Biological Forum – An International Journal **15(3): 696-712(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Taxonomy Studies: An Overview on Isolation and characterization of Available Resources of Marine Microalgae for Bio-fuel Production

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(Received: 19 January 2023; Revised: 24 February 2023; Accepted: 27 February 2023; Published: 22 March 2023)
(Published by Research Trend)

ABSTRACT: Microalgae have been observed as a viable biomass for the manufacture of biofuels, but the scaled-up culture of these organisms has challenges regarding freshwater resource use. To overcome the restrictions of water resources, it is essential to obtain robust marine strains that can produce triacylglycerols and high-value-added metabolites.

The present study was conducted to identify microalgae from the Indian Sea and its environs which included toxified sites, tenable for biofuel production. 4 stations belong to the Nellore district (S-1 Mypadu, S-2 Kothakoduru, S-3 Nelatru, S4- Krishna Patnam) with this district is special for aquaculture industries like shrimp and fish culture also more hatcheries and processing, and Nellore beaches located in the east coast of Bay of Bengal of India. Sixty-four (64) species of microalgae were acquired in 4 terminals. On further isolation, 19 species with a process-oriented pressure choice were developed through artificial insemination cultures. This basic research provides an overview of the isolation and characterization of available resources of marine microalgae for biofuel production.

Keywords: Marine Microalgae, Isolation, Taxonomy, Diatom, Bio-fuel production.

INTRODUCTION

Microalgae offer a feasible biomass feedstock to manufacture ethanol as the demand for biofuels rises internationally (Culaba *et al.,* 2020). One of the different feed stocks for biofuel production, microalgae have obtained great interest over the last few years being obligated to pay to their possible higher efficiencies, high materials of triacylglycerols as well as adaptability for growing on the non-arable structure. Having pointed out that, some vital specific problems need to have to must become addressed right before algal biofuel ends up being a company reality (Illman *et al*., 2010). As instance, considerable centered obstacles continue to live in the source of unique algae stress, greater group functionality, in addition to reduced mowing price (Schenk *et al*., 2009). On top of that, commercialization of algal biofuels is based upon restrictions of water sources and also furthermore company economics. As there are distinguishing uses for readily available freshwater, whereas saltwater is bountiful, it is a lot more practical to make use of microalgae adjusted to saltwater for biofuel production. None of the hit-and-miss campaigns today has possessed the ability to show efficient production of biofuel stemming from microalgae. Because of that, tensions with essential co-products make use of a method for expense decline (Lee and Lee 2001). New methods such as hybrid indoor helical-tubular photo bioreactor was just recently proposed for growing and cultivating microalgae (Hashemi *et al.,* 2020). Emerging technologies such as hydrothermal liquification methods are also employed to develop the biofuel microalge (Devi and Parthiban 2020). Since lot of bioactive items have been covered as possessing the potential to be practical co-products, such as pigments, antioxidants, eicosapentaenoic acid, docosahexaenoic acid, as well as biofertilizers (Radmann and Costa 2008). One of them, astaxanthin is an effective, high-value, organic antioxidant. Astaxanthin production alongside microalgae has achieved substantial commercial excellence. On top of that, picking up microalgae is one of more primary problems in the significant production of microalgae-derived biofuels as a result of the size of their small cells along with also minimized focus in cultures. It was predicted that the fee for accumulating algal biomass represents 20%-30% of the comprehensive production expenditures. Therefore, a worry alongside the parts of having greater biomass performance, greater body system fat deposits product, helpful co-products, efficient- harvesting option, and benefiting from saltwater is highly pleasing to help make biofuel generated stemming from microalgae attainable.

An included approach for a lot more affordable algae way of life is boosting the production of the greatest products. In various microalgal broad assortments, the amounts of groups, proteins, carbohydrates as well as also pigments are an assortment. It was pointed out that the microalgal rate of metabolic process is affected by some environmental variables including temp, understanding, pH, salinity, and likewise most visibly, sustenance simplicity of access. Makeover of a particular product like group and even pigment can be completed through marketing these elements. For the goal of seeking advantageous algal strains, 56 marine microalgae coming from Bohai Basin, China, were segregated. Some all of them, HA-1 possessed much higher biomass productivity. Atop that, the cover of HA-1 ended up being orange red progressively throughout farming, which connected the astaxanthin selection stage of your organization's freshwater microalga Haemapodous Pluvial is. As a result, the reason for the investigation study was really to look at the attributes of HA-1 in regard to oblige i.e., astaxanthin in addition to additional crowd buildup, along with exploration. Microalgae are microscopically little bits of photosynthetically active eukaryotic microorganisms alongside enhancing importance in the medical place. Distinguished to better floras they possess lots of perks, featuring faster growth, the prospective to find to be improved on non-arable land, and the ability to collect efficient points like groups and even pigments.

There are truly between 200,000 as well as variety of microalgal kinds are available. Although microalgae uncover a big biotechnological ability due to their biodiversity besides comfort, your service is currently using simply around 15 types for sizable-scale production biofertilizers (Radmann and Costa 2008). Because of the enriching requirement for microalgal biomass, a process-oriented pressure choice is essential to make sizable-scale production money-saving. Process-oriented tension choices help in taking advantage of either indigenous tension coming from that production website or even perhaps stress-adapted panic, which as a result of fast development expenses might outcompete killers or even pot algae. Besides light existence along with nutrient availability, the temperature influences advancement productivity. Due to much higher in addition to similarly beneficial lighting, the summer season commonly leads to high biomass performances (Li *et al.,* 2008). The most effective achievable growth temperature degree for normal lab species of microalgae varies arising from a few of the assortment of kinds nevertheless is normally indicated to come to be in between twenty as well as 30°C.

Higher temperature quantity complications in environment-friendly homes, as well as even outdoor farming bodies throughout summer months opportunity, can adversely impact the development of a large amount of microalgal vast assortments. In green properties temperature, degrees could cheer 55°C, inducing the greatest culture warmth levels outperforming 35°C. For outdoor farming equivalent temperatures going beyond 35°C and also 40°C was divulged. Whereas temperatures listed below are the perfect cause a cared for biomass production, temperature amounts over the ideal cause a higher reduction in productivity along with likely the unsuccess of the lifestyle. The degeneration, in addition to the inactivation of chemicals related to the photosynthetic strategy generated through heat fear, creates the hang up of development and also even set up cell death. Innovation of temperature level moderated atmospheres for the development of microalgae will certainly be well, however, has been revealed to undoubtedly not be lasting because of higher first assets as well as likewise method prices. As a result, obtaining microalgae tensions together with the functionality to set up along with improvement in these intense heat power problems, throughout summertime period temperature quantities, resides in the fact of utmost worth. Microalgal features that agree with this sort of procedure consist of quick era times, higher crowd content, inexpensive nutrient resources, the ability to cultivate in numerous natural surroundings with different forms of wastewater, as well as higher photosynthetic capability reviewed to terrene electricity crop plan Microalgal growth and performance are highly determined by organic as well as abiotic ecological factors. Algal biomass performance is the net outcome of photosynthesis, which is highly influenced by both temp and lighting. Microalgae have the prospective to accumulate as high as 30%-- 70% of their mobile dry weight as fat under several ailments. One of the vital factors that may likely determine cellular crowd build-up is temperature level.

