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An Overview of Algae Biofuel Production and Potential Environmental Impact

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ABSTRACT: Algae are among the most potentially significant sources of sustainable biofuels in the future of renewable energy. A feedstock with virtually unlimited applicability, algae can metabolize various waste streams (e.g., municipal wastewater, carbon dioxide from industrial flue gas) and produce products with a wide variety of compositions and uses. These products include lipids, which can be processed into biodiesel; carbohydrates, which can be processed into ethanol; and proteins, which can be used for human and animal consumption. Algae are commonly genetically engineered to allow for advantageous process modification or optimization. However, issues remain regarding human exposure to algae-derived toxins, allergens, and carcinogens from both existing and genetically modified organisms (GMOs), as well as the overall environmental impact of GMOs. A literature review was performed to highlight issues related to the growth and use of algal products for generating biofuels. Human exposure and environmental impact issues are identified and discussed, as well as current research and development activities of academic, commercial, and governmental groups. It is hoped that the ideas contained in this paper will increase environmental awareness of issues surrounding the production of algae and will help the algae industry develop to its full potential.

I. BACKGROUND

Algae belong to a large, diverse group of organisms ranging from unicellular to multicellular that produce complex organic compounds from basic inorganic molecules using energy from photosynthesis, inorganic chemical reactions, and heterotrophic fermentation.[1−3] The algae fossil record dates back approximately three billion years, well into the Precambrian period. Algae are ubiquitous within the biosphere and have generated a large fraction of the oxygen present in the earth's atmosphere and a large quantity of organic carbon in the form of coal and petroleum.[2] Algae's role in the development of the earth's biosphere was of unique importance.

The importance of algae has increased with the search for renewable energy sources. Even under highly unfavorable growth conditions, algae can thrive and produce valuable byproducts such as lipids (oils), carbohydrates, proteins, and various feedstocks that can be converted into biofuels and other useful materials.[4−6] Hu et al. (2008) projected a possible yield of 200 barrels of oil per hectare (2.47 acres) of land used for growing photosynthetic algae.[5] This theoretical maximum algae yield is 100 times greater than that for soybeans, a commonly used feedstock for biodiesel, and is greater than any demonstrated yield (by a factor of 10−20), but should be a goal for the rapidly developing algae industry.[5]

Algae-based biofuel production has a number of potential advantages:

- Biofuels and byproducts can be synthesized from a large variety of algae.
- Algae have a rapid growth rate.
- Algae can be cultivated in brackish coastal water and seawater.
- Some land areas that are unsuitable for agricultural can be used to cultivate algae.
- Algae nutrient uptake uses high nitrogen, silicon, phosphate, and sulfate nutrients from human or animal waste.
- Algae can sequester carbon dioxide (CO_2) from industrial sources.

Developing this technology into a commercial success, however, will be a challenge. Many issues must be addressed for the algae industry to advance from its current state to commercial success. Of these issues, environmental impact is paramount. Algae production has demonstrated many positive environmental effects as well as the potential for negative effects on human health and the environment.

Numerous benchtop experiments and pilot projects have been conducted and small-scale production facilities and theoretical process models and projections have been established.[5,7−10] However, little extensive information has been published detailing algae production processes or algae-derived biofuel research and development that has been put

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into practice. Often, the existing literature either is specific to a unique example or is broader in scope. Additionally, what little published information there is often fails to describe the importance of algae production, how that information fits into the present state of knowledge, or what environmental impacts might result from expansion of the algae industry.

This paper provides a review of algae generation, the products derived from algae, and the environmental and human health issues surrounding the production process. This review summarizes information related to the status of algae-based biofuel research and development efforts, including the efforts of a number of commercial biofuel companies that are using genetic advancements to move in new directions. These unique genetic advancements are fueling algae biofuel research and making it an ever-changing area of activity with enormous potential environmental consequences. In addition, the risk of exposure to humans and the environment posed by algae biofuel production is summarized and a number of prominent forms of algae production are addressed and compared.

1. Nature of Algae. Algae lack the many distinct organs and structures that characterize land plants, such as leaves, roots, a waxy cuticle, and other organs.[1,2] All algae contain green chlorophyll; however, they are masked by photosynthetic pigments that give them a distinguishing color that is used to identify key divisions.[1,2] These photosynthetic pigments are made of four different kinds of chlorophyll: blue, red, brown, and gold.[1,2] Some algae are microscopic and are able to float in surface waters (phytoplankton) due to their lipid content, while others are macroscopic and attach to rocks or other structures (seaweeds).[2] Algae range in size from less than the size of bacteria (0.5 μm) to over 50 m long.[2]

Algae are cultivated in a variety of aqueous systems, from open air ponds to closed photobioreactors with closely controlled environments.[2,11,12] The temperature range needed to support algal growth is specific to the species and strain cultured. The optimal temperature for phytoplankton is within the range of 20−30 °C. Temperatures lower than 16 °C will slow growth, and temperatures higher than 35 °C are normally lethal for a number of species.[2,13,14]

2. Constituents and Byproducts. The algal growth cycle has demonstrated considerable flexibility in the biochemical ability of algal cells to manufacture various useful compounds. Depending on the species and growing conditions, algae can yield a wide array of byproducts such as lipids, carbohydrates, and proteins.[15] Lipids are long carbon chain molecules that serve as a structural component of the algal cell membrane. The increased lipid content decreases the specific gravity, making the algal cell buoyant. The buoyant algal cell then moves up in the water column toward the solar energy source. Algal species vary in lipid production from 20 to 80% (dry weight (DW)). Adjusting the specific species growth requirements can affect the algal biomass lipid, protein, and carbohydrate content.[12] These adjustments include environmental culture conditions such as process velocity, length of the growth period, and nutrient supply.[15−17]

Becker listed the constituents of 17 common algal species.[16] The constituents of lipids, starches, and proteins are listed in Table 1. As the table shows, these constituents vary substantially depending on the substrate and environmental conditions of the algal biomass composition.[16]

Lipids extracted from microalgae can be used to produce biodiesel.[12] Once the lipids have been extracted, the leftover solids are composed of mostly carbohydrates and proteins. Carbohydrates such as starches and sugars can be fermented to produce ethanol.[18] If algal oil is extracted for production of biodiesel fuel, producing ethanol can facilitate this process by becoming a key component of the transesterification process. Transesterification of algal oil can be accomplished with ethanol and sodium ethanolate, which serves as a catalyst. The sodium ethanolate can be produced by reacting ethanol with sodium. The catalyst sodium ethanolate and ethanol react with the algal oil to produce biodiesel and glycerol.[18]

Table 1. Constituents of Algae (% of Dry Matter)[16]

algae	lipids	protein	carbohydrate
Anabaena cylindrica	4−7	43−56	25−30
Aphanizomenon flos-aquae	3	62	23
Arthrospira maxima	6−7	60−71	13−16
Botryococcus braunii	86	4	20
Chlamydomonas rheinhardii	21	48	17
Chlorella ellipsoidea	84	5	16
Chlorella pyrenoidosa	2	57	26
Chlorella vulgaris	14−22	51−58	12−17
Dunaliella salina	6	57	32
Euglena gracilis	14−20	39−61	14−18
Prymnesium parvum	22−38	30−45	25−33
Porphyridium cruentum	9−14	28−39	40−57
Scenedesmus obliquus	12−14	50−56	10−17
Spirulina maxima	6−7	60−71	13−16
Spirogyra sp.	11−21	6−20	33−64
Spirulina platensis	4−9	46−63	8−14
Synechococcus sp.	11	63	15

Microalgae belonging to several different families possess the ability to produce and accumulate a large fraction of their dry mass as lipids, as listed in Table 2.[7,17,19] In microalgae, high cellular lipid content is often achieved under environmental stress, which can be caused by limitations of nitrogen, phosphorus, silicon, salinity, and iron.[20−24] Lipid accumulation in the cells also depends on the growth phase of cells.[25−28]

Table 2. Oil Content of Microalgae[7,19]

microalgae	oil content (% dry weight)
Botryococcus braunii	25−75
Chlorella sp.	28−32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16−37
Dunaliella primolecta	23
Isochrysis sp.	25−33
Monallanthus salina	>20
Nannochloris sp.	20−35
Nannochloropsis sp.	31−68
Neochloris oleoabundans	35−54
Nitzschia sp.	45−47
Phaeodactylum tricornutum	20−30
Schizochytrium sp.	50−77
Tetraselmis sueica	15−23

Proteins are biochemical compounds made of one or more peptides (a single linear polymer chain of amino acids) folded into a solid or fibrous form. Studies have shown that algal proteins are of high nutritional quality and are comparable to the protein found in conventional vegetables. Forms of macroalgae are commonly used for human consumption, while microalgal preparations are marketed as health food, cosmetics, and animal feed.[15,16,29]

Algae production can also yield additional secondary benefits such as the generation of hydrogen or methane, which can be used for transportation fuels. Other benefits of algae production can be gained from the removal of nitrogen and phosphorus from the treatment of municipal, agricultural, and industrial wastewater; the absorption of carbon dioxide from industrial flue gas; the production of protein for human or animal consumption; and the production of compounds for pharmaceuticals, cosmetics, and aquaculture purposes.[1,2,30−34]

3. Environmental Conditions of Cultivation. Algae can be cultivated in either open ponds or photobioreactors (PBRs). Open pond systems are used for the majority of algae cultivation, especially those strains with high oil content.[25,35−37]

PBRs are closed, controlled systems with equipment that provides an ideal environment for high algae cultivation productivity.[25,35−37]

Open ponds are generally categorized as either natural waters, such as lakes, lagoons, and ponds, or artificial ponds or containers. These include shallow ponds and tanks that are circular or parallel raceway ponds (PRPs).[25,35−38] Major advantages of open ponds are that they are easy to construct and operate and their costs are minimal. However, major limitations of open ponds stem from the lack of control, which can result in poor light utilization by microalgal cells (low surface to volume ratio), evaporative losses, and poor diffusion of CO_2 to the atmosphere. These systems also require use of large land areas, and they are highly susceptible to environmental fluctuations such as swings in temperature and pH.

Microorganism contamination, such as the invasion of fast-growing heterotrophic algae and bacteria, poses a significant problem in open pond systems and has restricted their successful use for commercial production of algae.[25,35−38] The nature of open ponds and their susceptibility to contamination have limited this application to mainly three taxa: *Spirulina*, *Dunaliella*, and *Chlorella*.[39] Research efforts to deal with the problem of contamination involve the genetic modification of microalgae.[7,40−42] Processes using genetically modified microalgae are currently being developed by many biofuel industry participants.

