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Production of Biofuel using Diatoms: An Overview

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ABSTRACT: Diatoms belong to the class of microalgae which are ubiquitous across the planet. The unique property and application that draws the attention of scientists towards the diatoms is their ability to produce and store lipid in their body, which makes them a perfect candidate in the sector of bio-fuel industry. It is also very important to know and study the mechanism of the lipid production ability of diatoms which will eventually pave the way in the synthesis and production of lipid content. Bio-fuel produced from diatoms will eventually boost the clean energy sector and it will elevate the green economy. The biofuel produced from diatoms comes under the category of third generation bio-fuel. Although there are a lot of challenges regarding diatoms as they need to prove their mantle as an alternative to fossil fuel, because the quantum of energy produced by fossil fuel is huge, but in recent times diatoms has given a ray of hope in the sector of energy concerning sustainable development. This review mainly discusses about the applications of diatoms, the mechanism and process by which it synthesizes lipid content in their body and eventually its role in clean energy sector and green economy.

Keywords: Diatoms, bio-fuel, clean energy, green economy.

INTRODUCTION

Diatoms belong to the class of microalgae and they are present across all aquatic ecosystems encompassing from marine to fresh water ecosystems. Marine diatoms' community composition is done on the basis of phosphate present on surface layer of diatoms and also on the basis of salinity (Endo *et al.*, 2018). Generally diatoms are categorized into two classes *viz.* centric diatoms (which display radiate symmetry) and pinnate diatoms (which display binary symmetry) (Hussein & Abdullah 2020). The silicified cell walls present in diatoms act as a defense shield for the diatoms and also it provides structural support to the diatoms. This silicified cell wall present in diatoms varies across species and this depends upon the degree of predation of the predators that depend on diatoms for their food (Pančić *et al.*, 2019). There is a remarkable divergence in the photosynthetic pigments of diatoms which is a result of various factors like light exposure of diatoms based upon seasonal duration like long term and short-term light exposure, variable water column arising in different aquatic ecosystems as well as heat and nutrient gradient (Fisher *et al.*, 2020). Diatoms also play a pivotal role in the bio cycling of various nutrients and also it act as bio indicators in various aquatic ecosystems and simultaneously diatoms aid in the process of purification of contaminated water (Gügi *et al.*, 2015). From the studies of photosynthetic mechanism of diatoms, it was observed and studied that the assembly and the setup of the light harvesting complex (LHC) proteins in diatoms is quite different from that of higher plants, interestingly in case of diatoms, the complex via which light is harvested in diatoms is from fucoxanthin chlorophyll protein (FCP) rather than that of light harvesting complex (LHC) (Kuczynska *et al.*, 2015). The genome of diatoms is very complex which is responsible for encoding different pathways in diatoms viz. non canonical, nitrogen assimilation, managing different types of metabolites, urea cycle etc., (Benoiston *et al.*, 2017). Diatoms which are found in the benthic zone of deep oceans where penetration of light is negligible, happens to produce many anti fouling compounds specially of terpenoid derivatives and these terpenoid compounds are reported to be anti-microbial in nature and can act on various bacteria and fungi (Saha *et al.*, 2018). The silica layer of diatoms is associated with Cenozoic era, as there was a competition between the present-day marine diatoms versus radiolarians (protozoa), the competition was reported to be inter specific in nature (Cermeño *et al.*, 2015). Majority of the diatoms come under nano phytoplankton (2-20 micro meter) and rest comes under pico phytoplankton (less than 2 micro meter), which makes the study and characterization of diatoms very cumbersome, as for oceanic study models, organisms of at least size greater than 20 micro meters are required, for this reason only in order to study diatoms in aquatic ecosystem, support of large spring blooms of diatoms are required(Leblanc *et al.*, 2018). The trauma of diatoms is the zoosporic parasites as they infect the diatoms regardless of whichever ecosystem the diatoms are habitat to, moreover the identification and characterization of these zoosporic parasites are very cumbersome, hence making it vulnerable for diatoms (Scholz *et al.*, 2016).The major advantage of analyzing the diatoms is that they have the capability to produce bi-products of varied applications, other than producing primary products like bio-fuel and bio-hydrogen, however the major challenge in the production of bio-fuel from diatoms is the numerous steps of purification involved in generating the final product (Sivaramakrishnan *et al.*, 2022).