Microalgae are an especially varied group of microorganisms found in all communities. Several factors recommend that bioprospecting for aboriginal microalgae will be actually of wonderful advantage in creating local microalgal production for B-carotene (Hashemi *et al.,* 2020) biotechnological points, consisting of biofuel production. Furthermore, the use of locally sourced tensions will prevent restrictions that may be applied to imported stress, as well as will provide stress that is adjusted to local area ailments. This technique is especially likely for creating countries that possess considerable enthusiasm for renewable resource production for power security in addition to establishing neighborhood sources, whereas algal medical request in OECD (Organisation for Economic Co-operation and Progression) countries is in the palms of private enterprise. As an example, one recent job reviewed a few of the qualities of regional eco-friendly algae separated in Kerala, India with these points to consider in mind Algal biofuels' techno-economic analysis has been the focus of recent publications. A techno-economic analysis was conducted by Ahmad *et al.* (2020) on the commercialization of microalgae obliquus growth through an integrated fish and production of biofuels. Techno-economic analysis was used by Beckstrom *et al.* (2020) to assess the possibility of producing bioplastics and biofuels from microalgae. Rajesh Banu *et al.* (2020) used a techno-economic analysis to assess how well different biorefinery layouts produced algal biofuels. Algal biofuels must commercially compete with already available biofuels, which is one of their problems. For the better biofuel production identifying the better microalgae is essential, however the studies concerning their identification is challenging and the present study aimed to contribute in identifying unique microalgae to produce commercial biofuels.

ALGAE TOWARDS BIODIESEL FEEDSTOCK

Microalgae are prokaryotic and even eukaryotic photosynthetic bacteria that may grow rapidly along with stay in too many alkaline worries because of their unicellular or easy multicellular design. Circumstances of prokaryotic microalgae are really Cyanobacteria (Cyanophyceae) along with furthermore eukaryotic microalgae are eco-friendly algae (Chlorophyta) along with diatoms (Bacillariophyta). They are not just water having pointed out that additionally earthlike, meaning a sizable wide array of styles residing in a significant series of environmental conditions. It is truly anticipated that above 50,000 kinds exist, yet just a limited quantity, of around 30,000, has been researched as well as examined. Previous checks explained that do not compete with eatable veggie oils, the efficient and efficient biodiesel ought to be made arising from cheap feed items like non-eatable oils, taking advantage of frying oils, animal excess physical body fats, cleansing soap supplies as well as likewise better. However, the supplied volumes of terrible oils as well as additionally animal excess fats are insufficient to match the requirements for biodiesel. Subsequently change to 2nd creation biofuels, including microalgae, might similarly aid a reduction in land requirements because of their presumed a lot of godship yields every hectare aside from their non-need of cultivating the land. On top of that, biodiesel needs to have minimal environmentally friendly effects as well as also see to it the same amount of efficiency as existing gasoline. It resides still on its start even with it remains in simple fact the improving interest rate. Significant belonging in r & d along with appropriate preparing's and additional strategies are still called for to have, for all durations of the biofuels market price establishment, arising from basic materials production to distribution and furthermore utmost utilization. Among the numerous options, presently being investigated aside from carrying out at the flyer range or at the industrial range concerns prospective feed sales, together with the capacity of the microalgae.

Because of the truth that microalgae farming resides in simple fact not straight linked to personal application, they need to have a decreased region required for its quite own production. Illman *et al.* (2010) have illustrated that the microalgae recreate taking advantage of photosynthesis to change solar energy right into chemical elements and electrical power, accomplishing a whole growth style every handful of times.

Microalgae may give feedstock for numerous sorts of renewable resources featuring biodiesel, marsh gas, hydrogen, ethanol, etc. Alga biodiesel consists of no sulfur as well as in addition achieves as well as furthermore petroleum diesel-powered, while minimizing discharges of fragment fear, Carbon monoxide gas, hydrocarbons, along with furthermore Sox. (Illman *et al.*, 2010) explained that for existing 50 years, substantial assessment invites simple fact has been definitely executed on microalgae and additionally simply exactly how they might be utilized in a vast array of treatments to produce numerously favorable along with financially important points. The first significant range lifestyle of microalgae began in the early 1960s in Asia through Nihon Chlorella along with the lifestyle of Chlorella. Interest was made use of microalgae for renewable energy built up in the 1970s throughout the initial oil problem (Fig. 1).

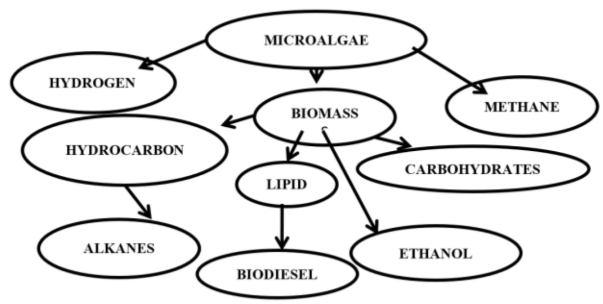


Fig. 1.

Microalgae Growth Requirement. The biochemical makeup of microalgae could be influenced via such variables as development expense, and ecological difficulties, in addition to the life cycle. Microalgal progression and also, in addition, chemical drug make-up is largely managed via light, temperature, rapidly readily available carbon dioxide, pH, as well as nutrients (Several various other variables, consisting of salinity, might be life-and-death to some styles. Development medium needs to make use of enough nutrients for micro-algal growth (Lee and Lee 2001) positioned that the excellent quality, as well as additionally quantity of crowds within the tissues, may conveniently contrast because of augmentations in advancement ailments (temperature and similarly light durability) or even possibly health and wellness as well as nourishment media qualities (the focus of nitrogen, phosphates and also iron). (Schenk *et al*., 2009) indicated that while large amounts of microalgae tension and also stress and anxiety normally have greater team details (20-70% on dry out physique weight procedure), it is, in fact, manageable to enrich the interest with increasing the advancement determining facet like the administration of nitrogen degree light size, temperature as well as likewise salinity, CO_2 focus and also furthermore compiling technique had evaluated that definitely not merely 100% all- natural carbon dioxide and also substrate (a CO_2 source like sugary foods items, well thought-out proteins alongside excess body fats), vitamins, sodium's and likewise a range of various other nutrients (Nitrogen and likewise Phosphorous) are crucial for algal advancement, nevertheless additionally balance in between working criteria (Oxygen, carbon dioxide, pH, temperature, light intensity, item as well as likewise through product removal). CO_2 is similarly related to the demand of pH as well as CO_2 equilibrium. Some algae may grow in extreme wellness as well as well-being ailments of pH. Investigates helped make understood the outcome of redesigns in pH impacts on team metabolic process in non-extremophiles. Alkaline pH worries as well as stress and also worry as well as stress developed TAG accumulation as well as a reduction in membrane level body fat in chlorella species.

Light. Making use of monocultures is needed in countless microalgal uses as well as also evaluated farming tools. This necessity has selected the innovation of protected photograph bioreactors. Dependable use of light is amongst the significant difficulties in microalgal health care, particularly when remodeling in the biomass turnout is meant. It has been accepted that eco-friendly aspects like temperature, light, and nutrients, determine the fat cosmetics of algae. Some pros took advantage of steady light for culturing *Botryococcus braunii*, and *Chlorella* sp. Numerous other experts administered black alongside light photoperiod for improving *Chlorella* sp. To offer black along with light photoperiod in the system, air airlift bioreactor stirred compartment photograph bioreactor and in addition, various kinds and photobioreactors were taken advantage of purity.

pH. The pH of the social tool is merely a number of the notable look at algal raising. the pH of the tool for algae growth is frequently neutral or a little bit acidic, primarily to steer clear of rainfalls of many notable components. The algae present a crystal-clear dependence on the pH of the growth tool and likewise, a variety of kinds vary greatly in their action to pH. The pH of algal societies may be figured out via a selection of variables such as cosmetics along with the buffering potential of the system, the volume of carbon dioxide diffused temperature (which manages Carbon Dioxide solubility) together with the metabolic job of the algal tissues. pH improves throughout the time to market values approximately 10, mostly because of the shortage of the anions NO - in addition to of Carbon monoxide gas developed in the tool, in addition, to also the discharging of OH- ions. Among the favored results observed in social media possessing pH absolutely worth over 9, remains, in fact, the storm of a lot of calcium mineral salts, i.e. carbonates, phosphates, and sulfates, leading to nutrient deficiencies and development reprieves or possibly algal flocculation, due to the precipitating minerals. The absolute best pH for many cultured algal designs ranges from 7 to 9, along with the excellent pH being 8.2-8.7. A complete lifestyle system crash due to the interruption of a variety of cellular phone strategies might quickly arise arising from malfunction to always keep a necessary pH. When its comes to higher immensity algal culture, the add-on of CO_2 permits correction for improved pH, which might reach limiting market values of pH during algal growth.

