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Algae for Biofuels – Production and Conversion

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The production of algae for renewable energy has garnered much attention since the beginning of the 21st century. Currently, the United States is rapidly developing green energy solutions to address rising energy costs and concerns related to dependence on foreign oil. Algae are primarily mentioned as a feedstock for biodiesel production but also provide material that could be converted to other renewable fuels such as ethanol and butanol. Algae have several advantages over agricultural crops used for biofuels that are often cited as a reason to invest in the development of algal biofuels, these advantages include:

1. Algae do not compete with food resources. Algae are not a significant food or feed resource like other agricultural crops, so redirecting a resource from the dinner table to the fuel tank is no longer a concern. Also, algae production does not compete for arable land resources since it can be grown in tanks or ponds over relatively small acreage.
2. Algae can yield more biomass per acre than any agricultural crop or woody biomass material. Between 1,000 to 6,500 gallons of plant oil can be produced per acre from algae based on a U.S Department of Energy estimate. This is far superior to soybeans and canola (rapeseed) which yield 48 and 120 gallons per acre per year (450 to 1,125 l/ha/yr), respectively.
3. Algae oils and biomass can support an entire value-added product chain. Algae have been produced for decades to support the needs of pharmaceutical, nutraceutical (food supplements with nutritional or medicinal benefit), and food processing needs. Building block organic acids, proteins, and amino acids can be extracted from algae cells. These high value products provide justification for algae-oil based business enterprises; however, the value of biofuel products is significantly less than these industrial compounds.

While algae show tremendous potential as a bioenergy feedstock, there are a number of hurdles and complications that must be addressed before commercial algae fuel production is a reality. The production of a monoculture species, algae harvest, algae drying, and oil extraction are among the chief concerns that must be addressed to make algal biofuels a reality. This factsheet discusses the types of algae used for industrial purposes, including biofuels production, how algae are cultivated, and what is required to refine algae into industrial products including fuel. The goal of this factsheet is to provide the reader with a basic understanding of algal production so they can make informed management decisions with regards to organizational plans for aquaculture production facilities.

This publication only discusses the production and conversion of algae into industrial products, for more information on the economic costs and environmental impact of algae production see SRAC Publication No. 4310, *Algae for Biofuels – Economic and Environmental Costs.*

Types of Organisms and Uses

Microalgae are photosynthetic microorganisms comprised of both eukaryotic (unicellular algae) and prokaryotic (blue green algae or cyanobacteria) members. They are considered to be one of the oldest life forms and

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are present in all known ecosystems on Earth. Microalgae are primitive plants lacking roots, stems, and leaves. It is estimated that over 50,000 distinct species exist, of which, approximately 30,000 have been studied. Microalgae are of great interest for their ability to grow rapidly to high biomass and because, depending on the species, they produce compounds of nutritive value or that can be used for fuel production. Key microalgal species that are cultivated for commercial purposes are listed in Table 1.

Eukaryotes

The eukaryotic microalgae contain membrane bound organelles (plastids, mitochondria, nuclei, golgi bodies, etc.) that control cell function and reproduction. Eukaryotic microalgae are grouped primarily based on their pigmentation, life cycle, and basic cellular structure. The eukaryotic microalgae groups that are most important for nutrition and fuel applications are the green algae *(Chlorophyta)* and red algae *(Rhodophyta).*

Prokaryotes

The prokaryotic microalgae are often referred to as cyanobacteria or blue-green algae. Unlike the eukaryotic microalgae, they lack membrane bound organelles and are actually bacteria rather than true algae. Cyanobacteria are primarily cultivated commercially as fish feed or as animal/human nutritional supplements. Transgenic (genetically modified) strains of certain species of cyanobacteria *(Synechococcus* and *Synechocystis)* have recently been developed for use in the production of fuel precursor compounds (Ruffing 2011).

It should be noted that the commercial use of the microalgae shown in Table 1 for human/animal nutrition and aquaculture applications are mature industries resulting in the production of ~250 to 3,000 tons (~227 to 2,720 mt) of dry algal cell weight per year. However, in comparison, the mass culture of microalgae for biofuel production is unknown as this is currently an emergent industry.

