International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 869-877
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Review Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Diatoms from Saline Ecosystems and Biotechnological Applications: An Overview

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Received: 10 Mar 2019 / Accepted: 9 Apr 2019 / Published online: 1 Jul 2019
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Abstract
Diatoms are unicellular, eukaryotic, microscopic algae that are abundant and flourish in diverse ecosystems. They are also interesting due to their cellular structure with deposition of silica that is due to biomineralization. There are many reports on the diversity of these unique microbes in both fresh water as well as marine / saline ecosystems. They belong to the phylum *Bacillariophyta* family *Bacillariophyceae*. The DiatomBase is an online free database that provides information on the taxonomy, diversity, recognized species, etc. Currently, there are 2,546 accepted species in this database. These unique phototrophs are also found in saline ecosystems and have been explored for basic as well as applied research. They have applications in oil exploration, climate change, biomineralization, designing, nanoscience, forensic science, etc. The present article is a review to present the current information on diatoms from saline environments and their applications in various areas.

Keywords
Biomineralization, Diatoms, Nanomaterials, Silicon Deposition Vesicle.

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INTRODUCTION
"Pastures of the sea" [1], "Jewels of the sea", "Living opal", "Silicon marvels" are some of the titles given to these one of a kind microalgae [2, 3]. Its unique feature is the fact that they synthesize and encase themselves in beautified walls of silicon called shell or frustules, (that looks like a clamp) having two valves that holdfasts together just like a lid to the bottom of a petri-dish [4]. It is made up of silicon nanostructures who impart structural coloration due to photonic nature. Hence, the aforementioned titles are justified and have also been an inspiration for architectural as well as other designs. This feature coincidently also has earned "diatoms" their name which is derived from the Greek words '*dia*' meaning "through" and '*temnein*' meaning "to cut in half". Diatoms are microscopic, eukaryotic algae that are diverse, abundant and widely distributed in nature. Taxonomically they belong to the Domain: *Eukaryota*; Phylum: *Ochrophyta*; Division: *Chrysophyta;* Class *Bacillariophyceae* [5]. The phylogenetic drift causing the evolution of diatoms occurred roughly 150-185 million years ago, this is supported by the first diatom fossil discovered [6].
Diatoms can be broadly classified into "Pennate", i.e., having bilateral symmetry; and "Centric", i.e., with radial symmetry (Figure 1). Diatoms having bilateral symmetry can either consist of openings into the valves called "raphes" (instantaneous genera include *Nitzschia, Phaeodactylum*, *Navicula*, etc.) or those without the raphes (for example, *Fragilaria, Synedra, Diatoma, etc.*). The genera included into those having radial symmetry are *Chaetoceros, Thalassiosira, Biddulphia,* etc. These

most common and abundant type of phytoplankton are inherent to terrestrial areas, Limnetic and benthic zones of lotic and lentic fresh and marine water bodies, in and under ice, salterns, mud flats and temperate coastal areas [5]. The present article deals with mainly diatoms found in saline ecosystems and their applications. They are useful in studying acidification and eutrophication in environment, biomineralization, oil exploration, forensic science, designs, nanomaterials, etc.

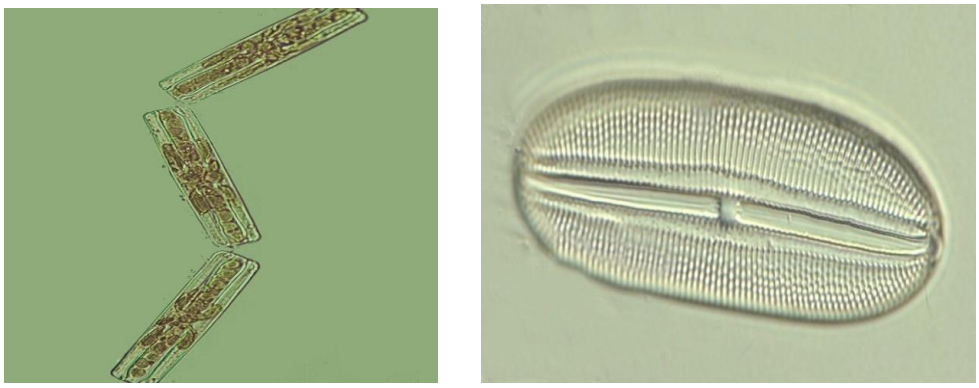

Figure 1. Photomicrographs of diatoms from saline ecosystem (a) *Tabelleria* sp. (b) *Nitzchia* sp. (Source: FWC Research licensed by CC BY-NC-ND 2.0)

