CHAPTER 6. Algal Oil Biodiesel

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6.1 Algae Background and Overview

6.1.1 Introduction

Algae form a large and diverse group of organisms that live in a wide variety of aqueous habitats. Under older scientific classification schemes (e.g., two-kingdom) they were commonly considered plants, but with the introduction of genetically-based phylogenic systematics, their taxonomical placement has been in a state of flux. Like plants, algae engage in photosynthesis, but they lack the complexity found in land organisms. Some taxonomists use the term "algae" to describe only eukaryotic organisms (those whose cells contain a membrane-bound nucleus) characterized by the presence of chloroplasts (membrane-bound structures within the cells (organelles) that perform photosynthesis). Blue-green algae, or Cyanobacteria, are prokaryotic (organisms without a cellular nucleus), and are considered by most sources to be more closely related to bacteria than eukaryotic algae. The genus Spirulina, which has been widely cultivated as a food source and a nutritional supplement, is a well known example of blue-green algae. One distinguishing feature of Cyanobacteria is that they are able to "fix" nitrogen, i.e. convert atmospheric dinitrogen (N₂) into ammonia-based products that can be absorbed by other organisms. None of the eukaryotic algae are known to have this capability and must obtain nitrogen in the form of ammonium, nitrite, or nitrate ions available in their immediate environment.

Eukaryotic algae may be divided into macroalgae and microalgae. Macroalgae, or seaweeds, exhibit a considerable amount of organization, with cells that are grouped into structures resembling leaves and stems observed in land plants. They are fast growing, marine and freshwater organisms, that can be quite large (up to 60 meters in length) (Sheehan *et al.*, 1998). Microalgae are typically microscopic, single-celled, photosynthetic organisms. Nearly all are less than 50 microns (50×10^{-6} meters) in diameter (smaller than the thickness of a human hair) and most are less than 20 microns (20×10^{-6} meters); however, a few species may reach sizes as large as 0.5 cm ($5 \times 10^{-3} \text{ m}$). As feedstock for biofuels, microalgae are thus the focus of this report.

6.1.1.1 Microalgae, General Form and Composition

Most microalgae are autotrophic (i.e., they generate their own source of energy through photosynthesis). As such, they are net producers of oxygen and net consumers of carbon dioxide (CO₂). There are a small number of heterotrophic species that can utilize organic carbon sources, such as glucose or acetate, to either supplement or replace photosynthesis. This type of organism has appeal from a cultivation standpoint because it can grow in the absence of light. However, if the energy required to grow "food" for these organisms is considered, the overall energy balance is likely to be unfavorable, especially when the objective is to produce a fuel feedstock. Microalgae are generally efficient converters of solar energy due to their simple cellular morphology as, unlike land-based plants, very little biomass is needed to perform structural functions. In addition, because they grow in aqueous suspension and have a greater surface area to mass ratio than land-based plants, algae have more efficient access to water, CO₂, and nutrients. The general form of microalgae is either that of a flagellate (round with one or

more tails or "whips" to control movement) or of an amoeboid (spheroids with slight bulges). It is believed that there are hundreds of thousands of species of microalgae, although only about 40,000 individual species have been described to date (Hu *et al.*, 2008).

Microalgae are composed primarily of three classes of organic compounds: proteins, carbohydrates, and lipids. Metabolic intermediates and ash are also present and may contribute up to 20% to the total biomass (Burlew, 1953; Neenan et al., 1986). Proteins, which include enzymes, make up nearly 50% of the dry mass of healthy algae (Burlew, 1953; Benemann and Oswald, 1996) and are the "engines" of the cell. They consist of chains of amino acids, which have the general formula H₂NCHRCOOH (where R is an organic substituent and COOH is a carboxylic acid). Synthesis of proteins is the primary driver for nitrogen uptake by the organism. Carbohydrates, the second class of organic compounds, contain only carbon (C), hydrogen (H), and oxygen (O). These constituents have a minimum of three carbons, include hydrogen and oxygen in a ratio of 2:1 (i.e., H_2O), and may be expressed as the general formula $C_m(H_2O)_n$, m>2. In simple terms, carbohydrates are starches and sugars that act as energy storage devices within algae cells. Lipids, the final type of major organic compounds, are commonly thought of as fats and oils. The building blocks of lipids are fatty acids, which consist of a long, unbranched, aliphatic (hydrocarbon) chain attached to a carboxylic acid (COOH). The chains of fatty acids are very similar to hydrocarbon forms found in petroleum and, in fact, are believed by many to be the source of crude oil. Fatty acids are synthesized in one or more chloroplasts (the organelle responsible for photosynthesis), using a single set of enzymes. The rate of fatty acid synthesis is controlled by acetyl CoA carboxylase (ACCase). Although fatty acids are formed within the chloroplast, most lipids are found elsewhere in the cell. Lipids may accumulate as pockets within the body of the algae cell, as a means of storing energy. However, in healthy algae cells, more than half (50 to 70%) the lipids (Neenan et al., 1986) are bound within cell membranes where they provide a physical-chemical barrier that functions as part of the membrane structure. A membrane encloses each organelle within the cell, including the chloroplast(s). The exterior surface of the cell is protected by both a membrane and, in most algae, an outer wall. In diatoms, one of the groups of microalgae that may be of interest as a fuel feedstock, this wall is made of silica (SiO₂).

All three major types of organic compounds found within algae cells (proteins, carbohydrates, and lipids) have the potential to serve as a source of food for humans and/or animals. Each also has the potential to produce fuel. Undifferentiated biomass can be gasified; carbohydrates may be fermented to produce ethanol; and all lipids, in theory, could be converted to liquid fuels. However, there is a significant amount of variability found in lipid molecules, including the number of unsaturated bonds and the presence of elements other than hydrogen and carbon (e.g., oxygen and phosphorus), that make it difficult to treat lipids as a single group. Consequently, the lipid extraction and feedstock conversion processes are expected to vary depending on the nature of the specific lipids. Only a portion of the total lipid content is likely to be useful for any given fuel type and associated production pathway.

Lipids found in algae tend to be much more heterogeneous than seed or vegetable oils, especially the individual free fatty acid components. Overall there is much greater variation in chain length and degree of saturation (lack of double bonds between carbon atoms). Lipids can be categorized generally as either polar or nonpolar (neutral). The polar lipids, which are found in membrane structures, consist primarily of glycolipids (attached to carbohydrates) and

phospholipids (integral to cell membrane structure). Polar lipids are critical to the structural integrity of cell membranes, as their polarity allows them to align in response to the chemical composition of the medium on either side of the membrane. The predominant neutral lipids are triglycerides, isoprenoids, and hydrocarbons. Neutral lipids collect as self-isolating pockets in the cytosol of the cell (Figure 6.1). Because they are not bound up in membrane walls, this feature makes physical extraction of neutral lipids, for use as a biodiesel feedstock, more viable than extraction of those that are membrane-bound. Minor lipids present in algae include monoglycerides, diglycerides, free fatty acids, wax esters, and sterols. For the interested reader, Thompson (1996) presents a thorough review of algal lipid composition.



Figure 6.1. The general morphology of a microalgae cell includes one or more membranebound chloroplasts and a nucleus. Neutral lipids form self-isolating concentrations in the cytosol; polar lipids are concentrated in membrane walls. In addition to a membrane, the cell is bound by a wall, which in diatoms contains silica.

The chemical composition of microalgae is not an intrinsic, fixed feature of a given species or even of an individual cell. It varies as a function of cultivation conditions such as temperature, illumination, pH, salinity, and nutrient availability, as well as age of the cell. Different species, however, can be characterized as to their general compositions and their response to environmental conditions. In particular, many algae produce more lipids, especially triglycerides (also triacylglycerols or TAG), under conditions of stress. Conditions which have been shown to increase lipid/TAG concentrations include increased light, increased temperature, and nutrient depletion. The exact mechanism for increased lipid production/TAG production is not entirely understood, but it is generally associated with a reduction in growth rate (reproduction) and biomass accumulation (Burlew, 1953; Sheehan, 1998, Figure II.B.3). Sriharan and others (1991) note a roughly 30% decrease in growth rate in nutrient-deprived diatoms. There is some evidence that this type of stress is correlative with the degradation of the chloroplast membrane and suppression of photosynthesis (Hu et al., 2008). Thompson (1996) suggests that algae cells under stress continue to produce fatty acids at a relatively constant rate while overall cell growth is depressed. Without growth, membranes cease to be formed and thus there is a reduced need for polar lipids. Accumulating fatty acids are stored as triglycerides so that they are readily available for use once conditions improve and cell growth resumes. In general, overproduction

of lipids can be described as a short term response that is resolved either when conditions improve or the algae cell dies. While the relative amount of lipids within an individual cell (lipid to biomass mass ratio) increases under conditions of stress, it does not appear that overall lipid production for a given population (lipid to growing-medium mass ratio) is significantly greater.

The most extensively studied stress trigger is nitrogen deprivation; however silicon-depleted environments for diatoms have also been explored (Sheehan et al., 1998). Lipid levels as high as 60% total biomass, 80% of which are TAG) have been reported in certain species under nitrogen starved conditions. In contrast, normal concentrations are about 20% lipids with less than half occurring as TAG (Neenan et al., 1986). Two recent papers have compiled data from the literature and found that the average reported lipid content relative to the dry cell mass for nitrogen-starved green microalgae (multiple species) is 41% (Griffiths and Harrison, 2009) and 45.7%, (Hu et al, 2008). Normal lipid contents, as reported in the literature, average between 23% (Griffiths and Harrison, 2009) and 25.5% (Hu et al., 2008) as a percent of dry weight. Thompson (1996) comments that algae that naturally produce high amounts of lipids are typically slow growers. Griffiths and Harrison (2009) state this correlation could not be determined based on published results. In general, most of these data are gathered from algae grown under laboratory conditions in batch cultures that have been allowed to develop past the logarithmic (exponential) phase of the growth curve. It has also been observed that lipid contents increase with age. Unknown, is how these features will translate to a continuously, or semi-continuously harvested open pond system, but it is expected that lipid contents will be lower.

6.1.1.2 Microalgae, Growing Conditions

The key inputs required to grow algae are sunlight, water, nutrients, carbon dioxide (CO_2), and a containment system. If one thinks of soil as a containment system, these requirements are no different than conventional land-based plants; however, the proportions are different. Cultivated algae, grown to produce significant amounts of biomass over short periods of time, need specific amounts of each and are much more sensitive to these levels than other crops. Too much or too little of any input can result in loss of biomass or significant decrease in per area yields.

6.1.1.2.1 Water Chemistry, Nutrients, and Amendments

Algae can grow in freshwater, sea water, and brackish water; but the ideal specific chemical characteristics of the water are relatively fixed for a given species; thus, in general, freshwater species cannot be grown in salt water, and vice versa. Both relative and absolute concentration of different chemical elements and compounds must be maintained during the cultivation process. The ability to tolerate fluctuations in water chemistry varies by species. Major alkali cations typically present in saline waters, including sodium (Na⁺), potassium (K⁺), calcium (Ca²⁺), and magnesium (Mg²⁺), as well as the anions chloride (Cl⁻) and sulfate (SO4²⁻), are known to be critical for algae growth, but little is known about optimal concentrations or how varying conditions and/or species affect the need for these elements. There is more information available with regard to the need for ions that supply nitrogen (N), phosphorous (P), and carbon (C), as these elements are incorporated directly into the algal biomass. These ions include nitrate (NO₃⁻), nitrite (NO₂⁻), ammonium (NH₄⁺), phosphate (PO₄³⁻), carbonate CO₃²⁻), and bicarbonate (or hydrogencarbonate, HCO₃⁻). Certain trace elements are vital to the survival and growth of

algal, but can be toxic if present in excess. These include copper (Cu), cobalt (Co), zinc (Zn), manganese (Mn), molybdenum (Mo), and vanadium (V). Other toxic elements, not used by algae but which may be present in groundwater include mercury (Hg), cadmium (Cd), and selenium (Se) (Neenan *et al.*, 1986; Burlew, 1953).

Source waters used to create the algae growing medium may provide some of the nutrients required for algae to thrive; however, these concentrations will change over time, either due to natural variation in the water supply or due to *in situ* changes in the cultivation pond in response to algae uptake and evaporation. Consequently, it is generally assumed that concentration levels will need to be monitored and adjusted. This may be accomplished through additions of commercial fertilizers, reclaimed post-harvest biomass and/or cultivation water, or other sources. It is essential that chemical assessments of the algae cultivation system consider the chemistry of all inputs (makeup water, return water, nutrient and "seeding" mediums), all outputs (water and biomass removed from the production stream), and the growing environment (biomass plus aqueous medium). This must be coupled with an understanding of both the range and optimal conditions for growing a specific algae cultivar. The most desirable source waters are those containing chemical constituents in amounts that are just below the optimum levels for the particular species of algae. In this case, only small volumes of supplementary materials need to be added in order to bring the culture medium up to minimum requirements and a slight increase in concentration of constituents due to evaporation will not harm the population. Care also must be taken to ensure that the source water does not contain any compounds that cannot be tolerated by the algae.

Carbon and oxygen (plus hydrogen) are elements vital to all three types of organic compounds (proteins, carbohydrates, and lipids). Roughly speaking, the amount of biomass increases proportionally with the amount of carbon available. In natural systems, carbon dioxide (CO_2) in the atmosphere and bicarbonate (HCO_3) in the water provide a sufficient amount of these two elements. The photosynthetic reaction makes carbon and oxygen available to the growing algae and releases excess oxygen as a waste product. Most healthy biomass, of any type, consists of about 50% carbon by mass, with a significant portion of the carbon stored in lipids. A "generic" fatty acid contains between 70 and 75% carbon by mass. In order to increase the content of biomass in general, and the lipid content in particular, to desired production levels during mass cultivation of algae, carbon dioxide, in excess of that naturally available in the atmosphere and the water, must be provided in large, reliable quantities.

Oxygen is the end product of photosynthesis and is released in a process known as photorespiration. Carbon dioxide, in addition to supplying a source of carbon, is necessary to offset high levels of oxygen that can occur in dense cultures. When the ratio of oxygen to carbon dioxide increases, photosynthetic reaction rates decrease. Addition of CO_2 minimizes this effect.

The concentration of carbon dioxide in the atmosphere, while high with respect to historical levels, is relatively low (less than 0.04% by volume in dry air) with respect to the amount that can be utilized by algae. Forced delivery of ambient air (e.g., through the use of bubblers) would require hundreds of cubic meters of air per square meter of culture per day (Neenan *et al*, 1986) and thus would be extremely energy intensive on a "per-molecule of CO_2 delivered" basis. Consequently, concentrated CO_2 must be added to algae cultivation systems in order to achieved targeted growth rates. Most conceptual designs of algae farms assume that nearly pure CO_2 is

delivered to the pond, although use of flue gas from coal-fired power plants, which typically has a CO_2 concentration of 9 to 14%, has been explored as well. The advantage of using flue gas directly is that the CO_2 does not need to be captured and concentrated, which, assuming systems available today, is energy intensive. However, there are also concerns about potentially toxic trace elements in the flue gas. This is of particular concern if the algae biomass will be used as a food product. The advantages of using highly concentrated CO_2 gas is that it requires a much lower volume of gas delivered to the system for the same number of carbon atoms; thus the amount of energy needed to transport the gas from its source to the algae pond is significantly reduced. Once on site, it is also more efficient to deliver pure CO_2 to the pond and the algae, on per molecule of CO_2 basis.

CO₂ delivered at the bottom of an aqueous medium will rise to the surface and outgas, potentially before it can be completely utilized by the algae. Assuming that the CO₂ is introduced at the base of the pond, the rate at which the CO₂ reaches the surface will vary depending on the system design. The primary design factors that need to be considered are the depth at which the CO₂ is released (deeper is better) and the size of the bubbles (smaller is better). Increase in depth is addressed through use of a sump that is approximately 1 meter deep; bubble size is minimized by maintaining a relatively low flow rate. Another option is to produce a CO₂ rich "blanket" between the surface of the water and a transparent cover. This is especially attractive when using dilute mixtures of CO₂, such as flue gas. Covers, however, create their own set of problems, including increased material usage, reduction in light, and the potential for excess temperatures. A small amount of carbon may be available as bicarbonate (HCO₃⁻) in some source waters, but the level of HCO₃⁻ is limited by water chemistries conducive to algae culture and is thus expected to be less than 10% of that required. Carbon may also be recovered from residual algal biomass but this approach will require additional material and energy resources and may or may not be compatible with the desired slate of products derived from the algae system. Detailed analyses of CO₂ delivery systems and mass transport within the algae pond are provided in Weissman and Goebel (1987).

6.1.1.2.2 Sunlight, Water Management, and Containment

It is assumed in this study that the microalgae to be used as a feedstock are autotrophic, that is, they make their own food through the process of photosynthesis, which requires light as an energy source. It is also assumed that providing light through artificial means would require significantly more energy than could be extracted from the algae. Therefore, access to natural sunlight is critical for growth of biomass and the vitality of the culture. Light penetration depth in water is quite shallow (100 mm or less), requiring that algae be grown either in shallow ponds with a significant surface area or else in vertical, transparent tubes no more than 200 mm (8 inches) in diameter, but typically smaller. The actual depth of the culture can be increased by approximately 50% (150 mm) through the use of circulation systems, but the extent to which this is practical is limited by the energy required to operate the circulation system and the potential to damage (shear) algae cells during agitation. In this analysis, only horizontal, outdoor ponds are Thus, the maximum amount of sunlight available is determined by the solar addressed. insolation at a particular location, which in turn is determined by degrees latitude and climate (primarily relative humidity and cloud cover). While too little light is detrimental, excess exposure can also be stressful to algal biomass and actually cause a decrease in photosynthesis (Benemann and Oswald, 1996, Figure 5.2).

Light utilization by algal mass cultures is in part determined by the photosynthetic efficiency of the individual algae cells, which is in turn related to the species and health of the algae. However, more important is the amount of incident light that actually reaches the algal cells. Losses of incident light can occur as the result of covering materials, reflection by the water surface, shadowing by walls and earthworks, and suspended particles in the culture medium. Total suspended solids that affect the amount of available light include the algae culture itself, dust/dirt, chemical precipitates, and nonalgal microorganisms, such as bacteria. Bacteria are most likely to be a problem when organic sources of nitrogen are used (such as in waste water) or when growing species of algae that produce relatively high amounts of bio-waste products. The ability of the algae to utilize an incremental amount of additional light decreases geometrically with the increase in total light (Burlew, 1953). Thus, in order to maximize optimal light exposure and biomass growth, algae cultures typically employ circulation systems that exchange algae cells at the surface (where light intensity is highest) with those at depth, where there may be little to no light, to the benefit of both groups of organisms.

Warm sunny climates, where algae are likely to have the highest growth rates, are also characterized by high evaporation rates. During evaporation, water is removed, preferentially leaving behind salts and other chemical compounds. There are three basic solutions to preventing the algal growing medium from becoming increasingly enriched in these substances: minimization of evaporation, continual replacement with water of the correct composition, and/or controlled additions of freshwater. All of these require monitoring and control systems and/or contrainment systems and the last two require pumping. It is likely that large quantities of both reclaimed water and naturally occurring source water (ground and/or surface) will be utilized. A process referred to as blowdown is performed in order to remove a portion of the algae culture medium (preferably after the harvest operation in order to minimize loss of product); the eliminated medium is subsequently replaced with an equivalent amount of virgin source water in order to maintain water quality. Although evaporation can be a problem, so too can precipitation, especially heavy rainfall which can quickly produce changes in overall water chemistry.

Culture mixing and aeration is required for mass production systems. This ensures that all algae receive optimal amounts of exposure to sunlight, that nutrients and CO_2 are delivered uniformly, and that the algae stay in suspension. During periods of maximum photosynthetic activity, the algal culture produces significant amounts of oxygen through photorespiration and culture concentrations can reach more than five times the equilibrium oxygen concentration if no mechanism of vigorous gas exchange is provided (Neenan *et al*, 1986). Circulation also minimizes temperature gradients within the pond.

The containment systems used to cultivate algae generally fall into one of two categories, enclosed bioreactors and shallow ponds. It is also possible that the two could be combined in order to create a hybrid system; some would argue that a covered pond is a hybrid system. A description of both types follows; however, only the shallow pond configuration is addressed in this analysis.

Shallow ponds are assumed to be in the form of "raceway" ponds, so named because their shape is similar to an oval racetrack. Each pond is likely to be between 1000 to $10,000 \text{ m}^2$ (0.1 to 1.0 hectares), although ponds as large as 10 hectares have been proposed (Benemann and Oswald,

1996). In mass culture enterprises, multiple ponds are placed immediately adjacent to one another, separated by walls and roads, in order to create a module of 10 or more ponds. An individual pond typically consists of an oval separated by a central barrier, which creates two elongated, connected channels. Each channel has an aspect ratio (length to width) of between 10:1 and 20:1. A paddle wheel is used to keep the water circulating around the "track". Circulation of the water (culture medium) is required in order to optimize light exposure and temperature for all of the algae cells in the pond. Circulation also keeps the algae in suspension, ensures adequate circulation of nutrients, and allows oxygen generated by the biomass to more efficiently outgas from the culture medium. Regardless of the depth of the culture medium (water), the depth of the algae culture is limited to between 10 and 20 cm, due to the physical limits of light penetration and the energetic limitations of circulating the culture medium. (High circulation rates not only require significant amounts of power, but may also result in shear stress and potentially lethal damage to the algae). The nominal water depth is expected to be maintained at 30 cm (Figure 6.2). The additional depth below the base of the algae population allows for lack of planarity in the bottom surface of the pond, fluctuations in water depth due to evaporation and harvesting, and sedimentation on the bottom. Depths greater than this are avoided because it would add to the amount of water required, putting strain not only on the water supply but also on the material and energy systems needed to maintain the quality of the water environment. Although most, if not all existing ponds are uncovered, proposals for covered systems have been made.



Figure 6.2. The cross-section of an open pond system illustrates the expected distribution of a nominal 0.15 m deep algae culture in a 0.3 m deep culture medium.

Bioreactors consist of tubes or bags made of transparent plastic through which the algae and growing medium are circulated. They are thought to be potentially more productive in terms of the biomass to culture medium ratio and, if constructed using vertical or semi-vertical systems, may require less land. It is also easier to avoid contamination from dust, predators, and invasive species. Because evaporative losses are much lower, water consumption is reduced and controlling the chemical composition of the growing medium is expected to be more straightforward. However, there are a number of problems with bioreactors that have yet to be addressed. Material consumption (and the embodied energy in these materials) is anticipated to be high. It is expected that greater amounts of direct energy will be required for circulation to account for vertical lift and the higher surface area to volume when using tubes. The amount of surface area means that the temperature of the growing medium will change in response to

ambient conditions much more quickly and therefore both cooling and heating of the system is expected to be required. The most critical issue is the potential to produce a build-up of oxygen in the bioreactor, which can essentially shut down the photosynthesis process. This analysis does not consider bioreactors, in part because there is little available data that address this design approach.

6.1.1.2.3 Cultivation Strategies

The primary culture management variables are water salinity, nutrient concentration, CO₂ concentration, culture mixing/aeration, and residence time of the population. Culture management strategies include timing and composition of additions to and subtraction from the culture medium in order to control water chemistry and volume; control of nutrient levels (potentially including deprivation); timing and method of algae removal from the primary cultivation pond (harvesting), and species selection. With respect to species selection, use of multiple species has been suggested for outdoor culture systems where light and temperature will vary. Thus when environmental conditions are optimal for one species it may thrive while the others may experience suppressed growth. Another strategy may be to have linked continuous cultures. The first pond would contain adequate nutrients for biomass development; the second would be nutrient starved to promote relative increases in lipid and TAG content. Assuming that total lipid content is maintained, reduction in biomass growth could actually be a benefit in that there would be less material handling required during the harvesting operation.

