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Journal of Water Process Engineering 39 (2021) 101705

Contents lists available at ScienceDirect

Journal of Water Process Engineering

journal homepage: www.elsevier.com/locate/jwpe

Diatoms recovery from wastewater: Overview from an ecological and economic perspective

Abhishek Saxena [a], Archana Tiwari [a,*], Rinku Kaushik [a], Hafiz M.N. Iqbal [b,*], Roberto Parra-Saldívar [b]

[a] Diatoms Research Laboratory, Amity Institute of Biotechnology, Amity University, Noida, UP, 201301, India
[b] Tecnologico de Monterrey, School of Engineering and Sciences, Monterrey, 64849, Mexico

ARTICLE INFO

Keywords:
Axenic culture
Diatoms
Microalgae
Bioindicators
Isolation
Wastewater

ABSTRACT

Alarming water pollution is toxic to the aquatic ecosystem leading to a sharp decline in species diversity. Diatoms have great potency to survive in contaminated water bodies, hence they can be compelling bioindicators to monitor the change in the environmental matrices effectively. Around the globe, researchers are intended to evaluate the impact of pollution on the diatoms recovery and techniques used for the assessment. The diatoms are precious for futuristic need viz. value-added products, energy generation, pharmaceuticals, and aquaculture feedstocks. All these applications led to a significant rise in diatoms research among the scientific community. This review presents different isolation practices, cultivation, and other challenges associated with the diatoms. A precise focus is given to diatoms isolation techniques from highly polluted water bodies with the main thrust towards obtaining an axenic culture to elucidate the significance of pure diatom cultures. Recovery of "*jewels of the sea*" from polluted water signifies the prospective ecological and economic aspects.

1. Introduction

Diatoms are the most successful contemporary group of photosynthetic eukaryotic microbes that inhabit almost every kind of aquatic ecosystem. They can occur as endosymbionts in dinoflagellates [1,2]. Diatoms have arisen because of an endosymbiotic event between red algae and heterotrophic flagellate approximately 240 million years ago [3]. Although there are several types of microalgae present in water, diatoms are the most important and unique as they are the only species with the presence of silica in their cell [3,4]. Diatoms are surrounded by a silica cell wall that allowing them to have varied shapes and sizes. Their silica cell wall is popularly known as frustules that offer protection from photoinhibition; supply appropriate nutrients uptake; manage sinking rate and playing a significant role as a mechanical barrier against grazers [5]. Their silica content varies from one species to another depending upon their size, growth pattern, and environmental variables such as light, temperature, salinity, nutrients, and growth phase [6]. There are more than 200,000 extant species of diatoms distributed to a different ecosystem [7]. They are widely present in all aquatic habitat constituting lentic, lotic habitat and sand. It is estimated that 70% of species occur in freshwater, and 83% are benthic [8].

Aquatic ecosystems such as rivers, oceans, lakes, etc. support the lives of a large variety of organisms but excessive urbanization and industrialization transform them into the heavily polluted zone [9,10]. The rising pollution level deteriorates the water quality index leading to a huge decline in species diversity due to untreated discharge of metals, pesticides, aromatic polycyclic hydrocarbons, etc., which slows down the survival of an aquatic species, thus posing a big threat [11]. Several organisms have been used as bioindicators such as protozoa, bacteria, fishes, zooplankton, algae, etc. However, diatom-based monitoring for the assessment of ecosystem remedy is in great demand [12]. Because diatoms produce organic matter to a large extent that permits natural inbuilt capacity to withstand toxicity levels in water bodies, extended survival rate, short regeneration time than microalgae, fishes, and other micro invertebrates thus making them one of the best candidate for water quality monitoring, and excellent bioindicators of aquatic biological integrity [12,13]. Water quality analysis is essential as it allowed to detect the habitat linked information of the related organisms [14]. As diatoms also produce eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and other nutritional compounds, they can be used as a food source to every aquatic organism such as zooplankton and fish. They constitute 40% of oxygen in water bodies, fix excessive chemical

* Corresponding authors.
E-mail addresses: panarchana@gmail.com (A. Tiwari), hafiz.iqbal@tec.mx (H.M.N. Iqbal).

https://doi.org/10.1016/j.jwpe.2020.101705
Received 26 May 2020; Received in revised form 9 September 2020; Accepted 19 September 2020
Available online 16 October 2020

compounds and inorganic nutrients in the water, such as carbon, phosphorous, silica etc., [15]. As fossil fuels are diminishing quickly, therefore, the demand for biofuel production has increased many folds since the last decades. The crop and food-producing plants will never be the top priority for biofuel production. In the future, diatoms will remain as the best candidate for biofuel production. Some strain of diatoms produces lipids as 50% to 80% of the dry weight that can be used in the production of biofuel thereby focusing on the role of diatoms as an ideal substitute for fossil fuels [16]. Despite having all these important characteristics, they are the least explored aquatic species because isolation and maintenance of axenic culture is a tedious job [17]. In this review, an effort has been put forward on the details related to the isolation of diatoms from polluted water bodies, the significance of diatoms, and their future potential.

2. Ecological significance of diatoms

Rapid industrialization and urbanization releasing a toxic substance into the aquatic environment. Thousands of chemicals without any proper risk assessment and legislation are being introduced, which are endangered to aquatic species [18]. Algae, bacteria, protozoa, fishes, micro invertebrates, and zooplankton have been extensively used in biomonitoring purposes, but recently, diatoms have gained much more attention because they are primary producers and plays a crucial role in biogeochemical cycles of the aquatic food web [11,12,19]. Diatoms survive well in a variety of habitat, such as oceans, lakes, estuaries, wetlands, etc. The use of diatoms in ecotoxicological studies is advantageous to examine any possible effects of toxic elements. This makes diatom a potential tool in bioassay than any other aquatic species because of its easy sampling and frustules identification [20,21]. Diatoms are highly sensitive to metal pollution than macroinvertebrates, which could very well explain gradation in metal composition, thus allows early identification of the presence of any heavy metal pollution in an aquatic ecosystem [12]. Diatom responds quickly to any organic matter or nutrient contamination, for example, metolachlor, atrazine, simazine, phenols, nitrate, phosphate, heavy metals, and PAHs [22,23]. Even though diatoms are ecologically diverse, their potential in biomonitoring is underutilized in evaluating any risk assessment because of traditionally available metrices such as cell density, biovolume, and relative abundance. New and advanced metrices are more advantageous in recognizing the dynamics of diatoms communities by demonstrating the sub-lethal effect that is not evident in diatom counts [19,24]. Nuclear abnormalities, cell membrane, cytoplasmic content, and photosynthetic apparatus alteration change in lipid content and diatoms classification using ecological status are the main parameters that were infrequently reported [25–27]. This behavior demonstrates sensitivity and efficiency in quantifying the toxic elements, stress detection after ultraviolet (UV) exposure, which remains unnoticed by the conventional method [28]. As reported, calculating live diatom species in the biofilms seems to be a lucrative bioassessment tool.

Similarly, for assessing weak cytoplasmic content of freshwater species, the ecological association of diatoms is examined for evaluating the environmental health of water bodies because these newer metrices are easy to acquire, rapid detection, low labor-intensive, good reproducibility, standardized and worldwide acceptance [23,28]. Diatoms as bioindicators check and observe the integrity of the precious environment by enumerating the change in water quality over large geological areas, provide variability, and any possible backdrop precondition [29]. Investigator worldwide have examined various water bodies using pollution tolerant diatom species to measure the pollution level by employing algal indices such as Palmer's Algal Index; Chlorophycean Index; Nygaard's phytoplankton indices to better realize the water quality. For example, some investigator utilized Nygaard's Algal Index to evaluate the trophic status of Hatia Dam revealing the oligotrophic condition of water bodies [30]. Mishra and co-workers studied the Bhoj Wetland, employing Nygaard's Algal Index to illustrate the wetland's eutrophic nature [31]. Palmer index was utilized in determining the seasonal variation in phytoplankton diversity of Pichhola lake of Udaipur, Rajasthan [32]. Sharan and Rekha used Nygaard's Algal Index in their evaluation of the trophic status of Hatia Dam, finding the waterbody to exhibit an oligotrophic status [33]. But still, diatoms potential is hardly exploited as bioindicators in determining the economic conditions as compared to other groups of microalgae [30].

