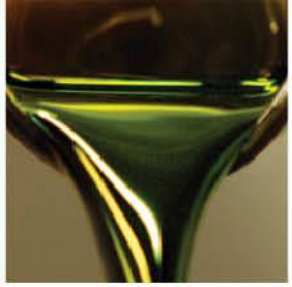









Growing the World's Fuels

Sapphire Energy®

BETO 2015 Project Peer Review

March 2015

Goal Statement

Overall objective: Sapphire Energy, Inc. is developing an end-to-end process to produce renewable, algae-based fuel that is fungible with existing refinery streams. This project aims to address three Priority Areas: (1) improve algal biomass productivity in outdoor cultivation environments relevant to commercial scales; (2) improve pre-processing technologies that can be integrated at scale with biomass production; and (3) successfully integrate Priority Areas 1 and 2 to ensure that target yields are met at a scale that enables production of cost-competitive fuels and products. In addressing each of these priority areas, we aim to demonstrate sufficient improvements in algal biomass yield at lab and outdoor pilot scales in the first 24 months to provide a positive indication towards the likely success of a 1-acre demonstration of 2,500 gallons per acre per year oil productivity by 2018.

Relevance and Outcomes for the United States: The advancement of the algal technology platform strategized in this project is aligned with the Bioenergy Technologies Office's mission to transform renewable biomass resources into commercially viable fuels. This would allow for a reduction in both greenhouse gas emissions relative to petroleum-based fuels along with a reduction in the United States' dependence on foreign oil

