A Decade of Algae Technology R&D: Hopes, Hypes and How Animal Ag May Benefit

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ABSTRACT

The high capital cost of large-scale algae cultivation and multiple biological risks remain major barriers to algae-based production of protein for animals and fuel feedstocks. We suggest that both barriers can be lowered by domesticating algae adapted to extreme conditions for energy-positive, wastewater treatment applications. Capital construction costs could be financed through energy savings relative to current municipal wastewater treatment technology. The same technology could be applied to animal waste treatment to generate energy savings.

INTRODUCTION

Algae are fast-growing photosynthetic microorganisms that absorb CO_2 from the atmosphere and produce a plethora of useful pigments, vitamins, and essential fatty acids. Based on their biochemical composition and fast growth rates, algae have been rightly touted as a new source of high-value biochemicals such as astaxanthin (Doan and Obbard, 2012), omega-3 fatty acids (Tonon et al., 2002), and vitamins (Vandamme, 1992). There is also good scientific data in support of the use of microalgae as a fish-meal replacement for the aquaculture industry (Harel et al., 2002). New studies on the utilization of algal protein by ruminant animals are appearing in respected animal science journals (Lodge-Ivey et al., 2014; Christaki et al., 2012). Food safety tests have been conducted on multiple products derived from *Chlorella protocothoides*, documenting the absence of any detrimental effects upon ingestion or significant potential for inducing food allergies (Day et al., 2009; Szabo et al., 2013; Szabo et al., 2012). There are hundreds of thousands of algal strains and powerful biotechnology tools available for genetic modifications. Recent interest and enhanced funding for algal research is driven by the possibility of producing renewable liquid fuels via cultivation of high-lipid algal species (Chisti, 2008). The future of algal biotechnology should be unlimited.

Nevertheless, algal biotechnology is severely limited by barriers to low-risk, large-scale cultivation of desirable algae strains. Discussions of algae as a new crop for farmers is primarily unsupported hype and hope. Claims that algae cultivation is like farming are not supported by the facts. Inoculum scale-up, harvesting, storage, transportation, quality control, and pest management strategies are substantially different than any current agronomic crop. Virtually all currently successful commercial enterprises utilize a small handful of strains cultivated on 1 to 100 ac plots for high-value products and the situation hasn't fundamentally changed in 25 yr (Becker, 1994). Larger scale commercial market opportunities awaiting breakthroughs in large-scale algae cultivation include:

- Fish meal replacement,
- Single-cell protein for animal feeds,
- Wastewater treatment, and

- Renewable biofuel production.

All of these fields depend on low risk methods of algal cultivation at scales of 1,000 to >100,000 ac. However, despite a decade of increased spending on algal research, the largest algae cultivation facilities in the world today barely approach 1,000 ac in size.

BACKGROUND

Scale-up Challenges Dominate the Market Potential of Algae

To understand the scale-up challenge, consider the following, it would take roughly 30,000 acres to produce 100 % of the yearly fuel feedstock for the smallest petroleum refinery in the state of California. In terms of protein production, a maximum of 130,000 metric tons of single-cell protein could be produced on 30,000 ac, assuming 30 % recoverable protein content and a conservative productivity rate of 10 g ash-free dry weight/m^2/d. Such year-around productivities are currently achievable only in the southeastern U.S. gulf coast region (Davis et al., 2012).

The potential benefits of large-scale algae cultivation are still largely theoretical for two major reasons:

a) The agronomics of large-scale production of elite algal strains, including crop protection strategies, have proven to be far more difficult than many imagined and
b) Strain improvement efforts thus far have focused on a few well-studied laboratory species in the absence of any proof those strains will produce well in large-scale, controlled monocultures.

Algae technology is likely to be limited to production of high-value products in photoautotrophic ponds and industrial fermenters until scale-up barriers are systematically surmounted. Specific barriers include control of invasive algae species and small invertebrate grazers, pathogenic fungi and bacteria, and algal viruses. Until solutions for these barriers are developed, strain improvement projects are likely to focus on high-value products produced in small-scale production systems by well-studied model algal species (Wijffels, 2013).

