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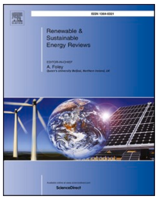

Sustainable Bioeconomy prospects of diatom biorefineries in the Indian west coast

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ABSTRACT

The rapidly depleting fossil fuel reserves with rising greenhouse gas levels (GHGs) in the atmosphere necessitate exploring alternate sustainable energy options. Biofuels from microalgae are emerging as a viable renewable energy resource owing to their inherent characteristics of higher biomass and lipid yield per hectare compared to other terrestrial bioenergy feedstocks. In this context, the present communication highlights the prospects of microalgal biofuel and other value-added products produced in a decentralized microalgal biorefinery in the flood plains (gazani lands) of the west coast of India. The spatial extent of potential sites for diatom cultivation estimated in three districts along the Indian west coast was 1940 ha. The opportunities for establishing biorefineries using diatoms as renewable bioenergy feedstocks were investigated through species prioritization, seasonal availability, tolerance, and biochemical composition analyses. *Nitzschia* and *Amphora* sp. were prioritized for lab-scale productivity studies based on their tolerance and macromolecular composition. When cultivated in a prototype biofilms-based bioreactor designed using gravel stones as substrates, *Amphora* sp. Yielded 16 times more productivity (0.56 g L^{-1}) than conventional shake flask cultures. Design of a diatom biorefinery and its mass budgeting considering 100 kg dry biomass yielded ~15–24 kg of biodiesel. Techno-economic assessment of biodiesel with value-added products of glycerol, biogas, and biofertilizer demonstrated a biodiesel production cost of 30.08–59.52 INR/kg of biodiesel. Harvesting cost in a hybrid mode using mechanized scrubbers and manual labour was estimated as 20 INR/kg of biomass.

1. Introduction

The burgeoning population with industrialization and globalization has intensified the greenhouse gas (GHG) footprint with higher utilization of fossil energy resources. The carbon dioxide (CO_2) level will reach 550 ppmv in 2050 [1,2]. The transportation sector accounts for about 21% of the current global CO_2 emissions, next to emissions from power generation [2]. Coupled with this, the dwindling stock of fossil fuels has posed severe challenges to energy security, which necessitated exploring viable, sustainable energy alternatives that are economically viable, technically feasible, environmentally sound, and socially acceptable. The recent decades have witnessed considerable efforts towards sustainability in renewable energy generation. The transition to utilizing non-conventional sources has successfully addressed energy security concerns, depleting fossil fuel reserves, global warming, and climate change by lowering atmospheric GHG emissions [3]. The Intergovernmental Panel on Climate Change [4] has projected a sea-level rise of about 50.8 cm (at the current rate of increase) by 2100 due to global warming, with a 2 °C rise in the worldwide temperature ascribed to a 2.5% increase in CO_2 emissions per year [5]. Hence, CO_2 sequestration using biomass and exploration of sustainable renewable energy alternatives gained impetus among other industrial modes of carbon capture.

During the last few decades, the development of technologies that capture, sequester, and biologically fix CO_2 has been on the rise. Such technologies are recognized as the most effective method of reducing CO_2 emissions [6]. The ability of microalgae to biologically fix CO_2 is ten to fifty times higher than that of land plants with CO_2 tolerance up to 40% [7]. Microalgal biorefineries are designed to reduce the costs involved in their production by maximizing resource recovery [8] and

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Abbreviations

GDP	Gross Domestic Product
GHG	Greenhouse gas
CO_2	Carbon dioxide
EBP	Ethanol blending programme
NBM	National biodiesel mission
SCO	Single cell oil
ASP	Aquatic Species Program
MPB	Microphytobenthos
LC-PUFA	Long-chain polyunsaturated fatty acid
DoE	Department of Energy
EPS	Exopolysaccharides
ATS	Algal turf scrubbers
GoI	Government of India
DBT	Department of Biotechnology
ICT	Institute of Chemical Technology
UCO	Used cooking oil
FAME	Fatty Acid Methyl Ester
HTL	Hydrothermal liquefaction
3G	Third generation
ABE	Acetone-Butanol-Ethanol
TAG	Triacylglycerol
ICTGEB	International Centre for Genetic Engineering and Biotechnology
C5	Five carbon sugars
MI	Mission Innovation
IOC	Indian Oil Corporation
IIT	Indian Institute of Technology
COD	Chemical Oxygen Demand
HRT	Hydraulic retention time
QGIS	Quantum Geographic Information System
ISRO	Indian Space Research Organization
NRDMS	Natural Resources Data Management System

efficiently processing biomass into energy, platform chemicals, food additives, among others [9]. Microalgae are considered as raw materials for biorefineries since they possess numerous bio components of interest, such as carbohydrates, pigments, and proteins that can be effectively utilized to minimize bio-residue.

1.1. Diatom as a source of oil

From the perspective of diatoms as a source of lipids (oil) for biofuel production, the contribution of the Aquatic Species Program [10] (ASP) is of paramount importance. It was one of the pioneering efforts in algal biotechnology with rigorous isolation of nearly 3000 microalgal species, especially from harsh environmental conditions like saline and brackish waters, and subsequent screening of algae for species with higher lipid content. The results of this program had provided valuable insights on physiology and biochemistry of algae, systems biology and algae production concepts using open ponds for mass cultivation, the chronology of research activities from 1980's – 1990's, outdoor testing and system analyses, along with cost-benefit analysis. The close-out report on ASP identified 50 species out of the complete set of isolated algae to be better lipid accumulators, out of which 60% were diatoms. These numbers best describe the importance of diatoms, among other microalgae, as a significant source of lipids for biodiesel production [11–13].

Diatoms are unicellular photosynthetic eukaryotes, playing a crucial role in biogeochemical cycles. Diatoms sequester carbon and constitute the major carbon sinks of oceans. They can be of freshwater or marine origin and are the most promising microorganisms for biofuel production [14,53,54]. They are the dominant primary producers of the oceans and efficient carbon sequesters, accounting for about 40% of the marine and 20% of the global primary production [15,16]. Diatoms are the most species-rich group among other microalgae with a reported 30,000–2,00,000 taxa. They can thrive in extreme geological conditions, from deep ocean thermal vents to icy polar regions of the Arctic and the Antarctic [17]. They are best characterized by their ornate cell wall with nano-structures and are made up of biomineralized hydrated silica ($SiO_2 . nH_2O$). Diatoms produce a polysaccharide -chrysolaminarin and storage lipid – triacylglycerol, TAG [12] in a significant quantity (25–45% on a dry weight basis) [18]. These storage lipids as energy reserves induce in diatoms buoyancy to keep the diatoms afloat in the euphotic zones of the open oceans.

1.2. Diatom cultivation

Analogous to microalgal cultivation, diatom cultivation has been demonstrated planktonically (as cell suspensions in culture medium) in open ponds and closed photobioreactors for more than 50 years [11]. However, the past decade witnessed significant efforts towards biofilm-based microalgae cultivation [19]. Biofilms are formed by a consortium of diverse microbes such as bacteria, protozoa, larvae, microalgae, microzooplankton, and macroalgae [20]. The biofilm formation process involves the initial adherence by the microbial cells onto a solid substratum through adsorption, which is usually reversible, followed by an irreversible adherence with the help of secretion of EPS [21]. Microalgal cells grown on biofilms exhibit higher resistance and improved resilience to extreme and hostile environments [22]. The EPS matrix serves dual roles of providing storage space for nutrients and water and defending the cells from environmental adversities [23]. Due to these qualities, there has been considerable attention and shift in research focus from algal cultivation in suspension forms in open/raceway ponds to biofilm cultivation. The biofilm cultivation systems are submerged biofilm systems that are either continuously or intermittently submerged and perfused systems. A porous substrate provides the moisture, and the system is exposed to the ambient gas phase [23]. Constantly inundated systems have been constructed as flow-ways with mechanized pumping systems where the flow ways are subjected to slight inclination in angle to enable gravity-induced flow [24]. The constantly submerged biofilm cultivation systems being used so far were designed using diverse construction materials such as polycarbonates [25], polyvinyl chloride [26], concrete [27], and glass fiber-reinforced plastics [21].

Biofilm in diatom cultivation offers multiple advantages: it has a lower carbon footprint area, economical, readily available with a reuseable supporting surface [19]; reduced harvesting costs, easy scalability, higher biomass productivity, lesser water requirement with bioremediation capability, increased light availability owing to the broader exposed surface area [28] and better control of cell growth area when cultivated in lagoons and open oceans [29]. Biofilm-based microalgal cultivation has substantially reduced harvesting costs, proving its economic viability [30]. Comparative assessment of the water requirement for the production of 1 kg of microalgal biomass shows a minimal water requirement of 17 kg, which is ~12 times lesser than the quantum of water required for the same quantity cultivated under suspended cultivation using open ponds/photobioreactors that require 12–2000 kg of water [31].

In biofilm cultivation, the colonizing tendency of benthic diatoms with EPS secretions has been exploited for successful biofuel production in biofilm bioreactors. The diatom strain *Haslea ostrearia*, grown in immobilized cell photobioreactor using agar gel layers, yielded a two-fold higher cell growth [32]. Earlier studies on algae production in the biofilm cultivation system showed dry biomass productivity as high as

12–45 g/m^2/day in different parts of USA [33–35]. The productivity reported in such an ecologically engineered system is five times more than that achieved in conventional microalgal cultivation (open pond/raceway pond) systems. Although biofilm cultivation is found to perform better than conventional microalgal cultivation in suspension mode, ash content of the biomass, washing off biofilms from the substrate, and light penetration hindrance in a matured biofilm are the areas that need significant research attention.

