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Different Approaches for Extraction of Oil from Diatoms for Biofuel Production: A Review

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ABSTRACT: The increase in the consumption of fossil fuels have resulted in reduction of natural resources. Thus, hunt for alternate energy sources is getting much attention now a days. Hence, diatoms a class of photosynthetic microalgae available naturally are best suited class of microorganisms utilized for the production of biofuels. Because of their universal presence, ability to grow rapidly, diatoms can be utilized for production of biofuel. One most useful outcome out of these is biodiesel. "Biodiesel" used as a form of sustainable diesel fuel is derived from natural sources. Conventional approach involved in lipid production from diatoms and later transforming it into bio-oil includes list of stages such as cell harvesting and also involves application of stressed condition so as to maximize the assembly of lipids, cell lysis to extract out the lipid content, transformation of lipid content into biodiesel by the method of transesterification. The process of Extraction initiates with disruption of diatom cell wall and further the lipid extraction process can be carried out by performing several processes. Namely spontaneous oozing pulsed electric field, mechanical pressure, High-pressure homogenization, microwave oven and ball mill and so forth. Out of all microwave oven and Solvent assisted ultrasound are considered as effective process for cell wall disruption however for extraction of lipids above mentioned procedures utilizes high energy input and also it is hard to scale up. Diatom-based biofuel comes under third-generation biofuel which ultimately contribute approximately 60 to 90% less greenhouse gases as compared to traditional fuel sources. Extraction steps in biofuel production needs to be renewed because it sometimes causes destruction in entire diatom biomass. The extraction and purification process yields organic wastes which results in demand of significant amount of energy inputs. So, it is preferable to develop eco-friendly purification processes in order to keep the diatom cells alive during extraction.

Keywords: Diatoms, Biofuels, Microalgae, Transesterification, lipid.

INTRODUCTION

"Mother of origin of life", the Ocean serves as the source for unlimited unique and precious organisms. Keeping this in mind Scientists and Researchers these days are looking forward to the solutions that satisfies human needs by making good use of the already available natural resources. The increase in human population and the expansion of these many growing populations towards the countryside adds up to a faster reduction in natural resources, which ultimately results in expanding the costs of these resources in commercial Market (Alexandratos and Bruinsma 2012). In order to deal with such hassle in growing populations and also reduction in the cost of resource, hunt for alternate pharmaceutical based product, food product, high value molecules (HVM), and also other energy sources, are getting much attention. Therefore, utilizing already available organisms such as diatoms are considered as remarkably promising source for production of biofuels out of all other microorganisms. There are also wide variety of characteristics which makes these diatoms better suited for the production of biofuel. Presence of diatoms globally (saline water, fresh water, in soil or on damp surfaces) gives diatom cells competitive advantage against all other microalgae, they are usually free living and unicellular, Diatom cells are surrounded by a rigid cell wall termed as frustule, Diatom cells show rapid growth under suitable conditions, biomass concentration doubles in a couple of hours, diatoms produce characteristic spores, Diatom growth can be controlled effortlessly by the accessibility of silicate, most importantly entire of their biomass can be utilized to productive use. Amongst all the advantages of a diatom-based, open pond system in production of biofuels are the instantaneous ability of utilizing available carbon dioxide and eliminating nutrients from various wastewater sources, although simultaneously, producing valuable fuels and other bio products (Wang and Seibert 2017). One most useful outcome out of these is biodiesel. Biodiesel which is used as a form of renewable diesel fuel, is mainly derived from natural sources such as fats and natural oils. Biodiesel additionally offers various benefits such as economic benefits, quality of fuel obtained, environment friendly, as well as energy safety benefits vs. petroleum diesel also named as petro diesel. Obtained Natural oils can be transformed into biodiesel with the help of a comparatively easy refining practice known as transesterification. Mentioned procedure utilizes sources such as animal fats, oil derived from vegetable, micro algal oils which are further forwarded for esterification process by utilizing alcohol (either one out of methanol or ethanol) in the existence of a catalyst (it can be either potassium hydroxide or sodium hydroxide) to further form fatty esters (either methyl ester or ethyl ester) (Vasudevan and Briggs 2008).