APPLICATIONS OF DIATOMS IN DIFFERENT FIELDS

Diatoms are ubiquitous, and being single-celled eukaryotic microalgal species gives them an advantage over all other photosynthetic organisms, making it an appealing living being for a wide range of applications, including ecological ones, as they are a crucial component of the food web in ocean environment and provide food for several life forms (Hildebrand, 2008). Additionally, diatoms drive the silicon cycle and undoubtedly contribute towards the nitrogen pool (25–30%), especially in large bodies of water like the deep seas (Serôdio & Lavaud, 2020). These phytoplanktons account for more than a quarter of the world's total primary production, and over forty percent of it if only the maritime ecosystem is considered. They also create a significant amount of oxygen as well as play a frequent role in carbon fixation (about 20%) (Serôdio & Lavaud 2020). As a result, diatoms are important for ecological maintenance because they supply carbon to a variety of organisms. Diatoms do have wide range of applications in different domains, including microbiological and nanotechnological fields, notably evidenced by ongoing research. Looking at current developments, there seem to be a variety of domains of life sciences where diatom studies is ongoing and highly valued, including bioengineering, nanotechnology, metabolomics, and indeed the modern fuel industry is showing great interest in this organism owing to its lipid synthesizing capabilities which can be exploited for bio-fuels (Bongale & Gautam 2012). Thus, diatoms have a wide range of uses contrasted to other microalgae.

Drug delivery vehicles – Most cancer therapies, notably the most often used one i.e., chemotherapy, destroy tumor cells while simultaneously causing severe harm to normal body cells, resulting in adverse complications in patients. Methods to deliver medications exclusively to specific cancer cells have been developed in order to limit these negative effects (Aw *et al.*, 2012). Researchers have had some success using silica-based nanomaterials in this area, but the manufacturing method is costly and requires harmful chemicals. Diatoms are emerging as a promising replacement for costlier synthetic silicon nanoparticles, and latest research demonstrated that *Thalassiosira pseudonana* species of diatom, can be genetically modified to exhibit antibody binding capacity, allowing it to convey drug-loaded silica-based nanoparticles to selectively targeted cells while leaving healthy cells out of the equation, which is revolutionary in the cancer field (Delalat *et al.*, 2015).

Environmental pollution indicators – Most of the species of diatoms are sensitive to the chemistry of the water in which they reside. Species will only thrive in specific pH and salt concentration ranges. Numerous environmental factors, such as nutrient content, floating debris, altitude, and various forms of human interference can also impact their growth and reproduction (Beyene *et al.*, 2009). Resultantly, diatoms are commonly used in environmental evaluation and monitoring. Furthermore, because they constitute a major source of carbon fixation on a worldwide scale, they are closely tied to climatic change in the form of acidification etc. (Battarbee *et al.*, 2014; Park *et al.*, 2020).

Palaeoecological studies – Diatoms in seawater and freshwater sediments could be utilized to evaluate historical circumstances because their silicon containing cell walls do not dissolve even in tough and hostile settings (Zalat, 1995). Since their valves are retained in such environments, both live and subfossil diatoms are used in this technique. Living cells are used by experts to learn about the external factors that affect newer existence and abundance (Battarbee *et al.*, 2014).

Biotechnological uses – They are utilized in industrial settings to manufacture a range of metabolites, including vital lipids, amino acids, medications, and nutritional supplements, all of which are highly popular these days (Park *et al.*, 2022). The creation of biosensing devices, which employs diatom frustules, is another area that is significantly expanding the future possibilities of these phytoplanktons. The optical characteristics of diatom frustules can aid biosensor development in a variety of domains (Bongale & Gautam, 2012).