2. Biofuel from diatoms: the current scenario

Diatoms are considered promising feedstocks for biofuel production worldwide due to their unique distribution of C14, C16, and C20 fatty acids, unlike other green algae and land plants [36]. Biofuel and biochemical potential of the marine diatom *Amphora coffeaformis* in open raceways [37], quality assessment of biodiesel from marine diatom *Navicula cincta* [36], exploration of biodiesel prospects of the diatom *Halamphora coffeaeformis* [38] are some of the global ventures on biofuel production from diatoms. In India, although large-scale production and utilization of diatoms as live feed in aquaculture has been in practice for more than five decades now, the mass cultivation of diatoms from a biofuel perspective is yet to be realized. The adversity of global warming and the imminent threat of freshwater scarcity have necessitated transitions to the microalgae of estuaries, backwaters, and open oceans. Moreover, it is desirable to choose microalgal strains optimally productive for a prolonged period under varying environmental conditions than a strain under controlled environmental conditions [12]. Thus, prioritizing microalgal strains for biofuel production would involve effective screening mechanisms that include critical factors such as abundance, tolerance, and higher resilience to fluctuating environmental conditions [39].

Exploring abundantly available microalgae strains at the local level, biochemical composition, understanding physiology under different growth conditions, and optimization of the downstream processes of lipid extraction/transesterification would aid toward commercial scale-up for industrial utilizations. Thus, habitat mapping and prioritization of microalgae species are necessary to understand the tolerance and sensitivity levels of different microalgae at the local level, leading to the selection of resilient diatom strains. This would help in addressing problems encountered with the open cultivation of microalgae, such as the difficulty in acclimatizing microalgae to unconducive open environments, contamination, and invasion by pests/pathogens [40]. The most pressing challenge in microalgae biofuel production is harvesting, which consumes up to 30% of the total capital investment spent in biodiesel production [41].

2.1. Scope for diatom cultivation along the west coast of India

A controlled ecosystem-based approach utilizing the free energies, entropy, and matter of nature (solar energy and nutrients from wastewater) with minimal interventions of human technology would certainly provide low-cost solutions to manage environmental and energy issues due to anthropogenic activities [33,42,43]. The remediation of wastewater coupled with bioenergy generation through constructed wetlands [3,19,44] is emerging as a viable ecological engineering solution. The constructed wetlands are tertiary treatment systems that utilize naturally occurring physical, chemical, and microbial processes to take up nutrients (NPK and micronutrients) and bioremediate wastewater [45] with the added benefit of biomass production [46]. Algal ponds with suitable locally-available substrates such as gravel stones along the flood plains (gazani lands) of the coast would aid in the phyco-remediation of surface run-off (including sewage from nearby localities) and sustainable biofuel production with the value-added products such as fodder for livestock and fertilizers for agricultural field [44,47]. The productivity reported in such ecologically engineered systems is five times (12–45 g/m^2/day) more than what is achieved in conventional microalgae cultivation (open pond/raceway pond) systems. Thus, the cultivation of diatoms by introducing low-cost substrate for algal cell attachment along the coastal regions of India would provide assured benefits to the coastal fishing community that practices aquaculture farming in flood plains (gazani lands). The construction of decentralized biofuel production units should consider critical aspects of habitat conditions and seasonality of diatoms, biochemical composition analyses, and appropriate choice of downstream processing. Each of these aspects is discussed in detail in the following sections.

2.1.1. Potential sites for diatom cultivation on the west coast of India

The spatial extent of flood plains (gazani lands) that could be considered as potential microalgae cultivation sites along the west coast coastal districts of Uttara Kannada, Udupi, and Dakshin Kannada was estimated as 1940 ha (Fig. 1). determined using remote sensing data (Google Earth) and QGIS (Quantum Geographic Information System) version 3.16. These three districts were chosen for the study as they fall under the administrative boundary of the Karnataka state. These flood plains that are under traditional shrimp cultivation or abandoned, integrated with constructed wetlands and biofilm-based algal cultivation ponds, provide multiple environmental and societal benefits such as (i) remediation of nutrient-rich wastewaters, (ii) GHG mitigation through carbon sequestration by microalgae, (iii) sustainable microalgal biomass production with less/no input energies or associated costs, (iv) prospects of local employment opportunities with the empowerment of women, (v) benefits from multiple products of bio-refinery including biodiesel, biogas [48], glycerol and fertilizers, (vi) minimal/no land-use changes as marginal lands are utilized and (vii) additional profits for gazani landowners. Similar studies on the scope of algal biofuel grown in urban wastewaters resulted in a lipid potential ranging from 1.94 to 6.52 t ha^{-1} yr^{-1} [49].

Thus, in the present study, the benthic diatoms, predominant in estuaries and marine environments along the Uttara Kannada coasts, were targeted as viable biofuel feedstocks for alternative renewable energy production. The current research involved habitat mapping, seasonal dynamics, biochemical composition analyses, techno-economic and lifecycle assessment of biofuel, and value-added products in the microalgal refinery at the abandoned flood plains, which enhances the scope of algal biorefinery along the Indian west coast. The large-scale microalgae cultivation integrated with wastewater remediation presents a technically feasible, economically viable, and environmentally sound proposition to address the challenges of dwindling stocks of fossil fuel and challenges of global warming through mitigation of the GHG footprint.

2.1.2. Habitat mapping and prioritization of diatoms

Understanding the spatial distribution of diatoms and species abundance with habitat mapping in fluctuating seasonal and hydro-ecological conditions helps to prioritize the resilient group of diatoms that are viable for industrial-scale exploitations. Habitat mapping of the diatoms carried out in different lentic, and lotic systems of the Aghanashini estuary resulted in twenty-seven tolerant diatom species belonging to the genera *Amphora*, *Nitzschia*, *Navicula*, *Cyclotella, Raphoneis,* and *Pleurosigma*. Statistical analyses revealed a strong correlation between the environmental conditions and possible lipid potential [39]. The seasonal dynamics, covering all three seasons (i.e., pre-monsoon, monsoon, and post-monsoon), were assessed through monthly field investigations in the intertidal regions along the shorelines of the Aghanashini estuary. Statistical analyses of field data through agglomerative hierarchical clustering and non-metric multidimensional scaling (n-MDS) show a distinct variation between the tolerant and sensitive species. *Melosira* sp., *Nitzschia* sp., *Cyclotella* sp., *Coscinodiscus subtilis*, *Navicula* sp., and *Achnanthes* sp. including few representation species like *Melosira lineatus*, *Nitzschia acicularis*, *N.obtusa*, *N.sigma*, *Pleurosigma balticum*, *P.angulatum*, *Amphora salina*, *A.ovalis*, *Cyclotella meneghiniana*, *C.operculata*, *Navicula forcipata*, *N. weisflogii*,

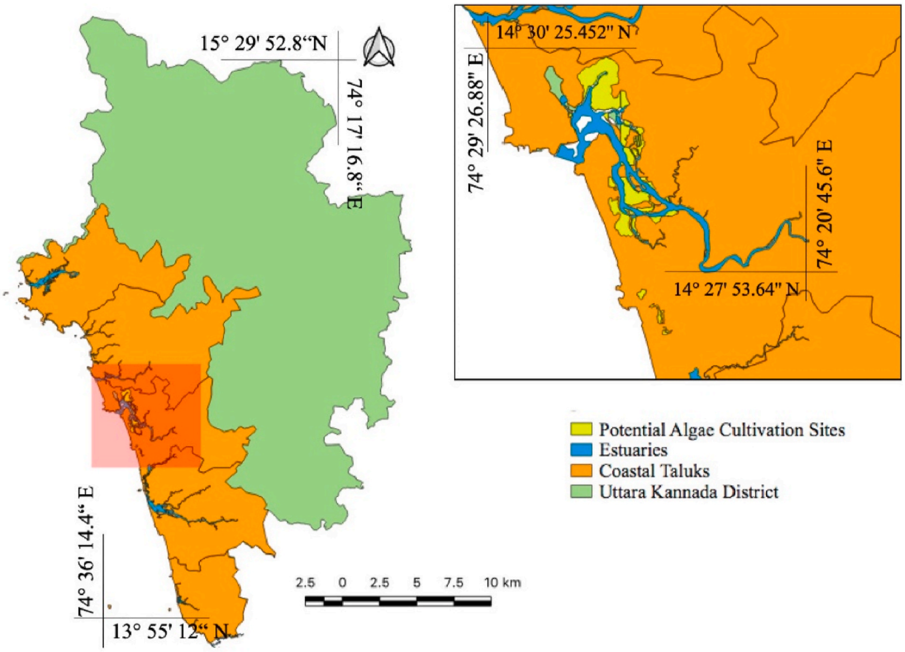

Fig. 1. Potential microalgae cultivation sites along Uttara Kannada coast.

N. amphisbaena, *N.scutelloids* were prevalent across all seasons with varying relative abundances, exhibiting higher tolerance and resilience to wide fluctuations in salinities and nutrient levels across seasons [50].