6.1.1.2.4 Reporting Conventions

The term "growth rate" is not used consistently in the literature. It may be used casually to refer to biomass productivity, which is expressed as mass per unit area (or volume) per unit of time. In a continuously harvested system at steady state (or a semi-continuous system at near-steady state), the harvest rate, the dilution rate (i.e. introduction of medium) and mean biomass productivity are approximately equal. "Growth rate" is also used to describe the exponential or logarithmic growth constant, which is the natural logarithm of the ratio between the number of cells (or other measure such as mass, volume, or optical density) at the end of a unit of time (e.g. a day) to that at the beginning of the time period (ln [Nt₁/Nt₀]). If the number of cells doubles in one day, the growth constant (k) would be ln [2/1] or 0.69. Other investigators use "growth rate" to mean the number of doublings in biomass per unit of time (e.g., doublings per day). The reciprocal, referred to as doubling time or generation time (time to achieve one doubling), is also a common expression. Most algae exhibit 1 to 2 doublings per day (k = 0.69 to 1.39, Td = 0.5 to 1.0 (Burlew, 1953; Sheehan *et al.*, 1998; Griffiths and Harrison, 2009).

Biomass productivity describes mass of algae produced per day as a function of either area or volume. This may also be referred to as yield, although technically, yield takes into account harvesting parameters in addition to growth parameters. The overwhelming factor that controls biomass productivity is the area illuminated. The same productivity per unit area can be obtained with any combination of volume, depth, and concentration as long as the depth and/or concentration are enough for optical extinction to occur (Burlew, 1953). Similar results were found in the SERI/NREL studies (Sheehan *et al*, 1998). Concentrations of laboratory batch cultures are typically reported in terms of mass per unit volume (grams per liter). This may in part be due to difficulties in determining the exact area illuminated (Burlew, 1953). In mass

cultivation systems (especially flat ponds), algae productivity is typically measured in terms of mass per unit area (grams per square meter), as the surface area of the water is assumed to be equal to the area illuminated. A rough estimate of pond productivity based on laboratory results can be achieved by assuming a culture depth of 0.15 meters in the pond (with circulation). Thus, a reported density of 100 mg/liter-day (g/m³-day) would translate to (approximately) 15 g/m^2 -day. In general, however, laboratory results reported in terms of volume are best used to compare variables within a single experimental design.

6.1.2 Historical Trends

The first interest in mass cultivation of algae occurred during World War II, when these organisms were investigated as the potential source of a number of products including antibiotics and food. In the late 1940's, a small culture unit was built at Stanford Research Institute, but closed in 1950 due to lack of funding. The work was subsequently reinitiated through funding made available by the Carnegie Institution of Washington to Arthur D. Little, Inc. in Cambridge, Massachusetts for construction of a pilot plant. Supplemental laboratory studies were conducted by Jack Meyers at the University of Texas at Austin, and Robert W. Krauss at the University of Maryland. The primary interest in developing mass cultivation systems was to produce algae as a food source for animals and/or humans. The report that was generated as a result of these studies (Burlew, 1953) remains a valuable source of data and information with respect to mass cultivation and general algae characteristics.

Commercial production systems for cultivation of algae were developed in Japan in the 1960's. These facilities used circular ponds to grow *Chlorella* for human consumption. Since the 1970's, the blue-green algae *Spirulina* has been grown commercially in Mexico, California, and Hawaii. It is used for human food supplement, aquaculture feed, and food coloring. The species *Dunaliella salina* is grown in Australia, the US, and Israel as a source of beta-carotene, which can be used as a food colorant as well as a nutritional supplement. Microalgae also have a significant role in waste water treatment plants, although in general, the algae are not harvested (Benemann and Oswald, 1996).

The concept of cultivating microalgae for conversion to fuel (biogas, rather than biodiesel) was, according to Sheehan and others (1998), first suggested by R. L. Meier in the early 1950's. Between 1978 and 1996, the US Department of Energy (DOE), funded a program to develop fuels from algae through the Solar Energy Research Institute (SERI), which in 1991, became the National Renewable Energy Laboratory (NREL). This effort is described in an NREL document entitled "A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae" (Sheehan *et al*, 1998). The Aquatic Species Program focused on production of biodiesel from naturally occurring oils within the algae. Historical information through 1996 is taken from the NREL report.

In the 1970's a number of projects, many of them at the University of California-Berkeley, were conducted to look at open algae ponds as a means of wastewater treatment. The removal of algal biomass (harvesting) was a significant part of this research, as water discharged from wastewater systems is required to have a low suspended solids content (i.e., no algal cells). It was recognized that with an adequate harvesting system the harvested algae could serve as fertilizer or as a fuel source. One of the problems with early harvesting systems was that the wastewater

treatment ponds made no attempt to control the species present and thus the size of the organisms was quite variable. Early attempts at identifying the most advantageous species to cultivate, therefore, were driven by the ease with which the species could be separated from the growing medium. As the work progressed, emphasis shifted towards identifying algal species that produced significant amounts of lipids and that could survive under a range of growing conditions, including extremes of temperature, pH, and salinity. After considering more than 3,000 organisms, the potential candidates were reduced to approximately 300 species, mostly green algae and diatoms.

The first studies conducted by SERI/NREL took place in California and Hawaii over a six-year period. Based on the results of these early explorations, two 0.1 hectare (1000 m²) test ponds were built in Roswell, New Mexico. The southwestern US was identified as a desirable location based on the availability of large areas of flat land not used for other human activities, high incident solar radiation (low latitude and high percentage of sunny days), and the presence of saline ground water, which would minimize competition for freshwater needed for human consumption and agriculture. The Roswell ponds were operated for one year and demonstrated the feasibility of reasonably controlling the algae species grown, along with the ability to achieve a 60% utilization of injected CO₂ (and the potential to use 90% with recycle). Other important studies included an evaluation of water circulation systems. The range in growth rates observed during this program was 5 to 50 grams of algae per square meter per day (g/m²-day) for a single day. The annual average growth rate was 16 g/m²-day.

The work conducted by the US Department of Energy (SERI/NREL) failed to support the "trigger" theory, which postulates that environmental stress increases overall lipid production (Sheehan *et al*, 1998). Instead, it appears that other cell component production ceases, or is greatly reduced, which increases the relative amount of lipids in the cells, but not overall lipid content in the population. In fact, these authors conclude that overall productivity decreases as higher levels of oil in the cells are more than offset by lower rate rates of cell growth.

6.2 Algae as a Potential Feedstock

Algal biomass contains three main components, carbohydrates, proteins, and natural oils (lipids), which are discussed in detail in section 6.1.1.1 of this report. Biodiesel production applies exclusively to the neutral oil fraction that consists of triacylglycerols (commonly referred to as triglycerides and abbreviated as TAG or TG). The remainder of the biomass could have other uses, including other energy products such as methanol from gasification of any residue, or ethanol from fermentation of the carbohydrates. Other lipids also have potential applications as fuel feedstocks, but would require additional treatments and/or something other than simple transesterification as part of the production pathway. In this analysis only triglycerides are considered as potential feedstock for biodiesel via transesterification.

There have been several well documented studies that have investigated the lipid and/or fatty acid profiles of algal lipids (c.f., Tornabene *et al.*, 1982). Most clearly differentiate between algae that have been grown in nutrient deficient or nutrient replete environments.

6.2.1 Current Supply

There are no algae mass cultivation systems within the US currently operating at a scale that could supply algae as feedstock for the production of biodiesel in commercial amounts.

6.2.2 Potential Supply

6.2.2.1 Available Land

The following scenario analysis is undertaken in an attempt to define a physical maximum to potential algal cultivation in the US, specifically for the purpose of producing biodiesel from algal oil through transesterification. In this exercise, the maximum available land area is considered to be that located where there is 1) adequate sunlight, 2) a growing season of at least 250 days under natural conditions, and 3) lack of significant topographical relief. Further limitations on land availability are imposed by using data from the Natural Resources Conservation Service (NRCS, 2007) and assuming that the percentage of land in different land use categories is evenly distributed across the state. All land is taken to be available for growing algae except for four categories: 1) existing cropland (excluding that in the Conservation Reserve Program (CRP)), 2) federal land, 3) developed land, and 4) water areas. Although realistically, much of this land would be not be suitable, it is, at least for this exercise, regarded as land on which algae conceivably could be grown, given strong enough market forces and/or policy.

The basic requirements for mass production of algal (using known species) limit high productivity cultivation to regions with relatively high solar insolation (Benemann et al., 1982). Photosynthesis, which requires sunlight, is the process through which algae biomass accumulates Fatty acids are synthesized in the chloroplasts (the site of and carbon is assimilated. photosynthesis) and are the building blocks of lipids. As carbon is the major elemental component of fatty acids, lipid accumulation is also related to photosynthetic efficiency. Optimal growth rates for most algae occur over a temperature range that many organisms would find comfortable (roughly 20° C \pm 10° C). While they may not die at temperatures below 10° C or above 30° C, most species are also unlikely to thrive and produce significant amounts of biomass. Assuming that large shallow ponds (rather than bioreactors) will be used to grow the cultures, the land upon which the ponds are located must be relatively flat. There are other significant, practical, limiting factors, such as access to water and CO₂ that would likely influence the location of mass cultivation facilities; however, climate and surface gradient are assumed to define the minimum limits for which accommodations cannot be made without significant expenditures of energy for lighting, temperature control, and/or creation of a level surface. It is assumed that any significant implementation of supplemental resources and activities would negate the energy value of the produced algae culture.

For the purpose of this analysis, the following limits for the above parameters were established based on the collective documentation of the studies conducted during the US Department of Energy's (US DOE) Aquatic Species Program (Sheehan, et al., 1998; Maxwell et al., 1985, Benemann et al., 1982, etc.) and on an Australian study by Qin (2005).

- Annual insolation, as measured using a fixed horizontal plate collector, must be at least 4.5 kWh/m²/day (equivalent to 387 langleys or 16.2 GJ/m²/day). Areas meeting these criteria were identified with maps supplied by Benemann et al. (1982) and the US Department of Energy (NREL, 2010).
- 2) The number of cold days must be fewer than those experienced at the US DOE test pond in Roswell, NM, where it was determined that an annual growing season of less than 250 days was too short to achieve desired biomass productivity levels. The US Department of Agriculture's Plant Hardiness Zone Map (USNA, 2003) was used to locate areas with fewer freeze days than Roswell, NM (Zones 7b or higher).
- 3) The number of days when temperatures exceed 30° C (86° F) must be less than 50% within a given year. The American Horticultural Society's Heat-Zone Map (AHS, 1997) was used to identify areas that experienced more than 180 days per year above 30° C (86° F) (Zones 11 and 12). While some algae grow well between 30 and 35° C, it is assumed that if the median high temperature is greater than 30° C, there are many days when the maximum temperature is significantly hotter than 35° C.
- 4) The slope of the land over the extent of the pond area should 0.5% or less. Hilly and mountainous areas (at a large-scale) were excluded using maps provided by Maxwell *et al.* (1985) as well as large-scale topographic maps of the US.

Making a gross-overlay of these maps and imposing the above limits, a crude estimate of the total land area available for mass algae cultivation in the US can be made. Based on insolation values only, there are eleven candidate states for algae production. Applying all the above imposed limits to these 11 states results in the following potential land areas:

90% of Louisiana (LA): 10% of the state is excluded based on insolation.

70% of Georgia (GA): 30% of the state is excluded based on insolation and cold.

65% of Florida (FL): There are no restrictions based on insolation, topography, or cold. Excessive heat excludes 35% of the state, a significant portion of which is located in the environmentally sensitive Everglades.

60% of South Carolina (SC): 40% of the state is excluded based on insolation.

55% of Texas (TX): There are no restrictions based on insolation; 10% of the state is too hot, 30% is too cold, and 10% of the remainder too hilly/mountainous.

50% of Alabama (AL): 50% of the state is excluded based on insolation

50% of Mississippi: 50% of the state is excluded based on insolation.

40% of North Carolina (NC): 60% of the state is excluded based on insolation and topography

30% of California (CA): 10% of the state is too hot; an additional 30% is too cold and/or has low insolation; 50% of the remainder is too hilly/mountainous.

15% of New Mexico (NM): 85% of the state is too cold and/or mountainous

10% of Arizona (AZ): 20% of the state is too cold; 30% is too hot, and 80% of the remainder is too hilly/mountainous

These are relatively crude estimates, but are believed to be no less accurate than currently available predictions regarding algal productivity and data regarding production requirements. These state-level area percentages are combined with land use data from the Natural Resources Conservation Service (NRCS, 2007) to estimate the maximum possible land area within the continental US for mass cultivation of algae (Table 6.1). Based on these criteria, there are a total of 181.6 x 10^6 acres (73.5 x 10^6 hectares) of available land where algae could be successfully grown.

Table 6.1. Land use and calculation of maximum available land for mass cultivation of algae in the continental US, assuming a land-use based on 2003 data from NRCS, 2007 and estimating the amount of land with adequate insolation, level surfaces, and minimal temperature extremes.

			Av	vailable Lar	nd			Unavail					
State	% of Area Meeting Minimum	CRP Land	Pasture- land	Range- land	Other Rural Land	Forest Land	Maximum Algae Land	Cropland	Developed, Federal, and Water Areas	Total Surface Area			
	Requirements		1000 Acres										
AL	50%	230	1,700	36	225	10,765	12,956	1,255	2,502	16,712			
AZ	10%	0	8	3,225	303	414	3,951	93	3,252	7,296			
CA	30%	40	357	5,327	1,387	4,171	11,282	2,840	16,330	30,453			
FL	65%	51	2,353	1,753	1,825	8,276	14,257	1,868	8,272	24,397			
GA	70%	206	1,959	0	598	15,325	18,087	2,906	5,425	26,418			
LA	90%	180	2,024	255	2,646	12,004	17,110	4,892	6,238	28,239			
MS	50%	395	1,612	0	214	8,377	10,598	2,488	2,178	15,264			
NM	15%	87	35	5,993	309	822	7,246	232	4,195	11,673			
NC	40%	35	733	0	349	6,182	7,300	2,205	3,979	13,484			
SC	60%	109	656	0	499	6,697	7,960	1,421	2,583	11,964			
тх	55%	2,196	8,710	52,860	1,257	5,837	70,861	14,059	9,158	94,079			
	1000 acres	3,529	20,147	69,451	9,611	78,871	181,609	34,259	64,111	279,979			
Total	1000 hectares	1,428	8,153	28,106	3,890	31,918	73,494	13,864	25,945	113,303			

6.2.2.2 Algae Productivity

Many projections have been made regarding the potential productivity of algae, in terms of both biomass yield and the amount of lipids and/or TAG that may be produced within the biomass. Several points are worth noting.

1) The variability that occurs in mass yield and chemical composition of algae is likely to resemble that of an agricultural system rather than a manufacturing system (in particular, if it is assumed that algae are grown in open ponds rather than in bioreactors). For example, cotton is grown in the US over a geographic extent that roughly corresponds to that being considered for algae. Almost all cultivated cotton (96%) consists of just one species, and most plants are genetically modified. The mean yield for cotton in 2007 was 994 kg/ha (based on county-level data in USDA, 2009). The range in yield at the 95% percentile was 540 to 1772 kg/ha, which is approximately plus or minus 50% of the mean, with a skew towards lower yield (log normal distribution). The variability over multiple years would be much greater. Outdoor cultivation of algae is likely to exhibit a

similar annual pattern from pond system to pond system. In addition, because algae are harvested on a daily rather than an annual basis, a similar type of variability would be expected within a single pond system, thus compounding the variance for the US algae production system as a whole.

- 2) Biomass yield numbers are expressed as mass per unit area when describing open ponds (e.g. 20 grams per meter squared per day (g/m²-day)). This area applies only to the surface area of the cultivation medium; it is not the full footprint of the algae farm or even the cultivation pond. It does not include area occupied by walls and dividers of the pond; nor does it include auxiliary ponds such as those used to grow the starter culture or for harvesting (inoculum or settling ponds). Land is also required for storage of culture medium, waste management, and control systems.
- 3) Yields must be expressed for one full calendar year. Thus if an average biomass yield is 50 g/m²-day, but growth occurs during only 182 days out of 365, then the yield for the purposes of this analysis are taken to be 25 g/m²-day. Most data in the literature represent yields observed over relatively short times under optimum temperature and other conditions. Extrapolations to annual yields may or may not accurately reflect environmental fluctuations that would occur over a full year.
- 4) Algae grown in any large system are almost certain to exhibit lower mean biomass productivities than those in smaller ones simply because it is harder to control conditions. This is especially true when attempting to translate laboratory results to outdoor ponds.
- 5) There are physical limits to the fractional amount of lipids that a single algal cell may contain. The maximum is unknown, but certain constituents cannot be eliminated, including the nucleus, the chloroplast(s), organelle and cell membranes, and some amount of proteins (including enzymes). An algae cell must contain at least 20 to 30% proteins and carbohydrates in order to survive, even for short periods of time. Normal functioning (active photosynthesis and reproduction) requires at least 50% non-lipid constituents. Assuming that cells are not "milked" for lipids, average amounts lipids greater than 70-80% of total biomass would seem to be a physical maximum.
- 6) There are physical limits to the biomass density. The most important factor is "shading" of algae furthest from the light source by those closest to it. The ability to overcome this by mixing and agitation is limited not only by the desire to minimize energy consumption, but also by the potential to inflict stress and strain damage to the cells during movement. Other factors such as excess oxygen, a waste product of photosynthesis, may also play a role in limiting population density.
- 7) Biomass productivity numbers do not account for losses that occur during harvesting. Some portion of the biomass will remain as residue on or in any equipment that is used to separate the biomass from the culture medium. Harvesting systems are still being developed so the relative amount of loss is unknown, but harvesting efficiency is a known challenge. In addition to loss of algal biomass, water that is removed with and within the algae biomass will be lost. The amount depends upon the nature and efficiencies of the harvesting and reclamation systems.

6.2.2.3 **Area Requirements**

The analysis presented here is intended to characterize likely yields of algal biomass, the lipid content of the harvested algae, and the weight percent triglycerides in the lipids. Under largescale production systems, these yields and chemical compositions need to represent the average values over a broad geographic area and over the full growing season, which even under the best climate conditions would be less than 365 days because of downtime and maintenance. The constraints applied above are expected to define areas that have a growing season of the equivalent of 10 months (300 days) \pm 1 month (30 days). This means that on average, 65 days out of 365 (17.8%) are non-productive due to 1) climatic/weather conditions, 2) system management (e.g. cleaning, chemical adjustments, etc.), or 3) system failure.

A number of studies have indicated that algal lipid and TAG content may be increased by inducing short-term environmental stress, particularly due to a sharp decrease in nutrient availability. However, it also has been shown that such treatments retard or even reverse biomass productivity (Sheehan et al., 1998). One proposed solution to this is to use two types of ponds in series, whereby biomass production is first optimized in a nutrient replete cultivation pond; algae are subsequently transferred to a separate induction pond with conditions selected for producing higher lipid and TAG contents within the existing biomass. Induction requires at least the same level of light exposure as the cultivation pond (Benemann and Oswald, 1996); therefore, the induction ponds would have the same footprint as the cultivation ponds, and an accurate reporting of biomass productivity needs to account for the surface area of both pond types. If biomass production ceases in the induction pond and retention time is equivalent, the true system-level biomass productivity is half that reported for the cultivation pond alone. An alternative approach would be to use a single pond for both phases (biomass growth and induction). However, this would require batch culture and likely double the amount of time per unit mass of algae produced (thus similarly reducing overall biomass productivity by 50%). A constant nitrogen limited environment in the cultivation pond does not produce the same effect as changing from a nutrient replete to a nutrient depleted environment (Benemann and Oswald, 1996). For this analysis, it is assumed that sustained, extreme levels of lipid and TAG production (as mass fraction of biomass) at very high biomass productivities are not possible with current levels of cultivation system and biotechnology.

Harvesting is taken to be semi-continuous, rather than batch, with one half of the algae-rich cultivation medium removed at sunset and an equivalent volume replaced before sunrise. This harvest rate assumes that the cell density doubles once per day, which is a common growth rate for microalgae (Sheehan et al, 1998). Such an approach approximates a two-day retention time for the algae and takes advantage of the lack of photosynthetic activity during the night. Harvesting frequently is thought to maximize biomass production by ensuring that the population is always in the exponential growth stage and by minimizing biomass density, and consequently competition for resources (especially light). Although growth rates are likely to be quite variable over the course of the year, the harvest system requires the handling of such large volumes that the system is best designed in response to pumping rates rather than biomass growth rates. The fact that algae do not grow at night also precludes the use of a truly continuous system.

The following analysis assumes that algae are contained within a shallow racetrack pond designed in a manner similar to that described by Benemann and Oswald (1996) and in the SERI/NREL reports summarized by Sheehan and others (1998). This may or may not reflect the design of any future systems, but there is currently no evidence that equally energy and material efficient designs are available. The ponds are thus open, land-based, and physically constrained (impermeable wherever the land is in contact with the algae culture medium). While algae have the potential to serve as feedstock for a number of fuel types, this analysis examines only the case where algae are grown as a feedstock for fatty acid methyl ester biodiesel (FAME). Biodiesel is assumed to be produced via transesterification of algal lipids that occur naturally in the algae biomass as TAG. There is no artificial light or temperature control in the growth ponds. The nominal depth of the algae biomass is 15 cm, plus or minus 5 cm, and the nominal depth of the culture medium, consisting primarily of water, is 30 cm (see Figure 6.2).

The analysis addresses six factors that are considered at three levels: low, nominal, and maximum. Low values represent a conservative number that has been demonstrated during pilot scale operations. Nominal values are those that have been achieved in a laboratory setting but which have not been demonstrated at scale. These are considered optimistic, but possibly achievable. Maximum values are meant to represent physical limits that are unlikely to be realized and that would require significant amounts of engineering. The output is the amount of water surface area required to produce a unit amount of algae biodiesel from TAG.

6.2.2.3.1 Growing Season

The effective growing season will vary depending upon local conditions. The 73 x 10^6 hectares located in the eleven states where climate and terrain are expected to be conducive for mass production of algae are characterized by a significant variety of climates and microclimates. Given the constraints placed on defining the locations where algae is likely to be cultivated, a lower limit of 250 growing days per year (not necessarily contiguous) is assumed. For both the nominal and maximum cases a total of 300 effective growth days per year is assumed.

6.2.2.3.2 Biomass Productivity

Benemann *et al.*, 1982, predict that a mean annual growth rate of 22.5 grams of algal biomass per square meter per day (g/m²-day) could be sustained over a 300 day growing season. The DOE test site in Roswell, NM produced an annual average of 16 g/m²-day over a season that was just under 250 days long (Weissman *et al.*, 1989). Benemann and Oswald (1996) project that a well-designed system could, under optimum conditions, produce 30 g/m²-day. The same authors give a theoretical maximum of 60 g/m²-day. (It is important to note the words "theoretical" and "maximum;" there is no documented evidence that such production levels can be sustained over extended periods of time in large-scale production systems). The geographic extent considered in this analysis means that there will be less than optimum conditions in many locations. Thus in this analysis, the nominal value for biomass productivity is taken to be 24 g/ m²-day. The range is taken to be \pm 33%, based on the variability observed in US cotton (see discussion above). Although the actual distribution is likely to be log normal, a normal distribution is assumed here for the sake of simplicity. The conservative range is taken to be 10 to 20 g/ m²-day (15 \pm 33%) and the maximum is taken to be 32 to 64 (48 \pm 33%); this places the absolute maximum slightly higher than the 60 g/ m²-day suggested by Benemann and Oswald (1996).

6.2.2.3.3 Percent Usable Oil

The lipid content of most eukaryotic microalgae grown in a nutrient replete environment is generally between 15 and 25% of the biomass and the percent of all lipids that occur as triglycerides is typically about 20% by mass (e.g.,. Benemann and Oswald, 1996, Table 6.1; Hu et al., 2008; Griffiths and Harrison, 2009). Note that these values are highly dependent on both the species and growing conditions; therefore these are intended to represent central tendencies rather than absolute values. The conservative mean value for each of these parameters (lipids as a percent of total dry biomass and TAG as a percent of total lipids) is therefore set at 20% \pm 10%, or 18 to 22%. When multiplied together, the likely TAG content, as a percent of total biomass, is 3 to 5% (18% * 18% for the lower end of the range and 22% * 22% for the upper end).

