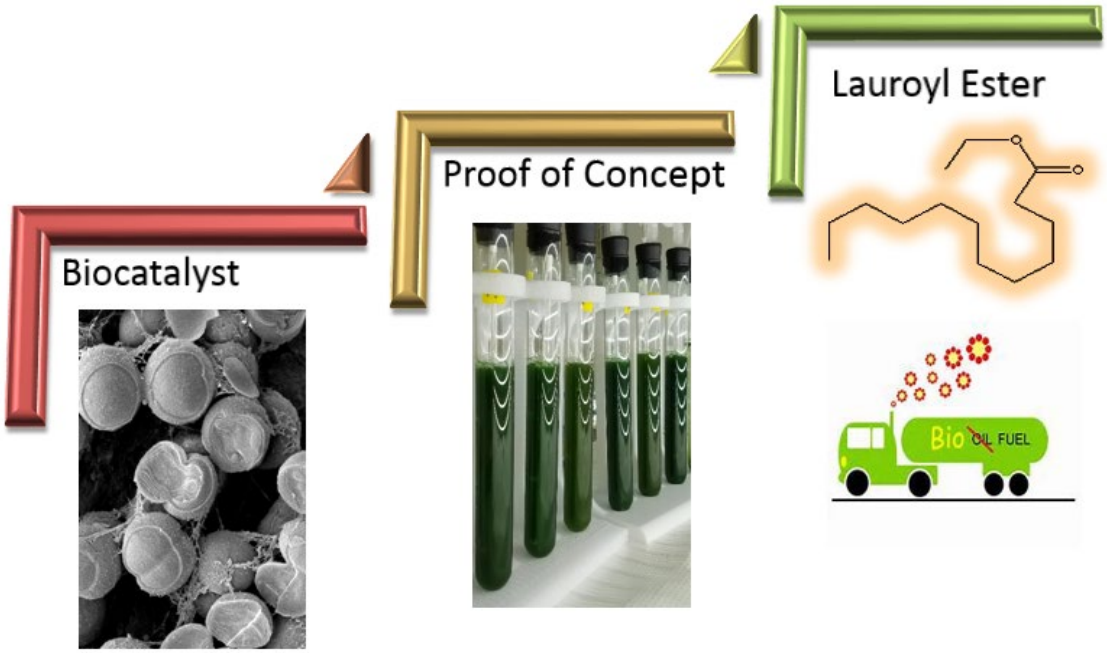
Final Technical Report



Energy Efficiency and Renewable Energy
Bioenergy Technologies Incubator 2
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Award:	DE-EE0007561
Lead Recipient:	Arizona State University
Project Title:	Direct Photosynthetic Production of Biodiesel by Growth-Decoupled Cyanobacteria
Principal Investigator:	Willem F.J. Vermaas
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Arizona State University

DE-EE0007561

BTI2 Final Scientific/Technical Report

Award #DE-EE0007561

Direct Photosynthetic Production of Biodiesel by Growth-Decoupled Cyanobacteria

I. Executive Summary

The ultimate goal of this project was the production and excretion of a lauroyl ester-based drop-in biofuel by building on the development of a cyanobacterial strain derived from *Synechocystis* sp. PCC 6803 that is efficient in using sunlight to reduce CO_2 from the atmosphere to produce and excrete laurate, a fatty acid (FA) that is easily used as a biofuel precursor. To achieve this goal the project focused on five primary objectives: (1) produce more laurate, (2) convert laurate to a lauroyl ester by introducing additional genetic modifications for the synthesis of fatty acid esters, (3) arrest cell growth but not metabolic activity, thereby increasing the availability of resources for ethyl laurate production, (4) reduce the native production of exopolysaccharides to increase the amount of fixed carbon available for biofuel production and to reduce the level of fixed carbon nutrients available in the medium as substrates for potential invasive microbes, and (5) perform a techno-economic analysis to evaluate the longer-term promise and viability of lauroyl ester production in cyanobacterial systems. A photosynthetically produced lauroyl ester could be used as a diesel replacement without the need for additional and costly downstream processing steps, a development of great importance to the mission of DOE with regard to biofuel production.