Diatoms are mostly unicellular, but they have been noted to form filaments. Diatoms form various shapes of diverse complexity ranging from bi-lobed to ribbons to even stars with size ranging from 2-200 micrometers [7]. The mechanism of morphogenesis of some shapes is explained by [8].

CULTIVATION OF HALOPHILIC DIATOMS

Diatoms can be collected from their natural aquatic habitat using nets. The minimum mesh size used for the collection of diatoms is 0.03-0.04 mm. For their cultivation, generally the natural marine samples are used. The algal cultivation media for example: Guillard's f/2 medium [9] and DM medium are used for their cultivation [10]. The major inorganic compounds used are: $MgSO_4.7H_2O$, $NaHCO_3$, $Ca(NO_3)_2.4H_2O$, KH_2PO_4, Na_2SiO_3, $Na_2.EDTA$, H_3BO_3, $MnCl_2.4H_2O$, vitamins such as vitamin B_{12} (1-3 µg L^{-1}) and biotin. Bacillariophycean medium contains $Ca(NO_3)_2.4H_2O$, K_2HPO_4, $MgSO_4.7H_20$, Na_2CO_3, $Na_2SiO_3.9H_2O$, Fe-citrate and citric acid as its major components. Another much widely known medium is the Enriched Seawater Artificial Water (ESAW) medium, which mimics the composition of seawater [11]. The illumination in lab ranges from 1000-5000 lux for approximately 18 hours/day. The pH is usually maintained in the range 8.0-9.0, and salinity depends upon the diatom to be cultured [12]. The cultivation can be carried out in airlift photobioreactors (ALPBR), glass bubble columns or even open ponds. The cultivation process can be carried out in batch fermentation or semi-continuous and continuous fermentation, in which case, the cells need to be harvested every 12 hours or so [13].

AND IDENTIFICATION METHOD OF DIATOMS

Similar to all the microorganisms, the primary method for identification of diatoms is based on studying its morphology by light or phase contrast microscopy. Due to their typical morphological characteristics, diatoms can be easily differentiated from the other algal species such as cyanobacteria. Natural auto-fluorescence can also be used. But morphological grouping based upon microscopy of diatoms alone, may result into false interpretations as similar morphological structures may not necessarily represent phylogenetically coherent cells due to the property of pleomorphism [14, 15, 16]. Additionally, it has been found that numerous diatoms contain hidden or "cryptic" species which are not readily recognized or differentiated with ease by classical microscopic tools [17, 18].

Hence, molecular tools are employed for further characterization of diatoms and to study their phylogenetic taxonomy [19]. Generally, a polyphasic identification process consisting of more than one step with each step representing one tool (microscopic, biochemical, molecular, etc.) is used for accurate identification. For instance, light microscopy along with 16S rRNA sequencing or any other culture independent molecular technique will help better decode its position in the microalgal domain. But certain diatom variants may exist which lack the signature sequences used for PCR amplification. Hence, results of 16S rRNA sequencing should be considered as the minimal approximation

of the present richness of diatoms in metagenomics studies [20]. Gene amplification using PCR technique can be conducted followed by analysis using denaturing gradient gel electrophoresis (DGGE) for identification purposes [21].

