Biodata of **Anju Dahiya**, author of "***Integrated Approach to Algae Production for Biofuel Utilizing Robust Algal Species***."

Dr. Anju Dahiya is President of General Systems Research LLC, a R&D business dedicated to algae biofuel and related software development. She is also affiliated with the University of Vermont as a biofuels instructor. She has been leading several algae-biofuel research projects funded through Department of Energy (VSJF), Environmental Protection Agency, NASA (VT-EPSCoR), and NSF (VT-EPSCoR) related to development of a robust system of algae-oil production that could be integrated with dairy farm and industrial wastewater treatment. As an algae oil scientist, she has been applying systems approach to develop a robust system of algal oil production. Her algae-biofuel works have been captured by VPT (PBS) TV channel (Emerging Science); Burlington Free Press; and long-standing TV program – Across the Fence (forthcoming). She has been presenting her work as an invited speaker in conferences, State programs, workshops, and published papers. In 2010, she successfully co-organized the "Algae for Energy in Northeast conference" to stimulate the research at regional level and invited speakers from DoE, national and regional universities, and government and private sectors that attracted a large number of participants from academic, government, and private sectors including energy-related farms: http://www.uvm.edu/~epscor/index.php?Page=events/2010_algae_for_energy_conference.php

E-mails: **adahiya@uvm.edu; adahiya@gensysresearch.com**

R. Gordon and J. Seckbach (eds.), *The Science of Algal Fuels*,
Cellular Origin, Life in Extreme Habitats and Astrobiology, 83–100
DOI 10.1007/978-94-007-5110-1_5, © Springer Science+Business Media Dordrecht 2012

INTEGRATED APPROACH TO ALGAE PRODUCTION FOR BIOFUEL UTILIZING ROBUST ALGAL SPECIES

ANJU DAHIYA[1,2]
[1]*General Systems Research LLC, Burlington, VT 05408, USA*
[2]*Plant and Soil Science Department, The University of Vermont, Burlington, VT 05405, USA*

1. Introduction

Algae as biofuel feedstock provide economically and environmentally beneficial option as follows: (a) algae do not compete with food and water resources; (b) algae grow significantly faster than land crops used for biodiesel and are reported to produce up to 300 times more oil than traditional crops on an area basis; (c) they can treat industrial, municipal, and agricultural wastewaters; (d) they are carbon-neutral and can capture carbon dioxide; (e) low-temperature fuel properties and energy density of algae fuel make it suitable as jet fuel; (f) they ensure a continuous supply; and (g) they can provide valuable by-products like protein-rich feed for farm animals, organic fertilizer, and feedstock for producing biogas.

The algae biomass research community acknowledges that integration of algae biomass production with fuel and waste treatment would be a cost-effective approach. However, algae-oil production at commercial scale is not yet a reality due to the challenges such as the availability of oleaginous (capable of producing oil) algae strains that could be massively cultured to utilize various waste streams as nutrients source. Over 40,000 algal species have been already identified (Hu et al., 2008); however, the list of oleaginous strains is only starting to emerge. To begin with, the Algae Species Program (ASP) sponsored by US Department of Energy (DoE) had identified 300 algae strains suitable for oil production out of the 3,000 collected from different regions in the United States (Sheehan et al., 1998). These strains have been maintained in repositories. Many of the algal strains in algal repository collections (such as the Culture Collection of Algae at the University of Texas, UTEX) have been cultivated for several decades, and as per DoE (2010) it is quite possible that these strains may have lost part of their original wild-type properties necessary for mass culture; for that reason, there is need of isolating novel, native strains directly from unique environments to obtain versatile and robust strains.

The goal of this paper is to look at the concept of algae species *robustness* in context of algae species selection for biodiesel production. Several aspects of species robustness have to be examined: how, for example, does the abundance of a dominating species or algae assemblages can affect many other species co-surviving in the same media; how it can outcompete non/low-lipid wild algae;

how a robust algal strain should respond to changing environmental conditions; and how high-end oleaginous algae species perform in low-quality wastewater streams (municipal, industrial, agricultural: dairy and piggery farms).

2. Robustness Concept as Applied to Algae Biofuel Research

2.1. ROBUSTNESS CHARACTERISTICS

Various desirable characteristics of algae for culturing at massive scales have been identified (Borowitzka, 1992; Chisti, 2007; Hu et al., 2008; Schenk et al., 2008; Griffiths and Harrison, 2009; DoE, 2010) as follows: potential to synthesize and accumulate large quantities of neutral lipids/oil (20–50% DCW); rapid growth rate (e.g., 1–3 doublings per day); large cell size (colonial or filamentous morphology); growth in extreme environments to thrive in saline/brackish water/coastal seawater for which there are few competing demands and tolerate marginal lands (e.g., desert, arid- and semi-arid lands) that are not suitable for conventional agriculture; wide tolerance of environmental conditions; utilize growth nutrients such as nitrogen and phosphorus from a variety of wastewater sources (e.g., agricultural runoff, concentrated animal feed operations, and industrial and municipal wastewaters) providing the additional benefit of wastewater bioremediation; tolerance of contaminants; CO_2 tolerance and uptake to recover carbon dioxide from flue gases emitted from fossil fuel-fired power plants and other; tolerance of shear force; no excretion of auto-inhibitors; high product content to produce value-added coproducts or by-products (e.g., biopolymers, proteins, polysaccharides, pigments, animal feed, fertilizer, and H_2); and grow in suitable culture vessels (photo-bioreactors) throughout the year with annual biomass productivity, on an area basis, exceeding that of terrestrial plants by approximately tenfold.

The important point to note is that robustness is achieved due to combinations of these desirable features. A single algal species is unlikely to excel in all categories; hence, prioritization of desirable features is required (Griffiths and Harrison, 2009). The two major criteria sought in algal culture for mass production are high "algal growth rate" resulting into increased biomass production and "lipid productivity." These points can serve as a foundation for defining first broad criterion of algae species robustness as follows: "an algal species or assemblage that can rapidly grow to produce biomass and accumulate significant amounts of lipid while aggressively out-competing a diverse array of other competing species when grown in cost effective systems."

