Diatoms for Carbon Sequestration

Subjects: Biology

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Carbon dioxide (CO2) is a major greenhouse gas responsible for climate change. Diatoms, a natural sink of atmospheric CO2, can be cultivated industrially in autotrophic and mixotrophic modes for the purpose of CO2 sequestration. In addition, the metabolic diversity exhibited by this group of photosynthetic organisms provides avenues to redirect the captured carbon into products of value. These include lipids, omega-3 fatty acids, pigments, antioxidants, exopolysaccharides, sulphated polysaccharides, and other valuable metabolites that can be produced in environmentally sustainable bio-manufacturing processes. To realize the potential of diatoms, expansion of our knowledge of carbon supply, CO2 uptake and fixation by these organisms, in conjunction with ways to enhance metabolic routing of the fixed carbon to products of value is required.

Keywords: carbon supply ; CO2 uptake ; carbon fixation ; CCM ; biomanufacturing ; diatoms

1. Introduction—The Carbon Calamity

Global anthropogenic activities are resulting in annual carbon dioxide (CO_2) emissions in excess of 40 $GtCO_2$ y^{-1} [1]. Over the past decade, there have been modest declines in CO_2 emission in the USA and the 28 (now 27) European Union countries, but increasing emissions in China, India and most developing countries have dominated global emission trends, resulting in a global increase in CO_2 emissions of 0.9% per year [2]. Even during the economic crisis of the COVID-19 pandemic in 2020, the unprecedented cessation of human activities has all but led to a small dent in the global energy use and resulting CO_2 emissions [3]. A slowdown in CO_2 emissions will only occur when fossil fuels, especially coal, are replaced by renewables, such as solar, wind, biomass and other sustainable alternatives, and conventional vehicles are replaced by an electric fleet that relies on renewable energy generation at point sources [2][4]. The world's oceans are the most heavily utilized carbon storage sites, and already contain 39 trillion metric tons of carbon, where sinking particles transport carbon to the seafloor and it is buried in the sediment. There is a limit to the CO_2 sequestration capacity of oceans, and it is projected that the pH of the oceans will further decrease by 0.3 to 0.4 units by the end of the century, which could dramatically alter marine food chains [5]. Therefore, there is an urgent necessity to develop feasible strategies for CO_2 sequestration to alleviate the concerns.

Current strategies to reduce CO_2 emissions include absorption, adsorption, membrane separation and cryogenic fractionation, and their limitations have been critically evaluated [6]. It has been identified that out of all the capture processes, post-combustion capture is the most relevant process that can be retrofitted to existing industrial infrastructures. The technology most explored to date for the sequestration of CO_2 is chemical-based sequestration, but it has its own set of challenges. Recent research on carbon capture has mostly focused on optimizing CO_2 absorption using amines, predominantly mono-ethanolamine (MEA) (a molecule developed in the 1970s), to minimize the energy consumption and to improve absorption efficiency. However, the process still remains energy intensive, and possible degradation reactions could lead to the formation of toxic compounds such as nitrosamines [7]. The ammonia-based CO_2 capture technology can be suitably utilized only where there is residual heat for generating low grade steam used to provide the regeneration energy. Furthermore, there are common issues such as ammonia slippage [8].

CO_2 sequestration by photosynthetic organisms can be a sustainable alternative when coupled to bioprocessing and biomanufacturing for value-addition. The photosynthetic production of molecular oxygen, otherwise known as oxygenic photosynthesis, was first observed in the ancestors of the present-day cyanobacteria, more than 2.7–3.7 billion years ago [9]. Microalgae are some of nature's finest examples of solar energy conversion systems. They convert carbon dioxide into complex organic molecules through photosynthesis, with theoretical efficiencies in the order of 8–10% of solar energy (biomass productivities of 280 ton dcw ha^{-1} y^{-1}), translating to 3% conversion efficiency in practice (biomass productivities of up to 146 ton dcw ha^{-1} y^{-1} in small scale cultivations and 60–75 ton dcw ha^{-1} y^{-1} in mass cultivations) [10][11]. It is well known that microalgae do not need arable land, and can be cultivated on marginal land, in deserts, in brackish water, or even in the open ocean, and thus do not compete with food crops for resources. Microalgae cultivations can use

