MICROALGAE BIOFUELS

MYTHS and RISKS





Prologue: Will Algae Save Us From Climate Chaos?

In mid-June 2017, ExxonMobil announced that together with biotechnology company Synthetic Genomics, it had achieved a major breakthrough in the development of algae biofuels.1 According to reports, Exxon-funded scientists used the CRISPR-Cas9 genome editing technique to engineer a microalgae able to produce 40 percent more lipids while maintaining growth rates, thereby addressing a major stumbling block in the commercial-scale production of algae biofuels.2 The announcement came eight years after ExxonMobil first announced its collaboration with Synthetic Genomics, and four years after then-CEO Rex Tillerson conceded that microalgae biofuels were further than 25 years away due to hurdles faced at the basic science level.3

Overall, Exxon's investment in algae biofuel production is relatively minor, and the prospects for commercial-scale production are dim at best. However, the company is using the partnership to great advantage, portraying itself as a committed friend of the environment as it faces scrutiny for its role in purposefully misleading the public about climate change.⁴

Exxon isn't the only oil major to invest in algae biofuels.⁵ The question remains: Is Big Oil's investment in algae biofuels based on confidence in a credible alternative to fossil fuels, or is it nothing more than a public relations stunt?

Microalgae play a key role in the regulation of earth systems. Their voracious appetite for CO₂ is thought to have played a significant role in drawing down atmospheric CO₂ levels in a previous spike around 50 million years ago. Microalgae allowed all higher life forms to evolve during the earth's history, by creating an atmosphere rich in oxygen. They still provide about half of the oxygen in our atmosphere.⁶

Microalgae also form the base of the marine

and freshwater food chains, and play a key role in nutrient cycling. They are found in marine and aquatic ecosystems, but also play a vital, if poorly understood, role in every single terrestrial ecosystem, including in soils.⁷ Even cloud formation is influenced by microalgae.⁸ Recent research indicates that the asteroid impact that wiped out the dinosaurs 65 million years ago may have also triggered a massive algae bloom resulting in a major marine extinction event.⁹

As the climate crisis unfolds, many scientists are also researching the complex responses of microalgae to ocean warming and acidification. These scientists have found a troubling decline in phytoplankton populations, which could over time lead to the loss of life supporting oxygen in the atmosphere. 11

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In sum, microalgae are key to a habitable earth. They are incredibly diverse, adaptable and ubiquitous. As the repository of hopes and ambitions for a "green and sustainable" biofuel alternative, algae have become the target of a rapid and risky advancement of biotechnology research and development, including both "traditional" genetic engineering and new "synthetic biology" techniques. The aim of these efforts is to engineer microalgae to produce biofuels, chemicals, pharmaceuticals, neutraceuticals, plastics, and lubricants, under the umbrella of an eco-friendly "bioeconomy" alternative to the fossil fuel economy.

Bioeconomy proponents claim that microalgae-derived biofuels and other compounds can provide "carbon negative" fuels and "recycle CO₂" from polluting industries. ¹² Some scientists even advocate using microalgae to "geoengineer" the earth's climate, for example, by fertilizing ocean waters with iron to stimulate microalgae blooms, which would presumably absorb CO₂ from the atmosphere and sequester it in the deep ocean after dying.

Ancient microalgae make up a significant portion of coal and oil deposits, the extraction and burning of which is the primary cause of contemporary climate change. Now, industry scientists are turning to living microalgae as a green alternative. In the process, the evolved biology and genetics of microalgae have come under siege, with enthusiasts eager to harness them for commercial and industrial applications.

This report looks at the hype about microalgae biofuels - how, even after hundreds of millions, if not trillions of dollars invested, woefully few cars, trucks or airplanes have been fueled.

The industry is increasingly shifting focus from biofuels to production of a slew of new consumer "bioproducts" derived from genetically engineered/

synthetic microalgae, many of which are already being marketed, with little regulatory oversight or evaluation of the potential risks.

The ongoing hype that a truly "green" and "sustainable" algae biofuel breakthrough lies just over the horizon perpetuates the myth that we can maintain our familiar (or even expanding) levels of fuel and resource consumption even in a climate changing world. The report concludes with a call for governments to end public-sector support for algae biofuels, and redirection of limited resources towards more promising solutions, especially those that do not introduce serious health and environmental risks.

Box A: What are algae?

Broadly, the term "algae" is used to refer to both microalgae (usually single cells) as well as macroalgae (seaweeds). Microalgae are the focus of most research for algal biofuels and bioproducts, and hence the focus of this report. The term microalgae in turn refers to both cyanobacteria, which are prokaryotes, meaning they have no cell nuclei or other specialized organelles (similar to bacteria), and photosynthetic protists, which are eukaryotes, and do have cell nuclei and specialized organelles (including chloroplasts). Though microalgae are single-celled organisms, they sometimes grow in chains or filaments. Like plants, microalgae are photosynthetic (autotrophic), though some species can also derive energy from other sources (heterotrophic). Unlike plants, they have no vascular structure, i.e. no connective tissues, roots, stems or leaves.

For most of us, microalgae are familiar as the green "scum" floating on lakes and ponds or as "phytoplankton" floating at or near the ocean surface. We may be familiar with harmful algal blooms, which can result in contamination of water and seafood. But microalgae populations, when in balance, play a key role in healthy ecosystems. They are in fact one of the most diverse groupings of organisms on earth, found in marine and freshwater ecosystems, cold Arctic and Antarctic waters, deserts, caves, soils, and beyond. The advent of molecular genetic techniques revealed that what we refer to as "microalgae" includes such divergent evolutionary lines that some organisms we lump together under the heading of "algae" are more different from each other than plants are from animals. While many microalgae appear morphologically similar, geneticists have found far more diversity in functional genes than is found in plants and animals. This genetic diversity is one reason that they are of such interest to biotechnologists.

It is estimated that there may be some eight hundred thousand species of microalgae, of which only about fifty thousand have so far been identified.¹³ Some of the genera that have emerged as potentially useful and have been the focus of ongoing research include strains of *Chlamydomonas*, *Chlorella*, *Haematococcus*, *Nannochloropsis*, *Dunaliella*, *Botryococcus*, and *Scenedesmus*, among others.

1. Algae Cultivation History and Methods

Scientists have conducted research and development into algae biofuels since the mid-20th century, while cultivation of some species – such as *spirulina* – for food has a much longer history. Most research on algae biofuels involves photosynthetic microalgae, which produce lipids that can in turn be converted to fuels and chemicals. Macroalgae, or "seaweeds", are the focus of some bioenergy research, primarily as a biomass feedstock for anaerobic digesters, although so far with limited success.

Cultivation methods hinge first of all on whether a species is autotrophic – producing its own food via photoysnthesis, and therefore requiring light exposure – or heterotrophic – meaning that it must be provided with sugars in order to grow, most commonly from sugarcane. Additionally, some species are capable of photosynthesis but will grow more efficiently when provided with sugars.

Microalgae are typically cultivated either in open ponds called raceways or in closed photobioreactors (PBR) – or in some cases, through hybrid systems.¹⁵

Open pond raceway systems rely upon paddlewheels and/or pumps to circulate algaeladen water. They must be kept shallow enough to allow adequate light penetration, which requires a larger land area, and also require a source of CO₂ addition known as a sparge. Research has shown that these systems are vulnerable to contamination by bacteria, parasites, and other strains of competing microalgae. They are also vulnerable to weather fluctuations, and water evaporation.

Microalgae are typically cultivated either in open ponds called raceways or in closed photobioreactors – or in some cases, through hybrid systems.

Photobioreactors are closed units that take a variety of forms, ranging from flat plates to tubular columns. The benefit of a PBR system is that it requires less land area and provides greater control and isolation from the external environment. However, PBR systems are typically more expensive than open pond raceways, requiring up-front investment in materials in addition to operational expenses such as cleaning and energy costs.

On the regulatory level, PBRs have an advantage over open pond systems in that they are considered "contained use," which exempts them from certain regulatory requirements when using GE microalgae. Overall, there are costs and benefits associated with both approaches. One comprehensive modeling study comparing the farm-level (scaled-up) economics of open ponds versus photobioreactors concluded that while neither approach was economically viable, PBR's performed better in spite of higher necessary capital expenditures.

