“ALGAL BIOPROCESSING FOR ENHANCED BIOFUEL AND BIOCHEMICAL PRODUCTION”

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DECLARATION

This is to declare that the work presented in this thesis entitled of "**Algal Bioprocessing for Enhanced Biofuel and Biochemical Production"** is original and has been carried out by me for the degree of Doctor of Philosophy under the supervision of **Dr. Navneeta Bharadvaja, Assistant Professor**, Department of Biotechnology. This thesis is a contribution to my original research work. Wherever research contribution of others is involved, every effort has been made to clearly indicate the same. To the best of my knowledge, this research work has not been submitted in part or full for the award of any degree or diploma in Delhi Technological University or in any other university/institution.

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CERTIFICATE

This is to certify that the thesis entitled **"Algal Bioprocessing for Enhanced Biofuel and Biochemical Production"** submitted to the Delhi Technological University, Delhi-110042, in fulfilment of the requirement for the award of the degree of **Doctor of Philosophy** by the candidate **Mr. Lakhan Kumar** (Reg. No. 2K17/PhD/BT/03) under the supervision of **Dr. Navneeta Bharadvaja, Assistant Professor**, Department of Biotechnology. It is further certified that the work embodied in this thesis has been neither partially nor fully submitted to any other university or institution for the award of any degree or diploma.

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Dedicated to the Almighty, my parents, my teachers, my siblings, and the love of my life

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"Creating a life that reflects your values and satisfies your soul is the real success."

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ABSTRACT

Scarce fossil fuels resources and environmental pollution due to their consumption is a major global concern nowadays. It has emphasized on exploring novel, ecofriendly, and renewable sources of energy. Microalgae have been established as a potential feedstock for biofuel production which is renewable as well as environmentally friendly. To date, numerous studies have been done to elucidate these organisms for large-scale biofuel and biochemical production. However, enhancing the target biomolecule (or say lipid in case of biodiesel) synthesis rate and reducing the production cost still remain a major bottleneck for its economic viability. Biofuel production from microalgae biomass is a multistep process and each process is specific to particular microalgae as well. High cost of oil extraction from algal biomass and its conversion into biodiesel poses a major challenge to its commercial viability. Recent studies on algal based biofuels production have suggested the need of extraction of other co-products like astaxanthin, lutein, β-carotene, polyhrdoxybutyrate, omega fatty acids, polysaccharides, and vitamins, from the same algal biomass following the concept of biorefinery analogous to petroleum refinery. Several studies have reported production of platform chemicals having therapeutics, nutraceuticals, and cosmetics value from the same algal biomass before and after algal oil extraction. To make microalgae-based biofuel production economic and sustainable; other microalgal high-value components extraction is termed as a promising measure.

Under this direction, this study explores the bioprospecting of microalgae for production of biofuels and biochemicals. Under the first objective of this study, microalgae were isolated and identified on the basis of morphological and molecular studies. Master culture of isolated microalgae were established and used for further investigations. Microalgae isolate which showed

maximum growth rate was chosen for further investigation. Out of all four *chlorella* strains, *chlorella sorokiniana* showed maximum growth rate. The amount of lipid content on cell dry weight basis was calculated for all these four select microalgae. The maximum lipid content (11% w/w dry cell weight) was found in *Chlorella sorokiniana*. Culture conditions including pH, temperature, and light intensity for its optimum growth rate were optimized. Also, effect of different carbon, nitrogen, and phosphorous sources were studied. *Chlorella sorokiniana* and *Chlorella minutissima* were found to have ability to grow in RO spent water facilitating the simultaneous wastewater treatment and biomass production for extraction for lipid and other valuable compounds. It can pave the way of replacing fresh water for preparation of growth medium with RO spent or reject water, thus overall reducing the cultivation cost. Further these microalgae strains were evaluated for production of polyhrdoxybutyrate, a type of bioplastic. The presence of PHB was confirmed by Sudan black staining in both *Chlorella sorokiniana* and *Chlorella minutissima.* Post confirmation, solvent extraction method was applied to quantify the amount of PHB in them. Extraction of PHB along with the lipid will enhance the economy of the algal based biodiesel production.

The microalgae can be exploited not only for its high lipid content useful in production of economical biofuels, but also for high yields of essential pigments with high nutraceutical values. Algae have a unique property of accumulating high amounts of carotenoids under unfavorable conditions. There are efficient methods of subjecting the algal strain to stresses, both biotic and abiotic, that enhances the pigment production in them. The isolation and identification of economic, fast-growing, and adaptable algal strains are important. Therefore, one of the objectives of this study was to isolate locally adapted microalgal strains for prospective β-carotene production. β-carotene was qualitatively and quantitatively estimated from a novel microalgae

Graesiella emersonii isolated from an industrial cement curing tank. We found high lipid content (up to 27% w/w dry cell weight) in normal growth condition. The amount of β-carotene was also higher. The co-extraction of both these chemicals can provide a sustainable algal based biofuel and biochemical production.

Further scope of synthesis of silver nanoparticles from algal biomass extract and their application in wastewater treatment was evaluated. Silver nanoparticles were synthesized using *Chlorella sorokiniana* biomass extract and characterized using UV-spectroscopy, XRD crystallography, and Scanning electron microscopy for their morphological and structural properties. Upon confirmation of their synthesis, these nanoparticles were used for degradation of four different dyes. Also, antibacterial properties of these nanoparticles against two bacteria were evaluated. The study concludes that algal biomass can be a suitable feedstock for biodiesel production and in order to make it commercially viable other compounds along with the lipid should be co-extracted. The algal extract post cell disruption followed by lipid extraction can be used for nanoparticle synthesis which can be used for wastewater treatment. Thus, algae can be used for providing a sustainable solution to energy and environmental problems together.

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List of abbreviations

ATP	: Adenosine Triphosphate
Au-NP	: Gold nanoparticles
BLAST	: Basic Local Alignment Search Tool
C/N	: Carbon/Nitrogen ratio
CO_2	: Carbon dioxide
Cr	: Chromium
CV	: Crystal violet
DHA	: Docosahexaenoic acid
DW	: Dry weight
EPA	: Eicosatetraenoic acid
EY	: Eosin yellow
FTIR	: Fourier Transform Infrared
G	: Gram
GLA	: gamma- Linolenic acid
HPLC	: High Performance Liquid Chromatography
IARI	: Indian Institute of Agricultural Research
MALDI-MS	: Matrix Assisted Laser Desorption/Ionization -Mass Spectroscopy
MB	: Methylene Blue
MC	: Market cost
MD	: Market demand
Mg l^{-1} d^{-1}	: milligram per litre per day
Mg/l	: milligram per litre
NaCl	: Sodium Chloride
NADP	: Nicotinamide Adenine Dinucleotide
NCBI	: National Centre for Biotechnology Information
Ni	: Nickel
NPK	: Nitrogen Phosphorous Potassium

OVAT	: Ove variable at a time
Pb	: Lead
PC	: Paper Chromatography
PCR	: Polymeric chain reaction
PHB	: Polyhydroxy butyrate
PPM	: Parts per Million
PUFA	: Polyunsaturated omega-3 fatty acids
RhB	: Rhodamine B
RO	: Reverse Osmosis
RT	: Room temperature
SDS-PAGE	: Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis
SEM	: Scanning electron microscope
SNPs	: Silver Nanoparticles
TAG	: Triacyclglycerols
TEM	: Transmission electron microscope
TLC	: Thin Layer Chromatography
UP	: Unit operations
USD	: United States Dollar
XRD	: X-ray Diffractogram
Zn- NP	: Zinc nanoparticles
ZP	: Zeta potential

CHAPTER 1

INTRODUCTION AND OBJECTIVES

1.1. Introduction

The application of fossil fuels has created serious and persistent threats to our environment in terms of pollution, acid rain, global warming, and climate change. Also, the reserves of fossil fuels are depleting very fast. It has created a need for identification, recovery and utilization of renewable and environment friendly energy sources. Microalgae have been established as economical and environment-friendly feedstock for viable and sustainable production of biofuels such as biodiesel or bioethanol along with other valuable co-products (Ismail et al. 2020). Biofuels, produced from microalgae, can be a potential measure to deal with energy security as well as a reduction in the consumption of fossil fuels (Moreno-Garcia et al. 2017). Microalgae, crowned as efficient cell factories, are photosynthetic microorganisms that sequester Carbon Dioxide from the environment (Subramanian et al. 2016). They fix CO_2 into sugars which eventually enter into central cellular metabolism for use as macromolecular building blocks (Mondal et al. 2017b). Other than microalgae oil, biodiesel industries exploit edible oils extracted from rapeseed, soybean, sunflower, palm, etc., and among non-edible sources Jatropha, Karanja, Tobacco seeds, Mahua, Neem oil (Meira et al. 2015). Algal biofuels can ensure stable and sustainable transport fuel supply. Algal biodiesel has been identified as an alternative to fossil based diesel or as blend with diesel in compression-ignition engines, gas turbines system and also as aviation fuel (Chiong et al. 2018). Besides biofuels, algal biomass has a diverse range of industrial and societal applications (Chew et al. 2017). It is now a proven fact that microalgal biomass can be used for the extraction of various therapeutically active compounds/biomolecules having health benefits, namely astaxanthin, lutein, canthaxanthin, docosahexaenoic acid (DHA), eicosatetraenoic acid (EPA), etc

(Yen et al. 2013). Natural resources based biologically active compounds that can efficiently act on molecular targets, involved in the treatment of various diseases are currently attracting a lot of attraction (Michalak and Chojnacka 2015).

Despite several advantages of using microalgae biomass as a feedstock for biodiesel production, it also presents a set of challenges. Currently, biofuel production houses are struggling hard to make the production economically viable (Wrede et al. 2014). Biofuel production from microalgae biomass is a multistep process. It includes mass cultivation systems, harvesting, drying, cell disruption, extraction and purification. Each step is specific to a particular microalgae type (Mallick et al. 2016). The amount of carbohydrate, lipids, proteins and other biomolecules in microalgae vary with species, strain, environmental growth conditions and extraction processes (Mendoza et al. 2015). The downstream processing costs approximately 50% of the total cost of microalgal biodiesel production. For the above-mentioned reasons, the algal biofuels industry is running hard after the development of new and economic down streaming processes starting from pre-treatment to process optimization for saccharification and fermentation in case of ethanol production and lipid extraction and its transesterification for biodiesel production, scale-up for algal biomass cultivation, harvesting and dewatering followed by drying. There is a range of methods for each step which are particular to microalgae strain and processing technologies (Chen et al. 2011). For reducing algal cultivation cost, wastewater treatment has been added to algae-based biofuel production to make it more cost-effective by reducing the need for nutrients and water sources (Zhou et al. 2016). It has been shown that heavy metals, dyes, nutrients such as carbon, nitrogen and phosphorous, xenobiotic compounds present in the wastewater, utilized or absorbed by microalgae as their growth requirements, can be utilized as cultivation medium (McGinn et al. 2011). Also, integration of microalgal biomass production with sugar mills,

chimneys, and other production houses can significantly reduce the cost of production and requirements of barren land for the purpose (Lohrey and Kochergin 2012). An economic cost-effective overall production system for algal biofuel is still a persistent challenge (Mondal et al. 2017b).

Recent studies on algal biodiesel production have suggested several interventions to make it economically viable. Apart from, using algal biomass for biodiesel production, the same biomass can be used for extraction of various high value biomolecules having nutraceutical, pharmaceutical values. To increase yield of lipid or any other biomolecule of interest, chemical precursors or genetic engineering are found to be promising. Some studies report to convert protein into higher alcohols using metabolic flux engineering. Milder cell disruption techniques can significantly improve the yield of high value algal metabolites with intact bioavailability. Extraction of algal components demands a high volume of organic solvents which can be replaced with alternative ionic liquids. Ionic liquids have several advantages over conventional organic solvents in terms of quantitative and qualitative aspects both.

Extensive literature review was carried out to identify research gaps for algal based biofuels and biochemical production. Bioprospecting of a smart microalgae strain i.e. selection and successful outdoor large scale cultivation of robust microalgae strain having high growth rate, high biomass productivity, high lipid content and immunity towards invasion of other microorganisms is highly recommended. Development of cost effective, energy efficient and easy to operate biomass harvesting methods and dewatering technologies are needed to make algal based biofuel and biochemical production a sustainable and economically viable business. In the present investigation following objectives are undertaken;

1.2. Objectives

- Collection of various microalgae found naturally in the Delhi and nearby areas and their identification.
- Biochemical characterization and their evaluation for biofuel and biochemical production
- Optimization of biomass and lipid productivity through biotic and abiotic means.
- Application and evaluation of isolated microalgae biomass for wastewater treatment.

1.3. Structure of thesis

The thesis has been divided into eight chapters. **Chapter 1** deals with the introduction and objectives of the study. The background of the work as well as the relevant literature survey including several reported applications of the algae presented in ***Chapter 2***. The collection, isolation, and identification of microalgae has been reported in ***Chapter 3***. The isolated microalgal cultures were characterized based on their morphological features as well as molecular identification studies. The ***Chapter 4*** deals with the selection of microalgae on the basis of growth rate and lipid content for rest of the experiments and optimization of necessary culture conditions light, pH, and temperature of the select microalgae. The ***Chapter 5*** reports bioprospecting microalgae for the production of lipid and β-carotene. A microalgae *Graesiella emersonii* was isolated, and the lipid and β-carotene were estimated. Further, to improve β-carotene accumulation different strategies were reported in this chapter. The ***Chapter 6*** reports the method for screening of polyhydroxy butyrate positive cells and their estimation in target microalgae cells. The ***Chapter 7*** reports the green synthesis of silver nanoparticles from select microalgae for their application photocatalytic dye degradation of four dyes and antibacterial activity against two different bacteria. The conclusions and the future scope of the work have been presented in the ***Chapter 8*** of the thesis.

CHAPTER 2

REVIEW OF LITERATURE

2.1. Microalgae-based biofuels and biochemical production

Biorefinery, in a similar fashion to petro-refinery of crude oils for petrol, diesel, wax, kerosene, etc production, facilitates extraction of different biomolecules e.g., lipid, protein, carbohydrates from same algal biomass. It can have promising deliverance against the demand for food, feed, fiber, pharmaceuticals and fodder (Bharadvaja and Kumar 2020a). Lipids from microalgae are the source of biofuels and platform chemicals for many industries dealing with nutraceuticals (Ambati et al. 2014). The fractionation of crude lipids into lipids for biodiesel production and a range of high-value chemicals, such as omega-3 free fatty acids, Docosahexaenoic acids (DHA), Eicosatetraenoic acid (EPA), γ-linolenic acid (GLA), etc. having anti-oxidant, anti-inflammatory, and anti-carcinogenic, etc., properties (Guedes et al. 2011). Food supplements based on these high-value chemicals have sound and proven effects on human health and thus are high in demand (Vadivelan and Venkateswaran 2014). Biomolecules derived from algal biomass like polysaccharides find their use in ethanol, nutraceuticals, pharmaceuticals, cosmetics and many industries. Carbohydrate extracted from the residual biomass post lipid extraction, if the concentration is high in the microalgae, can be extracted and subjected to fermentation for bio-ethanol production and generation of biohydrogen and other chemicals. Residues of the processed biomass can be used for protein extraction. Proteins, when purified, are used as therapeutics, feed, and food supplements, vaccines, pharmacy. Pigments and carotenoids find their application in pharmaceutical, cosmetic industries, fragrance, medicinal use, and nourishment (’t Lam et al. 2018). The final residual biomass can be applied as fertilizers in agricultural fields.

The world market for Lutein (Web-1), Astaxanthin (Web-2), and omega fatty acids (DHA, EPA, GLA and others)(Web-3) is estimated to be valued at USD 358 million by 2024, USD 814 million by 2022 and USD 19 billion by the year 2020 respectively. The total carotenoid market has been evaluated to reach at USD 1.53 billion by the year 2021 (Web-4). Algal biomass based polyhrdoxybutyrate are a sustainable raw material for biopolymer and bioplastic production (Kumar et al. 2020). Bioplastic market has been estimated to reach USD $43 billion by 2022 and the market price for bioplastic is USD 970/tonne (Tůma et al. 2020). So, algal biomass after extraction of high value products can be diverted to commercial bioplastic production. Algal biomass is also useful source of many other commercial products majorly agar agar, carrageenan, diatomite etc. This approach can pave the way for microalgal biomass-based energy generation economically viable and profitable business.

In general, lipids and proteins are the largest fractions of microalgal biomass while carbohydrates, pigments, and carotenoids are normally a minor part. Biochemical characterizations and elemental analysis exercised on a range of microalgae species have shown that the yield of biomolecules composition varies species to species and strain to strain and under different environmental conditions (Duong et al. 2012). Some microalgae have high concentrations of lipids such as *Botryococcus braunii* (up to 70% of total dry biomass) while some contain a high concentration of carbohydrates. Starch-less strains are the highest lipid producing ones (Banerjee et al. 2002). Apart from it, extraction methods, the sequence of extraction, solvents used for extraction, etc., affect the yield of a particular component. A correct sequence of product extraction from microalgae can bring advantages in terms of significant economic viability and stability to the microalgae-based biodiesel industry (Venteris et al. 2014). Many high value biomolecules of interest obtained from algal biomass have been discussed below.

2.1.1. Lipid

Microalgae cells contain large amounts of storage lipid primarily in the form of triacylglycerols (TAG) (Hu et al. 2008b). Lipid molecules having 14-20 carbons in their structure are used as a feedstock for biodiesel production. Further, the fatty acid profile is important in selecting microalgae lipid as a feedstock for biodiesel production (Halim et al. 2012). Lipids suitable to biodiesel are subjected to the transesterification process for biodiesel production. Several microalgae including *Chlorella minutissima, Chlorella vulgaris, Scenedesmus* sp. *Botryococcus braunii, Desmodesmus intermedius, Spirulina platensis, Isochrysis galbana* etc. have been explored for production of biodiesel (Thirugnanasambandham 2018).

Glycerol is a by-product, which finds its application in cosmetics industries. Microalgae lipid-based biodiesel production pathway starts from microalgae isolation and identification to cultivation to biomass harvest followed by lipid extraction and then its conversion to biodiesel. Lipid biosynthesis pathway and improvement strategies have been extensively studied by Wase *et al*. (Wase et al. 2018). Much has been discussed about microalgae biomass-based lipid extraction and its conversion into biodiesel using the transesterification process (Mubarak et al. 2015). Lipids having more than 20 carbons in their structure aren't suitable for biofuel production. They are a better source for polyunsaturated fatty acids such as DHA, EPA, etc., commonly used in nutraceutical and pharmaceutical industries (Hu et al. 2018). Algal lipids having carbon numbers more than 20 such as EPA, and DHA are potential valuable biochemicals extractable from microalgae biomass. They have been presented in the following section.

2.1.1.1. Docosahexaenoic acid or DHA for human consumption

Docosahexaenoic acid, or DHA, is a polyunsaturated omega-3 fatty acid (PUFA). Recent research findings suggest that DHA should be considered as a conditionally essential nutrient supplement

for humans. It is a major structural fat in the brain and retina. It accounts for up to 97% and 93% of the omega-3 fats in the brain and retina respectively. It is also a key component of membrane lipids, heart, and human nutrition. Potential therapeutic applications are majorly found in the treatment of lung and breast cancer, hypertension, depression, cardiac arrest, asthma, neurodegenerative diseases, chronic skin diseases, chronic inflammatory bowel disease and rheumatoid arthritis (Hu et al. 2018). Its intake helps in cognitive and physical improvements in persons with brain injuries and effective treatment of psoriasis (Lewis et al. 2013). Numerous studies have suggested that from infants to adults, everyone benefits from an adequate supply of DHA. It can be obtained directly through maternal milk; oils extracted from rich alpha-linolenic acid plants (flax, hemp, rapeseed, soya bean, walnut), fish oil (Herring, Mackerel, Sardine, and Salmon), fungi or can be extracted from microalgae. Consumers are aware of the importance of these nutrients. Several properties of microalgal oils are particularly appealing, such as their sustainability, high purity and quality, "vegetarian" origin, and improved organoleptic qualities when compared to animal or fish oils (Vadivelan and Venkateswaran 2014).

2.1.1.2. Eicosapentaenoic acid (EPA) for human consumption

Microalgae contain large quantities of high-quality EPA. They are considered a potential source of this important fatty acid (Cardozo et al. 2007). Culture age, cultivation conditions, nutritional and environmental factors influence the production of EPA production of microalgae. Similar to DHA, the recovery of EPA can be enhanced by manipulating culture conditions, the use of specialized cultivation vessels, etc. and by the use of metabolic and genetic engineering (Wen and Chen 2003). Specialized photobioreactors with optimum light intensity and better light penetration can effectively increase EPA production. The use of blue LED light for irradiation proved to be EPA yield improving strategy for microalgae *Nannochloropsis oceanica* CY2 (Chen et al. 2013).

Also, the application of photobioreactors found to be useful in enhancing the yield of EPA in *Nannochloropsis oceanica* CY2 (Chen et al. 2015). The current annual global market of both DHA and EPA is approximately 700 million USD (Bhalamurugan et al. 2018).

2.1.2. Pigments

The color of algal thallus varies in different classes of algae. It is due to the presence of definite compounds in the cells. These compounds are called pigments. They are categorized in three different classes- chlorophylls, carotenoids and phycobillins or billiproteins (Cardozo et al. 2007). These are further categorized into several subclasses- Chlorphylls (a,b,c,d, and e), carotenoids (carotene, carotenoid acids, and xanthophylls) and phycobillins (phycocyanin and phycoerythrin). Astaxanthin, lutein, fucoxanthtin, β-Carotene etc, are of prime interest (Guedes et al. 2011). Phycobillins show hepatoprotective, anti-inflammatory, immunomodulatory, anticancer and antioxidant properties. Phycocyanin is widely applied as food colorant nutraceutical and in immunodiagnostic applications.

2.1.2.1. Astaxanthin

Astaxanthin (approx. market price 1.8 USD/g) products are used for commercial applications in the dosage forms as tablets, capsules, syrups, oils, soft gels, creams, biomass, and granulated powders. Astaxanthin patent applications are available in food, feed and nutraceutical applications. It has a global market value in order of 240 million USD per annum (Barkia et al. 2019). Astaxanthin, a red fat-soluble pigment, found naturally in a diverse range of microorganisms, is termed as a more potent and biologically active xanthophylls carotenoid among all other carotenoids. Astaxanthin has its application as a nutraceutical, food supplement, antioxidant, anti-inflammatory and anti-cancer agent (Hu et al. 2018). It has proven therapeutic activity against

diabetes, cardiovascular ailments/diseases, and neurodegenerative diseases or disorders. It also stimulates immunization in animals and the human body. Studies conducted over a range of marine and freshwater microalgae such as *Haematococcus pluvialis*, *Chlorella zofingiensis*, *Chlorococcum*, and *Phaffia rhodozyma* provided information over its extraction, yield, bioavailability, activity, and stability. *Haematococcus Pluvialis* accumulates astaxanthin approximately 3.8% of its total dry biomass (Ambati et al. 2014). Astaxanthin production gets affected under environmental stress such as adverse light intensity and exposure, salinity, temperature, C/N ratio, nutrient deprivation such as NPK deficient medium, inoculums concentration and amount. These manipulations have increased the astaxanthin production up to 2.2 mg l^{-1} at large scale facilities while up to 11.5 mg $l^{-1}d^{-1}$at bench scale at Cyanotech, Aquaresearch and Micro Gaia. Light quality, not the quantity matters more in astaxanthin production. Flashing light increased the rate of astaxanthin production by at least 4-times as compared to continuous light sources (Vo et al. 2017). Optimization of astaxanthin production needs strain improvement, development of better growth media and induction conditions along with improved culture conditions. Photo-bioreactor design and its implementation for astaxanthin production must be given priority to reach on economic competence with other astaxanthin producing sources (Eonseon et al. 2006).

2.1.2.2. Lutein

Lutein (approx. market price 2.5 USD/g and global market value of 233 million USD per annum), a xanthophyllic compound, has extensive application in food, feed, nutraceutical, and pharmaceutical product formulations. It finds its application in the treatment of neurodegenerative diseases due to its high anti-oxidative activities. It is also used as colorant and food additives (Bhalamurugan et al. 2018). Lutein is predominantly found in fruits and flowers. Microalgae, due

to their proven advantages in terms of yield per unit area and water consumption, have started replacing conventional sources of lutein e.g., maize, egg yolk and petals of the marigold flower. The microalgae *Scenedesmus almeriensis* cultivated for lutein production yielded 4.77 mg-$l^{-1}d^{-1}$. Other prominent microalgae exploited for lutein production are *Scenedesmus almeriensis, Chlorella sp. (C. protothecoides, C. Zofingiensis), Chlorococcum citriforme, and Neospongiococcus gelatinosum,* etc. Lutein productivity can be enhanced by optimizing the growth conditions and introducing chemical and genetic engineering (Bhalamurugan et al. 2018). Apart from several other value-added compounds can be extracted from microalgal biomass including β-carotene approx. market price 0.6 USD/g and global market value of 261 million USD per annum), and phycobiliproteins (phycocyanin, allophycocyanin, phycoerythrin and phycoerythrocyanin) of global annual market value of 60 million USD. *Dunaliella salina, Dunaliella bardawil,* and *Scenedesmus almeriensis* have been exploited for β-carotene production while *Arthrospira platensis, Amphanizomenon floa-aquae,* and *Spirulina* sp., are major microalgae which can be used for Phycobiliproteins production (Barkia et al. 2019).

2.1.3. Vitamins

Vitamins trigger many metabolic pathways and also serve as a precursor for several enzyme co-factors. Higher organisms, due to absence of metabolic pathways that synthesize these enzyme co-factors, depend upon other organisms to fulfill their vitamins requirements. Algae are rich source of several vitamins including vitamin C, D and E. They contain almost all essential and non-essential kinds of vitamin (Ganesan et al. 2019). *Eisenia arborea, Nannochloropsis oceanic, Chlorella vulgaris* have been used for vitamin C, vitamin D, and Methylcobalamin (Vitamin B_{12}) production respectively. Several other biomolecules of industrial importance have been extracted

from different classes of algae including marine and freshwater, their industrial importance, market cost and demand have been presented in **Table 2.1.**

Table 2.1: Biomolecules extracted from algae and their industrial importance

Biomolecules	**Therapeutic Importance/Industrial significance**	**Market Cost (MC)/ Market Demand (MD)**	**Microalgae Species**	**Yield of Biomolecule/ Microalgae Growth/ Culture Conditions/ Other remarks**	**References**
9-cis β-Carotene	Activity against atherosclerosis, psoriasis, atherogenesis and retinitis pigmentosa	MC- USD 600,000/g	*Dunaliella salina*	Trans form of β-carotene market value: USD 7/g	(Harvey and Ben-Amotz 2020)
β-Carotene	Pharmaceutical	MC- USD 7/g	*Tetradesmus obliquus SGM19*	Yield: 0.67 mg/g dry biomass, Lipid content: 29 wt% of dry biomass.	(Singh et al. 2020)
Algal hydrolysates	Bioethanol	MD- USD 33.7 billion in 2020 to USD 64.8 billion by 2025	*Codium tomentosum*	58.7% of total carbohydrate	(Gengiah et al. 2020)
Alpha-linolenic acid	Hypocholesterolemic, hypolipidemic, neuroprotection, reduces skin's roughness and scaling, anti-depressant, anti-inflammatory	MC- USD 120/g	*Desmodesmus sp. MCC34*	Lipid productivity of 15.9 mg L^{-1} d^{-1}; Alpha- linolenic acid: 24% of total lipids.	(Nagappan and Kumar Verma 2018)
Amphidinol 2	Anti-Cancer potential, chemotherapeutic agent, Cytotoxic and Antifungal.	-	*Amphidinium klebsii*	These compounds are known to perforate the membrane via	(Espiritu et al. 2017)

				sterol interaction ultimately leading to pore formation and cell death.	
Arachidonic acid	Muscle development	-	*Chlorella*, *Spirulina*	Lower levels of AA contribute to Alzheimer and Autism.	(Andrade 2018)
Astaxanthin	Effective against heart diseases and cancer occurrence, strong immunity to different types of infections, dietary supplement.	MD: USD 1.5billion for 2020	*Haematococcus pluvialis*	Astaxanthin concentration up to 2.7% of dry algal biomass.	(Niizawa et al. 2018)
BPFS bioplastic feedstock	Bioplastic	BPFS cost-970 USD tonne−1	*Scenedesmus acutus (UTEX B72)*	Lipid content: 8% of the biomass by mass	(Beckstrom et al. 2020)
Triacylglycerols	Bioplastic	-	*Chlamydomonas reinhardtii*	Triacylglycerols biopolymers: In preparation of crude bioplastic-beads. Other products: Carotenoids.	(Kato 2019)
Carotenoids (Lutein, β-Carotene, Zeaxanthin)	Antioxidant activity	-	*Dunaliella salina*	β-Carotene (trans form): 138.65 mg/g algae, 9-cis β-Carotene: 124.65 mg/g algae, Zeaxanthin: 11.27 mg/g algae, Lutein: 6.55 mg/g algae.	(Hu et al. 2008a)
Carotenoids - (neoxanthin, 9′-cis-neoxanthin,	Antioxidant activities, inhibitory effects against enzymes α-amylase, α-	World market value of total carotenoids:	*Oedogonium intermedium*	Carotenoids (µg/g dry algal biomass) in Crude extracts: 3,411.2 ± 20.7;	(Wang et al. 2018)

loroxanthin, violaxanthin, lutein, α-carotene and β-carotene)	glucosidase, pancreatic lipase and hyaluronidase	USD 1.53 billion by the year 2021		Saponified extracts: 2,929.6 ± 5.9	
Chrysolaminarin-polysaccharides	Biological activities including anti-tumor, anti-oxidant, and immunomodulatory.	-	*Tribonema utriculosum*	Yield: 14.67% of Dry Biomass (DB), Lipid content: 25- 34% of DB.	(Wang et al. 2020)
Docosahexaenoic acid	Human nutrition		*Crypthecodinium cohnii*	DHA yield: 35.6% of total lipids.	(Chalima et al. 2020)
Docosahexaenoic acid	Food and nutraceutical industry, essential for fetal development, antioxidant activities, brain health supplement,	MD for Omega fatty acids (DHA, EPA, ALA etc.) for 2020: USD 18.95 billion	*Crypthecodinium cohnii*	DHA: 43.5% of total lipid. Lignocellulosic biomass used as growth media for production of omega-3 fatty acids- enzymatic hydrolysates.	(Karnaouri et al. 2020)
Eicosapentaenoic Acid	-	-	*Nannochloropsis gaditana*	EPA yield: 11.50 mg/g algal dry biomass.	(Molino et al. 2019)
Eicosapentaenoic Acid	Defense against inflammation, acts as a precursor for prostaglandin-3, thromboxane-3, leukotriene-5.	-	*Chlorella sorokiniana UTEX 2714*	EPA productivity- 4.338 mg L^{-1} d^{-1} ; Omega-3 FA productivity:26.08 ±2.47 mg L^{-1} d^{-1} ; Biomass productivity: 0.412 g L^{-1} d^{-1}	(Shim et al. 2020)
Fatty acids/Lipids	Biodiesel	-	*Chaetomorpha cf. gracilis*	-	(Sánchez-Borroto

					et al. 2018)
Fatty acids/Lipids	Biodiesel	-	*Isochrysis galbana*	Lipid content: 8.41 wt%, Maximum yield palmitic acid ($C_{16:0}$): 22.3%	(Silitonga et al. 2017)
Fatty acids/Lipids	Biodiesel	-	*Spirulina platensis*	Lipid content: 26.65% of total dry biomass; Average methane content: 62.38 ± 2.12%, wastewater was used a nutrient medium.	(Chavan and Mutnuri 2019)
Fatty acids/Lipids	Biodiesel	-	*Desmodesmus intermedius*	Lipid productivity: 37.5 mg L^{-1} d^{-1}	(El-Sheekh et al. 2019)
Fatty acids/Lipids	Biodiesel	-	*Botryococcus braunii*	-	(Prathima and Karthikeyan 2017)
Fatty acids/Lipids	Biodiesel	-	*Chlorophyta species*	Lipid content-18.29 ±0.4 wt.%	(Yusuff 2019)
Fucoxanthin and Eicosapentaenoic acid	Pharmaceutical and nutraceutical industries	-	*Thalassiosira weissflogi*	EPA productivity:33.4 mg L^{-1} d^{-1} ; Fucoxanthin-0.95% Dry Cell Weight	(Marella and Tiwari 2020)
Fucoxanthin, Biosilica and Protein	Antioxidants, feed	-	*Phaeodactylum tricornutum*	Fucoxanthin,-0.18, Biosilica-0.93 and Protein-6.95 ton·$year^{-1}$;	(Branco-Vieira et

				Other products: biodiesel-1.72, bioethanol-0.35 and biomethane-1361 (in m^3/year).	al. 2020)
Fucoxanthin, Chrysolaminarin, and Eicosapentaenoic acid	-	-	*Odontella aurita*	Fucoxanthin-6.01 mg L^{-1} d^{-1}, Chrysolaminarin (161.55 mg L^{-1} d^{-1} and EPA-9.37 mg L^{-1} d^{-1}	(Xia et al. 2018)
Fucoxanthin, Eicosapentaenoic acid and Chrysolaminarin	Chrysolaminarin-: anti-tumor activity, Counters growth and reproduction of Colon cancer cells.	-	*Phaeodactylum tricornutum*	Fucoxanthin-extensive pharmacological bioactivities.	(Zhang et al. 2018)
Fucoxanthinol, Fucoxanthin	Food and pharmaceutical applications.	-	*Nitzschia laevis*	Fucoxanthinol - 4.64 mg g^{-1} Dry Weight, Fucoxanthin: 1.68 mg g^{-1} DW.	(Sun et al. 2019)
Isobutanol and Isopentanol	Fuels and commodity chemical production from	-	*Microchloropsis salina*	-	(Liu et al. 2019)
Lipid	Biodiesel	-	*Scenedesmus obliquus*	Lipid productivity-10.5 mg L−1 day−1.	(Xia et al. 2020)
Lutein, β-carotene	Prevent diseases such as arteriosclerosis, cataracts, and macular degeneration	Lutein Cost-US$900-15,000 /kg	*Choricystis minor var. minor*	Lutein: 13.2 mg per g; β-carotene-0.9 mg per g of algal dry biomass	(D'Alessandro et al. 2020)
Carotenoids -(Lutein, β-Carotene, Neoxanthin, β-Cryptoxanthin,	Antioxidant activities, pro-vitamin A activity, health benefits towards macula and retina. Other	MD for Lutein: USD 357.7 million by 2024	*Ulva spp.*	Mixture of carotenoids produces strongest therapeutic effect.	(Eismann et al. 2020)

Violaxanthin, Antheraxanthin And Zeaxanthin)	benefits: Colorants, food additives, animal feed.			Total carotenoids yield: 0.005 to 800 mg.g−1 fresh weight basis.	
Methylcobalamin (vitamin B12)	Crucial for metabolic processes in humans; nutritional supplement.	-	*Chlorella vulgaris*	29.87 ± 2 l g/100 g dry weight.	(Kumudha et al. 2015)
Monoacylglycerides	Cytotoxic activity against the hematological cancer cell line U-937 and colon cancer cell lines.	-	*Skeletonema marinoi*	Mechanism of action: Induction of apoptosis through caspase 3/7 activation.	(Miceli et al. 2019)
Phoroglucinol	Activity against gastrointestinal disorders.	-	*Chlorella sp.*	Other uses: explosives.	(Andrade 2018)
Phycobiliproteins	Hepatoprotective, anti-inflammatory, immunomodulatory, anticancer and antioxidant properties	-	-	Other uses: used as labels for antibodies and receptors during fluorescence diagnosis.	(Andrade 2018)
Phycocyanin	Food colorant nutraceutical, immunodiagnostic applications.	-	*Spirulina platensis*	Phycocyanin: 67 mg/g of dry biomass; Other products: β-carotene: 4.66 mg/g of dry weight.	(Bachchhav et al. 2020)
Polyhydroxyalkanoates	Packaging material, biomedical implants	MD: USD 43 billion by 2022	*Gelidium sesquipedale*	Other product-Agar	(Price et al. 2020)
Polysaccharide	Antitumor activity	-	*Porphyridium cruentum*	-	(Gardeva et al. 2009)

Proteins	Emulsifying agent and antioxidant	-	*Chlorella pyrenoidosa*	Protein yield: 722.70 mg/g of dry biomass.	(Lu et al. 2019)
Scytovirin	Anti-HIV Protein/ anticytopathic activity	-	*Scytonema Varium*	Scytovirin yield: 0.03% of total algal dry biomass	(Boschesch et al. 2003)
Vitamin C	Improves immune defense system, activates intestinal absorption of iron.	-	*Eisenia arborea*	Vitamin C yield: 34.4 mg/100 g DW of algal biomass. Other advantages: Regenerates Vitamin E.	(Hernández-carmona et al. 2009)
Vitamin D3 (cholecalciferol)		-	*Nannochloropsis oceanica*	Productivity- 1 ± 0.3 µg/g DM	(Ljubic et al. 2020)
52 metabolites, including amino acids organic acids, sugars (α/β glucose), nucleosides, and other compounds, such as trigonelline, inositol, and choline	Hydrophilic crude extract fraction-bioactivity against two cells lines namely- Human Melanoma and Keratinicytes. Trigonelline: useful against diabetes and hyperlipidemia, cancer, and cardiovascular diseases.	-	*Staurosirella pinnata; Phaeodactylum tricornutum*	Other useful products- Eicosapentaenoic, hexadecenoic, and octadecanoic acid and Biomethane. Lipid Content: *S. pinnata*: 14.54% ± 1.61%, *P. tricornutum* :17.09% ± 1.50% (% dry weight of algal biomass)	(Savio et al. 2020)

2.2.Microalgal up and downstream processes for biofuel production and biorefinery

Microalgal biofuel and by-products extraction or biorefinery is a multistep process (**Fig. 2.1**). It starts from strain selection to mass cultivation of microalgal biomass to biofuel production (Halim

et al. 2012). To make biofuel production sustainable, renewable and commercially amenable, identification of cheap and abundant raw material, economically effective and environment friendly pre-treatment and hydrolysis techniques along with high yielding species strain-specific conversion and processing techniques still awaits many scientific breakthroughs (Kose and Oncel 2017). The reason for uneconomic microalgal biodiesel production lies in downstream processing methods. These methods have been developed for a particular product from a particular strain (’t Lam et al. 2018). Biodiesel production only focuses on the extraction of lipids while bioethanol production employs carbohydrate extraction. In each case, only one component gets extracted while others get rejected or their nutritional and functional property in foods such as taste, structure, and stability get destroyed. For maximum gain, protein and other water-soluble components should be removed from the cell lysate before lipid extraction. Research & Development on upstream and downstream processing of algal based biorefinery are inadequately addressed and explored (Singh and Olsen 2011). Simple, versatile and low-cost downstream processing strategies providing simultaneous extraction of various cellular components are highly required for sustainable microalgal biorefinery. Development in this direction is needed to make this technology attractive and promising for energy producers and industrialists (Brasil et al. 2017).The downstream processing (DSP) cost for microalgal biorefinery is estimated to be 50% of total production costs. DSP costs include the cost of equipment and operational expenditures on chemicals and energy. Specialty product processes require more unit operations (UPs) for example product purification. Current microalgal biorefinery systems are based on multiple selective unit operations (**Table 2.2**) including cultivation, harvesting, drying, cell disruption, extraction procedures, fractionation and purification (Kose and Oncel 2017). These are complex, specific, time and energy-consuming and labor-intensive in nature. Optimize each single UP to optimize

overall algal up and downstream processes are not advisable and rational in approach. Focus on improving one single UP concerning a benchmark may result in a more costly and complex affair. Reduction in the number of UPs is one of the best strategies towards cost-effective microalgal-biorefinery consuming lesser chemicals, labor, energy and treatment time. The integration approach for various downstream processes is required for better results. Their assessment and suitability for extraction and separation before their application to biorefinery must be conducted (’t Lam et al. 2018).