Nanotechnology field – Diatom cells produce valves of variety of shapes and sizes in a consistent and reliable manner, effectively allowing them to produce micro-scale or even nano-scale frameworks that could be used in a multitude of devices varying from optoelectronic devices to semiconductor nanofabrication (Mishra *et al.*, 2017). Moreover, effective artificial selection processes can open the way for diatoms that manufacture valves of certain shapes and sizes to be chosen and cultured in laboratories to produce nanoscale materials in surplus (Korsunsky *et al.*, 2020).

Solar energy – By replacing photosensitive TiO_2 for the SiO_2 that diatoms ordinarily utilize to build their cell walls, diatoms might be included as a material of photovoltaic modules. There have already been proposals for solar cells consisting of diatom components (Fuhrmann *et al.*, 2004). The naturally existing diatom shell, that comprises of two irregular ends with a split among them and encompasses many naturally present nm size pores throughout, was primarily used in this innovative diatom-based procedure to construct photovoltaic panels that could be up to three times as effective as traditional solar cells. To create novel dye-sensitized solar panels and nano-structured battery electrodes, these small holes can be utilized to incorporate additional metal oxide compounds like Ti or Ge dioxide (Fuhrmann *et al.*, 2004).

Bio-fuel production – Diatoms have already been mass produced in outdoor settings for purposes other than biofuel production, such as aquaculture, for many years. Algal biomass efficiency has been measured at a broad range of levels, which can be attributable to differences in cultivation circumstances. Factors of consideration include the light source and its intensity, mixing rate, pH, and essential nutrients. The content of triacylglycerol (TAG) grows when levels of chlorophyll diminish (symbolizing the beginning of the bloom's death), according to research (Mourya *et al.*, 2022). TAG accumulation happens prior to cell death in a biofuels production scenario, so damaging would not have much impact on the production(Hildebrand *et al.*, 2012). As a result, it makes sense to utilize them in the biofuel sector which will assist to clean up and green our environment.

The flow chart below shows all of the relevant industries in which they've been employed for different products (Fig. 1).