Following cultivation, a variety of additional steps are required, each of which require energy and raise the cost of production. In most processes the algae must be harvested, dewatered, and dried before the oils are extracted. In others where the algae directly secrete ethanol or chemicals, these must be separated from the seawater medium in an energy- and cost-intensive process.

Researchers have experimented with a variety of harvesting approaches, ranging from using chemicals to aggregate microalgae, to relying upon centrifuges, flocculation, ultrasound, and special filters. Extraction generally requires mechanical disruption (microwaves, pulsed electric field) as a pretreatment, followed by the use of chemical solvents to break down cell walls and release contents.

For the final step, biochemical and thermochemical means are used to convert the algae (extracted lipids or whole algae biomass) into the final fuel or chemical product. Lipids generally are processed using transesterification, while carbohydrates

and proteins are converted using fermentation. Additionally, there may also be further processes to make use of byproducts or contend with wastes.

2. Investment and Support for Algae Biofuels

Research on microalgae biofuels has been strongly supported in the United States (US) and Europe – the primary focus of this report – but also has significant support from the governments of China, Taiwan, India, South Korea, Japan, Canada, Mexico and Brazil.¹⁹

From 1978 to 1996, the US government funded research into algae-derived hydrogen and algae biodiesel under the umbrella of the Aquatic Species Program. Over its 18 years of existence, the scientists involved assessed nearly three thousand different species for their suitability to cultivation and genetic manipulation. However, it was ultimately defunded, concluding that the costs of production and other barriers offered little promise for success.

Starting during the oil price spike in the mid-2000's, venture capitalists and some oil companies began investing substantially into algae biofuel start-up companies. Growing awareness of climate change combined with high oil prices to revive the dream of microalgae biofuels as an alternative to imported petroleum.

What followed was a bubble of public and private sector investment. In the wake of the 2007 Energy Independence and Security Act, the US Department of Energy (DOE) started a microalgae program as part of the Bioenergy Technologies Office, publishing the first National Algal Biofuels Technology Roadmap in 2010.

In addition to the DOE, funding for microalgae biofuel research has come from the Department of Defense, National Science Foundation, US Department of Agriculture, DARPA and the US Airforce, as well as state and private sources. The American Recovery and Reinvestment Act of 2009 provided a massive cash infusion - \$97 million - for microalgae "integrated biorefinery" demonstration projects (including to the startup companies, Solazyme, Sapphire and Algenol).²⁰

The DOE supported the establishment of four public-private partnership research consortia, including the National Alliance for Advanced Biofuels and Bioproducts (NAABB), the Sustainable Algal Biofuels Consortium, the Consortium for Algal Biofuels Commercialization (CAB Comm) and the Cornell Consortium. Each of these included academic institutions, national laboratories, and private companies.

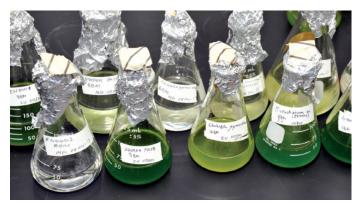


Photo: U.S. Air Force

It also funded the Microalgae Testbed Public-Private Partnership, led by Arizona State University, and the Regional Algal Feedstock Testbed, led by the University of Arizona, along with a series of projects aimed to improve yields and pilot new technologies. DOE also provided ongoing funding for research granted to the national laboratories.

Funding continues to flow: in June of 2016, DOE granted \$15 million to support algae research. Then in December, it announced an \$8 million funding opportunity for "productivity enhanced microalgae and tool kits," followed by another \$8 million in June of 2017.

Overall, many have made life-long careers, with lucrative grant supports for academics, the

national laboratories, and startup companies, with never ending calls for more research and little accountability or transparency regarding the amount of spending and the delivery of outcomes – commercial production of algae biofuels - from those grants.

European governments and industry associations have also invested significantly in algae biofuel research and development. The European Commission's Seventh Framework Programme has provided funding to projects such as the Biofuel Algae Technologies Project, while the European Union's Renewable Energy Directive (RED) includes algae under its list of feedstocks for "advanced biofuels.²¹ In the UK, the government launched an Algae Biofuels Challenge in 2008, only to cut funding for the program three years later.²²

A European Microalgae Biomass Association promotes the industry, and a "European Roadmap for a Microalgae Based Industry", was developed. A recent conference opened with overviews from ongoing major projects from across Europe: Pufa-Chain, BISIGODOS, D-Factory, InteSusAl, All GAS, BIOFAT, MIRACLES, SPLASH, FUEL4ME, MicroalgaeBioGas, PhotoFuel and others. The primary challenge identified in the outcome was to "make microalgae biomass cheap". Microalgae biofuels were relegated to a longer term (10 year) goal, while other products and services (animal feed, nutraceuticals, inks and dyes, and wastewater treatment) were identified as nearer term goals.²³



Algae raceway pond. Photo: JanB46

Doubts About the Reality of Commercial Algae Biofuels

In 2012, the US National Research Council released a report concluding that:

"The scale-up of algal biofuel production sufficient to meet at least five percent of U.S. demand for transportation fuels would place unsustainable demands on energy, water, and nutrients with current technologies and knowledge."²⁴

But this conclusion did not put an end to supports for research and development.

Shortly thereafter, in 2014, the National Alliance for Advanced Biofuels and Bioproducts (NAABB) published a report that appears specifically designed to counter the pessimism of the NRC report – but sends mixed messages.²⁵ The report notes several "advances and breakthroughs", and enthusiastically envisions "algal biofuels to be a viable competitor in the liquid transportation fuels market after a few more key improvements." At the same time, however, it concludes, "as the knowledge base continues to evolve and build on prior learnings, disruptive breakthroughs are going to be necessary to achieve cost-competitive and commodity-scale quantities of algal biomass for biofuel and bioproduct production." Those will presumably be forthcoming with more research funding.

Meanwhile, the European Energy Algae (Enalgae) project, which ran from 2011-2015, ultimately concluded.

"One of the major ideas enthusiastically considered 5 years ago was the potential role of microalgae in energy generation. With the barrel cost of oil almost halving, and revised estimates for the realistic potential for algal biofuels coming from the Enalgae project, it now looks highly unlikely that microalgae can contribute significantly to Europe's need for sustainable energy."²⁶

A 2017 International Energy Agency (IEA) report on algae biofuel technology states,

"Microalgae-based production to produce bioenergy products like liquid or gaseous fuels as primary products is not foreseen to be economically viable in the near to intermediate term."²⁷

Together, these reports shed light on an industry that has been unable to scale-up beyond laboratory scale – in many cases, surviving off of public-sector grants and venture capital. While oil prices have created a much more difficult playing field, ultimately the barrier to commercial-scale algae biofuel production stems from an impractical cost of production rooted in basic biological constraints.

Microalgae biofuels have arisen as the next bright alternative, a "savior" technology holding great promise, with the important breakthrough lying just beyond the horizon.

Still, overblown promises of "new breakthroughs on the horizon" continue, as does the funding. Even after years of investment with little to show by way of results, the US Department of Energy website still claims that microalgae will "ultimately be capable of producing billions of gallons per year of renewable diesel, gasoline, and jet fuels."²⁸ Some day!

Ongoing support for algae biofuels is also playing out against a backdrop of advancing supports and policies for first generation biofuels, such as ethanol from corn or sugarcane, and biodiesel from soy or palm oil. As the negative impacts of those fuels have become increasingly clear - including food price spikes, land grabs, and failure to reduce GHG emissions or deliver "energy independence" - public opinion towards those biofuels has soured.

Microalgae biofuels, in this context, have arisen as the next bright alternative, a "savior" technology holding great promise, with the important breakthrough lying just beyond the horizon, along with cellulosic and other "advanced" biofuels.

3. Myths and Realities of Algae Biofuels

The dream of microalgae biofuels is kept alive through the perpetuation of key myths that are persistently repeated by industry and the media, even though they have little basis in fact. These myths include:

- 1. Microalgae can produce biofuels with "nothing but sunlight, water and CO₂."
- 2. Microalgae are phenomenally productive and can produce massive amounts of fuel using very little land.
- 3. Microalgae fuels are a climate-friendly alternative to fossil fuels, and can be used to sequester or "recycle" carbon, or provide "carbon-negative" fuels.
- 4. Commercially viable microalgae biofuels are "just around the corner."