Many scientific and commercial algae production efforts use PBRs, which facilitate better control of the pure culture environment by providing optimal growth requirements such as amounts of carbon dioxide and water, temperature, exposure to light, mixing, culture density, pH levels, and gas supply and exchange rate.[43,44] As these systems are closed, all of the specific growth requirements are internally maintained.

PBRs pump the cultured organism and nutrient-laden water through plastic or glass tubes that are exposed to sunlight. The full spectrum of sunlight is generally not available to aquatic algae, especially in high-density cultures and algae contained within bioreactors.[43,44] Light penetrates only the exposed 7.6–10 cm due to turbidity caused by algae growth and media.[43−46]

A number of enclosed configurations, such as a helical-tubular photobioreactor designed by Briassoulis et al.,[47] are being used to optimize the limiting growth conditions for continuous production.[32−34,47−52] The following conditions are optimized in a PBR design:

- Volume size to surface area ratio (maximized for light penetration).
- Containment to control temperature and pure culture contaminants.
- Spatial distribution of fresh air and CO_2.
- CO_2 transfer rates (improved through extensive interface surface between air and culture in liquid medium).
- Novel automated flow-through sensors (used to provide continuous cell concentration monitoring).

PRP systems generally are perceived to be less expensive than PBRs due to their lower construction and operation costs. While these systems have been shown to be cost-effective for limited applications such as producing protein, their low cost is offset by their low biomass productivity and high vulnerability due to technical constraints in pond design, separation, and culture stability compared with PBRs.[5,7,8] PBR systems have higher construction and operational costs associated with the culture process, but their algal cultures are capable of producing a high lipid content biomass (40−55%).[5,7,8] Given the advantages and disadvantages of both systems, hybrid culture systems are sometimes used to integrate the best aspects of both systems.[5,7,8,53−55]

4. Genetically Modified and Enhanced Organisms. The need to enhance or optimize production of target products has led to the development of new forms of microalgae. Consequently, as new forms of microorganisms increase, the conversion steps necessary for biofuel production increase. In the last two decades, molecular methods have been developed to synthesize and clone genes and then transform or transfer them into living organisms.[56−59] All forms of microorganisms, including microalgae, have undergone experimental modifications resulting in what are called genetically modified organisms (GMOs), or genetically enhanced organisms (GEOs). These techniques are generally known as recombinant DNA technology, which creates new organisms with modified or novel traits.[56−62]

Biotechnology research is developing GMOs to generate biofuels directly or to produce intermediate organics that can be converted into a biofuel. Examples of the former include organics such as petroleum, ethanol, and hydrogen, while intermediate organics include bot lipids (for processing into biodiesel) and carbohydrates (for conversion into ethanol). GMOs have also been developed to promote the breakdown of plant fiber.[61] New varieties of GMOs are being developed to improve process speed, efficiency, and stability and simplify the process; to enhance enzyme production; and, in the field, to optimize plant feedstock production for use as energy crops.[61]

5. Growth Optimization. Research has also examined the environmental conditions affecting microalgae growth.[63,64] Many factors influence microalgae growth including irradiance, culture temperature, algal biomass density, uniformity of mixing, nutrient concentration, and culture age. Nutrients, for example, specifically nitrogen, phosphorus, and sulfur, are necessary for algae growth. Silica and iron, as well as several trace elements, are also considered important marine nutrients for growth. Unlike the growth of heterotrophic algae, which remains constant, the productive growth of autotrophic microalgae increases by daylight and decreases at night. The losses are caused by respiration of carbohydrates (starch) and glycogen, whereas the autotrophic input of metabolites is shut-down. Additionally, the rates of growth of all forms of microalgae are highly temperature dependent.[38]

Decreased irradiance and light penetration, which reduce algae growth within PBRs can be caused by turbulent streaming, transverse mixing of the suspension layer on the culture surface of thin-layer PBRs, increased viscosity of cell suspension, sedimentation buildup, or the sticking of cells on the surface of PBRs.[63] For autotrophic cultivation in Trebon, Czech Republic, with the maximum daily solar photosynthetic active radiation (PAR), Doucha and Livansky[63] obtained a near linear rate of growth as a function of time, as shown in Figure 1.

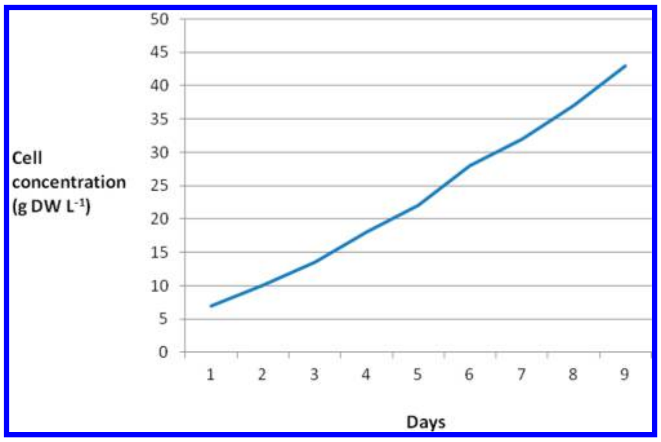

Figure 1. Algal mean cell concentration (DW) over time in a PBR.[63].

As an example of the relationship between algae growth and irradiance, *Cyanobacterium anabaena* (green-blue algae) was studied by Gao et al.[64] for growth responses under three different solar radiation exposure treatments: (1) constant low PAR (400−700 nm), (2) natural levels of solar radiation, and (3) natural levels of solar radiation but without UV radiation (290−400 nm). Although exposure to natural levels of PAR exhibited increased growth, solar UV inhibited the growth up to 40%.[64] This finding could lead to improved PBR design that filters or excludes UV penetration.

The internal cellular storage of energy as oil rather than as carbohydrates slows the reproduction rate of any algae. The higher oil strains of algae grow slower than low oil strains. The longer growth period makes the PRP culture process more susceptible to contamination, but favors the PBR process.[65] As previously stated, in trying to maximize oil production with algae, higher oil concentrations require longer growth periods followed by a period of stress requiring nutrient restrictions. However, the nutrient restrictions limit growth and the net photosynthetic efficiency. To balance the growth and high oil production, Huntley and Redalje[39] used a combination of PBR (for growth) and PRP (for nutrient stressing) using *Haematococcus pluvialis*. The average biomass energy production reported was 4542.5 L (1,200 gal) of biodiesel per acre-year, which is greater than conventional oil-bearing crops such as soybeans, which yield an average of 227 L (60 gal) of oil (or approximately 151 L (40 gal) of biodiesel) per acre-year.[39,65,66]

An example methodology is illustrated with *Neochloris oleabundans,* a freshwater microalga, which was grown in continuously stirred PBRs at 30 °C, with CO_2 supplementation and illuminated by six fluorescent lamps (Philips TL-DM36W/54-765).[17] The lipid biomass concentration reached 56% (DW) after 5 days of nitrogen starvation. An average lipid production of 37.7 mg L^{-1} day^{-1} was obtained in a highly controlled laboratory-scale setting.[17]

6. Extraction. After the growth of algae to their harvest concentration, separation and extraction of the microalgal biomass from the culture broth is required to use the oil, alcohol, or target product. Biomass can be separated from the broth by filtration, centrifugation, flocculation (colloidal separation), and other means.[55] In general, the cost of biomass recovery can be considerable.[7] A significant task involved with the cultivation of microalgae is the difficulty in economically harvesting and extracting the target product (such as oil) from a dilute suspension.[67] Depending on the specific algal organism and the process employed, a substantial percentage of the biomass generated can be in lipid oil.

The next step in the process involves the separation of lipid oil from the rest of the algae biomass.[55,68] One separation technique is by crushing with an oil press. Commercial manufacturers often use a combination of mechanical pressing and chemical solvents to extract algae oil. Two other methods, osmotic shock and ultrasonic extraction, cause the cell walls of cells in a solution to rupture and release the lipid contents into a solvent.[53−55]

Cyanobacteria (blue-green algae) are autotrophic microalgae that require sunlight, CO_2, and nutrients for energy and carbon. GMO versions of these organisms have been given the ability to transport primary metabolites such as ethanol or lipids across membranes and the cell wall. The unique ability of the GMO cyanobacteria cell to produce and secrete the target biofuel is an example of an induced separation technique. It also presents an overall strategy for making use of the organism over its entire lifespan.[69] Once the product is separated from the organism, it is then further separated from the culture mix by other techniques such as distillation for ethanol or oil−water separation.[66,68]

7. Heterotrophic Algae Respiration and Fermentation. As many as 121 strains of algae (e.g., *Chlamydomonas*) were studied by Gladue and Maxey[29] for their ability to ferment carbohydrates such as starches and sugars and produce lipids while growing in the dark. These algae are called *true heterotrophs*, because they use organic compounds as their energy source as opposed to light-dependent autotrophs. Solazyme, Inc., employs this heterotrophic algal culture to manufacture oil, which is then processed into either algal oil to yield biodiesel, HRF-76 jet fuel, or HRF-76 marine fuel. Solazyme has already produced thousands of gallons of algal oil and has refined algal oil into fuels that comply with applicable ASTM D-975 specifications.[70]

Heterotrophic growth of microalgae requires carbohydrates and oxygen (aerobic) and is typically one-third slower than autotrophic growth. However, unlike the spatial limitations found with PBRs regarding the transfer of light, heterotrophic cultures can be grown in large-volume, dark, continuously stirred tank reactors (CSTRs). Although heterotrophic CSTRs are often referred to as fermentors, the growth production process is a function of respiration and growth, not fermentation (such as the production of ethanol from the fermentation of sugars using yeast).[71,72]

In a bench-scale optimization study, Gao et al.[73] were able to generate a high lipid yield for biodiesel production using glucose as the carbon source and heterotrophic growth of the microalga *Chlorella protothecoides*. Using sorghum juice as the glucose source yielded a lipid content of 5.1 g L^{-1} DW and 52.5%.[73] These findings indicate that sweet sorghum juice is an effective substrate for algal lipid and biodiesel production.

