BIOPROSPECTING MICROALGAE FOR β-CAROTENE AND SYNTHESIS OF NANOPARTICLES FOR ENVIRONMENTAL APPLICATIONS - A ZERO WASTE APPROACH

A DISSERTATION
SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT
FOR THE AWARD OF THE DEGREE
OF

MASTER OF SCIENCE
IN
BIOTECHNOLOGY

SUBMITTED BY
RAKSHA ANAND
(2K20/MSCBIO/24)

UNDER THE SUPERVISION OF
DR NAVNEETA BHARADVAJA

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Bawana Road, Delhi-110042

MAY, 2022

CANDIDATE'S DECLARATION

I, Raksha Anand, hereby certify that the work which is being presented in the research work entitled "**Bioprospecting Microalgae for β-Carotene and Synthesis of Nanoparticles for Environmental Applications - A Zero Waste Approach**" in fulfillment of the requirement for the award of Degree of Masters of Sciences in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own work, carried during 4th semester of MSc. course, under the supervision of Dr. Navneeta Bharadvaja. The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other University. The work has been published and communicated in various journals under my name with the guide.

Place: Delhi
Date:

CERTIFICATE

This is to certify that the Project dissertation titled "**Bioprospecting Microalgae for β-Carotene and Synthesis of Nanoparticles for Environmental Applications - A Zero Waste Approach**" which is submitted by Raksha Anand, 2K20/MSCBIO/24, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Sciences, is a record for the project work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Dr Navneeta Bharadvaja
In-charge, Plant Biotechnology Laboratory
Department of Biotechnology
Delhi Technological University
Delhi, India 110042

Prof Pravir Kumar
Head of the Department
Department of Biotechnology
Delhi Technological University
Delhi, India 110042

Bioprospecting Microalgae for β-Carotene and Synthesis of Nanoparticles for Environmental Applications - A Zero Waste Approach

Raksha Anand*

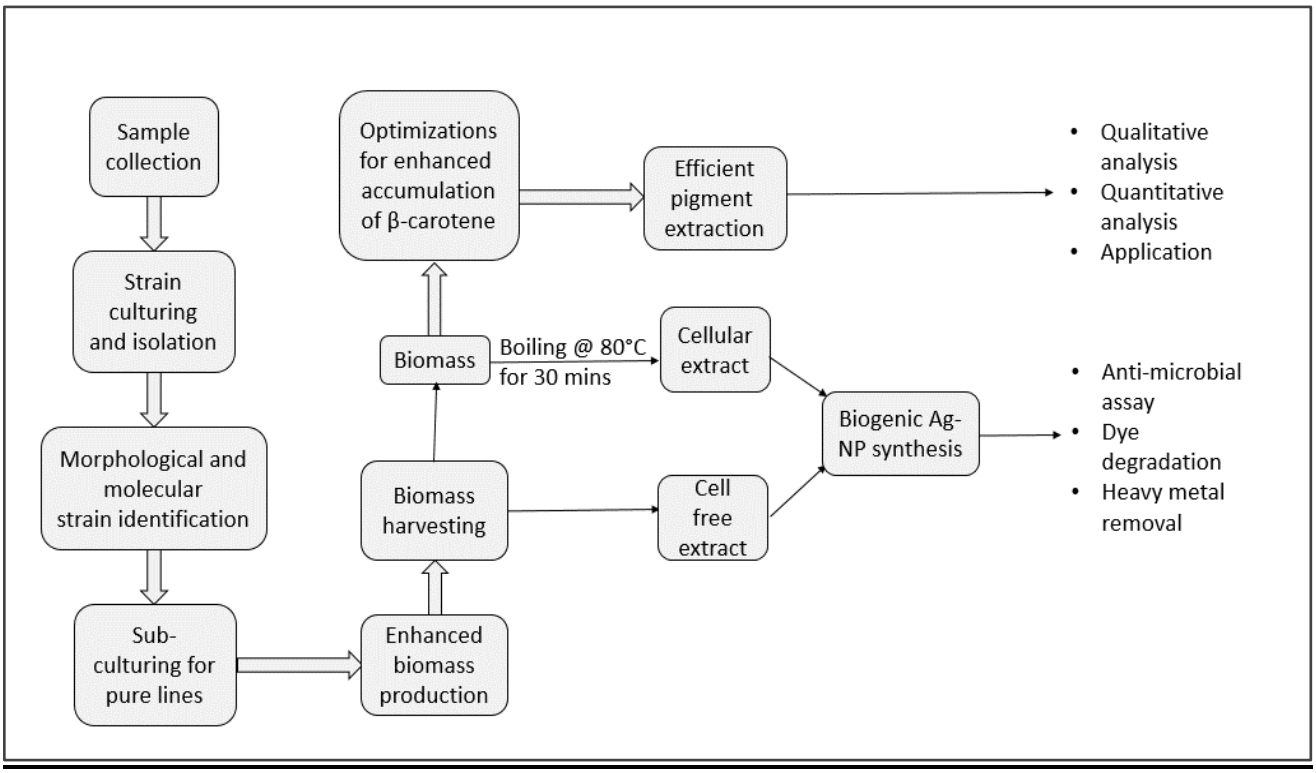
*Delhi Technological University, Delhi, India

Email: r27anand@gmail.com

Abstract:

The exponential rise in global population, technological development, and rise in lifestyle standards have brought a heavy load on natural resources to fulfill the demand, and it is increasing day by day. The conventional sources of food and energy are limited and are getting exhausted at a very fast rate. Environmental pollution is another concern due to increased anthropogenic activities. This has led to identification of novel sources for fulfilling the anthropogenic demands in terms of energy and food. Algae have been recently explored extensively as an alternative source for conventional ones. In this study, we have evaluated native microalgae for nutraceuticals and synthesis of nanoparticles for environmental pollution remediation. Microalgae isolate was identified as *Graesiella emersonii* based on morphological and molecular identification. Beta-carotene was extracted from this isolate. Various parameters were optimized for optimum extraction of beta-carotene. The beta-carotene was used for its antioxidant activity. The leftover algal extract was used for synthesis of silver nanoparticles. The formation of silver nanoparticles was confirmed using spectrophotometer. The synthesized silver nanoparticles were tested for their antibacterial against *Escherichia coli,* and were found to be effective. Further, silver nanoparticles were used for photocatalytic degradation of methylene blue with an efficiency of more than 88% in ten hours. In addition to this, the silver nanoparticles were tested as an adsorbent for removal of heavy metals from synthetic wastewater. They were found to remediate 64 % of chromium in 48 hours.

Graphical Abstract



ACKNOWLEDGMENT

A formal statement of acknowledgment will hardly meet the needs of justice in the matter of expression of deeply felt sincere and allegiant gratitude to all who encouraged and helped me in many ways throughout the dissertation of Master of Sciences. I would like to thank the Head of the Department, Prof Pravir Kumar for providing me with this opportunity. It is my privilege to express my profound sense of gratitude and indebtedness to my supervisor Dr. Navneeta Bharadvaja, Assistant Professor in the Department of Biotechnology, Delhi Technological University for her valuable guidance and consistent encouragement during the progress of the project work. The dissertation wouldn't be completed without her insightful suggestions and support. A special thanks to my senior, Mr. Lakhan Kumar for his constant support and immense faith in me. I have completed this project because of his guidance, inspiration, and motivation provided by him. I am equally grateful and wish to express my wholehearted thanks to the technical staff Mr. CB Singh and Mr. Jitendra K Singh, and Mr. Sandeep for their kind support. I would also like to thank other lab members Mr. Sidharth Sharma, Ms. Harshita Singh, Ms. Anuradha and Mr. Lalit Mohan, Ms. Neha Nanda, Mr. Shaubhik Anand and Mr. Vijay for their support and help in the course of my research work. I prevailed enough to experience a sustained enthusiasm and involved interest from their side. This fueled my enthusiasm even further and encouraged me to boldly step into what was totally dark and unexplored expanse for me. On a personal note, I wish to express my gratitude and affection to my family for their constant love and support.

Raksha Anand
2K20/MSCBIO/24

CONTENTS

List of Graphs/Figures

List of Tables

List of Abbreviations

ADMET – Adsorption, Distribution, Metabolism, Excretion and Toxicity

AgNPs – Silver Nano Particles

BG11 – Blue Green 11

BLAST - Basic Local Alignment Search Tool

CBPs – Carotene Breakdown Products

Chl – Chlorophyll

Crts – Carotenoids

DNA – deoxy-ribonucleic acid

DPPH - 2,2-diphenyl-1-picrylhydrazyl

GI – Gastro-Intestinal

HPLC – High Performance Liquid Chromatography

LC – Liquid Chromatography

LBDS – Lipid Based Delivery Systems

MB – Methylene Blue

NPs – Nano Particles

NREL – National Renewable Energy Laboratory

PBDS – Polymer Based Delivery Systems

PCR – Polymerase Chain Reaction

RNA – ribonucleic acid

RO – Reverse Osmosis

ROS – Reactive Oxygen Species

UKS – Unknown Strain

UV-Vis – Ultraviolet Visible

1. INTRODUCTION

Out of 2.1 million living species identified worldwide, up to 30,000 species have been identified as algae. These autotrophic organisms comprise of economically relevant group. These can be either uni- or multi-cellular photosynthetic organisms living in diverse environments. Algae can be micro- or macro-algae depending upon their size and morphology. The find applications in a wide range of fields, ranging from feedstock to medical and pharma industry, nutraceutical and cosmeceutical industry as well as modernly in bio-nanotechnology. These have been identified to be good sources of fuel in the form of biodiesel as well as high value-low yield bio-products like beta-carotene, xanthophylls and so on. Being easy to handle, algae are preferred over other green organisms for such applications.

The isolation and identification of microalgal species is done stepwise, through Morphological study using microscopy and molecular studies. The molecular study is done by sequencing the target nucleic acid sequence followed by construction of phylogenetic tree which leads to strain identification. Computational analysis or molecular level identification involves finding closest similarity species, tree making and phylogenetic analysis.

There are so many essential phyco-compounds targeted for recovery from algal species. Some of these are beta-carotene, xanthophyll, astaxanthin and so on. They are medically and industrially essential compounds which can be sustainably produced from algae. Chemical synthesis of such compounds produces compounds with similar efficacy however, its cost and treatment are not user-friendly. *Dunaliella* and *Chlorella* species are the most widely cultivated strains for the algae-based production of beta-carotene.

Since the culturing of microalgal cells requires liquid nutrient media, there exists a lot of strain on fresh, distilled water supply. The growth vessels are big enough to demand large amount of water as media component. This calls for the analysis of potential alternatives of distilled water. It has to be non-toxic, to make sure the compounds extracted are food-grade safe for consumption. There is scope for emphasis to be laid on waste-water remediation and reuse, attributing to the capacity of algae to reduce metal and dye contaminations.

Plants and algae are considered the best sources of bright orange-red colored pigments, such as β-carotene. β-Carotene ($C_{40}H_{56}$) is an oxygen lacking isoprene containing compounds, the characteristic color imparted due to the presence of double bonds. Due to the number of therapeutic

and preventive effects that β-carotene offers, it has been recognized as a functional component of food.

As per James Olson, the activity of carotenes can be studied better upon the categorization of its properties as; “functions, actions, and associations”. The equivalence among these three would imply greater confidence in studies [1]. Functions would be the indispensable role of β-carotene, to convert into vitamin A. Actions are demonstrations noted upon the administration of β-carotene in clinical subjects, minimized photosensitivity and so on. Associations could be simply understood as the correlation of β-carotene to any medical condition, like cancer or diabetes.

