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Applications of Diatoms as Potential Microalgae in Nanobiotechnology

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ABSTRACT

Introduction: Diatoms are single cell eukaryotic microalgae, which present in nearly every water habitat make them ideal tools for a wide range of applications such as oil exploration, forensic examination, environmental indication, biosilica pattern generation, toxicity testing and eutrophication of aqueous ecosystems. ***Methods:*** Essential information on diatoms were reviewed and discussed towards impacts of diatoms on biosynthesis and bioremediation. ***Results:*** In this review, we present the recent progress in this century on the application of diatoms in waste degradation, synthesis of biomaterial, biomineralization, toxicity and toxic effects of mineral elements evaluations. ***Conclusion:*** Diatoms can be considered as metal toxicity bioindicators and they can be applied for biomineralization, synthesis of biomaterials, and degradation of wastes.

Introduction

Diatoms are single cell eukaryotic microalgae, which are found in every habitat where water is present. Their abundance and wide distribution make them ideal tools for a wide range of applications both, as fossils and living organisms, (Atazadeh and Sharifi 2010). Examples of their wide range of applications include oil exploration, forensic examination and environmental indication. Today, the major emphasis are focused on their application in nanotechnology and biotechnology including nanofabrication techniques, chemo and biosensing, particle sorting, and control of particles in micro- and nano fluidics and also on their use in analyzing ecological problems; such as climate change, acidification, and eutrophication of aqueous ecosystems (Atazadeh and Sharifi 2010, Atazadeh *et al* 2007, Dolatabadi and de la Guardia 2011). Diatoms are highly robust organisms, and can inhabit virtually all photic zones from the equator to seemingly inhospitable sea ice where they are highly useful indicators of environmental conditions in their rapid response to environmental changes, including their capacity to react to sea ice freezing around them with their "natural antifreeze" ice-binding proteins (Chen *et al* 2010). Thus, in all climate zones, diatoms show an exceedingly high degree of flexibility that offer exciting possibilities in biotechnological applications despite challenging conditions.

The high degree of complexity and hierarchical structure displayed by diatom silica walls is achieved under mild physiological conditions. The biological processes that generate patterned biosilica are therefore of interest to the emerging field of nanotechnology. Biosilica and silicon, in their various forms, finds widespread use in electronic, optical, and structural materials. Research on uses of silicon and silica has been intense for decades, raising the question of how much diversity is left for innovation with this element (Dolatabadi and de la Guardia 2011, Losic *et al* 2009). Silica nanoparticles have proven to be important for several biotechnological and biomedical applications, such as biosensor design, drug delivery, cell labeling, cell separation, contrast agents for magnetic resonance and ultrasound medical imaging, and as a targeting and therapeutic platform for drug- or enzyme-released systems (Dolatabadi and de la

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Guardia 2011). Silica bodies are opal phytoliths (phyto means 'plant' and lithos means 'rock' in Greek) produced by plants when soluble silica from the ground water is absorbed by the roots and carried to different parts of the plant system through the vascular system. Some plants have nonsiliceous phytoliths, such as calcium oxalate. Furthermore, the diatoms frustules could be used as complex templates in the patterning of biomolecules both at the micro and the nanoscale due to their intricate structural geometries (Yuan *et al* 2006). Figure 1 shows the structure of diatoms and their frustule holes in different scales which owes different features.

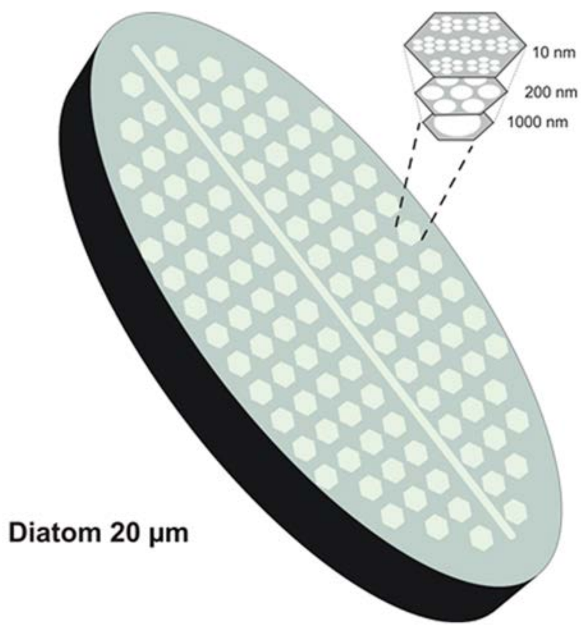

Fig.1. Schematic of the hierarchical structure of diatoms, showing different levels of structure from the nanoscale to the micrometer scales.

