



Diatoms Biotechnology: Various Industrial Applications for a Greener Tomorrow

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OPEN ACCESS

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Specialty section:

This article was submitted to
Marine Biotechnology,
a section of the journal
Frontiers in Marine Science

Received: 01 December 2020

Accepted: 26 January 2021

Published: 23 February 2021

Citation:

Sharma N, Simon DP,
Diaz-Garza AM, Fantino E,
Messaabi A, Meddeb-Mouelhi F,
Germain H and Desgagné-Penix I
(2021) Diatoms Biotechnology:
Various Industrial Applications
for a Greener Tomorrow.
Front. Mar. Sci. 8:636613.
doi: 10.3389/fmars.2021.636613

The benefits of the complex microscopic and industrially important group of microalgae such as diatoms is not hidden and have lately surprised the scientific community with their industrial potential. The ability to survive in harsh conditions and the presence of different pore structures and defined cell walls have made diatoms ideal cell machinery to produce a variety of industrial products. The prospect of using a diatom cell for industrial application has increased significantly in synch with the advances in microscopy, metabarcoding, analytical and genetic tools. Furthermore, it is well noted that the approach of industry and academia to the use of genetic tools has changed significantly, resulting in a well-defined characterization of various molecular components of diatoms. It is possible to conduct the primary culturing, harvesting, and further downstream processing of diatom culture in a cost-effective manner. Diatoms hold all the qualities to become the alternative raw material for pharmaceutical, nanotechnology, and energy sources leading to a sustainable economy. In this review, an attempt has been made to gather important progress in the different industrial applications of diatoms such as biotechnology, biomedical, nanotechnology, and environmental technologies.

Keywords: diatoms, microalgae biotechnology, metabolic engineering, metabarcoding, sustainable economy, biofuel, lipids

INTRODUCTION

The global trend of economy and society is shifting toward building a greener and more sustainable society to combat climate and health issues. This is a critical issue, which is being approached with various interdisciplinary strategies to produce a wide range of sustainable products. For instance, biotechnology research has invested a significant number of resources, time, and money in studying microorganisms to exploit them for human consumption in multiple ways. Furthermore, the decades of research and improvisation in cultivation strategies, extraction, and harvesting protocols strongly support a good return on investment in industrial applications of microbes. A pinch of soil and a drop of water contain a diversity of microbes that controls major biogeochemical cycles and subsequently have the potential of producing an abundance of sustainable products. Since the beginning of this century, a high amount of research work has been published on industrial applications of microbes such as bacteria, yeast, and microalgae (**Figure 1**). But, limited attention

has been paid to diatoms which have the potential of becoming a robust sustainable industry because diatoms can continuously grow with an average annual yield of 132 MT dry diatoms ha^{-1} over almost 5 years (Wang and Seibert, 2017).

Diatoms are dynamic microorganisms with rich diversity and detailed membrane design. They are the most dominating phytoplankton with an overall number of around 200,000 species having complex variability in dimensions and shapes (Round et al., 1990; Smetacek, 1999; Mann and Vanormelingen, 2013). Diatoms' distinctive characteristic compared to the phytoplankton community is their silica cell wall, known as a frustule. This innate ability to uptake silicon from the environment has made them an interesting community of microbes since the 19th century. Few studies have stated the role of frustule biosilicate as pH buffering material which facilitates shifting of bicarbonate to CO_2 dissolved in cell fluids (the latter is readily metabolized by diatoms) (Milligan and Morel, 2002).

The access to advanced microscopes and modern genetic tools enabled us to study the detailed frustule structure and validate metabolic pathways involved in absorption, transportation, and polymerization of silicon and other biomolecules like lipids (Knight et al., 2016; Zulu et al., 2018). Furthermore, this advanced knowledge of metabolic pathways and validation of diatom structure can be applied to produce a wide range of renewable products such as optoelectronics, biofuels, nutritional supplements, ecology tools, etc. (Marella et al., 2020).

Other common factors that have shaped the evolution of diatoms are their ability to adapt and grow in various natural resources; fresh and marine water, wastewater, rivers, and oceans. Their abundance and adaptability in a wide range of climate and geographical areas make them suitable for different applications (Jin and Agustí, 2018). It was reported that diatoms are responsible to produce yearly, 40% of the organic carbon and 20% of oxygen (Tréguer et al., 1995; Falkowski et al., 1998; Afgan et al., 2016). Besides, these photoautotrophic organisms are involved in biogeochemical cycles, which play a significant role in global carbon fixation, carbon sequestration, and silicon cycle. They are also suitable candidates to capture nitrogen and carbon from various sources, which can be exploited by waste management and the biofuel industry to create carbon-neutral fuels (Singh et al., 2017). Furthermore, these algae are used to produce nutraceutical compounds, such as vegetarian proteins, omega, and other essential fatty acids for pharmaceutical industries (Wen and Chen, 2001a,b).

Multiple epidemiological, clinical, and pre-clinical studies have shown that omega fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are useful in slowing down age-related diseases such as cardiovascular diseases and cancer (Cole et al., 2010; Dyal, 2015; Thomas et al., 2015; Wang and Daggy, 2017). The development of diatoms strains rich in omega fatty acids can replace the dependence on fish as a source of omega oils and reduce the problems associated with seasonal variations and ocean pollution which might affect the biochemical composition of fish oil (Alves Martins et al., 2013). Also, various marine diatoms are considered for the commercial production of antioxidant pigments such as fucoxanthin and other carotenoids. It has been reported that these pigments

exhibit various protective effects such as strong antioxidant activities (Xia et al., 2013).