Even being categorized as a micronutrient, due to its requirement in minute amounts as compared to other requirements of the body, β-carotene is considered to be very essential. β-Carotene conversion to vitamin A has moved from 6:1 to the current ratio of 28:1. The reason is the single nucleotide polymorphisms in the β-carotene-15’,15-monooxygenase (BCMO) enzyme, responsible for converting provitamin A into retinol. This means that, in the affected individuals, 28 mg of β-carotene is converted into 1 mg of retinol. This exposes the population to a greater risk of developing vitamin A deficiency and associated disorders, as well as the inability to utilize provitamin A from the diet [2]. This also calls for the need of beta-carotene in the form of supplements.

Some algal strains, as per literature, have been subjected to various physiological stresses to which the production and accumulation of target pigments like beta-carotene has been found to be functionally related. Hence, the identification of optimal stress conditions for efficient production and extraction could be figured out. Further, the application of these algal extracts as potential anti-oxidants can be accessed through several assays like DPPH.

Some of the physiological properties of β-carotene limit its effectiveness. It was reported its poor bioavailability in crystalline form; and is insoluble in water. Hence, its better delivery is obtained in oil/water emulsion, which also imparts the pigment its stability [3]. Its sensitivity to heat and light and liability to oxidation are other challenges that need to be addressed. The trend of involving low-on-fat products in food has been adversely affecting the absorption of β-carotene in the body. This might also be the reason for trial results not agreeing with the epidemiological observations. The span of the study, the dosage of β-carotene is all crucial in determining the pigment’s disease-preventing potential.

Various species of algae like *Chlorella* and *Graesiella*, *Rhodophyceae* and so on are utilized for the production of biogenic metal-nanoparticles that have found to be stable and very effective in applications like antibacterial assay, dye degradation as well as heavy metal remediation from waste water. More research needs to be done on the strategies development for recovery of nanoparticles from the treated wastewater.

These nano-particles can been tested for their antimicrobial properties through disc-diffusion, measurement of zone of inhibition and well diffusion methods. The photocatalytic dye degradation and stirred heavy metal removal capacity of nanoparticles has been found to be very efficient. The present study has been carried out with following objectives:

1. Isolation, identification and characterization of microalgae from industrial cement curing tank – Morphological, Molecular and Computational studies
2. Optimizations of culture conditions and selection of suitable factors for enhanced biomass production and accumulation of beta-carotene, and its applications.
3. Condition optimization for synthesis of biogenic silver nanoparticles using leftover microalgal biomass, its characterization and applications as antimicrobial, dye and heavy metal remediation agent.

2. REVIEW OF LITERATURE

Microalgae and its diversity

The photosynthetic unicellular microorganisms having high amounts of intracellular carbohydrates, proteins, lipids and psycho-compounds are simpler forms of plants. They however grow 25X faster than terrestrial plants. They require very little nutrients for growth. The algal biomass obtained can be used as food supplement, feedstock for fishes and animals, bio-composites as well as good sources of essential pigments and biofuels.

Graesiella emersonii

The microalgal strain used for this study is considered the best candidate for biodiesel production due to its high lipid content. It belongs to the family *Chlorellaceae.* Very less work has been published on pigment extraction from this species. However, it has been exploited for biodiesel production.

Factors affecting the growth of algae

- Abiotic and physicochemical factors like light (Intensity and duration), temperature, oxygen concentration, carbon source, nutrient concentration, pH, salinity and presence of toxins.
- Biotic factors like presence of pathogens (bacteria, fungi, viruses); interspecies competition, predators like zooplanktons.
- Operational factors like dilution rate, growth chamber dimensions, mixing and agitation, harvesting protocol and frequency.

Beta-carotene: The pigment

Carotenoids (commonly abbreviated as Crts) are the class of natural pigments with hefty scientific attention due to their substantial properties. Out of around 600 structurally and functionally diverse natural types; three major provitamin A are the *alpha*, *beta* and *gamma* isomers. The beta isomers get actively cleaved at the center in an O2-dependent manner to produce retinal [4]. Plants and algae are considered the best sources of these bright orange-red colored pigments, the β-carotene. These oxygen lacking pigments are fat-soluble and highly hydrophobic due to the conjugated double bonds and central symmetry [5]. These

have been reported to be safe for consumption, as nutritional supplements as well as food additives[6]. β-carotenes (C40H56) are 40-C isoprene-containing compounds, the characteristic color imparted due to the presence of double bonds. Both the ends of the molecule have cyclic rings. During isolation, cis-isomers of carotenoids are most common, however, they readily undergo cis↔trans isomerization in polar environments [7]. Due to the number of therapeutic and preventive effects that β-carotene offers, it has been recognized as the functional component of food.

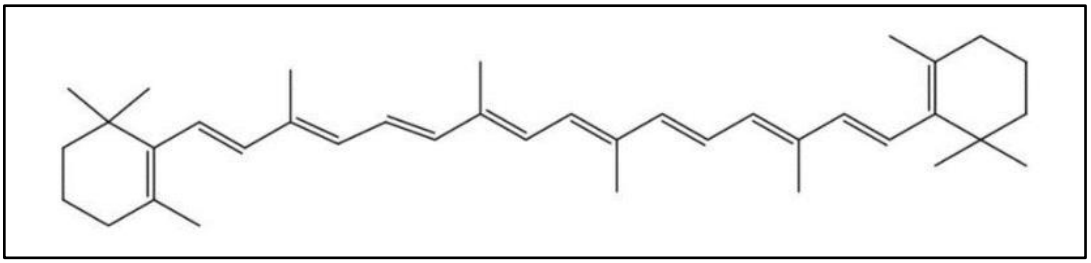


Figure 1: Structure of beta-carotene

Physicochemical properties of beta-carotene

β-Carotene, a type of secondary metabolite produced by plants and algae, is a dark red to brown colored solid substance. The brightness of the color depends upon the level of double bond cis↔trans isomerization, as a result of exposure to high temperatures [8]. The melting point of β-Carotene is around 178°C, having variable solubility. The substance is heat-labile and photosensitive, making the storage condition preferably in dark at 4°C. The pro-vitamin A characteristic of carotenoids is attributed to the β-ion ring in their structure [9]. The lipophilic properties of β-Carotene make it localized in lipoproteins and cell membranes. Hence the extracted c is also found dissolved in the lipid phase.

Sources of Beta-carotene

Mammals lack the de-novo carotenoid synthesis capability and hence totally depend on external sources for its supplementation[10]. These yellow, red, orange-colored pigments are naturally found in algae, higher plants (in fruits and flowers as esters), fungi and animals as illustrated in fig 4 [5]. Even human milk has a carotenoid component, which is easily alterable, depending on the manipulations in the mother's diet [11].

This intake of beta carotene is reflected in serum levels which is also an indicator of the health parameters. A natural pigment, so promising, should have been the center of the bio-pharmaceutical industry. However, it is not. Out of all the beta-carotene in the market, only 3% of it comprises the bio-synthesized type and the rest comes under the synthetic β-carotene category. There is no strict difference between the function of the two, natural and synthetic. But the latter costs more.

Switching to microalgae for β-carotene extraction is both scientifically and economically sensible. A lot of research has been done for the optimization of pigment in various species, along with special extraction techniques for cost-effective and enhanced yields [12].

Extraction of beta-carotene

There are several extraction methods of β-carotene, depending upon the source selected. A lot of research is involved in the optimization of pigment production as well as its extraction. From a broader perspective, the extraction methods can be divided into the organic solvent-based, enzymatic method as well as mechanical extraction. Some collaborative extraction techniques also exist which involve clubbing of the existing methods, as well as with some innovation [13].

Different modes of synthesis of β-carotene in different sources and their extraction methods with advantages has been presented in Table 1.

Table 1: Different modes of synthesis and extraction of β-carotene

	Method	**Condition/Protocol/ Advantages**	**Sources**	**Ref**
		Natural extraction		
	Organic solvents	Post-pre-treatment, the solubility of β-carotene in polar solvents like tetrahydrofuran,	Algae, fungi, plant	[13]

Solvent Based		hexane, acetone offers ease for extraction		
	Supercritical fluid extraction	Reduced extraction time, as well as enhanced efficiency involving supercritical carbon-dioxide	Plant, algae	[14]
Physical	Ultrasonic waves	To prevent thermal as well as chemical damage to pigment, extraction in solvent by ultrasonic waves	Algae, yeast, plants (Conditions optimizable as per source)	[15], [16]
	Bead milling			
	High-pressure homogenization			
Enzymatic	Pectinolytic and cellulolytic enzymes	Non-commercial enzymes used in combination with other extraction procedures, for better cell disruption	Carrot, Bagasse, apples and other juice extracted leftovers	[17]

	Collaborative	**approaches for**	**synthesis**	
Biotechnological and chemical synthesis	Genetic engineering	Insertional of functional genes like crtI, crtE, crtY and crtYB Blocking expression of repressor genes like crgA	Red yeast, fungus	[18], [19]
	Environment optimization	Organism based optimization of culture conditions for enhanced production and accumulation of β-carotene (like light intensity, salt stress, pH, carbon sources etc)	Microalgae, plant and fungi	[13]
	Construction of poly-ene chains	Wittig's reactions	Two 15-C containing phosphonium salt molecule + one 10-C containing	[20]

			dialdehyde molecule	
		Grignard's reaction	2 molecules of Methanol + di-ketone	[21]

Analytical methods for beta-carotene estimation

Spectrophotometric analysis method is an inexpensive, sensitive, simple and rapid procedure for the estimation of β-Carotene. This method employs the use of smaller amounts of toxic chemical solvent for the analysis of β-Carotene. Spectroscopic methods have widely been employed in commercial settings; however, this method faces certain disadvantages such as being applicable for certain food components only [22]. Recently many liquid chromatography (LC) techniques have also been developed for the analysis of β-Carotene. Amongst all the LC techniques, high performance liquid chromatography (HPLC) is the most suitable method for analysis (separation and quantification) of β-Carotene. HPLC has a requirement for high sample purification steps for the analysis of β-Carotene present in the sample [23]. Column chromatography is also one of the most important methods for the separation and detection of various carotenoids including β-Carotene. In case of column chromatography, silicic acid and alumina are used as stationary phase in order to separate different components from extract [24].

Enhancement of Beta-carotene content in algal cells

For beta-carotene production, *Dunaliella* is generally cultivated by means of a two-stage process. First, microalgae grow in a culture medium rich in nitrates, phosphates and other minerals to obtain optimal biomass production. Second, the cells are moved in a larger production pond characterized by high nutrient deficiency. Tafreshi et al. (2006) reported that, in addition to the medium poor of essential components, also a high concentration (2.5 M) of sodium chloride as a stress condition, promoted a further increase of beta-carotene in the algal biomass [25].

ADMET of beta-carotene

The lipophilic β-Carotene like all other lipids and lipid-associated compounds is absorbed in the mammalian small intestine for further transport to the peripheral tissues. Despite the presence of β-Carotene cleaving enzymes in the small intestine, about half of the un-cleaved β-Carotene enters the circulatory system. The readily available provitamin A absorbed by human intestinal epithelia can be estimated by the concentration of intact plasma-β-Carotene [26]. The other factors affecting the bioavailability of β-Carotene are: genetic factors like pigment-cleaving gene polymorphisms and mutations, the type and lipid content of the food and its matrix, its digestibility and interactions, as well as subjective variations referring to individuals' endogenous digestive enzymes [27].

Lipoproteins and cholesterols facilitate the transport and distribution of the non-polar β-Carotenes in the organism. They can be found in the hydrophobic cores of organic compounds like various subtypes of lipoproteins, cholesteryl-esters and so on. This flowing β-Carotene from blood can be taken up by tissues for either storage or metabolism. The most efficient reservoir of β-Carotene in the human body is the liver, followed by muscles, kidneys, skin, as well as glands like adrenal and mammary glands. It has also been found in the placenta and the yolk sac. Hence, it is comparatively very widely distributed in the body as compared to the other classes of carotenoids [28]. The cleavage, transport and distribution of β-Carotene in humans are similar to that in ruminants and hence they are considered a good study model. Being water-insoluble, the bioavailability of β-Carotene through the GI tract is very low. Due to its vulnerability to physio-chemical degradation during processing, storage and post-consumption, its protected delivery becomes necessary. Nano-technology offers better solubility, storage, target delivery, encapsulated protection stability and dispersion properties to the β-Carotene. Some such nano-engineered forms of β-Carotene are nanostructure- or solid-lipid-carriers, microemulsions, nano-spheres, -capsules and so on. These add to the physical and chemical properties of the pigment. The polymer-based and lipid-based delivery systems (PBDSs and LBDSs) are the most adopted delivery systems for β-Carotene. These nano-engineered pigments have to be targeted to the GI tract fluid for them to be absorbed by the enterocytes for subsequent assimilation. For this purpose, micelles and niosomes have been widely exploited. However, the interaction of these nano-pigments with the GI tract cells and the

environment within must be wisely researched before their incorporation into functional foods.