Diatoms have been used for toxicity testing (Atazadeh *et al* 2009, Florence and Stauber 1986). However, information obtained from such tests is based on the results for a few freshwater green algae that are easy to manipulate in culture (Mohan and Hosetti 1999). Although toxicity tests with isolated species can provide useful indications for environmental risk assessments of test compounds, they cannot predict changes at different organizational levels in natural communities (Berard *et al* 2002). Assessment of impacts of chemical contamination on the environment should take into account the natural variability of biological systems in space and time, and it is particularly noteworthy that any endpoint used to evaluate toxicity may be expected to vary in magnitude under different environmental and biological factors (Schindler 1987).

The taxonomic composition of benthic diatom communities has been widely used for monitoring water quality (Kelly 1998). Water quality monitoring programs have focused on comparisons of water chemistry with criteria derived ultimately from bioassays. However, assessment of community composition has several advantages over physical and chemical measurements of water quality and the use of biological communities as indicators of water quality is evolving as our understanding of the interactions between water quality and the integrity of biological communities improves (Atazadeh *et al* 2007).

In this paper, we try to show the feasibility of diatom frustules usage in biomineralization, synthesis of biomaterial, degrading waste and metal toxicity. Additionally, they have been used as bioindicators of water pollution in aquatic ecosystems.

Application of diatoms in biomineralization

Biomineralization is the process of producing mineral elements from organic compounds which is involved with biosystems. This process consists of methods, devices and requirements which could be supplied by microorganisms. To get this goal, bacteria, fungus, and diatoms are used largely. Diatoms due to their big plentiful are more commonly used than other organisms. In this case, diatoms can manufacture most of mineral materials. Biosilica, which is participate in diatoms structure, are being produced by diatoms, formed only in natural water and in the presence of sunlight without the need for high temperature or high-pressure treatment. Because of their unique structure, frustules find wide industrial application; they are used in water filters, building materials, and chromatography supports (Brunner *et al* 2009, Gutu *et al* 2009, Heredia *et al* 2008, Hildebrand 2008, Jeffryes *et al* 2008).

The production of platinum metal (Pt) has been recently showed by means of diatoms such as *Melosira nummuloides* in culture condition. In that study, the cultivation of *Melosira nummuloides* was demonstrated in the presence of dihydrogen hexachloroplatinate, hexahydrate in order to introduce Pt into frustules. The results indicated that Pt can be injected into frustules at the time of cultivation of living diatom cells (Yamazaki *et al* 2010).

Cadmium metal (Cd) is another element which can be produced by diatoms during their growth (Gutu *et al* 2009). Addition of cadmium sulphide to *Pinnularia sp.* Culture resulted in the appearance of Cd crystals in diatom's frustule. The results showed a simple and inexpensive chemical deposition process which can be utilized to coat intricately patterned diatom frustules biosilica with optoelectronic semiconductor thin films (Gutu *et al* 2009).

Application of diatoms in synthesis of biomaterial

Diatoms due to mentioned features and qualities are considered as unique machines in biosynthesis of biomaterial. Diatoms are ubiquitous and constitute an important group of the phytoplankton community having a major contribution to the total marine primary production. These products are being extracted and used in many cases. On the other hand, the more recent development of synthetic nanotechnology has been driven by the nearly insatiable global demand for ever smaller structures for electronic, optical, chemical, or biomedical devices (Bertrand 2010, Gordon *et al* 2005). Diatoms are considered as major group of phytoplankton that account for approximately 40% of the ocean carbon fixation and the vast majority of biogenic silica production through the construction of their cell walls. These microorganisms are used in synthesis of enzymes, biofuels, biosilica, and mineral materials, (Bertrand 2010, Haynes *et al* 2007, Schroder *et al* 2007). Common methods of silica synthesis mostly require high temperature conditions and extreme pH which imposes high costs to producers. However, microorganisms can manufacture silica and this process is well described in the most recent literature on the molecular biology of enzyme-mediated silica formation in marine and freshwater organisms, and the development of strategies towards the application of biocatalytically formed silica ("biosilica") that may be advantageous compared to silica synthesized by chemical methods. The synthesis of spicules is a rapid process. Studies on freshwater organisms revealed a growth rate of 5 μm per hour. Therefore, these microorganisms must accumulate large amounts of silicic acid from the surrounding water, which is undersaturated with respect to silicon, a process that obviously requires a lot of energy (Schroder *et al* 2007).