Studies have been generally using the term "robust" to indicate massive growth of certain algal strains or to indicate high lipid production as in algae biofuel research (e.g., Rodolfi et al., 2009). This term has also been used to indicate the massive growth of algae even leading to blooms. Robustness has been used to indicate algal "biomass–nutrient" relationships as demonstrated in many cross-sectional analyses of lakes and reservoirs (e.g., Smith, 2003). However, it is important to understand what robustness of a strain means in terms of algae biofuel. In this case, the robustness would be based on "biomass–lipid" relationships in general and "biomass–lipid–nutrients" relationships for integrated systems. It has been proven that in algae, the lipid content may be enhanced by nutrient stress or depletion especially nitrogen (Roessler, 1988), which means combining the algae culturing with nutrients recovery would be a difficult goal to achieve because in the latter case the objective would be to recover maximum nutrients. The algal growth rate is negatively affected by increase in lipid contents under nutrient limitations (Gouveia et al., 2009). To illustrate further, an example of environmental impact on naturally growing algae from a microalgal prospecting experiment follows in the next subsection.

2.1.1. Algal Robustness at Community Level and Lipid Production

Table 1 presents lipid content in algae assemblages including the respective predominant algal species and the suitable media. A comparison of lipid percentages found in different classes of algae shows the higher amount is bulked up by green algae and diatoms (Borowitzka, 1988; Hu et al., 2008). Specifically under the "normal" and the "stress" culture conditions, the oleaginous green algae or chlorophytes show average total lipid contents (dry cell weight) 25.5 and 45.7%, respectively under the two conditions, oleaginous diatoms 22.7 and 44.6%, respectively, and other oleaginous algae (including chrysophytes, haptophytes, eustigmatophytes, dinophytes, xanthophytes, or rhodophytes) 27.1 and 44.6%, respectively, whereas in cyanobacteria considerable amounts of total lipids have not been found, and the accumulation of neutral lipid triacylglycerols has not been observed in naturally occurring cyanobacteria (Hu et al., 2008). The final culture catalog prepared by the Algae Species Program (Sheehan et al., 1998) showed the collection consisted of predominantly green algae (chlorophytes) and diatoms – specifically out of the algae strains present, 26% were chlorophytes, 60% were diatoms, 8% were chrysophytes, and 6% were eustigmatophytes.

A typical algal assemblage varies from environment to environment depending on biotic and abiotic factors, as is shown in Table 1, algae assemblages vary in different sources of water producing different amounts of lipids or fatty acids. A remarkable unity is evident in the global response of algal biomass to nitrogen and phosphorus availability in lakes and reservoirs, wetlands, streams and rivers, and coastal marine waters; most importantly, the species composition of algal communities inhabiting the water column appears to respond similarly to nutrient loading, whether in lakes, reservoirs, or rivers (Smith, 2003).

Here is an example of environmental impact on naturally growing algae community containing lipids. Algae growing in diverse environments representing different nutrients settings, dairy farms (Fig. 1a, d), nondairy farm (Fig. 1c), and a composting site (Fig. 1b), were analyzed for microalgal prospecting (General Systems Research, 2011). The samples collected from these sites on same day were tested for abundance of (a) algae as marked by chlorophyll (Fig. 1) and (b) algae as marked by both chlorophyll and lipids (Fig. 2). Chlorophyll-containing algae

Table 1. Algae (multispecies) and percentage of lipid/fatty content (where available) as growing/cultured in different media.

Algae community	Lipid/fatty acids	Sources/media	Literature
Winter/spring, dominant diatom, *Melosira* sp.; summer/fall, dominant blue-green alga *Lyngbya* sp., green alga *Spirogyra* sp. Rest *Ulothrix, Microspora*, *Claophora*, and numerous pennate diatoms	0.34 ± 0.14	Patuxent River water (Chesapeake Bay)	Mulbry et al. (2010)
Filamentous diatoms and the green alga, *Enteromorpha* sp., dominated year-round	0.65 ± 0.21	Patapsco River water (Chesapeake Bay)	Mulbry et al. (2010)
Diatom *Melosira* sp., blue-green alga, *Lyngbya* sp., and green alga, *Spirogyra* sp., *Ulothrix, Microspora*, *Claophora*, and numerous pennate diatoms	0.51 ± 0.19	Bush River water (Chesapeake Bay)	Mulbry et al. (2010)
Aphanocapsa, *Asterionella, Navicula, Stephanodiscus, Tabellaria*, other diatoms and green algae	0.87–5.33	Enriched lake water (Champlain)	Dahiya et al. (2009, 2010)
Chlorella, *Micractinium*, and *Actinastrum* genera	4.9–11.3	Municipal wastewater	Woertz et al. (2009a)
Scenedesmus, *Micractinium*, *Chlorella*, and *Actinastrum*	10–29	Dairy manure wastewater	Woertz et al. (2009a)
Dominant filamentous *Rhizoclonium hieroglyphicum*. Other *Microspora willeana Lagerh., Ulothrix zonata* (Weber and Mohr) Kütz, *Rhizoclonium hieroglyphicum* (C.A. Agardh) Kütz *and Oedogonium* sp.	0.6–1.5	Dairy manure wastewater	Mulbry et al. (2008)
Chlorella, *Scenedesmus*, *Micractinium*	NA	Sewage (average load)	Oswald (2003)
Euglena, *Chlamydomonas*, and *Oscillatoria*	NA	Sewage (excessive load)	Oswald (2003)

