Chapter 1: Biology

Cellular and Molecular Biology

Convener: Stephanie Brunelle

Discussion:

The research goals for algal physiology and cell biology should include:

- High yield photosynthetic production
- "'OMICs" – capturing the whole genetic picture via genomics, proteomics, etc., and there is industry interest in doing so
- Cell cycle linked with lipid production
- Phenotypic stability
- The value chain of production of fuel
- Crop protection
- Bio-flocculation
- Genetic toolbox for commercial process; this could prove to be a big opportunity from an industry perspective
- Permitting requirements at the commercial level that could use basic research (i.e. Environmental Protection Agency [EPA] research as to whether aerosolization is true).

According to the discussion participants, BETO is not interested in funding basic research. But the discussion participants feel basic research is required to understand the algae organism. How can the industry use basic research to improve the economics of algae cultivation/algal oil production? Participants discussed possible basic research that they believe will improve strain selection.

If the Algae Roadmap has a basic research component to improve biology, why is it so difficult to get funding for the research? Should BETO engage the National Science Foundation or the DOE Office of Science to coordinate basic research for algae? What is DOE doing to get basic research complete?

Where does the money come from for basic research? An argument for funding basic research is that the funds will help bring new scientists into the field. DOE should make this a priority to nurture the future workforce. The field is lacking scientists focusing on photosynthesis, an example provided by a stakeholder.

There is a lack of algae literature. Basic research can help industry and advance the development curve.

When funding basic research at universities, resulting data and information become publicly available. Private companies are holding on to basic research results due to protection of intellectual property (IP) and in so doing are stalling progress for the rest of the field. Because they are not sharing their results, some private companies are performing redundant research.

DOE could create a forum for industry to discuss the major black boxes for the basic research community. Dr. Steve Mayfield would be a good broker of industry information to help direct the basic research for commercial applications.

Guiding Questions:

1. What is the time horizon for this topic or issue; will this impact algal biofuel production in the near, mid, or long term?
    - The timeframe for this topic is immediate.
2. Has this topic been included in the National Algal Biofuel Technology Roadmap; and if not, should it be?
    - Basic biology research is included in the Algae Roadmap.
3. What is the BETO Algae Program role in furthering this topic or addressing this issue?
    - Should fund basic research to nurture the workforce in the field
    - Should create a forum for industry basic research needs

Poster:

Research Goals:

- Improve photosynthetic productivity
- Cell cycle
- Lipid biogenesis/metabolism
- “Omic” Research/Systems biology
- Evolution (short term) -> Phenotypic Stability
- Cost Assessment of each step

1. Time horizon -> Now!
2. Covered but is not adequately funded
3. DOE Role – working w/Office of Science to make basic research a priority

Algal Genomics

Convener: Rose Ann Cattolico

Discussion:

A reference database for genetic sequences is critical to advancing a commercial algal biofuels field. The technology is available to generate genetic sequence data for underrepresented organisms and diversify the information available to researchers. Currently, only Chlamydomonas is well sequenced in a database. The field is having trouble annotating haptophytes. Annotation is important, and identifying symbiotic relationships among organisms has, if managed properly, the potential to increase the oil content of the culture. The value of the work can be findings of the cell wall structure, algae reproduction, oil production, promoter and transposable systems, organisms that have antibiotics, evolutionary divergence and other advances.

Through high throughput screening, the field can make determinations on important organism phenotypes and exploit model organisms. The field should define operational sequencing parameters and develop a central database of genomes and a genetic toolbox. This will require long-term research within a coordinated effort.