Diatoms: Structure and Evolutionary origin. Diatoms are a class of eukaryotic microalgae, photosynthetic in nature which are not just found in the *Bacillariophyta* family, they are also found in other families namely (*Chaetocerotaceae*, *Thalassiosiraceae, and Lithodesmiaceae etc.)* Commonly organisms belonging to class *Bacillariophyta* is known for the presence of cell wall made up of hydrated silica. About more than 200 genera of existing diatoms and about one hundred thousand of living species are recorded (Round *et al.,* 1990). Entire of diatom lineage is commonly distributed into two orders: centric diatoms which are symmetrically radial and the other order is of pennate diatoms which are symmetrically bilateral (d'Ippolito *et al.,* 2015). Centric diatoms are again subdivided into polar centric and non-polar centric, while the second order comprises of the classes namely *Fragilariophyceae* and *Bacillariophyceae* classified on the basis of the existence of raphe or lack of a raphe (Mann 1999). Diatoms characterized as a microalgae, eventually these diatoms are unique in different traits in relation to wide range of various eukaryotes which are photoautotrophic in nature (Armbrust *et al.,* 2004; Saade and Bowler 2009; Smith *et al.* 2012; Obata *et al.* 2013). The presence of cell wall in diatoms, which is considered as utmost characteristic cellular feature. Diatoms cell wall is made up of silica, with complicated designs and having different symmetry within their cell wall made up of silica. Usually, diatoms size ranges between 20-200 microns in length, mainly majority of the species ranges in between 10 and 50 μm (Hildebrand *et al.* 2012). It has already been proven earlier that diatoms have various characteristic features making them best alternative for large scale-based bio-fuel cultivation (Singha *et al.,* 2022).

The evolutionary story for diatoms is somewhat complex; early evidences puts forward that at a certain point in the evolution of plant cell progenitor, a Chlamydial invasion took place (Becker *et al.,* 2008). As reported at an earlier stage in the evolution of life on the earth, diatoms were believed to be originated from photosynthetic scale covered cells. Since then diatoms are further categorized into major morphological forms (an event occasionally known as the big-bang theory of evolution). Later on this diversification was followed by a relative stasis period till existing day (Round 1981). There are also evidences reporting that nuclear genes of green algal biomass are found in secondary endosymbiont (Moustafa *et al.* 2009), which proposes an existence of endosymbiotic event with green alga. Genomes of diatom additionally contain rich numbers almost up to 5% of genes which are from bacterial origin belonging to different bacterial classes, and nearly more than half of these genes are shared between two diatom groups which are evolutionarily diverse, namely *Thalassiosira pseudonana* and *Phaeodac tylumtricornutum* (Bowler *et al.,* 2008). Consequently, genomes of diatom, and their resultant metabolomes, are a complicated combination of components derivative of sources which are exceptionally dissimilar (Bowler *et al.* 2008; Finazzi *et al.,* 2010). The four groups of diatoms divided on the basis of their silica cell wall features are: 1) Radial centrics, 2) Bipolar centrics and multipolar centrics, 3) Araphid pennates, 4) Raphid Penates. Every single cell of these group arose and later differentiated sequentially under decreasing CO2 concentration and this event took place during Mesozoic era (Armbrust, 2009). Early fossil evidences shows that larger eukaryotic phytoplankton of the lineage red algae over the Mesozoic era, comprising coccolithophorids, diatoms, and dinoflagellates, banished a greater percentage of other algal group residing in the ocean, mainly very small green algae and cyanobacteria (Falkowski *et al.* 2004). Additionally, some initial reports recommended that the most important source of carbon for fossil fuels were diatoms. During the main carbon export period coccolitho-phorids in addition dinoflagellates were considered as the leading variety of phytoplankton. In recent times, diatoms are liable for an enormous part of the natural carbon covered on continental margins and are significant supporters of nascent petroleum reserves.

