Department of Energy National Algal Biofuels Roadmap Workshop, December 9-10 2008, University of Maryland Conference Center. Notes by Keith Cooksey

The following highlights only a few notes from of the meeting, which involved many concurrent ”breakout” sessions, which did not allow for a more comprehensive report. (Statements here are quoted without attribution to individuals or sources). A full workshop report is expected in the Spring.

1. The 2007 Energy Act: It mandates 36B gallons of renewable fuels produced in the US by 2022, with ethanol a major part of this renewable fuels target.

2. Continental airlines: With algal fuel supplied by Sapphire Energy, Continental will carry out a biojet fuel demonstration flight in 2009. (Note added: flight successful on January 7th with 2.5% algal jet fuel produced by UOP from algae oil procured by Sapphire from Cyanotech).

3. Ethanol vs. Biodiesel: Ethanol has a one third lower energy density (approx. 80 K BTU/gal) than algal biodiesel (about 120K BTU/gal). It is not possible to run diesel engines on ethanol. Algae oil can be converted to either biodiesel or jet fuels (through hydrothermal treatment, as UOP and others have demonstrated). Advantages of algal fuel also include its uniformity compared to petroleum feed stocks and the logistics of its use are manageable using current infrastructure. As the world’s stocks of light crude oil are depleted, remaining crude oil will be denser, but algal oil will be uniform, making refining easier.

4. Why the Interest in Algal Biofuels?: What has changed since the end of the DoE Aquatic Species Program (ASP) in the mid nineties? In 1996, DoE decided to devote its rapidly decreasing funding resources exclusively to cellulosic ethanol, and terminated algal biodiesel R&D. The new interest in algal biofuels is driven by the need for energy security, which is now universally recognized, and the need transportation fuels that do not increase the carbon dioxide content of the earth’s atmosphere. Also, it is being recognized that ethanol alone will not be able to solve these problems. Added to this is the increase in crude oil prices prior to mid 2008, as well as the increase in our knowledge of algal metabolism and improved design of photobioreactors. All these factors have increased the interest in algal biofuels.

5. ASP Culture Collection: Research carried out under the ASP found that of 3000 algal isolates studied, only about 300 had favorable characteristics for biofuel formation. This was achieved using light v. temperature gradient shaking tables. Notable genera of algae based on growth rates and triglyceride (TAG, oil) production were *Chlorella, Phaeodactylum* and *Amphora*. About 160 of these isolates still exist in the University of Hawaii culture collection. A major lesson from this research is that no strain was found to be ”perfect” for TG production.

6. Genetic Modification: *Cyclotella cryptica* was designated a model strain for genetic manipulation. Using this organism it was found that although the level of acetyl Coenzyme A carboxylase could be increased 2-3 fold by genetic manipulation, such cells did not increase their content of TAG. This finding highlighted the need for a better understanding of the control of TAG pathways.

7. Ponds vs. Photobioreactors: The ASP initially considered both open ponds and photobioreactors for algal growth, but then chose to work almost exclusively with open ponds for algal mass cultures. Ponds were susceptible to problems with the control of temperature,

oxygen inhibition of growth, respiratory loss, grazing organisms, take over of the ponds by algae that were not a part of the original inoculum and water loss by evaporation. However, photobioreactors were not investigated, thus a comparison with ponds was not possible.

8. Strain Selection: Although many of the organisms investigated in the lab grew well and had high TG content, when transferred to a pond, the growth yield was low, as was the TAG content.

9. Economics: The ASP estimated algal oil production costs of about $40-106/barrel. Productivity was considered to have the greatest influence on costs Many of the ASP research reports are now on the NREL web site.

10. Algal Biology: The number of species shown to be potential production candidates has increased. The application of the Nile Red method was credited with being a major influence on this increase. Should we be concerned with algal viruses which will in all probability colonize open ponds and may be bioreactors also? There are now 10 algal genome sequences known and two of these are diatoms (*Thalassiosira* and *Phaeodactylum*).

11. Harvesting: Methods are not well researched. Bioflocculation is a possibility as is flocculation by chemical means. Centrifugation is thought to be too expensive, but maybe the easiest method.

12. Extraction of algal oil from the cell biomass: The optimal methods for oil extraction are also not defined yet. A continuous *in situ* extraction method using a no-toxic solvent such as dodecane shows promise. Should methods to extract oil from whole cells be preferred or should the cells be broken first? The state of the algal biomass to be extracted is critical. Dried algal biomass is probably best, but there are no inexpensive drying methods.

13. Conversion of algal oil to biofuels: Two methods mentioned: direct methanolysis (transesterification) or decarboxylation (and hydrogenation, as needed) of fatty acids to hydrocarbons. The advantage of the hydrocarbon route is that those are the molecules currently in use by the petrochemical industry.