Guiding Questions:

1. What is the time horizon for this topic or issue; will this impact algal biofuel production in the near, mid, or long term?
    - The time horizon is immediate, especially as the cost of gene sequencing is decreasing.
2. Has this topic been included in the National Algal Biofuel Technology Roadmap; and if not, should it be?
    - Roadmap should include model systems for studying algae, genetic toolbox.
3. What is the BETO Algae Program role in furthering this topic or addressing this issue?
    - The DOE could fund continued genetic research. Genomics is critical because biology data for commercial strains is lacking and this information could reduce risk down the road for the field. DOE should fund establishment of a large sequencing database for new untapped organisms, establish an accessible database for the algal community. This could lead to potential high throughput screening, alternative model systems ('omics', including proteomics), genome-made association, and improved collaboration between field and lab analysis. If the DOE pushes to develop the database and advance the industry, the Algae Program could have an accomplishment similar to other major research efforts.

Poster:

1. Time horizon is immediate. The impact is now.

2. It is covered [in the roadmap] but not funded.
   - Base of the (biology algal biofuels) + critically needed

3. Provide funding for:
   - Sequencing of genomes (especially new organisms)
   - Development of databases (accessible for biofuel community)
   - High throughput sequencing for desirable traits
   - Genome wide association
   - Genetic toolbox
   - Alternative model systems (other ’omics’ studies)
   - Harmonize/collaboration between the field and applied/genomic tools/studies
   - Supply new scientist training (graduate and post-doctoral students)

Genetic Engineering and Advanced Biology

Convener: Jainping Yu

Discussion:

Will improved productivity from genetic engineering obviate oil production? New models and new genetic systems are needed to determine productivity; though some cyanobacteria models exist. To maximize the work funded by the DOE, new genetic systems should be based on systems being tested outside and new platform organism, such as Algae Testbed Private Public Partnership (ATP3)[3] strains.

Research needs include:

- Marker free transformation
- Traits to modify
    - Inducible flocculation
    - Self-separation
    - Pest resistance
- Homologous recombination
- Increased genetic information
- Functional annotation and ongoing curation
- Identify mating type genes and crossing them.

DOE could play a role in answering these research needs, but Energy Efficiency and Renewable Energy (EERE) and Office of Science (OS) should coordinate on this effort. More agency coordination is needed among EPA, USDA, and DOE. EPA needs to be looped in to approve genetically modified (GM) strains. The DOE could go through terra application process with EPA for ATP3.

Generating community buy-in is important in this area. Getting public buy-in could be a decade-long effort. The field should demonstrate GM strains at select sites in places that are friendly to the idea (i.e. Florida, which has already approved GM algae), then study impact and issues like contamination and spreading. Some further issues to consider include:

- Dispersion in an open system poses more of a threat.
- Deploying sites next to schools or neighborhoods poses more of a problem for public image.
- There is no current framework for making GM products work.
- The Algae Program needs to raise the priority of issues that comes out of this workshop.

[3] Algae Testbed Private Public Partnership (ATP3) is a geographically diverse network of algal biomass production facilities within the U.S., funded by the DOE Algae Program via the FY12 Achievements in Sustainable Algal Biomass Production Funding Opportunity. ATP3 enables the acceleration of applied algal research, development, investment, and commercial applications for biofuel feedstock by providing the public research community with access to facilities and services.

Guiding Questions:

1. What is the time horizon for this topic or issue; will this impact algal biofuel production in the near, mid, or long term?
   - This is a long-term issue.
2. Has this topic been included in the National Algal Biofuel Technology Roadmap; and if not, should it be?
   - This issue should be in the Roadmap.
3. What is the BETO Algae Program role in furthering this topic or addressing this issue?
   - The DOE currently supports this work at a small-scale.