In the statistical analysis using n-MDS, cumulative season-wise species abundance was determined to identify the most common species across different sampling locations. The n-MDS plot represented as ordination shells (can be found in Ref. [51]) formed for pre- post- and monsoon seasons across stations demarcated tolerant species recorded year-long from sensitive species that are discrete to a single station. For example, diatoms like *Cyclotella meneghiniana, Melosira* sp., *Coscinodiscus subtilis*, *Nitzschia obtusa*, *Gomphonema gracile* were present in the conjoining sections of all three ordination shells depicting tolerance with species presence in all three seasons at different sampling locations. Whereas diatoms *Melosira jurgensii Achnanthes brevipes and A. longipes*, *Amphora salina*, *Navicula amphisbaena*, were recorded only in monsoon and pre-monsoon seasons in one or more study stations. Fig. 2 represents diatom dynamics across seasons. A similar exercise of habitat mapping and species prioritization in other regions is required to implement decentralized biofuel production systems.

2.1.3. Biochemical composition analyses of diatoms

Diatoms *Nitzschia*, *Amphora*, and *Navicula* sp. were tolerant by exhibiting their presence in all seasons and showed dominance during the pre-monsoon season (estimated based on species richness) highest

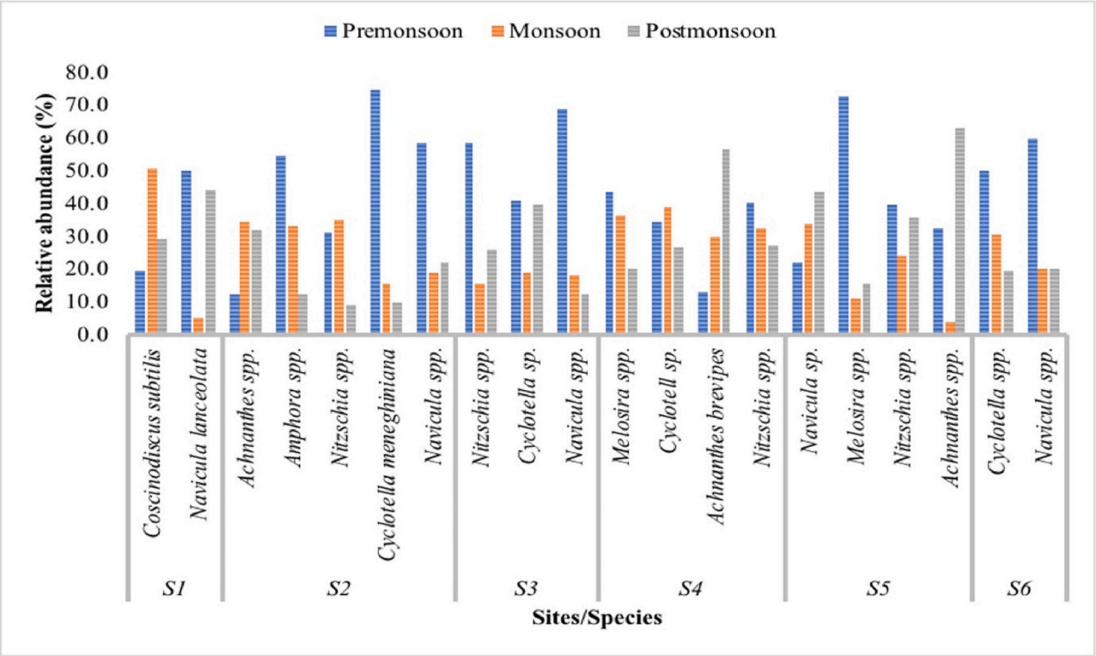

Fig. 2. Seasonality of diatoms across different seasons in the Aghanashini estuary.

lipid productivity (18–21%) under field conditions. Hence, among the three tolerant diatom species, *Nitzschia* and *Amphora* sp. were prioritized from the nutrient-rich regions of the Aghanashini estuary and were analyzed for their biochemical composition. The diatom samples collected during the pre-monsoon season were pre-treated and processed to obtain dry biomass in the laboratory. The lipid content of the prioritized diatoms was estimated using the modified Folch method [52]. Cells were harvested (on the onset of stationary phase), and the biomass was stored at −20 °C until further analysis. Lipids were extracted from dried biomass using the solvents chloroform, methanol, and water in the ratio of 2:1:0.8. Other macromolecular compositions of the biomass were analyzed by estimating carbon, hydrogen, and nitrogen content of the dried diatom biomass in a CHN elemental analyzer. Protein content was calculated by multiplying the nitrogen content by 6.28. The carbohydrate content in biomass was calculated by subtracting estimated percentages of lipids and proteins from a known quantity of biomass by heating the pre-weighed algal biomass to 575 °C for 4 h in a muffle furnace. After cooling crucibles to room temperature, the left-over ash was weighed and subtracted with dry weight the mineral (ash) content.

Fig. 3 illustrates the biochemical composition of the prioritized diatoms *Nitzschia* sp. and *Amphora* sp. The biochemical composition analysis of prioritized diatoms shows 27% lipids, 10% proteins, 53% carbohydrates, and 10% ash in *Amphora* sp., whereas *Nitzschia* sp. had 18% lipid content, 8% protein, 59% carbohydrates, and 15% ash. Nutrients used for diatom cultivation influence the fatty acid profiles of the diatoms, evident from higher proportions (up to 90%) of Monounsaturated fatty acids (palmitoleic and oleic acids) and their saturates (C16–C18), by subjecting diatoms to glucose as a carbon source (mixotrophy). Thus, understanding the biochemical composition helps in the target extraction of biomolecules of interest by altering growth conditions. The lipid extracted from diatom *Nitzschia* sp. was transesterified with a biocatalyst using lipase derived from *Cladosporium* sp. strain CS4. A biodiesel yield of 87.2% was achieved in the biocatalyst-based transesterification compared to 83.02% with the acid (2% H_2SO_4) catalyzed transesterification [53].

2.1.4. Prototype bioreactor

Lab-scale bioreactor (Fig. 4) was designed using polypropylene-based non-reactive plastic trays, each having a dimension of (51 × 27) cm with a footprint area of 0.1377 cm^2. Gravel stones were placed uniformly in the tray as substrates for diatom attachment.

Prioritized diatom *Amphora* sp. based on habitat mapping and seasonal dynamics were isolated and cultured under laboratory conditions using f/2 media (an enriched seawater medium, especially used for the growth of marine diatoms) [54]. The f/2 media used for diatom growth comprised of $NaNO_3$: 75 mg L^{-1}; $Na_2SiO_3.9H_2O$: 30 mg L^{-1}, $NaH_2PO_4.2H_2O$: 5 mg L^{-1}, 1 mL trace metals stock solution ($FeCl_3.6H_2O$: 3.5 g L^{-1}; $Na2EDTA.2H_2O$: 4.36 g L^{-1}; $CuSO_4.5H_2O$: 9.8 g L^{-1}; $MnCl_2.4H_2O$: 180 g L^{-1}; $Na_2MoO_4.2H_2O$: 6.3 g L^{-1}; $ZnSO4.7H_2O$: 22 g L^{-1}; $CoCl_2.6H_2O$: 10 g L^{-1}) and 0.5 mL vitamin stock solution (vitamin B1: 200 mg L^{-1}; Vitamin H 1 g L^{-1} and Vitamin B12: 1 g L^{-1}). The ambient conditions maintained for diatom culture were 25 ± 2 °C, salinity: 35 ppt (parts per thousand) and light intensity: ~360 μmol m^{-2} s^{-1} with 14:10 h light: dark cycle. Then pure culture isolates were sequentially sub-cultured to maintain cell viability. Comparative assessment on efficacy of harvesting and productivity assessment of reactors and suspended mode cultivation (in Erlenmeyer flasks) (where the cells are grown suspended in the water column) was performed. The scaled-up culture of one of the prioritized diatoms (*Amphora* sp.) with an initial biomass concentration of 0.187 g L^{-1} (grams per litre) was used as an inoculum. The experiment was conducted in batch mode with 3.5 L of media as working volume both in biofilm as well as suspended mode cultivation in Erlenmeyer culture flasks (control) of 5 L capacity.

Fig. 4. Prototype bioreactor.

Prototype substrate-based biofilm bioreactor was constructed at three different stations in the flood plains of the Aghanashini estuary (Fig. 5) by introducing gravel stones of irregular shapes and sizes, with flat surface area onto a platform made of polyvinyl chloride (PVC) mesh (1.21 m × 1.06 m) supported with a frame made of PVC pipes. This prevents sediment deposition on substrates, which is common during tidal undulations in the intertidal regions of estuaries. The height of the platform was 0.35 m above the ground level while ensuring adequate water contact with substrates. Two bioreactors were deployed at each location. Sixty granite stones of varying shapes, sizes with the flat surface area were deployed in each of the bioreactors, and the setup is given in Fig. 6. Bioreactors were continuously monitored for 25 days on every alternate day. Results were validated by repeating the experiment for another 15 days after 30 days interval.

Growth of the prioritized diatom strain *Amphora* sp. was cultivated

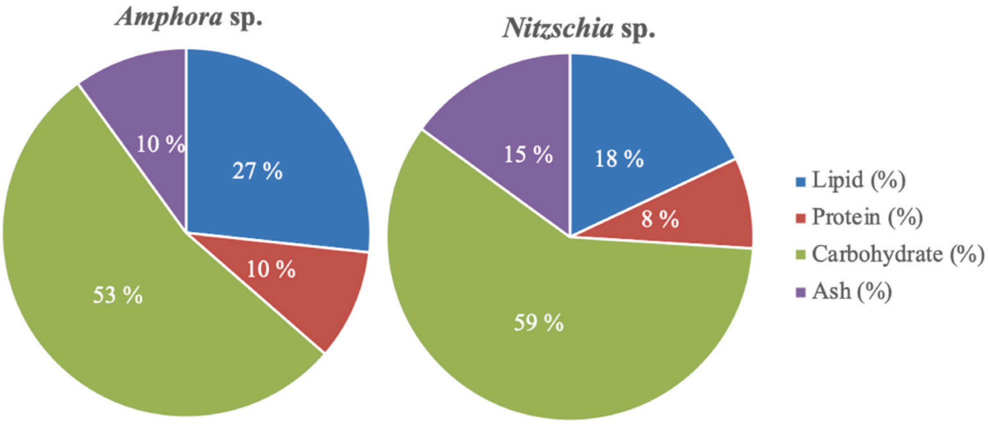

Fig. 3. Biochemical composition of the prioritized diatoms of the Aghanashini estuary.