The environmental conditions of the target species must be taken into account as understanding their habitat and mimicking them in the laboratory play a crucial role in obtaining pure strains. Each aquatic ecosystem has different attributes. River ecosystems may have less alkalinity than a marine ecosystem [34]. These attributes directly or indirectly affect the occurrence of diatoms in a diverse aquatic ecosystem. Thus, several factors, such as seasonal variation, pH, temperature, salinity, etc. should be considered as important factors [35]. The representatives of the genus *Gomphonemoid* can be examined from both marine and freshwater. *Gomphonemoid* is widely present often in freshwater epiphytic and epilithic communities [36]. *Gomphonema*, one of the most common diatom strains, its valve and girdle view, the heteropolar cuneate shape can be recognized easily. *Gomphonema* is attached to the substratum by a mucilage stalk, which assists in recognize strain from the rest of the other diatoms found in the freshwater ecosystem. The valves are wide at the head than foot pole with both uniseriate and biseriate nearby amid individual areolae cover-up externally by crescent-shaped volae [37–39]. The raphe is straight or sinuous with terminal fissures expanding both apices to the valve margin, and inner central endings turn aside to one or the same side, respectively. The raphe is always shorter from the central nodule to the head pole, thus extending towards the foot pole.

Consequently, a perforated distinct pole field of different structures is also present at the foot pole. In many species, isolated punctatum near the central nodule occurs. The cingulum is arranged into many open bands; every second band is shorter than the next one to form lingual at the foot pole. Each band is punctured by more than one row of pores. Many other diatom species with the same cuneate shape, growing in the marine ecosystem can be compared to *Gomphonema* by various workers [40].

Benthic diatoms are the significant controller in freshwater owing to their excellent adsorption, absorption, oxidation, decomposition, precipitation capability to store nutrients. They are very well utilized in biomonitoring purposes since the last decade because these diatoms provide crucial linkage in the aquatic ecosystem stipulating the flow of energy, cycling of materials, and reveal the water environment [41]. Diatom based indices were first produced by Kolkwitz & Marsson [42]. Since more than 20 indices have been created from 1975 to 2015 such as humic degree, trophic level indicators, eutrophication, biological diatom index to name a few. However, the impact of indices has become less focal owing to the differences between floral geographical area and environment fluctuations guiding species to adapt according to the water quality criteria of the particular region in a particular instance of time [43]. Isolation and identification of benthic diatoms are problematic in comparison with planktonic species due to difficulties in sample treatment, sampling, and microscopic observation though benthic diatoms play the main role as bioindicators in the aquatic ecosystem because they attached to the substratum with secreted mucilage from their cell wall [44,45]. The morphological features help in the classification of particular species. Morphological analysis by light microscope (LM) and electron microscope, for instance, transmission electron microscope (TEM), scanning electron microscopy (SEM) provides the better-detailed result of diatom biodiversity [46].

The detection of diatom communities on various substrata can be observed as the slimy layer of biofilm appears brown or yellow-brown. Although there are several types of substrata for isolating diatoms, the most preferred is from cobbles (Epilithon). Epiphytes also have diatoms attached to them [47]. The taxonomy of the epiphyte should be recorded as specific strains attach to specific macrophytes. A typical substratum

can be introduced to the water manually if the preferred substratum is absent. The isolated biofilm can be preserved or can be used for the preparation of axenic culture. A well-concentrated sample collected from a highly polluted habitat may have a more significant number of microalgae rather than diatoms. Sometimes an association between diatoms and other microalgae can be seen. It may also contain empty or broken frustules. Highly polluted water may have a high concentration of biogenic compounds, which may provide less nutrition or over nutrition. This may profit in the growth of those strain, which requires fewer nutrients or high nutrients, respectively. Salinity, pH, light, etc. factors also play a role in the growth of such diatoms [48].

The productivity of diatoms is higher than the other class of microalgae. They have undergone 2-4 cell divisions per day. They can survive in adverse climatic conditions since their silica cell wall is a nanoporous structure which allows them to act as a potential buffer system by improving carbonic anhydrase activity and helps in the transformation of bicarbonate to carbon-di-oxide (CO_2). They actively participate in wastewater remediation and quenching of heavy metal, leading to a degradation of environmental pollution [49]. Though bacteria, fungus, yeast, and algal biomass are commonly used in the treatment of heavy metal, diatoms are most preferred due to their large surface area, mucilage, nutritional requirement, and faster growth rate. Bioremediation of heavy metal involves biosorption to metal-binding ligands on the cell surface, followed by bioaccumulation consists of inorganic molecules and related enzymes. In biosorption, the uptake of heavy metals is either carried out by the physiochemical pathway devoid of ATP or during metabolism engaging ATP energy. This mode of heavy metal remediation is a cost-effective approach through filtration. It is an extracellular passive route of adsorption of heavy metal, which is an energy-independent process occurring at the cell surface in equilibrium [50]. Biosorption is dependent upon temperature, ionic strength, pH, contact time, biomass and metal concentration, the composition of the cell wall, and metallic ions complexation. The principle behind biosorption is that diatoms are equipped with polymers, their outer wall has a negative surface charge due to functional groups like carboxyl, phosphoryl, and amine. The heavy metal with a positive surface charge attracts a negative charge through electrostatic interaction, which finally supports biosorption. Bioaccumulation is an active intracellular process driven by energy. The heavy metals grow along with nutrients that assist in bioremediation. When heavy metals are accumulated, the microalgae produce reactive oxygen species (ROS) to control cellular metabolism. There is an underlying passive uptake followed by an active process that is brought about by the detoxification of heavy metal at the cell surface by dehalogenation and denitrification process. This triggers the exclusion of heavy metals to maintain equilibrium conditions, thus eliminate toxic effects. Despite this, a lot of work needs to be done in the treatment of wastewater by testing more species and study their capability of heavy metals removal. The biological degradation of heavy metal is quite challenging but not impossible [49,51].

3. Taxonomic studies of the target species

Previously, diatoms have been differentiating according to shape, size, pattern, and ultrastructure of their exoskeleton, which consists of silica, known as frustules. Microscopic identification is based on the shape of their frustules, but still, they are challenging to identify, and improper examination reflects misleading observation upon identification beyond the genus level [52–54]. To combat such a drawback, deoxyribonucleic acid (DNA) sequence analysis has become a more potent approach in diatom research, thus opens a new leaf into their systematic and evolution. The main aim of the taxonomic examination is to make a hierarchical classification accurately reflecting evolutionary relationship, which then converted into a hierarchy of names related to different levels in a hierarchy of taxonomic ranks. Recently, diatoms phylogenetic analysis at the generic and infrageneric levels require internal transcribed spacer (ITS) region of the 18S–5.8S–26S nuclear ribosomal DNA and among all, 18S ribosomal ribonucleic acid (rRNA) sequencing resulting in the largest reference sequence dataset among the different markers used for diatoms [55–57]. Microarray technology enables parallel analysis of many genes in a single reaction. Here, ordered matrices of DNA sequence marked on the glass slide used for hybridization experiment with fluorescently labeled target genes and have been extensively used in both basic and applied aspects of the biological sciences [58].

Though several papers related to taxonomy and most of them provide line drawings or light microscopic images that are unable to give enough ultrastructure details of the diatom cell [59]. A complete taxonomic survey can give the entire morphology of lesser-known microalgae or diatoms. As there can be a minor difference in the morphological structure of two or more organisms, such organisms are difficult to identify even under the LM. Organisms like *Cyclotella brebisson* may comprise more than complex 100 species, which includes small specimens with a diameter of ≤ 5 μm [60]. Based on frustules morphology, observation of these small specimens under a SEM provides notable interspecific similarities that help in taxonomic studies. As some species may have similar morphological characteristics which may be ecologically distinct, their identification should be fundamentally accurate as it can provide quite an information about biodiversity which can be represented as an essential tool for different kind of studies such as ecological and applied studies. *Proschkinia* is a rare diatom of the family Proschkiniaceae within the Naviculales. Based on light microscopy, it was classified as a relative of *Nitzschia*. The phylogenetic position of *Proschkinia* was resolved with sequencing the complete mitochondrial genome of *Proschkinia complanatoides* and compared the data set to other published diatom mitochondrial genes [61]. To differentiate between diatoms strains that have similar morphology, it is vital to know the taxonomy of the target species. The nutritional requirements of various species also vary. Some species require less nutrition composition, light temperature variables, while some require more. The taxonomy also helps in understanding the basic requirements of the target species [62]. Once the culture has been collected and identified, they will be preserved in the world federation of culture collection centers, which then made available to researchers and industry for intending applications and further experiments. Table 1 shows the list of available algae, microalgae, and diatom culture collection centers worldwide.