CO_2 from flue gases of power stations containing SOx and NOx, and can be coupled with wastewater treatment plants for the remediation of nitrates and phosphates, heavy metals in tertiary wastewater, and for removing secondary pollutants, e.g., pharmaceuticals [12]. Microalgae have been found to have a higher CO_2 uptake rate than forests [13]. Although large-scale microalgal cultivation for biofuels has been limited due to concerns of economic viability and sustainability, many companies are successfully producing biomass and added-value chemicals, such as pigments (β-carotene, astaxanthin, phycocyanin) and omega-3 fatty acids (docasahexaneoic acid and eicosapentaenoic acid). In addition, several companies are utilizing renewable energy for running the production plants, e.g., solar energy (AlgaTechnologies-Israel, Brevel-Israel, Simris-Sweden) and geothermal (Algalif-Iceland). The carbon content of microalgal cells typically ranges between 40–60% dcw. For a carbon content of 50% dcw, the amount of carbon potentially fixed with current biomass productivities in the range of 60–140 ton dcw ha^{-1} y^{-1} (see above) would be 30–70 ton C ha^{-1} y^{-1}. This translates to a potential CO_2 fixing capacity in the region of 100–250 ton CO_2 ha^{-1} y^{-1}. Although this would mean several hectares of cultivations to make an effective contribution to global CO_2 mitigation, every bit of contribution adds to the total and justifies development of strategies that maximize the potential of microalgal CO_2 sequestration.

Diatoms are a group of microalgae found in all aquatic environments, reportedly responsible for 20% of the global net primary production and 40% of marine primary production, in nature [14]. They have evolved from their ancestors, from about 250 to 190 MYA (million years ago) [15][16], and have become a highly diverse and biogeochemically relevant group of phytoplankton, and contribute significantly to the natural carbon sink [17]. Diatoms have many adaptations enabling them to thrive in the oceans. The diatomic silica cell wall may discourage ingestion by grazing organisms, provide necessary support for the large vacuole, facilitate light harvesting, increase nutrient uptake, and protect the cell against UV radiation [18]. Diatoms are favored over other phytoplankton groups in environments with fluctuating light, as occurs in non-stratified water columns, due to their favorable photo-physiology, as demonstrated for *Phaeodactylum tricornutum* [19] and *Thalassiosira weissflogii* [20]. Diatoms are well adapted to turbulence, and can be more productive in these environments compared to other microalgae [21][22]. They are an algal taxonomic group that offer a potential bio-based solution to rising CO_2 levels. *P. tricornutum* and *Thalassiosira pseudonana* are two of the most well characterized species of diatoms. Furthermore, diatoms are very adaptive and can serve as ideal candidates for manufacturing bulk commodity products (biomass, biofuels, protein and bioplastics) and specialty chemicals (eicosapentaenoic acid, docasahexaenoic acid, fucoxanthin and recombinant proteins, e.g., recombinant antibodies) as a viable cell factory, whilst enabling strategies to reduce CO_2 in the atmosphere [23].

2. Diatoms for Bio-Based Manufacturing

Diatoms are unicellular microalgae possessing a silicon-based cell wall, and belong to the class Bacillariophyceae. They are an ecologically successful taxonomic group of phytoplankton. They contribute heavily to the global primary productivity [17][24], and play fundamental roles in the global nutrient cycling of carbon, nitrogen, phosphorus, and silicon [25][26]. The silica exoskeleton provides diatoms with structural integrity and protection in the ocean environment. Silicification increases cell density, enabling the cells to sink; possibly a selective evolutionary trait to move the cell to more optimal growth environment deeper in the water column, and evolved as a selection pressure against parasitism [27] that can be useful in establishing cost-effective harvesting methods. Their ability to prosper in the natural environment indicates their suitability for large scale cultivations in less sterile environments, to enable viable industrial scale operations. Diatoms have considerable metabolic diversity attributable to their evolution that involved endosymbiosis of diverse lineages. As a result, they can be employed to produce diverse chemicals. Manipulation of CO_2 supply can also be used to improve the accumulation of both lipids and carbohydrates, as has been studied in *T. pseudonana*, *P. tricornutum*, *Asterionella formosa* and *Navicula pelliculosa* [28]. The presence of efficient uptake systems for CO_2 and bicarbonate (HCO_3^-) have been identified in the diatoms *T. weissflogii* and *P. tricornutum*, at concentrations typically encountered in ocean surface waters. The ability to adjust uptake rates to a wide range of inorganic carbon supply has also been reported [29]. Nevertheless, there is paucity of information and evidence regarding CO_2 uptake and there are many unanswered questions. In addition to photo-autotrophy, mixotrophic cultivation regimes can help yield higher biomass concentrations and productivities.