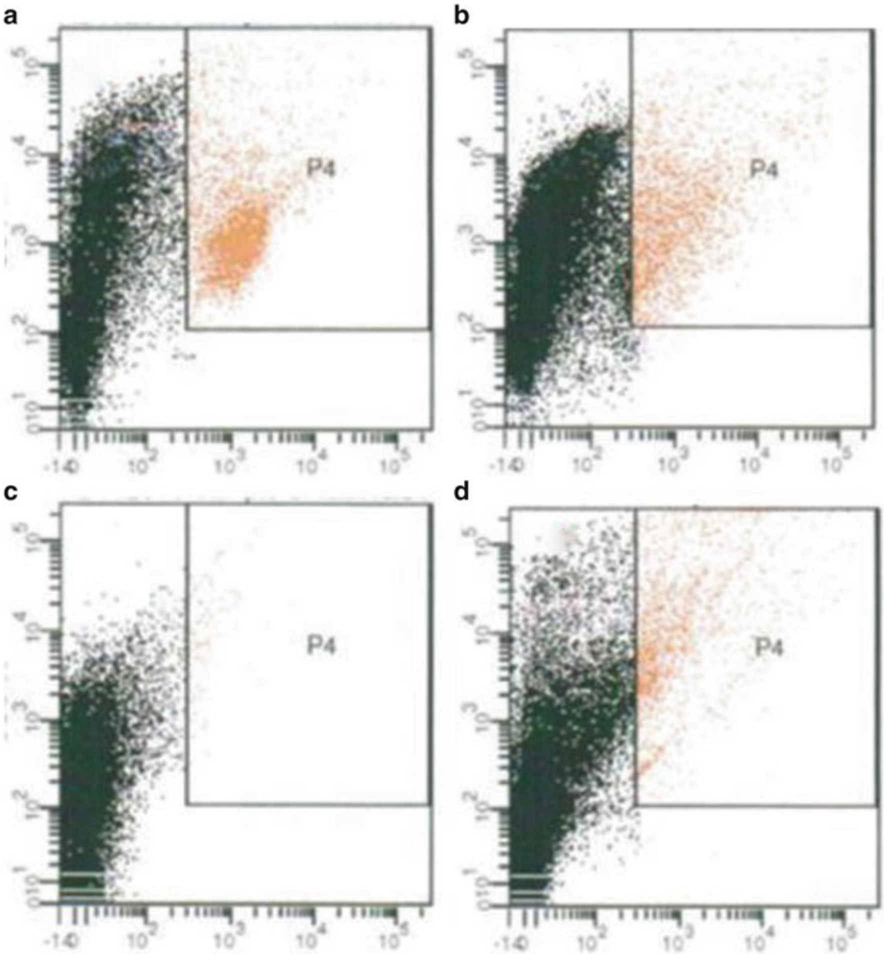

Figure 1. Cytograms showing chlorophyll containing algae (P4) from samples collected from four different sites (Source & © General Systems Research LLC).

populations surviving in these sites showed marked difference between the algal abundance at dairy farm/compost and nondairy farm. The respective samples were set with lipophilic dye and tested for the lipid containing algae populations. From this case, can we infer and generalize that the algae community at higher nutrient-containing sites (dairy farm and compost) is more robust than that at the nondairy farm site, especially for biofuel production, and that the algae cells from the nutrient-rich communities are more robust than the algae at the nondairy farm site? Can we expect the algae cells isolated from high-lipid populations and cultured to produce robust population than the other one? These aspects are important for robust algal strain isolation and selection as discussed in next sections.

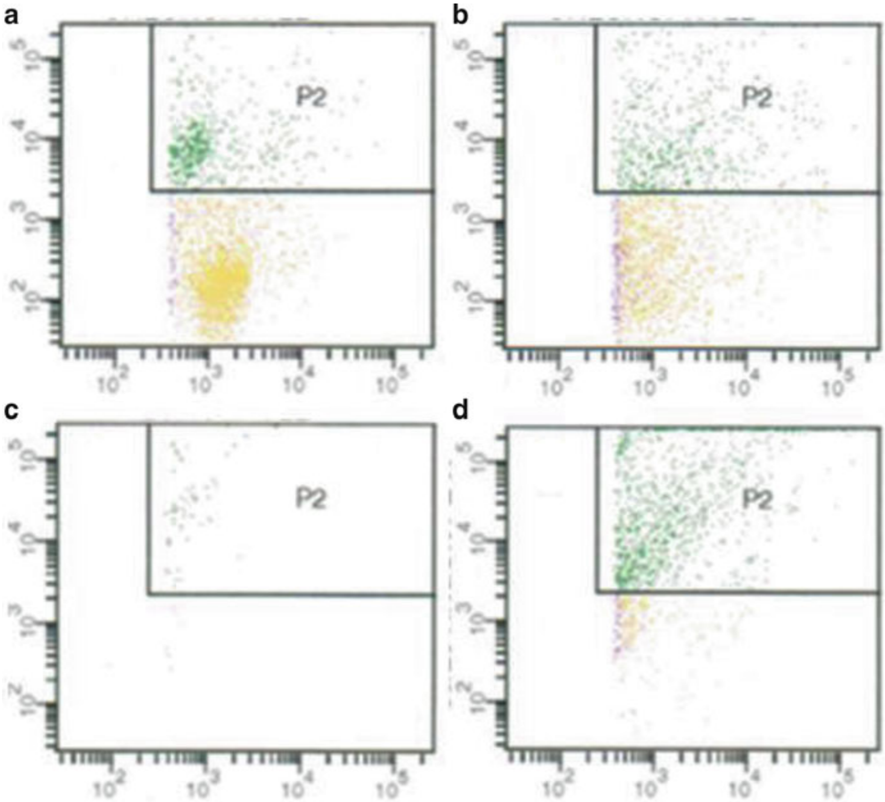

Figure 2. Cytograms showing both chlorophyll and lipid containing algae (P2) from samples collected from four different sites (Source & © General Systems Research LLC).

2.1.2. Algal Robustness at Species Level and High-End Lipid Content

Algal oil production rate at theoretical maximum was found to be 354,000 L·ha^{-1}·year^{-1} (38,000 gal·ac^{-1}·year^{-1}) of unrefined oil, while the best cases examined range from 40,700 to 53,200 L·ha^{-1}·year^{-1} (4,350–5,700 gal·ac^{-1}·year^{-1}) of unrefined oil (Weyer et al., 2009). The algal biofuel production would require synthesizing and accumulating large quantities of neutral lipids/oil (at least in the range of 20–50% dry cell weight), and the intrinsic ability to produce large quantities of lipid and oil is species/strain-specific rather than genus-specific (Hu et al., 2008).