4. Understanding diatoms: Isolation and investigations

4.1. Preparation for sample collection

The field procedures play a crucial role in the isolation of diatoms, microalgae, or other species. In a riverine environment, small boulders (rocks) and cobbles are the most favored substratum for diatoms screening. Almost all diatom indexed to the epilithion community found here where epilithion`s ecology is recognized better than any other group [63]. Bricks, concrete, bridge supports, canal walls, etc. may be used as an alternative substratum, whereas simulated substrate can be set up into the stream if pebbles, cobbles, boulders, or macrophytes do not exist from the site though sampling should be done if they have been plunged for several weeks [47]. Sampling is an important step towards the collection of diatoms as the whole isolation process depends on it. The collected water sample may contain dead cells or damaged cells, which is not considered good, so it becomes very crucial to ideally collect the sample and isolate the most viable cells at the earliest. Keeping the water sample for longer times is not recommended because environmental conditions change very frequently, which leads to cell death. Some species multiply quickly and suddenly die, in that case, isolation should be done rapidly. Sometimes, a planktonic net can be used to concentrate the sample as it removes other unwanted algal species, microorganisms, or debris but should be avoided as more concentrated sample leads to more damaged cells [47,63].

The collected sample should be kept according to the target species` environmental conditions. For example, several marine species are susceptible to sample concentration. For a sampling of these strains of

Table 1
Tabulated representation of available algae, microalgae and diatom culture collection centers worldwide.

S. No	Algae Collection Centre	Microalgae Collection Centre	Diatom Collection Centre
1.	NCMA WDCM2-Provasoli-Guillard National Center for Marine Algae and Microbiota Bigelow Laboratory for Ocean Sciences	MZCH-SVCKWDCM480-MicroalgaeandZygnematophyceae Collection Hamburg Universitat Hamburg	BCCM Diatom Collection Gent, Ghent University Belgium
2.	CALU WDCM 461-Collection of Algae St. Petersburg (Laningrad) State University Centre for Culture Collection of Microorganisms, Laboratory of Microbiology, Faculty of Biology, St. Petersburg state University	IPPAS WDCM 596-Culture Collection of Microalgae IPPAS, Institute of Plant Physiology, Russian Academy of Sciences	VBCCA WDCM 931-Visva-Bharati Culture Collection of Algae, Visva-Bharati Central University
3.	CAUP WDC 486-Culture Collection of Algae of Charles University in Prague Department of Botany, Faculty of Science, Charles University, Prague	PGC WDCM 641-Peterhof Genetic Collection of Microalgae Biological Research Institute of the Leningrad State University	
4.	NIVA CCA WDCM 498-NIVA ulture Collection of Algae Norwegian Institute for Water Research	IOUSP WDCM 728-Marine Microalgae Culture Collection IOUSP	——
5.	CCAP WDCM 522-culture Collection of Algae and Protozoa Scottish Association for Marine Science (SAMS Ltd)	CCMA-UFSCar WDCM 835-Freshwater Microalgae Collection Cultures University Federal of Sao Carlos	——
6.	ANACC WDCM 532-Australian National Algae Culture Collection CSIRO Marine and Australian National and Atmospheric Research	EGE-MACC WDCM 845-Ege-Microalgae Culture Collection Ege University Faculty of Engineering Department of Bioengineering	——
7.	IPPAS WDCM 596-Culture Collection of Microalgae IPPAS Institute of Plant Physiology, Russian Academy of Sciences	CWU-MACC WDCM 886-Herbarium of Kharkov University (CWU)-Microalgae Cultures Collection, V.N. Karazin Kharkov National University	——
8.	BOROK WDCM 602-The Collection of Algae Institute for Biology of Inland Waters Academy of Sciences of the Russia	KMMCC WDCM 894-Korean Marine Microalgae Culture Center Pukyong National University, Dept. of Aquaculture	——
9.	CPCC (formely UTCC) WDCM 605-Canadian Phycological Culture Centre (formerly University of Toronto Culture Collection of Algae & Cyanobacteria	Soley WDCM 979-Microalgae Culture Collection Soley Institute	——
10.	UTEX WDCM 606-The Culture Collection of Algae at the University of Texas Austin, University of Texas Austin	BCMD WDCM 1124-Freshwater Microalgae Collection Cultures FURG	——
11.	PGC WDCM 641-Pterhof Genetic Collection of Microalgae Biological Research Institute of the Leningrad State University	SYKOA WDCM1125 - Strain collection of microalgae and cyanobacteria from nothern and arctic regions in the Institute of Biology of Komi Scientific Centre	——
12	CCAC WDCM 807-Culture Collection of Algae at the University of Cologne CologneBiocenter/ Botany Department	MCC-MN WDCM1144 - Microalgae Culture Collection	——
13.	BEA WDCM 837-Banco Espanol de Algas-Spanish Bank of Algae, Fundacion Parque Cientifico, Tecnologico-University of Las Palmas de Gran Canaria	TAU-MAC WDCM1156 - Thessaloniki Aristotle University Microalgae and Cyanobacteria Collection Aristotle University of Thessaloniki-School of Biology	——
14.	FACHB WDCM 873-Freshwater Algae Culture Collection, Chinese Academy of Sciences, Institute of Hydrobiology, Chinese Academy of Sciences	MASUALGDMA WDCM 1166-Collection of Microalgae, Department of Mycology and algology, Faculty of Biology,MSU, M.V. Lomonosov Moscow State University	——
15.	ACOI WDCM 906-Algoteca de Coimbra/Coimbra Collection of Algae	IBSS WDCM 1201-Collection of Living cultures of plankton microalgae, The A.O.Kovalevsky Institute of Biology of the Southern Seas of RAS	——
16.	CCBA WDCM 914 –Culture Collection of Baltic Algae at the University of Gdansk Institute of Oceanography, University of Gdansk	CICCM WDCM 1042-Cawthron Institute Culture Collection of Microalgae Cawthron Institute	——
17.	SCCAP WDCM 935-Scandinavian Culture Collection of Algae & Protozoa Department of Biology, University of Copenhegan	——	——
18.	ACCS WDCM 936-Algae Culture Collection of Siberia Siberain Federal University	——	——
19.	CCCryo WDCM 940-Culture Collection of Cryophilic Algae Fraunhofer Institute for Immunology & Cell Therapy, Branch Bioanalytics& Bioprocesses (IZI-BB)	——	——
20.	ACKU WDCM 994-Culture Collection of Algae at Kyiv University Taras Shevchenko National University of Kyiv	——	——
21.	BCAC WDCM 1023-Bashkortostan Collection of Algae and Cyanobacteria Bashkir State Pedagogical University named after M. Akmullah	——	——
22.	UMACC WDCM 1059-University of Malaya Algae Culture Collection Institute of Ocean and Earth Sciences (IOES), C308, Institute of Graduate Studies Building, University of Malaya, 50603, Malaysia	——	——
23.	MACC WDCM 1150-Malaysia Algae Culture Collection Aquatic Botany Laboratory, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak	——	——
24.	CAMU WDCM 1158-Collection of Algae at Bohdan KhmelnytskyiMelitpol State Pedagogical University Bohdan KhmelnytskyiMelitopol State Pedagogical University	——	——

*Source: Copyright@2011. World Data Centre for Microorganisms “Culture Collections Information Worldwide”. World federation for Culture Collection.

species, special water bottles can be used. As several factors like water depth, pressure, temperature, or light influence the sample conditions, so instead of using a concentrated sample, a normal water sample taken in sterile containers can provide a better result. The collected sample should always be kept in sterile containers at a stable temperature [63].

Often sample collected from different water bodies contains zooplankton or other species which feed on algae or specifically on diatoms. So, it is essential to remove the unwanted organisms gently by filtering the sample at the sample site. However, sometimes tiny organisms may pass through the filter, which may pose threats to target

species, in that case, dilution methods can be used to isolate the target species. The health of species in nature also plays a crucial role as an isolated cell will have successful growth only if they were in a good state at the sample collection time. It is advised to collect the sample carefully. The use of sterile equipment is mandatory, as dirty equipment may lead to contamination. All the isolation techniques should be performed under sterile conditions [64]. A general idea about field procedure, laboratory procedure, culture preparation, and finally aim towards obtaining axenic culture is presented in Fig. 1.

4.2. Substrata Selection

The identification of a suitable substrate is a pivotal step to recognize the diatom communities in the natural environment. Diatom communities form a slimy layer or thin golden- brown layer on the substratum. It can be more noticeable by feel or touch and can be identified at a specific time depending on species. The substratum can be differentiated into a preferred substrate and an alternative substrate. Diatom attaches to different substrata or facilitates locomotion by releasing mucilage from various structures of the cell wall [65]. Diatom community composition is mainly influenced by chemicals present in water, water turbulence, temperature and light present in water, being eaten by large microorganisms, etc. The most preferred substratum is the solid substratum. It mainly includes cobbles/pebbles and small rocks (Epilithon). Fig. 2 depicts various substrates intended for diatoms sampling. They are widely available in almost every aquatic habitat and throughout the year. In the absence of a solid substrate, submerged or emerging macrophytic plants (epiphyte) should be sampled as it also provides a suitable habitat for diatom communities. It is ideal to note the microhabitat of diatoms species [35].