Temperature. The perfect temperature to boost *Scenedesmus* sp. is definitely in between 20-40°C (Lee and Lee 2001) analyzed *Scenedesmus* sp. at temperature volumes of 15 to 36°C as well as located at minimized temps, the chlorophyll and additionally well-balanced protein volumes were decreased, while amounts of carotenoids, saccharides, as well as fat deposits were boosted. They in addition viewed a growth of 30% of the glucose and likewise excess fats at excessive temperature levels (36°C).

MATERIALS AND METHODS

Description of the study stations. The study area is the SPSR Nellore coast of Andhra Pradesh stations, Southeast coastal region Investigation period, In July (Dry season), November (Rainy season) and March (summer season) Examining was done 3 possibilities at the seasons. The research internet site was accessed by a fiberglass boat using the fishermen to help us to collect at a different level. Checking out was carried out in 3s; one collection was kept with 5% Lugol's iodine for measurable range studies. The 2nd set was set apart as well as cultured, while the third assortment was nourished at 28°C for fourteen opportunities. Temperature, pH, and generally postpone solids and salinity dimensions for all the evaluated stations were videotaped. The physicochemical parameter is major key points Temperature, Salinity, pH was measured directly at the sampling stations. Send to samples immediately in the lab, culturing of species and finally identified under the microscope (Krammer and Lange-Bertalot 1986).

Fig. 2. Sampling site in a map.

Table 1: Site of sampling latitude and longitude with season.

Sampling Location	**Sampling period**	**Latitude (South)**	**Longitude (East)**	**Seasons**	**Climate indication 2021-2022**
S- 1 Mypadu	March 1 – May 31-2021	14.5053977	80.1806739	Spring	Hottest month-may (33 degree average)
S-2 Kothakoduru	June 1- August 31-2021	14.4229345	80.1745930	Summer	coldest month-January (25)
S-3 Nelaturu	Sept. 1-Nov 30-2021	14.3233103	80.1637542	Antumn (Fall)	Wettest month-October (241.4 mm avg)
S-4 Krishna patnam	Dec. 1-Feb. 28-2022	14.2462533	80.1601382	Winter	Windest month-May (8 km/h avg)
4 Stations	**12 months**	-	-	-	**Annual precipitation (1116.4 mm) per year.**

A sampling of micro-algal strain. Water examples were accumulated in three at the testing terminals for study. Surface area water examples were collected using a vessel. 40 liters of water was looked at 20µm mesh-size plankton net for interest to 50ml. The resultant focused plankton was sent to instance containers tagged along with date as well as the additionally tasting station, and additionally preserved in 5% Lugol's solution. Qualitative examples were kept in a cooler container after assortment and carried to the laboratory for additional evaluation.

Water Quality Parameter. Surface area water high-quality criteria were determined sitting at each terminal; pH was assessed using an electronic pH penetrating, the temperature was assessed using the YSI Version 550A, salinity was analyzed using a hand-held refractometer.

Table 2: Standard water parameters.

Parameters	**Salinity**	**Temperature**	**Light intensity**	**pH**	**Photo period**
Std Range	12-40 ppt	16-27°C	1,000-10,000 Lux	7-9std	16:8Min
Optimum range	20-24 ppt	18-24°C	2,500-5000 Lux	8.2-8.5	24 Max

Quantitative sample analysis. Hectic, 1ml aliquots of instances sustained in Lugol's iodine company were positioned on slides and additionally monitored under an inverted microscopic lens along with the matters of all viewed phytoplankton caught. The algal styles were realized utilizing the i.d. guidebook of marine microalgae.

Isolation and Purification. The algal examples for qualitative study experienced filtering with serial dip, add-on of prescription antibiotics, and also enhancement of decoration media complied with using culturing all of them in FAUCET, f/2 and additionally Walne media for microalgae along with nurtured at 25 ± 1° C under 1.2 + 0.2 k lux magnitude together with 16:8 humans resources light photoperiod. The purity of the culture was ensured by reduced below culturing in clean media and also frequent monitoring under the upside-down minuscule lense.

Although the marine centric diatoms, Chaetoceros Bacillariophyceae (Hernández-Becerril *et al.,* 2009) Thalassiosira Coscinodiscophyceae (Grunow) (Fryxell and Hasle 1977) Skeletonema Bacillariophyceae (Greville, 1865; Cleve, 1873) Green algae of Tetraselmis Prasinophyceae (Zhang and Hu 2002); (Stein, 1878), brown golden marine microalgae Isocrysis (Parke, 1949) fresh and sea water that related to diatoms and brown algae Nannochloropsis (Sukenik,1999 and were followed f/2 media by serial dilutions method from Nellore coast, east coast of India. We stored stock culture in Guillard f/2 media and certain methods follow and set out in Andersen, 2005) and Conway Medium (Tompkins *et al.,* 1995).

Table 3: Standard nutrient media.

Microalgae genera	Taxonomic class	Cell morphology	Minimum cell size	Maximum cell size
Tetraselmis sp.	Prasinophyceae (Zhang and Hu 2002)	Cells round, Ovoid, Elliptical, flattend, compressed on these shapes.	3.5 µm	25 µm
Isochrysis sp	Haptophyceae (Lacour *et al.,* 2012)	Ellipsoid shape	5 µm	6 µm
Nanochloropsis sp	Eustigmatopyceae (Hibbered 1981; Droop 1955)	Spherical and Ovoid	2 µm	4 µm
Chaetoceros sp.	Bacillariophyceae (Hernández-Becerril, 2009)	Cells are lack a raphe system &lack significant motility (strutted processes)	<10 um	50 um
Thalssiosira sp	Coscinodiscophyceae (Fryxell and Hasle 1977)	Cell shapes cylindrical,box shaped, drum shaped, discoid, coin shaped.	4 µm	32 µm
Skeletonema sp	Bacillariophyceae (Greville 1865; Cleve 1873)	Cylindrical shaped and silica frustule	2 µm	21 µm

