Ind. Eng. Chem. Res. **2009,** *48,* 8769–8788 **8769**

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

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In the face of increasing CO_2 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 diatom[26] (see Figure 1) has

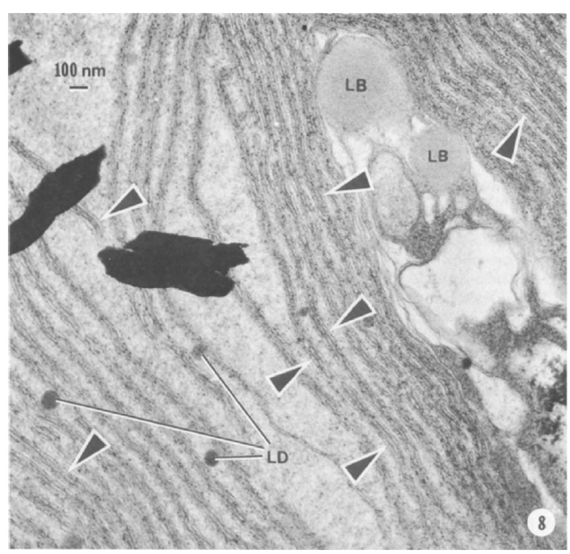


Figure 1. Lipid bodies (denoted as "LB") and lipid droplets (denoted as "LD") inside a chloroplast of the nuisance diatom *Didymosphena geminata*. Arrowheads indicate triple thylekoids. (Reprinted with permission from ref 26.)

10.1021/ie900044j CCC: $40.75
Published on Web 06/02/2009

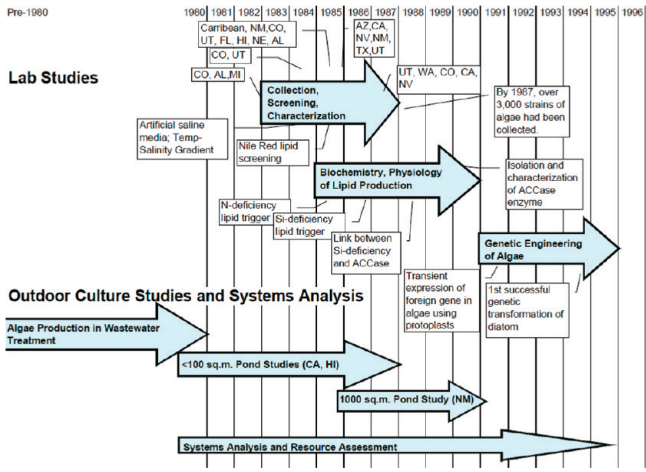


Figure 2. Partial history of attempts to use diatoms and other algae for biofuels. (Reprinted, with permission, from ref 21. Copyright 1998, National Renewable Energy Laboratory, Golden, CO.)

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 h[51] 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 C_3 photosynthesizers, and their photosynthetic efficiency is enhanced by concentrating CO_2 around Rubisco, diminishing photorespiration. It is estimated that diatoms are responsible for up to 25% of global CO_2 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 upwelling[56] 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 CO_2 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 crops[66] 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 manipulated[25] 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 genetic[70] and sedimentary[71] 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]

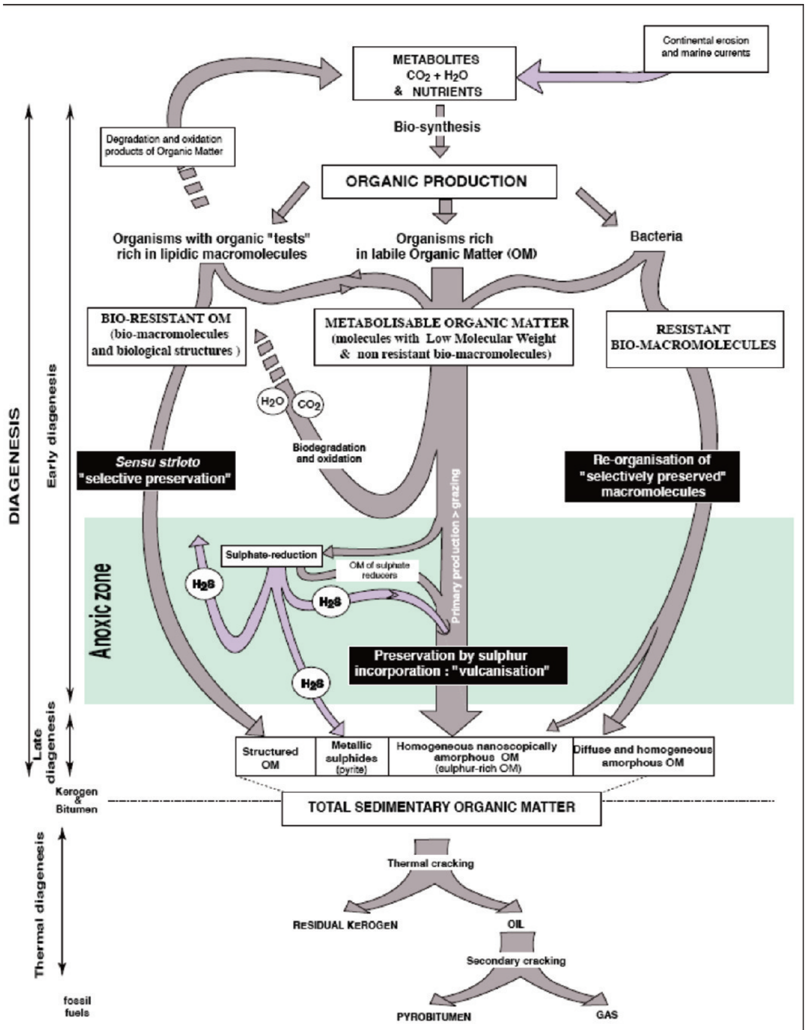


Figure 3. Diagrammed fossilization model for organic matter of petroleum source rocks. The “organisms rich in labile organic matter” are primarily diatoms. (Reprinted, with permission, from ref 399. Copyright 1997, Elsevier, Amsterdam, The Netherlands.)

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 forth[91] 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. Tolman[89] 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

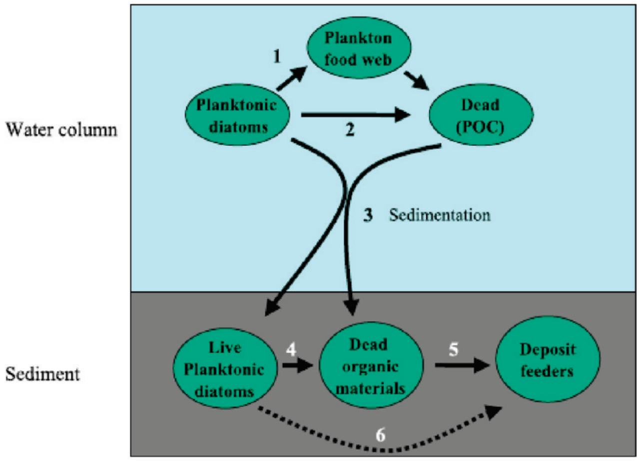


Figure 4. Schematic diagram of the role of planktonic diatoms in the benthic-pelagic coupling during the spring blooms. Major pathways and transport routes of diatom carbon to benthic deposit feeders are marked with solid lines. Processes marked with numbers: (1) Primary production by planktonic diatoms 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 diatoms[106] 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-asphaltenes[108]), 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 live[115] and land mostly intact.[116] The freshest arrivals[117,118]

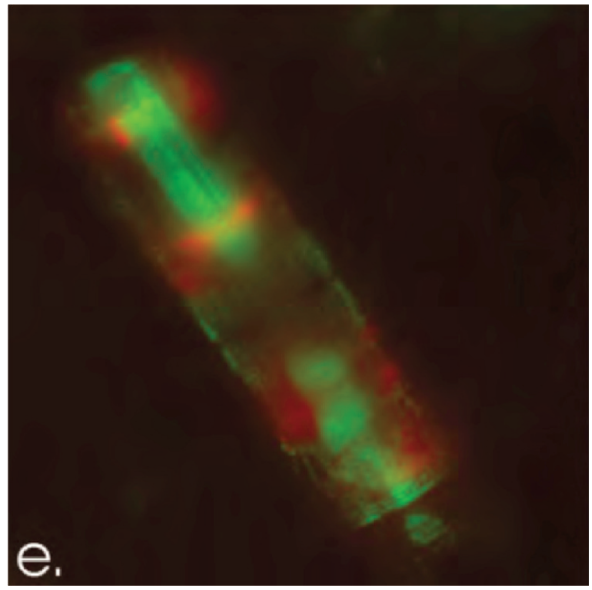


Figure 5. Intense red-fluorescing chlorophyllinite [fossil chlorophyll] preserved in association with green fluorescing diatom oils directly within diatom frustule [width 10 μm]; imaged using a scan of 363 nm excitation, with 510 ± 40 nm, 570 nm, and then >665 nm emission. (Reprinted, with permission, 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 spores[120] (although spores of some species sink more slowly than vegetative cells[107]). Here,[121] diatoms survive ingestion by pelagic deposit feeders (or maybe only diatom spores survive[107]), 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 silica[128] 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 rejuvenation[131] 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 0.81[144]) 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 crenation[146] 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 features have no influence whatsoever on the direction (sink or float), unless they ensnare material of different mean density.

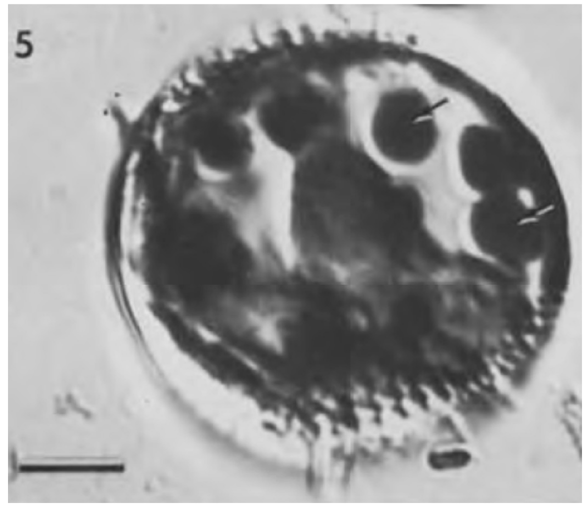


Figure 6. Light micrograph showing a *Thalassiosira antarctica* var. *antarctica* resting spore. This section is roughly parallel to the plane of the valve. Lipid inclusions are shown; two are marked with arrows. Scale bar = 5 μm. (Reprinted, with permission, from ref 172. Copyright 1985, Springer, Berlin,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

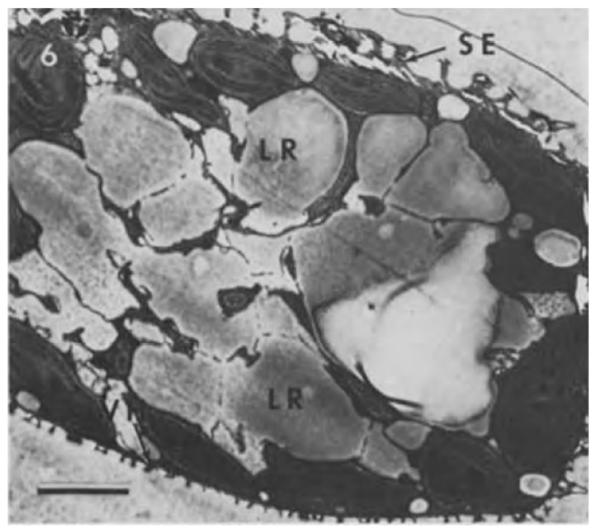


Figure 7. Transmission electron microscopy (TEM) micrograph of *Thalassiosira antarctica* var. *antarctica* with one vegetative valve (bottom, VH = vegetative hypotheca) and one spore valve (SE = spore epitheca). This TEM micrograph of a section shows "extensive lipid reserves (LR) ... accumulation of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

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

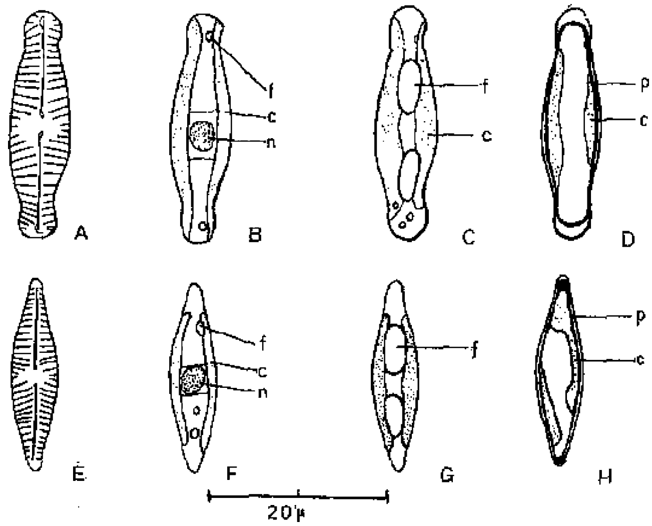


Figure 8. Sketches of diatom oil droplets or fat globules (denoted as "f") in diatoms: (A−D) *Pinnularia biceps f. minutissima* and (E−H) *Navicula cryptocephala*. The oil droplets are small in fresh diatoms (B,F) but enlarge on desiccation (C,G), perhaps resulting in a "peripheral fatty layer" (denoted 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.)

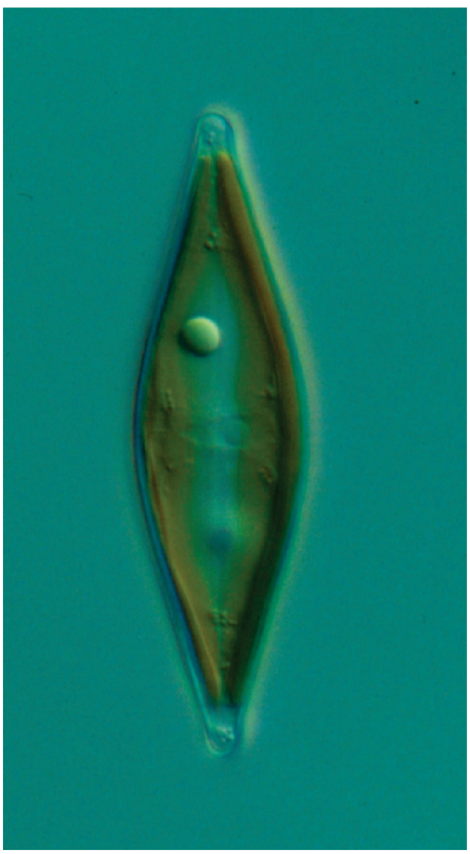


Figure 9. A pennate diatom, *Navicula sp.*, showing an oil droplet. (Photograph reproduced with permission; courtesy of Charles J. O'Kelly, http://www.bigelow.org/srs/okelly.html, accessed October 2004.)