SALT CONCENTRATION RANGE OF HALOPHILIC DIATOMS

Halophiles are the microorganisms which require comparatively higher salt (NaCl) concentration for growth and are distributed in all the three Domains: Bacteria, Archaea and Eukarya. They can be classified based upon the salt concentration requirement for growth. Based on their salt requirement (%w/v NaCl) they are classified as: Slight halophiles (1-5%); Moderate halophiles, (5-20%); and extreme halophiles grow optimally at salt concentration above 15-20% [22, 23]. The seawater generally contains 3.5 (% w/v) salt concentration and areas having a higher salt concentration than this, are considered as hypersaline environments. They have a general salt range from 4-30% [24]. Diatoms can be stenohaline or euryhaline, and diatoms having both of these properties predominate in the marine littoral zones [25, 26, 27]. However, in marine basins, generally euryhaline diatoms are noted. This property of euryhalinity might be an adaptation to tackle physical phenomena such as evapotranspiration, which increases the salinity or flooding of the basins by fresh water, which decreases salinity [28]. Halophilic diatoms can be found in all kinds of saline water bodies including oceans, sea, various inshore salt bodies such as soda lakes and saltpans. Diatoms can also be classified into slightly, moderate or extreme halophiles. The reported salt range of diatoms is 0.5-15.0 (%w/v) NaCl. Clavero found that the genus *Amphora* has members that are slightly halophilic, *Amphora arcus* (3.5%) as well as moderately halophilic: *Amphora coffeaeformis* (5.0%) and *Amphora cymbamphora* (5.2%). The species belonging to the genus *Nitzschia* are moderate halophiles for example, *Nitzschia rhopalodioides* (5.0%), *Nitzschia frustulum*(5.4%) and *Nitzschia fusiformis* (11.0%), etc. Nubel reported that the moderately halophilic benthic diatoms don't give lavish growth above 12.0 (% w/v NaCl) [21]. Nagasathya & Thajuddin found *Achnanthes hauckiana, Cyclotellastriata, Pseudonitzschia seriata* and *Thallassionema eccentrica*at 15.0% salinity [29]. Most common diatoms which have found a place in biotechnological applications are generally found in the salt range from 3.5 – 15 (% w/v) salt [30, 31, 32]. The sphere of extremely halophilic diatoms has not been properly explored to its full potential. *Chaetoceros calcitrans* remains one of the uncommon diatoms found in salinity up to 30% NaCl [33]. Degree of salinity tolerance is generally studied using clonal isolation technique [34, 35]. Here gDNA is fragmented and then various cells are transformed by one fragment each. Each transformed cell gives rise to a colony having genetically identical cells. Hence, the colony as a whole is a clonal isolate.

BIOMINERALIZATION AND FRUSTULES OF DIATOMS

Formation of the frustules is done by a systematic mechanism due to adsorption of undissociated monomeric silicic acid $Si(OH)_4$ which is formed by the dissolution of silicon dioxide in water [36]. Silicic acid by the help of intracellular 'nanobioreactors' called Silicon Deposition Vesicle (SDV), are precipitated into the diatoms in the form of amorphous silica [37]. The total silicic acid content in oceans is equal to 10^{17} moles of Si. The net annual net input of silicic acid roughly equals to 6.1×10^{12} moles of silicon, out of which 80% comes from rivers. Diatoms uptake and incorporate 10^{12}kgs of this silicon from their immediate environment [38]. This silicon is brought back into the ocean's inorganic nutrient pool as dead diatom-deposits which enrich the soil. Apart from being the prime representative quality of diatoms, these frustules are of uttermost importance in the field of biotechnology and nanotechnology as they can be manipulated and the dimensions along with the pores present in the valves can be specifically altered for applied biotechnological purposes [8, 39].

SCOPE FOR THE BIOTECHNOLOGICAL APPLICATIONS OF DIATOMS

Harnessing of halophilic diatoms for biotechnological proposes has proved to be cost efficient with easy handling. Acquisition from their natural habitat is easy and requires neither complex materials nor sophisticated instruments. The silica nanoparticles have found utilization in varied biotechnological and bio-therapeutic fields. This includes their use in bioprobes, drug and gene delivery, bioleaching, targeted remedy and controlled release of drugs [39]. This is due to their unique chemical, electronic, optical and mechanical properties.