Quad Chart Overview

Timeline

- Project start: Mar 2014
- Project end: Aug 2016
- Percent complete: 40%

Barriers addressed

- Unlock algal and terrestrial biomass productivity
- Algal feedstock processing

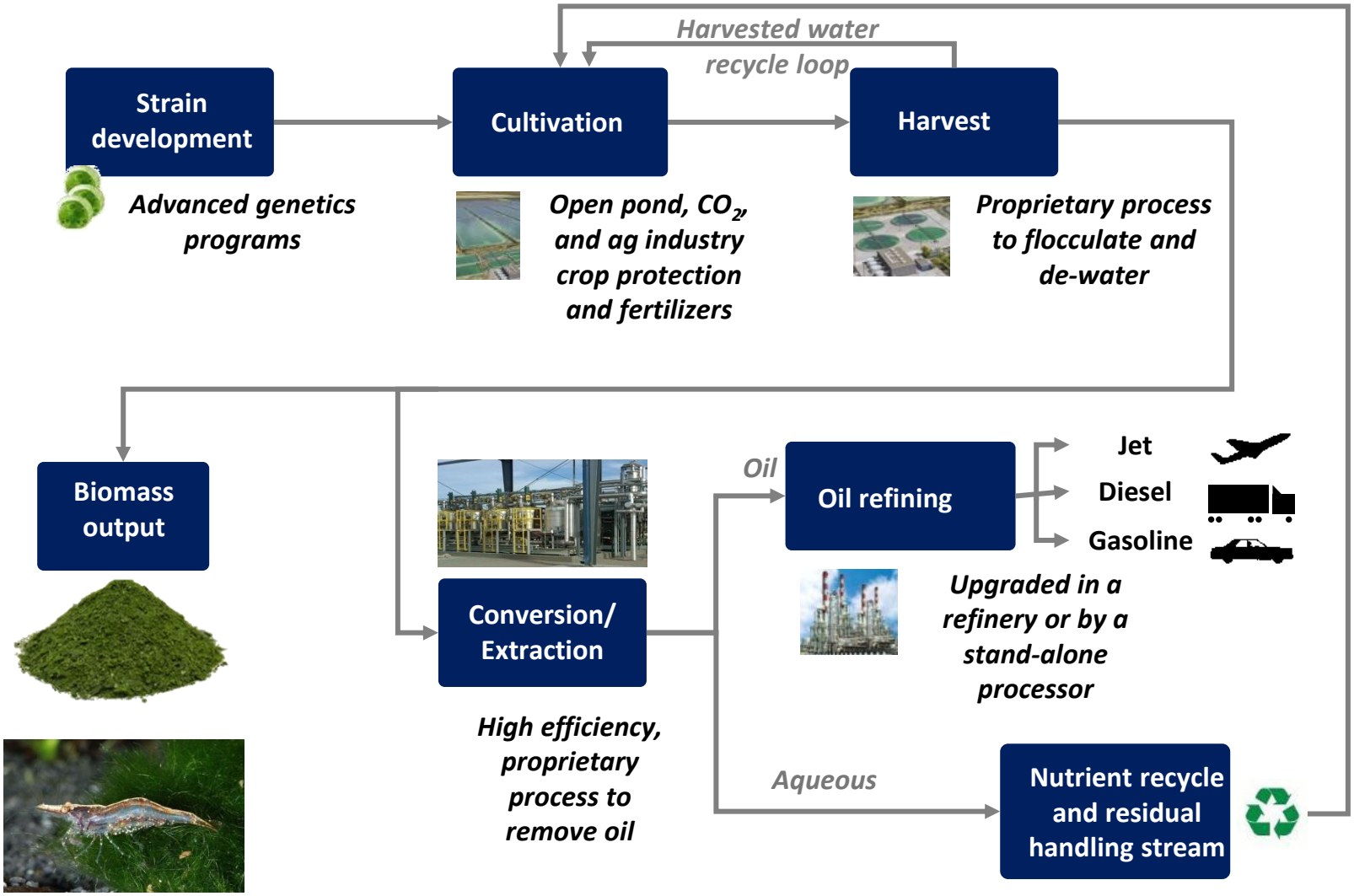
Recast budget

	FY 14 Costs ($000's)	FY 15 Costs ($000's)	FY 16 Costs ($000's)	Total Planned Funding ($000's)
DOE Funded	1,107	3,317	577	5,000
Project Cost Share	303	908	508	1,720

Technical partners

- Institute for Systems Biology: 32%
- NREL: 4%
- Penn State University: 2%
- University of Virginia: 1%

Overview of Sapphire Energy's process



High-level Objectives

Priority Area 1 – Improvements in algal biomass productivity

Objective 1: Improve biomass productivity of current production strains using Evolutionary Engineering (EE) approaches.

Objective 2: Improve biomass yield through rational strain design by (1) doing a complete functional annotation and comparative genomic analysis of the model algae *Chlamydomonas reinhardtii* and our production strains, (2) build a gene regulatory network model based on growth and environmental influences to understand transcriptional responses to environmental perturbations and integrate with a metabolic flux balance model, (3) use the integrated gene regulatory network-metabolic model to identify and rank target genes for optimization of fuel production, and, and (4) validate the model by incorporating target genes into field optimized strains to achieve higher yields.

Institute for Systems Biology

Objective 3: Understand the ecology of microbial communities under open-pond conditions and through engineering communities and establishing approaches for management of open pond ecosystems that confer improved performance and robustness across abiotic, biotic, and nutritional gradients, decrease the annual variance of algal biomass productivity.

High-level Objectives

Priority Area 2 – Improvements in pre-processing technologies

Objective 4: Develop methods to harvest algae to decrease cost.

Objective 5: Improve yield from hydrothermal treatment (HTT) process through (1) reducing water post-harvest/pre-HTT, (2) conducting detailed characterization of process oils and product streams and (3) developing a generalized, strain-independent kinetic reaction network model to better predict bio-crude yields as a function of HTT time and temperature.

High-level Objectives

Priority Area 3 – Technical advances that enable integration of algal biomass unit operations

Objective 6: Using strains in Objective 1 studies, maximize integration and techno-economics of the biocrude process through nutrient recycling.

