

Milking Diatoms for Sustainable Energy: Biochemical Engineering versus Gasoline-Secreting Diatom Solar Panels

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In the face of increasing CO₂ emissions from conventional energy (gasoline), and the anticipated scarcity of crude oil, a worldwide effort is underway for cost-effective renewable alternative energy sources. Here, we review a simple line of reasoning: (a) geologists claim that much crude oil comes from diatoms; (b) diatoms do indeed make oil; (c) agriculturists claim that diatoms could make 10–200 times as much oil per hectare as oil seeds; and (d) therefore, sustainable energy could be made from diatoms. In this communication, we propose ways of harvesting oil from diatoms, using biochemical engineering and also a new solar panel approach that utilizes genomically modifiable aspects of diatom biology, offering the prospect of “milking” diatoms for sustainable energy by altering them to actively secrete oil products. Secretion by and milking of diatoms may provide a way around the puzzle of how to make algae that both grow quickly and have a very high oil content.

Introduction: Diatoms as an Energy Source

The recent soaring and crashing of oil prices and diminishing world oil reserves, coupled with enhanced greenhouse gases and the predicted threat of climate change, have generated renewed interest in using algae as alternative and renewable feedstock for energy production.^{1–3} In fact, diatoms, which are single cell algae with silica shells,⁴ may have created much of the purported global warming crisis by providing us with a convenient fossil source of energy in the form of much of the crude oil used to produce gasoline.^{5,6}

*“The main primary producers within the phytoplankton today—in terms of net production—[and] ... contributors to sedimentary organic matter ... are the diatoms ...”*⁷

Therefore, living diatoms may also point the way to a sustainable source of oil. Diatoms have become central to a new direction in nanotechnology in which we grow and harvest them for their hard silica parts, with the result that our knowledge of diatoms is increasing rapidly.^{8–10} In our consideration of the question of exploiting diatoms for their oil, we will apply some of the concepts that are coming out of this new field of “diatom nanotechnology”.^{4,8–17}

The transparent diatom silica shell consists of a pair of frustules and a varying number of girdle bands^{18,19} that both protect and constrain the size of the oil droplets within, and capture the light needed for their biosynthesis.²⁰ We propose three methods: (a) biochemical engineering, to extract oil from diatoms and process it into gasoline; (b) a multiscale nanostructured leaf-like panel, using live diatoms genetically engineered to secrete oil (as accomplished by mammalian milk ducts), which is then processed into gasoline; and (c) the use of such a panel with diatoms that produce gasoline directly. The latter could be thought of as a solar panel that converts photons to gasoline rather than electricity or heat.

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Over the past few decades, several thousand species of algae including diatoms have been screened for high lipid content.^{1,21–23} It was found that, on average, polyunsaturated fatty acid, which has a lower melting point than saturated fats,²⁴ constitutes ~25% of algal mass. This content may vary noticeably between species, and, interestingly, the lipid content increases considerably (double or triples) when cells are subjected to unfavorable culture conditions, such as photo-oxidative stress or nutrient starvation.²⁵ This is due to the shift in lipid metabolism from membrane lipid synthesis to the storage of neutral lipids.²⁵ One lipid oil drop bearing a pennate diatom²⁶ (see Figure 1) has

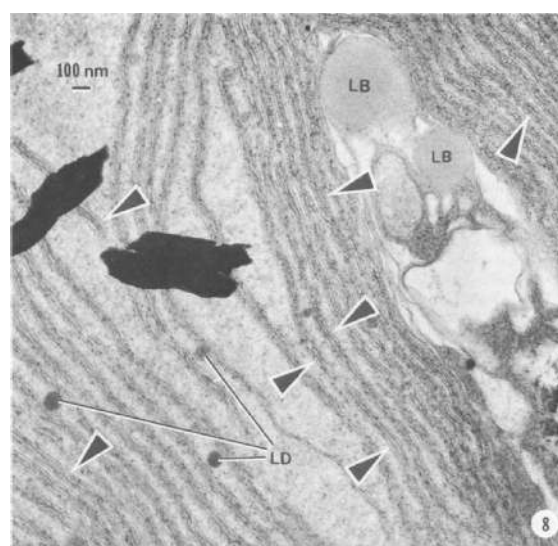


Figure 1. Lipid bodies (denoted as “LB”) and lipid droplets (denoted as “LD”) inside a chloroplast of the nuisance diatom *Didymosphenia geminata*. Arrowheads indicate triple thylakoids. (Reprinted with permission from ref 26.)

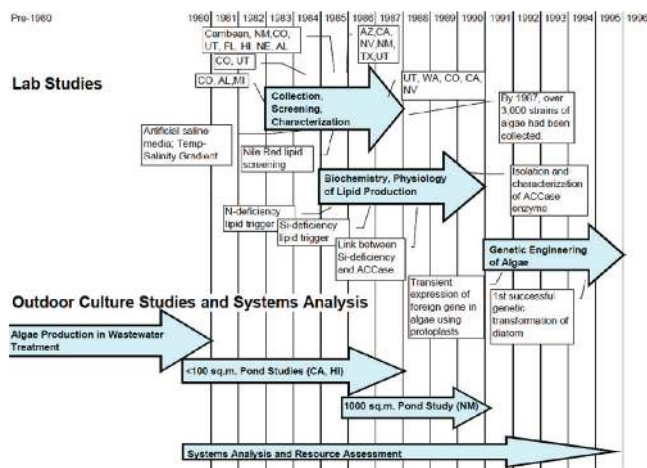


Figure 2. Partial history of attempts to use diatoms and other algae for biofuels. (Reprinted, with permission, from ref 21. Copyright 1998, National Renewable Energy Laboratory, Golden, CO.)

outwitted us by its extraordinary ability to proliferate; however, after we understand its secrets, we may be able to put it to work for us:

*“The diatom *Didymosphenia geminata* (Bacillariophyceae) has garnered increased attention as a nuisance and invasive species in freshwater systems. Historically described as rare yet cosmopolitan, a suspected new variant of *D. geminata* has the capacity to inundate kilometres of river bottom during a bloom. Unlike most other bloom-forming algae, *D. geminata* proliferates under high water quality (i.e., low turbidity and low nutrient) conditions”.*²⁷

Physiological and genetic manipulations of diatoms have the potential to bring the concept of “diatoms for oil”^{23,25,28,29} closer to commercial reality. Indeed, genetic transformation of diatoms started with the goal of improving oil production^{25,30–32} (see Figure 2), and it may be time to resume this line of research with the large range of genomics tools being developed for diatoms.^{33–44}

Agricultural oil crops, such as soybean and oil palm, are being used widely to produce biofuel; however, the amounts produced are <5% of the plants’ biomass. They also require high cropping area and extensive cultivation, with considerable concern about environmental impact and competition with food production and water use.^{22,45–48} Based on the photosynthetic efficiency and the growth potential of algae, theoretical calculations suggest that an annual oil production of >30 000 L (or ~200 barrels) of algal oil per hectare of land may be achievable in mass culture of oleaginous algae, which is 100–200 times greater than that of soybeans.^{21,23} Estimates vary:

*“The per unit area yield of oil from algae is estimated to be between 5000 and 20,000 gallons per acre [56 000 to 225 000 liters per hectare] per year, which is 7–31 times greater than the next best crop, palm oil”.*⁴⁹

Diatoms, unlike other oil crops, grow extremely rapidly,⁵⁰ and some can double their biomass within 5 h⁵¹ to 24 h.⁵² Even “in the wild”, doubling times can be 2–10 days,^{53,54} which includes photosynthesis and photorespiration periods. Diatoms have been regarded as C₃ photosynthesizers, and their photosynthetic efficiency is enhanced by concentrating CO₂ around Rubisco, diminishing photorespiration. It is estimated that diatoms are responsible for up to 25% of global CO₂ fixation.⁹ Benthic diatoms, under favorable conditions, migrate toward sunlight and bloom in the presence of ample nutrients.⁵⁵ Similarly, during unfavorable conditions they sink downward,

which constitutes the bulk of the organic flux. In addition, continuous upwelling⁵⁶ also replenishes nutrients, which end up in the next round of diatom blooms. Each diatom cell creates and then uses its own gas tank, so to speak, so perhaps diatoms could eventually keep our gas tanks full, and, of course, reabsorb CO₂ in the process.

Diatoms may have a major role to play in the coming years, with regard to the mass production of oil. This entails appropriate cultivation and extraction of oil, using advanced technologies that mimic the natural process while cutting down the time period involved in oil formation. Here, we consider a simple line of reasoning:

- (1) Geologists claim that much crude oil comes from diatoms.^{57,58}
- (2) Diatoms do indeed make oil.
- (3) Agriculturists claim that diatoms make 10 times as much oil per hectare as oil seeds,⁵⁹ with theoretical estimates reaching 200 times.^{21,23}
- (4) Therefore, sustainable energy could be made from diatoms.
- (5) We may be able to get diatoms to secrete their oil, perhaps even as gasoline, and therefore milk them, as we do cows.