BIOFUEL PRODUCTION FROM DIATOMS

Biofuels from diatoms, could be basically obtained by means of two routes. The first method is to thermochemically convert the entire biomass fraction into bio-crude oil such as crude oil and the next is directly extracting out lipid content and later sent for processing into biofuel. Though the second method is the conventional mode of technology so far, but the first route is attaining motion because it has some positive advantages. Another fact of concern, is: what is the percentage of oil content in a diatom cell? In order to find out the oil content the most important step is to find out everything related to available lipids including all unsaturated fatty acids forms and forms of saturated fatty acids, followed by extraction process, and lastly quantify the oil content (Jha and Zi-Rong 2004). Along with selecting the best approach to maximize bio products it is very important to select a suitable strain which can give high yield. It is most popular that by choosing specific species and by manipulating the provided supplements in growth medium, the oil production of microalgae can be affected. It has been found that under conditions where organisms grow under nitrogen starvation, *Chaetocerosgracilis triacylglycerols* can account for more than 70% of total cell volume (Syvertsen, 2001). Measurement done because of per weight is much preferable, but this discloses additional possible problem associated with prevailing processes of algal biofuel production. Algal cells growing under nutrient starved condition will support in obtaining maximum lipid content, but this is only possible if we extend microbial growth period. This affects the overall productivity of the manufacturing process per unit area over time. Algae that are not exposed to stressful conditions can grow rapidly, but the resulting lipid content is limited. Additionally, a large difference among lipids is establish in diatoms, few of them are extra chloroplastic phospholipids and membrane associated glycolipids. Significantly within a species the quantity of these lipids may differ, it can also be determined by the provided culture conditions along with the method of cultivation. Therefore, increasing the lipid content of diatoms, including other microalgae, is costly, which requires much more additional culture time to maximize the lipid content under stress conditions. Evidently, if our main aim relies upon bio crude production, then the organic carbon content of diatom must be exploited, and not essentially the lipid content (though content of lipid can affect bio crude quality, in algae at least). The warning is that bio crude would need to be moved up to a fuel-grade item (this should be possible in a petroleum processing plant, however, requires H2 input), though bio-oil (lipid that is upgraded) requires limited processing before it can be used as a fuel. In general, the method of lipid production from diatoms or algae and later conversion into bio-oil involves series of steps shown in Fig. 1.