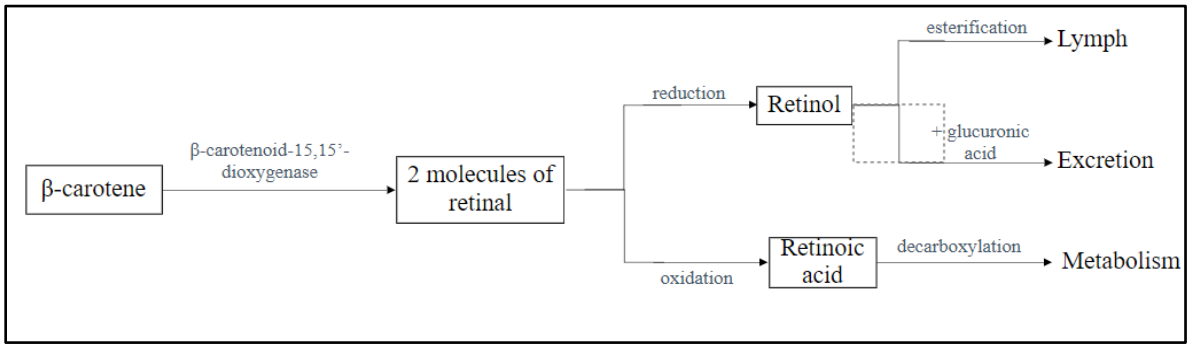


Figure 2: - Fate of ingested beta-carotene

Antioxidant properties of beta-carotene

Depending upon the extent of oxidative stress of the environment to which the pigment is exposed, the actions can be beneficial or damaging. The accumulation of very reactive products released upon carotenoid breakdown contribute to pro-oxidation. This involves the production of very reactive organic compounds like aldehydes. Such products are termed as carotene breakdown products (CBPs) [29]. A representation of the necessary balance of antioxidant properties of β-carotene is depicted. Hence, it can be summarized that the pigment offers several therapeutic effects in the context of acting as a pro-vitamin A: antioxidants neutralizing ROS, regulating connexin expression thus improved communications through gap junctions, activating macrophages, and triggering immune response [13].

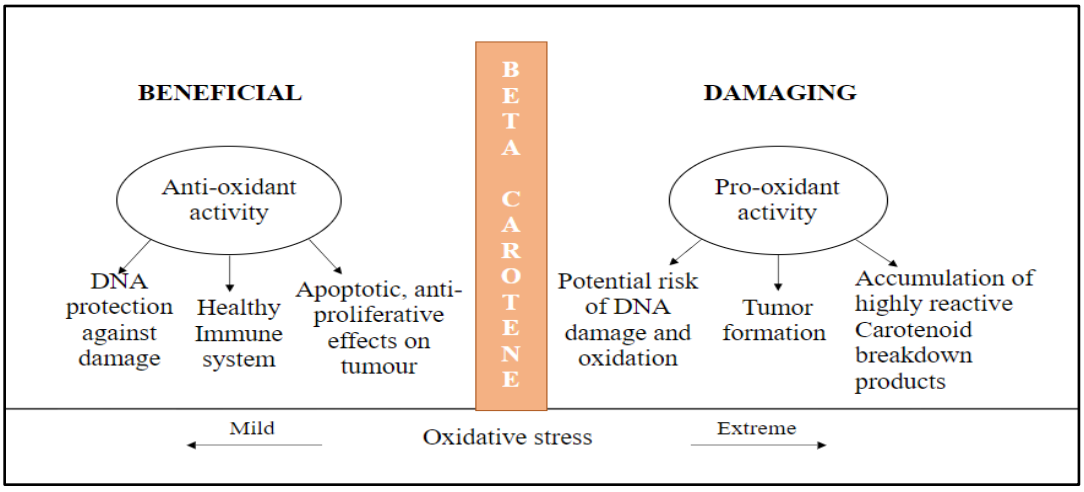


Figure 3: A representation of the necessary balance of antioxidant properties of β-carotene

Environmental nano-remediation

The environmental pollution caused due to the heavy metals and dyes produced by mining activities, industries and effluent discharge directly into the environment causes great environmental threats. These chemicals and metals get bio-accumulated in the food chain and are highly toxic for the higher organisms [30]. These toxic chemicals and metals get accumulated in higher concentrations in the body due to bioaccumulation capacity. There have been a number of treatment technologies adopted for the clearance of these pollutants which includes physical treatment methods such as ion exchange, precipitation, electrocoagulation, membrane filtration, electrodialysis, chemical treatments which include chemical washing, reduction and chelate flushing, and biological treatment methods such as biofiltration, bioremediation, biosorption, bioaugmentation, phytoremediation, phycoremediation, and microbial reduction of compounds [31], [32] . In addition to these traditional technologies, nanotechnology has also been used for the environment remediation and is gaining popularity due to its relative ease of operation and high efficiency.

What is Phyco-nano-remediation?

Despite numerous advantages, nanotechnology faces some drawbacks such as the traditional methods of synthesis of nanoparticles produces toxic by-products and requires a lot of energy input. So, a new methodology called “phyco-nano-remediation” is employed which involves the green synthesis of the nanoparticles and its use in the remediation process [33]. By adopting algae for the synthesis of nanoparticles, the toxic by-products and high energy consumption is almost eliminated. These nanoparticles can be synthesized either by intracellular or extracellular mode using algal biomass. Using algae; gold, silver, ZnO, iron, CdS and nanoparticles have been synthesized [34], [35]. The synthesized nanoparticles have been used to remediate lead, copper, mercury, and other heavy metals along with many dyes like methylene blue, congo red, malachite green, crystal violet and so on. However, nanoparticles synthesized from algae lack uniformity [36], [37]. Their shape and size depend on the conditions of the culture system provided during the synthesis of the nanoparticles. If synthesized intracellularly, the extraction of nanoparticles becomes a time and energy consuming job. There is a risk of cross-

contamination of the environment from the acting nanoparticles as their recovery is difficult due to their extremely small size [38]. Growing the algae at the site of discharge will provide a cost-effective remediation strategy while bridging the ends of lab-scale and large-scale application. **Phyco-nano-remediation** is advantageous as compared to **phyco-remediation** because it is independent of the growth of algal cells and the prevailing environmental conditions at the site of remediation. The remediation efficiency for phyco-remediation is dependent on the growth of the algal species under consideration whereas in case of phyco-nano-remediation, the remediation efficiency is independent of the environmental as well as algal growth conditions from which these nanoparticles have been derived from.

Why silver nanoparticles (AgNPs)?

The characteristics of nanoparticles depend upon various factors such as incubation conditions, molar concentration of metal precursor solution, pH and biogenic extract employed for synthesis. Silver has many inherent properties due to which it has been used over a period of time [39]. Biogenic silver nanoparticles have various applications in wide research areas including nanomedicines, biosensing and others. Biogenic silver nanoparticles have been extensively studied for its antimicrobial action and it has been found that they are highly effective against gram negative bacterial species as compared to gram positive ones [37]. They have been studied for their dye degrading and heavy metal removing capabilities and it has been found that they have high potency as a remediating agent against a wide range of environmental pollutants [40].

Algal AgNPs

Algae are the most abundant and easily available organism; this property of algae makes them the best candidates for nanoparticles' synthesis [41]. Algal mediated synthesis of silver nanoparticles involves three major steps;

(i) algal extract preparation in distilled water or in organic solvents by boiling at a particular temperature for a predetermined duration,

(ii) preparation of precursor metal molar salt solution, and;

(iii) incubation of algal extract with salt solution under controlled conditions [41], [42].

Various algal biomolecules such as polysaccharides, proteins, cytochromes and other pigments are responsible for the reduction and stabilization of metal ions [43]. Algal based NP synthesis takes a short time as compared to any other biogenic synthesis method [44]. Algal NP synthesis takes place both extracellularly and intracellularly [45]. Intracellular synthesis of NPs requires an additional step of recovery of synthesized NPs from within the cells [46] . Edison et al., synthesized spherical Ag-NPs of size ~25 nm employing *Caulerpa racemose* [47] . Ag-NPs of size range 8- 12 nm have been synthesized using algal extract of *Chlorella pyrenoidosa* [48]. Spherical NPs have been reported to be synthesized from *Jania rubins* [49]. Azizi et al., reported the synthesis of spherical Ag-NPs in size range 5-15 nm using the algal extract of *Sargassum muticum* [50].

Applications of Algal Silver Nanoparticles

- Ag-NPs of biological origin are emerging as new age antimicrobials and the research in this area has accelerated over the period of time due to the frequent occurrence of antimicrobial resistant strains [51]. They interact directly with bacterial cells and consequently overcome the antibiotic resistance mechanisms adopted by bacterial species [42]. Biogenic Ag-NPs are advantageous over conventional antibacterial agents as they prevent the emergence of antimicrobial resistance strains, are cheap and rapid to synthesize, and environment friendly [52]. These NPs are reported to use one of the three mechanisms or a combination of mechanisms for causing bactericidal effect:
  (i) increased production of reactive oxygen species;
  (ii) penetration within membrane, and;
  (iii) interaction with cellular components like DNA, RNA and cell organelles [53].
  Dhavale et al., synthesized Ag-NPs using *Amphiroa fragilissima* and its application as an antibacterial agent targeting *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus sp* [54]. Ag-NPs produced from *Chlorella vulgaris* extract was used as antibacterial agents against *E. coli* and *Pseudomonas aeruginosa* [55]. Other algal strains used for biogenic NP synthesis are *Chlorella sorokiniana*, *Graesiella emersonii* and so on.
- Nanoparticles are considered to be the best material for heavy metal remediation as they have high surface activity, large surface-to-volume ratio and unique physicochemical characteristics. Type of nanoparticles and their physical, chemical and magnetic properties

play a major role in the abatement of heavy metal [56]. Metal-based nanoparticles like silver, gold, iron, and metal-oxide nanoparticles are widely used for remediation of heavy metals like cadmium, copper, chromium, lead and mercury. Ag-NPs can remediate mercury, cadmium, chromium, cobalt, lead, etc [57]. It is observed that remediation ability of Ag-NPs is dependent on the reduction potential of heavy metals [58]. Thus, it can be concluded that for every different heavy metal, a different type of nanomaterial is required. Attasi and Nsiah reported that 20 nm Ag-NPs are able to remediate 92.92% lead and 53.34% cobalt within 14 days [59]. On the other hand, El-Tawil *et al*., observed that Ag-quartz nanocomposite enhances the removal efficiency of mercury to 96% within an hour [60]. Many algal species like *Sargassum muticum*, *Turbinaria ornate*, *Sargassum polycystum*, *Turbinaria conoides*, *Gilidiella acerosa*, *Sargassum wightiigrevilli*, *Padina pavonica*, *Colpmenia sinusa* are able to synthesize different size of Ag-NPs [45].