Sterility is a limiting factor in fermentor size and efficient production technique. The volumes of fermentors commonly used for industrial heterotrophic microalgal cultivation range from 80 to 200 m^3. These stirred-tank fermentors control temperature, pH, and oxygen while using the desired algae culture and carbohydrate. Sterility is first established by steam sterilization (at 121 °C for 20 min) of the fermentor vessel, as well as batch sterilization of all nutrient solutions, and use of a pure microalgae starter culture.[71,72]

Batch and continuous cultivation techniques have been demonstrated. The highest reported biomass culture densities in heterotrophic productions are approximately 120 g L^{-1} (DW) for a three-day process or 40 g L^{-1} day^{-1} (DW), with the average production being 70−100 g L^{-1} (DW) for a three-day process or 23−33 g L^{-1} day^{-1} (DW). Additional varieties of heterotrophic productivity are found in *Chlorella sorokiniana* (green alga) at 24 g L^{-1} day^{-1} (DW) and *Nitzschia alba* (diatom) at 19.2 L^{-1} day^{-1} (DW). Process productivity is not easily compared due to differences in substrates, end points, and product separation efficiencies.[71,72]

8. Biofuel Production. The potential for microalgae to generate biofuel is augmented by their fast growth, reaching maturity in as little as three days while producing in excess of half their weight in oil.[25,74] The production yield can vary greatly depending on the algal culture, method (such as open pond or PBR), reactor (batch, fed-batch, or continuous operation), and culture technique (autotrophic or heterotrophic).

Algae have been cultivated to produce feedstocks needed for the production of biofuels such as biodiesel, ethanol, and petroleum.[7] In fact, algae productivity is higher than that of many energy crops (6−12 times greater than the energy production for corn or switchgrass).[75] The advantage of high productivity is that the cultivation of algae requires a smaller land area.[7,76,77] High productivity and high lipid content make algae a potentially important future source of biofuel.[75]

9. Biodiesel. Biodiesel is an alternative fuel that can be produced from a variety of renewable sources. These sources include canola oil, soybean oil, sunflower oil, cottonseed oil, animal fats, and lipids produced by algae. Biodiesel is defined as a monoalkyl ester of a long-chain fatty acid derived from the oils listed above, which conform to American Society for Testing and Materials (ASTM) D6751 specifications for use in diesel engines. Typically, biodiesel is produced by mixing a lipid with an alcohol (usually methanol) and a basic catalyst (usually sodium hydroxide, 85% KOH) and then heating to approximately 70 °C (20 psi) for several hours in a process called transesterification. This process bonds the methanol to the oils to produce the monoalkyl esters.[7,17,19]

10. Alternative Biodiesel Processes. Stages of the standard process used to produce biodiesel, described in the previous section, have been studied to improve the overall process. Following are two examples of process optimization, which when combined with the lipid oil from algae, can contribute to an environmentally friendly fuel source.

One process improvement uses the enzyme lipase to eliminate the need for heating the oil in the transesterification process.[78−80] Researchers have identified that lipase made from the fungus *Metarhizium anisopliae* and two Gram-negative bacteria, *Aspergillus oryzae* (and *A. niger*) and *Chromobacterium viscosum,* can cause transesterification at room temperature.[78−80] This cuts the energy expenditure of biodiesel production, making the process more energy efficient and less sensitive to process problems than the standard process.[81]

Another possible process optimization effort involves using ethanol instead of methanol in the transesterification process. Methanol, a component often used in the production of biodiesel, is highly toxic. If ignited it burns invisibly and can be absorbed through the skin, making the risk of exposure and cleanup a serious problem.[81] Methanol is also often recovered from finished biodiesel for reuse. Using ethanol instead allows biodiesel fuel production entirely from renewable resources. The use of ethanol has advantages in vehicle emissions and in controlling microbial growth in storage containers. The storage of organic products such as petroleum-derived diesel, and especially biodiesel, can be problematic because organic products are susceptible to microbial growth when in the presence of water. The accumulation of water, storage tank slime, and sediment can harm an engine by clogging fuel filters and fuel injectors and can increase emissions by affecting engine performance.[78−80] Biodiesel that is combined with ethanol for the transesterification process or as a fuel additive can be used both as a component of a fuel blend (such as E10 gasoline) and as a biocide. A biocide can be any chemical that kills bacteria and mold growing in solution or on fuel tank surfaces without interfering with the combustion of fuel or the operation of the engine. Thus, the use of ethanol can control the problem of microbial contaminants while in storage and make the biodiesel fuel production process reliant on a renewable resource. While ethanol has distinct advantages in biodiesel production, it has not been used or studied as extensively as has methanol.[81]

11. Bioethanol. Cyanobacteria (blue-green algae), as mentioned above in the discussion of process extraction, have been studied to genetically enhance favorable traits. These algae are made up of autotrophic prokaryotes; they lack a cell nucleus and membrane-bound organelles. Deng and Coleman (1999)[82] genetically modified these algae to create a pathway for carbon utilization resulting in the direct generation of ethanol. The coding for ethanol synthesis from the bacterium *Zymomonas mobiliz* was cloned into a vector and then used to transform the cyanobacterium *Synechococcus* sp.[82] The GMO form of cyanobacteria directly synthesized ethanol, which diffused from the cells into the culture medium and the airspace above it. The growth requirements of this GMO form of cyanobacteria are light, CO_2, and inorganic nutrients such as that found in wastewater.[59,60,82,83]

Ongoing research aims to increase ethanol yields or optimize processes similar to that used by Deng and Coleman (1999).[82] Means to improve ethanol production include (1) further genetic modification; (2) manipulation of the growth conditions to lower the nutrient concentrations with the mature growth of cells; and (3) development of more efficient ethanol capture techniques, such as sequestering technologies, within the mixture of growth medium and gaseous headspace. Industrial efforts to produce bioethanol by using photoautotrophic organisms in PBRs are underway by Algenol Biofuels in cooperation with Dow Chemical Company, the Linde Group, the National Renewable Energy Laboratory (NREL), the Georgia Institute of Technology, and Membrane Technology and Research, Inc.[84]

12. Hydrogen Fuel. In addition to the algae-derived biofuels already mentioned, various petroleum-like products and end products can be generated from microalgae. An additional biofuel, for example, comes from the microalga *Chlamydomonas reinhardtii*, which has been demonstrated to grow in the laboratory to produce hydrogen.[30,85] While in a sulfur deprivation stage, *Chlamydomonas reinhardtii* stops producing oxygen and switches to the production of hydrogen.[30,85] *Cladophora fracta* and *Chlorella protothecoides* were also studied for biofuel production by Demirbas.[86] The generation of hydrogen was shown to be dependent on the growth media composition, culture conditions, and PBR design.[86] Hydrogen can be used to produce heat, electricity, or power (via fuel cells) for transportation.

13. Commercial Interests. Worldwide commercial applications of algae are vast and many products have been developed. However, the current focus of industrial expansion has shifted from food-related products to fuel as market forces emphasize the search for renewable fuels.[87] Commercial interests have created industries with a wide range of products using many forms of microalgae. One industry, Cyanotech Corporation based in Kona, HI, for example, uses *Chlorella* sp. for nutrition and health products.[87] More than 70 other companies worldwide manufacture *Chlorella* sp. for health food purposes. Another example, the Taiwan Chlorella Manufacturing Company, produces 400 tons of dry *Chlorella* sp. algal biomass per year.[42] *Spirulina* (*Arthrospira*) is another algae often produced for human nutrition due to its high protein content, health benefits, and overall nutritional value.[42]

The majority of commercial growth processes of algae use varieties of open ponds. These commercial facilities are located in lower latitudes where temperature, climate, and solar radiance are favorable for algae growth. Some of the primary commercial algae producers are described below.

Novozymes is an international leader in enzyme and pharmaceutical production technology and supplies over half the enzymes used to produce bioethanol in the United States.[88] Novozymes employs GMO cloning techniques and gene expression systems to manufacture enzymes for use in industrial biofuel generation.[88] Additionally, Novozymes assists companies in replacing process constituents with less environmentally adverse process constituents.[88]

As discussed above in the section on Heterotrophic Algae Respiration and Fermentation, Solazyme employs a heterotrophic method to produce a lipid byproduct that is converted into biodiesel. Solazyme was the first algae manufacturer to be approved for algae jet fuel production by ASTM.[89,90] Solazyme had a commercial plant by 2010. In 2009, it sold 20 000 gallons (at a cost of $8.5 million for 20 000 gallons or $425/gallon) of algae fuel to the U.S. Navy. In 2010 it received another purchase for 150 000 gallons (at a cost of $10 million for 150 000 gallons or $67/gallon). The initial cost of research and development was not applied to the second purchase.[89,90] Whether the price of Solazyme biodiesel fuel is $425 or $67 per gallon, this company has demonstrated the ability to deliver a product as contracted.

Based in San Diego, CA, Sapphire Energy is associated with Linde Industries. Sapphire Energy operates a facility in New Mexico that uses an open pond system with CO_2 injection that produces a lipid-like petroleum.[4] Sapphire Energy's goals are to produce 1 million gallons of algae-derived biodiesel and jet fuel by 2011, 100 million gallons by 2018, and 1 billion gallons by 2025.[4]

Synthetic Genomics, Inc. (SGI) received $600 million in 2009 from Exxon for research and development. SGI has developed a GMO strain of algae that can produce a petroleum-like lipid.[91]

Algenol Biofuels is using a GMO form of cyanobacteria (a form of blue-green microalgae grown in an autotrophic process within PBRs) to directly synthesize ethanol. The ethanol diffuses through the microalgae cell wall into the culture medium and the confined airspace of the PBR. The cyanobacteria require light, CO_2, and inorganic elements and are being grown in combination with municipal wastewater and commercial CO_2 effluent integrated in a pilot-scale biorefinery.[82] The U.S. Department of Energy (DOE) has selected Algenol for a $25 million grant to support this work. Algenol is also working in cooperation with Dow Chemical Company, the Linde Group, the National Renewable Energy Laboratory (NREL), the Georgia Institute of Technology, and Membrane Technology and Research, Inc.[84]

Cellana (formerly HR BioPetroleum, Inc., (HRBP)) was founded in Hawaii in 2004 to produce feedstocks for biofuels, personal care products, nutritional oils, renewable chemicals, and aquaculture and livestock feed. Cellana's patented Alduo technology (a combination PBR/PRP autotrophic process) uses industrial emissions of CO_2, sunlight, and a non-GMO strain of microalgae. HRBP and Royal Dutch Shell PLC formed Cellana, which operates a six-acre demonstration facility to produce marine algae and harvest oil for conversion into biofuel.[92]

Heliae Development, LLC, produces multiple algae strains and multiproduct refining including algae-derived jet fuels at its pilot facility in Gilbert, AZ. Azmark Aero Systems has tested algae-derived jet fuels in small gas turbine engines for use in military unmanned aerial vehicles. Heliae currently uses autotrophic non-GMO algal strains in PBRs while using CO_2 and wastewater.[93] This process using non-GMO algal strains has been developed in cooperation with Arizona State University to allow growth in a range of climates. Heliae's proprietary PBR is designed to maximize efficiency, production consistency, and per-acre yields.[93]

14. Economics of Algae. The fluctuating price of petroleum continues to set the economic standard that all biofuels must meet to be competitive. Microalgae lipid production has the greatest potential for the production of renewable fuels. Although many processes (with theoretical costs) use various forms of microalgae, information on algal biofuel production costs is limited to biodiesel. Additionally, the production costs discussed below do not have the benefit of economics of scale. Until an established plant is able to produce a minimum of 1 million gallons per year of biofuel, the cost projections will be based on assumptions. While useful, even the best assumptions are likely to be inaccurate. The ultimate costs, both economic and resource (land, water, and air) costs, of large-scale production are largely unknown.