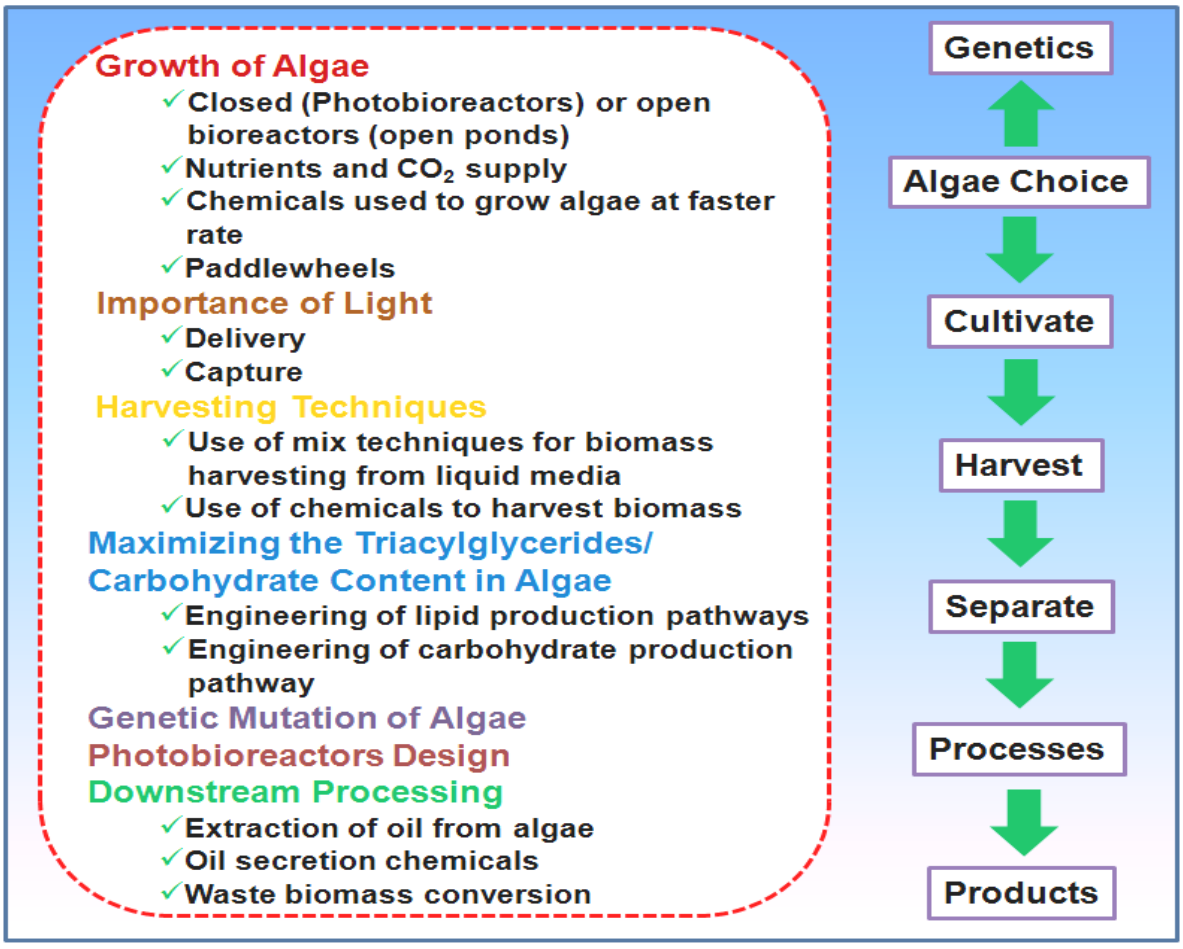


Fig. 2.1: A schematic diagram of microalgae-based biofuel and biochemical production and associated aspects including growth conditions, yield enhancement strategies and downstream processing

Mass cultivation performed on closed photobioreactors provides an economic advantage to the microalgal biorefinery. It also prevents any type of contamination and adequately addresses fluctuations in environmental conditions. Cell density is much higher than conventional ponds in closed PBRs thus it facilitates easy and less energy-intensive harvesting of algal biomass from the media (Chisti 2007). Closed PBRs for mass cultivation and centrifugation for their harvest from media (5-7% of total cultivation) have a significant advantage over algal biomass grown in algal ponds followed by harvest using flocculants (20-25% of total cultivation cost). Environmental factors play an important role in algal growth, biomolecules yield and also properties of the biodiesel prepared using the extracted oil. Kumar *et. al.* investigated the effect of exposure of ultraviolet light on growth, biomass, lipid accumulation and biodiesel properties of microalgae. UV light causes reduction in algal growth rate. FAME profile of the algal lipid also gets altered. UV treated algal cells mainly contains hexadecenoic acid (C_{16}), stearic acid (C_{18}) fatty acids. The amount of PUFA gets reduced as compare to the control. So, it is pertinent to identify the favorable growth conditions for cost-effective production of algal biofuels and other valuable chemicals (Kumar et al. 2018). CO_2 addition resulted into enhancement into algal growth rate and lipid yield. In addition to this, growth rate and lipid productivity significantly depend on temperature, nitrogen content, the interaction between temperature, light intensity, and concentration of available CO_2 (Chen et al. 2014). In different strategy, lipid accumulation was increased by using molasses and addition of Mg^{2+} in algae *Monoraphidium* sp. FXY-10 (Dong et al. 2020). Flocculants, either chemical or biological, are used for harvesting biomass from cultivation media in ponds or bioreactors. Their application increases the overall production cost. Moreover, their complete removal from harvested biomass is a serious challenge for many low volume high-value products

extracted from algal biomass. The presence of these flocculants decreases the economic value of the final product. The same is the case with the organic solvents used for the extraction process.

Table 2.2: Various methods adopted at each unit operation in a microalgal biorefinery

Cultivation	Open tank/pond, Photobioreactor, Hybrid system grown under the following three conditions: -Photoautotrophic -Heterotrophic -Mixotrophic
Harvesting or dewatering	Chemical-Flocculation, Electro-flocculation, Bio-flocculation, Filtration, Centrifugation, Sedimentation, Dissolved air-floatation, Filter press dewatering, Ultrasound
Drying methods	Drum drying, Spray drying, Sun drying, Solar drying, Cross-flow air drying, Vacuum-shelf drying, Freeze drying, Incinerator drying
Cell-wall disruption	High-pressure homogenization, Enzymatic lysis, Bead milling, Grinding, High-speed homogenization, Pulsed electric field, Ultrasonication, Microwave, Autoclave, Freezing, Osmosis, Acid-alkali treatment, Microbial degradation
Extraction	Organic solvents, Aqueous two-phase extraction, Alkali extraction, Supercritical extraction
Conversion techniques	Transesterification, Fermentation, Anaerobic digestion, Hydrothermal liquefaction (HTL), Catalytic hydrothermal gasification coupled with HTL[a]

a: converts algal biomass into bio-oil directly which can be used for feedstock for biofuel and other value-added product syntheses

2.3. Algal based nanoparticles for environmental remediation

2.3.1. Phycoremediation approach

Algae has the ability to accumulate the pollutants from the water as they have large surface area of absorption/adsorption(Parameswari et al. 2010). Application of algae for remediation is termed as Phycoremediation. It is eco-friendly and is more promising than any other remediation

technique as it is inexpensive and is in accordance with sustainable development. Algae can bind up to 10% of its biomass to metals. The metal removal is based on the principle of adsorption of metal on the surface of the algal cell surface and is independent of the metabolism while the absorption of metals by algal cells depends on metabolic activities. The efficiency of biosorption depends upon the bioavailability of the metals, surface area/volume ratio, presence of metal binding groups on algal cells, metal uptake and storage efficiency of the storage of metal (Ahmad et al. 2020).

There have been studies reporting the capability of various algae for the adsorption/absorption of heavy metals from the wastewater. *Anabaena variabilis* has been studied for its capability of removing Pb, Cr, Ni and Cd from the sewage water with an efficiency of removing 100% bioavailable concentration of these metals within 28 days and not only they eliminate these metals but also reduces the offensive odour from the treated water (Parameswari et al. 2010). In another set of studies it was reported that *Oscillatoria anguistissima* has the capability of effective adsorption of Cu^{2+} from the mine water (Ahuja et al. 1997). *Porphyra leucosticte* has been reported to remove 95% of Pb(II) from waste water (Ye et al. 2015). *Chlorella marina* has been reported to remove 89% Cr and 87% Pb from the targeted source (Dinesh Kumar et al. 2015). There have also been attempts to immobilize algal cells of *Anabaena doliolum* and *Chlorella vulgaris* on chitosan, alginate, agar and carrageenan to improve the efficiency of remediation of heavy metals such as Ni and Cr (Mallick and Rai 1994). *Dunaliella* alga has been demonstrated for the removal of heavy metals such as Cd, Hg, Pb present at higher concentrations (Imani et al. 2011).

Algae are also the promising agents for remediation of dyes. The surface of the algal cell wall surface contains many functional groups which help in chelation or electrostatic binding of

the dyes. This kind of removal is known as biosorption. The algae *Caulerpa scalpelliformis* removes basic yellow by biosorption (Aravindhan et al. 2007). Algae secrete extracellular biopolymers which coagulates the dye on their surface. The removal of pollutant by this method is termed as bio-coagulation. Algae *Spirogyra Rhizopus* exhibited potential for removal acid red 274 from the environment via bio-coagulation as was reported in the study (Özer et al. 2006). In another approach, termed as biodegradation, algal enzymatic system works on the pollutants and degrades them. For example, degradation of methyl red dye by the enzyme azo-dye reductase produced by *Nostoc linckia*(El-Sheekh et al. 2009).

The phycoremediation being eco-friendly and efficient also faces some challenges. Such as not the entire concentration of heavy metals is removed, but only the bioavailable amounts are only removed from the contaminated water. Also, surrounding conditions or algal growth conditions substantially control the phycoremediation efficiency. Moreover, the remediation of pollutant materials varies with strain used. So, it is imperative to prospect highly efficient algal species for remediation of each of pollutant material. To overcome this problem the focus of research community has been now shifted to nano-bioremediation approach. It addresses well the problems of pollution created during nanomaterials synthesis and growth dependent algal cells-based remediation of pollutants under diverse climatic conditions.

2.3.2. Algal nanomaterials for remediation: Phyconanoremediation approach

Nanoparticles have been used for the remediation of dyes from the environment in recent times. Being in the range of nanometers, nanoparticles provide an efficient adsorption due to the large surface. Nanoparticles can also degrade the pollutants into a simpler form. Silver nanoparticles (Ag-NP) are used to degrade dyes like Methylene blue, Congo Red, and Coomassie Brilliant Blue.

Similarly, Cu-NP are found to be promising for degradation of Methylene Orange, Zn-NP for Malachite Green, Brown CGG dye and Congo Red, and Au-NP for Methylene blue (Rauta et al. 2019). However, methods used for synthesis of nanoparticles are expensive, require heavy machinery and energy, may change the surface chemical and physical properties of the nanomaterials, and can cause secondary pollution due to the produced by-products (Khanna et al. 2019). To overcome these problems biosynthesis of nanoparticles from algae is considered to provide an eco-friendly and cost-effective remediation strategy for dyes. A schematic diagram of algal nanoparticle synthesis and their application in environmental remediation has been illustrated in below **Fig. 2.2**.

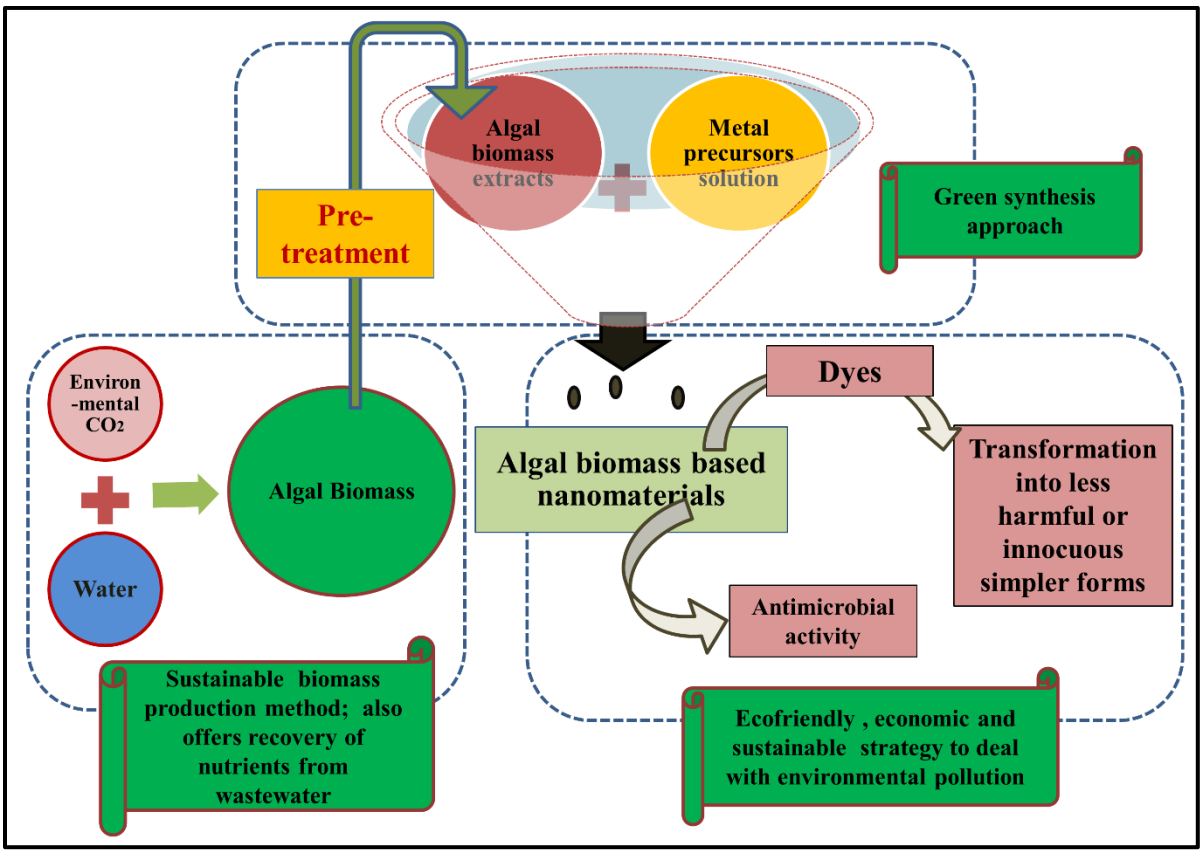


Figure 2.2: Algal nanoparticle synthesis and their application in environmental remediation

2.3.3. Algal biomass for synthesis of nanomaterials: an alternative to physicochemical methods of synthesis of nanomaterials

Even being more efficient, bottom-up methods require a lot of energy and cost. They can also lead to secondary pollution due to the by-products formed during the nanomaterial synthesis (Khanna et al. 2019). Algae can accumulate large amount of metal ions which are stabilized by the bioactive compounds present in the algae. Algal biomass extracts along with protein, carbohydrates and fatty acids contain a variety of numerous other bioactive compounds such as the pigments, chlorophylls, carotenoids, phycobilins, and antioxidants like terpenoids or polyphenol. These bioactive compounds also oxidized the metal ions to convert them into a malleable form. The synthesis of nanomaterials from algae requires algal extract and a molar solution of metal precursor. These two components are then mixed into a fixed proportion to initiate the synthesis of nanomaterials which can be identified by a change in colour of the mixture. The bioactive compounds present in the extract facilitate nucleation of the metal and the nucleonic particle self-assemble to form a thermodynamically stable nanomaterial (Sharma et al. 2016; Khanna et al. 2019). Algal biomass is a promising source of synthesis of nanomaterials as (a) they are abundantly available, (b) have a low cost of large-scale production with easy to harvest, (c) they have a short doubling time, (d) they have a simple yet well-developed systems, (e) they have a negative charge on the surface which enhances the process of nucleation and crystallization (Khanna et al. 2019). **Table 2.3** gives the list of nanomaterials synthesized by using algal biomass and their diverse applicability in the field of medicine, and remediation. There are several factors which effect algal nanomaterials synthesis. For example, concentration of the extract, pH, temperature, and contact time have a significant role in nanomaterials synthesis using algal biomass (Lengke et al. 2007).

Table 2.3: Utilization of algal biomass-based nanomaterials for various purposes

	Microalgae species	Nanomaterials	Application	Reference
Cyanobacteria	*Phormidiumvalderianum*	Au-NP	Gold nanorods for medical purpose	(Parial et al. 2012)
	Anabaena spp.	Au-NP	In-vivo self-reproducing bioreactor	(Rösken et al. 2016)
	*Microchaete*NCCU-342	Ag-NP	Degradation of methyl-red	(Husain et al. 2019)
	Anabaena strain L31	ZnO	Sunscreen filter	(Singh et al. 2013)
	Phormidium tenue NTDM05	CdS	Biolabeling	(MubarakAli et al. 2013)
Brown algae	*Turbinariaconoides*	Au-NP	Reduction of organic dyes	(Ramakrishna et al. 2016)
	Sargassumtenerrimum	Au-NP	Degradation of Rhodamine dye	(Ramakrishna et al. 2016)
	Padina gymnospora	Au-NP	Cancer therapeutic	(Singh et al. 2013)
	Sargassum muticum	Ag-NP	Antiviral, antifungal	(Azizi et al. 2013)
	Bifurcariabifurcata	CuO	Antibacterial	(Abboud et al. 2014)
Green algae	*Coelastrellaspp.*	Au-NP	Biolabeling	(MubarakAli et al. 2013)
	Caulerpa serrulata	Ag-NP	Remediation of congo red	(Aboelfetoh et al. 2017)
	Ulva lactuca	Ag-NP	Remediation of methyl orange	(Singh et al. 2013)
	Ulva faciata	Ag-NP	Antimicrobial	(El-Rafie et al. 2013)
	Chlorococcum sp. MM11	Fe-NP	Remediation of Cr (VI)	(Subramaniyam et al. 2015)
	Scenedesmus-24	PdS	Environmental remediation	(Jena et al. 2015)
Red algae	*Gracilariacorticata*	Au-NP	Ferric ion reduction	(Kumar et al. 2013a)
	Galaxauraelongata	Au-NP	Antibacterial	(Abdel-Raouf et al. 2017)
	Gracilariacorticata	Ag-NP	Antifungal	(Kumar et al. 2013a)

	Gracilaria dura	Ag-NP	Wound healing, Food preservation	(Shukla et al. 2012)
	Gracilaria edulis	ZnO	Anti-cancer against PC3 cell lines	(Priyadharshini et al. 2014a)

2.3.4. Mechanism of algal nanoparticle synthesis

To synthesize algal nanoparticles, metal concentrate is mixed with the algal biomass. To initiate this process metal ions adsorbs and accumulate on the algal surface. Then algae use different enzymes and metabolites like chlorophyll, phycobilin, polyunsaturated fatty acids, carbohydrates, polysaccharides, and minerals to remodel the metal ions into malleable form by reducing them. For example, brown algae use the hydroxyl group in their cell wall polysaccharide to synthesize Ag-nanoparticles. Reduction is the process which algae implies to covert the metal ions into precursors of nano-scaled particles. The functional group present in the metabolites produced by the algae helps in the reduction of metal ions as illustrated in **Table 2.4**. The step of reduction is marked by the change in colour of the solution(Ramakrishna et al. 2016; Gour and Jain 2019).

After reduction, the nanoparticles undergo nucleation, where they self organizes to achieve a thermodynamically stable appearance. The process of reduction and nucleation together marks the first phase of algal nanoparticle synthesis, called the activation phase. The second phase is called the growth phase, where the reduced and nucleated metal ions start to amalgamate with each other forming variable sized nanoparticles. During the growth phase to prevent the nucleated metal ions from forming large amalgamates and to provides them stability capping reagents are used. Capping reagents are amphiphilic substances like membrane polysaccharides which covalently binds to the nanoparticles. This binding induces stearic hindrance into the structure and thus provides stability. In the final stage or termination phase the stabilized nanoparticles undergo conditioning through

temperature, pH, and physical shearing with due course of time to attain a final refined shape and size (Ramakrishna et al. 2016; Gour and Jain 2019; Javed et al. 2020).

Table 2.4: List of various bioactive compounds found in algae and their function

Category of compound	**Bio-active compound**	**Function**	**Algae**	**Nanomaterial information**	**Reference**
Redox proteins	ATP synthase, superoxide dismutase, carbonic anhydrase, histones, ferredoxin-$NADP^+$ reductase. Identified using SDS-PAGE and MALDI-MS-MS.	Biosynthesis and stabilization	*Chlamydomonas reinhardtii*	Ag-NP. 15 nm.	(Barwal et al. 2011)
Amino acids	Glutamic acid. Identified using FTIR studies and GLC.	Capping	*Galaxaura elongate*	Au-NP. Spherical, few rods, hexagonal, triangular. 3.85-77.13 nm.	(Abd el-Raouf et al. 2017)
Protein	Amide-I and amide-II bonding. Identified using FTIR studies.	Capping agent	*Cystoseira baccata*	Prevent agglomeration of NP	(El-Kassas and El-Sheekh 2014; González-Ballesteros et al. 2017)
Polyphenols	Epigallocatechin Catechin and epicatechin gallate. Identified using FTIR studies.	Capping agent	*Galaxaura elongate*	Au-NP. Spherical, few rods, hexagonal, triangular. 3.85-77.13 nm.	(Abd el-Raouf et al. 2017)

Aromatic compounds/ alkanes/ amines	Primary and secondary amines. Identified using FTIR studies.	Capping agent	*Gelidiella acerosa*	Ag-NP. Spherical, 22 nm.	(Vivek et al. 2011)
Alcohol, Phenol, aliphatic amines, nitro-compound.	Identified using FTIR studies.	Capping agent	*Spirulina platensis*	AgO, Ag_2O-NP. Spherical, 14-48 nm.	(El-Sheekh et al. 2020 b)
Alcohol, carboxylic acid, amines, aliphatic amines, aldehydes.	Identified using FTIR studies.	Capping agent	*Spirulina platensis*	Au-NP. Triangular, pentagonal, octahedral. 15-77 nm.	(El-Sheekh et al. 2020 b)
Phenols and alcohols	Free O-H group. Identified using FTIR studies.	Capping and stabilizing agent	*Gracilaria corticate*	Ag-NP. Spherical, 51.82 nm.	(Kumar et al. 2013 b)
Soluble starch	Attachment to the hydroxyl group provides passiveness. Identified using FTIR studies.	Capping and stabilizing agent	*Phormidium tenue*	CdS and CdSe-NP. Spherical, 5 nm.	(Mubarak Ali et al. 2013)
Phenolic compounds, amines, aromatic rings	--	Capping and stabilizing agent	*Ulva lactuca*	Ag-NP. Spherical 48.59nm	(Kumar et al. 2013 a)
Thiocarbonyl derivatives	C=S stretches. Identified using FTIR studies.	Capping and tailing	*Chlorella vulgaris*	Au-NP Tetrahedral, icosahedral, and decahedral structure	(Castro et al. 2013)
Polysaccharide	Sulfonic group. Identified using FTIR studies.	Metal complexation	*Cystoseira baccata*		(González-Ballesteros et al. 2017)

Algal ETS	Present on thylakoid membrane. Identified by TEM.	Reducing agent	*Anabaena cylindrica*	Au-NP. Spherical, 10 nm.	(Rösken et al. 2016)
Polyphenols and polysaccharides	Hydroxyl functional group. Identified using FTIR studies.	Reducing agent	*Cystoseira baccata*	Spherical, polycrystalline NP Size- 8.4 nm	(El-Kassas and El-Sheekh 2014; González-Ballesteros et al. 2017)
Proteins	Amide -I. Identified using FTIR studies.	Reducing agent	*Lemanea fluviatilis*	Au-NP. Spherical, 35.8nm.	(Sharma et al. 2014)
Protein residue	Glutamic acid and aspartic acid carboxyl group and hydroxyl group of tyrosine. Identified by SEM and UV-Vis spectroscopy.	Reducing agent	*Oscillatoria sp. NCCU-369*	Ag-NP. Spherical, 80 nm.	(Zahoor et al. 2017)
Oxido-reductase, carbon-dependent rH_2.	Membrane bound enzymes. Identified by TEM.	Reducing agent	*Phormidium tenue*	CdS-NP. Spherical, 5 nm.	(Mubarak Ali et al. 2013)
Sulphated polysaccharide	Anionic disaccharides containing 3-lined-D-galactosyl residues flashing with 4-linked 3,6-anhydro-l-galactose and 6-sulfate residues. Identified using FTIR studies.	Reducing agent	*Porphyravietnamensis*	Spherical NP Size- 20 to 60nm	(Venkatpurwar and Pokharkar 2011; Chaudhary et al. 2020a)

NADH-based nitrate reductase	Reduces silver nitrate. Identified using FTIR studies.	Reducing agent	*Spirulina platensis*	AgO, Ag_2O-NP. Spherical, 14-48 nm.	(El-Sheekh et al. 2020 b)
ETS in thylakoids	Photosynthetic transport of electron on the thylakoid surface. Identified by TEM.	Reducing agent.	*Nostoc ellipsosporum*	Variable size and shape, 20-40 nm.	(Pari al et al. 2012)
Sesquiterpenes	Alloaromadendrene oxide. Identified using FTIR studies.	Reducing and stabilizing agent	*Galaxaura elongata*	Au-NP. Spherical, few rods, hexagonal, triangular. 3.85-77.13 nm.	(Abd el-Raouf et al. 2017)
Labdane diterpenoid lactone	Andrographolide. Identified using FTIR studies.	Reducing and stabilizing agent	*Galaxaura elongate*	Au-NP. Spherical, few rods, hexagonal, triangular. 3.85-77.13 nm.	(Abd el-Raouf et al. 2017)
APS-reductase and sulphite reductase	Reduction of sulphite to sulphide followed by electron transfer. Identified by SDS-PAGE.	Reducing and stabilizing agent.	*Chlamydomonas reinhardtii*	CdS-NP. Crystalline and spherical, 2-8 nm.	(Rao and Pennathur 2017)
Fatty acids	Hexadecanoic acid/ palmitic acid. Identified using FTIR studies.	Stabilizing agent	*Galaxaura elongate*	Au-NP. Spherical, few rods, hexagonal, triangular. 3.85-77.13 nm.	(Abd el-Raouf et al. 2017; Chaudhary et al. 2020 a)
Flavonoids	Gallic acid. Identified using FTIR studies.	Stabilizing agent	*Galaxaura elongate*	Au-NP. Spherical, few rods, hexagonal, triangular.	(Abd el-Raouf et

				3.85-77.13 nm.	al. 2017)
Algal LPS and reducing sugars	Prevent metal toxicity	Stabilizing agent	*Nostoc ellipsosporum*	Au-NP. Variable size and shape, 20-40 nm	(Pari al et al. 2012)

CHAPTER 3

COLLECTION OF MICROALGAE FROM DIFFERENT AREAS AND THEIR IDENTIFICATION

3.1 Introduction

Algae, tiny photosynthetic microorganisms are under investigation for commercial biofuels and biochemical production (Bharadvaja and Kumar 2020b). They comprise eukaryotes and prokaryotic cyanobacteria, also known as blue-green algae, capable of capturing atmospheric CO_2 and utilizing it to produce biomass more efficiently than plants. The capability of microalgae to accumulate large amounts of lipid bodies in the form of triacylglycerols (TAG) nominates them as efficient biofuel micro-factories (Lee et al. 2014)(Kumar and Bharadvaja 2020a). Applications of bioactive compounds of algal origin are now increasing for food, feed, fodder, fibre, cosmetics, nutraceutical and pharmaceuticals. Recent years have witnessed a major thrust moving towards a sustainable, biobased economy using a biorefinery concept. Biorefinery concept is based on obtaining a broad spectrum of products such as biodiesel, bioethanol, biogas, jet fuels and many value-added biobased products from renewable bioresources analogous to the petroleum refinery(Bharadvaja and Kumar 2020b). Microalgae have an inherent property of absorbing nutrients from the culture media, making it possible to use industrial and domestic wastewater as a nutrient source. This, in turn, also provides provisions for wastewater remediation (Chiellini et al. 2020).

Numerous algae and cyanobacteria have been isolated from diverse environmental locations and characterized for their potential application in biofuel production across the globe till date in order to provide an eco-friendly and economically sustainable and inexhaustible source of lipid, carbohydrate, protein along with many therapeutically active enzymes, pigments, sterols, and vitamins (Patel et al. 2022)(Zohir et al. 2022)(Ji et al. 2013)(Yu et al. 2012)(Wu et al.

2013)(Kim et al. 2015)(Song et al. 2014)(Shrivastav et al. 2010)(Kavitha et al. 2016)(Selvaraj et al. 2021). Algae can be a potential source of many bioactive compounds and thus has become potential and cost-effective raw material for the biorefinery. In a past few decades, numerous studies have been carried out to estimate the number of major macromolecules present in a diverse range of algae. Researchers have found that as many as 15000 novel compounds can be extracted from algal biomass. It has opened the door to a major untapped natural bioresource, low cost, an eco-friendly resource for valuable bioactive compound and biochemical production. Due to this proven ability, algae have become a major raw material for commercial biotechnological applications and pharmaceutical industries. Also, microalgae have been a potential bioremediator for wastewater treatment having an additional advantage in terms of utilization of its biomass for biofuels and value-added biochemical production(Kumar and Bharadvaja 2021). There is a huge scope for further investigation and exploitation of metabolites to be extracted from algal biomass and identification and validation of their pharmacological activities in the treatment and prevention of various diseases (Bharadvaja and Kumar 2020b).

The market for algal-based nutraceuticals and pharmaceuticals is growing as it has the ability to provide, irrespective of season and region for bioactive compounds of industrial importance. They do photosynthesis, very similar to plants, in the presence of light by sequestering Carbon Dioxide and water from their surroundings and produce biomass more efficiently and rapidly than terrestrial plants. They have been considered as a sustainable and carbon-neutral source, based on their fast growth rate, biomass productivity and compatibility for their conversion into different kinds of biofuels and biochemicals finding their application in food, feed, fodder, fertilizer, pharmaceuticals, nutraceutical and energy production. The objectives of this chapter were (i) isolation of microalgae and establishment of pure culture, (ii) identification of microalgae

on the basis of morphology and molecular studies (Fig. 3.1). The native microalgal species isolated were screened for biomass cultivation; as potential sources of essential phyco-compounds having nutraceutical values, microplastic, and lipids for bio-diesel.

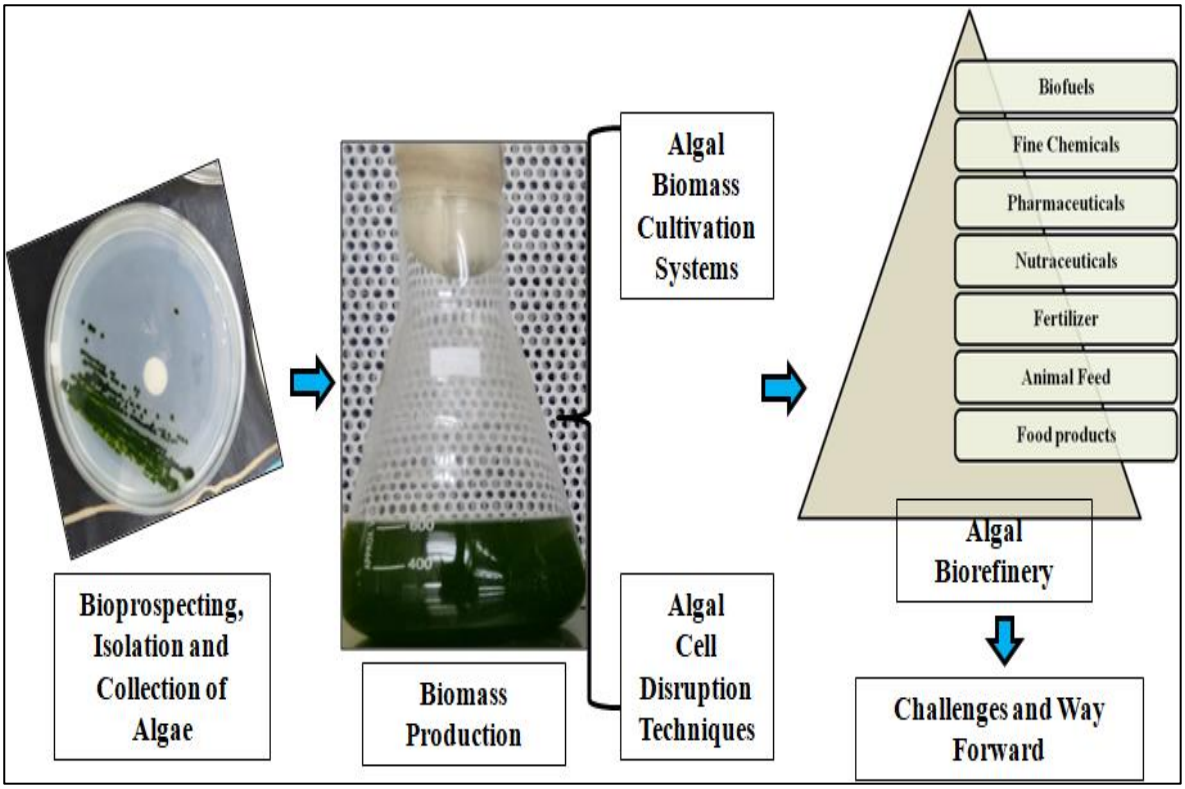


Figure 3.1: Bioprospecting microalgae for biofuels and biochemical production

3.2 Materials and methods

3.2.1 Sample collection and isolation of microalgae

The freshwater sample was collected from different sites for microalgae isolation. Microalgae containing sample were first filtered to remove solid and undesired particles by passing it through a filter paper followed by inoculated in BG-11 Medium (Kumar et al. 2017). This preparation was

incubated at in an artificial light chamber under illumination with a white fluorescent light on a photoperiod of 16L: 8D for growth to occur. The pH of the growth medium was kept 8.

After proper growth of microalgae was observed in the culture flask, a small volume was taken and spread onto BG11 Agar plate. The plate was kept at the same growth conditions. Isolated single colony was identified, picked up, and transferred onto a different agar plate. Colonies were sub-cultured repeatedly until a culture dominated by the pure strain was developed (Yun et al. 2020). The sub-cultured individual colonies were picked and transferred into the BG-11 medium and scaled up successfully. Thus, isolated and established pure culture were obtained. *Chlorella minutissima* was procured from Centre for Conservation and Utilisation of Blue Green Algae, IARI Delhi and cultivated in BG-11 medium for future applications.

The microalgal isolates were cultivated in BG-11 medium. The composition of media consists (gram/litre): ‘’Sodium nitrate ($NaNO_3$),1.5; ($K_2HPO_4.3H_2O$) 0.04; Magnesium Sulphate ($MgSO_4.7H_2O$), 0.075; Calcium Chloride ($CaCl_2.2H_2O$), 0.036; Citric Acid, 0.006; Ferric Ammonium Citrate, 0.006; EDTA (disodium magnesium salt), 0.001; Na_2CO3, 0.01; and one millilitre of A5 Solution which contains (gram/litre): H_3BO_3, 2.86; $MnCl_2.4H_2O$, 1.81; $ZnSO_4.7H_2O$, 0.222; $Na_2MoO_4.2H_2O$, 0.39; $CuSO_4.5H_2O$, 0.079; $Co(NO_3)_2.6H_2O$, 0.0494’’. The pH of medium was kept 8. All chemicals were of analytical grade and used as received.

3.2.2 Morphological Identification studies of microalgae isolates

Isolated microalgae cells were examined at different resolution under light microscope (10X, 20X, 40X, and 100X) and scanning electron microscope (5000X, 10000X, 20000X, and 50000X) for cell morphology examination (Kumar et al. 2017). SEM Microscope provides the morphological

details of the cells. This property is utilized for the characterization of the microalgae. SEM was performed for the determination of the size of the microalgae isolate cells. It scans solid samples with a beam of electrons, which interacts with the atoms that make up the sample to produce signals. These signals contain topographic information about the samples.

Fourier Transform Infrared (FTIR) spectroscopy is a highly advanced technique analytical tool. It can be used for rapid determination of the isolated microalgae. The FTIR is a non-destructive and rapid method. It helps in identification of the microalgae by providing vibrational structure of the cellular constituent materials which are species specific in nature. The IR spectrum of the microalgal sample was recorded under region 4000-400cm^{-1}. Analysis of composition, shape and intensity of absorption at specific wave numbers reveals information about functional groups and molecular structure that can then be used for identification (Vidyadharani and Dhandapani 2013).

3.2.3 Molecular Identification studies of microalgae isolates

For molecular identification of the microalgae isolates 18s rDNA molecular identification was performed. Isolated microalgae were grown in BG-11 medium in growth chamber under white fluorescent light. In their exponential phase, microalgae culture was collected for molecular identification studies. From this collected culture volume, DNA was extracted and its quality was evaluated on 1.2% agarose gel. A single band, of high molecular weight DNA, was observed against the ladder. The DNA was recovered from the gel and further processed for amplification. It was amplified with 18S rRNA Specific Primer (1Fand 4R) using Veriti®96well Thermal Cycler. A single discrete PCR amplicon band was observed. The PCR amplicon was subjected to enzymatic purification followed by Sanger Sequencing. Bi-directional DNA sequencing reaction of PCR amplicon was carried out with 1F and 4R primers using BDT v3.1 Cycle sequencing kit

on ABI 3730xl Genetic Analyzer. Consensus sequence of 863 (857 and 858) bp of 18s rDNA gene in SSU region was generated from forward and reverse sequence data using aligner software for algal isolate 1 (2 and 3 respectively). The 18s gene in SSU region sequence was used to carry out BLAST alignment search tool of NCBI GenBank database. Based on maximum identity score first Fifteen sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA softwarte.

3.3 Results and discussion

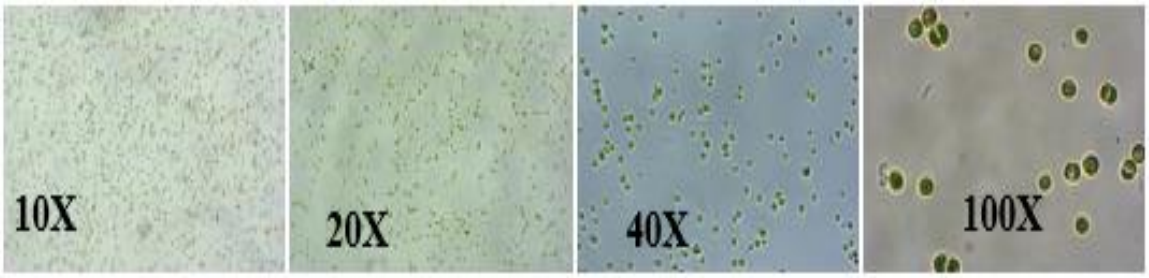
3.3.1 Collection and establishment of pure culture of microalgae

Three different microalgae were isolated, and their pure culture were established (Fig. 3.2). *Chlorella minutissima* was procured from CCUBGA, IARI, Delhi. All these four microalgae were cultivated in BG-11 suspension medium and used for morphological and molecular identification studies. The suspension culture was green in colour. The morphological features of the isolate have demonstrated its close similarity with green microalgae *Chlorella*(Ma et al. 2019)(Ani Azaman et al. 2017). In a recent study, Yun et al., reported isolation of *chlorella* sp., from an algal bloom and reported similar morphological characteristics(Yun et al. 2020).