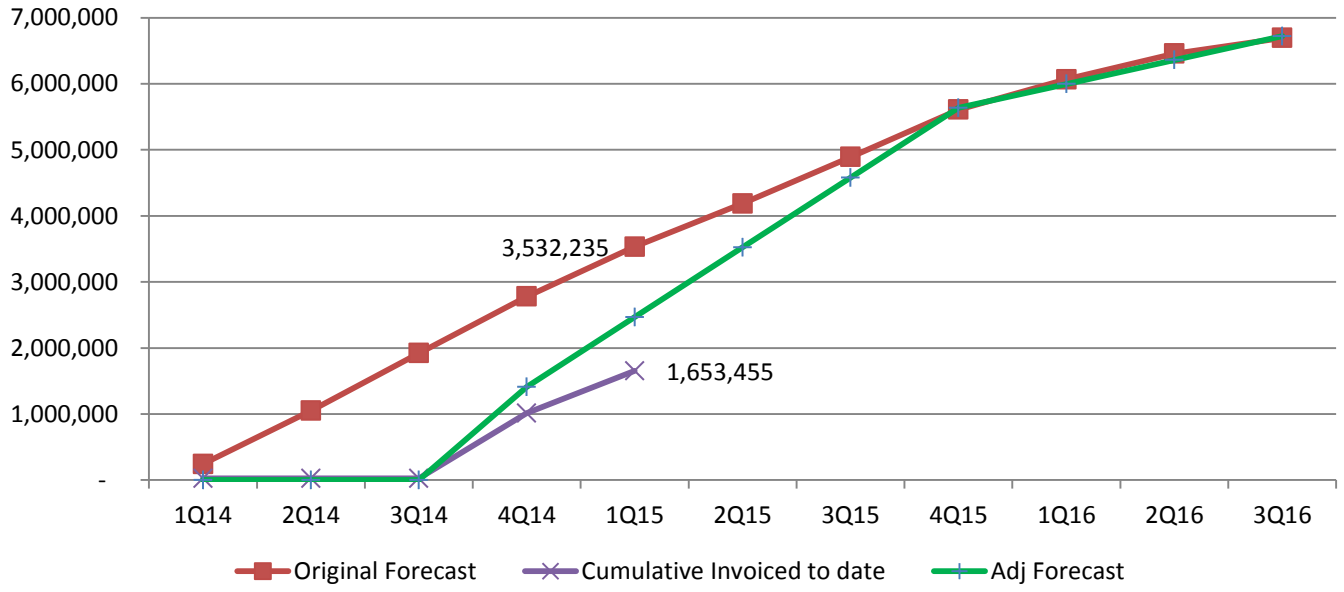
Objective 7: Expand the current techno-economic model—which incorporates cultivation, harvest, conversion, and extraction processes from end-to-end based on single sets of parameters—to allow process modeling over extended periods and with changing parameters.

Objective 8: Integration of life cycle assessment in process development and decision-making process for research options to ensure a maximum energy return on investment (EROI) and minimum life cycle greenhouse gas (GHG) emissions.

Project Start-up History

2013		2014				2015				2016		
3Q13	4Q13	1Q14	2Q14	3Q14	4Q14	1Q15	2Q15	3Q15	4Q15	1Q16	2Q16	3Q16
★ Original planned award notice												
		★ Modified projected award notice										
		Some activities started at risk →										
					★ Actual award notice							
						All priority areas work initiated/ongoing →						

Cash Flow Summary



Approach – Technical and Management

Technical approach

- Sapphire is employing three approaches to improve field biomass productivity
  - Streamlined non-GMO evolutionary engineering approaches
  - Ecological engineering for yield stability
  - Building an algal model to generate novel targets for GM-based yield increases
- Pre-processing and integration work streams are strategized to ensure that we are improving the entire technology platform and not only the biomass productivity piece