Biodiesel production can be achieved by both heterotrophic and autotrophic processes. The cost of feedstock or carbon source (carbohydrate) in the heterotrophic process accounts for 60–75% of the total cost of biodiesel.[6,48,49,91] The most recent price of biodiesel fuel produced and delivered by Solazyme was $67 per gallon. Although not competitive, Solazyme achieved a notable milestone in this rapidly developing market.[89,90]

Chisti[7] compared PBR and PRP facilities, which are detailed in Table 3. Both example techniques produce 100 tons of biomass and consume 183 333 kg of atmospheric CO_2. Both techniques are compared with optimal productivity and process concentrations that have been documented in actual large-scale PBRs and PRPs. The PBR technique for oil yield illustrated in the table has been shown to achieve a 38% greater oil yield per hectare compared with PRP techniques. Both PBR and PRP techniques are used extensively in commercial operations.[7,42,53,54,66,94,95]

Table 3. Comparison of Photobioreactor and Raceway Production Methods[7]

variable	PBR	PRP
annual biomass production (kg)	100 000	100 000
volumetric productivity (kg m^{-3} d^{-1})	1.535	0.117
areal productivity (kg m^{-2} d^{-1})	0.048[a] 0.072[c]	0.035[b]
biomass concentration in broth (kg m^{-3})	4.00	0.14
dilution rate (d^{-1})	0.384	0.250
area needed (m^2)	5681	7828
oil yield (m^3 ha^{-1})	136.9[d]	99.4[d]
	58.7[e]	42.6[e]
annual CO_2 consumption (kg)	183 333	183 333
system geometry	132 parallel tubes/unit; 80 m long tubes; 0.06 m tube diameter	978 m^2/pond; 12 m wide, 82 m long, 0.30 m deep
number of units	6	8

[a]Based on facility area. [b]Based on actual pond area. [c]Based on projected area of photobioreactor tubes. [d]Based on 70% by weight oil in biomass. [e]Based on 30% by weight oil in biomass.

As mentioned above, recovery of the microalgal biomass from the culture broth is the necessary first step for using the target product. The techniques for biomass separation are filtration, centrifugation, and other means.[55] The cost of biomass separation can be significant and is dependent on the technique employed. PBR recovery costs are typically less than PRP costs due to the higher biomass concentration (nearly 30 times greater than PRP).[7] The cost of producing a kilogram of microalgal biomass is estimated to be approximately \$2.95 for PBR and \$3.80 for PRP.[55,96] If the production scale is increased to an annual biomass capacity of 10 000 tons, the biomass per kilogram production cost decreases to approximately \$0.47 for PBR and \$0.60 for PRP.

With a conservative assumption of 30% oil extraction efficiency by weight of biomass, the cost of a liter of extracted oil would be \$1.40 for PBR (\$5.30/gal) and \$1.81 for PRP (\$6.85/gal).[7,55,96] The conversion to algae-derived biodiesel, assuming a standard 66% efficiency, would yield a cost of \$8.03/gal for PBR and \$10.38/gal for PRP.

Improvements in growth techniques, GMO efficiency and stability, and process efficiency have continued to evolve since the values above were current. These improvements will continue to drive the cost of biodiesel produced from algae oil closer to being competitive with other sources that are produced from feedstocks such as palm, soybean, canola, and petroleum.

Currently there are no commercial algae plants operating on a consistent schedule for the purpose of producing biofuel. Without a working example and an industry that is willing to share information on costs and revenues derived, the hypothetical example given above is the best speculative estimate available. It is conceivable that locating large scale algae plants which take advantage of available high nutrient wastewater and industrial sources of CO^2 and waste-heat can produce biodiesel for half the \$8.03/gal price derived above. At the approximate price of \$4/gal algae derived biodiesel would be competitive with other biodiesel feedstocks and petroleum derived diesel. This hypothetical scenario would have the additional benefits of reducing wastewater nutrient loads, minimizing the plant water demands, and sequestering CO^2. Additionally, the value of the nonlipid algae solid residue cannot be ignored. Depending on the actual content of the species utilized, high levels of carbohydrate could be used for ethanol generation and a high protein content could be used for animal feed.

15. Risk Association. Depending on the algae organism and the process used, the constituents of the algae biomass and process stream can vary. A typical process might involve microorganisms such as bacteria, mold, and yeast, including GMOs, and a wide variety of ingredients used to generate the algae and convert this biomass into a desired end product.[1,2,74] The contents could also have potential human health risks such as those from infection (bacteria, mold, yeast, and GMOs) and exposure to allergens, toxins, carcinogens (endotoxins, mycotoxins, proteins, and organic and inorganic chemicals), antibiotics (used to prevent unwanted biological growth), enzymes (used to hydrolyze cellulose), chemicals (process additives), and acidic and caustic materials (used to hydrolyze cellulose).[1,2]

The biofuel production industry is composed of many companies, each of which has adopted its own process and, for many, its own GMO form of algae. Each proprietary process design, and the reagents used (e.g., microorganisms, enzymes, chemicals), will determine the quantity and nature of waste produced. The various biological processes will amplify the microbial populations (including GMO varieties), algae, toxins, and enzymes that may be potentially hazardous to the environment and individuals. Each process could contain constituents that are potentially pathogenic, toxic, infective, or allergenic and that are of concern for affecting native microbial populations and, consequently, ecosystem balance. It is unclear what the impacts of release of these materials might be, but without a more complete understanding of the composition and amounts produced by the various processes, it is impossible to adequately estimate the risk associated with these materials.

Potential human and environmental risks exist in association with the numerous forms of GMO algae that are being developed for biofuel generation. The various risks are toxigenicity (from known and unknown GMO toxins), allergenic responses (from proteins and organic and inorganic chemicals), and unknown environmental effects that could potentially cause the unintended transfer of transgenes or cause the loss of flora and fauna biodiversity. An evaluation methodology is needed to better understand the GMO effects and their associated risks to the environment.

16. Toxicological Impacts. Algae populations can be affected by increased waterborne nutrient load caused by farming, growing populations, land use development, and trends causing increased environmental stress to wetlands and marshes. Freshwater algae, marine algae, and cyanobacteria all produce toxins. These toxins can induce dermatitis, neurological disruptions, and hepatotoxicity or liver failure.[46,97,98] Anthropogenic factors such as point and nonpoint source discharges into waterways can cause increased nutrient levels in marine and limnic environments triggering algae blooms and negatively impacting biodiversity with increased toxins and decreased dissolved oxygen levels.[97,99−101] Commonly referred to as fish kills, the effects can be widespread and environmentally detrimental. Increased incidents of toxic algae are being documented in more localities and at greater frequency and magnitude.[102]

Algae bloom formations are known to create high concentrations of toxins, which can be controlled by limited water volume, warm water temperature, high nutrient concentrations, high pH, low CO_2, and the low nutrient uptake rate demonstrated by zooplankton.[103] Numerous lakes, rivers, sounds, and oceans have experienced pollution from algal blooms that generate many toxins such as peptide hepatotoxin microcystin-LR. Human and animal exposures usually occur through drinking water ingestion, recreational activities, absorption by contact, or inhalation. Knowledge of the effects of many toxins of algal origin on humans and animals is limited, and knowledge of the effects of GMO microalgae is nonexistent.[98]

Marine microalgae can cause many human illnesses linked to the consumption of seafood and the inhalation of contaminated aerosolized toxins. They have also been responsible for the massive die-off of fish, shellfish, and marine vertebrates, as well as the corresponding mortality in seabirds, marine mammals, and other animals.[104] Marine microalgae produce toxins that cause 60 000 human intoxications (a physiological state of impairment) per year worldwide, with a mortality rate of 1.5% (or 900 fatalities).[104] Most fatalities are caused by ingestion of seafood containing saxitoxins, tetrodotoxins, and in rare cases, ciguatera and domoic acid.[71,105−107] Only acute intoxications have been studied for their toxicological or medical effects, while chronic or low-concentration exposures have not.[71,105−107]

Marine toxins are produced by two algal groups, dinoflagellates and diatoms. Of the 3000 species of dinoflagellates and diatoms, approximately 2%, or 60−80 species, are known to be toxic.[71,105−108] The majority of these toxins are temperature-stable neurotoxins, which eliminates cooking as a control measure.

In addition to human intoxications, marine toxins cause deaths to other forms of marine life and wildlife that are dependent on the aquatic food chain. Marine biotoxins, such as diatoms and red algae (*Chondria* spp.), are routinely monitored for toxins, including paralytic, neurotoxic, diarrhetic, and amnesic shellfish toxins, as well as compounds such as yessotoxins, specifically pectenotoxin and gymnodimine. Monitoring of algae blooms and other harmful algae is constantly reviewed in the light of new research that incorporates local knowledge of oceanographic and climatic conditions. Increased awareness, monitoring, surveillance, and identification of toxic algae blooms are the only means of control or avoidance.[109−112]

17. Pollution Control and Remediation. Algae can have both positive and negative environmental impacts. Among the positive effects of algae are removal of excessive amounts of nitrogen (N), phosphorus (P), and sulfur (S) from municipal and agricultural wastewater and the sequestration of CO_2 from stack emissions. A potential negative impact is the release of toxigenic, carcinogenic, and allergenic algal products as well as viable organisms, including GMOs, into the environment.