Sr. No.	Nutrients (Salts)	f/2 Medium (Guillard *et al.,* 1975)	Conway-Medium (Tompkins *et al.,* 1995) the modified Conway Medium (Walne, 1979)
1.	Nitrate	$NaNO_3$ (75g m^{-3})	KNO_3 (100g m^{-3})
2.	Phosphate	NaH_2PO_4 (5g m^{-3})	Na3PO$_4$ (20g m^{-3})
3.	Trace metals	Na_2H_2EDTA (4.36g m^{-3}) $FeCl_3.6H_2O$ (3.15g m^{-3}) $MnCl_2.4H_2O$ (180mg m^{-3}) $ZnSO_4$ (22.0mg m^{-3}) $CoCl_2.6H_2O$ (10.0mg m^{-3}) $CuSO_4.5H_2O$ (9.8mg m^{-3}) $Na_2MoO_4.2H_2O$ (6.3mg m^{-3}) Na_2SiO_3 (30g m^{-3})	1) $Na_2H_2EDTA.2H_2O$ (45g m^{-3}) $FeCl_3.6H_2O$ (1.3g m^{-3}) $MnCl_2.4H_2O$ (0.36g m^{-3}) $ZnCl_2$ (4.2g m^{-3}) $CoCl_2.6H_2O$ (4.0g m^{-3}) $CuSO_4.5H_2O$ (4.0g m^{-3}) $(NH_4)6Mo7O_{24}.4H_2O$ (1.8g m^{-3}) H_3BO_3(33.4g m^{-3}) 2) (mg.L^{-1}): Na_2EDTA, 45; $NaNO_3$, 100; H_3BO_3, 33.6; Na_2HPO_4, 20; $MnCl_2.4H_2O$, 0.36; $FeCl_3.6H_2O$, 1.3, 1mL L^{-1} of trace metal solution ($ZnCl_2$, 2,1 g.L^{-1}; $CoCl_2.6H_2O$, 2 g.L^{-1}; $(NH_4)6Mo7O_{24}.4H_2O$, 0,9 g.L^{-1}; $CuSO_4.5H_2O$, 2 g.L^{-1}) and vitamin solution 1 mL.L^{-1} (Thy-amina, 100 mg.L^{-1}; Cyano-cobalamina, 2 mg.L^{-1}; Biotina, 1 mg.L^{-1}). Growth media with Conway nutrients were prepared using either Fine-seawater (80% seawater, 20% millicue or dist-water)
4.	Vitamins	Thy-amine HCl (200mg m^{-3}) Cyano-cobalamin (10mg m^{-3}) Biotin (100mg m^{-3})	Thy-amine HCl (200mg m^{-3}) Cyano-cobalamin (10mg m^{-3})

Identification of Microalgal Strains. The washed monoalgal instances were noted under the upside downed microscopic lens and also the grammatical homes of the isolates were determined based on the guide. In additionally we have used reflection base microscope and stereo compound trinacular microscope Observed most common photosynthetic Algae species. A compound microscope as well as biological microscope, this microscope is used to research work Marine microalgae studies. Samples viewed under a compound microscope wit microscope slides using cover slip to flatten samples. View a of variety of samples such as algae, bacteria, parasites, cheek cells, tissue etc, the level of magnification in compound microscope is commonly 40X, 100X, 160X, 400X and 1000X.

Culture Maintenance. Uni-algal cultures of the microalgae stress remained and maintained in the F/2 along with Walne culture media. A decrease of the matching way of life was shielded aseptically making use of a germ-free micropipette right and suitable for fifty ml of the medium in sterilized fifty ml cone like flask. The cultures were nurtured in an algal growth area with consistent enlightenment at 110 µmol.m^{-2}/ s at 25°C. This method was carried out on regular a manner.

Microalgal Isolation and Purification. Cultures. This was carried out via isolation of the microalgae and also culturing all of them on audio and likewise the 3 dissolved media specifically; FAUCET, Walne as well as F/2.

Measurement of Growth rate. The growth expense of algae was determined via optical thickness at 660nm, 680nm, 760nm along with 780nm for 4weeks. Daily sizes were also looked at 7 opportunities. This is based on the introduction of growth substrate in this study. This Micro algal cells were divided into two groups followed by the addition of f/2 Medium (Guillard, 1975); Conway Medium (Tompkins *et al.,* 1995) as the nutrient supplement. This cultivation as well performed for a period of 7 days in order to reach a growth plateau, and the biomass then collected by centrifugation at 550 rad s^{-1} (Li and Deng 2012). Then, the cultures were maintained with the respective growth substrate in one liter sterile Erlenmeyer flasks for 30 days under illumination in an air-conditioned room at a temperature of 25 ± 2 °C before being subjected to the desired experimental condition.

Extraction of EPSs and Unialgal culture. The axenic lifestyle of D. salina, separated emerging coming from a risky salt ranch was expanded in five hundred ml of De Walne's way of life media having a vast selection of salt emphasis; 0.5, 1.0, 2.0, 3.0, 4.0 and also furthermore 5.0 M under determined lab problems at 25 ± 2 ° C under 12:12 h (light/dark pattern) in addition to white tinted fluorescent light of 38 photons m^{-2} s^{-1} light durability. Twenty possibilities enriched Dunaliella lifestyle was centrifuged at 15,000 g for twenty moments at 4 °C to deal with cells and also various other precipitates (clutter). The supernatant was filtered (Whatman, UK) 2 options and additionally centred to one-fourth volume on a magnetic stirrer at 60 °C for 10-12 h (Parikh and Madamwar 2006). The exopolymer was pre- speed up using notably consisting of the identical volume of chilly methanol to centered supernatant together with additionally continual 4 ° C for 12 h. After extraction of proceeding methanol, the precipitate was cleaned in addition to comprehensive ethanol in addition to likewise redissolved in Milli-Q water (Millipore, U.S.A.). Diffused EPSs were dialysed versus faucet water for 2 opportunities, noticed using distilled water for 1 chance to perform away with ions in addition to sodium. Dialysed EPSs were icy up at twenty ° C alongside lyophilized at 70 °C f. C for 10-12 h.

Emulsifying activity. Lyophilized EPSs (1 milligram), liquified in 0 5 ml deionized water, was warmed at one hundred ° C for around 15-20 min along with being made achievable to cool to the location temperature (25 ± 2 ° C). The amount was made up to 2 ml making use of phosphate-buffered saline (PBS). The case was vortexed for 1 second after the add-on of 1 ml hexadecane. The absorbance at 540nm read immediately before and also furthermore after vortexing (A0). The sign up with absorbance was snatched after maternity at region temperature for half an hour (At). The investment was cared for together with 2 ml PBS in addition to 1 ml hexadecane. The emulsification work was shared as the per-cent retentiveness of answer throughout reproduction for chance t: At/A0 one hundred.

Fourier-transformed infrared spectroscopic. The crucial quality workers of purified EPSs were found out making use of Fourier transformed infrared (FT-IR) spectroscopy. Pellets for infrared evaluation were safeguarded along with grinding a mix of 2 milligrams EPSs with 200 milligrams dries out KBr, stuck to along with pushing the mix right into a 16-mm-diameter mould. The FT-IR selections were wrapped around 4000-- 400 centimetres- 1 on a Perkin-- Elmer vast collection GX TOOTSIES- IR device (Perkin-- Elmer, UNITED CONDITIONS).

Table 4: Marine Diatoms Systematic analysis.