- Biogenic NPs have been considered for their potential dye degradation capacity from contaminated water bodies. NPs help in effective adsorption of these dyes owing to the large surface area of NPs [61]. NPs are also known to degrade dyes into simpler non-toxic forms. Ag-NPs can degrade dyes such as Congo Red, Coomassie blue, Methyl orange, Malachite green, and so on [62]. Ag-NPs derived from *Microchaete sp* have been reported to demonstrate 84.6% removal efficiency of Methyl red in contaminated water sources [59] [62]. Aziz et al., used *Chlorella pyrenoidosa* extracts and reported the synthesis of Ag-NPs that could efficiently degrade methylene blue from wastewater [48]. Ag-NPs have been synthesized using algal extracts of *Ulva lactuca* and were subsequently used for the degradation of methyl orange from contaminated water sources [63]. So, silver nanoparticles can be employed for treating effluents from dyeing and textile industries at a larger scale in an economic and environment friendly manner.

3. MATERIALS AND METHODOLOGY

Bioprospecting microalgae: sample collection, enrichment, isolation, and identification

Sample water containing target microalgae was collected from an industrial run-off cement curing tank and brought to the laboratory. A certain volume of the sample water was then mixed with microalgae culture media BG-11 and kept for growth in incubator chamber at 25±2°C in 16:08 photoperiod. After a week, this preparation was used for isolation of microalgae using BG-11 agar plates.

After establishment of pure culture, morphological and molecular identification of the isolated microalgae strain was performed. For morphological identification, isolate was examined on light microscope. For molecular identification, 18S rRNA gene molecular identification was performed. DNA was extracted from the culture and the target DNA sequence was amplified and subjected to agarose gel electrophoresis. After sequencing of the strain, computational works (Basic Local Alignment Search Tool and Phylogenetic analysis) were performed to identify the closely related species.

Cultivation and biomass harvesting

The isolated strain was cultivated in BG11 medium at 25±2°C in 16:08 photoperiod in a growth chamber. For growth measurement, sample from 10% starter culture was inoculated in fresh media, was collected and observed under UV spectrophotometer @ 690nm for 12 days at a regular interval. The growth curve was prepared for identification of suitable time period for biomass harvesting. The biomass was harvested using centrifuge (5000 rpm for 10 mins). Thus, harvested biomass was dried in a hot air oven and stored for future studies.

The biomass productivity of strains can be calculated by using the following formula.

$$\text{Biomass productivity (mg/l-d)} = \frac{\text{Total dry weight (mg)}}{\text{Number of days of cultivation(days) X Volume(ml)}}$$

$$Biomass\ concentration = \frac{Weight\ (mg)}{Volume\ of\ culture\ (L)}$$

Total carotenoids estimation

Carotenoids estimation was done by 1:2 hexane-ethanol method. A slight modification was exercised in the protocol provided by NREL for total carotenoids estimation, beta-carotene analysis and chlorophyll measurement. 10 ml culture of microalgae was taken and subjected to centrifugation for 10 minutes at 10,000 rpm. The harvested biomass was washed twice with distilled water and resuspended in 10 ml (X ml) of distilled water. Cell disruption techniques were performed on the preparation. Then sample was allowed to cool and 3X ml of 1:2 hexane-ethanol solution was added in it. The preparation was subjected to centrifugation and supernatant was collected. The absorbance of collected supernatant was taken for chlorophyll a, chlorophyll b and total carotenoids estimation. The following formula was used for the estimation:

$$C_a = 12.23A_{663} - 2.81A_{646}$$

$$C_b = 20.13\ A_{646} - 5.03\ A_{663}$$

$$C_t = \frac{1000A470 - 3.27Ca - 104Cb}{198}$$

Where C_a, C_b, and C_t are chlorophyll a, chlorophyll b and total carotenoids respectively in µg/ml.

Qualitative and quantitative estimation of beta-carotene

Paper chromatography and Thin-layer chromatography were performed to confirm the presence of beta-carotene in algal extracts. Samples were run against beta-carotene standard dissolved in 95% ethanol. For quantification, the extracts were subjected to UV-Vis spectrophotometry. Beta-carotene, as per literature, gives peak at 450 nm.

Quantification of beta-carotene from algal sample was done using the following formula.

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

Factor optimization for efficient Beta-carotene production and accumulation

Several abiotic factors influencing the production and accumulation of beta-carotene in algal cells were performed.

The algal culture was subjected to light stress, with light intensity variations of normal incubator (2000 Lux), in modified lighting incubator (10,000 Lux) and natural sunlight (last week of March 2022, Delhi; 50,000 Lux) respectively.

A salt stress using NaCl salt varying from 0M, 0.1M, 0.2M…1M was performed to the growing algal culture. Suitable salt and light stress were identified using the standard beta-carotene extraction protocol.

Growth media for algal culture were varied. The cells were grown in normal BG11 media, in RO spent water and D-Glucose supplemented BG11 media.

Factor optimization for efficient Beta-carotene extraction from algal cells

The standard protocol for beta-carotene extraction from algal cells is subjective to the cell morphology and hence variations to cell disruption levels were studied. Different cell disruption techniques including bead milling (3 mm diameter glass beads + high speed vortexing), ultrasonication, heat treatment (boiling@60°C) and centrifugation were performed.

The solubility of beta-carotene in solvents vary, from being soluble in lipids, some organic solvents including hexane, chloroform. Beta-carotene is sparingly soluble in ethanol, methanol and insoluble in water. Hence, a set of organic solvents were tried as beta-carotene extraction solvents for efficient recovery.

The sonication ranges have also been varied to study the extraction efficiency. Cycles (0.5 to 1), amplitude (25, 50 and 100) and sonication times (0, 15, 30, 45, 60, 90, 120, 150 mins) were performed.

Biomass type, wet and oven-dried were also studied for profitable beta-carotene extraction, for identification of suitable type of biomass. Wet and Dry biomass from all the three types of cultures, from normal BG11, BG11 supplemented with glucose and BG11 in 50% RO spent water were taken.

For beta-carotene recovery from dry biomass, Soxhlet extraction was performed, using Hexane: Ethanol (1:2) and the resultant sample was studied with UV-Vis spectrophotometer.

Applications of beta-carotene (DPPH Assay)

Beta-carotene has anti-oxidative properties that were accessed through 2,2-diphenyl-1-picrylhydrazyl (DPPH assay). 0.2mM DDPH in ethanol was prepared and its initial absorbance (A_o) at 517 nm was taken. To 4 ml of the ethanolic DPPH was added 0.2 ml of sample and incubated in dark for 40 mins. The final absorbance at 517 nm (A_1) was noted.

$$\text{DPPH scavenging effect (\%) Inhibition} = \left(\frac{Ao - A1}{Ao}\right) * 100$$

Synthesis, characterization and applications of silver nanoparticles from residual biomass

The residual algal biomass, post beta-carotene extraction was used for the synthesis of biogenic silver nanoparticles. Synthesis of nanoparticle was carried out using silver salt as a precursor. Different formulations (1:10, 1:1, 10:1) of silver salt in 10mM strength against algal extract at varying pH (5-10) was performed to identify optimum conditions for silver nanoparticle synthesis using algal extract.

The synthesized silver nano particles were characterized using UV-Vis spectrophotometer (200-700 nm).

The silver nanoparticles synthesized were preserved for antibacterial, photocatalytic dye degradation and heavy metal removal.

Antibacterial assay of synthesized silver nanoparticles

- 0.1 ml of overnight grown bacterial culture *Escherichia coli* was spread using a spreader on the nutrient agar plate.
- Circular disks were cut from filter paper under aseptic conditions
- Four filter-paper disks were taken. 1 dipped in solution, other three dropped with 10, 15 and 20 µl of synthesized Ag-NP solution and placed carefully on the nutrient agar plates containing *E. coli* cultures.
- The plates were incubated in upright manner for 24 hrs.
- The plates were checked for zone of inhibition around the disks and was measured carefully using a millimetre scale for determination of antibacterial activity.

Photocatalytic degradation of Methylene blue dye using Ag-NPs synthesized using *Graesiella emersonii* extract

- Standard curve of different concentration of Methylene blue (MB) dye was prepared and absorbance at 663 nm was noted for each dilution.
- 10 mL of 10 and 100 PPM solution of MB was taken and 1 mL of biogenic Ag-NPs was mixed.
- Absorbance was noted after mixing Ag-NPs with the MB solution.
- The solutions were incubated in the sunlight and absorbance was noted after every hour.
- Removal efficiency was calculated using the standard curve.

Removal of Chromium using Ag-NPs synthesized using *Graesiella emersonii*

- Take 2 mL of biogenic Ag-NP solution in a centrifuge tube
- Centrifuge at 10,000 rpm for 10 mins
- Discard the supernatant and collect the pellet
- Prepare different PPM solution of Chromium (30 and 100 PPM)
- Mix the biogenic Ag-NP pellet with different concentration solution of Chromium
- Incubate at rotatory shaker overnight
- Absorbance was noted at 350 nm at 0 min, 24 and 48 hours of incubation by centrifuging the solution at 10,000 rpm for 10 mins.
- Removal efficiency was calculated using the concentration after removal using standard curve.

4. RESULTS AND DISCUSSION

Morphological studies: Sample from an industrial run-off cement curing tank was isolated and cultured for strain isolation. The isolated microalgae were studied by light microscope. The morphology was circular as seen under the microscope. The colonies initially are dark green 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. Based on the molecular identification study, the strain was found to be *Graesiella emersonii*.

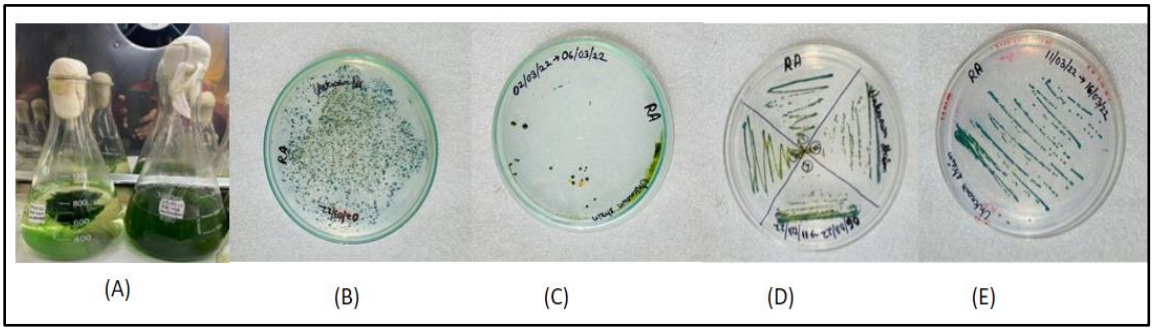


Figure 4: Isolated colonies of microalga in agar plate in order to establish pure culture

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.

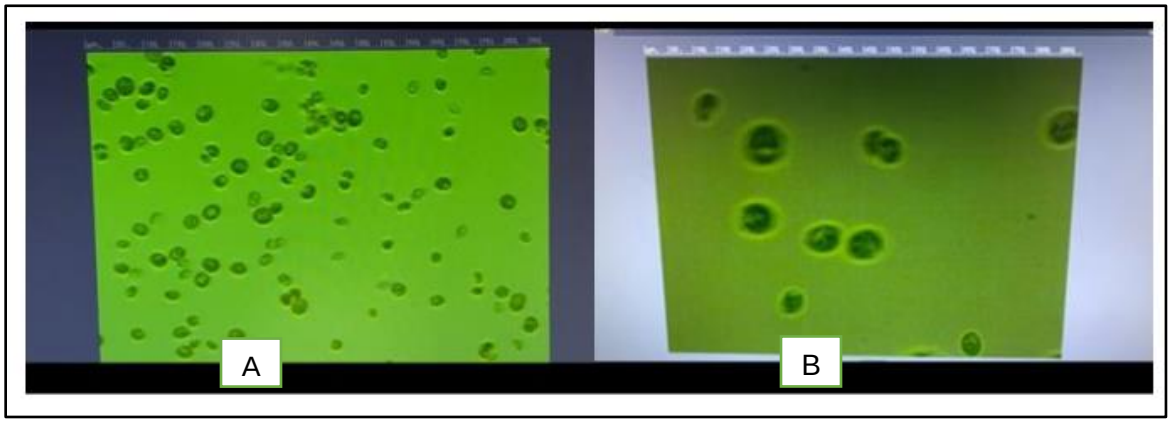


Figure 5: Microscopic view of isolated pure culture. A- 20X and B- 40X magnification under light microscope

Molecular identification studies: Genomic DNA of the sample was isolated using the phenol-chloroform method. The purity of extracted genetic matter was confirmed using Nano Drop 2000 Spectrophotometer. PCR was run, which gave a single ~700 bp PCR product of the 18S rRNA gene.