The biomass of microalgae contains approximately 50% carbon, which is obtained from the atmosphere or from commercial sources of CO_2. Carbon sequestration from industrial sources has been demonstrated to increase algae yield.[87,112−114]

The wide range of algae growth requirements means that not all algae are readily adaptable to the conditions within a wastewater treatment plant. Research performed by Bhatnagar and Bhatnagar demonstrated that *Chlorella minutissima* has a positive impact in municipal wastewater remediation.[115,116] In this study, *C. minutissima* removed 75% of the biochemical oxygen demand (BOD_5), 41% N, 30% P, and 30% S.

A benchtop study performed by Woertz et al.[117] examined algae lipid productivity using municipal and agricultural wastewater. The municipal wastewater stream was populated by the algae genera *Chlorella, Micractinium,* and *Actinastrum,* and the lipid contents ranged from 9.7 mg L^{-1} day^{-1} (with the addition of air sparge and a 3-day hydraulic residence time [HRT]) to 24 mg L^{-1} day^{-1} (with the addition of CO_2 sparge and a 3-day HRT). Lipid production using dairy wastewater was populated by the algae genera *Scenedesmus, Micractinium, Chlorella,* and *Actinastrum* and demonstrated a maximum value of 17 mg L^{-1} day^{-1}.[117] Although this work is preliminary, the results indicate a fundamental positive correlation with nutrient removal and lipid production with the additional improvement of CO_2 uptake.

Bench-top research conducted by Kong et al.[67] used *Chlamydomonas reinhardtii* in both artificial media and wastewater (influent, effluent, and concentrate) over a 10 day period. The amount of algae growth and pH were measured with the addition of CO_2. The experiment demonstrated results of 0.82 g L^{-1} day^{-1} in flask reactors and 2.0 g L^{-1} day^{-1} in PBRs.[67] The wastewater contained in flasks exhibited a 42.2−55.0% removal of N (as NH_4) and 12.5−15.4% removal of P (as PO_4). In the PBR, approximately 83.0% of the N and 14.45% of the P were removed from the wastewater.[67] The 10 day yield for *C. reinhardtii* was 25.25% oil in the PBR and 16.6% in the flask reactors.[67]

Algae play an integral role in the earth's carbon cycle, using many sources of carbon but depending on CO_2 as the main source. Sparging concentrated CO_2 emissions (such as those found in flue gas) into the algal culture can increase the dissolved CO_2 concentration above ambient (0.036%) and elicit an increase in algae growth.[118] Temperature also regulates cellular metabolic functions that can increase algae production. The beneficial increase in metabolism caused by increasing temperature is limited to the point when protein synthesis is affected.

Chlorella vulgaris was studied by Chinnasamy et al.[118] for optimizing algae growth conditions using wastewater and increased levels of temperature and CO_2. The findings showed that at a temperature of 30 °C and with an elevated CO_2 concentration of 6% algae biomass increased 114% DW (210 μg mL^{-1}) more than at ambient. However, at CO_2 concentrations greater than 6%, *Chlorella vulgaris* demonstrated a decrease in growth rate with decreasing pH levels.[118] The increase in biomass was observed for protein and carbohydrate, whereas the lipid concentration decreased by 5.8%.[118] This benchtop research demonstrates a useful methodology needed to find the optimal algae growing conditions.

A benchtop study conducted by Francisco et al.[119] tested six strains of microalgae in a PBR employing CO_2 sequestration. *Chlorella vulgaris* demonstrated the best biomass productivity of 20.1 mg L^{-1} h^{-1} and lipid content of 27.0% at 5.3 mg L^{-1} h^{-1} for biodiesel production.

Theoretical models published by Pokoo-Aikins et al.[9] and Rosenberg et al.[10] use CO_2 sequestration as an integral part of generating algae for biodiesel and ethanol biorefineries, respectively. Research continues to provide examples of biofuel generation using wastewater nutrients and CO_2 sequestration. These examples provide a wealth of knowledge to guide industrial applications. However, the status of current industrial applications is not routinely made known but rather is treated as proprietary.

It is difficult to predict the environmental impact of industrialized algae cultivation. The use of algae-derived sustainable biofuels such as biodiesel and ethanol rather than petroleum-based fuels such as gasoline and diesel will have a very positive effect on air quality.[120] When algae production is also combined with wastewater treatment and CO_2 sequestration, the potential for environmental benefits are great.

18. Promotion Authority. Both the U.S. DOE and the U.S. Department of Agriculture (USDA) have provided funding mechanisms for research and development of algae biofuel production as a means to stimulate commercial involvement. This section provides some examples of those provisions meant to initiate commercial applications of algae-derived biofuel ventures. Except for Solazyme's site in Riverside, PA, all of these examples are located in southern latitudes, presumably to take advantage of the moderate climate, temperature, and solar radiance.

DOE has announced three grants totaling $24 million for three research groups addressing specific projects on algae-based biofuels.[121] These DOE-funded projects are as follows:

- Sustainable Algal Biofuels Consortium of Mesa, AZ, has received a DOE grant of $6 million.[121] The project is directed by Arizona State University and will focus on testing the acceptability and comparing algal biofuels as replacements for petroleum-based fuels.[121] Biological pathways using algae to generate biofuels are also being studied.[121,122]
- The Consortium for Algal Biofuels Commercialization is directed by the University of California, San Diego, and has received a $9 million DOE grant.[121] This work involves developing biofuel feedstocks, nutrient use, process stream recycling, and genetic enhancement for culture optimization and process stabilization.[121]
- Cellana, LLC Consortium of Kailua-Kona, HI, is directed by Cellana, LLC, and has received a $9 million DOE grant.[121] This work involves developing enhanced-scale biofuel production of marine algae in seawater.[121] Additionally, research and development of feedstock byproduct use for aquaculture is being conducted.[121]

To assist commercial algae biorefinery projects through the construction and operation of pilot-scale demonstrations, three industries were selected to receive nearly $100 million in grants from USDA's American Recovery and Reinvestment Act.[123] Additionally, the Biorefinery Assistance Program, which is part of the 2008 Farm Bill, provides funds for technology development and guarantees loans to develop, construct, and retrofit working commercial-scale biorefineries.[123,124] The three industries receiving grants and loan guarantees are as follows:

- Algenol Biofuels, Inc., of Freeport, TX, received a DOE grant of $25 000 000 and $33 915 478 in nonfederal funding. Algenol will generate ethanol directly from an autotrophic microalgae process using carbon dioxide and seawater. This pilot-scale facility will have the capacity to produce 100 000 gallons of fuel-grade ethanol per year.[123,125]
- Solazyme, Inc., of Riverside, PA, received a DOE grant of $21 765 738 and $3 857 111 in nonfederal funding. Solazyme will investigate the economic feasibility of large-scale algae biofuel production.[123,125]
- Sapphire Energy, Inc., of Columbus, NM, received a DOE grant of $50 000 000 and $85 064 206 in nonfederal funding. Sapphire will focus research on pond production of algae-derived biofuels.[123,125]

In March 2011, USDA, Rural Business-Cooperative Service, Rural Utilities Service, announced that $85 million in grant funding was available to biofuel producers.[126] The funding is to "support and ensure expanding production of advanced biofuels."[126] Applications were taken and awards are expected to be announced.

19. Regulatory Authority. The USDA regulates GMOs from the standpoint of preventing the spread of pests, weeds, and diseases under the Federal Plant Pest Act (FPPA). USDA also regulates the spread of new varieties of feedstock whether they are developed by selection or hybridization, or are genetically modified. Crops that are bioengineered for pest resistance could have a number of advantages, such as increased yield and reduced or eliminated use of insecticides.[127] Hundreds of field trials of GMO plants are now being carried out each year with only researcher notification, as is required by the USDA.[127]

The U.S. Food and Drug Administration (FDA) has the authority to regulate manufactured products containing GMOs. Examples of FDA product responsibility are the safety of food, food additives, livestock feed, and medical products. Few products, however, have been identified as requiring agency approval under the Food, Drug and Cosmetic Act (FDCA). "If the gene-modified organism expresses a pesticide or functions as a pesticide, the Environmental Protection Agency (EPA) regulates it under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)."[101] Additionally, under the Toxic Substances Control Act (TSCA), EPA also controls GMOs that have no pesticide functions. An example is a bacterium engineered to produce ethanol from residue carbohydrate.[127] The discharge of pollutants into surface waters of the United States is regulated by the Clean Water Act, specifically through the National Pollutant Discharge Elimination System (NPDES) permit program.[101,128]

The regulatory distinction of monitoring, treating, and ultimately controlling GMO discharges by NPDES permitting is unclear. Beyond nutrient load, GMOs contained in NPDES discharges have the potential to impact drinking water supplies as well as the environment at large. Genetically modified or enhanced organisms could possess characteristics such as fast growth rate or tolerance to contaminants that would threaten the stability of natural ecosystems. When a new GMO is introduced, there is a need to be sure of the safety related to possible exposure. The scope of any GMO study ideally will cover the potential for any unforeseen problems that its introduction could cause.[62,128]

The Energy Independence and Security Act of 2007 (Pub. L. 110−140) was created by an Act of Congress with the stated purpose "to move the United States toward greater energy independence and security, to increase the production of clean renewable fuels, to protect consumers, to increase the efficiency of products, buildings, and vehicles, to promote research on and deploy greenhouse gas capture and storage options, and to improve the energy performance of the Federal Government".[129] EISA set forth improved vehicle fuel economy targets, biofuels production standards, and energy savings standards, and specified research and development of solar energy, geothermal energy, and marine and hydrokinetic renewable energy technologies and federal research on carbon sequestration technologies.[129]

II. SUMMARY AND FUTURE CONTRIBUTION OF ALGAE

The process of generating biofuel from algae involves the growth, concentration, separation, and conversion of microalgae biomass, some of which can be genetically altered. After separating the desired biofuel product or products from the microalgae biomass, a significant portion of byproduct remains. It is important that the remaining byproducts have a useful and safe purpose for the economic feasibility and environmental sustainability of the process.

If ethanol is a desired product from algae carbohydrate, the process can also involve bacteria, mold, and yeast, some of which may be genetically modified.[128] The waste streams may include these microorganisms as well as the biological toxins, allergens, and carcinogens produced by these microorganisms; antibiotics; enzymes; chemicals (e.g., wastewater high in nutrients, BOD); as well as acids and bases. Exposure to GMOs carries possible human and environmental health risks. Risks to humans include toxigenicity and allergenic responses. Environmental impacts of GMOs include their potential to cause the unintended transfer of transgenes or to cause the loss of flora and fauna biodiversity. An evaluation methodology should be employed to better understand the content of algae production and waste streams and the associated risks to humans and the environment.