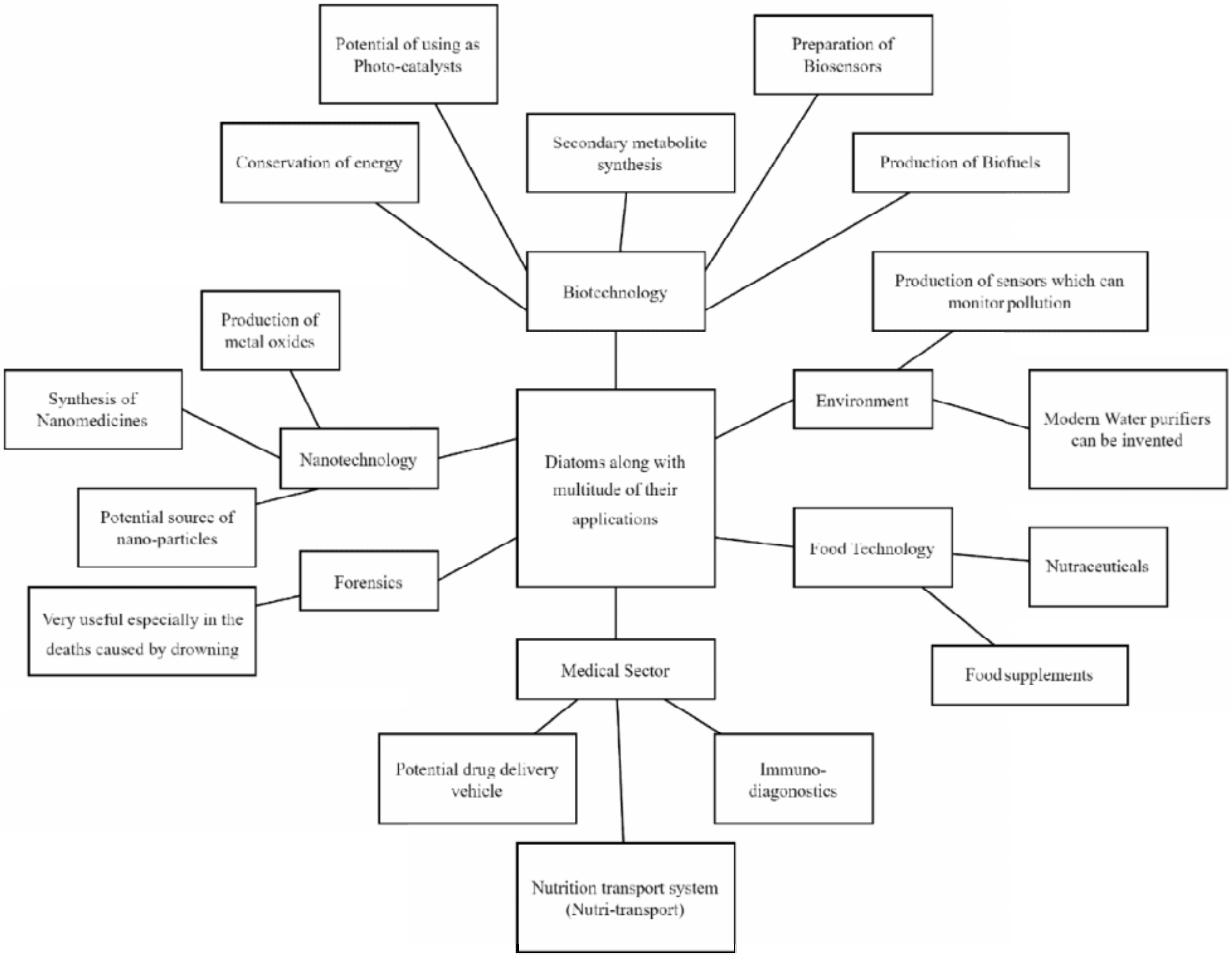

Fig. 1. Depicts the fields in which diatoms can be used and in what aspects.

Hence, it's indeed easy to conclude that these phytoplanktons not only play important roles in all aspects of life, but they also play a critical role in maintaining the planet's ecological stability (Hildebrand, 2008). Diatoms are distinguished out of other species by their silicon containing cellular membrane, which makes it a perfect candidate for widening applications; ranging from nanoscale-particles generation to delivery systems for drugs; industry-based applications to biomedical values (Bongale & Gautam 2012). They're multipurpose in nature. Of all the applications discussed above, the production of bio-fuel from diatoms is the need of the hour. In recent times, apart from synthesizing and harnessing the lipid produced from diatoms, a lot of work is done on improving and enhancing the lipid content in diatoms using various chemical and biotechnological methods. In chemical method, nitrogen deprivation in diatoms to elevate the lipid content is worth mentioning. In biotechnological method, using CRISPR, gene editing of lipid producing genes in diatoms can be done to increase the lipid content (Chen *et al.*, 2022).

MOLECULAR MECHANISM FOR LIPID SYNTHESIS IN DIATOMS

The amount of information available regarding lipogenesis and its metabolism in diatoms is currently quite restricted, and much more study is needed in this area. If contrasted to flora, fauna, or phytoplankton, diatoms' lipid metabolism is strikingly comparable to them (especially in the synthesis of fatty acids) (Zulu *et al.*, 2018). Thus, the underlying mechanisms for lipid synthesis in diatoms will be explained using some knowledge of such pathways involved in case of angiosperms and microalgae (Khozin-Goldberg, 2016). Diatoms, like flowering plants, contain both eukaryotic as well as prokaryotic lipid production routes.