We will review the evidence for these statements that is in the public domain. While some companies are forming to produce oil from unspecified “algae”,^{60,61} diatoms have received scant mention,^{62,63} except by some hobbyists.⁶⁴ What we leave for a future study is a critical comparison of diatoms with other energy alternatives, such as nondiatom algae (cf. refs 23 and 65), other biofuels, solar, wind, tidal, geothermal, hydrogen, hydroelectric and nuclear power. It is possible that, unlike crop biofuels, diatoms would not compete with food crops⁶⁶ for arable land.

Clearly, if diatoms could be used to make gasoline, then we could continue using our gasoline-based motor vehicles without a major change in technology or our way of life. The private automobile becomes a sustainable proposition. We could continue to use the combustion engine, which would then remain a major competitor to other propulsion technologies. It sounds like an easy resolution to the current situation, a way to “have our cake and eat it too”. Thus, in this regard, diatoms are worthy of serious consideration. Let us see what we find, beyond a recent review:

*“The characteristics of algal oil are similar to those of fish and vegetable oils, and can thus be considered as potential substitutes for the product of fossil oil. In the late 1940s, lipid fractions as high as 70–85% on a dry weight basis [were] ... reported in microalgae Nevertheless, only a few authors have reported lipid valorisation as biodiesel using diatoms with *Hantzschia DI-60*⁶⁷ and with *Chaetoceros muelleri*.⁶⁸ A maximum yield of 400 mg total lipid L⁻¹ in nitrogen-replete cultures was obtained. In the aim to produce biodiesel from microalgae, *Cyclotella cryptica* and *Navicula saprophila* were genetically manipulated²⁵ to optimise lipid production”.*⁶⁹

The optimization of resource production via genetic manipulation seems to be appropriate for making diatom biotechnology viable and economically lucrative.

Does Crude Oil Come from Diatoms?

The recent genetic⁷⁰ and sedimentary⁷¹ evidence suggests an origin of diatoms in the early Jurassic period, 185 million years ago.⁷⁰ Medlin et al. have suggested that their origin may be related to the end-Permian mass extinction, after which many marine niches were opened.⁷²

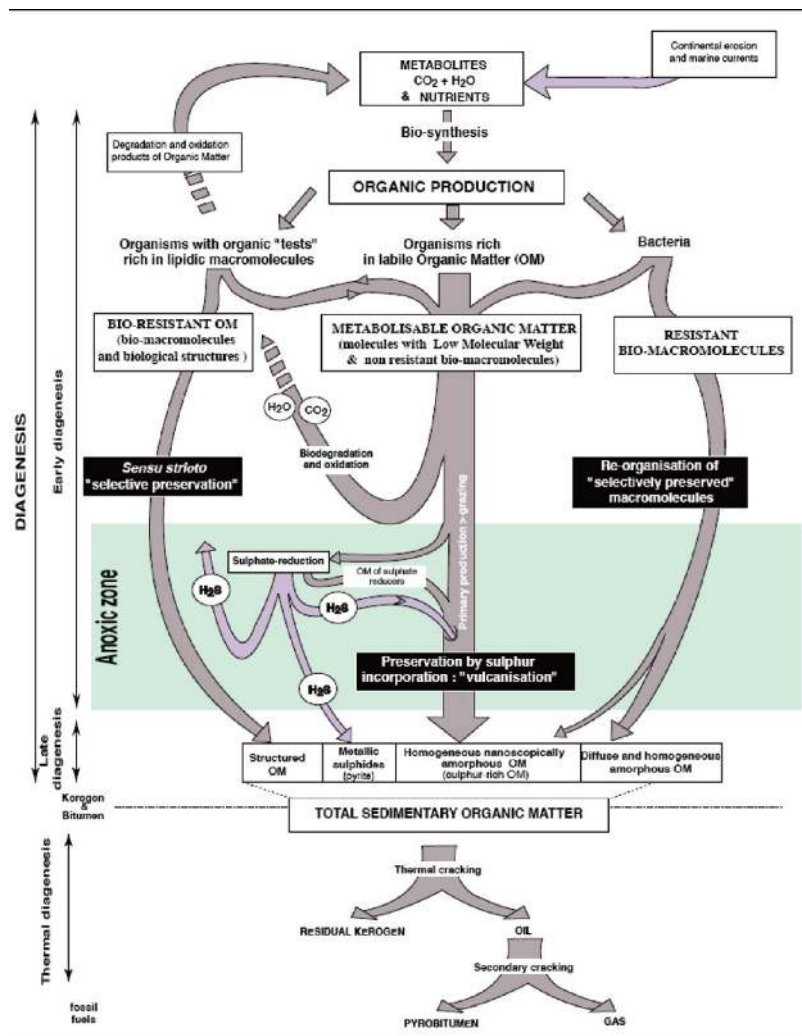


Figure 3. Diagrammed fossilization model for organic matter of petroleum source rocks. The “organisms rich in labile organic matter” are primarily diatoms. (Reprinted, with permission, from ref 399. Copyright 1997, Elsevier, Amsterdam, The Netherlands.)

The basic line of reasoning of geologists in attributing crude oil to diatoms is that they comprise the bulk of the ocean phytoplankton, so they must be a major source of the oil.^{73–75} This view must be tempered by a comparison of the geological record of diatoms with the estimated ages of oil deposits. Clearly, those that preceded diatoms could not have been generated by them,¹ and, indeed, a wide variety of prehistoric organisms are suggested as sources of crude oil.⁷ The pattern of fatty acids in recent sediments matches that of diatoms,⁷⁶ and the 24-norcholestane biomarkers are indicators of diatom-formed oil,^{77,78} as are many others.^{79–84}

The notion that diatoms are major contributors to crude oil deposits has been around since 1839,^{85–90} with evidence put forth⁹¹ and then denied.⁹² The upshot is that this period left us with uncertainty in the matter:

“The abundance of diatoms in parts of the Monterey formation has suggested that these organisms may have been the source of much of the oil of the formation. Tolman⁸⁹ has emphasized this view, and he had the charge of a research project of the American Petroleum Institute for the investigation of diatoms as a source of oil, but a final report on this work has not appeared.”⁹³

However, recent work seems to have firmly reinstated this hypothesis, based on the organic molecules (biomarkers) with

over 20 C atoms that are often unique to diatoms.^{76–84,94,95} Nevertheless, this may not account for the bulk of diatom oil, because many of these molecules may be membrane components,^{95,96} rather than the bulk fluid inside intracellular oil droplets. Indeed, “storage components, such as neutral lipids, and membrane-associated structural components, such as glycolipids and phospholipids, varied independently”.⁹⁷ Stratigraphic correlations of oil with diatoms are strong:⁹⁸

“... diatomaceous sediments may themselves be important sources for petroleum,^{99,58} and there is now an “overwhelming consensus in the literature that the OM [organic matter] in the biosiliceous unit of the MF [Monterey Formation of California] is of marine origin”.¹⁰⁰

Despite all the evidence that diatoms are major contributors to crude oil formation, doubts continue with language such as: “A number of organic-rich sediments occur in the sedimentary record which contain abundant organic matter presumed to be of diatom origin”.¹⁰¹ The root cause of this has been attributed to authoritarian pronouncements,⁸⁹ which may continue through our education system. On the other hand, a definitive story that both makes a definitive analysis of the oil in diatoms and evaluates the contribution of diatom oil, modified or not, to the worldwide petroleum reserves, apparently has not been written yet, although diatom biomarkers (24-norcholestanes) have been