Poster:

- New genetic systems, ATP3 strains
- Compliant genetic tools
- Inducible flocculation
- Self-separation
- Pest resistance
- Classical genetics mating
- Homologous recombination in nuclear genome
- High-throughput (HTP) screening
- Function/annotation of genes; ongoing citation
- Agency coordination: EERE/Office of Science/DOE/EPA/USDA
- Ecological evaluation of GMOs

Deployment of Superior Strains

Convener: Philip Pienkos

Discussion:

The topic of deploying superior strains is directly relevant to the workshop goals as biological productivity has been identified by the Algae Roadmap and the Aquatic Species Program as one of the most important parameters in bringing down the current estimate of $20/gallon gasoline equivalent (gge) to the DOE goal of $3/gge, and in "replacing the whole barrel" with non-fuel products. The goal of this breakout session is to help guide research, or provide the DOE with some sense of how to do this work. It is important, however, to work effectively to get where we want to go and meet the goals for cost and performance. For example, how do we talk about replacing petroleum when we do not want to talk about new products that are currently being produced by petroleum?

The definition of "superior" relates to robustness, ability to be productive in wide temperature ranges and variable pHs, and pump-ability. Productivity can be defined by concentration and growth rate.

Research objectives should include:

- How will superior strains be generated?
- How do we characterize a superior strain? What traits make a strain superior?
    - Size of alga (larger are easier to harvest), cell wall thickness, extractability.
- How does a polyculture affect the superiority of the strain?
- How do we ensure phenotypic stability and storability of a superior strain? This may change the focus of research from the maximum production range to the most stable range of algae in terms of density of yield as well as seasonality.
- The industry needs high productivity strains in order to move to the more stable range of the curve, more consistently. The asymptote is not good enough; more engineering is needed to push the cost/greenhouse gas (GHG) curve down.
- An algae strain most likely will not have everything that you would like. What is good enough, and what constitutes an improvement? How do you determine if your strains are worth deploying?

Genetic engineering may be the only way to get to the superior region of the curve needed for commercial deployment, though it might be possible to get there with directed evolution. If you want to make biofuels for a lower cost, you have to make genetic improvements. You will need to stack traits to get these improvements, and that has to come from biological enhancements. We are looking for a better biological seed to achieve what is needed.

However, no matter what you start with, you are going to end up with a polyculture when you take the strain outside.

Bio-prospecting is a possible way to get improvements. After starting with bio-prospecting you would likely need to use genetic modification to advance from there. The field needs to get better at advancing a robust strain to get superior characteristics. There needs to be a genetic toolbox that will work across a large variety of strains. Algae are far behind all other industrial strains like yeast and E. coli. Cyanobacteria is simpler to genetically modify, but is harder to grow at larger scales. It takes energy to fix nitrogen, which has benefits, but there are trade-offs. Fixing nitrogen is a possible crop defense strategy. There is a wealth of knowledge emerging regarding the genomic toolbox of green algae. There is currently a focus on a couple of industrial strains, and then making improvements from there based on those platform organisms. There are two approaches to this research: get one strain that grows as fast and as cheap as possible, and the other is to get a strain that produces the most oil possible. If genetic engineering advances, you could choose which direction you want your platform species to go in.

Barriers to deployment:
The regulatory environment is uncertain for GMOs. In order to choose platform strains, the first issue is proving the superiority of the strain and then people need to be willing to share that information. Avoiding licensing fees requires you to either ignore the risk, or to find a work-around if you can't afford it. When you find your own strain you spend time trying to convince people your strain is superior and the time spent is going towards a platform strain and the information doesn't become helpful to the rest of the industry. DOE could provide funding to groups that are willing to share information about a platform species in order to increase the knowledge base. There needs to be a more focused effort on fewer strains to develop the genetic background information necessary to create the toolbox. There needs to be more people working on the platform species and sharing information to improve the knowledge base/GMO toolbox.

Biodiversity laws make bio-prospecting difficult. The ease of species movement could be a factor in choosing platform strains. States/regions will vary in their willingness to be welcoming to different strains/GMOs.

Phenotypic shift is a barrier. Selection is difficult as algae adapt to their environment and select naturally for traits.