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 oil[177] 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 photosynthetic 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 palmiloteleic acid.[180]

Diatoms of genera *Haslea* and *Rhizosolenia* biosynthesize a series of C_{25} 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 C_{25} (haslene) and C_{30} (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 C_{26} 24-norcholestanes[77] 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

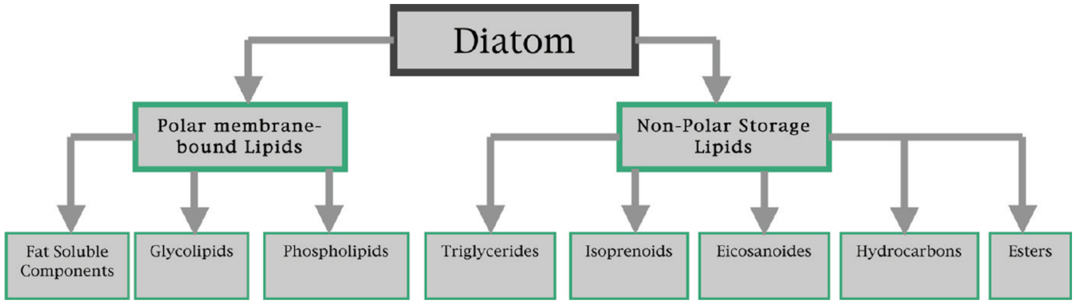


Figure 10. Classification of diatom lipids.

(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-60[67] 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]

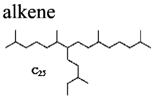
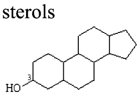
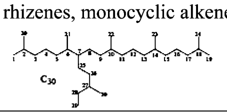
Fuel oil formation can be achieved by base-catalyzed transesterification, acid-catalyzed transesterification (with simultaneous esterification of free fatty acids), and noncatalytic conversion via transesterification and esterification under supercritical alcohol conditions.[240–243] The main process 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:

$$\text{triglycerides} + \text{free fatty acids} + \text{alcohol} \rightarrow \text{alkyl esters} + \text{glycerin}$$

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 °C, 200−420 bar) or supercritical (400−500 °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, napthenes, and alkyl benzenes[5] 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_3Br), methyl chloride (CH_3Cl)	196
2	ethane(C_2H_6), ethane(C_2H_4), trichloroethene(CHC_{l3}), 1.1-dichloroethane(CH_2C_{l2}-CH_4), 1.2-dichloroethane (CH_3Cl-$ClCH_3$)	196-198
3	propane(C_3H_8), propene(C_3H_6)	197, 198
4		
5	isoprene (2-methyl-1,3-butadiene)	196, 197, 199, 200
6	n-hexane(C_6H_{14})	197
7	heptadienal(C_2H_5CH=CHCH=CHCHO)	201-203
8	octenal($C_8H_{14}O$), octatrienal($C_8H_{10}O$), octadienal($C_8H_{12}O$)	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:

$$\underset{\text{lipids}}{C_{50}H_{92}O_6} \rightarrow \underset{\text{hydrocarbon}}{C_XH_Y} + \underset{\text{carbon dioxide}}{nCO_2}$$

From this, it is evident that the direct extraction of diatom lipids is a more-efficient method for obtaining energy than fermentation. This can occur by having solvents such as CH_2Cl_2 (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.

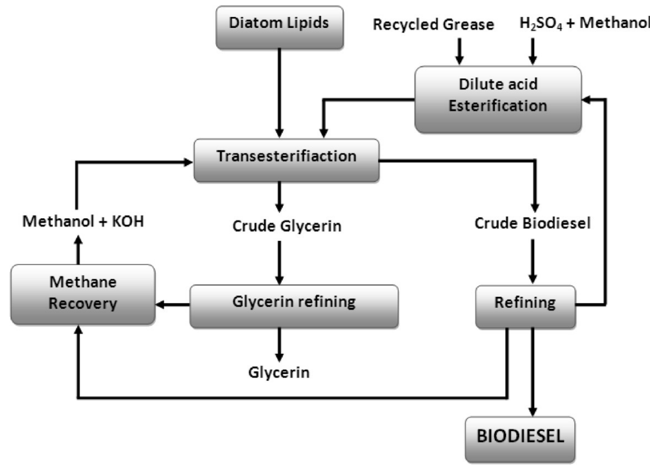


Figure 11. Process of extraction of biodiesel from diatom lipids.

Environmental and Genetic Manipulation of Diatoms

Could we manipulate diatoms, by optimizing their environment[255] 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 C_{25} haslenes varies with temperature.[223]

• Growth rate or salinity can affect C_{25} and C_{30} production,[225] and there is an optimum salinity for C_{20}.[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 obligatory[139] 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.

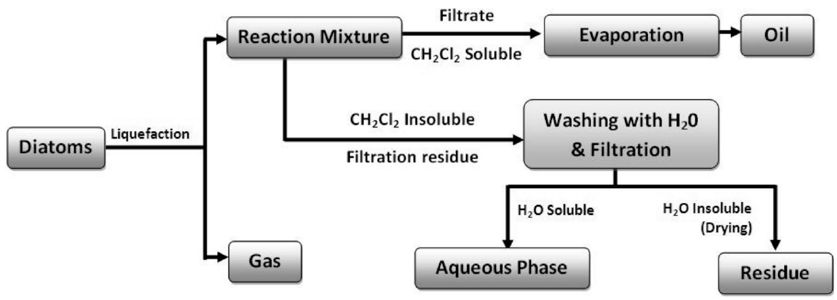


Figure 12. Thermal liquefaction accompanied by the separation of fuel oil by solvent extraction.

"'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 exponental 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 environments[15] 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 dodecane[283] from the unicellular green alga *Dunaliella*, perhaps accelerated from the natural exocytosis mechanism of this species[284] by the presence of the dodecane.[283] Higher plants have oil secretion glands,[285–288] and diatoms already exocytose the silica contents of the silicalemma,[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 production[292] 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 layer[294] 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-

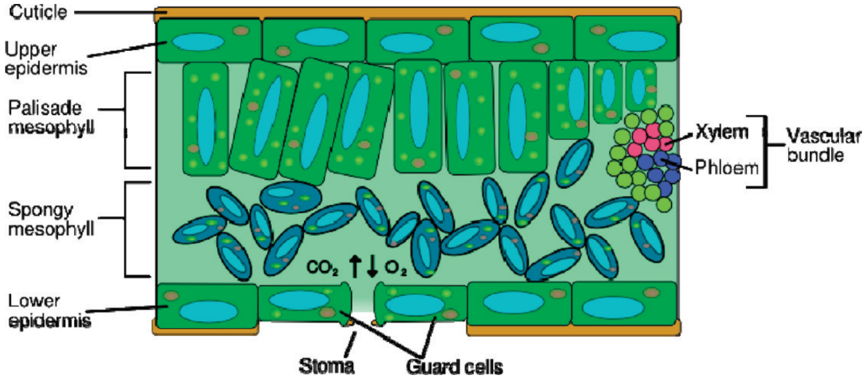


Figure 13. Anatomy of an angiosperm leaf. Diatoms could possibly occupy the air space and/or replace palisade or spongy mesophyll cells. Such an arrangement might provide gas exchange, temperature, and humidity control. (Image reproduced with permission, under the Creative Commons Attribution ShareAlike 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 CO_2, 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 colonies[18] 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 linkages[18] 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 focus[20] and redistribute[332] 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 scaffolds[339] 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 phototaxic motility[342–344] of raphid diatoms, to allow them to build their own optimal, perhaps constructal 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 heterovalvy[18]) 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 heterotrophy[352] or light level adaptation.[353] Low light may imitate conditions of nitrogen deprivation[354] 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 production[21] 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 C_2−C_6 hydrocarbons in diatoms unfortunately are treated only in a single report:[197]

> *"Laboratory experiments have been carried out[197] 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 C_2-C_4 alkanes in ocean surface waters,[359] along with isoprene (C_5), also attributed to algae[360] (mostly diatoms), and direct laboratory observation of isoprene production by diatoms.[199]

In the range of C_7-C_{12}, $\sim$1/3 of the tested diatom strains produced $\alpha,\beta,\gamma,\delta$-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 story[366]*]"*[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 decompartmentation and mixing of preexisting enzymes and requires hydrolysis of chloroplast-derived glycolipids to feed the downstream activities of C_{16} and C_{20} 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 $\sim$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 EJ[387] 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 automobile[392] 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 1980–1990s[21] (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 2030",[393] 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 work[401] suggests that diatoms can triple the efficiency of electrical solar panels, an efficiency that should also apply to gasoline secreting solar panels.

Acknowledgment

We would like to thank Janhavi S. Raut and R. Venkataraghavan (Hindustan Unilever, Ltd.) for creating the opportunity that brought us together and led to this review. We also thank Ian R. Jenkinson and the referees for helpful comments. This work is not funded by any grant agency or commercial establishment, so we have no conflicts of interest to declare.

Literature Cited

(1) Guliyev, I. S.; Feizulayev, A. A.; Huseynov, D. A. Isotope geochemistry of oils from fields and mud volcanoes in the South Caspian Basin, Azerbaijan. *Pet. Geosci.* **2001**, *7* (2), 201–209.

(2) Fortman, J. L.; Chhabra, S.; Mukhopadhyay, A.; Chou, H.; Lee, T. S.; Steen, E.; Keasling, J. D. Biofuel alternatives to ethanol: pumping the microbial well. *Trends Biotechnol.* **2008**, *26* (7), 375–381.

(3) Gordon, R. The Glass Menagerie: Diatom Nanotechnology and Its Implications for Multi-Scale Manufacturing and Oil Production. In *2nd International Conference on Multi-Scale Structures and Dynamics of Complex Systems: Processes & Forces for Creation of Designer Materials with Multi-Scale Structures*, September 4−5, 2008, Bangalore, India; Raut, J. S., Venkataraghavan, R., Eds.; Unilever: Bangalore, India, 2008.

(4) Gordon, R.; Drum, R. W. The chemical basis for diatom morphogenesis. *Int. Rev. Cytol.* **1994**, *150*, 243–372, 421−422.

(5) Aoyagi, K.; Omokawa, M. Neogene diatoms as the important source of petroleum in Japan. *J. Pet. Sci. Eng.* **1992**, *7*, 247–252.

(6) Chisti, Y. Biodiesel from microalgae. *Biotechnol. Adv.* **2007**, *25* (3), 294–306.

(7) Killops, S. D.; Killops, V. J. *Introduction to Organic Geochemistry*, 2nd Edition; Blackwell Publishing: Malden, MA, 2005.

(8) Gordon, R.; Sterrenburg, F. A. S.; Sandhage, K. A Special Issue on Diatom Nanotechnology. *J. Nanosci. Nanotechnol.* **2005**, *5* (1), 1–4.

(9) Gordon, R.; Losic, D.; Tiffany, M. A.; Nagy, S. S.; Sterrenburg, F. A. S. The Glass Menagerie: Diatoms for Novel Applications in Nanotechnology. [Invited Paper]. *Trends Biotechnol.* **2009**, *27* (2), 116–127.

(10) Gordon, R. Diatoms and nanotechnology. [Invited Paper]. In *The Diatoms: Applications for the Environmental and Earth Sciences*, 2nd Edition; Smol, J. P., Stoermer, E. F., Eds.; Cambridge University Press: Cambridge, U.K., in press.

(11) Gordon, R.; Aguda, B. D. Diatom morphogenesis: Natural fractal fabrication of a complex microstructure. In *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Part 1/4: Cardiology and Imaging*, New Orleans, LA, Nov. 4−7, 1988; Harris, G., Walker, C., Eds.; Institute of Electrical and Electronics Engineers: New York, 1988; pp 273−274.

(12) Parkinson, J.; Gordon, R. Beyond micromachining: the potential of diatoms. *Trends Biotechnol.* **1999**, *17* (5), 190–196.

(13) Drum, R. W.; Gordon, R. Star Trek replicators and diatom nanotechnology. *Trends Biotechnol.* **2003**, *21* (8), 325–328.

(14) Gebeshuber, L. C. Biotribology inspires new technologies. *Nano Today* **2007**, *2* (5), 30–37.

(15) Sterrenburg, F. A. S.; Gordon, R.; Tiffany, M. A.; Nagy, S. S. Diatoms: living in a constructal environment. In *Algae and Cyanobacteria in Extreme Environments*; Seckback, J., Ed.; Cellular Origin and Life in Extreme Habitats and Astrobiology, Vol. 11; Springer: Dordrecht, The Netherlands, 2007; pp 141−172.

(16) Grachev, M. A.; Annenkov, V. V.; Likhoshway, Y. V. Silicon nanotechnologies of pigmented heterokonts. *BioEssays* **2008**, *30* (4), 328–337.

(17) Kröger, N.; Poulsen, N. Diatoms: from cell wall biogenesis to nanotechnology. *Annu. Rev. Genet.* **2008**, *42*, 83–107.

(18) Round, F. E.; Crawford, R. M.; Mann, D. G. *The Diatoms, Biology & Morphology of the Genera*; Cambridge University Press: Cambridge, U.K., 1990.

(19) Cox, E. J. *Identification of Freshwater Diatoms from Living Material*; Chapman & Hall: London, 1996.

(20) De Stefano, L.; Rea, I.; Rendina, I.; De Stefano, M.; Moretti, L. Lensless light focusing with the centric marine diatom *Coscinodiscus walesii*. *Opt. Express* **2007**, *15* (26), 18082–18088.

(21) Sheehan, J.; Dunahay, T.; Benemann, J.; Roessler, P. *A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae*, Close-Out Report [NREL/TP-580−24190], National Renewable Energy Laboratory, Golden, CO, 1998.

(22) Dismukes, G. C.; Carrieri, D.; Bennette, N.; Ananyev, G. M.; Posewitz, M. C. Aquatic phototrophs: efficient alternatives to land-based crops for biofuels. *Curr. Opin. Biotechnol.* **2008**, *19* (3), 235–240.

(23) Hu, Q.; Sommerfeld, M.; Jarvis, E.; Ghirardi, M.; Posewitz, M.; Seibert, M.; Darzins, A. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J.* **2008**, *54* (4), 621–639.

(24) Imahara, H.; Minami, E.; Saka, S. Thermodynamic study on cloud point of biodiesel with its fatty acid composition. *Fuel* **2006**, *85* (12−13), 1666–1670.

(25) Dunahay, T. G.; Jarvis, E. E.; Dais, S. S.; Roessler, P. G. Manipulation of microalgal lipid production using genetic engineering. *Appl. Biochem. Biotechnol.* **1996**, *578*, 223–231.

(26) Moffat, M. C. An ultrastructural study of *Didymosphenia geminata* (Bacillariophyceae). *Trans. Am. Microsc. Soc.* **1994**, *113* (1), 59–71.

(27) Kirkwood, A. E.; Shea, T.; Jackson, L.; McCcauley, E. *Didymosphenia geminata* in two Alberta headwater rivers: An emerging invasive species that challenges conventional views on algal bloom development. *Can. J. Fish. Aquat. Sci.* **2007**, *64* (12), 1703–1709.

(28) Nagle, N.; Lemke, P. Production of methyl ester fuel from microalgae. *Appl. Biochem. Biotechnol.* **1990**, *24−25* (1), 355–361.

