U.S. Department of Energy (DOE)
Bioenergy Technologies Office (BETO)
2017 Project Peer Review

Integrated Low Cost and High Yield Microalgal Biofuel Intermediates Production

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Advanced Algal Systems

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Goal Statement

The project goals are to meet DOE-BETO's 2020 targets of 3,700 gallons per acre-year of biofuel intermediates and $3 per gallon gasoline equivalent (gge) by 2022. This will be achieved by:

(1) Selecting and developing improved algal strains with higher productivities of lipids and/or carbohydrates using enrichment/selection cultures

(2) Using genomics to identify the genetic basis of improved strains

(3) Outdoor open pond cultivation of selected improved strains on **reclaimed** wastewater

(4) Incorporating mixotrophy in algae cultivation processes

(5) Converting algal biomass to biofuel intermediates through a variety of processes: oil extraction of lipids, fermentations of carbohydrates to ethanol and proteins to fusel alcohols and conversion to 'biocrude' of the biomass residuals with HTL.

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Quad Chart Overview

Timeline

- Start: October 1, 2016 (Validation to Dec 31, 2016)
- End: September 30, 2020
- As of 1/31/17: 3% complete

Budget

	Total Costs FY 12 –FY 14	FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 17-Project End Date)
DOE Funded	$0	$0	$0	$4,999,958
Project Cost Share Heliae	$0	$0	$0	$725,293
Project Cost Share Cal Poly	$0	$0	$0	$437,221
Project Cost Share MicroBio Engineering Inc.	$0	$0	$0	$128,800

Barriers

The goal to produce 3,700 gal/acre-y of biofuel intermediates and achieve $3/gal gasoline equivalent by 2020, requires:

- Using selection to develop improved algal strains with high oil or carbohydrate content
- Increased biomass productivity in open ponds, including mixotrophic processes
- Converting the entire biomass to biofuel intermediates by lipid extractions, protein / carbohydrate fermentations and HTL

Partners (% of total Project)

MicroBio Engineering Inc. ABY2 partners:

- Cal Poly (30%) enrichment cultures for high C-storage algal strains
- Heliae (18%) – mixotrophic production
- Sandia (19%) - protein fermentations; genomics
- PNNL (3%) - HTL of biomass and residues after extractions/fermentations
- Collaboration (1%) J. Polle, Consultant

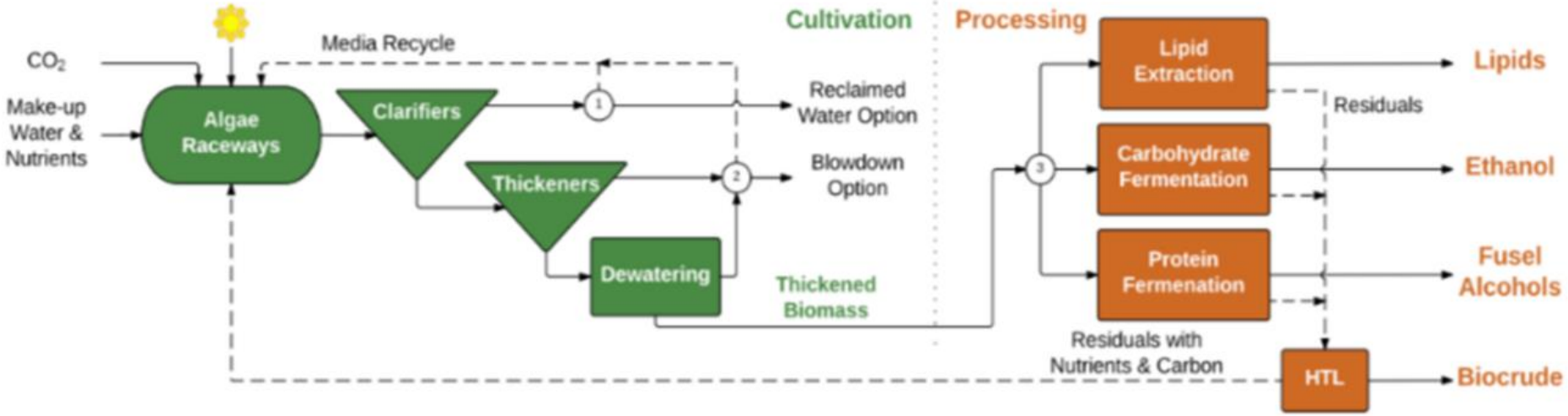
1 - Project Overview

The major objective of this project is to increase algal biomass lipid and/or carbohydrate content and overall productivities through:

1. Strain improvements by mutagenesis followed by cultivation in laboratory continuous cultures designed to provide environmental conditions that select (enrich) for strains with desired traits, for higher content of carbon storage products (lipids and/or carbohydrates).
2. Isolation of improved strains from such enrichment cultures and the application of genomics to identify genetic differences between selected strains and original wild type stock.
3. Cultivation of selected strains in outdoor ponds on wastewater and comparison to wild-type for productivity, lipid/carbohydrate content and culture stability.
3. Use of mixotrophic cultivation to further increase algal productivity.
4. Conversion of algal biomass to biofuel intermediates through extraction of oils, fermentations of carbohydrates and proteins and HTL of residues

1 - Project Overview

Full-scale process flow diagram



Algal strain laboratory and outdoor culturing facilities:
flasks, strain selection PBR (new design being built), panels, ponds




2 – Approach (Management)

Dr. John Benemann, PI, is responsible for project direction and workflow, supervises MicroBio Engineering Inc. staff, and coordinates subcontractors research through regular meetings. Team members and research areas:

MicroBio Engineering, Inc. – John Benemann, Project management and guidance, also TEA / LCA, open pond cultivation, PBR design & assembly.

Cal Poly - Dr. Tryg Lundquist, supervision and operations; Dr. Aubrey Davis algal cultures, PBR enrichment cultures, strain isolation. Ms. Ruth Spierling carbohydrate fermentations, pond operations supervision.

Heliae – Dr. Ravi Vannela and Dr. Eneko Ganuza, mixotrophic cultivation.

Sandia – Dr. Ryan Davis, protein fermentations; Dr. Todd Lane, genomics.

PNNL - Dan Anderson, HTL conversion of biomass to 'biocrude'

Consultant – Dr. Juergen Polle, algal strains, cultures, genomics.

2 – Approach (Technical)

1. Algal strains with higher contents of carbon storage products (lipids or carbohydrates) will be developed through selective enrichment cultures. Random mutagenesis will be followed by laboratory continuous cultures operated to select for desired traits. An initial objective is increased productivity of lipids or carbohydrates.

2. Strains from enrichment cultures will be isolated and compared to wild type in laboratory cultures for growth rate and lipid and carbohydrate content.

3. Specific mutations in selected strains identified by genomic analysis.

4. Cultivation of selected strains in outdoor ponds, compared to wild type.

5. Further increases in productivity through mixotrophic processes.

6. Conversion of algal biomass harvested from ponds to biofuel intermediates by fermentation of carbohydrates to ethanol, proteins to fusel alcohols, lipid extraction, conversion of residues to HTL oil.

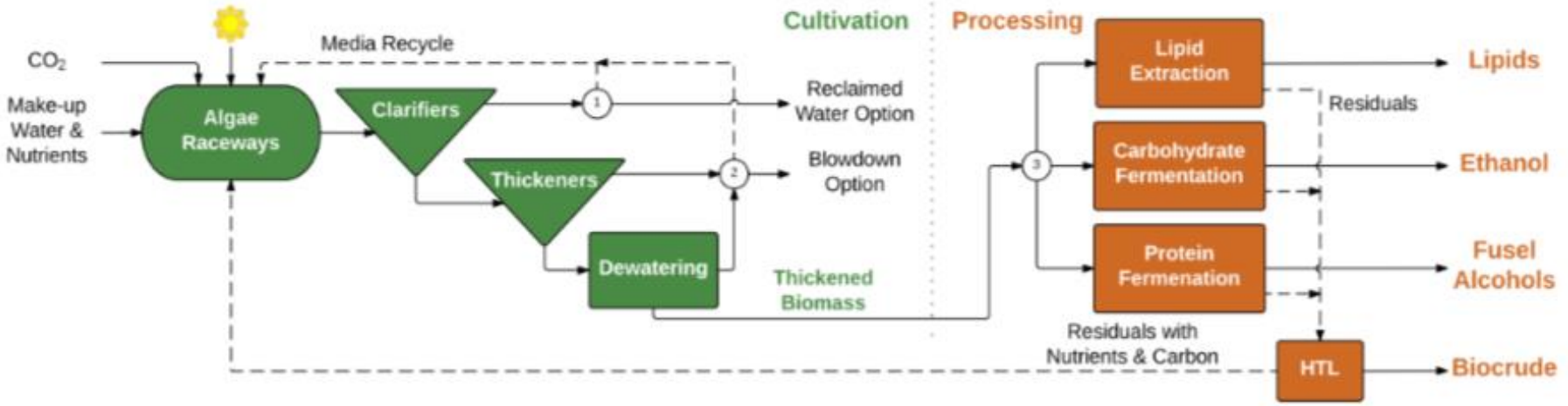
These approaches can advance relatively independently in the initial stages and will be integrated as research advances.