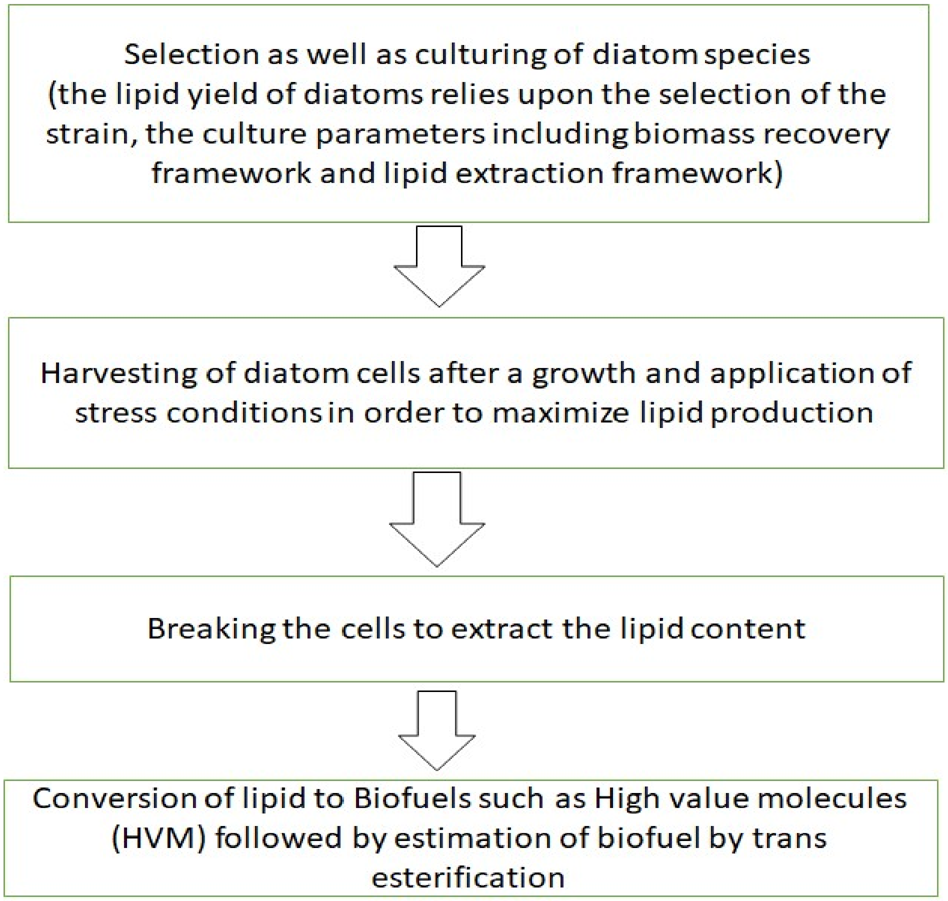

Fig. 1. Series of steps involved in extraction of oil from diatoms for biofuel production.

EXTRACTION OF OIL FROM DIATOMS FOR BIOFUEL PRODUCTION

Plastic Bubble Wrap approach for culturing diatom cells: Recently studied on Employing newly developed plastic bubble wrap technique for biofuel production from diatoms cultivated in discarded plastic waste and concluded that diatoms serves as the best source for Diafuel *i.e.* biofuel extracted from diatoms. The main aim of the mentioned study was to culture diatom cells in a closed system and this was done by tightly sealing the reactor rim with plastic bubble wrap and cheap priced plastic material which are disposed of in a lodging and transportation of goods. In order to optimize it, different plastic wraps disposed of from a plastic industry were sent first for their permeability test to gases and impermeability to water loss. As a result it was found that among all the plastic bubble wrap varieties LDPE (low density poly ethylene) used for sealing glass containers as photo bioreactors allowed harvest highest cell count of (1152×10^2 cells mL^{-1}), maximum Diafuel (37%), lipid (35 $\mu g mL^{-1}$), maximum

CO_2 absorbance (0.084) with nutrient uptake for 40 days and nearly no water loss was observed. In order to check the usability of Low density poly ethylene on other microalgae *Haematococcus pluvialis* example of red green microalgae demonstrated scope to be extended for production of *astaxanthin* utilizing disposed bubble wrap plastic. Results of this study may be beneficial for a new way to decrease plastic disposal and the application of diatoms for biofuel production (Khan *et al.,* 2022).

The extraction as well as fatty acid and lipid analysis from diatoms varies in comparison to other entities, such as foods and vegetable oils, because they possess stiff cell wall, and variety among different classes of fatty acids as well as lipids. Therefore, precise approaches should exist for cell lysis and to release out the lipids and then converting these lipid content into biofuels. The method for lipid extraction must be speedy, effective and subtle so that degradation of lipid content can be reduced and also have to be feasible economically. The process of biofuel extraction begins with the destruction of the diatom cell wall, and the lipid extraction process can be carried out in a variety of ways. The cytolysis process is an essential step in extracting oil from diatoms for biofuel production.

Spontaneous oozing: Researchers has already discovered a diatom strain known as *Diadesmis confervacea* that not only accumulates high amount of oil (14.6%) but also extracts oil naturally almost near the 31st day of culturing in in- vivo conditions, when the cells attain maturity which is very important in the area of economical biofuel production (Bongale and Gautam 2012). It was also proposed that diatoms be confined at stationary phase of growth in solar panels in which they would keep on delivering organic compounds appropriate for biofuel in the form of droplets of oil (Ramachandra *et al.,* 2009), otherwise known as lipid droplets or oleosomes. Spontaneous oozing significantly reduces the cost of algal fuels, as separation of the oil from the diatom cells has previously been an expensive as well as fuel requiring process, and now because of spontaneous oozing it is easier to design diatom biofuel solar panels. The overall protocol for spontaneous oozing can be carried out by Sample collection followed by culturing (the water sample should be first studied for its species richness and further inoculation onto cultured media such as f/2 solid agar medium, appearance of mixed colonies under different cultured conditions, the mixed colonies of cultured diatoms further serially diluted to obtain axenic cultures. Every day the respective plates observed for the presence and size of oil globules in the diatom cells. The exocytosis of oil from the diatom cells is looked for on each day after their inoculation. Cell counting followed by estimation of oil content with the help transesterification method. Fatty acid methyl esters shortly known as (FAME) can be analyzed by GCMS and tested using TLC (Bongale and Gautam 2012).