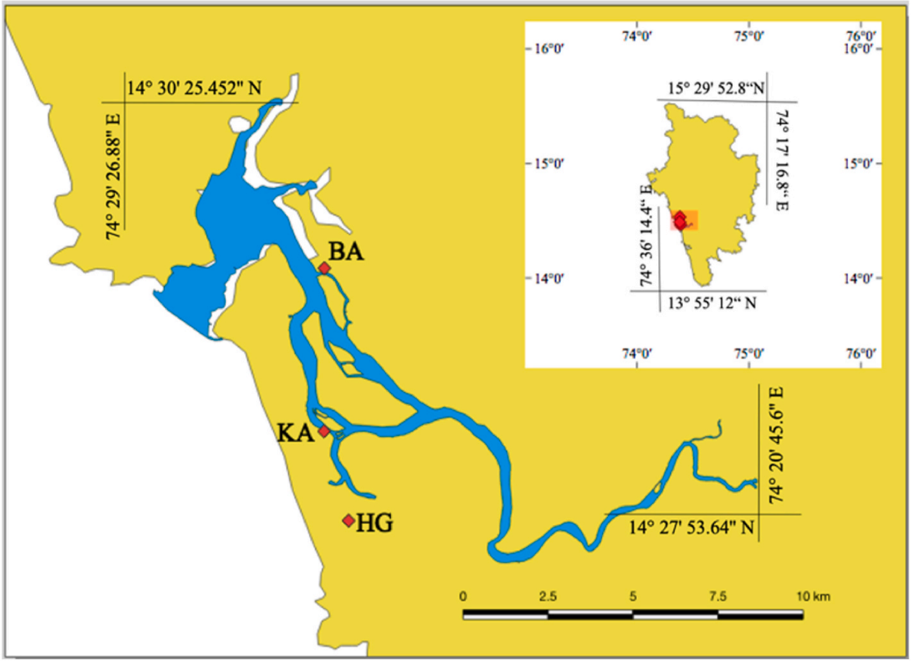

Fig. 5. Bioreactor deployed locations in the flood plains of the Aghanashini estuary.

Fig. 6. Substrate-based bioreactor deployed at field.

and monitored in a bioreactor at laboratory conditions. The growth rate of *Amphora* sp. was determined by measuring chlorophyll-a concentration every three days during the growth period. Chlorophyll and biomass productivity estimations were carried out following the protocols [55]. In addition to chlorophyll estimation, the viability of the cells was also monitored by visual examination under a high-resolution microscope Olympus BX 51. *Amphora* sp. followed a lag, exponential and stationary phase for 12 days growth cycle. The trend of variations in chlorophyll content of biomass in bioreactors (gravel stone substrate with biofilm) and conical flasks (suspended cultivation) are given in Fig. 7. The growth of diatoms in the bioreactor exhibited a lag phase till the third day, then entered the log phase and exhibited a higher growth rate than suspended cultivation. Fig. 8 depicts the light and SEM micrographs of *Amphora* sp.

The algal (diatom) biomass harvested after 12 days of cultivation in the bioreactor (with substrates) and suspended cultivation was 565.4 mg L^{-1} and 33.37 mg L^{-1}, respectively, within biomass productivity of 47.11 mg $L^{-1}d^{-1}$ and 2.78 mg $L^{-1}d^{-1}$, respectively. The increase in biomass concentration of *Amphora* sp. in biofilm-based cultivation was ~16 times more than that of the suspended cultivation. The aerial biomass concentration and productivity obtained in substrate-based

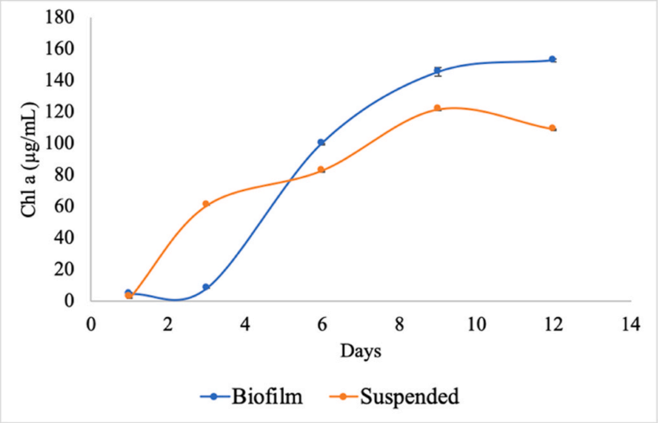

Fig. 7. Comparison of chlorophyll content in biofilm and suspended Cultivation modes.

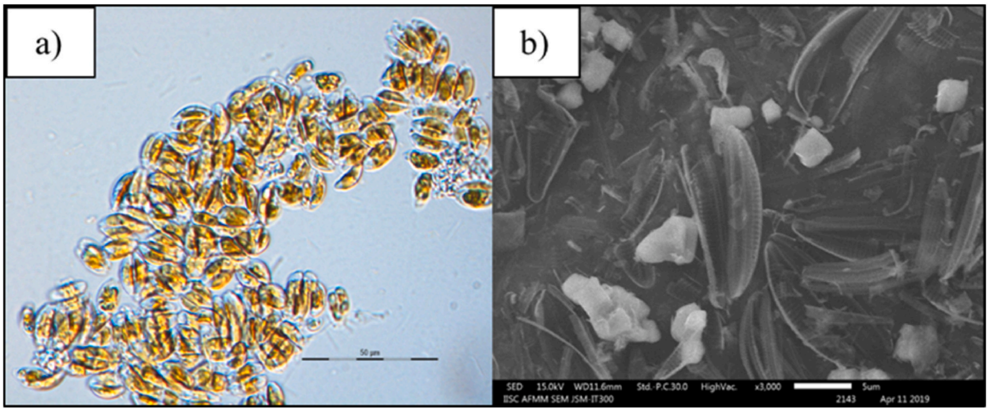

Fig. 8. Light microscopic image (a) and SEM image (b) of *Amphora* sp.

cultivation with biofilm are about 4.10 g m^{-2} and 0.34 g m^{-2} d^{-1}, respectively. This result substantiates the advantages of the substrate with biofilm for feedstock cultivation over conventional suspended mode cultivation. Elemental analysis of the harvested dried algal biomass of different cultivation modes through EDS FE SEM (Energy Dispersive X-Ray Spectroscopy in Ultra55 FE-SEM Karl Zeiss EDS at low KV after gold sputtering algal biomass, showed a higher carbon content (43.23%) for substrate-based cultivated biomass compared to that under the suspended cultivation mode (38.04%). Higher carbon content in biofilm cultivation with lesser carbon content (36.3%) would ensure a higher calorific value of the biomass than suspended cultivation obtained biomass. However, the mineral ash content in the form of chloride (4.76%) and calcium (2.53%) found in biofilm cultivated biomass was absent in biomass of suspended cultivation, thus reinstating higher ash content encountered in biofilm cultivation. Earlier studies on the green microalga *Chlorella vulgaris* had shown an ash content of 6.03% when cultivated in a rotating algal biofilm [56] which is comparable with the present study. Fig. 9 illustrates the elemental composition acquired in SEM-EDS. Table 1 list the elemental composition of algal biomass in different cultivation modes.

Table 1
Elemental composition of algal biomass cultivated using different treatments.

Element	Attached cultivation (with biofilm)		Suspended cultivation	
	Weight%	Atomic%	Weight%	Atomic%
C K	43.23	54.9	38.04	48.52
O K	36.3	34.61	42.81	41
Na K	2.83	1.88	2.07	1.38
Mg K	2.45	1.54	2.78	1.75
Si K	4.05	2.2	9.91	5.41
P K	2.45	1.21	1.12	0.54
S K	1.4	0.67	3.27	1.41
Cl K	4.76	2.05	–	–
Ca K	2.53	0.96	–	–
Total	100		100	

*K after each element refers to the ‘n’ value that electrons in the shell have K electrons.