4.3. Elimination of contaminants from collected sample

The most effective method to eliminate contaminants is achieved by various techniques such as filtration, sedimentation, and centrifugation as gravity separation. The most successful technique is filtration as it removes all unwanted microorganisms or other zooplankton, which feed on diatoms from the sample. In this, a planktonic net of a specific size is used, which does not allow any other organism to pass through except the target species. In gravity separation, diatoms settle down as sediments while leaving other microalgae in the suspension, which can be discarded further [66].

4.4. Health and safety tips

Field operators should always wear thigh waders for the protection of feet. They should wear life jackets while sampling. They should never go in-depth for sampling. They should wear globes when sampling heavily polluted water bodies. They should be very careful in habitats dominated by dangerous animals posing a threat to people and blood-

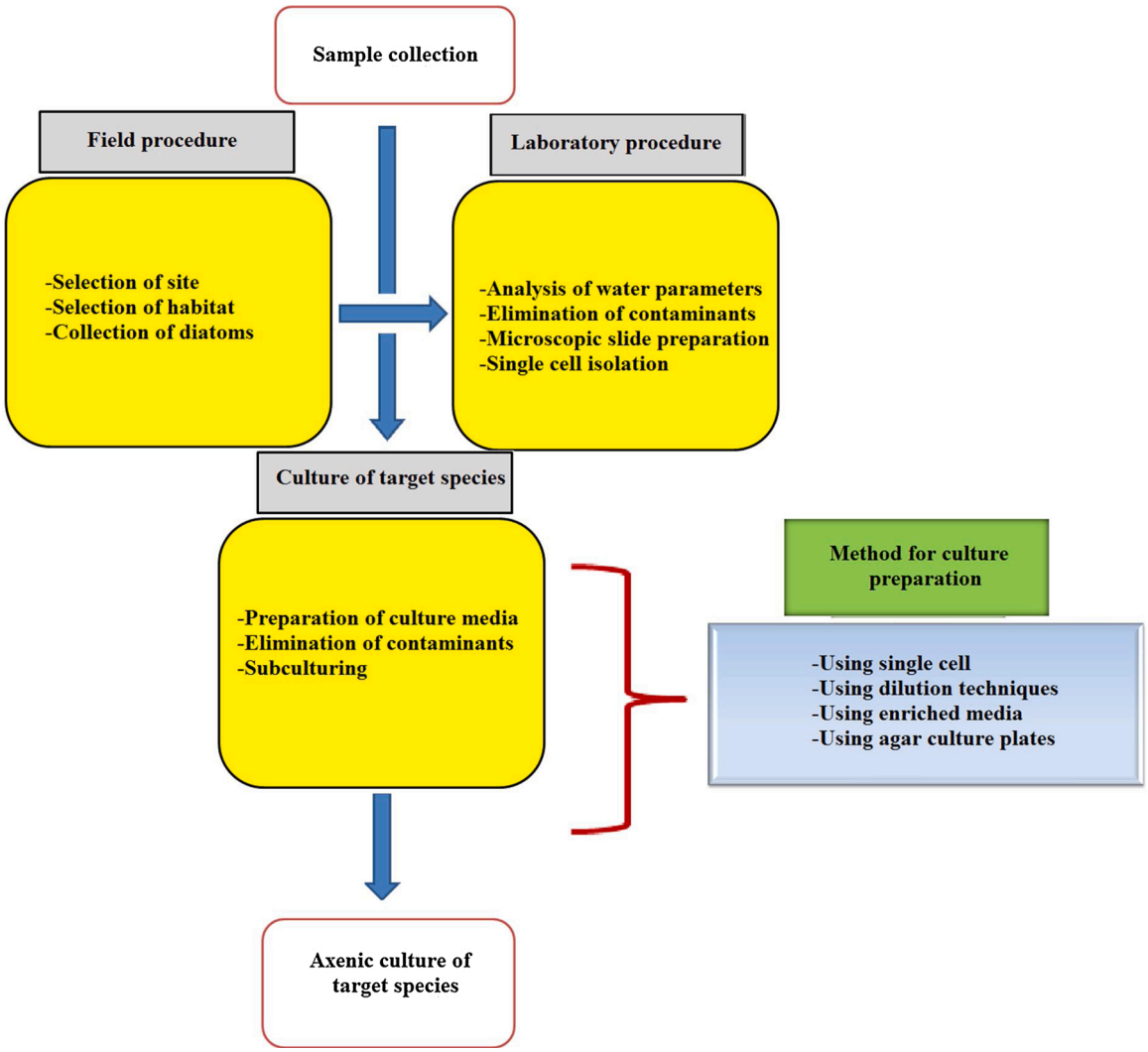

Fig. 1. Flow-chart depicting isolation of diatoms from sample collection procedure to obtaining axenic culture of target species.

sucking worms like leeches [65].

4.5. Water quality analysis

Anthropogenic pressure from the urbanized area causes changes in the water quality of a river and leads to the eutrophication of reservoirs and the proliferation of algae. The informal settlements without sanitary infrastructure aggravate the deterioration of water quality in urban water sources [67]. The water quality index, conducted by using the planktonic diatom index (PDI) for monitoring the Lake Erie's nearshore pelagic zone, provides a robust assessment of water quality [68]. The collected water sample should be adequately analyzed as it gives full information about the diatom habitat and its environment. Several factors should be measured. Hydrological characteristics of the stream, which mainly examined with stream velocity and channel depth and channel breadth. Physical variables of water mainly involve water temperature and turbidity. Physico-chemical variables are examined with pH, conductivity/total dissolved solids. Water contains nutrients such as Orthophosphate-phosphorus (PO_4-P), Total phosphate (TP), Ammonium-nitrogen (NH_4-N), Nitrite-nitrogen (NO_2-N), Nitrate-nitrogen (NO_3-N), which should be further analyzed. Major Cation/Anions such as Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Sodium (Na^{+}), Chloride (Cl-), Sulphates (SO_4) should also be estimated in water. Parameters for Oxygen and Organic matter like Oxygen saturation, Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Total Organic Carbon (TOC) should be analyzed. All physicochemical parameters of water should be tested as per the American Public Health Association (APHA) standards [69]. For monitoring the pollution of water bodies, several indices have been developed, such as the index of Saprobity-Eutrophication (IDSE/5), which utilizes the diatom population in terms of organic pollution [70]. Water can be divided into two types such as groundwater and surface water and both are at the risk of pollutants ranges from heavy metals, pesticides, fertilizers, hazardous chemicals, etc. An accepted water quality criterion according to American Public Health Association (APHA), the World Health Organization (WHO), Indian Standard Institution (ISI), Indian Council of Medical Research (ICMR), and Central Pollution Control Board (CPCB) consisting of various parameters are presented in Table 2 [71].

5. Laboratory procedures

Examination of the collected sample from different substrata should be done as quickly as possible to make sure that diatom assemblage contains live cells and not dead ones. Sample with more dead cells should be discarded, and sampling should be performed again. The environmental conditions of samples collected from various aquatic habitats should be mimicked in a laboratory. It should be noted that all equipment like glass-slide, coverslip, pipette, forceps, inoculating loop, centrifuge tube, glass test tube, watch glass, etc. must be sterilized before use. Uncleaned equipment may lead to contaminations that further result in the death of cells. After examination of diatoms in the collected sample, standard isolation methods should be performed [72].

5.1. Standard isolation methods

As the collected sample contains various microorganisms and other microalgae, these standard isolation methods play a crucial role in the isolation of target species of diatom. The isolator should focus on finding