Nutrient stressed algae may produce excess total lipids in the range of 40 to 60%, with up to 80% neutral lipids, over short periods of time; however, total biomass is also reduced (Sheehan et al, 1998). Long-term mass culture of algae that produces acceptably high biomass densities and a higher percentage of neutral lipids has yet to be demonstrated. Algae need some amount of non-lipids (proteins and carbohydrates) in order to maintain basic cell functions, including photosynthesis. Polar lipids are required to keep membranes intact. The maximum percentage of non-polar lipids (primarily TAG) reported, based on laboratory studies, is 80% of total lipids. Data in the literature are not as clear as to the maximum amount of lipids that can be present, but 80% is a generous maximum (it is likely closer to 65%). There may be an as yet unidentified or yet to be engineered species that can produce lipids and TAG at these levels while still maintaining a viable culture, but 80% is assumed to be the absolute physical maximum for each of these. As some amount of variability is to be expected, the mean value for each of these is set at 73% \pm 10%, to give a range of 66 to 80%. When multiplied together, the range in maximum TAG content possible, as a percent of total algae biomass, is 43 to 64%.

The nominal values for lipid and TAG content selected for this analysis are set at a mid-point between observed values in healthy algae (i.e., those grown in a resource-replete environment) and environmentally stressed algae. It is thought that this could be achieved through species selection and/or engineering, tightly controlled environmental conditions that induce only minor stress, and well-timed harvesting. The average lipid content is taken to be $35\% \pm 10\%$, and the average %TAG within the lipid content at $40\% \pm 10\%$. When multiplied together, the range in usable oil (TAG as a percent of total biomass), is 11 to 17%.

6.2.2.3.4 Harvest and Extraction Efficiencies

Harvest and extraction processes for microalgae are currently in the research and development phase. Centrifuges may achieve as high as 90% recovery, but for relatively low starting concentrations, 70% is more typical (Benemann and Oswald, 1996). Sedimentation is expected to result in 60 to 80% recovery, depending upon whether flocculation agents are used and their effectiveness. Neenan *et al*, 1986 suggest a range in harvest efficiencies of 50% to 98% with 90% as the nominal value. For this analysis, 85% is taken to be the nominal value, 65% is the conservative value, and 95% is taken to be the theoretical maximum.

61,200 kilograms/hectare-year

(6.1)

The extraction methods that are likely to be used are not defined. Efficiencies of 80%, 90%, and 95% are chosen to represent three levels, but these are assumed to be illustrative rather than representative of any particular technology selection.

A table of all factors, the range in values assumed, and results are presented in Table 6.2.

Overall, the potential average annual US algal yield in grams per hectare-year is estimated to be

$$24 \text{ g/m}^2$$
-day * 300 days / yr * 10^4 m^2 / ha * $1 \text{ kg} / 10^3 \text{ g}$ * $0.85 =$

If the harvested algae have a triglyceride mass fraction of 0.14, the resulting TAG yield would be 8,568 kg/ha-yr. It is assumed that 90% of this, or 7,711 kg/ha-yr can be effectively extracted and used for feedstock in a 1 to 1 conversion to FAME biodiesel. At a density of 3.36 kilograms per gallon, this is equivalent to 2,294 gallons (8,685 liters) per hectare-year. Conversely, 436 hectares (1077 acres) of cultivation pond surface area is required per 10^6 gallons of triglycerides. If a lower heating value of 33.32 MJ / liter (ANL, 2009) is assumed, this equates to an energy production rate of 289,379 MJ /ha-yr.

The amount of water surface area required for a given volume of triglycerides is extremely sensitive to the assumptions made regarding biomass yield, lipid yield, TAG content, harvest efficiency, and extraction efficiency. None of the values used in equation 6.1 have been demonstrated in a mass-cultivation system over a time period of at least a year; however, laboratory works suggests that these numbers are achievable. They are, therefore considered optimistic, but potentially realistic.

Table 6.2 .	Factors and	d assumptions	used to	estimate	requirements	for water su	rface area	of an
algae cultiv	ation pond.							

Factor	Units		Low	Nominal	Maximum	
Biomass Proc	luction	Calculation / Assumptions	conservative/ demonstrated at pond scale	optimistic/ observed at limited scale	expected physical limit	
Biomass productivity	g/m²-day	mass of algae produced daily during the growing season per unit of surface area of water (cultivation ponds only) - mean	15	24	48	
(daliy)		estimated range	10 - 20	16 - 32	32 to 64	
Growing season	days/year	growing season over identified geographic area - mean	250	300	300	
		estimated range	220 - 280	270 - 330	270 - 330	
Annual mass	$a/m^2 vr$	= biomass productivity * growing season	3,750	7,200	14,400	
cultivated	g/m -yr	estimated range	2,200 - 5,600	4,320 - 10,560	8,640 - 21,120	
Harvest efficiency	% of mass cultivated	accounts for losses due to residues on harvest equipment and unrecovered algae in pond	65%	85%	95%	
Biomass	g/m²-yr	= harvest efficiency * annual mass cultivated	2,438	6,120	13,680	
naivesteu		estimated range	1,430 - 3,640	3,672 - 8,976	8,208 - 20,064	
Triglyceric	les	Calculation / Assumptions	Low	Nominal	Maximum	
% Lipids	wt% of	dry weight biomass that consists of polar and neutral lipids - mean	20%	35%	73%	
-	DIOITIASS	estimated range	18% - 22%	32% - 39%	66% - 80%	
% TAG	wt% of	percent lipids that consist of TAG - mean	20%	40%	73%	
% TAG	lipids	estimated range	18% - 22% 32% - 39% 66% - 80 mean 20% 40% 73% 18% - 22% 36% - 44% 66% - 80 4% 14% 53% 3% - 5% 11% - 17% 43% - 64	66% - 80%		
% Llooblo oil	wt% of	= % lipids * % TAG	4%	14%	53%	
	biomass	estimated range	3% - 5%	11% - 17%	43% - 64%	
	g/m²-yr	= usable oil * biomass harvested	98	857	7,290	
TAG harvested	ka/bo vr	= unit conversion	975	8,568	72,901	
	ку/па-уг	estimated range	463 - 1762	4,164 - 15,205	35,430 - 129,374	
TAG extraction efficiency	wt% of TAG	accounts for losses due to inability to separate TAG from biomass and other lipids	80%	90%	95%	
Feedstock	kg/ha-yr	= TAG extraction efficiency * TAG harvested	780	7,711	69,256	
production		estimated range	371 - 1,409	3,748 - 13,685	33,658 - 122,906	
Area Require	ments	Calculation / Assumptions	Low	Nominal	Maximum	
Conversion efficiency	biodiesel / TAG (w/w)	common assumption	1.0	1.0	1.0	
Biodiesel density	kg/gallon	from ANL, 2009	3.361	3.361	3.361	
Annual volume of biodiesel per unit		 = conversion efficiency * biodiesel density * feedstock production 	232	2,294	20,606	
surface area of cultivation pond water	gal/ha-yr	estimated range	110 - 419	1,115 - 4,072	13,621 - 32,468	
	ha-yr/gal	= reciprocal	0.00431	0.00044	0.00005	
Water surface	ha-yr/10 ⁶	= unit conversion	4,309	436	49	
area per unit volume of	gal	estimated range	2,385 - 9,068	245 - 897	31 - 73	
biodiesel	ha-yr/10 ⁶	= unit conversion	1,138	115	13	
	liters	estimated range	630 - 2,395	65 - 237	8 - 19	

Yields of algal biomass, as well as lipids and triglycerides, are expressed as functions of the surface area of the water in the cultivation pond only. The total amount of land needed for each pond is greater than the water area, and facilities other than the cultivation ponds are required to support a complete operation. Although there are a number of construction options for algae ponds, the most likely scenario is one that is similar to rice cultivation, where earthwork berms or levees are used as barriers within and between ponds and to form the perimeter around the ponds. Flattened crowns on the crest of the berms provide access to the ponds by foot or light vehicle. Benemann and Oswald (1996) present design factors for a large scale pond constructed in such a manner. Using the dimensions described for berm construction by these authors, it is estimated that the total amount of land needed for each cultivation pond, including the walls and channel dividers is 25% greater than the active, aqueous surface area of the pond itself. In addition to the cultivation pond, settling ponds are assumed to be used in the harvesting process. If half the volume of each cultivation pond is harvested per day (i.e., assuming a 2 day retention time), settling ponds that are capable of containing half the volume of water are required. Benemann and Oswald (1996) refer to a design described by Benemann et al, 1982, where settling ponds are 8 foot deep straight-walled structures that occupy an area equal to 14% of surface area of the ponds. In addition to harvesting areas, land will be required for inoculum pond(s), control facilities and infrastructure. These are estimated to require an additional area equal to approximately 15% of the harvest ponds (Weissman and Goebel, 1987). The area required for inoculum development is assumed to be 10% of the cultivation area (Benemann and Oswald, 1996). In total, it is estimated that the land required for a complete algae cultivation facility will equal approximately 165% of the water surface area of the cultivation pond: (the exact proportion is dependent upon the size and design of the system). Therefore, it is projected that 719 hectares (1,777 acres) of land will be occupied per 10^6 gallons of triglycerides produced.

The energy content of methyl-ester biodiesel, measured as the lower heating value (LHV) is 37.53 MJ/kg, or slightly less than the value for petroleum diesel, which has an LHV of 42.79 MJ/kg (ANL, 2009). Thus, 1 gallon of biodiesel would displace 0.88 gallons of petroleum diesel. Assuming a 1 to 1 conversion rate of triglycerides to biodiesel, meeting the 2008 US motor distillate demand of 43.8×10^9 gallons using algae-derived biodiesel would result in the use of 35.8×10^6 hectares (88.4×10^6 acres) of land.

719 ha /
$$10^6$$
 gal biodiesel * 43.8 x 10^9 gal diesel / $0.88 = 35.8 \times 10^6$ hectares (6.2)

This is nearly half (49%) of the 73 x 10^6 hectares of land in the US estimated to be physically capable, at the most basic level, of supporting mass cultivation of algae (Table 6.1) and is approximately equal to the amount of US land planted in corn each year. (Corn is currently the most significant crop, in terms of land use, grown in this country.) As there are no existing facilities, all of this area would undergo land-use change.

Design and material changes could result in a lower overall footprint for the pond system, but the surface area of the cultivation ponds establishes an absolute minimum land area requirement. If 436 hectares (1077 acres) of cultivation pond surface area is required per 10^6 gallons of triglycerides this minimum value can be calculated as

436 ha /
$$10^6$$
 gal biodiesel * 43.8 x 10^9 gal diesel / $0.88 = 21.7 \times 10^6$ hectares (6.3)

This suggests that the surface area of water required to grow enough algae to fully replace petroleum motor diesel in the US is slightly less than that of the Great Lakes, which cover 24.4 x 10^6 hectares. Total existing surface area of waterways for the regions presented in Table 6.1 is just over 4.7 x 10^6 hectares suggesting that potential harvesting of wild algae for biodiesel could displace only a small portion of US demand for transportation diesel. Cultivation of saline algae is in coastal waters has also been proposed. This would minimize the impact on land use and allow pumping of surface water rather than from saline aquifers. The length of the general coastline in the southern US (i.e., Virginia to Texas, plus California) is 6,036,649 meters (CRS, 2006). Providing the 21.7 x 10^6 hectares estimated in equation 6.3, would require continuous algae cultivation areas along the southern US coast, 35.9 kilometers (22.3 miles) out to sea. It is important, however, to note that these numbers are extremely sensitive to assumptions about yields and efficiencies at every process step.

The area of developed land in regions conducive to mass algae propagation is equal to 25.9×10^6 hectares. It is assumed, as a rough estimate, that a maximum of 50% of developed land consists of impervious cover. For the purposes of this exercise, the existing impervious cover is assumed to be 12×10^6 ha. Algae ponds are expected to be built using an impermeable barrier between the culture medium and the surrounding land; thus the minimum surface area of impervious cover for algae ponds is equal to the surface area of the water (i.e., the pond bottoms). Total impervious cover is estimated to be roughly half-way between the minimum and maximum land occupation values or approximately 29×10^6 hectares. Thus building of algae ponds to support biodiesel at present petroleum diesel consumption levels would result in a nearly 250% increase in impervious cover in an area of the country that relies on heavily on ground water and thus recharging of aquifers. This does not include paving due to support systems for the algae ponds and infrastructure (including roads) for access. One means of minimizing this dilemma would be to use covered ponds and a rainwater collection system that then could be used to supply makeup water to the ponds. This means the covers need to be not only transparent, but also relatively rigid (thick).

6.3 Algal Oil Biodiesel, Life Cycle Assessment

The life cycle assessment approach taken is that of an attributional rather than consequential LCA and evaluates the typical practices in the United States in the year 2007 (approximately). A description of life cycle assessment, and in particular, its application to transportation fuels is addressed in Chapter 1 of this report. A simplified process flow, illustrating the overall life cycle of biodiesel produced from algae oil feedstock is presented in Figure 6.3.

Analysis of Innovative Feedstock Sources and Production Technologies for Renewable Fuels EPA: XA-83379501-0



Figure 6.3. Biodiesel as FAME (fatty acid methyl ester) produced from algal oil can be characterized by three life cycle stages each potentially separated by a transportation event.

6.3.1 Algal Oil Biodiesel, LC Stage 1, Raw Material Acquisition: Land Preparation, Propagation, Nurturing, and Harvest

6.3.1.1 General

6.3.1.1.1 System Boundaries

The first life cycle stage in the production of biodiesel from algae is the acquisition of algae mass through an open-pond growing system located in the US. This entails preparation of the site including construction of containment, harvesting, and inoculum ponds, introduction of an inoculum or "starter" algae, tending of the live algae, and removal of the algae from the pond (harvesting). Because there are virtually no commercial scale data for this operation, all values are estimates derived from information in publicly available reports and the peer reviewed literature. The system includes the consumption of raw materials, energy, land, and water, as well as captured waste and emissions to air. Not included in the system are infrastructure (other than the ponds), equipment manufacture, and emissions to water and soil. Upstream energies associated with production of energy and nutrients are included, but development of infrastructure and manufacture of farm equipment are not (Figure 6.4). The downstream system boundaries are terminated with the harvesting of the crop, thus transport and storage activities from and off the algae farm are included in the next life cycle stage (stage 2). This decision is driven primarily by the change in reference flow from a unit area of land in life cycle stage one to a unit mass of algae biomass in life cycle stage two and the recognition that activities for transportation and storage are better modeled in units of mass.



Figure 6.4. The above shows a simplified process flow and system boundaries for algal oil biodiesel life cycle stage 1 (raw material acquisition), which includes land preparation, propagation, tending, and harvest.

6.3.1.1.2 Units

The basis for the first stage of the life cycle is one hectare of harvested area per year (1 ha-yr). In the case of algae, this is taken to be equal to the surface area of the cultivation ponds. This approach is taken because most of the inputs are independent of biomass yield. A final transformation to algae produced per harvested area per year (kg/hectare-yr) is performed at the end of this stage, along with the embodied inventories, for input into the second stage of the life cycle where the basis is one kilogram (1 kg) of algae mass with variances noted as a function of harvest yield.

6.3.1.1.3 Resources

Growing algae requires, as do all agricultural products, land, sunlight, water, and nutrients. The amount of land that must be committed (actively managed) in order to produce a hectare of algae is greater than the cultivated area. Additional land is required for propagation, harvesting, water storage, and waste management. The total amount of land suitable for growing algae is limited by climate, terrain, and competing demands from other land uses. Sunlight is limited by location of the site (degrees latitude) as well as cloud cover and wind (which causes water to ripple and results in a decrease in available light). Although it is expected that a significant volume of water will be reclaimed and reused, all water is expected to be sourced initially through pumping

from surface water or groundwater systems. Rain, unless collected and added under controlled conditions, could be detrimental rather than beneficial, as it will alter the chemistry of the growing medium. Nutrients naturally available in the makeup water are insufficient for commercially viable production, thus these also must be supplied. Equipment, buildings, and energy in the form of electricity and liquid fuel are required to manage these resources. A containment system for the algae and its growing medium are absolutely essential for the assumed cultivation model. Unless the pond is located in an area of impermeable clay (expected to be a very small portion of the total available land), the surface of the containment system must be lined in order to avoid the loss of water and to avoid biological and chemical contamination. In some instances a clear plastic cover may be used to control the growing environment.

6.3.1.1.4 System Design

The following pond system design is based primarily on ideas and information presented in Benemann and Oswald (1996) and Weissman and Goebel, 1987. The basic assumptions are as follows. The pond system includes a minimum of 10 cultivation (growth) ponds, placed side-byside. Each individual cultivation pond consists of two elongated channels, separated by a divider. A single paddlewheel located at the end of one of the channels is used to circulate the water down each channel and around curved connecting ends to create a continuous loop (Figure 6.5). The channels are designed to hold the algae culture medium (primarily water) at a nominal depth of 0.3 meters. The walls of the pond are higher, with the crown at 0.7 m above the bottom surface of the pond. The aspect ratio for each channel (length to width) may be as low as 10:1 or as high as 20:1. For every cultivation pond, there is a deep (3 m) harvest pond associated with it that is capable of holding at least half the cultivation pond volume. An inoculation system, similar to that described by Benemann and Oswald (1996) is located on site; it consists of 1 open pond, equivalent in scale and design to a cultivation pond, for every 10 cultivation ponds; a covered pond with an area and volume equal to 1% of the cultivation pond system; and a system of small-scale bioreactors. Shallow evaporation ponds are used to precipitate out salts from water released during the blowdown process.



Figure 6.5. Each individual pond is assumed to consist of two channels connected together to form a continuous loop. The channels are separated by a divider. A single paddlewheel is used to circulate the culture medium around the racetrack configuration. The length (L) to width (W) ratio of the channels may range from 10:1 to 20:1.

The harvest ponds are assumed to be constructed with concrete. The racetrack ponds (both cultivation and inoculum) are assumed to be formed using earthworks construction, employing berms (walls and levees) made of compacted soil. The sides and bottom are lined with plastic, or alternatively, as proposed by Benemann and Oswald (1996), the slopes are stabilized using a

geotextile and the bottom is sealed using clay. The specific cross-sectional dimensions proposed by Benemann and Oswald (1996) are illustrated in Figure 6.6 and are taken to be representative of earthworks ponds, regardless of the areal dimensions of the pond.



Figure 6.6. The cross-section for a set of earthwork ponds is assumed to have the dimensions proposed by Benemann and Oswald (1996), independent of the areal size of the pond. A plastic liner may replace the geotextile on the sides and clay surface on the bottom.

Benemann and Oswald (1996) propose a 10 hectare pond design with channels that are 1000 meters (1 kilometer) long. The argument for such an aggressive design rule is that it will minimize the amount of land required for building supporting walls. However, the land area savings between a 500 meter long pond and a 1000 meter long pond is rather small (a total difference of 11%). The concern with an extremely long pond is that not only would it be difficult to complete the original land grading with the required planarity (less than 10 cm deviation along the entire length), but this tolerance must be maintained over the life of the pond (10 or more years), which for an earthworks pond seems doubtful. A plot of the factor increase in total land area required for an individual pond (surface area plus channel divider plus walls) as a function of surface area of water in the pond is given in Figure 6.7. It can be seen that any advantage in decreased pond footprint as a factor of the pond surface area quickly declines for pond sizes greater than 1 hectare and the benefit is relatively small for ponds with between 0.5 and 1.0 hectares of water area.



Figure 6.7. The walls and channel dividers add to the total pond footprint, but it is most pronounced when the surface area of the pond drops below 0.5 hectares. Above 1.0 hectares, the rate at which the factor increases with increasing pond size is small.

A one-hectare pond (water surface area) is assumed for this analysis. The channel dimensions in each of the straight sections are 18 x 245 m, which for two channels gives a surface area of 8820 m². The additional 1180 m² is provided by the curved ends, which are assumed to have a radius of 19.4 m (the width of the channel plus one half the width of the channel divider). The total land area required for each pond is 12,565 m² (or 126% of the area covered by water).

The pond system is taken to be a total of 20 growth ponds, 20 harvesting ponds, 3 inoculum ponds, and several small inoculum bioreactors. Two of the inoculum ponds are equal in size to the cultivation ponds; the third has 0.2 hectares (2000 m²) of surface area (using 8 x 108 m channels). Each cultivation pond is serviced by a harvest pond that is 3 meters deep, designed to hold a water depth of 2.5 ± 0.25 meters and a volume equal to half the cultivation pond volume (1500 m³). The areal dimensions of each harvest pond are roughly 20 x 30 meters. The bioreactor area is estimated to require 250 m². A possible layout for such a system is presented in Figure 6.8. Not shown are control stations and storage ponds that could be placed along the midway. The total area for the system is 32.2 hectares, or 161% of the total production area (cultivation pond water surface) of 20 hectares. A small amount of additional land likely would be required for infrastructure.



Figure 6.8. A possible layout for a 20-pond system including inoculum, harvest, and blowdown evaporation ponds is diagramed. Depths of all pond types are fixed, thus areas of non-cultivation ponds will scale with size of the cultivation ponds.

6.3.1.2 Unit Operations and Activities

The unit operations involved in the growing of algae include: site preparation, cultivation of an inoculum and inoculation, tending of the growing algae, and harvesting. For the purpose of this analysis, the specific list of activities that are performed within these unit operations and their descriptions are taken from reports from the US Department of Energy (DOE) SERI/NREL Aquatic Species Program, summarized by Sheehan and others (1998) and in a separate DOE report by Benemann and Oswald (1996).

6.3.1.2.1 Site Preparation

6.3.1.2.1.1 General Description

The following site preparation assumes a "typical" algae farm using a system of open raceway ponds. It is composite of information presented in Benemann et al. (1982), Neenan et al. (1986), Weissman and Goebel (1987), Weissman et al. (1989), and Benemann and Oswald (1996), but draws primarily on Benemann and Oswald (1996). The layout is taken to approximate that diagramed in Figure 6.8. Hydraulics and the ability to grade land uniformly over extended areas limit the maximum dimensions of the individual ponds. The analysis is based on a system of twenty cultivation ponds that have 1.0-ha (10,000 m²) of surface area. Each pond is 43 by 292 meters (including earthworks) and 0.7 meters deep (from crown to floor).

Site preparation occurs in three stages. The first requires that the overall site be rough-graded. The actual equipment used and total volume and mass of the material that will need to be moved will vary significantly from site to site. Trees may need to be uprooted and rocks removed. Relatively deep excavations will be required for harvest ponds, trenches for laying of pipe, and foundation footings for any pads that are installed in support of control systems and other infrastructure. The second stage will require precision grading of the land in order to maintain the hydraulics for a flowing, 30 cm deep body of water, over a length of 200 to 300 meters (or more). The ability to meet the grading requirements and the amount of energy required to do so will in part depend upon the soil type. The final stage is preparation of the interface between the land and the algae culture medium. If this interface is permeable or subject to leaking, it can lead to the loss of water, product (algae), and/or by-products (including waste); it also increases the risk of culture contamination due to introduction of undesirable materials and organisms.

The harvest ponds are assumed to be constructed of concrete. The cultivation and inoculum ponds may be constructed from a variety of materials. While a small number of sites may be able to take advantage of existing impermeable soils and clays, it is expected that if algae cultivation occurs at large scale, pond interfaces will be constructed from man-made materials, (e.g., plastic liners or concrete). Even in areas where soils are reasonably self-sealing, the top level is at risk for erosion due to the pond circulation system. This will not only degrade the surface of the pond bottom but may also lead to shifting of the soil such that it adversely affects the hydraulic design of the pond. Erosion also increases the level of suspended solids in the pond, which results in decreased light penetration and loss of biomass productivity. A thin layer of gravel or crushed rock can be added to minimize erosion, but this may not be desirable for circulation efficiencies and creates sites for potentially undesirable biologic activity. If plastic liners are used, they must be thick enough and/or flexible enough to withstand punctures from the underlying surface (e.g., rock fragments in the soil). They also must be resistant to chemical and or biological degradation. Even the most resistant plastic is likely to have a significantly shorter lifespan than concrete and therefore need to be replaced several times over the life of the pond. As an alternative to plastic liners, it has been suggested that clay obtained through conventional mining operations (i.e., from a location other than the algae cultivation site) could be deposited in a thin layer at the bottom of the ponds.

6.3.1.2.1.2 Activities

It is assumed that the entire site will be subjected to some degree of earthmoving and that the unit operation will occur in a series of steps. Based on the pond system layout diagramed in Figure 6.8, the area of land that must be prepared (i.e., hectares affected by the unit operation) is 1.61 times the total surface area of the cultivation ponds and the area of the cultivation ponds is taken to be equivalent to harvested hectares.