Diatom samples were collected by dislodging the cells from the introduced substrates (stones) in the field bioreactor deployed at different stations, and microscopic examination of the processed cells was carried out to understand the variations in species richness and diversity. At station Bargi (BA), the diatom *Mastogloia* sp. and *Epithema gibberula* were the most abundant, with a cell density of 1.27 × 10^4 cells mL^{-1} and 2.23 × 10^4 cells mL^{-1}, respectively. At Kagal (KA), the

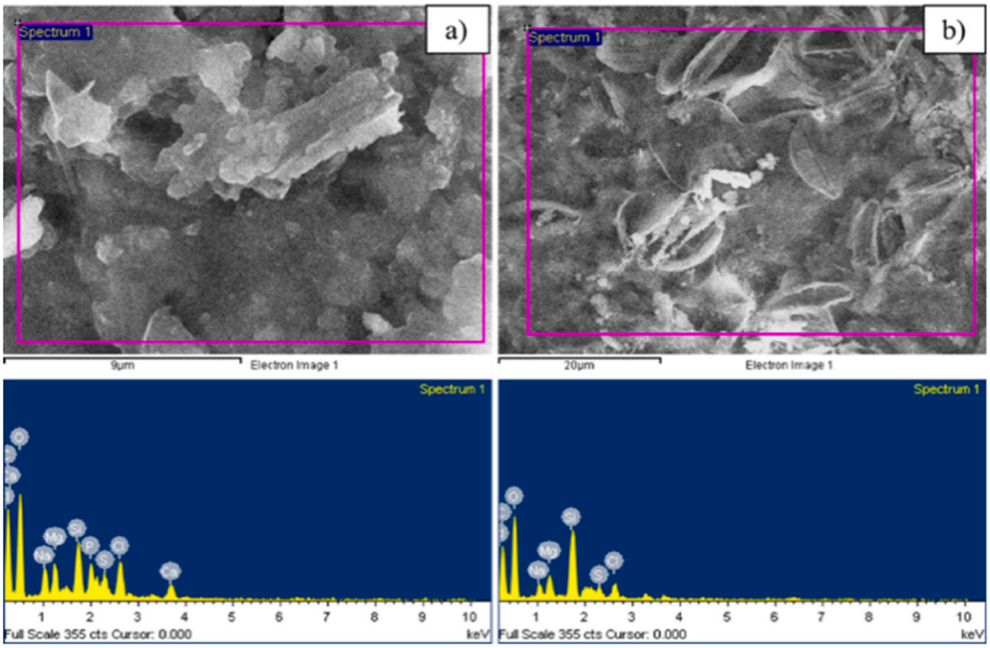

Fig. 9. EDS FE SEM analysis of attached (a) and suspended (b) algal biomass.

predominant species was *Navicula forcipata* with a cell density of 7.1 × 10^3 cells mL^{-1}, followed by *Nitzschia panduriformis* with 2.6 × 10^3 cells mL^{-1}. Wider variations in cell density were observed among triplicate samples (stones) collected at each station. BA station showed the highest species diversity with 15 species, followed by KA with 12 species. Station HG was observed to have the least species diversity with ten different species with a maximum cell density of 1.4 × 10^3 cells mL^{-1} recorded for the diatom *Nitzschia sigma*. Fig. 10 lists the optical microscopic images of diverse diatoms that are present in the biofilm formed at different stations.

Species composition of the daily sample exhibited significant variation in the species types and cell density across stations. The cell density of *Mastogloia* sp., at station BA was found to be maximum on Day 15 with a measured cell density of ~2.6 × 10^4 cells mL^{-1} followed by the diatom *Epithema gibberula* with ~1.45 × 10^5 cells mL^{-1}. The station KA showed a dominance of the diatom *Navicula forcipata* (~7.5 × 10^4 cells mL^{-1}) with maximum cell density recorded on the 17th day of the field sampling. In the station HG, the diatom Navicula cincta showed its predominance with 4.5 × 10^4 cells mL^{-1} on day 15. Fig. 11 lists the daywise variations in species composition at different study stations.

Substrate-based field bioreactors' biomass productivity and yield show variations across stations primarily influenced by local environmental conditions. The biomass at BA, KA, and HG was 22.23, 12.84, and 1.91 g m^{-2} after 18 days of introducing substrata with average biomass productivity of 12.3 g m^{-2} (Fig. 12). The biomass productivity values were calculated on an ash-free dry weight (AFDW) basis, showing areal biomass productivity of 1.24, 0.71, and 0.11 g m^{-2} d^{-1}. Total average productivity in the aluminium-based Algal Turf Scrubbing (ATS) system showed 14 g m^{-2}. The wooden scrubbing system showed algal productivity of 11.7 g m^{-2} [57], comparable to the present study.

Dry microalgal (diatom) biomass (about 1.2 kg) harvested from the field after pre-treatment was subjected to direct transesterification using hexane, methanol, and H_2SO_4 in various batches yielded ~ 230 mL of microalgal biodiesel. The biodiesel property of diatom oil extracted from the algal biomass cultivated with biofilm was determined through standard protocol - Hoekman's equation [58]. Critical fuel parameters of extracted microalgal biodiesel (230 mL) like density, viscosity, pour point, flash point, and calorific content was assessed at the CSIR- Indian Institute of Chemical Technology (biofuel testing laboratory) Hyderabad. Fig. 13 represents the microalgal biodiesel extracted from field harvested diatom biomass. Table 2 and Table 3 compare the produced biodiesel quality with the International and Indian biodiesel quality standards.

These preliminary pilot demonstration results on biofilm cultivation of diatoms showed promising scope for a decentralized biorefinery in the abandoned flood plains of the central west coast. Species composition and biofilm formation studies on the field-deployed substrate-based bioreactor showed the influence of environmental parameters on diatom species composition. Biodiesel quality testing on the field harvested biomass-derived fuel showed critical quality parameters to fall well within the specification limits of the biodiesel standards, thus proving its compatibility as diesel fuel. When scaled up to an industrial level, this pilot design would fetch assured benefits of generating decentralized bioenergies at local levels.

2.1.5. *Techno-economic and lifecycle assessment*

The techno-economic (TE) analysis of a decentralized microalgal biorefinery was done considering a 1-ha plot of abandoned gazani land (flood plain) situated in the coastal regions of Karnataka. Discounted cash flow model was used to determine the financial feasibility of the proposed microalgal cultivation system. The model considered 60% of the capital investments as project financing loans from the National Bank for Agriculture and Rural Development (NABARD) with an annual loan repayment determined by a 4.5% interest rate for a loan tenure of 5 years and the remaining 40% of farmer's investment share. Loan options are available for micro, small and medium enterprises (MSME) under the supervision of the ministry of micro, small and medium enterprises, a branch of the Government of India (GoI).

The capital and operating (fixed and variable) costs were determined by considering the material and energy inputs at each stage (Table 4). The capital costs were estimated to evaluate the different processes considered with equipment, transportation costs, and raw material requirement incurred under each unit operation. For cost estimations, a currency rate of 1 USD to 72.52 INR, according to the Reserve Bank of India exchange rate as of March 2021, was used.where,

$$C_{TOC} = C_{FOC} + C_{VOC};\ C_{FOC} = C_{LV} + C_{LR} + C_{ML};\ C_{VOC} = C_{EC}$$

$$Payback\ period = 1 + n_y - \frac{n}{p} \tag{2}$$

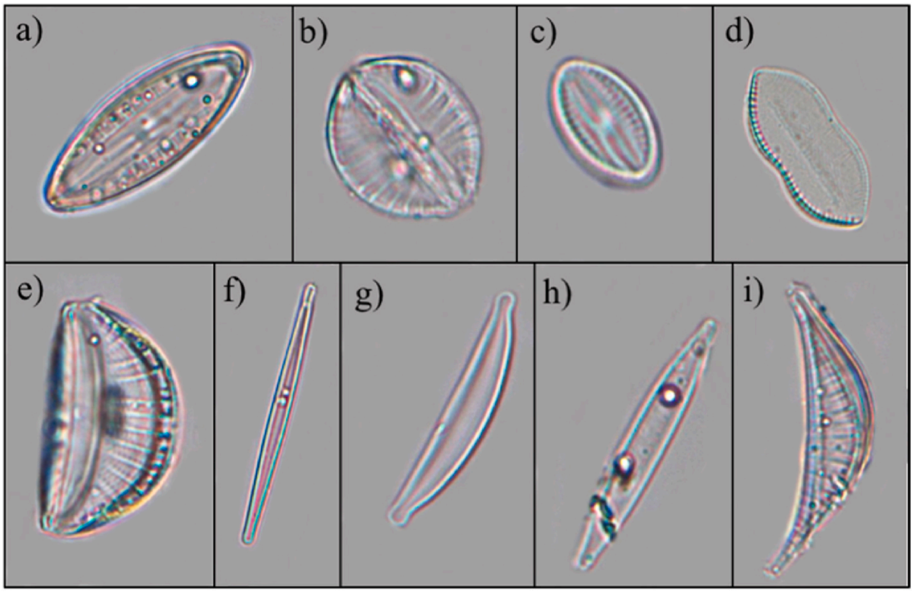

Fig. 10. Light micrographs of processed diatom frustules *a) Mastogloia* sp. b) *Epithema gibberula* c) *Navicula forcipata* d) *Nitzschia panduriformis* e) *Epithema* sp. f) *Nitzschia* sp. g) *Cymbella* sp. I) *Nitzschia linearis* j) *Epithema* sp.