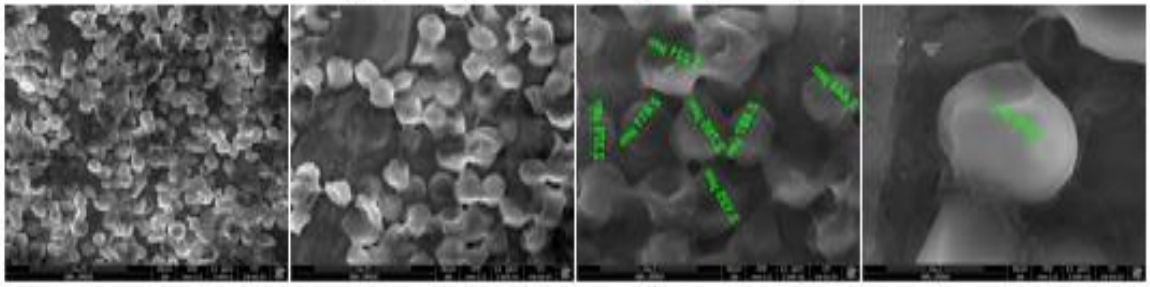
Figure 3.2: Master culture of all four microalgae (L to R: *Chlorella minutissima*, isolate 1 (D), isolate 2 (S), isolate 3 (R))

3.3.2 Identification of algal isolates: morphological and molecular studies

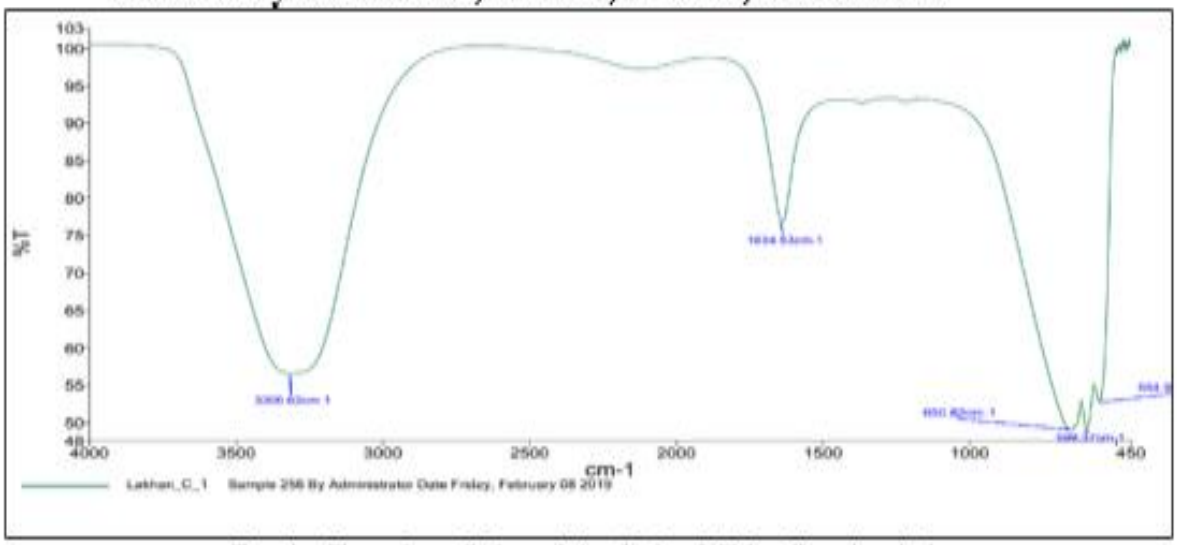
Pure cultures of algal isolates were examined under light microscope as well as scanning electon microscope. Colonies grown on BG-11 agar plates were green and spherical and the average cell size ranges between 2-6 µm when observed under microscope which were in agreement with studies reported in the past(Ma et al. 2019)(He et al. 2021)(El-Sheekh et al. 2020a). Also, the cells were spherical with as reported in our study. Scanning electron microscopic studies performed to find the effect of cultivation of C*hlorella* sp., under different culture conditions revealed the size of the cells varies from 2-6 µm with a spherical structure in appearence(Ani Azaman et al. 2017). Molecular identification based on 18S rDNA sequence revealed that these algal isolates showed closed similarity with *Chlorella* sp. GC (Accession Number: KF773743.1), *Chlorella sorokiniana* isolate 19-4 (Accession Number: KU948990.1), and *Chlorella sorokiniana* strain Icheon4 (Accession Number: KF864476.1) respectively. Morphological and molecular identification results (Figure (3.3- 3.12, and Table (2-4)) have been presented below:



Light Microscopy analysis at 10X, 20X, 40X, and 100X



SEM analysis at 5000X, 10000X, 20000X, and 50000X



Fourier Transform Infrared Analysis of *Chlorella minutissima*

Figure 3.3: Light Microscope image, Scanning Electron Microscope image, and FTIR spectra of *Chlorella minutissima*

3.3.2.1 FTIR spectroscopic analysis of *Chlorella* isolate

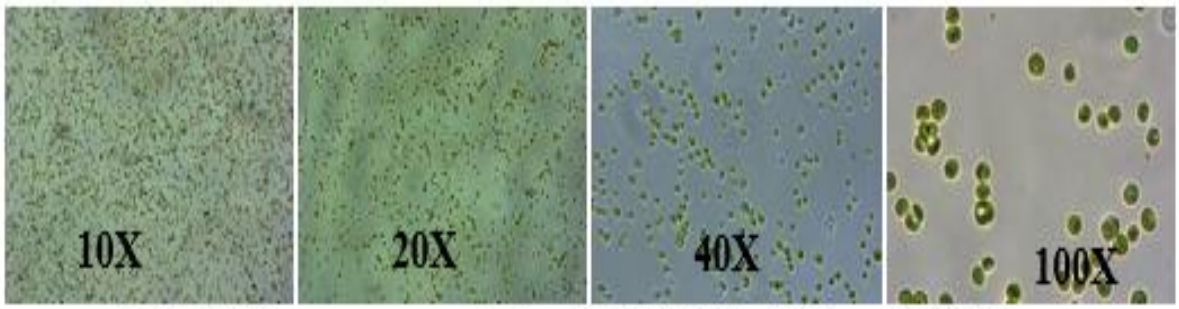
FTIR spectroscopy has been widely used to provide information on a range of vibrationally active functional groups including 'O–H', 'N–H', 'C=O', '=C–H',' –CH2',' –CH_3', 'C–O–C' and '

>P=O' in biological samples (Vidyadharani and Dhandapani 2013). Functional groups have their own characteristics spectra assigned to them (Table 3.1) and this spectra varies species to species and it is the basis for the characterisation of biological samples(Dean and Sigee 2006). In this study, several peaks for Wave number (cm^{-1}) vs % Transmittance were recorded for test microalgae *Chlorella minutissima* and other three microalgae isolate which shows presence of functional groups, based on the frequency peak measured, including Aliphatic groups: 400-800 cm^{-1}; Phenol and alcoholic group: 1000-1400 cm^{-1}; Carbonyl group: 1500-1700 cm^{-1}; Hydroxyl group: 3200-3400 cm^{-1}; Carbohydrate and Protein (1800-800 cm^{-1}); O-H and N-H stretching: 3700-3300 cm^{-1}. In a recent study on *Chlorella* sp., several functional groups were reported, alcohols and phenols (O-H stretching free hydroxyl: 3786-3351 cm^{-1}), nitrite (C-H stretching: 2924-2856 cm^{-1}), α,β unsaturated aldehydes ketones (C=O stretching: 1743-1647 cm^{-1}), aliphatic amines (C-N stretching: 1024-875 cm^{-1}), and alkanes (C-H stretching: 702-581 cm^{-1})(Narayanan et al. 2021).

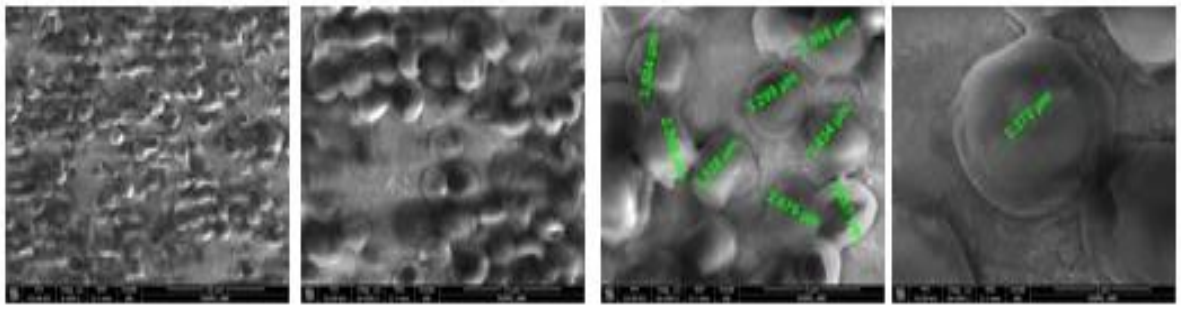
Table 3.1: Details of functional groups by FTIR analysis for *Chlorella* sp.

Frequency peak (cm^{-1})	**Typical bond**	**Functional groups**
3786-3351 cm^{-1}	O-H stretching free hydroxyl	Alcohols and Phenols
2924-2856 cm^{-1}	C-H stretching	Nitrite
1743-1647 cm^{-1}	C=O stretching	α, β unsaturated aldehydes ketones
1024-875 cm^{-1}	C-N stretching	Aliphatic amines
702-581 cm^{-1}	C-H stretching	Alkanes

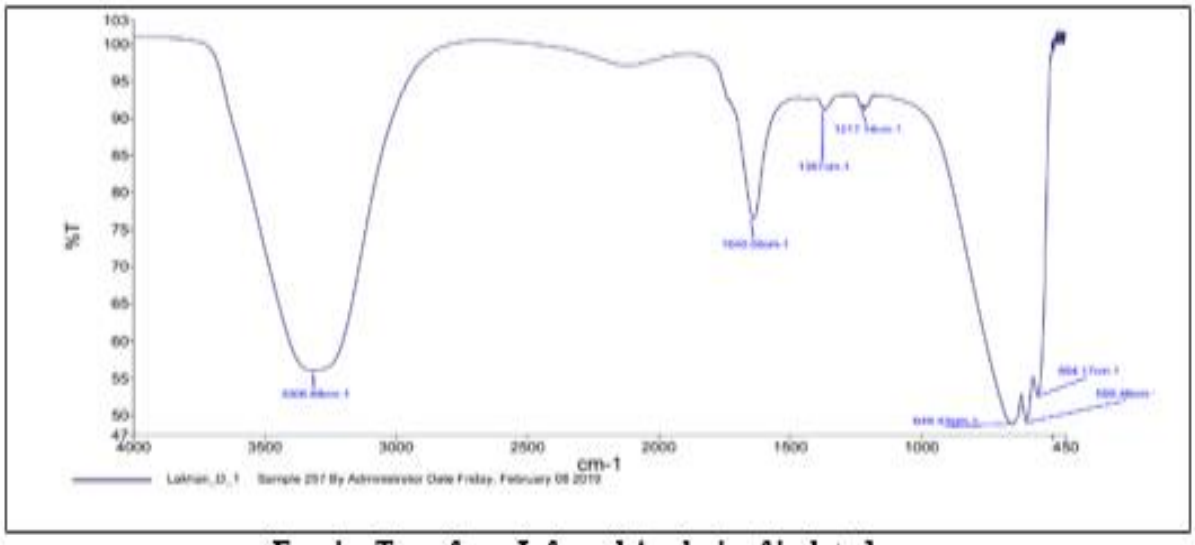
Morphological studies of isolate 1



Light Microscopy analysis at 10X, 20X, 40X, and 100X



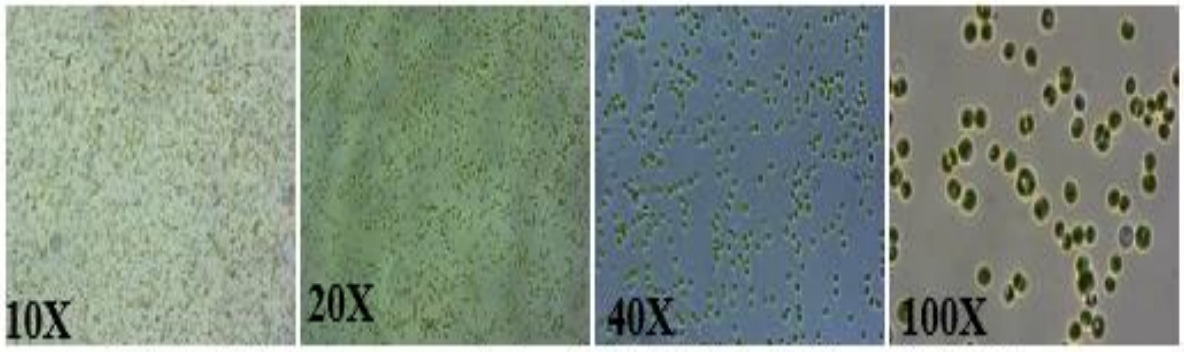
SEM analysis at 5000X, 10000X, 20000X, and 50000X



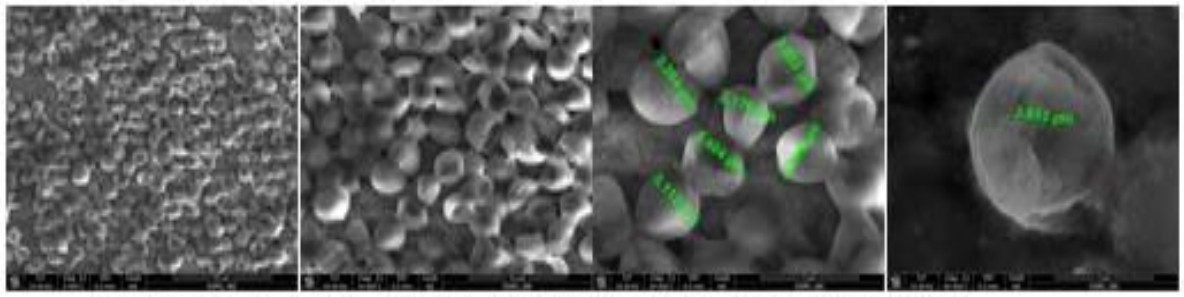
Fourier Transform Infrared Analysis of isolate 1

Figure 3.4: Light Microscope image, Scanning Electron Microscope image, and FTIR spectra of isolate 1

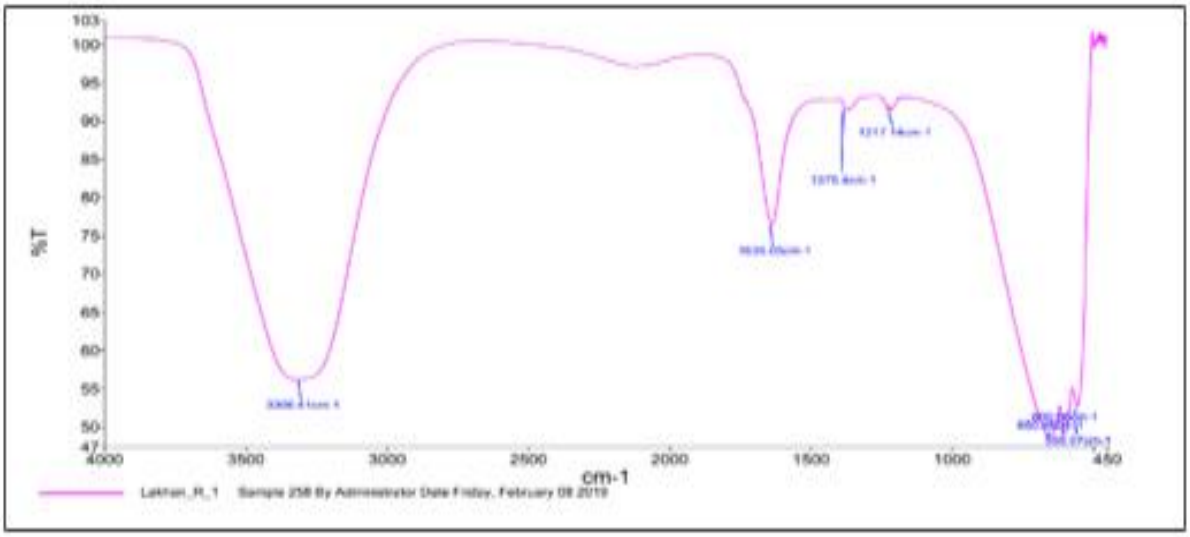
Morphological studies of isolate 2



Light Microscopy analysis at 10X, 20X, 40X, and 100X



SEM analysis at 5000X, 10000X, 20000X, and 50000X



Fourier Transform Infrared Analysis of isolate 2

Figure 3.5: Light Microscope image, Scanning Electron Microscope image, and FTIR spectra of isolate 2

Morphological studies of isolate 3

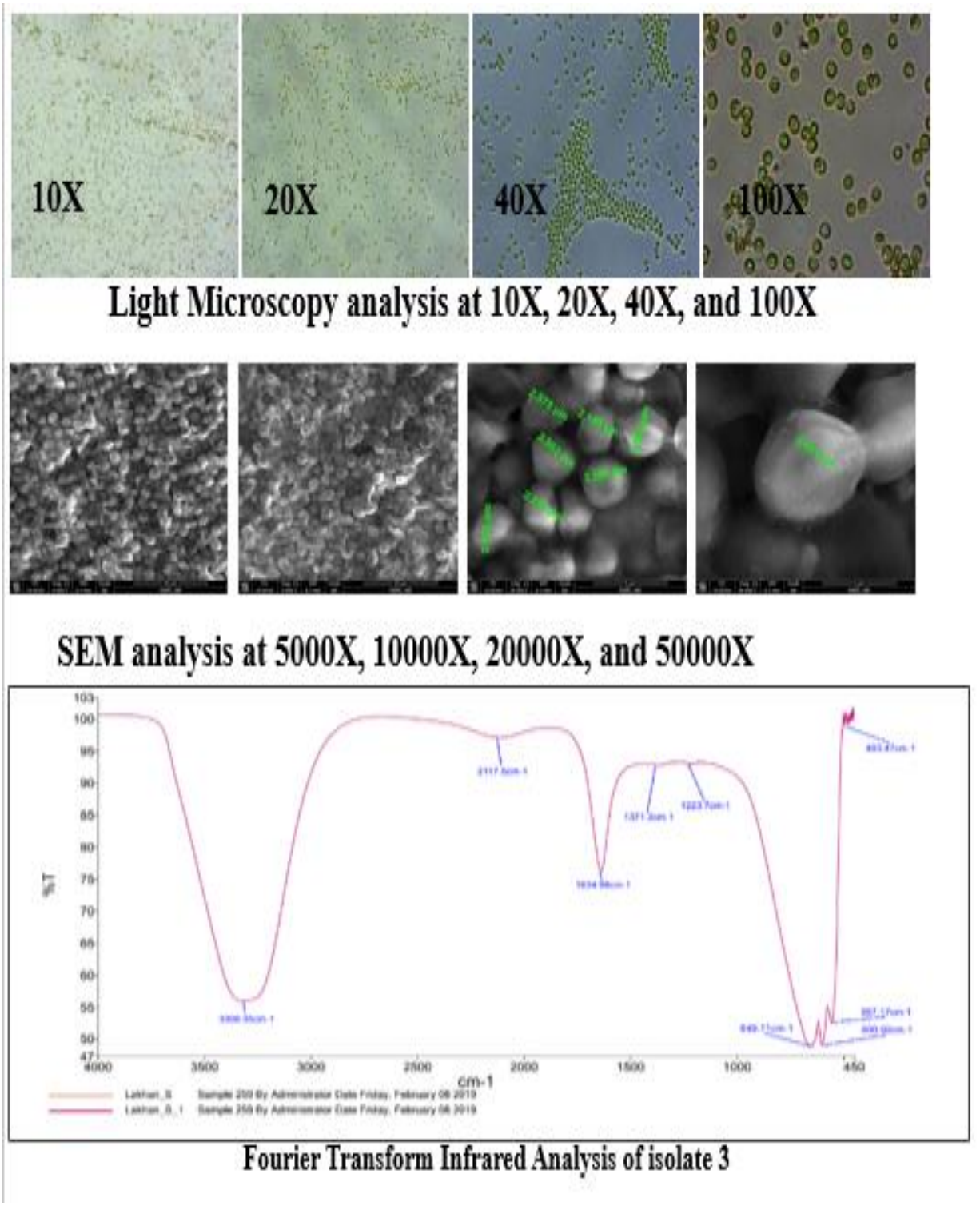


Figure 3.6: Light Microscope image, Scanning Electron Microscope image, and FTIR spectra of isolate 3

Comparison of light microscopic images of Chlorella minutissima and other three isolates

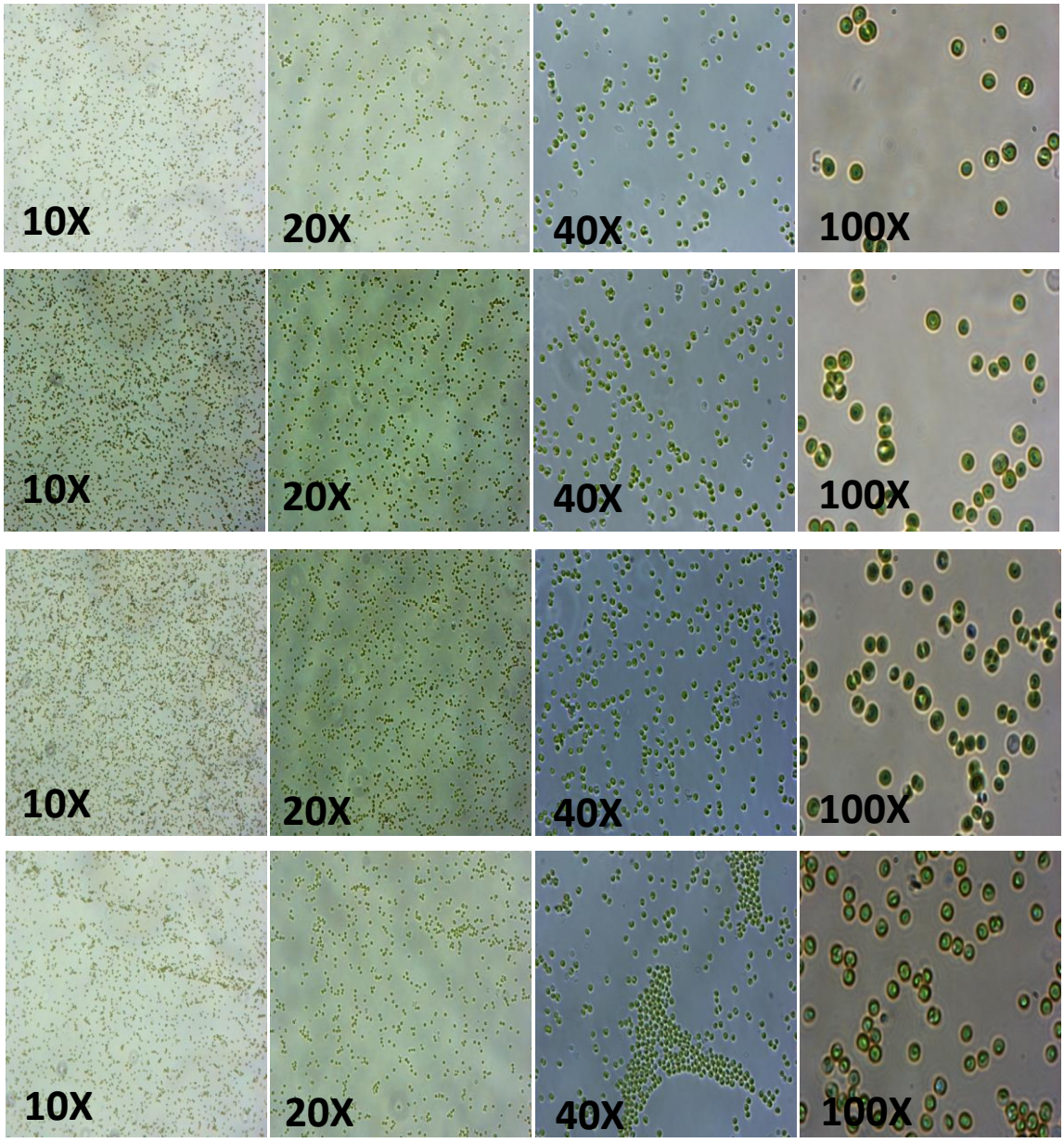
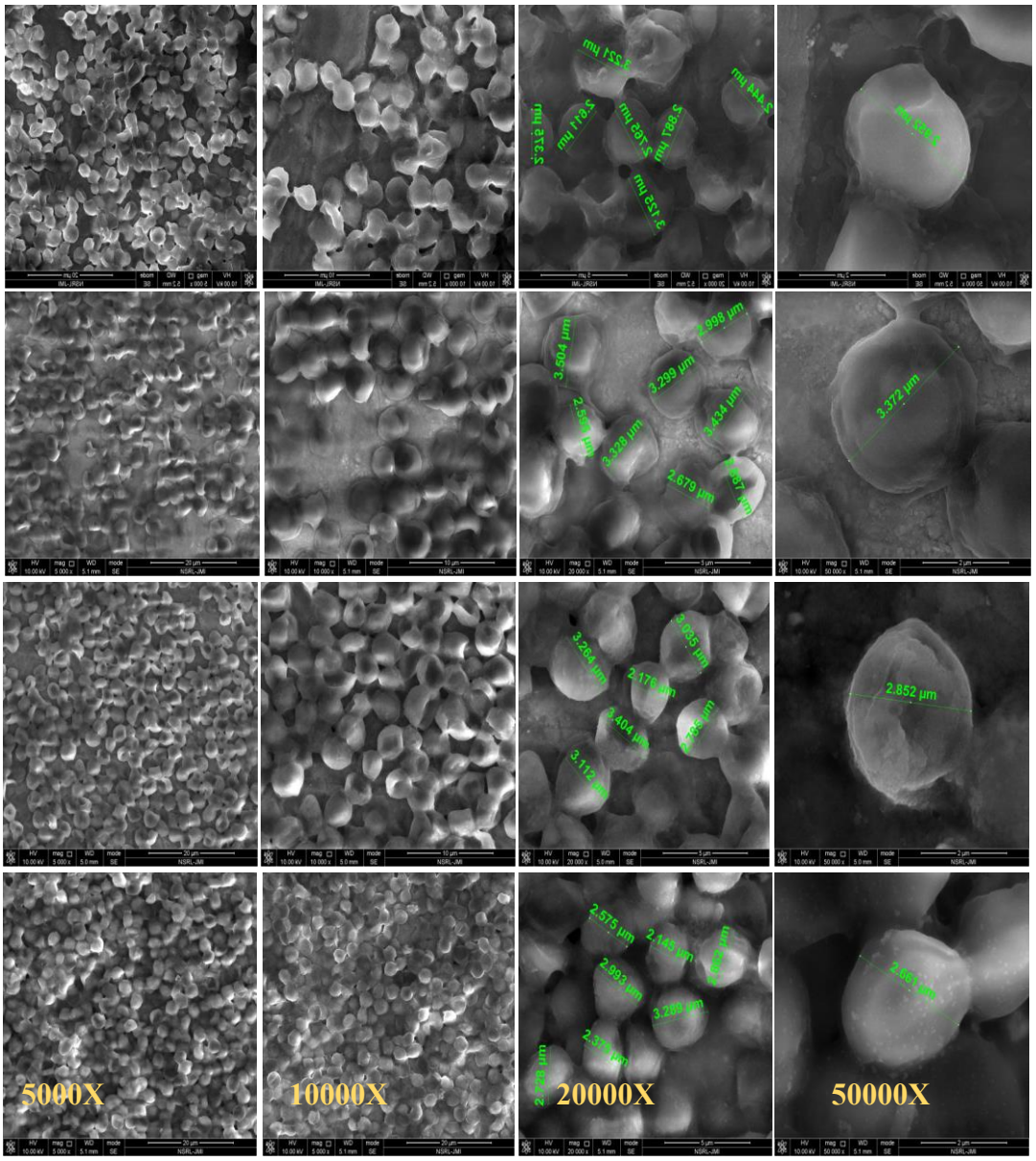


Figure 3.7 (a): All three microalgae isolates (isolate 1, isolate 2, and isolate 3) and *Chlorella minutissima* obtained from IARI Delhi were observed under light microscope at different resolutions (10x, 20x, 40x and 100x).

Comparison of SEM images of Chlorella minutissima and other three isolates



Chlorella minutissima

Isolate 1

Isolate 2

Isolate 3

Figure 3.7 (b): All three microalgae isolates (isolate 1, isolate 2, and isolate 3) and *Chlorella minutissima* obtained from IARI Delhi were observed under Scanning electron microscope at different resolutions (5000x, 10000x, 20000x and 50000x).

3.3.3 Molecular identification of algal isolates

Molecular identification of isolate 1

The 18s rDNA molecular identification revealed that the microalgae isolate is closely similar to *Chlorella* sp.

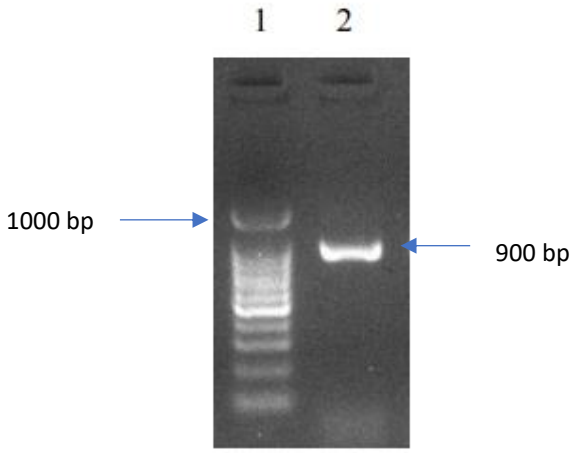


Figure 3.8: 1.2% Agarose gel showing 900bp amplicon (SSU region) of 18S rDNA. Lane 1: 1000bp DNA Ladder and, Lane 2: 900bp amplicon (SSU region) of 18S rDNA.

Table 3.2: Sequences producing significant alignments. Nearest relatives (Accession Number, % Identicity) observed in GenBank when BLAST was performed with microalgae consensus sequence. Based on maximum identity score first Fifteen sequences were selected and aligned using multiple alignment software program Clustal W

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
MH725912.1	Chlorella sp. DBRF-1	1576	1576	99%	0.0	99.77%
MK235183.1	Chlorella sorokiniana strain ACSSI 198	1572	1572	99%	0.0	99.65%
LC425389.1	Chlorella sorokiniana NIES-4216	1572	1572	99%	0.0	99.65%
KY054944.1	Chlorella sorokiniana strain NZmm3W1	1572	1572	99%	0.0	99.65%
KY303731.1	Chlorella sorokiniana voucher BR001 s	1572	1572	99%	0.0	99.65%
KU948990.1	Chlorella sorokiniana isolate 19-4	1572	1572	99%	0.0	99.65%
KR904895.1	Chlorella sorokiniana strain UTEX 1230	1572	1572	99%	0.0	99.65%
KR936170.1	Micractinium sp. IPOME-2	1572	1572	99%	0.0	99.65%
KM243322.1	Micractinium sp. KNUA032	1572	1572	99%	0.0	99.65%
KF773743.1	Chlorella sp. GC	1572	1572	99%	0.0	99.65%
KF673387.1	Chlorella sorokiniana strain SAG 211-31	1572	1572	99%	0.0	99.65%
KF879593.1	Chlorella sp. Sp12	1572	1572	99%	0.0	99.65%
KF879580.1	Chlorella sp. I5	1572	1572	99%	0.0	99.65%
KF879579.1	Chlorella sp. I4	1572	1572	99%	0.0	99.65%
KF879578.1	Chlorella sp. D1	1572	1572	99%	0.0	99.65%

Phylogenetic analysis

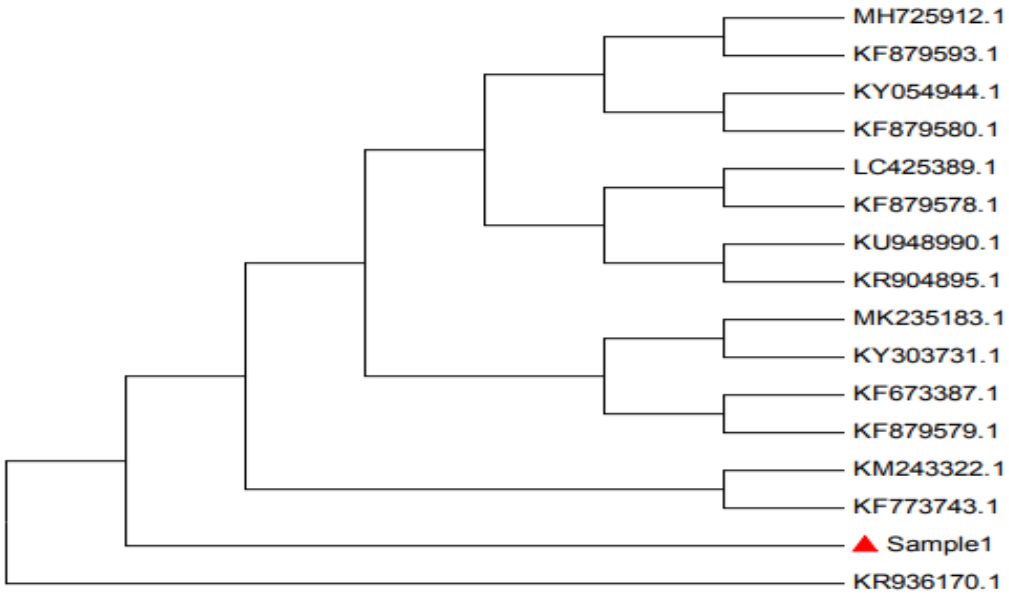


Figure 3.9: The evolutionary history was inferred using the Neighbor-Joining method. The phylogenetic tree was constructed using MEGA .

Molecular identification of Isolate 2

The 18s rDNA molecular identification revealed that the microalgae isolate is closely similar to *Chlorella sorokiniana.*

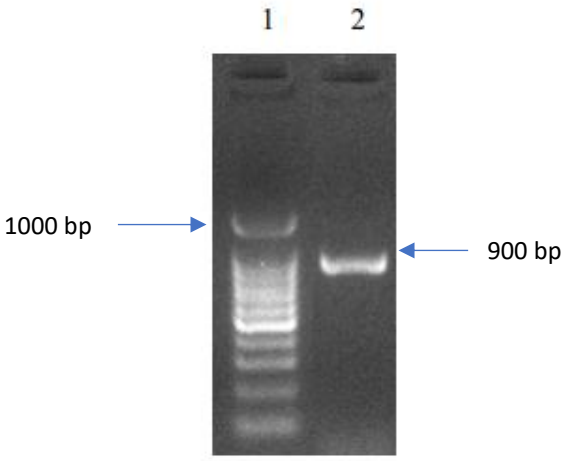


Figure 3.10: 1.2% Agarose gel showing 900bp amplicon (SSU region) of 18S rDNA. Lane 1: 1000bp DNA Ladder and Lane 2: 900bp amplicon (SSU region) of 18S rDNA.

Table 3.3: Sequences producing significant alignments. Nearest relatives (Accession Number, % Identicity) observed in GenBank when BLAST was performed with microalgae consensus sequence. Based on maximum identity score first Fifteen sequences were selected and aligned using multiple alignment software program Clustal W.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
MK235183.1	Chlorella sorokiniana strain ACSSI 198	1578	1578	99%	0.0	100%
LC425389.1	Chlorella sorokiniana NIES-4216	1578	1578	99%	0.0	100%
KY054944.1	Chlorella sorokiniana strain NZmm3W1	1578	1578	99%	0.0	100%
KY303731.1	Chlorella sorokiniana voucher BR001	1578	1578	99%	0.0	100%
KU948990.1	Chlorella sorokiniana isolate 19-4	1578	1578	99%	0.0	100%
KR904895.1	Chlorella sorokiniana strain UTEX 1230	1578	1578	99%	0.0	100%
KM243322.1	Micractinium sp. KNUA032	1578	1578	99%	0.0	100%
KF773743.1	Chlorella sp. GC	1578	1578	99%	0.0	100%
KF673387.1	Chlorella sorokiniana strain SAG 211-31	1578	1578	99%	0.0	100%
KF879593.1	Chlorella sp. Sp12	1578	1578	99%	0.0	100%
KF879580.1	Chlorella sp. I5	1578	1578	99%	0.0	100%
KF879579.1	Chlorella sp. I4	1578	1578	99%	0.0	100%
KF879578.1	Chlorella sp. D1	1578	1578	99%	0.0	100%
KF864476.1	Chlorella sorokiniana strain Icheon4	1578	1578	99%	0.0	100%
KF864471.1	Chlorella sorokiniana strain YeoJu4	1578	1578	99%	0.0	100%

Phylogenetic analysis

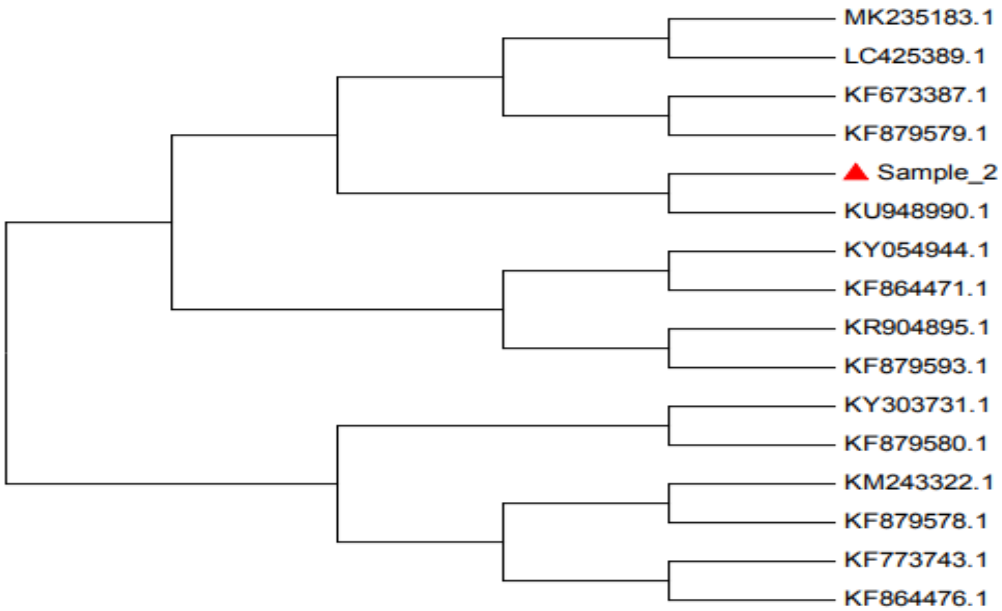


Figure 3.11: The evolutionary history was inferred using the Neighbor-Joining method. The phylogenetic tree was constructed using MEGA.

Molecular identification of Isolate 3

The 18s rDNA molecular identification revealed that the microalgae isolate is closely similar to *Chlorella sorokiniana.*

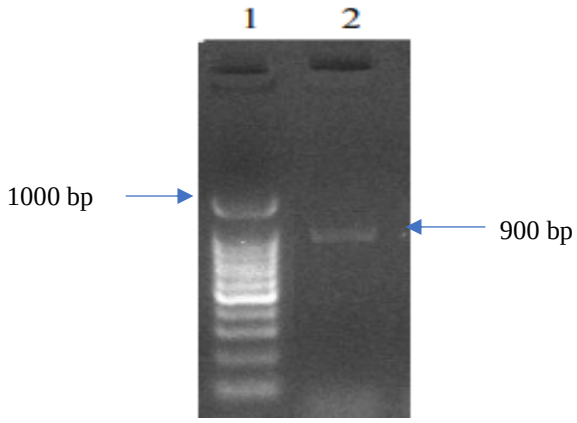


Figure 3.12: 1.2% Agarose gel showing 900bp amplicon (SSU region) of 18S rDNA. Lane 1: 1000bp DNA Ladder and, Lane 2: 900bp amplicon (SSU region) of 18S rDNA.

Table 3.4: Sequences producing significant alignments. Nearest relatives (Accession Number, % Identicity) observed in GenBank when BLAST was performed with microalgae consensus sequence. Based on maximum identity score first Fifteen sequences were selected and aligned using multiple alignment software program Clustal W.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
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LC425389.1	Chlorella sorokiniana NIES-4216	1574	1574	98%	0.0	99.54%
KY054944.1	Chlorella sorokiniana strain NZmm3W1	1574	1574	98%	0.0	99.54%
KY303731.1	Chlorella sorokiniana voucher BR001	1574	1574	98%	0.0	99.54%
KU948990.1	Chlorella sorokiniana isolate 19-4	1574	1574	98%	0.0	99.54%
KR904895.1	Chlorella sorokiniana strain UTEX 1230	1574	1574	98%	0.0	99.54%
KM243322.1	Micractinium sp. KNUA032	1574	1574	98%	0.0	99.54%
KF773743.1	Chlorella sp. GC	1574	1574	98%	0.0	99.54%
KF673387.1	Chlorella sorokiniana strain SAG 211-31	1574	1574	98%	0.0	99.54%
KF879593.1	Chlorella sp. Sp12	1574	1574	98%	0.0	99.54%
KF879580.1	Chlorella sp. I5	1574	1574	98%	0.0	99.54%
KF879579.1	Chlorella sp. I4	1574	1574	98%	0.0	99.54%
KF879578.1	Chlorella sp. D1	1574	1574	98%	0.0	99.54%
KF864476.1	Chlorella sorokiniana strain Icheon4	1574	1574	98%	0.0	99.54%
KF864471.1	Chlorella sorokiniana strain YeoJu4	1574	1574	98%	0.0	99.54%

Phylogenetic analysis

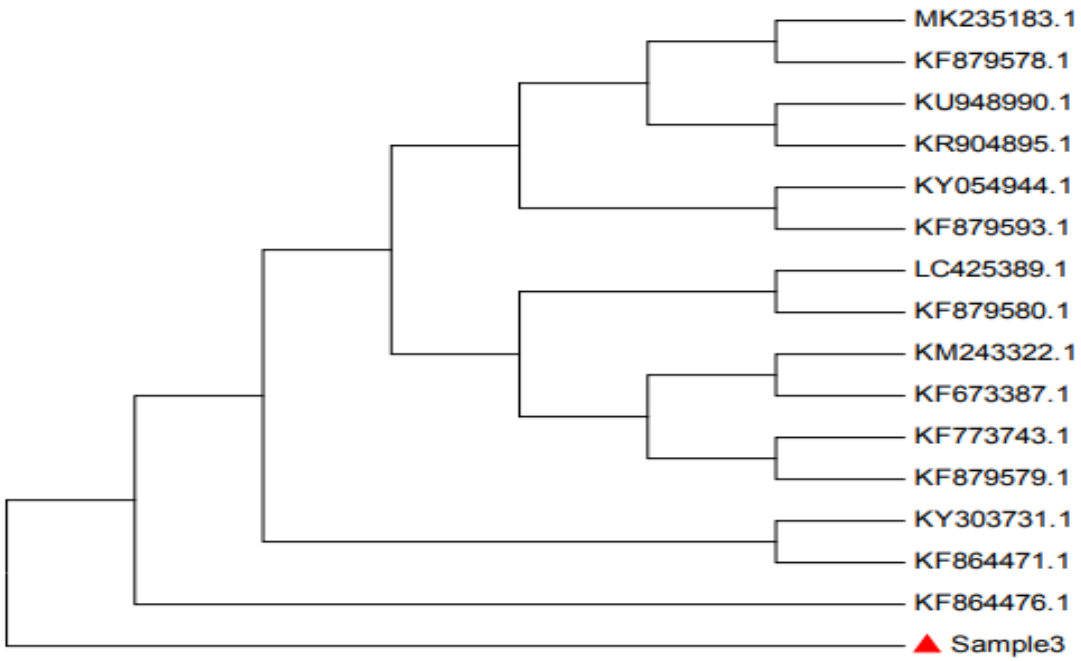


Figure 3.13: The evolutionary history was inferred using the Neighbor-Joining method. The phylogenetic tree was constructed using MEGA.

3.4 Conclusion

Three microalgae strains were isolated from different water resources. Isolated microalgae strains were grown on BG-11 agar plate at incubated at 27±2ºC in an artificial light chamber under illumination with a white fluorescent light at 4000 lux on a photoperiod of 16L: 8D. Colonies were green and spherical and the average cell size, for each isolate, ranges between 2-6 µm. Molecular identification based on 18S rDNA sequence revealed that these algal isolates showed closed similarity with *Chlorella* sp. GC (Accession Number: KF773743.1), *Chlorella sorokiniana* isolate 19-4 (Accession Number: KU948990.1), and *Chlorella sorokiniana* strain Icheon4 (Accession Number: KF864476.1) resoectively. *Chlorella minutissima* was procured and sub-cultured and

scaled up in the laboratory. Thus, based on morphology and molecular examination, microalgae isolated were identified as *Chlorella* sp. (D), *Chlorella sorokiniana* strain 1 (R), and *Chlorella sorokiniana* strain 2 (S). *Chlorella minutissima (cm)* procured from CCUBGA IARI, Delhi. Culture bank of all these four microalgae were established for the further use.