Table 1: Key microalgal species and their commercial applications[1]

Microalgal species	Type of cultivation system	Productivity of Biomass (g/m^2/day, unless otherwise stated)	Commercial use
Green Algae			
Botryococcus braunii	Fresh water open ponds	3	Algal oil[2]
Chlorella sp.	Fresh water open ponds	11-32	Human nutrition, cosmetics, aquaculture, algal oil
Dunaliella salina	Salt water open ponds	20-38	Human nutrition, cosmetics, β-carotine, algal oil
Haematococcus pluvialis	Fresh water open ponds	15	Aquaculture astaxanthin
Nannochloropsis sp.	Fresh water open ponds Flat plate reactor	2-5 0.3 (g/L/day)	Algal oil
Scenedesmus sp.	Fresh water open ponds	2-13	Algal oil
Red Algae			
Crypthecodinium cohnii	Salt water continuous culture reactor	10 (g/L/day)	DHA[3] oil
Galdieria sulphuraria	Salt water continuous culture reactor	50 (g/L/day)	C-phycocyanin
Cyanobacteria			
Aphanizomenon flos-aquae	Fresh water open ponds	30	Human nutrition
Spirulina sp.	Fresh water open ponds	12-19	Human and animal nutrition, cosmetics, phycobiliproteins
Synechococcus sp.	Fresh/Salt water batch-fed	0.1 (g/L/day)[4]	N-alkanes[5], ethanol, Isobutanol, Hydrogen

[1] Productivity data taken from Brennan and Owende, 2010; Mata et al., 2010
[2] Use of microalgae strains to produce algal oil has been demonstrated at lab-scale but the microalgae have not yet been cultivated at industrial scale for commercial purposes
[3] Fatty acid docosahexaenoic acid
[4] WT refers to wild type, non-genetically modified Synechococcus
[5] Products listed are all from different transgenic Synechococcus strains

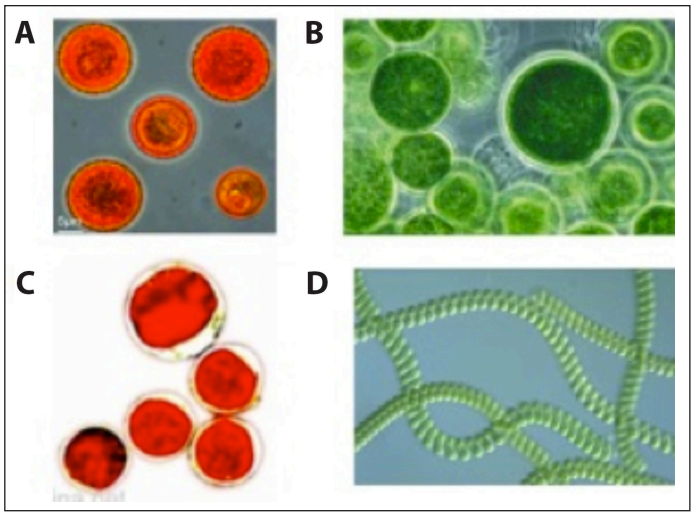

Figure 1. Key microalgae species. A. *Dunaliella salina,* B. *Chlorella* sp., C. *Crypthecodinium cohnii,* D. *Spirulina* sp.

Common Cultivation Considerations

Nutrients and Energy (freshwater vs. saltwater)

Algae can be either photoautotrophic or heterotrophic. Photoautotrophic growth requires only inorganic carbon (CO_2 or soluble carbonates), salts (to provide a source of iron, magnesium, manganese, nitrogen, sodium, potassium, phosphate, sulfate, zinc), and a source of light. Heterotrophic growth requires an organic carbon source (typically sugars, carboxylic acids, or amino acids) in addition to the salts to provide the energy required for metabolism. In some instances microalgae can both photosynthesize and use organic compounds as energy sources. In the case of autotrophic algae, photosynthesis is critical for converting light, water, and CO_2 into fixed carbon (sugars), adenosine triphosphate (ATP), and into oxygen.

Under natural growth conditions, photoautotrophic microalgae absorb sunlight and take in CO_2 from the air and nutrients dissolved in the water in their environment. Some microalgae that come from marine habitats can only grow in cultures with at least 3 percent salt (NaCl) whereas freshwater microalgae often cannot tolerate salt concentrations above 3 percent. When culturing microalgae, attempts to replicate and optimize natural growth conditions should be the goal.

An attraction of using natural conditions for culturing phototrophic miocroalgae is the ability to use sunlight as a free natural resource; however, there can also be inefficiency in relying solely on sunlight, as sunlight can be limiting for culture productivity due to diurnal cycles and seasonal variation. To avoid light limitation, mass culture systems often augment sunlight with artificial light sources (fluorescent or LEDs), but the energy required for providing additional light needs to factored in to the overall efficiency of the system.