Enormous study findings recently described the methods of carotenoid biosynthesis in diatoms; these carotenoids consist of b-carotene, violaxanthin (Vio), diadinoxanthin (Ddx) and diatoxanthin (Dtx) and the chlorophyllous pigments, i.e. chlorophyll a, c1, c2 (Bertrand 2010). The carotenoid amount can be modified according to the environment and density of these compounds can be found by spectrochemical and chemical analysis. Besides, the biosynthetic pathway used to synthesis carotenoids is now quite well established in higher plants whilst many information are missing in diatoms (Bertrand 2010).

Evaluation of toxicity

Several mineral elements are essential for living organisms at very low concentrations, but at high concentrations most of them are toxic and have a direct and adverse influence on various physiological and biochemical processes. These elements belong to the category of essential micronutrients and participate in growth, metabolism and enzyme activities. In contrast, other elements are basically toxics and they cause many problems and difficulties in natural media for the normal growth of organisms. One of the most studied toxic effects of mineral elements on microorganisms is growth inhibition. The degree of growth inhibition can be affected in different ways, depending on the strain of microalgae and metal concentration added in solution (Hess 2010, Liu *et al* 2010, Navarro *et al* 2008).

Application of diatoms in waste degradation

Wastes are unwanted substances invariably produced during daily activities. Depending on their physical state, they are classified as solid and liquid wastes and gaseous emissions. Sanitary refuse is the process of collection, transportation and disposal of solid wastes in a systematic, economic and hygienic way. Industrial wastes are the pollutant delivered out of a particular industry. The quality and quantity of wastes depend on the nature of industry, raw materials used, manufacturing housekeeping and process. Environment pollution is defined as contamination of water or alteration of the physical, chemical or biological properties of natural water. Water or other sources are said to be polluted when they change their quality or composition either naturally or because of human activities, thus becoming unsuitable for domestic, agricultural, industrial, recreational uses and for the survival of wildlife. Environment pollutant can be defined as an agent affecting aesthetic, physical, chemical and biological quality and wholesomeness of water (Ashok 2009, Evans and Furlong 2003, Pepper and Gerba 2005, Srinivas 2008, Yamazaki *et al* 2010).

These materials should be managed and controlled. Some methods are used in all over the world to overcome this problem. These methods consist of degradation physically, chemically or biologically. Because of releasing materials, chemically and physically methods do not be considered as a suitable for getting rid of these pollutants. Therefore, biological methods and their unique features qualified them to use in biodegradation of wastes. Biological methods involve the transformation or mineralization of contaminants to less toxic, more mobile, or more toxic but less mobile, forms. The main advantages of these methods are their ability to destroy a wide range of organic compounds, their potential benefit to soil structure and fertility and their generally nontoxic, 'green' image. Biodegradation process involves using bacteria, algae, and diatoms. Diatoms due to their unique structure and biological aspects are used largely in waste degradation (Glazer and Hiroshi 2007, Marchetti and Cassar 2009, Saade and Bowler 2009, Srinivas 2008).

As diatoms respond quickly to environmental changes and reflect both, physical and chemical characteristics of the overlying water masses, they are particularly useful for paleoecological reconstructions. These frustules accumulate and are partially preserved in the ocean sediments (Gordon *et al* 2005, Marchetti and Cassar 2009, Saade and Bowler 2009).

Diatoms, because of their structural and physiological features, can be used in material degradation. For example, some diatoms are removing the waste in environment and pollutants. In Malaysia, the degradation of aquatic pollution in the Pinang River Basin through diatoms was investigated (Maznah and Mansor 2002). In this investigation, different species of diatoms on different samples which gathered from many stations was employed. The results showed that the diatom community structure and the specific sensitivity of certain diatom species could be related to the degree of water quality in Pinang River Basin. The abundance of certain diatom species could be used as biological indicators to measure impacts of river pollution (Maznah and Mansor 2002).