Guiding Questions:

1. What is the time horizon for this topic or issue; will this impact algal biofuel production in the near, mid, or long term?
    - Superior strain development is a long term problem. First, the genetic toolbox needs to be developed, then the application of the desired traits needs to be pursued. Identifying platform strains is a short term goal, but improving them to the point you want is long term.
    - Short Term
        - Bio-prospecting
        - What does success look like, what is the goal oil, biomass or secreted product?
        - Stack traits
    - Long Term
        - Integration

2. Has this topic been included in the National Algal Biofuel Technology Roadmap; and if not, should it be?
    - Topic is covered well in the Algae Roadmap, but deployment wasn't really considered and it should be. The Roadmap did not frame the question well, and ways to actually reduce the cost of algal biofuels needs to be included.
3. What is the BETO Algae Program role in furthering this topic or addressing this issue?
    - The DOE should focus funding around developing platform organisms with specific objectives to help the toolbox, and use the toolbox to begin addressing the superior qualities that are desired. All this information should then be freely available so that companies cannot keep it proprietary, hurting its platform status. The DOE needs to help determine what the platform organisms will be, and possibly develop a consortium/program specifically focused on determining the platform species. A high throughput literature search will be necessary to identify starting points, as a comprehensive review of what has already been done. DOE can help identify the best pathways to move forward with thermochemical conversion, bio-oil, biomass, products or some combination, as well as develop standard methodologies of validating performance and metrics.

Poster:

Define Superiority

- Productive under process conditions -> Technoeconomic Analysis (TEA)/Life-cycle Analysis (LCA) Guide
- Cultivation robustness (resistance to crash)
- Harvestability/extractability
- Phenotypic stability

Where do superior strains come from?

- Genetic engineering (trait stacking)
- Random mutants
- Bio-prospecting
- Need robust platform strains with broad effort to accelerate toolbox development

Barriers

- Regulatory
- Choice and sharing of platform strain
- Not enough pressure to focus
- Too much work on unproductive strains
- Biodiversity laws
- Intellectual property

Algal Polyculture—Planktonic and Benthic Systems

Convener: Dean Calahan

Discussion:

Algal polyculture systems (Planktonic and/or Benthic) are complex ecological systems, but opportunities do exist for developing these types of systems to increase algae production rates and reduced system costs. With most of the current efforts focused on monoculture systems and mitigating the risks associated with aggressive pest strains, the participants see the development of polyculture strains as a portfolio edge that offers low risk, but high potential investment awards. The convener, Dean Calahan from the Smithsonian Institute, started the session by sharing a photo presentation of polyculture algae/planktonic systems that were developed by a Smithsonian Institute colleague known as "Algal Turf Scrubbing Technology" that incorporates primarily filamentous algae of many genera and species to capture the energy of sunlight and build algal biomass from CO_2 and nutrients from fresh, brackish, and sea water, and a wide variety of waste and industrially-polluted waters.

Key issues and realizations:

- Understanding the complex ecology of the algal polyculture systems to enable development of scalable engineered 3D matrix systems for high-throughput, high-quality algae production and biofuel processing.
- Understanding the conditions necessary for high-quality algae production with respect to water quality, climate, insolation, temperature, and thermal gradient and buffering.
- Engineering appropriate systems and optimized operation parameters to achieve high-throughput, high-quality algae production from polyculture systems.
- Engineering scalable systems to enable cost reductions.
- Understanding the risk trade-offs of paraphyletic systems.
- Feasibility of downstream processing.

Next steps:

- Add polyculture algae/planktonic system production systems to the BETO Algae Program Portfolio as a low risk, high-reward opportunity. It serves as a risk hedge for monoculture cultivation strategies.
- Conduct fundamental and applied research to better understand and develop polyculture algae/planktonic system production capabilities.
- Investigate business case/model for expanding polyculture algae/planktonic system production as a feedstock for biofuel production.
- Study the potential for cultivating polyculture algae for both thermal and biochemical conversion processing (can this be a model for planktonic systems?).