Species name	Length	Width	Fibulae	Striae	Reference
Nitzsczia Sigma	30 – 200 µm	4– 13 µm	7-12 µm	9 – 24 µm	(Smith, 1853)
Climacos elongata	700 – 780 µm	26 – 28 µm	9 µm	21 – 25 in 10 µm	(Ehrenberg, 1844)
Pluerosigma directum	238 – 518 µm	39-56um	10um	28-29um	(Cleve and Grunow 1880; Subrahmanyan, 1946)
Chaetoceros compressus	5– 40 µm	7-24um	17-21um	9-10um	(Pavillard, 1916; Hustedt 1927, 1930)
Asterionella japonica	60-85um	2-4um	Not available	Not available	(Hassall, 1850)
Arachnoidiscus	200-265u	34-46um	Not available	Not available	(Brown, 1933)
Tabellaria	103um	2.1-6.9um	Araphid	14-19um	(Koppen, 1975)
Hemidiscus	125– 400 µm	140– 200 µm	Not available	Not available	(Wallich, 1860)
Coscinodiscus	100-300um	-	Cylindrical discoid	-	(Ehrenberg, 1844)
Rhizosolenia curvata	412-668um	6-31um	10um	12-16um	(Brighwell, 1858)
Skeletonema costatum	70-200um	30-50um	10um	12-15um	(Greville, 1865)
Ditylum Brightwelli	105-152um	-	Not available	-	(Van Heurck, 1885)

RESULT AND DISCUSSION

In this work investigate and data was carried with next step to study molecular techniques DNA isolation and identification of species and present taxonomic studies along general morphology of microalgae along bio-fuel production. This study is combined with Nellore coastal region 5 study station of Andhra Pradesh. We are collecting the raw sea water sample through plank net. In this paper subjugated microscopic observation and identification, analysed for the prevalence of microalgae (Diatoms). The field conducted during March 1st 2021 to February 28th 2022. However we reported 64 isolates of 12 diatom genera was recorded during this study (Plate 3 and 4). We colorized different morphology new species (Plate 1 and 2) 136 isolates of 21 species and repeated microalgae species 5.

Microscopy identification. The samples were collect and analyses for the popularity of diatom. The total Number of isolates 200 and 33 genera representing belonging to 21 families have recorded (table: 5) during March 2021 to February 2022. Here 33 genera centrales and pennales which introduce *Coscinodiscus* sp. in march to may 2 isolates, june to august 3 isolates, September to November 2 isolates and December to February high peak 6 isolates followed by *Gamphonema* spp. march to may 3 isolates, June to august 2 isolates, September to November 4 isolates and December to February low peak 1 isolates Continued given the Table 7. Status does not show statistically analytical method why because its significantly different. Almost the diatom found in the study area of east cost off on June to August is significantly different. During December 2021, to February 2022 only 12 isolates of 2 diatom genera that two orders belongs to two families were recorded. This study only the presence of 24 families of diatom which includes name of genus, species, belongs order and phylum see here in the Table 6.

Table 5: List of Diatom taxa found in Nellore coast of Andhra Pradesh (Plate 3 & 4).

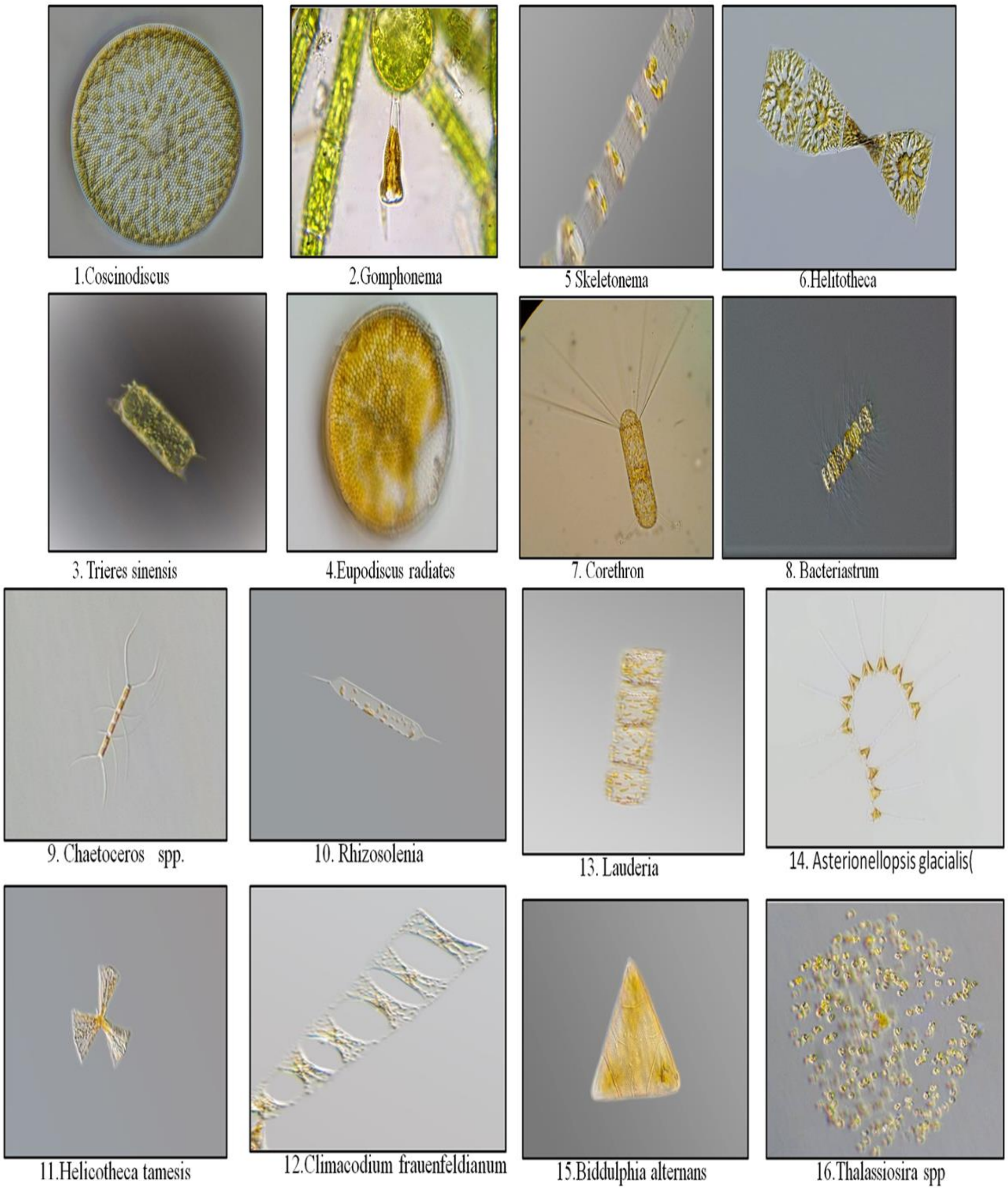
Sr. No.	DIATOM	March 1 – May 31-2021	June 1- August 31-2021	Sept 1-Nov 30-2021	Dec 1-2021 To Feb 28-2022
1.	**Genus:** ***Coscinodiscus* sp.** **Phylum:** Bacillariophyta **Sub phylum:** Heterokonta **Order:** Coscinodiscales **Family:** Coscinodiscaceae **Subfamily:** Coscinodiscaceae **(Ehernberg, 1839)**	2	3	2	6
2.	**Genus:** ***Gamphonema*** **Phylum:** Gyrista **Sub phylum:** Ochrophytina **Order:** Cymbellales **Family:** Gamphonemataceae **(Ehernberg, 1832)**	3	2	4	1
3.	**Genus:** ***Eupodiscus*** **Species:** Eupodiscusradiatus **Phylum:** Ochrophyta **Sub phylum:** Khakista **Order:** Triceratiales **Family:** Triceratiaceae **Class:** Bacillariophyceae **(Bailey, 1851)**	2	2	1	3
4.	**Genus:** ***Odontella*** **Species:** Triereschinensis **Phylum:** Bacillariophyta **Order:** Biddulphiales **Family:** Eupoodiscaeae **Class:** Bacillariophyceae **(Graville, 1866)**	1	1	2	1
5.	**Genus:** ***Skeletonema*** **Species:** *Skeletonema coscatum* **Phylum**: Gyrista **Subphylum**: Ochrophytina **Order**: Talassiosirales Family: Skeletonemataceae Class: Bacillariophyceae **(Graville, 1865)**	4	4	2	6
6.	**Genus:** ***Helicotheca*** **Species:** *Helicotheca* **Phylum:** Ochrophya **Subphylum:** Khakista **Order:** Lithodesmiales **Family:** Lithodesmiaceae **Class:** Bacillariophyceae **(Ricard, 1987)**	1	1	3	2
7.	**Genus:** ***Corethron*** **Species:** *Corethron* sp. **Phylum:** Bacillariophyta **Subphylum:** Bacillariophytina **Order:** Corethrales **Family:** Corethraaceae **Class:** Coscinodiscophyceae **(Catracane, 1886)**	1	2	1	3
8.	**Genus:** ***Bacteriastrum*** **Species:** *B. delicatulam*	3	1	1	1