How do these myths hold up to scrutiny? Let's evaluate each in turn.

Myth 1: Microalgae can produce biofuels with "nothing but sunlight, water and CO₂".

"Fuel from thin air." That was the title of a 2015 article about Joule, a microalgae biofuel startup that had just received \$40 million in investment.²⁹ Joule has yet to produce commercial-scale microalgae biofuels, and has since acquired Red Rock Biofuels, a company proposing a very unpromising wood "gasification" biofuel project in Oregon.³⁰

Algenol, another algae biofuel startup, formerly described itself as "harnessing the sun to fuel the

world" with a simple childlike graphic of the sun, CO_2 and water ("there's plenty of it") converted via a scribbled arrow pointing to ethanol, biomass and oxygen. The website has since been changed but still conveys the same basic message: "Algenol uses its patented microalgae technology platform for the production of biofuels using proprietary microalgae, sunlight, carbon dioxide and saltwater, all on non-arable land."³¹

Similarly, in the June 2017 ExxonMobil announcement referenced earlier, Synthetic Genomics CEO Oliver Fetzer made algae biofuels sound easy: "The major inputs for phototropic algae production are sunlight and carbon dioxide, two resources that are abundant, sustainable and free." 32

Simple, appealing and clean as these claims sound, there are many resources in addition to sunlight, water and CO_2 that are required to produce microalgae biofuels. Even providing adequate sunlight, water and CO_2 (in concentrated form) are challenging and also energy-intensive. What follows is a quick review of some of these necessary inputs:

Nutrients

Microalgae growth is regulated by available nutrient quantity and quality, including nitrogen, phosphorus, potassium, iron, sulphur and various micronutrients. Optimizing access to these nutrients is key to productivity.

The production of synthetic nitrogen fertilizers is energy-intensive and costly, and results in significant GHGs that can impact a particular fuel's life cycle analysis (LCA). Meanwhile, phosphorus availability is increasingly limited, even as demand for producing agricultural crops for food is rapidly expanding. The National Research Council estimates that replacing just five percent of US transport fuel demand with algal biofuels would require 6-15 million metric tons of nitrogen, and 1-2 million metric tons of phosphorus – representing 44-107 percent of total nitrogen use, and 20-51 percent of total phosphorus use in the US.³³

It is widely acknowledged that large-scale cultivation of microalgae cannot be sustainable



Tubular glass photobioreactor. Photo: IGV Biotech

unless methods for acquiring and recycling of nutrients are established. Attempts to circumvent this problem include colocation of microalgae ponds adjacent to nutrient-rich wastewater streams, and using seawater (for marine species), and finding ways to recycle nutrients from algae biomass residuals after processing. Wastewater treatment using consortia of algae has been proven effective for removing nutrients from wastewater streams.³⁴ However, this process is not necessarily compatible with biofuel production, which requires monocultures as well as controlled conditions to maximize growth while minimizing exposure to contaminants that are common in wastewaters

Water

Microalgae cultivation on a large scale requires massive quantities of water. Water quality is also key to success as microalgae are highly sensitive to salinity, pH and contaminants. Some studies estimate that over three thousand liters of water are necessary to produce just one liter of water – meaning that it would require at least 123 billion liters of water to replace just five percent of US transport fuels with algal fuels.³⁵ Furthermore, the regions where sunlight and temperature are most suited for algae cultivation, such as deserts, tend to be places where water availability is limited.

In open pond systems, water evaporation is a significant issue, and where saltwater is used, salinity increases as water evaporates, requiring periodic additions of fresh water. Photobioreactors do not entail as much evaporation, but the reactors must be periodically flushed and cleaned. Marine microalgae species might be cultivated using seawater, but this can introduce competitors, predators and pests as well as limit the location of facilities to near the coast.

Energy

Energy inputs necessary for successful microalgae cultivation have a major impact on overall greenhouse gas emissions as well as production costs. Direct energy inputs are necessary to run

circulation pumps, provide and regulate light and temperature, separate microalgae from the water medium, dry and extract oils, and convert algae to fuels. Energy is also required for the manufacture and delivery of nutrients, management of the residual biomass, production of co-products, as well as the energy embodied in materials such as PBRs. Because of this, biogas derived from anaerobic digestion of residual algal biomass is generally assumed to be an essential energy source in the process.³⁶

"The scale-up of algal biofuel production sufficient to meet at least five percent of U.S. demand for transportation fuels would place unsustainable demands on energy, water, and nutrients with current technologies and knowledge." - US National Research Council

Net energy analyses for microalgae biofuels measuring the energy outputs relative to energy inputs vary widely, and are tightly linked to algae productivity. Meanwhile, estimates of productivity in the literature vary by a factor of 60.³⁷ This is in part due to extrapolation from laboratory research to commercial production conditions, which is not at all straightforward and leads to wildly misleading outcomes for projected yields and lifecycle assessments.³⁸ In a laboratory setting, it is far easier to optimize conditions for microalgae growth, and hence to optimize yields relative to inputs, yet those conditions are extremely difficult to duplicate at a scale large enough for commercial cultivation.

Lifecycle analyses from actually existing production systems have generally not been favorable, indicating that algal biofuels produced using photobioreactors require more overall energy inputs than the process itself delivers (energy return on energy investment = <1).³⁹

Murphy et al. report that energy demands related to water management alone are around seven times greater than the energy contained in algal biofuels at the end of the process. Clarens et al. reports that microalgae biodiesel lifecycle assessments are poorer than switchgrass, canola or corn processes, largely due to fertilizer and CO₂ input requirements.⁴¹ Dassey et al. analyzed the lifecycle for a system operating in Louisiana and reported that energy inputs exceeded outputs by 53 percent under even ideal circumstances.⁴² Grierson et al. analyzed a system that would entail using the residual biomass for biochar production, reporting that fertilizer and energy demands ultimately undermine any gains from the process.⁴³

Overall, lifecycle analyses are notoriously tricky, with outcomes highly dependent upon assumptions, assigned values, and included processes.⁴⁴ Not surprisingly, when industry performs their own lifecycle assessments, highly unrealistic, promising outcomes are often the result, and should be viewed with skepticism.

Carbon Dioxide

Providing adequate quantities of CO_2 to support maximal microalgae growth rates adds an additional challenge. While CO_2 in the atmosphere is a leading cause of global warming, it is mixed with other atmospheric gases in very diluted form (about .04 percent) – not enough to support industrial-scale microalgae growth (an estimated 1.6 - 2.0 grams of CO_2 are required per gram of algae biomass cultured).

Industrial cultivation requires either concentrated CO₂ gas, or soluble inorganic carbonates such as sodium bicarbonate, which can be costly. 46 Furthermore, as microalgae undergo photosynthesis, the pH of the surrounding water increases, a process that in turn alters the chemical availability of CO₂ and adversely affects microalgae growth. Achieving both adequate CO₂ supply (in concentrated form) and controlling pH is a difficult balancing act for successful cultivation. Hooking up algae cultivation to the smokestacks

of large industrial power plants or cement manufacturing facilities that emit CO₂ is a focus of research. However, there are numerous very substantial hurdles that may prove insurmountable – discussed further below.

Myth 2: Microalgae are phenomenally productive and can produce massive amounts of fuel using very little land.

In 2009, Biofuels Digest Magazine editor Jim Lane predicted a billion gallons of algae biofuels would be in production by 2014.⁴⁷ The following year, in 2010, the Prometheus Institute projected that 40 billion gallons per year of microalgae biofuels would be in production by 2022.⁴⁸

In 2009, algae biofuel company Sapphire also claimed that:

"By 2011, Sapphire Energy will be producing 1 million gallons of diesel and jet fuel per year, double its initial estimates. By 2018, the number, increases to more than 100 million gallons annually; and by 2025, the company will be producing up to 1 billion gallons of fuel per year. This means Sapphire alone will be supplying enough fuel to meet approximately 3 percent of the country's 36 billion gallon renewable fuel standard."49



Aerial view of Cyanotech microalgae ponds in Hawaii. Photo: Cyanotech (CC)

These are just some examples of what have proven to be wildly overoptimistic projections about the production capacity of algae biofuels – and yet, these projections still continue.