For culturing algae at massive scales, *high-end* lipid content would be required. Under normal conditions of nutrient regimes, larger than 20% lipid content in algae species has already been found in the algal species growing under laboratory conditions or isolated from naturally growing algae assemblages. This percentage can be increased under stress conditions. The *high-end* oleaginous algae

Table 2. *High-end* oleaginous algae (single species) showing lipid content (percentage dry weight) grown in different media types.

Algae	Lipid %	Media	Literature
Botryococcus braunii	86		Brown et al. (1969) and Wolf (1983)
	80[a]	AM (mod. Chu)	
	>75		Brown et al. (1969)
	63		Banerjee et al. (2002)
	25–75		Metzger and Largeau (2005)
			Chisti (2007)
Schizochytrium sp.	50–77		Chisti (2007)
Nitzschia species:			
N. dissipata	66	AM	Sheehan et al. 1998
N. palea	40	AM	Shifrin and Chisholm (1981)
Boekelovia hooglandii	59	Urea enriched AM	Sheehan et al. (1998)
Monallantus .salina	41–72	AM	Shifrin and Chisholm (1981)
Navicula species:			
N. saprophila	58	AM	Sheehan et al. 1998
N. acceptata	47	AM	Shifrin and Chisholm (1981)
N. pelliculosa	45	AM	Sheehan et al. (1998)
N. pseudotenelloides	42	AM	Sheehan et al. (1998)
Chlorella species:			
C. minutissima	57	AFW	Shifrin and Chisholm (1981)
C. vulgaris	41	AFW	Shifrin and Chisholm (1981)
C. pyrenoidosa	36	AFW	Shifrin and Chisholm (1981)
Dunaliella Sp.	45–55	AM	Sheehan et al. (1998)
Neochloris oleoabundans	35–54	AM	Sheehan et al. (1998)
Monoraphidium sp.	52	AM	Sheehan et al. (1998)
Amphora	51	AM	Sheehan et al. (1998)
Ourococcus	50	AM	Shifrin and Chisholm (1981)
Nannochloris sp.	48	ASW	Shifrin and Chisholm (1981)
	35	ASW ** 45	Sheehan et al. (1998)
			and Rodolfi et al. (2009)
Nannochloropsis salina	46	ASW	Sheehan et al. (1998)
Scenedesmus	45	AFW	Sheehan et al. (1998)
Scenedesmus obliquus	41	AFW	Shifrin and Chisholm (1981)
Ankistrodesmus	40	AM	Sheehan et al. (1998)
Chaetoceros species:			
C. calcitrans	40	ASW	Rodolfi et al. (2008)
C. muelleri	39	ASW	Sheehan et al. (1998)
Cyclotella cryptica	37	AM	Shifrin and Chisholm (1981)
Amphiprora hyalina	37	AM	Sheehan et al. (1998)
Cylindrotheca sp.	16–37		Chisti (2007)
Pavlova lutheri	36	ASW	Rodolfi et al. (2008)

AM Artificial media, *AFW* Artificial freshwater, *ASW* Artificial seawater f2
[a] Unsaponifiable lipids
** higher content cited by others

species should contain at least 35% lipid content (average of 20–50%). Table 2 presents such species grown in different media types and nutrient conditions including nutrient stress. To illustrate this point, based on the rationale that the oil content of 35% has already been observed in nutrient-sufficient cultures of some species, for mass scale algae biomass and oil production operations in Hawaii, Huntley and Redalje (2007) chose target oil content of 35% even though higher values have been attained.

Many algal species show prominent biomass–lipid relationships. Analysis of microalgal lipid content, biomass productivity, and their combination to yield lipid productivity in 55 species of microalgae (including 17 Chlorophyta, 1 Bacillariophyta, and 5 Cyanobacteria as well as other taxa) showed high lipid productivity ranging from 97 to 160 mg L^{-1} day^{-1} in certain species including *Amphora*, *E. oleoabundans*, *A. falcatus*, *C. sorokiniana*, and *T. suecica* (Griffiths and Harrison, 2009).

The biomass–lipid relationship in the most promising algae strain is not very strong. In terms of lipid production, the green colonial unicellular microalga, *Botryococcus braunii*, is considered to be hydrocarbon-rich alga. It can produce C_{21}–C_{33} odd-numbered n-alkadienes, mono-, tri-, tetra-, and pentaenes, even C_{40} isoprenoid hydrocarbon (Metzger and Largeau 2005; Dayananda et al., 2007), and can reduce nitrate, phosphate, and ammonia–nitrogen concentrations in the waste effluent (seafood processing effluent) by 73, 74, and 79%, respectively (Dumrattana and Tansakul, 2006), and in piggery wastewater effluent ammonia-nitrogen 98% and total nitrogen 43% (An et al., 2003). Very slow rate of growth (Wolf, 1983), with typical doubling time 72 h (Sheehan et al., 1998), makes *B. braunni* unpopular for mass culturing and for integrating this system with waste-water treatment.

In certain species, biomass–lipid–nutrient relationships are strongly demonstrated such as in *Chlorella* species. Since the turn of the twentieth century, *Chlorella* species have been extensively studied for their growth at various nutrients concentrations and/or for food potential, wastewater treatment, etc. (for instance, Eyster et al., 1958; Myers and Graham, 1971; Hills and Nakamura, 1978; Oswald et al., 1953; Oswald, 2003; Huang et al., 1994; Tam and Wong, 1996; Tam et al., 1994), and these species are considered good candidate for biofuel due to their oil accumulation potential. *Chlorella* species are considered robust due to following reasons: (1) they can accumulate lipids up to 50% of their dry weight, thereby making them good candidates for biodiesel production, and as the popular choice because very high biomass production combined with biodiesel production potential so as to produce at the rate of 3,200 GJ/ha/year projected to replace reliance on fossil fuel by 300 EJ/year besides eliminating CO_2 emission by 6.5 Gt/year by the year 2050 (Wang et al., 2008); (2) these species are well-known for use in wastewater treatment (removal of nitrogen and phosphorus) (Oswald et al., 1953; Tam et al., 1994; Gonzalez et al., 1997) as well as for their symbiotic relation with many kinds of naturally occurring bacteria, which is known since 1920s, for example, *Azotobacter chroococcum* (Lipman and Teakle, 1925;