Production yields are key to bringing any biofuel to market; thus, maximizing the laurate yields in this *Synechocystis* biocatalyst platform was the primary goal of Task 1. The starting point was a laurate-producing and laurate-excreting strain of *Synechocystis* sp. PCC 6803 generated in the Vermaas group by introduction a codon-optimized thioesterase gene (*fatB1*) and by deletion of a gene necessary for the organism's innate ability to reincorporate the free fatty acid into the lipid biosynthesis pathway. Variants of this strain also were developed to improve the strain's ability to resist the destructive effects of reactive oxygen species. Laurate yields were increased by ~25%, achieving the targets set for the primary objective of this task, using a multi-pronged approach that included: 1) optimization of culturing conditions, 2) deletion and overexpression of targeted genes involved in the FA biosynthesis pathway, and 3) introduction of an orthogonal FAS I (FA synthase type I) FA biosynthesis pathway. A novel outcome of this task was the first known development of a *Synechocystis* strain with a functional bacterial FAS I complex, for which a provisional patent application has been filed.

The secondary goal of Task 1 was developing a high-throughput assay for rapid detection of laurate overproducers to speed up the genetic engineering process, and for identification of isolates that, through reversion or secondary mutations, decreased their laurate output. The milestone for this task was successfully completed with the development of a fluorescence-based 96-well plate screening method with moderately high throughput and with the ability to distinguish between newly transformed fatty acid over-producers as well as to identify possible revertants.

The focus of Task 2 was development of a lauroyl ester-based biofuel, which is a drop-in-ready fuel molecule requiring little or no downstream processing and that is less desirable to heterotrophic scavengers, thus minimizing the chances of culture contamination. The first milestone for this task, demonstrating ethyl laurate production by a laurate-producing strain grown in the presence of exogenous ethanol, was successfully completed as planned. Unfortunately, problems related to genetic

instability and/or toxicity of one of the ethyl laurate pathway enzymes did not allow for development of a *de novo* ethyl-laurate producing *Synechocystis* strain. As often happens in research, the team pivoted to an alternative approach, namely development of a *de novo* methyl-laurate producing *Synechocystis* strain. Two such strains were successfully constructed with methyl-laurate yields of one of the strains comparable to the laurate yields of the parental strain. This was another novel outcome as this was the first known production of methyl laurate by a photosynthetic organism, resulting in the filing of the second provisional patent application from this project.

While biomass yields are important in biofuel production systems based on eukaryotic algae, in the case of a biocatalyst platform, biomass production is a significant competitor for available carbon and energy resources that could ideally be directed towards biofuel production instead. Thus, the third major part of this project and the primary focus of Task 3 was decoupling *Synechocystis* growth from laurate production. Toward this end a *Synechocystis* laurate-producing strain with a functional, inducible gene repression system was developed. The strain was used to test for repression of native and essential gene targets involved in different aspects of cell function such as cell division, transcription and translation. Unfortunately, none of the targets proved successful in decoupling growth from laurate production. While this was disappointing, the potential of the technology for growth decoupling remains as the tested targets were not exhaustive of the possibilities.

Another objective of Task 3 was to integrate the most effective growth-decoupling strategy with lauroyl-ester production and demonstrate growth in a photobioreactor (PBR). As growth-decoupling strategies were not ready for integration into a lauroyl-ester production strain yet, scale-up cultivation in PBRs was performed with the methyl-laurate producing strain and we did successfully demonstrate growth while methyl laurate was produced at apparently modest levels.

About 20% of the total organic carbon found in *Synechocystis* cultures is in the form of attached and soluble exopolysaccharides (EPS), which are a drain on fixed carbon that otherwise could be used for biofuel production, and which may be a feedstock for undesired contaminants that are practically unavoidable in large-scale cultures. The objective of Task 4 was to delete key genes involved in EPS biosynthesis in order to reduce EPS levels in *Synechocystis* cultures. A total of five reduced-EPS laurate-producing strains were developed that produced up to 40% less EPS than the parental strain. The milestone for this task was to generate at least one lauroyl-ester-producing *Synechocystis* strain with reduced EPS that also should be growth-arrest-inducible. Despite challenges with the development of the growth-decoupling system, two reduced-EPS laurate/methyl-laurate producing strains were produced, completing the milestone to the extent possible.