CHAPTER 4

OPTIMIZATION OF CULTURE CONDITIONS FOR BIOMASS AND LIPID PRODUCTIVITY FOR SELECT MICROALGA

4.1. Introduction

Microalgae are diverse group of prokaryotic and eukaryotic organisms ranging from unicellular genera such as *Chlorella* and diatoms to multi-cellular forms such as giant kelp, a large brown alga (50m in length). They are termed as sustainable and renewable source of biomass-based biofuel production due to high yield and low cost as compared to other feedstocks/terrestrial plants. Algal cell contains high amount of carbohydrates, proteins and lipids which can be converted into biofuels by using suitable conversion techniques. They are environment friendly; sequester CO_2 from the environment and fix it into biomass in presence of sunlight. It helps in reduction in other greenhouse gases. These photosynthetic microorganisms, grows 10-25 times faster than any other terrestrial plants. Also, they require less land as compare to other oil producing plants. In case of ethanol production, for producing same amount, microalgae require only 4% of land as compare to corn. The main advantage of algae-based biofuel is the biomass production from wastewater discharged by domestic and industrial sources as it grows on wastewater by utilizing nitrogen, phosphorous, and other elements present in it(Kumar et al. 2017)(Bharadvaja and Kumar 2020b)(Kumar and Bharadvaja 2020a)(Kumar and Bharadvaja 2021).

Microalgae based biodiesel production has been termed as a sustainable alternative to fossil fuels. In this direction, a range of process technologies involving microalgae cultivation followed by lipid extraction to its conversion into biodiesel have been established. But the entire algal cultivation to biodiesel production isn't economic and this its industrial level utility remains to be exploited. One of the major bottlenecks is low biomass yield. Overcoming these challenges are essential to economic and ecofriendly algal based biodiesel and biochemical production. Algal

cultivation primarily depends upon the availability of light i.e., when cultivated photo-autotrophically. Light transmittance reduces as the cell density increases in the culture medium thus dramatic reduction in algal growth and biomass productivity is observed. Under this condition, exogeneous supply of carbon sources have been reported to maintain the growth rate and gain maximum biomass productivity(Chai et al. 2018). Similarly, effect of several phytohormones, nitrogen and phosphorous sources have been reported to promote growth rate of algae as well as enhancement of the target biomolecule(Mostert and Grobbelaar 1987)(Kozlova et al. 2017)(Han et al. 2018)(Yaakob et al. 2021). Also, a variety of alterations in culture conditions including light intensity, light period, light color, pH of the medium, temperature, culture vessel, aeration and agitation, shaking period, inoculum size, etc., have been tested to increase the overall biomass and lipid productivity of the target microalgae(Kumar et al. 2017)(G et al. 2016)(Zhang et al. 2014)(Abedini Najafabadi et al. 2015)(Mondal et al. 2017a)(Ugwu et al. 2007)(Kazbar et al. 2019). For example, Increased frequencies of the light/dark cycles may considerably enhance productivity and photosynthetic efficiency of algae(Grobbelaar 2009).

The present study investigates the effects of different physico-chemical parameters including pH, temperature and light intensity for the growth of *Chlorella sorokiniana* and subsequently determination of lipid content of its biomass. Microalgae cultivation under heterotrophic mode to explore and establish novel strategies of increasing biomass productivity and target biochemical has brought new insight to sustainable commercial biodiesel production. To understand the relationship between exogenous nutrient supplementation and algal biomass productivity, the effects of different carbon, nitrogen, and phosphorous sources on the growth of *Chlorella sorokiniana* was carried out. In addition to this, the growth of *Chlorella sorokiniana* in spent or

reject water of reverse osmosis-based water purifier has been conducted in order to check its suitability for using it as mass scale cultivation medium for algal biomass production.

4.2 Materials and methods

4.2.1 Cultivation and growth medium, and microalgal growth analysis

The microalgae were cultivated in the BG-11 medium in a growth chamber at 27(±2) ºC temperature, and light intensity of 4000 lux for the 16h/8h light period for 12 days in a conical flask (Fig. 4.1). Flasks were manually agitated twice or thrice a day in order to ensure proper mixing and prevention of deposition of cells in the flask. The initial algal inoculums were having absorbance 0.455 at 690nm. The microalgal growth was analysed spectrophotometrically by measuring the absorbance at 690nm on a regular interval. Cultivation lasted for up to three weeks with samples being taken on a period of 48 hours. Samples were analysed on spectrophotometer as well as by calculating dry cell mass. The dry cell weight of the microalgal culture was estimated by taking a certain volume of culture and centrifuging it at 4000rpm for 10 minutes. Thus, separated biomass was dried in a hot air oven until the constant weight was achieved. Dry algal biomass powder was used for further experimental works. Optimization of culture conditions such as light (intensity), pH of medium, temperature for the optimum growth rate and biomass yield was carried out for select microalgae. Also, the effect of different carbon, nitrogen and phosphorous sources was determined on select microalgae growth and productivity.

4.2.2 Biochemical composition Determination: Lipid content determination by modified Bligh and Dyer method

For lipid estimation from microalgae cells a protocol developed by Kumar *et al*., was followed with a slight modification(Kumar et al. 2014b). A certain amount of dry microalgal biomass

powder (105 mg) was mixed with a certain volume (12ml) of Chloroform: Methanol mixture (1:2 v/v). Mixture was vortexed for 10 minutes and kept on incubation at 50ºC in an ultrasound bath for 30 minutes. Further, 4ml chloroform and 6 ml water was added and vortexed for 10 minutes. The final preparation was centrifuged at 5000rpm for 5 minutes. Collected the lipid containing chloroform layer in a pre-weighed glass vials and transferred in a hot air oven and allowed to dried overnight. Lipid content was calculated by subtracting the blank weight of vial (w_1) from the dried lipid containing glass vial (w_2). Weight ratio of lipid to the dried algal biomass was then determined by following formula:

$$\% \text{ lipid} = \{(w_2 - w_1/105) * 100\} \%$$

4.2.3 Elemental analysis: CHNS analysis

Simultaneous determination of major elemental components such as carbon, hydrogen, sulfur and nitrogen were carried out by combustion in CHNS analyzer. Algal dried biomass powder was used for the elemental analysis.

Figure 4.1: Microalgae culture established for experimentation work

4.3 Results and discussion

4.3.1 Microalgal growth analysis

The microalgal culture growth was analysed on a regular interval by measuring the absorbance at 690nm performed on a spectrophotometer. Growth chart was prepared and presented in Fig. 4.2.

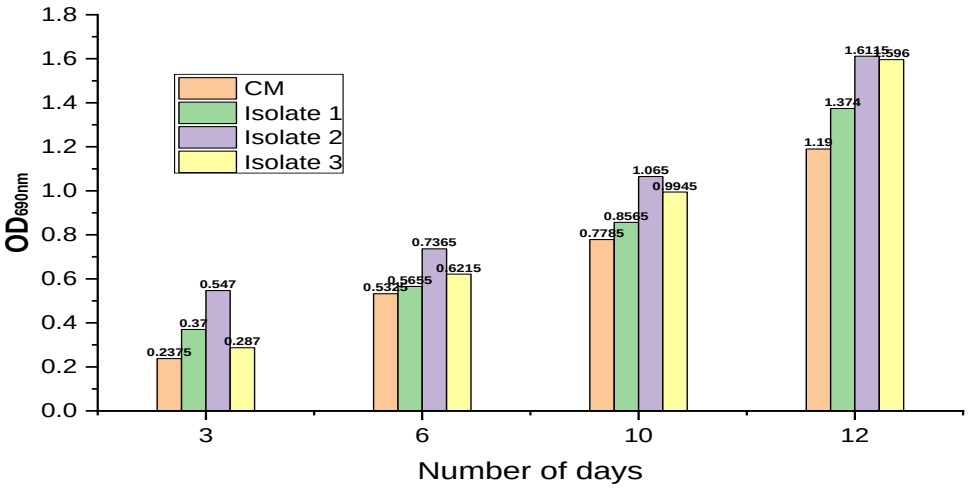


Figure 4.2: Growth characteristics of all four microalgae culture

4.3.2 Biochemical Composition determination of algal isolates: Lipid content

Microalgae culture thus isolated was then subjected to a process of lipid content estimation and elemental analysis. The biochemical constituents of the microalgal cell, lipid, was estimated by performing lipid extraction by Bligh and Dyer method as presented in **Table 4.1.**

Table 4.1: Biochemical composition: Lipid content (% dry cell weight)

S. No.	Biomolecules	*C. minutissima*	isolate 1	isolate 2	isolate 3
1.	Lipids	13%	10%	11%	10%

4.3.3 Elemental composition of microalgae cells: CHNS analysis

Elemental composition was determined by subjecting dried algal biomass powder to CHNS analyzer. C, H, N and S content of all four algal isolate was determined. Results were found in agreement with previously reported studies(Kumar et al. 2014c)(Rizzo et al. 2013)(Jabeen et al. 2020). Elemental composition in terms of C, H, N, and S for algal isolates have been presented in **Table 4.2**.

Table 4.2: Elemental composition of microalgae cells

	CM	Isolate 1	Isolate 2	Isolate 3
C	46	44	44	46
H	6	8	8	9
N	8	8	9	7
S	1	1	1	1
Others	39	39	38	37

Based on the cell composition and growth characteristics microalga isolate 2 was opted for the culture conditions optimization for optimum biomass and lipid production.

4.3.4 Optimization of culture conditions for optimum biomass production by microalgae isolate 2

Microalgae has been recognized as a potential feedstock for production of a range of biofuels and biochemicals and several processes to achieve this have been developed in recent times. There are several unit operations involved from algal strain selection to cultivation to biomass harvesting to extraction of target cell component or biomolecule of interest. The algal cultivation is one of the important steps of algal based biofuel and biochemical production. The algal biomass productivity

depends upon the culture conditions including availability of light, growth medium, pH of the medium, temperature, dissolved oxygen, aeration and many others. Algal biochemical composition's amount may be influenced and increased by changes in the surrounding environment. Here, we have optimized three culture conditions for *Chlorella sorokiniana* in order to get maximum growth rate and thus the optimum biomass concentration in the suspension culture.

pH: Microalga growth was observed under different pH regimes. pH ranges from 6 to 10 were used under white light with a light period of 16L: 8D at room temperature for the growth of microalgae. BG11 medium was prepared and pH was set and autoclaved before the inoculation.

Temperature: Four different temperature regimes 20(±1) ℃, 25(±1) ℃, 30(±1) ℃, 35(±1) ℃ and 40(±1) ℃ were provided to the microalgae culture and allowed to grow under white light with a light period of 16L: 8D at room temperature for the growth of microalgae isolate.

Light intensity: Five different light intensity regimes (3000, 4500, 6000, 7500, and 9000 in Lux) were tested for optimal growth of microalga isolate.

In this study, the most significant culture conditions including pH, temperature, and light intensity for *Chlorella sorokiniana* was optimized (Fig. 4.3, table 4.3). Alkaline pH was found more suitable for algal growth. The microalgae grow more rapidly at pH 8. There was no or less growth observed in growth medium at pH 5 and 11, 12. It was similar to previously reported studies conducted on *Chlorella sorokiniana*(Kumar et al. 2014c)(Goldman et al. 1982)(Kumar et al. 2014a). Most suitable temperature for microalgae growth was found to be 35(±1) ℃. There was no growth observed below 20°C for this microalga. The microalgae showed almost similar growth profile for the temperature range 30-40°C. It was in agreement with the previously reported

studies(Kumar et al. 2014c)(Cordero et al. 2011). Light intensity plays a significant role in algal growth. In our study, most suitable light intensity was 4500Lux for cultivation of this microalgae.

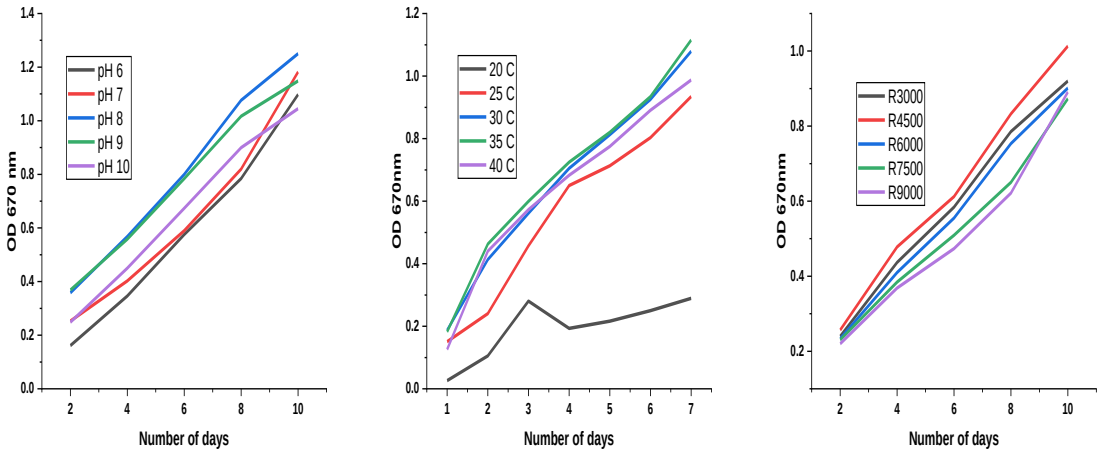


Figure 4.3: Optimization of culture conditions for optimum biomass production by microalgae isolate 2

Table 4.3: Culture conditions for optimum biomass production

S. No.	Optimum culture conditions	Isolate 2
1.	Light Intensity	4500 lux
2.	pH of medium	Alkaline (8)
3.	Temperature	35±1°C

4.3.5 Optimization of Carbon, Nitrogen, and Phosphorous sources for optimum biomass production by microalgae isolate 2

Algal isolate 2 was cultivated in 100ml BG-11 (pH 8, 4000 Lux, 16:08, 30(±2) ºC) supplemented with different carbon, nitrogen, and phosphorous sources under following conditions:

- Six different carbon sources (1g/l) including glucose, glycerol, sucrose, sodium acetate, sodium carbonate, and glycine (C1-C6)

- Four different nitrogen sources including ammonium chloride (1.5g/l), urea (0.75g/l), sodium nitrate (1.5g/l), and potassium nitrate (1.5g/l) (N1-N4)
- Four different phosphorous sources (0.1g/l) including potassium dihydrogen phosphate, disodium hydrogen phosphate, dipotassium hydrogen phosphate, and sodium dihydrogen phosphate (P1-P4)

In a recent study Chai *et al.*, characterize growth properties of *Chlorella sorokiniana* under carbon supplemented growth medium (Fig. 4.4). Glucose was found to have caused in 2-fold increase in OD_{750} value and 6fold increase in biomass productivity. Also, the lipid productivity increased by 12 times in glucose supplemented medium as compared to autotrophic cultivation mode(Chai et al. 2018). Zhang et al, reported stronger stimulatory effect of carbon supplementation on algal growth(Zhang et al. 2014). In this study, glucose was found to be causing optimal growth rate as compared to other tested carbon sources including glycerol, sucrose, sodium acetate, sodium carbonate, and glycine. Saxena et al., studied the influence of different carbon, nitrogen, and phosphorous sources on growth and productivity of *Chlorella minutissima* and found that glycerol as carbon source, sodium nitrate as nitrogen source, and disodium hydrogen phosphate as phosphorous source were best CNP sources for optimum growth of the microalga(G et al. 2016). For the optimum growth for Chlorella sorokiniana, sodium nitrate was found a suitable source while for potassium, potassium dihydrogen phosphate was found to cause optimal growth among tested nitrogen and phosphorous sources.

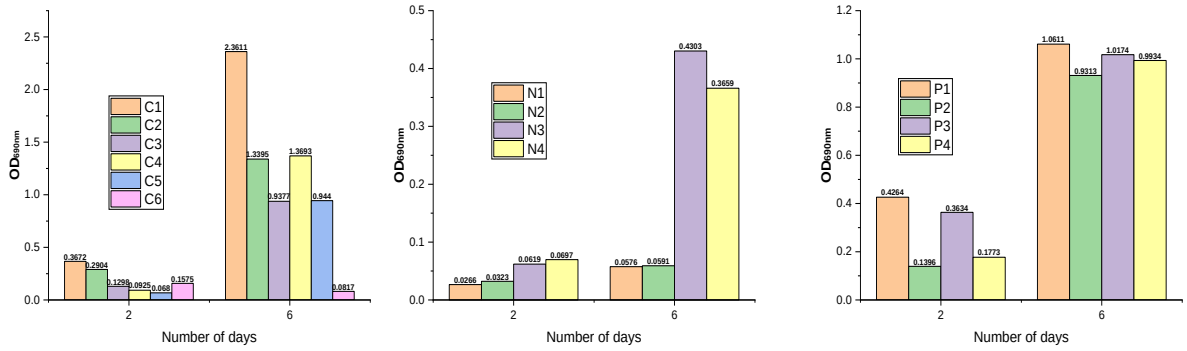


Figure 4.4: Optimization of Carbon, Nitrogen, and Phosphorous sources for optimum biomass production by microalgae isolate 2

Thus, the study concludes that the most suitable CNP sources among tested ones for the optimum growth of *Chlorella sorokiniana* are Glucose, sodium nitrate, and potassium dihydrogen phosphate

4.3.6 RO Spent water as a nutrient medium for algal cultivation

Algal based wastewater treatment and the biomass produced as a product of this process has been attracted a lot of scientific interest in recent times. Wastewater contains several organic and inorganic substances which are utilized by algae for their nutrient requirements. The wastewater thus can be used as an alternative to growth mediums for algal cultivation(Al-Jabri et al. 2021)(Yu et al. 2022)(El Ouaer et al. 2022). Spent water or reject water from reverse osmosis plants have been explored for algal biomass cultivation in order to tackle the problem of wastewater generation from reverse osmosis (RO) plants generally used for drinking water supply in households. The potential of RO reject water as cultivation medium has been presented in articulated manner elsewhere(Mamta et al. 2021). In this study, RO spent water collected from a domestic water cooler was collected and stored for its utilization as nutrient medium for cultivation of select microalgae. The RO Spent water characteristics was measured through HACH- Multimeter and noted.

RO Spent water characteristics: Parameter Analysis of RO Spent water using HACH-Multimeter and presented in **Table 4.4.**

Table 4.4: RO Spent water characteristics

Parameter	Obtained Values
Nitrate (NO_3^-)- Nitrogen	6.71 mg/L
pH	8.4
TDS	549 mg/L
Conductivity	1113 µs/cm
Salinity	0.55%
Total Ammonia	196 mg/L

Experiment details: Inoculum size 5%; Total medium – 100ml, Glucose (1g/l) supplemented BG-11 medium for the cultivation of microalgae;

Four different set ups of RO spent water was prepared:

(i) RO 0%- RO 0ml+ Bg-11 100ml;
(ii) RO 25%- RO 25ml + BG-11 75ml;
(iii) RO 50%- RO 50ml+ BG-11 50ml; and
(iv) RO100%- RO 100ml + BG-11 0ml

For growth measurement OD was taken at 690 nm for both *Chlorella minutissima* and *Chlorella sorokiniana.*

The optical density of the culture was reached to 1 in a span of two days of cultivation. RO spent water, wastewater, can be a potential nutrient source for algal cultivation. RO 50% and RO 25% provided highest growth for the microalgae *Chlorella minutissima* and *Chlorella sorokiniana.* RO 50% and RO 25% have comparable growth characteristics (Fig. 4.5). Alone RO spent water

also found to have potential for algal cultivation. In a recent study which tested the efficiency of microalga *Chlorella pyrenoidosa* to utilize the RO reject water as growth or nutrient medium reported (1.27-1.37) g/L of biomass production with (25-50)% RO reject water supplemented growth media. The biochemical constituents mainly lipid, were also increased in RO supplemented growth medium as compared to the BG-11 alone. The study concluded that RO reject water from drinking water purifiers can efficiently be utilized for lipid-rich microalgal biomass production(Bhandari and Prajapati 2022). In another study by the same group, the drinking water purifier reject water was used as cultivation medium for four different microalgae, *Chlorella sorokiniana*, *Scenedesmus obliquus*, *Scenedesmus* sp., and one native microalgae strain. Among all four tested microalgae, *Scenedesmus obliquus* was reported to have highest biomass productivity (84.38±2.3 mg $l^{-1}d^{-1}$) while *Chlorella sorokiniana* has the highest lipid productivity (19.37±1.04 mg $l^{-1}d^{-1}$)(Bhandari et al. 2023). Besides, microalgae were able to remove nitrate (up to 78%) and phosphorous (up to 83%) from the RO reject-based growth media. These findings suggest that the dependency on freshwater resources for mass scale microalgae cultivation through recycling of RO reject can be reduced.

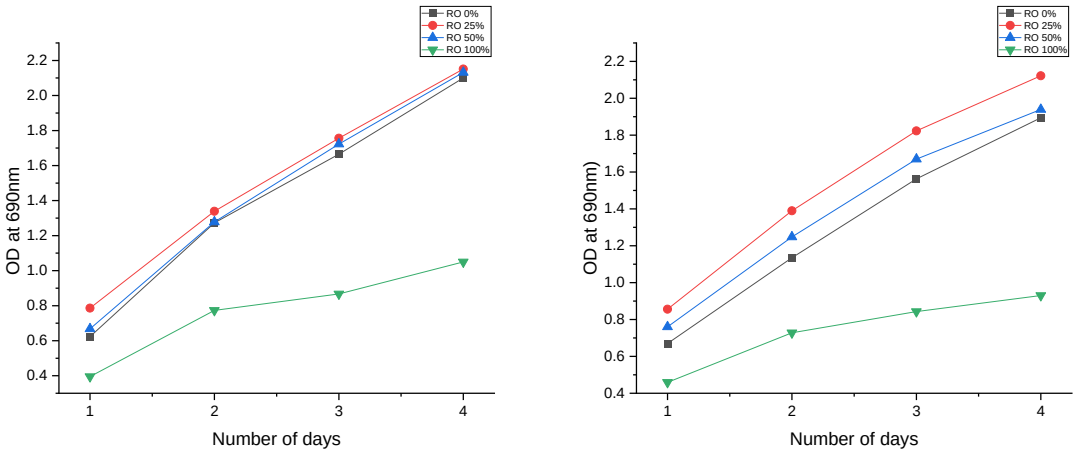


Figure 4.5: Growth pattern of *Chlorella minutissima* and *Chlorella sorokiniana* over four days on different proportions of BG-11 and RO spent water

4.4 Conclusion

Highest growth rate was observed for microalga isolate 2 on the basic of absorbance taken on a UV-Vis spectrophotometer. Lipid content of microalga *Chlorella sorokiniana* (isolate 2) was determined to be approximately 11% of dry algal biomass powder. Elemental analysis revealed the content of C, H, N and S to be 44%, 8%,9% and 1% respectively for microalga isolate 2. Optimization of culture conditions including light intensity, pH, and temperature was carried out. The best suitable light intensity, pH, and temperature was 4500 lux, pH 8, and 35ºC for optimal growth of microalga isolate 2. The microalga showed almost similar growth profile in alkaline pH range 7 to 9. No visible growth at pH 5, 11,12. Also, the microalga isolate exhibited similar growth profile for the temperature range (30-40) ºC. Most suitable carbon, nitrogen, and phosphorous (CNP) sources among tested ones were found to be glucose, sodium nitrate, and potassium dihydrogen phosphate for microalga isolate. RO spent water can be used as a nutrient source for algal cultivation for *Chlorella minutissima* and microalga isolate 2.

CHAPTER 5

BIOPROSPECTING MICROALGAE FOR THE PRODUCTION OF LIPID AND β - CAROTENE

5.1. Introduction

Microalgal biomass has contributed to the economy as a sustainable source of biofuels, feedstock, biopharmaceuticals, nutraceuticals, cosmeceuticals, and so on, making it a preferred industrial choice for production. The strain *Graesiella emersonii*, despite having a high potential for industrial exploitability, has been subjected to a knowledge gap of less taxonomical and biochemical information availability. This study explored an axenic unicellular green microalga isolated from a highly nutritive cement water open pond in India for its morphological, molecular, and biochemical characteristics. Literature supports the combined production of lipid and β-carotene, a high-value product from microalgae, through equipped biorefinery procedures. Prior studies reported the strains *G. emersonii* as inhabitants of freshwaters only. However, a recent study by Kang *et al.*, revealed the strain distribution over other habitats, including soil, brackish waters, and waterbodies of varied salinities(Kang et al. 2022a).

The rapid adaptability that microalgae have towards biotic and abiotic stresses accounts for the production of secondary metabolites. These compounds having unique chemical structures are the defense factors against the reactive oxygen species and damages that are caused to the microalgal cell in response to such stresses. The inter- and extra-thylakoid β-carotene are responsible for protecting algal cells from irradiation-induced damage. Despite having a short lifetime, the pigment quenches damaging oxygen species and excited chlorophyll molecules. Algae having highly accumulated β-carotene is resistant to photoinhibition when blue light (having wavelength absorbable by pigment) is induced on them.

β-carotene, a non-polar carotenoid, is readily soluble in non-polar solvents. Hence, hexane, and ethyl-acetate are preferred extraction solvents(Rivera and Canela 2012). Other solvents like ethanol and methanol find application in extraction because they affect cell wall permeability(Lucini et al. 2012). The cell walls of different algal strains vary in their polysaccharide conditions and hence, it is important to optimize the cell disruption and extraction strategy. The method of extraction, and extraction solvent if applicable, is also dependent on the nature of the compound targeted. Literature also states that the number of bioactive compounds in a particular strain also depends on the harvesting season(Garcia-Vaquero et al. 2021). Hence, to recover the best yields of a particular phyco-compound from the strain, there is a need for a monitored culturing pattern and harvesting season, along with strategized extraction technique.

The green microalgae, *Graesiella emersonii*, isolated and tested in this study have reported high amounts of lipid, nominating it as an efficient resource for biofuel production(Santhana Kumar et al. 2022). The FAME analysis of biodiesels produced from *G. emersonii* strains have Iodine Value (IV) and Cetane index (CN) values somewhat outside the standard range ($IV \leq 120$ and $CN \geq 47$). Thus, it is a good blend with fossil diesel for efficient automotive fuels(Kousoulidou et al. 2010). Recently, France has approved the use of *Graesiella emersonii* as a food supplement, and other European countries, including Austria, Germany, and Switzerland, use *Graesiella emersonii* as raw food supplements(European Commission, Joint Research Centre, Araújo, R., Peteiro 2021). Hence, other countries might see the use of *G. emersonii* as a food supplement in the near future. The altogether objective of this study was to report the estimated and recovered highly economical carotenoid, β-carotene, along with its anti-oxidant property, which otherwise is limited information in the scientific literature. The β-carotene content in algal cells majorly depends on the growth conditions and nutrient availability in the medium, a set of experiments

have been performed to assay the effect of light and salt stress on the accumulation of the pigment. This chapter facilitates the methods of bioprospecting microalgae for lipid and β-carotene production for economically sustainable algal based biofuel and biochemical production.

5.2. Materials and Methods

5.2.1. Bioprospecting microalgae: Collection, isolation and identification of microalgae

Microalgae containing fresh water sample was taken from an industrial cement pipe curing tank. After passing it through a filter paper, to remove solid and undesired particles, a certain volume of this filtrate was taken and transferred into BG-11 culture medium (pH 8) in a culture flask. It was placed in an incubator chamber at and allowed to grow. After a week, when growth of microalgae was prominent, a small volume of this preparation was taken and spread over on BG-11 agar plates to isolate microalgae. Colonies were grown on the agar plate. A single individual colony of the isolate is needed for the establishment of pure culture. Colonies were taken from the agar plate and transferred to another aseptically until single individual cell-based colony was achieved. morphological and molecular identification of the isolated microalgae strain was performed. Light microscopic observation was exercised for the morphological identification of the isolate. For molecular identification, 18S rDNA gene molecular identification was performed.

5.2.2 Cultivation condition of microalgal isolates

The isolated strain was cultivated in BG11 medium for growth. The culture was incubated at temperature 25±2°C, under white fluorescent light (4000 lux) and a photoperiod of 16:08. For growth measurement, the sample from pure culture was inoculated in a fresh media, and observed by UV spectrophotometer at 690nm for 12 days. The growth curve was prepared for the identification of a suitable time period for biomass harvesting. The biomass was harvested using a

centrifuge (5000 rpm for 10 mins). Thus, harvested biomass was dried in a hot air oven and stored for future studies.

5.2.3 Lipid estimation for *Graesiella emersonii*

105 mg dry *Graesiella emersonii* biomass powder was mixed with a certain volume (12ml) of Chloroform: Methanol mixture (1:2 v/v). Mixture was vortexed for 10 minutes and kept on incubation at 50ºC in an ultrasound bath for 30 minutes. Further, 4ml chloroform and 6 ml water was added and vortexed for 10 minutes. The final preparation was centrifuged at 5000rpm for 5 minutes. Collected the lipid containing chloroform layer in a pre-weighed glass vials and transferred in a hot air oven and allowed to dried overnight. Lipid content was calculated by subtracting the blank weight of vial (w_1) from the dried lipid containing glass vial (w_2). Weight ratio of lipid to the dried algal biomass was then determined by following formula:

$$\% \text{ lipid} = \{(w_2 - w_1/105) * 100\} \%$$

5.2.4 β-carotene extraction for *Graesiella emersonii*

A certain amount of dry biomass powder (100mg) was dissolved in 10ml of extracting solvents in a capped glass bottle. Acetone: Hexane (1:4) was used as extracting solvent. The solvent and algal biomass powder was vortexed for 10 minutes and kept in a water bath at 70ºC for 30 minutes. The mixture was allowed to cool at room temperature. To above mixture, 3.3ml of KOH (5% in Methanol) was added. This mixture was further kept in water bath at 70ºC for 30 minutes. After 30 minutes, the mixture was taken from water bath and allowed to cool at room temperature. Two separate layers were developed at the end (upper layer consisting β-carotene). The upper layer containing β-carotene was transferred in a glass bottled and stored for HPLC analysis. The UV-

Vis spectrophotometer reading of upper phase was observed at 450nm. Quantification of β-carotene from algal sample was done using the following formula.

Total β-carotene (µg/ml) = $25.2*A_{450}$

5.2.5 Qualitative and Quantitative estimation of β-carotene extraction

A standard curve for HPLC grade β-carotene purchased from Sigma Aldrich was prepared in 1:2 v/v Hexane-Ethanol. For quantification, the extracts from algal biomass were subjected to UV-Vis spectrophotometry (Lambda 365, Perkin Elmer). Pure Beta-carotene gives a peak at 450 nm. Quantification of beta-carotene from the algal sample was done using the following formula:

Total beta-carotene (µg/ml) = $25.2*A_{450}$

For qualitatively determining the presence of pigment in the selected sample, paper chromatography and thin-layer chromatography were performed. The pigment standard for reference and the algal extract was prepared in 95% ethanol. The mobile phase selected for paper chromatography was 9:1 v/v petroleum ether: acetone. Thin Layer Chromatography of beta-carotene standard and the algal extract was performed with 3:7 v/v Acetone-hexane mixture as mobile phase. Saponification of the algal extract was first carried out before performing High-Pressure Liquid Chromatography (HPLC) using acetonitrile as solvent(Aluç et al. 2018)·(Kumar et al. 2014d).

5.2.6 Enhancement of β-carotene content in microalgal cells through abiotic stresses

The algal culture was subjected to light and salt stress in order to check the influence of abiotic factors on the accumulation of β-carotene. Biomass growth and pigment accumulation were assayed at three different light intensities, 4000 Lux in a light incubator having 16:8 light-dark

photoperiod; continuous 10,000 Lux using artificial white light; and 50,000 Lux from direct sunlight, respectively. The strain was also subjected to salt stresses by adding 10% v/v NaCl for 0.1M, 0.2M, 0.4M, 0.6M, 0.8M, and 1.0M in the BG-11 medium, and 0M as control. The samples were isolated at regular intervals and checked for their β-carotene levels.

5.3 Results and Discussion

5.3.1 Isolation, selection, and identification of potential microalgae isolate

A pure culture of the isolated microalga was established in BG-11 medium. The colonies were grown onto the solid surface of the Bg-11 agar plate. The color of the colonies was green and they were spherical in shape. The isolated microalgae cells were examined under light microscope. The morphology was circular as seen under the microscope. The colonies initially are dark green (Fig.5.1) in color similar to the liquid media culture and are a vigilantly growing species. However, they turn brown after 10-12 days of culture inoculation.

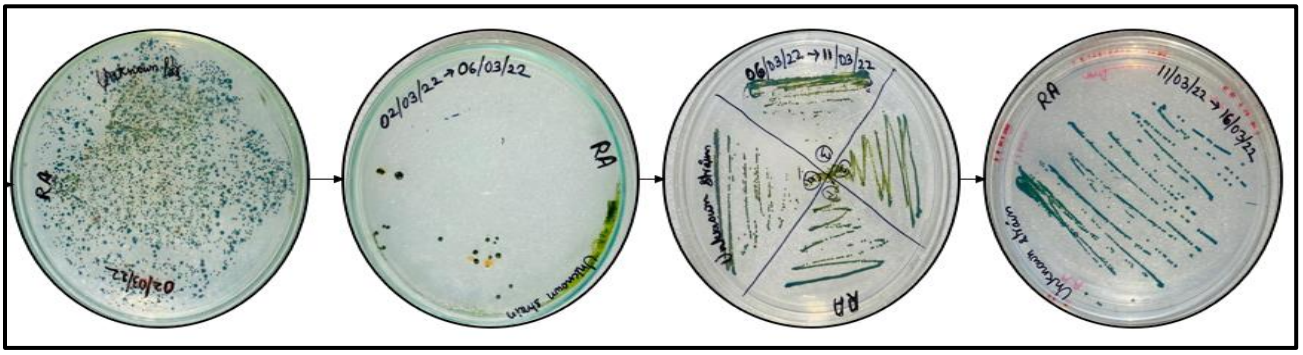


Figure 5.1: Sample isolation and establishment of pure culture achieved through serial dilution and sub-plating on BG11 Agar plates

5.3.2 Morphological examination

The slides were prepared and viewed under the light microscope at 20X and 40X magnification. The cells are prominently visible with the organelles stained. They have morphological resemblance with the scientifically described structures. The microscopic observation of the isolated microalgae indicated the close resemblance with the green microalga (Fig.5.2). As the culture aged, a color shift from green to orangish-red was observed. This is due to the accumulation of intracellular lipid bodies in this rapidly growing strain(Wen et al. 2016).

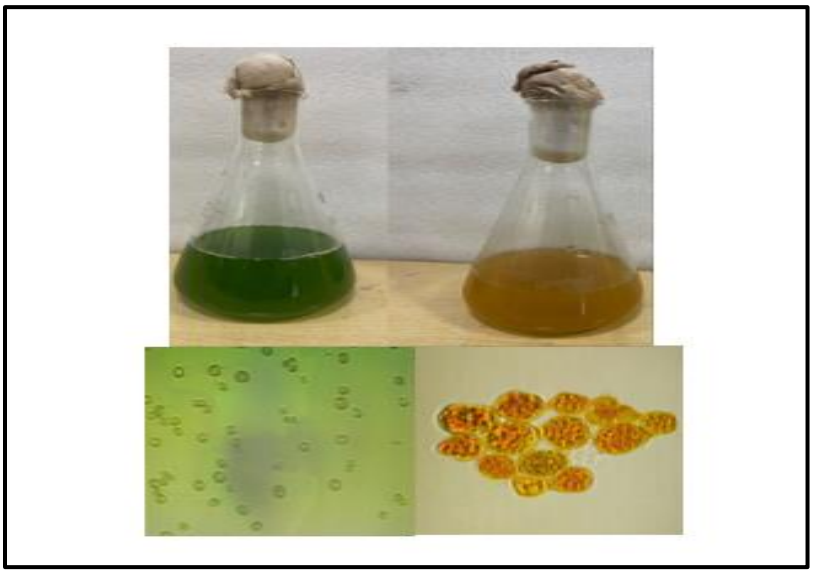


Figure 5.2: The observable colour shift of 7-day-old culture from green to reddish-orange, confirmed under a light microscope

5.3.3 Molecular Identification of strain

The genomic DNA of the sample was isolated using the phenol-chloroform method. The purity of extracted genetic matter was confirmed using NanoDrop 2000 Spectrophotometer. PCR was run, which gave a single ~700 bp PCR product of the 18S rDNA gene. The gene sequence was used to carry out BLAST in the nucleotide database. Based on the identity score, the top 15 sequences

(reported in Table 1) were selected and aligned. Later, the neighbor-joining phylogenetic tree was constructed using the software MEGA 11 (Fig.5.3, Table 5.1).

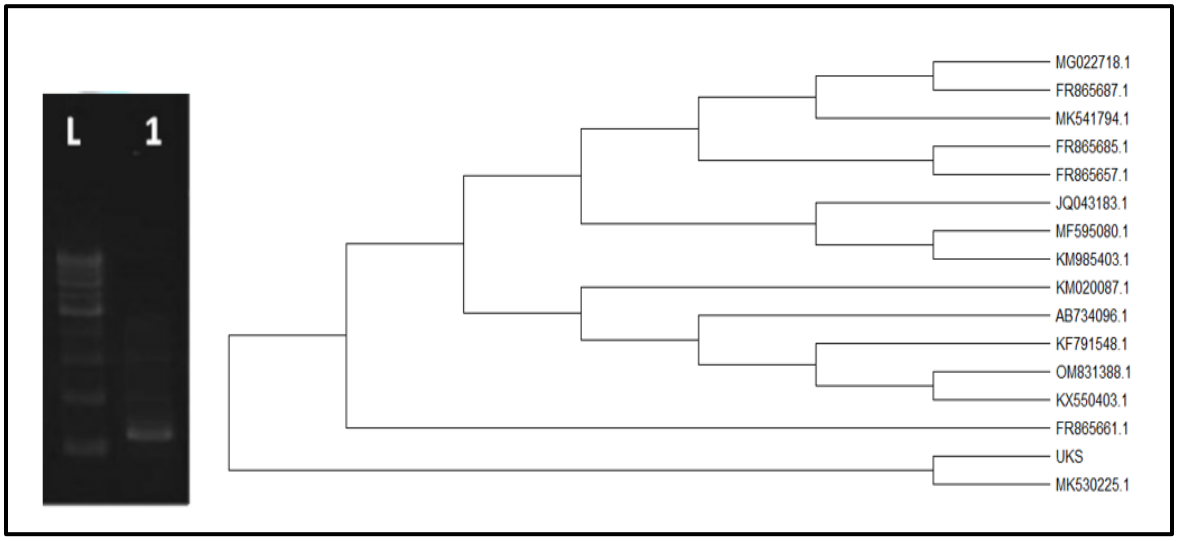


Figure 5.3: a) 1.2% agarose gel run of the PCR product showing 700 bp amplicon, with Lane L as the 500 bp DNA ladder and Lane 1 as the 700 bp amplicon product and phylogenetic analysis .

Table 5.1 The list of top 15 nearest relatives, having sequences producing significant alignments as observed through NCBI BLAST

Accession	**Description**	**Max Score**	**Total Score**	**Query Cover**	**E value**	**Per. ident**
MK541794.1	Graesiella emersonii strain CCAP 211/11N	948	948	100%	0	99.43
MK530225.1	Graesiella emersonii isolate JS04	948	948	100%	0	99.43
MF595080.1	Asterarcys sp. SA-1702	948	948	100%	0	99.43
MG022718.1	Graesiella emersonii isolate CCAP 211/8H	948	948	100%	0	99.43
KX550403.1	Coelastrella sp. Z2	948	948	100%	0	99.43
KM985403.1	Chlorella sp. QUCCCM36	948	948	100%	0	99.43

OM831388.1	Coelastrella sp. isolate S3A	948	948	100%	0	99.43
KM020087.1	Coelastrella sp. SAG 2471	948	948	100%	0	99.43
KF791548.1	Scenedesmus sp. JB11	948	948	100%	0	99.43
AB734096.1	Scenedesmus sp. Ki4 gene	948	948	100%	0	99.43
JQ043183.1	Asterarcys quadricellulare strain KNUA020	948	948	100%	0	99.43
FR865687.1	Graesiella emersonii genomic DNA 211/8P	948	948	100%	0	99.43
FR865685.1	Graesiella vacuolata genomic DNA 211/8C	948	948	100%	0	99.43
FR865661.1	Chlorella emersonii genomic DNA 211/15	948	948	100%	0	99.43
FR865657.1	Chlorella emersonii genomic DNA 211/11M	948	948	100%	0	99.43

5.3.4 Growth curve of *Graesiella emersonii*

Morphological and molecular studies confirmed that the isolated microalgae have maximum similarity with *Graesiella emersonii.* A study conducted by reported the similar morphology for the *Graesiella emersonii*(Kang et al. 2022b). The growth curve (Fig.5.4) of the strain was prepared to access its growth at regular intervals through UV-Vis spectrophotometry at 690 nm wavelength. The graph was plotted as shown below. The OD for culture was recorded till the value reached 1.