As a sustainable source of energy, algae and the feedstocks they produce have great potential to meet the demands of replacing petroleum-based fuels. The versatility of algae to produce lipids, carbohydrates, and protein will be needed to create multiple products in multiple markets to successfully satisfy economic demand. Currently, biotechnology firms and the algae industry are focused on producing relatively low volumes of high-value products such as pharmaceuticals or nutritional supplements. These same industries must refocus on high volumes of biofuel production at low, competitive prices, as well as using byproducts such as the protein for distiller's grains and carbohydrates for ethanol.[130] Postextraction byproducts must be used efficiently and completely.

Algae-derived biofuel will directly impact the generation of transportation fuels (biodiesel, ethanol, and petroleum), and as part of the future of renewable fuel it will also impact many environmental and economic resources. Examples of these impacts are the treatment of wastewater; capture of carbon dioxide from power plants; production of human and animal food, pharmaceuticals, cosmetics, and organic fertilizers; aquaculture; and soil nutrient recovery. Ultimately, the need to decrease fossil fuel dependence makes it imperative that algae and algae-derived products are safe to humans and the environment. The rapid commercial expansion of the algae biofuels industry is an excellent example of sustainable product development with dramatic future potential for contributions to fuel supplies, yet many questions regarding algae production remain unanswered. The state of knowledge regarding the potential environmental impact of the production of algae and algae-derived biofuels continues to be incomplete, fragmented, and largely obscured by proprietary concerns. This knowledge is, however, changing rapidly, facilitated by research and industry and driven by economics.

Commercialization of the production of algae derived biofuels as part of the overall biofuel industry will have a profound future impact on society. Waste products that are currently discharged into the environment as contaminants will be utilized to produce much needed renewable energy sources. Now is the time to initiate the development of an algae industry evaluation methodology that allows for the advancement of knowledge and evaluation tools for authorities to best understand the potential implications.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Evangelista, V., Barsanti, L., Frassanito, A. M., Passarelli, V., Gualieri, P. Algal toxins: Nature, occurrence, effect and detection . In *Proceedings of the NATO Advanced Study Institute on Sensor Systems for Biological Threats*; The Algal Toxins Case: Pisa, Italy, 2008; ISBN: 978-1-4020-8479-9.

(2) Graham, L. E.; Graham, J. E.; Wilcox, L. W. *Algae*, 2nd ed.; Benjamin-Cummings Publishing: Menlo Park, CA, 2008; ISBN: 0321559657.

(3) Li, X.; Xu, H.; Wu, Q. Large-scale biodiesel production from microalga *Chlorella protothecoides* through heterotrophic cultivation in bioreactors. *Biotechnol. Bioeng.* **2007**, *98*, 764−771.

(4) A. France-Presse, Sapphire Energy, Linde unveil CO_2 algae-to-fuel deal: German industrial gas giant will deliver carbon dioxide, *Industry Week*, 2011. http://www.industryweek.com/articles/sapphire_energy_linde_unveil_cosub2/sub_algae-to-fuel_deal_24600.aspx?ShowAll=1&SectionID=36 (accessed December 14, 2011).

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

(6) Hughes, E.; Benemann, J. R. Biological fossil CO_2 mitigation. *Energy Convers. Manage.* **1997**, *38*, S467−S473.

(7) Chisti, Y. Biodiesel from microalgae. *Biotechnol Adv.* **2007**, *25*, 294−306.

(8) Hu, Q., Zhang, C., Sommerfeld, M. Biodiesel from algae: Lessons learned over the past 60 years and future perspectives. In *Annual Meeting of the Phycological Society of America*, Juneau, AK, July 7−12, 2006, pp. 40−41.

(9) Pokoo-Aikins, G.; Nadim, A.; El-Halwagi, M.; Mahalec, V. Design and analysis of biodiesel production from algae grown through carbon sequestration. *Clean Technol. Environ. Policy* **2010**, *12*, 239−254.

(10) Rosenberg, J. N.; Mathias, A.; Korth, K.; Betenbaugh, M. J.; Oyler, G. A. Microalgal biomass production and carbon dioxide sequestration from an integrated ethanol biorefinery in Iowa: A technical appraisal and economic feasibility evaluation. *Biomass Bioenergy* **2011**, *35*, 3865−3876.

(11) Lehr, F. Posten, Closed photo-bioreactors as tools for biofuel production. *Curr. Opin. Biotechnol.* **2009**, *20*, 280−285.

(12) Shen, Y.; Pei, Z.; Yuan, W.; Mao, E. Effect of nitrogen and extraction method on algae lipid yield. *Int. J. Agric. Biol. Eng.* **2009**, *2* (1), 51−57.

(13) Andersen, T.; Andersen, F. O. Effects of CO_2 concentration on growth of filamentous algae and Littorella uniflora in a Danish softwater lake. *Aquat. Bot.* **2006**, *84*, 267−271.

(14) Hanagata, N.; Takeuchi, T.; Fukuju, Y.; Barnes, D. J.; Karube, I. Tolerance of microalgae to high CO_2 and high temperature. *Phytochemistry* **1992**, *31*, 3345−3348.

(15) Adams, J. M.; Gallagher, J. A.; Donnison, I. S. Fermentation study on *Saccharina latissima* for bioethanol production considering variable pre-treatments. *J. Appl. Phycol.* **2009**, *21*, 569−574.

(16) Becker. Micro-algae as a source of protein. *Biotechnol. Adv.* **2007**, *25*, 207–210.

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

(18) Coppola, F.; Simonciniand, E.; Pulselli, R. M. Bioethanol potentials from marine residual biomass: An energy evaluation. *Energy Environ.* **2009**, *122*, 379–387, 0013-936X.

(19) Meng, X.; Yang, J.; Xu, X.; Zhang, L.; Nie, Q.; Xian, M. Biodiesel production from oleaginous microorganisms. *Renew. Energy* **2009**, *34*, 1–5.

(20) Li, Q.; Du, W.; Liu, D. Perspectives of microbial oils for biodiesel production. *Appl. Microbiol. Biotechnol.* **2008**, *80*, 749–756.

(21) Li, Y.; Horsman, M.; Wang, B.; Wu, N.; Lan, C. Q. Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. *Appl. Microbiol. Biotechnol.* **2008**, *81*, 629–636.

(22) Li, X.; Hu, H. Y.; Gan, K.; Yang, J. Growth and nutrient removal properties of a freshwater microalga *Scenedesmus* sp. LX1 under different kinds of nitrogen sources. *Ecol. Eng.* **2010**, *36*, 379–381.

(23) Li, Y.; Han, D.; Sommerfeld, M.; Hu, Q. Photosynthetic carbon partitioning and lipid production in the oleaginous microalga *Pseudochlorococcum* sp. (*Chlorophyceae*) under nitrogen-limited conditions. *Bioresour. Technol.* **2011**, *102*, 123–129.

(24) Liang, Y.; Cui, Y.; Trushenski, J.; Blackburn, J. W. Converting crude glycerol derived from yellow grease to lipids through yeast fermentation. *Bioresour. Technol.* **2010**, *101*, 7581–7586.

(25) Briggs, M. *Widescale Biodiesel Production from Algae*; University of New Hampshire Biodiesel Group, 2004.

(26) Laurens, L. M. L.; Wolfrum, E. J. Feasibility of spectroscopic characterization of algal lipids: Chemometric correlation of NIR and FTIR spectra with exogenous lipids in algal biomass. *Bioenergy Res.* **2011**, *4*, 22–35.

(27) Reitan, K. I.; Rainuzzo, J. R.; Olsen, Y. Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. *J. Phycol.* **1994**, *30*, 972–979.

(28) Renaud, S. M.; Thinh, L. V.; Lambrinidis, G.; Parry, D. L. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. *Aquaculture* **2002**, *211*, 195–214.

(29) Gladue, R. M.; Maxey, J. E. Microalgal feeds for aquaculture. *J. Appl. Phycol.* **1994**, *6*, 131–141.

(30) Beer, L. L.; Boyd, E. S.; Peters, J. W.; Posewitz, M. C. Engineering algae for biohydrogen and biofuel production. *Curr. Opin. Biotechnol.* **2009**, *20*, 264–271.

(31) Clarens, A. F.; Nassau, H.; Resurreccion, E. P.; White, M. A.; Colosi, L. M. Environmental impacts of algae-derived biodiesel and bioelectricity for transportation. *Environ. Sci. Technol.* **2011**, *45* (17), 7554–7560.

(32) Kübler, J. E.; Johnston, A. M.; Raven, J. A. The effects of reduced and elevated CO_2 and O_2 on the seaweed *Lomentaria articulata*. *Plant Cell Environ.* **1999**, *22*, 1303–1310.

(33) Logothetis, K.; Dakanali, S.; Ioannidis, N.; Kotzabasis, K. The impact of high CO_2 concentrations on the structure and function of the photosynthetic apparatus and the role of polyamines. *J. Plant Physiol.* **2004**, *161*, 715–724.

(34) Tisserat, B. Influence of ultra-high carbon dioxide levels on growth and morphogenesis of Lamiaceae species in soil. *J. Herbs Spices Med. Plants* **2002**, *9*, 81–89.

(35) González-Fernández, C.; Molinuevo-Salces, B.; García-González, M. C. Nitrogen transformations under different conditions in open ponds by means of microalgae–bacteria consortium treating pig slurry. *Bioresour. Technol.* **2011**, *102*, 960–966.

(36) Venkata Mohan, S.; Mohanakrishna, G.; Raghuvulu, S. V.; Sarma, P. N. Enhancing biohydrogen production from chemical wastewater treatment in anaerobic sequencing batch biofilm reactor (AnSBBR) by bioaugmenting with selectively enriched kanamycin resistant anaerobic mixed consortia. *Int. J. Hydrogen Energy* **2007**, *32*, 3284–3292.

(37) Venkata Mohan, S.; Bhaskar, Y. B.; Krishna, T. M.; Chandrasekhara Rao, N.; Lalit Babu, V.; Sarma, P. N. Biohydrogen production from chemical wastewater as substrate by selectively enriched anaerobic mixed consortia: Influence of fermentation pH and substrate composition. *Int. J. Hydrogen Energy* **2007**, *32*, 2286–2295.

(38) Sukenik, A.; Levy, R. S.; Levy, Y.; Falkowski, P. G.; Dubinsky, Z. Optimizing algal biomass production in an outdoor pond: A simulation model. *J. Appl. Phycol.* **1991**, *3*, 191–201.

(39) Huntley, M. E.; Redalje, D. G. CO_2 mitigation and renewable oil from photosynthetic microbes: A new appraisal. *Mitigation Adaptation Strat. Global Change* **2007**, *12*, 573–608.