(29) Roberts, K.; Granum, E.; Leegood, R. C.; Raven, J. A. C_3 and C_4 pathways of photosynthetic carbon assimilation in marine diatoms are under genetic, not environmental, control. *Plant Physiol.* **2007**, *145* (1), 230–235.

(30) Dunahay, T. G.; Jarvis, E. E.; Zeiler, K. G.; Roessler, P. G.; Brown, L. M. Genetic engineering of microalgae for fuel production: scientific note. *Appl. Biochem. Biotechnol.* **1992**, *34−35*, 331–339.

(31) Roessler, P. G.; Brown, L. M.; Dunahay, T. G.; Heacox, D. A.; Jarvis, E. E.; Schneider, J. C.; Talbot, S. G.; Zeiler, K. G. Genetic engineering approaches for enhanced production of biodiesel fuel from microalgae. *ACS Symp. Ser.* **1994**, *566*, 255−270.

(32) Dunahay, T. G.; Jarvis, E. E.; Roessler, P. G. Genetic transformation of the diatoms *Cyclotella cryptica* and *Navicula saprophila*. *J. Phycol.* **1995**, *31* (6), 1004–1012.

(33) Apt, K. E.; Kroth-Pancic, P. G.; Grossman, A. R. Stable nuclear transformation of the diatom *Phaeodactylum tricornutum*. *Mol. Genet. Genomics* **1996**, *252* (5), 572–579.

(34) Falciatore, A.; Casotti, R.; Leblanc, C.; Abrescia, C.; Bowler, C. Transformation of nonselectable reporter genes in marine diatoms. *Mar. Biotechnol.* **1999**, *1* (3), 239–251.

(35) Zaslavskaia, L. A.; Lippmeier, J. C.; Kroth, P. G.; Grossman, A. R.; Apt, K. E. Transformation of the diatom *Phaeodactylum tricornutum* (Bacillariophyceae) with a variety of selectable marker and reporter genes. *J. Phycol.* **2000**, *36* (2), 379–386.

(36) Scala, S.; Bowler, C. Molecular insights into the novel aspects of diatom biology. *Cell. Mol. Life Sci.* **2001**, *58* (11), 1666–1673.

(37) Zaslavskaia, L. A.; Lippmeier, J. C.; Shih, C.; Ehrhardt, D.; Grossman, A. R.; Apt, K. E. Trophic conversion of an obligate photoautotrophic organism through metabolic engineering. *Science* **2001**, *292* (5524), 2073–2075.

(38) Scala, S.; Carels, N.; Falciatore, A.; Chiusano, M. L.; Bowler, C. Genome properties of the diatom *Phaeodactylum tricornutum*. *Plant Physiol.* **2002**, *129* (3), 993–1002.

(39) Grossman, A. R. Paths toward algal genomics. *Plant Physiol.* **2005**, *137* (2), 410–427.

(40) Poulsen, N.; Kröger, N. A new molecular tool for transgenic diatoms. Control of mRNA and protein biosynthesis by an inducible promoter−terminator cassette. *FEBS J.* **2005**, *272* (13), 3413–3423.

(41) Poulsen, N.; Chesley, P. M.; Kröger, N. Molecular genetic manipulation of the diatom *Thalassiosira pseudonana* (Bacillariophyceae). *J. Phycol.* **2006**, *42* (5), 1059–1065.

(42) Kroth, P.; León, R.; Gaván, A.; Fernández, E. Molecular biology and the biotechnological potential of diatoms. *Adv. Exp. Med. Biol.* **2007**, *616*, 23–33.

(43) Kroth, P. G. Genetic transformation: a tool to study protein targeting in diatoms. *Methods Mol. Biol.* **2007**, *390*, 257–268.

(44) Sakaue, K.; Harada, H.; Matsuda, Y. Development of gene expression system in a marine diatom using viral promoters of a wide variety of origin. *Physiol. Plant* **2008**, *133* (1), 59–67.

(45) Koh, L. P.; Ghazoul, J. Biofuels, biodiversity, and people: Understanding the conflicts and finding opportunities. *Biol. Conserv.* **2008**, *141* (10), 2450–2460.

(46) Müller, A.; Schmidhuber, J.; Hoogeveen, J.; Steduto, P. Some insights in the effect of growing bio-energy demand on global food security and natural resources. *Water Policy* **2008**, *10* (S1), 83–94.

(47) Zhu, L. Impacts of food and energy price hikes and proposed coping strategies. *Chin. World Econ.* **2008**, *16* (6), 35–45.

(48) Hoogeveen, J.; Faurès, J. M.; Van de Giessen, N. Increased biofuel production in the coming decade: To what extent will it affect global freshwater resources. *Irrig. Drain.* **2009**, *58* (S1), S148−S160.

(49) Demirbas, A. Progress and recent trends in biodiesel fuels. *Energy Convers. Manage.* **2009**, *50*, 14–34.

(50) Tadros, M. G.; Johansen, J. R. Physiological characterization of six lipid-producing diatoms from the southeastern United States. *J. Phycol.* **1988**, *24* (4), 445–452.

(51) Furnas, M. J. Net in situ growth rates of phytoplankton in an oligotrophic, tropical shelf ecosystem. *Limnol. Oceanogr.* **1991**, *36* (1), 13–29.

(52) Lewin, J.; Hellebust, J. A. Heterotrophic nutrition of the marine pennate diatom *Navicula pavillardi* Hustedt. *Can. J. Microbiol.* **1975**, *21* (9), 1335–1342.

(53) Brzezinski, M. A.; Villareal, T. A.; Lipschultz, F. Silica production and the contribution of diatoms to new and primary production in the central north Pacific. *Mar. Ecol.: Prog. Ser.* **1998**, *167*, 89–104.

(54) Shipe, R. F.; Brzezinski, M. A.; Pilskaln, C.; Villareal, T. A. *Rhizosolenia mats*: An overlooked source of silica production in the open sea. *Limnol. Oceanogr.* **1999**, *44* (5), 1282–1292.

(55) Furnas, M. J. In situ growth rates of marine phytoplankton: Approaches to measurement, community and species growth rates. *J. Plankton Res.* **1990**, *12*, 1117–1151.

(56) Dugdale, R. C.; Wilkerson, F. P. Silicate regulation of new production in the equatorial Pacific upwelling. *Nature* **1998**, *391*, 270–273.

(57) Breger, D. Petroleum Education, Hydrocarbon Systems. Step 1−Energy Capture. Available via the Internet at http://www.priweb.org/ed/pgws/systems/energy_capture/capture.html, 2001.

(58) Krebs, W. N., Gladenkov, A. Y., Jones, G. D. Diatoms in oil and gas exploration. In *The Diatoms: Applications for the Environmental and Earth Sciences*, 2nd Edition; Smol, J. P., Stoermer, E. F., Eds.; Cambridge University Press: Cambridge, U.K., in press.

(59) Whittington, T. *Biodiesel Production and Use by Farmers: Is it Worth Considering?*, Technical Report, Department of Agriculture and Food, Government of Western Australia, Perth, Australia, 2006.

(60) Associated Press. Algae Emerges as a Potential Fuel Source. Available via the Internet at http://www.nytimes.com/2007/12/02/us/02algae.html_r=2&oref=slogin&oref=slogin, 2007.

(61) Reuters. Sapphire Raises Over US$100 Mln for Algae Crude. Available via the Internet at http://www.planetark.com/dailynewsstory.cfm/newsid/50277/story.htm, 2008.

(62) Aguilera, M. C. Green Bullet: Scientists take aim with tiny algae and their giant promise as the biofuel solution of the future. Available via the Internet at http://explorations.ucsd.edu/Features/2008/Green_Bullet/images/12_2008_Feature.pdf, 2008.

(63) Santhanam, N. Oilgae: Biodiesel from Algae Oil−Info, Resources, News & Links. Available via the Internet at http://www.oilgae.com/, 2008.

(64) Fossil Freedom. Algal BioButanol Conquers Colorado Continental Divide. Available via the Internet at http://www.fossilfreedom.com/, 2008.

(65) Shifrin, N. S.; Chisholm, S. W. Phytoplankton lipids: interspecific differences and effects of nitrate, silicate and light-dark cycles. *J. Phycol.* **1981**, *17* (4), 374–384.

(66) Berndes, G.; Hoogwijk, M.; van den Broek, R. The contribution of biomass in the future global energy supply: A review of 17 studies. *Biomass Bioenergy* **2003**, *25* (1), 1–28.

(67) Sriharan, S.; Bagga, D.; Sriharan, T. P. Effects of nutrients and temperature on lipid and fatty-acid production in the diatom Hantzshia DI-60. *Appl. Biochem. Biotechnol.* **1990**, *24−25* (1), 309–316.

(68) McGinnis, K. M.; Dempster, T. A.; Sommerfeld, M. R. Characterization of the growth and lipid content of the diatom *Chaetoceros muelleri*. *J. Appl. Phycol.* **1997**, *9* (1), 19–24.

(69) Lebeau, T.; Robert, J. M. Diatom cultivation and biotechnologically relevant products. Part II: current and putative products. *Appl. Microbiol. Biotechnol.* **2003**, *60* (6), 624–632.

(70) Kooistra, W. H.; Medlin, L. K. Evolution of the diatoms (*Bacillariophyta*). IV. A reconstruction of their age from small subunit rRNA coding regions and the fossil record. *Mol. Phylogenet. Evol.* **1996**, *6* (3), 391–407.

(71) Schieber, J.; Krinsley, D.; Riciputi, L. Diagenetic origin of quartz silt in mudstones and implications for silica cycling. *Nature* **2000**, *406*, 981–985.

(72) Medlin, L. K.; Kooistra, W.; Gersonde, R.; Sims, P. A.; Wellbrock, U. Is the origin of the diatoms related to the end-Permian mass extinction. *Nova Hedwigia* **1997**, *65* (1−4), 1–11.

(73) Levorsen, A. I.; Berry, F. A. F. *Geology of Petroleum*, 2nd Edition; W. H. Freeman and Co.: San Francisco, CA, 1967.

(74) Hunt, J. M. *Petroleum Geochemistry and Geology*; W. H. Freeman and Co.: San Francisco, CA, 1979.

(75) North, F. K. *Petroleum Geology*; Allen & Unwin: Boston, 1985.

(76) Volkman, J. K.; Johns, R. B. The geochemical significance of positional isomers of unsaturated acids from an intertidal zone sediment. *Nature* **1977**, *267* (5613), 693.

(77) Holba, A. G.; Tegelaar, E. W.; Huizinga, B. J.; Moldowan, J. M.; Singletary, M. S.; McCaffrey, M. A.; Dzou, L. I. P. 24-norcholestanes as age-sensitive molecular fossils. *Geology* **1998**, *26* (9), 783–786.

(78) Rampen, S. W.; Schouten, S.; Abbas, B.; Panoto, F. E.; Muyzer, G.; Campbell, C. N.; Fehling, J.; Sinninghe Damsté, J. S. On the origin of 24-norcholestanes and their use as age-diagnostic biomarkers. *Geology* **2007**, *35* (5), 419–422.

(79) Volkman, J. K.; Barrett, S. M.; Dunstan, G. A.; Jeffrey, S. W. Geochemical significance of the occurrence of dinosterol and other 4-methyl sterols in a marine diatom. *Org. Geochem.* **1993**, *20* (1), 7–15.

(80) Volkman, J. K.; Barrett, S. M.; Blackburn, S. I.; Mansour, M. P.; Sikes, E. L.; Gelin, F. Microalgal biomarkers: A review of recent research developments. *Org. Geochem.* **1998**, *29* (5−7), 1163–1179.

(81) Xu, Y. P.; Jaffe, R.; Wachnicka, A.; Gaiser, E. E. Occurrence of C_{25} highly branched isoprenoids (HBIs) in Florida Bay: Paleoenvironmental indicators of diatom-derived organic matter inputs. *Org. Geochem.* **2006**, *37* (7), 847−859.

(82) Belt, S. T.; Masse, G.; Rowland, S. J.; Poulin, M.; Michel, C.; LeBlanc, B. A novel chemical fossil of palaeo sea ice: IP25. *Org. Geochem.* **2007**, *38* (1), 16−27.

(83) Rampen, S. W.; Schouten, S.; Hopmans, E. C.; Abbas, B.; Noordeloos, A. A. M.; Geenevasen, J. A. J.; Moldowan, J. M.; Denisevich, P.; Sinninghe Damsté, J. S. Occurrence and biomarker potential of 23-methyl steroids in diatoms and sediments. *Org. Geochem.* **2008**, *40* (2), 219–228.

(84) Shiine, H.; Suzuki, N.; Motoyama, I.; Hasegawa, S.; Gladenkov, A. Y.; Gladenkov, Y. B.; Ogasawara, K. Diatom biomarkers during the Eocene/Oligocene transition in the Il'pinskii peninsula, Kamchatka, Russia. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **2008**, *264* (1−2), 1–10.

(85) Ehrenberg, C. G. Ueber die Dysodil genannte Mineralspecies also ein Pruduct aus Infusorienschalen. *Ann. Phys. (Weinheim, Ger.)* **1839**, *48* (4−12), 573–575.

(86) Whitney, J. D. *Geological Survey of California. Vol. 1, Geology*; Sherman and Co.: Philadelphia, 1865.

(87) Whitney, J. D. On the fresh water infusorial deposits of the Pacific coast, and their connection with the volcanic rocks. *Proc. Calif. Acad. Nat. Sci., Ser. 1* **1867**, *3*, 319–324.

(88) Anderson, F. M. Origin of California petroleum. *Bull. Geol. Soc. Am.* **1926**, *37*, 585–614.

(89) Tolman, C. F. Biogenesis of hydrocarbons by diatoms. *Econ. Geol.* **1927**, *22* (5), 454–474.

(90) Hanna, G. D. An early reference to the theory that diatoms are the source of bituminous substances. *Bull. Am. Assoc. Petrol. Geol.* **1928**, *12*, 555–556.

(91) Phleger, F. B., Jr.; Albritton, C. C., Jr. Diatoms as a source for California petroleum: A summary review. *Field Lab.* **1937**, 25–32.

(92) Ver Wiebe, W. A. *How Oil is Found*; Edward Brothers: Wichita, KS, 1951.

(93) Bramlette, M. N. *The Monterey Formation of California and the Origin of Its Siliceous Rocks* [Professional Paper 212]; U.S. Geological Survey, United States Department of the Interior: Washington, DC, 1946.

(94) Rowland, S. J.; Robson, J. N. The widespread occurrence of highly branched acyclic C_{20}, C_{25} and C_{30} hydrocarbons in recent sediments and biota—A review. *Mar. Environ. Res.* **1990**, *30* (3), 191–216.

(95) Johns, L.; Belt, S.; Lewis, C. A.; Rowland, S.; Massé, G.; Robert, J. M.; König, W. A. Configurations of polyunsaturated sesterterpenoids from the diatom *Haslea ostrearia*. *Phytochemistry* **2000**, *53* (5), 607–611.

(96) Gotoh, M.; Miki, A.; Nagano, H.; Ribeiro, N.; Elhabiri, M.; Gurnienna-Kontecka, E.; Albrecht-Gary, A. M.; Schmutz, M.; Ourisson, G.; Nakatani, Y. Membrane properties of branched polyprenyl phosphates, postulated as primitive membrane constituents. *Chem. Biodiversity* **2006**, *3* (4), 434–455.