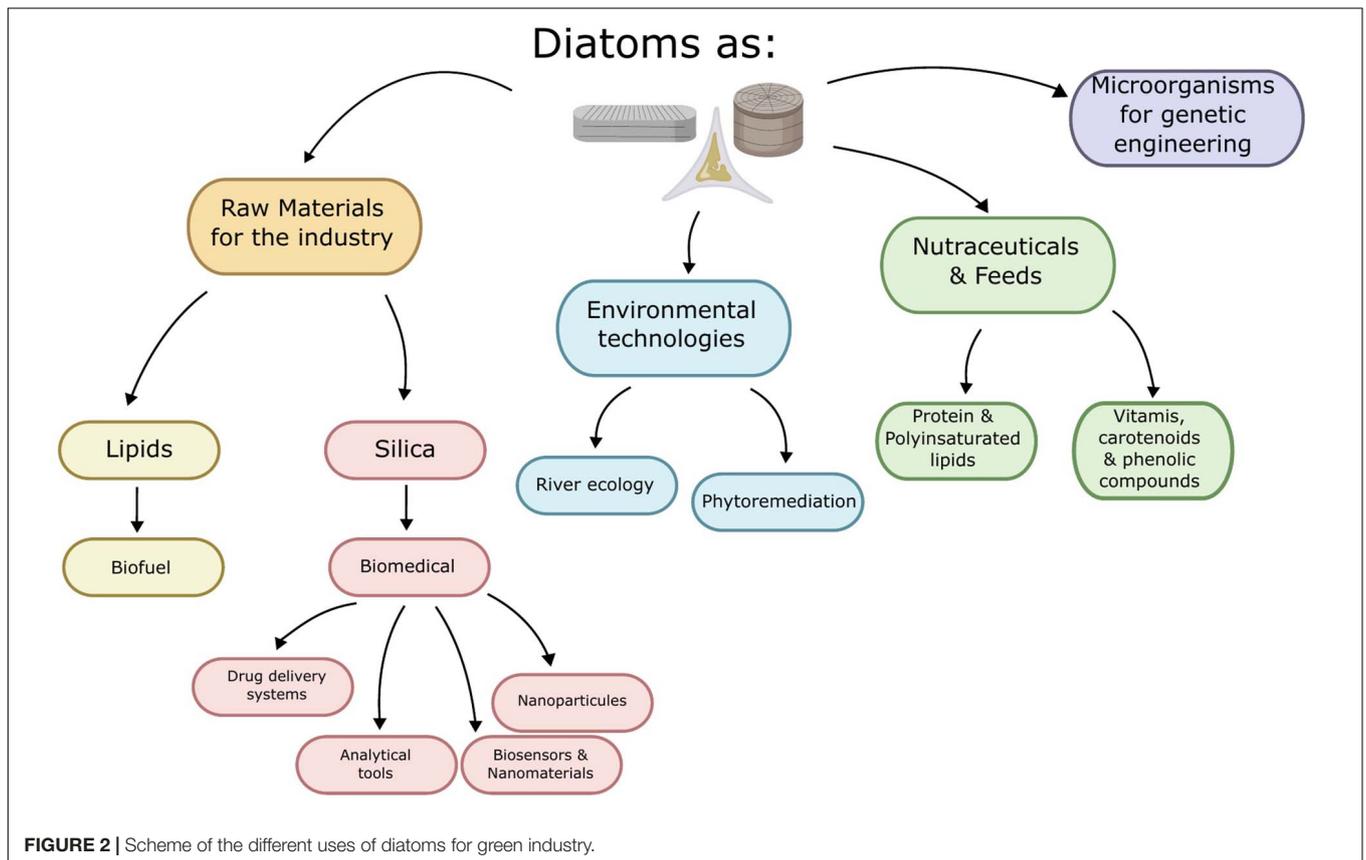
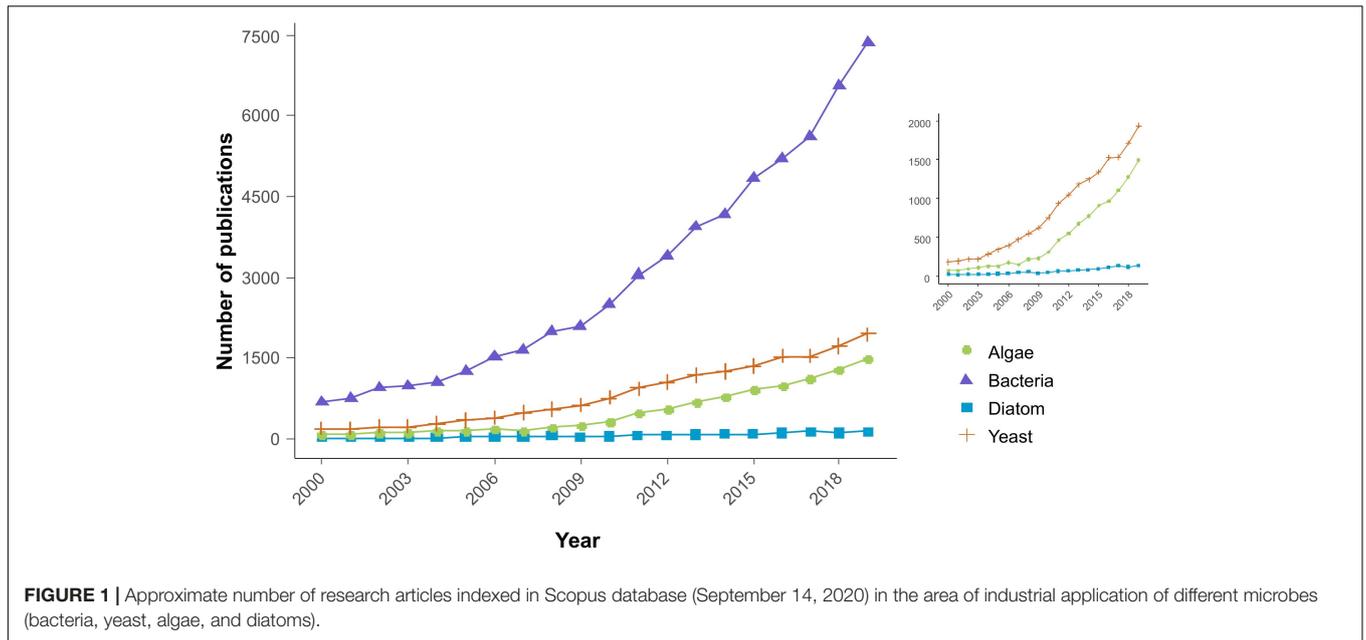
Thus, the flexible and complex nature of diatoms offers immense possibilities to develop a wide range of sustainable products and contributes to carbon neutrality. Because of its dimensions, pore distributions, and geometries, it is studied to develop tools for nanotechnology and biomedical industry such as nanofabrication techniques, chemo and biosensing, particle sorting, and control of particles in micro- and nano-fluidics (Mishra et al., 2017). Silica and biosilica can be used to develop advanced nanomaterial for electronic and optical technologies which can be employed for ultra-sensitive detection of biological compounds (Dolatabadi et al., 2011).

Recent accomplishment in diatoms metabarcoding, a reference database of the global population of diatoms, has advanced its use extensively in studying ecological problems such as climate change, acidification, and eutrophication (Nanjappa et al., 2014). Because of its robust nature and potential to inhabit different photic regions, from the equator to the poles, diatoms offer the potential to develop tools and products for all geographical regions (Medlin, 2016). The technological and infrastructure advancements of diatoms-based applications are at a new level. Besides, it requires different kinds of optimization either in laboratory or large-scale research such as energy utilization for different steps, financial modeling, and collaborating with different industries to make diatom-based products commercially successful. However, the standardization at various levels such as optimization of culture conditions, genetic tools, genome and transcriptome sequencing make diatoms based products commercially viable.

Therefore, this review aims to provide a better understanding of the potential of diatoms research at a laboratory scale. We have tried to provide comprehensive information on a variety of diatoms applications such as energy, biomedical products, and environment monitoring which are being investigated at different levels. All these applications have the potential to contribute toward a greener tomorrow. The purpose of the research is to increase the sustainable economy while reducing the dependence on non-renewable resources. Therefore, recovering and producing various sustainable products like biofuels, feed, bioactive molecules, and services like environment monitoring embedded in diatoms is a promising opportunity to be seized as shown in **Figure 2**.

BIOFUEL INDUSTRY

Fast globalization and industrialization have impacted the ecosystem widely but shutting or slowing down the globalization is not the solution. At the moment, almost 95% of all the transportation industry is based on a non-renewable source of energy (Rodrigue and Notteboom, 2013). Therefore, developing sustainable and carbon-neutral fuels could reduce the existing dependence on fossil fuels and contribute to bringing back harmony in nature without disrupting the existing economic development. Few economic aspects of biofuel production from microalgae such as biodiesel productivity, land use, and oil



yield support the use of microalgae for commercial production as compared to corn and other food crops. The oil yield for microalgae with high oil content is almost 15-fold more as compared to corn. Whereas, the land use for corn and maize is 66-fold more as compared to microalgae (Brocks et al., 2003).

The microalgae such as diatoms are the promising feedstock to replace non-renewable sources of energy. It has been proven by geochemists that algal lipids are the major feedstocks of petroleum and these lipids act as the biomarker remaining stable for millions of years (Brocks et al., 2003). The main biomarker

for the diatoms is the ratio of C28 and C29 steranes and highly branched isoprenoid alkenes which are found in high-quality oil fields around the globe (Katz et al., 2004).

Moreover, targeting the diatom lipids by manipulating and optimizing the growth and culture conditions such as light, stress, and nutrients can provide an interesting alternative to help meet the existing demands of commercial production of biofuel. Knowing the potential of diatoms to accumulate high lipids and varied compositions of fatty acids, diatoms are an underexploited area of the biofuel industry. The most predominant saturated and unsaturated fatty acids in diatom species are 14:0; 16:0, 16:1, 16:2, 16:3, 18:1, 18:2, 18:3, 20:4, and 20:5 (Dunstan et al., 1993; Sharma et al., 2020). Various reports have been published on different species of diatoms regarding the lipid yield and triacylglycerol accumulation (TAG) under different treatments as shown in **Table 1**.