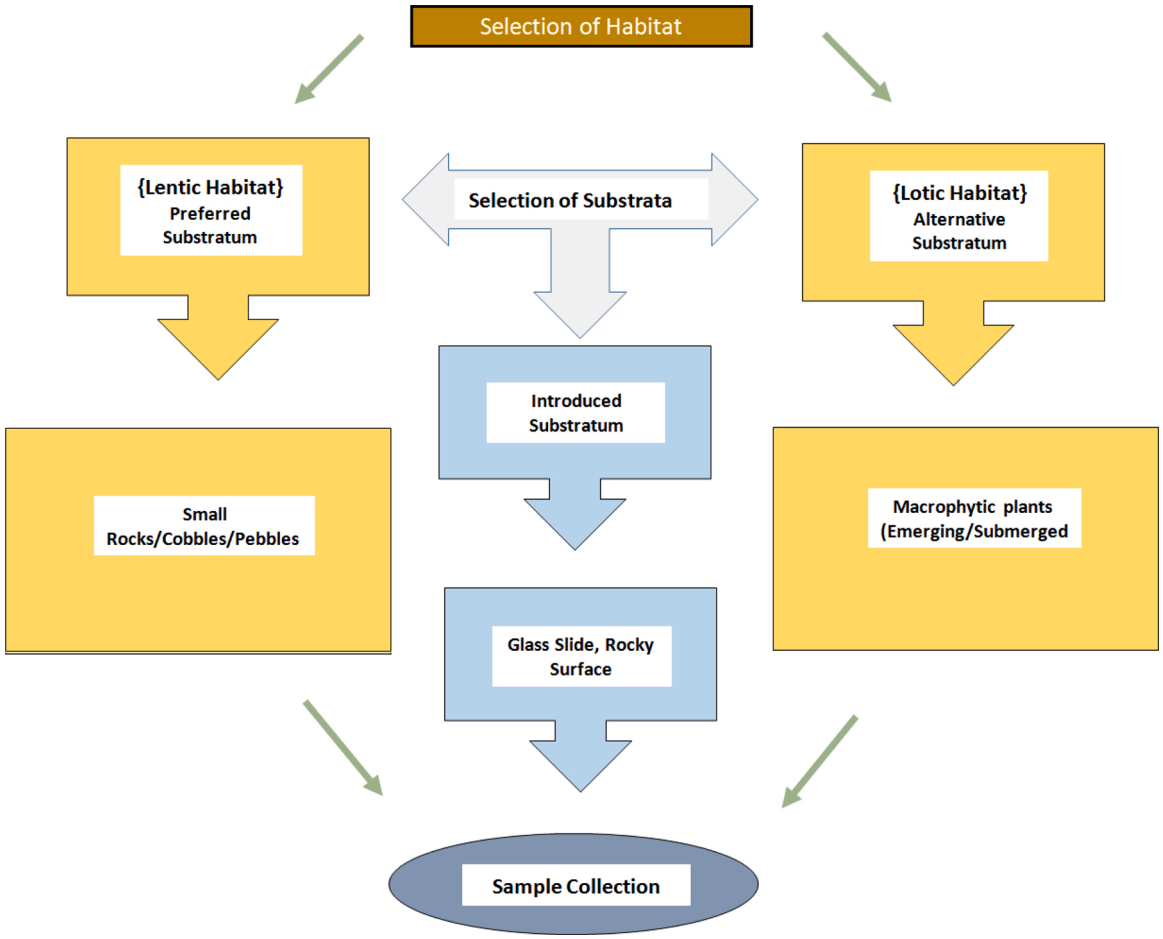

Fig. 2. Decision tree for sampling diatoms from various habitat.

the target species in the collected sample. Viable cells should be cultured as soon as possible while avoiding contaminants. All the following methods should be performed carefully [72].

5.1.1. Single-cell isolation: Capillary action

The simplest method of single-cell isolation is performed with the help of micropipette, first attempted by Zumstein (1900) later improved by Pringshiem (1949) [17,73,74]. A capillary is attached to the Pasteur pipette to make it ideal for single-cell isolation. This method requires a lot of practice. As diatoms have a minimal size, one single cell can also be picked separately with the help of a micropipette. Single-cell isolation with micropipette is to pick a single cell of target species without getting it damage and avoiding contaminants and deposit it into a sterile tube or watch glass and then transfer it to a culture tube. The whole process is done with the help of a microscope, especially an inverted microscope, as it allows the isolator to work conveniently [75,76].

5.2. Dilution techniques

This method is very useful while isolating cells from very contaminated samples with a tiny microorganism, which is harder to separate from the sample. The aim of this method to separate one single cell of target species and deposit it in a culture test tube. Usually, dilution is repeated serially up to 1:10 [77].

5.3. Isolation using agar

It is probably the oldest and standard method available to isolate microorganisms. Most of the diatom species usually grow well in agar. Isolation using agar can be used with both “streaking” and “spreading” methods. Later single cells or colonies can be picked from the culture plate and observed under the microscope. To study the colony/diatom community characteristics, agar plate method is advantageous. The agar concentration should be between 0.8% to 1.5% and 2.0%. While observing glass slide under a microscope, if it contains a single diatom cell, it can be replicated on the agar plate for culture. Agar pour plate method can also be used to isolate cells that do not grow on the surface of the agar plate [78].

5.4. Density centrifugation

This is a physical technique for the pre-separation of single cells of diatoms from polluted water. The Centrifugal technique applies gravity settling to isolate more prominent species from the microalgae. Low dense cells, for example, microscopic organisms and others present in the supernatant, are emptied while diatoms species stay at the base as a pellet. The speed and time of centrifugation vary depending upon the target microalgal species. Even though reasonably successful, however, it might harm delicate cells through sheer pressure [79].

5.5. Enrichment culture

This method involves the use of enrichment media. Enrichment media is rich in one constitute which provides nutrition to only one/two types of cells (species) while inhibiting the growth of other cells (microorganisms). For example, it has been noted that the presence of silica in a culture media boost the growth of diatoms but inhibit the growth of other microalgae. Sometimes even a trace element makes an enormous difference in the growth of species. So, it is necessary to know the nutritional requirement of the target species, and it should be reached as it will improve the growth of the target species [76,85].

5.6. Membrane filtration

This is a pre-isolation step towards the separation of diatoms. Samples can break into two portions depend upon particle size difference. Sometimes enrichment media can also be utilized for better outcomes while separating fungal cells from diatoms. Bigger species can be held at the membrane however, diatom cells pass through the filter quickly. This strategy is helpful, advantageous, and very adaptable [81].

5.7. Isolation with alginate beads

Another technique where alginate beads were utilized to isolate the algal species from the mixed algal culture [82]. In this method, the contaminated water is placed in nutrient media and incubate for one week or more to affirm the development of different species. A known concentration of sodium alginate solution containing a mixed culture of

Table 2
Different parameters of water quality analysis as per approved guidelines

S.No.	Parameters	APHA	WHO	ISI	ICMR	CPCB	References
1.	pH	6.6 – 8.5	6.5 – 8.5	6.5-8.5	6.5 – 9.2	6.5 – 8.5	[71]
2.	Turbidity (NTU)	–	–	10	25	10	[71]
3.	Conductivity (mg/l)	–	–	-	–	2000	[71]
4.	Alkalinity (mg/l)	–	–	-	–	600	[71]
5.	Total hardness (mg/l)	–	500	300	600	600	[71]
6.	Iron (mg/l)	–	0.1	0.3	1	1	[71]
7.	Chloride (mg/ml)	250	200	250	1000	1000	[71]
8.	Nitrate (mg/l)	–	–	45	100	100	[71]
9.	Sulfate (mg/l)	–	–	150	400	400	[71]
10.	Residual free chlorine (mg/l)	–	–	0.2	–	–	[71]
11.	Calcium (mg/l)	–	75	75	200	200	[71]
12.	Magnesium (mg/l)	–	50	30	–	100	[71]
13.	Copper (mg/l)	1.5	1.0	0.05	1.5	1.5	[71]
14.	Fluoride (mg/l)	4.0	1.5	0.6-1.2	1.5	1.5	[71]
15.	Mercury (mg/l)	0.002	0.001	0.001	0.001	No relaxation	[71]
16.	Cadmium (mg/l)	0.005	0.005	0.01	0.01	No relaxation	[71]
17.	Seleneium (mg/l)	0.05	0.01	-	-	No relaxation	[71]
18.	Arsenic (mg/l)	0.05	0.05	0.05	0.05	No relaxation	[71]
19.	Lead (mg/l)	-	0.05	0.10	0.05	No relaxation	[71]
20.	Zinc (mg/l)	-	5.0	5.0	0.01	-	[71]
21.	Chromium (mg/l)	0.1	-	0.05	-	No relaxation	[71]
22.	*E. coli* (MPN/100 ml)	-	-	-	-	No relaxation	[71]

*No relaxation = No permissible limits

diatoms is added drop by drop to a calcium chloride solution to form alginate beads and kept for 6-8 h to make beads stronger, stiff, and firm. The beads transferred to a 96 well enzyme-linked immunosorbent assay (ELISA) microplate (one bead per well), smashed partially containing standard culture media and incubated under controlled conditions in the presence of light for one week to confirm the growth of isolated diatom species. The trapped diatom species in the beads viewed microscopically for the confirmation of isolated species. This methodology is simple, easy, cost-effective compared with existing methods and can be easily applied for the mass cultivation of specific species [17].

5.8. Other methods

There are other different strategies for the isolation of diatom species like anti-microbials, UV radiation. Anti-microbial treatment hinders growth & development by eliminating microorganisms, thus help with getting the confined unadulterated culture of diatoms. UV radiation act as a disinfectant that prevents bacteria and other contaminants since diatoms are better resistant to UV radiation over bacterial cells [81]. Photoinhibition is an immediate strategy that harms biomolecules by engrossing UV light, which prompts the loss of natural capacity of microscopic organisms [17].