2 – Approach (Technical)

TEA/LCA Modeling Approach

Use MicroBio Engineering's Microalgae Production Model (MPM) with financial assumptions based on NREL's Harmonization Report and:

Facility Size:	**400 ha of pond surface area**
Location:	**San Joaquin Valley, CA**
Annual Average Productivity:	**33 g/m^2-d** (mixotrophic growth)
Peak Hourly Productivity:	**3 g/m^2-hr** (for CO2 supply design)
Growth Media:	**Wastewater** (reclaimed, primary)
Influent Flow:	**9 MGD**
Biofuel Intermediates:	**Ethanol/fusel alcohols; lipids; HTL of residuals to 'biocrude'**



2 – Approach (Technical)
Critical Success Factors

- **To improve biomass composition and productivity to meet DOE-BETO's target of 3,700 gal/ac-y biofuel intermediates by 2020 and 2022 goal of $3/gallon of gasoline equivalent (gge).**
- **Technical Challenges:**
  - Selection of improved algal strains through enrichment cultures
  - Maintenance of desired traits in open pond cultivation
  - Increase productivity through mixotrophic pond cultivation
  - Conversion algal biomass to biofuel intermediates

Go-No Go decision criteria at end of year 2:

Do selected strains have higher lipids/carbohydrate content and/ or productivity? Can these be cultivated in outdoor ponds? Does the yield of biofuel intermediates achieve the project goals?

If yes: develop additional improved strains and longer-term study of selected strains compared to wild-type.

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4 – Relevance

Increase algal biofuels intermediate production through strain selection, mixotrophic cultivation and complete conversion of the biomass to biofuel intermediates.

- Addresses BETO's 2020 target for the production of 3,700 gal/acre/yr of biofuel intermediates
- Addresses BETO's 2022 target of $3/gasoline gallon equivalent (GGE) through higher productivity and more efficient conversion of algal biomass to biofuel intermediates
- Combines technologies of strain selection and mixotrophic production with oil extraction, carbohydrate and protein fermentations, and HTL to achieve high productivities and yields of biofuel intermediates.

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5 – Future Work

Budget Period 1 (1/1/2017 to 12/31/2018)