Pulsed electric field (PEF): PEF also termed as Pulsed electric field uses high voltage, short electrical pulses and a uniquely planned treatment chamber to permeabilize cell membranes. There are two particular uses of PEF to algal development and processing - extraction of intracellular material and microalgae predator population control. Pulse electric field processing can possibly give lower costs and higher efficiency for the process of biofuel production, high-esteem specialty chemical compounds from large scale farms cultivating algae, also animal and human feed and nutritional supplements.

Algal products mostly nowadays depend on solvent extraction processes and drying process to reach out final commercial end product. Extraction processes such as freeze drying and separation done with the help of supercritical Carbon dioxide are intrinsically energy intensive and costly also, restricting the market for microalgae items. Pulsed electric field has been demonstrated by various analysts to lyse various number of microalgae species via electroporation, which delivers their intracellular substance into the neighbouring solution. The main advantage of algal cell wall lysis through Pulsed electric field is to form those intracellular materials that might include lipids, proteins and different chemicals, accessible for downstream processing into precise products. It is very important to adjust the physical parameters as the outcome of pulsed electric field is dependent on cell size (the smaller the cell size will be, the stronger the electric treatment should be) (Coustets *et al.,* 2015; Sixou and Teissié 1990; Bellard and Teissie 2009).

PEF won't fundamentally help in the separation or extraction of intracellular compounds that are found within the cell wall, nonetheless, such extraction process needs a mixture of concentration (to eliminate the water from the algal development media), drying, and chemical treatments. The step performed for drying is very energy consuming and in this way expensive process. Leaving this drying step, and empowering wet extraction, is one of the essential advantages of PEF.

Mechanical Stress: We can exert outward mechanical pressure on Algal cells lacking a natural oozing mechanism, it can be either done by applying ultrasound or touch, which helps in forcing High Value Molecule to come out of the cell. Ultrasound has been utilized in order to improve extraction processes of carotenoids *Haematococcus pluvialis* (Ruen-ngam *et al.,* 2010; Zou *et al.,* 2013), *Dunaliella* (Macías-Sánchez *et al.,* 2009; Pasquet *et al.,* 2011), chlorophyll *Chlorella* sp. (Kong *et al.,* 2014) and lipid *Chlorella vulgaris* (Araujo *et al.,* 2013). As ultrasound effects can be harmful sometimes and can cause death (Rajasekhar *et al.,* 2012) or stimulate programmed cell death (Broekman *et al.,* 2010), nonviable cellular damage one of the features of ultrasound treatment ought to be selected for keeping the cells alive and, hence, be competent for incorporation in a milking protocol. Diatom cells are known for their unique features of being enclosed in a hydrated cell wall made of silicon dioxide which is denoted as frustule. Also every diatom cell possess imperfect bilateral symmetry subsequently resulting in one of the frustules somewhat bigger than the other, allowing one valve to fit inside the edge of the other. Due to this and frustule robustness, mechanical strategies could provide an exceptionally strong strategy that supports the discharge of high value molecule outside the cell surface.

In order to carry out this option, selected diatom cells should be first harvested and further positioned on a stiff surface in water and place a 18×18 mm^2 coverslip, slightly put some stress with the help of a microbial needle (Vinayak *et al.,* 2015). Insure that stress should be applied on the coverslip mid region till water comes out. Water content that flows out persisted at the coverslip edge on the slide. Perform Visual screening of the diatom population residing under the coverslip and document the variations (cell wall lysis, release of oil outside the cell surface). Alive diatom cells are not damaged visibly with the application of mechanical stress in the cover slip and oil discharge from certain species like *Terpsinoë musica* is already documented and as a result it was found *Terpsinoë musica* cells were kept in incubater for 7 days and after putting some mechanical stress oil came out of the cell. The observations reported by the mentioned study shows that oil is released out of the cell via apical pore field, from where release of carbohydrates also takes place (Bahulikar and Kroth 2007).