Triacylglycerol (TAG) would be the main emphasis because it is the most prevalent lipid in the cells of several microalgal species and perhaps the most important component when discussing biofuels among all the other lipids existing in the microalgae. Ergo, TAGs are indeed the primary lipid constituents utilized in the production of renewable fuels, and it's essential to comprehend the TAG biosynthesis process in diatoms in order to employ them as an economical biofuel contributor in the future (Mourya *et al.*, 2022). The production process of TAG is divided into three basic phases, each of which is controlled separately viz. i) Fatty acid (FA) biogenesis, ii) Glycerol-lipids creation, iii) Packaging into lipid droplets (LDs)

In diatoms, fatty acid production is comparable to that in angiosperms i.e., acetyl-CoA serves as a precursor molecule in Bacillariophyceae as well. In eukaryotes, acetyl-CoA is produced in mitochondria, peroxisomes, and plastids cellular organelles (Baba & Shiraiwa, 2013). In case of plastids, acetyl-CoA can be formed from unbound acetic acid in an ATP-dependent mechanism mediated by acetyl-CoA synthetase, or removal of carboxyl group from pyruvate, catalyzed by a pyruvate dehydrogenase complex (present in plastids) could also result in the production of acetyl-CoA (Jaramillo-Madrid *et al.*, 2019). ATP-citrate lysate, that uses citric acid (generated from the TCA cycle) as a substrate, builds the mitochondrial reserve of acetyl-CoA in the cytoplasm. Numerous enzyme complexes are involved in this procedure, which comprises several phases (Chen & Wang 2021). First of all, acetyl-CoA carboxylase (ACCase) enzyme catalyzes the conversion of acetyl-CoA into malonyl-CoA and the aforementioned process is driven by an ATP-dependent carboxylation of the precursor molecule itself which in this case is acetyl-CoA (Zulu *et al.*, 2018). The FA synthase complex also known as FAS complex catalyzes another few stages of FA synthesis within plastids, which include serial condensation of two-carbon units. This FAS complex is comprised of two main classes of FAS and these are - FAS I and FAS II.

FAS II complexes can be found in both prokaryotic cells and in organelles considered to be prokaryotic in nature such as mitochondria and plastids(Guerra *et al.*, 2013). As a result, FAS II is the primary FAs source in diatom plastids. This consists of four enzyme processes, all of which adds two carbon atoms to the expanding acyl chain, while malonyl-ACP serves as the reaction's substrate. Ketoacyl-ACP synthases (KAS) catalyze the above processes, which result in 16:0-ACP synthesis in diatoms(Zhu *et al.*, 2011). The elimination of the acyl group from ACP and transfer of the same to glycerol-3-phosphate by acyl-ACP:G-3-P acyltransferase enzyme (in plastids) is by far the most critical element in the synthesis of active FAs. FA biosynthesis comes to an end at this step. However, it is hypothesized that another reaction occurs, namely the hydrolysis of acyl-ACP catalyzed by an acyl-ACP thioesterase, resulting in the synthesis of free FAs in the plastid's internal envelope but this has yet to be experimentally shown, therefore research is still ongoing (Liu & Benning, 2013). FFAs can then be transferred via the membranes of the plastids and utilized as TAG substrates. Furthermore, the process by which FAs in diatoms are transported from the plastid is unclear.