The focus of Task 5 was performing a techno-economic analysis (TEA) to evaluate the long-term viability of this biocatalyst platform for lauroyl ester production. Costs and production revenue sources were analyzed with consideration to the unique aspects of this technology as compared to algal biomass-based production approaches. These unique features are (1) production of two products, methyl laurate and dewatered *Synechocystis* biomass, (2) extensive downstream processing is not needed, and (3) the typical low-cost algal harvest system cannot be used. Taking these aspects into account, the completed TEA and sensitivity analysis concluded that if model assumptions for costs and performance were met, excretion of methyl laurate by this *Synechocystis* biocatalyst platform could be a profitable enterprise.

The goals and accomplishments of this project are summarized in the 'Table of Goals/Objectives vs. Actual Accomplishments' in Section II below. Despite challenges posed by known and unforeseen risks,

for the most part the milestones and deliverables were successfully met. In addition, the project led to the development of two patentable inventions based on (1) a *Synechocystis* strain with a functional FAS I complex, and (2) photosynthetic production of methyl laurate.

II. Goals and Accomplishments

Table of Goals/Objectives vs. Actual Accomplishments:

Goals and Objectives	Actual Performance
Task 1: Increase Laurate Production:	
1.1. Increase metabolic flux through the fatty acid biosynthesis pathway by:	
a) Overexpression of enzymes involved in fatty acid biosynthesis (acetyl-CoA carboxylase genes (*acc*) and fatty acid biosynthesis genes (*fab*)).	Developed three laurate-producing strains overexpressing *acc* genes in different arrangements and two *fab* overexpression strains. One *acc* strain and both *fab* strains showed increased laurate production as compared to the parental strain.
b) Deletion of a regulatory protein thought to negatively regulate acetyl-CoA carboxylase (ACC) (Milestone 1.1.1).	Developed two laurate-producing deletion strains, successfully completing the milestone for this subtask.
c) Overexpressing acyl carrier protein (ACP) to reduce palmitoyl-ACP accumulation thought to cause feedback inhibition of the fatty acid biosynthesis pathway.	Developed the laurate-producing ACP overexpression strain, which could not be segregated and showed decreased laurate production as compared to the parental strain.
Other strategies explored for increasing laurate production beyond those listed in the original proposal included:	
a) Optimization of culturing methods.	Improved culturing methods increased laurate production to 40 mg/L/day, which was the target set for the project in the original proposal.
b) Overexpression and deletion of a transport protein.	Developed laurate-producing strains carrying the appropriate deletion or overexpression construct.
c) Increase the NADPH pool.	Preliminary data from initial attempts to increase NADPH concentrations showed no effect on laurate production.

d) Introduction of a microbial FAS I fatty acid biosynthesis pathway.	Developed three strains with a functional FAS I complex, two in wild-type *Synechocystis*, and one in the laurate producing strain.
1.2. Develop a rapid assay to detect free fatty acid produced by *Synechocystis:*	
a) Milestone 1.2.1: Identify best method for rapid laurate detection and use method to identify at least one genetically modified *Synechocystis* strain with improved laurate production.	Developed a 96-well plate screening method for identifying the best laurate-producing strains successfully completing this milestone.
Task 2: Engineer ethyl laurate biosynthesis:	
2.1 Introduce ethyl laurate biosynthesis capability into *Synechocystis*:	
a) Milestone 2.1.1 and Go/No-Go Decision Point: Demonstrate ethyl laurate production by laurate-producing *Synechocystis* carrying the appropriate enzymes when supplied with exogenous ethanol.	Successfully completed this milestone by demonstrating ethyl laurate production by a laurate-producing strain grown in the presence of exogenous ethanol.
2.2 Demonstrate *de novo* ethyl laurate biosynthesis:	
a) Milestone 2.2.1: Demonstrate the *de novo* photosynthetic production of ethyl laurate by *Synechocystis*.	Development of a *de novo* ethyl-laurate producing *Synechocystis* strain was hampered by apparent instability and/or toxicity issues related to one of the introduced enzymes. This led to an alternative approach toward photosynthetic endogenous production of a lauroyl ester, namely methyl laurate.
2.3 Introduce methyl laurate biosynthesis capability into *Synechocystis*.	Developed two endogenous methyl-laurate producing strains.
Task 3: Decouple *Synechocystis* growth and lauroyl ester production	
3.1 Inhibit cell growth but not metabolic function and investigate the effect on laurate production:	
a) Milestone 3.1.1: Identify genes and conditions necessary to effectively halt *Synechocystis* growth within 6 h without a	Developed a *Synechocystis* laurate-producing strain amenable to specific gene repression and tested multiple targets but none proved