6. Advanced isolation techniques

6.1. Micromanipulation

Diatoms are valuable for the aquatic food chain and give significant bioactive compounds to human prosperity. Segregating unadulterated species or getting axenic culture from a polluted water source is a complicated and tedious procedure with the current strategies. Hence, it becomes necessary to search for other advanced isolation techniques. Micromanipulation is one such method permitting refined, confining single species in a more cleansed manner. Prior fine capillary tubes were used focusing on target cells under microscopic observation, which is a laborious task and threat to contamination [83]. With the arrival of a modern, sophisticated micromanipulator exploiting micromanipulator and stereomicroscope, a high level of precision can be achieved for screening and isolation of species of interest [80]. A cell of interest can be captured utilizing a focused laser and transferred to a sterile media of interest. This system is still in its early stages because of the high state of expertise and time required [84].

6.2. Automated techniques

The requirement for cutting edge new methods push researcher to redesign the flow cytometer coupling FACS (Fluorescence-Activated Cell Sorting). The premise of this procedure is light scattering and fluorescence [84]. Cells absorb the laser beam transmit fluorescence and give information on cell size, pigments, and reliability of species, which is identified with the morphology and other characteristics of the species accordingly, thus allowing characterization of up to 10,000 cells in a fraction of second [85]. This technique can be employed to build up an axenic culture and get rid of any bacterial contamination. In some cases, mixed culture or aggregation of cells may make issues in the precise identification of cells, which can be overcome by cell disruption through appropriate sonication under controlled conditions. This technique is generally excellent for quick screening of organisms overproduces metabolites of interest in conjunction with fluorescent dyes. For example, Bodipy or Nile red facilitates the selection of desired mutants from a mixed population [86]. An overview of the main techniques used to establish axenic diatom cultures is presented in Table 3.

6.3. Importance of correct isolation in diatom research

Diatoms, the most productive phytoplankton found all over the planet from antarctic glaciers to brick walls, have drawn a tremendous awareness in the research field, i.e., for the quantitative reconstructions of ocean surface conditions to establish palaeoceanographic records [87]. They are one of the most promising candidates for various applications such as pharmaceuticals, bioenergy, industrial chemicals, nutraceuticals, and aquaculture [88,89]. To make most out of it, an axenic culture of diatom must be a prerequisite bearing in mind that a pure culture of diatoms is undoubtedly required in genome sequencing [90], to identify the producer of any novel bioactive compound for large scale manufacturing of nutraceutical [91], building a consortium for bioremediation [92] and to elucidate the relationship between other microalgae using omics tool [93]. Maintaining an axenic culture for a longer duration is very difficult because bacteria are vulnerable, which frequently attacks the diatom. The primary focus is to isolate pure species of diatoms and their maintenance [17]. Nevertheless, an axenic strategy is dependent upon contamination and desirable organisms disclosing the possible relationship between them leading to the understanding, selecting, and developing an axenic culture, which is a critical step, thus alleviate the cultivation method. Mimicking the natural environment for optimum growth under laboratory conditions facilitates the development of an axenic culture, but this step requires the correct isolation plan and approach [94]. The fundamental question is, Are axenic culture genuine.? What is the acceptability of their purity level.? With the direct symbiotic association between diatoms and other aquatic organisms, the development of a new advanced technique to assess the purity level cannot be ruled out. Therefore, emphasis should be given towards improvement and innovation in isolation methods, to standardize and establish the correct isolation practices to solve future energy crises, nutritional requirement, nutraceuticals, pharmaceuticals by choosing the right technique for right diatom species for the benefit to the mankind, society and last but not least the ecological balance [95]. Therefore, constant effort to develop a new scientific method will surely pave the way towards the isolation of diatoms from mixed culture and maintaining their purity level to a greater extent.

Diatoms-virus interactions are rather difficult in obtaining pure culture. The virus kills diatoms, thus benefitting other algae. The limitation in silicon levels in the oceans facilitates infection by the virus. Accelerating diatoms mortality due to viral infection is a major concern affecting the carbon cycle leading to global warming and ultimately changes climatic conditions drastically. Also, ocean study is a difficult task. The main emphasis is given towards the marine environment rather than terrestrial causing disparity about diatoms-virus interactions. As a result, more isolations techniques and characterization is necessary for controlling the action of the virus in the regulation of host populations [96].

Several species of diatoms are found in a highly acidic environment, and they continue to grow consistently near or in the acidic conditions both in marine and freshwater ecosystems. They exhibited a significant response to the alterations in the growth conditions. They are capable of integrating numerous physiological as well as morphological adaptations, which favors their persistence. Diatoms growth inhibited majorly in silica limitations than any other nutrients because cell division cannot sustain for a more extended period under silica deprived conditions [97]. Highly acidic conditions resulted in decreased Si, indicating that close to the end of this century ocean acidification might persuade the C and Si cycle and alter the composition of diatoms. [34]. Considerable human interference is turning aquatic bodies towards the acidic zone, and many countries have seen ocean acidification even in extreme low-temperature conditions such as North America, Canada, and Italy. Due to the high concentration of hydrogen ions, diatoms flora like Nitzschia, Pinnularia, Eunotia, and Frustularia are exceptionally rich in habitats within the pH range of 4.5-5 [98]. In an acidic environment, a higher cell volume, chlorophyll, and productivity were observed due to a change in water chemistry because in acidic conditions number of grazing macroinvertebrates and microheterotrophs were less in number [99]. However, it is unattainable to understand the natural influx of

Table 3
An overview of available techniques to establish pure cultures of diatoms.

S. No.	Name of Technique	Advantage	Disadvantage	Reference
1.	Single cell isolation using microscopic mediated capillary action	Time saving, contamination free technique. Easy and clean handling.	High personnel skillsare required. Unsuitable for tiny cells that have similar shape and size. Elimination of bacterial contamination is a major issue. Excessive cell damage	[17, 73–76]
2.	Dilution techniques	A simple isolation method to obtain pure diatoms species from water sample.	Unsuitable for species which are scarce. Ineffective for rare species.	[77]
3.	Isolation using Agar	Simple method to obtain pure culture and can be establish without any prior treatment. Easy to quantify cells. Highly sensitive with appropriate media.	High risk of contaminations and time consuming technique. High skill expertise is required for best results. High purity agar is obligatory for delicate marine species.	[78]
4.	Density centrifugation	A very simple isolation technique to concentrate a target species. A flexible approach with high level of performance.	Inappropriate for whirl cells. High yield low purity. Low specificity. Not suitable for algae contamination.	[79]
5.	Enrichment culture	Used to isolate desired species from mixed cuture. Highly specific media is required for the growth of target species. Media should be prepared according to the nutritional requirement of the species.	High risk of bacterial growth. Difficult to isolate different type of diatoms species from mixed population. Not cost effective.	[76,80]
6.	Membrane filtration	Very easy and handy portable method. More effective because small pores has been tested to eliminate contaminants with selective enrichment.	Filter gets clogged up so require regular change. Sometimes very small virus or mycoplasma may pass through besides absorbing large amount of filtrate.	[81]
7.	Isolation with alginate beads	A novel value addition isolation technique. Limited loss of activity. Can be recycled, regenerated and reused. Highly economical technique.	Low surface area for large sized species. Yield is low due to inactivation and desorption.	[17,82]
8.	Micromanipulator	Pure culture comes from single cells and one can obtain strains within the species.	Very expensive equipment. A tedious technique which requires a skilled operator.	[83,84]
9.	Fluorescenceactivated cell sorting (FACS)	Ideal method to sort multiple population with positive selection. Highly sensitive in selection of cells from heterogenous population. High precision, resolution & powerful. Greater than 95% cell purity can be achieved	Mechanical sheering leads to cell damage. High initial cell count with low efficiency. Potential cell damage by rapid flow.	[85,86]

pre-acidification, natural fluctuations in conditions, and the level of nutrients that tend to vary from habitat to habitat. Abundance and fall in diatom species can be explained based on physiological pH, availability of the essential nutrients, and biological interactions, which could be the factor for the productivity of diatoms in extreme conditions. Very little is known about the influence of acidification on diatoms. Hence the effect of acidic environment on diatoms changes from the open sea, near to the sea and deep-sea, and indeed not a decrease in diatoms productivity and growth [100]. Since diatoms nurture associated aquatic species to a greater extent within the marine environment so achieving utmost purity and break communication with unwanted organisms is a significant challenge. Therefore, emphasis should be given to improve and channelize knowledge towards proper isolation techniques that will separate diatoms from contamination and any possible intervention to promote appropriate growth and development of diatoms [101,102]. An outline of isolation of pure diatom species getting affected by the surrounding contaminants is challenging since they get heavily occupied with different interfering organisms, which pose a significant threat in obtaining axenic culture, as presented in Fig. 3.