Transport vehicles for genes and drugs

Chemically inert hard biosilica frustules with homogeneous pore size are biocompatible in nature. Hence, they can be used as drug and non-viral gene delivery vehicles [40], for example *Thalassiosiraweiss flogii* can be used for this purpose [41,42]. The rate of drug release will be directly proportional to the pore size. Hence, a variety of diatom frustules can be used for different drugs with varying doses. Drug-

loaded diatoms can be used for site-directed delivery using ferromagnetic elements and magnets [43]. This technology is used to remediate gastrointestinal diseases using oral drugs. Gene delivery systems make use of cationic conveyance [44]. Hence the inert silica frustule nanoparticles need to be modified for this purpose. Nevertheless, after modification, they can be harnessed and can even be tracked intracellularly using fluorescent dyes [45]. In one instance, diatoms have also been used for the targeted delivery of siRNA into tumor cells as a form of therapy [46].

Bioprobes, Biomarkers and Photoluminescence

Detection and registration of individual diatom cell under microscope is possible due to fluorescence imaging using irradiation of high-pressure mercury lamps [47]. This is possible due to the photonic nature of frustules and the event of antibody-antigen complex formation as a change in the diatom's spectrum of photoluminescence; this was done in experiments on *Thalassiosira rotula.* Furthermore, several scientists were able to link and optimize antibody-functionalized diatoms biosilica frustules. This equipped scientist with a novel range of label-free microscale biosensors which are able to detect immunocomplex formation based on photoluminescence [48, 47, 49]. *Concinodiscus concinnus*is an example of a halophilic diatom, its frustules have been chemically modified to attach antibodies for the formation of selective bioprobes [50]. Analogous to Sandwich ELISA, Sandwich-type of immuno-sensors can also be prepared using diatoms [51]. For this, diatom-synthesized mesoporous silica nanoparticles were used along with horseradish peroxidase (HRP). Moreover, to improve the sensitivity of this amperometric immunosensor, anti-human IgG was further developed. This type of diatom-based biotechnologically synthesized immuno-sensor showed satisfactory level of selectivity, stability and reproducibility.

Halophilic Diatoms in Bioleaching and Earth Filters

During lab cultivations, alkoxysilane precursors are incorporated into the medium to facilitate the synthesis of biosilica. Alterations in the type of pre-added precursors can help gain desired products such as those with bioleaching property [52]. For instance, for the production of halophilic diatom *Thalassiosiraweiss flogii*, adding organo-alkoxysilane precursors along with alkoxysilane precursors gave cells with modified frustules having thiol moiety. This enabled scientist to leach out heavy metals from water samples [53]. Additionally, for the preparation of diatomaceous earth filters, live diatom cells with controlled growth by biotechnological fermentation and monitoring have benefits over fossilized diatoms. This is due to the fact that cultures originated from individual cells ensure uniform pore size and permeability [54].

Biotherapeutic and Metabolite Production

A wide range of metabolites are produced by halophilic diatoms. These metabolites are vital from the industrial and therapeutic point of view. Hence, they are employed in biotechnological processes for biotherapy (Table 1).

Table 1: Metabolites from halophilic diatoms and their biotherapeutic applications

Metabolite	Function	Diatom	Reference
Antioxidants	Scavenges radicals and decreases their adverse effects	*Chaetoceros* sp., *Odontella aurita*	[71,72]
Aqueous extracts	Anti-tumoral, lung cancer	*Hasleaos trearia*	[73,74,75]
Arachidonic acid	Boosts immunization	*Nitschia* sp.	[76]
Aspartic acid	Synthesis of other amino acids; muscle mass Biosynethesis of proteins	*Chaetoceros calcitrans* and *S. costatum*	[77]
Domoic acid	Anti-helminthic and insecticidal	*Amphora coffeaeformis*	[78]
Eicosapentaenoic acid (EPA)	Treatment of heart problems, cancer, asthma, lung disease, menstrual problems and kidney diseases (Along with Docosahexaenoic acid- DHA)	*Nitzschia laevis; Nitzschia inconspicia*	[79,80]
Glumatic acid	Brain development and function	*Thalassiosria* sp.	[77]
Isoleucine	Biosynthesis of proteins	*Chaetoceros calcitrans* and *S. costatum*	[77]
Isoprenoid polyenes	Anti-tumor	*Haslea* spp.	[81]