The first step in land preparation is coarse grading and rough formation of the earthworks used to define the pond structure. A simple, generic model is used to model this activity based on the use of diesel-powered, earthmoving equipment. The typical piece of equipment used to perform this function is taken to have a 300 HP diesel engine, a fuel use rate of 60 liters per hour, and a performance rating of 1.2 hours per hectare per pass. It is assumed that an average number of 3 passes is required to complete the grading for an effective completion rate of 3.6 hours per hectare. Given a lower heating value (LHV) energy content of 35.8 megajoule (MJ) per liter of

petroleum diesel (ANL, 2009), the total amount of energy required per hectare of harvested hectare is thus calculated as

1.61
$$ha_{op} / ha_{harv} * 35.80 \text{ MJ/liter} * 3.6 \text{ hr}/ha_{op} * 60 \text{ liter} / \text{hr} =$$

12,450 MJ / ha_{harv} (6.4)

where

ha op is area operated upon (coarse-graded) in hectares; equal to the entire site area

ha harv is area harvested, in hectares; equal to cultivation pond water surface area

Total fuel consumption for coarse grading is thus 348 liters / ha harv.

The second step in land preparation is fine-grading and leveling of the ponds. The total area affected by this operation is equal to the surface area of the cultivation ponds plus the inoculum ponds, which are estimated to be 11% of the area of the cultivation ponds. The activities associated with this process are modeled using formation of rice cultivation fields as a surrogate (Table 6.3). The basis is the set of land preparation data presented in cost and return budgets for water planted rice in Louisiana (Salassi and Deliberto, 2010). While earthworks ponds can last for 20 years or more, the size of these ponds, the relatively small tolerance with respect to surface gradients within the ponds, and concerns with leaks and contaminations suggests that the ponds will have to be re-graded more frequently. The model assumes that fine-grading will occur every 10 years, while course-grading will last over the life of the system.

Table 6.3. Equipment used for fine grading and leveling with Louisiana rice field preparation used as a surrogate (based on Salassi and Deliberto, 2010).

	Size/	Unit	Fuel U	lse Rate	Performance Rate		Times Over	ha-pass / ha	Fuel Consumption	
Equipment	Unit	Power (HP)	gal/ hr	liters/ hr	hr/ ac	hr/ ha	per Operation	cultivated	liters/ ha cultivated	
Levee plow	8 ft	300	15.44	58.45	0.05	0.12	2	2.20	15.89	
Blade scraper	10 ft	150	7.72	29.22	1.18	2.91	0.18	0.20	16.81	
Ditcher		150	7.72	29.22	0.02	0.05	2	2.20	3.18	
Backhoe		150	7.72	29.22	0.50	1.24	0.05	0.06	1.99	
Level	24 ft	300	15.44	58.45	0.15	0.37	1.67	1.83	39.45	
TOTAL									77.32	

The final step is to apply plastic liners (if used) and pour concrete. Plastic liners are modeled as high density polyethylene (HDPE); however, the exact material used may differ. Concrete is modeled as ordinary concrete made with Portland cement.

An optional design feature is the use of a transparent cover which would be made of plastic supported by ribs. This is considered only for one inoculum pond.

6.3.1.2.1.3 Direct Material and Energy Flows

The direct material and energy flows associated with the land preparation unit operation include land, diesel fuel to power the grading equipment, concrete, and plastic for liners. As discussed above, the total land required based on bottom-up calculations for this particular pond design is estimated to be 161% of the cultivation pond surface area. Top down calculations based on proportions given in the SERI/NREL literature produced a multiplier of 1.65; this value is suggested for the more general case. For the current analysis, land requirements are taken to be equal to 1.6 ha per hectare harvested (surface area cultivated).

6.3.1.2.1.3.1 Fuel Use

All of the fuel use associated with land preparation is due to the creation of ponds and earthworks. Coarse grading and excavation requires 348 liters of diesel per hectare cultivated; fine grading and leveling requires 77.3 liters per hectare cultivated. The former is performed once in the life of the pond system; the latter is assumed to be required every 10 years. A pond system lifetime of 20 years is used in this exercise. Thus total diesel use is equal to 25.1 (348/20 + 77.3/10) liters/ha-yr.

6.3.1.2.1.3.2 Materials

Concrete will be used, at a minimum, for the harvest ponds. Each harvest pond is assumed to be 3 meters deep; the area is equal to 6% of the cultivation pond area or 600 m² per hectare. The dimensions are taken to be 20 by 30 meters for a 1-ha pond system. The thickness of the concrete is assumed to be 0.15 m. Thus the total volume of concrete per harvest pond is equal to

$$20 \text{ m} * 30 \text{ m} * 0.15 \text{ m} + 2 * 3 \text{ m} * 0.15 \text{ m} * (20 \text{ m} + 30 \text{ m}) =$$

$$135 \text{ m}^3 / \text{hectares cultivated}$$
(6.5)

A typical density for concrete is 2,380 kg/ m^3 which results in a use of 321,300 kg of concrete per hectare harvested. This can be amortized over the lifetime of the pond, (20 years) giving a final material use rate of 6.8 m^3 /ha-yr or 16,065 kg/ha-yr.

The base system is assumed to consist of cultivation ponds that are constructed using earthworks; however, the amount of concrete required to build walls for these ponds is determined here for reference. Regardless of whether the walls are constructed of soil or concrete, the floor of the pond is assumed to be compacted soil, with some type of lining material. The primary advantage to concrete walls is that they require less land area. Problems with them include potential leaking at joints, difficulty in forming curved ends for optimization of the hydraulic system, and, because of the rough surface, more energy to power the circulation system.

Concrete walls and dividers, are assumed to have footings that are 45 cm, tapering to 15 cm or less towards the top of the wall; an average thickness of 0.3 m is assumed. For the sake of simplicity, a rectangular pond that makes use of baffles within the pond structure is assumed (rather than curved walls). The height of the walls and dividers is taken to be 0.5 m above grade and 0.1 m below, for a total height of 0.6 m. In the case of concrete ponds, it is beneficial (from

a material consumption standpoint) to maximize the pond area, as the ratio of perimeter length to area decreases significantly; however, as this design option is considered only for purposes of comparison, a 1 hectare pond is assumed. The channel divider is 245 meters in length; the side and end walls are 278 and 36 m long respectively. Thus total volume of concrete per cultivation pond is

$$245 \text{ m} * 0.6 \text{ m} * 0.15 \text{ m} + 2 * 0.6 \text{ m} * 0.15 \text{ m} * (278 \text{ m} + 36 \text{ m}) =$$

$$78.6 \text{ m}^3 / \text{hectares cultivated}$$
(6.6)

When amortized over the lifetime of the pond, assumed to be 20 years, the final material use rate is 3.9 m^3 / ha-yr.

Earthworks ponds will require, at a minimum, that the sides be stabilized with a geotextile or plastic sheet. Benemann and Oswald (1996) suggest the use of TenCate Polyfelt, a mechanically bonded, non-woven fabric made of UV stabilized polypropylene filament. It is 2.2 mm thick and has a very low density of 118 kg/m³. However, this material is permeable and the manufacturer recommends that it be used with an underlayer, such as a 2 mm thick polypropylene. In addition, it is also not recommended for slopes. For these reasons, it is not considered here. Although there are a number of suitable material options available (including vinyl and synthetic rubber), the current model, assumes the use of sheets of high density polyethylene (HDPE). The modeled 1-ha earthworks pond has a channel divider that is 245 meters in length with 2 sides and two (approximately) 3-meter ends for a total length of 896 m. The total sidewall length is 612 m. Based on the profile illustrated in Figure 6.5, 2-meter widths would be adequate to cover the sloping sides while leaving enough material for anchoring. The total area of textile required for the sides of each pond is thus 3016 m^2 (2 x (896 + 612). Allowing extra for overlap and fitting, this is rounded to 3100 m^2 per 1-ha pond. The thickness of the plastic is assumed to be 2 millimeters (2 mm) and the density of HDPE is 950 kg/m³. The mass of the plastic required to cover the walls is thus

The lifetime of the plastic on the walls is expected to be less than that used on the bottom of the ponds due to much greater exposure to sunlight, thermal cycling, and pests (insects, rodents, and weeds). It is estimated that this material will need to be replaced once every 5 years, thus the use rate is equal to 1,178 kg/ha-yr.

The bottom of the pond will also need to be lined with either plastic or clay. Concrete is another option, but the mass required would be significant and the surface is not especially conducive to energy efficient mixing. Gravel is even more problematic and is not considered here.

A 5% penalty is applied for overlap and fitting, for a required total of 10,500 m^2 of plastic per hectare. The mass of the HDPE liner needed to cover the bed of the pond is thus calculated as

The bottom of the pond is relatively protected and the plastic liner is expected to last 10 years before being replaced. The use rate is therefore estimated to be 1,995 kg/ha-yr.

A bottom liner made of clay is another option. If a 0.05 m (2 inch) were used, the mass required, assuming a density of 2000 kg/m³ is:

$$2000 \text{ kg/m}^3 * 10,000 \text{ m}^2/\text{ha} * 0.05 =$$

$$1,000,000 \text{ kg / hectares cultivated}$$
(6.9)

The energy of the circulation system will cause some portion of the clay to be physically and/or chemically eroded. The exact rate at which this will occur is highly dependent upon the water chemistry and the composition of the clay. It is estimated that perhaps 0.005 m (5 mm) per year may need to be replaced, which is the equivalent of a full replacement every ten years. Thus the use rate for a clay bottom is taken to be 100,000 kg/ha-yr.

A covered inoculum pond is included in the design. The size in terms of water surface area would be 20% of a single cultivation pond. Although it would not scale perfectly, it is assumed for this analysis that it does in terms of estimating material and energy flows. It differs from the other ponds in that it is protected with a transparent plastic cover. The cover is assumed to be made of 0.1mm (4 mil) thick HDPE and to be 10% larger than the area of the pond. It would be replaced once per year. The material requirement per hectare of cultivation pond area is calculated as:

The cover will require a support structure of ribs. It is thought that the material content of these ribs will be low and that they will not require frequent replacement. In addition, the exact design and material content is unknown. For these reasons they are not included in the analysis.

Materials will also be required for paddlewheels and pumps. As the total mass is expected to be relatively small over the life of the pond and the designs are highly uncertain, these are not considered here.

6.3.1.2.1.3.3 Emissions to Air

Emissions to air include criteria air pollutants (or precursors thereof) as well as greenhouse gas emissions. All of the criteria pollutants result from the burning of diesel for earthmoving equipment. Greenhouse gas emissions are released both from burning of diesel as well as from the land use change that would result from building the pond system. The latter is not addressed here as the type of land likely to be used is unknown.

Criteria Air Pollutants

Criteria air pollutants that result from the burning of diesel fuel in the field equipment listed in Table 6.3 are calculated based on the formulas and emission factors used in the US Environmental Protection Agency's NONROAD model (EPA, 2004; EPA, 2005). A generic piece of earthmoving equipment is also modeled. Although there was no large-scale mass cultivation of algae in 2007, the technology profiles selected for the pieces of equipment are based on this assumption, as that was the assumption for the other feedstocks addressed in this report.

The profile for each piece of equipment is estimated to be 30% Tier 1, 60% Tier 2, and 10% Tier 3 technology. The resulting emissions in grams per liter of fuel burned and grams per hectareyear are given in Table 6.4.

Table 6.4. Emissions in grams per liter (g/liter) of diesel fuel burned and grams per hectare (g/ha) for the land preparation unit operation (based on emission factors from the NONROAD model (EPA, 2004; 2005).

F aulian ant	Unit	Fuel Use		Emissions g/ha-yr								
Equipment	(HP)	liters/ ha-yr	VOC	со	NOx	PM	SOx	VOC	со	NOx	PM	SOx
Levee plow	300	1.59	1.71	6.18	21.35	0.97	0.83	2.71	10.6	132.0	20.7	0.8
Blade scraper	150	1.68	1.86	7.17	21.76	1.33	0.83	3.13	13.4	155.9	28.9	1.1
Ditcher	150	0.32	1.86	7.17	21.76	1.33	0.83	0.59	13.4	155.9	28.9	1.1
Backhoe	150	0.20	1.86	7.17	21.76	1.33	0.83	0.37	13.4	155.9	28.9	1.1
Level	300	3.95	1.71	6.18	21.35	0.97	0.83	6.74	10.6	132.0	20.7	0.8
Generic earthmoving equipment	300	17.39	1.66	6.02	20.80	0.95	0.81	28.94	10.0	125.2	19.7	0.8
TOTAL		25.13						42.49	71.2	857.0	147.8	5.7

Greenhouse Gas Emissions

Greenhouse gas emissions from burning of diesel fuel are estimated using IPCC emission factors (IPCC, 2006a). The default carbon dioxide (CO₂) emission rate for agricultural diesel operations is 74.1 kilograms (kg) of CO₂ per gigajoule (GJ) of fuel burned. If diesel is assumed to have a lower heating value (LHV) energy content of 0.0358 GJ/liter (ANL, 2009), this is equivalent to 2.65 kg CO₂ per liter of diesel burned. Similarly, the IPCC default value for methane (CH₄) is 4.15 kilograms per terajoule (TJ) and the default value for N₂O is 28.6 kg/TJ. These equate to emissions of 0.149 and 1.02 grams of CH₄ and N₂O respectively per liter of diesel combusted. Applying these values to the total fuel consumed (25.13 liters per hectare-year), the operation of diesel powered equipment during the land preparation unit operation results in per hectare-year emissions of 67 kg of CO_2 , 0.0037 kg of CH_4 , and 0.026 kg of N_2O_2 .

6.3.1.2.1.3.4 Land Use and Summary

The area required to support cultivation of algae is greater than the harvested area; the latter is equal to the water surface area of the cultivation pond. The burdens associated with land preparation activities (1.61 for coarse grading and 1.11 for fine grading) are accounted for in the
calculations of fuel use and associated emissions to air and are therefore not repeated here. With the exception of concrete used for harvest ponds, the estimated materials use rates are calculated per hectare-yr for any racetrack pond, which in the modeled design includes inoculum ponds as well as cultivation ponds. The inoculum ponds require 11% additional area and this burden is included as appropriate. A summary of the land preparation material and energy flows is given in Table 6.5.

Resource	Calculation	Value	Units				
Land	1.0 hectares / 1 hectare-year	1	1/yr				
Diesel							
Volume	1.0 / year * 25.1 liters / hectare	25	L/ha-yr				
Mass ¹	25.1 liters / hectare-year * 0.837 kilograms / liter	21	kg/ha-yr				
Energy ¹	25.1 liters / hectare-year * 35.8 megajoules / liter	899	MJ/ha-yr				
Materials							
Concrete	1.0 / year * 16,065 kilograms / hectare	16,065	kg/ha-yr				
HDPE	1.11 / year * (1,178 + 1,995 + 209)	3,754	kg/ha-yr				
Clay ²	1.11 / year * 100,000 kilograms / hectare	111,000	kg/ha-yr				
Criteria Air Pollutants and Precu	irsors						
VOC	1.0 / year * 0.042 kilograms / hectare	0.04	kg/ha-yr				
со	1.0 / year * 0.071 kilograms / hectare	0.07	kg/ha-yr				
NO _X	1.0 / year * 0.86 kilograms / hectare	0.86	kg/ha-yr				
PM	1.0 / year * 0.15 kilograms / hectare	0.15	kg/ha-yr				
SO ₂	1.0 / year * 0.01 kilograms / hectare	0.01	kg/ha-yr				
Greenhouse Gases							
CO ₂	1.0 / year * 67 kilograms / hectare	67	kg/ha-yr				
CH ₄	1.0 / year * 0.0037 kilograms / hectare	0.004	kg/ha-yr				
N ₂ O	1.0 / year * 0.026 kilograms / hectare	0.026	kg/ha-yr				
¹ Energy content and density of liquid fuels, default inputs to GREET (ANL, 2009)							
² Clay is not part of the base ca	se; it is included for informational purposes only						

Table 6.5. Direct material and energy flows for land preparation for mass cultivation of algae

6.3.1.2.2 Seeding and planting:

6.3.1.2.2.1 General Description

The algae mass cultivation system modeled in this analysis is assumed to be semi-continuous. In this approach, the amount of algae removed daily is equal to the growth rate. However, as the algae culture propagates only during day-light hours, all harvesting takes place at night in order to maximize growth. The model assumes that the mean growth rate is one doubling per day; thus the representative harvesting process over the course of the growing season is one that is described as half the culture medium (along with its resident algae) being removed at the end of each day, leaving the remaining half the biomass in the growth pond to act as a starting population at dawn the following day. (The harvesting process is discussed in further detail in section 6.3.1.2.4 of this report.) In principle, harvesting at the growth (or dilution) rate produces a near steady-state system that does not require inoculation. However, because cultivation

occurs in an outdoor pond, without supplemental lighting or temperature control, there are likely to be periods when growth rates are not sufficient to maintain a minimum population density. There is also risk of population loss due to extreme weather events, predators, disease, or invasion by an undesirable, more competitive species. In addition, it is expected that ponds will periodically need to be drained for cleaning and maintenance. It is therefore assumed that an inoculation system will be required.

The inoculum system assumed in the model is based loosely on the example presented in Benemann and Oswald (1996). The concept is to use sequential stages that increase geometrically in size while decreasing in complexity. Only the initial culture, produced in a laboratory, is grown off-site. The culture received from the laboratory is expanded in size by a factor of 10 at each step through a series of bioreactors. When the size of the culture is 1% of the final desired mass, it is placed in a covered raceway pond. After a 10-fold increase 2 full-scale inoculum ponds are inoculated with biomass from the covered pond. These ponds are then used to supply 20 cultivation ponds with inoculum as needed.

6.3.1.2.2.2 Activities

All of the activities related to development of an inoculum culture are accounted for relative to the cultivation ponds and are addressed in the tending unit operation.

6.3.1.2.1.3 Direct Material and Energy Flows

All of the direct material and energy flows related to development of an inoculum culture are accounted for relative to the cultivation ponds and are addressed in the tending unit operation.

6.3.1.2.3 Tending:

6.3.1.2.3.1 General Description

The tending unit operation applies to both the cultivation ponds and the inoculum ponds. The ponds must be filled with water and an inoculant introduced in order to initiate growth of algae in the pond. For freshwater cultures, the water used in the initial fill of the pond is taken from either surface or shallow groundwater or both. Water for saline cultures is assumed to be pumped from groundwater located in relatively deep aquifers. After the ponds are filled, the water is circulated around the pond using a paddlewheel. Other systems have been explored, but the paddlewheel offers the most energy efficient process with the least risk of physical damage to the algal cells. Nutrients are added to the ponds along with the fill water. Carbon dioxide (CO₂) is pumped into the pond on a continuous basis during daylight hours. Pure CO₂ may be pumped into a sump (a deep trench running perpendicular to the sidewalls of the pond) using a sparger. The increased depth of the sump allows for more efficient mass transport of the CO₂ by increasing the distance between the injection point and the surface of the water. Alternatively, flue gas, containing between 9-14% CO₂ may be introduced either as a blanket between the surface of the water and a cover overlying the pond surface or through a sparger, as for pure CO₂. Only the pure CO₂ system is considered in the current analysis.

Algae cultivated in an outdoor pond may be grown either in a batch system or in a semicontinuous mode. In a batch culture system, the culture is permitted to grow past the logarithmic portion of its growth curve before being harvested. This maximizes both biomass and lipid content, but it requires more time, and for a given productivity, more pond area. A continuous system would involve removing the culture at a rate that matched its growth rate. Because algae grow only during daylight a truly continuous process is not possible. Instead, a semi-continuous approach is used, whereby algae is allowed to grow during the day and is harvested at night. Since many species exhibit a doubling of the population once per day, a simple approach is to harvest half the pond each evening and replace the algae-laden culture medium with an equal volume of water, perhaps containing a small amount of algae from an inoculum pond. Thus the biomass density is decreased by half each night and returns to the same density over the course of the following day. This has the effect of providing a 2-day retention time for the algae. The water that is used to replenish the ponds will, to the extent possible, consist of water that has been reclaimed from the harvest operation. Some portion of the makeup water will, however, have to be supplied from the same sources used to supply the original fill of the pond. The inoculum ponds are filled only with new source water and medium from upstream inoculum ponds or bioreactors.

6.3.1.2.3.2 Activities

6.3.1.2.3.2.1 Water Supply to the Cultivation Ponds

There are two basic factors that must be considered in estimating the volume of water necessary to grow algae in a mass cultivation system. The first is the amount of water needed to support a culture at a target biomass productivity level at any given time. The second is the amount required to replace water that is removed from the cultivation ponds either as a function of system design, or due to natural losses.

The amount of water needed to fill a cultivation pond is simply equal to the depth of the water times its surface area. The optimum water depth for an outdoor pond is considered to be 0.3 meters (30 cm). Although the algae culture itself is expected to average only 15 cm (Figure 6.2), the extra water depth allows for unevenness in grade (especially in very large ponds), as well as variations in the culture depth. Therefore, the amount of water required to fill the pond is 0.3 meters x 10,000 m² per hectare or 3000 m³ (3.0 x 10^6 liters) / hectare multiplied by the total number of times per year that the pond is completely drained and refilled. It is anticipated that cultivation ponds will be emptied and cleaned on a regular basis (e.g., at the end of each growing season). This is necessary, as both biological and mineral sediment will collect on the bottom of the pond, decreasing the effective water depth and adversely affecting the hydraulics. Sediments and precipitates may also cause undesirable shifts in the overall chemistry of the pond. Ponds are likely to be refilled at least once a year (during the winter); thus, the minimum, base supply of water is estimated to be 3.0×10^6 liters/hectare-year. This is the assumption made in this analysis; however, the actual frequency with which ponds are drained and refilled is likely to be situation specific. If on average, the pond is drained and refilled more often than once per year, this amount will increase proportionally. The total volume of inoculum ponds relative to cultivation ponds is 11%, thus the volume required to fill the inoculum ponds is 0.33×10^6 liters per hectare of cultivation pond surface area. Inoculum ponds need to be more tightly controlled with respect to biomass content and water chemistry, therefore it is assumed that they are drained and cleaned twice per year. Consequently, the total volume required to fill drained inoculum ponds is estimated to be 0.66×10^6 liters/ha-yr.

Water, in excess of that used to fill the pond, will be required throughout the year in order to offset losses due to evaporation, leaks, blowdown, and harvesting. In the case of inoculum ponds, "harvesting" is the process of providing feedstock to the cultivation ponds. This water is lost from the ponds but not from the system. The blowdown process releases a portion of the inoculation or cultivation pond water; an equal volume of new makeup water is added to the pond in order to control the water chemistry. In this analysis, blowdown replacement water is taken to be fresh for both fresh and saline systems. It is assumed that all water used as input to inoculum ponds is newly sourced (fresh or saline, depending upon the type of algae). Inputs to the cultivation ponds will include newly sourced water, plus feed from the inoculum ponds, plus reclaimed water from settling ponds.

Total water inputs on an annual basis are calculated as:

$$V_{TOTAL input} = V_{pond} * freq_{empty} + V_{evap loss} + V_{leaks} + V_{blowdown} + V_{harv}$$
(6.11)

where

- $V_{TOTAL input}$ is the volume of water per unit area that must be added to the pond annually (liters / ha-yr)
- V_{pond} is the nominal volume of water in the pond per unit area (liters/ha)
- *freq empty* is the frequency at which the pond is emptied for cleaning or maintenance expressed as number of complete volumetric turnovers per year (year⁻¹)
- *V*_{evap loss} is the annual evaporative loss of water (equal to pan evaporation minus precipitation) per unit area of pond surface during the growing season (liters/ha-yr)
- V_{leaks} is the annual volumetric loss of water due to leaks at the interface between the culture medium and the containment system (liters / ha-yr)
- *V*_{blowdown} is the volume of water removed annually per unit area in order to control water chemistry (liters / ha-yr)
- V_{harv} is the volume of water removed from cultivation ponds during the harvesting process (liters / ha-yr), or in the case of inoculum ponds, the volume fed to the cultivation ponds.