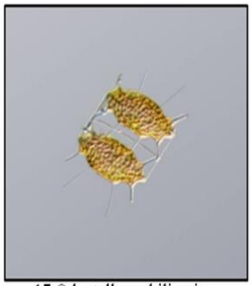
	Phylum: Gyrista **Subphylum:** Ochrophytina **Order:** Incertaesedis **Family:** Cheatocerotaceae **Class:** Bacillariophyceae **(Shadbold, 1854)**				
9.	**Genus:** ***Chaetoceros*** **Species:** *C. furcellatus* **Phylum:** Gyrista **Subphylum:** Ochrophytina **Order:** Incertaesedis **Family:** Cheatocerotaceae **Class:** Bacillariophyceae **(Ehrenberg, 1844)**	6	2	5	1
10.	**Genus:** ***Rhizosolenia*** **Species: -** **Phylum:** Gyrista **Subphylum:** Ochrophytina **Order:** Rhizosoleniales **Family:** Rhizosoleniaceae **Class:** Bacillariophyceae **(Brightwell, 1858)**	1	1	1	1
11.	**Genus:** ***Helicotheca*** **Species:** *Helicotheca tamesis* **Phylum:** Ochrophya **Subphylum:** Khakista **Order:** Lithodesmiales **Family:** Lithodesmiaceae **Class:** Bacillariophyceae **(Ricard, 1987)**	1	1	1	1
12.	**Genus:** ***Climacodium*** **Specie:** *C. frauenfeldianum* **Phylum:** Ochrophya **Subphylum:** Khakista **Order:** Hemiaulales **Family:** Hemiaulaleceae **Class:** Bacillariophyceae **Subclass:** Coscinodiscophyacea **(Grunow, 1867)**	1	1	1	1
13.	**Genus:** ***Lauderia*** **Species:** *Lauderia* **Phylum:** Ochrophya **Subphylum:** Khakista **Order:** Thalssiosirales **Family:** Lauderiaceae **Class:** Bacillariophyceae **Subclass:** Coscinodiscophyacea **(Cleve, 1873)**	1	1	1	1
14.	**Genus:** ***Asterionellopsis*** **Species:** *Asterionellopsis glacialis* **Phylum:** Ochrophya **Subphylum:** Khakista **Order:** Fragilariales **Family:** Fragilariaceae **Class:** Bacillariophyceae **Subclass:** Fragilariophycidae **(Round *et al.*, 1990, Crawford and Mann 1990)**	1	1	1	1
15.	**Genus:** ***Biddulphia*** **Species:** *Biddulphia alternans* **Phylum:** Ochrophya **Subphylum:** Khakista **Order:** Bidduphiales **Family:** Bidduphiaceae **Class:** Bacillariophyceae **Subclass:** Coscinodiscophyacea **(Bailey, 1851)**	1	1	1	1
16.	**Genus:** ***Thalassiosira*** **Species:** *Thallssiosira weigfloggi* **Phylum:** Bacillariophyta **Subphylum:** Bacillariophytina **Order:** Thalassiosirales **Family:** Thalassiosiraceae **Class:** Mediophyceae **Subclass:** Thalassiosirophycidae (**Cleve, 1873)**	2	1	1	1
17.	**Genus:** ***Odontella*** **Species:** *Odontella mobiliensis* **Phylum:** Ochrophyta **Subphylum:** Khakista **Order:** Triceratiales **Family:** Triceratiaceae **Class:** Bacillariophyceae	1	1	1	-

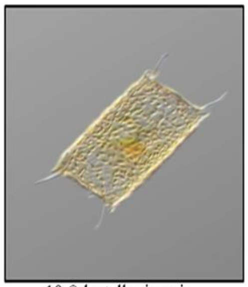
	Subclass: Coscinodiscophycidae **(Grunow, 1884)**				
18.	**Genus:** ***Planktoniella*** **Species:** Planktoniella sol **Phylum:** Ochrophyta **Subphylum:** Khakista **Order:** Thalassiosirales **Family:** Thalassiosiraceae **Class:** Bacillariophyceae **Subclass:** Coscinodiscophycidae **(Wallich 1860; Schutt, 1892)**	2	1	1	1
19.	**Genus:** ***Stephanopyix*** **Species:** *Stephanopyrix palmariana* **Phylum:** Ochrophyta **Subphylum:** Khakista **Order:** Melosirales **Family:** Stephanopyixdaceae **Class:** Bacillariophyceae **Subclass:** Coscinodiscophycidae **(Ehrenberg 1845; Grunow, 1884)**	1	1	1	1
20.	**Genus:** ***Pleurosigma*** **Species:** *Pleurosigma directum* **Phylum:** Ochrophyta **Subphylum:** Khakista **Order:** Naviculales **Family:** Pluerosigmataceae **Class:** Bacillariophyceae **Subclass:** Bacillariophycidae **(Smith, 1853)**	2	1	1	1
21.	**Genus:** ***Melosira*** **Species:** *Melosira* **Phylum:** Gyrista **Subphylum:** Ochrophytina **Order:** Melosirales **Family:** Melosiraceae **Class:** Bacillariophyceae **Subclass:** Cascinodiscophycidae **(Agardh, 1824)**	1	1	1	1
List of Diatom taxa found in Nellore coast of Andhra Pradesh (Plate 1 &2)					
1.	**Genus:** ***Nitzschia*** **Species:** *Nitzschia sigma* **Phylum:** Gyrista **Subphylum:** Ochrophytina **Order:** Bacilariales **Family:** Bacillariaceae **Class:** Bacillariophyceae **Subclass:** Bacillariophycidae	1	1	-	-
2.	**Genus:** ***Climatophenia*** **Species:** *Climatophenia elongeta* **Phylum:** Bacillariophyta **Subphylum:** Bacillariophytina **Order:** Ardissoniales **Family:** Ardissoneaceae **Class:** Mediophyceae **Subclass:** Biddulpiophycidae	1	2	-	1
3.	**Genus:** ***Pleurosigma*** **Species:** *Pleurosigma directum* **Phylum:** Ochrophyta **Subphylum:** Khakista **Order:** Naviculales **Family:** Pluerosigmataceae **Class:** Bacillariophyceae **Subclass:** Bacillariophycidae **(Smith, 1853)**	1	-	-	-
4.	**Genus:** ***Chaetoceros*** **Species:** *C. compressus* Phylum: Gyrista **Subphylum:** Ochrophytina Order: Incertaesedis **Family:** Cheatocerotaceae **Class:** Bacillariophyceae **Subclass:** Chaetocerophyceadae **(Ehrenberg, 1844)**	4	1	2	1
5.	**Genus:** ***Asterionella*** **Species**: *Asterionella japonica* **Phylum:** Ochrophyta **Subphylum:** Khakista **Order:** Fragilariales **Family:** Fragilariaceae **Class:** Bacillariophyceae	1	1	1	1