(97) Palmisano, A. C.; Lizotte, M. P.; Smith, G. A.; Nichols, P. D.; White, D. C.; Sullivan, C. W. Changes in photosynthetic carbon assimilation in Antarctic sea-ice diatoms during spring bloom—Variation in synthesis of lipid classes. *J. Exp. Mar. Biol. Ecol.* **1988**, *116* (1), 1–13.

(98) Gürgey, K. Correlation, alteration, and origin of hydrocarbons in the GCA, Bahar, and Gum Adasi fields, western South Caspian Basin: Geochemical and multivariate statistical assessments. *Mar. Pet. Geol.* **2003**, *20* (10), 1119–1139.

(99) Mertz, K. A., Jr. Origin of hemipelagic source rocks during the early and Middle Miocene, Salinas Basin, California. *AAPG Bull.: Am. Assoc. Pet. Geol.* **1989**, *73* (4), 510–524.

(100) Johnson, K. M.; Grimm, K. A. Opal and organic carbon in laminated diatomaceous sediments: Saanich Inlet, Santa Barbara Basin and the Miocene Monterey Formation. *Mar. Geol.* **2001**, *174* (1−4), 159–175.

(101) Gelin, F.; Volkman, J. K.; Largeau, C.; Derenne, S.; Sinninghe Damsté, J. S.; de Leeuw, J. W. Distribution of aliphatic, nonhydrolyzable biopolymers in marine microalgae. *Org. Geochem.* **1999**, *30* (2−3), 147–159.

(102) Garrison, D. L. Monterey Bay phytoplankton: II. Resting spore cycles in coastal diatom populations. *J. Plankton Res.* **1981**, *3* (1), 137–156.

(103) Eilertsen, H. C.; Sandberg, S.; Tøllefsen, H. Photoperiodic control of diatom spore growth; a theory to explain the onset of phytoplankton blooms. *Mar. Ecol.: Prog. Ser.* **1995**, *116* (1−3), 303–307.

(104) Wetz, M. S.; Wheeler, P. A.; Letelier, R. M. Light-induced growth of phytoplankton collected during the winter from the benthic boundary layer off Oregon, USA. *Mar. Ecol.: Prog. Ser.* **2004**, *280*, 95–104.

(105) Suto, I. The explosive diversification of the diatom genus *Chaetoceros* across the Eocene/Oligocene and Oligocene/Miocene boundaries in the Norwegian Sea. *Mar. Micropaleontol.* **2006**, *58* (4), 259–269.

(106) McGee, D.; Laws, R. A.; Cahoon, L. B. Live benthic diatoms from the upper continental slope: Extending the limits of marine primary production. *Mar. Ecol.: Prog. Ser.* **2008**, *356*, 103–112.

(107) Hargraves, P. E.; French, F. W. Diatom resting spores: significance and strategies. In *Survival Strategies of the Algae*; Fryxell, G. A., Ed.; Cambridge University Press: Cambridge, U.K., 1983; pp 49−68.

(108) Stasiuk, L. D.; Snowdon, L. R. Fluorescence micro-spectrometry of synthetic and natural hydrocarbon fluid inclusions: Crude oil chemistry, density and application to petroleum migration. *Appl. Geochem.* **1997**, *12* (3), 229–241.

(109) Bourdon, S.; Laggoun-Défarge, F.; Disnar, J. R.; Maman, O.; Guillet, B.; Derenne, S.; Largeau, C. Organic matter sources and early diagenetic degradation in a tropical peaty marsh (Tritrivakely, Madagascar). Implications for environmental reconstruction during the Sub-Atlantic. *Org. Geochem.* **2000**, *31* (5), 421–438.

(110) Stasiuk, L. D.; Sanei, H. Characterization of diatom-derived lipids and chlorophyll within Holocene laminites, Saanich Inlet, British Columbia, using conventional and laser scanning fluorescence microscopy. *Org. Geochem.* **2001**, *32* (12), 1417–1428.

(111) Vargas, G.; Ortlieb, L.; Pichon, J. J.; Bertaux, J.; Pujos, M. Sedimentary facies and high resolution primary production inferences from laminated diatomacous sediments off northern Chile (23°S). *Mar. Geol.* **2004**, *211* (1−2), 79–99.

(112) Michalopoulos, P.; Aller, R. C.; Reeder, R. J. Conversion of diatoms to clays during early diagenesis in tropical, continental shelf muds. *Geology* **2000**, *28* (12), 1095–1098.

(113) Wakeham, S. G., Lee, C. Production, transport, and alteration of particulate organic matter in the marine water column. In *Organic Geochemistry: Principles and Applications*; Engel, M., Macko, S. A., Eds.; Plenum Press: New York, London, 1993; pp 145−169.

(114) Harvey, H. R.; Macko, S. A. Kinetics of phytoplankton decay during simulated sedimentation: changes in lipids under oxic and anoxic conditions. *Org. Geochem.* **1997**, *27* (3−4), 129–140.

(115) Hayakawa, K.; Handa, N.; Ikuta, N.; Fukuchi, M. Downward fluxes of fatty acids and hydrocarbons during a phytoplankton bloom in the austral summer in Breid Bay, Antarctica. *Org. Geochem.* **1996**, *24* (5), 511–521.

(116) Hein, J. R.; Scholl, D. W.; Barron, J. A.; Jones, M. G.; Miller, J. Diagenesis of late Cenozoic diatomaceous deposits and formation of the bottom simulating reflector in the southern Bering Sea. *Sedimentology* **1978**, *25* (2), 155–181.

(117) Colombo, J. C.; Silverberg, N.; Gearing, J. N. Amino acid biogeochemistry in the Laurentian Trough: Vertical fluxes and individual reactivity during early diagenesis. *Org. Geochem.* **1998**, *29* (4), 933–945.

(118) DeMaster, D. J.; Dunbar, R. B.; Gordon, L. I.; Leventer, A. R.; Morrison, J. M.; Nelson, D. M.; Nittrouer, C. A.; Smith, W. O., Jr. Cycling and accumulation of biogenic silica and organic matter in high-latitude environments: The Ross Sea. *Oceanography* **1992**, *5* (3), 146–153.

(119) Josefson, A. B.; Hansen, J. L. S. Quantifying plant pigments and live diatoms in aphotic sediments of Scandinavian coastal waters confirms a major route in the pelagic-benthic coupling. *Mar. Biol.* **2003**, *142* (4), 649–658.

(120) McQuoid, M. R.; Nordberg, K. Composition and origin of benthic flocculent layers in Swedish fjords following the spring bloom—Contribution of diatom frustules and resting stages. *Nova Hedwigia* **2006**, *83* (1−2), 1–16.

(121) Zgurovskaya, L. N. Species composition and distribution of planktonic algae in bottom sediments of Black Sea. *Okeanologiya* **1978**, *18* (4), 716–721.

(122) Hamm, C. E.; Merkel, R.; Springer, O.; Jurkojc, P.; Maier, C.; Prechtel, K.; Smetacek, V. Architecture and material properties of diatom shells provide effective mechanical protection. *Nature* **2003**, *421* (6925), 841–843.

(123) Itakura, S.; Imai, I.; Itoh, K. "Seed bank"' of coastal planktonic diatoms in bottom sediments of Hiroshima Bay, Seto Inland, Japan. *Mar. Biol.* **1997**, *128* (3), 497–508.

(124) Lewis, J.; Harris, A. S. D.; Jones, K. J.; Edmonds, R. L. Long-term survival of marine planktonic diatoms and dinoflagellates in stored sediment samples. *J. Plankton Res.* **1999**, *21* (2), 343–354.

(125) McQuoid, M. R. Pelagic and benthic environmental controls on the spatial distribution of a viable diatom propagule bank on the Swedish west coast. *J. Phycol.* **2002**, *38* (5), 881–893.

(126) Hansen, J. L. S.; Josefson, A. B. Ingestion by deposit-feeding macro-zoobenthos in the aphotic zone does not affect the pool of live pelagic diatoms in the sediment. *J. Exp. Mar. Biol. Ecol.* **2004**, *308* (1), 59–84.

(127) Durbin, E. G. Aspects of the biology of resting spores of *Thalassiosira nordenskioeldii* and *Detonula confervacea*. *Mar. Biol.* **1978**, *45*, 31–37.

(128) Gendron-Badou, A.; Coradin, T.; Maquet, J.; Fröhlich, F.; Livage, J. Spectroscopic characterization of biogenic silica. *J. Non-Cryst. Solids* **2003**, *316* (2−3), 331–337.

(129) Iijima, A.; Tada, R. Silica diagenesis of Neogene diatomaceous and volcaniclastic sediments in northern Japan. *Sedimentology* **1981**, *28* (2), 185–200.

(130) Henchiri, M. Sedimentation, depositional environment and diagenesis of Eocene biosiliceous deposits in Gafsa basin (southern Tunisia). *J. Afr. Earth Sci.* **2007**, *49* (4−5), 187–200.

(131) Sicko-Goad, L.; Stoermer, E. F.; Fahnenstiel, G. Rejuvenation of *Melosira granulata* (Bacillariophyceae) resting cells from the anoxic sediments of Douglas Lake, Michigan. I. Light microscopy and ^{14}C uptake. *J. Phycol.* **1986**, *22* (1), 22–28.

(132) Nipkow, F. Ruheformen planktischer Kieselalgen in Geschichteten Schlamm Des Zürichsees. *Schweiz. Z. Hydrol.* **1950**, *12*, 263–270.

(133) Stockner, J. G.; Lund, J. W. G. Live algae in postglacial lake deposits. *Limnol. Oceanogr.* **1970**, *15* (1), 41–58.

(134) Davis, J. S. Survival records in the algae, and the survival role of certain algae pigments, fat, and mucilaginous substances. *The Biologist* **1972**, *54* (2), 52–93.

(135) Zgurovskaya, L. N. Effect of addition of nutrients on growth of spores and division of planktonic algae from bottom sediments. *Okeanologiya* **1977**, *17* (1), 119–122.

(136) McQuoid, M. R.; Hobson, L. A. Diatom resting stages. *J. Phycol.* **1996**, *32* (6), 889–902.

(137) White, A. W. Uptake of organic compounds by two facultatively heterotrophic marine centric diatoms. *J. Phycol.* **1974**, *10* (4), 433–438.

(138) White, A. W. Growth of two facultatively heterotrophic marine centric diatoms. *J. Phycol.* **1974**, *10* (3), 292–300.

(139) Li, C.-W.; Volcani, B. E. Four new apochlorotic diatoms. *Br. Phycol. J.* **1987**, *22*, 375–382.

(140) Belt, S. T.; Allard, W. G.; Rintatalo, J.; Johns, L. A.; van Duin, A. C. T.; Rowland, S. J. Clay and acid catalysed isomerisation and cyclisation reactions of highly branched isoprenoid (HBI) alkenes: Implications for sedimentary reactions and distributions. *Geochim. Cosmochim. Acta* **2000**, *64* (19), 3337–3345.

(141) Doucette, G. J.; Fryxell, G. A. *Thalassiosira antarctica*: Vegetative and resting stage chemical composition of an ice-related marine diatom. *Mar. Biol.* **1983**, *78* (1), 1–6.

(142) Hawes, I.; Schwarz, A. M. Photosynthesis in an extreme shade environment: Benthic microbial mats from Lake Hoare, a permanently ice-covered Antarctic lake. *J. Phycol.* **1999**, *35* (3), 448–459.

(143) Michels, P. C.; Clark, D. S. Pressure dependence of enzyme catalysis. *ACS Symp. Ser.* **1992**, *498*, 108–121.

(144) Nelson, D. M.; Treguer, P.; Brzezinski, M. A.; Leynaert, A.; Queguiner, B. Production and dissolution of biogenic silica in the ocean: Revised global estimates, comparison with regional data and relationship to biogenic sedimentation. *Global Biogeochem. Cycle* **1995**, *9*, 359–372.

(145) Sverdrup, H. U.; Johnson, M. W.; Fleming, R. H. *The Oceans, Their Physics, Chemistry and General Biology*; Prentice−Hall: New York, 1942.

(146) Gross, D.; Zeuthen, E. The buoyancy of planktonic diatoms: a problem of cell physiology. *Proc. R. Soc. Edinb.* **1948**, *135*, 382–389.

(147) Waite, A. M.; Thompson, P. A.; Harrison, P. J. Does energy control the sinking rates of marine diatoms? *Limnol. Oceanogr.* **1992**, *37* (3), 468–477.

(148) Post, A. F.; Dubinsky, Z.; Wyman, K.; Falkowski, P. G. Kinetics of light-intensity adaptation in a marine planktonic diatom. *Mar. Biol.* **1984**, *83* (3), 231–238.

(149) Collyer, D. M.; Fogg, G. E. Studies on fat accumulation by algae. *J. Exp. Bot.* **1955**, *6* (2), 256–275.

(150) Badour, S. S.; Gergis, M. S. Cell division and fat accumulation in *Nitzschia* sp grown in continuously illuminated mass cultures. *Arch. Mikrobiol.* **1965**, *51* (1), 94–102.

(151) Werner, D. Die Kieselsäure im Stoffwechsel von *Cyclotella cryptica* Reimann, Lewin und Guillard. *Arch. Mikrobiol.* **1966**, *55* (3), 278–308.

(152) Coombs, J.; Darley, W. M.; Holm-Hansen, O.; Volcani, B. E. Studies on the biochemistry and fine structure of silica shell formation in diatoms. Chemical composition of *Navicula pelliculosa* during silicon-starvation synchrony. *Plant Physiol.* **1967**, *42* (11), 1601–1606.

(153) Richardson, T. L.; Cullen, J. J. Changes in buoyancy and chemical composition during growth of a coastal marine diatom: Ecological and biogeochemical consequences. *Mar. Ecol.: Prog. Ser.* **1995**, *128* (1−3), 77–90.

(154) Waite, A.; Harrison, P. J. Role of sinking and ascent during sexual reproduction in the marine diatom *Ditylum brightwellii*. *Mar. Ecol.: Prog. Ser.* **1992**, *87* (1−2), 113–122.

(155) Belt, S. T.; Massé, G.; Allard, W. G.; Robert, J. M.; Rowland, S. J. Effects of auxosporulation on distributions of C_{25} and C_{30} isoprenoid alkenes in *Rhizosolenia setigera*. *Phytochemistry* **2002**, *59* (2), 141–148.

(156) Jacobs, W. Das Schweben der Wasserorganismen. *Ergeb. Biol.* **1935**, *11*, 131–218.

(157) Smayda, T. J.; Boleyn, B. J. Experimental observations on the flotation of marine diatoms. I. *Thalassiosira CF. Nana Thalassiosira rotula* and *Nitzschia seriata*. *Limnol. Oceanogr.* **1965**, *10* (4), 499–509.

(158) Villareal, T. A. Buoyancy properties of the giant diatom *Ethmodiscus*. *J. Plankton Res.* **1992**, *14*, 459–463.

(159) Villareal, T. A.; Lipschultz, F. Internal nitrate concentations in single cells of large phytoplankton from the Sargasso Sea. *J. Phycol.* **1995**, *31* (5), 689–696.