The net amount of water lost to due evaporation depends on the climate, particularly temperature, humidity, precipitation, and wind velocity. Each of these will vary from site to site and over the course of a year. Neenan and others (1986) estimate that evaporative losses (evaporation pan data less precipitation) in the southwestern US range from 0.002 to 0.010 meters per day, with a mean of 0.0035 m/day during the growing season. This is taken to represent the upper limit for evaporative losses was estimated by evaluating evaporation pan and precipitation data collected over a 10-year period during all 12 months of the year in the Everglades region of Florida (SWFMD, 2010). Although this region is not likely to be an area where mass cultivation of algae occurs, it is characterized by very high humidity, rainfall and wet ground surfaces, all of which would minimize net evaporation. The average value of 0.0017 m/day for this area is therefore estimated to represent the lower end of potential evaporative losses. The mean value for US algae cultivation is taken to be 0.0025 m/day, the approximate

mid-point of these two extremes. An average evaporative loss of 0.0025 m/day is consistent with a GIS evaluation completed at Sandia National Laboratory (under the US Department of Energy), which evaluated the average annual free water surface evaporation from shallow lakes in the continental United States. Based on these results, the regions where algae is likely to undergo mass cultivation are characterized by evaporation rates that range from 30 to 60 inches per year (0.0021 to 0.0042 m/day) with most of the land area typified by rates of approximately 30 to 40 inches per year (0.0021 to 0.0028 m/day) (Pate, 2008).

At 0.0025 m/day, the annual loss of water from the cultivation ponds due to evaporation over a 1-hectare area during a 300 day growing season, would equal

$$300 \text{ days/year } * 0.0025 \text{ meters/day } * 10,000 \text{ m}^2/\text{hectare} = 7,500 \text{ m}^3/\text{hectare-year}$$

$$= 7.5 \text{ x } 10^6 \text{ liters /hectare-year}$$
(6.12)

The minimum and maximum values for evaporative losses from cultivation ponds are similarly calculated to be 5.1×10^6 liters /ha-yr and 10.5×10^6 liters /ha-yr, respectively. In the design assessed here, the total surface area of the uncovered inoculum ponds is 10% that of the cultivation ponds. Applying a multiplier of 1.1, the typical evaporative losses for the inoculum ponds are thus estimated to be 0.75×10^6 liters per hectare of cultivation pond area per year. This is meant to broadly characterize evaporation in the region under consideration. The amount of variability would be greater if considered on a site-specific basis and there are much more precise means of predicting evaporative losses at a particular, known location.

Another potential source of water loss is leaks from the pond to the surrounding soil, although the degree to which this phenomenon is inevitable is not known. Weissman and others (1989) report loses of 0.11 to 0.36 cm/day from a lined pond and 0.40 to 0.79 cm/day in an unlined pond. The unlined pond eventually self-sealed to give a leak rate of 0.4 cm/day. These numbers equate to a range of 3.3 to 23.7 x 10^6 liters /hectare-year. In this analysis, it is assumed that better designs and materials can minimize this problem and the lowest value, 3.3 x 10^6 liters per hectare-year is assumed for lined cultivation ponds. A total of 15 x 10^6 liters /hectare-year is assumed for unlined ponds. These values are multiplied by a factor of 0.11 to account for inoculum ponds to give an annual rate of 0.36×10^6 liters per cultivated pond surface hectare for lined pond systems and 1.7×10^6 liters/ha-yr in the case of unlined ponds.

Uncovered ponds lose a significant amount of water through evaporation and act as solar concentrators of dissolved salts. This effect may be counteracted with a process referred to as blowdown, in which the culture medium is removed from the pond and replaced with freshwater (as that is what is preferentially evaporated). Even freshwater algae will require this process, although on a smaller scale than species adapted to saline environments. Weissman and Goebel (1987) specify a blowdown rate equal to 14% of evaporative losses for saline species and 2% for freshwater species. For average evaporative losses of 7.5 x 10^6 liters/hectare-year, water removed due to blowdown is thus estimated to be 1.1×10^6 liters /ha-yr for saline species and 0.15×10^6 liters /ha-yr for freshwater species. The total surface area of the uncovered inoculum ponds is modeled as 10% of the cultivation pond surface area; thus the water that must be replaced due to blowdown in these ponds is equal to 0.11×10^6 liters per hectare of cultivation

(6.13)

pond surface area per year (ha-yr) for saline species and 0.015×10^6 liters /ha-yr for freshwater species.

The amount of water removed from the cultivation ponds (and which therefore must be replaced) during the harvesting operation depends upon the harvesting strategy used. In the current model, it is assumed that algae are grown in a semi-continuous mode in which half the pond volume is removed after sunset and an equal amount of water is delivered to the pond within a two hours after sunrise. The total water that must be replaced due to harvesting is, therefore, half of 3000 m^3 (3.0 x 10⁶ liters) or 1500 m³/ hectare-day, which is equal to 450,000 m³ (450 x 10⁶) liters) hectare-year for a 300 day growing season. The total amount of water removed from inoculum ponds to provide daily feed to downstream inoculum ponds and cultivation ponds is equal to 11% the volume harvested or 49.5 x 10^6 liters per cultivation pond area (as measured in hectares) per year. This water, however, is not lost to the system and is accounted for as input streams to the downstream ponds.

The average volume of water per unit area that must be added annually to lined cultivation ponds growing a saline water species of algae ($V_{TOTAL input}$, per 6.11) is equal to

$$(3.0 * 1 + 7.5 + 3.3 + 1.1 + 450) \times 10^{6}$$
 liters/hectare-year =
465 x 10⁶ liters/hectare-year (6.13)

Freshwater ponds will require 1.0×10^6 liters/ha-yr less, or 464 x 10^6 liters/hectare-year.

The average volume of water per unit area that must be added annually to lined inoculum ponds, growing a saline water species of algae, with the small inoculum pond(s) covered (V TOTAL input, per 6.11) is equal to

$$(0.33 * 2 + 0.75 + 0.36 + 0.11 + 49.5) \times 10^{6}$$
 liters/hectare-year =
51.4 x 10⁶ liters/hectare-year (6.14)

Newly sourced water is expected to be used to fill both cultivation and inoculum ponds after being drained for cleaning or maintenance. In order to protect the viability of the culture and the chemistry of the medium, all of the makeup water added to the inoculum ponds to offset that removed during inoculation of the cultivation ponds is also assumed to be newly sourced. In both cases the water will be drawn from sources with a salinity that corresponds to that needed to grow the desired species of algae. For both saline and freshwater species, water lost to evaporation or removed during the blowdown process will require additions of newly sourced fresh water.

A portion of the input water that flows into the cultivation ponds will include that which is reclaimed from the harvest operation; the remainder is expected to be pumped from local surface or groundwater sources. In most locations available surface water will be fresh, thus if the algae are to be grown in a saline environment, water will have to be obtained from subsurface aquifers. The amount of water that is withdrawn is presumed to be equal to the amount consumed; (rainfall is accounted for in the budget in the estimation of net evaporative losses).

Figure 6.9 illustrates the overall flow of water and algae growth medium assumed in this model and used to determine the water balance for the algae cultivation system. A summary of estimated water use for cultivation and inoculum ponds during the tending unit operation is presented in Table 6.6.



Figure 6.9. The flow of water and algae growth medium used to determine the water balance for the modeled algae cultivation system is shown above, where "inoc" refers to inoculum ponds; "cult" refers to cultivation ponds; "settle" refers to harvest ponds; "new" refers to newly sourced water; and "conc" refers to medium that has been concentrated with respect to algae.

		Lawre	Createll ²		Saline Algae System				Fresh Water Algae System			
Output / Losses	Cultivation Ponds	Large Inoculum ponds	Inoculum ponds	Inputs / Sources	Cultivation Ponds	Large Inoculum ponds	Small Inoculum ponds	TOTAL	Cultivation Ponds	Large Inoculum ponds	Small Inoculum ponds	TOTAL
		10 ⁶ L/ ha-yr						10 ⁶ L/	ha-yr			
				New fresh water	0	0	0	0	3	0.6	0.06	3.66
Drain pond	3.00	0.60	0.06	New saline water	3	0.6	0.06	3.66	0	0	0	0
Evaporative losses	7.5	0.75	0	New fresh water	7.5	0.75	0	8.25	7.5	0.75	0	8.25
				New fresh water	0	0	0	0	3.3	0.33	0.033	3.663
Leaks	3.3	0.33	0.033	New saline water	3.3	0.33	0.033	3.663	0	0	0	0
Blowdown ¹	1.1 (0.15)	0.11 (0.015)	0	New fresh water	1.05	0.105	0	1.155	0.15	0.015	0	0.165
				New fresh water	0	0	0	0	0	40.5	4.5	45
				New saline water	0	40.5	4.5	45	0	0	0	0
Harvest / Feed	450	45	4.5	Upstream inoculum	45	4.5	0	49.5	45	4.5	0	49.5
			Reclaimed harvest water ³	405	0	0	405	405	0	0	405	
TOTAL ¹	464.90 (463.95)	46.79 (46.70)	4.593	Total water pumped	464.85	46.785	4.593	516.23	463.95	46.695	4.593	515.24
				Newly sourced fresh water	8.55	0.855	0	9.405	13.95	42.195	4.593	60.738
				Newly sourced saline water	6.3	41.43	4.593	52.323	0	0	0	0

Table 6.6. Water balance for the tending unit operation in a lined pond system; units of per ha-yr refer to cultivation pond surface area harvested per year

¹ Values for fresh water algae systems given in parentheses when not equal to saline water algae systems

² Small inoculum ponds are covered

³ Allowing up to 90% recovery of water at harvest after newly sourced water and inoculation minimums are met

Final Report

Based on the above analysis, saline systems will require withdrawals of 52.3×10^6 liters/hectareyear of saline water and 9.41×10^6 liters/hectare-year of freshwater. Freshwater systems will require withdrawals of 60.7×10^6 liters/hectare-year of freshwater, where the units of hectareyear refer to the surface area of the cultivation ponds (harvested area). Adjustments to the chemistry of the return water from harvest are likely to use additional amounts of freshwater, but specific volumes will depend upon the particular system.

The 2008 Farm and Ranch Irrigation Survey (USDA, 2010) was used to estimate the amount of energy that will be required to pump from both surface and groundwater sources using electric and diesel pumps. The average cost per acre to pump water using each of these energy sources at the state level was divided by the average acre-inches pumped in order to obtain the average cost per acre-inch of water supplied for diesel and electric pumping systems. Prices of \$0.10 per kilowatt-hour (kWh) and \$2.50 per gallon of diesel were assumed. The values were then weighted by the relative amount of land within each state that is suitable for algae cultivation (based on land areas given in Table 6.1) and by the proportion of electric and diesel and pumps employed. It is assumed that all water for saline systems is groundwater. The amount of energy estimated applies to pumping from freshwater aquifers with an average well depth of 281 ft (86 m). The depth to saline aquifers in the southern US is significantly greater than this. Based on a US Geological Survey map presented by Alley (2003) (taken from Feth and others (1965)), the average depth to the top of saline aquifers sources is estimated to be at least 1000 feet (305 m) below the surface, which is at least 4 times deeper than currently used freshwater sources; therefore, it is estimated that the pumping energy requirements for saline water will be 400% of that for fresh. The existing mix of surface and groundwater sources is assumed for freshwater algae cultivation systems with no adjustment for deeper groundwater sources. A summary by state is given in Table 6.7.

Saline systems are expected to require withdrawals of 52.3 x 10^6 liters/hectare-year of saline water plus 9.46 x 10^6 liters/hectare-year of fresh water (Table 6.6). The diesel component of each is, per Table 6.7, 0.121 liters of diesel per m³ of saline ground water and 0.0267 liters per m³ of freshwater. The amount of electricity required to pump source water is anticipated to be 1.173 kWh/m³ and 0.280 kWh/m³ for saline systems and freshwater, respectively. These inputs must be added together to account for the total energy used for pumping.

Electricity use to withdraw water for a saline system is calculated as:

52.3 x
$$10^3$$
 m³/ha-yr * 1.173 kWh/m³ + 9.41 x 10^3 m³/ha-yr * 0.280 kWh/m³ = 64,008 kWh/ha-yr (6.14)

Diesel use for a saline system is determined as:

52.3 x
$$10^3$$
 m³/ha-yr * 0.121 liters/m³ + 9.41 x 10^3 m³/ha-yr * 0.0267 liters/m³ =
6,582 liters of diesel per ha-yr (6.15)

The total energy necessary to acquire source water for a saline algal system is thus equal to

		1	<u> </u>			<u> </u>					
			Well Depth	Fre Groun	esh dwater	Sa Ground	line Iwater ¹	Fresh and Gro	Surface undwater	Surface	e Water nly
State	Area %	Elec: Diesel	ft	kWh/ acre-in	gal/ acre-in	kWh/ acre-in	gal/ acre-in	kWh/ acre-in	gal/ acre-in	kWh/ acre-in	gal/ acre-in
Alabama	7%	0.5	180	58.8	3.8	235.3	15.2	49.6	3.9	44.1	3.8
Arizona	2%	8.3	521	20.0	0.7	79.9	3.0	15.8	0.4	5.1	0.3
California	6%	4.7	380	29.7	1.4	118.8	5.7	21.5	1.0	11.9	0.6
Florida	8%	0.3	493	30.1	1.5	120.4	6.0	24.0	1.1	9.1	0.4
Georgia	10%	1.5	338	35.1	3.2	140.3	12.8	34.7	3.2	38.7	3.0
Louisiana	9%	0.3	133	19.9	1.5	79.6	6.1	19.7	1.5	14.8	1.1
Mississippi	6%	0.4	107	14.6	1.2	58.5	4.6	14.3	1.2	12.0	0.7
New Mexico	4%	20.3	261	35.5	1.2	142.1	4.9	28.1	1.3	12.4	0.0
North Carolina	4%	0.5	203	63.2	4.1	252.6	16.5	53.5	2.8	51.2	2.6
South Carolina	4%	5.0	293	28.0	2.3	111.9	9.2	31.9	2.4	35.7	2.3
Texas	39%	7.6	284	58.6	2.7	234.4	10.9	57.9	2.0	50.2	0.9
Weighte	d average	Э	281	30.1	0.8	120.5	3.3	28.8	0.7	24.6	0.6
kWh/m3 or liters	of diesel/	[/] m ³ water				1.173	0.121	0.280	0.027	0.239	0.021
parti	tioned M.	I/10 ⁶ liters				4,221	4,332	1,007	957	860	747
т	OTAL M.	J/10 ⁶ liters				8,5	554	1,9	964	1,6	808

Table 6.7. Estimated energy requirements to supply source water to cultivation ponds

¹ Assumes depth to saline aquifers is 4 times that of current fresh water; thus requiring 400% of the energy required to pump

Freshwater systems are expected to require withdrawals of 60.74×10^6 liters/hectare-year of fresh water (Table 6.6). The diesel component, per Table 6.7, is 0.0267 liters per m³ and the electricity component is 0.280 kWh/m³. These inputs must be added together to account for the total energy used for pumping.

The amount of electricity used for freshwater systems is calculated as

$$60.74 \text{ x } 10^3 \text{ m}^3/\text{ha-yr} * 0.280 \text{ kWh/m}^3$$

$$17,007 \text{ kWh/ha-yr} \tag{6.17}$$

The total amount of diesel used for freshwater systems is calculated as

 $60.74 \text{ x } 10^3 \text{ m}^3/\text{ha-yr} * 0.0267 \text{ liters/m}^3$

1,622 liters of diesel per ha-yr (6.18)

The total energy necessary to acquire source water for a freshwater algal system is equal to

6.3.1.2.3.2.2 Water Pumping In and Out of Cultivation Ponds

A total of 515 to 516 x 10^6 liters of water per hectare-year will need to be pumped into the cultivation and inoculum ponds (Table 6.6); roughly 90% of this water is sourced from other ponds within the system. An additional 450 x 10^6 liters/ha-yr will need to be removed for harvesting. In order to avoid diluting the harvested volume, it is assumed that the harvest and replenishment do not occur simultaneously; and to maximize production, all pumping is completed between sunset and within 2 hours of sunrise. In the southern US, the shortest nights are taken to be 10 hours and the system is designed for this minimum (i.e., 10 hours of darkness plus 2 hours of early morning light for a total of 12 hours). In order to allow time for introduction of the floculant and for settling to occur, the medium to be harvested is removed from the cultivation ponds during the first 5 hours after sunset. There are thus 1500 pumping hours per calendar year (300 days x 5 hr/day) during which time 450 x 10^6 liters/ha-yr of algae medium must be transferred from the cultivation ponds to the harvest ponds.

The required pumping rate for harvesting is calculated as

$$450 \times 10^{3} \text{ m}^{3}/\text{ha-yr} / 1.5 \times 10^{3} \text{ hours} =$$

$$300 \text{ m}^{3}/\text{hr}$$
(6.20)

Water reclaimed from the harvest ponds (the supernatant) is returned to the cultivation ponds during the settling process, which occurs in the final 7 hours of the nocturnal cycle. A total of 405 x 10^6 liters/ha-yr must be pumped over 2100 hours (300 days x 7 hr/day). The required pumping rate for water return, calculated as in equation 6.20, is equal to 193 m³/hr.

Culture medium (algae plus water) is assumed to be "harvested" from the large inoculum ponds and delivered to the cultivation ponds in the 2 hours following harvesting of the cultivation ponds. This requires that 45×10^6 liters/ha-yr must be pumped over 600 hours (300 days x 2 hr/day) at a rate of 75 m³/hr. Similarly, small inoculum ponds are "harvested" and the inoculum delivered to the large inoculum ponds. If 4.5×10^6 liters/ha-yr are pumped over a 1 hour time period this equates to a pumping rate of 15 m^3 /hr. Newly sourced water (61.73×10^6 liters/ha-yr in the case of saline systems) is assumed to be delivered to all pond types over a 4 hour time period (after all harvesting has occurred) at a rate of 51.44 m^3 /hr.

The energy required to pump this water is estimated by assuming a differential head of 25 meters (requiring an operating pump pressure of 35 psi). This is consistent with the sizing of discharge pumps used in US agriculture (USDA, 2010, Table 19) and accounts for friction losses as well as small (less than 3 m) differences in elevation. All pumping is assumed to be completed using an electric pump with an efficiency of 50%, the density of the medium is assumed to be the same as water. Power requirements are calculated as

$$P = q_{medium} * \rho_{medium} * g * dh_{system} / \mu_{system}$$
(6.21)

where

P is the power required to pump water in and out of the ponds per hectare-year

 q_{medium} is the flow rate of the culture medium in terms of mass per unit of time

 ρ_{medium} is the density of the culture medium

g is the gravity constant

dh system is the differential head of the system in units of length

 μ_{system} is the efficiency of the system

Required pumping power for q_{medium} equal to 300 m³/hr (0.0833 m³/sec) is thus calculated as

$$0.0833 \text{ m}^3/\text{sec} * 1000 \text{ kg/m}^3 * 9.81 \text{ m/s}^2 * 25 \text{ m} / 50\% =$$

40.9 kW (6.22)

Energy requirements are calculated over the total time of 1500 hours to give 61,288 kWh/ha-yr to pump 450 x 10^6 liters out of the cultivation ponds, which is equal to 220,637 MJ/ha-yr (490.3 MJ/ 10^6 liters) in order to harvest the cultivation ponds. As a comparison, the estimated mean energy used to pump irrigation water, from surface water sources only, averages 1,608 MJ/ 10^6 liters (Table 6.7).

The above calculations are repeated for transferring reclaimed harvest water to the cultivation ponds, transferring feedstock from the inoculum ponds, and providing newly sourced water as inputs to both the cultivation and inoculum ponds. A summary of the volumes and energy required for interpond and other lateral water and algae transfer within the system is presented in Table 6.8.

 Table 6.8.
 Pumping and energy requirements for lateral water and algae transfer within the system

Pumpin	Volume *	Time	Pumping rate	Power	Electricity *	Energy *	
From	То	10 ⁶ L/yr	hr/yr	m3/s kW		kWh/yr	MJ/yr
Cultivation ponds	Settling ponds	450.0	1,500	0.0833	40.88	61,313	220,725
Large inoculum ponds	Cultivation ponds	45.0	600	0.0208	10.22	6,131	22,073
Small inoculum ponds	Large inoculum ponds	4.5	300	0.0042	2.04	613	2,207
Settling ponds	Cultivation ponds	405.0	2,100	0.0536	26.28	55,181	198,653
Newly sourced water	Inoculation and cultivation ponds	61.7	1,200	0.0143	7.01	8,410	30,278
	TOTAL					131,649	473,935

* per hectare of cultivation pond surface area

6.3.1.2.3.2.3 Water Circulation in the Cultivation Pond

The model pond is configured in a racetrack, through which water is circulated at a moderate rate using a motorized paddlewheel. Circulation helps ensure optimum exposure to light, but also

acts to retain culture suspension, distribute nutrients, and minimize thermal gradients. Paddlewheels are relatively energy efficient as compared to other pumping systems and induce minimal mechanical damage to the algal cells. Airlift pumps and direct culture bubbling have also been used, but are generally less energy efficient and may cause shear stress (Weissman and Goebel, 1987; Weissman *et al.*, 1989). The amount of energy required to power the paddlewheel used for mixing depends upon the area and depth of the water, the roughness of the pond surface, the nominal mixing speed, and the efficiency of the pump.

The general formula used to determine the amount of power required is based on Weissman et al, 1989 and is given as:

$$P / A_{pond} = g * \rho_{culture} * (v_{mixing})^3 * n^2 / (\delta_{water})^{0.33} * \mu_{mixing}$$
(6.23)

where

 P/A_{pond} is power in watts per meter squared (W/m²) required to operate the paddlewheel

- g is the gravity constant in meters per seconds squared (9.81 m/s²)
- $\rho_{culture}$ is the density of the algae culture in kilograms per meter cubed (kg/m³), which is estimated to be equal to the density of water (1000 kg/m³).
- v_{mixing} is the mixing velocity in meters per second (m/s)
- *n* is the Manning coefficient in seconds per cube root meter $(s/m^{0.33})$
- δ_{water} is the depth of the water in meters (m)
- μ_{mixing} is the overall efficiency of the mixing system

The nominal system assumes a mixing velocity, v_{mixing} , of 0.3 m/sec. This has been determined by researchers at The University of Texas to be the minimum required to keep the algae in suspension. Typical values for the Manning coefficient, *n*, per Weissman and others (1989) are 0.010 for very smooth surfaces such as plastic, 0.014 for unfinished concrete, 0.017 to 0.025 for smooth earth, and 0.029 for gravel. The depth of the water is assumed to be 0.3 meters. The overall efficiency of the mixing system is estimated to be 20% based on investigations completed at The University of Texas. Assuming a plastic liner and inserting these values into 6.14, the estimated required mixing power per unit area is

$$(9.81 * 1000 * 0.3^3 * 0.010^2) / (0.3^{0.33} * 20\%) = 0.197 \text{ watts/m}^2$$
 (6.24)

Converting this to kilowatts per hectare gives 1.97 kW/ha. A similar calculation gives a power requirement of 3.86 kW/ha for unfinished concrete 5.69 to 12.3 kW/ha for smooth earth, and 16.6 kW/ha for gravel. If the circulation system is run an average of 12 hours per day, 300 days per year (3600 hours per year), the total energy required ranges from 25,536 MJ/ha-yr for a plastic lined pond to 214,760 MJ/ha-yr for a gravel surface (Table 6.9). The relationship between material type (as indicated by the Manning coefficient) and mixing power requirements is shown graphically in Figure 6.9.

Pond surface	Manning coefficient	Power (watts/m ²)	Energy (kWh/m ² -yr) ¹	Energy (MJ/ha-yr)
Plastic	0.010	0.20	0.71	25,536
Concrete	0.014	0.39	1.39	50,051
Smooth Earth min	0.017	0.57	2.05	73,800
Smooth Earth max	0.025	1.23	4.43	159,602
Gravel	0.029	1.66	5.97	214,760

Table 6.9. Electricity for paddlewheel operation as a function of pond surface material

¹ 300 days per year, 12 hours per day



Figure 6.9. The mixing power requirement for a gravel lined pond is 8.4 times greater than for a plastic lined pond, while concrete is roughly double.