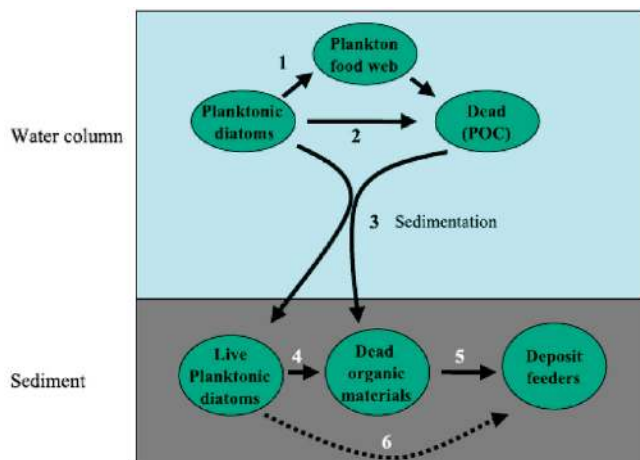


Figure 4. Schematic diagram of the role of planktonic diatoms in the benthic-pelagic coupling during the spring blooms. Major pathways and transport routes of diatom carbon to benthic deposit feeders are marked with solid lines. Processes marked with numbers: (1) Primary production by planktonic diatoms during the spring bloom is partly grazed in planktonic food web and turned into dead particulate organic carbon (POC). (2) Part of the diatoms die without being grazed. (3) Sedimentation of aggregates of live diatoms and miscellaneous dead POC add material to the pool of live diatoms and to a pool of dead labile detritus in the sediment. (4) Diatoms gradually decay, thereby becoming a part of the pool of dead material. (5) Benthic deposit feeder utilizes the pool of dead organic material. (6) Direct utilization of live planktonic diatoms by deposit feeders is questioned. (Reprinted, with permission, from ref 126. Copyright 2004, Elsevier, Amsterdam, The Netherlands.)

found in 109 crude oil basins.⁷⁷ Two recent models for the role of diatoms in producing sedimentary organic matter are shown in Figures 3 and 4, the latter of which indicates live diatoms in the sediment. What should be added to these flow diagrams is the upwelling^{102–104} that leads to “the return of favorable conditions” for germination of the spores,¹⁰⁵ perhaps triggered by changes in day length,¹⁰³ at least of neritic diatoms. Also missing are the obligate benthic diatoms¹⁰⁶ and the possibility that some sinking diatoms or spores stop at a pycnocline.¹⁰⁷

Perhaps the best “smoking gun” is the presence of oil inside fossil diatoms in sediments (see Figure 5):

“Based on experimental correlations between fluorescence microspectrometry and crude oils of known gross chemical composition (i.e., saturates, aromatics and resin-asphaltenes¹⁰⁸), the ‘pure’ diatom oil contains an estimated 60–70% saturates. The diatom oils are likely to be rich in fatty acids, which during early diagenesis, reportedly transform into condensed lipids.”^{101,109,110}

Nevertheless, the sedimentation of organic matter with diatom valves may be species- and season-dependent.¹¹¹ In any case, to date, no one has directly compared the oil in droplets inside fossil versus living diatoms, to determine how much diagenetic change, if any, has occurred. This could be tested in the laboratory, where diagenesis of the silica occurs within two years.¹¹²

Some of the contradictions may be resolved by noting that, although much of the organic material is reprocessed as it sediments down through the water column,^{113,114} “... most of the unsaturated [fatty] acids will be deposited directly into the sediment when the diatoms die, and hence the acids will largely escape the aerobic conditions at the surface. Preservation is then aided by the anaerobic conditions below the surface.”⁷⁶ Approximately 1% of the living diatoms in the ocean contribute to the sediment below, where the frustules travel downward live¹¹⁵ and land mostly intact.¹¹⁶ The freshest arrivals^{117,118}

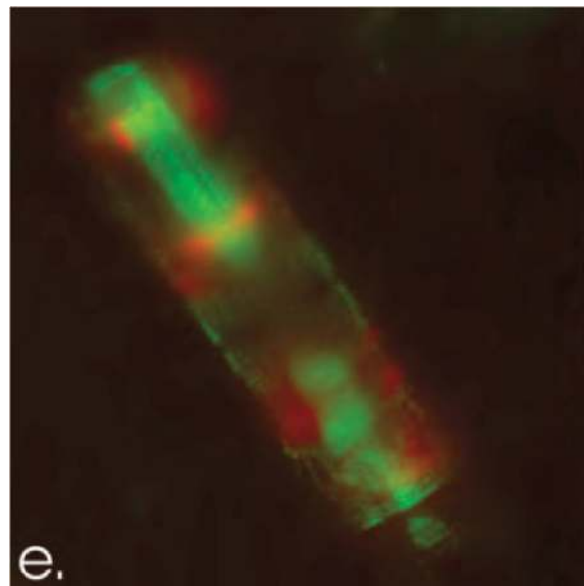


Figure 5. Intense red-fluorescing chlorophyllite [fossil chlorophyll] preserved in association with green fluorescent diatom oils directly within diatom frustule [width 10 μm]; imaged using a scan of 363 nm excitation, with 510 ± 40 nm, 570 nm, and then >665 nm emission. (Reprinted, with permission, from ref 110. Copyright 2001, Elsevier, Amsterdam, The Netherlands.)

include the cell contents, protected by two valves, consisting of live vegetative and spore cells (recall Figure 4). Perhaps even dead diatoms protect their oil contents from bacteria. Despite the darkness of the benthic zone, “... diatoms to a much greater extent than other phytoplankton groups escape degradation by heterotrophs [and] ... to a greater extent than other groups sink out of the productive zone to the bottom ... particularly ... in shallow boreal areas.”¹¹⁹ After a “bloom, a large proportion of diatoms sink to the benthos” as spores¹²⁰ (although spores of some species sink more slowly than vegetative cells¹⁰⁷). Here,¹²¹ diatoms survive ingestion by pelagic deposit feeders (or maybe only diatom spores survive¹⁰⁷), which, perhaps, is testament to their mechanical strength,¹²² and “... some cells survive several years^{123–125,126} especially at low temperatures.¹²⁷ As the sediment piles up on the diatoms, the pressure and time gradually transform the silica¹²⁸ until the diatom structure is no longer recognizable.^{116,129,130} However, the record survival time under the accumulating sediment has not yet been established:

“In freshwater systems, Aulacoseira granulata resting cells that had been in anoxic sediments for 20 years were still able to germinate,¹³¹ and other species have been found to survive in sediments from one to many decades.^{132,133} [cf. ref 134]. Marine species have been germinated from depths of up to 50 m in sediments,¹³⁵ but the amount of time these cells had been buried is uncertain.”¹³⁶

These long-term survivors rapidly form storage products upon rejuvenation¹³¹ and survive through heterotrophy.^{137,138} Some nonphotosynthetic diatoms are obligately heterotrophic.¹³⁹

The selective loss of unsaturated lipids during simulated diagenesis^{114,140} suggests that these lower-melting-point organics would best be recovered from fresh or live diatoms. In any case, the inefficiency of natural sedimentation in capturing diatom oil is apparent,¹³⁰ and if we attribute substantial crude oil deposits to diatoms, it is clear that living diatoms have even more to offer. The live diatom cells at the bottom of the sea may survive on their stored oil and/or may be heterotrophic, if they are not in suspended animation. Diatom spores contain oil

droplets (“more or larger vesicles of storage product^{107,141,136}), which is consistent with the idea that their “primary role is probably related to nutrient stress”.¹²⁷ Up to 92% of cells form spores.¹²⁷ If, as suggested,¹²⁷ the survival time of spores is inversely related to temperature, because metabolism slows, then we would propose that death occurs after the reserve of oil inside spores is depleted via respiration,¹⁰⁷ perhaps delayed by an ability of diatoms to photosynthesize efficiently at low light levels.^{106,142} Metabolism under the high pressure of a water column may also be altered.¹⁴³

Why Do Diatoms Make Oil Droplets?

It would have seemed obvious that the function of oil droplets in diatoms would be first and foremost to counter the weight of the dense silica shell (for Si/C mole ratios up to 0.81¹⁴⁴) and provide buoyancy:

*“The specific gravity of diatoms may also be lessened by the presence of an oil in the cells. Oil has been noted to accumulate in diatoms late in the vegetative period and, in addition to providing a food reserve, may also result in keeping them afloat while waiting return of conditions favorable for multiplication”.*¹⁴⁵

However, this simple hypothesis is not the prevailing one for buoyancy, which suggests that ionic pumps instead somehow maintain buoyancy.^{146,147} Apart from that, extracellular secretions and the incorporation of the diatom cells in suspended organic matter may govern their buoyancy. Earlier, there were explanations invoking the importance of the carbohydrate pool as reserve products in light dark cycles.¹⁴⁸

Indeed, nitrate depletion, which causes diatoms to increase oil production,^{149–152} changes one species, *Thalassiosira weissflogii*, from neutrally buoyant to sinking,¹⁵³ and another to enter a faster sinking sexual phase,¹⁵⁴ so the correlation between oil and buoyancy is not so clear. Therefore, while auxospore formation is correlated with oil production,^{110,155} some auxospore sinks nevertheless.^{146,154} It may be that some diatoms respond by crenation¹⁴⁶ and others by oil droplet formation, or both occur, during auxospore formation. The problem here is that the oil content has never been quantified and compared to the mass and density of the other components (silica shell, cytoplasm, nucleus, vacuoles, and attached organic coat and extracellular material) for any diatom.