(160) Villareal, T. A.; Woods, S.; Moore, J. K.; Culver-Rymsza, K. Vertical migration of *Rhizosolenia* mats and their significance to NO_3^- fluxes in the central North Pacific gyre. *J. Plankton Res.* **1996**, *18* (7), 1103–1121.

(161) Villareal, T. A.; Joseph, L.; Brzezinski, M. A.; Shipe, R. F.; Lipschultz, F.; Altabet, M. A. Biological and chemical characteristics of the giant diatom *Ethmodiscus* (Bacillariophyceae) in the central north Pacific gyre. *J. Phycol.* **1999**, *35* (5), 896–890.

(162) Lancelot, C.; Mathot, S. Biochemical fractionation of primary production by phytoplankton in Belgian coastal waters during short-term and long-term incubations with ^{14}C-bicarbonate. 1. Mixed diatom population. *Mar. Biol.* **1985**, *86* (3), 219–226.

(163) Gautier, C.; Livage, J.; Coradin, T.; Lopez, P. J. Sol-gel encapsulation extends diatom viability and reveals their silica dissolution capability. *Chem. Commun. (Cambridge, U.K.)* **2006**, (44), 4611–4613.

(164) Tesson, B.; Gaillard, C.; Martin-Jézéquel, V. Brucite formation mediated by the diatom *Phaeodactylum tricornutum*. *Mar. Chem.* **2008**, *109* (1−2), 60–76.

(165) Faraloni, C.; De Philippis, R.; Sili, C.; Vincenzini, M. Carbohydrate synthesis by two *Navicula* strains isolated from benthic and pelagic mucilages in the Tyrrhenian Sea (Tuscan Archipelago). *J. Appl. Phycol.* **2003**, *15* (2−3), 259–261.

(166) Wustman, B. A.; Lind, J.; Wetherbee, R.; Gretz, M. R., III. Organization of fucoglucuronogalactans within the adhesive stalks of *Achnanthes longipes*. *Plant Physiol.* **1998**, *116* (4), 1431–1441.

(167) McConville, M. J.; Wetherbee, R.; Bacic, A. Subcellular location and composition of the wall and secreted extracellular sulphated polysaccharides/proteoglycans of the diatom *Stauroneis amphioxys* Gregory. *Protoplasma* **1999**, *206* (1−3), 188–200.

(168) Gordon, R.; Drum, R. W. A capillarity mechanism for diatom gliding locomotion. *Proc. Nat. Acad. Sci. U.S.A.* **1970**, *67* (1), 338–344.

(169) Gordon, R. A retaliatory role for algal projectiles, with implications for the mechanochemistry of diatom gliding motility. *J. Theor. Biol.* **1987**, *126*, 419–436.

(170) Haynes, K.; Hofmann, T. A.; Smith, C. J.; Ball, A. S.; Underwood, G. J.; Osborn, A. M. Diatom-derived carbohydrates as factors affecting bacterial community composition in estuarine sediments. *Appl. Environ. Microbiol.* **2007**, *73* (19), 6112–6124.

(171) Lee, J. B.; Hayashi, K.; Hirata, M.; Kuroda, E.; Suzuki, E.; Kubo, Y.; Hayashi, T. Antiviral sulfated polysaccharide from *Navicula directa*, a diatom collected from deep-sea water in Toyama Bay. *Biol. Pharm. Bull.* **2006**, *29* (10), 2135–2139.

(172) Doucette, G. J.; Fryxell, G. A. *Thalassiosira antarctica* (*Bacillariophyceae*): Vegetative and resting stage ultrastructure of an ice-related marine diatom. *Polar Biol.* **1985**, *4* (2), 107–112.

(173) Fahl, K.; Kattner, G. Lipid-content and fatty-acid composition of algal communities in sea-ice and water from the Weddell Sea (Antarctica). *Polar Biol.* **1993**, *13* (6), 405–409.

(174) Wilhelm, C.; Büchel, C.; Fisahn, J.; Goss, R.; Jakob, T.; Laroche, J.; Lavaud, J.; Lohr, M.; Riebesell, U.; Stehfest, K.; Valentin, K.; Kroth, P. G. The regulation of carbon and nutrient assimilation in diatoms is significantly different from green algae. *Protist* **2006**, *157* (2), 91–124.

(175) Waller, R. F.; Keeling, P. J.; Donald, R. G.; Striepen, B.; Handman, E.; Lang-Unnasch, N.; Cowman, A. F.; Besra, G. S.; Roos, D. S.; McFadden, G. I. Nuclear-encoded proteins target to the plastid in *Toxoplasma gondii* and *Plasmodium falciparum*. *Proc. Nat. Acad. Sci. U.S.A.* **1998**, *95* (21), 12352–12357.

(176) Armbrust, E. V.; Berges, J. A.; Bowler, C.; Green, B. R.; Martinez, D.; Putnam, N. H.; Zhou, S.; Allen, A. E.; Apt, K. E.; Bechner, M.; Brzezinski, M. A.; Chaal, B. K.; Chiovitti, A.; Davis, A. K.; Demarest, M. S.; Detter, J. C.; Glavina, T.; Goodstein, D.; Hadi, M. Z.; Hellsten, U.; Hildebrand, M.; Jenkins, B. D.; Jurka, J.; Kapitonov, V. V.; Kröger, N.; Lau, W. W.; Lane, T. W.; Larimer, F. W.; Lippmeier, J. C.; Lucas, S.; Medina, M.; Montsant, A.; Obornik, M.; Parker, M. S.; Palenik, B.; Pazour, G. J.; Richardson, P. M.; Rynearson, T. A.; Saito, M. A.; Schwartz, D. C.; Thamatrakoln, K.; Valentin, K.; Vardi, A.; Wilkerson, F. P.; Rokhsar, D. S. The genome of the diatom *Thalassiosira pseudonana*: Ecology, evolution, and metabolism. *Science* **2004**, *306* (5693), 79–86.

(177) Princen, L. H. Alternate industrial feedstocks from agriculture. *Econ. Bot.* **1982**, *36* (3), 302–312.

(178) Neenan, B.; Feinberg, D.; Hill, A.; McIntosh, R.; Terry, K. Fuels from Microalgae: Technology Status, Potential, and Research Requirements. Publication No. SERI/SP-231-2550; Solar Energy Research Institute: Golden, CO, 1986.

(179) Meier, R. L. , Biological cycles in the transformation of solar energy into useful fuels. In *Solar Energy Research*; Daniels, R., Duffie, J. A., Eds.; University of Wisconsin Press: Madison, WI, 1955; pp 179−184.

(180) Formo, M. W. , Physical properties of fats and fatty acids. In *Bailey's Industrial Oil and Fat Products*, 4th Edition; Swern, D., Ed.; Wiley: New York, 1979; Vol 1, pp 177−190.

(181) Cheremisioff, N. P.; Cheremisinoff, P. N.; Ellerbusch, F. *Biomass: Applications, Technology, and Production*; Marcel Dekker: New York, 1980; p vii (221 pp).

(182) Hall, D. O.; House, J. I. Biomass: A modern and environmentally acceptable fuel. *Sol. Energy Mater. Sol. Cells* **1995**, *38*, 521–542.

(183) Opute, F. I. Lipid and fatty-acid composition of diatoms. *J. Exp. Bot.* **1974**, *25* (87), 823–835.

(184) Ahlgren, G.; Lundstedt, L.; Brett, M.; Forsberg, C. Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. *J. Plankton Res.* **1990**, *12* (4), 809–818.

(185) Renaud, S. M.; Parry, D. L.; Thinh, L. V. Microalgae for use in tropical aquaculture I: Gross chemical composition and fatty acid composi-

tion of twelve species of microalgae from the Northern Territory, Australia. *J. Appl. Phycol.* **1994**, *6* (3), 337–345.

(186) Chen, G. Q.; Jiang, Y.; Chen, F. Fatty acid and lipid class composition of the eicosapentaenoic acid-producing microalga *Nitzschia laevis*. *Food Chem.* **2007**, *104* (4), 1580–1585.

(187) Belt, S. T.; Allard, W. G.; Massé, G.; Robert, J. M.; Rowland, S. J. Structural characterisation of C_{30} highly branched isoprenoid alkenes (rhizenes) in the marine diatom *Rhizosolenia setigera*. *Tetrahedron Lett.* **2001**, *42* (32), 5583–5585.

(188) Belt, S. T.; Massé, G.; Rowland, S. J.; Rohmer, M. Highly branched isoprenoid alcohols and epoxides in the diatom *Haslea ostrearia* Simonsen. *Org. Geochem.* **2006**, *37* (2), 133–145.

(189) Kooistra, W. H. C. F. The evolution of the diatoms. In *Handbook of Biomineralization*; Baeuerlein, E., Behrens, P., Epple, M., Pickett-Heaps, J., Eds.; Wiley−VCH: Weinheim, Germany, 2007; pp 95−112.

(190) Sinninghe Damsté, J. S.; Muyzer, G.; Abbas, B.; Rampen, S. W.; Massé, G.; Allard, W. G.; Belt, S. T.; Robert, J. M.; Rowland, S. J.; Moldowan, J. M.; Barbanti, S. M.; Fago, F. J.; Denisevich, P.; Dahl, J.; Trindade, L. A. F.; Schouten, S. The rise of the rhizosolenid diatoms. *Science* **2004**, *304* (5670), 584–587.

(191) Volkman, J. K.; Barrett, S. M.; Dunstan, G. A. C_{25} and C_{30} highly branched isoprenoid alkenes in laboratory cultures of two marine diatoms. *Org. Geochem.* **1994**, *21* (3−4), 407–413.

(192) Sinninghe Damsté, J. S.; Schouten, S.; Rijpstra, W. I. C.; Hopmans, E. C.; Peletier, H.; Gieskes, W. W. C.; Geenevasen, J. A. J. Structural identification of the C_{25} highly branched isoprenoid pentaene in the marine diatom *Rhizosolenia setigera*. *Org. Geochem.* **1999**, *30* (12), 1581–1583.

(193) Belt, S. T.; Allard, W. G.; Massé, G.; Robert, J. M.; Rowland, S. J. Highly branched isoprenoids (HBIs): Identification of the most common and abundant sedimentary isomers. *Geochim. Cosmochim. Acta* **2000**, *64* (22), 3839–3851.

(194) Grossi, V.; Beker, B.; Geenevasen, J. A. J.; Schouten, S.; Raphel, D.; Fontaine, M. F.; Sinninghe Damsté, J. S. C_{25} highly branched isoprenoid alkenes from the marine benthic diatom *Pleurosigma strigosum*. *Phytochemistry* **2004**, *65* (22), 3049–3055.

(195) Wraige, E. J.; Johns, L.; Belt, S. T.; Massé, G.; Robert, J. M.; Rowland, S. Highly branched C_{25} isoprenoids in axenic cultures of *Haslea ostrearia*. *Phytochemistry* **1999**, *51* (1), 69–73.

(196) Colomb, A.; Yassaa, N.; Williams, J.; Peeken, I.; Lochte, K. Screening volatile organic compounds (VOCs) emissions from five marine phytoplankton species by head space gas chromatography/mass spectrometry (HS-GC/MS). *J. Environ. Monitor.* **2008**, *10* (3), 325–330.

(197) McKay, W. A.; Turner, M. F.; Jones, B. M. R.; Halliwell, C. M. Emissions of hydrocarbons from marine phytoplankton—Some results from controlled laboratory experiments. *Atmos. Environ.* **1996**, *30* (14), 2583–2593.

(198) Ratte, M.; Bujok, O.; Spitzy, A.; Rudolph, J. Photochemical alkene formation in seawater from dissolved organic carbon: Results from laboratory experiments. *J. Geophys. Res., [Atmos.]* **1998**, *103* (D5), 5707–5717.

(199) Moore, R. M.; Oram, D. E.; Penkett, S. A. Production of isoprene by marine phytoplankton cultures. *Geophys. Res. Lett.* **1994**, *21* (23), 2507–2510.

(200) Milne, P. J.; Riemer, D. D.; Zika, R. G.; Brand, L. E. Measurement of vertical distribution of isoprene in surface seawater, its chemical fate, and its emission from several phytoplankton monocultures. *Mar. Chem.* **1995**, *48* (3−4), 237–244.

(201) Pohnert, G. Diatom/copepod interactions in plankton: The indirect chemical defense of unicellular algae. *ChemBioChem* **2005**, *6* (6), 946–959.

(202) Fontana, A.; d'Ippolito, G.; Cutignano, A.; Romano, G.; Lamari, N.; Gallucci, A. M.; Cimino, G.; Miralto, A.; Ianora, A. LOX-induced lipid peroxidation mechanism responsible for the detrimental effect of marine diatoms on Zooplankton grazers. *ChemBioChem* **2007**, *8* (15), 1810–1818.

(203) Wichard, T.; Gerecht, A.; Boersma, M.; Poulet, S. A.; Wiltshire, K.; Pohnert, G. Lipid and fatty acid composition of diatoms revisited: Rapid wound-activated change of food quality parameters influences herbivorous copepod reproductive success. *ChemBioChem* **2007**, *8* (10), 1146–1153.

(204) Barofsky, A.; Pohnert, G. Biosynthesis of polyunsaturated short chain aldehydes in the diatom *Thalassiosira rotula*. *Org. Lett.* **2007**, *9* (6), 1017–1020.

(205) Garg, A.; Bhosle, N. B. Abundance of macroalgal organic matter in biofilms: Evidence from *n*-alkane biomarkers. *Biofouling* **2004**, *20* (3), 155–165.

(206) Tornabene, T. G.; Kates, M.; Volcani, B. E. Sterols, aliphatic hydrocarbons, and fatty acids of a nonphotosynthetic diatom *Nitzschia alba*. *Lipids* **1974**, *9* (4), 279–284.

(207) Volkman, J. K.; Eglinton, G.; Corner, E. D. S. Sterols and fatty acids of the marine diatom *Biddulphia sinensis*. *Phytochemistry* **1980**, *19* (8), 1809–1813.

(208) Nichols, D. S.; Nichols, P. D.; Sullivan, C. W. Fatty-acid, sterol and hydrocarbon composition of Antarctic sea-ice diatom communities during the spring bloom in McMurdo Sound. *Antarctic Sci.* **1993**, *5* (3), 271–278.

(209) Viso, A. C.; Marty, J. C. Fatty acids from 28 marine microalgae. *Phytochemistry* **1993**, *34* (6), 1521–1533.

(210) Suzuki, T.; Matsuyama, Y. Determination of free fatty acids in marine phytoplankton causing red tides by fluorometric high-performance liquid chromatography. *J. Am. Oil Chem. Soc.* **1995**, *72* (10), 1211–1214.

(211) Whyte, J. N. C.; Ginther, N. G.; Townsend, L. D. Formation of domoic acid and fatty acids in *Pseudonitzschia pungens f* multiseries with scale of culture. *J. Appl. Phycol.* **1995**, *7* (2), 199–205.

(212) Reuss, N.; Poulsen, L. K. Evaluation of fatty acids as biomarkers for a natural plankton community. A field study of a spring bloom and a post-bloom period off West Greenland. *Mar. Biol.* **2002**, *141* (3), 423–434.