6.3.1.2.3.2.2 Nutrients and Carbon Dioxide

Algae in a natural setting typically derive nutrients from the water in which they are growing and from other organisms present in the ecological system. In a large monoculture, symbiotic relationships will be largely absent, and supplemental additions of critical elements will be required to support high biomass densities. Both major and minor nutrients are required; however, this is an area that is not particularly well known and the exact nutrient mix will depend on the particular algae species being cultivated. The current analysis uses a rough elemental mass balance in order to estimate the requirements for the major elements nitrogen (N), phosphorous (P), potassium (K), and carbon (C). A generic elemental composition for healthy algae is assumed as 50% carbon, 8% nitrogen, 0.6% phosphorus, and 0.3% potassium. This is based on values reported in the Phyllis database (ECN, 2010), with modifications made based a general overview of the literature. Reported values of nitrogen range from 6% to approximately 8.5%, but the former represent organisms grown in nitrogen depleted environments. Weissman et al., 1989 defined a nitrogen-replete environment as one where the

algae biomass contained greater than 8% N. Carbon content varies by species and environment with a general range of between 45% and 55%; 50% is assumed to be typical for non-stressed organisms.

The nominal biomass productivity is taken to be 24 g/m²-day. In a 30 cm pond, a 24 g/m²-day growth rate corresponds to 80 g/m³-day; thus the culture medium needs to contain more than 6.4 g/m³ N, 0.4 g/m³ P and 0.2 g/m³ K. With perhaps the exception of potassium, mid- to deep-level groundwater is expected to have levels much lower than these. Surface water, or shallow groundwater supplies, especially those affected by agricultural runoff, may have significant amounts of nitrogen or phosphorus, as would waste water; however, this analysis does not address such sources. Deep groundwater may contain bicarbonate (HCO₃⁻), although not in amounts that would contribute significantly to the overall carbon balance. In this analysis, all nutrients are provided through supplemental additions to the source water.

The total amount of nutrients and carbon dioxide additions required depends not only that taken up by the algae, but also the amounts removed from the system through precipitation and/or sedimentation, and that lost through the harvesting operation. Some fraction of nitrogen and carbon will also be lost via outgassing at the interface between the upper surface of the pond and the atmosphere. It is estimated that a minimum of 10% of the water will not be returned from the harvesting operation. Carbon dioxide is relatively insoluble in water and very little is expected to be present in the harvest water. However, both N and P are highly soluble in water and the amount of these elements lost will be proportional to the amount of water that is unreturned. Because harvesting occurs at the end of the day, the culture will be somewhat depleted with respect to these nutrients, but the specific amount will depend upon the degree of system control that is both achievable and employed. As a first approximation, it is estimated that 20% of the total N and P will be removed from the system each day due to harvest and chemical precipitation within the cultivation pond. In addition, Weissman and Goebel (1987) report up to a 30% loss of N to the atmosphere in a 20-cm deep, well-mixed pond. A value of 20% N outgassed is used here, as the assumed pond depth is 0.3 m rather than 0.2 m. In total, utilization rates are estimated to be 60% and 80% for nitrogen (N) and phosphorus (P), respectively. Potassium (K) is assumed to occur in the source water in excess of that required. Other elements, such as Fe may also need to be provided to the algae culture, but are not addressed here.

Weissman and others (1989) demonstrated 90% injection efficiency of CO₂, but the overall utilization efficiency ranged from only 32 to 65% of CO₂. It was projected that with use of a biomass recycle system, that overall efficiencies could reach 90%. As this option is not included in the current analysis, a value of 60% utilization of CO₂ is assumed. The amount of each of the three main elements that must be provided per unit of biomass produced is therefore equal to 0.13 kg of N per kg of algae (8% of biomass /60% utilization), 0.0075 kg of P per kg of algae (0.6% of biomass/80% utilization), and 0.83 kg of C per kg of algae (50% of biomass/60% utilization. When expressed as carbon dioxide, this is equal to 3 kg of CO₂ per kg of algae. Note that these inputs are all higher than estimates made by Weissman and Goebel (1987), even without recycle, but the basis of their estimates is not discussed in the report and from the standpoint of a simple mass balance, it is not clear how the values assumed by these authors can be justified.

All CO_2 is taken to be provided through supplemental additions of CO_2 gas using spargers (bubblers) that inject CO₂ located near the bottom of a sump (as described in Weissman and Goebel (1987) and Weissman et al. (1989)). The bubble size is targeted at 1 mm in order to maximize CO₂ transfer efficiency; this requires a very low flowrate. Because of its low solubility in water, CO₂ must be added continuously and in very large ponds, multiple injection stations may be required. Carbon dioxide is supplied in pure form through the use of spargers as described in Weissman and Goebel (1987). The pure CO₂ gas is delivered under pressure and current estimates account for no additional energy inputs in the delivery process. However, it is unclear as to whether this is a valid assumption or not. Flue gas from power stations (containing 9 to 14% CO₂, with the remainder primarily N₂) has been proposed as a substitute for pure CO₂. However, for both the gas as a whole and per molecule of CO₂ delivered, flue gas will require more energy to transport and compress and/or pump than pure CO₂. Nitrogen does not compress easily and approximately 10 times more gas would need to be delivered to the ponds as a carbon source for the algae. It is likely that use of flue gas would require a cover over the algae pond, which would add significantly to the material intensity of the system and decrease available light. There are also concerns about the presence of toxic materials in flue gas, particularly if sourced from coal plants. This is an issue not only because of the potential effect on algae productivity, but also because it could compromise the use of residual algal biomass as a food source. For these reasons, the flue gas option is not considered here.

Nitrogen is assumed to be supplied as ammonia (NH₃) and phosphorus as phosphate (P₂O₅). Both nutrients are added along with the makeup water at the beginning of each day. Because they are both highly soluble in water, one addition per day is expected to be adequate. With the use of a paddlewheel circulation system, no additional mechanism for distribution is required. A portion of the nutrients will be recycled along with the harvest water. One means of reducing overall nutrient demand (including carbon) would be anaerobic digestion of biomass residue. Weissman and Goebel (1987) estimate that this approach has the potential to reduce overall additions of nitrogen supplements by 75%, additions of phosphorus by 50%, and additions of CO_2 by 30%. This does, however, make the biomass unavailable for other, potentially high value, uses and is not considered in the current analysis.

6.3.1.2.3.3 Direct Material and Energy Flows

6.3.1.2.1.3.1 Fuel and Electricity Use

Diesel fuel is assumed to be used only for pumping of source water to both cultivation and inoculum. While it could be used for other applications such as pumping water between ponds, that scenario is not considered here. In the current analysis, the total amount of diesel for pumping source water is estimated to be 6,584 liters of diesel per harvested hectare-year for saline systems and 1,622 liters of diesel per ha-yr for freshwater systems, as calculated in expressions 6.15 and 6.18. The total energy from diesel is equal to 235,707MJ/ha-yr (6,584 liters/ha-yr * 35.8 MJ/ liter) for saline systems and 58,068 MJ/ha-yr (1,622 liters/ha-yr * 35.8 MJ/ liter) for freshwater systems.

Electricity is used for pumping of source water, pumping of water between ponds, and operation of the paddlewheel. The amount of energy required for pumping water into and between all ponds is estimated to be 131,649 kWh/ha-yr (473,935 MJ/ha-yr) and that to operate the

paddlewheel in a plastic lined cultivation pond is 7,093 kWh/ha-yr (25,535 MJ/ha-yr). Assuming the same system is used for the inoculum ponds, an 11% burden must be applied to give a total energy input per harvested hectare-year of 7,873 kWh/ha-yr (28,344 MJ/ha-yr) for water circulation in plastic-lined ponds; for clay-lined ponds a total of 22,755 kWh/ha-yr (81,918 MJ/ha-yr) will be required for circulation. Electricity use is summarized in Table 6.10.

		Sali	ne		Fresh				
Electricity Energy Input	Plastic		Clay		Pla	stic	Clay		
	kWh/ ha-yr	MJ/ ha-yr							
Source water pumping (electric fraction)	64,008	230,430	64,008	230,430	17,007	61,225	17,007	61,225	
Pump water between ponds	131,649	473,936	131,649	473,936	131,649	473,936	131,649	473,936	
TOTAL water pumping	195,673	704,423	195,673	704,423	148,656	535,162	148,656	535,162	
Paddlewheel	7,873	28,344	22,755	81,918	7,873	28,344	22,755	81,918	
Total	203,530	732,710	218,412	786,284	156,529	563,505	171,411	617,079	

Table 6.10.	Electricity	consumption	per harvested	hectare-year	during the	tending unit c	peration
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6.3.1.2.1.3.2 Water

The water balance is presented in Table 6.6. It is assumed that all water that is withdrawn is consumed (a total of 60.7 to 61.7 x 10^6 liters/ha-yr for freshwater and saline systems, respectively). The total amount of water engaged is 515 to 516 x 10^6 liters/ha-yr. The delta, 454 x 10^6 liters/ha-yr, is water that is at risk of being consumed and/or degraded.

6.3.1.2.1.3.2 Materials

The amount of nitrogen that must be added to the cultivation ponds, as determined in section 6.3.1.2.3.2.2 of this report, is equal to 13% of the algae biomass. Required additions of phosphorus are estimated to equal 0.075% of the biomass, but as phosphorus is accounted for as P_2O_5 , the latter would be added at a rate of 0.17%. Carbon requirements are 83% of the biomass, with corresponding carbon dioxide (CO₂) requirements equal to 300% of the biomass. It is assumed that inoculum ponds would be treated at the same rate, thus total values are burdened by 11%. Totals per harvested hectare-year for each of the biomass productivity scenarios are given in Table 6.11.

Biomass Productivity		Ν	Р	P_2O_5	С	CO ₂	% of US electricity
g/m ² -day	kg/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr	CO ₂ per 10 ⁶ ha
16	48,000	7,104	400	915	44,400	162,800	6.75%
24	72,000	10,656	599	1,373	66,600	244,200	10.12%
48	144,000	21,312	1,199	2,746	133,200	488,400	20.24%

Table 6.11. Nutrients required for algae cultivation and inoculum ponds per harvested hectareyear

Biomass uptake: 8% N, 0.6% P, 50% C

% Utilization: N = 60%, P = 80%, C = 60%

 CO_2 emissions from electricity generation in 2007 = 2,412 Tg (EPA, 2010)

For the nominal case (biomass productivity of 24 g/m²-day) the amount of CO₂ required per 10^6 hectares of algae cultivation, would equal 10% of all CO₂ emitted by US electric power plants in 2007 (based on data in EPA, 2010). Assuming all CO₂ is from electricity generating sources and no change in the mix of electricity generation power, places an upper limit of 10 x 10^6 hectares of production for the nominal case. Based on the estimates made in section 6.2.2.3.4 of this report (Eqn 6.2), this would support production of enough algae feedstock to displace only one-third of US motor diesel consumed in 2008.

6.3.1.2.1.3.4 Emissions to Air

Criteria Air Pollutants

Diesel powered pumps used to bring groundwater to the surface release emissions. Criteria air pollutants and their precursors are determined using EPA AP 42 guidelines for diesel engines (EPA, 1996, Table 3.3-1). Total emissions for both freshwater and saline systems are presented in Table 6.12.

Table 6.12. Emissions of criteria pollutants and their precursors in grams per liter (g/liter) of diesel fuel burned and grams per hectare-year (g/ha-yr) for the tending unit operation

Fue	Fuel Use			Emissions g/liter					Emissions g/ha			
Diesel use	e (liters/ha-yr)	VOC	со	NOx	PM	SO ₂	VOC	со	NOx	PM	SO ₂	
saline	6,582						35,873	96,166	446,470	31,397	29,357	
fresh	1,622	5.45	14.61	67.83	4.77	4.46	8,838	23,693	110,000	7,736	7,233	

Greenhouse Gas Emissions

Greenhouse gas emissions from burning of diesel fuel using a stationary source in an agricultural environment are estimated using IPCC emission factors (IPCC, 2006a, Table 2.5). The default carbon dioxide (CO₂) emission rate for agricultural diesel operations is 74,100 grams (g) of CO₂ per gigajoule (GJ) of fuel burned. If diesel is assumed to have a lower heating value (LHV) energy content of 0.0358 GJ/liter (ANL, 2009), this is equivalent to 2,653 g CO₂ per liter of diesel burned. Similarly, the IPCC default value for methane (CH₄) is 10 g/GJ and the default value for N₂O is 0.6 g/GJ. These equate to emissions of 0.358 and 0.021 grams of CH₄ and N₂O

respectively per liter of diesel combusted. Applying these values to the total fuel consumed (1,622 liters per hectare-year for freshwater and 6,582 liters for saline), the operation of diesel powered pumps during the tending unit operation results in per hectare emissions of 4,302 kg of CO_2 , 0.581 kg of CH_4 , and 0.0.035 kg of N_2O for freshwater and 17,461 kg of CO_2 , 2.356 kg of CH_4 , and 0.141 kg of N_2O for saline (Table 6.13).

Table 6.13. Emissions of greenhouse in grams per liter (g/liter) of diesel fuel burned and kilograms per hectare-year (g/ha-yr) for the tending unit operation

		CO2	CH4	N ₂ O
g/GJ fuel		74,100 10		0.6
g/L		2,653 0.358		0.021
1. m/h. m m	saline	17,461	2.356	0.141
кg/na-yr	fresh	4,302	0.581	0.035

The use of nitrogen as a nutrient will contribute to emissions of nitrous oxide (N₂O), but the emission factors are unknown for this system. Given that the system is largely contained, all emissions of nitrous oxide (N₂O) due to use of nitrogen fertilizer are treated as indirect emissions and addressed in section 6.3.1.3.4.2.3 of this report.

6.3.1.2.1.3.5 Land Use and Summary

The tending unit operation applies to both cultivation and inoculum ponds. A summary of the tending material and energy flows for both a saline and a freshwater system using plastic lined ponds are given in Tables 6.14 and 6.15, respectively. Only the nominal biomass productivity case (24 g/m^2 -day) is considered for nutrient use. All of the calculations completed in the above sections are expressed as inoculum plus cultivation requirements per harvested hectare-year, thus no further burden is required. The land use rate in Tables 6.14 and 6.15, reflecting the tending unit operation, is therefore 1.0.

Resource	Calculation	Value	Units
Land	1.0 hectares / 1 hectare-year	1.0	1/yr
Diesel			
Volume	1.0 / year * 6,582 liters / hectare	6,582	l/ha-yr
Mass ¹	6,582 liters / hectare-year * 0.837 kilograms / liter	5,507	kg/ha-yr
Energy ¹	6,582 liters / hectare-year * 35.8 megajoules / liter	235,650	MJ/ha-yr
Electricity	1.0 / year * 271,895 kWh / hectare	203,530	kWh/ha-yr
	equivalent to	732,710	MJ/ha-yr
Materials			
Nitrogen (N)	1.0 / year * 10,656 kilograms / hectare	10,656	kg/ha-yr
Phosphorus (P ₂ O ₅)	1.0 / year * 1,373 kilograms / hectare	1,373	kg/ha-yr
Carbon (CO ₂)	1.0 / year * 244,200 kilograms / hectare	244,200	kg/ha-yr
Water			
Freshwater withdrawn	1.0 / year * 9.41 x 10 ⁶ liters / hectare	9	10 ⁶ liters/ha-yr
Freshwater consumed	1.0 / year * 9.41x 10 ⁶ liters / hectare	9	10 ⁶ liters/ha-yr
Freshwater engaged ²	1.0 / year * 9.41 x 10 ⁶ liters / hectare	9	10 ⁶ liters/ha-yr
Saline water withdrawn	1.0 / year * 52.3 x 10 ⁶ liters / hectare	52	10 ⁶ liters/ha-yr
Saline water consumed	1.0 / year * 52.3 x 10 ⁶ liters / hectare	52	10 ⁶ liters/ha-yr
Saline water engaged ²	1.0 / year * 516.2 x 10 ⁶ liters / hectare	516	10 ⁶ liters/ha-yr
Criteria Air Pollutants and Precu	ursors		
VOC	1.0 / year * 35.87 kilograms / hectare	36	kg/ha-yr
со	1.0 / year * 96.17 kilograms / hectare	96	kg/ha-yr
NOx	1.0 / year * 446.47 kilograms / hectare	446	kg/ha-yr
PM	1.0 / year * 31.40 kilograms / hectare	31	kg/ha-yr
SO ₂	1.0 / year * 29.36 kilograms / hectare	29	kg/ha-yr
Greenhouse Gases			
CO ₂	1.0 / year * 17,461 kilograms / hectare	17,461	kg/ha-yr
CH ₄	1.0 / year * 2.356 kilograms / hectare	2.356	kg/ha-yr
N ₂ O	1.0 / year * 0.141 kilograms / hectare	0.141	kg/ha-yr

Table 6.14 .	Direct materia	al and energ	v flows for	tending algae	in saline	water system
1 abic 0.14.	Direct materia	an and energ	y 110 w 3 101	tenung argae	in same	water system

¹ Energy content and density of liquid fuels, default inputs to GREET (ANL, 2009)

² Water engaged is that is managed and therefore at risk for consumption or degradation

Resource	Calculation	Value	Units
Land	1.0 hectares / 1 hectare-year	1.0	1/yr
Diesel			
Volume	1.0 / year * 1,622 liters / hectare	1,622	l/ha-yr
Mass ¹	1,622 liters / hectare-year * 0.837 kilograms / liter	1,357	kg/ha-yr
Energy ¹	1,622 liters / hectare-year * 35.8 megajoules / liter	58,059	MJ/ha-yr
Electricity	1.0 / year * 123,504 kWh / hectare	156,529	kWh/ha-yr
	equivalent to	563,504	MJ/ha-yr
Materials			
Nitrogen (N)	1.0 / year * 10,656 kilograms / hectare	10,656	kg/ha-yr
Phosphorus (P ₂ O ₅)	1.0 / year * 1,373 kilograms / hectare	1,373	kg/ha-yr
Carbon (CO ₂)	1.0 / year * 244,200 kilograms / hectare	244,200	kg/ha-yr
Water			
Freshwater withdrawn	1.0 / year * 60.74 x 10 ⁶ liters / hectare	61	10 ⁶ liters/ha-yr
Freshwater consumed	1.0 / year * 60.74 x 10 ⁶ liters / hectare	61	10 ⁶ liters/ha-yr
Freshwater engaged ²	1.0 / year * 515.2 x 10 ⁶ liters / hectare	515	10 ⁶ liters/ha-yr
Criteria Air Pollutants and Precu	irsors		
VOC	1.0 / year * 8.84 kilograms / hectare	9	kg/ha-yr
со	1.0 / year * 23.69 kilograms / hectare	24	kg/ha-yr
NO _x	1.0 / year * 110.0 kilograms / hectare	110	kg/ha-yr
PM	1.0 / year * 7.73 kilograms / hectare	8	kg/ha-yr
SO ₂	1.0 / year * 7.23 kilograms / hectare	7	kg/ha-yr
Greenhouse Gases			
CO ₂	1.0 / year * 4,302 kilograms / hectare	4,302	kg/ha-yr
CH ₄	1.0 / year * 0.581 kilograms / hectare	0.581	kg/ha-yr
N ₂ O	1.0 / year * 0.035 kilograms / hectare	0.035	kg/ha-yr
¹ Energy content and density of	liquid fuels, default inputs to CREET (ANI 2000)		

Table 6.15. Direct material and energy flows for tending algae in a freshwater system

² Water engaged is that is managed and therefore at risk for consumption or degradation

6.3.1.2.4 Harvesting (separation of target material from growing medium):

6.3.1.2.4.1 General Description

Harvesting of algae from the cultivation pond is the portion of the raw material acquisition stage that is least well understood. Even at the conceptual level, it is not clear that there is an optimal design or set of technologies. There are three main challenges for harvesting algae. First, large volumes of water expected to contain less than 0.05% algae dry mass (less than 0.25% wet mass) must be removed from the cultivation ponds. This can require significant amounts of pumping, potentially at relatively high rates. Second, wet algal biomass, consisting of individual microscopic cells must be separated from the growth medium (principally water). Finally, as much water as possible must be returned to the production system in order to minimize overall water withdrawals and consumption in support of the cultivation pond. Any harvesting system will require equipment and process designs that are tailored to match the species that is being cultivated. There is no known universally applicable approach for harvesting eukaryotic

microalgae, independent of species and growing conditions, that is not temporally, materially, and/or energy intensive. Because of the significant volumes of aqueous medium involved, it is also likely that the harvesting process will be the rate limiting step, which means that the cultivation process may need to operate at less than maximum production levels.

Physical separation processes typically depend on either size (filtration) or density differences (gravity or centrifugation). Because algae consist of roughly 80% water, there tend to be very small density differences between the organisms and the growing medium. In addition, most microalgae considered for fuel production are small, 20 microns (10⁻⁶ m) or less and do not tend to form filaments or mats, Consequently, they do not lend themselves to most filtration devices without some mechanism (natural or induced) that causes them to aggregate into larger entities (flocculate). The current analysis assumes the use of a flocculation agent introduced in a pond that is separate from the cultivation pond, followed by settling. This is defined as stage one of the harvesting process. The supernatant from the top of the settling pond is returned to the growth (cultivation) pond and the "bottoms" of the settling pond are further separated, typically with a centrifuge, although other systems such as belt filtration have been proposed.

A second factor that must be considered in the harvest system design is whether an induction phase will be used to foster increased lipid content. Studies indicate that the two most important factors in encouraging concentration of neutral lipids are removal of nutrients (specifically, silicon for diatoms and nitrogen for all other algae) and an increase in available light (Benemann and Oswald, 1996). If this approach were to be used, it is assumed that induction would take place in separate ponds that are similar to cultivation ponds but which are shallower, unmixed, and not supplied with supplemental nitrogen. The purpose of these ponds would be to maintain total biomass while increasing relative lipid content within the biomass. Although the pond dimensions would be different, the process parameters of induction pond would be the same as a harvesting (settling) pond (no mixing, no addition of nutrients); thus, use of such a configuration may not require a separate harvesting pond. By virtue of being unmixed and nitrogen starved, algae are likely to settle on the bottom of this pond; however, it is expected that a flocculation agent would still be required in order to increase the settling rate. Use of induction ponds is expected to require a nearly two-thirds increase in land area and is not modeled in the current analysis.

Growth (cultivation) ponds are assumed to be operated in a semi-continuous manner. All transfer of the culture (cultivation medium plus algal biomass) from the growth ponds to the harvesting stations (either in the form of settling or induction/settling ponds) occurs during the first half of the night after all photosynthetic activity has ceased. The growth ponds are refilled during the second half of the night. Allowing for shorter summer nights, the design must allow for complete transfer of water in 5 hours (300 minutes) from the cultivation ponds to the harvest ponds. One half the volume of water is removed each evening unless the growth rate in the previous day was below some established minimum. If the algae experienced one doubling per day and the growth rate were perfectly constant, this would result in a uniform starting and harvest density each day. For example, if the starting density is 80 grams of biomass per meter cubed (equal to 24 g/m² in a 0.3 m deep pond) and the growth rate is 24 g/m²-day (where day equals hours of sunlight), the ending density would be 160 g/m³. Half the biomass is removed along with half the growth medium and replaced with growth medium nominally containing no biomass, bringing the starting density back to 80 g/m³. In actuality, the densities would not be

uniform from day to day; these values are intended to represent the average conditions. If there is minimal growth on a given day, harvesting may be avoided. Such circumstances are accounted for in assuming 300 growth days per year. System monitoring and control may allow for more sophisticated harvested timing schemes, but they are not evaluated here.

One of the process objectives during the harvest unit operation is to reclaim water for return to the cultivation ponds, thus minimizing the amount of newly sourced water that must be delivered to the system. Simply stating the percentage of harvested water that is reclaimed can be misleading, as this is driven not only by separation efficiencies but also by the "as harvested" biomass density of the medium and the absolute maximum biomass densities achievable for a given separation process. The more dilute the harvested medium (i.e., the lower the starting biomass density), the greater the ease with which water can be separated from the algae and returned to the cultivation pond. This results in a greater percentage of water reclaimed, but not a reduction in overall water demand. In addition, because of the need to maintain chemical and biological integrity in the inoculation and cultivation ponds, a certain amount of newly sourced water will be required regardless of the potential to reclaim harvested culture medium. In the current model, it is assumed that input into the inoculum ponds as well as that used to replenish water lost to blowdown, evaporation, and leaks is not reclaimed water.