High-pressure homogenization: HPH is also known as the French press method. This cytolysis procedure utilizes hydraulic shear force which is produced when high-pressure biomass is sprayed down a narrow tube. (Kim *et al.,* 2013; Dong *et al.,* 2016). Because this process is a heat generating process, so it puts forward risk of average energy consumption, thermal degradation, and probability of scale up process.

This method involves high pressure homogenizer, where two liquids are dispersed one is considered as aqueous phase and the other one is oily phase. Or finely divided solids in liquid is attained by pushing their combination via an inlet orifice small in size along with high pressure almost 500 to 5000 psi, which put through the product into strong turbulence and hydraulic shear resulting in enormously fine particle of suspension. Depending upon the cell wall rigidity efficiency of high pressure homogenization in diatom cells differs between different species and may drop (Dong *et al.,* 2016).

Ball Mill: A ball mill is made up of its hollow spinning metal cylinder filled of magnetic beads that serves as a crushing frame. As a result of this framework harm the cell wall, due to collision or friction, caused by rapid rotation of metallic beads. Harm that is brought by metallic beads can cause cell lysis within minutes without the use of any biomass preparation. While working open the lid of metallic cylinder and fill in the feedstock or cells of interest into the chamber (diatom cells should cover almost 60 percent of the cylindrical volume), cover almost 40 to 30 percent of the volume of cylinder with stainless metal balls, close the lid of cylindrical chamber, adjust the mill at critical speed that is 2/3rd centrifugation speed and switch on the mill to rotate. (In case of high speed the metallic balls will be thrown towards the wall of the chamber and no grinding will take place due to the action of centrifugal force whereas in case of low speed the ball mass will slide up into one another which may cause an inconsequential amount of size reduction). After size reduction of feed stop the mill, separate balls from desired product and finally recover desired product. Numerous factors that affect the rupture performance and the power intake of the method, *e.g.*, the stirring speed, the form of the container, the size, type, and the amount of sphere. This method has benefits due to the simplicity of the tools and the quickness of the procedure. However, its scheduling necessitates an extensive cooling device simply to keep away from thermal degradation of the lipids (Kim *et al.,* 2013; Mubarak *et al.*, 2015; Prabakaran and Ravindran 2011; Halim *et al.,* 2012).

Accomplishment of ball mill as a pre-treatment process for extraction of lipid content is useful for few microalgae species. As recorded, extraction process carried out by ball mill method gave over 28% of lipids from *Botryococcus* sp.*,* hence was found 20% superior as compared to ultrasound method along with solvent extraction.

Microwave oven: Fundamentally, microwaves are electromagnetic waves with wavelengths between one metre and one millimetre and frequency between 300 MHz and 300 GHz. Though, small-scale microwaves, approximately of range 2450 MHz are preferable for causing cell lysis in microwave oven (Kim *et al.,* 2013; Balasubramanian *et al.,* 2013). Microwaves used for lipid extraction from microbial cells is done with the help of waves causing cell wall breakage by inducing heat and interacting with molecules hence resulting in lipid molecules to drained from the cell (Mubarak *et al.,* 2015; Pragya *et al.,* 2013). Microwave aided heating system is quicker as compared to traditional heating because transmission of heat happens due to radiation rather than conduction/convection. This process is better option for polar solvents such as water that produces steams resulting in cell wall breakage, later releasing intracellular contents (Dong *et al.,* 2016) and results to an effective lipid withdrawal technique. Due to generation of high temperature, certain products such as fatty acids, lipids can sometimes result in degradation. In that situation, it is essential to reduce process time and use cooling system which can avoid several bio products degradation. By means of microwave we can achieve a method with less demand for solvents and reduced extraction time, but needs high energy cost seeing its scale up process (Halim *et al.*, 2012; Pohndorf *et al.,* 2016).