(213) Pel, R.; Floris, V.; Gons, H. J.; Hoogveld, H. L. Linking flow cytometric cell sorting and compound-specific ^{13}C-analysis to determine population-specific isotopic signatures and growth rates in cyanobacteria-dominated lake plankton. *J. Phycol.* **2004**, *40* (5), 857–866.

(214) Massé, G.; Belt, S. T.; Rowland, S. J.; Rohmer, M. Isoprenoid biosynthesis in the diatoms *Rhizosolenia setigera* (Brightwell) and *Haslea ostrearia* (Simonsen). *Proc. Nat. Acad. Sci. U.S.A.* **2004**, *101* (13), 4413–4418.

(215) Sinninghe Damsté, J. S.; Rijpstra, W. I. C. Identification of a novel C_{25} highly branched isoprenoid thiophene in sediments. *Org. Geochem.* **1993**, *20* (3), 327–331.

(216) Summons, R. E.; Barrow, R. A.; Capon, R. J.; Hope, J. M.; Stranger, C. The structure of a new C_{25} isoprenoid alkene biomarker from diatomaceous microbial communities. *Aust. J. Chem.* **1993**, *46* (6), 907–915.

(217) Belt, S. T.; Cooke, D. A.; Robert, J. M.; Rowland, S. Structural characterisation of widespread polyunsaturated isoprenoid biomarkers: A C_{25} triene, tetraene and pentaene from the diatom *Haslea ostrearia* Simonsen. *Tetrahedron Lett.* **1996**, *37* (27), 4755–4758.

(218) Wraige, E. J.; Belt, S. T.; Lewis, C. A.; Cooke, D. A.; Robert, J. M.; Massé, G.; Rowland, S. J. Variations in structures and distributions of C_{25} highly branched isoprenoid (HBI) alkenes in cultures of the diatom *Haslea ostrearia* Simonsen. *Org. Geochem.* **1997**, *27* (7−8), 497–505.

(219) Wraige, E. J.; Belt, S. T.; Massé, G.; Robert, J. M.; Rowland, S. J. Variations in distributions of C_{25} highly branched isoprenoid (HBI) alkenes in the diatom *Haslea ostrearia*: Influence of salinity. *Org. Geochem.* **1998**, *28* (12), 855–859.

(220) Johns, L.; Wraige, E. J.; Belt, S. T.; Lewis, C. A.; Massé, G.; Robert, J. M.; Rowland, S. J. Identification of a C_{25} highly branched isoprenoid (HBI) diene in Antarctic sediments, Antarctic sea-ice diatoms and cultured diatoms. *Org. Geochem.* **1999**, *30* (11), 1471–1475.

(221) Sinninghe Damsté, J. S.; Rijpstra, W. I. C.; Schouten, S.; Peletier, H.; van der Maarel, M.; Gieskes, W. W. C. A C_{25} highly branched isoprenoid alkene and C_{25} and C_{27} *n*-polyenes in the marine diatom *Rhizosolenia setigera*. *Org. Geochem.* **1999**, *30* (1), 95–100.

(222) Belt, S. T.; Masse, G.; Allard, W. G.; Robert, J. M.; Rowland, S. J. C-25 highly branched isoprenoid alkenes in planktonic diatoms of the Pleurosigma genus. *Org. Geochem.* **2001**, *32* (10), 1271–1275.

(223) Rowland, S. J.; Belt, S. T.; Wraige, E. J.; Massé, G.; Roussakis, C.; Robert, J. M. Effects of temperature on polyunsaturation in cytostatic lipids of *Haslea ostrearia*. *Phytochemistry* **2001**, *56* (6), 597–602.

(224) Massé, G.; Belt, S. T.; Allard, W. G.; Lewis, C. A.; Wakeham, S. G.; Rowland, S. J. Occurrence of novel monocyclic alkenes from diatoms in marine particulate matter and sediments. *Org. Geochem.* **2004**, *35* (7), 813–822.

(225) Rowland, S. J.; Allard, W. G.; Belta, S. T.; Massé, G.; Robert, J. M.; Blackburn, S.; Frampton, D.; Revill, A. T.; Volkman, J. K. Factors influencing the distributions of polyunsaturated terpenoids in the diatom *Rhizosolenia setigera*. *Phytochemistry* **2001**, *58* (5), 717–728.

(226) Rampen, S. W.; Volkman, J. K.; Hur, S. B.; Abbas, B. A.; Schouten, S.; Jameson, I. D.; Holdsworth, D. G.; Bae, J. H.; Sinninghe Damsté, J. S. Occurrence of gorgosterol in diatoms of the genus *Delphineis*. *Org. Geochem.* **2009**, *40* (1), 144–147.

(227) Sicko-Goad, L. M.; Schelske, C. L.; Stoermer, E. F. Estimation of intracellular carbon and silica content of diatoms from natural assemblages using morphometric techniques. *Limnol. Oceanogr.* **1984**, *29* (6), 1170–1178.

(228) Mann, A. The economic importance of the diatom. *Ann. Rept. Smithsonian Inst.* **1916**, 386–397.

(229) Fritsch, F. E. *The Structure and Reproduction of the Algae, Vol. 1: Introduction, Chlorophyceae, Xanthophyceae, Chrysophyceae, Bacillariophyceae, Cryptophyceae, Dinophyceae, Chloromonadineae, Euglenineae, Colorous Flagellata*; Cambridge University Press: Cambridge, U.K., 1935.

(230) South, G. R.; Whittick, A. *Introduction to Phycology*; Blackwell Scientific: Oxford, U.K., 1987.

(231) Coulon, F. Über die Fettbildung und den Plastidenformwechsel bei *Nitzschia palea*. *Arch. Protistenk.* **1956**, *101*, 443–476.

(232) Gibbs, S. P. The ultrastructure of the pyrenoids of algae, exclusive of the green algae. *J. Ultrastr. Res.* **1962**, *7* (3−4), 247–261.

(233) Drum, R. W. The cytoplasmic fine structure of the diatom *Nitzschia palea*. *J. Cell Biol.* **1963**, *18*, 429–440.

(234) Fogg, G. E. Photosynthesis and formation of fats in a diatom. *Ann. Bot.* **1956**, *20* (78), 265–285.

(235) Strickland, J. D. H.; Holm-Hansen, O.; Eppley, R. W.; Linn, R. J. The use of a deep tank in plankton ecology. I. Studies of the growth and composition of phytoplankton crops at low nutrient levels. *Limnol. Oceanogr.* **1969**, *14*, 23–34.

(236) Smith, A. E.; Morris, I. Synthesis of lipid during photosynthesis by phytoplankton of the Southern Ocean. *Science* **1980**, *207* (4427), 197–199.

(237) Palmisano, A. C.; Sullivan, C. W. Physiology of sea ice diatoms. I. Response of 3 polar diatoms to a simulated summer-winter transition. *J. Phycol.* **1982**, *18* (4), 489–498.

(238) Allen, A. E.; Vardi, A.; Bowler, C. An ecological and evolutionary context for integrated nitrogen metabolism and related signaling pathways in marine diatoms. *Curr. Opin. Plant Biol.* **2006**, *9* (3), 264–273.

(239) Feinberg, D. A. Technical and economic analysis of liquid fuel production from microalgae. In *Symposium Papers: Energy from Biomass and Wastes IX*, Lake Buena Vista, FL, January 28−February 1, 1985; Klass, D. L., Ed.; Institute of Gas Technology: Chicago, 1984; pp 1225−1244.

(240) Ma, F. R.; Hanna, M. A. Biodiesel production: A review. *Bioresour. Technol.* **1999**, *70* (1), 1–15.

(241) Kusdiana, D.; Saka, S. Methyl esterification of free fatty acids of rapeseed oil as treated in supercritical methanol. *J. Chem. Eng. Jpn.* **2001**, *34* (3), 383–387.

(242) Kusdiana, D.; Saka, S. Kinetics of transesterification in rapeseed oil to biodiesel fuel as treated in supercritical methanol. *Fuel* **2001**, *80* (5), 693–698.

(243) Gercel, H. F.; Pütün, A. E.; Pütün, E. Hydropyrolysis of extracted *Euphorbia rigida* in a well-swept fixed-bed tubular reactor. *Energy Sources* **2002**, *24* (5), 423–430.

(244) Otera, J. Transesterification. *Chem. Rev.* **1993**, *93* (4), 1449–1470.

(245) Lotero, E.; Liu, Y. J.; López, D. E.; Suwannakarn, K.; Bruce, D. A.; Goodwin, J. G. Synthesis of biodiesel via acid catalysis. *Ind. Eng. Chem. Res.* **2005**, *44* (14), 5353–5363.

(246) Kusdiana, D.; Saka, S. Effects of water on biodiesel fuel production by supercritical methanol treatment. *Bioresour. Technol.* **2004**, *91* (3), 289–295.

(247) López, D. E.; Goodwin, J. G.; Bruce, D. A.; Lotero, E. Transesterification of triacetin with methanol on solid acid and base catalysts. *Appl. Catal., A* **2005**, *295* (2), 97–105.

(248) Bunyakiat, K.; Makmee, S.; Sawangkeaw, R.; Ngamprasertsith, S. Continuous production of biodiesel via transesterification from vegetable oils in supercritical methanol. *Energy Fuels* **2006**, *20* (2), 812–817.

(249) van Kasteren, J. M. N.; Nisworo, A. P. A process model to estimate the cost of industrial scale biodiesel production from waste cooking oil by supercritical transesterification. *Resour., Conserv. Recycl.* **2007**, *50* (4), 442–458.

(250) Enssani, E. A method for the extraction of liquid hydrocarbons from microalgal biomass. In *Proceedings of the 25th Intersociety Energy Conversion Engineering Conference (IECEC-90)*, August 12−17, 1990; IEEE: Piscataway, NJ, 1990; Vol. 6; pp 250−255.

(251) Ishiwatari, R.; Ishiwatari, M.; Kaplan, I. R.; Rohrback, B. G. Thermal alteration of young kerogen in relation to petroleum genesis. *Nature* **1976**, *264*, 347–349.

(252) Espitalié, J.; Madec, M.; Tissot, B. Role of mineral matrix in kerogen pyrolysis: Influence on petroleum generation and migration. *AAPG Bull.: Am. Assoc. Pet. Geol.* **1980**, *64* (1), 59–66.

(253) Takeda, N.; Asakawa, T. Study of petroleum generation by pyrolysis—I. Pyrolysis experiments by Rock-Eval and assumption of molecular structural change of kerogen using ^{13}C-NMR. *Appl. Geochem.* **1988**, *3* (5), 441–453.

(254) Demirbas, A. Global biofuel strategies. *Energy Educ. Sci. Technol.* **2006**, *17* (1−2), 27–63.

(255) Chelf, P. Environmental control of lipid and biomass production in two diatom species. *J. Appl. Phycol.* **1990**, *2* (2), 121–129.

(256) Clarke, H. T.; Mazur, A. The lipids of diatoms. *J. Biol. Chem.* **1941**, *141* (1), 283–289.

(257) Chen, G. Q.; Jiang, Y.; Chen, F. Salt-induced alterations in lipid composition of diatom *Nitzschia laevis* (Bacillariophyceae) under heterotrophic culture condition. *J. Phycol.* **2008**, *44*, (5), 1309−1314.

(258) Smith, G. M. *Cryptogamic Botany, Vol. 1. Algae and Fungi*, 2nd Edition; McGraw−Hill: New York, 1955.

(259) Wainman, B. C.; Smith, R. E. H.; Rai, H., Furgal, J. A. Irradiance and lipid production in natural algal populations. In *Lipids in Freshwater Ecosystems*; Arts, M. T., Wainmann, B. C., Eds.; Springer: Berlin, 1999; pp 45−70.

(260) Evans, J. H. The survival of fresh-water algae during dry periods. Part I. An investigation of the algae of 5 small ponds. *J. Ecol.* **1958**, *46* (1), 149–167.

(261) (a) Evans, J. H. The survival of fresh-water algae during dry periods. Part II. Drying experiments. *J. Ecol.* **1959**, *47* (1), 55–70. (b) Evans, J. H. The survival of fresh-water algae during dry periods. Part III. Stratification of algae in pond margin litter and mud. *J. Ecol.* **1959**, *47* (1), 55–81.

(262) Chaturvedi, R.; Uppalapati, S. R.; Alamsjah, M. A.; Fujita, Y. Isolation of quizalofop-resistant mutants of *Nannochloropsis oculata* (Eustigmatophyceae) with high eicosapentaenoic acid following *N*-methyl-*N*-nitrosourea-induced random mutagenesis. *J. Appl. Phycol.* **2004**, *16* (2), 135–144.

(263) Wen, Z. Y.; Chen, F. Heterotrophic production of eicosapentaenoic acid by microalgae. *Biotechnol. Adv.* **2003**, *21* (4), 273–294.

(264) Jones, G. J.; Nichols, P. D.; Johns, R. B.; Smith, J. D. The effect of mercury and cadmium on the fatty-acid and sterol composition of the marine diatom *Asterionella glacialis*. *Phytochemistry* **1987**, *26* (5), 1343–1348.

(265) Jha, A. UK announces world's largest algal biofuel project: Carbon Trust launches £26m project to develop transport fuels made from algae by 2020. Available via the Internet at http://www.guardian.co.uk/environment/2008/oct/23/biofuels-energy/print2008.

(266) Tempelman, R. J. Generalized linear mixed models in dairy cattle breeding. *J. Dairy Sci.* **1998**, *81* (5), 1428–1444.

(267) Dahl, G. E.; Buchanan, B. A.; Tucker, H. A. Photoperiodic effects on dairy cattle: A review. *J. Dairy Sci.* **2000**, *83* (4), 885–893.

(268) Dahl, G. E.; Petitclerc, D. Management of photoperiod in the dairy herd for improved production and health. *J. Animal Sci.* **2003**, *81* (Suppl. 3), 11–17.

(269) Stockdale, C. R. Influence of milking frequency on the productivity of dairy cows. *Aust. J. Exp. Agric.* **2006**, *46* (6−7), 965–974.

(270) Hejazi, M. A.; Wijffels, R. H. Milking of microalgae. *Trends Biotechnol.* **2004**, *22* (4), 189–94.

(271) Hejazi, M. A.; Holwerda, E.; Wijffels, R. H. Milking microalga *Dunaliella salina* for β-carotene production in two-phase bioreactors. *Biotechnol. Bioeng.* **2004**, *85* (5), 475–481.

(272) Andersen, F. O. Fate of organic carbon added as diatom cells to oxic and anoxic marine sediment microcosms. *Mar. Ecol.: Prog. Ser.* **1996**, *134* (1−3), 225–233.

(273) Mock, T.; Kruse, M.; Dieckmann, G. S. A new microcosm to investigate oxygen dynamics at the sea ice water interface. *Aquat. Microb. Ecol.* **2003**, *30* (2), 197–205.

(274) Carter, C. M.; Ross, A. H.; Schiel, D. R.; Howard-Williams, C.; Hayden, B. In situ microcosm experiments on the influence of nitrate and light on phytoplankton community composition. *J. Exp. Mar. Biol. Ecol.* **2005**, *326* (1), 1–13.