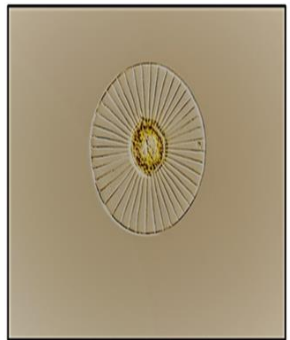
	Subclass: Fragilariophyceadae **(Clave and Möller 1882)**				
6.	**Genus:** ***Arachnoidiscus*** **Species**: *Arachnoidiscus major* **Phylum:** Ochrophyta **Subphylum:** Khakista **Order:** Archnoidiscales **Family:** Archnoidiscaceae **Class:** Bacillariophyceae **Subclass:** Coscinodiscophycidae **(Brown, 1933)**	1	1	1	1
7.	**Genus:** ***Tabellaria*** **Species**: - Tabellaria **Phylum:** Ochrophyta **Subphylum:** Khakista **Order:** Tabellariales **Family:** Tabellariaceae **Class:** Bacillariophyceae **Subclass:** Fragilariophyceadae (**Ehrenberg 1844; Kützing 1844)**	1	1	1	1
8.	**Genus:** ***Hemidiscus*** **Species**: - Hemidiscus **Phylum:** Ochrophyta **Subphylum:** Khakista **Order:** Coscinodiscales **Family:** Hemisdiscaceae **Class:** Bacillariophyceae **Subclass:** Coscinodiscophycidae (**Wallich, 1860)**	1	1	1	1
9.	**Genus:** ***Coscinodiscus*** **Species:** *Cosinodiscus centrails* **Phylum:** Bacillariophyta **Sub phylum:** Heterokonta **Order:** Coscinodiscales **Family:** Coscinodiscaceae **Subfamily:** Coscinodiscaceae **Class:** Bacillariophyceae **Subclass:** Coscinodiscophycidae **(Ehernberg, 1844; Hasle and Lange, 1992)**	1	1	1	1
10.	**Genus:** ***Rhizosoeina*** **Species:** *Rhizosoeina curvata* **Phylum:** Ochrophyta **Subphylum:** Khakista **Order:** Rhizosoleniales **Family:** Rhizosoleniaceae **Class:** Bacillariophyceae **Subclass:** Coscinodiscophycida **(Brightwell, 1858)**	1	1	1	1
11.	**Genus:** ***Skeletonema*** **Species:** ***Skeletonema* spp.** **Phylum:** Gyrista **Subphylum:** Ochrophytina **Order:** Talassiosirales **Family:** Skeletonemataceae **Class:** Bacillariophyceae **Subclass:** Coscinodiscophycidae **(Graville, 1865)**	4	2	16	1
12.	**Genus:** ***Ditylum*** **Species:** *Ditylum brightwellii* **Phylum:** Ochrophyta **Subphylum:** Khakista **Order:** Lithodesmiales **Family:** Lithodesmiaceae **Class:** Bacillariophyceae **Subclass:** Coscinodiscophycidae **(Van Heurck, 1880)**	1	1	1	1

The generation of micro-algae studies is a Major source of controlling greenhouse gases and global warming. In the Algae-based oil production process, a high yield of lipids (record showing the biomass yield high production of lipid content) is the primary goal, production of costs is expensive and support economically. High lipids content improves the high efficiency of biomass process oil extracting. Several productivity stands based on climatic conditions (solar radiation and alternate temperature) only suitable for culture management, also selected organisms to need necessary requirements. Outdoor culture must need high thick silica cell wall made of cell structure micro-algal strain produced high lipid content, along with essential lipid high productivity complete to respond to considerable accretion nutrient deficiency, fit and sufficiently to withstand mingle cause tangential tension, with pliable then extremely through modify in the inaccessible substitute and water parameters in outdoor natural habitat. Own manner the native isolated strains are credible and useful, also not much focus lead and developed much strength and the lifestyle of algae, therefore, local algae cells have struggled at contact previously and high range environmental conditions in outdoor mass culture. These studies were the isolation of 21 microalgae done experiments in the laboratory production of oil potential. While awaited, extraction of the lipids-rich source of strains appears in generic lower biomass productivity. We did not expect what we have known for some time; High lipid content production high yield oil in the preference of quality biodiesel production from microalgae, move collectively complete version (Sheehan *et al.*, 1998). Although, certain encouraging aspirants, both fresh and marine water, remained to initiate and look over more. In biotechnology and industrial find the not at all algal oils/ biodiesels only gained satisfactory and cooperative along with engine preowned present. Biodiesel needs completely outstanding (e.g., Bureau of Indian Standard (BIS- IS 15607:2005). Which important rule of limitation is the extent of the un-saturation value (iodine limits) here over all the content of fatty acid additionally making four double bonds (Chisti, 2007).



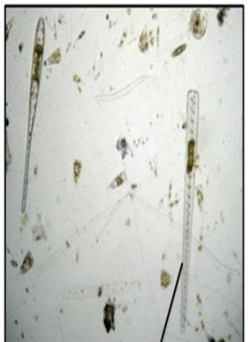

17.Odontella mobiliensis


18.Odontella sinensis


21.Planktoniella sol


22.Stephanopyix spp


19.Tripos spp.