The puzzle was stated succinctly 4 decades ago, and it still seems to be unsolved:

*“The nature of the problem is easily grasped. The specific weight of a diatom test [silica shell] has been reported to be 2.07, and that of protoplasm to range from 1.02 to 1.15.¹⁵⁶ The specific weight of seawater usually ranges from 1.020 to 1.028. It is evident that diatoms are heavier than seawater and will ordinarily sink rapidly unless adaptations to minimize the high frustule [shell] and protoplasmic weight load occur... Our knowledge of diatom sinking rates is still inadequate to evaluate critically the various theories of adaptive flotation mechanisms possessed by diatoms”.*¹⁵⁷

The mean density of a diatom is the weighted sum of the densities of its components, where the mathematical weights are the volumes of the components. Whether it floats or sinks is dependent only on the difference of this mean density from that of the surrounding water (unless it is attached to material of a different buoyancy). Large marine diatoms can be positively or negatively buoyant.^{158–161} Although shape, including spines and colony formation, can influence the rate of sinking, these

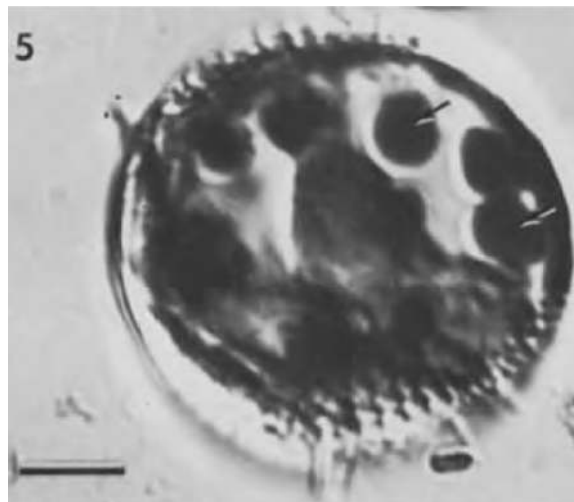


Figure 6. Light micrograph showing a *Thalassiosira antarctica* var. *antarctica* resting spore. This section is roughly parallel to the plane of the valve. Lipid inclusions are shown; two are marked with arrows. Scale bar = 5 μm . (Reprinted, with permission, from ref 172. Copyright 1985, Springer, Berlin, Germany.)

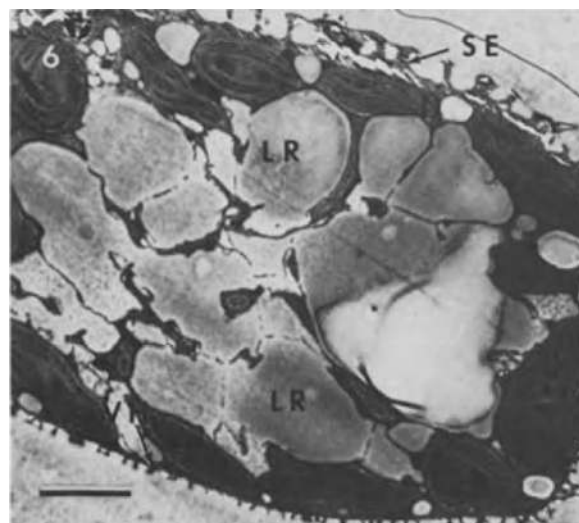


Figure 7. Transmission electron microscopy (TEM) micrograph of *Thalassiosira antarctica* var. *antarctica* with one vegetative valve (bottom, VH = vegetative hypotheca) and one spore valve (SE = spore epitheca). This TEM micrograph of a section shows “extensive lipid reserves (LR) ... accumulation of lipid reserves can occur before completion of spore formation”. Scale bar = 2 μm . (Reprinted, with permission, from ref 172. Copyright 1985, Springer, Berlin, Germany.)

features have no influence whatsoever on the direction (sink or float), unless they ensnare material of different mean density.

Again, one would suppose that the oil in diatoms might be a food store. However:

*“Catabolism rate of lipids does not seem to be very important: 1 to 2% h^{-1} , respectively, for the light and dark periods. On the other hand, significantly higher rates of catabolism of polysaccharides are found for both the light [20% h^{-1}] and dark periods [8% h^{-1}]. This ... suggests that reserve products of this diatom population are mainly composed of polysaccharides”.*¹⁶²

Perhaps the correct conclusion is that oil droplets assist the long-term survival of poor environmental conditions, whereas polysaccharides are for daily fare. However, polysaccharides are also secreted for microenvironment alteration,^{163,164} adhesion,¹⁶⁵ colony support,^{166,167} motility,^{168–170} and possibly

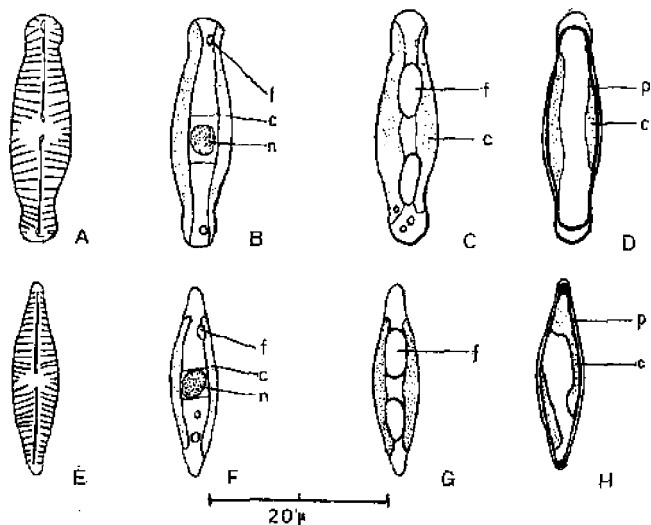


Figure 8. Sketches of diatom oil droplets or fat globules (denoted as “f”) in diatoms: (A–D) *Pinnularia biceps f. minutissima* and (E–H) *Navicula cryptocephala*. The oil droplets are small in fresh diatoms (B,F) but enlarge on desiccation (C,G), perhaps resulting in a “peripheral fatty layer” (denoted as “p”) surrounding the cytoplasm (denoted as “c”) and a vacuole. (Reprinted, with permission, from ref 297. Copyright 1960, Kluwer Academic Publishers, Dordrecht, The Netherlands.), accessed October 2004.)



Figure 9. A pennate diatom, *Navicula sp.*, showing an oil droplet. (Photograph reproduced with permission; courtesy of Charles J. O’Kelly, http://www.bigelow.org/srs/okelly.html, accessed October 2004.)

antiviral activity.¹⁷¹ While spores generate their own substantial quantities of oil (see Figures 6 and 7),¹⁷² so do some vegetative cells (see Figures 8 and 9), with “the oil droplets comprising most of the cell volume”.¹⁷³

However, the “reason” an organism does something can be quite different from our initial guesses:

“Laboratory studies showed that polar diatoms ... contain high levels of polyunsaturated fatty acids, which may help to maintain membrane fluidity at low temperatures. Preliminary data suggest that they react to changes in temperature by changing their fatty acid profiles (Lange, unpublished observations) ...”.¹⁷⁴

Finally, we must keep in mind that oil production is far more ancient than diatoms:

“All plastid-containing organisms examined so far carry out *de novo* biosynthesis of fatty acids within the plastid via the type II fatty acyl synthase.¹⁷⁵ Diatoms can contain large proportions of polyunsaturated fatty acids. Armbrust *et al.*¹⁷⁶ identified a complete pathway for polyunsaturated fatty acid biosynthesis. After export from the plastid, lipid or oil droplets often accumulate in the vacuoles or cytoplasm under nutrient-limited conditions”.¹⁷⁴

What Do We Know about Diatom Oil?