It is possible to improve the quality of biodiesel by optimizing the content of different fatty acids that impacts biodiesel properties; cetane number, level of emissions, cold flow, oxidative stability, viscosity, and lubricity (Knothe, 2005). Fatty acids with chain lengths from C16 to C18 should contribute the maximum amount in the final product (Knothe, 2009). Some researchers have reported that a high percentage of mono-unsaturation is also desirable for biodiesel (Knothe, 2012). Thus, optimizing the

fatty acid profile along with increased biomass will significantly enhance their economic value.

Statistical analyses predicted that 100 mt/ha/year biomass of diatoms is required for commercial biofuel production (Gallagher, 2011). Over 10 years, productivity range was observed to be between 29 and 142 mt/ha/year (Sheehan et al., 1998; Huesemann and Benemann, 2009), these values motivate the researchers and industry experts to study diatom cell in-depth for the biofuel industry in both lab-scale and large scale.

Furthermore, the availability of advanced genetic tools can help to achieve the missing targets in developing diatoms cells as biofuel machinery (Radakovits et al., 2010; Tibocha-Bonilla et al., 2018). Based on theoretical calculations about the land area, lipid production, and photosynthetic energy conversion, the biofuel demand of the complete United States population could be met using only 5% of United States land (Levitan et al., 2014). Although various other factors that define the efficacy of biodiesel such as engine performance, that is based on (cylinder pressure, brake mean effective pressure, frictional mean effective pressure, power, torque, brake specific fuel combustion, brake thermal efficiency). The statistical data supports the use of microalgae-based biofuel but there are various limitations at a technological level for large-scale implementation of this project. Therefore, one of the alternatives is to use the blended form of

TABLE 1 | Lipid content and productivities of different microalgae diatom species (-: no data).

Microalgae	Culture condition	Lipid (% dry weight)	Lipid productivity mg L ⁻¹ day ⁻¹	TAG productivity μ mol L ⁻¹ day ⁻¹	% of TAG	References
<i>Thalassiosira weissflogii</i> P09	-	29.94 ± 1.17	7.27 ± 0.28	-	51.0 ± 3.2	d'Ippolito et al. (2015)
	Nitrogen limitation	-	-	19 (+20%)	-	d'Ippolito et al. (2015)
<i>Thalassiosira weissflogii</i> CCMP 1010	-	38.84 ± 0.78	4.87 ± 0.10	-	53.0 ± 1.9	d'Ippolito et al. (2015)
<i>Thalassiosira pseudonana</i> CCMP 1335	-	29.33 ± 1.17	1.72 ± 0.07	-	19.0 ± 0.9	d'Ippolito et al. (2015)
	High CO ₂ 20,000 ppm	-	-	45.5 ± 26 (exponential) (+285%)	-	Jensen et al. (2020)
<i>Cyclotella cryptica</i> CCMP 331	-	41.97 ± 1.26	2.98 ± 0.09	-	55.0 ± 2.1	d'Ippolito et al. (2015)
	Nitrogen limitation	-	-	45 (+20%)	-	d'Ippolito et al. (2015)
<i>Phaeodactylum tricornutum</i> CCMP 632	-	9.32 ± 0.28	2.09 ± 0.06	-	19.0 ± 0.6	d'Ippolito et al. (2015)
	Tn19745_1 strain + nitrogen limitation	-	-	-	45-fold increase	Daboussi et al. (2014)
	Dark	+2.3-fold	-	-	-	Bai et al. (2016)
textit <i>Navicula pelliculosa</i> (marine)	High CO ₂	-	-	75.7 ± 9 (stationary) (+50%)	-	Jensen et al. (2020)
	High CO ₂	-	-	158.4 ± 29 (stationary) (+35%)	-	Jensen et al. (2020)

biodiesel. It would be more efficient to make a blended version of petro-diesel and microalgae/diatoms based fuel for large-scale operation. The comparative studies of blended (20% microalgae fuel plus 80% petrodiesel) and 100% petrodiesel have no major performance variations. Furthermore, it was reported that there was a reduction in the CO, unburnt HC, and smoke emissions in blended form as compared to pure diesel (Soni et al., 2020).

BIOMEDICAL INDUSTRY

Drug Delivery Systems

The cost required to bring a new drug to the market has been estimated by the Tufts Centre for the Study of Drug Development at approximately 2.6 billion dollars (DiMasi et al., 2016). In addition, the current drug delivery systems have limited solubility, poor bio-distribution, lack of selectivity, premature degradation, and unfavorable pharmacokinetics (Aw et al., 2011a,b). Therefore, these limitations have motivated the research and development of alternative drug delivery systems to improve the performance of existing drugs (i.e., increasing bioavailability), while reducing undesirable effects. There is no doubt that existing biomedical technologies have increased the life span but the human society wants to improve the quality of life further by adopting environment friendly methods. Therefore, we should speed up the process and conduct in-depth research on using diatom frustules, even other bio-inspired alternatives for biomedical applications.

Among the available drug delivery tools (liposomes, nanogels, carbon nanotubes), the intricate frustule characteristics of diatoms such as specific surface area, thermal stability, biocompatibility, and alterable surface chemistry, have attracted attention for its use in drug and gene delivery. It took million years of evolution for diatoms to manufacture this level of complex and delicate structure to protect from the unwanted conditions like high temperature and variable light fluctuations. 3-D section analyses of diatom frustules have shown the availability of multiple pore patterns that range from nanometer to micrometer (Chandrasekaran et al., 2014; Cicco et al., 2015; Ragni et al., 2017). These characteristics are sufficient to explore alternative and low-priced silica-based materials for the biomedical industry (Mishra et al., 2017; Terracciano et al., 2018). Diatoms' frustule structure changes its homogenous nature, space, and intricate nature according to various environmental factors and silicon uptake efficiency (Knight et al., 2016). This ability can be used to change the frustule shape and pore size, which has multiple applications in the biomedical and nanotechnology industry. The process of biosilicification in diatoms is quite complex, it includes the role of silicic acid transporters, transportation of silica, and polymerization of silica monomers among other processes that have been extensively explained (Martin-Jézéquel et al., 2000; Knight et al., 2016). Moreover, a detailed investigation is being conducted to make the natural 3D porous structure an efficient substitute for delivery systems attributed to its chemical and mechanical features. For instance, some diatom species such as *Coscinodiscus concinnus* sp. (Gnanamoorthy et al., 2014), *Thalassiosira weissflogii* sp. (Aw et al., 2011a) are

potential drug carriers candidates due to their amorphous nature and morphology. Additionally, various studies have shown that diatoms microcapsules are effective carriers for poorly soluble and water-soluble drugs, which can be applied in both oral and implant applications (Aw et al., 2011a; Ragni et al., 2017).

The defined structural architecture of diatoms, such as pore volume and controllable particle size, allows the synthesis of biomolecules at the micro- to nano-scale (Losic et al., 2005, 2010; Slowing et al., 2008). The growth of fibroblast and osteoblast has been observed on functionalized frustules supporting the idea of using biosilica from diatoms as smart support for cell growth (Ragni et al., 2017). Regarding modified diatoms, Losic et al. (2010) have designed the magnetically guided drug carrier via a functional surface of diatoms with dopamine-modified iron oxide. This modification has shown the capability of sustained release of poorly soluble drugs for 2 weeks, presenting an enhanced performance for drug delivery (Losic et al., 2010). Moreover, genetically modified biosilica has been used to selectively deliver anticancer drugs to tumor sites (Delalat et al., 2015). Overall, these findings have opened the doors to novel drug delivery systems using renewable material. Therefore, all properties of diatoms such as uniform pore structure, chemically inert and biocompatible, non-toxic, easy to transport, filtration efficiency, and specific drug delivery make it a potential model for drug delivery tools (Curnow et al., 2012; Milović et al., 2014; Rea et al., 2014; Vasani et al., 2015).

Analytical Tools

The controlled production of nanostructured silica is possible through chemical and mechanical treatment for a wide range of applications. This nanopore structure has a huge potential to attach the desired biomolecule (enzymes, DNA, antibodies) and develop label-free analytical tools or enhance the catalytic properties. It has also been shown that enzymes and DNA (oligonucleotides) can be conjugated to silica (Losic et al., 2005; Zamora et al., 2009). The encapsulation of enzymes in diatom biosilica exhibits improved enzymatic properties as compared to other immobilization technologies (Kato et al., 2020).