Besides light, another important factor influencing microalgae productivity is inorganic carbon availability. Microalgae typically use CO_2 from three different sources: CO_2 naturally existing in the atmosphere (360 parts per million per volume), CO_2 discharge from industrial sources (power plants, cement factories, etc.), or from soluble carbonate sources. Since most microalgae can use CO_2 at levels much greater than that available naturally in the atmosphere (up to 150,000 parts per million per volume), and increased biomass yields can result from higher CO_2, mass culture of algae often make use of CO_2 supplementation either with industrial gas discharges or soluble carbonates.

Initial attempts at developing artificial culture media for microalgae growth made use of soil water extracts. Years of subsequent study have resulted in the development of a list of general requirements that form the basis of microalgae culture media formulations and are summarized below:

1. Total salt content of the media needs to be matched to the habitat from which the microalgae originate (marine microalgae require salt concentrations at 3 percent or above and freshwater microalgae at 3 percent or below).
2. Potassium, magnesium, sodium, calcium, phosphate, sulfate, and chloride need to be provided as macronutrients.
3. Dedicated nitrogen sources are needed (generally in the form of nitrate, ammonium, or urea).
4. Inorganic carbon is supplied either as atmospheric CO_2 or soluble carbonates.
5. The media need to have an appropriate pH for the particular microalgae to be cultured and buffering capacity needs to be provided in the media (usually in the form of phosphate or carbonate buffers).
6. Trace elements and vitamins should be supplied as needed.

For each microalgal strain, optimal levels of inorganic carbon, nitrogen, macronutrients, trace elements, vitamins, and pH levels need to be established, and those levels may change if the goal of the microalgal cultivation is for the greatest biomass production or is for increased specific metabolite production (algal oil, β-carotene, phycobiliproteins, etc.).

Climate

Climate control for light transfer (intensity, spatial distribution, adsorption by cells, absorption, and scattering) and temperature are critical factors to algal growth and product formation. It is important to select culturing locations and systems accordingly to meet productivity requirements for the species of interest and remain within the tolerances. Seasonal and day to day variations in climate throughout the year can have a significant impact on performance and success of outdoor systems.

Mixing

Mixing of the algae culture suspension can impact how well the cells are distributed, affecting accessibility of nutrients, CO_2, and light, dissipation and removal of O_2, transfer of heat in and out of the system, gradients in pH, as well as stress placed on the algae cells. The mode of mixing and flow patterns created need to be calculated and selected within the property limitations of the cells to avoid cellular and overall loss in productivity of the cultivation system.

Contamination

Biological contamination can be a common and restrictive problem to mass cultivation of microalgae. Contaminants can be other algae, bacteria, fungi, viruses and predatory protozoans. Cross contamination by other microalgae can be due to direct contact where collisions between competing algal species can lead to inhibited growth. Other mechanisms are related to competition of nutrients and other resources where the dominant species is not the production strain and detrimental allelopathy that produces chemicals that interfere and inhibit growth of the desired algae. Bacterial contaminants can limit algae growth by lysing the cells and destroying the integrity of the cell wall through direct or indirect attack. Direct attack is through cell to cell contact whereas indirect attack is related to secretion of inhibitory compounds. Infection by viruses can spread within days and destroy an algae population quickly. Protozoa and zooplankton can graze on microalgae lowering cell concentrations and product formation in culture systems. Control measures on a large scale outside of strains that grow in highly selective environments are critical to a successful process. Filtration and/or disinfection of water, gas and material surface transmission points need to be evaluated and incorporated into design of a cultivation system.

Land Use

Microalgae production systems are designed to maximize surface area where light can reach the cells for photosynthesis. Closed systems have a higher productivity than open systems because of the increased surface area provided by the enclosure. Even open systems though are designed to optimize photosynthesis potential and have depths of around 4.72 inches (0.12 m). It is common for publications to mention the reduced land use requirement of oil produced from microalgae compared to that produced from terrestrial plants (Table 2). This is usually proceeded by some statement about land use change, available cropland, and required land to meet biofuel production standards. Though this is true, cropland is not required for algal production, former industrial sites, and rooftops work fine for algae cultivation. Cost and environmental impacts need to be taken into account not just the amount of land that it takes to grow a crop.