(275) Cozar, A.; Echevarria, F. Size structure of the planktonic community in microcosms with different levels of turbulence. *Scientia Marina* **2005**, *69* (2), 187–197.

(276) Kress, N.; Thingstad, T. F.; Pitta, P.; Psarra, S.; Tanaka, T.; Zohary, T.; Groom, S.; Herut, B.; Mantoura, R. F. C.; Polychronaki, T.; Rassoulzadegan, F.; Spyres, G. Effect of P and N addition to oligotrophic Eastern Mediterranean waters influenced by near-shore waters: A microcosm experiment. *Deep-Sea Res., Part II* **2005**, *52* (22−23), 3054–3073.

(277) Scarratt, M. G.; Marchetti, A.; Hale, M. S.; Rivkin, R. B.; Michaud, S.; Matthews, P.; Levasseur, M.; Sherry, N.; Merzouk, A.; Li, W. K. W.; Kiyosawa, H. Assessing microbial responses to iron enrichment in the Subarctic Northeast Pacific: Do microcosms reproduce the in situ condition. *Deep-Sea Res., Part II* **2006**, *53* (20−22), 2182–2200.

(278) Heid, H. W.; Keenan, T. W. Intracellular origin and secretion of milk fat globules. *Eur. J. Cell Biol.* **2005**, *84* (2−3), 245–258.

(279) Mather, I. H.; Keenan, T. W. Origin and secretion of milk lipids. *J. Mammary Gland Biol. Neoplasia* **1998**, *3* (3), 259–273.

(280) Deyrup-Olsen, I.; Luchtel, D. L. Secretion of mucous granules and other membrane-bound structures: A look beyond exocytosis. *Int. Rev. Cytol.* **1998**, *183*, 95–141.

(281) Patton, S. Milk secretion at the cellular level: a unique approach to the mechanism of exocytosis. *J. Dairy Sci.* **1978**, *61* (5), 643–650.

(282) Boutry, J. L.; Bordes, M.; Février, A.; Barbier, M.; Saliot, A. Marine diatom, *Chaetoceros simplex calcitrans* Paulsen and its environment. IV. Relation to culture medium: Study on hydrocarbons. (In Fr.) *J. Exp. Mar. Biol. Ecol.* **1977**, *28* (1), 41−51.

(283) Hejazi, M. A.; Kleinegris, D.; Wijffels, R. H. Mechanism of extraction of β-carotene from microalga *Dunaliellea salina* in two-phase bioreactors. *Biotechnol. Bioeng.* **2004**, *88* (5), 593–600.

(284) Ginzburg, M.; Ginzburg, B. Z.; Wayne, R. Ultrarapid endocytotic uptake of large molecules in *Dunaliella* species. *Protoplasma* **1999**, *206* (1−3), 73–86.

(285) Croteau, R. Oil-filled glands on a leaf. *Trends Plant Sci.* **2001**, *6* (9), 439.

(286) Lange, B. M.; Wildung, M. R.; Stauber, E. J.; Sanchez, C.; Pouchnik, D.; Croteau, R. Probing essential oil biosynthesis and secretion by functional evaluation of expressed sequence tags from mint glandular trichomes. *Proc. Nat. Acad. Sci. U.S.A.* **2000**, *97* (6), 2934–2939.

(287) Sharma, S.; Sangwan, N. S.; Sangwan, R. S. Developmental process of essential oil glandular trichome collapsing in menthol mint. *Curr. Sci.* **2003**, *84* (4), 544–550.

(288) Wagner, G. J.; Wang, E.; Shepherd, R. W. New approaches for studying and exploiting an old protuberance, the plant trichome. *Ann. Bot. (Oxford, U.K.)* **2004**, *93* (1), 3–11.

(289) Schmid, A. M. M.; Eberwein, R. K.; Hesse, M. Pattern morphogenesis in cell walls of diatoms and pollen grains: A comparison. *Protoplasma* **1996**, *193* (1−4), 144–173.

(290) Dugdale, T. M.; Willis, A.; Wetherbee, R. Adhesive modular proteins occur in the extracellular mucilage of the motile, pennate diatom *Phaeodactylum tricornutum*. *Biophys. J.* **2006**, *90* (8), L58–L60.

(291) Higgins, M. J.; Crawford, S. A.; Mulvaney, P.; Wetherbee, R. Characterization of the adhesive mucilages secreted by live diatom cells using atomic force microscopy. *Protist* **2002**, *153* (1), 25–38.

(292) Fernández, F. G.; Pérez, J. A.; Sevilla, J. M.; Camacho, F. G.; Grima, E. M. Modeling of eicosapentaenoic acid (EPA) production from *Phaeodactylum tricornutum* cultures in tubular photobioreactors. Effects of dilution rate, tube diameter, and solar irradiance. *Biotechnol. Bioeng.* **2000**, *68* (2), 173–183.

(293) Raniello, R.; Iannicelli, M. M.; Nappo, M.; Avila, C.; Zupo, V. Production of *Cocconeis neothumensis* (Bacillariophyceae) biomass in batch cultures and bioreactors for biotechnological applications: light and nutrient requirements. *J. Appl. Phycol.* **2007**, *19* (4), 383–391.

(294) Schlichting, H.; Gersten, K. *Boundary Layer Theory*, 8th Revision and English Edition; Springer−Verlag: Berlin, 2000; p xxiii (799 pp).

(295) Barry, P. Thinning fuel before injection boosts efficiency. *Sci. News* **2008**, *174* (9), 9.

(296) Tao, R.; Huang, K.; Tang, H.; Bell, D. Electrorheology leads to efficient combustion. *Energy Fuels* **2008**, *22* (6), 3785–3788.

(297) Evans, J. H. Further investigations of the algae of pond margins. *Hydrobiologia* **1960**, *15* (4), 384–394.

(298) Hoekema, S.; Douma, R. D.; Janssen, M.; Tramper, J.; Wijffels, R. H. Controlling light-use by *Rhodobacter capsulatus* continuous cultures in a flat-panel photobioreactor. *Biotechnol. Bioeng.* **2006**, *95* (4), 613–626.

(299) Hoekema, S.; Douma, R. D.; Janssen, M.; Tramper, J.; Wijffels, R. H. Erratum: Controlling light-use by Rhodobacter capsulatus continuous cultures in a flat-panel photobioreactor (Vol. 95, pg 613, 2006). *Biotechnol. Bioeng.* **2008**, *99* (1), 249–249.

(300) Carvalho, A. P.; Meireles, L. A.; Malcata, F. X. Microalgal reactors: A review of enclosed system designs and performances. *Biotechnol. Prog.* **2006**, *22* (6), 1490–1506.

(301) Shi, D. J.; Hall, D. O. The *Azolla-Anabaena* association: historical perspective, symbiosis and energy metabolism. *Bot. Rev.* **1988**, *54* (4), 353–386.

(302) Lee, J. J. Algal symbiosis in larger foraminifera. *Symbiosis* **2006**, *42* (2), 63–75.

(303) Pienaar, R. N.; Sakai, H.; Horiguchi, T. Description of a new dinoflagellate with a diatom endosymbiont *Durinskia capensis sp. nov.* (Peridiniales, Dinophyceae) from South Africa. *J. Plant Res.* **2007**, *120* (2), 247–258.

(304) Cerrano, C.; Calcinai, B.; Cucchiari, E.; Di Camillo, C.; Totti, C.; Bavestrello, G. The diversity of relationships between Antarctic sponges and diatoms: the case of *Mycale acerata* Kirkpatrick, 1907 (Porifera, Demospongiae). *Polar Biol.* **2004**, *27* (4), 231–237.

(305) Inagaki, Y.; Dacks, J. B.; Doolittle, W. F.; Watanabe, K. I.; Ohama, T. Evolutionary relationship between dinoflagellates bearing obligate diatom endosymbionts: Insight into tertiary endosymbiosis. *Int. J. Syst. Evol. Microbiol.* **2000**, 2075–2081.

(306) Janson, S.; Wouters, J.; Bergman, B.; Carpenter, E. J. Host specificity in the *Richelia*-diatom symbiosis revealed by *hetR* gene sequence analysis. *Environ. Microbiol.* **1999**, *1* (5), 431–438.

(307) Lee, J. J.; Reimer, C. W.; McEnery, M. E. Identification of diatoms isolated as endosymbionts from larger foraminifera from the Gulf of Eilat (Red Sea) and the description of two new species *Fragilaria shiloi sp nov* and *Navicula reissii sp nov*. *Bot. Mar.* **1980**, *23* (1), 41–48.

(308) Peyton, K. A.; Hanisak, M. D.; Lin, J. D. Marine algal symbionts benefit benthic invertebrate embryos deposited in gelatinous egg masses. *J. Exp. Mar. Biol. Ecol.* **2004**, *307* (2), 139–164.

(309) Hasle, G. R. *Navicula endophytica sp. nov.*, a pennate diatom with an unusual mode of existence. *Br. Phycol. Bull.* **1968**, *3* (3), 475–480.

(310) Buck, K. R.; Bentham, W. M. A novel symbiosis between a cyanobacterium *Synechococcus sp*., an aplastidic protist, *Solenicola setigera*, and a diatom, *Leptocylindrus mediterraneus*, in the open ocean. *Mar. Biol.* **1998**, *132* (3), 349–355.

(311) Bavestrello, G.; Arillo, A.; Calcinai, B.; Cattaneo-Vietti, R.; Cerrano, C.; Gaino, E.; Penna, A.; Sarà, M. Parasitic diatoms inside antarctic sponges. *Biol. Bull.* **2000**, *198* (1), 29–33.

(312) Jolley, E. T.; Jones, A. K. Interaction between *Navicula muralis* Grunow and an associated species of *Flavobacterium*. *Br. Phycol. J.* **1977**, *12* (4), 315–328.

(313) Fogg, G. E. Some speculations on the nature of the pelagic mucilage community of the northern Adriatic Sea. *Sci. Total Environ.* **1995**, *165* (1−3), 59–63.

(314) Okamoto, N.; Nagumo, T.; Tanaka, J.; Inouye, I. An endophytic diatom *Gyrosigma coelophilum sp nov.* (Naviculales, Bacillariophyceae) lives inside the red alga *Coelarthrum opuntia* (Rhodymeniales, Rhodophyceae). *Phycologia* **2003**, *42* (5), 498–505.

(315) Booth, W. E. *Navicula climacospheniae sp-nov*, an endophytic diatom inhabiting the stalk of *Climacosphenia moniligera* Ehrenberg. *Nova Hedwigia* **1986**, *42* (2−4), 295–300.

(316) Drum, R. W. Endophytic diatoms in cortex of submerged *Salix* roots. *J. Phycol.* **1967**, *3* (Suppl.), 4.

(317) Biebl, R.; Kusel-Fetzmann, E. Observations on the occurrence of algae at thermal sites in Iceland. (In Ger.) *Öesterr. Bot. Z.* **1966**, *113* (3/4), 408−423.

(318) Jha, M. Hydrobiological studies on Suraj Kund and Chandrama Kund, hot-springs of Rajgir, Bihar, India. *Int. Rev. Gesamten Hydrobiol.* **1992**, *77* (3), 435–443.

(319) Mpawenayo, B.; Cocquyt, C.; Nindorera, A. Diatoms (Bacillariophyta) and other algae from the hot springs of Burundi (Central Africa) in relation with the physical and chemical characteristics of the water. *Belg. J. Bot.* **2005**, *138* (2), 152–164.

(320) Brown, P. B.; Wolfe, G. V. Protist genetic diversity in the acidic hydrothermal environments of Lassen Volcanic National Park, U.S.A. *J. Eukaryotic Microbiol.* **2006**, *53* (6), 420–431.

(321) Bryanskaya, A. V.; Namsaraev, Z. B.; Kalashnikova, O. M.; Barkhutova, D. D.; Namsaraev, B. B.; Gorlenko, V. M. Biogeochemical processes in algal-bacterial mats of the Urinskii alkaline hot spring. *Microbiology* **2006**, *75* (5), 611–620.

(322) Jones, B.; Renaut, R. W. Growth of siliceous spicules in acidic hot springs, Waiotapu geothermal area, North Island, New Zealand. *Palaios* **2006**, *21* (5), 406–423.

(323) Schinteie, R.; Campbell, K. A.; Browne, P. R. L. Microfacies of stromatolitic Sinter from acid-sulphate-chloride springs at Parariki stream, Rotokawa Geothermal Field, New Zealand. *Palaeontol. Electron.*, **2007**, *10*, (1). (Available via the Internet at http://palaeo-electronica.org/2007_1/sinter/index.html.)

(324) Owen, R. B.; Renaut, R. W.; Jones, B. Geothermal diatoms: a comparative study of floras in hot spring systems of Iceland, New Zealand, and Kenya. *Hydrobiologia* **2008**, *610*, 175–192.

(325) Fairchild, E.; Sheridan, R. P. Physiological investigation of hot spring diatom *Achnanthes exigua* Grün. *J. Phycol.* **1974**, *10* (1), 1–4.

(326) Zijffers, J. W. F.; Janssen, M.; Tramper, J.; Wijffels, R. H. Design process of an area-efficient photobioreactor. *Mar. Biotechnol.* **2008**, *10* (4), 404–415.

(327) Zijffers, J. W. F.; Salim, S.; Janssen, M.; Tramper, J.; Wijffels, R. H. Capturing sunlight into a photobioreactor: Ray tracing simulations of the propagation of light from capture to distribution into the reactor. *Chem. Eng. J.* **2008**, *145* (2), 316–327.

(328) MNP Group. Fiber Optics and Algae. Available via the Internet at http://www.myninjaplease.com/green/p=696, 2007.

(329) Wohlgeschaffen, G. D.; Rao, D. V. S.; Mann, K. H. Vat incubator with immersion core illumination: a new, inexpensive setup for mass phytoplankton culture. *J. Appl. Phycol.* **1992**, *4* (1), 25–29.

(330) Weiss, P. Soaking up rays, a primitive marine creature has natural-glass fibers that hint at high tech. *Sci. News* **2001**, *160* (5), 77–79.

(331) Bismuto, A.; Setaro, A.; Maddalena, P.; De Stefano, L.; De Stefano, M. Marine diatoms as optical chemical sensors: A time-resolved study. *Sens. Actuators B* **2008**, *130* (1), 396–399.

(332) Mitchell, E. Diatom nanostructures bend light. Available via the Internet at http://news.bbc.co.uk/1/hi/sci/tech/7608369.stm, 2008.

(333) Engel, E.; Michiardi, A.; Navarro, M.; Lacroix, D.; Planell, J. A. Nanotechnology in regenerative medicine: the materials side. *Trends Biotechnol.* **2008**, *26* (1), 39–47.

(334) Venugopal, J.; Low, S.; Choon, A. T.; Ramakrishna, S. Interaction of cells and nanofiber scaffolds in tissue engineering. *J. Biomed. Mater. Res. B: Appl. Biomater.* **2008**, *84* (1), 34–48.

(335) Guda, T.; Appleford, M.; Oh, S.; Ong, J. L. A cellular perspective to bioceramic scaffolds for bone tissue engineering: The state of the art. *Curr. Top Med. Chem.* **2008**, *8* (4), 290–299.