Productivity is key to virtually all aspects of making microalgae cultivation viable, and along with cultivation technology, determines the amount of land area required. Misrepresentations and claims of outlandishly high productivity have been key to attracting investment. However, microalgae face fundamental barriers to productivity: unlike agricultural crops, we do not have hundreds or even thousands of years of experience with large-scale cultivation to draw on.

Like agricultural crops, microalgae do not naturally grow in vast monocultures, but rather in multispecies interactive assemblages. Monocultures are more vulnerable to contamination by pests, predators and competitors. In the case of microalgae cultivation, these may be introduced via water, air or animal vectors and are especially problematic in open pond raceways where a large portion of the microalgae may be lost.⁵⁰

Misrepresentations and claims of outlandishly high productivity have been key to attracting investment in microalgae biofuels.

Light exposure is key to productivity. Microalgae have very efficient and broad-spectrum light harvesting capabilities, a trait evolved for life in aquatic environments where light penetration is often limited. However, under crowded conditions of mass cultivation, upper layers of microalgae can effectively shade out the cells further below, thereby limiting growth. Meanwhile, if exposed to *too much* light, algae cells are damaged. Achieving the right exposure to maximize productivity has thus proven challenging.⁵¹

Another issue involves trade-offs between growth and lipid production. Most microalgae species, given access to sufficient nutrients, will grow and reproduce prolifically, converting assimilated carbon into proteins. When stressed, and especially when nutrients become limited, they switch gear, directing carbon into the production of carbohydrates and lipids for energy storage.

For biofuel production, the goal is to produce lipids, but not at expense of the ongoing growth of the culture. This very fundamental trade-off between growth and lipid production remains one of the most significant roadblocks to commercial production, and much research has focused on understanding and overcoming it.⁵²

ExxonMobil/Synthetic Genomics June 2017 claim (cited above) of a lipid production breakthrough (with *Nannochloropsis gaditana*) was by no means the first announcement of this kind. For example, earlier in 2017, researchers had reported a breakthrough in lipid production in *Chlamydomonas*.⁵³ And in 2013, researchers at Scripps Institution of Oceanography announced a similar breakthrough with *Thalassiosira pseudonana*.⁵⁴

Even more fundamental than all of the above are limitations imposed by the biochemistry of photosynthesis itself. A 2015 review concluded, "At present, photosynthetic microbial biofuels are not viable in energy terms due to intrinsic inefficiencies in photosynthesis." Flynn et al. illustrate that the performance of the enzyme RuBisCo, which is central to photosynthesis, is the factor that ultimately defines the limits of productivity. This is a key reality check for any claims about productivity, among other reasons, because as the authors conclude: "In a commercial microalgal setting, the assumption of implausible specific growth rates leads to implausible business projections."

One of the main advantages claimed for microalgae biofuels is the capacity to produce very large amounts of fuel on relatively small land area – hence avoiding competition with food production or biodiversity. That claim is clearly not applicable to cultivation of heterotrophic microalgae, which must be provided with feedstocks produced

from land-based monoculture crops. Solazyme/ TerraVia is an example of a company that used heterotrophic microalgae supplied with sugarcane, rather than producing their own sugars via photosynthesis. Sugarcane cultivation is a major driver of deforestation and land degradation, as well as being an industry notorious for violent displacement of people from their lands, and slave labor conditions.⁵⁷

Even cultivation of photosynthetic microalgae requires large areas of land. To understand why, begin with the fundamental limitations of RuBisCo and photosynthesis, which imply that there is a limit to the amount of carbon that can be absorbed by microalgae in a cultivation facility, per unit of area, per day – that rate is about 5 grams of carbon per square meter, per day.⁵⁸ This scientific reality translates into a logistical headache for projects seeking to absorb CO₂ from large industrial facilities, constraining developers to large tracts of land located directly adjacent to the facility.

Myth 3: Microalgae fuels are a climate-friendly alternative to fossil fuels, and can be used to sequester or recycle carbon, or provide "carbon-negative" fuels.

Over the past few years, policy-makers have spun up the idea of "negative emissions technologies" as the latest silver bullet to address the climate crisis without challenging corporate power – that is, without challenging the roots of the crisis. Algae biofuel proponents are attempting to tap into this narrative, building off of the notion that algal fuels can be produced with just sunshine, water, and CO_2 .

The US-based Algae Biomass Organization promotes microalgae Carbon Capture and Utilization (CCU), a designation it won under the Clean Power Plan – a set of regulations passed under the Obama administration and anticipated

to be rolled back under the Trump administration. Rather than relying upon the "hammer" of regulation, the organization states:

"A new crop of microalgae technologies can... [convert] CO₂ into valuable commodities for trillion dollar industries, thus turning a problem – the high cost of compliance – into an opportunity – an ongoing revenue stream." ⁵⁹

The organization goes on to state that:

"Beneficial utilization of CO_2 is the only option to turn the market forces and economics of waste CO_2 into a ROI-driven, growth industry that will turn a huge problem into an economic opportunity. In doing so, we can achieve a rare trifecta – the reduction of emissions, the creation of jobs and economic development across the country, and a contribution to our food and energy security."

How is it possible not to be enthused by such hype?

Many algae biofuel companies have attempted to hook up algae cultivation to industrial power plants that provide CO₂. Pond Technologies is one such company, which has three pilot facilities aimed at producing algae-derived bioproducts from the steel, cement, oil and gas, and power generation industries. Similarly, the Tata Steel manufacturing facility in Port Talbot (UK) has partnered with the UK EnAlgae program to test the use of flu gases for algae cultivation.⁶⁰



Port Talbot steel plant. Photo: Grubb

But growing algae on flu gases is fraught with problems. For one thing, there is much more than CO_2 emerging from those smokestacks, including a variety of toxins that can inhibit growth or are lethal to microalgae. Species that can tolerate the industrial flu gas environment are not necessarily those that are generally of commercial interest. Furthermore, as discussed above, the limits of photosynthesis dictate the amount of carbon that can be absorbed per day. For a facility dumping hundreds of thousands of tons of carbon from a smoke stack, providing enough area for algae cultivation to absorb even just a portion of the output would require a vast area of land directly adjacent to the facility.

From a larger perspective, while hooking up microalgae production to polluting power plants could be viewed as "cleaning up" those facilities, it could also be viewed as perpetuating and greenwashing dirty processes that should be halted altogether.

Taking the concept of microalgae carbon capture a step further, many proponents now refer to producing "carbon negative" fuels. The idea is based off of the notion that more carbon is sequestered during microalgae (or plant) growth - or otherwise captured during production and combustion of the fuel - than is emitted into the atmosphere when the fuel is used. Thus, the net impact would be that CO₂ is not only recycled, but actually removed from the atmosphere by using the fuel. This fanciful notion, which would allow the perpetual use of large amounts of fuel, completely sidesteps reality by defying the basic laws of physics.



Arnot coal plant, South Africa. Photo: Gerhard Roux

Algae Systems is one such company touting "carbon negative fuels", stating on their website:

"The fuel we need for the future we want is a fuel that lowers atmospheric CO₂ levels with every gallon consumed, and fits within today's existing infrastructure...Algae Systems' partnership with Global Thermostat enables us to produce truly carbon negative fuels—feed our microalgae pure CO₂, sequestered directly from the air using Global Thermostat's revolutionary technology, to produce biochar, diesel and jet fuels that actually emit less CO₂ when burned than is fixed in growing the microalgae."⁶¹

Taking the algae carbon fix logic yet even a step further, there are those who consider algae as potentially useful for "climate geoengineering" – i.e. readjusting the concentration of greenhouse gases in the atmosphere on a global scale, by cultivating massive amounts of algae.

Myth 4: Commercially viable microalgae biofuels are "just around the corner."

It appears to be taking several decades to get around this corner. Given the fundamental barriers to commercial production of biofuels from microalgae, even after so much investment and effort, it seems clear this claim should be relegated to the dustbin.

Exxon and Synthetic Genomics are currently hyping their algae biofuel partnership. Yet only a few years ago, when the first phase of the partnership had proven unproductive, then-CEO Rex Tillerson acknowledged that microalgae biofuels were at least 25 years away, stating that, "what we've come to understand is that the hurdle is pretty high, and the hurdle seems to come at the basic science level which means it's even more difficult to solve."62

Box B: Geoengineering the Climate with Algae?