Hills and Nakamura, 1978), *Pseudomonas diminuta*, and *P. vesicularis* (Mouget et al., 1995). *Chlorella pyrenoidosa*, a species commonly found near wastewater treatment ponds, grows vigorously in sterile sewage (Oswald et al., 1953). The *Chlorella* sp. were able to produce more than 3.2 g/m^2-day with lipid contents of about 9% dry weight, while treating dairy farm wastewater and removing upwards of 90% of the total phosphorus and 79% of the total nitrogen contained within the wastewater (Johnson, 2009); (3) addition of an external carbon source induces heterotrophic growth in *Chlorella protothecoides* and increases both growth rate and lipid production, resulting in greater than 50% dry weight lipid (Xu et al., 2006); *Chlorella* has a capacity to produce by-products such as 10–20 times as much protein per unit area per year as cereal crops (Boersma and Barlow, 1975).

2.1.3. Evolutionary Forces to Shape Oil Production by Algae

Can the naturally growing algae assemblages or controlled monocultures be forced toward oil production? Specifically, can the high-end rapidly growing oleaginous strains be evolved to bulk up lipids and compete with the slow-growing hydrocarbon-rich *B. braunii*, or vice versa: can *B. braunii* be evolved to grow rapidly like *Chlorella* species? Importance of these aspects lies in the fact that now geologists and scientists are unanimous about algae being surviving on earth for over 500 million years and that the oil and coal reserves are their direct products. *B. braunii* provides such evidence. The highly resistant nature of the *B. braunii* algae to degradation allows it to be selectively preserved during fossilization, leading to fossil *B. braunii* remains, a major contributor to a number of high oil potential sediments (Simpson et al., 2003).

The evolutionary equilibrium strategies allow the most successful species to follow opportunistic evolutionary pathways (Riley, 1979). The concepts behind evolutionarily stable strategies (ESS) (Maynard Smith and Price, 1973) have been demonstrated by Geritz et al. (1998), who used game theory principles to predict adaptive evolution. Klausmeier and Litchman (2001) modeled these strategies for pelagic and benthic algal systems subject to the light and nutrient competition so that the motile phytoplankton can form a thin layer under poorly mixed conditions, and under the assumption of a thin layer, competition for light from above and nutrients from below can be thought of as a game, with the depth of the phytoplankton layer as the strategy. In this case, the ESS is a depth that prevents growth in the rest of the water column, which is determined in this model. The environmental conditions to force algal evolution to oil production traits require light and dark cycles intertwined with low- and high-nutrient conditions typically found in pelagic ecosystems; however, 50% light and dark regimes tested to evolving the naturally growing lake algae toward producing lipids did not produce immediate results (Dahiya et al., 2010); this is a work in progress.

The effect of light–dark (L–D) cycles on algal photosynthetic activity has been extensively researched for mass culturing of algae for biodiesel production. For instance, Janssen et al. (2001) and Barbosa (2003) found that the light/dark cycles affect the biomass yield and specific growth rate of algae. Little information is available as to how much time the algal cells should be in the light and dark, and as such there is no consensus on what is an appropriate light/dark cycle (Kommareddy and Anderson, 2004). However, based on the flashing light effect (Laws et al., 1983) tested in ASP studies, the optimized transfer of cells from dark zones to bright zones and vice versa has been tried in the closed bioreactors in order to harness the flashing light effect by inducing low light/high light cycle (Meiser et al., 2004). The frequencies of these cycles is kept at 10 Hz or faster with the dark period lasting up to ten times longer than light period (Janssen et al., 2001). We have a long way to go before the robust oleaginous algae strains or assemblages are evolved for culturing in either open or closed systems. Next section deals with the challenges with integrated systems.

3. Robust Algae and Integrated System Challenges and Solutions

"Integrated systems" are referred to as treatment plants (e.g., sewage) integrated with communities in such a way that maximum benefits are attained by population at least cost without compromising health or welfare (Oswald, 2003). The industries and dairy farms are required to meet regulatory standards for handling and recycling of nutrients including nitrogen and phosphorus, but as per United States Department of Agriculture (USDA) report, the commonly used plants, the anaerobic digesters, for the treatment of wastewaters are effective only in treating the biochemical oxygen demand (BOD) but not nutrient removal (Liebrand and Ling, 2009). The wastewater coming out of biodigester normally needs to be further treated before it can be safely discharged into the water streams. Algae can efficiently utilize the wastewater effluents and recover nitrogen, phosphorus, potassium, heavy metals, and other organic compounds (Oswald et al., 1953; Oswald, 1990; Wilkie and Mulbry, 2002; Kebede-Westhead et al., 2003; Pizarro et al., 2006; Mulbry et al., 2008). This task calls for robust algae strains. Selecting indigenous algae with intrinsic characteristics amenable to bioresource production and waste mitigation – phycoprospecting – is the most sustainable path forward for widespread algae-based bioresource development (Wilkie et al., 2011).

Attempts in growing algal monocultures in high-rate algal ponds for over 3 months have not succeeded primarily due to contamination by wild algae and grazing by zooplankton (Sheehan et al., 1998). Algae-based treatment of wastewater has been shown to be 40% more cost-effective than the best conventional means (Downing et al., 2002), but availability of fast growing oleaginous algae species for treating waste is limited. Integrated algae-oil production and waste treatment (wastewater and/or CO_2) has been identified a cost-effective approach (Sheehan et al., 1998; Lundquist, 2008; DoE, 2010).

Whether monoculture or polyculture, the algae will have to potentially grow symbiotically with other organisms present in the wastewater. An instance of algae assemblage includes *Sargassum natans*, *Ascophyllum rodosurm*, and *Flucus* *vesiculosus* growing with the bacteria *Bacillus subtilis* and *Bacillus licheniformis* (Mulligan and Gibbs, 2003), and an instance of algal monoculture is *Chlorella sorokiniana* grown with *Rhodobacter sphaeroides* (Ogbonna et al., 2000). Algae assemblages commonly form in wastewaters. In waste treatment ponds, there is minimum control over the algae species that grow, but some limits could be imposed through pond operations, such as residence time, depth, and mixing, as very often the species of *Chlorella*, *Scenedesmus*, and *Micractinium* are commonly found in these ponds, and other species including *Euglena*, *Chlamydomonas* and *Oscillatoria* may occur in ponds with excessive loadings or long residence times (Oswald, 2003).