(40) Becker, E. W., *Microalgae: Biotechnology and Microbiology*; Cambridge University Press, 1994; ISBN 978-0-521-06113.

(41) Sheehan, J.; Dunahay, T.; Benemann, J.; Roessler, P. *A Look Back at the U.S. Department of Energy's Aquatic Species Program—Biodiesel from Algae*; National Renewable Energy Laboratory (NREL): Golden, CO, 1998.

(42) Spolaore, P.; Joannis-Cassan, C.; Duran, E.; Isambert, A. Commercial applications of microalgae. *J. Biosci. Bioeng.* **2006**, *101*, 87–96.

(43) Lee, C. G. Calculation of light penetration depth in photobioreactors. *Biotechnol. Bioprocess Eng.* **1999**, *4*, 78–81.

(44) Mussgnug, J. H.; Thomas-Hall, S.; Rupprecht, J.; Foo, A.; Klassen, V.; McDowall, A.; Schenk, P. M.; Kruse, O.; Hankamer, B. Engineering photosynthetic light capture: Impacts on improved solar energy to biomass conversion. *Plant Biotechnol. J.* **2007**, *5*, 802–814.

(45) Muller, C.; Reuter, W.; Wehrmeyer, W. Adaptation of the photosynthetic apparatus of *Anacystis nidulans* to irradiance and CO^2-concentration. *Bot. Acta* **1993**, *106*, 480–487.

(46) Rapala, J.; Sivonen, K. Assessment of environmental conditions that favor hepatotoxic and neurotoxic *Anabaena* spp. strains cultured under light limitation at different temperatures. *Microb. Ecol.* **1998**, *36*, 181–192.

(47) Briassoulis, D.; Panagakis, P.; Chionidis, M.; Tzenos, D.; Lalos, A.; Tsinos, C.; Berberidis, K.; Jacobsen, A. An experimental helical-tubular photobioreactor for continuous production of *Nannochloropsis* sp. *Bioresour. Technol.* **2010**, *101*, 6768–6777.

(48) Borowitzka, M. A. Microalgae for aquaculture: Opportunities and constraints. *J, Appl. Phycol.* **1997**, *9*, 393–401.

(49) Brennan, L.; Owende, P. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable Sustainable Energy Rev.* **2010**, *14*, 557–577.

(50) Kodama, M.; Ikemoto, H.; Miyachi, S. A new species of highly CO_2-tolerant fast growing marine microalga suitable for high density culture. *J. Mar. Biotechnol.* **1993**, *1*, 21–25.

(51) Watanabe, Y.; Ohmura, N.; Saiki, H. Isolation and determination of cultural characteristics of microalgae which functions under CO_2 enriched atmosphere. *Energy Convers. Manage.* **1992**, *33*, 545–552.

(52) Yue, L.; Chen, W. Isolation and determination of cultural characteristics of a new highly CO_2 tolerant fresh water microalgae. *Energy Convers. Manage.* **2005**, *46*, 1868–1876.

(53) Molina Grima, E., Microalgae, mass culture methods. In *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis and Bioseparation*Flickinger, M. C.; Drew, S. W., Eds.; Wiley, 1999; Vol. 3, pp 1753–69.

(54) Molina Grima, E.; Acién Fernández, F. G.; García Camacho, F.; Chisti, Y. Photobioreactors: Light regime, mass transfer, and scaleup. *J. Biotechnol.* **1999**, *70*, 231–247.

(55) Molina Grima, E.; Belarbi, E. H.; Acién Fernández, F. G.; Robles Medina, A.; Chisti, Y. Recovery of microalgal biomass and metabolites: Process options and economics. *Biotechnol Adv.* **2003**, *20*, 491–515.

(56) Centers for Disease Control and Prevention (CDC). *National Center for Environmental Health, Investigation of Human Health Effects Associated with Potential Exposure to Genetically Modified Corn*; A Report to the U.S. Food and Drug Administration from the Centers for Disease Control and Prevention: Atlanta, GA, 2001.

(57) Miraglia, M.; Berdal, K. G.; Brera, C.; Corbisier, P.; Holst-Jensen, A.; Kok, E. J.; Marvin, H. J. P.; Schimmel, H.; Rentsch, J.; van Rie, J. P. P. F.; Zagon, J. Detection and traceability of genetically modified organisms in the food production chain. *Food Chem. Toxicol.* **2004**, *42*, 1157−1180.

(58) Sicard, T. E. L. Between development and environment: Uncertainties of agrofuels. *Bull. Sci. Technol. Soc.* **2009**, *29*, 226−235.

(59) U.S. Department of Energy (DOE), Office of Science, Genomics: GTL Systems Biology for Energy and Environment, Cellulosic Ethanol: Fuel Ethanol Production, 2008. http://genomicsgtl.energy.gov (accessed).

(60) Demain, A. L.; Newcomb, M.; Wu, J. H. D. Cellulase, *Clostridia*, and ethanol. *Microbiol. Mol. Biol, Rev.* **2005**, *69*, 124−154.

(61) Kuiper, H. A.; Kleter, G. A.; Noteborn, H. P.; Kok, E. J. Assessment of the food safety issues related to genetically modified foods. *Plant J.* **2001**, *27*, 503−528.

(62) Letourneau, D. K.; Robinson, G. S.; Hagen, J. A. *Bt* crops: Predicting effects of escaped transgenes on the fitness of wild plants and their herbivores. *Environ. Biosafety Res.* **2003**, *2*, 219−246.

(63) Doucha, J.; Livansky, K. Outdoor open thin-layer microalgal photobioreactor: Potential productivity. *J. Appl. Phycol.* **2009**, *21*, 111−117.

(64) Gao, K.; Yu, H.; Brown, M. T. Solar PAR and UV radiation affects the physiology and morphology of the cyanobacterium *Anabaena* sp. PCC 7120. *J. Photochem. Photobiol. B* **2007**, *14*, 117−124.

(65) Vasudevan, P. T.; Briggs, M. Biodiesel production-current state of the art and challenges. *J. Ind. Microbiol. Biotechnol.* **2008**, *35*, 421−430.

(66) Tredici, M. R., Bioreactors. In *Encyclopedia of Bioprocess Technology: Fermentation Biocatalysis and Bioseparation*; Flickinger, M. C., Drew, S. W., Eds.; Wiley, 1999; pp 395−419.

(67) Kong, Q.; Li, L.; Martinez, B.; Chen, P.; Ruan, R. Culture of microalgae *Chlamydomonas reinhardtii* in wastewater for biomass feedstock production. *Appl. Biochem. Biotechnol.* **2010**, *160*, 9−18.

(68) Beychok, M. R., *Aqueous Wastes from Petroleum and Petrochemical Plants*, 1st ed.; John Wiley and Sons, 1967; LCCN 67019834.

(69) Niederholtmeyer, H.; Wolfstädter, B. T.; Savage, D. F.; Silver, p. a.; Way, J. C. Engineering cyanobacteria to synthesize and export hydrophilic products. *Appl. Environ. Microbiol.* **2010**, *76*, 3462−3466.

(70) LaMonica, M.; , Solazyme's algae diesel ready to hit the road, 2008. http://news.cnet.com/8301-11128_3-9965683-54.html#ixzz1MpaL1xHy (accessed December 20, 2011).

(71) Burkholder, J. M. Implications of harmful microalgae and heterotrophic dinoflagellates in management of sustainable marine fisheries. *Ecol. Appl.* **1998**, *8*, S37−S62.

(72) Wageningen University, Research on microalgae within Wageningen UR, 2011. http://www.algae.wur.nl/UK/technologies/production/heterotrophic_organisms/ (accessed December 20, 2011).

(73) Gao, C.; Zhai, Y.; Ding, Y.; Wu, Q. Application of sweet sorghum for biodiesel production by heterotrophic microalga *Chlorella protothecoides*. *Appl. Energy* **2010**, *87*, 756−761.

(74) Demirbas, M. F. Biofuels from algae for sustainable development. *Appl. Energy* **2011**, *88*, 3473−3480.

(75) Bajpai, D.; Tyagi, V. K. Biodiesel: Source, production, composition, properties and its benefits. *J. Oleo Sci.* **2006**, *55*, 487−502.

(76) Dismukes, G. C.; Carrieri, D.; Bennette, N.; Ananyev, G. M.; Posewitz, M. C. Aquatic phototrophs: Efficient alternatives to land-based crops for biofuels. *Curr. Opin. Biotechnol.* **2008**, *19*, 235−240.

(77) Sandefur, H. N.; Matlock, M. D.; Costello, T. A. Seasonal productivity of a periphytic algal community for biofuel feedstock generation and nutrient treatment. *Ecol. Eng.* **2011**, *37*, 1476−1480.

(78) Adachi, D.; Hama, S.; Numata, T.; Nakashima, K.; Ogino, C.; Fukuda, H.; Kondo, A. Development of an *Aspergillus oryzae* whole-cell biocatalyst coexpressing triglyceride and partial glyceride lipases for biodiesel production. *Bioresour. Technol.* **2011**, *102*, 6723−6729.

(79) Fiametti, K. G.; Sychoski, M. M.; De Cesaro, A.; Furigo, A.; Bretanha, L. C.; Pereira, C. M. P.; Treichel, H.; de Oliveira, D.; Oliveira, J. V. Ultrasound irradiation promoted efficient solvent-free lipase-catalyzed production of mono- and diacylglycerols from olive oil. *Ultrason. Sonochem.* **2011**, *18*, 981−987.

(80) Talukder, M. R.; Das, P.; Shu Fang, T.; Wu, J. C. Enhanced enzymatic transesterification of palm oil to biodiesel. *Biochem. Eng. J.* **2011**, *55*, 119−122.

(81) Lam, M. K.; Lee, K. T. Mixed methanol−ethanol technology to produce greener biodiesel from waste cooking oil: A breakthrough for SO_4^{2-}/SnO_2-SiO_2 catalyst. *Fuel Process. Technol.* **2011**, *92*, 1639−1645.

(82) Deng, M. D.; Coleman, J. R. Ethanol synthesis by genetic engineering in cyanobacteria. *Appl. Environ. Microbiol.* **1999**, *65*, 523−528.

(83) Badger, P. C., Ethanol from cellulose: A general review, In *Trends in New Crops and New Uses*; Janick, J., Whipkey, A., Eds.; ASHS Press: Alexandria, VA, 2002; pp 17−21.