Additionally, luminescent nano- and micro-particles have gained the attention of the interdisciplinary scientific community (biology, chemistry, and physics). Current available fluorescent labeling agents are quantum dots, lanthanide-doped compounds, and organic fluorophore-tagged nanobeads, which offer good optical properties and a broad excitation spectrum. However, these agents have limitations in properties such as photobleaching and biocompatibility. For instance, De Stefano et al. (2009) studied diatoms' potential to incorporate fluorophores with increased stability used to study the molecular event of antibody-antigen identification. Moreover, molecular recognition between antibody and antigen was observed in relation to the change in the photoluminescence spectrum of diatoms. Concluding that diatom's frustules, due to their high sensitivity, low-cost, and availability are ideal alternative candidates for lab-on-particle applications (De Stefano et al., 2008, 2009).

There is no concrete evidence of diatoms' presence in land animal bodies. Although, various studies showed the presence of diatoms in the internal organs and circulatory system of alive or dead animals in an aquatic environment (Ludes et al., 1996; Lunetta et al., 1998; Hürlimann et al., 2000; Lunetta and Modell, 2005; Horton et al., 2006; Levkov et al., 2017). The siliceous cell wall of this organism is resistant to degradation even under high acidic conditions for a long period (Lunetta and Modell, 2005). The investigation on the occurrence of these organisms inside dead bodies of aquatic environment that died from different causalities opened up a new possibility of forensic analysis through the examination of diatoms called 'diatom axiom' or 'diatom test' (Lunetta et al., 1998). The diatom test is based on the hypothesis that the microalgae will not enter the systemic circulation and reach other internal organs and tissues such as bone marrow unless the circulation is functional. A forensic examiner can determine whether the individual was alive when it was entering the water by checking the presence of diatoms in various organs and tissues (Levkov et al., 2017). In addition, since diatoms are highly sensitive to environmental conditions, different water bodies have different diatom species abundance which allows forensics to identify the drowning site (Zhou et al., 2020).

Despite being a distinguishable method, the diatom test has limitations also. One of the major issues is the occurrence of diatoms in a drowning medium. The absence or low presence of diatoms in a water body can lead to a false positive or negative result. The presence of diatoms in different layers (water base, deeper, and surface) of the water body also can be varied (Levkov et al., 2017). Rapid death is another situation where the diatom test can be wrong. Instant death when an animal or human enters the water body for various reasons such as cold shock and cardiac diseases will give a negative result in the diatom test (Smol and Stoermer, 2010). The use of alcohol or drugs is another factor that can mislead in the diatom test (Ago et al., 2011). Recent advances in DNA Barcoding and pyrosequencing opened the possibility of increasing the accuracy of the diatom test by checking the presence of plankton specific genes (e.g., Rubisco gene) in animal tissue (Fang et al., 2019).

Biosensors and Nanomaterials

The advances in biotechnological tools have made it effective to characterize the frustules of diatoms for the fabrication of optoelectronics. The uptake of various elements such as zinc and germanium by diatom like *Stephanodiscus hantzschii*, *Thalassiosira pseudonana*, etc. to change the pore size, shape, and other characteristics which are being studied for a variety of functions such as paleolimnological indicator and photonic device application (Qin et al., 2008; Jaccard et al., 2009). It has been reported a relationship between the amount of Zn/Si (zinc/silicon) and free zinc ions which can be used as a proxy of paleolimnological indicators (Jaccard et al., 2009). The studies have raised intriguing questions about the uptake and the process of various elements which need detailed validations. Although, they have reported that they could only detect Zn and Fe as chemical elements. The analysis of various trace elements could be used as an environmental indicator which indeed will

reduce the total workload needed to monitor large water bodies (Ellwood and Hunter, 2000).

The complex nanobiochemical machinery of diatoms can be exploited to fabricate a wide range of nanostructures with diverse optical and electronic properties (Rorrer et al., 2007). The ability to manufacture different pore size nanostructure molecules has inspired many research groups and industries to use diatoms in biosensing (De Tommasi, 2016). The incorporation of chemical elements such as germanium significantly affects the structure and size of frustule pores. A study tested the possibility of using Si-Germanium composite material in living diatoms in a two-stage photobioreactor cultivation process which reduced the pore size without disturbing the morphology (Rorrer et al., 2007). Another study reported that insertion of germanium in *Nitzschia frustulum* induces the nanocomb structure with blue photoluminescence (Qin et al., 2008). These nanostructure materials exhibit optical properties suitable for use in semiconductors and optoelectronics. Manufacturing of these materials combined with the silica frustule will improve the overall durability and range of applications in nanotechnology industries. These lab-scale scientific discoveries have shown that it is possible to create advanced nanomaterials in living diatoms.

Nanoparticles

The development of well-defined, advanced, and eco-friendly nanoparticles has attracted the attention of many researchers in the area of nanotechnology and its applications. Nanoparticles can be applied to study antimicrobial activity, catalyst, and filtering waste and chemical compounds. Biosynthesis of metallic nanoparticles in photoautotrophic organisms has gained the attention of nanotechnology researchers. Various approaches such as the sol-gel process, atomic layer deposition, chemical bath deposition, and inkjet printing process, have been used to modify the chemical composition of frustules. In this regard, an inexpensive chemical deposition technique was tested to deposit cadmium sulfide (CdS) on the surface on *Pinnularia* sp. without changing its morphology, since CdS has a wide range of applications in photodetectors and solar cells (Gutu et al., 2009).

Recently, it has been reported that diatoms can biosynthesize the nanoparticles such as gold and silver which has shown strong cytotoxicity against harmful microorganisms. Additionally, a highly ductile and malleable metal platinum (Pt) has been introduced in presence of dihydrogen hexachloroplatinate (IV) hexahydrate (DHH) in the living diatom *Melosira nummuloides*, without interfering the native morphology (Yamazaki et al., 2010). This is due to the platinum's excellent resistance to corrosion and stability at high temperatures, hence having application in a broad spectrum of industries, besides biomedicine. Other various examples of the on-going investigation of diatoms silica-based materials and their applications in biomedicine are shown in **Table 2**.

We have discussed the major application of diatoms for established industries such as biofuels, nanomaterials, and biomedicine. However, diatoms also have other fascinating applications in environment monitoring, animal feed, and aquaculture, which indeed have a huge potential considering

climate change and devastating impacts of globalization on ecology and environment.

ENVIRONMENTAL TECHNOLOGIES

River Ecology

Environment monitoring is an important aspect that is considered a necessity to deal with irregular changes or disturbances in our ecosystem. Therefore, researchers are developing tools using biotechnology and informatics to monitor the environment cost-effectively. Water resources are always under the influence of damaging anthropogenic pressures such as plastic waste and industrial sewage, which ultimately change or disturb the biogeochemical cycles and biodiversity. Besides, water is a universal solvent that holds the industries and economies together.

It is a well-established fact that diatoms hold the primary role in maintaining the aquatic ecosystem. Therefore, biodiversity assessment of diatom species in an environmental sample is one of the well-known strategies for biomonitoring. Presently, morphological assessment of the diatoms using microscopy is largely used which is time-consuming and requires special expertise (Larras et al., 2014). However, environmental metabarcoding has opened a quick way of analyzing the microbial DNA diversity in a natural environment such as flora and fauna (Bik et al., 2012; Taberlet et al., 2012). The metabarcoding approach is based on DNA sequencing a specific region (barcode) of the whole DNA extracted from an environmental sample (eDNA). For example, the sequencing data obtained from diatom metabarcoding are then used to assign precise taxonomic identification of the diatoms present in the eDNA sample, which are further compared with the conventional morphological database to confirm the efficacy of metabarcoding results. Diatoms metabarcoding tool has been optimized significantly to quantify the diversity of diatoms at the genus and species level (Vasselon et al., 2017; Kelly et al., 2018).