major negative effect on laurate production.	successful in decoupling growth from laurate production.
3.2 Integrate the most effective growth decoupling strategy with lauroyl ester production, and demonstrate growth in photobioreactors:	
b) Milestone 3.2.1: Demonstrate a positive effect of growth decoupling on lauroyl ester production without major accumulation of laurate or lauryl-CoA, and demonstrate success in maintaining photobioreactor (PBR) cultures with suppressed growth and reduced exopolysaccharides (EPS) while retaining lauroyl ester production.	Despite challenges with development of the growth decoupling system reduced EPS capabilities were introduced into the methyl laurate production strains and methyl-laurate production at the 55-L PBR scale was demonstrated.
Task 4: Reduce exopolysaccharide production:	
4.1 Milestone 4.1.1: Generate one or more mutants of the growth-arrest-inducible, lauroyl ester producing *Synechocystis* strain with reduced exopolysaccharides (EPS).	Despite challenges with the growth decoupling system, two reduced-EPS laurate/methyl-laurate producing strains were produced.
Task 5: Perform a techno-economic analysis on lauroyl ester production in cyanobacteria:	
5.1 Milestone 5.1.1: Demonstrate a techno-economic model of lauroyl ester production in cyanobacteria based on the results obtained in this project.	Developed a techno-economic model of lauroyl ester production showing that, if model assumptions for costs and performance are met, secretion of methyl laurate could be a profitable enterprise.

III. Project Summary

Task 1 – Increase Laurate Production:

The primary goal of Task 1 was to increase laurate yields in our laurate-producing *Synechocystis* sp. PCC 6803 strains. The initial strategies for increasing laurate yields in these strains included: 1) maximizing carbon flux through the fatty acid (FA) biosynthesis pathway by overexpression of acetyl-CoA carboxylase (*acc*) and the FA biosynthesis (*fab)* genes along with deletion of a regulatory protein thought to downregulate ACC activity; and 2) minimizing feedback or other inhibition of the FA biosynthesis pathway. To assist in this process a secondary goal of this task was to develop a high-throughput assay to rapidly identify newly developed high laurate producers.

Task 1 milestones and deliverables were successfully completed per project plan. Milestone 1.1.1 was achieved with the development of two laurate-producing regulatory protein deletion strains, one of

which showed significantly higher laurate production than the parent strain, and development of a 96-well plate screening method for identifying the best laurate-producing strains successfully completed Milestone 1.2.1. Three laurate-producing strains overexpressing *acc* genes in different arrangements and two *fab* overexpression strains were developed. One *acc* strain and both *fab* strains showed increased laurate production as compared to the parental strain. Other genetic strategies explored for increasing laurate production such as overexpression of the acyl carrier protein (ACP), the addition of a second thioesterase gene, deletion and overexpression of a transport protein, and attempts to increase the pool of NADPH by deleting genes to divert glycolysis towards the oxidative pentose phosphate pathway and the Entner-Douderoff pathway did not improve laurate yields. However, by modifying growth conditions and nutrient availability laurate production was increased to 40 mg/L/day, which was the target set for the project in the original proposal. Researchers also introduced a type I fatty acid synthesis (FAS I) biosynthesis pathway into *Synechocystis* in hopes of increasing laurate production by bypassing the native fatty acid regulatory system. Three strains with a functional FAS I complex, two in wild-type *Synechocystis*, and one in the laurate producing strain were developed, for which a patent application has been filed.