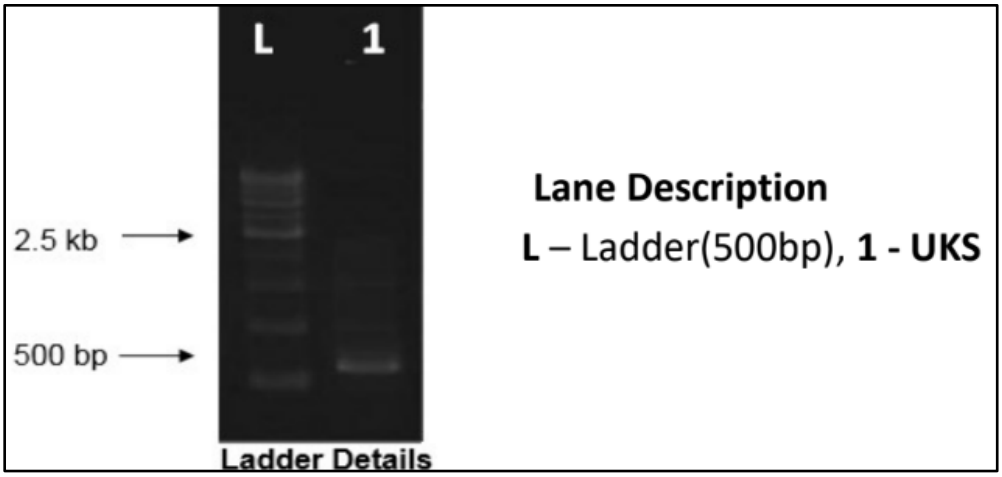


Figure 6: Gel electrophoresis of microalgal gDNA amplicons

FASTA sequence of the strain

>UKS-Microalgal-1F.ab1

ATTAAGGCCATGCATGTCTAAGTATAAACTGCTTATACTGTGAAACTGCGAAT
GGCTCATTAAATCAGTTATAGTTTATT
TGGTGGTACCTTACTACTCGGATAACCGTAGTAATTCTAGAGCTAATACGTGC
GTAAATCCCGACTTCTGGAAGGGACGT
ATATATTAGATAAAAGGCCGACCGGGCTTTGCCCGACCCGCGGTGAATCATG
ATATCTTCACGAAGCGCATGGCCTTGCG
CCGGCGCTGTTCCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAG
AGGCCTACCATGGTGGTAACGGGTGAC
GGAGGATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACAT
CCAAGGAAGGCAGCAGGCGCGCAAATTA
CCCAATCCTGATACGGGGAGGTAGTGACAATAAATAACAATACCGGGCATTT
AATGTCTGGTAATTGGAATGAGTACAAT
CTAAATCCCTTAACGAGGATCCATTGGAGGGCAAAGTCTGGTG

This gene sequence was used to run the Basic Local Alignment Search Tool (BLAST) with the GenBank database. The top 10 hits with maximum similarity are shown below.

Table 2: Sequences producing significant alignments. Nearest relatives, Accession Number and % Identity observed in GenBank when BLAST was performed with microalgae consensus sequence. Microalgae displayed the maximum identity with *Graesiella emersonii* MK541794.1 with 99.64% identity.

Sl.No.	Organism Name	Accession No	% Match
1	Graesiella emersonii strain CCAP211/11N small subunit ribosomal RNA gene	MK541794.1	99.64%
2	Graesiella emersonii isolate CCAP211/8H small subunit ribosomal RNA gene	MG022718.1	99.64%
3	Uncultured Chlorophyta clone 3-9 18S ribosomal RNA gene	JF720730.1	99.64%
4	Coelastrella sp. SAG 2471 18S ribosomal RNA gene	KM020087.1	99.63%
5	Scenedesmus sp. Ki4 gene for 18S rRNA	AB734096.1	99.63%
6	Scenedesmus sp. JB11 small subunit ribosomal RNA gene	KF791548.1	99.63%
7	Graesiella emersonii genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, culture collection CCAP 211/8P	FR865687.1	99.63%
8	Graesiella vacuolata genomic DNA containing 18S rRNA gene, ITS1, 5.8SrRNA gene, ITS2, 28S rRNAgene, culture collection CCAP 211/8C	FR865685.1	99.63%
9	Chlorella emersonii genomic DNA containing 18S rRNA gene, ITS1, 5.8SrRNA gene, ITS2, 28S rRNA gene, culture collection CCAP 211/11M	FR865657.1	99.63%
10	Coelastrella sp. A2 18S ribosomal RNA gene	MF677854.1	99.46%

The phylogenetic tree gives a distance matrix resembling the closest species, as shown below.

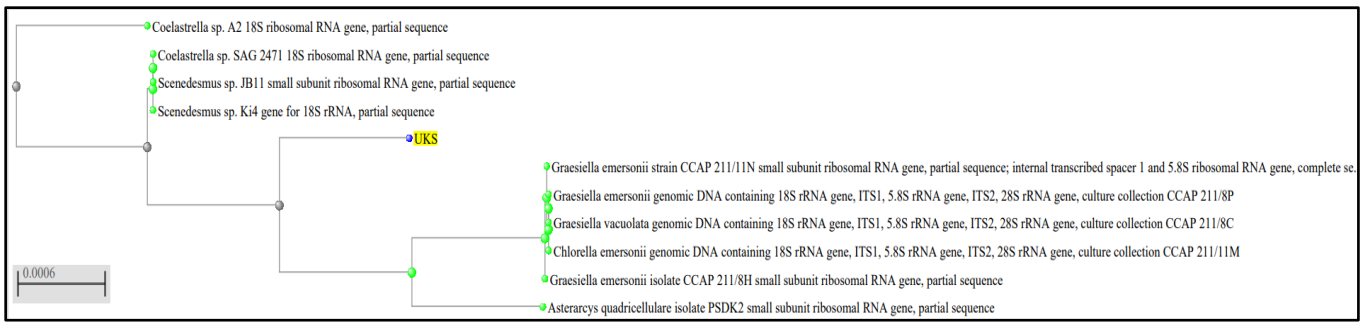


Figure 7: The evolutionary history was inferred using the phylogenetic tree construction.

Growth medium and algal biomass production

The growth curve of the strain was prepared accessing its growth on regular intervals through UV-Vis spectrophotometry at 690 nm wavelength. The graph was plotted as shown below. Generally, the OD for cultures is recorded till the value reaches 1.

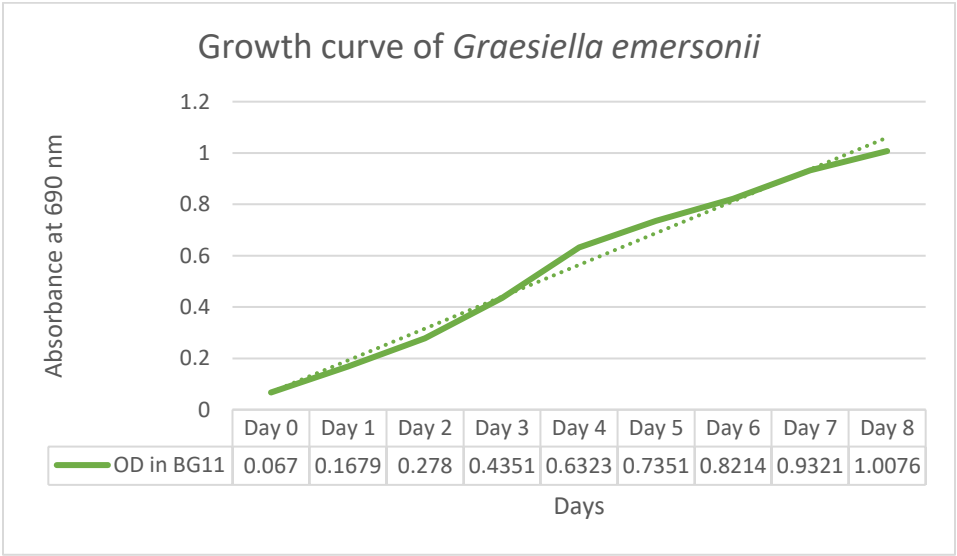


Figure 8: Growth curve of *Graesiella emersonii* with 10% starter culture inoculum (Fast growing strain)

The standard of beta-carotene from Sigma Aldrich was dissolved in 1:2 Hexane-Ethanol and absorbance was measured at 450 nm. The readings were plotted on a graph for reference standards. This graph will work as the standard reference for determination of effective concentration with respect to absorbance at 450 nm.

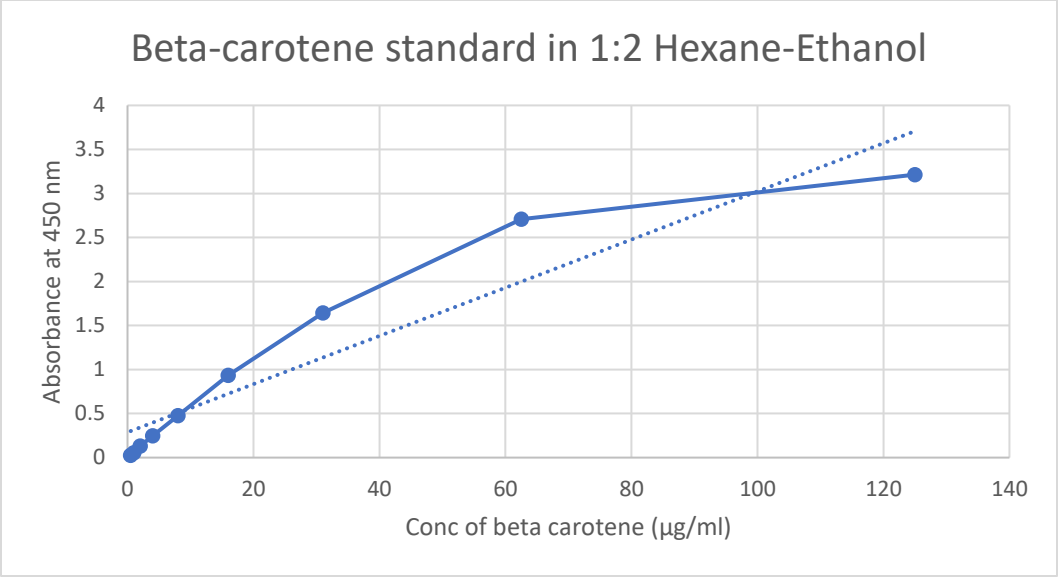


Figure 9: Absorbance pattern of beta-carotene standard made in 1:2 Hexane: Ethanol

Assessment of different solvents for beta-carotene extraction from *Graesiella emersonii*

The solubility of beta carotene is different in various solvents and hence extraction efficiency is different. Nano-technology offers better solubility, storage, target delivery, encapsulated protection stability and dispersion properties to the β-carotene. Some such nano-engineered forms of β-carotene are nanostructure- or solid-lipid-carriers, microemulsions, nano-spheres, and capsules, inter alia.