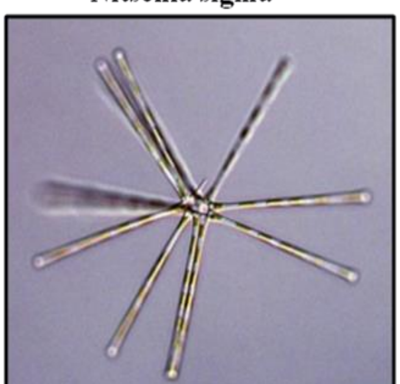

20. Climacosphenia elongeta

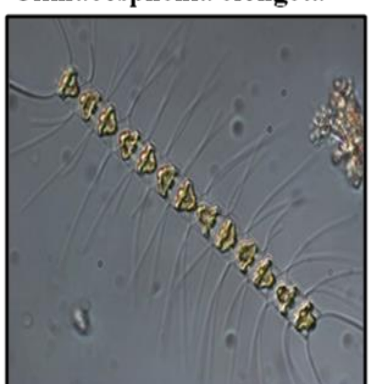

23.Pleurosigma

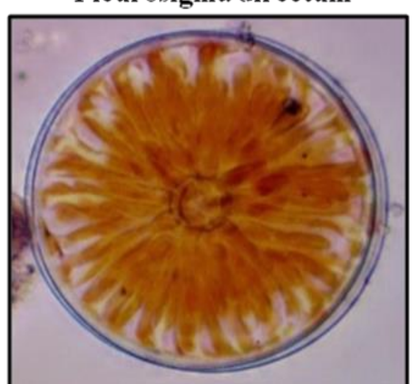

Nitschia sigma

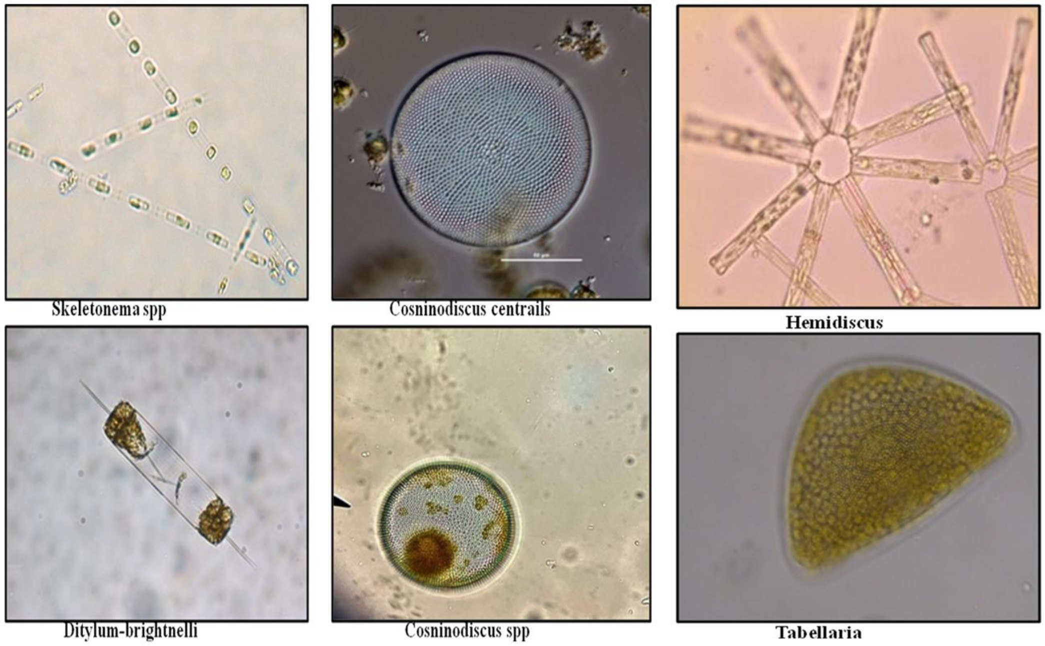

Climacosphenia elongeta


Pleurosigma directum


Asterionella japonic


Arachnoidiscus


Chaetoceros compressus



23 strains, listed microalgae in Table 8 along with native of habit and origin, were must analyses structure improvement and quality lipid content. Total 21 types species we haves cultured in average nutrient medium in 2000-ml Erlenmeyer flasks 1000 mil.lit of standard pure algal continue cautious in an orbital incubator flushed with air/ CO_2 (95/5, v/v) at a temperature of 258^o C, under continuous illumination (100 µmol PAR photons/m^2 /s) provided by daylight fluorescent tubes. Each strain was cultivated in a single batch for about 2 weeks.

Table 6: Extraction lipids from microalgae 2000-ml Erlenmeyer flask.

Micro algae	Micro algae	Biomass production (g/L/D)	Lipid productivity(mg/L/D)	Oil content (% dry w/w)
Botryococusbraunii	*Botryococusbraunii*	0.02	5-15	25-75
Chlorella sps	*Chlorella* sps	0.02-2.5	2-1200	10-48
Chlorella emersonii	*Chlorella emersonii*	0.036-0.041	10.3-50	25-63
Chlorella sorokiniana	*Chlorella sorokiniana*	0.24-1.46	43.0-323.3	19-22
Chlorella protothecoides	*Chlorella protothecoides*	2.00-7.71	1214	14.6-57.9
Chlorella pyrenoidosa	*Chlorella pyrenoidosa*	2.91-3.65	58-72.8	2.0
Dunaliella salina	*Dunaliella salina*	0.22-0.34	13.2-85	6.0-25.0
Dunaliella primolecta	*Dunaliella primolecta*	0.20-0.33	12.4-84	5.0-23.0
Euglena gracilis	*Euglena gracilis*	7.70	1078-1540	14-20
Isochrysis sp.	*Isochrysis* sp.	0.03-3.0	23-33	50
Nannochloropsis sp.	*Nannochloropsis* sp.	0.17-1.43	20.4-757.9	12.0-53.0
Nannochloris sp.	*Nannochloris* sp.	0.16-1.42	20.1-750.0	20-35
Nitzshia sp.	*Nitzshia* sp.	0.20	22.0	45-47
Phaeodactylum tricornutum	*Phaeodactylum tricornutum*	0.003-1.9	0.54-1083	20.0-57.0
Scenedesmus sp.	*Scenedesmus* sp.	0.03-0.26	5.88-54.6	19.6-21.1
Senedesmus obliquus	*Senedesmus obliquus*	0.004-0.75	0.44-409	11.1-55.1
Schizochytrium sp.	*Schizochytrium* sp.	0.20	42	50-77
Spirulina maxima	*Spirulina maxima*	0.20-0.26	0.84-2.26	4.1-9.1
Spirulina plantensis	*Spirulina plantensis*	0.5-4.4	2.4-714.0	4.1-16.7
Tetrasemis sp.	*Tetrasemis* sp.	0.29	43.4	12.5-14.9
Tetraselmis suecica	*Tetraselmis suecica*	0.25	40.0	15.23

Essential sets of restrictions in micro-algae development are also good and only used various processes which instruct to be worth and fixed oil-wealthy algae species never change throughout culture management. A single mixture appears and entire the culture is hardly managed, with prohibitive costs and spends inexpensive methods Photo Bioreactor PBR, Moreover, this method never guarantees of long stable culture and standard procedure. Both open and closed systems sometimes supply excellent data and solutions to acquire cultural strength at comparatively low cost. Integrate the Photo-Bioreactor open pond cultivation method and procedure done for the past experiments (Pushparaj *et al*., 1997). Here also the production of astaxanthin and oil production through 'The haemapodous pluvial (Huntley *et al.,* 2007). In the necessary condition 'N-sufficient' phase could be carried out in the photo-bioreactor to primary direct produce the inoculums in the 'N-starved' phase extraction and leads to oil collection via open ponds (Helisch and Keppler 2020). Advanced technology is developed nowadays, in the strategy presents that comparing old one, in the open pond in few days only; letter it never considers in schedule how to prevent while contaminant. In the final stage, how to operate the open culture system means while the process is perfect high mass production yield, since the open pond is inoculated, and culture diluted, past present and feature N-starvation, a light intensity lux increasing the cell population and yield high concentrate oil lipid content, this study how to increase lipid productivity in microalgae open pond culture system. Feature aspects need to continue isolating marine diatom and culture in open pond culture system production cell density in high mass lipids extraction.

CONCLUSIONS

From the present study the marine microalgae in the SPSR-Nellore district of Andhra Pradesh is distinctive in their quality and was determined to be a potentially economical feedstock for biofuel production further they could be cultured in seawater with high lipid yield. This paper provided an overview of the isolation and characterization of available resources of marine microalgae for biofuel production.

FUTURE SCOPE

Further studies are warranted to support the findings of current study, especially for large-scale algal biofuel production and it needs to become strengthened using assortment and hereditary techniques. Innovations and advancements in areas including boosting the functionality of algae to use nutrients successfully or design concepts to lessen handling demands possess the prospective to greatly boost the power balance as well as improve the general sustainability of algal biofuels. Advanced studies such as gene sequencing 18s rRNA along with DNA Bar-coding are required to be conducted for the better understanding of the usage of the mentioned species for the production of the biofuel.

Acknowledgement. The authors are grateful to the head of the biotechnology department at Vikrama Simhapuri University in Nellore, Andhra Pradesh, India for providing the necessary resources. We also appreciate the head of the marine biology department at Vikrama Simhapuri University in Nellore, Andhra Pradesh, India for allowing us to use their facility.

Conflict of Interest. None.

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How to cite this article: Fairoz Basha M. and Shrikanya Rao K.V.L. (2023). Taxonomy Studies: An Overview on Isolation and characterization of Available Resources of Marine Microalgae for Bio-fuel Production. *Biological Forum – An International Journal, 15*(3): 696-712.