TAG formation in diatoms appears to be particularly happening via two distinct processes at the endoplasmic reticulum/lipid droplet interface. They are, i) Acyl-CoA-dependent pathway, ii) Acyl-CoA-independent pathway

In these phytoplanktons, the former pathway, otherwise referred as the Kennedy pathway, is the primary route for TAG creation(Zulu *et al.*, 2018). The addition of acyl group to glycerol-3-phosphate (G-3-P) and production of lysophosphatidic acid (LPA) are the first events inside this pathway, which are catalyzed by the enzymatic activity of acyl-CoA:glycerol-3-phosphate acyltransferase. Phosphatidic acid (PA) can then be synthesized via the use of acyl-CoA:LPA acyltransferase. Phosphatidic acid phosphatase (PAP) dephosphorylates the PA generated, resulting in diacylglycerol (DAG) (Chen & Wang, 2021). This DAG is transformed to TAG by integrating the third acyl-CoA, this step is aided by acyl-CoA:DAG acyltransferase, and this is termed as the last stage of acylation. Similarly, a route from G-3-P to DAG has been identified in chloroplasts; however, whether DAG generated in chloroplasts adds value to TAG production has yet to be determined, and research is still in progress in this particular aspect (Guerra *et al*., 2013).

While, in the latter pathway, for the synthesis of TAG from DAG, phosphatidylcholine (PC) acts as an acyl donor and phospholipid:DAG acyltransferases (PDATs) uses it and catalyzes the reaction (Jaramillo-Madrid *et al.*, 2019). Ultimately, TAG is organized into LDs, that have a uniform design throughout all kingdoms and comprise of a phospholipid monolayer along with a hydrophobic core where neutral lipids (typically TAGs) are contained (Leyland *et al.,* 2020). This is the whole mechanism of lipid biosynthesis in diatoms and the potential of use of diatom manufactured lipids in the biofuel industry is huge and it will be utilized in recent years.

ROLE OF DIATOMS IN BIO-FUEL INDUSTRY AND GREEN ECONOMY

Quick development and industrialization ought to affect the environment; generally closing or going slower in globalization isn't the result. Right now, practically 95% about the total conveyance manufacturers, rest upon a non-inexhaustible origin of power (Rodrigue & Notteboom, 2013). With collective facts and figures on genomic plus transcriptome records being assimilated, there is a lot of extensive advancement in explanation of various parts about the diatom isoprenoid bio-combination (Keeling *et al*., 2014). It is presently conceivable to constantly develop diatoms as well as control the existence based on intrusive genus devoid of biochemical substance at a normal yearly produce of 132 MT dried diatoms ha−1 above a time based on right around 5 centuries, despite these facts keeping up with the dominancy of the ideal diatom breed at a cyclical premise (Wang & Seibert 2017). The third-era bio-fuels depend on enhancements in the improvement of biomass. They rely on uniquely designed energy harvests, for example, green growth as their energy source (Mourya *et al.*, 2022). Regardless of being a discernable strategy, the diatom trial has controls moreover. One of these significant concerns is the occurrence of diatoms in a immerse environment. The non-appearance or fewer occurrences of diatoms now in a marine group could prompt misleading affirmative or else adverse outcome. The manifestation of diatoms from various layers (water bed rock, further, in addition to shallow) of the marine group likewise could be fluctuated (Levkov *et al.*, 2017). Fast demise, what is going on where the diatom trial can be off-base. Moment demise when a creature or humanoid enters the water body for different causes, for example, frosty shock and cardiovascular illnesses will give an adverse outcome in the diatom trial (Smol & Stoermer 2010).