Guiding Questions:

1. What is the time horizon for this topic or issue; will this impact algal biofuel production in the near, mid, or long term?

- The time frame for polyculture algae/planktonic systems spans the near, mid, and long term, though there are relatively near-term opportunities that exist based on "Algal Turf Scrubbing" technology that incorporates attached, primarily filamentous algae of many genera and species to capture the energy of sunlight and build algal biomass from CO_2 and nutrients from fresh, brackish, and sea water, and a wide variety of waste and industrially-polluted waters. Based on a preliminary presentation provided by the convener and high-density algae production opportunities, the group viewed this as an opportunity that could potentially impact the 2016/2017 BETO cost targets.

2. Has this topic been included in the National Algal Biofuel Technology Roadmap; and if not, should it be?
   - The development of polyculture algae/planktonic systems is not included in the Roadmap. After the discussions in this session, the participants noted that this should be added to the Roadmap.
3. What is the BETO Algae Program role in furthering this topic or addressing this issue?
   - BETO should add polyculture algae/planktonic systems to its Research and Development (R&D) portfolio, and should engage EPA in discussions on issues of water quality and methodologies for reducing energy use for water quality improvements from industrial waste streams and polluted waters.

Poster:

This is one of the more complex ecologies, could be a lower cost system
Key challenges:

- Understanding productivity and algae quality issues with respect to water quality, climate, insolation, temperature, thermal buffering, etc.
- Scalability for cost reduction
- Risk tradeoffs of paraphyletic systems
- Feasibility of downstream processing
- Understanding system design operations to optimize system
- Understanding the ecology

Next Steps:

- Fundamental and applied research
- Closer look at business models to expand feedstocks for biofuel production
- Add to the portfolio as risk mitigation hedge
- Study potential for cultivation and conversion processing; thermal and biological (can this be a model for planktonic systems?)

Q1: Relatively near-term, but also longer-term. Low risk/high reward.
Q2: Not in the roadmap, should be added.
Q3: DOE could add to the portfolio and involve EPA and states in discussions on water quality. This is a potential low risk/high reward opportunity; this could help meet near-term production and cost targets. Opportunity for thermochemical pathway. Could help to meet 2017 targets.

Managing Pond Ecology

Convener: Dan Anderson

Discussion:

What are our current crop protection strategies? Reliable crop protection requires studying ecology of the ponds and finding ways to stabilize and manage the biology. The more stable the system, the less action will be needed to correct it. However, the field still requires a lot more tools to manage these ponds at a large scale.

There is a section in the Algae Roadmap on pond management, but there is not a robust representation in the BETO R&D portfolio. The DOE-funded National Alliance of Advanced Biofuels and Bioproducts (NAABB) consortium looked at ecological aspects of management and peptides through a GMO strategy. The Pond Crash Forensics project at DOE looked at how and why ponds fail, and implemented a crash detection device. The project was able to gather some data on microbiology associated with the pond. Additional work is underway on probiotic effects of the pond ecology and biocomplexity for biopredator/pathogen effects.

Early warning systems can assist in identifying indicators of infection and bad actors within ponds, and there is the potential for looking for nonspecific indicators that can be more generally applied to mitigate the costs associated with robust testing. The field can develop better culture surveillance tools, but first indication is typically visual. Pond monitors use indicators such as odor or fluorescence as well, but a better method is still needed in the field, especially at scale. Expensive assays would not be practical, so a non-specific indicator system would be more economical.

How do you develop predation resistant ponds? Investigators are researching concepts around biocontrol. Some predators are selective, and others are not, making predation interruption complex. The field requires good genetics research to produce robust strains, not just strains that are highly lipid productive, but also resistant to predation and disease.

The field also needs to control the pond media including temperature and preconditioning with additives, rather than only reacting. Preconditioning with biological controls could include fungicide, viruses, or using rotifers to prey on everything in the culture that is not the robust and desired strain. No one in the room has found a virus to be the cause a pond crash outdoors, although a virus has been found to crash chlorella in a lab as part of an investigation as a harvesting strategy. The field is lacking information on viruses.