6.3.1.2.4.1.1 Commercially Demonstrated Harvesting Methods

General descriptions of harvesting methods used at existing large-scale commercial microalgae facilities are provided by Benemann and Oswald (1996). A summary of those descriptions is as follows. *Chlorella*, which occur as small (less than $5 \ge 10^{-6}$ m) spherical cells, are typically harvested using a series of centrifuges. *Spirulina* are blue-green algae and therefore not currently under consideration as a feedstock for biodiesel production. It is filamentous in nature and thus forms masses that may be separated using screening methods (including microstrainers and belt vacuum filters). Harvest efficiency is estimated to be less than 50% with remaining biomass returned to the production pond. This approach has not been successful with other species. *Dunaliella* can be harvested by centrifuge, but because it lacks a cell wall, must be done at low throughputs in order to minimize shear. In addition, the high salinity of the medium can result in corrosion of the centrifuges, thus ceramic linings may be required.

6.3.1.2.4.1.2 Harvesting Options

General descriptions of different harvesting options are provided by Benemann and Oswald (1996) and summaries of each are provided below.

Centrifugation

Centrifugation is a commonly used process in microbial processes (such as fermentation). It relies on density differences between suspended particles and the medium that contains them and high centrifugal forces in rotating chambers. The amount of energy required for separation is strongly dependent upon the total volume handled and the solids concentration (positively correlated with the first factor and negatively correlated with the second). As microalgae concentrations are expected to be low and volumes large, the anticipated energy requirement for this approach, on a per unit mass of algae basis, is high. Throughput for an algal input of

 200 g/m^3 is estimated to be 1000 liters per minute, with a harvest efficiency of 70% and require 3 kWh per kg of algae. Centrifugation has an assumed concentration factor of 10 to 20X.

Filtration

Filtration of unicellular algae is difficult, primarily because of their small size; most conventional screening materials cannot be made to provide uniform openings at this scale. The approximately spherical shape and conformal (gelatinous) nature of microalgae also tends to produce clogging of the filtration system. Filtering, therefore, is most appropriate for filamentous or colonial algae. At a minimum, it is expected that at least some agglomeration, such as settling in the presence of a flocculation agent, will have to occur prior to the filtration processes in order for this to be a viable approach for most eukaryotic microalgae. Filtration processes include microstrainers, continuous belt vacuum filtration, and tangential or cross-flow filtration.

Settling

Settling, or gravity sedimentation, requires very little energy input. Only relatively large (greater than 100×10^{-6} m) aggregates will settle in a reasonable time period, thus a foreign substance that causes the algae to agglomerate (flocculate) is typically used. A minimum acceptable rate is considered to be 10 cm/hr, but most investigators target rates between 30 and 50 cm/hr. The process typically employs deep (2 to 3 meters) settling ponds. The concentrated algae biomass is collected off the bottom, while the remaining medium (the supernatant) is siphoned off the top to be returned to the cultivation ponds.

The surface of an algae cell naturally carries a negative charge. One means of inducing flocculation is to add a substance that will dissociate to form cations capable of neutralizing the algae surface, thus allowing them to coagulate. Inorganic substances, including sulfates or hydrates such as alum, ferrous sulfate (FeSO₄), and/or lime (Ca(OH)₂), can be effective flocculants; however, they typically require relatively high use rates (100's of grams per m³ of medium), and recovery of the additives is difficult. As a consequence, the process produces a chemical-algae sludge that that is not usable as animal feed and which must be disposed of. Another approach is the use of organic cationic polyelectrolytes. These are similar in function to inorganic chemical flocculation substances, but require much smaller amounts of flocculant (less than 10 grams per m³ of medium). Polyelectrolytes are polymers that contain an electrolyte group that dissociates in aqueous solutions to form charged particles with a high molecular weight. Positively charged (cationic) polymers act to neutralize the negative surface in the same manner as inorganic substances; they may also act to form "bridges" between the algae cells. In addition to having a lower concentration in the residual biomass, there is greater likelihood that they will be compatible with downstream uses of the residue including feedstock for fuel or animal feed (a number of polyelectrolytes are derived from plant or animal matter).

Algae cells may also be induced to flocculate without the introduction of a foreign substance. This typically occurs in response to chemical changes in the medium, including decreased nitrogen or carbon, increased light, and precipitation of calcium and phosphate ions. The currently understanding of this process is limited.

6.3.1.2.4.2 Activities

The harvesting process that is assumed in this analysis is a two-staged one. Beginning at dusk, half the volume of each cultivation ponds is emptied into settling ponds to a depth of 2.5-meters. The pumping process is expected to require 5 hours. The assumed concentration of the harvested culture is 160 grams of algae (dry mass) per m^3 of medium.

A polyelectrolyte flocculation agent is added to the settling ponds near the end of the pumping cycle at a rate of 1 kg per 500 kg of algae dry mass. This is assumed to produce a settling rate of 0.4 meters per hour. Thus after 0.5 hour, the top 20 cm of the settling pond would be relatively free of algae and pumping of the supernatant could begin. Removal of the supernatant would continue for 7 hours, at a rate that maintained a minimum 20 cm buffer between the pumping surface and the top of the settling algae column. The final concentration after settling is assumed to be 0.2% dry biomass, which is roughly one-third to one-fourth the concentration obtainable via microstrainer separation methods that are capable of producing a mixture consisting of 0.75% dry algal mass (Weissman and Goebel, 1987).

Pumping would ultimately reduce the level of the settling pond by 2.3 meters (from 2.5 to 0.2 m above the base). This would remove 92% of the volume in the settling pond (2.3/2.5) and increase the concentration of the dry mass of the algae to 2000 g/m³. Based on restrictions in the amount of reclaimed water that is suitable for input into the inoculation and cultivation ponds (section 6.3.1.2.3.2.2 of this report) only 90% of the harvested medium be returned as growth medium. The remaining 2% could be used for some other application or else sent to a waste stream. The energy requirements for returning the supernatant are addressed in the tending unit operation.

The concentrated "bottoms" of the settling pond as well as any excess supernatant must be removed from the settling pond during the day in order to ready the pond for the next evening's harvest. The total volume of the concentrated algae plus non-recycled supernatant is 10% of the original settling pond volume, which in turn is 50% of the cultivation pond volume of 3000 m³ per hectare. This means that 150 m³ per hectare of cultivation pond area must be pumped out of the harvest ponds per day, 300 days per year, for a total of 45,000 m³ per hectare-year. The average number of daylight hours is taken to be 12 hours; thus, there are 3600 pumping hours per calendar year (300 days x 12 hr/day) and the required pumping rate is 12.5 m³ / hr (0.0035 m³/sec).

The energy required to pump this water is estimated by assuming a differential head of 25 meters. All pumping is assumed to be completed using an electric pump with a 50% efficiency and the density of the concentrated medium is taken to be the same as water. Power requirements are calculated as per (6.21) or

$$0.0035 \text{ m}^3/\text{sec} * 1000 \text{ kg/m}^3 * 9.81 \text{ m/s}^2 * 25 \text{ m} / 50\% =$$

1.70 kW (6.25)

Energy requirements are calculated over the total time of 3600 hours to give 6,131 kWh/ha-yr required to pump concentrate out of the settling ponds, which is equal to 22,073 MJ/ha-yr.

In the current model, the concentrate is sent to a centrifuge, although alternative systems, such as belt filtration, are also an option. Benemann and Oswald (1996) give centrifuge energy requirements of 3 kWh per kg of algae, when the concentration is about 200 g/m³. This is equivalent to 0.6 kWh per m³ of medium. The concentration of the medium removed from the bottom of the settling ponds is expected to be 2000 g/m³ (therefore more viscous) and to require more energy to separate. As a very crude estimate 2.0 kWh per m³ of medium is assumed. The total volume sent to centrifuge is 8% of the original settling pond volume, or 36,000 m³ per hectare-year. The electricity required to produce a concentrate of 3.0% algae (15X concentration) through centrifugation is estimated to be 72,000 kWh/ha-yr.

Following the centrifuge separation process, the $36,000 \text{ m}^3$ per hectare-year of medium that is pumped in, must be pumped out of the centrifuge. Algae concentrate (2,400 m³/ha-yr) is sent to a natural gas dryer and the supernatant (33,600 m³/ha-yr) is sent either to waste or to some other purpose outside the immediate system. It is assumed that 12 hours per day are available to pump these streams. Per equation 6.21, the total amount of electricity required to pump both the concentrate and the effluent is 4,905 kWh/ha-yr, which is equal to 17,658 MJ/ha-yr.

A natural gas dryer is used to remove additional water to bring the final algal dry mass concentration to 10%. The amount of water evaporated is equal to

$$m_{evap} = m_{bmc} * [1 - (C_1/C_2)]$$
(6.26)

where

 m_{evap} is the mass removed through evaporation

 m_{bmc} is the mass of biomass concentrate that is subject to drying

 C_1 is the starting concentration of algae (dry mass)

 C_2 is the final concentration of algae (dry mass)

The mass of water removed per kilogram of biomass concentrate from the centrifuge is thus

1 kg * (1 - 0.03/0.10)

0.70 kg water per kg of concentrate (6.27)

The heat of vaporization for water at 100°C is 2.26 MJ/kg; therefore, 1.58 MJ is required to "dry" (dewater) 1 kg of 3% biomass concentrate to produce a 10% algae slurry. Note that the exact amount of drying that required will depend upon the tolerance of the lipid extraction process to the presence of water, thus this amount of energy input is sensitive to downstream process design. Natural gas has an energy content of 36.6 MJ/m³ (ANL. 2009). Assuming that a 10% concentration is appropriate, 0.043 m³ of natural gas will be required per kilogram of centrifuge effluent.

Based on the harvest process used in the model and described above, a volume of 2,400 m³/ha-yr will be subjected to the dewatering process. If the biomass concentrate is assumed to have the same density as water (1000 kg/m³), the amount of natural gas (NG) required for drying is equal to

$$0.052 \text{ m}^3 \text{ NG} / \text{kg} * 1000 \text{ kg} / \text{m}^3 * 2,400 \text{ m}^3/\text{ha-yr} =$$

124,800 m³ natural gas / ha-yr (6.28)

This is equal to 4,567,680 MJ/ha-yr.

Finally, the dewatered algae concentrate must be pumped from the dryer. The total volume is expected to be 720 m³/ha-yr and the pumping rate is estimated at 0.2 m³/hr. Per equation 6.21, the required electricity to perform this function is 98 kWh/ha-yr or 353 MJ/ha-yr.

6.3.1.2.4.3 Direct Material and Energy Flows

6.3.1.2.4.3.1 Fuel and Electricity Use

Electricity is assumed to be used for pumping and operation of the centrifuge. Energy required for centrifugation is estimated to be 72,000 kWh/ha-yr (259,200 MJ/ha-yr). An estimated 11,134 kWh/ha-yr is required to pump concentrate and waste (or by-product) supernatant out of the settling ponds, centrifuges, and dryers, which is equal to 40,084 MJ/ha-yr. A summary of these activities is presented in Table 6.16. Pumping of the supernatant that is returned to the cultivation ponds is accounted for in the tending unit operation. Natural gas is used for drying. If a 10% algae slurry is desired, 124,800 m³ natural gas / ha-yr (4,567,680 MJ/ha-yr) will be required.

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Table 6 16	Nummary (nt num	nino e	enerov re	nurements	durino	harvest	unit on	eration
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		Outflow		Pumping of Outflow		
	Concentrate	Waste (by-product)	Algae (dry mass)	Electricity	Energy	
Activity	m3/ ha-yr	m3/ ha-yr	% of concentrate	kWh/ ha-yr	MJ/ ha-yr	
Settling *	36,000	9,000	0.20%	6,131	22,073	
Centrifuge	2,400	33,600	3.0%	4,905	17,658	
Dryer	720	1,680	10%	98	353	
			TOTAL	11,134	40,084	

* water reclaim accounted for in tending unit operation

6. 3.1.2.4.3.2 Water

No additional water, beyond that which is discussed in the tending unit operation, is required.

6. 3.1.2.4.3.3 Materials

The only materials required are polyelectrolytes of unknown composition. They are assumed to be added at a rate of 1 g per 500 g of algae. The nominal case of 24 g/m²-day is expected to deliver a total of 72,000 kg/ha-yr of algae to the harvest system, thus requiring 144 kg/ha-yr.

6. 3.1.2.4.3.4 Emissions to Air

Criteria air pollutants and their precursors (Table 6.17) as well as greenhouse gas emissions (Table 6.18) from the burning of natural gas for drying are determined using EPA AP 42 guidelines for natural gas combustion (EPA, 1998).

Table 6.17. Emissions of criteria pollutants and their precursors in grams per unit of fuel and grams per hectare-year for natural gas fueled dewatering, as part of the harvest unit operation

Fuel Use	Emissions g/scm of natural gas					Emissions g/ha-yr				
scm/ ha-yr	VOC	CO *	NO _X *	PM	SO ₂	SO ₂ VOC CO NO _X PM				
124,800	0.09	1.35	1.6	0.12	0.01	11,232	168,480	199,680	14,976	1,248

* Assume small uncontrolled boiler

Table 6.18. Emissions of greenhouse gases in grams per unit of fuel and kilograms per hectareyear for natural gas fueled dewatering, as part of the harvest unit operation

Fuel Use	Emission	s g/scm of na	atural gas	Emiss	Emissions kg/ha-yr			
scm/ ha-yr	CO ₂	CH4	N ₂ O	CO ₂	CH₄	N ₂ O		
124,800	1,922	0.037	0.035	239,866	4.62	4.37		

6. 3.1.2.4.3.5 Land Use and Summary

The harvest unit operation material and energy flows are calculated per harvested hectare-year, thus no further burden is required. The land use rate in Table 6.19 is therefore 1.0.

Resource	Calculation	Value	Units	
Land	1.0 hectares / 1 hectare-year	1.0	1/yr	
Natural Gas				
Volume	1.0 / year * 124,800scm / hectare	124,800	scm/ha-yr	
Mass ¹	124,800 m3 / hectare-year * 0.777 kilograms / m3	96,960	kg/ha-yr	
Energy ¹	124,800 m3 / hectare-year * 36.6 megajoules / m3	4,570,868	MJ/ha-yr	
Electricity	1.0 / year * (11,134 + 72,000) kWh / hectare	83,134	kWh/ha-yr	
	equivalent to	299,282	MJ/ha-yr	
Materials				
polyelectrolytes	1.0 / year * 144 kilograms / hectare	144	kg/ha-yr	
Criteria Air Pollutants and Prec	Criteria Air Pollutants and Precursors			
VOC	1.0 / year * 11.23 kilograms / hectare	11	kg/ha-yr	
СО	1.0 / year * 168.48 kilograms / hectare	168	kg/ha-yr	
NO _x	1.0 / year * 199.68 kilograms / hectare	200	kg/ha-yr	
PM	1.0 / year * 14.98 kilograms / hectare	15	kg/ha-yr	
SO ₂	1.0 / year * 1.248 kilograms / hectare	1	kg/ha-yr	
Greenhouse Gases				
CO ₂	1.0 / year * 239,866 kilograms / hectare	239,866	kg/ha-yr	
CH ₄	1.0 / year * 4.6 kilograms / hectare	5	kg/ha-yr	
N ₂ O	1.0 / year * 4.4 kilograms / hectare	4	kg/ha-yr	

Table 6.19. Direct material and energy flows for harvesting and dewatering algae

¹ Energy content and density of liquid fuels, default inputs to GREET (ANL, 2009)

6.3.1.2.7 Waste Management

An estimated 42,600 m3/ha-yr of excess water will be removed from the settling ponds and centrifuges. In order to maintain the biological and chemical quality of the inoculation and cultivation ponds, this water is not returned within the cultivation system. There may be unidentified uses for this outflow, but if not, it must be treated as a waste stream.

The salt balance in the cultivation and inoculum ponds is governed by the evaporation rate as well as the salinity of the source water. Removal of excess salt is achieved by a process termed "blowdown" in which a portion of the water in the culture is released from the ponds and replaced with an equal volume of freshwater. Water for blowdown is ideally removed downstream of the harvesting point in order to minimize loss of algal biomass. The blowdown water is released into evaporation ponds and salt precipitated out. The salts collect at the base of the pond and must be disposed of. One simple solution may be to release them into the ocean; however, as the precipitates will also include excess nutrients, there are concerns with potential eutrophication if this is done at significant scale. The mass of material that must be removed and the distance that it must be transported will be highly dependent on the chemistry of the algae system and its location. The presence of toxic materials, residual live algae, and flocculants also complicates the disposal process. These issues are not accounted for in this analysis, but potentially have significant energy and environmental impacts.

6.3.1.3 Environmental Metrics

Four categories of environmental metrics are considered in the study: land use, net energy, water use, and emissions to air. Land use includes a quantitative assessment of the total amount of land required to support production of algae. Net energy is the quantity of energy required to produce the product less the quantity of energy generated. Water use includes both consumption and withdrawals (i.e., that lost to evaporation and that returned to the source in an altered state). Emissions to air that are considered in the analyses include carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), carbon monoxide (CO), particulate matter (PM), volatile organic compounds (VOCs), oxides of nitrogen (NO_X), and oxides of sulfur (SO_X). This section is also used to account for indirect flows and embodied inventories that occur due to activities upstream from the reference flow of cotton agriculture. In the case of the latter, only energy and emissions to air are considered.

6.3.1.3.1 Land Use, Area Requirements

The reference flow for the first life cycle stage in the production of biodiesel from algal oil is one hectare of land. The amount of algae that is produced on one hectare of land is primarily dependent on available light, measured in terms of surface area of the cultivation pond. Productivity is likely to vary by location, as for conventional crops, but in the absence of significant mass-cultivation, it is difficult to predict what the variability would actually be. Pilotscale production suggests that a daily average of 15 grams of algae per meter squared of pond area can be produced over the course of a 250 day growing season. Laboratory-scale investigations suggest that this number could be increased to 24 g/m^2 -day over a 300 day annual season. Benemann and Oswald (1996) state that the maximum physical limit is 60 g/m^2 -day for an open pond. This is taken to be the upper limit of a distribution with an average of 48 g/m²day. The total amount of land required is estimated to be between 160% and 165% of the surface area of the cultivation ponds. This allows for space required for walls and channel dividers in an earthwork constructed pond system, inoculum ponds, settling ponds, and blowdown evaporation ponds. Additional area, not accounted for, might include infrastructure and access. A possible log-normal yield distribution, assuming a modest skew, is shown graphically in Figure 6.10. The approximate 5^{th} and 95^{th} percentiles, in terms of amount of total land producing at a given yield, are determined to be at 32 and 45 Mg (megagrams, also metric tons) of algae biomass per hectare-year, respectively. The median (50th percentile) is equal to 38 Mg/ha-yr hectare-year. Note that the corresponding daily yields would exhibit a much wider distribution.



Figure 6.10. The median yield for algae is projected to be 38 Mg/ha-yr for an average 24 g/m^2 -day biomass productivity. This assumes a 60% burden for land requirements based on the need for non-cultivation ponds and for walls and channel dividers.

6.3.1.3.2 Water Use

All of the direct water use is accounted for in the tending unit operation and water balances are presented in Table 6.6.

Water is used in the production of concrete, but the amount is very small relative to other water used. Embodied water is 186 kg per m³ of ordinary concrete (SimaPro, 2007). Total use of concrete for settling ponds, amortized over 20 years is estimated to be 6.8 m³/ha-yr. Thus total embodied water is 1264 kg (1.3 x 10^3 liters) per ha-yr, one-thousandth of the freshwater sourced for saline ponds.

6.3.1.3.3 Net Energy

There are no energy products produced during this stage of the life cycle, therefore, net energy is equivalent to all the direct energy inputs to algae aquaculture, plus the upstream energy required to generate the direct energy, as well as the embodied energy in the nutrients supplied to the pond and the materials used to construct the ponds.

6.3.1.3.3.1 Direct Energy

The direct energy inputs are the sum of those due to the land preparation, tending, and harvesting unit operations; those from the seeding and planting operation (i.e., that related to the inoculum ponds and bioreactors) is accounted for by scaling the other unit operations. Total energy is equal to the sums presented in Table 6.5 (land preparation), Table 6.14 (tending of saline systems) or Table 6.15 (tending of freshwater systems), plus Table 6.19 (harvest). These are summarized in Table 6.20.

		F	Direct Energy Use MJ/ha-yr			
Unit Operation	ACTIVITY	Energy Source	Saline	Fresh		
Land Preparation	earthworks	Diesel	899	899		
		Diesel	235,650	58,059		
	pump source water	Electricity	230,430	61,224		
Tending	inter-pond pumping	Electricity	473,936	473,936		
	paddlewheel	Electricity	28,344	28,344		
	Tending TO	TAL	968,360	621,563		
	pumping	Electricity	40,084	40,084		
Lienvest	centrifuge	Electricity	259,200	259,200		
Harvest	dewatering	Natural Gas	4,570,868	4,570,868		
	Harvest TO	TAL	4,870,152	4,870,152		
	TOTAL without dewatering		1,268,543	921,746		
	TOTAL with dewatering		5,839,411	5,492,614		

 Table 6.20.
 Summary of direct energy use in cultivation of algae

6.3.1.3.3.2 Upstream Energy Use

Energy is required to generate and deliver fuels and electricity for direct use within the pond system. The upstream energy inputs for energy production are taken from the GREET model (ANL, 2009). The multipliers, based on the sum of the energy used to produce the feedstock plus the fuel, are applied to the energy used to cultivate algae in the form of electricity and fossil fuels. The inputs and calculated upstream energy requirements are shown in Table 6.21.

Table 6.21. Upstream energy required to produce electricity and fossil fuels (based on ANL, 2009)

Energy Source	Direct Energy	Use MJ/ha-yr	Upstream Energy	Upstrear MJ/ł	n Energy na-yr	TOTAL Energy to Supply Energy		
2	Saline	Fresh Factor		Saline	Fresh	Saline	Fresh	
Electricity	1,031,993	862,788	2.565	2,647,063	2,213,050	3,679,057	3,075,838	
Diesel	236,549	58,958	0.180	1,215	10,612	237,764	69,571	
Natural Gas	4,570,868	4,570,868	0.130	594,213	594,213	5,165,081	5,165,081	
TOTAL	5,839,411	5,492,614		3,242,491	2,817,875	9,081,902	8,310,489	
TOTAL excluding NG	1,268,543	921,746		2,648,278	2,223,663	3,916,821	3,145,408	

The amount of energy consumed in the production of material inputs is also significant. In this analysis, the nutrients as well as the pond construction materials are included. The GREET model (ANL, 2009) is used to determine the values associated with the manufacture and transportation of nitrogen and phosphorus. The upstream energy required to produce compressed CO_2 is based on currently existing technologies proposed for CO_2 capture and compression. The inputs are based on a life cycle assessment completed by Koornneef and others (2008) that compares 600 MW state-of-the-art coal-fired electricity generating units with and without carbon capture. In the system studied, the energy burden for capture of CO_2 gas using an MEA (monoethanolamine) solvent system is equal to 5.95 MJ/kg of CO_2 . This is determined by dividing the decrease in power (145 MW) times the number of operating hours per year (7800) by the amount of CO_2 captured (605 kg/MWh). Subsequent compression of the

 CO_2 gas is calculated by the authors to equal 111 kWh per Mg of CO_2 , which is equal to 0.4 MJ/kg of CO_2 . Compression energy required to inject the CO_2 into underground storage is calculated separately by the authors and is not included in the energy requirements estimated for this analysis. Combining these two energy requirements (capture and compression) gives a total energy demand of 6.35 MJ/kg of CO_2 . As the estimate does not include energy required to deliver the compressed CO_2 to the algae ponds, total energy requirements are expected to be slightly higher. Total estimated upstream energies for each are presented in Table 6.22.