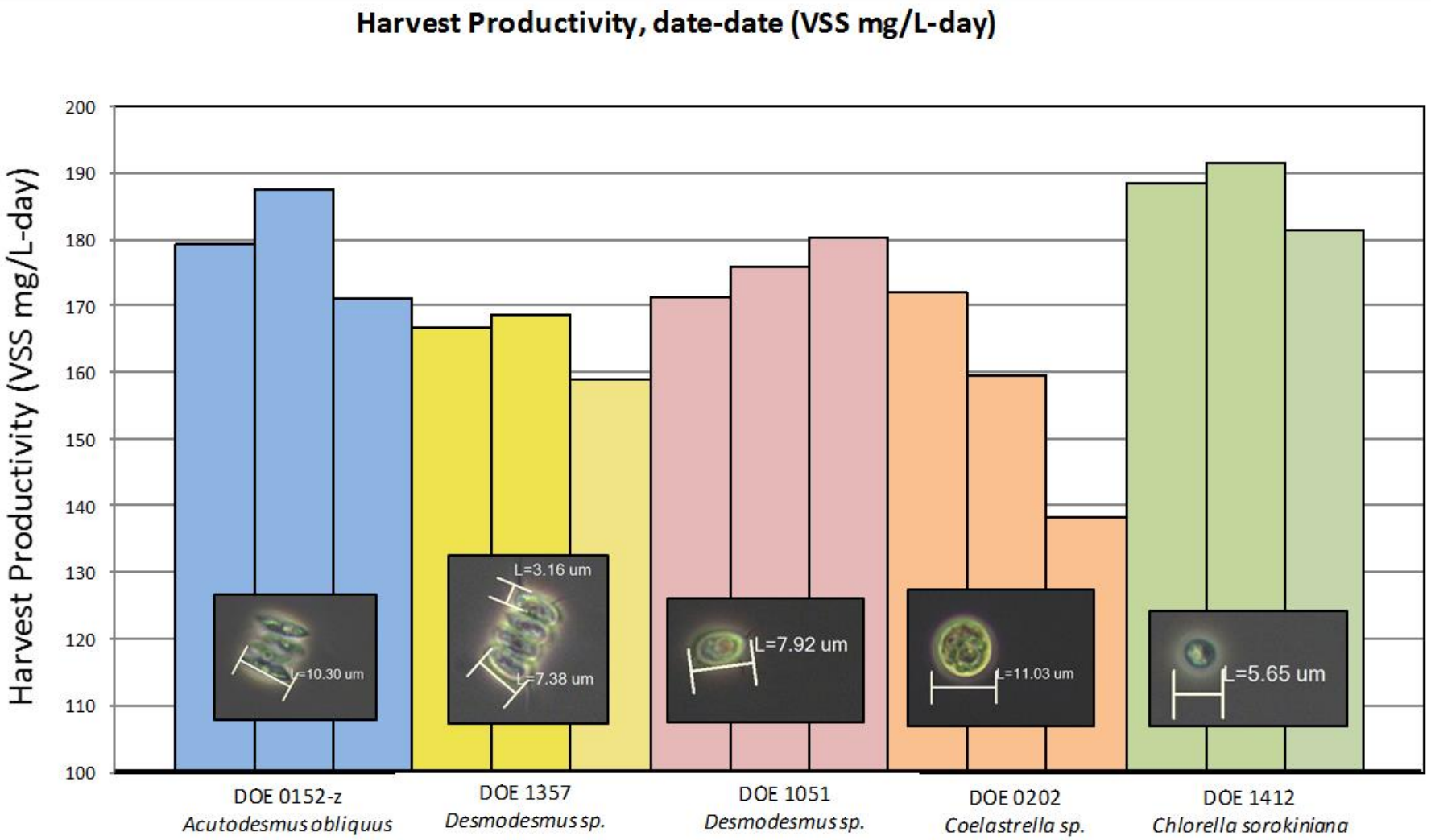
- Baseline laboratory data for biomass and C storage (carbohydrates, lipids) productivity of six wild type strains; choose two for further work
- Determine growth potential and stability in outdoor wastewater ponds of chosen strains (Milestone 2.1.1)
- Update TEA and LCA protocols and procedures (Milestone 2.1.2)
- Develop mutagenesis method (UV, chemical)
- Bioreactors assembled and enrichment protocols for strain selection developed (Milestone 2.1.3)
- Ethanol fermentation studies initiated (Milestone 2.2.1)
- HTL work carried out with algal biomass from pond cultures
- Cell growth and product formation kinetics and yields for AY3 on *Scenedesmus* sp. hydrolysates (Milestone 2.2.2)

Budget Period 2 (1/1/2019 to 9/30/2020)

- Initiate long-term study of selected strains; comparison with wild-type and polyculture (Milestone 2.3.2)
- TEA/LCA sensitivity methodology (Milestone 2.3.3)
- Complete fermentation studies (Milestone 2.4.2)