Leucine	Regulation of blood sugar levels	*C. calcitrans*	[77]
Organic acids	Food preservation, flavours, pharma formulations, etc.	*Skeletonem acostatum*	[73]
Ornithine	Hepatic encephalopathy	*S. costatum*	[77]
Serine	Biosynthesis of purines and pyrimidines	*Thalassiosria* sp.	[77]
Squalene	Medicinal compound and precursor to steroids	*Amphora coffeaeformis*	[82]
Tyrosine	Protein building; brain function	*Thalassiosria* sp.	[77]
Various fatty acids	Antibacterial and antifungal	*Chaetoceros* spp.	[83,84]

Nanoparticles and Metal Oxides Synthesis

Halophilic diatoms can also be used as 'microfactories' to biofabricate oxides and nanoparticles for biotechnological purposes [55]. *Nitzchia frustulum* has been used to produce germanium oxides though artificially synthesizing Si-Ge nanocomposites in the frustules. This was possible by first starving the cells from silicon, then growing them in $Ge(OH)_4$ and $Si(OH)_4$ (germanium) rich media in a photobioreactor [56]. *Navicula atomus, Diadesmis gallica* have been used to produce gold nano particles [57]. *Pinnularia* sp. has been used as a platform to produce silica-titania composites by manipulating cells to incorporate TiO_2 into them [58]. This titania could be converted to crystalline form by thermal annealing. Biotechnologically synthesized diatoms having nanostructured titanium in their frustules can be applied for optoelectronic and light capturing processes [59].

Biofuels

Biofuels from diatoms can be of two types: Naturally forming biofuels and biofuels made in fermentation processes. In nature, halophilic diatoms generally draw-in and store CO_2. This causes the diatoms to sink. Over time, they get conserved to form petroleum [60]. Diatoms can also be exploited to make biofuels in large scale biotechnological fermentation processes. This is due to the large range of fatty acids and hydrocarbons production by them. *Thalassiosira antarctica* var. *antarctica, Thalassiosira weissflogii, Pinnularia biceps F. minutissima, Navicula cryptocephala, Hasleaostrearia, Rhizosolenia* and *Pleurosigma* [61,62,63] are some of the diatoms whose potentials can be harnessed to form biofuels. The biotechnological applications of halophilic diatoms are summarized in Table 2.

Table 2: Other biotechnological applications of halophilic diatoms.

Biotechnological application	Diatom	Salt concentration (%w/v)	Reference(s)
Antibody tethering	*Thalassiosira roluta*	15	[47]
Biofuels	*Thalassioria; Pinnularia; Navicula; Haslea; Rhizosolenia; Pleurosigma*	15	[61,62,63]
Bioleaching	*Thalassiosira weissflogii*	12-15	[43]
Bioprobe	*Concinodiscus concinnus*	15-30	[50]
Drug / gene vehicle	*Thalassiosira weissflogii*	12-15	[41]
Metabolites	*Nitzschia; Amphora; Haslea; Skeletonem*, etc.	3.5-5.4	see Table 1
Nanoparticles	*Nitzschia frustulus,*	5.4-15	[56,57,58]
Si-Ge nanoparticles	*Navicula atomus, Diadesmis gallica, Pinnularia*	5-10	
Gold nanoparticles			
Titanium			

FUTURE ASPECTS

The utilization of halophilic diatoms for biotechnological applications needs strong fundamental research in the field of eco-physiology to better understand their basic requirements [64]. Genetic manipulation is possible due to a number of reasons. Firstly, diatoms undergo frequent recombination as they are able to reproduce sexually. The recombination process can be further manipulated by genetically reprogramming diatoms using nucleases. Recombination also allows the alteration of biochemical pathways for their novel

biotechnological processes. Varying the salinity has an effect on lipid production, which can in turn affect the metabolite production such as proline, fatty acids and biofuel production [65, 66, 67]. The salt concentration can also be used to govern the valve formation. This property is of prime importance as it can be used to make valves with desired pore size [68, 69, 70]. This could be the fundamental property for advantageous targeted and delayed drug delivery, gene delivery and even for encapsulation of enzymes and hormones. The research on diatoms has full potentials to explore/exploit their biotechnological applications, discover novel pathways and metabolites.

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