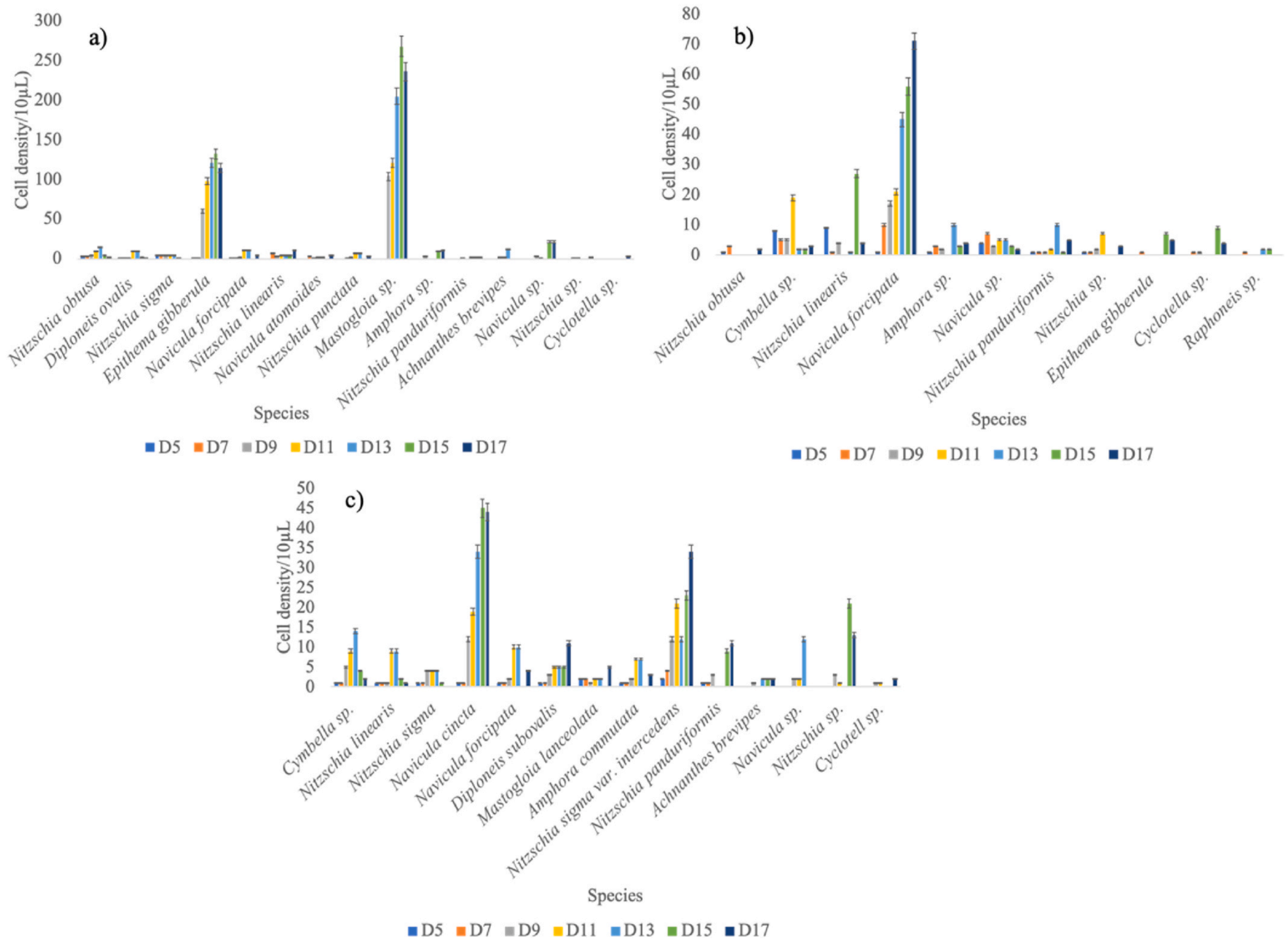

Fig. 11. Species composition dynamics of stations a) BA, b) KA, and c) HG.

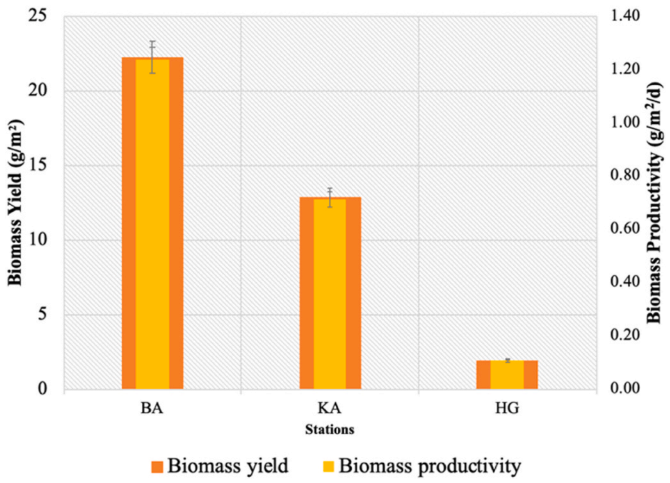

Fig. 12. Biomass productivity and yield of the field photobioreactor.

Fig. 13. Biodiesel extracted from the field harvested microalgal biomass.

where,

$n = no\ of\ years$

$n_y = The\ year\ at\ which\ the\ last\ cumulative\ cash\ flow\ occurs$

$p = cumulative\ value\ at\ first\ positive\ cash\ flow$

$$\text{Return on investment } ROI = \frac{Total\ revenue}{Total\ operating\ cost} \quad (3)$$

Table 2
Comparison of biodiesel quality with ASTM and EN biodiesel standard.

Fuel property	Algal biodiesel			Biodiesel standard	
	BA	KA	HG	ASTM D6751	EN 14214
Cetane number	62.4	62.2	62.5	47	51–120
HHV (MJ/kg)	38.7	38.7	38.6	–	–
Iodine number (gI_2/100 g)	17.8	20.4	17.4	–	<120
Specific gravity (kg L^{-1})	0.9	0.9	0.9	–	0.86–0.90
Density (kg/m^3)	900	900	900	–	860–900
Cloud point (°C)	19.1	18.6	19.2	LD[a]	–
Kinematic viscosity 40 °C (mm^2 S^{-1})	5.2	5.1	5.2	1.9–6.0	3.5–5.0
Avg. degree of unsaturation	0.07	0.1	0.06	–	–

[a] LD – Location dependent.

Table 3
Analysis of basic biodiesel quality parameters as per Indian biodiesel standards.

Test	Test Method IS:1448	Result	Specification limits
Kinematic viscosity @40 °C, cSt	ISO 3104	5.16	3.5–6.0
Density at 15 °C, g/cm^3	ISO 3675	0.8830	0.86–0.9
Flashpoint, °C (PMCC)	ISO 2719	115	101 (min)
Pour Point, °C	P-10	0	–
Copper strip corrosion for 3 h @50 °C	ISO 2160	[a]1a	–

[a] 1a – Light orange, almost the same as freshly polished strip.

Table 4
Methods considered for techno-economic assessment.

$$Biodiesel\ cost = \frac{Total\ operating\ cost\ (C_{TOC})(INR)}{Biodiesel\ production\ volume\ (L)} \tag{1}$$

S. No	Methods used for calculating techno economics of the microalgal biorefinery	
	Parameter	Calculation methods
	Facility lifetime	30 years
Capital cost		
1.a	Bioreactor material/fermenter procurement	Actual prices from manufacturers.
1.b	Pitching, bunding, sluice gate installation cost	Compiled through interviews and interaction with landowners and shrimp farmers.
1.c	Labour cost for land preparation	Fixed as per the minimum wages act after confirming the same with the current scenario in the study region.
Operating cost		
Fixed operating cost		
2.a	Gazani land lease value	It was fixed as per the current lease trend in the study region.
2.b	Labour cost for harvesting	Same as 1.c.
2.c	Loan repayment cost	Calculated by considering 4.5% interest rates on the principal for a loan tenure of 5 years.
Variable costs		
2.d	Cost of lime fertilizer and solvents	Actual prices of chemicals, fertilizers, and solvents.
2.e	Biodiesel production and other downstream processing costs	Fixed as per Karnataka Electricity Regulation Commission's standard power tariffs for industrial uses.

Assuming facility lifespan of 30 years, biodiesel production cost (INR/kg), payback period (years), return on investment, and annual profit (INR/ha/yr.) was calculated using equations (1)–(4) respectively.

$$Profit = Revenue - Cost \tag{4}$$

The three varying nutrient input scenarios (no nutrient input, wastewater input, and fertilizer input) yielded a yearly profit ranging between 35,296 INR/ha and 2,09,190 INR/ha based on the level and type of inputs during cultivation. Land preparation (pitching) entails leveling the land, which requires five persons working on the activity for 3-human days (7 h a day) per hectare. Microalgae attached to substrates in a bioreactor are predominantly diatoms confirmed with the field experiments. This microalgal cultivation facility can operate for 224 days (32 weeks), excluding the torrential monsoon period due to salinity dilutions, which would affect microalgal growth, and also water turbulence affects the initial colonization of the microalgal community (2 weeks). A hybrid version involving mechanized scrubbers and manual labor was considered for harvesting the microalgal biomass from the substrata. A total of four people working in a 1-ha plot would require two to two and half days to gather the biomass. The cost of harvesting is estimated as ~3 INR/m^2or 20 INR/kg of microalgal biomass. In order to dry the algal biomass, i) direct solar drying; ii) filter press and subsequent solar drying were considered. Manual washing and sonication were the two pre-treatment methods considered with biodiesel production from dried algal biomass using direct transesterification in the presence of acid- and biocatalyst's FAME conversion efficiencies ranging between 83 and 87% (based on conversion efficiencies of pilot lab-scale experiments). The biofuel production cost in a bioreactor (hectare) was estimated, considering all cost inputs, which vary between 30.08 INR/kg to 59.52 INR/kg of biodiesel.

The environmental assessment was performed using a cradle-to-gate Life cycle assessment (LCA) approach considering each process, right from preparation of flood plains for microalgal cultivation to end product (biodiesel) production. LCA considered both energy and GHG emissions footprints. The LCA's functional unit was defined as the algal biomass achievable in a 1-ha plot in the coastal flood plains of the Uttara Kannada district in Karnataka. Three different nutrient input scenarios, i.e., i) without any external nutrient inputs, (ii) in wastewater (gives an additional scope to assess bioremediation potential with biofuel production) and (iii) with external inputs–synthetic fertilizer, were considered for the environmental loading assessment. The likely energy expenditure and Carbon-di-oxide equivalent emissions in kilograms were computed considering 1 kg of biodiesel as the basis. (Further details in Ref. [59]). Life cycle assessment revealed a conventional fuel requirement of 3.6–5.7 MJ/kg and lifecycle emission (as kg CO_{2e} emissions) that varied between 0.85 and 1.46 kg $CO_{2eq}.kg^{-1}$ of biodiesel.