Currently, this approach is still in development, since various questions have been raised especially when deciding which are

the most suitable barcodes. The barcodes that had been used are the ribosomal small subunit, cytochrome c, and the internal transcribed spacer region combined with the 5.8S rRNA gene (Zimmermann et al., 2011; Luddington et al., 2012).

Another main issue is processing the sequencing output data through computing. This method must be consistent with government policies for environmental regulation. For instance, MOTHR is a comprehensive and efficient platform to study microbial diversity, but there are other bioinformatics software such as R, QIIME2 (Caporaso et al., 2010), LotuS (Hildebrand et al., 2014), and PIPITS (Gweon et al., 2015) that can be used to process a larger amount of data.

Additionally, various other research studies have supported the use of the diatoms metabarcoding approach as an alternative strategy to monitor river ecology on a timely basis. The results provide an estimated number of abundant and scarce species in samples obtained from different locations. Also, they give great insights into the fundamental status of the aquatic ecosystem (Larras et al., 2014). For instance, detailed evidence has been published by the Environmental Agency of the United Kingdom using diatoms indexes for river classification (Kelly et al., 2018). A similar study on detailed information on diatom biodiversity using metabarcoding has been conducted using environmental samples from Mayotte Island, France (Vasselon et al., 2017). Moreover, a recently published work studied the impact of treated effluents on benthic diatom communities that showed a systematic change in diatom community composition (Chonova et al., 2019). Concluding that detailed information about diatom diversity will give in-depth insights into climate change, micropollutants, and other organic pollutants, to study the disturbing effects of anthropogenic pressure on rivers. The use of metabarcoding for analyzing biodiversity is rapidly increasing and has been adopted by academic institutes and various companies/industries like Spygen (Canada), Naturemetrics (United Kingdom), IGAtch (Italy), Sinsoma (Austria), to name a few. This particular strategy has been adopted by public authorities as well and has shown the potential to be used as an additional screening tool to replace the existing methods, which require excessive

TABLE 2 | Biomedical applications of diatom silica-based materials using different diatom species.

Application	Organism	References
Specific nanoporous biosilica delivery system of chemotherapeutic drug, consisting in the attachment of antibodies and hydrophobic drug molecules, without using cross-linking, to the diatoms biosilica.	<i>T. pseudonana</i>	Delalat et al. (2015)
Modified frustule with self-assembled antibacterial aromatic amino acid conjugates Tyr–Zn ^{II} as a zinc carrier for its controlled release to bacteria and inhibiting the bacterial growth.	<i>N. palea</i>	Singh et al. (2020)
Genetically modified frustule with chimeric fusion proteins: diatom-derived silica targeting peptide Sil3T8 and a small synthetic antibody derivative to detect <i>Bacillus anthracis</i>	<i>T. pseudonana</i>	Ford et al. (2020)
Rapid and selective detection of typhoid using cross-linked amine-functionalized diatom photoluminescent biosensor.	<i>Amphora</i> sp.	Selvaraj et al. (2018)
Nano composite of nanoporous diatom-ZrO ₂ selective and highly sensitive sensor for non-enzymatic detection of methyl parathion.	<i>P. tricornutum</i>	Gannavarapu et al. (2019)
Biomaterial for negative electrode composed by a 3D-structured diatom biosilica for lithium-ion batteries, showing increased charge capacity compared to graphite.	<i>P. trainorii</i>	Nowak et al. (2019)
Improved capacitor performance of <i>in situ</i> coating of FeOx on live diatoms as a potential material for super capacitor electrodes.	<i>P. tricornutum</i>	Karaman et al. (2019)

infrastructure and human resources. It is indeed possible to make it a primary and permanent tool for river monitoring with advancements in sequencing, big data science, and artificial intelligence tools.

Phytoremediation

Besides the monitoring of river quality, water treatment is one of the major concerns for many countries around the world. In fact, human consumption has undoubtedly increased in the last few decades, subsequently, incrementing waste products presence in aquatic communities (Walker, 1983). Globally, almost 80% of the wastewater generated worldwide is discharged on rivers creating health and environmental hazards. The rise of nutrient accumulation in the aquatic system needs to be neutralized to maintain the balance in the environment. Increasing of pollution is disturbing the basic biogeochemical cycles, killing fish, depleting the dissolved oxygen, and producing different toxins, i.e., neurotoxins (Boyd, 1990). Hence, there is an urgent need to explore new ways and upscale the existing systems to test reports and mitigate pollution from rivers and lakes worldwide.

The use of microalgae for wastewater treatment has been a subject of research for a long period which could be applied in collaboration with small- and large-scale industries. The excess of industrial waste discharged in the aquatic system can be used as nutrient supply by diatoms. Different kinds of wastewater such as brewery (Choi, 2016), aquaculture (Tossavainen et al., 2019), and textile (El-Kassas and Mohamed, 2014) have been studied for phytoremediation capability and have shown interesting results. The published studies have established that diatoms and microalgae can treat the wastewater to an extent, therefore, it would be less damaging to treat the wastewater with microalgae/diatoms before discharging in water bodies. In addition, use the harvested biomass for different industrial products such as biofuel. It is safe to assume that it is possible to develop small scale business in collaboration with restaurants, breweries, textile industries, to name a few, to treat wastewater, and use the biomass

for the production of valuable products such as fertilizers (Suleiman et al., 2020).

Heavy metal pollution is one of the major challenges which comes from the industries working with chemicals and dyes. Diatoms species are desirable organisms to study heavy metal pollution because of the simplicity of metal exposure, absorption, and detoxification of metal ions by single cells. This is a unique detoxification process of diatoms and microalgae due to metal-binding peptides known as phytochelatins (PCs) that protect photosynthetic organisms from heavy metals (Grill et al., 1985). Some intracellular PCs have been characterized in cultures of *P. tricornutum* exposed to different metals such as Cd, Pb, or Zn. Besides, they are used widely in waste degradation considering the unique structure of diatoms and their ability to respond to the changing environment (Glazer and Nikaido, 2007).

A study published in 2015 have reported a novel diatom *Bacillariophyta* sp. (BD11ITG) from petroleum biorefinery wastewater that can degrade phenol in a concentration range of 50–250 mg/L in Fog's media (Das et al., 2016). Another example of the degradation of toxic molecules like phenylalanine hydroxylase into less toxic compounds using simple enzymatic oxidation has been identified in diatoms during the metabolism of phenanthrene and pyrene (Wang and Zhao, 2007). These results are relevant considering that around seven billion kg of phenol is produced for oil refining, pesticide production, and to use in the pharmaceutical industry. Traditional phenol removal techniques involve several steps including the generation of by-products, which increments the cost of the treatment (Senthilvelan et al., 2014). However, there are very few reports available on exploiting the potential of diatoms in biodegrading waste materials. It is interesting to note that the studies have shown interesting results but the field of algae biotechnology requires more entrepreneurs to join the pieces of industrial and academic research to build a successful circular economy. Furthermore, there are some upcoming and growing ventures and companies in microalgae working in diverse applications and producing valuable products such as healthcare, animal feed, water management, chocolates, etc. (Table 3).

TABLE 3 | Different industries producing variety of products from microalgae and diatoms around the world.

Company	Products/services	Country	Website
Algae Biotechnologia	Wastewater treatment, animal nutrition, carbon dioxide fixation, biofuels, human health	Brazil	http://www.algae.com.br/site/pt/
Algae Farm	Omega3, diatom, water treatment and reuse, nutraceuticals, cosmeceuticals, algae based solar fuels cell, die sensitized solar panel, bioplastics	Canada	https://www.algaefarm.us/
Algorigin	Nutritional supplements	Switzerland	https://algorigin.com/en/
Algaetoomega	Omega 3, astaxanthin, animal feed	United States	https://algae2omega.com/
Algae Control Canada	Pond and lake water management	Canada	https://www.algaecontrol.ca/
The Algae Factory	Chocolate	Netherlands	http://thealgaeactory.com/
Algae Health	Antioxidants	United States	https://www.algaehealthsciences.com/
Swedish Algae Factory	Personal care products	Sweden	https://swedishalgaeactory.com/
Sabrtech	Recombinant proteins, fuel, nutraceuticals, aquaculture, etc.	Canada	https://www.sabrtech.ca/
Pondtech	Astaxanthin, aquaculture	Canada	https://www.pondtech.com/

DIATOMS AS NUTRACEUTICALS AND FEEDS

Multiple epidemiological and clinical trials have shown the health benefits of omega fatty acids from fish oils and algae extracts (Cole et al., 2010; Cottin et al., 2011; Thomas et al., 2015; Wang and Daggy, 2017). Besides, there are few publications on cardio-protective and cognitive performance of omega fatty acids which have led to the commercial production of infant foods, infant formula, fortified snack bars, and other dairy products supplemented with omega fatty acids (Arterburn et al., 2007; Cottin et al., 2011).