Success factors

- Sapphire's research and development process is conducted alongside a sophisticated techno-economic model
- Many outputs are important for this project (e.g., biomass productivity, gallons of Green Crude per acre per year)
- Ultimate metric is cost per barrel of Green Crude assuming production in a commercial facility

Challenges

- State of progress on non-project work (*e.g.*, oil content, crop protection)
- Continued interest from investors to develop an algae-to-oil process
- Translation of experiences and approaches to the commercial strain
- Delayed start to the majority of grant work
- Risk management – if decision point targets are not met (but close) review mitigation plans and advance into next stage gate if possible

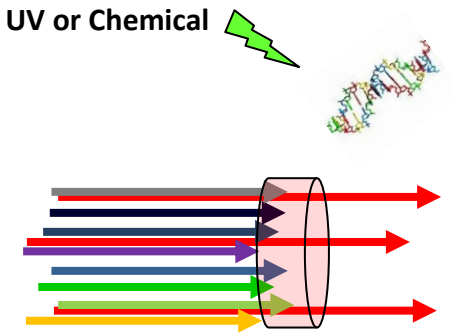
Technical progress - Overview

Priority Area/Work Stream		1Q14			2Q14			3Q14			4Q14			1Q15			2Q15			3Q15			4Q15			1Q16			2Q16			3Q16			4Q16		
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
	Quarterly DOE Meeting				◆			◆			◆			◆			◆			◆			◆			◆			◆			◆					
1.1 Evolution-based strain engineering	Phase Complete							Candidate strains			Phase I ★														Phase II ★												
1.2 Systems biology	Phase complete										Candidate genes						Phase I ★						Phase II ★						Phase III ★							
1.3 Pond Ecology	Phase complete				Review data ★			Phase I									Phase II ★																				
2.1 Harvest	Phase complete													Assess particle size, settling, etc. ★			Phase I			Pilot process			Phase II ★						★			Phase III					
2.2 Hydrothermal treatment (HTT)	Phase complete							Reaction modeling Decrease water						Phase I ★						Phase II ★																	
2.3 Oil analysis	Phase complete										Develop analysis method			★			Phase I																		Phase II ★			
3.1 Nutrient recycling	Phase complete							Analyze raffinate Lab experimentation						Phase I ★						Phase II ★																	
3.2 Process modeling	Phase complete																Improve process model			Phase I ★														Phase II ★		
3.3 Life cycle analysis	Phase complete													Baseline LCA Integrate into review process			Phase I ★			★			Phase II													

Technical progress – Priority area 1

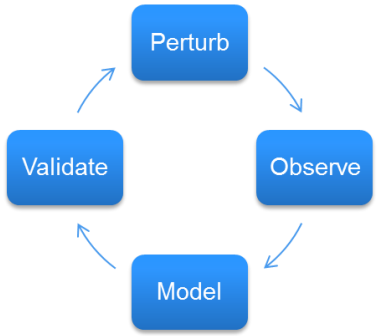
Evolutionary engineering

- Further refinement of the evolutionary engineering platform is complete
- Novel tracking process eliminates the need for a artificially created marker strain
- We have the capability to take any new strain and improve its growth rate



Systems biology

- Experiments have been conducted under various environmental conditions
- Expression data have been collected and are currently being integrated into the model

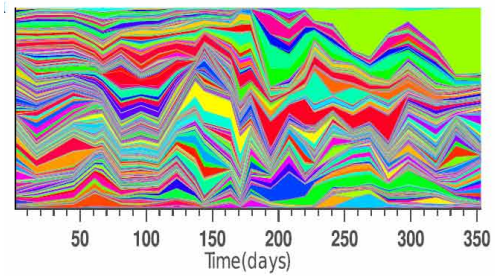


Institute for Systems Biology

- 311 new genes have been added to the existing metabolic model
- Putative yield genes will be identified by Q2 2015