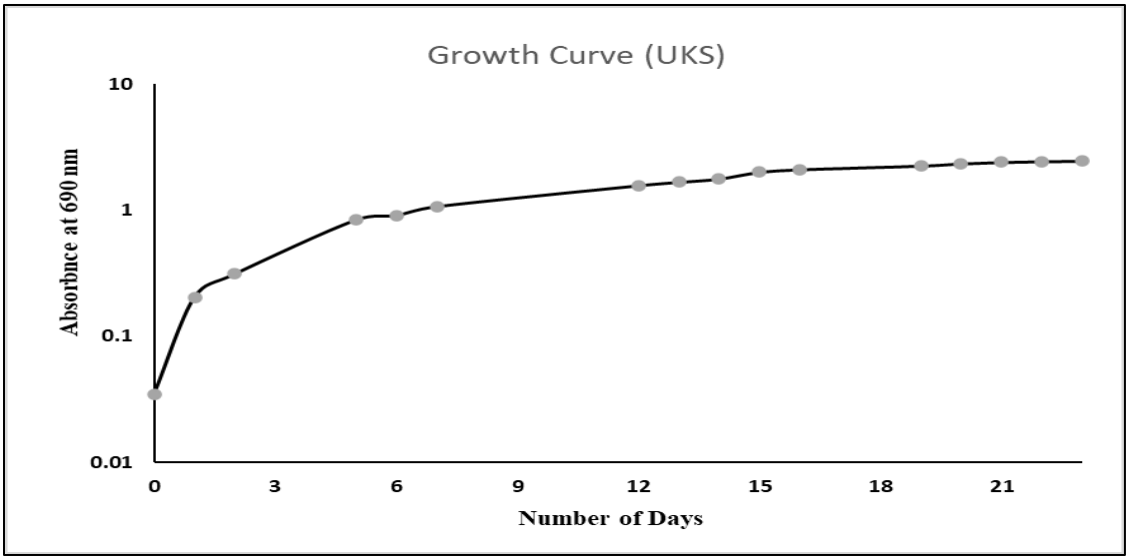


Figure 5.4: Growth curve of *Graesiella emersonii*, entering the late exponential phase in 7 days

Since the strain enters the stationary phase after 7 days, as depicted in the growth curve (Fig. 5), the strain may be identified as rapid growing. The color shift in the stationary phase can be attributed to the accumulation of lipid bodies and other high value pigmented products. The re-inoculation of the orangish-red culture is also red for the initial days and sometimes turns green. This could be due to the growth and division of young cells and them taking over the media. Literature and handling in such conditions suggest that it's important to re-inoculate the strain while its green to preserve the strain.

5.3.5 Lipid estimation for *Graesiella emersonii*

Weight ratio of lipid to the dried algal biomass was then determined by following formula: % lipid $=\{(w_2-w_1/105) *100\}$ %

Lipid content in *Graesiella emersonii* was estimated following Bligh and Dyer method with a slight variation (Fig.5.5, Table 5.2).

% Lipid content= 24.43% of dry cell weight

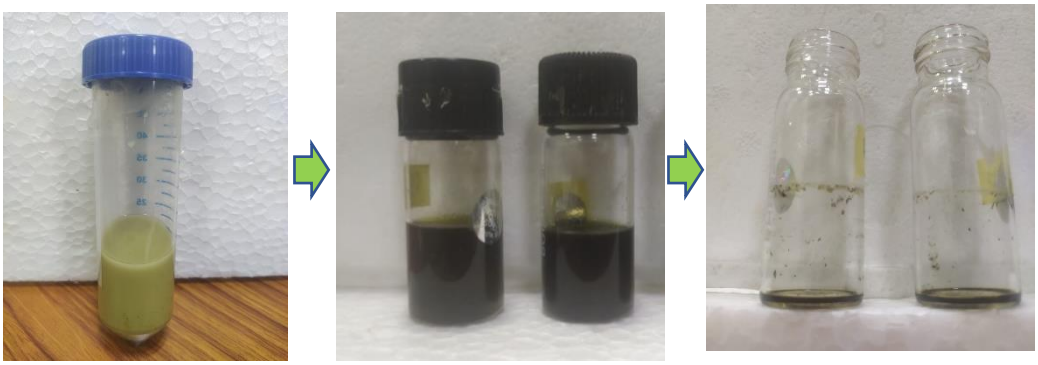


Figure 5.5: Algal biomass + Solvent mixture, Lipid containing chloroform layer, Algal lipid

Table 5.2: Lipid estimation from *Graesiella emersonii*

Amount of dried biomass	**Blank (w_1) mg**	**Lipid + Blank (w_2) mg**	**Lipid ($w_2.w_1$) mg**	**Lipid content {(w_2-w_1/105) *100} %**
105 mg	13083.3	13109.2	25.9	24.66%
105 mg	13470.8	13496.2	25.4	24.19%

In the present study, *Graesiella emersonii* grown in BG-11 medium under 4000Lux at 16:08 light period at 28±2º C contained lipid approximately 24.5% of cell dry biomass. In a recent study, phytohormones were added into growth medium to maintain the growth rate under nitrogen limiting conditions. They reported 2.3- and 2.5-fold increase in biomass and lipid yield for *Graesiella emersonii* compared to nitrogen limited conditions. The study also reported that the addition of phytohormones increased the lipid productivity up to 1.6-fold as compared to standard condition i.e., when microalgae was grown in BG-11 medium only. The lipid content was approximately 30% of total dry cell weight(Mandal et al. 2020). So, addition of phytohormones can be a strategy for enhancement of lipid in isolated microalgae to make it a suitable feedstock

for economic sustainable biodiesel production. The microalgal cells turns from green to red after 7 days of inoculation when cultivated in BG11 media under same culture conditions. This can be due to the accumulation of carotenoids in the cells in the stationary phase. A recent study reported a range of carotenoids including chlorophyll a, chlorophyll b, α-carotene, β-carotene, neoxanthin, lutein and zeaxanthin in the *Graesiella emersonii* cells(Kang et al. 2022b). They reported 0.84 mg g^{-1} DW of β-carotene in *Graesiella emersonii* cells. In another study, the amount of β-carotene in three different *Graesiella emersonii* strains were reported to be 0.08 to 1.15 mg/g of dry cell weight(Paliwal et al. 2016). We investigated for the presence of β-carotene in the microalgal cells as it is a high value compound of industrial importance and its co-extraction with lipid from the isolated microalgae would open the avenue for possible potential use of an easy growing comparatively sturdy strain of *Graesiella emersonii* for sustainable algal biofuel and biochemical production.

5.3.6 Qualitative and Quantitative Analysis of extracted β-carotene

The standard of beta-carotene from Sigma Aldrich was dissolved in 1:2 v/v Hexane-Ethanol, and absorbance was measured at 450 nm **Fig 5.6**. The readings were plotted on a graph for reference calibration curve (R^2=0.99). This graph will work as the standard reference for the determination of effective concentration with respect to absorbance at 450 nm. The amount of β-carotene was calculated by using following formula (Table 5.3):

Total β-carotene (μg/ml) = $25.2*A_{450}$

Table 5.3: β-carotene estimation from *Graesiella emersonii* strain

Samples	A_{450}	β-carotene(µg/ml) = 25.2*A_{450}	β-carotene (µg/ml)	β-carotene (µg/mg dry algal biomass)[#]
S1	0.319	25.2*0.319	8.038	0.643
S2	0.322	25.2*0.322	8.114	0.649
S3	0.329	25.2*0.329	8.291	0.661

[#]Considering 100% efficient extraction

The approximate β-carotene was estimated to be 0.652 mg/g of dry algal biomass powder.

Qualitative analysis

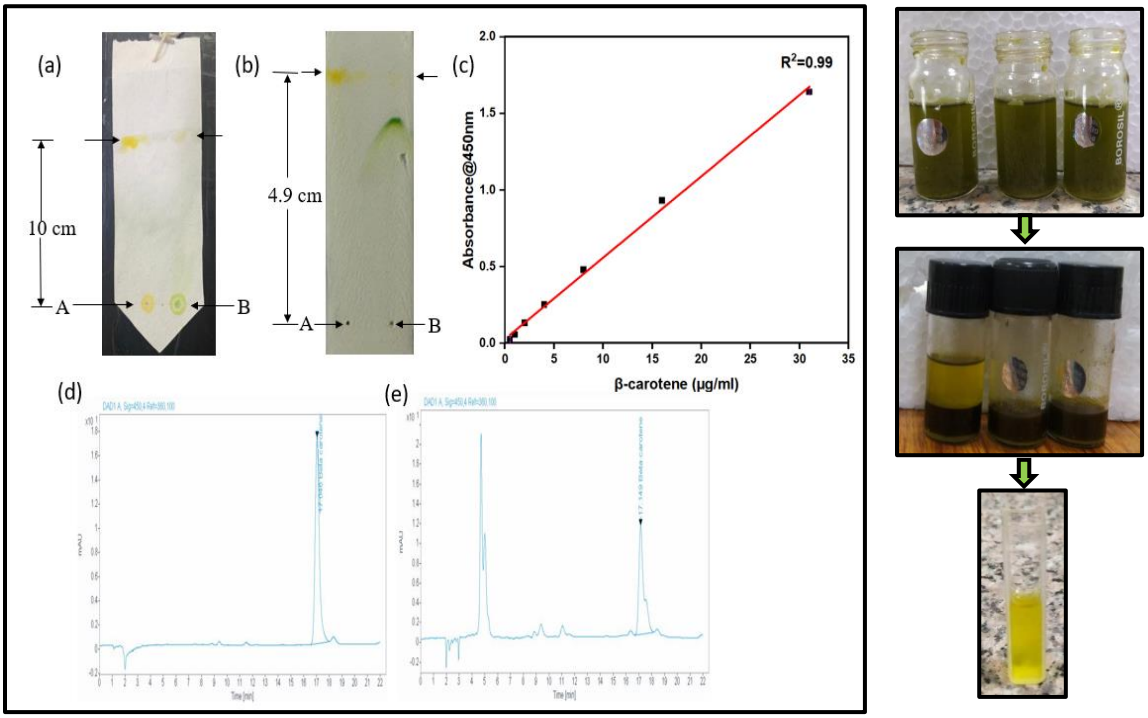


Fig. 5.6: Qualitative and quantitative estimation of β-carotene; a) through paper chromatography with the pigment travelling 10 cm on stationary phase; b) on thin-layer chromatography with the

pigment moving 4.9 cm; c) the calibration curve of standard β-carotene with R^2=0.99; d) the HPLC pattern of standard beta carotene in acetonitrile through C_{18} column; and e) the HPLC peak of algal extract.

The distance of sample run in paper chromatography and thin layer chromatography was measured to calculate the R_f value. Since the compounds like carotenoids and polyphenols are found in tight association with carbohydrates, they generally get co-extracted. Hence, it becomes important to eliminate these polysaccharides in order to improve the degree of purification of the extract. This is carried out by saponification of the algal extract. The C18 column is highly efficient in separating stereoisomers.

5.3.7 Enhancement of β-carotene content in microalgal cells through abiotic stresses

The biomass productivity and accumulation of antioxidants like β-carotene are observed to increase under high intensities of light as stress and salt stress(Minhas et al. 2016). Being a secondary metabolite in the algal lifecycle, β-carotene accumulation within the growth-arrested cell's lipid bodies of chloroplast is high under stress conditions(Lamers et al. 2012)(Del Campo et al. 2007)(Rabbani et al. 1998). Since the fatty acid and biochemical profile of algal strains largely depends on the cultivation techniques, nutritional status, and other physiochemical conditions like carbon-nitrogen ratio and stresses, further studies are required to promote the qualities and properties of the isolate. The optimal cultivation conditions for the microalgae isolate were determined under laboratory-scale conditions by varying salinity and light stress. The time-dependent high β-carotenogensis induction of salt and light stresses on the strain were assayed following the protocol followed in a recent study(Nguyen et al. 2016). The dependence on the salt content of the culture medium for improved lipid concentration within the cell for biofuel

production is another industrial application of the strain's tolerance against salinity. An overall range of tolerable temperature for *G. emersonii* is 25°C–42°C, making it possible for large-scale outdoor cultivations(Mandal and Chaurasia 2021). The isolate in this study demonstrated the best growth at 4000 Lux light intensity (16:8 light–dark photoperiod) and temperature 28±2°C at a pH of 8.

Under our study, the strain thrived at 10,000 Lux light intensity and withstood a NaCl stress of up to 1.0 M, accumulating up to 15.8 µg β-carotene per microlitres of culture with light stress of 4000 Lux of white light having 16:8 light-dark photoperiod over a span of 10 days. A salt stress of 0.2M NaCl accumulated about 9.2 µg β-carotene per microlitres of culture in the same time span. The β-carotene composition of *G. emersonii* varies from 0.08 to 0.85 mg g^{-1} DCW as per the available literature(Kang et al. 2022a)(Paliwal et al. 2016). A light intensity of 4000 lux provided in the incubator with a 16:8 light-dark photoperiod was most suitable for β-carotene accumulation as compared higher light intensities of 10,000 Lux and 50,000 Lux. For a strain isolated from open-pond water, the exposure of the pure strain to direct sunlight led to the death of biomass and hence proves the co-living of strains in consortium. This is shown by the reduced bars in 50,000 Lux as shown in **Fig 5.7**.

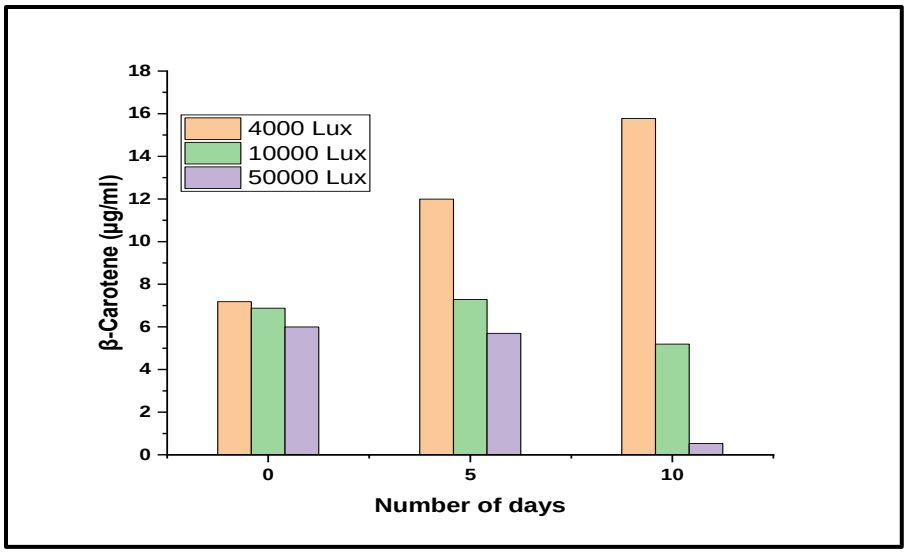


Figure 5.7: The effect of light stress on the accumulation of β-carotene

It was found that a 0.2M NaCl supplemented BG-11 was most suitable salt stress followed by 0.1M NaCl for the accumulation of target pigment in the longer run. There is also a different in the colour of the culture media with high salt concentration as shown in **Fig 5. 8**.

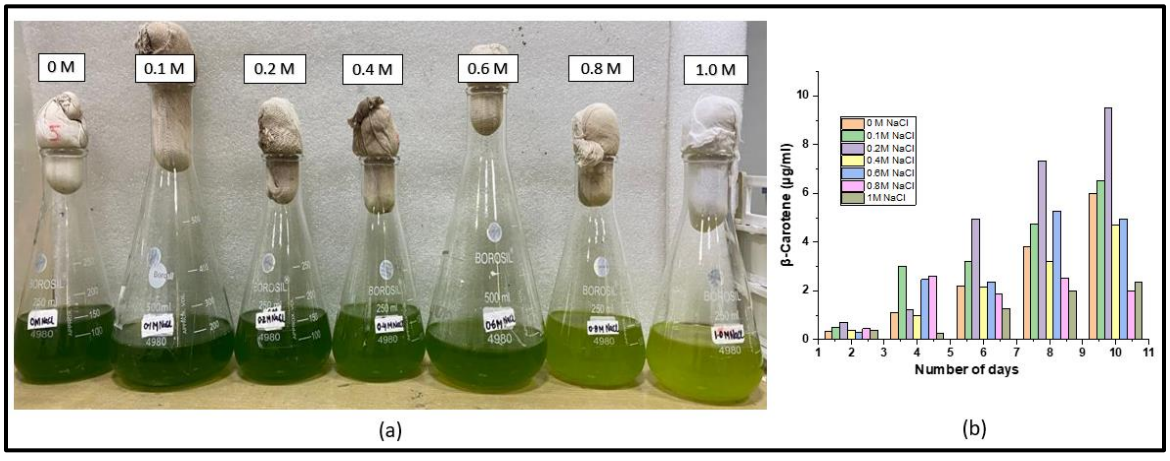


Figure 5.8 (a) The effect of salt stress on culture, with 0M as control and subsequent increase in NaCl molarity with 0.1M, 0.2M, 0.4M, 0.6M, 0.8M and 1.0M (b) The effect of salt stress on β-carotene accumulation

5.4 Conclusion

The microalgae can be exploited not only for its high lipid content useful in production of economical biofuels, but also for high yields of essential pigments with high nutraceutical values. Algae have a unique property of accumulating high amounts of carotenoids under unfavourable conditions. There are efficient methods of subjecting the algal strain to stresses, both biotic and abiotic, that enhances the pigment production in them. The isolation and identification of economic, fast-growing, and adaptable algal strains are important. Therefore, the objective of this study was to isolate locally adapted microalgal strains for prospective β-carotene production.

Based on the morphological and molecular identification studies, the strain was found to be *Graesiella emersonii*. Green, spherical cells of size 3-6µm. The color of the algal culture broth was initially green (for first 5-7 days) but later on it turned orange-red. It signifies the presence of pigments like astaxanthin, β-carotene and other high value chemicals. Lipid content in *Graesiella emersonii* was estimated to be 24.43% of dry cell weight. The presence of β-carotene in algal extract was confirmed by PC, TLC, and HPLC. β-carotene content varied to 0.643-1.26 mg/g dry algal biomass. 0.2M NaCl and 4000 lux light intensity were found to be best among tested strategies for enhancement of β-carotene accumulation in the algal cells.

CHAPTER 6

BIOPROSPECTING MICROALGAE FOR POLYHYDROXY BUTYRATE (PHB) PRODUCTION

6.1. Introduction

Plastics are high-molecular-weight polymers synthesized chemically from petrochemicals and their derivatives for numerous anthropogenic purposes. Their mass production began in the 1940s. More than 20 types of plastic are widely used by the current human population. For the military, households, production houses, construction, the automobile in- dustry, the medical sector, and many more, plastic is an essential component. Packaging and storage is a major sector that uses plastic extensively. The light weight, low production cost, and most importantly, all-weather durability of plastic make it the first choice of consumers for various needs. To date, 8300 million metric tons of plastics have been produced worldwide, and the world had discarded approximately 6300 million metric tons of plastic waste by 2015. An equivalent of 4% of world oil production was used as feedstock for the production of plastic, and a similar amount of energy was consumed during production processes. At present, global plastic production stands between 360 and 380 million metric tons(Kumar et al. 2022b)(Lear et al. 2021)(Paço et al. 2019).

In 1970, environmental pollution due to plastics was reported for the first time(Carpenter and Smith 1972). Plastics are versatile, nonreceptive, and above all, nonbiodegradable. Henceforth, there has been a remarkable expansion in plastic wastage, which has since been perceived as a worldwide ecological danger. The worldwide plastic market size was estimated at 569 billion USD (2019), and from 2020 to 2021, it was estimated to have increased by 3.2% (Amobonye et al. 2021). It is expected that as much as 26 billion tons of plastic wastes will be delivered by 2050, and the greater part will be discarded into landfills, which leach into ecospheres

like lakes and seas, prompting serious environmental contamination(Kumar et al. 2022b). Thus, there is a need for reduction in usage of plastics as well as their remediation and transformation into lesser or no harmful substances. Biobased plastics can be an alternative to this problem. Bioplastics are a family of plastics that is either biodegradable, bio-based or both. They are termed so because they are produced from renewable 'biomass' like bacteria, fungi, and microalgae (Trakunjae et al. 2021)(Getachew and Woldesenbet 2016)(Luengo et al. 2003)(Roy Chong et al. 2022)(Abdo and Ali 2019)(Rahman and Miller 2017). For example, polyhrdoxybutyrate (PHB), an aliphatic polyester with thermoplastic properties, a type of bioplastic is naturally produced by certain microorganisms as storage compound which are completely biodegradable in nature. Due to their hydrophobicity, complete biodegradability, and biocompatibility, they have become a promising alternative to common plastics (Selvaraj et al. 2021).

At present bioplastics comprise only 1% of the global plastic market. Due to environmental concern and thus global pressure on 'go green' and carbon neutral approach, the bioplastic market is increasing its share in the total plastic market (2016). Thus, there is a dire need of identification of high yielding bioplastic producing biological agents and also the development of extraction, purification and product formulation of several bioplastics from these sources and their deployment and demonstration at larger scale to make its implementation as an alternative to conventional plastics a reality. In recent past, several studies have demonstrated the quantitatve and qualitative analysis of bioplastics from different biological agents(Trakunjae et al. 2021)(Getachew and Woldesenbet 2016)(Abdo and Ali 2019)(Selvaraj et al. 2021). Bacterial bioplastic prduction is costly affair, due to the high cultivation cost, thus limiting its industrial level production and consumption in general. In contrast, Algae can be considered as an alternative source of PHB production due to their low cultivation cost, year round availability and ability to

grow in wide range of environment and nutrient recovery from wastewater as well (Selvaraj et al. 2021). Microalgae, have gained attention for food, fuel, fertilizer, animal feed, and source for several nutraceuticals and pharmaceutical products in current times. Several microalgae species including *Synechocystis* sp., *Scytonema* sp., *Synechococcus elongatus*, *Nostoc muscorum, Cupriavidus necator, Haloferax mediterranei, Scytonema geitleri, Spirulina subsalsa, otryococcus braunii,* and *Chlorella* sp., have been investigated for bioplastic like polyhydroxybutyrate production (Selvaraj et al. 2021)(Kamravamanesh et al. 2017)(González-Resendiz et al. 2021)(Singh et al. 2019)(Atlić et al. 2011)(Shrivastav et al. 2010)(Ansari and Fatma 2016)(Kavitha et al. 2016). More information on algal based PHB production has been summarized and presented elsewhere(Balaji et al. 2013)(Amadu et al. 2021)(Khyalia et al. 2022).

In this section, we have performed screening of two *Chlorella* sp., for extraction of polyhrdoxybutyrate. Sudan black dye test has been performed to identify PHB producing microalgae strains followed by solvent extraction method to extract and quantify PHB from the select microalgae isolate. The amount of the PHB has been estimated using commercial PHB as standard. Also, effect of salt and light stress has been tested in order to enhance the PHB accumulation in the select microalgae strains.

6.2. Materials and methods

6.2.1. Screening of PHB Positive microalgae Strain

Chlorella minutissima and *Chlorella sorokiniana* two microalgae strains grown in BG-11 medium (250ml Erlenmeyer flask) at 27±2ºC in an artificial light chamber under illumination with a white fluorescent light at 4000 lux on a photoperiod of 16L: 8D were used in this study. The cultivation flask was manually agitated twice a day to ensure proper nutrient mixing. Microalgal cells in

exponential phase were taken and screened for presence of polyhydroxy butyrate. For screening of PHB accumulation in the strains, Sudan Black B staining was performed as illustrated in a previous studies (Kavitha et al. 2016). 0.3 gm of the stain was dissolved in 100 ml 0f 70% ethanol. Microalgae cells were heat-fixed on a clean glass slide followed by staining with 3% solution of the stain. This slide was kept undisturbed for 15 minutes followed by immersed in xylene until decolorization and counterstained with 0.5% w/v Safranin. Thus, prepared slide was examined under light microscope for screening of PHB containing microalgal cells. Microalgae cells appearing blue-black under microscope were termed as PHB positive strains. After screening, extraction of PHB from microalgae biomass was performed using solvent extraction method in the following section.

6.2.2 Extraction of Polyhydroxy butyrate (PHB) from microalgae cells

Microalgal cell suspension centrifuged at 3000 rpm for 10 min at room temperature. The supernatant was discarded and pellet was washed twice with distilled water to remove medium nutrients. PHB extraction was performed using a solvent extraction method with slight modification in the protocol described in recent studies(Das et al. 2018)(Martins et al. 2017)(Roja et al. 2019). Thus, obtained pellet was suspended in a mixture of 4% sodium hypochlorite and chloroform (1:1) and incubated at room temperature for two hours. Post incubation, the mixture was centrifuged at 8000rpm for 10 min. Three different layers were formed -upper layer containing sodium hypochlorite, middle layer containing undisrupted cells, cell debris, and non-polymeric cell materials, and the bottom layer which contains the PHB, the component of interest. The bottom layer was collected for the recovery of PHB followed by its quantification. For recovery of PHB from chloroform, water and acetone (1:1) was added to PHB containing chloroform for non-solvent precipitation of PHB. Sulphuric acid digestion method was followed to quantify the

amount of PHB present in the microalgae(Wicker et al. 2022). PHB present in the sample converted into the Crotonic acid by treating it with Sulphuric acid at 100°C. Amount of Crotonic acid released is proportional to PHB concentration extracted. PHB extracted was characterized by UV-Visible spectroscopy (as it gives optimum absorbance at 230-240 nm, corresponding to Crotonic acid). The absorbance value of PHB converted Crotonic acid for the test microalgal strains was compared with the standard graph of commercially available PHB for quantification of PHB.

6.2.3 Standard curve for Polyhydroxy butyrate

A standard graph of Crotonic acid was prepared for commercially available PHB as method described by Abdo *et al*(Abdo and Ali 2019). A certain amount of PHB was mixed with H_2SO_4 to prepare several dilutions (20-200 µg/ml) and subjected to heat in a water bath at 100°C for 20 min. PHB on heat treatment converted to Crotonic acid. Absorbance was taken at 235nm using H_2SO_4 as blank and standard graph was prepared.

6.3. Results and discussion

6.3.1 Screening of PHB Positive microalgae Strain

Both select microalgae strains *Chlorella minutissima* and *Chlorella sorokiniana* were stained with Sudan black B and examined under light microscope. Lipid rich PHB fractions in the cell appeared black as they retained the Sudan Black dye. Black rings appeared along the intracellular boundaries against reddish background due to counter staining with Safranin (Fig. 6.1). It revealed both stains are PHB positive.

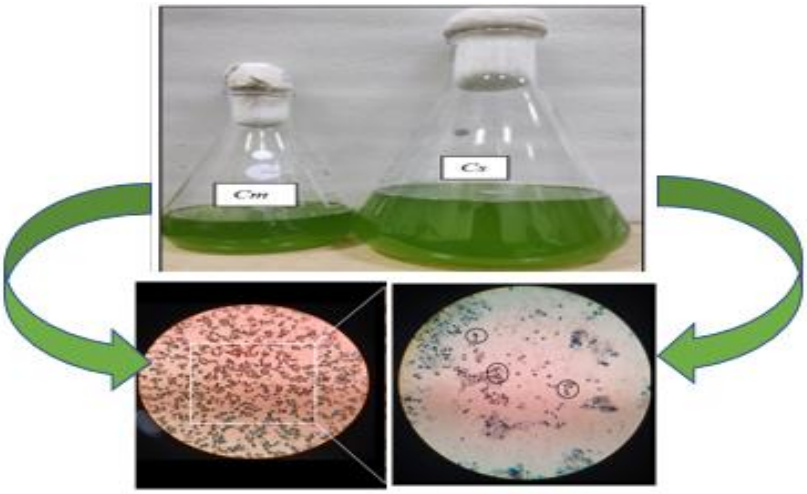


Figure 6.1: Screening of *Chlorella minutissima* and *Chlorella sorokiniana* for polyhrdoxybutyrate

6.3.2 Standard curve for Polyhydroxy butyrate

Commercially available polyhrdoxybutyrate was used as received to prepare several dilutions of PHB in Sulphuric acid (Fig. 6.2 & Fig. 6.3). On heat treatment, PHB gets converted into Crotonic acid. Amount of Crotonic acid released is proportional to PHB concentration in the sample. The standard curve plotted by taking absorbance value of different PHB dilutions indicated a linear trend with correlation coefficient of 0.99.

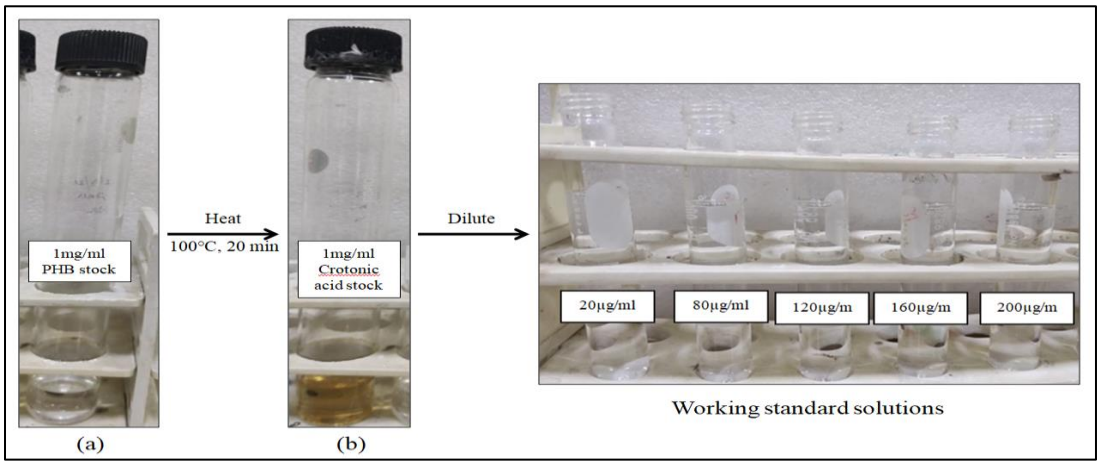


Figure 6.2: Preparation of Crotonic acid dilutions

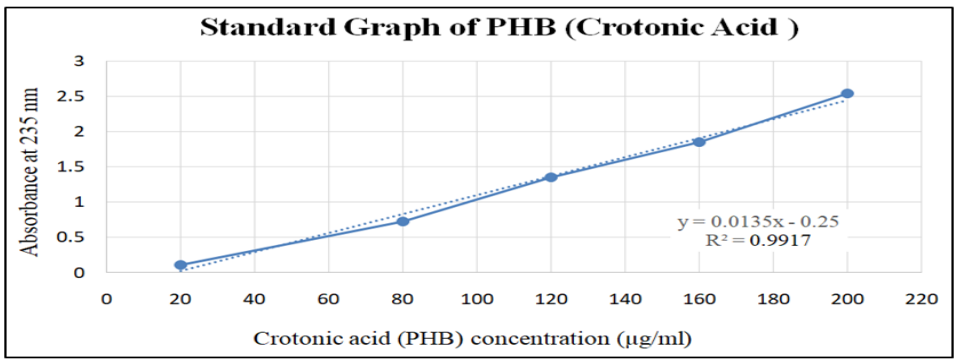
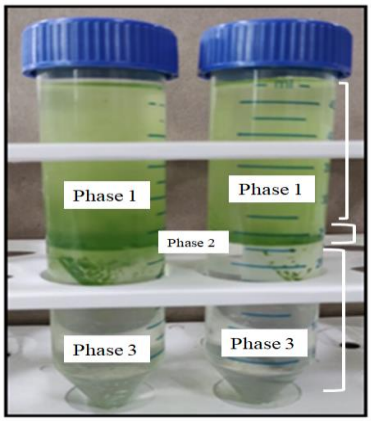


Figure 6.3: Standard curve of Polyhydroxy butyrate (converted to Crotonic acid;

Y= 0.0135*X – 0.25, Where Y denotes absorbance taken at 235nm, and X represents Crotonic acid (PHB) concentration in µg/ml.

6.3.3 Estimation of Polyhydroxy butyrate using commercially available PHB as standard

100ml culture broth of both strains were centrifuged and thus collected algal biomass pellet was washed with distilled water twice. Upon treatment with sodium hypochlorite and chloroform, three phases were found at the end as shown in below fig. 6.4. The bottom layer containing the desired PHB component was separated using a mixture of water and acetone and digested with Sulphuric acid. On Sulphuric acid treatment, the PHB converted into Crotonic acid (brownish color) as shown in Fig 6.5.

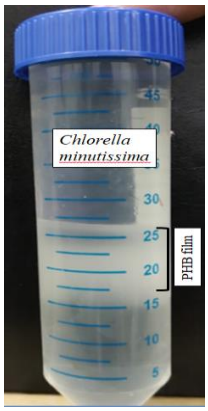


Separation into 3 layers following centrifugation after

Upper layer (Phase 1): Sodium hypochlorite solution

Middle layer (Phase 2): Undisrupted cells and cell debris

Bottom layer (Phase 3): PHB containing chloroform



Presence of biofilm layer, separated from chloroform

Figure 6.4: PHB extraction using chloroform and its precipitation using Water: Acetone (1:1)

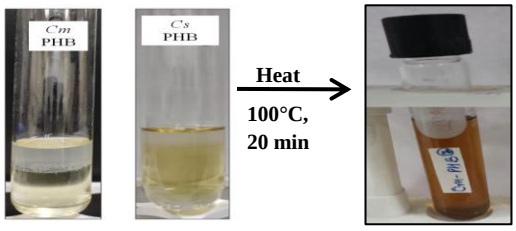


Figure 6.5: PHB containing biofilm on digestion with Sulphuric acid

Amount of Crotonic acid released is proportional to PHB concentration extracted. The acid digested PHB which turned into Crotonic acid was then analyzed under UV-Vis spectroscopy and observation was recorded as follows:

- For *Chlorella minutissima* peak was observed at 240 nm with an absorbance value of 4.78
- For *Chlorella sorokiniana* peak was observed at 235.65 nm with an absorbance value of 4.69

Using equation Y= 0.0135*X – 0.25 from the standard graph, the amount of PHB in microalgae cultures were calculated as follows (Where Y denotes absorbance taken at 235nm, and X represents Crotonic acid (PHB) concentration in µg/ml, and assuming 100% efficient extraction and conversion of PHB into crotonic acid):

Microalgae	**A_{235}**	**PHB Content (in µg/ml)**
Chlorella minutissima	4.78	374
Chlorella sorokiniana	4.69	367

About 140 million tons of annual plastic consumption necessitates processing of 150 million tons of fossil fuels. PHB is an aliphatic polyester with thermoplastic properties which is 100% biodegradable. Bacterial PHB production is not widespread in part due to the cost of the carbon substrate(Selvaraj et al. 2021). Microalgae based PHB production has attracted substantial interest

because of its simple cellular structure, photoautotrophic nature, minimal nutrient requirements, diversities and characteristics(Kumari et al. 2022). Algal-based bioplastic can be a significant replacement for traditional plastics that is both environmentally benign and biodegradable(Dhivya et al. 2023). *Chlorella minutissima* and *Chlorella sorokiniana* were screened for the presence of PHB accumulation the cells using Sudan Black B staining. On confirmation PHB extraction was performed by solvent extraction method. The amount of PHB in *Chlorella minutissima* and *Chlorella sorokiniana* was estimated to be 374 mg/µl and 367mg/µl of algal suspension culture considering the complete extraction efficiency of the process applied. In a recent study on PHB extraction from chlorella sorokiniana reported the accumulation of PHB in the range 150-280 mg/liter under varying conditions(Kumari et al. 2022). A study conducted to extract PHB from 14 days old *Chlorella pyrenoidosa* reported that the cells contain PHB up to 27% of their total dry cell weight(Das et al. 2018). Costa et.al., utilized lipid extracted residual *Chlorella fusca* biomass powder for PHB extraction(Cassuriaga et al. 2018). They reported 17.4% w/w of PHB accumulation in the cells, which is quite high and facilitates a sustainable way towards algal based biodiesel and other valuable by-products production. Jayaseelan *et.al.,* reported 41% PHB recovery from defatted algal biomass facilitating an economically viable algal biomass based biopolymer and biodiesel production (Arun et al. 2022). *Chlorella vulgaris* was reported to have upto 37% PHB of total dry cell weight(Setyorini and Dianursanti 2021). In our study, the total amount of PHB was estimated to be 374 mg/µl and 367mg/µl for *Chlorella minutissima* and *Chlorella sorokiniana* respectively while the lipid content was 13% and 11% when grown in normal BG-11 medium. These strains can be used for co-production of biodiesel and biopolymer production facilitating a algal based biorefinery for biodiesel and biochemical production.

6.4 Conclusion

Poly-β-hydroxybutyrate (PHB), a type of Polyhydroxyalkanoates, is considered as one of the most promising bioplastics materials which is readily biodegradable in nature. Several microbes including bacteria, and microalgae have been used for production of PHB. In this direction, we explored the possibility of using microalgae *Chlorella minutissima* and *Chlorella sorokiniana* for production of PHB. In our study, Sudan Black B dye staining confirmed that both *Chlorella minutissima* and *Chlorella sorokiniana* are PHB accumulating strains. Black ring around the intracellular boundaries against a reddish background on staining with Sudan black dye followed by counterstaining with safranin was observed under light microscope. These microalgae cells were subjected to solvent extraction for estimation of PHB accumulated in the cells. The amount of PHB in *Chlorella minutissima* and *Chlorella sorokiniana* was estimated to be 374 mg/µl and 367mg/µl of algal suspension culture considering the complete extraction efficiency of the process applied. The study concludes that these microalgae can be used as suitable feedstock for renewable, sustainable, and ecofriendly bioplastic production.

CHAPTER 7

BIOSYNTHESIS, CHARACTERIZATION, AND EVALUATION OF ANTIBACTERIAL AND PHOTOCATALYTIC DYE DEGRADATION ACTIVITIES OF SILVER NANOPARTICLES BIOSYNTHESIZED BY *CHLORELLA SOROKINIANA*

7.1.Introduction

Due to increased industrialization and urbanization by anthropogenic activities, several pollutants have been gradually detected and quantified in and around the human being and their environment. Being xenobiotic and persistent, Dyes are one of the prominent pollutants having proven harmful effects on public and environmental health (El-Sheekh et al. 2009). Out of the 60,000 tons of dye being discharged into waterbodies worldwide each year, almost 80% comprises the azo-dyes. The printing and dyeing industry run-offs are complex, difficult to bio-degrade, and highly concentrated in color containing a variety of organics. Such wastes are considered to be mutagenic, carcinogenic, and teratogenic. The principal organic components of dye industry wastewater are benzene, anthraquinone, and so on. The chromatic pollution of dyes is attributed to the chromophore-molecular group in its structure. Hence, individual treatments of these wastewaters are not suggested. The wastewater containing such a high “color index” requires dedicated yet comprehensive treatments to match the stringent standards of discharge (Liu 2020). Health hazards of dyes on flora and fauna health, the microbial community, and water bodies are a rising concern (Kumar and Bharadvaja 2020b).

Likewise, antibiotic-resistant bacterial infections are creating substantial distress to human health and economy. The indiscriminate and overuse of antibiotics, and other possible genetic causes, including mutations, genetic transfer, and acquisition leading to resistant genes is resulted in the development of microbial populations adapting to tolerate, exist, and propagate in the presence of a range of antibiotic agents (Fatima et al. 2021)(Murray et al. 2022). The antimicrobial

resistance led to the increasing of death cases numbers which is a growing concern worldwide today and it has facilitated the exploration of novel antimicrobial agents (Serwecińska 2020).

In recent times, nano-dimensional particles are under investigation for a range of applications including environmental remediation, antimicrobial agents, drug delivery, biosensors, cosmetics, and others (Kumar et al. 2021). Nanoparticles, due to their nanoscale dimension, increased surface area, shape, and inherent properties, have been proven as an alternative to conventional antibiotics (Gupta et al. 2019)(Makabenta et al. 2021). Metal nanoparticles including silver, zinc, copper, titanium, gold, iron, magnesium, and gold, metal oxide nanoparticles, and several other organic and inorganic nanoscale agents have been reported to have the ability to combat different pathogens (Fatima et al. 2021)(Truu et al. 2022)(Abdolalian and Taghavijeloudar 2022)(Chen et al. 2019)(Azizi-Lalabadi et al. 2019)(Soo et al. 2020)(Arakha et al. 2015)(Shamaila et al. 2016). Silver nanoparticles, in particular, are tested against a range of bacterial infections due to their potent antimicrobial activities via cell wall membrane defragmentation, apoptosis, alteration in the enzymatic pathway and cellular pathways, and synergy of several known and unknown mechanisms. Therefore, economical and eco-friendly routes of silver nanoparticle synthesis for diverse applications has received continued interest (Yun and Lee 2017).

The synthesis of nanomaterials via conventional physicochemical methods isn't economic and ecofriendly. In recent times, green synthesis or biological synthesis route of nanomaterials has gained momentum. Plants and microbes, including bacteria, algae, and fungi, have been explored for the synthesis of nanomaterials. The bioactive compounds present in plant materials and microbial biomass extracts act as reducing, capping, and stabilizing agents during synthesis of nanomaterials. In this direction, algae-based silver nanoparticle synthesis is a novel and sustainable approach (Kumar et al. 2022a). The biogenic synthesis is considered to occur in phases, namely

the activation, growth, and termination phases. The metal ion from the precursor salt gets reduced, leading to a colorimetric change in the activation phase. In the growth phase, the nucleation products fuse, forming nanoparticles of diverse morphologies. The nanoparticles attain their final morphologies in the termination phase (El-Sheekh and El-Kassas 2016)(Chaudhary et al. 2020b).