Task 2 – Engineer and optimize lauroyl ester biosynthesis:

The goal of Task 2 was to expand the *Synechocystis* laurate-secreting platform by engineering a lauroyl ester-production strain, which presents practical advantages over laurate production. The first target molecule was ethyl laurate, which required introduction and optimization of three key elements: 1) conversion of laurate to lauroyl-CoA, 2) ethanol biosynthesis, and 3) condensation of lauroyl-CoA and ethanol via an alcohol O-acyltransferase. Introduction of this multi-step metabolic pathway proved to be more difficult than anticipated, resulting in a shift in focus from ethyl laurate to methyl laurate as production of this lauroyl ester required the addition of only a single enzyme and proved to be much more amenable to *Synechocystis* physiology.

Task 2 Milestone 2.1.1, which was also the Go/No-Go Decision Point, was successfully completed per the project plan. Researchers demonstrated ethyl laurate production by a laurate-producing strain carrying a fatty acid acyl-CoA ligase and an alcohol-O-acyltransferase (also called a wax synthase) grown in the presence of exogenous ethanol. While development of a *de novo* ethyl-laurate producing *Synechocystis* strain was hampered by apparent instability and/or toxicity issues related to one of the introduced enzymes, researchers were able to develop two endogenous methyl-laurate producing strains, which essentially achieved the goal of Milestone 2.2.1.

Task 3* – Decouple *Synechocystis* growth and lauroyl ester production*:

The primary objective of Task 3 was to engineer *Synechocystis* to enable it to halt cell growth at the desired time while continuing to produce laurate and a lauroyl ester. The end result would be a novel two-stage biocatalyst system, in which: 1) *Synechocystis* first accumulates to a desired cell density and then 2) its growth is 'switched off' to preserve carbon/energy resources for enhanced lauroyl ester production. Four largely complementary approaches were simultaneously investigated to achieve this dynamic growth decoupling.

Task 3 milestones and deliverables proved a bit more challenging to achieve. We were largely unsuccessful in inhibiting cell growth without impairing metabolic viability of the cells. Researchers did develop a *Synechocystis* laurate-producing strain amenable to specific gene repression and tested multiple targets but none proved successful in decoupling growth from laurate production leaving Milestone 3.1.1 partially complete. The second objective of this task, Milestone 3.2.1, was to integrate

the most effective growth decoupling strategy into a lauroyl-ester production strain, and demonstrate growth in large-scale photobioreactors. Despite challenges with development of the growth decoupling system, researchers were able to incorporate reduced EPS capabilities (see Task 4) into the methyl-laurate production strains and demonstrate methyl-laurate production at the 55-L PBR scale.

Task 4: Reduce exopolysaccharide production:

Task 4 was focused on reducing the amount of organic, non-biofuel material in the culture medium to help prevent product losses by opportunistic heterotrophic organisms and to maximize the amount of fixed carbon that can be used for product formation by deleting genes involved with formation of exopolysaccharides (EPSs), which are high-molecular-weight sugar polymers released from and attached to the surface of a microorganism. Three *Synechocystis* genes that were key in EPS biosynthesis were the deletion targets. Five reduced-EPS laurate-producing strains and two reduced-EPS laurate/methyl-laurate-producing strains were developed. The milestone for this task was to generate one or more mutants of the growth-arrest-inducible, lauroyl ester producing *Synechocystis* strain with reduced exopolysaccharides (EPS), and it was completed with regard to EPS reduction and methyl laurate production. Unfortunately, as described in Task 3, the inducible-growth-arrest element did not develop as planned and could not be incorporated into the strain.