Diatoms can be cultivated in both indoor and outdoor settings, as suspension cultures (in open ponds, flat panel, tubular and airlift photobioreactors (PBRs)), as well as immobilized cultivation systems to avoid dewatering costs. *P. tricornutum* biomass productivity was found to be doubled in high-technology photobioreactors to 21 ± 2.3 g m^{-2} d^{-1}, compared to cultivation in open ponds, and resulted in a CO_2 fixation rate of 35.5 g^{-1} m^{-2} [30]. Overall, this gives flexibility in cultivation, as different cultivation methods can be used to enhance productivity. Novel culturing media, such as FDMed medium, have been used for high biomass, fucoxanthin and EPA production yields in freshwater diatoms, such as *Sellaphora minima* and *Nitzschia palea* in autotrophic batch cultures [31]. Media for cultivation of freshwater diatoms

include: FDMed medium [31], WC [32] and modified COMBO (MCOMBO) (modified COMBO (MCOMBO)) medium of the UTEX Culture Collection of Algae). F/2 media [33], DAM (diatom artificial medium; [34]), ASW (artificial sea water; [35]), Walne media [36] can be used for the culturing of marine diatoms. Yeast extract supplementation of F/2 media has been reported to result in increased biomass concentration (3.48 fold), TAG content (2.13 fold) and fucoxanthin content (1.7 fold) in the stationary phase [37].

Optimization of operational conditions has been shown to be useful in increasing product yields. Several such studies have been reported with the model diatom, *P. tricornutum*, for example, light shift with tryptone addition to improve fucoxanthin production [38], UV mutagenesis to improve EPA productivity by 33% [39], adaptive laboratory evolution to improve neutral lipid and carotenoid accumulation [40]. Marginal improvement in total lipid contents, in association with reduced poly unsaturated fatty acids, have been observed in *Cyclotella cryptica* as a result of silicate deprivation [41]. In the case of *P. tricornutum*, a weakly silicified diatom, the required quantities of silicon can be obtained from silicon dissolved from glass vessels in alkaline culture media [42]. *P. tricornutum* grown in the presence and absence of silicon showed little difference in growth, except under low light and green light conditions [43].

3. Carbon Assimilation in Diatoms

Carbon can be found in many forms in the natural environment. In the oceans, the dynamics of chemical dissolution of CO_2 and its biological uptake creates an interplay between chemical and biological equilibria that requires further elucidations and understanding. For terrestrial photosynthetic organisms, atmospheric CO_2 is the main form of inorganic CO_2 assimilated, but in water, the dissolution of CO_2 results in carbonic acid, which dissociates into bicarbonate and carbonate. In the oceans, 90% of inorganic carbon is in the form of bicarbonate [44]. Prior to the industrial revolution, CO_2 concentrations in the atmosphere were ~280 ppm [45], but today they have increased to ~420 ppm in 2020 (https://www.co2.earth/), with an increasing proportion of CO_2 sequestered in the oceans and on land. At pre-industrial concentrations of atmospheric CO_2, the seawater concentration of bicarbonate was 1757 µmol kg^{-1}, but elevated levels of bicarbonate are now being observed, contributing to ocean acidification and a higher solubility of carbonate [44].

The effect of increasing CO_2 concentration supply to diatoms leads to increased growth and biomass production, under growth optimal conditions. Carbon capture in *P. tricornutum* happens predominantly in the form of bicarbonates with bicarbonate transporters [46], and as mentioned above, CO_2 fixation rates of 35 g m^{-2} d^{-1} have been reported [30]. When cultivating *P. tricornutum* in air sparged cultures, a CO_2 consumption rate of 1 g g^{-1} DW, at pH 7.2 and 0.8 g g^{-1} DW, at pH 9, both resulting in 0.06–0.08 g CO_2 uptake per day (removal of 50–65% of CO_2 from the air), has been reported [47]. It has been identified that the optimal CO_2 concentration for biomass accumulation is in a narrow range, between 1% and 1.25% CO_2 in air (v/v), at a gas supply rate of 0.66 vvm and light intensity of 1000 µmol m^{-2} s^{-1} (16 h light period), 90% of CO_2 supplied leaving the medium unused [48]. When *P. tricornutum* was provided with bicarbonate as an inorganic carbon source, between 73–99.9% of the bicarbonate was consumed or remained dissolved in the medium, resulting in a CO_2 consumption rate of 0.31 g d^{-1} (2.3 g CO_2 g^{-1} biomass), albeit at the cost of reduced growth and biomass production [47]. Cultivations of *P. tricornutum* (PHAEO2) in modified F/2 seawater (enriched four-fold with nitrogen and phosphorus) with 15% CO_2 have been shown to increase biomass productivity to 0.15 g L^{-1} d^{-1}, whilst consuming 0.28 g L^{-1} d^{-1} of CO_2 in a batch operation [49]. A comparative assessment of CO_2 concentration mechanisms (CCMs) in a handful of freshwater and marine diatoms (*P. tricornutum*, *As. formosa*, *N. pellicosa*, *T. pseudonana*, *T. weissflogii*) revealed that, for all the species, at 20,000 ppm, the affinity for DIC was lower than at 400 ppm CO_2 (atmospheric concentrations), and the reliance on CO_2 was higher, and that species-specific differences were greater than environmental differences, in determining the effectiveness of the CCMs [50]. Negative effects of CO_2 on growth have also been recorded. For example, *Attheya longicornis* growth was hampered by high levels of CO_2 supply [51]. Another factor affecting marine species is temperature. Rising temperatures may also have a negative effect on the CO_2 uptake rate by diatoms. In *Navicula distans*, rising temperature and pCO_2 resulted in a reduction of diatom cell size, which inevitably relates to the ecological and physiological functions of diatoms, such as nutrient diffusion, intake and requirements, and even the metabolic rate [52]. There are also some cases recorded where no reaction to increased CO_2 levels could be observed, as seen with *Chaetoceros brevis* cultures supplemented with pCO_2 (750 ppmv (2 × ambient) and 190 ppmv (0.5 × ambient) CO_2), where little or no significant effect was observed on the diatom growth, pigment content and composition, photosynthesis, photoprotection and RuBisCO activity [53].