Table 2. Annual oil production for biodiesel feedstocks (Source: Lardon et al. 2009)

Feedstock	ton/acre/yr
Algae	11.6
Soybean	0.19
Rapeseed	0.53
Palm Tree	1.9

Production/Cultivation Systems

In addition to selection of microalgae strain and identification of product(s) of interest, production of algae biomass requires a cultivation system to support growth and product formation, harvest operations to separate the algae solids from water, and extraction to remove the product of interest if not the algae as a whole (Fig. 2).

A number of different cultivation systems are used for algae production and are often classified as either open systems or closed systems.

There are four major types of open culture systems and include 1) shallow big ponds, 2) tanks, 3) circular ponds, and 4) raceways ponds constructed as an endless loop or series of loops. These systems are typically used outdoors to take advantage of natural sunlight and are located on extensive land areas (2 acres to > 600 acres/pond; 0.81 to > 242 ha/pond), offering large surface area for light penetration with ponds typically less than 11.8 inches (30 cm deep). Open culture systems face challenges in climate control which can make growth temperatures and light delivery variable and lead to evaporation of water and

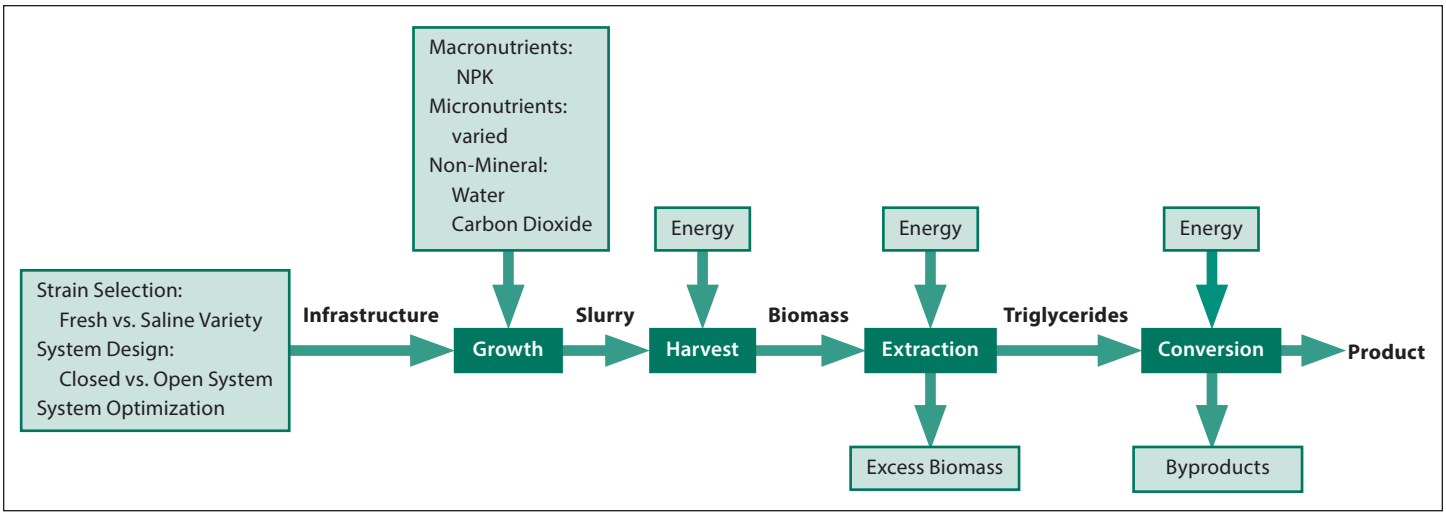

Figure 2. Schematic of the general process used to grow algae for industrial uses.

Figure 3. Section of a raceway pond and paddle wheel mixing. Photo courtesy of Nature Beta Technologies LTD, EI.

modification of nutrient concentrations. In addition, issues with contamination risks are high and difficult to control with exposure to ambient conditions, affecting quality and consistency of the final algal biomass. Although some mixing is achieved through stirring of circular ponds and tanks by a rotating arm, by paddle wheel in raceways ponds (Fig. 3), and pumping in inclined systems, mixing to achieve uniform distribution of nutrients, CO_2 and light exposure is usually considered poor in open systems.