3.1. NUTRIENT RECOVERY CORRELATED WITH LIPID CONTENT

"Advanced Integrated Wastewater Pond Systems" designed by Oswald's group have been efficiently used for municipal sewage treatment that could remove over 90% of total nitrogen in the wastewater stream (Oswald, 1990). Natural or wastewater-grown algal assemblage or polyculture is low in lipid contents compared to monocultures (Tables 1 and 2), although it is highly efficient in recovering nutrients. The algal assemblage used in algal turf systems (ATS) for treating dairy and swine wastewater had fatty acid contents ranging from 0.6 to 1.5% of dry weight that recovered over 95% of the nitrogen and phosphorous from agricultural manure wastewater (Mulbry et al., 2008). The fatty acid (FA) content of ATS harvested material from three Chesapeake Bay rivers was 0.3–0.6% of dry weight (Mulbry et al., 2010). Woertz et al. (2009a) reported lipid productivities of 9.7 mg/L/day (air sparged) to 24 mg/L/day (CO_2 sparged), and over 99% of both the ammonium and orthophosphate removed in municipal wastewater, whereas 2.8 g/m^2 per day of lipid productivity in dairy wastewater. Currently, the use of high-end oleaginous species in treatment of wastewater is limited, and lots of research is required in that direction.

3.2. CARBON-DIOXIDE UTILIZATION COMBINED WITH NUTRIENT RECOVERY FOR WASTEWATER TREATMENT

CO_2 addition can also impact lipid production. Gouveia et al. (2009) showed a slight increase in lipid contents when CO_2 was added to the algae culture media. The problem with wastewater treatment ponds is that they are limited in providing carbon for algal growth. The heterotrophic oxidation of organic material by bacteria is one way that CO_2 is made available to algae (Oswald et al., 1953). Addition of CO_2 has been shown to increase algae biomass (Benemann, 2003; Woertz et al., 2009a). High-rate algae ponds fed clarified domestic wastewater and CO_2-rich flue gas are expected to remove nutrients to concentrations similar to those achieved in mechanical treatment technologies, such as activated sludge; however, the energy intensity of wastewater treatment with CO_2-supplemented high-rate ponds (HRPs) would be less than that of mechanical treatments (Woertz et al., 2009b). This approach would require robust oleaginous strains capable of tolerating high CO_2 concentrations. Some studies have shown that *Cyanidium caldarium* can tolerate 100% CO_2 concentration (Seckbach et al., 1971), *Scenedesmus* sp. 80%, *Chlorella* sp. 40%, and *Eudorina* spp. 20% CO_2 concentrations (Hanagata et al., 1992).

Many studies have utilized either wastewater or CO_2 from the respective point sources as nutrient streams for growing algae biomass. Very few studies have actually taken advantage of both and actually combined CO_2 and wastewater from point sources for algae production. For instance, Yun et al. (1997) cultured *Chlorella vulgaris* (inoculant prepared in 5% (v/v) CO_2) in steel manufacturing wastewater effluent under high concentrations of 15% (v/v) CO_2 supply captured from a power plant and removed 0.92 g. m^{-3} h^{-1} ammonia and 26 g m^{-3} h^{-1} CO_2. One of the possible reasons behind lack of combining CO_2 and wastewater supplies from point sources in culturing algae is the distance between the locations of respective point sources that may not be found close enough to each other, and as such, if an algae production facility is utilizing CO_2/flue gas from a point source, hauling wastewater from a distant location would increase the costs of biomass production. This area of research needs special attention.

3.3. VALUED BY-PRODUCTS

It is estimated that besides integrating algae biomass production for oil with waste treatment, the valued by-products especially from the algae cake leftover from oil extraction, such as feedstock for biogas, organic fertilizer, and proteinaceous feed for animals, can offset the cost of algae biomass production.

The value of algae as food was explored as early as 1950s by Burlew (1953). Later on, Dugan et al. (1972) demonstrated the concept by raising baby chickens to adults on 20% algae-fortified feed, and also he grew the algae used on pasteurized chicken manure. The antibiotic chlorellin extracted from *Chlorella* during World War II marked the start of algae-based pharmaceutical and nutraceutical industry that led to the Japanese *Chlorella* production facilities during 1960s (Oswald, 2003), further leading to current production of *Chlorella*, *Spirulina*, *Dunaliella*, and *Haematococcus* at commercial scales. Fresh dewatered biomass could potentially be mixed in with animal feed (and substituted on a protein basis for soybeans) (Wilkie and Mulbry, 2002). Studies have also focused on use of the dried biomass as an organic fertilizer and demonstrated that it was equivalent to a commercial organic fertilizer with respect to plant mass and nutrient content (Mulbry et al., 2005). Further research is required for utilization of leftover high-end oleaginous algae for fertilizer, animal feed, and possible feedstock for biogas production for systems based on single species or algal assemblages.

To achieve cost-effective algae biomass production for oil research is needed to isolate and test the high-end oleaginous algae species. The robustness of species is important as the starting point in algae species selection that will yield significant improvements in biomass productivity besides offsetting the production costs when integrated with waste treatment systems and valued by-product production. The continuing efforts to search an ideal robust oleaginous algae strain should be combined with other aspects of algae production.

4. Acknowledgments

Funding provided by Environmental Protection Agency (EPA), Department of Energy (via VT Sustainable Jobs Fund), NASA-EPSCoR (VT), and NSF-EPSCoR (VT) to General Systems Research LLC is greatly appreciated.