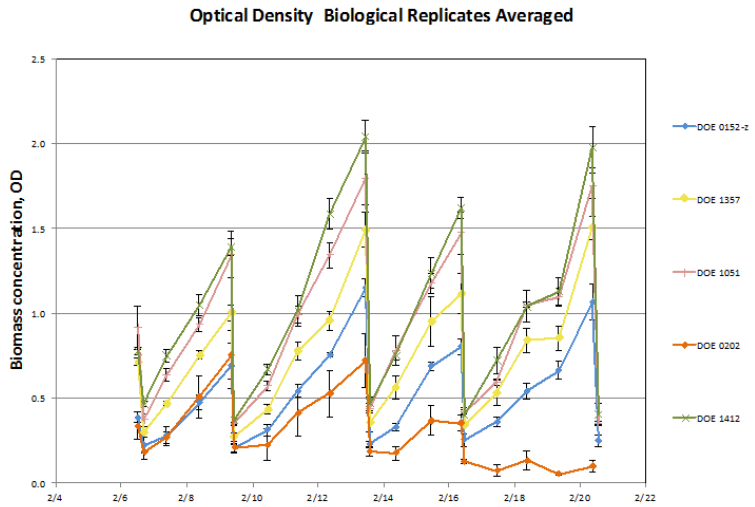
5 – Future Work

- Baseline laboratory data for biomass and carbon storage (carbohydrates, lipids) productivity of six wild type strains; choose two for further work



5 – Future Work

- Lab selective enrichment and selection. **Isolate strains, compare lab & pond cultivation, genomic analysis (Sandia)**
  - End of project performance
    - 33 g/m^2-day auto and mixotrophic and either:
    - 40% lipid (strain 1)
    - 60% carbohydrate (strain 2)
    - 40% protein (strain 3)
  - Current activities
    - Harmonize work with project partners in similar reactors with a common strain.
    - Develop and test UV mutagenesis method
    - Measure baseline productivity and biomass composition.



5 – Future Work

- Determine growth potential and stability in outdoor wastewater ponds of selected stains and wild type (Milestone 2.1.1)

Plan:
-Establish baseline productivity of wild type on **reclaimed** wastewater.

-Test selective pressures (high dilution, N limitation on schedules) in outdoor ponds.

Six 10,000-L ponds

Ten 1,000-L ponds for experiments and inoculum production

End of project performance

- 33 g/m^2-day auto and mixotrophic and
- 40% lipid (strain 1)
- 60% carbohydrate (strain 2)
- 40% protein (strain 3)

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5 – Future Work

- Perform TEA and LCA
  - Based on project data
  - End of project performance
    - $4.96/GGE
- Ethanol fermentation studies
  - Carbohydrate rich biomass will be harvested, thickened, and disrupted. Algal carbohydrates will be measured and fermented to ethanol. Carbohydrate destruction and ethanol yields will be measured.
  - End of project performance
    - 0.252 g EtOH/g AFDW

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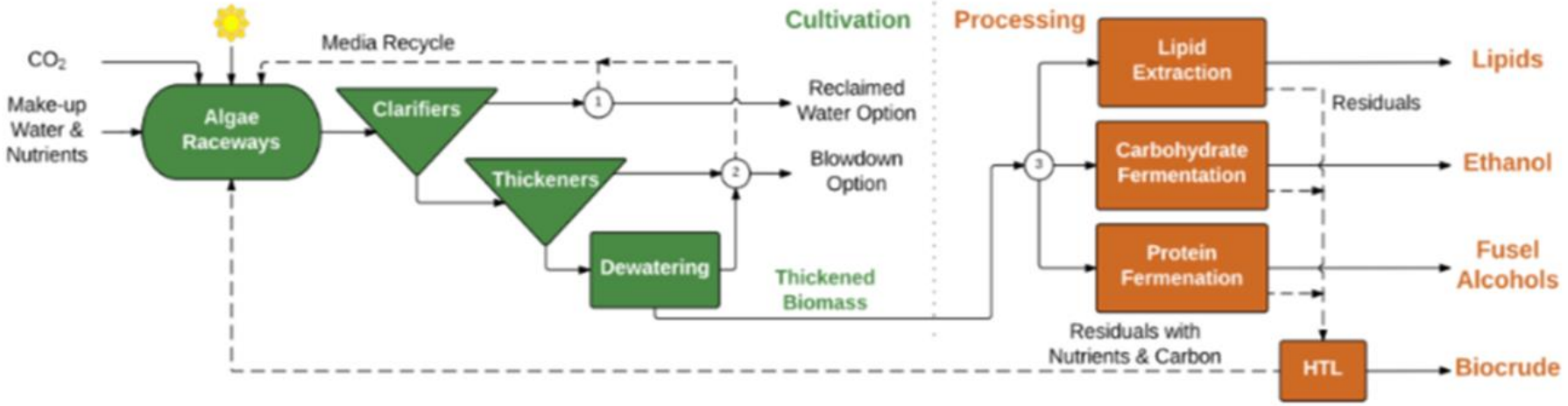
5 – Future Work