Closed culture systems are best suited for microalgae that do not grow in highly selective environments to protect them from potential contamination by environmental elements (e.g. heavy metals, chemical application runoff) and other microbial species. Closed systems are highly controlled and can support algae that grow on light and CO_2 (photoautotrophs) as well as algae that use acetate or glucose for energy (heterotrophs). When light is not necessary for growth, algae can be produced in sterile stirred tank reactors, similar to a conventional fermentation vessel. When light is part of the growth requirements, the closed culture systems are referred to as photo-bioreactors (PBRs). Control over culture conditions, minimized contamination risks, and reduced losses in water and CO_2 for closed systems allows for greater productivity (g/L/d and $g/m^2/d$) over open culture systems.

There are many designs of PBRs with respect to shape, light paths and mixing with three main categories of design: 1) flat pate, 2) tubular, and 3) vertical column. Flat plate and tubular designs are suitable for outdoor use with large surface areas for light penetration. Flat plate systems are made out of transparent materials to enhance light distribution that are positioned vertically. Sparging (i.e. bubbling a gas through the water) of CO_2 is used to supply carbon energy and provide mixing. Baffles can also be added to these systems to enhance mixing and conduction of light. Tubular reactor designs are made of glass or plastic and have diameters ranging from 1 to 6 cm with lengths typically up to several hundred meters. The tubes are arranged in parallel and often found in horizontal, vertical, and inclined forms. The tubes can be straight or serpentine with arrangements determined to enhance light distribution (Fig. 4). Mixing and CO_2 supply and degassing is often accomplished by air circulators, centrifugal pumps, or air lift (compressed air) systems, yet gradients in pH and gasses often develop along the tubes in addition to growth of algal cells on the tube walls. Outdoor PBRs have been placed in greenhouse spaces to assist with temperature control, and both tubular and flat plate systems require significant amounts of material and operating units as well as land space with scale up.

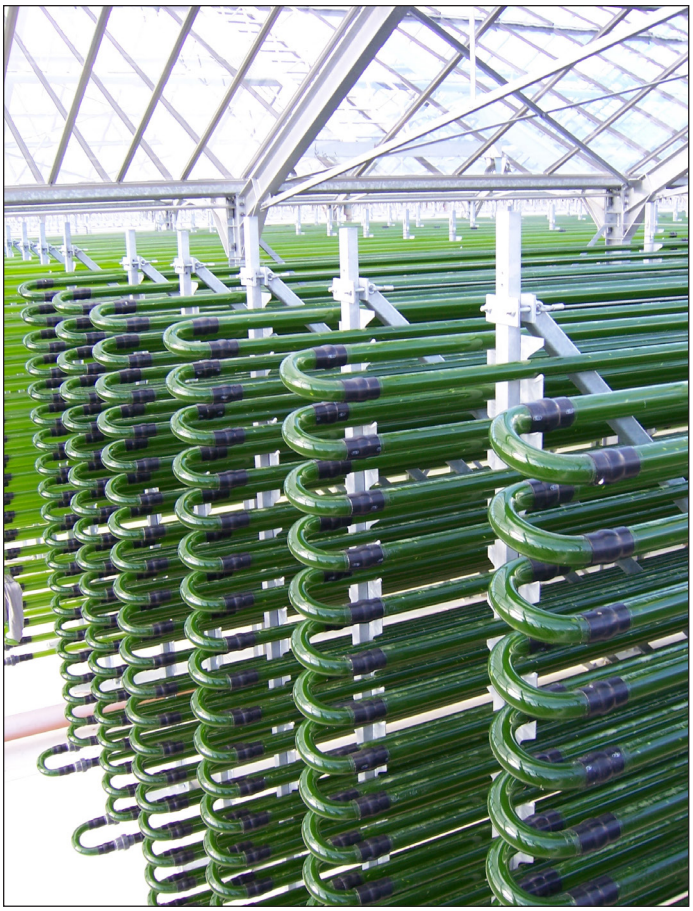

Figure 4. A tubular closed-loop system arranged as a serpentine. Photo courtesy of Jörg Ullmann, Roquette Klötze GmbH & Co. KG.

Vertical column PBRs are generally used for large lab-scale and indoor experiments and have diameters of 7.9 inches (20 cm) or more (Fig. 5). Bubble columns and air-lift cylinders are common designs that provide good axial gas transfer and improved radial movement. These systems generally have small illumination surface areas and require sophisticated materials for construction. Although mixing assists with photoinhibition, the middle of the reactor often becomes light limited, contributing little to the overall productivity of the system. As a result annular columns (hollow center) have been developed that improve light saturation and reduce light exposure fluctuations.