Task 5 – Perform a techno-economic analysis on lauroyl ester production in cyanobacteria:

The focus of this program element was to evaluate the longer-term promise and viability of lauroyl ester production in cyanobacterial systems by performing a techno-economic analysis (TEA) based on lauroyl ester yield and productivity data. Towards that end this techno-economic analysis (TEA) evaluates scenarios where the sale of a combination of biomass and methyl laurate at appropriate selling prices could create a positive net present value (NPV). This analysis differs from traditional TEAs on algal biomass production in that with a biocatalyst platform such as this productivity of the excreted product does not depend on biomass production and typical microalgae harvest methods are not appropriate. In addition, typical cost models based on average biomass productivities ($g/m^2/d$) are likely to be inaccurate. Thus, in this TEA we make every attempt to calculate correct process flows, economic inputs/outputs, and outcomes for a unique production process and we rely heavily on published TEA papers to estimate the costs of capital items such as the construction costs of well-researched separation technologies or cultivation techniques (raceways/PBRs). As the TEA was discussed in some detail in quarterly reports, only brief highlights will be presented here.

The process flow model (Figure 1) is based on 1,000 hectares of cultivated area in uncovered raceway ponds mixed by a fixed paddlewheel, which are drained, harvested, and re-inoculated every 30 days. The assumption is that most water will be recovered, nutrients such as nitrogen (N), phosphorus (P), and potassium (K) are provided via agricultural commodities and

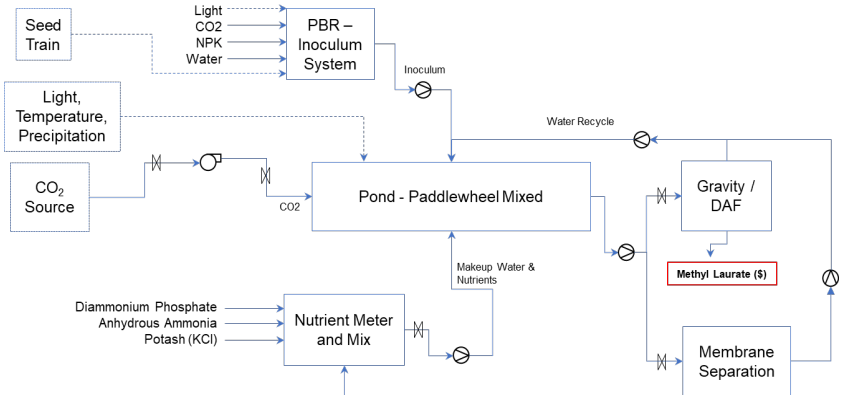

Figure 1: Process flow diagram for the production of biomass and excreted laurate/methyl laurate.

leave the production system primarily via harvest of biomass, carbon dioxide (CO_2) gas is delivered via highly efficient sump spargers and all dissolved inorganic carbon (DIC) will be recovered. Methyl laurate will be harvested by a gravity/DAF process based on mature wastewater oil and grease separation techniques and biomass will be harvested via a membrane separation system based on the Global Algae Innovations process documented in Davis et al. (2016).

Costs were estimated for the capital and operational expenses of each installed system including anticipated maintenance charges and commonly consumed items. Compared to typical algal biomass or bio-product TEAs, costs related to product separation were high because there are two products to separate and a large volume of culture to process. The result is a total Installed Capital to cultivated area ratio of ~$60,000 per acre, compared to $47,500 per acre in Davis et al. (2016).

The energy-based production model (Robertson et al. (2011) and Weyer et al. (2010)) uses the hourly Global Horizontal Irradiance (GHI) (Direct + Diffuse) at a given geography (Corpus Christi, TX) on an hourly basis, and then evaluates the total energy that would be allocated for cell metabolism and maintenance, biomass growth, and methyl-laurate secretion. We modeled a single pond inoculated at a cell density of 0.1 g/L and grown at a specific growth rate determined by the hourly insolation received with the energy split between biomass and methyl-laurate production, resulting in peak productivities of 14.7 g/m^2/d and 13.6 g/m^2/d, respectively (Toulopakis et al., 2016).

The financial analysis method applies "Required Return on Capital Employed" (ROCE) to compensate investors for the "privilege" of using their capital, a commonly used engineering economics methodologies for process industries (Hastak, 2015; Peters et al., 2002). In this case, a rate of 9% of all capital employed was used in the operation of the facility, including Capital Expenses, Contingency, Construction Expenses, Working Capital, and Land. Comparing revenue and production cost we found the fixed and variable costs, including depreciation, were lower than anticipated revenue, even when lower product values were considered. Thus, if the assumptions for costs and performance are met secretion of methyl laurate could be a profitable enterprise.