Even if diatoms could produce enough oil¹⁷⁷ to satiate our drive for transportation, is it the right type of oil, or would it require much chemical processing before we would want to burn it? Microalgae generally are a potential source of energy, because the energy-rich storage lipid product of these plants is among the more useful natural products for conversion to alternate energy such as gasoline and diesel fuel,¹⁷⁸ besides being extremely efficient biomass producers with high photo-synthetic efficiencies of 12%–16% and possessing often 50%–60% of their biomass weight in the form of lipids.^{178–182} Lipid fractions as high as 70%–85% (on a dry weight basis) in microalgae have been reported.¹⁷⁷

Certain microalgae cultures could result in higher quantities of storage lipids, such as triglycerides.¹⁸³ Diatoms have been regarded as useful neutral lipid sources of liquid-fuel precursors.¹⁸⁴ Besides high lipid and fatty acid content, there is an abundance of eicosapentaenoic acid (polyunsaturated fatty acids (PUFAs)) in diatoms.¹⁸⁵ *Nitzschia laevis* is a potential producer of eicosapentaenoic acid, as shown by extracting the lipid and analyzing it via thin layer chromatography (TLC) and gas chromatography (GC).¹⁸⁶ The lipids present are neutral lipids (accounting for ~75%), glycolipids, and phospholipids (see Figure 10). Fatty acids that dominate the organisms include tetradecanoic acid, hexadecanoic acid, and palmiloteic acid.¹⁸⁰

Diatoms of genera *Haslea* and *Rhizosolenia* biosynthesize a series of C₂₅ highly branched isoprenoid (HBI) alkenes or haslenes, which evolved ~90–146 million years ago in the Cretaceous period.^{187–189} Isoprenoid-derived alkenes and alkanes are important fuel sources that are found in a wide variety of recent and ancient sediments and in some crude oils.^{94,190} Many of the C₂₅ (haslene) and C₃₀ (rhizene) alkenes are biosynthesized by a restricted number of diatom genera, particularly some species of *Haslea*, *Rhizosolenia*, *Pleurosigma*, and *Navicula*.^{187,190–194} In *H. ostrearia*, highly branched isoprenoid alkene (haslene) biosynthesis proceeds even under axenic conditions, indicating *de novo* biosynthesis.¹⁹⁵ Interestingly, HBI biosynthesis seems to have evolved at least twice in geological time in separate diatom genera.¹⁹⁰ The C₂₆ 24-norcholestanes⁷⁷ mark an increase in diatoms in the Oligocene–Miocene.⁷⁸ The spectrum of fatty acid and related molecules reported in diatoms is given in Table 1.

How Much Oil Do Diatoms Produce?

Diatoms would seem to fare, in average dry weight that they can synthesize as lipids, only a little better than green algae

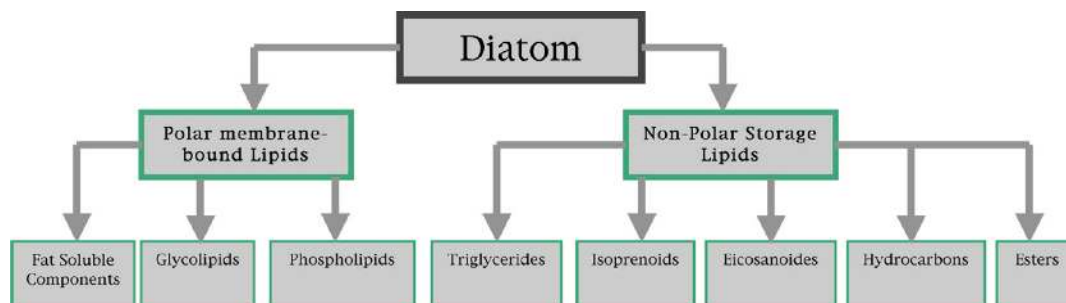


Figure 10. Classification of diatom lipids.

(24.5% vs 17.1%), although their average dry weight is enhanced by a factor of 2 to 3 by nitrogen deprivation.⁶⁵ However, the dry weight of silica in diatoms averages 60%.²²⁷ Therefore, the corrected dry weight percentage of organic material in diatoms that is lipids is 24.5%/40% = 61%. This compares favorably to the record-holding nonsiliceous alga *Botryococcus braunii* (70%).⁶⁵ If we take the “stressed” lipid content of 44.6%, we end up with the apparent contradiction of >100% lipid organic matter,²³ which means that we cannot compute using cross-species averages. Nevertheless, this suggests that if we discount the silica shells, some diatoms may be far more productive for oil than other organisms.

Let us compare these calculations to actual observations:

*“Living diatom plants will always be found to contain from two to ten shining oil globules. The bulk of this oil, in proportion to the size of the diatom, rarely falls below 5 percent; and the author has samples of diatom material in which a careful measurement of the contained oil shows a proportion of 50 percent”.*²²⁸

*“... some diatoms... are known to produce anomalous oils and fats during periods of high nutrient availability (e.g., 37% lipids^{229,230}), apparently after bloom growth and reproduction have ceased”.*¹¹⁰

Oil droplets with diameters of 100–200 μm have been observed in and near chloroplasts in some diatoms, which suggests that they are synthesized within the chloroplasts.^{26,231–233} In the stationary phase, because of nitrogen depletion (but cf. ref 234) “the oil droplets could be clearly seen filling nearly the whole cell”, whereas the resumption of exponential growth led to a reduction in fats.¹⁵⁰ The source of nitrogen may matter, because, for *Ditylum brightwellii*, growth in the nitrate almost doubled lipid production, compared to ammonia, perhaps because “The most striking aspect was the use of nearly all ammonia before any nitrate was assimilated”.²³⁵ Perhaps nitrate without ammonia present is a trigger for impending nitrogen depletion? On the other hand, a “... dramatic synthesis of lipid in the waters around Antarctica ... [with] no evidence for nitrogen deficiency” has been observed.²³⁶ The factors involved may be decreased temperature and irradiance, accompanied by increased salinity,^{172,236,237} i.e., there may be multiple stimuli to lipid synthesis. Combining all these ups and downs of oil production versus nitrogen supply into a rational physiology is a task for the future:

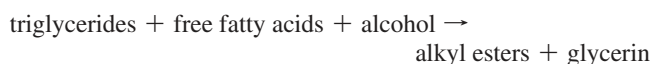
*“A major research challenge is to conceptually integrate photorespiratory metabolism in diatoms into the framework of overall cellular energy balance, stress management, and C and N status and turnover”.*²³⁸

Industrial Processing of Diatom Oil

Lipid valorization as biodiesel using diatoms was reported with *Hantzschia* DI-60⁶⁷ and *Chaetoceros muelleri*.⁶⁸ The

production of fuel (diesel, gasoline) through the transesterification and catalytic cracking of lipids accumulated in algal cells has been reported,²³⁹ including diatoms.²⁸ The main raw material for diatom-based biodiesel is the enormous range of triglycerides (monoglycerides, diglycerides, and triglycerides), which are indeed compounds of fatty acids and glycerol. In the transesterification process, an alcohol (such as methanol) reacts with the triglyceride oils that are contained in diatom fats, forming fatty acid alkyl esters (biodiesel) and glycerin.⁶⁹

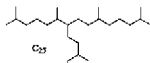
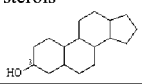
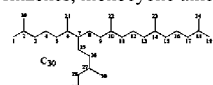
Fuel oil formation can be achieved by base-catalyzed transesterification, acid-catalyzed transesterification (with simultaneous esterification of free fatty acids), and noncatalytic conversion via transesterification and esterification under supercritical alcohol conditions.^{240–243} The main process that can be used for biodiesel production from organisms is base-catalyzed transesterification.²⁴⁴ The reaction requires heat and a strong base catalyst, such as sodium hydroxide or potassium hydroxide. The simplified transesterification reaction is depicted in Figure 11:



This chemical process converts the triglycerides (TGs) found in plants, microalgae, and animal fats to fatty acid methyl esters (FAMES) in a multistep synthesis, with glycerol being liberated as a byproduct.^{240,245} More recently, it has been demonstrated that supercritical alcohol^{246,247} can esterify free fatty acids and transesterify triglycerides simultaneously with virtually no sensitivity to water content. A major advantage is that simultaneous esterification and transesterification makes use of diatoms, with elevated levels of free fatty acids,^{248,249} in less time.

Liquefaction of algal diatom biomass can be accomplished by natural, direct and indirect thermal, and fermentation methods. Liquid hydrocarbon yields of close to 50% have been obtained using a very-high-temperature, high-pressure, catalyzed hydrogenation process. Reacting organic diatom biomass with near-critical (320–390 $^{\circ}\text{C}$, 200–420 bar) or supercritical (400–500 $^{\circ}\text{C}$, 400–550 bar) aqueous phases can altogether transform the organic compounds over short time periods (time frames from minutes to hours). The reductive process is conducted under anaerobic or near-anaerobic conditions. The lipids can be converted to a hydrocarbon mixture that is similar to crude petroleum, along with volatile alkane and alkene gases (C_2 – C_5). This conversion allows the generation of a burnable fuel of very high calorific value.²⁵⁰ Kerogens are converted to an oily substance under conditions of high pressure and temperature.^{251–253} Besides *n*-alkanes, aromatics, naphthenes, and alkyl benzenes⁵ were found in the hydrocarbons produced from kerogen. The