Because of their capacity to uptake carbon, algae have become a focus of research for "climate geoengineering", with the goal of reducing the atmospheric concentration of greenhouse gases on a global scale.

The algae-based approaches under discussion include "ocean iron fertilization", which involves dumping iron into the sea to stimulate algae blooms. The algae, it is hyphothesized, should grow prolifically in the presence of iron, which is a limiting nutrient in some cases, fixing carbon in the process, and then die and sink to the ocean floor where the carbon will remain sequestered away from the atmosphere. Ocean iron fertilization is subject to both the International Law of the Sea (where it is regulated as ocean dumping) and also under a moratorium put in place under the UN Convention on Biological Diversity.⁶³



Algae blooms off the US west coast. Photo: NASA

Deciding who can implement ocean iron fertilization, where, and at what scale remains seriously problematic as indicated by the uproar created by a recent unauthorized test in the Pacific Northwest.⁶⁴

Others suggest that large scale "ocean afforestation" would be an effective way to remove CO₂ from the atmosphere. This would involve planting giant swathes of kelp or other macroalgae, much as industrial tree plantations are installed on land to sequester carbon. ⁶⁵ There are however serious challenges associated with such largescale cultivation.

The IPCC, in their fifth assessment report, incorporated hypothetical "negative emissions" technologies into the models they assessed for achieving target stabilization goals. ⁶⁶ Those technologies are ways to not only slow the flow of additional greenhouse gases into the atmosphere, but also to remove gases already in the atmosphere. The only "near term available" technology they refer to are bioenergy with carbon capture and storage (BECCS) and largescale afforestation. BECCS is based on the assumption that bioenergy results in no net emissions, and that capturing the carbon emissions and sequestering them underground would render them "negative" emissions. Yet, current large scale bioenergy includes ethanol production and burning wood for electricity, both of which have been shown to result in even more, rather than less greenhouse gas emissions along with land use, human rights and biodiversity concerns. Hence scaling up bioenergy would only worsen those concerns and increase rather than reduce emissions. Further, the feasibility of effective below-ground storage of CO₂ is highly questionable. Nonetheless, some enthusiastic proponents now argue in favor of using algae biomass for BECCS and to provide algae based "negative emissions". ⁶⁷

Another proposed algae based approach to climate geoengineering involves using carbon captured by "direct air capture" to supply CO₂ to support microalgae cultivation, and then convert the algae to biofuels.

Box B (Continued)

A couple of startup companies including Kilimanjaro, Global Thermostat and Carbon Engineering, are working to develop direct air capture, but it has so far proven very energetically demanding and costly. Developers are looking at ways to profit from the sale of the carbon that is captured. Current demand for concentrated CO_2 is primarily for the oil industry which is seeking cheap sources to pump into depleted oil wells to extract remaining oil (aka "enhanced oil recovery"). Algae producers also require concentrated CO_2 to support growth. Since algae can be grown on less concentrated CO_2 , which is cheaper (costs go up as concentration goes up), the use of more dilute captured CO_2 for algae cultivation is considered to be the "most beneficial near-term applications for utilization of CO_2 from Direct Air Capture."

In a recent article, Greene et al enthusiastically report that:

"As microalgae have much higher primary production rates than terrestrial plants, they require much less land area to produce the same amount of bioenergy and/or food. On a global scale the avoided emissions resulting from displacement of conventional agriculture may exceed the benefits of microalgae biofuels in achieving climate stabilization goals".

Such statements are essentially fantasy, far removed from the realities on-the-ground and serve only to distract attention from real solutions. The time frame for addressing greenhouse gas emissions is far too short to accommodate wishful thinking.⁷⁰

Industry changing course:

Most algae biofuel companies, after failing to produce commercially viable biofuels, are either struggling financially or have gone bankrupt. Many are turning to other products and co-products such as food additives, animal feed, flavorings, nutraceuticals and cosmetics, as well as CO₂ sequestration and water treatment. These can be produced in lower volume, and sell at higher prices, making the economics of microalgae cultivation more viable.

Some companies are marketing co-products in the short term, while continuing to pursue biofuels in the long term, for which the market could potentially be vast. In industry parlance, this is a "biorefinery" approach that makes use of multiple products and coproducts. As Sapphire founder Stephen Mayfield articulated, "the only way you make money on a pig is if you sell everything but the oink."⁷¹

Most algae biofuel companies, after failing to produce commercially viable biofuels, are either struggling financially or have gone bankrupt.

These products include Omega-3's, astaxanthin and betacarotenes, polyunsaturated fatty acids DHA and EPA, coenzyme Q10, ACE inhibitor for blood pressure control, various pharmaceuticals including proteins, antimicrobials, antivirals and antifungals and neuroprotective products, cosmetics including anti-cellulite and alguronic acid, hydrocolloids including agar, alginate and carrageenan, biofertilizers, biopolymers and bioplastics, animal and fish feed (especially as replacement for fish meal in aquaculture and livestock.

Box C: From Algae Biofuels to Consumer Bioproducts

Solazyme/TerraVia initially aimed to produce biofuels using heterotrophic microalgae (*Prototheca moriformis*) raised on sugarcane feedstocks. Over the years, the company partnered with Chevron, UP, and Honeywell, and won a contract to supply the US Navy with microalgae based "drop-in" fuels at a cost of \$400 per gallon. Solazyme/TerraVia established a joint venture with Bunge in Brazil; partnered with Dow and Unilever (to produce the oils in Lux soap); and released a microalgae-derived lubricant for use in industrial drilling, including horizontal drilling (fracking) operations.⁷² In early 2016, burdened by significant debt, it split into TerraVia (food and cosmetics) and Solazyme Industrials (fuels and chemicals). Faced with the failure of its biofuels production, the company attempted to pivot by selling an anti-wrinkle cream, and won FDA approval for a line of specialty foods derived from non-GE microalgae, but was unable to overcome its debt.⁷³ Solazyme/TerraVia filed for bankruptcy in August 2017.⁷⁴

Algenol developed a GE *Synechocystis* species that directly secrete ethanol. The company received between \$30 and \$55 million in US state and federal funding on the basis of its claim to be able to produce ethanol, gasoline, jet, and diesel fuel, "for around \$1.30 per gallon each using proprietary microalgae, sunlight, carbon dioxide and saltwater at production levels of 8,000 total gallons of liquid fuel per acre per year." Algenol has since shifted to production of nutraceuticals (Omega oils) and microalgae carbon capture.⁷⁵

Sapphire Energy, a company closely affiliated with the University of California-San Diego, set out to produce microalgae biofuels and won supports from DOE and USDA, as well as tens of millions in venture capital. The company accrued a vast and diverse collection of patents, and established partnerships with Monsanto and Phillips 66, as well as Linde and Chinese petroleum giant Sinopec. Sapphire constructed a 2,200 acre microalgae farm in New Mexico along with two other facilities. After selling small volumes of "green crude" (at \$26 per gallon), however, it shifted focus to the production of omega-3 oils and animal feed ingredients. In May of 2017, the company announced the results of the first EPA-approved open pond test of GE microalgae, *Acutodesmus dimorphus*.⁷⁶

Aurora Biofuels developed GE *Nannochloropsis* species for open pond cultivation. The company shifted focus from fuel to food production, but went bankrupt and closed down in 2015.⁷⁷

OriginOil changed name to OriginClear and changed focus from fuel production to water treatment.⁷⁸

Solix started out to produce microalgae biofuels but now produces astaxanthin, and DHA Omega-3.⁷⁹

Synthetic Genomics works on a wide variety of biotechnology processes and organisms, and has a partnership with ExxonMobil to develop microalgae biofuels.⁸⁰ In May 2014, SGI announced a partnership with Archer Daniels Midland to produce Omega-3 from (non GMO) microalgae.⁸¹

Joule won EPA advanced biofuel pathway approval in 2016 for fuels produced with GE cyanobacteria, (*Synechococcus*), but after failing to secure new rounds of investement, the company folded in the summer of 2017.^{82,83}

Cellana operates a demonstration scale project in Hawaii to produce microalgae biofuels. They signed joint agreement with Neste Oil in 2013 for microalgae crude production, using a hybrid PBR/open pond system and non-GE microalgae. Cellana is producing omega oils and animal feed, and recently signed an agreement to collaborate in production of microalgae derived inks.⁸⁴

4. Biotechnology to the Rescue?

As ecologists, geneticists, and students of nature understand, predictability and control are far more elusive than the practitioners of "engineering biology" will acknowledge. Nature in the real world is messy.