A sensitivity analysis was also performed on the production model. The tornado chart in

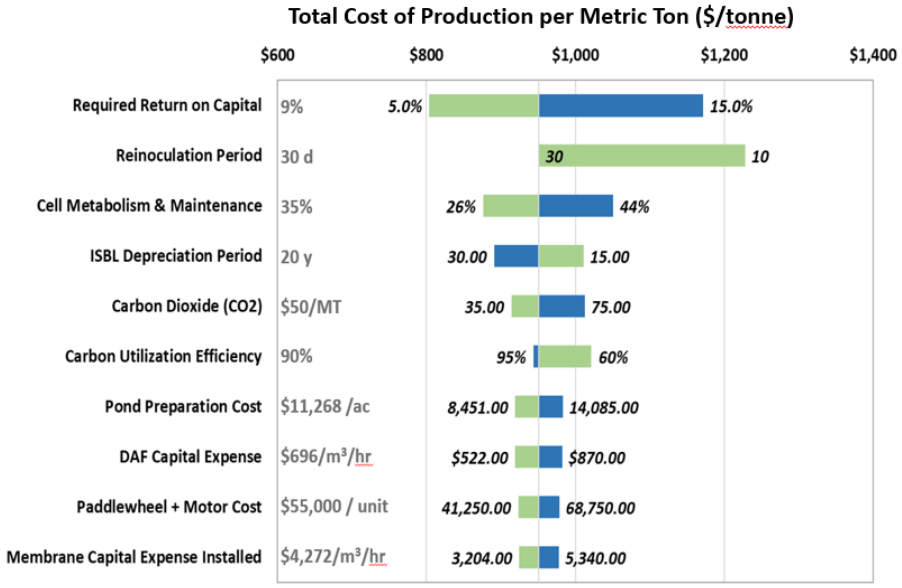

Figure 2: Tornado chart of key parameters influencing total cost of biomass and methyl laurate production. DAF Capital Expense in the chart refers to the Dissolved Air Floatation equipment. This is the modeled separation process for most of the methyl laurate. ISBL Depreciation Period specifies the depreciation period for the assets used in the direct production process (*e.g.*, ponds, separation equipment) and excludes indirect assets like warehouses and office space. Membrane Capital refers to separation equipment used in biomass membrane separation processes.

Figure 2 shows the most influential assumptions impacting total cost of production, of 1 metric ton ($/tonne) of "product". The baseline assumptions are shown in gray text to the right of the named assumption. The "product" is actually a hypothetical mass of two separated products, methyl laurate (460 kg) and biomass (540 kg). This was done for ease of analysis when the team varies the energy allocated to each of the two products. A similar analysis was conducted on profit per metric ton ($/tonne) using revenue assumptions for each of the two sub-products (Q1 FY2019 RPPR Report).

In another analysis the cost and revenues of the product mix was examined as a function of the energy allocation to each respective product. The model assumes 35% of available energy was allocated to cell metabolism and maintenance, leaving 65% for allocation to either biomass growth or methyl laurate synthesis. As this 65% is allocated to these two products, costs and revenues will vary with the ratio between the two products. The analysis showed that costs and revenues were fairly stable unless only very small allocations were made to biomass growth (Q1 FY2019 RPPR Report).

Based on the sensitivity analysis the following observations are noteworthy: 1) the biomass separation is a limiting factor for economical harvests of cyanobacteria, 2) limiting pond drainage and re-inoculation is important to keep operational costs reasonable in this paradigm, and 3) short- and mid-term profitability of commercial ventures will be dependent on finding high-revenue markets for the biomass and methyl laurate produced.

The project faced several challenges including delays in developing transformation vectors for *fab* gene expression, discovery that *de novo* ethyl-laurate production was not compatible with *Synechocystis*'s physiology, and inability to develop non-growing strains that remained fully metabolically viable. Despite these setbacks, in most cases researchers found work-around solutions or other approaches that led to successful completion of project objectives, including two patentable discoveries, a *Synechocystis* strain with a functional FAS I complex, and efficient photosynthetic production of methyl laurate.