Some researcher has been reported that certain freshwater green algae (e.g. *Chlorella vulgaris*, *Scenedesmus platydiscus*, *Scenedesmus quadricauda* and *Selenastrum capricornutum*) are capable of uptaking and degrading polycyclic aromatic hydrocarbons (PAHs) (Keum *et al* 2006, Li *et al* 2006, Luan *et al* 2006, Seo *et al* 2006, Seo *et al* 2007). *Laminaria joapnica*, a famous macroalgae, is growing in the coastal waters along the east coast of China uptakes and degrades of two PAHs phenanthrene and pyrene (Wang and Zhao, 2007). This macroalgae grown under different phenanthrene and pyrene concentrations showed variable stress responses as determined by their enzymatic activities (Wang and Zhao, 2007). This study finding showed that the *Laminaria joapnica* has a strong capacity to tolerate and uptake PAHs from seawater. Some recent studies have also reported that phenanthrene and pyrene could be transformed by micro-organisms into soluble diols, phenols, lactones, naphthoic acid and phthalic acid that could be excreted into water (Keum *et al* 2006, Li *et al* 2006, Luan *et al* 2006, Seo *et al* 2006, Seo *et al* 2007, Wang and Zhao 2007). The metabolism of phenanthrene and pyrene in diatoms was carried mainly by the enzyme-oxidation process converting PAHs to less or non-toxic forms of compounds (Wang and Zhao 2007). The application of diatoms in biosynthesis, biodegradation and biomineralization was gathered in Table 1.

Diatoms and metal toxicity

Soil and water contamination with toxic metal have become increasingly widespread and result in posing a global threat, which need more attention by scientists. Toxic metal pollution is a focus point of serious concern and the examination and monitoring water quality are becoming essential procedures (Ferreira da Silva *et al* 2009, Magnusson *et al*, Rudolph *et al* 2009, Wang and Wang 2008). Subcellular partitioning of metals may provide a mechanistic approach to investigate metal toxicity and tolerance. The presence of metal-tolerant populations at metal-contaminated sites has been shown for a number of genera of freshwater algae, including blue-green algae, filamentous greens and diatoms (Ivorra *et al* 2002). Diatoms are important bioindicators to monitor the metal concentrations in diverse habitats (Ferreira da Silva *et al* 2009, Magnusson *et al* Rudolph *et al* 2009, Wang and Wang 2008). If the purpose of toxicity tests is to predict the quantitative effect of a metal under particular field conditions, then the tests need to be refined. The assay medium should be similar to water at the field site with respect to factors such as chelating agents which influence toxicity (Whitton and Kelly 1995). The approach was applied by some researches to indicate sensitivity of microalgae for some ions shows the microalgae present in young biofilms were more sensitive to Zn or Cd than microalgae in old biofilms, the difference being apparent even when both types of biofilm were taken from a clean stream (Ivorra *et al* 2000). To examine seasons effects on the toxicity tolerance of microalgae to toxicants, in the River Ter, N-E. Spain microalgal communities at two different seasons (spring and summer) were tested for copper toxicity. Results of this study showed the tolerance to copper in microalgal communities was lower in spring than summer (Navarro *et al* 2002).

Table 1. Diatom applications in biosynthesis, biodegradation and biomineralization

Algal species	Biosynthesis	Biodegradation	Biomineralization	Reference
Achnanthes oblongella Oestrup	-------	waste	-------	(Maznah and Mansor 2002)
Cocconeis placentula Ehr.	-------	waste	-------	(Maznah and Mansor 2002)
Fragilaria capucina Desm.	-------	waste	-------	(Maznah and Mansor 2002)
Chaetoceros cryptica	Carotenoids	-------	-------	(Bertrand 2010)
Haslea ostrearia	Marennine	-------	-------	(Robert *et al* 2002)
Melosira nummuloides	-------	-------	Platinum	(Yamazaki *et al* 2010)
Pinnularia sp.	-------	-------	Titanum	(Jeffryes *et al* 2008)
Pinnularia sp.	-------	-------	Cadmium	(Gutu *et al* 2009)