14. Plant siting: many things to consider, including availability of carbon dioxide. Availability of real estate may play a dominant role.

15. Roadmaps for the way forward: There have been several attempts to define these. Two were mentioned. An earlier effort (Benemann, 2003) discussed as research and development goals: raceway ponds (stated as the only feasible method to produce algal biofuels, others disagree), stability of strains in pond mass culture strain (e.g. ability to out compete contaminants), control of pH fluctuations and high efficiency of CO2 utilization, ability to increase the light saturation level for photosynthesis, control of respiratory losses, induction of TAGs, etc.
At a recent (February 2008) workshop (by the Air Force Office of Scientific Research and the National Renewable Energy Laboratory, report in preparation) the importance of setting up a biofuel database was stressed. Content would include names of strain, growth rate, percentage dry weight of lipid (TAG) able to be accumulated and some information on the general physiology of the alga. The need to increase efforts in: algal molecular biology, annotation of existing algal genomes, carbon partitioning studies (e.g. what controls carbon flow to TAG or carbohydrate in algae through metabolic flux studies), cellular regulation in general, to attempt to increase the photosynthetic efficiency of cells in culture.

16. “Breakout sessions”. These were focused sessions on all topics of importance. Mentioned here are only the ones I attended: Screening of Isolates for Production of Oil and Algal Cell Biology.

17. Screening of Isolates: Although seemingly a straight forward exercise, this turned out to promote considerable discussion. What is the goal? It should be high lipid content with high biomass production leading to high lipid accumulation in the culture. To achieve this we have to define what lipid we are seeking, how that lipid will be detected and how the dry weight of the biomass will be measured. Neutral lipid as TAG is the most directly useful as a biofuel and the Nile Red method gives a good indication of the relative content of this lipid in cells. The results of chemical analysis of extracted lipid however will depend on the method of extraction and the solvents used. Furthermore there are no accepted methods to define dry weight. It was pointed out that the ABO with its Standards Committee is making attempts to define all these parameters.

18. Cell Biology of Algae: There is disagreement on the means by which the lipid content of algae increases with time in culture. In one explanation, it is thought that nutritional stress causes phospholipid (PL) and glycolipid (GL) to be converted to TAG and the TAG accumulates. An alternative explanation is that anything that causes the natural cell cycle to be inhibited does not allow precursor fatty acids (16:0 and 18.1) to be converted to PL or GL and TAG so these acids accumulate. Supporting this latter explanation is that progress through the cell cycle involves TAG utilization, so that its inhibition will also result in TAG accumulation. The group agreed that a production system which decouples lipid synthesis from algal growth is required. This has been achieved by several investigators. Other needs for research identified by this Break Out group were: lipid synthesis pathway information, metabolic flux analysis, controlling dark respiration (which reduces lipid yield), and minimizing photorespiration (which reduces overall photosynthetic efficiency).

19. “Omics”. Since the end of the ASP, the various “omics” (genomics, proteomics, metabolomics, lipidonomics, etc.) have been developed. However they have not generally been applied to algae. These techniques could be of help in understanding metabolic regulation. For instance, if two isolates which accumulate differing amounts of lipid are found, can “omics” tell us why one is better than the other? This field is lacking in a critical mass of investigators. As the field is however growing, is it possible to use the DoE (organizers of this meeting) to try to influence the Joint Genome Program to sequence more potential biofuel production algae species. As part of the effort to bring more people into the algal field, the formation of a program to provide training grants was suggested to DoE managers. The plan would be similar to that already run by DoD. The University of Texas Culture Collection now runs a training course for people wishing to grow algae.

20. Lipid Analysis. It was generally recognized by members of the Cell Biology Break Out group that there is a need to have faster methods for algal lipid analysis. The current state of the art is Nile Red staining of cells followed by GC analysis of fatty acids methyl esters produced from the extracted lipid. There was a report that there will shortly be a method that depends on solid state NMR of whole algal cells.

21. Political Efforts: There is a DoE group working with the Obama transition team to promote algal energy production as it is recognized that lack of energy independence is the number one problem for the US. The DoE is also working with China using a Memorandum of Understanding to set up collaborations at all levels of research. China is the second largest oil importer, the US is number one. Cellulosic ethanol will continue as DoE’s major effort in

biofuels, but algae will play an important part in the overall effort ($2B). This spending is distributed as follows: Industry, 33%; Academia, 27% and DoE Labs, 33%. Part of this effort should include a demonstration project where all aspects of algal oil production are covered without an initial consideration of production costs.