Mass budgeting of microalgal biorefinery by-products from microalgal biomass was carried out by assuming a 100 kg dry algal biomass. Lipid ranging between 18 and 26% would yield 14.94–22.62 kg biodiesel through transesterification with a by-product crude glycerol of 1.49–2.26 kg [60]. The spent algal biomass subjected to anaerobic digestion in the anaerobic digester would provide 24.48 m^3 biogas at a production rate of 0.272 m^3/kg of biomass [61,62]. The raw biogas is purified/upgraded by passing on to a CO_2 stripper absorption column or can directly be used for domestic cooking/heating applications. About 10% biomass loss was considered after lipid extraction using direct transesterification for accounting for the losses encountered during biomass processing, handling transportation, and storage of the residual biomass. The solid digestate in the form of a slurry (~55–70 kg) is rich in nitrogen is useful as organic biofertilizers in crop fields. These results substantiate the scope of setting up a decentralized microalgal biorefinery along the Indian west coasts.

2.2. *Diatom biorefineries*

The burgeoning wastewater generation with 50–80% of wastewater being discharged to water bodies is of serious pollution concern worldwide [63]. Thus, resource recovery from wastewater using

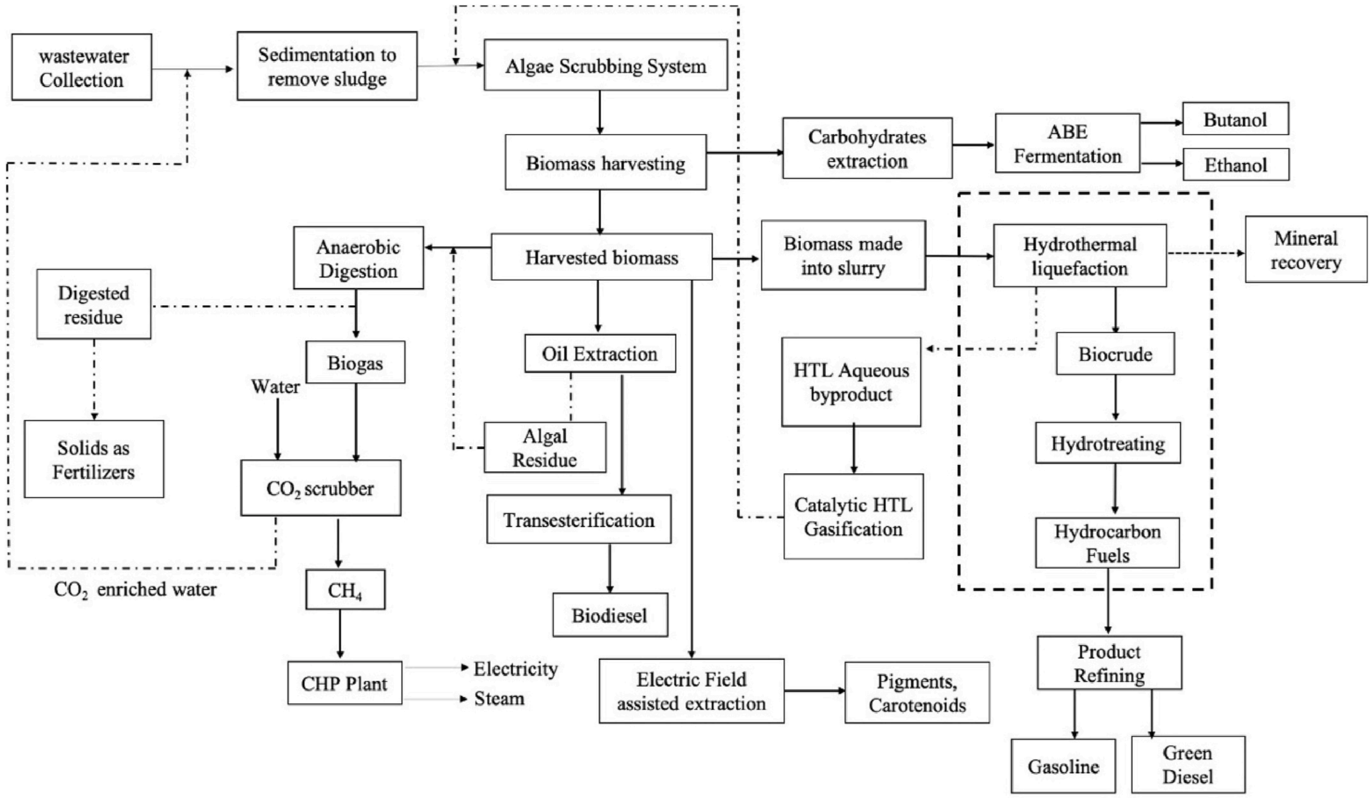

Fig. 14. Possible sustainable options of utilizing microalgal biomass.

microalgal production systems is predicted as highly advantageous in establishing a circular bioeconomy [64]. The diatom biomass with carbohydrates, phycobiliproteins, and essential fatty acids forms a viable feedstock for bioenergy and value-added products that warrants a circular bioeconomy by manifesting the basic concept of reducing, reuse, and recycling [65,66]. Fig. 14 illustrates pathways to utilizing algal biomass grown using low-cost sustainable algal production systems. Algae are rich in carbohydrates, which could be converted into a range of bioenergy components such as biogas and liquid biofuels through different biological processes. When subjected to anaerobic digestion, the carbohydrate-rich biomass would yield biogas, butanol, and ethanol [67]. The acetone-butanol-ethanol (ABE) fermentation [68] is an anaerobic fermentation process that is carried out using a gram-negative bacterium called *Clostridium beijerinckii.* Butanol is gaining prominence due to its superior fuel value and better storage characteristics. The hydrothermal liquefaction (HTL) provides a direct pathway for liquid biocrude production from wet algal biomass with medium temperature (350 °C) and pressure (20 MPa) conditions [69]. At specified operating conditions, the liquid present in the algal biomass (maintained at sub-critical levels) would aid as a catalyst for biocrude production. Efforts are being made [70–72] to produce biocrude through the HTL pathway as lower energy is spent on biomass harvesting and drying. The harvested biomass, when subjected to oil extraction and subsequent transesterification, results in biodiesel. The microalgal biomass rich in pigments such as carotenoids is useful as a feedstock for bioactive/value-added product synthesis.

3. Policy stimulus to biofuel sector in India

India is currently engaged in accelerated research, development, and demonstration of sustainable and clean energy initiatives, increasing awareness of energy-related opportunities and challenges. The Department of Biotechnology (DBT), Government of India (GoI) has established four bioenergy centres i) DBT-ICT Centre for Energy Biosciences at the Institute of Chemical Technology (ICT), Mumbai; ii) DBT-IOC Centre for Advanced Bioenergy at the Indian Oil Corporation (IOC) at Faridabad, Haryana; iii) DBT-ICGEB Centre for Advanced Bioenergy Research at the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi and iv) DBT Pan-IIT Centre for Bioenergy – a nodal centre having a consortium of premier Indian Institute of Technology institutes across different states, with a vision to promote biofuel technology and imbibe clean and renewable energy alternatives. These four initiatives have provided an impetus to cutting-edge research focusing on: i) new renewable feedstock development; ii) waste (municipal solid waste) to bioenergy; iii) microalgal strain improvement to enhance biomass productivity; iv) development of enzymes or microorganisms to achieve higher yields of biofuels; v) advanced biofuel and allied value-added products development; vi) fermentation technologies for ethanol production; vii) cellulolytic enzyme for C5 ethanol production and viii) cyanobacterial bioethanol.

The biofuel research and development had a further boost with the revised biofuel policy of GoI (2018), of 20% blending of ethanol and 5% biodiesel blending by 2030. The DBT Pan-IIT Centre for Bioenergy has been working towards efficient bioprocess development and scale-up by integrating primary and translational science capabilities in algal biotechnology, synthetic biology, enzymes, fermentation technologies, and separation technologies for the production of drop-in biofuels such as biobutanol, bio hydrocarbons, aviation fuels, advanced biofuels and other value-added products (proteins, lipids, organic acids, and carotenoids). The Mission Innovation (MI) – a global intergovernmental initiative for accelerating the clean energy revolution, involving 24 countries and the European Commission (on behalf of the European Union) – was initiated in 2015. It aimed to identify the potential areas of clean energy research, revise policies for developing these potential sectors, actively bring in breakthroughs/achievements through public-private industrial collaboration and bi/multilateral international collaborations. In this regard, the focus of algal biofuel research is to bring down the costs of algal biofuel by five-folds by using robust, preferably marine algae grown along the seacoast to achieve i) higher productivity; ii) tolerance to stress; iii) low capital costs; iv) low energy consumption and v) efficient harvesting and conversion process.