Diatoms have an immense nutritional value that can be used to produce novel compounds such as antioxidants, vitamins, animal feed, and vegetarian protein supplements. Several photosynthetic pigments have been identified in diatoms including carotenoids such as fucoxanthin (Kuczynska et al., 2015). Additionally, *Nitzschia laevis*, *Nitzschia inconspicua*, *Navicula saprophila*, and *Phaeodactylum tricornutum* extracts have a noticeable amount of EPA and DHA that can be used as a nutritional feed in human diet and animal feed (Kitano et al., 1997; Wen and Chen, 2001a,b; Wah et al., 2015; Tocher et al., 2019).

Moreover, diatoms are known to have diverse defense mechanisms in form of chemical substances for them to be protected against pathogens. For instance, *P. tricornutum* has a high amount of omega-7 monounsaturated fatty acids such as palmitoleic acid (C16:1) and other bioactive compounds that are active against gram-positive pathogens (Desbois et al., 2009). Furthermore, the EPA-rich marine diatom, *Odontella aurita*, used as a dietary supplement has shown antioxidant effects in rats (Haimeur et al., 2012). *O. aurita* has been approved to be commercialized as food in France by following EC regulation 258/97 in 2002 (Pulz and Gross, 2004; Buono et al., 2014).

Increasing the content of these bioactive molecules in diatoms has attracted a large amount of research. Some studies have managed to enhance the production of flavonoid and polyphenol content by culture modifications, for instance, cultivation temperature and nutrient supplementation in *Amphora* sp. (Chtourou et al., 2015). The general tendency when changing the culture temperature is an increase in lipid content in most species, while the chemical composition varied between species (Renaud et al., 2002). For example, the total amount of saturated

and monounsaturated fatty acids increases with temperature in *Rhodomonas* sp. (NT15) and *Cryptomonas* sp. (CRFI01). Whereas, there was a comparative decrease in polyunsaturated fatty acids in both *Rhodomonas* sp. (NT15) and *Cryptomonas* sp. (CRFI01) (Renaud et al., 2002).

GENETIC ENGINEERING OF DIATOMS

The debate on using genetically modified microalgae and diatoms is on-going. However, it is a more controlled alternative for the production of recombinant proteins or any precursor molecules, considering the use of bioreactors for their production. The employment of genetic engineering tools in diatoms, to produce or increase the yield of compounds, allows the companies to optimize their use in the applications mentioned above. Therefore, genetic engineering is a promising method and an important branch to be used in the diatoms industry to further enhance the economic value of diatoms. However, it comes with two big challenges, firstly, to redesign the natural metabolic pathways in order to increase the production of desired endogenous compounds, and secondly, producing new heterologous compounds.

In the last 20 years, several projects have shown that these challenges can be solved at lab scale, by optimization of transformation methods, utilization of different gene promoters, expression of recombinant proteins, gene silencing, and genome editing methods; such as targeted mutagenesis techniques using meganucleases, gene knockouts, TALENS, and CRISPR/Cas9. Marketable bioproducts like lipids, pigments, nanomaterials, food supplements, fuel, syntheses of chemicals, drugs, and metabolites have been produced in *P. tricornutum*, *T. pseudonana*, and other diatoms species. While most of these analyses are related to lipid production for biofuel or bioenergy purposes, other studies showed that diatoms are biological factories that can generate a wide range of products from food to pharmaceuticals biomaterial industry (Lauritano et al., 2016; Mishra et al., 2017; Slattery et al., 2018; Dhauadi et al., 2020; Sharma et al., 2020). In addition, there are few companies such as Algenol Biofuels, Synthetic Genomics, which have reported the use of genetically modified microalgae for the production of biofuels.

TABLE 4 | Sequence Database of different diatoms species.

Species	Genome database
<i>Phaeodactylum tricornutum</i> CCAP 1055/1	http://protists.ensembl.org/Phaeodactylum_tricornutum/Info/Index
<i>Thalassiosira pseudonana</i> CCMP 1335	https://genome.jgi.doe.gov/Thaps3/Thaps3.home.html
<i>Thalassiosira oceanica</i> CCMP 1005	https://genome.jgi.doe.gov/Thaoce1/Thaoce1.info.html
<i>Thalassiosira weissflogii</i> CCMP1030	https://genome.jgi.doe.gov/portal/
<i>Fragilariopsis cylindrus</i> CCMP 1102	https://genome.jgi.doe.gov/Fracy1/Fracy1.info.html
<i>Pseudo-nitzschia multiseriata</i> CLN-47	https://genome.jgi.doe.gov/Psemu1/Psemu1.home.html
<i>Pseudo-nitzschia multistriata</i> B856	http://apollo.tgac.ac.uk/Pseudo-nitzschia_multistriata_V1_4_browser/sequences
<i>Seminais robusta</i> D6	https://genome.jgi.doe.gov/portal/Semrobnscripome/Semrobnscripome.info.html
<i>Fistulifera solaris</i> JPCC DA058	https://trace.ddbj.nig.ac.jp/DRASearch/submission?acc5DRA002403
<i>Cyclotella cryptica</i> CCMP332	http://genomes.mcdb.ucla.edu/Cyclotella/download.html

TABLE 5 | Diatoms genetic engineering.