Commonly Used Algae and Cyanobacteria

The term *algae* commonly refers to both prokaryotic cyanobacteria and eukaryotic microalgae found in aquatic and soil habitats. Many thousands of algal species have been described and several dozen whole genome sequencing projects have been published or are in the works. By far the most well-studied eukaryotic green alga is *Chlamydomonas reinhartii*, a model species representative of organisms that grow in freshwater environments at neutral pH and mild temperatures (Merchant et al., 2007). Well studied marine algae include several *Nannochloropsis* species (Vieler et al., 2012; Radakovits et al., 2012) that have been adapted to growth in large-scale cultures and shown resistance to *take-over* by competitor strains (Quinn et al., 2012). *Nannochloropsis* strains have above average lipid content, useful for the production of biofuel precursors (Sudasinghe et al., 2014; Patil et al., 2012). With respect to cyanobacteria, the most well studied species are *Arthrospira platensis* (Klanchui et al., 2012), *Synechococcus* (Holtman et al., 2005) and *Synechocystis* (Kaneko and Tabata, 1997). Cyanobacteria may be particularly useful platforms for the production of

secreted small molecules including ethanol, butanol, organic acids, and fatty acids (Wijffels et al., 2013).

NEW APPROACHES TO LARGE-SCALE CULTIVATION

The key three most pressing issues for high-volume, low-margin commodity production using algae are:

i) High productivity potential,
ii) Low risk of culture failure in large-scale monocultures, and
iii) A long-term market need that justifies financing expensive new cultivation infrastructure.

Large-scale algae cultivation is typically accomplished in open *raceway* ponds with mixing provided by a paddlewheel driving an open 10 - 30 cm depth waterway around in a circular or serpentine pathway (Becker, 1994). Current capital costs for raceway pond systems are difficult to find in the literature, but estimates from $50,000 to $150,000/ac are not uncommon.

One of the innovative approaches to sustainable cultivation of eukaryotic microalgae involves the use of high-density fermentation on glucose to produce large amounts of inoculum for mixotrophic algae like *Chlorella sorokiniana* followed by shorter term photoautotrophic growth to decrease the risk of invasive species and maintain higher quality harvests (Fan et al., 2012; Zheng et al., 2012). This approach is restricted to those algae that can be grown in the dark on a suitable carbon source, but fortunately, this phenotype is well represented in both eukaryotic algae and cyanobacteria (Goksan et al., 2012; Graneli, 2006; Tittel et al., 2003).

By far the most successful cyanobacterium for large-scale cultivation is *Arthrospira platensis* (Spirulina). The robust phenotype is likely due to a combination of high productivity (Qiang and Richmond, 1996), ability to grow at extremely high pH values (up to 11), and its helical morphology that makes it difficult for invertebrate grazers to consume.

LARGE SCALE ALGAE CULTIVATION FOR ECOSYSTEM SERVICES

Our approach to the cultivation scale-up problem was motivated by two factors. First we wanted to build a cultivation system deployable in the desert southwest that would also reduce the cultivation risk associated with invasive species. The strategy was to identify an *extremophile* able to grow at both high temperatures and extreme pH values where competitors would be rare. We were motivated to seek heat tolerant strains, because evaporative water losses over thousands of acres would be prohibitive in the desert southwest. Even the use of brackish and saline ground water would eventually require the use of freshwater to make up for evaporative losses that approach 7 ft/y in southern New Mexico and west Texas. Prevention of evaporation requires closed cultivation systems achieved with plastic enclosures. Solar heat gain in such systems generate internal temperatures of 50-55 °C in the summer months.

Second, we reasoned that municipal wastewater treatment (**WWT**) represents the most accessible market opportunity for large-scale algal biotechnology. This is because current WWT is mandated by law, supported by user fees and taxes, and is energy intensive even with anaerobic digestion and co-generation (McCarty et al., 2011). The high capital cost of algal

cultivation could be financed through energy savings, if an energy positive WWT technology could be devised. To accomplish this task, we also needed to identify a strain that could grow photosynthetically and utilize a wide variety of organic molecules for growth. This is because WWT must perform three functions:

i) Remove organic molecules responsible for biological oxygen demand,
ii) Remove organic and inorganic nitrogen, and
iii) Remove phosphorus.

The stoichiometric ratio of carbon to nitrogen to phosphorus in wastewater is deficient in carbon relative to the C:N:P ratio in activated sludge organisms and algae. However, algae can utilize photosynthetic CO_2 fixation to balance that ratio, generating more biomass than current WWT bacteria. The large amount of algal biomass relative to the traditional activated sludge process will be converted into energy products to create a cash-flow from WWT. Those products could be bio-crude oil, bio-gas, or electricity via hydrothermal liquefaction, catalytic hydrothermal gasification, and co-generation (Zhou et al., 2013; Elliott, 2008).