References:

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Weyer, K.M., Bush, D.R., Darzins, A., Willson, B.D. (2010) Theoretical maximum algal oil production. Bioenergy Research 3, 204-213.

IV. Products Developed

a. Publications, conference papers or other public releases of results:

Burch, T.A., Brune, D.C., Wang, X., Nielsen, D., Vermaas, W. (2018) Optimization of parameters for cyanobacterial growth and production. Poster presentation at the 27th Western Photosynthesis Conference, Oracle, AZ.

Burch, T.A., Vermaas, W. (2019) Photosynthetic production of fatty acyl-CoA via a heterologous type I fatty acid synthase in *Synechocystis* sp. PCC 6803. Poster presentation at the 28th Western Photosynthesis Conference, Friday Harbor, WA.

Holland, S., Wang, X., Vermaas, W. (2018) Increasing NADPH availability in fatty acid producing strains of *Synechocystis* sp. PCC 6803. Poster presentation at the 27th Western Photosynthesis Conference, Oracle, AZ.

Jones, C.M., Nielsen, D.R. (2018) Nickel-Inducible CRISPRi in Synechocystis sp. PCC 6803. Poster presentation at the 27th Western Photosynthesis Conference, Oracle, AZ

Jones, C.M., Pfleger, B.F., Nielsen, D.R. (2018) Advancements in gene expression control in cyanobacteria. Poster presentation at the Synthetic Biology Engineering, Evolution, and Design (SEED), Scottsdale, AZ

Li, S., Dookeran, Z., Flores, A., Wang, X., Nielsen, D., Vermaas, W. (2018) Ethyl laurate production in cyanobacteria. Poster presentation at the 27th Western Photosynthesis Conference, Oracle, AZ.

Swenson, W., Holland, S., Keilty, A., Wang, X., Nielsen, D., Vermaas, W. (2018) Strategies to increase metabolic flux through the fatty acid biosynthesis pathway in *Synechocystis* sp. PCC 6803. Poster presentation at the 27th Western Photosynthesis Conference, Oracle, AZ.

Swenson, W., Holland, S., Wang, X., Vermaas, W. (2019) Improving fatty acid biosynthesis from photosynthetically fixed carbon in cyanobacteria. Oral presentation at the 28th Western Photosynthesis Conference, Friday Harbor, WA.

b. Web site or other Internet sites:

None to report at this time.

c. Networks or collaborations fostered:

None to report at this time.

d. Technologies/Techniques:

None to report at this time.

e. Invention Disclosures/Patent Applications, licensing agreements:

Burch, T., and Vermaas, W. (2018) US Provisional Patent application No. 62/780,164, Washington, DC: U.S Patent and Trademark Office. (DOE iEdison no. S-151,021)

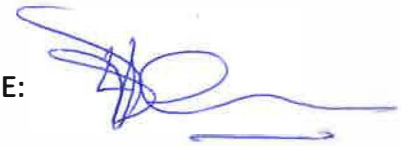
Vermaas, W. (2019) US Provisional Patent application No. 62/814,035, Washington, DC: U.S Patent and Trademark Office. (DOE iEdison no. S-150,963)

f. Other products, such as data or databases, physical collections, audio or video, software or netware, models, educational aid or curricula, instruments or equipment:

None to report at this time.

XII. Certification of Compliance

I have the authority to make the following certification on behalf of the Lead Recipient, Arizona State University. On behalf of the Lead Recipient, I certify that this project - i.e., the entirety of the work performed under the Award - is in compliance with the "Performance of Work in the United States" requirements set forth in the Award Special Terms and Conditions, Term 9. On behalf of the Lead Recipient, I further certify that the information provided in this Research Performance Progress Report is accurate and complete as of the date shown below.

SIGNATURE: 

DATE: 03/30/19

TYPED NAME: Willem F. J. Vermaas

TITLE: Foundation Professor

ORGANIZATION: Arizona State University