Table 1. Fatty Acids and Related Organic Molecules Reported in Diatoms^a

# C Atoms	Sample Organic Compounds in Diatoms	References
1	methyl bromide (CH ₃ Br), methyl chloride (CH ₃ Cl)	196
2	ethane(C ₂ H ₆), ethane(C ₂ H ₄), trichloroethane(CHCl ₃), 1,1-dichloroethane(CH ₂ Cl-CH ₂ Cl), 1,2-dichloroethane (CH ₂ Cl-ClCH ₂)	196-198
3	propane(C ₃ H ₈), propene(C ₃ H ₆)	197, 198
4		
5	isoprene (2-methyl-1,3-butadiene)	196, 197, 199, 200
6	n-hexane(C ₆ H ₁₄)	197
7	heptadienal(C ₇ H ₁₂ , CH=CHCH=CHCHO)	201-203
8	octenal(C ₈ H ₁₄ O), octatrienal(C ₈ H ₁₆ O), octadienal(C ₈ H ₁₂ O)	201-204
9	9-oxo-nonadiynoic acid	201
10	decatrienal, decadienal	201, 203, 204
11	hormosirene	201
12	12-alkane [dodecanoic acid (lauric acid) 12:0], 12-oxo-dodecatrienoic acid	201, 203, 205
13		
14	fatty acid, normal olefin, 14-alkane CH ₃ (CH ₂) ₁₂ COOH	186, 203, 205-210
15	fatty acid, 15-alkane[CH ₃ (CH ₂) ₁₃ COOH (pentadecanoic acid)]	205-207, 209
16	fatty acid, normal olefin, 16-alkane[hexadecanoic acid (palmitic acid) 16:0], epoxy alcohol	186, 202-213
17	fatty acid	209
18	fatty acid, 18-alkane [octadecanoic acid (stearic acid) 18:0]	186, 203, 205, 206, 209
19		
20	fatty acid, 20-alkane [eicosanoic acid (arachidic acid) 20:0], epoxy alcohol	186, 202-206, 209, 210, 212
21	phytol (C ₂₀ H ₄₀ O)	214
22	fatty acid, 22-alkane[docosanoic acid (behenic acid) 22:0]	203, 205, 209
23		
24	fatty acid, 24-alkane[tetracosanoic acid (lignoceric acid) 24:0]	205, 209
25	highly branched isoprenoid (HBI) C ₂₅ pentaene, unsaturated haslenes, monocyclic alkene 	82, 94, 155, 194, 195, 214-224
26		
27	C ₂₇ n-polyene	221
28	normal olefin	206
29	sterols 	208
30	C ₃₀ highly branched isoprenoid (HBI) alkenes, rhizenes, monocyclic alkene, gorgosterol 	155, 214, 224-226
>30		

^a Blank lines mean no instances were found in our literature survey.

production of diesel fuel and gasoline through the transesterification and catalytic cracking of lipids accumulated in algal cells has been reported,²³⁹ as has the production of energy from diatom biomass through fermentation and also via thermochemical liquefaction.²⁵⁴

Lipids in diatoms are converted to hydrocarbons:



From this, it is evident that the direct extraction of diatom lipids is a more-efficient method for obtaining energy than fermenta-

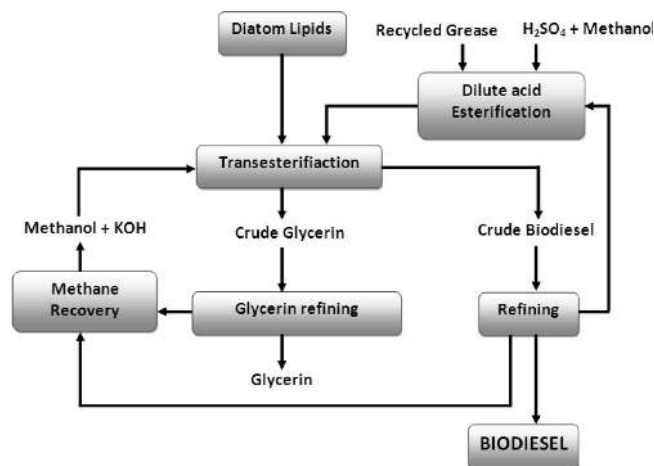


Figure 11. Process of extraction of biodiesel from diatom lipids.

tion. This can occur by having solvents such as CH₂Cl₂ (dichloromethane), through the direct expression of the liquid lipids, or a combination of both methods (see Figure 12). The thermochemical liquefaction process often results in a heavy oily or tarry material that is then separated into different fractions by catalytic cracking. As with hydrocarbons derived from other forms of renewable biomass, microalgal diatom lipids can be converted to suitable gasoline and diesel fuels through transesterification.

Environmental and Genetic Manipulation of Diatoms

Could we manipulate diatoms, by optimizing their environment²⁵⁵ or genetics, to produce the type of oil that we want? Here are a few of the factors that have already been manipulated:

- The aging of a sample of diatoms is reported to increase hydrocarbon content.²⁵⁶
 - The degree of unsaturation of C₂₅ haslenes varies with temperature.²²³
 - Growth rate or salinity can affect C₂₅ and C₃₀ production,²²⁵ and there is an optimum salinity for C₂₀.²⁵⁷
 - Diatoms kept in the dark produce more oil droplets.²⁵⁸
- However, in the wild, there is “a small loss from lipid, during the night”^{162, 259}
- Nitrogen depletion increases “fat” production,^{149,150,255} sometimes within a day.^{151,152}
 - Drying or desiccation increases oil production,^{260,261} and slower drying allows cells to survive better.²⁶¹
 - Mutants of fatty acid synthesis have been attained.²⁶²
 - Diatom species optimal for “ω-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)”²⁶³ have been selected.
 - Organic mercury and cadmium inhibit fatty acid and sterol production in diatoms.²⁶⁴

There is much variability between diatom species, such as, for instance, in their response to desiccation,²⁶¹ so the choice of optimal species for oil production is necessary.

An obligate photoautotrophic diatom has already been genetically transformed to a heterotroph.³⁷ Most, but not all, diatoms are photosynthesizers.¹³⁹ Regardless of whether one has obligatory¹³⁹ or facultative heterotrophs,^{137,138} the heterotrophic diatoms could then use other energy sources to make oil. An alternative point of view is that we may wish to use an obligate photoautotrophic diatom,³⁷ because it could possibly be engineered to avoid using its stored oil.

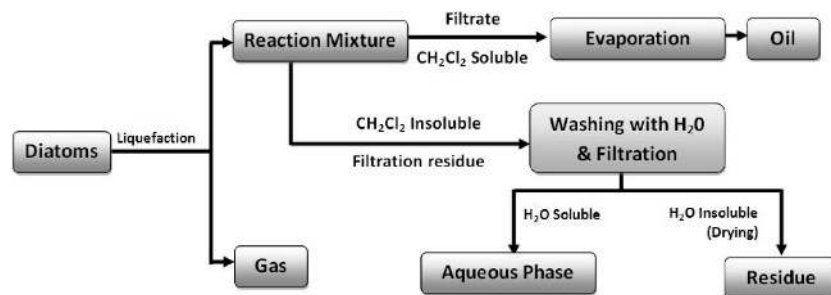


Figure 12. Thermal liquefaction accompanied by the separation of fuel oil by solvent extraction.

“You can make algae with a very high oil content and you can make algae that grows very quickly and, at the moment, no one can do both,” said Robert Trezona, R&D director at the Carbon Trust.²⁶⁵ However, the answer to this may merely be switching the diatoms from exponential growth to “stress” that induces oil formation, both of which they can do rapidly. Nevertheless, there is a caveat:

“Prior to this program [Figure 2], little work had been done to improve oil production in algal organisms. Much of the program’s research focused attention on the elusive ‘lipid trigger.’ (Lipids are another generic name for TAGs [triacylglycerols], the primary storage form of natural oils.) This ‘trigger’ refers to the observation that, under environmental stress, many microalgae appeared to flip a switch to turn on production of TAGs. Nutrient deficiency was the major factor studied. Our work with nitrogen-deficiency in algae and silicon deficiency in diatoms did not turn up any overwhelming evidence in support of this trigger theory. The common thread among the studies showing increased oil production under stress seems to be the observed cessation of cell division. While the rate of production of all cell components is lower under nutrient starvation, oil production seems to remain higher, leading to an accumulation of oil in the cells. The increased oil content of the algae does not lead to increased overall productivity of oil. In fact, overall rates of oil production are lower during periods of nutrient deficiency. Higher levels of oil in the cells are more than offset by lower rates of cell growth.”²¹

Thus, discussions so far highlight that diatoms are a potential source of oil and have the ability to grow rapidly. However, there is a need for technically sound methodologies that can serve as the infrastructure for implementing diatoms as a comprehensive source of energy in the form of oil. In the next section, a new solar panel approach that utilizes genomically modifiable aspects of diatom biology, offering the prospect of “milking” diatoms for sustainable energy, is discussed.