Biofuel production may still be the "holy grail" of the microalgae industry, because of the potentially massive market that it would serve, but these other microalgae-derived bioproducts provide a financial lifeline and are increasingly dominating the agenda for producers. The common thread between most bioproducts is a very heavy dependence upon biotechnology to engineer microalgae genetics suitable for industrial purposes.

Meanwhile, the tools available for manipulating genetics have greatly advanced. Recent developments include a suite of new approaches sometimes referred to as "synthetic biology," or "new breeding techniques." These have arisen with the advent of "omics" capabilities, which involve very rapid gene screening for function and characteristics, as well as laboratory synthesized genes and sequences which can then be compiled and inserted into organisms, creating radically altered "synthetic organisms." The primary orientation of synthetic biology has been to engineer "cell factories" intended to secrete chemicals, compounds and molecules with utility for commercial and industrial applications.⁸⁵

Traditional genetic engineering, known as transgenics, mostly involved the transfer of genes from one organism into another unrelated organism, and proved to be slow and laborious with very limited rates of success. The new approaches provide vastly increased speed with

which genes and genomes can be screened, as well as lab synthesized and modified. Some synthetic biology approaches do not involve introduction of foreign genes at all - for example, "directed mutagenesis" which forces mutations on existing genes.

One new technique that has gained much attention is "genome editing" using CRISPR/Cas9 or CRISPR/Cpf1 (CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats). While only introduced a few years ago, genome editing has since been applied to a wide array of organisms, including microalgae.86 Genome editing is referred to as "more precise" - providing capability to tightly control the "editing" of gene sequences, analogous to the use of a word processor for writing. This representation, however, is misleading. Critics point out that genome editing is only "precise" in the most superficial sense given many unintended and off-target effects, and a lack of knowledge about the impacts of "precise edits" on the behavior and physiology of the organisms.87,88

Genetic engineering and synthetic biology are founded on a reductionist view of nature and genetics. The engineering mindset is, however, poorly reflected in the real biological world, where genes can have multiple effects, depending on the activity of other genes, the organism as a whole, and the environment, all of which change over time. As ecologists, geneticists, and students of nature understand, predictability and control are far more elusive than the practitioners of "engineering biology" will acknowledge. Nature in the real world is messy.

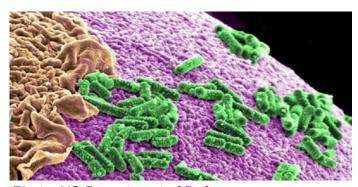


Photo: US Department of Defense

Many regulators have not kept up with the potential risks posed by new synthetic biology techniques, resulting in an erosion of oversight that the industry is more than willing to take advantage of. As national governments and multilateral institutions like the United Nations Convention on Biological Diversity are debating how to regulate synthetic biology, many civil society groups are calling for the application of the precautionary principle as a foundation of regulation and governance.⁸⁹ Industry, however, is plowing forward at its own pace, with little regard to these processes.

In the US, microalgae and other micro-organisms are regulated by the EPA through the Toxics Substance Control Act (TSCA). In 2015, the Obama Administration called for an overall reform of the regulatory framework for biotechnology, prompting the EPA to engage in public consultation focused on risk assessments for engineered microalgae. Under the current Trump administration, the very limited regulations that already exist are likely to be reduced or eliminated altogether – a troubling development at a moment when the speed and pace of new applications for commercial deregulation are dramatically escalating.

In 2015 the EPA called together a public meeting and opportunity for comment on revision of regulatory guidance. This was partly spurred by the very rapid increase in applications received.

Under the current Trump administration, the very limited regulations that already exist are likely to be reduced or eliminated altogether – a troubling development at a moment when the speed and pace of new applications for commercial deregulation are dramatically escalating.



US EPA headquarters. Photo: NRDC via Flickr

EPA had received about 5 applications per year for engineered microalgae between 2003-2011. In 2015 they had already received 42 applications by the month of June. 91

Current EPA regulations exempt almost all research and development activities, even if ultimately targeted for commercial application. The agency exempts pilot- and demonstrationscale projects, so long as they don't involve open pond cultivation (which requires the applicant to submit a TSCA Experimental Release Application, or TERA). Commercial production involves more reporting and oversight, requiring applicants to submit a Microbial Commercial Activity Notice, or MCAN. As of August 2017, three companies that use engineered microalgae have been reviewed and permitted for commercial use by the EPA: Solazyme, Joule, and Algenol. Solazyme has also won approval from the Food and Drug Administration for high oleic oils, protein powder and flour derived from modified microalgae.

In May 2017, researchers associated with UCSD and Sapphire Energy published their results from the first EPA-permitted open pond test of engineered microalgae, *Acutodesmus dimorphus*, as part of the "Consortium on Microalgae Biofuel Commercialization." They reported that GE microalgae did in fact move out into the environment, finding its way into ponds at various distances from the test site.

After only two months of observation, the researchers concluded that the engineered traits were maintained in open pond cultivation, and that those algae that did escape did not outcompete or otherwise interfere with native microalgae. The very short time period of the study, however, makes conclusions tentative at best.

Scientists have used a wide range of techniques to modify microalgae, including random mutagenesis and recombinant nucleic acid technologies, directed evolution, as well as synthetic biology techniques such as genome-editing technologies such as TALENS (transcription activator-like effector nucleases), ZFNs (zinc-finger nucleases) and CRISPR/Cas-based RNA guided DNA endonucleases.⁹³

Given the long history of research into bacterial genetics, genetic engineering of (prokaryotic) cyanobacteria is more advanced than eukaryotic microalgae, however, the latter is receiving much attention.

What are the characteristics being engineered in microalgae?

"Improved" Photosynthesis:

A major focus of current research involves controlling photosynthesis in order to maximize productivity. Scientists aim to increase or target microalgae's light gathering capacity as well as extend the range of the light spectrum they can utilize. ⁹⁴ This includes efforts to manipulate RuBisCo, the enzyme responsible fixing carbon from CO₂ into energy rich molecules (such as glucose), and hence ultimately responsible for determining productivity.

Maximize lipid production

Another major goal of biotechnology researchers is overcoming the trade-off between growth and lipid production through manipulating controls that enable the algae to "overproduce" lipids without killing the organisms.





Photo: Pixabay

This is the basis for the "fat algae" breakthrough reported by Exxon/SGI, and the above mentioned 2016 report on targeting the role of salinity as a factor controlling the switch from starch to lipid production for *Chlamydomonas* microalgae.⁹⁵

Redirection of metabolic pathways to directly produce specific kinds of lipids, fuels and chemicals

Manipulating cell biochemistry independent of growth mechanisms, yet again, remains a challenge. Scientists have been able to genetically engineer microalgae to produce alkanes, ethanol, acetone, ethylene, isoprene, isobutyraldehyde, isobutanol, 2,3 butanediol, 1-butanol, 2-methyl-1-butanol, and various fatty acids in controlled laboratory conditions, on small scales. 96 However, transitioning from laboratories to large-scale commercial production has so far not been possible.

Tolerance to conditions of mass cultivation, including resistance to contamination and predators

Just as herbicides such as glyphosate are used to control invasive weeds in agricultural crops, researchers are investigating chemical controls for large-scale microalgae cultivation, including engineering resistance to glyphosate and other herbicides and pesticides.⁹⁷ This is alarming as it indicates that large-scale microalgae

cultivation could follow a similar path to that of industrial agriculture. By far, the most widespread GE terrestrial crops are those engineered for resistance to glyphosate (Roundup).⁹⁸

Hydrogen production

Some microalgae species naturally produce hydrogen, but can only do so under particular conditions – namely, lack of sulfur availability and cessation of photosynthesis, meaning that they cannot grow and produce hydrogen simultaneously. Efforts are underway to engineer "optimal" hydrogen productivity.⁹⁹

Will it ever be possible to engineer microalgae such that they can efficiently and sustainably produce significant quantities of biofuels? This seems unlikely, at least any time in the foreseeable future. As former ExxonMobil CEO Rex Tillerson noted, the barriers exist at the basic science level.