Could the field use chemical controls from agriculture? These chemicals are tightly regulated and are not meant to be put into water. The regulatory pathway towards using chemical controls is tightly controlled. One company is currently operating under experimental use amounts. Scheduled chemical doses are easier (preconditioning), but responsive doses are cheaper. However, in conditioning the ponds, breeding resistance should be avoided. The same chemicals should not be used repeatedly because organisms will evolve to adapt to them. If you add an agent to deal with a pest, the EPA defines it as a pesticide. For example, if you add ammonia as a fertilizer, it is acceptable under the regulation; however, if you add it to the culture to kill rotifers, then it is a pesticide and subject to regulation.

Consortia research has found beneficial responses from bacteria and other symbiotic relationships. It is unknown whether these relationships are species specific or broadly applicable.

There is not a lot of research into the "edibility" of algae. Some strains are avoided by predators while some species are targets for predation. Is predation the primary problem as opposed to infection or something else? High lipid content algae are targets for predators because these are highly caloric.

Can we do this screening before we scale? It is difficult to predict what will happen within cultures once the strain is taken outside.

There is potential for genetic engineering in this area, such as looking at extremophiles and adaptation (i.e. tolerance to a heavy metal).

Strains could be pretreated with initial stress to then aid with later and different stresses. However, this is difficult to scale.

What is the role of crop rotation in introducing more robust strains? All biomass can be harvested from the pond when preprocessing with hydrothermal liquefaction (HTL), including predators. Crop rotation could be used as both a preventative measure as well as a reactive measure, or it could be combined with adaptation. When scaling, it is no longer a true batch process, so it will be difficult to shock and rotate strains and subsequent cycles will see adaptive resistance.

One can use dilution rates to get rid of undesirables, or one can select for un-harvestable strains.

Guiding Questions:

1. What is the time horizon for this topic or issue; will this impact algal biofuel production in the near, mid, or long term?
    - Research on this issue is needed now; assuming there are resources to tackle the problem. The issue is intractable, it will always be a problem, and, therefore, it is a long term issue as well. Pond ecology strategies can fold into the GMO strategy.
2. Has this topic been included in the National Algal Biofuel Technology Roadmap; and if not, should it be?
    - Pond ecology should be emphasized more in the Roadmap. Growing productive strains outdoors is a significant barrier to full scale production.
3. What is the BETO Algae Program role in furthering this topic or addressing this issue?
    - The DOE would likely have little role for investing in this, but it is going to be a major factor in scaling. It could be so strain specific that we would need to only concentrate on a few strains.

- DOE could assist crash research at the testbeds. Ponds are going to crash at the testbeds, so they could send crashed samples to these researchers. Sharing information/data about these reagents is done very broadly. It is also very time dependent, so it needs to be shared immediately to get the snap shot of the biology at the crash. An effort is underway to try to standardize how to take these samples throughout the lifecycle, not just post-crash.
- The testbeds are set up to do just that. Those protocols need to be provided to inform this specific research.
- Pond ecology strategies are very adaptable to best practices of performers.
- USDA also has a role in funding this. There are many biologists funded in this effort, and algae needs to be treated as a crop. This issue may need to be raised to the interagency level and included in conversations among agencies.
- Farmers need to be engaged in growing algae, but the USDA requires finished cultivation strategies to be able to incorporate algae into their purview. This leads to a chicken and egg scenario.

Poster:

Approaches/Needs

Early warning systems	Growing Consortia
Good robust strains	Determine strain robustness
Biological controls	Shocking Cultures
Chemical controls	Strain rotation

1. Long-term R&D required to develop/demonstrate methods
2. In DOE roadmap, but not being addressed with focused R&D
3. BETO should use testbeds to study crashes, develop best practices, encourage users with technology.