In the present study, *Chlorella sorokiniana* extract has been to synthesize silver nanoparticles (SNPs). *Chlorella sorokiniana* belongs to *Chlorella*, an important genus of the Chlorophyta phylum, which can efficiently grow in a wide range of temperature and light intensities and has been extensively in application for wastewater treatment (Ziganshina et al. 2022)(Taghavijeloudar et al. 2021). It is a fast-growing green microalga. It can grow autotrophically, heterotrophically, and mixotrophically depending upon the water used, nutrients provided, and the surrounding conditions. It is an essential source of biomass as well as biological components, including proteins, carbohydrates, lipids, pigments, and vitamins (Cazzaniga et al. 2014). The cellular extract of the microalgae contains different enzymes and metabolites like carotenoids, polysaccharides, amino acids, polyphenols, aromatic compounds, minerals, etc., which function as reducing, capping, and stabilizing agents during nanoparticle synthesis (Kumar et al. 2022a). *Chlorella sorokiniana* mediated synthesized nanoparticles were characterized and tested for their photocatalytic degradation potential against four different dyes, including methylene blue, crystal violet, eosin Y, and rhodamine B, and bactericidal effect against two bacteria, *Escherichia coli* and *Staphylococcus aureus*. Also, the influence of certain physicochemical parameters, including pH, cell disruption methods, temperature, extract to precursor metal salt solution ratio, salt strength, biomass concentration, and incubation conditions on SNPs synthesis, has been studied.

7.2. Materials and methods

7.2.1. Microalgae extract preparation and silver nanoparticles synthesis

Microalgae culture was cultivated in BG-11 medium under 4000 Lux light intensity at 27±1°C and 16:08h photoperiod in an algal growth chamber. Biomass harvested at the stationary phase was mixed into miliQ water and boiled for 20 minutes at 80°C to prepare the microalgae extract. This extract contains a range of bioactive compounds, including protein, carbohydrates, fatty acids, and pigments like chlorophylls, carotenoids, phycobilin, and antioxidants like terpenoids or polyphenol. For biosynthesis of SNPs, a certain volume of microalgae extract was mixed with freshly prepared silver nitrate solution and kept at room temperature for the reaction to progress. SNPs formation was indicated by a gradual change in the color, from light green to dark brown of the aqueous solution. This preparation was kept under observation for a period of three days, and nanoparticles synthesis was monitored using a UV-Vis spectrophotometer.

Silver nitrate ($AgNO_3$) was purchased from SRL, India. All other reagents were of analytical grade and used as received. All the solutions were freshly prepared with double distilled water for the experimental procedure and were kept in the dark to avoid any photochemical reaction.

7.2.2. Optimization of parameters influencing green synthesis of silver nanoparticles

Microalgae-mediated synthesis of SNPs depends on several conditions, including the strength of metal precursors used, the ratio of salt to microalgae extract, pH of the microalgae extract, temperature, incubation condition, and period. Here in this study, we have optimized the abovementioned parameters to get the optimum synthesis of the silver nanoparticles. One condition was varied at a time, and rest other conditions were kept constant. This method is called "One Variable At a Time" or the OVAT method. Different ratios pertaining to extract to salt ratio

(20:1, 10:1 , 1:1, and 1:10), metal precursor salt strength (1mM , 2mM , 5mM, 10mM , and 20mM), pH (6-10), biomass concentration (0.5g/100ml, 1 g/100ml, 2g/100ml, and 4 g/100ml), cell disruption techniques (autoclaving, boiling, and sonication) temperature (room temperature (30°C and 60°C), incubation conditions (light and dark), and incubation period (up to 72 hours) were optimized for efficient green synthesis of SNPs using microalgae extract. Three cell disruption methods, including boiling for 20 minutes at 80°C, autoclaving at 121°C for 20 minutes, and Sonication (0.5 cycle, 50% amplitude) for 20 minutes, were tested for the selection of suitable cell disruption methods.

7.2.3. Characterization of Silver nanoparticles (SNPs) synthesized using algal extract

The formation of SNPs is time-dependent and confirmed by the development of brownish color solution as the reaction progresses. Initial characterization was performed using UV-Vis spectrophotometry (Lambda 365, Perkin Elmer) by measuring the absorbance (200-700nm) of the samples taken at regular intervals. The morphology of the synthesized SNPs was obtained by Scanning Electron Microscopy FESEM, (ZEISS Microscope). Dynamic Light Scattering (DLS) technique (Zetasizer nano series ZS- Malvern panalytical) and X-Ray diffraction analysis (Bruker D8 Advance) were performed to find the average size and stability of nanoparticles and the crystalline nature of the nanoparticles formed respectively. For SEM analysis, an aliquot of SNPs suspension was placed on a quartz thin film and allowed to settle, followed by SEM images obtained for morphological analysis.

7.2.4. Antibacterial activity of silver nanoparticles

To determine the antibacterial activity, the synthesized SNPs were tested against *Escherichia coli* and *Staphylococcus aureus* by well diffusion method. Overnight grown bacterial cultures were

spread on the nutrient agar plate. Wells were created using a well borer. At a concentration of 40 µg/mL, a colloidal solution of silver nanoparticles, and a certain volume of synthesized SNPs solution (10µl, 20µl, and 25 µl) was added to the wells. The algal extract and silver nitrate ($AgNO_3$) solutions were also placed in the well as control. Then the plates were kept in incubation overnight. The antibacterial activity was determined by observing and measuring the presence or absence of a zone of inhibition (millimeters or mm) around the well compared to the control. The diameter of such zones of inhibition was measured using a meter ruler, and the mean value for each microorganism was recorded and expressed in millimeters. The experiment was performed in triplicates, and the zone of inhibition was measured after overnight incubation.

7.2.5. Photocatalytic degradation of select dyes

The photocatalytic degradation of four different dyes Eosin yellow (EY), Methylene blue (MB), Crystal violet (CV), and Rhodamine B (RhB), using SNPs as a catalyst, synthesized in this study, was carried out under natural sunlight. A 5-ppm dye solution was prepared by mixing the required amount of dye in water. 0.5 ml of biogenic nanoparticles solution (40 µg/mL) was added into dye solutions (5 ppm, 20ml) and kept under sunlight for degradation to proceed. The degradation of dyes was monitored using UV-Vis spectrophotometry by taking samples at regular intervals. The percentage of decolorization is calculated by the following formula:

$$\% \text{ Degradation} = (C_0\text{-}C_t/C_0) *100$$

Where C_0 = absorbance of dye at time 0 min, C_t= absorbance of the dye after the photocatalytic degradation.

7.3. Results and discussion

This study aimed to determine the antibacterial activity and photocatalytic dye degradation potential of silver nanoparticles (SNPs) synthesized using *Chlorella sorokiniana* extract. The green synthesized SNPs used in this study were characterized by UV-vis spectroscopy, scanning electron microscope, X-ray diffraction pattern, and zeta potential analyzer. Certain parameters affecting the SNP synthesis have been optimized and presented in the following section.

7.3.1. Optimization of SNPs synthesis parameters using *Chlorella sorokiniana* biomass

In the current study, different parameters for the synthesis of SNPs were considered and optimized. These included pH conditions, extract-to-salt ratios, the concentration of precursor salt, cell disruption methods, biomass concentration, incubation conditions, and method of extract preparation from *Chlorella sorokiniana*. Screening of optimum conditions for synthesis was done by keeping only one condition dynamic at a time, rest of other conditions were kept constant throughout the study (OVAT method). The final synthesis was performed at the combination of all the optimum parameters. UV-Vis spectrum representing stability and synthesis of SNPs synthesis by *C. sorokiniana* at different conditions has been illustrated in **Fig. 7.1**. It was observed that the synthesis of SNPs took place at a slower pace in the initial hours; the synthesis rate increased and attained a maximum at 24 hours. The brownish color of the solution was found to be increasing upon increase in the incubation time up to 24 hours. This increase in color intensity with time indicates SNPs synthesis. Similar results were reported for the SNPs synthesis using marine algae, *Caulerpa racemosa* (Kathiraven et al. 2015). The environmental factors, including pH, reaction time and temperature, precursor metal salt concentration, etc., have significant role to play during synthesis and morphology of silver nanoparticles. The spectrophotometric analysis

recorded a strong Surface Plasmon Response band between 400 nm and 440 nm at different physiochemical parameters tested in this study, as shown in **Fig. 7.1**. Literature review also confirm that the occurrence of the peak in the range of 410- 450 nm in the formulation indicates the presence of SNPs in the solution (Vivek et al. 2011; Kannan et al. 2013; WA 2016; Bhuyar et al. 2020; Rani et al. 2020).

pH has a profound effect on the morphology and structural build of the synthesized nanoparticles (Rajkumar et al. 2021). The considered range of pH for the optimization of synthesis of biogenic NPs was chosen from 6 to 10. SNPs formed at this pH range were stable while those formed at pH (5, 11, 12) were highly unstable. Fig.1(a) shows the spectrophotometric spectra of synthesized SNPs at different pH values. The most stable SNPs being synthesized, with a strong SPR, at a pH of 7 amongst all other tested pH values. This indicates the higher activity of reducing, capping, and stabilizing agents present in the extracts of *Chlorella sorokiniana* (Anigol et al. 2017). Lower pH favors large-sized SNPs formation while high pH facilitates a comparatively faster formation of small-sized and highly dispersed SNPs in the synthesis medium (Ahmed and Mustafa 2020).

At higher concentrations of metal precursor salts, the formation of SNPs increases. Along with this, the aggregation of synthesized nanoparticles also increases. In order to get nanoparticles stable for a longer duration, optimization of precursor metal salt strength is essential (Aryan et al. 2021). In this study, the ratio of algal extract to precursor salt solution was varied at 20:1, 10:1, 1:1, and 1:10. The best synthesis occurred when the algal extract and precursor metal salt solution were mixed in the ratio of 10:1. The SNPs synthesized using this ratio traced a broader peak around 430-440 nm as evident in Fig.1(b). This can be attributed to a rapid rate of reduction of Ag+ ions with the optimal presence of secondary algal metabolites having high electron density (Shaik et al.

2018)(Rajkumar et al. 2021). Ahmed and Mustafa reported that the particle size decreases with the increasing amount of extract (Ahmed and Mustafa 2020). Different concentrations of precursor metal salt solutions ($AgNO_3$); 1 mM, 2 mM, 5 mM, 10 mM and 20 mM; were prepared. It was confirmed using UV-Vis spectroscopy that a 10 mM concentration of $AgNO_3$ was optimum for the synthesis of SNPs using *Chlorella sorokiniana* extract. A prominent surface plasmon response peak was observed at 10 mM silver nitrate solution, as illustrated in Fig.1(c). A recent study reported similar findings for synthesizing SNPs using different molar strengths of metal precursor salts (Anigol et al. 2017).

For the extraction of algal bioactive compounds, different cell disruption techniques such as sonication, boiling and autoclave were performed. The prominent SPR was observed for SNPs prepared using algal extract obtained after boiling algal biomass as compared to the algal extract obtained after autoclaving and sonicating the biomass, as indicated in Fig.1(d). Autoclaving of the algal biomass leads to degradation of cellular metabolites due to very high operational temperature and pressure, whereas in the case of sonication, complete algal cell wall disruption could not be achieved. Thus, the concentration of metabolites in the extract was low, which could not enable a rapid or stable synthesis of SNPs.

A 100 ml volume with varying algal biomass concentration (0.5g/100ml, 1 g/100ml, 2g/100ml, and 4 g/100m) were tested for the synthesis of SNPs. The UV-Vis spectrophotometric analysis was recorded and presented in Fig.1(e). Extract prepared using 2 gm biomass of *Chlorella sorokiniana* per 100ml distilled water facilitated optimum concentration of reducing, caping and stabilizing phyco-compounds. These agents contributed in efficient bio-reduction of Ag+ ions present in the reaction mixture leading to the synthesis of SNPs.

The mixture of algal extract and precursor metal salt was incubated in two different conditions; light and dark. The results from visual and UV-Vis analysis indicated that the SNPs synthesis in light conditions is more favorable than the rate of synthesis in dark conditions. Fig.1(f) presents the UV-Vis spectrophotometric analysis of SNPs formation under both light and dark conditions. This observation can be attributed to the activation of various compounds under light conditions, which aid in the synthesis process. A similar observation was reported by a recent study on silver nanoparticle synthesis using *Kalanchoe* leaves extract (Aryan et al. 2021).

The favorable incubation temperature for the synthesis of SNPs was found to be 30^{o}C. A recent study on *Chlorella vulgaris* mediated green synthesis of silver nanoparticles reported a similar observation which corroborates our results (Rajkumar et al. 2021). The algal extract and silver salt mixture were incubated at 30^{o}C (RT) and 60^{o}C. It was observed that the rate of synthesis was much higher when incubated at 60^{o}C as compared to the samples incubated at 30^{o}C, as illustrated in Fig.1(g). High temperature accelerated the synthesis of the SNPs, but the particles were highly unstable. It was further noted that the SNPs synthesized at higher temperatures were not stable for a longer time than those synthesized at lower temperatures. The optimum incubation temperature for precursor metal salt solution and algal extract for the synthesis of SNPs was observed at 30°C (RT). Furthermore, any increase or decrease in the incubation temperature led to the broadening of peak values, indicating an increase in the size of SNPs as observed by (Amin et al. 2012)(Pastoriza-Santos and Liz-Marzán 2002).

SNPs were synthesized at optimum conditions, and the stability of SNPs was checked using UV-Vis at 24 hours, 48 hours, 72 hours, 96 hours, 168 hours, and after 180 days of synthesis and it was observed that the SNPs prepared at optimum conditions are highly shelf stable as illustrated by Fig.1(h). The optimized condition for *Chlorella sorokiniana* mediated synthesis of SNPs was

extract to salt ratio 10:1 with 10mM strength of precursor metal salt $AgNO_3$ at pH 7 under light conditions at room temperature (30°C). Algal extract prepared by boiling algal biomass (2g/100ml) at 100°C for 20 minutes gave better green synthesis of biogenic SNPs.

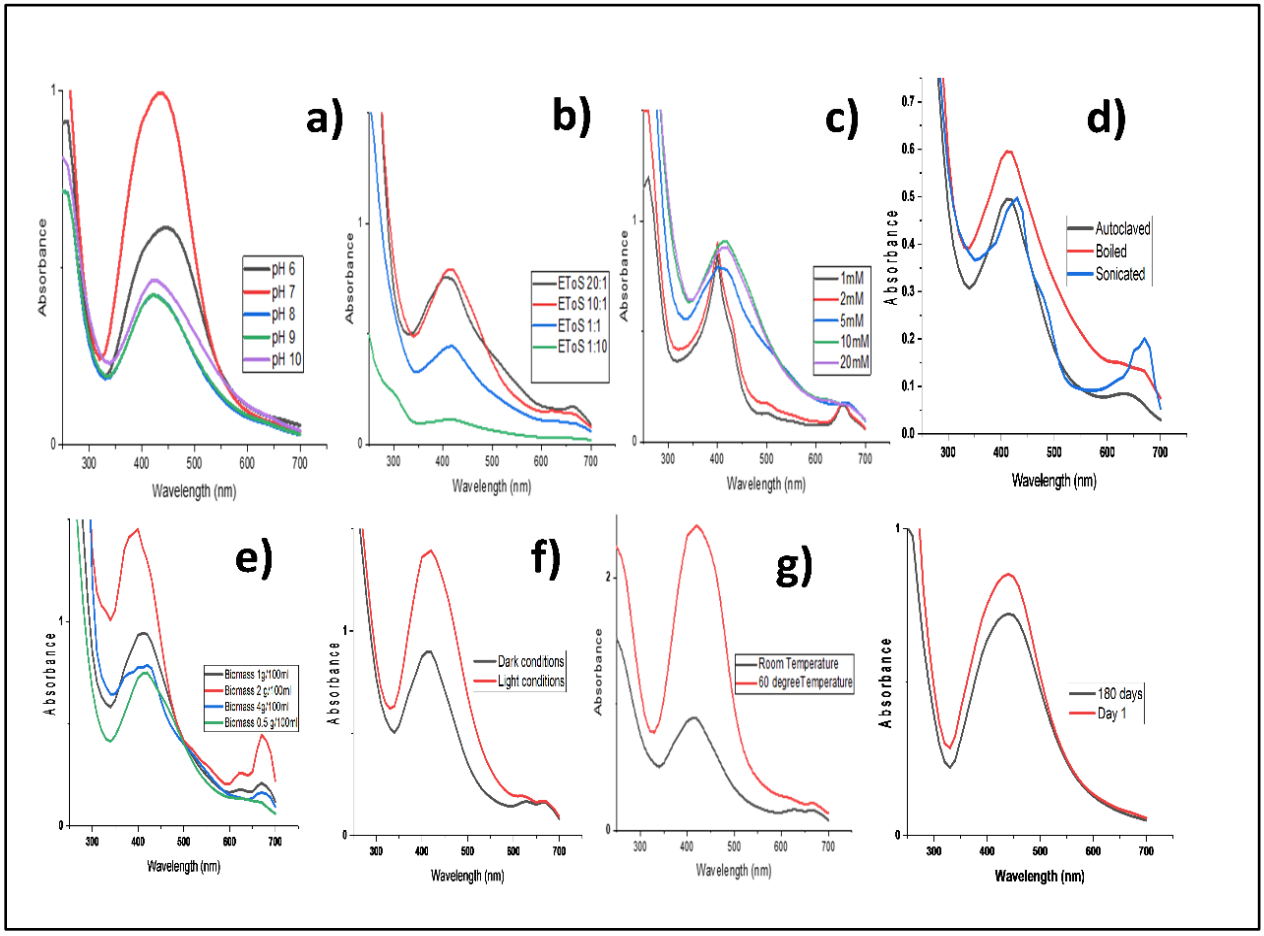


Fig. 7.1 UV-Vis spectrum representing stability and synthesis of SNPs synthesis by *C. sorokiniana* at different conditions: (a) pH range from 6-10; (b) different extract to precursor metal salt ratios; (c) different strengths of silver nitrate solution; (d) different methods of cell disruption; (e) different concentration of algal biomass; (f) incubation in light and dark; (g) incubation temperature and; (h) synthesis of SNPs at optimized conditions and stability of SNPs at different periods of incubation

7.3.2. Characterization of SNPs synthesized from *Chlorella sorokiniana*

Silver nanoparticles synthesized at optimum conditions using *Chlorella sorokiniana* extract were characterized primarily by UV-Vis spectrophotometer. A change in color from light green to

brown was observed with time as the reaction progressed. The UV-Vis absorption spectrum of the synthesized SNPs at optimized conditions showed a broad peak at 441 nm, a characteristic band for SNPs. Further, the synthesized particles were characterized using SEM, XRD, and Zetasizer, as illustrated in **Fig. 7.2**. The XRD patterns for silver nanoparticles indicated the crystalline nature of the synthesized nanoparticles. The XRD showed four prominent diffraction peaks observed at 2θ values of 38.23°, 44.37°, 64.57°, and 77.51°, which correspond to the (111), (200), (220), and (311) crystallographic planes of face-centered cubic silver (Ag) crystals respectively. It was similar to the planes of the cubic structure of SNPs presented in JCPDS, file No. 04-0783)(Meng 2015)(Priyadharshini et al. 2014b).

The zeta potential of synthesized SNPs in aqueous solution was −23.7 mV, indicating higher stability of SNPs in colloidal solution. It is known that zeta potential indicates the stability of SNPs containing aqueous suspension and particle morphology as well (Sankhla et al. 2016). This parameter shows the degree of repulsion between the charged particles in the dispersion. ZP values are typically in the range of +100 to −100 mV. High ZP implies highly charged particles, which prevents aggregation of the particles due to electric repulsion. If the ZP is low, attraction overcomes repulsion, and it is likely that the mixture would form coagulate(Shnoudeh et al. 2019). It is the measurement of immensity and type of surface charges corresponding to double layers all over the particles with high positive/negative values of zeta potential. Accordingly, ±30 mV is considered a more stable colloidal solution due to electrostatic attraction/repulsion among the particles (Xu 2008). It is related to particle size and environmental factors like pH, ionic strength, and the type of ions present in suspension (El Badawy et al. 2010).

Scanning electron microscope (SEM) was used to determine the shape and size of the synthesized SNPs in this study. An aqueous solution of SNPs was added dropwise to a quartz thin

film and allowed to settle, followed by SEM images being captured. The shape of SNPs observed from SEM was spherical, as shown in Fig. 2 (i). A broad size distribution of silver nanoparticles was observed. A similar study agreed with the observed absorption and Zetasizer (Pesika et al. 2003). A comparatively lower value of zeta potential for the silver nanoparticles suggests that SNPs have a tendency to aggregate to form bigger-sized particles (Satapathy et al. 2015).

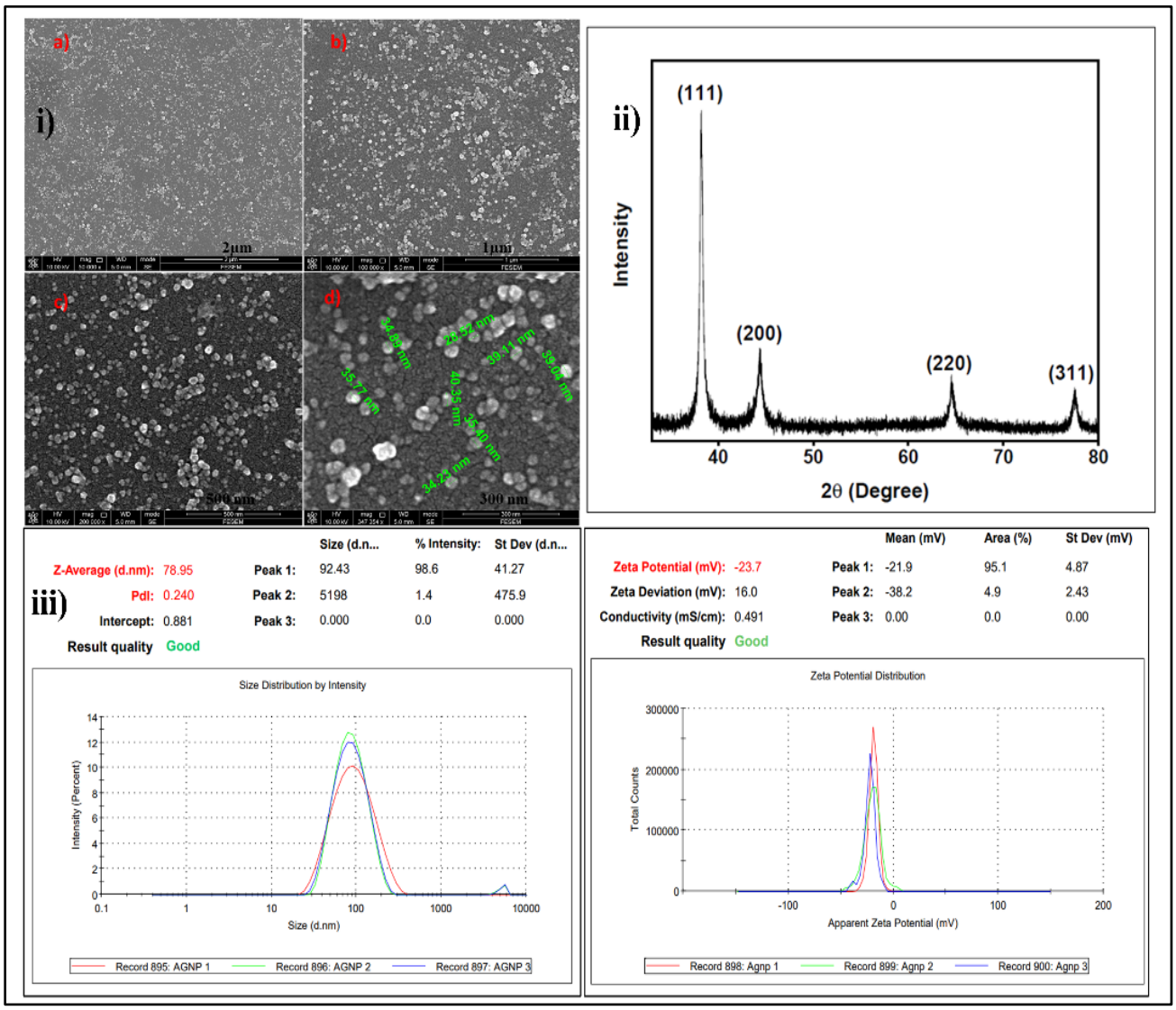


Fig. 7.2 Characterization of green synthesized silver nanoparticles using FESEM, XRD, and Zetasizer

7.3.3 Antibacterial effect of SNPs produced using *Chlorella sorokiniana*

Table 7.1 Zone of inhibition (mm) depicted by synthesized SNPs against *E. coli* and *S. aureus*

Test solution (in µL)	**Zone of inhibition (in millimetres)**	
	E. coli	S. aureus
Algal Extract	0 mm	0 mm
SNPs (10µL)	11±1 mm	7±1 mm
SNPs (20µL)	17±1 mm	11±1 mm
SNPs (25µL)	20±1 mm	15±1 mm

In the present study, the antibacterial activity of SNPs synthesized using *Chlorella sorokiniana* extract was tested against *E. coli* (gram-negative) and *S. aureus* (gram-positive) using the agar well diffusion technique. *E. coli* and *S. aureus*, considered as potential human pathogens (de Aragão et al. 2016). The result of the study indicated that the maximum zone of inhibition was observed at higher concentrations (25 µL) of SNPs as compared to lower concentrations (**Fig. 7.3**). The algal extract exhibited poor or no killing activity against any of the tested bacteria, thus indicating that the killing mechanism is solely due to the synthesized SNPs and not due to any of the bioactive compounds present in the algal extracts. As shown in **Table 7.1**, SNPs exhibited profound antibacterial activity against *E. coli* (20±1 mm) and were found to be moderately effective against *S. aureus* (15±1 mm). This difference in the effectiveness of SNPs can be attributed to the differential composition of cell walls of gram-positive and gram-negative bacterial

species. SNPs exhibited a lower antibacterial activity against *S. aureus* due to the thick peptidoglycan layer in the cell wall of gram-positive bacteria, which hinders the absorption of SNPs in the cell cytoplasm leading to a lower or ineffective killing activity (Mohandass et al. 2013; de Aragão et al. 2016). The profound killing activity against *E. coli* was due to a thin peptidoglycan layer present in the cell wall of gram-negative bacterial species allowing the absorption of SNPs into the cell cytoplasm (Peiris et al. 2018).

The antibacterial activity of SNP is associated with the nanoscale hydrodynamic diameters of the particles, which enable easy uptake by the cells for acting against bacterial cells (de Aragão et al. 2016). It has been reported that smaller the size of nanoparticles, larger is the surface area, enabling a greater contact with the cells, thus is enhanced the killing efficacy (Wang et al. 2017). The antibacterial properties of nanoparticles can be attributed to one or a combination of the following mechanisms: induction of oxidative stress amongst the bacterial cells, the release of metal ions that interact with cells and their components through various non-oxidative mechanisms (Nagy et al. 2011; Leung et al. 2014). Some studies have reported that SNPs are responsible for neutralizing the charge on the bacterial cell wall leading to changes in permeability and ultimately causing cell death (Wang et al. 2017).

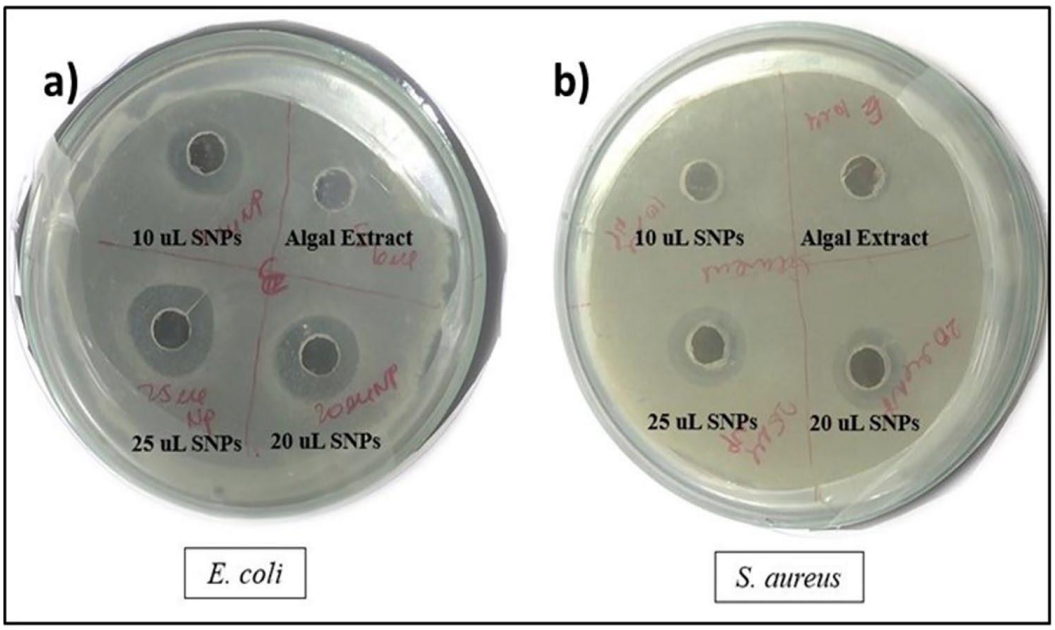


Fig. 7.3 Nutrient Agar plates depicting zone of inhibition caused due to the activity of SNPs (a) zone of inhibition caused due to varied concentration of SNPs against *E. coli* and; (b) zone of inhibition caused due to varied concentration of SNPs against *S. aureus*

7.3.4 Photocatalytic dye degradation using SNPs produced using *Chlorella sorokiniana*

The photocatalytic dye degradation was observed due to the excitation of surface plasmon response in SNPs. The electrons in the valance band move towards the conduction band, causing a hole in the lower band, and shifting the electrons in the upper band when exposed to sunlight. Excited electrons at nanoparticle surfaces interact with oxygen molecules and produce superoxide ions and hydroxyl radicals. The nanoparticles and hydroxyl radicals react with target dyes and break them into smaller fragments, thus facilitating their degradation (Aryan et al. 2021)(Kang et al. 2000)(Karthik et al. 2017a)(Khare et al. 2018)(Singh and Mehata 2019). In the current study, the photocatalytic degradation of 5 PPM solution of Eosin yellow (EY), methylene blue (MB), crystal violet (CV), and Rhodamine B (RhB) dye was carried out using *Chlorella sorokiniana* mediated SNPs (**Fig. 7.4**). MB is one of the most prominent water pollutants as it finds its use in the textile industry for various purposes. CV belongs to the group of triaryl methane dye and finds its application in the paper industry, textile dying, and other purposes (Abdel Azeem et al. 2021). EY

finds its use in histology for cell staining and visualization. RhB is commonly used for industrial purposes such as printing, dyeing, paints, and leather and is known to cause various health problems such as eye and skin irritation (Thakur and Kaur 2016). It was noted that after 18 hours of incubation in the sunlight, 0.5 mL of the biogenic SNPs per 20 mL of dye solutions were able to photo-catalytically degrade 97.04% of crystal violet, followed by 95.75% of methylene blue and 94.9% of eosin yellow. The degradation of EY reached 92.97% in 4 hours, after which there was only a little increment in degradation efficiency till 18 hours (**Fig.7.5**). The least degradation efficiency was found for rhodamine blue (56.05%). Vanaja et al. reported 95% degradation of MB using biogenic SNPs after 72 hours of incubation in sunlight (Vanaja et al. 2014). A study conducted by Karthik et al. reported that biogenic SNPs were able to degrade about 97% of EY after 1 hour of irradiation (Karthik et al. 2017b). Studies performed by Azeem et al., reported 89% degradation of crystal violet after 6 hours of incubation (Abdel Azeem et al. 2021). The degradation efficiency of SNPs under UV irradiation was found to be 86.51% after 90 minutes of incubation in the case of RhB, as demonstrated by Shaikh et al. in their study (Shaikh et al. 2020). In a different study conducted by Aryan et al. on SNPs based RhB degradation, 83% degradation within 45 minutes under UV irradiation was reported (Aryan et al. 2021). In this study, *C. sorokiniana* mediated SNPs were able to degrade 56.05% of RhB in presence of sunlight making this experimental setup cost-effective due to non-requirement of UV light. In another study, photocatalytic degradation of Rhodamine B by different metal nanoparticles, i.e., zinc oxide nanoparticles synthesized using the leaf extract of *Cyanometra ramiflora,* was reported with 98% degradation efficiency under direct sunlight (Varadavenkatesan et al. 2019). The photocatalytic ability refers to the creation of electron-hole pairs in a material when exposed to sunlight. SNPs have been widely studied as a photocatalytic agent for the degradation of dyes for the treatment of

wastewater. The use of solar energy for photocatalytic degradation is gaining importance. The photocatalytic activity in the presence of sunlight is highly dependent upon the size of SNPs, morphology, and crystalline structure of SNPs (Kumar 2021).

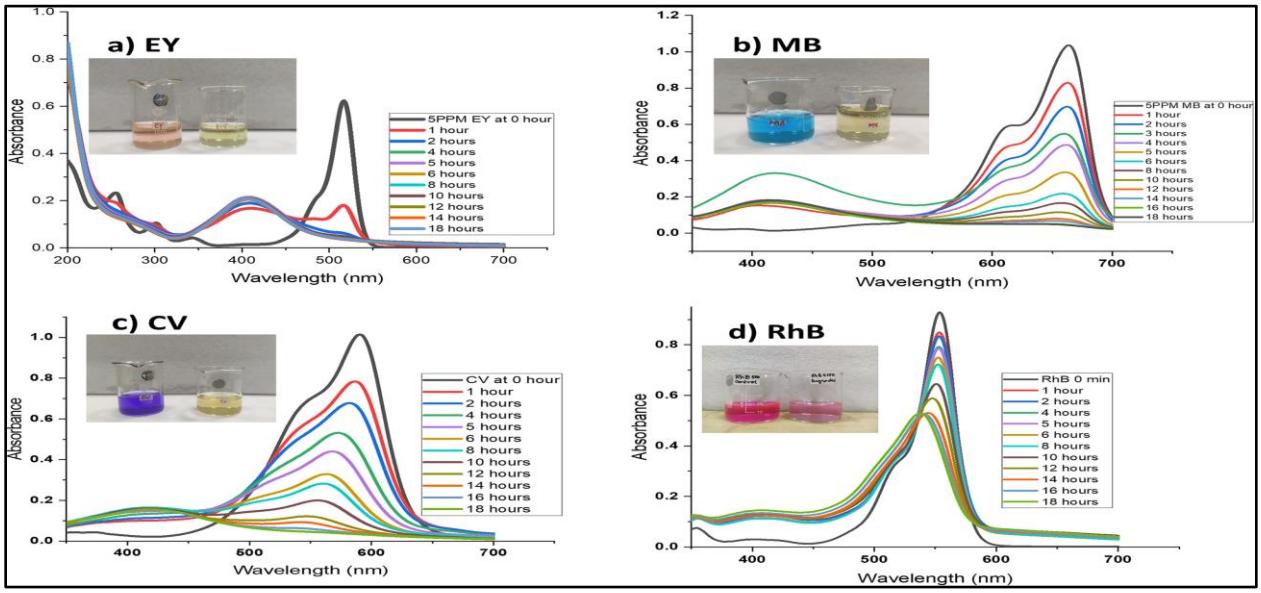


Fig. 7.4 Absorption spectrum and visual color change after dye degradation over a period of 18 hours of incubation in light condition: (a) EY; (b) MB; (c) CV and; (d) RhB

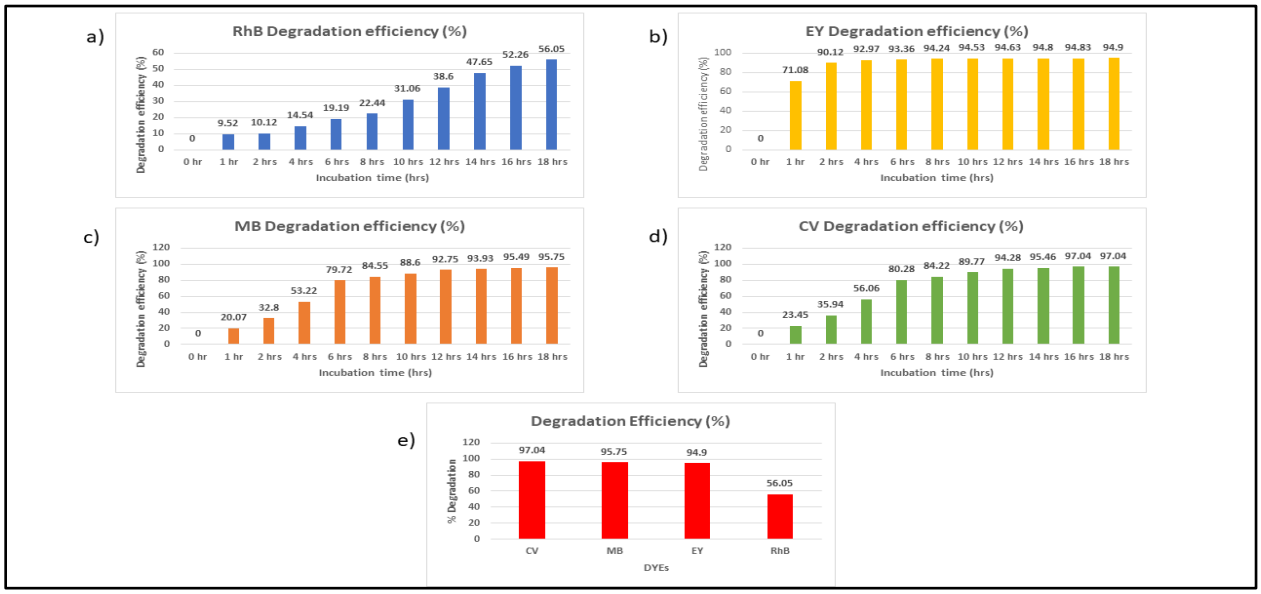


Fig. 7.5 Photocatalytic degradation efficiency using SNPs as catalysts against (a) Rhodamine B (RhB) dye; (b) Eosin Yellow (EY); (c) Methylene Blue (MB); (d) Crystal Violet (CV), and; (e) comparative degradation efficiency of four dyes after 18 hours of exposure to sunlight

7.4 Conclusion

Nanoparticle synthesis by physiochemical methods is highly expensive and uses hazardous chemicals, and leaves behind toxic by-products. Thus, green or biological synthesis has emerged as an alternative for the same. The present study investigated the prospect of using a green microalga *Chlorella sorokiniana* extract for silver nanoparticle synthesis. Optimum SNPs yield was achieved at pH 7, with a Metal precursor strength of 10mM with extract to the salt ratio of 10:1. Boiling biomass at 80°C for 20 minutes to prepare extract is a better strategy than sonication and autoclave for cell disruption. SNPs were synthesized optimally at room temperature under light conditions. It was observed that the SNPs synthesis rate was quite higher at 60°C temperature compared to room temperature, but the SNPs synthesis at the former condition was highly unstable. A color change from light green to dark brown initially confirmed the formation of SNPs. The UV-Vis absorption spectrum of the synthesized SNPs at optimized conditions showed a broad peak at 441 nm, a characteristic SNPs band. FESEM and XRD analysis showed that the SNPs were spherical and crystalline in nature, whereas the significant value of zeta potential indicates their high stability over a longer period of time. SNPs thus prepared at optimum conditions exhibited potency against two bacteria, gram-negative *Escherichia coli*, and gram-positive *Staphylococcus aureus* with zones of inhibition up to 20 ± 1 and 15 ± 1 mm respectively. Also, these SNPs were observed to catalyze the degradation of crystal violet by 97.04%, followed by methylene blue (95.75%), eosin Y (94.9%), and the least efficiency was observed for rhodamine B (56.05%); at the end of 18 hours under direct exposure of sunlight. It is evident from the study that *Chlorella sorokiniana* extract-mediated green SNPs can be considered a promising agent for the degradation of dyes in an aqueous solution as well as an adjuvant for the treatment of microbial infections**.**

CHAPTER 8

CONCLUSIONS AND FUTURE SCOPE OF THE WORK DONE

8.1. Summary and conclusion

Three microalgae strains were isolated from different water resources. Isolated microalgae strains were grown on BG-11 agar plate at incubated at 27±2ºC in an artificial light chamber under illumination with a white fluorescent light at 4000 lux on a photoperiod of 16L: 8D. Colonies were green and spherical and the average cell size, for each isolate, ranges between 2-6 µm. Molecular identification based on 18S rDNA sequence revealed that these algal isolates showed closed similarity with *Chlorella* sp. GC (Accession Number: KF773743.1), *Chlorella sorokiniana* isolate 19-4 (Accession Number: KU948990.1), and *Chlorella sorokiniana* strain Icheon4 (Accession Number: KF864476.1) respectively. *Chlorella minutissima* was procured and sub-cultured and scaled up in the laboratory. Thus, based on morphology and molecular examination, microalgae isolated were identified as *Chlorella* sp. (D), *Chlorella sorokiniana* strain 1 (R), and *Chlorella sorokiniana* strain 2 (S). *Chlorella minutissima (cm)* procured from CCUBGA IARI, Delhi. Culture bank of all these four microalgae were established for the further use.