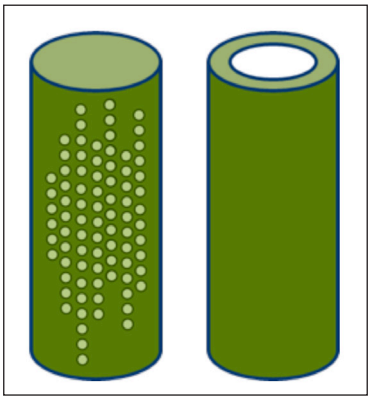

Figure 5. Schematic of the general geometry of vertical column photobioreactors: bubble column (left) and annular column (right).

Bag systems are also options for closed photoautrophic systems operated at large scale. Bag systems that have been successful are sterile (~ 19.7 inches (0.5 m) diameter) with an aeration system in place. Most systems are run in batch, yet require indoor climate control and artificial light, limiting light distribution. Bag systems also face challenges of inadequate mixing.

Despite the increased control over climatic conditions and regulation of growth and product formation, overheating, oxygen accumulation, cell damage from modes of mixing, wall growth, stability of photo-stage materials, and scale-up difficulties are limitations common to PBRs. Currently, large commercial systems use open air systems. The select properties of those algal species and the high value products of interest make that possible. Additional advancements in PBR technology and knowledge of algal culture performance is needed to make closed systems commercially viable options for relatively low value products required in large volumes/mass.

Every system configuration has its own advantages and disadvantages. When it comes to algae, there is no one size fits most approach. The system put in place is highly dependent on the algal species and product of interest, as the conditions required for best growth and yield can vary significantly, as well as the geographic location and production costs.

Commercial Production

Microalgae have been grown commercially for over 40 years for high value products for food, feed, nutraceuticals, pharmaceuticals, and cosmetics. Four algal species in particular have been successfully produced in large scale culture systems, *Chlorella* and *Spirulina* for health food and human nutrition, *Dunaliella salina* for β-carotene and *Haematococcus pluvialis* for astaxanthin as an aquaculture feed additive, food colorant, and antioxidant dietary supplement. Aside from *Haematococcus,* these algae have unique characteristics that allow them to be grown in open air systems with limited risk of contamination. These properties include high pH, very high salinity, and relatively high concentrated nutrient media.

The fatty acid docosahexaenoic acid (DHA) commonly used as a supplement in infant formula and foods supporting brain function and nervous system and vision development is currently commercially produced using algae. This market is currently supported primarily by Martek using a heterotrophic algae, *Crypthecodinium cohnii* in closed fermenters.

Large scale commercial production of algae primarily takes place outside the U.S. (e.g. European Union, Asia, New Zealand, Mediterranean, Australia). Some closed

systems for high value products (e.g. food supplements, aquaculture feed) and selection of algae from natural systems have been developed in the U.S. Algae feedstock production for biofuels has been limited and most success has been achieved at the pilot scale. Companies in the U.S. are developing new technologies to overcome some of the hurdles tied to economically viable production of algae and conversion to fuel, however challenges remain. The idea of farms providing the tonnage of algae required to support alternative sources of oil for a sustainable biofuels industry is still in the early development stages.

Harvest

Harvest of algae biomass on a large scale requires 1) separation of the cells from the bulk suspension, 2) dewatering or cell concentrating, and 3) product extraction (e.g. oils, proteins, carbohydrates) depending on the form of product that needs to be transferred from the culture site or farm. Flocculation processes can be used to pull cell particles together to form larger particles, which can enhance settling of algae by gravity sedimentation for solids collection. This aggregation of cells can occur naturally due to the presence of carbonate salts and elevated pH in the bulk medium, through the addition of chemicals (organic or inorganic) that affect the negative charge of microalgae, and/or by an electrolytic process that applies an electric charge to the culture suspension to alter the surface charge of the cells and how they move.

Sedimentation and flotation by gas bubbles are gravity based processes used to capture algae biomass of various particles sizes. Filtration under vacuum or pressure and screen separation by microstrainers are methods used to isolate algae biomass and more effectively remove water. Centrifugation is a way to recover algae under relatively high gravitational forces in short periods of time from a bulk liquid, with the option to recycle the culture medium. Additional water can be removed by drying (e.g. spray-, drum-, freeze-, sun-drying) while creating a more stable biomass feedstock.