Species/strain	Genetic and molecular tools				
	Transformation methods and target compartment	Promoters: (S) strong, (I) inducible and (H) heterologous	Reporters (R) and resistance (Re) genes	Expression of recombinant proteins	Genome editing methods and gene silencing
<i>Phaeodactylum tricomutum</i> CCAP 1055/1	Biolistic (Cho et al., 2015) Electroporation (Niu et al., 2012) Conjugation (Zaboikin et al., 2017) Nuclear and chloroplast transformation (Xie et al., 2014)	(S): Lhcf (Fcp), light responsive (Karas et al., 2015), EF-1a, 40SRPS8, g-tubulin, RBCMT (Erdene-Ochir et al., 2019) and EF2 (Nymark et al., 2013), h4 (Fabris et al., 2020), HASP1 (De Riso et al., 2009). (I): rbcL (Xie et al., 2014), NR, low NO ₃ induce (Schellenberger Costa et al., 2012), V-ATPase C, AP1 low P induce (Lin et al., 2017) Fbp1, Fld, Isi1 iron-responsive (Yoshinaga et al., 2014) ca1, ca2 CO ₂ -responsive (Harada et al., 2005; Tanaka et al., 2016), U6, RNA polymerase III transcribed (Nymark et al., 2016) (H): CdP1, CIP1, CIP2, TnP1, TnP2 (Erdene-Ochir et al., 2016), CMV, RSV-LTR, PCMV, CaMV35S (Sakaue et al., 2008)	(R): GUS, GFP (Zhang and Hu, 2014), YFP, CFP (Zaboikin et al., 2017) cat (Karas et al., 2015), LUC (Cho et al., 2015), Aequorin (Falcatore et al., 2000) (Re): Zeocin and Phleomycin/sh ble, Nourseothricin/nat, Blastidicin-S/bsr, Streptothricin/sat, Neomycin/nptII (Karas et al., 2015)	Expression of Acyl-ACP thioesterases, increased accumulation of shorter chain (Radakovits et al., 2011). Malic enzyme (Trentacoste et al., 2013). G6PD (Wu et al., 2019), enhanced lipid productivity. Heterologous biosynthesis of the MIAs by CrGES expression under phototrophic conditions (Slattery et al., 2018), Vanillin production (Erdene-Ochir et al., 2019). PHBs for Bioplastics production (Hempel et al., 2011a). Human IgGαHBsAg:(Hempel et al., 2011b) and IgG1/kappa Ab CL4mAb: antibody to hepatitis B virus surface protein against the nucleoprotein of Marburg virus (Hempel and Maier, 2012). Over expression of DXS increased fucoxanthin synthesis (Eilers et al., 2016).	Targeted mutagenesis methods: meganucleases, gene knockouts, TALENS, and CRISPR/Cas9 (Poulsen and Kröger, 2005). Development of auxotrophic strains of <i>P. tricomutum</i> by CRISPR/Cas9 (Sakaguchi et al., 2011). A lipid producing strain through the disruption of the UDP-glucose pyrophosphorylase gene (Daboussi et al., 2014).
<i>Thalassiosira pseudonana</i> CCMP 1335	Biolistic (Poulsen et al., 2006) Electroporation (Buggé, 2015) Conjugation (Zaboikin et al., 2017)	(S): Lhcf9 (I): nr (161) SIT1, Si-starvation inducible (Davis et al., 2017), Thaps3_9619, Si-starvation inducible (Shrestha and Hildebrand, 2017), U6, RNA polymerase III transcribed (Weyman et al., 2015)	(R): YFP (Zaboikin et al., 2017) (Re): sh ble, nat (Poulsen et al., 2006)	Overexpression a multiple plasmids can be cotransformed; cloning multiple genes of interest Secretion of recombinant proteins has been shown. Localization of SiMat1-GFP (Kotzsch et al., 2016). Expression of the protective HslbpA DR2 antigen for the production of a vaccine against bovine respiratory disease (Davis et al., 2017), scFvTNT scFv and sdAbEA1 to detected <i>Bacillus anthracis</i> (Ford et al., 2016).	Targeted mutagenesis methods: meganucleases, TALENS, and CRISPR/Cas9 (Weyman et al., 2015). Gene silencing and gene knockouts are well established (Shrestha and Hildebrand, 2015).
<i>Thalassiosira weissflogii</i> (CCMP1030)	Biolistics (Cho et al., 2015)	(S): Lhcf2 (Cho et al., 2015)	(R): GUS (Cho et al., 2015)		

(Continued)

TABLE 5 | Continued

Species/strain	Genetic and molecular tools				
	Transformation methods and target compartment	Promoters: (S) strong, (I) inducible and (H) heterologous	Reporters (R) and resistance (Re) genes	Expression of recombinant proteins	Genome editing methods and gene silencing
<i>Pseudo-nitzschia multistriata</i> B856	Biolistics (Sabatino et al., 2015)	(S): h4 (Sabatino et al., 2015)	(Re): sh ble (Sabatino et al., 2015)		
<i>Pseudo-nitzschia arenysensis</i> B858	Biolistics (Sabatino et al., 2015)		(R): GUS, GFP (Sabatino et al., 2015)		
<i>Fistulifera solaris</i> JPCC DA058	Biolistics (Muto et al., 2015)	(S): Lhcf2 and h4 (H): RSV and CaMV35S (Muto et al., 2015)	(R): GFP (Re): nptII (Muto et al., 2015)	Overexpression of the endogenous GK improve lipid productivity (Muto et al., 2015)	
<i>Cylindrotheca fusiformis</i> CCAP 1017/2 –CYL	Biolistics (Kong et al., 2019)	(I): nr (Kong et al., 2019)	(R): GFP (Re): sh ble (Kong et al., 2019)		
<i>Navicula saprophila</i> NAVICI	Biolistics (Dunahay et al., 1995)	(S): ACCase (Dunahay et al., 1995)	(Re): nptII (Dunahay et al., 1995)		
<i>Chaetoceros gracilis</i> UTEX LB2658	Biolistics (Ifuku et al., 2015)	(S): Lhcr5 (I): nr (Ifuku et al., 2015)	(R): GFP, LUC (Re): nat (Ifuku et al., 2015)		

DXS, 1-deoxy-D-xylulose 5-phosphate synthase; *40SRPS8*, 40S ribosomal protein S8; *ACCCase*, acetyl-CoA carboxylase; *Acyl-ACP thioesterases*, acyl-acyl carrier protein thioesterases; *AP1*, alkaline phosphatase 1; *bsr*, blasticidin-S resistance gene; *Ca1*, carbonic anhydrase 1; *CaMV35S*, cauliflower mosaic virus 35S; *CdP*, *Chaetoceros debilis*-infecting DNA virus; *CIP*, *Chaetoceros lorenzianus*-infecting DNA virus; *cat*, chloramphenicol acetyl transferase conferring resistance to chloramphenicol; *CRISPR*, clustered regularly interspaced short palindromic repeats; *CFP*, cyan fluorescent protein gene; *CMV*, cytomegalovirus; *Fcp*, diatom light-regulated promoters of the fucoxanthin chlorophyll a/c-binding protein genes *Lhcf*; *EF-1 α* , elongation factor 1 alpha; *EF2*, elongation factor 2; *Fbp1*, ferrichrome binding protein1; *Fld*, flavodoxin; *CrGES*, *Catharanthus roseus* geraniol synthase; *G6PD*, glucose-6-phosphate dehydrogenase; *GK*, glycerol kinase; *GFP*, green fluorescent protein gene; *HASP1*, highly abundant secreted protein 1; *h4*, histone H4; human *IgG α HbSAg*, antibody against hepatitis B virus surface IgG1/kappa Ab CL4mAb; *HslbpA DR2*, *lbpA DR2* antigen from *Histophilus somni*; *Isi1*, iron-starvation-induced gene 1; *MIA*s, monoterpene indole alkaloids; *nptII*, neomycin phosphotransferase II; *NR*, nitrate reductase; *nat*, nourseothricin acetyl transferase; *P*, phosphate; *PHBs*, polyhydroxybutyrate; *PCMV*, promoter sequences of the cytomegalovirus; *psba*, *PSII* reaction center core 2 quinones are associated with D1; *Lhcr5*, red algal-like LHCs; *RBCMT*, ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit N-methyltransferase I; *RSV-LTR*, Rous sarcoma virus long terminal repeat; *rbCL*, Rubisco large subunit; *SiMat1*, silica matrix protein; *SIT1*, silicon transporter; *scFvTNT*, single chain antibodies; *sdAbEA1*, single domain antibodies; *U6*, small nuclear RNA of the U6 complex; *sh ble*, *Streptoaloteichus hindustanus* bleomycin resistance gene; *TnP*, *Thalassionema nitzschoides*-infecting DNA virus; *TALENs*, transcription activator-like effector nucleases; *sat*, treptothricin acetyl transferase; *TAG*, triacylglycerol; *g-tubulin*, tubulin gamma chain; *GUS*, *uidA b-glucuronidase*-encoding gene; *V-ATPase C*, vacuolar H⁺-ATPase; *YFP*, yellow fluorescent protein gene.

Moreover, the approach of synthetic biology along with high throughput sequencing technologies open the doors to understanding the whole genome, the proteins that it encodes, and the regulatory elements of the cell during cellular growth and division (Hildebrand and Lerch, 2015; Huang and Daboussi, 2017). Several sequencing projects have been performed in *P. tricornutum* and *T. pseudonana* strains (Armbrust et al., 2004; Bowler et al., 2008; Koester et al., 2018; Rastogi et al., 2018), generating the transcriptomic and proteomic data sets that make possible precise reconstructions of metabolic networks (Fabris et al., 2012; Levering et al., 2016). Recently, the Synthetic Diatoms Project website has been launched as a platform to provide information to grow, transform, edit, and analyze *P. tricornutum* and *T. pseudonana*¹. These projects have been used as a springboard to facilitate genome annotation for other diatoms species: *T. oceanica*, *T. weissflogii*, *Fragilariopsis cylindrus*, *Pseudo-nitzschia multiseriata*, *Pseudo-nitzschia multistriata*, *Seminavis robusta*, *Fistulifera solaris*, *Cyclotella cryptica* (Table 4).