In "Photosynthetic constraints on fuel from microbes", the authors conclude:

"At present, photosynthetic microbial fuels are not viable in energy terms. This is related to intrinsic inefficiencies in photosynthesis, and thus research has been directed to improving photosynthesis. A brief survey indicates that most suggested modifications would be beneficial only under restricted culture conditions. Controlled growth in bioreactors may then be required but this will incur a significant energy cost. Which, at this point, is much bigger than the engineered efficiency gain." 100

Nonetheless, spending on research and development continues, and meanwhile with the new tools that are available, researchers argue that it is increasingly possible to quickly, inexpensively and profoundly alter genetics. But at what risks to health and the environment?

5. Risks and Threats of Microalgae Biofuels

In order to evaluate the potential risks of GE or synthetic microalgae, we have to first recognize what we know and also, especially, what we do not know. Scientists know that microalgae are enormously diverse. There are an estimated 800 thousand species, of which only perhaps 50 thousand have even been described. Microalgae species thrive in oceans, freshwater, soils, and tree barks, as symbionts with various animals (for example in the fur of sloths), and in many other environments. We know next to nothing about most species, and even for familiar species, we know little about their natural history or behavior in nature.

In "Genetically Engineered Microalgae for Biofuels: A Key Role for Ecologists" the authors ask:

"How frequently would GE microalgae escape from cultivation and processing facilities? This could occur through aerosolization, wildlife vectors, turbulent weather that damages or destroys these facilities, accidents, human error, or other events. How far would GE microalgae disperse, and how long would they survive? Could transgenes designed to enhance the growth and fitness of released GE microalgae subsequently spread across metapopulations, species, habitats, and regions, and, if so, at what scales and over what time frames?" 101

Scientists, policy-makers, and the public at large do not have the answers to these questions. We do, however, know that some species can easily become invasive under favorable conditions, and

Many researchers assume that microalgae under cultivation simply cannot be prevented from escaping containment.

that others secrete toxins capable of causing illness and even death in humans and other species. We also know that many ecosystems depend upon a delicate balance of species, including in some cases dynamic multi-species aggregations of microalgae. Our lack of knowledge means we cannot really predict or model what would occur with the introduction of non-native, genetically modified or synthetic microalgae into the mix. There are simply too many "unknowns" and "unknown unknowns" to adequately assess the risks – a disturbing reality given the pace of research and development. We can, however, make some assumptions and extrapolations from what is already known about microalgae.



Photo: Robert Kerton, CSIRO

Release into the wild is inevitable. Microalgae simply cannot be contained, especially in commercial and industrial facilities.

Many researchers **assume** that microalgae under cultivation simply cannot be prevented from escaping containment. Their very small size (single cells) means they can easily escape via minute spills and accidents and through a variety of vectors, such as clothing or in air vents. Accidents are inevitable. Leaks from ponds or reactors, spills during harvesting, dewatering or extraction processes, or during transportation of materials, as well as incomplete sterilization processes all provide opportunities for microalgae to escape. Some species can remain dormant for



Algae covered pond. Photo: Dave Shafer

long periods, while others can be transported over long distances by becoming airborne. ¹⁰³ Based on these realities, it should be considered a given that cultivated microalgae, whether native, nonnative, GE or synthetic, will escape from cultivation facilities. ¹⁰⁴ While major accidents resulting from natural disasters are probable, long-term, low-level releases from ongoing operations are also likely to have significant ecological impacts.

In "Cultivated Microalgae Spills: Hard to Predict/ Easier to Mitigate", the authors state:

"Cultivating microalgae on a large scale will inevitably lead to spills into natural ecosystems...spills of non-native microalgae in aquatic (or terrestrial) ecosystems may have massive ecological repercussions regardless of whether the microalgae are genetically modified...it will be very difficult or even impossible to make firm predictions about the risks of non-native microalgae based on algal fitness characteristics determined in laboratory experiments or in modeling studies."

In "Large scale cultivation of genetically modified microalgae: a new era for environmental risk assessment", the authors state:

"It is generally accepted that the deliberate release of GMOs into the environment, is in most cases, a necessary step in the development of new products derive from or containing GM algae, and that these organisms, whether release into the environment in large or small amounts, may survive, reproduce and spread, and that the effects of such releases on the environment may be irreversible."¹⁰⁶

Many claim that microalgae engineered for industrial uses would not likely survive in the wild. However, microalgae are being engineered in some cases for the exact traits that could make them outcompete native species.

These traits include abnormally prolific growth rates, which can be achieved in part by outcompeting wild species over access to nutrients, as well as resistance to contamination by wild species (which can be achieved by engineering microalgae to secrete toxins lethal to invaders).

In a paper entitled "Monster Potential Meets Potential Monster," Flynn et al. elaborate that while it is likely possible to engineer microalgae to increase productivity fivefold, doing so would alter the stoichiometry of the microalgae in a manner that would make them unappetizing to the predators that normally are key in keeping microalgae blooms in check. The authors conclude:

"The spread of GM microalgae of the type of configuration we identify would be effectively impossible to halt. As GM factors likely affecting palatability of microalgae is already being conducted in the name of biofuels production, there is a real risk that the genie is already part way out of the bottle. If GM biofuels-optimized microalgae were to destroy fisheries then a main driver for microalgae biofuels research, the argument that such biofuels would not compete with production of biomass for food, may prove to be totally misplaced. Accordingly a strong argument can be made for the regulation of GM microalgae at an international level, because the potential for damage could have global consequences, echoing recent

concerns over geoengineering. Whether against arguments for sovereign fuel security, regulation could be enforced is a dilemma that society may soon have to face up to."107

Microalgae's high rate of productivity means mistakes spread quickly.

Henley et al. state:

"GM organisms that survive in natural ecosystems are potentially unlimited in time or space. Algal populations can grow explosively and episodically through asexual and in many cases sexual reproduction. Indeed, rapid growth is one of the primary advantages of microalgae over plants for biomass production. But it also may represent a larger ecological risk." 108

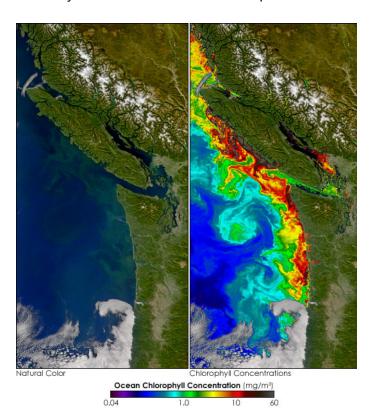
Harmful microalgae blooms (HABs)

HABs are a phenomenon where conditions favor proliferation of particular microalgae species that secrete toxins. These toxins can result in massive fish kills, and death to marine birds and mammals as well as illness and even death to humans. 109 Some are neurotoxins; others affect the liver or cause blood poisoning. Studies suggest that exposure to toxins released by some cyanobacteria may be linked to diseases like Alzheimer's and Parkinson's disease. 110 HABrelated incidents have risen very sharply with pollution and warming of waterways as well as the transport and introduction of various microalgae species in ballast waters among other vectors.111 In particular, climate change is considered a catalyst that is resulting in dramatic increases in HABs and in shifting microalgae population dynamics. 112

In addition to secreting toxins, microalgae blooms result in hypoxia (depleted oxygen) in waterways, such as oceanic "dead zones" caused by microalgae blooms driven by an influx of nutrient runoff from upstream agriculture. Contamination of drinking water supplies and recreational waterways are of particular concern along with the economic impacts of microalgae blooms, estimated to range

up to over \$2 billion per year in the US.¹¹³ Given the risks, mass cultivation of toxin-producing species would clearly be a potential disaster – however, a precautionary approach to non-native, GE, or synthetic microalgae is also necessary, given the lack of knowledge about many species and their ecology. Engineering microalgae specifically to secrete toxins (i.e. as a means to deter pathogen contamination in cultivation processes) would, of course, be especially risky.