A Diatom Solar Panel

We do not harvest milk from cows by grinding them up and extracting the milk. Instead, we let them secrete the milk at their own pace, and selectively breed cattle and alter their environment to maximize the rate of milk secretion.^{266–269} We do not simultaneously attempt to maximize their rate of reproduction. Perhaps we could do the same with diatoms. The milking of algae has been done by solvent extraction methods that do not kill the cells,^{270,271} but in which they are otherwise passive. Here, we propose altering cells so that they actively secrete their oil droplets.

Some diatoms are tough extremophiles that reside in assorted harsh environments¹⁵ and diatoms have been raised in various

microcosms;^{272–277} therefore, it is plausible to consider confining a diatom colony to a solar panel. Unlike ordinary solar panels that produce electricity, a diatom solar panel would produce oil for us; therefore, in designing it, we would have to solve various optical and mass transport problems. We pose this here as an engineering and genomics challenge, rather than presuming to give a complete solution. Let us consider some of the questions and opportunities involved:

- Mammalian milk contains oil droplets that are exocytosed from the cells lining the milk ducts.^{278–281} It may be possible to genetically engineer diatoms so that they exocytose their oil droplets. This could lead to continuous harvesting with clean separation of the oil from the diatoms, provided by the diatoms themselves. The reverse process of the uptake of hydrocarbons from the environment has already been demonstrated,²⁸² although we do not know if it occurs via endocytosis. Exocytosis of β -carotene globules has been hypothesized as the mechanism of extraction into the biocompatible hydrophobic liquid dodecane²⁸³ from the unicellular green alga *Dunaliella*, perhaps accelerated from the natural exocytosis mechanism of this species²⁸⁴ by the presence of the dodecane.²⁸³ Higher plants have oil secretion glands,^{285–288} and diatoms already exocytose the silica contents of the silicium,^{9,289} adhesion and motility proteins, and polysaccharides,^{166,168,290,291} so the concept of secretion of oil by diatoms is not far-fetched.

- The concept of milking diatoms adds a third dimension to the inverse relationship between biomass and oil production. Therefore, the optimization of oil production²⁹² must be reconsidered. For example, a system that is closed except for oil secretion and gas exchange may be able to conserve micronutrients,²⁹³ especially if little or no net growth of cells is occurring. This might solve the productivity gap by getting 10–200 times more oil, compared to oilseed crops.^{23,59}

- With at least a boundary layer²⁹⁴ of water on the diatoms, secreted oil droplets would separate under gravity, rising to the top of a tilted panel, forming an unstable emulsion, which should progressively separate. The oil could then be removed, very similar to the cream that rises to the top of mammalian milk that has not been homogenized. The maximum size of the droplets might be limited by the diameters of the pores of the diatom valves (shells) or the width of the raphes.¹⁶⁶ It may prove wise to keep diatom oil in its natural droplet form, rather than produce a bulk liquid, because small droplets increase energy efficiency.^{295,296}

- Diatoms require water, or at least high humidity.²⁹⁷ Thus, we either need a water-impermeable chamber or a source of additional water for the panel. Diatoms can survive desiccation, and, indeed, drying or desiccation increases oil production.^{260,261} Because slower drying allows cells to survive better,²⁶¹ one could surmise that this gives time for more oil production: “fat-

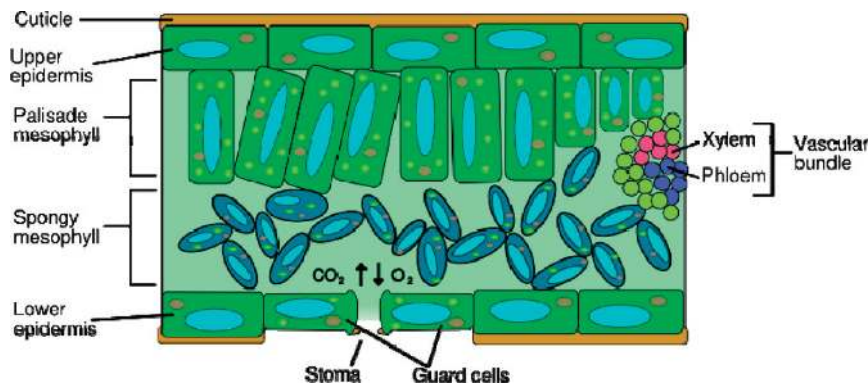


Figure 13. Anatomy of an angiosperm leaf. Diatoms could possibly occupy the air space and/or replace palisade or spongy mesophyll cells. Such an arrangement might provide gas exchange, temperature, and humidity control. (Image reproduced with permission, under the Creative Commons Attribution ShareAlike 2.5 License, from ref 400. Copyright 2006, H. McKenna.)

containing cells are more likely to survive drying than fat-free cells".²⁹⁷ Thus, wet/dry cycling might be a key to oil production.

- If we decide to keep the water in while allowing the entrance of CO₂, then we either must use real stomata or design a replacement for them. After all, a solar panel with live diatoms inside (cf. refs 298 and 299) is not too different in structure and function from a plant leaf (see Figure 13), from which we could learn much.

- Of course, many leaves have air spaces within them; therefore, contrary to the usual practice of growing diatoms immersed in water,³⁰⁰ we might find it better to grow them on surfaces in air with high humidity. This also might enhance gas exchange.

- We could go one step further and use actual leaves to support diatoms inside in their internal air spaces, similar to the relationship between a blue green alga (cyanobacterium) *Anabaena* and the plant *Azolla*.³⁰¹ The leaf would then provide and control water and gas exchange, temperature control, and directional control of light by solar tracking. Perhaps diatoms could substitute for the palisade mesophyll and/or spongy mesophyll (see Figure 13). Diatoms have entered naturally into many associations and symbioses,^{18,302–316} so the creation of such a new angiosperm leaf–diatom endophytic symbiosis may prove to be possible.

- Of course, solar panels are subject to solar heating. This could be alleviated as a problem by starting with thermophilic diatoms, some of which have been reported to exist at temperatures up to 76 °C.^{317–324} Temperature ranges for growth and photosynthesis of diatoms have only occasionally been investigated,³²⁵ let alone for oil production.

- Can we take advantage of the optical properties of diatoms and diatom colonies¹⁸ to increase the photosynthetic yield of oil? The delivery of light to algal cultures has been addressed in a variety of ways, including fiber optics^{326–328} and immersion optics,³²⁹ however, in all cases, the algae have been passively involved. Diatoms may make use of natural light pipes, such as sponges do with spicules,³³⁰ and colonial diatoms that are attached to each other in chains via silica or polysaccharide linkages¹⁸ may share the light by having the entire colony acting as a light pipe.⁹ In this case, we would be utilizing the multiscale optics of the diatoms themselves,³³¹ including their ability to focus²⁰ and redistribute³³² light, which may partially explain why diatoms photosynthesize efficiently at low light levels.¹⁴² Diatoms can probably be oriented on grooved surfaces,³³³ so that the deliberate optimization of light delivery may be achievable. The engineering of three-dimensional tissue scaffolds^{334–338} may help contribute to optimizing the growth

of adherent diatoms. Microfluidic scaffolds³³⁹ could be designed to deliver time-cycled nutrients and perhaps remove oil.

- Some motile colonial diatoms build their own tubular scaffolds, within which they orient themselves,^{340,341} perhaps forming dynamically adaptive light pipes. We could take advantage of the phototactic motility^{342–344} of raphid diatoms, to allow them to build their own optimal, perhaps constructural branched architectures.^{15,345,346} Such self-assembled multiscale structures might simultaneously produce the optical properties needed, as well as the nutrient flow and oil removal microfluidics.

- If our diatom panel is to have diatoms attached to a surface, then a monoraphid diatom^{347,348} might be best. This would be asymmetric (an example of heterovalvy¹⁸) and we might be able to arrange oil secretion from the araphid side, because of possible polarity to the cytoskeleton.³⁴⁹

- It is generally assumed that more light means more productivity for photosynthesizing organisms; however, because diatoms flourish at low light levels,^{293,350–352} optimizing either their growth or oil production may require taking this into account. This ability, in nature, may be due to their facultative heterotrophy³⁵² or light level adaptation.³⁵³ Low light may imitate conditions of nitrogen deprivation³⁵⁴ and therefore improve the amount of oil per cell. On the other hand, we might want to select for diatoms that lack heterotrophic ability, if that ability uses part of the oil that they produce.

- At the other extreme, excess and fluctuating light leads to stress and photoprotection reactions in diatoms;^{355,356} however, the relation of the “rapid (seconds to tens of minutes) regulation of a switch from a light harvesting to a photoprotecting state”,¹⁷⁴ to the postulated “switch” to oil production²¹ seems unexplored. A full inquiry into the existence (and, if so, mechanism) of the “oil trigger”²¹ is long overdue.