5. References

An J-Y, Sim S-J, Lee JS, Kim BW (2003) Hydrocarbon production from secondarily treated piggery wastewater by the green alga *Botryococcus braunii*. J Appl Phycol 15:185-191

Banerjee A, Sharma R, Chisty Y, Banerjee UC (2002) *Botryococcus braunii*: a renewable source of hydrocarbons and other chemicals. Crit Rev Biotechnol 22(3):245–279

Barbosa M (2003) Microalgal photobioreactors: scale-up and optimization. PhD thesis, Wageningen University, Wageningen

Benemann JR (2003) Biofixation of CO2 and greenhouse gas abatement with microalgae – technology roadmap. Prepared for the U.S. Department of Energy National Energy Technology Laboratory, No. 7010000926

Boersma L, Barlow EWR (1975) Animal waste conversion systems based on thermal discharges special report# 416. Agricultural Experiment Station Oregon, State University, Corvallis

Borowitzka M (1988) Fats, oils and hydrocarbons. In: Borowitzka MA, Borowitzka LJ (eds) Microalgal biotechnology. Cambridge University Press, Cambridge, pp 257–287 University, The Netherlands (2003)

Borowitzka MA (1992) Algal biotechnology products and processes— matching science and economics. J Appl Phycol 4:267–279

Brown AC, Knights BA, Conway E (1969) Hydrocarbon content and its relationship to physiological state in the green alga *Botryococcus braunii*. Phytochemistry 8:543–547

Burlew JS (ed) (1953) Algae culture from laboratory to pilot plant. Carnegie Institute of Washington Publication No. 600, Washington, DC

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25(3):294–306

Dahiya A, Boumans R, McInnis A (2009) Effects of light and dark cycles on algae biomass grown in open pond system for oil production. American Ecological Engineering Society, 24–26 June 2009 at Oregon State University in Corvallis, Oregon

Dahiya A, Boumans R, McInnis A (2010) Algae production design for oil production project. Project report to University of Vermont Office of Technology

Dayananda C, Sarada R, Kumar V (2007) Isolation and characterization of hydrocarbon producing green alga *Botryococcus braunii* from Indian freshwater bodies. Electron J Biotechnol 10:78–91

DoE (2010) National algal biofuels technology roadmap. US Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program. http://www1.eere.energy.gov/biomass/pdfs/algal_biofuels_roadmap.pdf. Accessed 30 June 2010

Downing JB, Bracco E, Green FB, Ku AY, Lundquist TJ, Zubieta IX, Oswald WJ (2002) Low cost reclamation using the advanced integrated wastewater pond systems technology and reverse osmosis. Water Sci Technol 45:117–125

Dugan GL, Golueke CG, Oswald WJ (1972) Recycle system for poultry wastes. J Water Pollut Control Fed 44:432–444

Dumrattana P, Tansakul P (2006) Effect of photoperiod on growth and hydrocarbon content of *Botryococcus braunii* cultured in effluent from seafood processing plant. Songklanakarin J Sci Technol 28:99–105

Eyster HC, Brown TE, Tanner HA (1958) Mineral requirements for *Chlorella pyrenoidosa* under autotrophic and heterotrophic conditions. In: Lamb CA, Bentley OJ, Beattie JM (eds) Trace elements. Academic, New York, pp 157–191

General Systems Research LLC (2011). www.GenSysResearch.com

Geritz SAH, Metz JAJ, Kisdi E, Mesze´NA G (1998) Evolutionarily singular strategies and the adaptive growth and branching of the evolutionary tree. Evol Ecol 12:35–57

Gonzalez LE, Cañizares RO, Baena S (1997) Efficiency of ammonia and phosphorus removal from Colombian agroindustrial wastewater by the microalgae Chlorella vulgaris and Scenedesmus dimorphus. Bioresour Technol 60:259–262

Gouveia L, Marques AE, da Silva TL, Reis A (2009) Neochloris oleabundans UTEX #1185: a suitable renewable lipid source for biofuel production. J Ind Microbiol Biotechnol 36:821–826

Griffiths M, Harrison S (2009) Lipid productivity as a key characteristic for choosing algal species for biodiesel production. J Appl Phycol 21:493–507

Hanagata N, Takeuchi T, Fukuju Y, Barnes DJ, Karube I (1992) Tolerance of microalgae to high CO2 and high temperature. Phytochemistry 31(10):3345–3348

Hills CB, Nakamura H (1978) Food from sunlight: planetary survival for hungry people, how to grow edible algae and establish a profitable aquaculture. World Hunger Research Project. University of the Trees Press, Boulder Creek

Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J 54:621–639

Huang B, Hong H, Chen L (1994) The physiological effects of different N-P ratios on algae in semicontinuous culture. Asian Mar Biol 11:137–142

Huntley M, Redalje D (2007) CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. Mitig Adapt Strateg Glob Change 12:573–608

Janssen M, Slenders P, Tramper J et al (2001) Photosynthetic efficiency of Dunaliella tertiolecta under short light/dark cycles. Enzyme Microb Technol 4–5:298–305

Johnson MB (2009) Microalgal biodiesel production through a novel attached culture system and conversion parameters. Master thesis, Virginia Polytechnic Institute and State University

Kebede-Westhead E, Pizarro C, Mulbry WW, Wilkie AC (2003) Production and nutrient removal by periphyton grown under different loading rates of anaerobically digested flushed dairy manure. J Phycol 39:1275–1282

Klausmeier CA, Litchman E (2001) Algal games: the vertical distribution of phytoplankton in poorly-mixed water columns. Limnol Oceanogr 46:1998–2007

Kommareddy A, Anderson G (2004) Study of light requirements of photobioreactor. 2004 CSAE/ASAE North-Central Intersectional Meeting. Paper No: MB04-111

Laws EA, Terry KL, Wickman J, Challup MS (1983) A. simple algal production system designed to utilize the flashing light effect. Biotechnol Bioeng 25:2319–2336

Liebrand CB, Ling KC (2009) Cooperative approaches for implementation of dairy manure digesters. Research report 217. United States Department of Agriculture (USDA), Washington, DC

Lipman CB, Teakle LJH (1925) Azotobacter chroococcum and nitrogen fixation. Available from www.jgprupress.org

Lundquist TJ (2008) Production of algae in conjunction with wastewater treatment. Paper presented at the National Renewable Energy Laboratory-Air Force Office of Scientific Research joint workshop on algal oil for jet fuel production, 19–21 Feb 2008, Arlington, VA