For lipid based products such as those used as feedstocks for biodiesel or aviation fuels, algae oil can be extracted using a number of different processes. Extraction can be achieved using a press to expel the oil under pressure, with solvents where the oils have a higher solubility in organic solvents, by supercritical fluid extraction to rupture cells under high temperatures and pressures without hazardous solvents, and through ultrasound. In the later, ultrasonic waves disrupt the algae cell walls and help release the internal products of interest into solution. Aside from oils, other products can be recovered through mechanical (e.g. homogenization, bead mills) or non-mechanical (e.g. freezing, osmotic shock, enzymatic reactions) cell disruption.

The process operations selected for bulk harvest and concentration of a microalgae culture are highly dependent on the physical properties of the cells, the liquid medium in which they are suspended, and the value and quality necessary for the final product. Aside from investment and operational costs, size, density, charge, and sensitivity to mechanical stress are the most influential cell properties that factor in to the development and design of effective harvest systems.

Several costs associated with algae biomass cultivation need to be considered in estimating financial commitments to production, including the unit operations for product, initial investment costs, and recurring operation and maintenance costs. These costs are tied to growth, harvest, and processing through dewatering and extraction systems. Additional costs may include engineering, permits, infrastructure preparation, equipment acquisition and installation, and contractor fees. Operational expenses for nutrients, CO_2, water to address evaporative losses, utilities, labor, equipment maintenance, and land use either through ownership or lease also need to be factored in.

Is Growing Microalgae for Feedstocks Right for You?—Questions to Ask

Assessing if microalgae are an appropriate aquacultural species for a production system is a difficult decision. Given the complexity of harvesting, refining, and marketing algal oils, most growers will contract with a larger biorefining company to provide much of the services required to make a final product from the algae. Many of the technologies required to develop an algae bioenergy business are too complex or too costly to use at the farm-scale so contracting with a technology supplier will be necessary. In order to make an informed decision from a production standpoint, the following questions should be evaluated. These questions are critical for assessing the likelihood for success if a microalgae production enterprise is selected for an aquaculture facility.

1) *What species of algae will be grown?*
   When approached by an algal bio-oil proprietor it is important to understand the rationale for selecting the algae species that will be grown and what properties of this algal species lend it to biofuels production. Try to determine what properties of the algal species lend itself to the biofuel market.

2) *Why was this species chosen and does it have proven markets in this industrial area?*
Determine if the algal bio-fuel proprietor has any experience growing algae, working in the biofuels arena, or developing new agricultural/aquacultural enterprises. There are a number of intricacies to growing algae for industrial uses so it is important to make sure the person guiding the enterprise is experienced. Also, make sure they have worked in the biofuels industry before as it is a volatile and somewhat risky business for the inexperienced businessperson.

3) *What is the largest scale system in which the interested party has grown this species of algae?*
Similar to question 2, a potential investor should make sure the proprietor directing the new algal oil business has experience growing algae at a large scale for industrial uses. As a potential investor, placing money or resources into an unproven, pilot-scale, or first generation system could lead to economic ruin.

4) *Can you visit the current production site?*
Again, as a potential investor you want to make sure the algal bio-oil company has experience growing algae and has a proven system to grow, harvest, and extract oil from the aquatic organism. Beware of any 'black box' processes which simply show a raw material going in and a final product coming out. A non-disclosure or confidentiality agreement can be drafted to allow the investor to see every part of the system without the bio-oil company risking a loss of information or technology. Also, if a company has a unique algae production system, inquire about its patent status and if they did not patent the technology it is important to determine why.

5) *What system will be used for production and why?*
This factsheet discusses numerous systems that can be used to produce algae for industrial uses. Inquire about the system chosen for any enterprise opportunity that is presented by an algal bio-oil proprietor. The better they can describe their system and discuss the reason for choosing it over other systems shows technical competency in the field.

6) *What are the potential problems with this production system?*
Every crop production system has problems that will arise when it is put into practice. Any system that is touted as being "worry-free" is probably not being represented correctly. Make sure the potential problems and previous operating issues are known before investing in a system. Insuring the resources are available to address any cultural management concerns is critical. In identifying potential problems, also make sure also make sure the market for the algal oil is well-developed, stable, and capable of surviving downturns in the marketplace.

Additional Resources

This list represents a truncated version of the references used in the development of this publication. This reduction was necessary to meet SRAC formatting requirements. A fully cited version of this publication is available from the authors.

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USDA United States Department of Agriculture National Institute of Food and Agriculture

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