(336) Glowacki, J.; Mizuno, S. Collagen scaffolds for tissue engineering. *Biopolymers* **2008**, *89* (5), 338–344.

(337) Dunn, J. C. Analyses of cell growth in tissue-engineering scaffolds. *Regen. Med.* **2008**, *3* (3), 421–424.

(338) Arumuganathar, S.; Jayasinghe, S. N. Living scaffolds (specialized and unspecialized) for regenerative and therapeutic medicine. *Biomacromolecules* **2008**, *9* (3), 759–766.

(339) Wang, G. J.; Ho, K. H.; Hsu, S. H.; Wang, K. P. Microvessel scaffold with circular microchannels by photoresist melting. *Biomed. Microdevices* **2007**, *9* (5), 657–663.

(340) Drum, R. W. Light and electron microscope observations on the tube-dwelling diatom *Amphipleura rutilans* (Trentepohl) Cleve. *J. Phycol.* **1969**, *5* (1), 21–26.

(341) Lobban, C. S. Environmental factors, plant responses, and colony growth in relation to tube-dwelling diatom blooms in the Bay of Fundy, Canada, with a review of the biology of tube-dwelling diatoms. *Diatom Res.* **1989**, *4* (1), 89–109.

(342) Nultsch, W. Phototactic and photokinetic action spectra of the diatom *Nitschia comunis*. *Photochem. Photobiol.* **1971**, *14* (6), 705–712.

(343) Nultsch, W. Phototaxis and photokinesis. In *Primitive Sensory and Communication Systems: The Taxes and Tropisms of Micro-Organisms and Cells*; Carlile, M. J. , Ed.; Academic Press: New York, 1975; pp 29−90.

(344) Cohn, S. A.; Weitzell, R. E., Jr. Ecological considerations of diatom cell motility: I. Characterization of motility and adhesion in four diatom species. *J. Phycol.* **1996**, *32* (6), 928–939.

(345) Bejan, A. *Shape and Structure, from Engineering to Nature*; Cambridge University Press: Cambridge, U.K., 2000.

(346) Bejan, A. The constructal law of organization in nature: Tree-shaped flows and body size. *J. Exp. Biol.* **2005**, *208* (Part 9), 1677–1686.

(347) De Stefano, M.; De Stefano, L. Nanostructures in diatom frustules: Functional morphology of valvocopulae in *Cocconeidacean monoraphid taxa*. *J. Nanosci. Nanotechnol.* **2005**, *5* (1), 15–24.

(348) Kingston, J. C. Araphid and monoraphid diatoms. In *Freshwater Algae of North America. Ecology and Classification*; Wehr, J. D., Sheath, R. G., Eds.; Academic Press: New York, 2003; pp 595−636.

(349) Boyle, J. A.; Pickett-Heaps, J. D.; Czarnecki, D. B. Valve morphogenesis in the pennate diatom *Achnanthes coarctata*. *J. Phycol.* **1984**, *20*, 563–573.

(350) Goldman, J. C.; McGillicuddy, D. J. Effect of large marine diatoms growing at low light on episodic new production. *Limnol. Oceanogr.* **2003**, *48* (3), 1176–1182.

(351) Karsten, U.; Schumann, R.; Rothe, S.; Jung, I.; Medlin, L. Temperature and light requirements for growth of two diatom species (Bacillariophyceae) isolated from an Arctic macroalga. *Polar Biol.* **2006**, *29* (6), 476–486.

(352) Tolomio, C. Experimental approach for the analysis of diatoms in Venice Lagoon sediment, Italy. (In Ital.) *Diatom Res.* **2004**, *19* (1), 81−101.

(353) Friedman, A. L.; Alberte, R. S. Biogenesis and light regulation of the major light harvesting chlorophyll-protein of diatoms. *Plant Physiol.* **1986**, *80* (1), 43–51.

(354) Bartual, A.; Gálvez, L. A. Growth and biochemical composition of the diatom *Phaeodactylum tricornutum* at different pH and inorganic carbon levels under saturating and subsaturating light regimes. *Bot. Mar.* **2002**, *45* (6), 491–501.

(355) Lohr, M.; Wilhelm, C. Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. *Proc. Nat. Acad. Sci. U.S.A.* **1999**, *96* (15), 8784–8789.

(356) Coesel, S.; Obornik, M.; Varela, J.; Falciatore, A.; Bowler, C. Evolutionary origins and functions of the carotenoid biosynthetic pathway in marine diatoms. Available via the Internet at http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0002896, 2008.

(357) Lee, R. F.; Baker, J. Ethane and ethylene production in an estuarine river-formation from the decomposition of polyunsaturated fatty acids. *Mar. Chem.* **1992**, *38* (1−2), 25–36.

(358) Muñoz, J.; Mudge, S. M.; Sandoval, A. Effects of ionic strength on the production of short chain volatile hydrocarbons by *Dunaliella salina* (Teodoresco). *Chemosphere* **2004**, *54* (8), 1267–1271.

(359) Bonsang, B.; Al Aarbaoui, A.; Sciare, J. Diurnal variation of non-methane hydrocarbons in the subantarctic atmosphere. *Environ. Chem.* **2008**, *5* (1), 16–23.

(360) Broadgate, W. J.; Liss, P. S.; Penkett, S. A. Seasonal emissions of isoprene and other reactive hydrocarbon gases from the ocean. *Geophys. Res. Lett.* **1997**, *24* (21), 2675–2678.

(361) Kim, J. D.; Lee, C. G. Systemic optimization of microalgae for bioactive compound production. *Biotechnol. Bioprocess Eng.* **2005**, *10* (5), 418–424.

(362) Vidoudez, C.; Pohnert, G. Growth phase-specific release of polyunsaturated aldehydes by the diatom *Skeletonema marinoi*. *J. Plankton Res.* **2008**, *30* (11), 1305–1313.

(363) Ribalet, F.; Wichard, T.; Pohnert, G.; Ianora, A.; Miralto, A.; Casotti, R. Age and nutrient limitation enhance polyunsaturated aldehyde production in marine diatoms. *Phytochemistry* **2007**, *68* (15), 2059–2067.

(364) Wichard, T.; Poulet, S. A.; Halsband-Lenk, C.; Albaina, A.; Harris, R.; Liu, D.; Pohnert, G. Survey of the chemical defence potential of diatoms: Screening of fifty one species for $\alpha,\beta,\gamma,\delta$-unsaturated aldehydes. *J. Chem. Ecol.* **2005**, *31* (4), 949–958.

(365) Pohnert, G.; Boland, W. The oxylipin chemistry of attraction and defense in brown algae and diatoms. *Nat. Prod. Rep.* **2002**, *19* (1), 108–122.

(366) Wichard, T.; Poulet, S. A.; Boulesteix, A. L.; Ledoux, J. B.; Lebreton, B.; Marchetti, J.; Pohnert, G. Influence of diatoms on copepod reproduction. II. Uncorrelated effects of diatom-derived $\alpha,\beta,\gamma,\delta$-unsaturated aldehydes and polyunsaturated fatty acids on *Calanus helgolandicus* in the field. *Prog. Oceanogr.* **2008**, *77* (1), 30–44.

(367) Ianora, A.; Casotti, R.; Bastianini, M.; Brunet, C.; d'Ippolito, G.; Acri, F.; Fontana, A.; Cutignano, A.; Turner, J. T.; Miralto, A. Low reproductive success for copepods during a bloom of the non-aldehyde-producing diatom *Cerataulina pelagica* in the North Adriatic Sea. *Mar. Ecol. (Berlin, Ger.)* **2008**, *29* (3), 399–410.

(368) Poulet, S. A.; Escribano, R.; Hidalgo, P.; Cueff, A.; Wichard, T.; Aguilera, V.; Vargas, C. A.; Pohnert, G. Collapse of *Calanus chilensis* reproduction in a marine environment with high diatom concentration. *J. Exp. Mar. Biol. Ecol.* **2007**, *352* (1), 187–199.

(369) Fontana, A.; d'Ippolito, G.; Cutignano, A.; Miralto, A.; Ianora, A.; Romano, G.; Cimino, G. Chemistry of oxylipin pathways in marine diatoms. *Pure Appl. Chem.* **2007**, *79* (4), 481–490.

(370) van Tamelen, E. E.; Webber, B. D. Hydrogenative deoxygenation of organic compounds: Direct conversion of amides to alkanes. *Proc. Nat. Acad. Sci. U.S.A.* **1981**, *78* (3), 1321–1322.

(371) van Tamelen, E. E.; Gladysz, J. A. Direct conversion of aldehydes, esters, and 1,2-oxides to alkanes with carbon skeleton preservation. *J. Am. Chem. Soc.* **1974**, *96* (16), 5290–5291.

(372) Mojaat, M.; Foucault, A.; Pruvost, J.; Legrand, J. Optimal selection of organic solvents for biocompatible extraction of β-carotene from *Dunaliella salina*. *J. Biotechnol.* **2008**, *133* (4), 433–441.

(373) Antiæ, M. P.; Jovanèvi, B. S.; Ili, M.; Vrviæ, M. M.; Schwarzbauer, J. Petroleum pollutant degradation by surface water microorganisms. *Environ. Sci. Pollut. Res.* **2006**, *13* (5), 320–327.

(374) Villareal, T. A. Positive buoyancy in the oceanic diatom *Rhizosolenia debyana* Peragallo H. *Deep-Sea Res. Part A* **1988**, *35* (6), 1037–1045.

(375) Moore, J. K.; Villareal, T. A. Buoyancy and growth characteristics of three positively buoyant marine diatoms. *Mar. Ecol.: Prog. Ser.* **1996**, *132* (1−3), 203–213.

(376) Eppley, R. W. Sinking rates of ocean phytoplankton-A. Citation Classic commentary on sinking rates of marine-phytoplankton measured with a fluorometer. *Current Contents* **1990**, *5*, 18.

(377) Eppley, R. W.; Holmes, R. W.; Strickland, J. D. H. Sinking rates of marine phytoplankton measured with a fluorometer. *J. Exp. Mar. Biol. Ecol.* **1967**, *1* (2), 191–208.

(378) Rai, A. N.; Söderbäck, E.; Bergman, B. Cyanobacterium−plant symbioses. *New Phytol.* **2000**, *147* (3), 449–481.

(379) Carpenter, E. J.; Janson, S. Intracellular cyanobacterial symbionts in the marine diatom *Climacodium frauenfeldianum* (Bacillariophyceae). *J. Phycol.* **2000**, *36* (3), 540–544.

(380) Carpenter, E. J.; Montoya, J. P.; Burns, J.; Mulholland, F.; Subramaniam, A.; Capone, D. G. Extensive blooms of N_2-fixing diatom/cyanobacterial association in tropical Atlantic Ocean. *Mar. Ecol.: Prog. Ser.* **1999**, *185*, 273–283.

(381) Coolen, M. J. L.; Volkman, J. K.; Abbas, B.; Muyzer, G.; Schouten, S.; Sinninghe Damsté, J. S. Identification of organic matter sources in sulfidic late Holocene Antarctic fjord sediments from fossil rDNA sequence analysis. *Paleoceanography* **2007**, *22* (2), PA2211 (DOI: 10.1029/2006PA001309).

(382) Jarvis, E. E.; Dunahay, T. G.; Roessler, P. G.; Zeiler, K. G.; Brown, L. M. Genetic engineering of microalgae for biodiesel production. *Abstr. Pap. Am. Chem. Soc.* **1994**, *207*, 187-BIOT.

(383) Roessler, P. G.; Bleibaum, J. L.; Thompson, G. A.; Ohlrogge, J. B. Characteristics of the gene that encodes acetyl-CoA carboxylase in the diatom *Cyclotella cryptica*. *Ann. N.Y. Acad. Sci.* **1994**, *721*, 250–256.

(384) Rosenberg, J. N.; Oyler, G. A.; Wilkinson, L.; Betenbaugh, M. J. A green light for engineered algae: Redirecting metabolism to fuel a biotechnology revolution. *Curr. Opin. Biotechnol.* **2008**, *19* (5), 430−436.

(385) Gordon, R. Computer controlled evolution of diatoms: Design for a compustat. *Nova Hedwigia* **1996**, *112*, 213−216. (Festschrift for Prof. T. V. Desikachary.)

(386) Ruddiman, W. E. How did humans first alter global climate. *Sci. Am.* **2005**, *292* (3), 46–53.

(387) Heinloth, K. *Die Energiefrage: Bedarf und Potentiale, Nutzen, Risiken und Kosten*, Vieweg, Wiesbaden, Germany, 2003; XXXIII, 597 S.

(388) Martinot, E. *Renewables 2007 Global Status Report*; Worldwatch Institute: Washington, DC, 2008.

(389) Schmalensee, R.; Stoker, T. M.; Judson, R. A. World carbon dioxide emissions: 1950−2050. *Rev. Econ. Stat.* **1998**, *80* (1), 15–27.

(390) Soares, M. *The Ocean, Our Future*; Cambridge University Press: Cambridge, England, 1998; 248 pp.

(391) World Coal Institute. Coal Facts 2008. Available via the Internet at http://www.worldcoal.org/pages/content/index.asp?PageID=1882008.

(392) Dyer, G. End of oil coming faster than we think. *Winnipeg Free Press* **2008** (December 23), A11.

(393) Shafiee, S.; Topal, E. An econometrics view of worldwide fossil fuel consumption and the role of U.S. *Energy Policy* **2008**, *36* (2), 775–786.

(394) Pickens, T. B. America is addicted to foreign oil. Available via the Internet at http://www.pickensplan.com/pdf/ThePlan_0710_01.pdf, 2008.

(395) Meng, Q. Y.; Bentley, R. W. Global oil peaking: Responding to the case for 'abundant supplies of oil'. *Energy* **2008**, *33* (8), 1179–1184.

(396) Mowat, F. *Sea of Slaughter*; McClelland and Stewart−Bantam: Toronto, Canada, 1984.

(397) Daly, H. E.; Cobb, J. B., Jr.; Cobb, C. W. *For the Common Good: Redirecting the Economy Toward Community, the Environment, and a Sustainable Future*, 2nd Edition; Beacon Press: Boston, 1994; p viii (534 pp).

(398) Thorpe, M.; Mitra, S. Growing economic interdependence of China and the Gulf Cooperation Council. *Chin. World Econ.* **2008**, *16* (2), 109–126.

(399) Boussafir, M.; Lallier-Vergès, E. Accumulation of organic matter in the Kimmeridge Clay formation (KCF): An update fossilisation model for marine petroleum source−rocks. *Mar. Pet. Geol.* **1997**, *14* (1), 75–83.

(400) McKenna, H. Anatomy of a leaf. Avalable via the Internet at http://commons.wikimedia.org/wiki/File:Leaf_anatomy.svg, 2006.

(401) Stauth, D.; Rorrer, G. Media Release: Ancient Diatoms Lead to New Technology for Solar Energy. http://oregonstate.edu/dept/ncs/newsarch/2009/Apr09/diatoms.html, 2009 (accessed April 30, 2009).

Received for review January 11, 2009
Revised manuscript received April 14, 2009
Accepted April 15, 2009

IE900044J