Ecology

- Studied changes of diversity from year-long growth in Las Cruces, NM



- High throughput platform was developed for evaluating consortia
- Screened a panel of strains across temperature and nutrient gradients
- No consortia was found that outperforms the best single strain
- Several consortia have decreased variance across the gradients explored

Technical progress – Priority area 2

Harvest

- The fundamental objective of this effort is to decrease cost
- Baseline technology utilizes polymer-based dissolved air flotation strategies
- Successfully demonstrated a proof of concept-scale capability to take a 0.05-0.1% algae culture to almost 3%
- Given that the polymer-based dissolved air flotation process achieves approximately the same density, this would significantly decrease both capital expenditure and possibly also operating expenditure.
- Next steps include piloting the new process

Oil characterization

- Due to the polar nature of biocrudes, the relationship between boiling point and molecular weight derived from traditional petroleum crudes do not hold
- Gel permeation chromatography conditions have largely been completed at Sapphire
- High resolution mass spectrometry work is underway at the National Renewable Energy Laboratory in the laboratory of Lieve Laurens

NREL
NATIONAL RENEWABLE ENERGY LABORATORY

Hydrothermal liquefaction

- Significant progress was made in the HTL area between the submission and funding
- Effort is being re-scoped to ensure that we wisely use DOE funds

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Technical progress – Priority area 3

Nutrient recycle

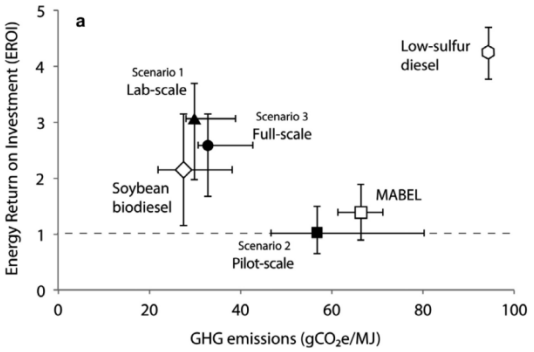
- Significant progress was made in the HTL area between the submission and funding
- We are no longer evaluating several nutrient recycle process options
- The non-oil stream is an aqueous stream that contains some solids
- We have demonstrated nutrient recycle using both the non-oil solid stream and the non-oil aqueous stream

Process modeling

- Process modeling is an important piece of the project with the intent of increasing the resolution of the model and the connectivity with commercial viability
- Included strain options, site location settings, monthly productivity resolution, labor, nutrient cost.
- Included different harvest options, ash content
- Included solvent options, HTL process conditions

Life cycle assessment

- Collaboration with Professor Andres Clarens and Professor Lisa Colosi
- Baseline LCA was conducted prior to the award



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Relevance

Meeting the platform goals of BETO MYPP

The described project will directly feed into the Bioenergy Technologies Office Mission and Goals since the objective of the project is to increase the fuel productivity of an integrated process not simply in a laboratory atmosphere. Success will help in laying the foundation of a sustainable, nationwide production of biofuels that is commercially viable.

Application in the emerging bioenergy industry

Performance period 1 is dedicated in developing and piloting the technologies required for improving biomass productivity. All efforts converge into the anticipated performance period 2 where the entire process is to be piloted at a 1 acre scale for an entire year.

Impact on commercial viability

Demonstrating at lab and pilot scale in performance period 1 the necessary performance metrics (e.g., extractable oil fraction, biomass productivity, etc.)

Future work

Next 18 months

Sapphire is working with the DOE on re-scoping the project to ensure the best use of the funds with a continued mindset of progressing the technology of an algae-to-oil platform

Highlight key future milestones

These are dependent on re-scoping discussions

Summary

- Sapphire has made significant progress in strain improvement approaches
- Novel approach for the first step of harvest would have a massive cost impact
- Re-scoping discussions are ongoing to make sure the project is strategically aligned with the objective of the FOA, the mission of BETO, and the core business of Sapphire