Maynard Smith J, Price G (1973) The logic of animal conflicts. Nature 246:15–18

Meiser A, Schmid-Staiger U, Trosch W (2004) Optimization of eicosapentaenoic acid production by Phaeodactylum tricornutum in the flat panel airlift (FPA) reactor. J Appl Phycol 16:215–225

Metzger P, Largeau C (2005) Botryococcus braunii: a rich source for hydrocarbons and related ether lipids. Appl Microbiol Biotechnol 66:486–496

Mouget JL, Dakhama A, Lavoie MC, De la Noüe J (1995) Algal growth enhancement by bacteria: is consumption of photosynthetic oxygen involved? FEMS Microbiol Ecol 18:35–44

Mulbry W, Kebede-Westhead E, Pizarro C, Sikora LJ (2005) Recycling of manure nutrients: use of algal biomass from dairy manure treatment as a slow release fertilizer. Bioresour Technol 96: 451–458

Mulbry W, Kondrad S, Buyer J (2008) Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates. J Appl Phycol 20:1079–1085

Mulbry W, Kangas P, Kondrad S (2010) Toward scrubbing the bay: nutrient removal using small algal turf scrubbers on Chesapeake Bay tributaries. Ecol Eng 36:536–541

Mulligan CN, Gibbs BF (2003) Innovative biological treatment processes for wastewater in Canada. Water Qual Res J Can 38:243–266

Myers J, Graham J-R (1971) The photosynthetic unit in chlorella measured by repetitive short flashes. Plant Physiol 48:282–286

Ogbonna JC, Yoshizawa H, Tanaka H (2000) Treatment of high strength organic wastewater by a mixed culture of photosynthetic microorganisms. J Appl Phycol 12:277–284

Oswald WJ (1990) Advanced integrated wastewater pond systems. Paper presented at the supplying water and saving the environment for six billion people 1990 ASCE convention EE Div/ASCE, 5–8 Nov 1990, San Francisco, CA

Oswald WJ (2003) My sixty years in applied algology. J Appl Phycol 15:99–106

Oswald WJ, Gotaas HB, Ludwig HF, Lynch V (1953) Algae symbiosis in oxidation ponds: III. Photosynthetic oxygenation. Sew Ind Wastes 25:692–705

Pizarro C, Mulbry W, Blersch D, Kangas P (2006) An economic assessment of algal turf scrubber technology for treatment of dairy manure effluent. Ecol Eng 26:321–327

Riley JG (1979) Evolutionary equilibrium strategies. J Theor Biol 76(2):109–123

Rodolfi L, Zittelli GC, Bassi N, Padovani G, Biondi N, Bonini G, Tredici MR (2009) Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng 102(1):100–112

Roessler PG (1988) Effects of silicon deficiency on lipid composition and metabolism in the diatom Cyclotella cryptica. J Phycol 24:394–400

Schenk P, Thomas-Hall S, Stephens E, Marx U, Mussgnug J, Posten C, Kruse O, Hankamer B (2008) Second generation biofuels: high-efficiency microalgae for biodiesel production. BioEnergy Res 1:20–43

Seckbach J, Gross H, Nathan MB (1971) Growth and photosynthesis of *Cyanidium caldarium* cultured under pure CO2. Israel J Bot 20:84–90

Sheehan J, Dunahay T, Benemann J, Roessler P (1998) A look back at the U.S. Department of Energy's aquatic species program: biodiesel from algae. National Renewable Energy Laboratory, Golden

Shifrin NS, Chisholm SW (1981) Phytoplankton lipids: interspecific differences and effects of nitrate, silicate and light–dark cycles. J Phycol 17:374–384

Simpson AJ, Zang X, Kramer R, Hatcher PG (2003) New insights on the structure of algaenan from *Botryococcus braunii* race A and its hexane insoluble botryals based on multidimensional NMR spectroscopy and electrospray-mass spectrometry techniques. Phytochemistry 62:783–796

Smith Val H (2003) Eutrophication of freshwater and coastal marine ecosystems. A global problem. ESPR – Environ Sci Pollut Res 10(2):126–139

Tam NFY, Wong YS (1996) Effect of ammonia concentrations on growth of Chlorella vulgaris and nitrogen removal from media. Bioresour Technol 57:45–50

Tam NFY, Lau PS, Wong YS (1994) Wastewater inorganic N and P removal by immobilized Chlorella vulgaris. Water Sci Technol 30:369–374

Wang B, Li Y, Wu N, Lan CQ (2008) CO2 bio-mitigation using microalgae. Appl Microbiol Biotechnol 79(5):707–718

Weyer KM, Bush DR, Darzins A, Willson BD (2009) Theoretical maximum algal oil production. BioEnergy Res. Online first [Open Source] 3:204–213

Wilkie AC, Mulbry WW (2002) Recovery of dairy manure nutrients by benthic freshwater algae. Bioresour Technol 84:81–91

Wilkie AC, Edmundson SJ, Duncan JG (2011) Indigenous algae for local bioresource production: phycoprospecting. Energy Sustain Dev 15:365–371

Woertz I, Feffer A, Lundquist T, Nelson Y (2009a) Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. J Environ Eng 135:1115–1122

Woertz IC, Fulton L, Lundquist TJ (2009b) Nutrient removal & greenhouse gas abatement with CO2-supplemented algal high rate ponds. Paper written for the WEFTEC annual conference, Water Environment Federation, 12–14 Oct 2009, Orlando, FL, p 13

Wolf FR (1983) Botryococcus braunii an unusual hydrocarbon-producing alga. Appl Biochem Biotechnol 8:249–260

Xu H, Miao X, Wu Q (2006) High quality biodiesel production from a microalga chlorella protothecoides by heterotrophic growth in fermenters. J Biotechnol 126:499–507

Yun Y-S, Lee Sun Bok, Jong Moon Park, Choong-Il Lee, Ji-Won Yang (1997) Carbon dioxide fixation by algal cultivation using wastewater nutrients. J Chem Technol Biotechnol 69:451–455