7. Steps for obtaining axenic culture

7.1. Preparation of culture media

Although there are several culture media are available, but the most recommended culture media for isolation and growth of diatoms are f/2-Si, PM, and WC media. The composition of each culture media is prescribed according to the selection of diatoms for culturing for the desired application. f/2-Si culture media is widely used as the most effective media, whereas PM media is utilized for culturing diatoms in an acidic environment. WC media is generally used for culturing diatoms existing in an alkaline environment [6]. All these culture media differ in their composition of major and minor nutrients and provide proper nourishment to diatom culture. f/2-Si media is required for marine species while PM and WC media supports the cultivation of freshwater species. For marine, it is advised to prepare both media with seawater and for freshwater prepare media with distilled water [103]. The chemical composition of different culture media for diatoms cultivation are presented in Table 4.

7.2. Elimination of contaminants from culture

Elimination of contaminants is important to maintain the pure culture of isolated species. As stated, the above contamination can be eliminated with gravity separation, dilution techniques, etc. In the case of bacterial contamination, antibiotics should be used in a specific amount in culture. Every equipment, glassware used in isolation or culture should be sterilized before performing experiments. Culture should be maintained in aseptic and optimum conditions [104].

7.3. Sub-culturing

After preparing the axenic culture, it should be subcultured after over a specific period. Subculturing should be done before the decline phase of the primary culture. Each subculture should be prepared in aseptic conditions. After each subculture, it should be observed for contaminations, and in case of occurrence of contamination that specific subculture should be discarded, and the process is repeated [105].

7.4. Preparation of axenic culture

An axenic culture contains only one target species and is free of all contaminations. After isolation of target species of diatoms, it is necessary to maintain the culture free from contaminants so that a pure culture can be established. Pure isolated cultures can be obtained from a combination of techniques such as flow sorting, Pasteur pipette, and agar plate methods. The polluted water collected from various habitat will be immediately examined by monitoring pH and temperature at the site and will be carried to the laboratory in 2-3 liters plastic bottles. The raw wastewater was filtered twice with a 0.45 μm pore size Whatman filter paper to remove the large suspended solids particles and debris. The wastewater will be autoclaved for sterilization and further used for the cultivation of the microalgae. The Physico-chemical parameters of wastewater will be characterized as per the standard procedure of APHA guidelines [69,106]. Initially, the water sample will be serially diluted in a microwell plate and test tube to get a pure culture and observed under the microscope to confirm the presence of microalgae species. Isolation will also be carried out by spreading or streaking wastewater in a solid agar medium supplemented with silica. Once confirmed, the microalgae species were transferred slowly and gradually in different volumes of Erlenmeyer flasks containing artificial seawater enriched with F/2-Si media at pH 8.3 and maintained in the culture room at 12 h dark/light diurnal cycle with a desired luminous intensity at 22-23 °C. Finally, strains will be identified, and their taxonomic classification will be established. Fig. 4 shows the overview of obtaining pure culture diatoms from polluted water bodies.

7.5. Future perspectives and commercial value of diatoms

Diatoms are very young as compared to other phytoplanktons but have evolved rapidly over some time. A decline return on investment in research and development (R&D) and slow-growing companies accelerating the demand for diatoms cultivation serving as a superfood. The term nutraceutical refers to nutrition that provides physiological benefits coupled with the protection and prevention of disease. These functional foods promote health by adding novel ingredients that are similar to conventional foods but with rich nutrition or bioactive compounds that may target the physiological mechanism of our body as characterized by the US Department of Agriculture, Agricultural Research Service

Table 4
Chemical composition of standard culture media for diatom cultivation.

	f/2-Si Culture media (mg/L)	PM Culture media (mg/L)	WC Culture media (mg/L)	Reference: [6]
Major Nutrients				
$CaCl_2$	–	0.37	36.76	
K_2HPO_4	–	2.90	08.71	
$MgSO_4.7H_2O$	–	3.70	36.97	
$Na_2SiO_3.9H_2O$	30.00	14.21	28.42	
$NaH_2PO_4.H_2O$	5.00	–	–	
$NaHCO_3$	–	3.15	12.60	
$NaNO_3$	75.00	56.70	85.01	
Minor Nutrients				
Na_2EDTA	4.360	2.180	4.360	
$FeCl_3.6H_2O$	3.150	1.580	3.150	
$CuSO_4.5H_2O$	0.010	0.005	0.010	
$MnCl_2.4H_2O$	0.180	0.090	0.180	
$ZnSO_4.7H_2O$	0.022	0.011	0.022	
$CoCl_2.6H_2O$	0.010	0.005	0.010	
$Na_2MoO_4.2H_2O$	0.006	0.003	0.006	
H_3BO_3	–	0.500	1.000	
Vitamins				
B_1	x 10^{-1}	1.0×10^{-1}	1.0×10^{-1}	
H	x 10^{-4}	5.0×10^{-4}	5.0×10^{-4}	
B_{12}	5.0×10^{-4}	5.0×10^{-4}	5.0×10^{-4}	

[107]. On account of large content of polyunsaturated fatty acids (e.g., PUFAs n3 and n6), essential amino acids (e.g., leucine, isoleucine, and valine) and pigments (e.g., lutein and β-carotene) and vitamins (e.g., B12), the diatoms biomass have gained much popularity across the globe [6].

Nutraceutical industries manufacturing products on a large scale in collaboration with the food and pharmaceutical industry, thus benefitting consumers. Diatoms are emerging as leading nutraceutical ingredients from a biotechnological point of view since they are equipped with polyunsaturated fatty acids, essential amino acids, pigments, and vitamins. According to the World Health Organization WHO, the most severe challenge of the 21 st century is a lifestyle disease, i.e., non-communicable disease. The current focus is to develop functional food and their products, which combat the disease development [107]. Diatoms are underutilized among the microalgae, and only a few species have been characterized thoroughly. Diatom based products are an

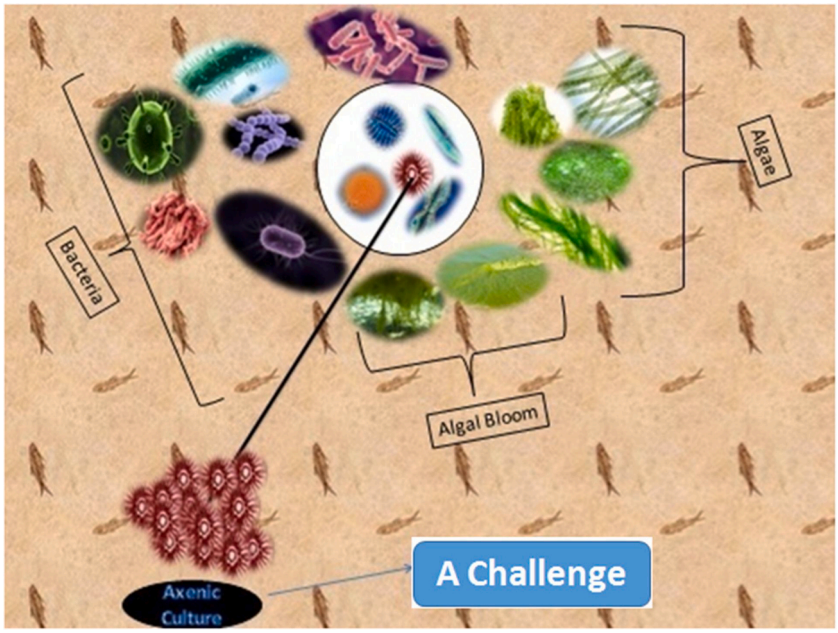

Fig. 3. Shows the main interfering agents or treats in obtaining axenic culture of diatoms.

excellent source for multifaceted use covering the health and nutraceutical sector. Therefore, the cultivation of diatoms under different cultural conditions helps to understand their biochemistry to a large extent [108].

Diatoms cells, after degradation, settled down in the form of silica, which is known as diatomaceous earth. These death remains have tremendous applications for industrial and agriculture purposes. Ongoing Covid-19 impacted the world economy. The diatomite industry has also suffered significantly but successful in maintaining an optimistic growth for four years. The average annual growth rate of the diatomite industry will reach millions USD in 2019. Probably by 2024, the market potential will see a significant expansion [109]. Diatomaceous earth is one of the major causes of their successful existence on the globe and also act as carbon dioxide sequester on the ocean floor. However, more information is needed about nutrient upwellings and the causes of their bloom degradation in their natural habitats.

Further, a more in-depth study on their self-defense mechanisms will reveal the unfolded truth about the interaction of diatoms and their feeders in the food web. To overcome the constraints of fossil fuels, the generation of bioenergy makes renewable energy particularly interesting. Nowadays, renewable resources are contributing 35% of the total energy of the world. Diatoms synthesize oil for human consumption, which is rich in nutrition showing a promising alternative to meet the demand of future growing populations. We need to emphasize the latest, more productive technologies to overcome the cost of their cultivation and harvesting for optimum utilization of diatoms as biofuels, valuable products, wastewater remediation, and aquaculture [110].