Human activities have been increasing the cadmium levels in soils and waters, disturbing many organisms in the primary trophic levels such as microalgae. Cadmium toxicity is due to Zn and Cu displacement in metalloenzymes and to the formation of reactive oxygen species (ROS), leading to oxidative stress (Goncalves *et al* 2009). Above normal concentration, ROS are potentially toxic and can react with lipids, proteins, photosynthetic pigments and nucleic acids (Moller *et al* 2007), leading to lipid peroxidation, decrease in chlorophyll and accessory pigment content (Sabatini *et al* 2009), membrane damage, inactivation of enzymes, DNA alterations and oxidation of proteins, thus affecting cell growth and viability (Qiu *et al* 2008). Cadmium (Cd) is a priority pollutant, and its toxicity is mainly related to binding to sulfhydryl groups of proteins or displacement of essential metals in metalloenzymes. Recently, a new approach, namely the subcellular partitioning model (SPM), which takes into account the cellular fates of metals, has been proposed to predict metal toxicity in aquatic organisms. The bioaccumulation, subcellular distribution, and toxicity of Cd in a marine diatom, *Thalassiosira nordenskioeldii*, under different irradiance levels was examined (Wang and Wang 2008). The findings showed that intracellular soluble Cd may be the best predictor of Cd toxicity under different nutrient conditions (Wang and Wang 2008). Besides, results of other studies showed that most Cd was distributed in the insoluble fraction (a combination of metal-rich granules, cellular debris, and organelles) in the diatom *Thalassiosira weissflogii* (Miao and Wang 2006). A good toxicity predictor should be rather constant under different environmental/ physiological conditions. Although good correlation between [Cd^{2+}] and growth inhibition was observed among the different irradiance treatments, suggesting that differences in Cd toxicity cannot be entirely explained by [Cd^{2+}], primarily because the diatoms displayed different bioaccumulation potentials (Desai *et al* 2006, Horvatic and Persic 2007, Miao and Wang 2006, Wang and Wang 2008).

Copper toxicity has recently been studied by many of researchers (Debelius *et al* 2009a, Debelius *et al* 2009b, Wang and Zheng 2008). Copper belongs to the category of "essential metals" and participates in growth, metabolism and enzyme activities. However, at high concentrations is toxic and have a direct and adverse influence on various physiological and biochemical processes (Debelius *et al* 2009a, Debelius *et al* 2009b). One of the most studied toxic effects of metals on microorganisms is growth inhibition. Attempts to standardize growth inhibition tests with microalgae for regulatory purposes have revealed a number of methodological problems. Most researchers suggested that the metal concentration that affects growth in microalgae, is largely variable and depends on the species used, cell density, composition of medium or physical culture conditions (Franklin *et al* 2002a, Franklin *et al* 2002b, Wang and Zheng 2008).

Recently, copper toxicity in five marine microalgae, *Tetraselmis chuii, Rhodomonas salina, Chaetoceros sp., Isochrysis galbana and Nannochloropsis gaditana* were studied because they represent different classes of marine microalgae and are all easy to culture (Debelius *et al* 2009a). The results demonstrated that the degree of growth inhibition was affected in different ways, depending on the strain of microalgae and metal concentration added in solution. All of the strains studied showed an increase in the signals of side-angle light scatter, corresponding to an increase in cell size or change in shape, also could reflect cell complexity when exposed to the highest copper and lead concentrations of their study (Debelius *et al* 2009a, Debelius *et al* 2009b, Wang and Zheng 2008).

Significant relationships were detected between a number of measures of the diatom community and various heavy metals in the environment. The comparison between the reference and affected sites showed a shift from a diatom community dominated by *Nitzschia palea*, *Achnanthes minutissima* and *Amphora pediculus* to one dominated by *Nitzschia palea* and *Gomphonema parvulum* (Whitton and Kelly 1995).

All the results obtained from researches proved that toxicity of different metals and their effects on diatoms, cause to change in biological, physiological, and morphological changes in diatoms which can be used as a unique indicator for determination and investigation of metal toxicity.

Conclusion

As it has been evidenced in the recent literature, diatoms are important synthesis microalgae for biomaterials. Additionally, they are appropriate indicators that can provide green tools in detecting contamination problems in aquatic environments because they are very sensitive to environmental stress. They also can be used in rehabilitation of suffered environment. However, until now, the aforementioned aspects remain unexplored for their quantitative analytical use and, as for example; it could be interesting to try the use of diatoms for trace element preconcentration media and as a cleaning step added after analyte determinations.

Ethical issues

No ethical issues to be promulgated.

Conflict of interests

No conflict of interests to be declared.

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