Direct Production of Gasoline by Diatoms

Low-molecular-weight C₂–C₆ hydrocarbons in diatoms unfortunately are treated only in a single report.¹⁹⁷

“Laboratory experiments have been carried out¹⁹⁷ in order to assess and quantify the role of marine phytoplankton in the production of nonmethane hydrocarbons. They provided evidence that supports the hypothesis that some short-chain hydrocarbons are produced during diatom and dinoflagellate lifecycles. Their results suggest that ethane, ethene, propane and propene are produced during the autolysis of some phytoplankton, possibly by the oxidation of polyunsaturated lipids released into their culture medium. In contrast, isoprene and hexane appear during phytoplankton growth and are thus most likely

produced either directly by the plankton or through the oxidation of exuded dissolved organic carbon. *Studies*³⁵⁷ suggested that ethylene produced in estuarine water is produced biotically by phytoplankton and abiotically by the photolysis of polyunsaturated lipids in particulate and dissolved organic matter. They predicted that high concentrations of ethylene are produced in areas with high primary productivity.³⁵⁸

This is supported by observations of C₂–C₄ alkanes in ocean surface waters,³⁵⁹ along with isoprene (C₅), also attributed to algae³⁶⁰ (mostly diatoms), and direct laboratory observation of isoprene production by diatoms.¹⁹⁹

In the range of C₇–C₁₂, ~1/3 of the tested diatom strains produced α,β,γ,δ-unsaturated aldehydes.²⁰¹ With some optimism about the power of systems biology and how malleable microalgae might be,³⁶¹ perhaps we could engineer diatoms that would make these compounds, or the lower-molecular-weight alkanes and alkenes, in great quantities. The biochemical pathways of these polyunsaturated aldehydes are being worked out,^{203,204} and their production correlates with the onset of culture “decline”,³⁶² giving us another possible insight (cf. ref 363) into the long sought “switch” to oil production.²¹ Thus, again, “stress” plays a role,^{363,364} especially mechanical damage,³⁶⁵ which is imitated in the laboratory by sonication:²⁰⁴

“...LOX [lipoxygenase] activation in marine diatoms triggers a multiphase mechanism that is responsible for the synthesis of at least two classes of proapoptotic and teratogenic products, FAHs [fatty acid hydroperoxides] and hROS [highly reactive oxygen species], which can mimic the toxic effects of the microalgae on grazer copepods.”²⁰²

“Although the effects of such toxins are less dramatic than those inducing direct poisoning and death of predators, they are nonetheless insidious, as they affect the future generation of grazers, inducing abortions, birth defects and reduced larval survivorship [though there is more to this story³⁶⁶].”³⁶⁷ ...

“... using chemical warfare [cf. ref 368] ... these ... cells produce a plethora of oxylipins [oxygenase-mediated oxygenated derivatives of fatty acids], including short-chain unsaturated aldehydes, hydroxyl-, keto-, and epoxyhydroxy fatty acid derivatives The biochemical process involved in the production of these compounds shows a simple regulation based on compartmentation and mixing of preexisting enzymes and requires hydrolysis of chloroplast-derived glycolipids to feed the downstream activities of C₁₆ and C₂₀ lipoxygenases.”³⁶⁹

Given that pathways exist for the production of many alkanes, starting with 12-alkane (see Table 1), the production of shorter alkanes within genetically manipulated diatoms might be plausible. If not, we could fall back on known organic chemistry reactions^{370,371} to convert the natural products to alkanes. Of course, we may have to select diatoms that can survive the very products that we are asking them to produce for us, but given the fact that extraction with biocompatible organic solvents works for some algae^{270,271,283,372} and diatoms can degrade petroleum hydrocarbons,³⁷³ this should be possible.

Conclusion

Despite ~170 years of research on the relationship between diatoms and crude oil, we still know very little about the oil inside diatoms itself. Therefore, we have collected some images from the literature, so we all know what we are looking for (recall Figures 1, 5, 6, 7, 8, and 9). To conduct a proper chemical analysis of the

oil inside diatom oil droplets, a method for separating out oil droplets inside diatoms from the shell and cytoplasm must be developed. Sonication, centrifugation, freezing and crushing, extraction, induced exocytosis, etc. must be attempted.

With more than 200 000 species from which to choose, and all the combinatorics of nutrient and genome manipulation, finding or creating the “best” diatom for sustainable gasoline will be quite a task. Nevertheless, some guidelines for starting species can be guessed from our survey:

- Choose planktonic diatoms with positive buoyancy^{158,374,375} or at least neutral buoyancy.^{153,235,376,377}
- Choose diatoms that harbor symbiotic nitrogen-fixing cyanobacteria,^{306,378–380} which should reduce nutrient requirements.
- Choose diatoms that have high efficiency of photon use, perhaps from those that function at low light levels.^{106,142}
- Choose diatoms that are thermophilic,^{317–324} especially for solar panels subject to solar heating.
- Consider those genera that have been demonstrated by paleogenetics to have contributed to fossil organics.³⁸¹
- For motile or sessile pennate diatoms that adhere to surfaces, buoyancy may be much less important than survival from desiccation, which seems to induce oil production.^{260,261} Therefore, the reaction of these diatoms to drying is a place to start. The reaction of oceanic planktonic species to drying has not been investigated, although one would anticipate that they have no special mechanisms for addressing this (for them) unusual situation.
- Genetic engineering of diatoms to enhance oil production has been attempted, but it has not yet been successful.^{25,30,31,382–384}
- We could use a compustat for mutation and selection of diatoms that maximize oil droplet size and/or number.^{9,385}
- Generally, cell proliferation seems to be counterproductive to oil production on a per-cell basis, which is a problem that has been expressed as an unsolved Catch-22.⁶² However, this balance may shift in our favor when we start milking diatoms for oil instead of grinding them.

The need of the hour is to develop technologies for efficient, safer, less-polluting alternative oil sources, because the present stock of fossil oil is fast dwindling and its burning has accentuated human-caused global warming, which started 8000 years ago.³⁸⁶ The mechanisms of crude oil formation by natural phenomena are now partially understood, and technology for crude oil synthesis is in the budding stage; however, because the majority of petroleum has its origins in algae, diatoms may play a vital role in sustainable oil production. The manipulation of microalgal or diatom lipid quantity and quality could be very significant and help us in effectively using this renewable resource as energy.

Energy is the prime mover for economic development. The global annual consumption of energy is now over 400 EJ³⁸⁷ and the major share (79%) comes from fossil fuels.³⁸⁸ Fossil fuels are nonrenewable resources that are used in the production of energy, and recent history shows that they have been consumed at increasing rates.³⁸⁹ Fossil fuels include coal, natural gas, and oil, and the term might be extended to methyl hydrate.³⁹⁰ During the 20th century, oil replaced coal as the dominant fossil fuel. Despite oil reserves that have been estimated to last until the year 2050,³⁹¹ the switch to an alternative energy source may occur as rapidly as the switch from the horse to the automobile³⁹² and, thus, may not take another generation. Therefore, it would seem wise to proceed with a rapid increase in biofuel use, from 0.3%³⁸⁸ to the majority, well before the oil supply is depleted, picking up from the major aborted program of the 1980–1990s²¹ (see Figure 2). On the other hand, estimates of fossil fuel reserves are “ambiguous” and “... the world consumption of oil, coal and

gas has increased over the last half century. This trend is forecast to continue to 2030³⁹³, so that if we put up with the predicted global warming, the pressure for biofuel use may prove more geopolitical than anything else. Certainly, the sale of crude oil has empowered some nations far beyond the role they would otherwise have in the world. We conclude that biofuel investment is a gamble in the short term, except for countries that are willing to eschew imported oil,³⁹⁴ despite the latter's possibly continued lower cost for a while. On the other hand, countries that master sustainable large-scale biofuel production now will be in a long-term position to sell the technology to others and ignore geopolitical pressures from fossil-oil-producing countries. "In [global oil] peaking there is certainly cause for significant concern, but not in our view for panic".³⁹⁵ We have an opportunity to plan ahead, or not. If each approach to sustainable energy sources, such as diatoms, must compete in price with fossil fuels before its proper investment occurs, we shall have to wait for the last drop of easily accessible oil to be extracted out of the ground, which is an approach to problem solving that has, for example, significantly reduced biodiversity.³⁹⁶ If we subsidize the development of sustainable energy sources for reasons other than price,³⁹⁷ there will be a gnashing of teeth and undermining of the effort by the oil-producing countries and those countries that maintain a dependence on them,³⁹⁸ however, in the long run, we will have done the right thing. Just announced work⁴⁰¹ suggests that diatoms can triple the efficiency of electrical solar panels, an efficiency that should also apply to gasoline secreting solar panels.

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