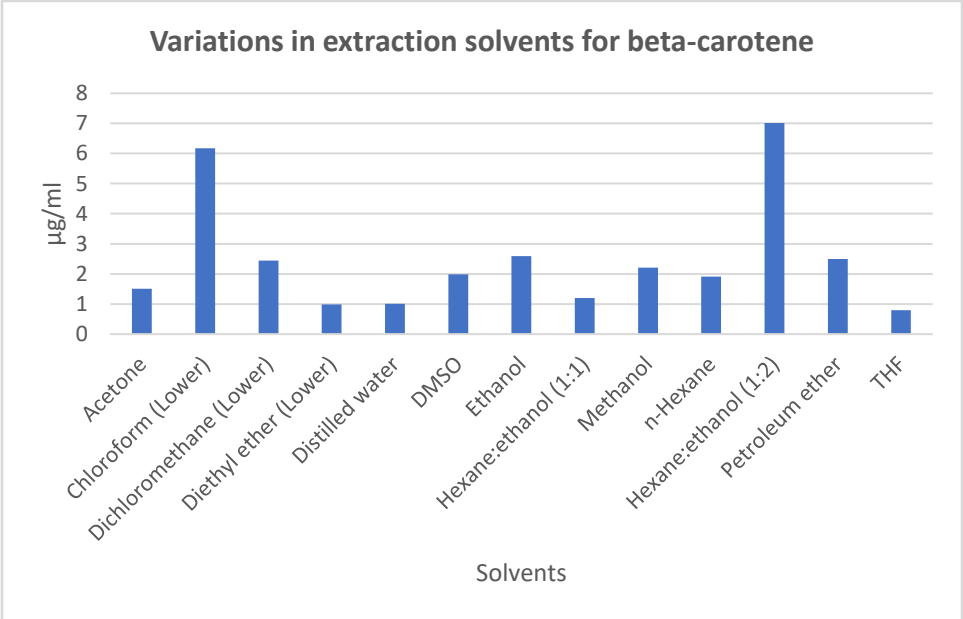


Figure 10: Comparison of different organic solvents for efficient beta-carotene extraction

Pigment estimation for algae under light stress

Table 3: Effect of environmental conditions and light stress on pigment accumulation in microalgae

Samples	B-Crt	Chl A	Chl B	Total Crt
Day 0				
1 - Incubator	7.192	4.4013	0.8347	0.454
2 - Sunlight	5.987	4.1599	0.7236	0.355
3 - Modified light stress	6.894	4.7390	0.9134	0.382
Day 4				
1 - Incubator	11.982	6.0096	1.7288	0.648
2 - Sunlight	5.702	2.9499	1.2455	0.239
3 - Modified light stress	7.280	4.089	1.0451	0.367
Day 10				
1 - Incubator	15.785	11.057	1.7968	1.007
2 - Sunlight	0.519	0.1534	0.0854	0.035
3 - Modified light stress	5.198	2.457	0.6757	0.316

Results and observations: The culture at 2000 Lux kept in the incubator at temperature 28°C and light period 16:8 showed highest accumulation of beta-carotene. The axenic algal culture kept in natural sunlight lost its pigmentation and ultimately entered the decline phase, in a very short span of time, probably due to isolation from mutualistic relations. The other factors could be temperature fluctuations and photo period. The highest accumulation of b-carotene was found in normal incubator (15.785µg/ml) at the end of 10 days as compared to culture kept at other two conditions sunlight (0.519 µg/ml) and modified chamber (5.198µg/ml).

Pigment estimation for algae under salt stress

Table 4: Effect of salt stress on pigment accumulation in microalgae over a period of time

Samples	B-Crt	Chl A	Chl B	Total Crt
Day 2				
0 M	0.3160	0.104199	0.017624	0.0324

0.1 M	0.5027	0.175968	0.041723	0.0351
0.2 M	0.7056	0.205437	0.160259	0.0358
0.4 M	0.3840	0.106831	0.058253	0.0276
0.6 M	0.3013	0.110333	0.034088	0.0199
0.8 M	0.441	0.178812	0.061077	0.0252
1.0 M	0.3691	0.144948	0.044159	0.0242
Day 4				
0 M	1.0949	0.314595	0.084271	0.1004
0.1 M	1.7032	0.542028	0.381265	0.0672
0.2 M	1.2423	0.415386	0.331114	0.0436
0.4 M	0.9704	0.327003	0.241699	0.0378
0.6 M	2.4575	0.889029	0.803135	0.0558
0.8 M	2.5915	0.881673	1.017291	0.0370
1.0 M	0.2696	0.126219	0.02321	0.0244
Day 6				
0 M	2.2090	0.838739	0.503814	0.0845
0.1 M	3.1878	1.128517	0.912896	0.0972
0.2 M	1.9429	0.77503	0.395392	0.0904
0.4 M	2.1682	8.306616	1.99289	0.9255
0.6 M	2.3564	0.964605	0.494179	0.1224
0.8 M	1.8516	0.665003	0.548647	0.0650
1.0 M	1.2461	0.47557	0.311544	0.0618
Day 11				
0 M	3.8278	1.65393	0.737959	0.1827
0.1 M	4.7376	1.797515	1.213417	0.1570
0.2 M	2.3133	1.29626	0.496796	0.1023
0.4 M	1.2020	0.6157	0.309218	0.0385
0.6 M	5.2794	2.275975	1.70965	0.1606
0.8 M	2.5275	1.02836	0.934535	0.0462
1.0 M	1.9882	0.79994	0.810174	0.0134

Day 19				
0 M	6.0026	4.225065	0.707096	0.3866
0.1 M	6.5192	4.22812	1.137262	0.2966
0.2 M	9.5230	5.98122	1.660055	0.4467
0.4 M	4.7149	2.782165	1.255747	0.1386
0.6 M	4.9518	3.12503	0.607436	0.3126
0.8 M	1.9731	1.172885	0.280015	0.1165
1.0 M	2.3688	1.33997	0.86337	0.0268

Results and observation: In the longer run, a salt stress of 0.1M to 0.2M was found to be the best for beta-carotene accumulation. For a duration of few days to a week, highest accumulation of beta-carotene was found to be in 0.6M to 0.8M. This is in accordance with the published literature on the pattern of pigment accumulation of algal cells.

Assessment of various cell disruption techniques on algal cells for beta carotene extraction

Results and observation: Out of all the cell disruption techniques, heat treatment and bead milling were found to be the best. However, as per industrial relevance, sonication is considered the best option due to cost-effectiveness and treatment effects.

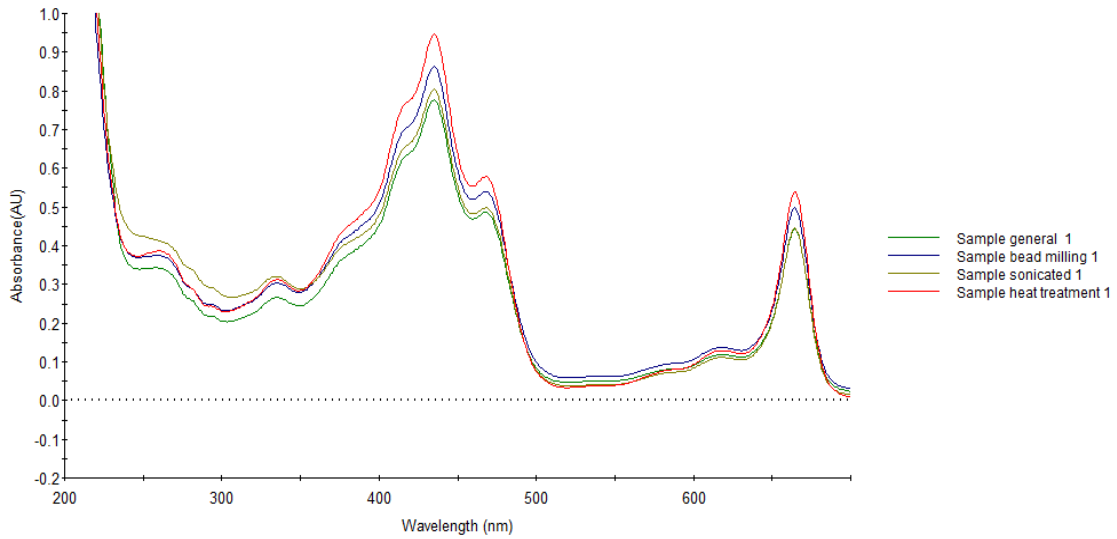


Figure 11: Comparison of different cell disruption techniques for efficient beta-carotene extraction

Assessment of sonication time ranges (0.5 cycle; 50 amplitude)

Table 5: Assessment of sonication range with 0.5 cycle; 50 amplitude for beta carotene extraction

Sonication time	beta carotene (µg/ml)	Chl A	Chl B	Total carotenoids
0 min	0.64512	0.367305	0.299281	0.0101112
15 min	7.4844	4.95004	1.235296	0.3163449
30 min	9.17784	6.376255	1.075143	0.5727280
60 min	10.3068	7.22719	1.074206	0.8434093
90 min	11.13588	4.449255	1.135207	0.9528385
120 min	18.24228	5.42709	2.282906	1.5896562
150 min	22.48344	7.115095	2.176367	1.9946804
180 min	25.27056	5.44213	2.23529	2.5832324

Results and observation: The extraction of beta carotene has been found to be linearly increasing up to 60 mins of sonication time. It was also found that sonication in distilled water was more effective as compared to sonication in extraction solvent. Because, organic solvents vaporize quickly, leading to pigment loss and adding to operating cost.

Assessment of wet biomass in culturing media variants for efficient beta-carotene extraction in Hexane: Ethanol (1:2)

The amount of beta-carotene was found to be higher in dried biomass for culture in RO water. However, the amount of beta-carotene is comparable in wet biomass of strain grown in RO water.

Table 6: Study of beta-carotene extraction on biomass type and cell disruption type

Samples	**Beta carotene**	**Chl A**	**Chl B**	**Total Carotenoids**
Wet Biomass				
GE in BG11				
Normal	11.64744	3.802065	1.918736	1.135613225
Sonicated	13.3686	4.005105	1.326458	1.579448152
Bead milling	12.40596	3.59926	1.19953	1.477039374
Heat treated	15.246	4.752875	1.355968	1.945529421
GE in BG11 + Glucose				

Normal	7.735896	1.78704575	0.92838285	0.9107623
Sonicated	8.006544	2.693194	0.950041	0.905035737
Heat treated	7.275744	1.925167	1.0078954	0.831573504
Bead milling	8.8263	2.95781575	1.06122125	0.976926645
GE in RO spent water				
Normal	6.96528	2.2629325	1.0378525	0.708731056
Bead milling	6.89724	2.1522475	0.8809395	0.74892477
Heat treated	7.6356	1.7109175	0.5806635	0.975992107
Sonicated	6.9426	2.121815	0.952668	0.732759052

Assessment of dry biomass in culturing media variants for efficient beta-carotene extraction using Hexane: Ethanol (1:2)

Table 7: Comparison of beta-carotene accumulation levels in media type variation study

Samples	**Beta carotene**	**Chl A**	**Chl B**	**Total Carotenoids**
in BG11	19.40778	0.516953	1.0627014	2.676531378
in BG11 + Glucose	4.842432	1.44948	0.173127	0.775472327
in RO water	32.93136	1.88329	0.970693	4.752469258

Results and observation: Glucose supplementation in media is not suggested for beta-carotene extraction. However, RO water supplementation gives better pigment extraction from dried biomass.

Qualitative estimation of beta-carotene in algal extract using paper chromatographic technique: Mobile phase of 9:1 petroleum ether: acetone. Standard and extract made in 95% ethanol to perform the paper chromatography to determine the presence of the beta-carotene in algal extract. The presence of beta-carotene was confirmed against the standard as shown in figure 12.

