for soluble sugars*. Metab Eng, 2013. **16**: p. 56-67.
3. Guerra, L.T., Y. Xu, N. Bennette, K. McNeely, D.A. Bryant and G.C. Dismukes, *Natural osmolytes are much less effective substrates than glycogen for catabolic energy production in the marine cyanobacterium Synechococcus sp. strain PCC 7002*. Journal of Biotechnology, 2013. **166**: p. 65– 75.
4. McNeely K., Kumaraswamy G.K., et al. (2014). Metabolic Switching of Central Carbon Metabolism in Response to Nitrate: Application to Autofermentative Hydrogen Production in Cyanobacteria. *Journal of Biotechnology*, 182-183, 83-91.
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7. Krishnan, A., Kumaraswamy, G.K., Vinyard, D. J., Gu, H., Ananyev, G., Posewitz, M. P. and Dismukes, G.C. (2015) Metabolic and photosynthetic consequences of blocking starch biosynthesis in the green alga *Chlamydomonas reinhardtii* sta6 mutant. Submitted to *The Plant Journal*.
8. Xiao Qian, Min Kyung Kim, G. Kenchappa Kumaraswamy, Ananya Agarwal, Desmond S. Lun, and G. Charles Dismukes. Beyond flux balance analysis of

photoautotrophic metabolism: Carbon partitioning into different biopolymers. Submitted.

9. Four more articles are in preparation for publication.

12. Key Scientific Results/Achieved Goals

1. Publication 4 show that the presence of NO_3 in the fermentative media, switch the carbohydrate catabolism through OPP pathway, to meet out the NADPH demand for nitrate reduction. Under nitrate presence, NAD(P)H are largely used for reducing nitrate, rather than H_2 . Nitrate presence resulted in 3-fold higher NAD(P)H consumption. Cells largely excrete H_2 and lactate under no nitrate, while CO_2 and nitrite when nitrate present.
2. Publications 6 show that the metabolic pathways for carbohydrate catabolism, especially OPP could yield higher NAD(P)H per glucose catabolised (during autofermentation). This revealed from five cyanobacterial mutant strains studied for their fermentative metabolism during dark/anaerobic conditions. Metabolic engineering of these glycolysis/OPP pathways of carbohydrate catabolism, along with the elimination of competing pathway of lactate production, resulted in enhanced NAD(P)H yields and in turn higher H_2 yields. Further we showed that elimination of H_2 in the headspace by either increasing the headspace volume (dilution) or by electrochemical milking shifted the equilibrium of bidirectional hydrogenase towards H_2 production rather than consumption.
3. Publication 7 show that during nutrient replete culturing, *sta6* does not redirect metabolism to make more proteins or lipids, and accumulates 20% lower biomass. Above a threshold light intensity, photosynthetic electron transport rate (water $\rightarrow$ CO_2) slows in *sta6* due to attenuated rate of NADPH reoxidation, without affecting photosystem I or II (no change in isolated PET). Large accumulations of carbon metabolites were measured that are precursors for biosynthesis of lipids, amino acids and sugars/starch, indicating system-wide consequences of slower NADPH reoxidation. The greatly slower flux through carbon fixation resulted in imbalances in both redox and adenylate energy.
4. Publication 8 presents the experimentally tested most comprehensive genome-scale flux balance model for photoautotrophic growth of the cyanobacterium, *Synechococcus sp.* PCC 7002, which accounts for 768 genes and 769 metabolites. Our model incorporates a variable biomass objective function (vBOF) in which stoichiometries of major biomass components vary according to change in light intensity. Modification of the BOF was constrained by measurements of biomass composition under different light conditions. The model provides rigorous agreement to experimentally measured growth rates, inorganic carbon uptake rates and the carbohydrate/protein content as a function of light intensity under photoautotrophic growth. Incorporation of vBOF adds greater metabolic flexibility for simulation of more realistic compositional changes in response to light stresses, thus providing more reliable prediction of growth rate and carbon partitioning. Moreover, this model predicts that there exists a gluconeogenesis-OPP hybrid pathway that flux the fixed CO_2 into glycogen.