(84) Algenol Biofuels, 2011. http://www.algenolbiofuels.com (accessed December 20, 2011).

(85) Mullner, K.; Happe, T. Biofuel from algae-photobiological hydrogen production and CO^2-fixation. *Int. J. Energy Technol. Policy* **2007**, *5*, 290−295.

(86) Demirbas, M. F. Biofuels from algae for sustainable development. *Appl. Energy* **2011**, *88*, 3473−3480.

(87) Milledge, Commercial application of microalgae other than as biofuels: A brief review, *Rev. Environ. Sci. Biotechnol.*, **2011**, *10*, 31−41.

(88) Phillips, T., Novozymes: A Case Study on Sustainability, 2011. http://biotech.about.com/od/biotechindailylife/a/Novozymes.htm (accessed December 20, 2011).

(89) Rapier, R., Solazyme CEO Clarifies Costs, Consumer Energy Report, 2010. http://www.consumerenergyreport.com/2010/10/09/solazyme-ceo-clarifies-costs/ (accessed December 20, 2011).

(90) BrighterEngergy.org, Solazyme wins 150,000 gallon Navy order for algal biofuel, *Clean Transport News*, 2010. http://www.brighterenergy.org/16288/news/transport/solazyme-wins-150000-gallon-navy-order-for-algal-biofuel/ (accessed December 20, 2011).

(91) Howell, K., Exxon sinks $600M into algae-based biofuels in major strategy shift, Energy and Environment, *The New York Times*. http://www.nytimes.com/gwire/2009/7/14/14greenwire-exon-sinks-600m-into-algae-based-biofuels-in-33562.html (accessed December 20, 2011).

(92) Cellana, 2011. http://www.cellana.com (accessed December 20, 2011).

(93) Heliae Development, LLC, , 2011. http://www.heliae.com (accessed December 20, 2011).

(94) Pulz, O. Photobioreactors: Production systems for phototrophic microorganisms. *Appl. Microbiol. Biotechnol.* **2001**, *57*, 287−293.

(95) Terry, K. L.; Raymond, L. P. System design for the autotrophic production of microalgae. *Enzyme Microb. Technol.* **1985**, *7*, 474−487.

(96) Humphreys, K., *Jelen's Cost and Optimization Engineering*, 3rd ed.; McGraw-Hill, 1991.

(97) Kotak, B. G.; Lam, A. K-Y.; Prepas, E. E.; Kenefick, S. L.; Hrudey, S. E. Variability of the hepatotoxin microcystin-Lr in hypereutrophic drinking water lakes. *J. Phycol.* **1995**, *31*, 248−263.

(98) Algae and cyanobacteria in fresh water. In *Guidelines for Safe Recreational Water Environments, Coastal and Fresh Waters*; World Health Organization (WHO): Geneva, Switzerland, 2003.

(99) Barinova, S. S.; Petrovand, A.; Nevo1, E. Comparative analysis of algal biodiversity in the rivers of Israel. *Cent. Eur. J. Biol.* **2011**, *6*, 246−259.

(100) Markina, Z. V.; Aizdaicher, N. A. *Phaeodactylum tricornutum* Bohlin bioassay of water quality of Amur Bay (the Sea of Japan). *Contemp. Probl. Ecol.* **2011**, *4*, 74−79.

(101) U.S. Environmental Protection Agency (EPA). National Pollutant Discharge Elimination System (NPDES), Clean Water Act (section 303(b)(1)(c)) and NPDES regulations (40 CFR 122.44(d)), 1977.

(102) Verity, P. G. Expansion of potentially harmful algal taxa in a Georgia Estuary (USA). *Harmful Algae* **2010**, *9*, 144−152.

(103) Zurawell, R. W.; Chen, H.; Burke, J. M.; Prepas, E. E. Hepatotoxic cyanobacteria: A review of the biological importance of microcystins in freshwater environments. *J. Toxicol. Environ. Health* **2005**, *8*, 1−37.

(104) Van Dolah, F. M. Marine algal toxins: Origins, health effects, and their increased occurrence. *Environ. Health Perspect.* **2000**, *108*, 133−141.

(105) Landsberg, J. H.; Balazs, G. H.; Steidinger, K. A.; Baden, D. G.; Work, T. M.; Russel, D. J. The potential role of natural tumor promoters in marine turtle fibropapillomatosis. *J. Aquat. Anim. Health* **1999**, *11*, 199−210.

(106) Edmunds, J. S.; McCarthy, R. A.; Ramsdell, J. S. Ciguatoxin reduces larval survivability in finfish. *Toxicon* **1999**, *37*, 1827−1832.

(107) Landsberg, J. H.; Balazs, G. H.; Steidinger, K. A.; Baden, D. G.; Work, T. M.; Russel, D. J. The potential role of natural tumor promoters in marine turtle fibropapillomatosis. *J. Aquat. Anim. Health* **1999**, *11*, 199−210.

(108) Landsberg, J. H., Steidinger, K. A. A historical review of *Gymnodinium breve* red tides implicated in mass mortalities of the manatee (*Trichechus manatus latirostris*) in Florida, USA. In *Proceedings of the 8th International Conference on Harmful Algae*; Reguera, B., Blanco, J., Fernandez, M., Wyatt, T., Eds.; Xunta de Galicia-IOC/Unesco: Vigo, Spain, 1998; pp 97−100.

(109) Anderson, D. M., Toxic algal blooms and red tides: A Global Perspective. In *Red Tides: Biology, Environmental Science and Toxicology*; Okaichi, T., Anderson, D. M., Nemoto, T., Eds.; Elsevier: New York, 1989; pp 11−16.

(110) Smayda, T. J., Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic. In *Toxic Marine Phytoplankton*; Graneli, E., Sundstrom, B., Edler, L., Anderson, D. M., Eds.; Elsevier: New York, 1990; pp 29−40.

(111) Rhodes, L. L.; Mackenzie, A. L.; Kaspar, H. F.; Todd, K. E. Harmful algae and mariculture in New Zealand. *J. Mar. Sci* **2001**, *58*, 398−403.

(112) Weissman, J. C., Benemann, J. R., Comparison of Marine Microalgae Culture Systems, *Second National Conference on Carbon Sequestration*, 2003. http://www.netl.doe.gov/publications/proceedings/03/carbon-seq/PDFs/217.pdf (accessed December 20, 2011).

(113) Benemann, J., Pedroni, P. M., Davison, J., Beckert, H., Bergman, P., Technology roadmap for biofixation of CO_2 and greenhouse gas abatement with microalgae. In *Second National Conference on Carbon Sequestration*, 2003. http://www.netl.doe.gov/publications/proceedings/03/carbon-seq/PDFs/017.pdf (accessed December 20, 2011).

(114) Chisti, Y. Microalgae as sustainable cell factories. *Environ. Eng. Manage. J* **2006**, *5*, 261−274.

(115) Bhatnagar, A., Bhatnagar, M.. In *Innovative Approaches in Microbiology*; Maheshwari, D. K., Dubey, R. C., Eds.; India, Bishen Singh Mahendra Pal Singh: Dehra Dun, 2001; pp 379−403.

(116) Bhatnagar, A.; Bhatnagar, M.; Chinnasamy, S.; Das, K. C. *Chlorella minutissima*—A promising fuel alga for cultivation in municipal wastewaters. *Appl. Biochem. Biotechnol.* **2010**, *161*, 523−536.

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

(118) Chinnasamy, S.; Ramakrishnan, B.; Bhatnagar, A.; Das, K. C. Biomass production potential of a wastewater alga *Chlorella vulgaris* ARC 1 under elevated levels of CO_2 and temperature. *Int. J. Mol. Sci.* **2009**, *10*, 518−532.

(119) Francisco, E. C.; Neves, D. B.; Jacob-Lopes, E.; Franco, T. T. Microalgae as feedstock for biodiesel production: Carbon dioxide sequestration, lipid production and biofuel quality. *J. Chem. Technol. Biotechnol.* **2010**, *85*, 395−403.

(120) Hemmingsen, J. G.; Møller, P.; Klenø Nøjgaard, J.; Roursgaard, M.; Loft, S. Oxidative stress, genotoxicity, and vascular cell adhesion molecule expression in cells exposed to particulate matter from combustion of conventional diesel and methyl ester biodiesel blends. *Environ. Sci. Technol.* **2011**, *45* (19), 8545−8551.

(121) Energy.Gov, Department of energy announces $24 million for algal biofuels research, 2010. http://energy.gov/articles/department-energy-announces-24-million-algal-biofuels-research (accessed December 21, 2011).

(122) National Renewable Energy Laboratory (NREL), Algal Biofuels R&D at NREL, 2010. http://www.nrel.gov/docs/fy10osti/49123.pdf (accessed December 21, 2011).

(123) U.S. Department of Agriculture (USDA) Rural Development, Section 9005, Bioenergy Program for Advanced Biofuels Payments to Advanced Biofuel Producers. http://www.rurdev.usda.gov/BCP_Biofuels.html (accessed December 21, 2011).

(124) Energy. Gov., Secretaries Chu and Vilsack announce more than $600 million investment in advanced biorefinery projects, 2009. http://energy.gov/articles/secretaries-chu-and-vilsack-announce-more-600-million-investment-advanced-biorefinery (accessed December 21, 2011).

(125) Algae Industry Magazine.Com, Money, U.S. DOE Awards $100 million to three algae biorefineries, 2011. http://www.algaeindustrymagazine.com/u-s-d-o-e/ (accessed December 21, 2011).

(126) Federal Register, Vol. 76, No. 48, Friday, March 11, **2011**, Notices, 13345, Department of Agriculture, Rural Business-Cooperative Service, Rural Utilities Service, Notice of Contract Proposal (NOCP) for Payments to Eligible Advanced Biofuel Producers. http://edocket.access.gpo.gov/2011/pdf/2011-5573.pdf (accessed December 21, 2011).

(127) Hileman, B., Views differ sharply over benefits, risks of agricultural biotechnology, *Chem. Eng. News*, 1995.

(128) Menetrez, M. Y. The potential environmental impact of waste from cellulosic ethanol production. *J. Air Waste Manage. Assoc.* **2010**, *60*, 245−250, DOI: 10.3155/1047-3289.60.2.245.

(129) Energy Independence and Security Act of 2007 (EISA 2007), Pub. L. 110−140.

(130) Foley, P. M.; Beach, E. S.; Zimmerman, J. B. Algae as a source of renewable chemicals: Opportunities and challenges. *Green Chem.* **2011**, *13*, 1399−1405.