The generation of bioenergy from novel sources like diatoms is incredibly significant as they contribute towards carbon dioxide mitigation generation of renewable energy concomitant with a plethora of value-added products. Due to their unique evolutionary history and their adaptations to varied environmental conditions, diatoms have spread all around the world. Undoubtedly, diatoms are playing a significant role in the reduction of global warming gases such as carbon dioxide and provide possibilities to change the present climatic conditions. But still more researches are needed on their biogeographic distribution and to learn more about factors which are responsible for their more successful existence. Diatoms cells, after degradation, settled down

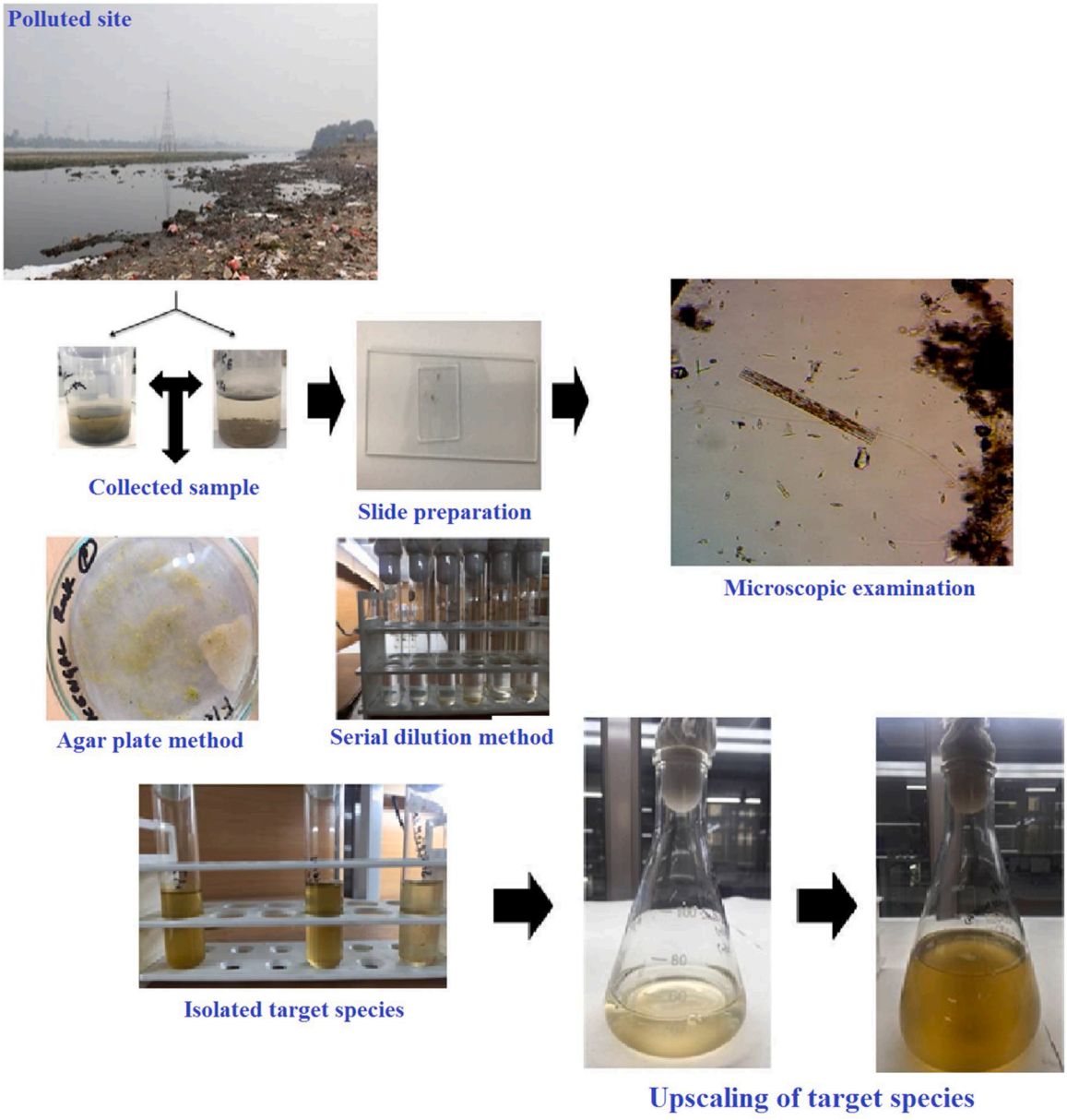

Fig. 4. Protocol for adopting isolation strategies employed for diatom axenic culture.

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They are the primary food of most of the herbivores in freshwater, as well as in marine water. Furthermore, being the rich source of Poly Unsaturated Fatty Acid (PUFA), they have a great scope in aquaculture. Therefore, diatoms are the cost-effective approach which sequesters CO_2, excess amount of heat, reduce the extra amount of nutrients, and remove metal contaminations from their habitats. They could be a possible solution to energy savers and to reduce the threat of global warming. Conversely, they may be a viable source of food and feeder for living beings. Diatoms and wastewater are an advanced integrated system that, on the one hand, utilize the potential of diatoms in purifying the wastewater thus, it helps in restoring the original quality of habitat. On the other hand, their biomass can be utilized in the production of antiobesity, antibacterial, antioxidant, biofuels, anticancerous, antiviral compounds.

Diatoms have received great attention because they are the significant producer of lipids and biomass for various commercial applications such as biofuels comprising of biodiesel and aviation fuel. They participate in cleaning the environment by fixing the atmospheric CO_2 and contributes to the reduction of greenhouse gas (GHG) [112]. Diatoms based biofuels are projected to be economically beneficial in the future, but still, some ambiguity exists whether they equate with fossil fuels or not is remain a question. Obtaining a biofuel require several steps like harvesting, extraction, conversion of biomass to target biofuel is economically not feasible. One of the key bottlenecks is cell density, which is similar to the water and negative surface charge that put off settling owing to gravity, thus makes harvesting a most crucial and challenging step. There is an urgent need for an efficient harvesting technique to improve the economics and efficiency of the whole downstream process [113,114]. At present, the technologies of the water purification industry have been used for harvest and recovery of microalgae, but still, some technical glitch needs to address that are sole to microalgae harvest. Firstly, harvesting techniques must be species-specific. Secondly, a blend of different harvesting techniques must result in synergistic outcomes. Thirdly, a complete cell separation from a diluted suspension to lower the cost of the downstream process. Fourthly, steps such as lipid extraction and biofuel conversion should be minimized. Finally, to come up with the idea of more advanced harvesting techniques in the future [114].

The ultimate goal of economic feasibility is the conversion method by utilizing inexpensive chemicals and the release of the least toxic waste. Microalgae biomass is the best option for fossil fuels for transportation, but the commercialization of microalgae-based biofuel is a significant task. New strategies should encourage innovative elements to the existing downstream process that can appreciably reduce the cost towards the realization of biofuel commercially and dispose of harmful CO_2 production. To sum up, harvest, extraction, and conversion steps must be environment friendly, while making biofuels as future transportation fuels [113,114]. All the above-mentioned factors enhancing the popularity of diatoms globally strengthen their market potential and growth in the coming years. Their medicinal characteristics are likely to provide more interest in diatoms cultivation with a rising population. Considering health benefits, the global diatoms market is expected to witness as one of the most emerging economies across the world.

8. Conclusions

A general question raised by everyone about diatom`s attractive potential and advantages but still exploring their true value is a long journey because high rich diversity limits their studies making it obscure and inaccurate. It is high time to investigate new class and species of diatoms and understand their mechanism, research models, and the potential role for future perspectives. The past few decades have seen a tremendous rise in diatoms research, as reflected in the publication. An undercurrent excitement is moving around, but challenges are roaring high. Practically, a good isolation practice is the only solution that will fill this void. Smart isolation practices are prerequisites that must help quick isolations and speedy recovery of diatoms from polluted water. Diatoms`axenic culture is the first step towards a complete study of an organism and maintaining their pure culture can be further used in different applications such as bioindicators, nutraceuticals, cosmetics, phycoremediation, aquaculture, etc. Thus, growing diatoms is the most sustainable and economical solution to meet the future demand of energy crises. Therefore, a thorough study of diatoms for various applications is necessary.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

Dr. Archana Tiwari is thankful to the Department of Biotechnology (DBT), New Delhi, India for providing financial support under project Grant No: BT/PR/15650/AAQ/3/815/2016. All listed authors are grateful to their representative universities for providing the literature access.

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