We have identified a eukaryotic red algae that evolved in geothermal hot springs that had the desired combination of phenotypes. *Galdieria sulphuraria* grows at pH 0-4 in volcanic hot springs at temperatures from 20-55 °C, conditions inhospitable to the growth of most organisms (Toplin et al., 2008). Both growth experiments and the genome sequence of *G. sulphuraria* revealed that this species is capable of growth on an exceedingly large number of organic molecules including sugars, sugar alchohols, organic acids, amino acids, and oligosaccharides (Schonknecht et al., 2013).

We have tested this strain for its ability to remove nitrogen and phosphorus from primary settled municipal wastewater (Selvaratnam et al., 2014). Seven-day removal efficiencies were 88.3 % for ammoniacal-nitrogen and 95.5 % for phosphates; corresponding removal rates were 4.85 and 1.21 mg/L/d. The temperature profile for outdoor cultures grown in late May and early June in Las Cruces, NM is shown in Figure 1. The culture system was a plastic-enclosed, paddlewheel-driven photobioreactor, with a 10 cm culture depth (100 L/m^2). The operating pH was 2.5 with 2 - 3 % CO_2 supplementation (vol/vol). The plastic enclosure was inflated with the air/CO_2 mixture delivered at less than 1 PSI and gas leakage at 1 - 2 L/min. These parameters lead to extremely low evaporation rates and high rates of CO_2 utilization. Diurnal temperatures ranged from 26 to 53 ^oC and the final cell concentration was over 2.5 g ash-free dry weight/L; roughly three times the value normally achieved in open raceway ponds with a variety of different algal species.

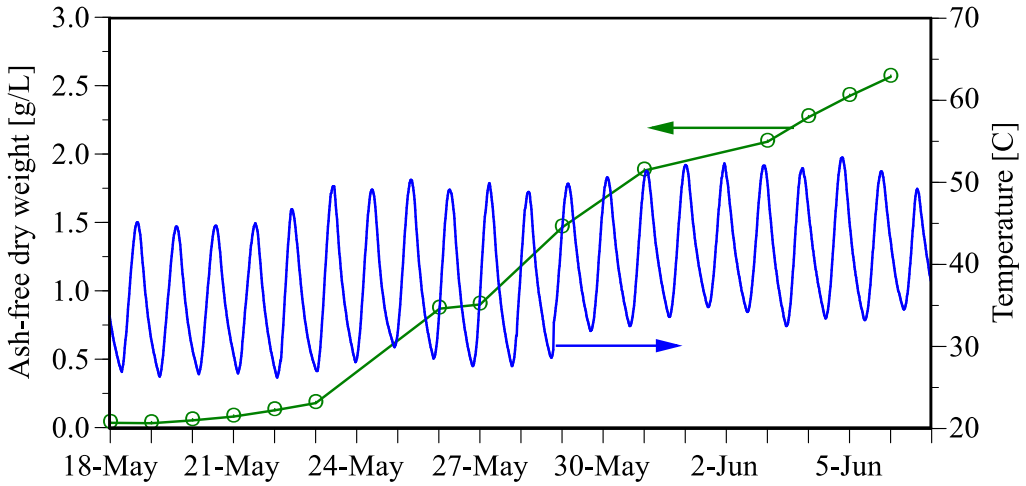

Figure 1. *G. sulphuraria* growth and temperature variations in an enclosed photobioreactor. The results show the large diurnal temperature variations that occurred (—line) and document the ash-free dry weight of the *G. sulphuraria* culture as a function of time (-o- line). Arrows point to appropriate axis.

CONCLUSIONS AND FUTURE DIRECTIONS

Beyond municipal WWT applications, we see several opportunities for applying this technology for beneficial use in the dairy and feedlot industries. First, single cell protein production, coupled to ecosystem services has been addressed by others (Wong and Chan, 1980; Ozyurt and Deveci, 2004). The scale of the WWT is compatible with large scale protein production, if quality control targets are achieved. Wastewater treatment services for a southwestern city of 1 million people would require about 10,000 ac. Hydrothermal liquefaction can be used to reclaim and recycle sterile forms of nitrogen and phosphorus nutrients (Zhou et al., 2013). Those nutrients could be sold as fertilizer or used with a different water source to produce more algal biomass. Other groups have reported on methods for production of single cell protein for animal feed from well studied algae including *Chlorella* and *Euglena* strains (Chae et al., 2006; Kuhad et al., 1997; Mahasneh, 1997). Finally, the WWT process could be adapted to processing wastewater from dairy and feedlot operations, swine and poultry production, and aquaculture to generate energy products as described previously.

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