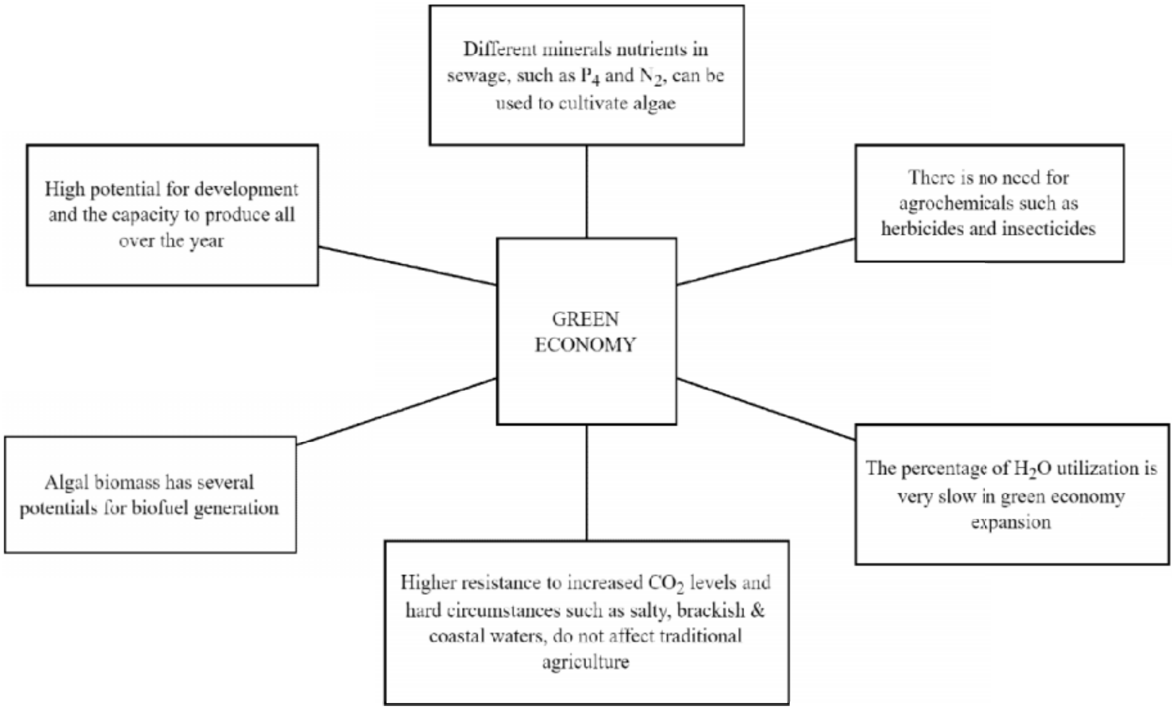

Fig. 2. Assets of Biofuel invention in green economy.

Complete ignition of biofuel happens giving it a cleaner consume. Their quick development rate prompts the high per section of land yield, which remains 7-31 times additional noticeable than each resulting finest yield, palm lubricant. Their high photosynthetic proficiency, more straightforward handling steps, quick development rate, yearly biomass usefulness, constant all year creation makes them a preferred option over the global yields (Levitan *et al.*, 2014). The potential role and applications of diatoms in green economy is shown in the figure below (Fig. 2).

CONCLUSION

Diatoms play a pivotal role in the bio-geo chemical cycling of the marine and fresh water ecosystem. The remarkable fact about diatoms is that it can be cultured very easily with minimal resources and in quick time, that is harvesting diatoms is very economic as well as time saving. From the ecological perspective also, the role of diatoms is note-worthy as it produces quarter of the total net primary productivity of the entire globe. Out of the many applications of diatoms, its drug delivery application is quite remarkable as it has embarked a new horizon in the avenue of precision medicine. Its unique role in the sector of clean energy is setting new standards in the bio-fuel industry and green economy. The property of diatoms to synthesize and store lipid in them is the crown jewel of all the properties it possesses. The most important aspect of study of diatoms is from the environmental aspect as it has given a glimpse of hope in context of the rapid depletion in the fossil fuels. The bio-fuel produced from diatoms is environment friendly and it can be a potential alternative to fossil fuels.

FUTURE SCOPE

The study and research of microalgae including diatoms is still in its initial stage. Diatoms have the capability to produce High Value Molecules (HVM) which includes bio-fuel too. Considering the constant depletion in fossil fuel around the globe, there is a very bright scope and opportunity to study diatoms from the context of bio-fuel and bio-energy (Vinayak *et al.*, 2015). Gene editing technologies like CRISPR, TALENs can be explored to maximize the lipid content enhancement in diatoms as well as other HVM (Chen *et al.*, 2022).

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Conflict of Interest. None.

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