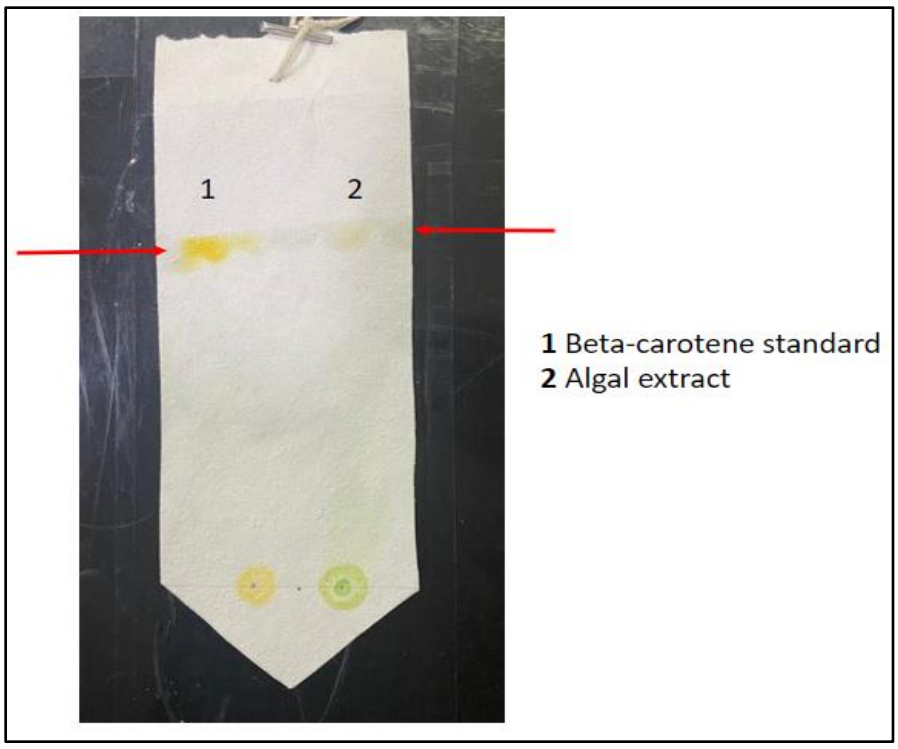


Figure 12: Paper chromatography of beta carotene standard with algal extract, indicating the presence of beta carotene in the sample.

Qualitative estimation of beta-carotene in algal extract using Thin Layer Chromatography

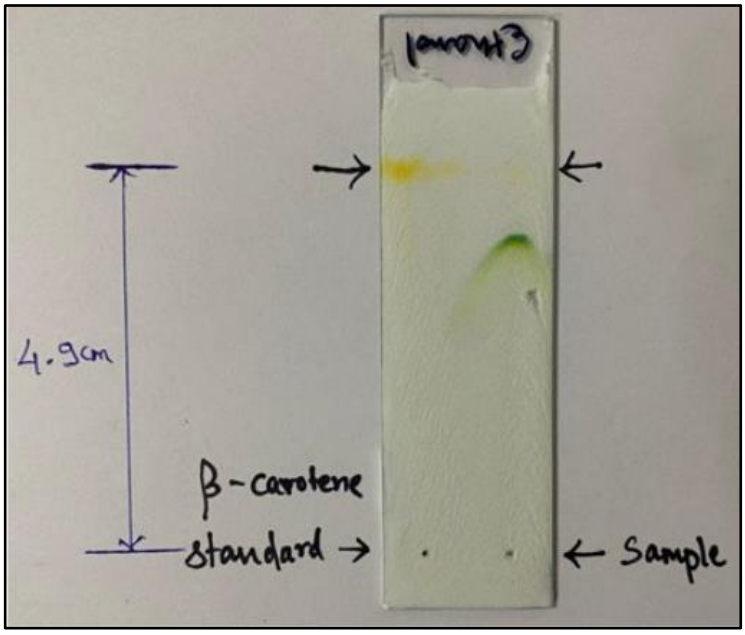


Figure 13: Thin Layer Chromatography of beta-carotene standard and algal extract prepared in 95% ethanol. Mobile phase of Acetone-hexane in 3:7 ratio prepared.

Applications of extracted beta-carotene:

The Radical scavenging capacity of algal extract was found to be around 28.9% and hence was found to be an effective anti-oxidant.

The initial absorbance at 517 nm for control was 0.5222.

Table 8: DPPH assay on beta carotene standard and algal extract for antioxidant activity

Sample	**A_{517} after 40 mins**	**R coefficient**
Control	0.4075	21.96476
Beta-carotene standard	0.2899	44.48487
Algal extract	0.3714	28.87782

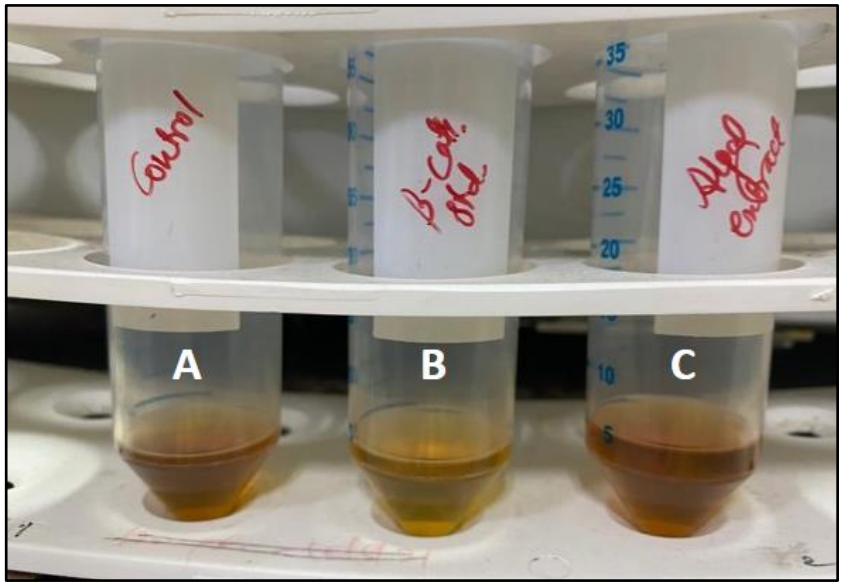


Figure 14: DPPH assay samples A - 4 ml of Ethanolic DPPH + water (Control) B - 4 ml Ethanolic DPPH + Beta-carotene standard (Reference) C - 4 ml of Ethanolic DPPH + algal extract (Sample)

Soxhlet extraction

The Soxhlet apparatus is effective for essential oil extraction from solid, dry biomass. It was found to be effective for beta-carotene extraction as well from dried algal biomass using 1:2 hexane:

ethanol mixture solution. The target extract is washed repeatedly under reflux and is further condensed and collected for characterization and application.

Figure 15: Soxhlet set-up for dry algal biomass in 1:2 hexane-ethanol solvent

Table 9: Pigment concentration post Soxhlet extraction

Beta carotene (µg/ml)	**Chl A (µg/ml)**	**Chl B (µg/ml)**	**Total Carotenoids (µg/ml)**
83.20536	36.87667	8.620181	10.18549439

Silver Nanoparticle synthesis from algal extracts

The cellular and cell free extracts were compared at different pH for nanoparticle synthesis. The particles synthesized at pH 10 in cellular extract was found to be stable.

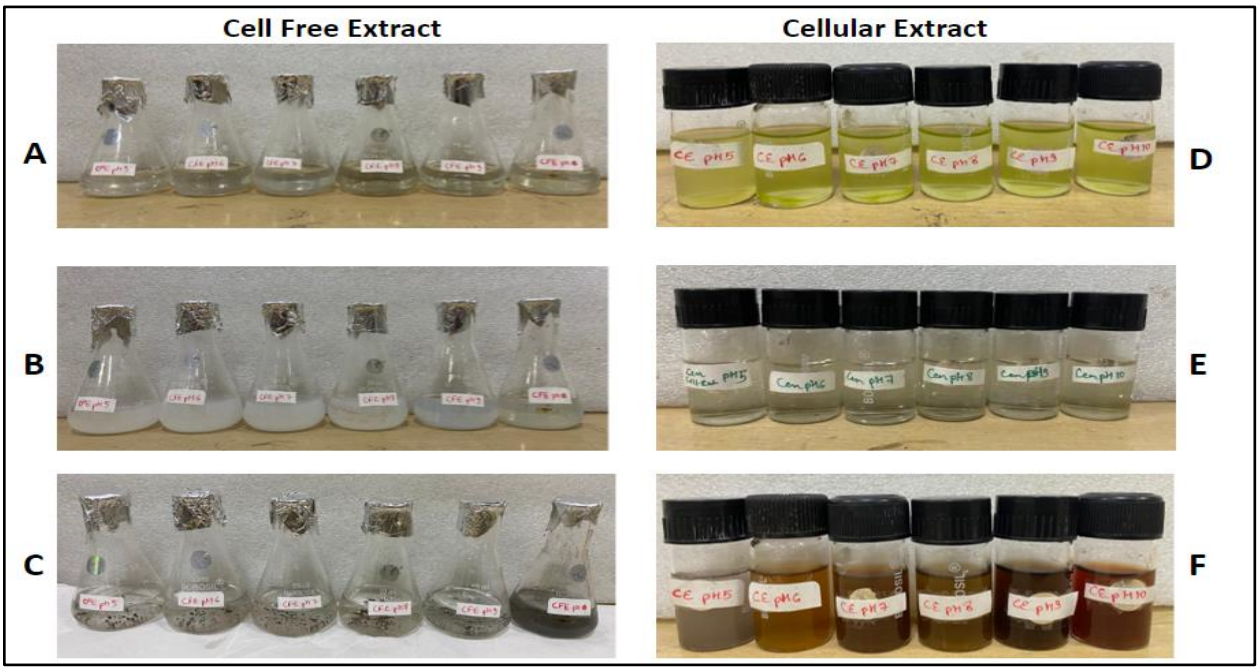


Figure 16: pH 5-10 range optimization for nanoparticle synthesis from cell free and cellular extract of *Graesiella emersonii*

The Extract: Salt ratio variations were checked for best results. Variants of 1:10, 1:1 and 10:1 were studied. The ratio of 10:1 extract: salt solution was found to be best suited for nanoparticle synthesis.

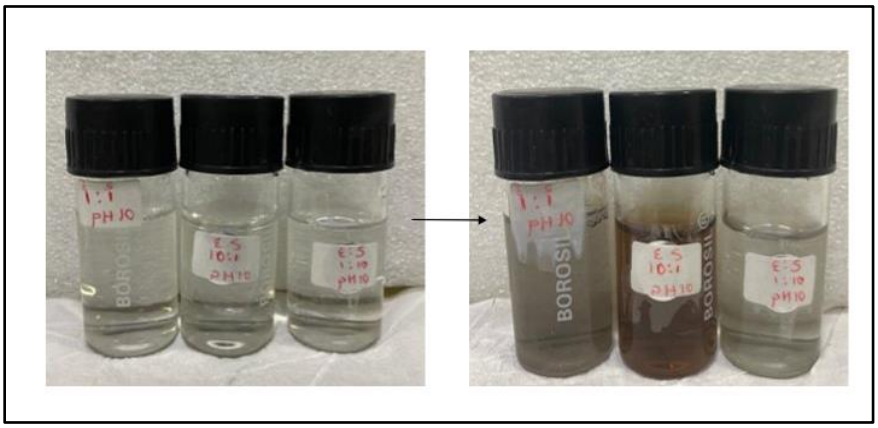


Figure 17: Extract: Salt solution ratio variation optimization for nanoparticle synthesis from cellular extract of *Graesiella emersonii*

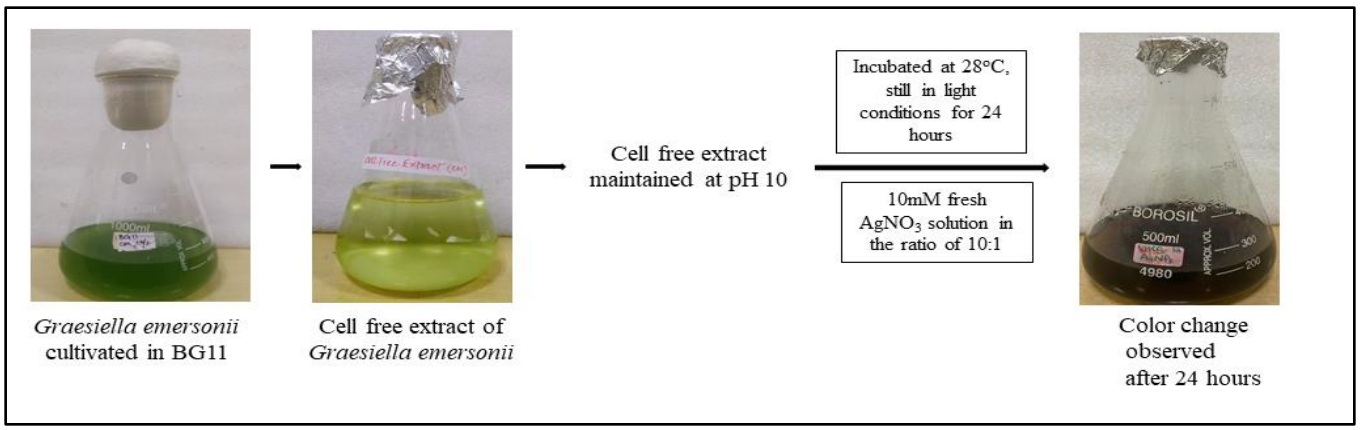


Figure 18: Schematic representation of algal silver nanoparticle synthesis

Results and observation: Cellular extract (biomass boiled in distilled water @ 80°C for 30 mins) was mixed with 10mM $AgNO_3$ precursor salt in 10:1 ratio respectively and maintained at pH 10. The preparation is left in light for 24 hours. Colorimetric changes resemble the synthesis of stable silver nanoparticles.