CO_2 uptake in the aquatic photosynthetic organisms, such as diatoms, cyanobacteria and other microalgae, take place with the involvement of the CCM. Carbon metabolism pathways in diatoms, like in plants and other algae, require the transportation of CO_2 across intracellular compartments like the peroxisomes, chloroplasts, mitochondria, endoplasmic reticulum and the cytosol, with concentration at the site where RuBisCO is located for CO_2 fixation (**Figure 1**). This arrangement gives flexibility to the cell to adjust the carbon flux, enhance the concentration of CO_2 in a stepwise manner

from low concentrations on the outside to levels required for RuBisCO activity and hence fix CO_2 [54][55]. There is limited information available on carbon metabolism in several diatoms, as of now. In order to obtain the appropriate design of the carbon flow in diatom cells under different conditions, information regarding the localization and functionality of the component diatom enzymes is a necessity. The cellular machinery involved in diatom photosynthesis includes the chloroplasts, carbonic anhydrases (CAs), RuBisCO, Calvin Benson Bassham (CBB) cycle proteins, transporters, phosphoglycerate kinase (PGK), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), CP12, fructose-1,6-biphosphatase (FBPase), sedoheptulose-1,7-biphosphatase (SBPase), phosphoribulokinase (PRK), basic leucine zipper (bZip) bZIP transcription factors family, and others [24][46][50][56][57]. Diurnal rhythms also affect the TCA Cycle and that influences the amount of CO_2 that is absorbed. Moreover, the bZIP14 protein family members are involved in CO_2 sensing and blue light signaling [58]. Our current knowledge of diatom CCMs is discussed in the section below, an understanding of which will help in devising strategies to maximize uptake of CO_2 by diatoms.

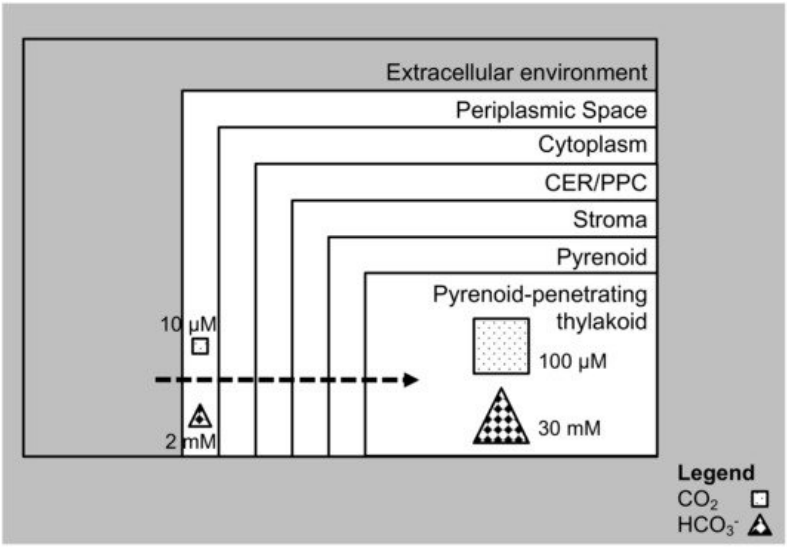

Figure 1. Carbon dioxide (CO_2) enrichment in cell organelles. In diatoms, there are different membranes for CO_2 to cross, and it has to be enriched from low to concentrated levels near RuBisCO to enable CO_2 fixation. The periplasmic space faces the extracellular environment. Adjacent to the periplasmic space is the cytoplasm. Further inward is the chloroplastic endoplasmic reticulum (CER)/periplastidal compartment (PPC). The stroma is the layer beyond the CER/PPC. The innermost layer is the pyrenoid where in embedded is the pyrenoid penetrating thylakoid. This usually happens in general, with CO_2 concentration mechanisms (CCM) in microalgae.

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