Microbial lipids further used for the biofuel production via conversion process: Direct Transesterification

Direct transesterification has been examined as a procedure of biodiesel producing strategy without even performing the steps of extracting and purifying oil (Liu *et al.,* 2015; Griffiths *et al.,* 2010; Zhang *et al.,* 2015). Done on the presence of algal biomass, a catalyst and an alcohol, commonly acid, when combined together and heated at ultra-high temperature. Extraction process of lipid as well as transesterification process take place at the same time, and results in the production of biodiesel (Mubarak *et al.,* 2015; Singh *et al*., 2014;

Velasquez-Orta *et al.,* 2012; Ehimen *et al.,* 2010). Mentioned procedure can be performed out with dry or else with wet biomass (Liu *et al.,* 2015; Suh *et al.*, 2015). Direct transesterification process reduces the stages of biodiesel production, moreover decreases protocol cost as well as final cost. Left over biomass concentration separation from cell debris and biodiesel, additional alcohol and glycerol is done by via centrifugation or filtration methods (Chen *et al.,* 2015). Direct transesterification can be utilized for finding out the composition of fatty acids and profile of fatty acids in lesser microalgae sample (Liu *et al.,* 2015). The drawback of this process lies while conversion of lipids into fatty acids, lipids cannot be classified and evaluated into various classes, *e.g.*, phospholipids, triacylglycerols, as well as glycolipids. If it is necessary to differentiate lipids into various classes, solvent extraction have to be performed (Griffiths *et al*., 2010).

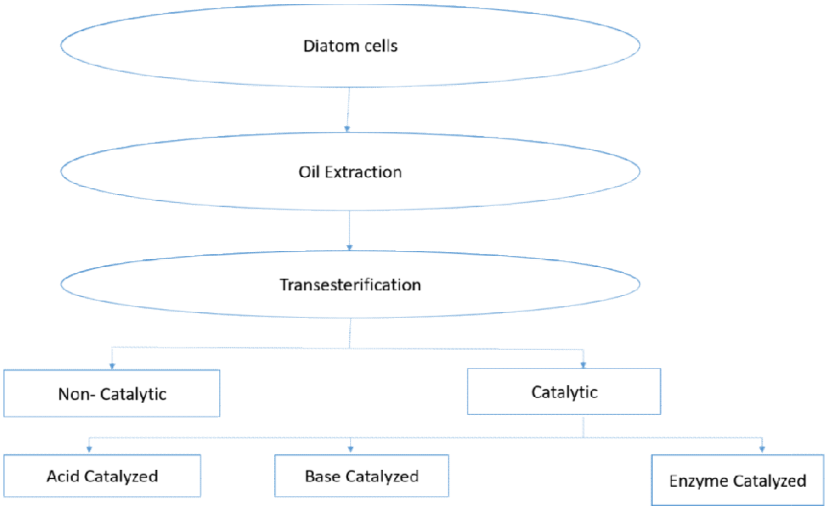

Fig. 2. Process outline of oil extraction from diatoms for synthesis of biofuel (subdivision of Transesterification that is performed for estimation of biodiesel).

ADVANTAGES AND DISADVANTAGES OF USING DIATOMS AS BIOFUEL

Studies that specifically aims to find out the viability of diatoms for industrial scale biofuel production are confined and their efficiency varies by different ecological factors, quite a few comparative outcomes are existing under favourable culture conditions. Researches that are performed to compare the productivity of diatom cells at favourable culture conditions that varies in different ecological conditions are important to gather information on the ecological condition-driven biases and possibility of culture success. Very few information is available on particular strains that can be used for biofuel production. Media required for culturing diatom cells utilizes silica which ultimately results in increment of costs (Marella *et al.*, 2019; Tan *et al.,* 2018). Moreover, the soluble silica is found in the form of silicic acid in aqueous conditions. Which may result in the formation of complexes with metal ions, of which magnesium silicate forms a precipitation that adversely disturbs the culture system.