Table 6.22.	Upstream	n ener	gies a	associat	ted with	the	manufa	acture an	d tra	nsportation c	of nitrogen,
phosphorus	(based on	data	from	ANL,	2009),	and	carbon	capture	and	compression	(based on
data from Ke	oornneef e	t al., 2	2008)								

			MJ/kg	nutrient	Total Upstream Energy
Scenario	Nutrient	kg/ha-yr	Feedstock + Production or Capture	Transportation or Compression	MJ/ha-yr
	Nitrogen (N) as ammonia	7,104	43.98	1.03	319,735
	Phosphate (P ₂ O ₅)	915	13.05	0.93	12,797
15 g/m ⁻ -day	Carbon (CO ₂)	162,800	5.95	0.40	1,033,780
	TOTAL				1,366,312
	Nitrogen (N) as ammonia	10,656	43.98	1.03	479,602
	Phosphate (P ₂ O ₅)	1,373	13.05	0.93	19,196
24 g/m ⁻ -day	Carbon (CO ₂)	244,200	5.95	0.40	1,550,670
	TOTAL				2,049,468
	Nitrogen (N) as ammonia	21,312	43.98	1.03	959,205
	Phosphate (P ₂ O ₅)	2,746	13.05	0.93	38,391
48 g/m ² -day	Carbon (CO ₂)	488,400	5.95	0.40	3,101,339
	TOTAL				4,098,935

Upstream energy to produce concrete for the settling ponds is taken from Prusinski and others (2004). The ponds are assumed to be constructed of a 20 MPa (3,000 psi), general use, Portland cement concrete. The mix is 14.6% cement, 45.9% coarse aggregate, 33.8% fine aggregate, and 5.7% water. Total upstream energy requirements are 1.675 GJ per m³ concrete. As determined in expression 6.5 of this report, a total of 135 m³/ha of cultivated pond area is needed to build the harvest ponds which are amortized over 20 years. This gives an effective use rate of 6.8 m³/ha-yr. Thus upstream energy is equal to 11,390 MJ/ha-yr.

The upstream energy to produce the plastic liners for the cultivation and covers for inoculum ponds is determined based on data in Hischier (2007). The energy requirements (cradle-to-gate) for a high density polyethylene (HDPE) liner or cover is estimated to be 101.0 MJ/kg lining material. The use rate for HDPE in the algae cultivation system is calculated as 3,754 kg/ha-yr (Table 6.5). Thus the upstream energy to produce HDPE is equal to 379,051 MJ/ha-yr.

6.3.1.3.3.3 Total Energy Inputs

The total energy required to cultivate algae is taken to be the sum of direct inputs and upstream inputs. Direct process energy consumption as well as the upstream energy required to produce

and deliver that energy are presented in Table 6.21. The total amount of process energy required for a saline system (direct plus upstream) without the dewatering step is 3,916,821 MJ/ha-yr; with dewatering this amount rises to 9,081,902 MJ/ha-yr. Additional upstream energy inputs are used in the production of nutrients (2,049,468 MJ/ha-yr and pond construction materials (379,051 MJ/ha-yr for plastic liners, 11,390 MJ/ha-yr for concrete). Total energy inputs are thus 11,521,811 MJ/ha-yr with the dewatering step or 6,356,730 MJ/ha-yr without dewatering.

The nominal production values for algae biodiesel from the system modeled in this analysis (Table 6.2) are optimistic, in that they have yet to be demonstrated at scale; yet with the proper strain of algae and cultivation strategies it is hoped that these levels may be achievable. Based on these assumptions, it is estimated that 7,711 kg of algae biodiesel could be produced per hectare of cultivation pond area per year. With an LHV (lower heating value) energy content for biodiesel of 37.53 MJ/kg (ANL, 2009) this suggests a potential energy output of 289,379 MJ/ha-yr. At these productivity levels and using the process design described in this analysis 20 to 40 times more energy will be required to produce the algae feedstock (with no accounting for the energy required for oil separation or fuel production) than the energy content of the potential algae biodiesel fuel produced by transesterification of neutral algal lipids (Figure 6.11).



Figure 6.11. The energy budget for cultivation of saline algae to be used as feedstock for biodiesel via transesterification, suggests that it will require at least 20 times more energy input than the energy content of the potential fuel.

The key driver for energy consumption, in the process assumed for this analysis, is the amount of water associated with algae cultivation. The most significant use of energy (more than 60%) is due to dewatering (not shown in Figure 6.11), which could be eliminated or reduced either by development of new post-harvest separation processes or through the introduction of a downstream oil extraction process that is relatively insensitive to water content in the algae slurry. The second largest determinant of energy consumption is water pumping and circulation. Reducing the harvest frequency has the potential to significantly decrease process energy requirements, but even a 90% reduction (e.g., using a batch system and harvesting once every

10-days) would still consume more energy than the energy content of the produced biodiesel. Such a strategy is also likely to result in lower biomass yields (kg/ha-yr) due to increased algal density in the cultivation ponds (and thus increased shading) and/or increased land area requirements. A more effective approach would be to identify a technology that minimized the water fraction removed from the cultivation ponds during the harvesting process.

Another significant source of energy inputs is the upstream burden associated with nutrient production. Biological sources of nutrients, including wastewater, could be used to lessen this quantity, however such feedstreams are likely to increase the opacity of the cultivation medium and thus reduce photosynthetic capability of the target algae species. In addition, harvesting processes may be adversely affected and biologic and chemical quality control of the culture medium would be more challenging. The mass of nutrients supplied relative to the algae biomass produced could be decreased (along with upstream energy burdens) by increasing the volumetric density of the biomass in the cultivation pond, but again, this is expected to result in a reduction in biomass productivity and yields.

A final approach to improving the potential net energy for mass cultivation of algae is to increase the total energy output. Increasing the lipid production rate, however, may prove difficult, as the assumed neutral lipid production rate in the above analysis is already greater than that demonstrated to date at scale. Additional energy products (e.g., diesel from lipids other than TAG, ethanol from carbohydrates) could also improve the overall energy balance.

- 6.3.1.3.4 Emissions to Air
- 6.3.1.3.4.1 Criteria

6.3.1.3.4.1.1 Direct Emissions of Criteria Pollutants and Precursors

Criteria pollutants and their precursors are released during the operation of earthmoving equipment, pumping of source water, and dewatering as a result of diesel and natural gas combustion. Total direct emissions are summarized in Table 6.23.

		<u>v</u>				
	Land Preparation	Tending, Saline	Tending, Fresh	Harvest	TOTAL, Saline	TOTAL, Fresh
Criteria Air Pollutants and Precursors	kg/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr
VOC	0.04	36	9	11	47	20
со	0.07	96	24	168	265	192
NO _x	0.86	446	110	200	647	311
РМ	0.15	31	8	15	47	23
SO ₂	0.01	29	7	1	31	8

Table 6.23. Direct emissions produced during life cycle stage one of biodiesel from algae

6.3.1.3.4.1.2 Upstream Emissions of Criteria Pollutants and Precursors

Emissions of criteria air pollutants and their precursors that are released during the production and delivery of energy and nutrients used in algae aquaculture are estimated only as functions of the energy required to produce and deliver these resources. The GREET model (ANL, 2009) is used to calculate values for energy, nitrogen and phosphorus. Emissions from CO_2 capture and compression are estimated from Koornneef and others (2008). Emissions for concrete production are from Prusinski and others (2004) and those for HDPE are from Hischier (2007). Results are presented in Tables 6.24, 6.25, and 6.26.

			Total Upstream Emissions							
Energy Source	System Type	Energy Use	VOC	СО	NO _x	PM	SOx			
		MJ/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr			
-	Saline	1,031,993	18	53	220	281	74			
Electricity	Fresh	862,788	15	45	184	235	62			
	Saline	236,549	2	3	9	2	1			
Diesel	Fresh	58,958	0	1	2	0	0			
Natural Gas		4,570,868	25	35	99	4	50			
	Saline	5,839,411	45	91	328	287	125			
TOTAL	Fresh	5,492,614	41	80	285	239	112			
	Saline	1,268,543	20	56	229	283	75			
TOTAL w/o NG	Fresh	921,746	16	45	186	235	62			

Table 6.24. Upstream criteria air pollutants and precursors from production of energy

Table 6.25. Upstream criteria air pollutants and precursors from production of nutrients

			Total Upstream Emissions							
Scenario	Nutrient / Amendment	Nutrient Use	VOC	СО	NO _x	PM	SO _x			
		kg/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr			
15 g/m²-day	Nitrogen (N) as NH ₃	7,104	41.9	38.4	15.0	3.6	1.2			
	Phosphate (P ₂ O ₅)	915	0.3	1.2	6.6	1.6	1.0			
	Carbon dioxide (CO ₂)	162,800	11.1	107.1	110.3	5390.7	39.8			
	TOTAL		53.3	146.6	131.9	5395.9	42.0			
24 g/m²-day	Nitrogen (N) as NH ₃	10,656	62.8	57.6	22.6	5.4	1.9			
	Phosphate (P ₂ O ₅)	1,373	0.5	1.7	9.9	2.4	1.4			
	Carbon dioxide (CO ₂)	244,200	16.6	160.7	165.5	8086.1	59.8			
	TOTAL		80.0	220.0	197.9	8093.9	63.1			
48 g/m²-day	Nitrogen (N) as NH ₃	21,312	125.6	115.1	45.1	10.8	3.7			
	Phosphate (P ₂ O ₅)	2,746	1.0	3.5	19.7	4.7	2.9			
	Carbon dioxide (CO ₂)	488,400	33.3	321.3	331.0	16172.2	119.5			
	TOTAL		159.9	439.9	395.8	16187.7	126.1			

Table 6.26.	Upstream	n criteria air	pollutants and	precursors from	production of materials
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Matarial	Material Use Rate		Emission Factor (kg/unit)					Total Upstream Emissions (kg/ha-yr)				
Material			VOC	СО	NO _x	PM	SOx	VOC	СО	NO _X	PM	SO _X
HDPE	3,754	kg/ha-yr	0.004	0.013	0.004	0.001	0.005	17	48	15	4	20
20 MPa concrete	6.8	m3/ha-yr	0.031	0.322	0.713	1.090	0.545	0.2	2.2	4.8	7.4	3.7
TOTAL								17	50	19	12	24
6.3.1.3.4.2 Greenhouse Gases

6.3.1.3.4.2.1 Direct Emissions of Greenhouse Gases

The direct net emissions of greenhouse gases released due to algae cultivation are summarized in Table 6.27. Most of the CO_2 emissions are from the use of natural gas for dewatering in the harvest unit operation, followed by diesel pumping of water for saline systems under the tending unit operation.

		0				0	
	Land Preparation	Land Tending, Tending, paration Saline Fresh		Harvest	TOTAL, Saline	TOTAL, Fresh	
Greenhouse Gases	kg/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr	
CO ₂	67	17,461	4,302	239,866	257,393	244,234	
CH4	0.00	2.36	0.58	4.62	6.98	5.20	
N ₂ O	0.03	0.14	0.03	4.37	4.54	4.43	

Table 6.27. Direct emissions produced during life cycle stage one of biodiesel from algae

6.3.1.3.4.2.2 Upstream Greenhouse Gas Emissions

Emissions of greenhouse gases that are released during the production and delivery of energy and nutrients used in algae aquaculture are estimated only as functions of the energy required to produce and deliver these resources. The GREET model (ANL, 2009) is used to calculate values for energy, nitrogen and phosphorus. Emissions from CO_2 capture and compression are estimated from Koornneef and others (2008). Emissions for concrete production are from Prusinski and others (2004) and those for HDPE are from Hischier (2007). Results are presented in Tables 6.28, 6.29, and 6.30.

		E a servicio a	Total Upstream Emissions					
Energy Source	System Type	Energy Use	CO ₂	CH₄	N ₂ O			
	System Type	MJ/ha-yr	kg/ha-yr	kg/ha-yr	kg/ha-yr			
Electricity	Saline	1,031,993	208,268	281	2.75			
Electricity	Fresh	862,788	174,120	235	2.30			
Diesel	Saline	236,549	3,232	23	0.05			
	Fresh	58,958	806	6	0.01			
Natural Gas		4,570,868	22,778	851	0.38			
TOTAL	Saline	5,839,411	234,278	1,155	3.17			
TOTAL	Fresh	5,492,614	197,704	1,091	2.68			
	Saline	1,268,543	211,500	304	2.80			
TOTAL W/0 NG	Fresh	921,746	174,926	241	2.31			

Table 6.28. Upstream greenhouse gas emissions from production of energy

			Total Upstream Emissions					
Scenario Nutrie Nitroge 15 g/m ² -day 24 g/m ² -day	Nutrient / Amendment	Nutrient Use	CO ₂	CH4	N ₂ O			
		kg/ha-yr	Total Upstream Emiss CO2 CH4 kg/ha-yr kg/ha-yr 18,082 17.7 2,330 2.3 185,412 116.5 205,824 136.4 63 57.6 1 1.7 278,119 174.7 278,182 234.0 126 115.1 3.5 556,237 349.4 556,364 468.0 165.1	kg/ha-yr				
	Nitrogen (N) as NH ₃	7,104	18,082	17.7	0.13			
	Phosphate (P ₂ O ₅)	915	2,330	2.3	0.02			
15 g/m⁻-day	Carbon dioxide (CO ₂)	162,800	185,412	116.5	1.75			
	TOTAL		205,824	136.4	1.90			
	Nitrogen (N) as NH ₃	10,656	63	57.6	22.57			
2 -	Phosphate (P ₂ O ₅)	1,373	1	1.7	9.86			
24 g/m²-day	Carbon dioxide (CO ₂)	244,200	278,119	174.7	2.63			
	TOTAL		278,182	234.0	35.06			
	Nitrogen (N) as NH₃	21,312	126	115.1	45.13			
	Phosphate (P ₂ O ₅)	2,746	1	3.5	19.73			
48 g/m²-day	Carbon dioxide (CO ₂)	488,400	556,237	349.4	5.26			
	TOTAL		556,364	468.0	70.12			

Table 6.29. Upstream greenhouse gas emissions from production of nutrients

Table 6.30. Upstream greenhouse gas emissions from production of materials

Matarial	Motorial Llas Rate		Emis	sion Factor (kg/unit)	Total Upstream Emissions (kg/ha-yr)			
Material	Materi	al Use Rate	CO2	CH4	N2O	CO2	CH4	N2O	
HDPE	3,754	kg/ha-yr	2	0.015	0.00001	8,049	57	0.02683	
20 MPa concrete	6.8	m3/ha-yr	228	0.011	nd	1,550	0.075	nd	
TOTAL			230	0.026	0.00001	9,600	57	0.02683	

6.3.1.3.4.2.3 Indirect Emissions of Greenhouse Gases

Indirect emissions of N_2O are likely to occur as the nitrogen added as a nutrient during the tending unit operation is volatilized to form NH_3 or oxides of nitrogen (NO_X) and subsequently deposited either in its gaseous form or as NH_4^+ and NO_3^- onto surrounding soil, water, or plant surfaces. Nitrogen may also be released beyond the pond boundaries if culture medium is lost due to leaks from the ponds or when water is released from the pond system, such as in the blowdown process. The potential magnitude of N_2O emissions due to leaks and releases from ponds is unknown and is likely to be extremely dependent on the specific site and design of the cultivation system. It is not accounted for here, but is noted as a potential source of uncertainty.

The indirect emissions of nitrous oxide due to volatilization from the pond surface are accounted for using IPCC guidelines (IPCC, 2006b). Sources of nitrogen that contribute to indirect emissions of N_2O are expected to occur primarily from added nitrogen (assuming low amounts of secondary biomass). IPCC Equation 11.9 accounts for N_2O emissions that result from atmospheric deposition of volatilized N (IPCC, 2006b).

After eliminating factors that are expected to be equal to zero for algae cultivation and converting nitrogen to N_2O , IPCC Equation 11.9 can be written as

$$N_2O_{atm dep} = N_{fert, N} * fraction_{GASF} * EF_{atm dep} * N_2O_{mw} / (2 * N_{aw})$$
(6.29)

where

- $N_2O_{atm dep}$ is the mass of annual nitrous oxide emissions per unit area produced from atmospheric deposition of nitrogen volatilized from the surface of the pond
- $N_{fert, N}$ is the mass of nitrogen fertilizer, as nitrogen, applied annually per unit area
- *fraction* _{GASF} is the fraction of synthetic fertilizer that volatilizes, assumed to be 0.20 per Weissman and Goebel (1987) and Neenan et al, (1986).
- $EF_{atm dep}$ is the mass of annual direct nitrous oxide emission per unit area due to the application of nitrogen fertilizer; assumed to be 0.01 per IPCC guidelines (IPCC, 2006b, Table 11.3, EF_4).
- N_2O_{mw} /(2 * N_{aw}) is the conversion factor for nitrogen to nitrous oxide, equal to 44/28

Substituting in the rate of nitrogen fertilizer application, as nitrogen, for the nominal case (Table 6.23), the expression becomes

$$10,656 \text{ kg/ ha-yr} * 0.20 * 0.01 * 44/28 =$$

$$33.49 \text{ kg N}_2\text{O} / \text{hectare-year}$$
(6.30)

6.3.1.3.4.2.4 Net Greenhouse Gas Emissions

Calculation of greenhouse gas emissions for the purpose of estimating total global warming potential requires that the each gas be scaled according to its global warming potential relative to carbon dioxide as given in the Fourth Assessment Report of the IPCC (IPCC, 2007). These factored emissions are then summed to give total greenhouse gas emissions in terms of carbon dioxide equivalents (CO₂e). In addition, CO and VOCs are assumed to oxidize readily to CO2. VOCs are taken to have a relatively low molecular weight and consist of 83 wt% C (e.g., pentane). The sum of the mass of greenhouse gas emissions expressed as CO_2 equivalents is thus calculated as:

$$GHG = \sum_{k} E_{k} * GWP_{k} + [E_{CO} * CO_{2 mw} / CO_{mw}] + [0.83 * E_{VOC} * CO_{2 mw} / C_{aw}] \quad (CS.21)$$

where:

GHG is the sum of the mass of greenhouse gas emissions expressed as CO₂ equivalents

 $E_{\rm k}$ is the mass of emissions of GHG species k

 GWP_{k} is the global warming potential for GHG species k (IPCC, 2007)

*E*_{CO} is the mass of CO emissions

CO_{2 mw} / CO mw is the conversion factor for CO to CO₂, equal to 44/28

 E_{VOC} * is the mass of VOC emissions

 $CO_{2 mw} / C_{aw}$ is the conversion factor for C to CO₂, equal to 44/12

The nominal biomass productivity of 24 g/m²-day is equal to 72 Mg/ha-yr; however, if total land occupation is taken into account (estimated to be 160% of the cultivation area), the effective biomass production is 45 Mg/ha-yr. Assuming 50% carbon in the biomass, the total CO₂ uptake will equal the mass ratio of carbon dioxide to carbon (44/12) times 0.5 x 45 or 82.5 Mg of CO₂ per hectare occupied per year. The construction of algae ponds will prevent anything else from growing on site. The minimum amount of plant matter that would be displaced is estimated based a conservative switchgrass yield of 5 Mg/ha-yr. If a carbon uptake for switchgrass is 50%, this is equivalent to 9.2 Mg of CO₂ per hectare, for a difference (benefit of growing algae over switchgrass) of 73.3 Mg of CO₂ per hectare-year.

Total emissions of species contributing to the greenhouse gas inventory are given in Table 6.31 as net emissions, as well as in terms of CO_2 equivalents. The analysis shown is for a saline system at the nominal biomass productivity rate of 24 g/m²-day. As can be seen in Table 6.31, nearly two-thirds (67%) of all the greenhouse gas emissions can be attributed to upstream emissions (Figure 6.12), with slightly more due to production and distribution of materials rather than energy production.

Because natural gas used for dewatering dominates energy usage, a scenario where it is not included is presented in Table 6.32. The overall reduction in greenhouse gas emissions under these circumstances is 28% (Figure 6.13). In addition, this would require advances in technology that would permit oil extraction in the presence of significant amounts of water (>90%) and/or a physical harvesting method that can produce a concentrate of greater than 3%.

Table 6.31. Greenhouse gas emissions (net and as carbon dioxide equivalents (CO₂e)) in kilograms per hectare-year (kg-ha-yr) released during cultivation of algae, in a saline system with nominal biomass productivity of 24 g/m²-day.

Saline System,	Net Emissions of GHG					Emissions of GHG in CO2e					
dewatering, 24g/m2- day		kg	g/ha-yr				k	g/ha-yr			
Source	CO2	CH4	N2O	со	VOC	<u> </u>	CH4	NO	<u> </u>	VOC	TOTAL
GWP (CO2e factor)	1	25	298	1.57	3.04	002	014	N2O	00	VUC	CO2e
Direct emissions	257,393	7	5	265	47	257,393	174	1,351	416	143	259,479
Indirect emissions	0	0	33	0	0	0	0	9,980	0	0	9,980
Upstream emissions - energy	234,278	1,155	3	91	45	234,278	28,869	946	143	138	264,374
Upstream emissions - nutrients	278,182	234	35	220	80	278,182	5,850	10,448	346	243	295,068
Upstream emissions - materials	9,600	57	0	50	17	9,600	1,430	8	79	51	11,167
TOTAL	491,671	1,162	41	356	93	779,453	36,322	22,733	983	576	840,068
Differential CO2 uptake	73,300									NET	766,768



Figure 6.12. Two-thirds (67%) of greenhouse gas emissions are due to upstream activities associated with the production and delivery of energy and materials.

Table 6.32 .	Greenhouse g	gas emissio	ns (net	and as	carbon	dioxide	equivalen	ts (CO_2e)) in
kilograms per	hectare-year	(kg-ha-yr) 1	eleased	during	cultivat	ion of al	gae, in a	saline sys	tem
with nominal l	piomass produ	ctivity of 24	g/m ² -d	ay, excl	uding na	tural gas	dewaterin	ıg.	

Saline System,		Net Emis	sions of G	HG			Emissions	of GHG in	CO2e		
dewatering, 24g/m2- day		kg	ı/ha-yr				k	g/ha-yr			
Source	CO2	CH4	N2O	со	VOC	<u> </u>	CH4	NOO	<u> </u>	VOC	TOTAL
GWP (CO2e factor)	1	25	298	1.57	3.04	002	CH4	N20	0.0	VUC	CO2e
Direct emissions	17,528	2	0	96	36	17,528	59	50	151	109	17,897
Indirect emissions	0	0	33	0	0	0	0	9,980	0	0	9,980
Upstream emissions - energy	211,500	304	3	56	20	211,500	7,602	834	88	62	220,085
Upstream emissions - nutrients	278,182	234	35	220	80	278,182	5,850	10,448	346	243	295,068
Upstream emissions - materials	9,600	57	0	50	17	9,600	1,430	8	79	51	11,167
TOTAL	229,027	306	36	152	56	516,809	14,940	21,319	664	465	554,198
Differential CO2 uptake	73,300									NET	480,898





6.3.1.4 By-Products

The current model assumes that there are no by-products associated with algae cultivation.

6.4 Algal Oil Biodiesel Glossary

AFDW: ash free dry weight

berm: a low earthen barrier or wall adjacent to a ditch

blowdown: The process of removing a portion of the water from the algae culture; the eliminated water is replaced with an equivalent amount of virgin source water in order to maintain water quality.

culture density: the mass of algae cells per volume of aqueous culture medium, typically measured in units of grams per liter)

cytosol: intracellular fluid that surrounds the organelles and nucleus; it consists of a complex mixture of substances dissolved in water.

inoculum: A small population of microalgae used to initiate mass growth of that particular species of algae in the pond (the culture medium).

insolation: A measure of solar radiation received on a given surface in a given amount of time

lipid: A broad group of naturally occurring molecules that include free fatty acids, triglycerides, and phospholipids. Their main biological functions are energy storage and structural components of cell membranes

National Renewable Energy Laboratory (NREL): A national lab within the U.S. Department of Energy (DOE), managed for DOE by the Alliance for Sustainable Energy, LLC.

NREL: National Renewable Energy Laboratory

PAR: photosynthetically active radiation

photosynthetically active radiation (PAR): wavelengths of sunlight between 400 and 700 nanometers (nm)

SERI: Solar Energy Research Institute

Solar Energy Research Institute (SERI): The original name of what was to become the National Renewable Energy Laboratory (NREL) in 1991.

sparger: a device used to inject an inert gas into a liquid, typically creating bubbles during the process.

sump: a low space used to collect liquids

TAG: triacylglycerol

Triacylglycerol (**TAG**): A glyceride formed by a single molecule of glycerol esterified with three fatty acids.

TSS: Total suspended solids

triglyceride: Common name for triacylglycerol

6.5 Algal Oil Biodiesel References

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