Palyotoxin (PITX), a compound produced by dinoflagellates and cyanobacteria, is an extremely potent neurotoxin whose mechanism of exposure was reviewed by David Haberman. 114 Exposure, which can occur from consuming contaminated seafood and inhalation or skin contact, can cause serious illness, blindness or death. 115 Haberman warned of the serious risks posed by GE microalgae. 116 Presenting at a 2013 International Biodefense and Natural Disaster conference, he referred to genetically modified microalgae as a potential "poor terrorist's bioweapon" based on the similarity between PITX and the compound "ricin"



Toxic pseudo-nitzschia bloom off the US/Canadian west coast, September, 2004. Photo: NASA

(recognized in the US as a second-priority potential bioweapon). 117

Horizontal Gene Transfer (HGT)

Cyanobacteria, also known as blue-green algae, very frequently engage in horizontal gene transfer, a characteristic that has played a major role in their evolutionary history. 118 HGT refers to transmission of genes not just to direct progeny via sexual reproduction, but to other even unrelated individuals, and even unrelated species. Some species can take up "naked DNA" from their surroundings and import/export DNA using viral or other vectors. Studies indicate HGT between cyanobacteria and their predators, called cyanophages, is so pervasive that it "performs the driving functions in adaptive microevolution."119 HGT could result in the transmission of DNA into edible aquatic species and ultimately via the food chain, into humans.

In an analysis of factors for risk assessment of engineered microalgae, Beacham et al state:

"A major concern for GM microalgae use therefore is that the modifications created may be transferred from the GMO via HGT into natural algae, bacteria or virus species in the environment and thereby cause damage to ecosystems via selective advantage conferred by the transferred genes."

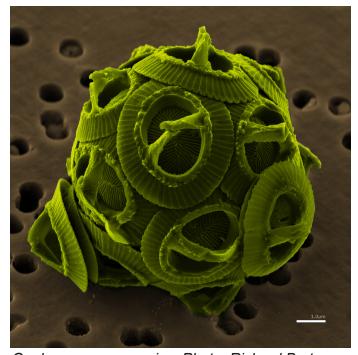
The authors address the possibility that genes may transfer to decomposer bacteria, raising concerns about using waste algae as fertilizer. In sum, the exchange and transfer of genetic material among these organisms is not straightforward and predictable, enabling a far-reaching dissemination of traits into the environment.

Instability of engineered traits

Engineered traits may not be retained over time due to very high rates of mutation, unstable expression and gene silencing. Researchers have found that cultures that are initially identical may over time become differentiated. ¹²⁰ In "Genetic

Instability in Cyanobacteria" this problem is referred to as "the elephant in the room," with the authors noting that:

"[f]or continuous production from cyanobacterial strains, culture stability remains a challenge with peak titers occurring after a week in many cases. Loss of production may be due to genetic instability, as carbon diversion creates a selective pressure for spontaneous mutants with an inactive pathway."¹²¹



Gephyrocapsa oceanica. Photo: Richard Bartz

More use of toxins

Microalgae monocultures are vulnerable to invasion from competing wild species, predatory organisms and fungal infections. As mentioned, this vulnerability has led researchers to prioritize "crop protection," engineering microalgae to be resistant to herbicides such as glyphosate and other toxins. ¹²² In 2012, Sapphire Energy was awarded a patent on glyphosate-resistant microalgae strains. ¹²³ Glyphosate application in terrestrial agriculture has skyrocketed with widespread adoption of crop varieties engineered for resistance. ¹²⁴ Meanwhile, glyphosate has been recognized as a "probable carcinogen", along with

causing numerous other health and environmental damages including destruction of beneficial soil microbes and the plummeting of monarch butterfly populations. Large scale cultivation of microalgae could result in yet another uptick in use of engineered pathogen resistance. But given the rapid evolution of pathogens, this would require continual modification of new strains. Algae with engineered pathogen resistance would have a clear advantage over their wild counterparts, should they be released into the environment.

Herbicide, and fungicide, and antibiotic resistance genes are also used as selective markers during engineering. These markers are inserted into the genome along with the genes for the trait of interest – often remaining there – and are favored for monoculture cultivation due to their resistance to pests and competitors. Protein products resulting from these genes could pose harm to people or



Plastic plate photobioreactor. Photo: IGV Biotech

animals that consume or come into contact with the algae. These genes could also lend a competitive advantage if released among wild counterparts, especially in waterways rich in herbicide, antibiotic or pesticide residues. The potential for horizontal gene transfer to other microorganisms in the environment poses a serious concern, given the recent evolution of antibiotic-resistant "superbugs," combined with the diminishing effectiveness of vital antibiotics.¹²⁵

Even non-GE, non-native species pose risks

Beacham et al point out that:

"[w]hilst there is currently very little regulatory control over the importation and release of nonnative algal strains into the environment, such as in the use of microalgae in aquaculture, the risks associated with non-native invasion should also be considered. The actual environmental risk associated with large algae spills therefore will not be limited to the GM aspect of these organisms but rather to a combination of factors including the fitness of the invading algae, the fitness of the indigenous alga populations, modes of competition for the resident and invading species, and intricacies and population stability characteristics of the disrupted ecological system. That said, successful environmental invasion and establishment does not necessary require rapid growth rate of the invader or even population dominance, just a low level persistence or a potential for gene flow, which will be determined by the difference in relative resource limitation between the alien and native species."126

In "Cultivated microalgae spills: hard to predict, easier to mitigate risks," the author states that

"[c]ultivating algae on a large scale will inevitably lead to spills into natural ecosystems. Most risk analyses have dealt only with transgenic algae, without considering the risks of cultivating the corresponding nontransgenic wild type species. This is despite the long-studied 'paradox of the plankton', which describes the unsuitability of laboratory experimentation or modeling to predict the outcome of introducing non-native algae into a new ecosystem."

Microalgae are fundamental to ecosystems and to regulation of biogeochemical cycles, hence there is potential for far-reaching and serious harms.

Microalgae are the source of much of the oxygen that makes earth habitable for humans and most other species, which means that their presence and population dynamics have broad-ranging consequences. They also form the base of the aquatic food chain; hence the composition of algal communities is a defining feature of ecosystems. Given the lack of knowledge about most microalgae species' basic biology and genetics, it is impossible to fully predict or control the impacts associated with the introduction of GE (or non-GE, non-native) microalgae into natural ecosystems. In addition to the vast genetic variation among microalgae species and populations, there is also much variation in species composition within ecosystems, which responds over time to shifting conditions including nutrient availability, temperature, light, water currents, and the presence of predators and pathogens, among other factors.

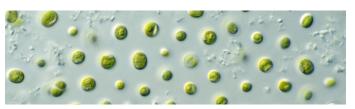
Humanity faces an unpredictable future with escalating pollution, fast-paced climate warming and ocean acidification that is already causing major shifts in microalgae communities and a dramatic increase in the incidence of harmful microalgae blooms. Most scientists assume that microalgae will inevitably escape from industrial cultivation systems – not only open ponds, but also photobioreactors, during industrial cultivation. The magnitude of potential negative impacts from the introduction of GE microalgae should be cause for serious concern and precaution. While wellmeaning, recommendations to monitor the area surrounding cultivation facilities are ultimately an exercise in futility given the lack of effective means to respond once a release occurs. With this in mind, a truly precautionary approach would be to altogether avoid the cultivation of GE or non-native microalgae species.

Conclusion: End Subsidies for Algae Biofuels

Even after decades of well-financed efforts, microalgae biofuels remain elusive. This is due to barriers at the biological level that make it difficult to achieve the productivity levels necessary to cultivate microalgae at economically viable costs. Faced with this reality, microalgae producers are turning to high-value niche coproducts for which lifecycle assessments and costs are not an issue.

However, the ongoing focus of research on GE microalgae introduces serious health and environmental risks that are under-researched and inadequately regulated. Given their fundamental role in earth systems, it seems particularly unwise to manipulate and engineer microalgae for commercial and industrial uses when "containment" in production facilities is essentially impossible.

The risks associated with large-scale microalgae cultivation – and especially engineered microalgae - must be weighed against a realistic assessment of potential benefits. Some products and services derived from microalgae - such as key nutraceuticals, omega 3 fatty acids, feeds for fish farming, or wastewater treatment – could supply broader societal benefits, provided they are produced with non-GE, preferably native species and with the highest precaution in cultivation methods. However, the ongoing investment of taxpayer dollars into the risky production of microalgae biofuels is a clear dead end, and a dangerous distraction from real solutions to the climate crisis. Governments must redirect efforts to more promising approaches to transportation in a warming world – ones that pose less risk to public and environmental health, and address the root causes of the crisis.



Nannochloropsis sp. Photo: CSIRO

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