Diatom cells are effectively developed in the aquaculture industry. Hence, making diatom cultivation for production of biofuel. Some research studies recommend that diatom based biofuel can be utilized in modern vehicles and engines also can be stored and burned in the same way as traditional fossil fuels (Eva-Mari, 2016; Bhagea *et al.,* 2019). Enormous utilization of diatom based biofuel will give energy security, especially when fossil fuel supply is disrupted and affected. This can additionally help in bringing improvement to energy balance through domestic energy crops. Transportation leads to higher greenhouse gas emissions because of pollutants in fossil fuels, while diatom based biofuels can potentially address significant challenges with respect to emission and fuel quality. Diatom based biofuel is supposed to decrease emissions of cancer-causing compounds upto a range of 75% - 85% and is considered as more nontoxic to handle as compared to conventional fossil fuel because of its low volatility (Sharma *et al.,* 2021).

Silica from diatom can be obtained from a monoculture for additional useful properties that can have applications in nanotechnology. Diatoms are known to make nanostructured silica in variable shapes (Zgłobicka *et al.,* 2021). Silica obtained from diatom cells in a production system can serve as a useful marketable product. Hence, along with the production of biofuel, diatom might give added benefits such as filtering materials it is because of porous nature of diatom frustules that makes it convenient sieving materials that can be used for separating very minute particles, silica known for its hygroscopic nature is utilized as neutral wormicides and natural insecticides (the contact of ghygroscopis silica with cuticle causes dehydration in the insect along with perforating the cuticle with sharply edged tiny particles, diatoms are also utilized in cosmetic industry prospected for amino acid synthesis, the abrasive nature of diatoms makes them useful for toothpaste, metal polish etc (Ahirwar *et al.,* 2021). Hence, cultivation of diatoms in large scale and diatom based biofuel production can introduce multiple benefits in today's demanding market.

CONCLUSION AND FUTURE PROSPECTS

It has been acknowledged world-wide that first generation and second-generation biofuels, which are predominantly produced by terrestrial crops are insufficient to fulfil the universal energy demand, and hence researchers are in search for alternative sources for production of biofuel. Also for growing terrestrial crops the need for agricultural land will increase as well as demand for fertilizers will increase accordingly. Recently, third-generation biofuels that can be gained from microalgae are graving a lot of attention because of its capability to propagate on non-arable land and also gives high oil yield per area. Morphological as well as physiological variation among diatom species permits these cells to react rapidly to any kind of stress condition. The oil content of diatom cells relies upon the selection of the strain or species to be used for cultivation purpose, the culture parameters, such as nitrogen stress condition provided, intensity of light required for growth, framework selected for recovering biomass concentration, and particularly protocol selected for extraction of oil. Effectiveness of the withdrawal strategy relies upon various elements which have impact for the selection of best technique for cell lysis, plus the strain selected and nature of their cell wall, whether nonpolar or polar lipid present , beyond operational cost and energy costs. Microwave oven and Solvent assisted ultrasound are accounted as proficient process for cell disruption and how ever biofuel extraction process utilizes highly energy input as well as hard to perform scale up process. Primary method for developing biofuel production strategy from diatom lipids is to obtain it at inexpensive cost, via the selection of finest strain for cultivation and development of better cultivation strategy that permits maximum lipid yield. Terrestrial yields like corn, rapeseed, and soybean acts as primary feedstock for production of biodiesel. Sadly, utilization of food yields for the production of biodiesel causes competition among utilization of agricultural land and its utilization for producing biological fuel with a subsequent growth in food costs and potential biodiversity and habitat loss.

For obtaining sustainable as well as cleaner diatom fuels of profitable feasibility, attention must be set to extraction procedures which are eco-friendly also, with a smaller amount of solvent utilization, by increasing the biofuel quality and limiting energy as well as time utilization and steps for downstream processing. Solvents must be precisely evaluated keeping in view about effective way for extracting out lipid content to make the bio refinery techniques more practical, constructing bio-economy based on renewable resources.

Therefore, production of bioethanol and biodiesel both the processes can be coordinated. This way, biodiesel can be obtained from fatty acids of microalgae and bioethanol can be obtained from de-fatted biomass (methyl solvent is swapped by ethyl solvent during transesterification process), rather than utilizing solvents derived from petroleum. Hence, the utilization of green solvents for microalgae extraction and biofuel production is significant for a successful economic, ecological, and such approaches can be useful in social production of multiple products over individual processes, under the idea of a bio refinery.

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Conflict of interest. None.

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