Highest growth rate was observed for microalga *Chlorella sorokiniana* (isolate 2) on the basic of absorbance taken on a UV-Vis spectrophotometer. Lipid content of microalga *Chlorella sorokiniana* was determined to be approximately 11% of dry algal biomass powder. Elemental analysis revealed the content of C, H, N and S to be 44%, 8%,9% and 1% respectively. Optimization of culture conditions including light intensity, pH, and temperature was carried out. The best suitable light intensity, pH, and temperature was 4500 lux, pH 8, and 35ºC for optimal growth of *Chlorella sorokiniana*. The microalga showed almost similar growth profile

in alkaline pH range 7 to 9. No visible growth was observed at pH 5, 11,12. Also, the microalga isolate exhibited similar growth profile for the temperature range (30-40) ºC. Most suitable carbon, nitrogen, and phosphorous (CNP) sources among tested ones were found to be glucose, sodium nitrate, and potassium dihydrogen phosphate for microalga isolate. RO spent water can be used as a nutrient source for algal cultivation for *Chlorella minutissima* and *Chlorella sorokiniana.* It can reduce the exploitation of freshwater for cultivation of microalgae thus facilitating the availability of more drinking water and also the solution to RO spent or reject water.

Since the microalgae can be exploited not only for its high lipid content useful in production of economical biofuels, but also for high yields of essential pigments with high nutraceutical values. Algae have a unique property of accumulating high amounts of carotenoids under unfavourable conditions. There are efficient methods of subjecting the algal strain to stresses, both biotic and abiotic, that enhances the pigment production in them. The isolation and identification of economic, fast-growing, and adaptable algal strains are important. Therefore, the objective of this study was to isolate locally adapted microalgal strains for prospective β-carotene production. *Graesiella emersonii was* isolated from an industrial cement curing tank. The morphological and molecular identification studies confirmed that the strain is similar to *Graesiella emersonii*. It was green in colour, and spherical in shape with average cell size of 3-6µm. The color of the algal culture broth was initially green (for first 5-7 days) but later on it turned orange-red. It signifies the presence of pigments like astaxanthin, β-carotene and other high value chemicals. Lipid content in *Graesiella emersonii* was estimated to be 24.43% of dry cell weight. The presence of β-carotene in algal extract was confirmed by PC, TLC, and HPLC. β-carotene content varied to 0.643-1.26 mg/g dry algal biomass. 0.2M NaCl and 4000 lux light intensity

were found to be best among tested strategies for enhancement of β-carotene accumulation in the algal cells.

Poly-β-hydroxybutyrate (PHB), a type of Polyhydroxyalkanoates, is considered as one of the most promising bioplastics materials which is readily biodegradable in nature. Several microbes including bacteria, and microalgae have been used for production of PHB. In this direction, we explored the possibility of using microalgae *Chlorella minutissima* and *Chlorella sorokiniana* for production of PHB. In our study, Sudan Black B dye staining confirmed that both *Chlorella minutissima* and *Chlorella sorokiniana* are PHB accumulating strains. Black ring around the intracellular boundaries against a reddish background on staining with Sudan black dye followed by counterstaining with safranin was observed under light microscope. These microalgae cells were subjected to solvent extraction for estimation of PHB accumulated in the cells. The amount of PHB in *Chlorella minutissima* and *Chlorella sorokiniana* was estimated to be 374 mg/µl and 367mg/µl of algal suspension culture considering the complete extraction efficiency of the process applied. The study concludes that these microalgae can be used as suitable feedstock for renewable, sustainable, and ecofriendly bioplastic production.

Nanoparticle synthesis by physiochemical methods is highly expensive and uses hazardous chemicals, and leaves behind toxic by-products. Thus, green or biological synthesis has emerged as an alternative for the same. The present study investigated the prospect of using a green microalga *Chlorella sorokiniana* extract for silver nanoparticle synthesis. Optimum SNPs yield was achieved at pH 7, with a Metal precursor strength of 10mM with extract to the salt ratio of 10:1. Boiling biomass at 80°C for 20 minutes to prepare extract is a better strategy than sonication and autoclave for cell disruption. SNPs were synthesized optimally at room temperature under light conditions. It was observed that the SNPs synthesis rate was quite higher

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8.2. Future scope

The pursuit of renewable, sustainable and viable energy sources has explored deeper and steered the algal biotechnology to, a petro-refinery similar, biorefinery system. Microalgal biomass-based biorefinery is still in its nascent phase and needs for public and private policies along with high investments in research and developments, large-scale demonstrations and deployment strategies. A large-scale microalgal biomass-based biorefinery facility coupled with a wastewater treatment plant might reduce the cost of biofuel production and make it economically viable and environmentally sustainable. Applications of chemical genetics along with genetic

and metabolic engineering coupled with Omics of microalgae can push forward the development of commercially sustainable microalgal biorefineries and thus facilitating a way forward to a carbon-neutral society. Several innovations are still needed to overcome the issue of strain selection and development, cultivation systems, harvesting methods, dewatering and drying and conversion technologies along with analysis of environmental risk, resource management and life cycle analysis of microalgal biorefineries. Several combinations of processing techniques can be made for biorefinery. But, their applicability towards efficiency, energy consumption and scalability remain less suitable due to their underdeveloped nature or maturity. Scalable and economic extraction methods are highly needed for the rapid development of microalgal biorefinery. PEF, a mild, low cost, and low energy, technique for cell disruption followed by extraction using ionic liquids could be a better strategy for microalgal biorefinery. Ionic liquids can be used as an alternative to conventional organic solvents as they play a significant role in the separation of both hydrophilic and hydrophobic cellular components. However, research on various aspects of algal based bioactive compound extraction is in nascent phase and requires bioprospecting of high yielding native algal species, development and deployment of mass cultivation strategies, process optimization for harvest and cell disruption techniques followed by efficient biomolecule extraction procedures to make algal biorefinery sustainable and commercially viable in nature. Here are some following future scope of the work:

- Rapid methods of bioprospecting of algal strains with high yield of biomolecules of interest.
- Application of chemicals and genetic engineering to improve biosynthesis of a particular cell component/ high-value biomolecules
- Maintaining the stability of the high-value chemicals and their bioavailability is a major concern while adopting the biorefinery approach. There is a need for identification of milder

cell disruption technologies and lipid extraction methods to ensure the stability, functionality, and bioavailability of various high-value cellular components are retained if not enhanced.

- A correct sequence of product extraction from microalgae can bring advantages in terms of significant economic viability and stability to the microalgae-based biodiesel industry.
- Photo-bio-electrochemical systems for integration of wastewater treatment plants, microbial fuel cells and algal biofuels production.
- Versatile and low-cost downstream processing strategies providing simultaneous extraction of various cellular components.
- Ionic liquids as an alternative to conventional organic solvents
- Research, Development, Demonstrations and Deployment of technologies on upstream and downstream processing of algal based biorefinery.

REFERENCES

't Lam GP, Vermuë MH, Eppink MHM, et al (2018) Multi-Product Microalgae Biorefineries: From Concept Towards Reality. Trends Biotechnol 36:216–227. https://doi.org/10.1016/j.tibtech.2017.10.011

Abboud Y, Saffaj T, Chagraoui A, et al (2014) Biosynthesis, characterization and antimicrobial activity of copper oxide nanoparticles (CONPs) produced using brown alga extract (Bifurcaria bifurcata). Appl Nanosci 4:571–576. https://doi.org/10.1007/s13204-013-0233-x

Abdel-Raouf N, Al-Enazi NM, Ibraheem IBM (2017) Green biosynthesis of gold nanoparticles using Galaxaura elongata and characterization of their antibacterial activity. Arab J Chem 10:S3029–S3039. https://doi.org/10.1016/j.arabjc.2013.11.044

Abdel Azeem MN, Hassaballa S, Ahmed OM, et al (2021) Photocatalytic activity of revolutionary galaxaura elongata, turbinaria ornata, and enteromorpha flexuosa's bio-capped silver nanoparticles for industrial wastewater treatment. Nanomaterials 11:. https://doi.org/10.3390/nano11123241

Abdo SM, Ali GH (2019) Analysis of polyhydroxybutrate and bioplastic production from microalgae. Bull Natl Res Cent 43:1–4. https://doi.org/10.1186/s42269-019-0135-5

Abdolalian S, Taghavijeloudar M (2022) Performance evaluation and optimization of ZnO-PVP nanoparticles for photocatalytic wastewater treatment: Interactions between UV light intensity and nanoparticles dosage. J Clean Prod 365:132833. https://doi.org/10.1016/j.jclepro.2022.132833

Abedini Najafabadi H, Malekzadeh M, Jalilian F, et al (2015) Effect of various carbon sources on biomass and lipid production of Chlorella vulgaris during nutrient sufficient and nitrogen starvation conditions. Bioresour Technol 180:311–317. https://doi.org/10.1016/j.biortech.2014.12.076

Aboelfetoh EF, El-Shenody RA, Ghobara MM (2017) Eco-friendly synthesis of silver nanoparticles using green algae (Caulerpa serrulata): reaction optimization, catalytic and antibacterial activities. Environ Monit Assess 189:. https://doi.org/10.1007/s10661-017-6033-0

Ahmad S, Pandey A, Pathak VV, et al (2020) Phycoremediation: Algae as Eco-friendly Tools for the Removal of Heavy Metals from Wastewaters. Bioremediation Ind Waste Environ Saf 53–76. https://doi.org/10.1007/978-981-13-3426-9_3

Ahmed RH, Mustafa DE (2020) Green synthesis of silver nanoparticles mediated by traditionally used medicinal plants in Sudan. Int Nano Lett 10:1–14. https://doi.org/10.1007/s40089-019-00291-9

Ahuja P, Gupta R, Saxena RK (1997) Oscillatoria anguistissima: A promising Cu2+ biosorbent. Curr Microbiol 35:151–154. https://doi.org/10.1007/s002849900229

Al-Jabri H, Das P, Khan S, et al (2021) Treatment of wastewaters by microalgae and the potential applications of the produced biomass—a review. Water (Switzerland) 13:27

Aluç Y, Başaran Kankılıç G, Tüzün İ (2018) Determination of carotenoids in two algae species from the saline water of Kapulukaya reservoir by HPLC. J Liq Chromatogr Relat Technol 41:93–100. https://doi.org/10.1080/10826076.2017.1418376

Amadu AA, Qiu S, Ge S, et al (2021) A review of biopolymer (Poly-β-hydroxybutyrate) synthesis in microbes cultivated on wastewater. Sci Total Environ 756:143729. https://doi.org/10.1016/j.scitotenv.2020.143729

Ambati RR, Moi PS, Ravi S, Aswathanarayana RG (2014) Astaxanthin: Sources, extraction, stability, biological activities and its commercial applications - A review. Mar Drugs 12:128–152. https://doi.org/10.3390/md12010128

Amin M, Anwar F, Janjua MRSA, et al (2012) Green synthesis of silver nanoparticles through reduction with Solanum xanthocarpum L. berry extract: Characterization, antimicrobial and urease inhibitory activities against Helicobacter pylori. Int J Mol Sci 13:9923–9941. https://doi.org/10.3390/ijms13089923

Amobonye A, Bhagwat P, Singh S, Pillai S (2021) Plastic biodegradation: Frontline microbes and their enzymes. Sci. Total Environ. 759:143536

Andrade LM (2018) Chlorella and Spirulina Microalgae as Sources of Functional Foods, Nutraceuticals, and Food Supplements; an Overview. MOJ Food Process Technol 6:45–58. https://doi.org/10.15406/mojfpt.2018.06.00144

Ani Azaman SN, Nagao N, Yusoff FM, et al (2017) A comparison of the morphological and biochemical characteristics of chlorella sorokiniana and chlorella zofingiensis cultured under photoautotrophic and mixotrophic conditions. PeerJ 2017:e3473. https://doi.org/10.7717/peerj.3473

Anigol LB, Charantimath JS, Gurubasavaraj PM (2017) Effect of Concentration and pH on the Size of Silver Nanoparticles Synthesized by Green Chemistry. Org Med Chem Int Journal 3:1–5. https://doi.org/10.19080/OMCIJ.2017.03.555622

Ansari S, Fatma T (2016) Cyanobacterial polyhydroxybutyrate (PHB): Screening, optimization and characterization. PLoS One 11:e0158168. https://doi.org/10.1371/journal.pone.0158168

Arakha M, Pal S, Samantarrai D, et al (2015) Antimicrobial activity of iron oxide nanoparticle upon modulation of nanoparticle-bacteria interface. Sci Rep 5:1–12. https://doi.org/10.1038/srep14813

Aravindhan R, Rao JR, Nair BU (2007) Kinetic and equilibrium studies on biosorption of basic blue dye by green macro algae Caulerpa scalpelliformis. J Environ Sci Heal - Part A Toxic/Hazardous Subst Environ Eng 42:621–631. https://doi.org/10.1080/10934520701244383

Arun J, Vigneshwar SS, Swetha A, et al (2022) Bio-based algal (Chlorella vulgaris) refinery on de-oiled algae biomass cake: A study on biopolymer and biodiesel production. Sci Total

Environ 816:151579. https://doi.org/10.1016/j.scitotenv.2021.151579

Aryan, Ruby, Mehata MS (2021) Green synthesis of silver nanoparticles using Kalanchoe pinnata leaves (life plant) and their antibacterial and photocatalytic activities. Chem Phys Lett 778:138760. https://doi.org/10.1016/j.cplett.2021.138760

Atlić A, Koller M, Scherzer D, et al (2011) Continuous production of poly([R]-3-hydroxybutyrate) by Cupriavidus necator in a multistage bioreactor cascade. Appl Microbiol Biotechnol 91:295–304. https://doi.org/10.1007/s00253-011-3260-0

Azizi-Lalabadi M, Ehsani A, Divband B, Alizadeh-Sani M (2019) Antimicrobial activity of Titanium dioxide and Zinc oxide nanoparticles supported in 4A zeolite and evaluation the morphological characteristic. Sci Rep 9:1–10. https://doi.org/10.1038/s41598-019-54025-0

Azizi S, Namvar F, Mahdavi M, et al (2013) Biosynthesis of silver nanoparticles using brown marine macroalga, Sargassum muticum aqueous extract. Materials (Basel) 6:5942–5950. https://doi.org/10.3390/ma6125942

Bachchhav MB, Kulkarni M V., Ingale AG (2020) Process-intensified extraction of phycocyanin followed by β-carotene from Spirulina platensis using ultrasound-assisted extraction. Sep Sci Technol 55:932–944. https://doi.org/10.1080/01496395.2019.1580293

Balaji S, Gopi K, Muthuvelan B (2013) A review on production of poly β hydroxybutyrates from cyanobacteria for the production of bio plastics. Algal Res 2:278–285. https://doi.org/10.1016/j.algal.2013.03.002

Banerjee A, Sharma R, Chisti Y, Banerjee UC (2002) *Botryococcus braunii* : A Renewable Source of Hydrocarbons and Other Chemicals. Crit Rev Biotechnol 22:245–279. https://doi.org/10.1080/07388550290789513

Barkia I, Saari N, Manning SR (2019) Microalgae for high-value products towards human health and nutrition. Mar Drugs 17:1–29. https://doi.org/10.3390/md17050304

Barwal I, Ranjan P, Kateriya S, Yadav SC (2011) Cellular oxido-reductive proteins of Chlamydomonas reinhardtii control the biosynthesis of silver nanoparticles. J Nanobiotechnology 9:1–12. https://doi.org/10.1186/1477-3155-9-56

Beckstrom BD, Wilson MH, Crocker M, Quinn JC (2020) Bioplastic feedstock production from microalgae with fuel co-products: A techno-economic and life cycle impact assessment. Algal Res 46:101769. https://doi.org/10.1016/j.algal.2019.101769

Bhalamurugan GL, Valerie O, Mark L (2018) Valuable bioproducts obtained from microalgal biomass and their commercial applications: A review. Environ Eng Res 23:229–241. https://doi.org/10.4491/eer.2017.220

Bhandari M, Kharkwal S, Prajapati SK (2023) Recycling drinking water RO reject for microalgae-mediated resource recovery. Resour Conserv Recycl 188:106699. https://doi.org/10.1016/j.resconrec.2022.106699

Bhandari M, Prajapati SK (2022) Use of reverse osmosis reject from drinking water plant for

microalgal biomass production. Water Res 210:117989. https://doi.org/10.1016/j.watres.2021.117989

Bharadvaja N, Kumar L (2020a) Algal biorefinery for extraction of bioactive compounds. Curr Bioact Compd 16:. https://doi.org/https://doi.org/10.2174/1573407216999200630115417

Bharadvaja N, Kumar L (2020b) Algal biorefinery for extraction of bioactive compounds. Curr Bioact Compd 16:. https://doi.org/10.2174/1573407216999200630115417

Bhuyar P, Rahim MHA, Sundararaju S, et al (2020) Synthesis of silver nanoparticles using marine macroalgae Padina sp. and its antibacterial activity towards pathogenic bacteria. Beni-Suef Univ J Basic Appl Sci 9:. https://doi.org/10.1186/s43088-019-0031-y

Bokesch HR, O'Keefe BR, McKee TC, et al (2003) A potent novel anti-HIV protein from the cultured cyanobacterium Scytonema varium. Biochemistry 42:2578–2584. https://doi.org/10.1021/bi0205698

Branco-Vieira M, San Martin S, Agurto C, et al (2020) Biotechnological potential of Phaeodactylum tricornutum for biorefinery processes. Fuel 268:117357. https://doi.org/10.1016/j.fuel.2020.117357

Brasil BSAF, Silva FCP, Siqueira FG (2017) Microalgae biorefineries: The Brazilian scenario in perspective. N Biotechnol 39:90–98. https://doi.org/10.1016/j.nbt.2016.04.007

Cardozo KHM, Guaratini T, Barros MP, et al (2007) Metabolites from algae with economical impact. Comp Biochem Physiol Part C Toxicol Pharmacol 146:60–78. https://doi.org/10.1016/J.CBPC.2006.05.007

Carpenter EJ, Smith KL (1972) Plastics on the Sargasso sea surface. Science (80-) 175:1240–1241. https://doi.org/10.1126/science.175.4027.1240

Cassuriaga APA, Freitas BCB, Morais MG, Costa JAV (2018) Innovative polyhydroxybutyrate production by Chlorella fusca grown with pentoses. Bioresour Technol 265:456–463. https://doi.org/10.1016/j.biortech.2018.06.026

Castro L, Blázquez ML, Muñoz JA, et al (2013) Biological synthesis of metallic nanoparticles using algae. IET Nanobiotechnology 7:109–116. https://doi.org/10.1049/iet-nbt.2012.0041

Cazzaniga S, Dall'Osto L, Szaub J, et al (2014) Domestication of the green alga Chlorella sorokiniana: Reduction of antenna size improves light-use efficiency in a photobioreactor. Biotechnol Biofuels 7:1–13. https://doi.org/10.1186/s13068-014-0157-z

Chai S, Shi J, Huang T, et al (2018) Characterization of chlorella sorokiniana growth properties in monosaccharide-supplemented batch culture. PLoS One 13:e0199873. https://doi.org/10.1371/journal.pone.0199873

Chalima A, Taxeidis G, Topakas E (2020) Optimization of the production of docosahexaenoic fatty acid by the heterotrophic microalga Crypthecodinium cohnii utilizing a dark fermentation effluent. Renew Energy 152:102–109. https://doi.org/10.1016/j.renene.2020.01.041

Chaudhary R, Nawaz K, Khan AK, et al (2020a) An overview of the algae-mediated biosynthesis of nanoparticles and their biomedical applications. Biomolecules 10:1–35. https://doi.org/10.3390/biom10111498

Chaudhary R, Nawaz K, Khan AK, et al (2020b) An overview of the algae-mediated biosynthesis of nanoparticles and their biomedical applications. Biomolecules 10:1–35

Chavan R, Mutnuri S (2019) Tertiary treatment of domestic wastewater by Spirulina platensis integrated with microalgal biorefinery. Biofuels 10:33–44. https://doi.org/10.1080/17597269.2018.1461509

Chen C-Y, Chen Y-C, Huang H-C, et al (2013) Engineering strategies for enhancing the production of eicosapentaenoic acid (EPA) from an isolated microalga Nannochloropsis oceanica CY2. Bioresour Technol 147:160–167. https://doi.org/10.1016/j.biortech.2013.08.051

Chen C-Y, Chen Y-C, Huang H-C, et al (2015) Enhancing the production of eicosapentaenoic acid (EPA) from Nannochloropsis oceanica CY2 using innovative photobioreactors with optimal light source arrangements. Bioresour Technol 191:407–413. https://doi.org/10.1016/j.biortech.2015.03.001

Chen C-Y, Yeh K-L, Aisyah R, et al (2011) Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. Bioresour Technol 102:71–81. https://doi.org/10.1016/J.BIORTECH.2010.06.159

Chen H feng, Wu J juan, Wu M yue, Jia H (2019) Preparation and antibacterial activities of copper nanoparticles encapsulated by carbon. New Carbon Mater 34:382–389. https://doi.org/10.1016/S1872-5805(19)30023-X

Chen JJ, Wong SL, Li YR, et al (2014) Optimization of microalgae cell conditions for lipid production. Energy Sources, Part A Recover Util Environ Eff 36:1596–1603. https://doi.org/10.1080/15567036.2011.555355

Chew KW, Yap JY, Show PL, et al (2017) Microalgae biorefinery: High value products perspectives. Bioresour Technol 229:53–62. https://doi.org/10.1016/j.biortech.2017.01.006

Chiellini C, Guglielminetti L, Sarrocco S, Ciurli A (2020) Isolation of Four Microalgal Strains From the Lake Massaciuccoli: Screening of Common Pollutants Tolerance Pattern and Perspectives for Their Use in Biotechnological Applications. Front Plant Sci 11:. https://doi.org/10.3389/fpls.2020.607651

Chiong MC, Chong CT, Ng JH, et al (2018) Liquid biofuels production and emissions performance in gas turbines: A review. Energy Convers Manag 173:640–658. https://doi.org/10.1016/j.enconman.2018.07.082

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25:294–306. https://doi.org/10.1016/J.BIOTECHADV.2007.02.001

Cordero BF, Obraztsova I, Couso I, et al (2011) Enhancement of lutein production in Chlorella sorokiniana (chorophyta) by improvement of culture conditions and random mutagenesis.

Mar Drugs 9:1607–1624. https://doi.org/10.3390/md9091607

D'Alessandro EB, Soares AT, Lopes RG, et al (2020) Lutein and biodiesel sequential production from microalga using an environmentally friendly approach. Chem Eng Commun 0:1–11. https://doi.org/10.1080/00986445.2020.1722654

Das SK, Sathish A, Stanley J (2018) Production of Biofuel and Bioplastic from Chlorella Pyrenoidosa. In: Materials Today: Proceedings. Elsevier, pp 16774–16781

de Aragão AP, de Oliveira TM, Quelemes PV, et al (2016) Green synthesis of silver nanoparticles using the seaweed Gracilaria birdiae and their antibacterial activity. Arab J Chem 12:4182–4188. https://doi.org/10.1016/j.arabjc.2016.04.014

Dean AP, Sigee DC (2006) Molecular heterogeneity in Aphanizomenon flos-aquae and Anabaena flos-aquae (Cyanophyta): A synchrotron-based Fourier-transform infrared study of lake micropopulations. Eur J Phycol 41:201–212. https://doi.org/10.1080/09670260600645907

Del Campo JA, García-González M, Guerrero MG (2007) Outdoor cultivation of microalgae for carotenoid production: Current state and perspectives. Appl. Microbiol. Biotechnol. 74:1163–1174

Dhivya M, Mirunalini A, P TV (2023) Isolation , purification and characterization of polyhydroxyalkanotes from green algae (Chlorella sp .). Res J Biotechnol 18:94–99

Dinesh Kumar S, Santhanam P, Jayalakshmi T, et al (2015) Excessive nutrients and heavy metals removal from diverse wastewaters using marine microalga Chlorella marina (Butcher). Indian J Geo-Marine Sci 44:97–103

Dong X, Huang L, Li T, et al (2020) The enhanced biomass and lipid accumulation in algae with an integrated treatment strategy by waste molasses and Mg2+ addition. Energy Sources, Part A Recover Util Environ Eff 42:1183–1192. https://doi.org/10.1080/15567036.2019.1602227

Duong VT, Li Y, Nowak E, et al (2012) Microalgae Isolation and Selection for Prospective Biodiesel Production. Energies 5:1835–1849. https://doi.org/10.3390/en5061835

Eismann AI, Perpetuo Reis R, Ferreira da Silva A, Negrão Cavalcanti D (2020) Ulva spp. carotenoids: Responses to environmental conditions. Algal Res 48:1–18. https://doi.org/10.1016/j.algal.2020.101916

El-Kassas HY, El-Sheekh MM (2014) Cytotoxic activity of biosynthesized gold nanoparticles with an extract of the red seaweed Corallina officinalis on the MCF-7 human breast cancer cell line. Asian Pacific J Cancer Prev 15:4311–4317. https://doi.org/10.7314/APJCP.2014.15.10.4311

El-Rafie HM, El-Rafie MH, Zahran MK (2013) Green synthesis of silver nanoparticles using polysaccharides extracted from marine macro algae. Carbohydr Polym 96:403–410. https://doi.org/10.1016/j.carbpol.2013.03.071

El-Sheekh M, Abu-Faddan M, Abo-Shady A, et al (2020a) Molecular identification, biomass, and biochemical composition of the marine chlorophyte Chlorella sp. MF1 isolated from Suez Bay. J Genet Eng Biotechnol 18:1–10. https://doi.org/10.1186/s43141-020-00044-8

El-Sheekh MM, El-Kassas HY (2016) Algal production of nano-silver and gold: Their antimicrobial and cytotoxic activities: A review. J. Genet. Eng. Biotechnol. 14:299–310

El-Sheekh MM, Eladel HM, Abomohra AEF, et al (2019) Optimization of biomass and fatty acid productivity of Desmodesmus intermedius as a promising microalga for biodiesel production. Energy Sources, Part A Recover Util Environ Eff 00:1–14. https://doi.org/10.1080/15567036.2019.1673851

El-Sheekh MM, Gharieb MM, Abou-El-Souod GW (2009) Biodegradation of dyes by some green algae and cyanobacteria. Int Biodeterior Biodegrad 63:699–704. https://doi.org/10.1016/j.ibiod.2009.04.010

El-Sheekh MM, Shabaan MT, Hassan L, Morsi HH (2020b) Antiviral activity of algae biosynthesized silver and gold nanoparticles against Herps Simplex (HSV-1) virus in vitro using cell-line culture technique. Int J Environ Health Res 1–12. https://doi.org/10.1080/09603123.2020.1789946

El Badawy AM, Luxton TP, Silva RG, et al (2010) Impact of environmental conditions (pH, ionic strength, and electrolyte type) on the surface charge and aggregation of silver nanoparticles suspensions. Environ Sci Technol 44:1260–1266. https://doi.org/10.1021/es902240k

El Ouaer M, Turki N, Ibrahim C, et al (2022) Insights into the use of landfill leachate to grow Chlorella sp. for lipid and fatty acids production. Clean Technol Environ Policy 1:1–12. https://doi.org/10.1007/s10098-022-02460-5

Eonseon J, Lee C-G, Polle JEW (2006) Secondary carotenoid accumulation in Haematococcus (Chlorophyceae) : Biosynthesis, regulation, and biotechnology. J Microbiol Biotechnol 16:821–831

Espiritu RA, Tan MCS, Oyong GG (2017) Evaluation of the anti-cancer potential of amphidinol 2, a polyketide metabolite from the marine dinoflagellate Amphidinium klebsii. Jordan J Biol Sci 10:297–302

European Commission, Joint Research Centre, Araújo, R., Peteiro C (2021) Algae as food and food supplements in Europe, Publications Office

Fatima F, Siddiqui S, Khan WA (2021) Nanoparticles as Novel Emerging Therapeutic Antibacterial Agents in the Antibiotics Resistant Era. Biol. Trace Elem. Res. 199:2552–2564

G S, L K, SM H, et al (2016) Identification of Potential Culture Conditions for Enhancing the Biomass Production of Microalga Chlorella minutissima. Expert Opin Environ Biol s1:. https://doi.org/10.4172/2325-9655.S1-005

Ganesan AR, Tiwari U, Rajauria G (2019) Seaweed nutraceuticals and their therapeutic role in

disease prevention. Food Sci Hum Wellness 8:252–263. https://doi.org/10.1016/j.fshw.2019.08.001

Garcia-Vaquero M, Rajauria G, Miranda M, et al (2021) Seasonal Variation of the Proximate Composition, Mineral Content, Fatty Acid Profiles and Other Phytochemical Constituents of Selected Brown Macroalgae. Mar Drugs 19:204. https://doi.org/10.3390/MD19040204

Gardeva E, Toshkova R, Minkova K, Gigova L (2009) Cancer protective action of polysaccharide, derived from red microalga porphyridium cruentum—a biological background. Biotechnol Biotechnol Equip 23:783–787. https://doi.org/10.1080/13102818.2009.10818540

Gengiah K, Moses GLP, Baskar G (2020) Bioethanol production from Codium tomentosum residue . Energy Sources, Part A Recover Util Environ Eff 00:1–10. https://doi.org/10.1080/15567036.2020.1771481

Getachew A, Woldesenbet F (2016) Production of biodegradable plastic by polyhydroxybutyrate (PHB) accumulating bacteria using low cost agricultural waste material. BMC Res Notes 9:1–9. https://doi.org/10.1186/s13104-016-2321-y

Goldman JC, Azov Y, Riley CB, Dennett MR (1982) The effect of pH in intensive microalgal cultures. I. Biomass regulation. J Exp Mar Bio Ecol 57:1–13. https://doi.org/10.1016/0022-0981(82)90140-X

González-Ballesteros N, Prado-López S, Rodríguez-González JB, et al (2017) Green synthesis of gold nanoparticles using brown algae Cystoseira baccata: Its activity in colon cancer cells. Colloids Surfaces B Biointerfaces 153:190–198. https://doi.org/10.1016/j.colsurfb.2017.02.020

González-Resendiz L, Sánchez-García L, Hernández-Martínez I, et al (2021) Photoautotrophic poly(3-hydroxybutyrate) production by a wild-type Synechococcus elongatus isolated from an extreme environment. Bioresour Technol 337:125508. https://doi.org/10.1016/j.biortech.2021.125508

Gour A, Jain NK (2019) Advances in green synthesis of nanoparticles. Artif Cells, Nanomedicine Biotechnol 47:844–851. https://doi.org/10.1080/21691401.2019.1577878

Grobbelaar JU (2009) Upper limits of photosynthetic productivity and problems of scaling. J Appl Phycol 21:519–522. https://doi.org/10.1007/s10811-008-9372-y

Guedes AC, Amaro HM, Malcata FX (2011) Microalgae as sources of carotenoids. Mar Drugs 9:625–644. https://doi.org/10.3390/md9040625

Gupta A, Mumtaz S, Li CH, et al (2019) Combatting antibiotic-resistant bacteria using nanomaterials. Chem. Soc. Rev. 48:415–427

Halim R, Danquah MK, Webley PA (2012) Extraction of oil from microalgae for biodiesel production: A review. Biotechnol Adv 30:709–732. https://doi.org/10.1016/J.BIOTECHADV.2012.01.001

Han X, Zeng H, Bartocci P, et al (2018) Phytohormones and effects on growth and metabolites of microalgae: A review. Fermentation 4:25

Harvey PJ, Ben-Amotz A (2020) Towards a sustainable Dunaliella salina microalgal biorefinery for 9-cis β-carotene production. Algal Res 50:102002. https://doi.org/10.1016/j.algal.2020.102002

He Y, Ma M, Hu Q, Gong Y (2021) Assessment of NH4HCO3 for the control of the predator flagellate Poterioochromonas malhamensis in pilot-scale culture of Chlorella sorokiniana. Algal Res 60:102481. https://doi.org/10.1016/j.algal.2021.102481

Hernández-carmona G, Carrillo-domínguez S, Arvizu-higuera DL, et al (2009) Monthly variation in the chemical composition of Eisenia arborea J . E . Areschoug. J Appl Phycol 21:607–616. https://doi.org/10.1007/s10811-009-9454-5

Hu CC, Lin JT, Lu FJ, et al (2008a) Determination of carotenoids in Dunaliella salina cultivated in Taiwan and antioxidant capacity of the algal carotenoid extract. Food Chem 109:439–446. https://doi.org/10.1016/j.foodchem.2007.12.043

Hu J, Nagarajan D, Zhang Q, et al (2018) Heterotrophic cultivation of microalgae for pigment production: A review. Biotechnol Adv 36:54–67. https://doi.org/10.1016/J.BIOTECHADV.2017.09.009

Hu Q, Sommerfeld M, Jarvis E, et al (2008b) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J 54:621–639. https://doi.org/10.1111/j.1365-313X.2008.03492.x

Husain S, Afreen S, Hemlata, et al (2019) Cyanobacteria as a bioreactor for synthesis of silver nanoparticles-an effect of different reaction conditions on the size of nanoparticles and their dye decolorization ability. J Microbiol Methods 162:77–82. https://doi.org/10.1016/j.mimet.2019.05.011

Imani S, Rezaei-Zarchi S, Hashemi M, et al (2011) Hg, Cd and Pb heavy metal bioremediation by Dunaliella alga. J Med Plants Res 5:2275–2780

Ismail MM, Ismail GA, El-Sheekh MM (2020) Potential assessment of some micro- and macroalgal species for bioethanol and biodiesel production. Energy Sources, Part A Recover Util Environ Eff 00:1–17. https://doi.org/10.1080/15567036.2020.1758853

Jabeen S, Gao X, Altarawneh M, et al (2020) Analytical Procedure for Proximate Analysis of Algal Biomass: Case Study for Spirulina platensis and Chlorella vulgaris. Energy and Fuels 34:474–482. https://doi.org/10.1021/acs.energyfuels.9b03156

Javed R, Zia M, Naz S, et al (2020) Role of capping agents in the application of nanoparticles in biomedicine and environmental remediation: recent trends and future prospects. J Nanobiotechnology 18:1–15. https://doi.org/10.1186/s12951-020-00704-4

Jena J, Pradhan N, Aishvarya V, et al (2015) Biological sequestration and retention of cadmium as CdS nanoparticles by the microalga Scenedesmus-24. J Appl Phycol 27:2251–2260. https://doi.org/10.1007/s10811-014-0499-8

Ji F, Hao R, Liu Y, et al (2013) Isolation of a novel microalgae strain Desmodesmus sp. and optimization of environmental factors for its biomass production. Bioresour Technol 148:249–254. https://doi.org/10.1016/j.biortech.2013.08.110

Kamravamanesh D, Pflügl S, Nischkauer W, et al (2017) Photosynthetic poly-β-hydroxybutyrate accumulation in unicellular cyanobacterium Synechocystis sp. PCC 6714. AMB Express 7:1–12. https://doi.org/10.1186/s13568-017-0443-9

Kang NS, Cho K, An SM, et al (2022a) Taxonomic and Biochemical Characterization of Microalga Graesiella emersonii GEGS21 for Its Potential to Become Feedstock for Biofuels and Bioproducts. Energies 15:8725. https://doi.org/10.3390/en15228725

Kang NS, Cho K, An SM, et al (2022b) Taxonomic and Biochemical Characterization of Microalga Graesiella emersonii GEGS21 for Its Potential to Become Feedstock for Biofuels and Bioproducts. Energies 15:8725. https://doi.org/10.3390/en15228725

Kang SF, Liao CH, Po ST (2000) Decolorization of textile wastewater by photo-fenton oxidation technology. Chemosphere 41:1287–1294. https://doi.org/10.1016/S0045-6535(99)00524-X

Kannan RRR, Arumugam R, Ramya D, et al (2013) Green synthesis of silver nanoparticles using marine macroalga Chaetomorpha linum. Appl Nanosci 3:229–233. https://doi.org/10.1007/s13204-012-0125-5

Karnaouri A, Chalima A, Kalogiannis KG, et al (2020) Utilization of lignocellulosic biomass towards the production of omega-3 fatty acids by the heterotrophic marine microalga Crypthecodinium cohnii. Bioresour Technol 303:122899. https://doi.org/10.1016/j.biortech.2020.122899

Karthik R, Govindasamy M, Chen SM, et al (2017a) Biosynthesis of silver nanoparticles by using Camellia japonica leaf extract for the electrocatalytic reduction of nitrobenzene and photocatalytic degradation of Eosin-Y. J Photochem Photobiol B Biol 170:164–172. https://doi.org/10.1016/j.jphotobiol.2017.03.018

Karthik R, Govindasamy M, Chen SM, et al (2017b) Biosynthesis of silver nanoparticles by using Camellia japonica leaf extract for the electrocatalytic reduction of nitrobenzene and photocatalytic degradation of Eosin-Y. J Photochem Photobiol B Biol 170:164–172. https://doi.org/10.1016/j.jphotobiol.2017.03.018

Kathiraven T, Sundaramanickam A, Shanmugam N, Balasubramanian T (2015) Green synthesis of silver nanoparticles using marine algae Caulerpa racemosa and their antibacterial activity against some human pathogens. Appl Nanosci 5:499–504. https://doi.org/10.1007/s13204-014-0341-2

Kato N (2019) Production of crude bioplastic-beads with microalgae: Proof-of-concept. Bioresour Technol Reports 6:81–84. https://doi.org/10.1016/j.biteb.2019.01.022

Kavitha G, Kurinjimalar C, Sivakumar K, et al (2016) Biosynthesis, purification and characterization of polyhydroxybutyrate from Botryococcus braunii kütz. Int J Biol Macromol 89:700–706. https://doi.org/10.1016/j.ijbiomac.2016.04.086

Kazbar A, Cogne G, Urbain B, et al (2019) Effect of dissolved oxygen concentration on microalgal culture in photobioreactors. Algal Res 39:762–768. https://doi.org/10.1016/j.algal.2019.101432

Khanna P, Kaur A, Goyal D (2019) Algae-based metallic nanoparticles: Synthesis, characterization and applications. J Microbiol Methods 163:105656. https://doi.org/10.1016/j.mimet.2019.105656

Khare P, Singh A, Verma S, et al (2018) Sunlight-Induced Selective Photocatalytic Degradation of Methylene Blue in Bacterial Culture by Pollutant Soot Derived Nontoxic Graphene Nanosheets. ACS Sustain Chem Eng 6:579–589. https://doi.org/10.1021/acssuschemeng.7b02929

Khyalia P, Gahlawat A, Jugiani H, et al (2022) Review on the use of Microalgae Biomass for Bioplastics Synthesis: A Sustainable and Green approach to control Plastic Pollution. Pollution 8:844–859. https://doi.org/10.22059/POLL.2022.334756.1273

Kim JH, Affan MA, Jang J, et al (2015) Morphological, molecular, and biochemical characterization of astaxanthin-producing green microalga Haematococcus sp. KORDI03 (haematococcaceae, chlorophyta) isolated from Korea. J Microbiol Biotechnol 25:238–246. https://doi.org/10.4014/jmb.1410.10032

Kose A, Oncel SS (2017) Algae as a promising resource for biofuel industry: facts and challenges. Int J Energy Res 41:924–951. https://doi.org/10.1002/er.3699

Kousoulidou M, Fontaras G, Ntziachristos L, Samaras Z (2010) Biodiesel blend effects on common-rail diesel combustion and emissions. Fuel 89:3442–3449. https://doi.org/10.1016/j.fuel.2010.06.034

Kozlova TA, Hardy BP, Krishna P, Levin DB (2017) Effect of phytohormones on growth and accumulation of pigments and fatty acids in the microalgae Scenedesmus quadricauda. Algal Res 27:325–334. https://doi.org/10.1016/j.algal.2017.09.020

Kumar B (2021) Green synthesis of gold, silver, and iron nanoparticles for the degradation of organic pollutants in wastewater. J Compos Sci 5:. https://doi.org/10.3390/jcs5080219

Kumar K, Banerjee D, Das D (2014a) Carbon dioxide sequestration from industrial flue gas by Chlorella sorokiniana. Bioresour Technol 152:225–233. https://doi.org/10.1016/j.biortech.2013.10.098

Kumar K, Dasgupta CN, Das D (2014b) Cell growth kinetics of Chlorella sorokiniana and nutritional values of its biomass. Bioresour Technol 167:358–366. https://doi.org/10.1016/j.biortech.2014.05.118

Kumar K, Dasgupta CN, Das D (2014c) Cell growth kinetics of Chlorella sorokiniana and nutritional values of its biomass. Bioresour Technol 167:358–366. https://doi.org/10.1016/j.biortech.2014.05.118

Kumar K, Dasgupta CN, Das D (2014d) Cell growth kinetics of Chlorella sorokiniana and nutritional values of its biomass. Bioresour Technol 167:358–366.

https://doi.org/10.1016/j.biortech.2014.05.118

Kumar L, Bharadvaja N (2020a) A review on microalgae biofuel and biorefinery: challenges and way forward. Energy Sources, Part A Recover. Util. Environ. Eff. 1–24

Kumar L, Bharadvaja N (2021) Algal-Based Wastewater Treatment and Biorefinery. In: SHAH MP, Sarkar A, Mandal S (eds) Wastewater Treatment: Cutting-Edge Molecular Tools, Techniques and Applied Aspects, 1st edn. Elsevier Inc., pp 413–432

Kumar L, Bharadvaja N (2020b) Microorganisms: A remedial source for dye pollution. In: Removal of Toxic Pollutants Through Microbiological and Tertiary Treatment. Elsevier, pp 309–333

Kumar L, Mohan L, Anand S, et al (2022a) Phyconanoremediation: a sustainable approach to deal with environmental pollutants heavy metals and dyes. Vegetos 2022 1–16. https://doi.org/10.1007/S42535-022-00399-Y

Kumar L, Ragunathan V, Chugh M, Bharadvaja N (2021) Nanomaterials for remediation of contaminants: a review. Environ. Chem. Lett. 19:3139–3163

Kumar L, Roy A, Saxena G, et al (2017) Isolation, Identification and biomass productivity analysis of microalga Scenedesmus rubescens from DTU Lake. J Algal Biomass Utln 8:56–67