- Mixed alcohol fermentations (SNL)
  - Determine cell growth kinetics and yields for YH83 and AY3 E. *coli* on *Scenedesmus* sp. hydrolysates. Track algae genotypes in Cal Poly enrichment cultures.
  - End of project performance
    - 0.29 g alcohol/g protein
- Hydrothermal liquefaction studies (PNNL)
  - HTL will be performed on residuals from fuel processes
  - End of project performance
    - 0.35 g biocrude/g residual



5 – Future Work

- Study O_2 evolution and consumption in mixotrophy (Heliae)
  - Determine relative contribution of phototrophic and heterotrophic growth in a photo-heterotrophic (i.e., mixotrophic) system;
  - Investigate whether to encourage one over the other is beneficial to the combined process measured as biomass and/or biofuel intermediate
- Develop mixotrophic culture management strategies (Heliae)
  - Study the ratio of primary vs secondary production will be studied with simulated wastewater (e.g.: sugars, acetic acid and glycerol blends).
  - End of project performance
    - 0.25 g BFI/ g Carbon substrate

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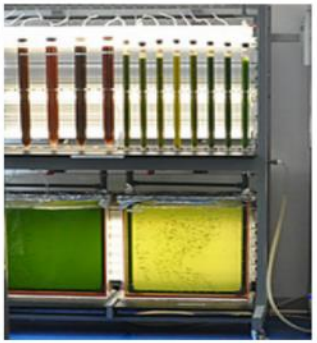
Summary

- Innovative approaches are needed to reach DOE-BETO's goal of producing 3,700 gal/ac/yr of biofuel equivalents by 2020 and $3/gallon of gasoline equivalent by 2022. To accomplish this, we will:
  - Develop and demonstrate innovative strain selection methods to yield improved strains with higher lipid/carbohydrate productivity in both laboratory and outdoor pond cultures.
  - Develop mixotrophic growth techniques on wastewater
  - Advance algal biomass to biofuel intermediates conversions
  - Update and apply LCA and TEA based on project results

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Additional Slides

Responses to Previous Reviewers' Comments -1

Reviewer comment: It is unclear what strains will be examined in this study and if they are the same strains as studied in the previous ABY or other projects listed.

Response: We will screen several strains of *Scenedesmus,* ***Chlorella*** and *Desmodesmus* suitability in the continuous cultures and select two for initiating the research plan. Other species and polycultures may be used in the future, such as isolates from outdoor raceways.

Reviewer comment: It is unclear if selective enrichment will be performed in actual wastewater or in defined media and if these cultures will maintain these qualities when transferred to other media.

Response: The selective enrichment cultures will be carried out with defined media. The cultures are expected to maintain their genotype/phenotype in reclaimed water, because it is low in organics just as defined media.

Responses to Previous Reviewers' Comments -2

Reviewer comment: response from applicant of risks in mixotrophy failed to convince reviewer that they have these risks fully under control despite a PI's long experience in growing algae on waste water.

Response: Heliae has developed extensive knowledge in the field of large scale, outdoor, high cell density mixotrophic cultures with effective contamination treatment strategies (that prevented culture crashes and production losses)

Reviewer comment: It is unclear what harvesting method will be utilized, or what harvesting method was used for baseline calculations.

Response: The baseline harvesting method was bioflocculation and sedimentation. This method and cost was not changed for the intermediate and long-term cases in the Tech Fin