3.1. *Biofuel initiative at federal levels*

The Karnataka State Biofuel Development Board (KBDB established in 2009 by the Government of Karnataka) and the Karnataka State Biofuel Taskforce (an independent organization), in collaboration, are actively involved in promoting biofuel from the second generation (non-edible plant-based oil) feedstock-derived biodiesel and bioethanol from beetroots, sugarcane peels, and green waste molasses. The non-edible plant-based seeds that are well-suited for the agroclimatic condition of Karnataka are Jatropha, Pongamia, Neem, Simarouba, and Mahuva. In this regard, biofuel parks have been established at various locations to develop different plant varieties and manage multiple model processing units. The economic analysis considering 1-ha bioreactor revealed the cost-effectiveness of microalgal biofuel compared to Jatropha and Pongamia biodiesel. The Pongamia (*Pongamia pinnata*) plantation takes ten years to fully mature, yielding seeds from the fifth year. The average yield of Pongamia is about 3–5 t ha^{-1}; it can yield up to ~120 kg ha^{-1} yr^{-1} of biodiesel, which is equivalent to 130.4 L ha^{-1} yr^{-1} along with a crude glycerol yield of ~76 kg ha^{-1} as a by-product. The calorific value of Pongamia was estimated to be 36.5 MJ. The current market rate of Pongamia biodiesel is 40 INR L^{-1} that could fetch a monetary benefit of 5216 INR yr^{-1}. Jatropha (*Jatropha curcas*) yields a biodiesel quantity of 656 kg ha^{-1} yr^{-1} [73]. Considering the present Jatropha biodiesel cost of 40.5 INR L^{-1}, the yearly benefit from Jatropha could be 26,568 INR $yr^{-1,}$ whereas the profit from diatom-based biodiesel is 81,162 INR yr^{-1}; these values are estimated based on field biofilm experiments and highlight the economic viability of third-generation biofuels.

3.2. *Integrated biomass and biofuel production system*

The microalgal biofuel-based large-scale commercial applications are usually limited by their higher operating costs related to nutrient inputs, low biomass production, and high energy requirement during harvest [74]. The US Department of Energy (DoE) suggests economic feasibility by coupling wastewater treatment with microalgal biofuel production. The efficacy of wetland systems was evident in removing significant amounts of organic matter, suspended solids, nitrogen, and phosphorus from nutrient-rich wastewaters [75]. The primary nutrients required for microalgae cultivation are carbon (organic and inorganic forms), nitrogen, and phosphorus [76]. Forty to fifty percent of dry microalgae biomass is composed of carbon followed by nitrogen (1–10%), contributing to protein production. Phosphates are vital for photosynthetic energy transfer and the synthesis of DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) [77]. The nutrient concentrations of aquaculture wastewater, depending on the type of fish/shrimp grown and its stocking density, typically range between 0 and 7 mg L^{-1} ammoniacal nitrogen (NH_4^+), 1–25 mg L^{-1} nitrate-nitrogen (NO_3^-), and 5–15 mg L^{-1} ortho-phosphates ($PO4^{3-}$); a chemical oxygen demand (COD) of 150–250 mg L^{-1} causes eutrophication, dissolved oxygen depletion, and other associated environmental disruptions when discharged directly into water bodies.

The constructed wetland (consisting of local aquatic plant species) is integrated with a biofilm-based algal cultivation system as given in Fig. 15. It offers a low-cost and straightforward sustainable solution to moderate nutrient input to the system for optimal microalgal biomass productivity. The proposed model consists of a flood plain with cascaded sections of salt marsh sedges followed by a biofilm section with flat gravel stones. The effluent from the aquaculture ponds with typical nutrient concentrations of 0–7 mg L^{-1} ammonia, 1–25 mg L^{-1} nitrates, 5–15 mg L^{-1} phosphates, and chemical oxygen demand (COD) of 150–250 mg L^{-1} drain into these flood plains either through the natural gradient or using solar pumps. Initially, the wastewater is subjected to sediment filtration and grit removal to reduce particulate and suspended solids. Then, the effluent passes through the wetland (consisting of a row of mangroves followed by salt marsh sedges such as *Porteresia coarctata* and *Cyperus malaccensis* (salt-tolerant sedges that are native to the Uttara Kannada district), and enters the biofilm cultivation pond. A hydraulic retention time (HRT) of 2–5 days in the wetlands would help remove nutrients and suspended solids. Partially treated effluent enters the biofilm cultivation pond with gravel stones as substrates; this helps in the abundant production of microalgal biomass is to be carried out every 3–5 days based on the prevailing environmental conditions. The higher levels of nutrients would lead to higher ammonia concentrations and species succession by blue-green algae. The favorable nutrient concentration levels in the biofilm cultivation pond are $\sim < 2$ mg L^{-1} NO_3^- and <1.5 mg L^{-1} PO_4^{3-}, which is achieved by draining the aquaculture effluent through the constructed wetlands salt marsh sedges, paddy, and grass. Earlier studies on estuarine wetlands using the estuarine sedge

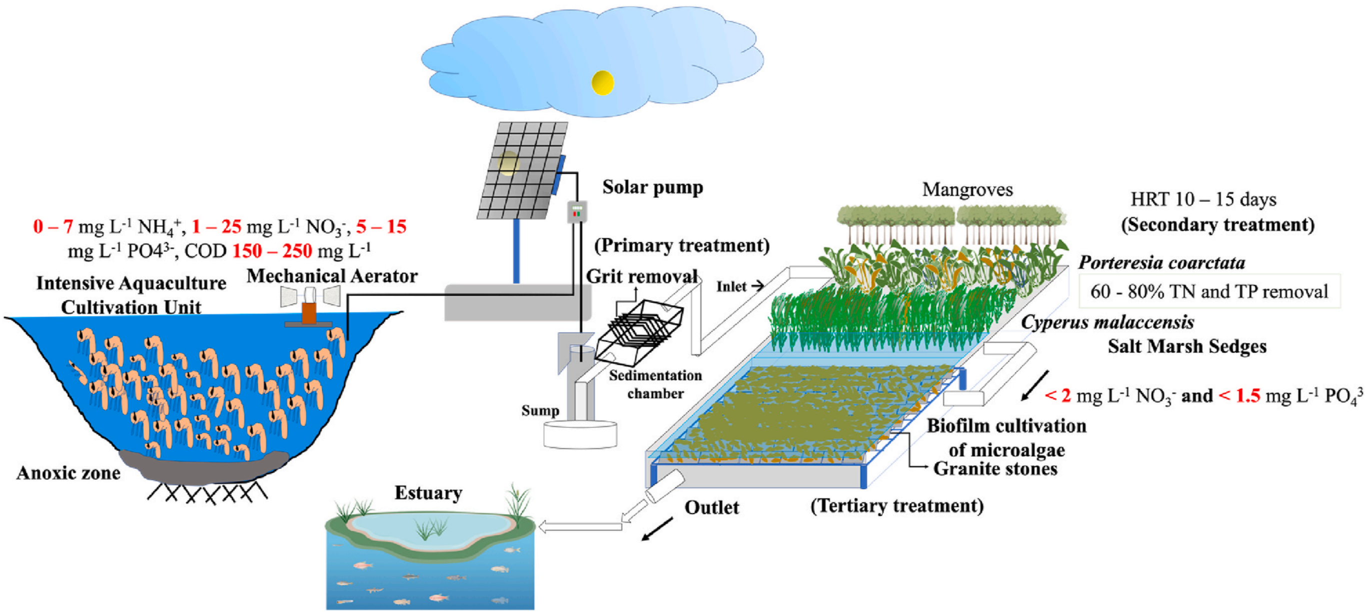

Fig. 15. Integrated algal refinery: constructed wetland coupled with the microalgal biofilm cultivation system.

Juncus kraussii to treat saline aquaculture wastewater indicate a TN (total nitrogen) and TP (total phosphorous) removal of 69% and 88.5%, respectively [78].

4. Conclusion

Sustainability in microalgal production systems depends on three critical factors: (i) efficient cultivation with a reduced ecological water footprint, (ii) economical harvesting, and (iii) appropriate downstream processes with a minimal environmental burden. Seawater usage for microalgae cultivation has been a better substitute for scarce freshwater resources and higher biodiesel yields [79] and has shown positive effects on sustainable cultivation. Moreover, there have been improvements in biodiesel yield of up to 4.17,10^5 ML/year [80] using saline water, apart from reducing the freshwater footprint. In conventional microalgae production systems, harvesting accounts for about 20–30% of biomass production costs [81,82].

The present work emphasizes the merits of attached diatom (with biofilm) cultivation using low-cost substrates such as gravel stones, producing ~ 5 times more biomass than suspended cultivation along with a considerable reduction in the harvesting costs, estimated as 20 INR per kg. This has been done through lab and field experimentation and is a first-of-its-kind study on diatoms, focusing on mitigation of the bottlenecks encountered in harvesting. The techno-economic analysis conducted to determine the possible monetary benefits in establishing an environment-friendly sustainable microalgal production system demonstrated significant economic benefits through biomass, biofuel, and other value-added products. In addition, environmental lifecycle assessment of biodiesel production through enzymatic transesterification using biocatalysts (lipase) showed potential to be an appropriate downstream process with minimal ecological burdens. Implementing such measures in future microalgal biofuel production systems would render substantial energy with cost reductions and remediation, leading to a holistic approach towards sustainable third-generation biofuel production.

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Authors contribution

Sustainable Bioeconomy with Decentralized Microalgal Refineries. T V Ramachandra – Concept, design of experiments, data analyses, validation, review and fine-tuning the manuscript. Saranya G – design of experiments, field data collection, data analyses, writing, implementing review suggestions.

Research ethics

The publication is based on the original research and has not been submitted elsewhere for publication or web hosting.

Animal ethics

The research does not involve either humans, animals, or tissues.

Declaration of competing interest

We have no competing interests, either financial or non-financial.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rser.2022.112399.

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