Kumar L, Saha A, Khushbu, Warkar SG (2022b) Biodegradability of automotive plastics and composites. In: Biodegradability of Conventional Plastics: Opportunities, Challenges, and Misconceptions. pp 221–242

Kumar M, Sun Y, Rathour R, et al (2020) Algae as potential feedstock for the production of biofuels and value-added products: Opportunities and challenges. Sci Total Environ 716:137116. https://doi.org/10.1016/j.scitotenv.2020.137116

Kumar P, Govindaraju M, Senthamilselvi S, Premkumar K (2013a) Photocatalytic degradation of methyl orange dye using silver (Ag) nanoparticles synthesized from Ulva lactuca. Colloids Surfaces B Biointerfaces 103:658–661. https://doi.org/10.1016/j.colsurfb.2012.11.022

Kumar P, Senthamil Selvi S, Govindaraju M (2013b) Seaweed-mediated biosynthesis of silver nanoparticles using Gracilaria corticata for its antifungal activity against Candida spp. Appl Nanosci 3:495–500. https://doi.org/10.1007/s13204-012-0151-3

Kumar V, Nanda M, Kumar S, Chauhan PK (2018) The effects of ultraviolet radiation on growth, biomass, lipid accumulation and biodiesel properties of microalgae. Energy Sources, Part A Recover Util Environ Eff 40:787–793. https://doi.org/10.1080/15567036.2018.1463310

Kumari P, Ravi Kiran B, Venkata Mohan S (2022) Polyhydroxybutyrate production by Chlorella sorokiniana SVMIICT8 under Nutrient-deprived mixotrophy. Bioresour Technol 354:127135. https://doi.org/10.1016/j.biortech.2022.127135

Kumudha A, Selvakumar S, Dilshad P, et al (2015) Methylcobalamin - A form of vitamin

B12identified and characterised in Chlorella vulgaris. Food Chem 170:316–320. https://doi.org/10.1016/j.foodchem.2014.08.035

Lamers PP, Janssen M, De Vos RCH, et al (2012) Carotenoid and fatty acid metabolism in nitrogen-starved Dunaliella salina, a unicellular green microalga. J Biotechnol 162:21–27. https://doi.org/10.1016/j.jbiotec.2012.04.018

Lear G, Kingsbury JM, Franchini S, et al (2021) Plastics and the microbiome: impacts and solutions. Environ. Microbiomes 16:1–19

Lee K, Eisterhold ML, Rindi F, et al (2014) Isolation and screening of microalgae from natural habitats in the midwestern United States of America for biomass and biodiesel sources. J Nat Sci Biol Med 5:333–339. https://doi.org/10.4103/0976-9668.136178

Lengke MF, Fleet ME, Southam G (2007) Synthesis of palladium nanoparticles by reaction of filamentous cyanobacterial Biomass with a palladium(II) chloride complex. Langmuir 23:8982–8987. https://doi.org/10.1021/la7012446

Leung YH, Ng AMC, Xu X, et al (2014) Mechanisms of antibacterial activity of mgo: Non-ros mediated toxicity of mgo nanoparticles towards escherichia coli. Small 10:1171–1183. https://doi.org/10.1002/smll.201302434

Lewis M, Ghassemi P, Hibbeln J (2013) Therapeutic use of omega-3 fatty acids in severe head trauma. Am J Emerg Med 31:477–479. https://doi.org/10.1016/j.immuni.2010.12.017.Two-stage

Liu F, Lane P, Hewson JC, et al (2019) Development of a closed-loop process for fusel alcohol production and nutrient recycling from microalgae biomass. Bioresour Technol 283:350–357. https://doi.org/10.1016/j.biortech.2019.03.006

Liu Q (2020) Pollution and Treatment of Dye Waste-Water. In: IOP Conference Series: Earth and Environmental Science. IOP Publishing, p 052001

Ljubic A, Jacobsen C, Holdt SL, Jakobsen J (2020) Microalgae Nannochloropsis oceanica as a future new natural source of vitamin D3. Food Chem 320:126627. https://doi.org/10.1016/j.foodchem.2020.126627

Lohrey C, Kochergin V (2012) Biodiesel production from microalgae: Co-location with sugar mills. Bioresour Technol 108:76–82. https://doi.org/10.1016/J.BIORTECH.2011.12.035

Lu K, Zhao X, Ho SH, et al (2019) Biorefining and the Functional Properties of Proteins from Lipid and Pigment Extract Residue of Chlorella pyrenoidosa. Mar Drugs 17:. https://doi.org/10.3390/md17080454

Lucini L, Pellizzoni M, Baffi C, Molinari G Pietro (2012) Rapid determination of lycopene and β-carotene in tomato by liquid chromatography/electrospray tandem mass spectrometry. J Sci Food Agric 92:1297–1303. https://doi.org/10.1002/jsfa.4698

Luengo JM, García B, Sandoval A, et al (2003) Bioplastics from microorganisms. Curr. Opin. Microbiol. 6:251–260

Ma M, Wei C, Wang H, et al (2019) Isolation and evaluation of a novel strain of Chlorella sorokiniana that resists grazing by the predator Poterioochromonas malhamensis. Algal Res 38:101429. https://doi.org/10.1016/j.algal.2019.101429

Makabenta JM V., Nabawy A, Li CH, et al (2021) Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. Nat. Rev. Microbiol. 19:23–36

Mallick N, Bagchi SK, Koley S, Singh AK (2016) Progress and Challenges in Microalgal Biodiesel Production. Front Microbiol 7:1019. https://doi.org/10.3389/fmicb.2016.01019

Mallick N, Rai LC (1994) Removal of inorganic ions from wastewaters by immobilized microalgae. World J Microbiol Biotechnol 10:439–443. https://doi.org/10.1007/BF00144469

Mamta, Rana MS, Sharma AK, et al (2021) Potential of reverse osmosis reject water as a growth medium for the production of algal metabolites–A state-of-the-art review. J Water Process Eng 40:101849. https://doi.org/10.1016/j.jwpe.2020.101849

Mandal MK, Chanu NK, Chaurasia N (2020) Exogenous addition of indole acetic acid and kinetin under nitrogen-limited medium enhances lipid yield and expression of glycerol-3-phosphate acyltransferase & diacylglycerol acyltransferase genes in indigenous microalgae: A potential approach for biodie. Bioresour Technol 297:122439. https://doi.org/10.1016/j.biortech.2019.122439

Mandal MK, Chaurasia N (2021) De novo transcriptomic analysis of Graesiella emersonii NC-M1 reveals differential genes expression in cell proliferation and lipid production under glucose and salt supplemented condition. Renew Energy 179:2004–2015. https://doi.org/10.1016/j.renene.2021.07.141

Marella TK, Tiwari A (2020) Marine diatom Thalassiosira weissflogii based biorefinery for co-production of eicosapentaenoic acid and fucoxanthin. Bioresour Technol 307:. https://doi.org/10.1016/j.biortech.2020.123245

Martins RG, Gonçalves IS, De Morais MG, Vieira Costa JA (2017) New technologies from the bioworld: Selection of biopolymer-producing microalgae. Polimeros 27:285–289. https://doi.org/10.1590/0104-1428.2375

McGinn PJ, Dickinson KE, Bhatti S, et al (2011) Integration of microalgae cultivation with industrial waste remediation for biofuel and bioenergy production: opportunities and limitations. Photosynth Res 109:231–247. https://doi.org/10.1007/s11120-011-9638-0

Meira M, Quintella CM, Ribeiro EMO, et al (2015) Overview of the challenges in the production of biodiesel. Biomass Convers Biorefinery 5:321–329. https://doi.org/10.1007/s13399-014-0146-2

Mendoza H, Carmona L, Assunção P, et al (2015) Variation in lipid extractability by solvent in microalgae. Additional criterion for selecting species and strains for biofuel production from microalgae. Bioresour Technol 197:369–374. https://doi.org/10.1016/J.BIORTECH.2015.07.094

Meng Y (2015) A sustainable approach to fabricating ag nanoparticles/PVA hybrid nanofiber and its catalytic activity. Nanomaterials 5:1124–1135. https://doi.org/10.3390/nano5021124

Miceli M, Cutignano A, Conte M, et al (2019) Monoacylglycerides from the Diatom Skeletonema marinoi Induce Selective Cell Death in Cancer Cells. Mar Drugs 17:1–16. https://doi.org/10.3390/md17110625

Michalak I, Chojnacka K (2015) Algae as production systems of bioactive compounds. Eng Life Sci 15:160–176. https://doi.org/10.1002/elsc.201400191

Minhas AK, Hodgson P, Barrow CJ, Adholeya A (2016) A review on the assessment of stress conditions for simultaneous production of microalgal lipids and carotenoids. Front. Microbiol. 7:546

Mohandass C, Vijayaraj AS, Rajasabapathy R, et al (2013) Biosynthesis of silver nanoparticles from marine seaweed Sargassum cinereum and their antibacterial activity. Indian J Pharm Sci 75:606–610

Molino A, Martino M, Larocca V, et al (2019) Eicosapentaenoic acid extraction from nannochloropsis gaditana using carbon dioxide at supercritical conditions. Mar Drugs 17:. https://doi.org/10.3390/md17020132

Mondal M, Ghosh A, Tiwari ON, et al (2017a) Influence of carbon sources and light intensity on biomass and lipid production of Chlorella sorokiniana BTA 9031 isolated from coalfield under various nutritional modes. Energy Convers Manag 145:247–254. https://doi.org/10.1016/j.enconman.2017.05.001

Mondal M, Goswami S, Ghosh A, et al (2017b) Production of biodiesel from microalgae through biological carbon capture: a review. 3 Biotech 7:99. https://doi.org/10.1007/s13205-017-0727-4

Moreno-Garcia L, Adjallé K, Barnabé S, Raghavan GSV (2017) Microalgae biomass production for a biorefinery system: Recent advances and the way towards sustainability. Renew Sustain Energy Rev 76:493–506. https://doi.org/10.1016/J.RSER.2017.03.024

Mostert ES, Grobbelaar JU (1987) The influence of nitrogen and phosphorus on algal growth and quality in outdoor mass algal cultures. Biomass 13:219–233. https://doi.org/10.1016/0144-4565(87)90061-8

Mubarak M, Shaija A, Suchithra T V. (2015) A review on the extraction of lipid from microalgae for biodiesel production. Algal Res 7:117–123. https://doi.org/10.1016/j.algal.2014.10.008

MubarakAli D, Arunkumar J, Nag KH, et al (2013) Gold nanoparticles from Pro and eukaryotic photosynthetic microorganisms-Comparative studies on synthesis and its application on biolabelling. Colloids Surfaces B Biointerfaces 103:166–173. https://doi.org/10.1016/j.colsurfb.2012.10.014

Murray CJ, Ikuta KS, Sharara F, et al (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399:629–655. https://doi.org/10.1016/S0140-

6736(21)02724-0

Nagappan S, Kumar Verma S (2018) Co-production of biodiesel and alpha-linolenic acid (omega-3 fatty acid) from microalgae, Desmodesmus sp. MCC34. Energy Sources, Part A Recover Util Environ Eff 40:2933–2940. https://doi.org/10.1080/15567036.2018.1514434

Nagy A, Harrison A, Sabbani S, et al (2011) Silver nanoparticles embedded in zeolite membranes: release of silver ions and mechanism of antibacterial action. Int J Nanomedicine 6:1833–1852. https://doi.org/10.2147/ijn.s24019

Narayanan M, Prabhakaran M, Natarajan D, et al (2021) Phycoremediation potential of Chlorella sp. on the polluted Thirumanimutharu river water. Chemosphere 277:130246. https://doi.org/10.1016/j.chemosphere.2021.130246

Nguyen A, Tran D, Ho M, et al (2016) High Light Stress Regimen on Dunaliella Salina Strains For Carotenoids Induction. Integr Food, Nutr Metab 3:. https://doi.org/10.15761/ifnm.1000158

Niizawa I, Espinaco BY, Leonardi JR, et al (2018) Enhancement of astaxanthin production from Haematococcus pluvialis under autotrophic growth conditions by a sequential stress strategy. Prep Biochem Biotechnol 48:528–534. https://doi.org/10.1080/10826068.2018.1466159

Özer A, Akkaya G, Turabik M (2006) The removal of Acid Red 274 from wastewater: Combined biosorption and biocoagulation with Spirogyra rhizopus. Dye Pigment 71:83–89. https://doi.org/10.1016/j.dyepig.2005.06.004

Paço A, Jacinto J, da Costa JP, et al (2019) Biotechnological tools for the effective management of plastics in the environment. Crit Rev Environ Sci Technol 49:410–441. https://doi.org/10.1080/10643389.2018.1548862

Paliwal C, Ghosh T, George B, et al (2016) Microalgal carotenoids: Potential nutraceutical compounds with chemotaxonomic importance. Algal Res 15:24–31. https://doi.org/10.1016/j.algal.2016.01.017

Parameswari E, Lakshmanan A, Thilagavathi T (2010) Phycoremediation of heavy metals in polluted water bodies. Electron J Environ Agric Food Chem 9:808–814

Parial D, Patra HK, Dasgupta AKR, Pal R (2012) Screening of different algae for green synthesis of gold nanoparticles. Eur J Phycol 47:22–29. https://doi.org/10.1080/09670262.2011.653406

Pastoriza-Santos I, Liz-Marzán LM (2002) Formation of PVP-protected metal nanoparticles in DMF. Langmuir 18:2888–2894. https://doi.org/10.1021/la015578g

Patel AK, Vadrale AP, Tseng YS, et al (2022) Bioprospecting of marine microalgae from Kaohsiung Seacoast for lutein and lipid production. Bioresour Technol 351:126928. https://doi.org/10.1016/j.biortech.2022.126928

Peiris MMK, Fernando SSN, Jayaweera PM, et al (2018) Comparison of Antimicrobial

Properties of Silver Nanoparticles Synthesized from Selected Bacteria. Indian J Microbiol 58:301–311. https://doi.org/10.1007/s12088-018-0723-3

Pesika NS, Stebe KJ, Searson PC (2003) Relationship between Absorbance Spectra and Particle Size Distributions for Quantum-Sized Nanocrystals. J Phys Chem B 107:10412–10415. https://doi.org/10.1021/jp0303218

Prathima A, Karthikeyan S (2017) Characteristics of micro-algal biofuel from Botryococcus braunii. Energy Sources, Part A Recover Util Environ Eff 39:206–212. https://doi.org/10.1080/15567036.2016.1222466

Price S, Kuzhiumparambil U, Pernice M, Ralph PJ (2020) Cyanobacterial polyhydroxybutyrate for sustainable bioplastic production: Critical review and perspectives. J Environ Chem Eng 8:104007. https://doi.org/10.1016/j.jece.2020.104007

Priyadharshini RI, Prasannaraj G, Geetha N, Venkatachalam P (2014a) Microwave-Mediated Extracellular Synthesis of Metallic Silver and Zinc Oxide Nanoparticles Using Macro-Algae (Gracilaria edulis) Extracts and Its Anticancer Activity Against Human PC3 Cell Lines. Appl Biochem Biotechnol 174:2777–2790. https://doi.org/10.1007/s12010-014-1225-3

Priyadharshini RI, Prasannaraj G, Geetha N, Venkatachalam P (2014b) Microwave-Mediated Extracellular Synthesis of Metallic Silver and Zinc Oxide Nanoparticles Using Macro-Algae (Gracilaria edulis) Extracts and Its Anticancer Activity Against Human PC3 Cell Lines. Appl Biochem Biotechnol 174:2777–2790. https://doi.org/10.1007/s12010-014-1225-3

Rabbani S, Beyer P, Lintig J V., et al (1998) Induced β-Carotene Synthesis Driven by Triacylglycerol Deposition in the Unicellular Alga Dunaliella bardawil. Plant Physiol 116:1239–1248. https://doi.org/10.1104/pp.116.4.1239

Rahman A, Miller CD (2017) Microalgae as a Source of Bioplastics. In: Algal Green Chemistry: Recent Progress in Biotechnology. Elsevier, pp 121–138

Rajkumar R, Ezhumalai G, Gnanadesigan M (2021) A green approach for the synthesis of silver nanoparticles by Chlorella vulgaris and its application in photocatalytic dye degradation activity. Environ Technol Innov 21:. https://doi.org/10.1016/j.eti.2020.101282

Ramakrishna M, Rajesh Babu D, Gengan RM, et al (2016) Green synthesis of gold nanoparticles using marine algae and evaluation of their catalytic activity. J Nanostructure Chem 6:1–13. https://doi.org/10.1007/s40097-015-0173-y

Rani P, Kumar V, Singh PP, et al (2020) Highly stable AgNPs prepared via a novel green approach for catalytic and photocatalytic removal of biological and non-biological pollutants. Environ Int 143:105924. https://doi.org/10.1016/j.envint.2020.105924

Rao MD, Pennathur G (2017) Green synthesis and characterization of cadmium sulphide nanoparticles from Chlamydomonas reinhardtii and their application as photocatalysts. Mater Res Bull 85:64–73. https://doi.org/10.1016/j.materresbull.2016.08.049

Rauta PR, Mohanta YK, Nayak D, et al (2019) Nanobioremediation. Nanotechnol Biol Med 245–257. https://doi.org/10.1201/9780429259333-21

Rivera S, Canela R (2012) Influence of sample processing on the analysis of carotenoids in Maize. Molecules 17:11255–11268. https://doi.org/10.3390/molecules170911255

Rizzo AM, Prussi M, Bettucci L, et al (2013) Characterization of microalga Chlorella as a fuel and its thermogravimetric behavior. Appl Energy 102:24–31. https://doi.org/10.1016/j.apenergy.2012.08.039

Roja K, Ruben Sudhakar D, Anto S, Mathimani T (2019) Extraction and characterization of polyhydroxyalkanoates from marine green alga and cyanobacteria. Biocatal Agric Biotechnol 22:101358. https://doi.org/10.1016/j.bcab.2019.101358

Rösken LM, Cappel F, Körsten S, et al (2016) Time-dependent growth of crystalline Au0-nanoparticles in cyanobacteria as self-reproducing bioreactors: 2. Anabaena cylindrica. Beilstein J Nanotechnol 7:312–327. https://doi.org/10.3762/bjnano.7.30

Roy Chong JW, Tan X, Khoo KS, et al (2022) Microalgae-based bioplastics: Future solution towards mitigation of plastic wastes. Environ Res 206:112620. https://doi.org/10.1016/j.envres.2021.112620

Sánchez-Borroto Y, Lapuerta M, Melo-Espinosa EA, et al (2018) Green-filamentous macroalgae Chaetomorpha cf. gracilis from Cuban wetlands as a feedstock to produce alternative fuel: A physicochemical characterization. Energy Sources, Part A Recover Util Environ Eff 40:1279–1289. https://doi.org/10.1080/15567036.2018.1476931

Sankhla A, Sharma R, Yadav RS, et al (2016) Biosynthesis and characterization of cadmium sulfide nanoparticles - An emphasis of zeta potential behavior due to capping. Mater Chem Phys 170:44–51. https://doi.org/10.1016/j.matchemphys.2015.12.017

Santhana Kumar V, Das Sarkar S, Das BK, et al (2022) Sustainable biodiesel production from microalgae Graesiella emersonii through valorization of garden wastes-based vermicompost. Sci Total Environ 807:150995. https://doi.org/10.1016/j.scitotenv.2021.150995

Satapathy S, Shukla SP, Sandeep KP, et al (2015) Evaluation of the performance of an algal bioreactor for silver nanoparticle production. J Appl Phycol 27:285–291. https://doi.org/10.1007/s10811-014-0311-9

Savio S, Farrotti S, Paris D, et al (2020) Value-added co-products from biomass of the diatoms Staurosirella pinnata and Phaeodactylum tricornutum. Algal Res 47:101830. https://doi.org/10.1016/j.algal.2020.101830

Selvaraj K, Vishvanathan N, Dhandapani R (2021) Screening, optimization and characterization of poly hydroxy butyrate from fresh water microalgal isolates. Int J Biobased Plast 3:139–162. https://doi.org/10.1080/24759651.2021.1926621

Serwecińska L (2020) Antimicrobials and antibiotic-resistant bacteria: A risk to the environment and to public health. Water (Switzerland) 12:3313. https://doi.org/10.3390/w12123313

Setyorini AI, Dianursanti (2021) Isolation and optimization of polyhydroxybutyrate from Chlorella vulgaris using NaClO and ultrasonication aided chemical pretreatment methods. In: AIP Conference Proceedings. AIP Publishing LLC AIP Publishing, p 020018

Shaik MR, Khan M, Kuniyil M, et al (2018) Plant-Extract-Assisted green synthesis of silver nanoparticles using Origanum vulgare L. Extract and their microbicidal activities. Sustain 10:1–14. https://doi.org/10.3390/su10040913

Shaikh WA, Chakraborty S, Islam RU (2020) Photocatalytic degradation of rhodamine B under UV irradiation using Shorea robusta leaf extract-mediated bio-synthesized silver nanoparticles. Int J Environ Sci Technol 17:2059–2072. https://doi.org/10.1007/s13762-019-02473-6

Shamaila S, Zafar N, Riaz S, et al (2016) Gold nanoparticles: An efficient antimicrobial agent against enteric bacterial human pathogen. Nanomaterials 6:71. https://doi.org/10.3390/nano6040071

Sharma A, Sharma S, Sharma K, et al (2016) Algae as crucial organisms in advancing nanotechnology: a systematic review. J Appl Phycol 28:1759–1774. https://doi.org/10.1007/s10811-015-0715-1

Sharma B, Purkayastha DD, Hazra S, et al (2014) Biosynthesis of fluorescent gold nanoparticles using an edible freshwater red alga, Lemanea fluviatilis (L.) C.Ag. and antioxidant activity of biomatrix loaded nanoparticles. Bioprocess Biosyst Eng 37:2559–2565. https://doi.org/10.1007/s00449-014-1233-2

Shim SJ, Hong ME, Chang WS, Sim SJ (2020) Repeated-batch production of omega-3 enriched biomass of Chlorella sorokiniana via calcium-induced homeoviscous adaptation. Bioresour Technol 303:122944. https://doi.org/10.1016/j.biortech.2020.122944

Shnoudeh AJ, Hamad I, Abdo RW, et al (2019) Synthesis, Characterization, and Applications of Metal Nanoparticles. In: Biomaterials and Bionanotechnology. Academic Press, pp 527–612

Shrivastav A, Mishra SK, Mishra S (2010) Polyhydroxyalkanoate (PHA) synthesis by Spirulina subsalsa from Gujarat coast of India. Int J Biol Macromol 46:255–260. https://doi.org/10.1016/j.ijbiomac.2010.01.001

Shukla MK, Singh RP, Reddy CRK, Jha B (2012) Synthesis and characterization of agar-based silver nanoparticles and nanocomposite film with antibacterial applications. Bioresour Technol 107:295–300. https://doi.org/10.1016/j.biortech.2011.11.092

Silitonga AS, Masjuki HH, Ong HC, et al (2017) Optimization of extraction of lipid from Isochrysis galbana microalgae species for biodiesel synthesis. Energy Sources, Part A Recover Util Environ Eff 39:1167–1175. https://doi.org/10.1080/15567036.2017.1310957

Singh A, Olsen SI (2011) A critical review of biochemical conversion, sustainability and life cycle assessment of algal biofuels. Appl Energy 88:3548–3555. https://doi.org/10.1016/j.apenergy.2010.12.012

Singh M, Kalaivani R, Manikandan S, et al (2013) Facile green synthesis of variable metallic gold nanoparticle using Padina gymnospora, a brown marine macroalga. Appl Nanosci 3:145–151. https://doi.org/10.1007/s13204-012-0115-7

Singh MK, Mehata MS (2019) Phase-dependent optical and photocatalytic performance of synthesized titanium dioxide (TiO2) nanoparticles. Optik (Stuttg) 193:163011. https://doi.org/10.1016/j.ijleo.2019.163011

Singh MK, Rai PK, Rai A, et al (2019) Poly-β-hydroxybutyrate production by the cyanobacterium scytonema geitleri bharadwaja under varying environmental conditions. Biomolecules 9:198. https://doi.org/10.3390/biom9050198

Singh N, Batghare AH, Choudhury BJ, et al (2020) Microalgae based biorefinery: Assessment of wild fresh water microalgal isolate for simultaneous biodiesel and β-carotene production. Bioresour Technol Reports 11:100440. https://doi.org/10.1016/j.biteb.2020.100440

Song M, Pei H, Hu W, et al (2014) Identification and characterization of a freshwater microalga Scenedesmus SDEC-8 for nutrient removal and biodiesel production. Bioresour Technol 162:129–135. https://doi.org/10.1016/j.biortech.2014.03.135

Soo JZ, Chai LC, Ang BC, Ong BH (2020) Enhancing the Antibacterial Performance of Titanium Dioxide Nanofibers by Coating with Silver Nanoparticles. ACS Appl Nano Mater 3:5743–5751. https://doi.org/10.1021/acsanm.0c00925

Subramanian G, Yadav G, Sen R (2016) Rationally leveraging mixotrophic growth of microalgae in different photobioreactor configurations for reducing the carbon footprint of an algal biorefinery: a techno-economic perspective. RSC Adv 6:72897–72904. https://doi.org/10.1039/C6RA14611B

Subramaniyam V, Subashchandrabose SR, Thavamani P, et al (2015) Chlorococcum sp. MM11—a novel phyco-nanofactory for the synthesis of iron nanoparticles. J Appl Phycol 27:1861–1869. https://doi.org/10.1007/s10811-014-0492-2

Sun P, Wong CC, Li Y, et al (2019) A novel strategy for isolation and purification of fucoxanthinol and fucoxanthin from the diatom Nitzschia laevis. Food Chem 277:566–572. https://doi.org/10.1016/j.foodchem.2018.10.133

Taghavijeloudar M, Yaqoubnejad P, Amini-Rad H, Park J (2021) Optimization of cultivation condition of newly isolated strain Chlorella sorokiniana pa.91 for CO2 bio-fixation and nutrients removal from wastewater: Impact of temperature and light intensity. Clean Technol Environ Policy 1:1–13. https://doi.org/10.1007/s10098-021-02199-5

Thakur A, Kaur H (2016) Removal of hazardous Rhodamine B dye by using chemically activated low cost adsorbent : Pine cone charcoal. Int J Chem Phys Sci 5:17–28

Thirugnanasambandham K (2018) Biodiesel production from Cholrella minutissima microalgae: Kinetic and mathematical modeling. Energy Sources, Part A Recover Util Environ Eff 40:1461–1468. https://doi.org/10.1080/15567036.2018.1477872

Trakunjae C, Boondaeng A, Apiwatanapiwat W, et al (2021) Enhanced polyhydroxybutyrate

(PHB) production by newly isolated rare actinomycetes Rhodococcus sp. strain BSRT1-1 using response surface methodology. Sci Rep 11:1–14. https://doi.org/10.1038/s41598-021-81386-2

Truu M, Ligi T, Nõlvak H, et al (2022) Impact of synthetic silver nanoparticles on the biofilm microbial communities and wastewater treatment efficiency in experimental hybrid filter system treating municipal wastewater. J Hazard Mater 440:129721. https://doi.org/10.1016/j.jhazmat.2022.129721

Tůma S, Izaguirre JK, Bondar M, et al (2020) Upgrading end-of-line residues of the red seaweed Gelidium sesquipedale to polyhydroxyalkanoates using Halomonas boliviensis. Biotechnol Reports 27:. https://doi.org/10.1016/j.btre.2020.e00491

Ugwu CU, Aoyagi H, Uchiyama H (2007) Influence of irradiance, dissolved oxygen concentration, and temperature on the growth of Chlorella sorokiniana. Photosynthetica 45:309–311. https://doi.org/10.1007/s11099-007-0052-y

Vadivelan G, Venkateswaran G (2014) Production and Enhancement of Omega-3 Fatty Acid from *Mortierella alpina* CFR-GV15: Its Food and Therapeutic Application. Biomed Res Int 2014:1–9. https://doi.org/10.1155/2014/657414

Vanaja M, Paulkumar K, Baburaja M, et al (2014) Degradation of methylene blue using biologically synthesized silver nanoparticles. Bioinorg Chem Appl 2014:. https://doi.org/10.1155/2014/742346

Varadavenkatesan T, Lyubchik E, Pai S, et al (2019) Photocatalytic degradation of Rhodamine B by zinc oxide nanoparticles synthesized using the leaf extract of Cyanometra ramiflora. J Photochem Photobiol B Biol 199:111621. https://doi.org/10.1016/j.jphotobiol.2019.111621

Venkatpurwar V, Pokharkar V (2011) Green synthesis of silver nanoparticles using marine polysaccharide: Study of in-vitro antibacterial activity. Mater Lett 65:999–1002. https://doi.org/10.1016/j.matlet.2010.12.057

Venteris ER, Wigmosta MS, Coleman AM, Skaggs RL (2014) Strain Selection, Biomass to Biofuel Conversion, and Resource Colocation have Strong Impacts on the Economic Performance of Algae Cultivation Sites. Front Energy Res 2:37. https://doi.org/10.3389/fenrg.2014.00037

Vidyadharani G, Dhandapani R (2013) Fourier transform infrared (FTIR) spectroscopy for the analysis of lipid from Chlorella vulgaris. Appl Biol 61:16753–16756

Vivek M, Kumar PS, Steffi S, Sudha S (2011) Biogenic silver nanoparticles by gelidiella acerosa extract and their antifungal effects. Avicenna J Med Biotechnol 3:143–148

Vo T, Tran S, Nguyen P, Mai T (2017) Growth , Carotenoid Production , Antioxidant Capacity and Lipid Accumulation of Haematococcus sp . Under Different Light Intensities. 2:142–147. https://doi.org/10.11648/j.ajpb.20170204.15

WA F (2016) Green Biosynthesis of Silver Nanoparticles Using Marine Red Algae Acanthophora specifera and its Antibacterial Activity. J Nanomed Nanotechnol 07:7–10.

https://doi.org/10.4172/2157-7439.1000409

Wang F, Gao B, Dai C, et al (2020) Comprehensive utilization of the filamentous oleaginous microalga Tribonema utriculosum for the production of lipids and chrysolaminarin in a biorefinery concept. Algal Res 50:101973. https://doi.org/10.1016/j.algal.2020.101973

Wang L, Hu C, Shao L (2017) The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomedicine Volume 12:1227–1249. https://doi.org/10.2147/IJN.S121956

Wang N, Manabe Y, Sugawara T, et al (2018) Identification and biological activities of carotenoids from the freshwater alga Oedogonium intermedium. Food Chem 242:247–255. https://doi.org/10.1016/j.foodchem.2017.09.075

Wase N, Black P, DiRusso C (2018) Innovations in improving lipid production: Algal chemical genetics. Prog Lipid Res 71:101–123. https://doi.org/10.1016/j.plipres.2018.07.001

Web-1 Lutein Market by Form (Powder and Crystalline, Oil Suspension, Beadlet, Emulsion), Source (Natural, Synthetic), Application (Food, Beverages, Dietary Supplements, Animal Feed), Production Process, and Region - Global Forecast to 2022. https://www.marketsandmarkets.com/Market-Reports/lutein-market-69753879.html. Accessed 16 Jul 2020

Web-2 Astaxanthin Market by Source (Plant, Yeast and Microbes, Marine, Petroleum), Form (Dry, Liquid), Method of Production (Biological Process, Chemical Process), Application (Feed, Supplements, Food, Cosmetics), and Region - Global Forecast to 2022. https://www.marketsandmarkets.com/Market-Reports/astaxanthin-market-162119410.html. Accessed 16 Jul 2020

Web-3 Omega-3 PUFA Market by Type (DHA, EPA, ALA), Application (Dietary Supplements, Functional Foods and; Beverages, Pharmaceuticals, Infant Formula), Source (Marine, Plant), Sub-source) and Region - Global Forecasts to 2020. https://www.marketsandmarkets.com/Market-Reports/omega-3-omega-6-227.html. Accessed 16 Jul 2020

Web-4 Carotenoids Market by Type (Astaxanthin, Beta-Carotene, Canthaxanthin, Lutein, Lycopene, and; Zeaxanthin), Source (Synthetic and Natural), Application (Supplements, Food, Feed, and Cosmetics), and; by Region - Global Trends and; Forecasts to 2021. https://www.marketsandmarkets.com/Market-Reports/carotenoid-market-158421566.html. Accessed 16 Jul 2020

Wen X, Du K, Wang Z, et al (2016) Effective cultivation of microalgae for biofuel production: A pilot-scale evaluation of a novel oleaginous microalga Graesiella sp. WBG-1. Biotechnol Biofuels 9:123. https://doi.org/10.1186/s13068-016-0541-y

Wen Z-Y, Chen F (2003) Heterotrophic production of eicosapentaenoic acid by microalgae. Biotechnol Adv 21:273–294. https://doi.org/10.1016/S0734-9750(03)00051-X

Wicker RJ, Autio H, Daneshvar E, et al (2022) The effects of light regime on carbon cycling,

nutrient removal, biomass yield, and polyhydroxybutyrate (PHB) production by a constructed photosynthetic consortium. Bioresour Technol 363:127912. https://doi.org/10.1016/j.biortech.2022.127912

Wrede D, Taha M, Miranda AF, et al (2014) Co-Cultivation of Fungal and Microalgal Cells as an Efficient System for Harvesting Microalgal Cells, Lipid Production and Wastewater Treatment. PLoS One 9:e113497. https://doi.org/10.1371/journal.pone.0113497

Wu Y, Guan K, Wang Z, et al (2013) Isolation, Identification and Characterization of an Electrogenic Microalgae Strain. PLoS One 8:. https://doi.org/10.1371/journal.pone.0073442

Xia S, Gao B, Fu J, et al (2018) Production of fucoxanthin, chrysolaminarin, and eicosapentaenoic acid by Odontella aurita under different nitrogen supply regimes. J Biosci Bioeng 126:723–729. https://doi.org/10.1016/j.jbiosc.2018.06.002

Xia T, Liu Z, Pan X, et al (2020) Effects of dilution ratio on nutrient removal, sedimentation efficiency, and lipid production by Scenedesmus obliquus in diluted cattle wastewater. Energy Sources, Part A Recover Util Environ Eff 42:121–130. https://doi.org/10.1080/15567036.2019.1587060

Xu R (2008) Progress in nanoparticles characterization: Sizing and zeta potential measurement. Particuology 6:112–115. https://doi.org/10.1016/j.partic.2007.12.002

Yaakob MA, Mohamed RMSR, Al-Gheethi A, et al (2021) Influence of nitrogen and phosphorus on microalgal growth, biomass, lipid, and fatty acid production: An overview. Cells 10:1–19

Ye J, Xiao H, Xiao B, et al (2015) Bioremediation of heavy metal contaminated aqueous solution by using red algae Porphyra leucosticta. Water Sci Technol 72:1662–1666. https://doi.org/10.2166/wst.2015.386

Yen H-W, Hu I-C, Chen C-Y, et al (2013) Microalgae-based biorefinery – From biofuels to natural products. Bioresour Technol 135:166–174. https://doi.org/10.1016/J.BIORTECH.2012.10.099

Yu H, Zhuang LL, Zhang M, Zhang J (2022) The mechanism study of attached microalgae cultivation based on reverse osmosis concentrated water (WROC). Resour Conserv Recycl 179:106066. https://doi.org/10.1016/j.resconrec.2021.106066

Yu X, Zhao P, He C, et al (2012) Isolation of a novel strain of Monoraphidium sp. and characterization of its potential application as biodiesel feedstock. Bioresour Technol 121:256–262. https://doi.org/10.1016/j.biortech.2012.07.002

Yun HS, Kim YS, Yoon HS (2020) Characterization of Chlorella sorokiniana and Chlorella vulgaris fatty acid components under a wide range of light intensity and growth temperature for their use as biological resources. Heliyon 6:e04447. https://doi.org/10.1016/j.heliyon.2020.e04447

Yun JE, Lee DG (2017) Silver Nanoparticles: A Novel Antimicrobial Agent. In: Antimicrobial Nanoarchitectonics: From Synthesis to Applications. Elsevier, pp 139–166

Yusuff AS (2019) Extraction, optimization, and characterization of oil from green microalgae Chlorophyta species. Energy Sources, Part A Recover Util Environ Eff 00:1–12. https://doi.org/10.1080/15567036.2019.1676327

Zahoor M, Irshad M, Rahman H, et al (2017) Alleviation of heavy metal toxicity and phytostimulation of Brassica campestris L. by endophytic Mucor sp. MHR-7. Ecotoxicol Environ Saf 142:139–149. https://doi.org/10.1016/j.ecoenv.2017.04.005

Zhang W, Wang F, Gao B, et al (2018) An integrated biorefinery process: Stepwise extraction of fucoxanthin, eicosapentaenoic acid and chrysolaminarin from the same Phaeodactylum tricornutum biomass. Algal Res 32:193–200. https://doi.org/10.1016/j.algal.2018.04.002

Zhang W, Zhang P, Sun H, et al (2014) Effects of various organic carbon sources on the growth and biochemical composition of Chlorella pyrenoidosa. Bioresour Technol 173:52–58. https://doi.org/10.1016/j.biortech.2014.09.084

Zhou W, Wang Z, Zhu S, et al (2016) Culture of four microalgal strains for bioenergy production and nutrient removal in the meliorative municipal wastewater. Energy Sources, Part A Recover Util Environ Eff 38:670–679. https://doi.org/10.1080/15567036.2012.754517

Ziganshina EE, Bulynina SS, Ziganshin AM (2022) Growth Characteristics of Chlorella sorokiniana in a Photobioreactor during the Utilization of Different Forms of Nitrogen at Various Temperatures. Plants 11:. https://doi.org/10.3390/plants11081086

Zohir WF, Kapase VU, Kumar S (2022) Identification and Characterization of a New Microalga Dysmorphococcus globosus-HI from the Himalayan Region as a Potential Source of Natural Astaxanthin. Biology (Basel) 11:. https://doi.org/10.3390/biology11060884

(2016) European Bioplastics e.V. https://www.european-bioplastics.org/. Accessed 30 Dec 2022

LIST OF PUBLICATIONS

Journal Articles:

1. **Kumar, Lakhan,** and Navneeta Bharadvaja. "A review on microalgae biofuel and biorefinery: challenges and way forward." Energy Sources, Part A: Recovery, Utilization, and Environmental Effects (2020): 1-24. ***(IF- 2.9; SCI-Indexed)***
2. **Kumar, Lakhan**, and Navneeta Bharadvaja."Biosynthesis, characterization, and evaluation of antibacterial and photocatalytic dye degradation activities of silver nanoparticles biosynthesized by Chlorella sorokiniana."Biomass Conversion and Biorefinery (2022) (Accepted on October 16, 2022). ***(IF- 4.05; SCI-Indexed)***
3. **Kumar, Lakhan**, Ragunathan, V., Chugh, M., & Bharadvaja, N. (2021). Nanomaterials for remediation of contaminants: a review. *Environmental Chemistry Letters*, *19*(4), 3139-3163. ***(IF-13.6, SCI-Indexed)***
4. **Kumar, Lakhan,** Mohan, L., Anand, S., Bhardwaj, D., & Bharadvaja, N. (2022). Phyconanoremediation: a sustainable approach to deal with environmental pollutants heavy metals and dyes. *Vegetos*, 1-16. ***(Scopus- Indexed)***
5. Bharadvaja, N., **Kumar, Lakhan.** (2021). Algal Biorefinery for the Extraction of Bioactive Compounds. *Current Bioactive Compounds*, *17*(4), 280-288. ***(Scopus- Indexed)***
6. **Kumar, Lakhan,** Anand, R., Shah, M. P., & Bharadvaja, N. (2022). Microalgae biodiesel: A sustainable source of energy, unit operations, technological challenges, and solutions. Journal of Hazardous Materials Advances. 100145
7. **Kumar, Lakhan,** et al. "A review on unit operations, challenges, opportunities, and strategies to improve algal based biodiesel and biorefinery." *Frontiers in Chemical Engineering* 4 (2022): 90.
8. **Kumar, Lakhan,** & Bharadvaja, N. (2021). Algal-Based Wastewater Treatment and Biorefinery. In *Wastewater Treatment* (pp. 413-432). Elsevier.
9. Ahuja, S., Roy, A., **Kumar, Lakhan,** & Bharadvaja, N. (2020). Media optimization using Box Behnken design for enhanced production of biomass, beta-carotene and lipid from Dunaliella salina. Vegetos, 33(1), 31-39. ***(Scopus- Indexed)***

10. **Kumar, L**, Anand R, Nanda, N, and Navneeta Bharadvaja." Microbial Carbon-capture Cells for wastewater treatment: A step towards Environmental."Biomass Conversion and Biorefinery (2022) (Accepted on October 16, 2022). ***(IF- 4.05; SCI-Indexed)***
11. **Kumar, Lakhan**., Saha, A., Khushbu, & Warkar SG.(2022). Biodegradability of automotive plastics and composites. In *Biodegradability of Conventional Plastics Opportunities, Challenges, and Misconceptions* (pp. 231-242). Elsevier.

Publications in conference/workshop proceeding:

1. **Kumar, Lakhan** and Navneeta Bharadvaja. "Progress in microalgae biomass-based Biorefinery". (2018) BITS International Conference on Life Science Research & its Interface with Engineering and Allied Sciences (LSRIEAS 2018), at BITS Pilani, Rajasthan, India.
2. **Kumar, Lakhan** and Navneeta Bharadvaja. "An overview of strategies for remediation of environmental pollutants using novel nanomaterials for public health safety". (2020) 2nd International conference on Future Aspects of Sustainable Technologies (FAST 2.0), at CIT Kokrajhar, Assam, India.