Diatoms are a robust model for genome editing and cell transformation. Optimized methods of DNA delivery have been developed using biolistic or via electroporation. In both techniques, the transgenes are randomly integrated into the genome, with multiple integration events, variable transgene copy numbers, and chromosomal positions. The biolistic gene transfer method affects genome integrity due to the break and repair of the DNA double-strand by non-homologous end joining (NHEJ) (Zaboikin et al., 2017). However, this method is needed if the aim is to transform the chloroplast genome. An alternative transformation technique is the extrachromosomal-based expression approach that depends on vectors containing a yeast-derived sequence, which can be delivered through bacterial conjugation using *E. coli* (Karas et al., 2015).

An important element for genetic engineering is the promoter. The most commonly used are the light-regulated promoters of the fucoxanthin chlorophyll a/c-binding protein genes *fcxA/B/C/D* (LHCF) (Zaslavskaja et al., 2000; Nymark et al., 2013). Alternatively, the elongation factor 2 (EF2) promoter sequence is a constitutive promoter (Seo et al., 2015). Recently, the most abundant secreted protein in *P. tricornutum* was identified, named “highly abundant secreted protein 1” (HASP1), and the activities of its promoter and the signal peptide were characterized using green fluorescent protein (GFP) as a reporter (Erdene-Ochir et al., 2019). A couple of inducible promoters have been reported: like nitrate reductase (NR) and alkaline phosphatase gene promoters in *P. tricornutum*, which are induced under nitrogen or phosphate starvation respectively (Slattery et al., 2018; Fabris et al., 2020) and glutamine synthetase gene promoter, induced by a blue light pulse (De Riso et al., 2009; Erdene-Ochir et al., 2016). In addition, promoter regions containing diatom-infecting viruses (DIVs) mediated a significantly higher level expression of the reporter gene in cells in the stationary phase compared to the exponential phase of growth (Kadono et al., 2015). Other elements needed for genetic engineering are reporter genes and selection markers. Among

reporter genes, beta-glucuronidase *uidA* (GUS), fluorescent proteins like GFP/YFP/CFP, chloramphenicol acetyltransferase conferring resistance to chloramphenicol (CAT) and luciferase (LUC) are the most employed, other reporter proteins are listed in Table 5. The classic selection markers in diatoms are genes that confer resistance to zeocin, phleomycin, and nourseothricin, as shown in Table 5 are the most used. An alternative to using selective markers is the use of auxotrophic strains, such as uracil, histidine, and tryptophan auxotrophs (Sakaguchi et al., 2011; Slattery et al., 2020). Moreover, it is considered that the urease gene, either in an inactive or edited form, is an interesting tool for the selection of *P. tricornutum* and *T. pseudonana* strains (Weyman et al., 2015; Hopes et al., 2016; Slattery et al., 2018). An endogenous selectable marker in diatoms was generated by point mutations at a conserved residue Gly290 to Ser/Arg in the phytoene desaturase (PDS1) gene, which confers resistance to the herbicide norflurazon (Taparia et al., 2019).

Concerning heterologous recombinant protein expression, diatom gene codon optimization is required for optimal expression; to avoid silencing expression and better protein translation. Although it has not been reported in diatoms, different projects which were done in green algae, have shown that including introns in the expression cassette can increase transcript abundance (Baier et al., 2018, 2020; Kong et al., 2019). In addition, 5'-UTR and 3'-UTR of nitrate reductase (NR) allow the control of timing and level of transgene expression in *C. fusiformis* (Poulsen and Kröger, 2005). Down-regulation of gene expression can be achieved through silencing by expressing antisense repeat sequences of target genes (Table 5).

Industrial processes using diatoms are cost-effective and have performed well in large-scale cultures (Benedetti et al., 2018). This is supported by the plasticity to adapt to extreme environmental conditions of diatoms, making them great candidates for sustainable biofactories (Kung et al., 2012; Cho et al., 2015; d'Ippolito et al., 2015). Altogether, these developments in metabolic pathways and synthesis of heterologous compounds represent promising insights for the improvement of yield, quality of products, and sustainability in the use of diatoms as cell factories.

CONCLUSION AND FUTURE PERSPECTIVES

The documented studies stated the astounding nature and possible all-round use of diatoms. This is one of the approaches to increase human consumption of renewable products and contributes toward reducing carbon emissions. Although the commercial application of diatoms still needs improvements, it is indeed a crucial research area for human wellbeing. For example, developments in diatoms research can lead to innovative products in domains of drug delivery, sensing, and detection parts to build complex biomedical devices and nanoparticles for waste degradation. Moreover, recent advancements in sequencing technology and processing large biological datasets

¹<https://www.syntheticdiatoms.org/>

have made it possible to label and store the global biodiversity of diatoms in all geographical locations.

One of the major challenges in diatom-based industries is scaling up the process for large-scale manufacturing which is dependent on many micro and macro factors such as cultivation, harvesting, drying, genetic modification, lack of genomic, proteomic, and metabolic information, etc. However, it is possible to overcome these challenges in near future with advancements in genetic tools, bioreactors, and other infrastructure changes. In general, there are many challenges in bio-based industries at different levels; academic/industrial research, infrastructure, policies, education, and information gaps. The advancements in academic research and discoveries are consistent considering the publications but it requires support from other domains such as the development of infrastructure, reducing the knowledge gaps between scientific researcher and entrepreneurs, changes in the policies at both national and international level. And to conclude, the recent research phenomenon blasted in the last decade, which is diatoms' industrial potential, still leaves many unsolved questions. Major questions will involve studying the extent of genetic or artificial manipulation without compromising its intact structure and delicate silica pattern. The unfolding of various missing links in genetic engineering, cultivation, and harvesting will make it possible to replicate complex plant pathways in diatoms. These tools have opened the door to study diatoms for eco-friendly processes.

Although the use of silica for food and agriculture has been approved by the FDA and is also labeled/classified as non-carcinogenic by the International Agency for Research on Cancer, this could be a big step toward accelerating its use at the biomedical level. It is not yet approved for biomedicine as it requires long-term evidence (Terracciano et al., 2018). All the biomedical inventions are scrutinized by multiple stakeholders like research leaders, public authorities such as provincial and federal government, before they reach the stage of commercial distribution. It is understandable considering that it will be used directly in the human body. Therefore, an innovative and different approach is required to bring in the academic researchers and bio-entrepreneurs to

speed up the innovation rate in biomedical industry without harming the screening process set by public health authorities. The collaboration between entrepreneurs and researchers will allow thorough evaluation of the market for new inventions, manufacturing, investment, and globalization of the product. It seems plausible considering the rapid advancements in the biomedical infrastructure around the world. This has been demonstrated by the quick inventions in response to COVID-19 and should be adopted to be applied in other biotech based industries (Harris et al., 2020). The simultaneous advancements in the use of silica-based support system for drug delivery along with the change in infrastructure in pharmaceutical industries and hospitals to deliver these technologies to the users is possible in the near future. The other requirement is to join the gap of vast and complex scientific information and knowledge between entrepreneurs and academic researchers.

AUTHOR CONTRIBUTIONS

NS and ID-P conceived, designed, and led the study. NS, DS, AD-G, EF, and AM collected and analyzed the data, and prepared the figures and tables. NS, DS, AD-G, EF, AM, FM-M, HG, and ID-P authored and reviewed the drafts of the manuscript, and approved the final manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We acknowledge that financial support for this review was funded by the Canada Research Chair on plant specialized metabolism Award No. 950-232164 to ID-P. Thanks are extended to the Canadian taxpayers and to the Canadian Government for supporting the Canada Research Chairs Program. Additional support in the form of scholarships to NS, DS, AD-G, EF, and AM from Mitacs—Acceleration program grants nos. IT12310 and IT16463 to ID-P is also acknowledged.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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