Antibacterial assay of synthesized silver nanoparticles

Table 10: Zone of inhibition of synthesized nanoparticles on *E. coli*

S. no	Quantity	Zone of inhibition
1	Dip	13.5 mm
2	10 µl	12.9 mm
3	15 µl	13.6 mm
4	20 µl	15.7 mm

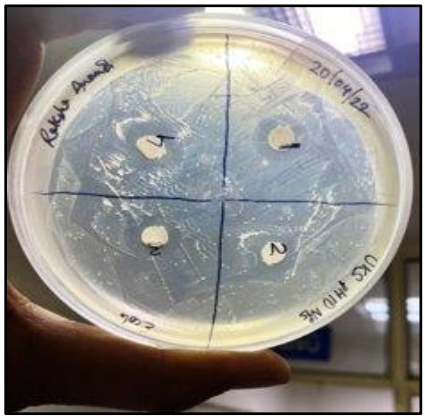


Figure 19: Nutrient agar plate showing zone of inhibition on *E. coli*

culture due to the activity of Ag-NPs produced by extract of *Graesiella emersonii* at pH 10

Observation and Results: Ag-NPs synthesized from *Graesiella emersonii* extract was found to be effective against *E. coli* and can be used as an effective agent to control the spread of the bacterium. The nanoparticles synthesized at pH 10 in extract: salt ratio 10:1 gave good antibacterial results against *E. coli*. The dipped disc, 10µl, 15µl and 20µl doses were used. 20µl gave the best zone of inhibition.

Photocatalytic degradation of Methylene blue dye using Ag-NPs synthesized using *Graesiella emersonii* extract

The photocatalytic dye degradation efficiency of AgNPs synthesized using *Graesiella emersonii* was found to be 88.80% after incubation of 10 hours in sun light as depicted in figure below.

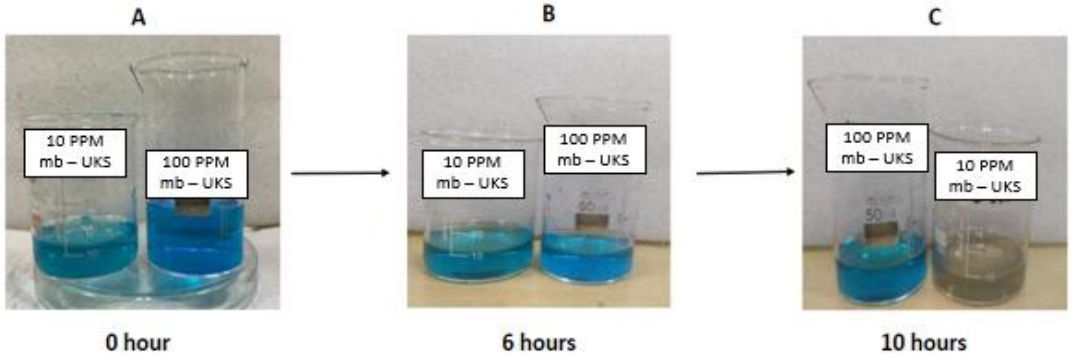


Figure 20: Photocatalytic degradation efficiency assay of synthesized AgNPs on Methylene blue dye (10 and 100 ppm)

Table 11: Absorbance range of dye degradation assay of synthesized nanoparticles

Time	**A_{663}**
0 hour	0.8976
1 hour	0.8185
2 hours	0.7005
3 hours	0.5715
4 hours	0.5082
5 hours	0.432

6 hours	0.3328
7 hours	0.2804
8 hours	0.2685
9 hours	0.2098
10 hours	0.1005

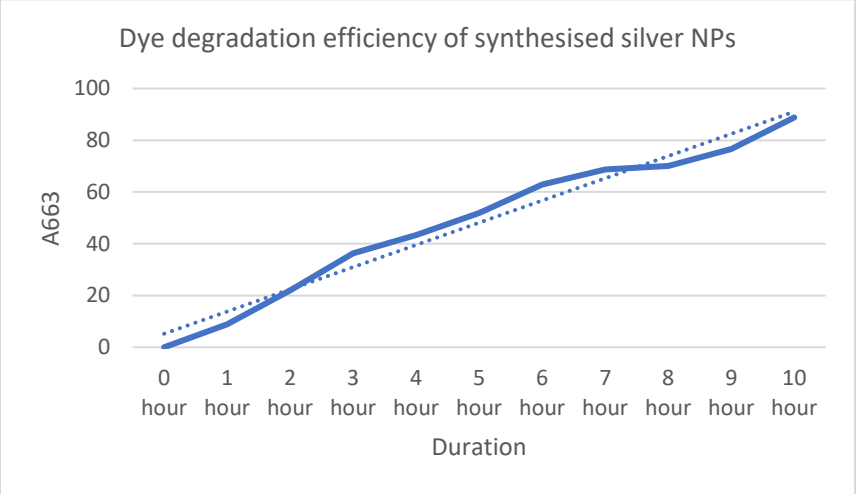


Figure 21: Dye degradation efficiency graph of synthesized silver nanoparticles

Degradation efficiency (%) = [(Initial Concentration – Final concentration)/Initial concentration] * 100

Removal of Chromium using Ag-NPs synthesized using *Graesiella emersonii*

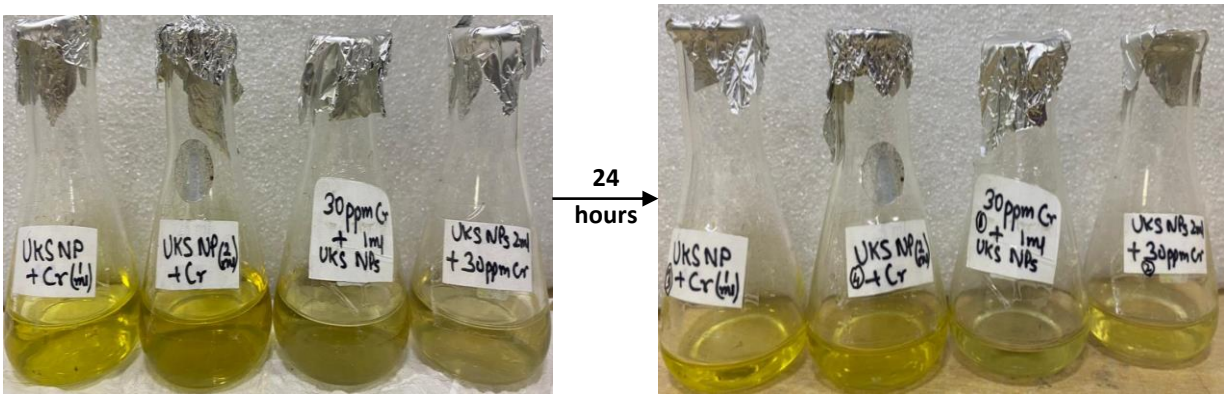


Figure 22: Heavy metal Chromium (30 and 100 ppm) remediation assay of the synthesized nanoparticles

Table 12: Efficiency of heavy metal removal assay performed using synthesized nanoparticles

Sample	0 min	24 hours	48 hours	Efficiency after 24 hours (%)	Efficiency after 48 hours (%)
100 ppm Cr	4.2564	4.2564	4.2564	0	0
100 pm Cr + 1 ml NP	4.2542	2.9622	2.3462	30.40597688	44.87830091
100 ppm Cr + 2 ml Cr	4.2638	2.0325	2.001	52.24837891	52.98844094
30 ppm	1.0389	1.0389	1.0389	0	0
30 pm Cr + 1 ml NP	1.0405	0.8213	0.5796	20.94523053	44.21022235
30 pm Cr + 2 ml NP	1.0469	0.6996	0.3771	32.65954375	63.70199249

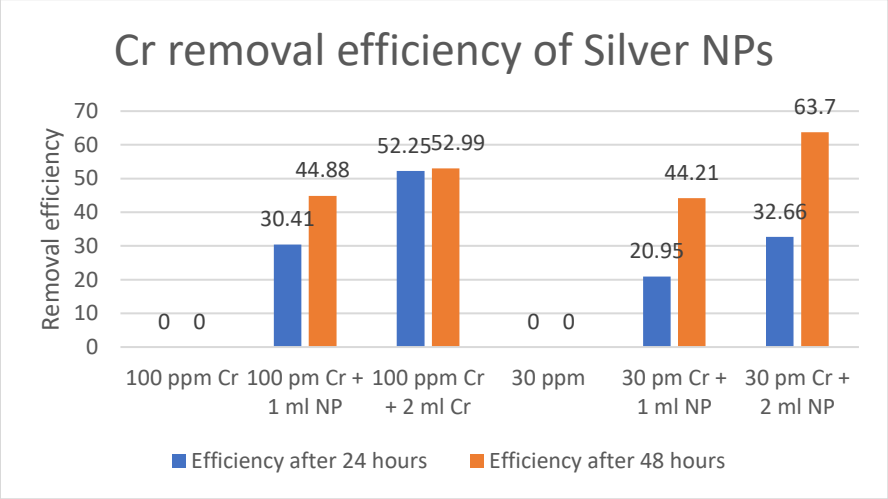


Figure 23: Heavy metal removal efficiency of synthesized nanoparticles

Observation and Results: Ag-NPs synthesized from *Graesiella emersonii* extract was found to be effective in removing smaller concentrations of Chromium. We performed heavy metal adsorption using two different concentration (30 ppm, and 100ppm) and two different dosages 1ml biogenic SNPs, and 2ml biogenic SNPs for two days. At 30 ppm concentration, silver nanoparticles were found to be effective for removal of chromium. At the interval of 24hours, 32.66% removal, and at the end of 48hours almost 64% removal was achieved.

CONCLUSION

The unsubstantiated healing roles of edible 'nutraceuticals' awaits acknowledgement. They have the potential to cure as well as preclude innumerable chronic diseases. These diseases even include outcomes of the modern, desk-bound sedentary lifestyle. The incline towards mainstream medicine and its associated side-effects calls for the unveiling of extraordinary attributes the natural products have to offer. The mode of action of these nutraceuticals is yet to be confirmed, along with legitimation for commercial acceptance. Some lifestyle associated medical conditions like hypertension, cardiovascular diseases and diabetes are treatable with remedial nutraceuticals. Such compounds might be slow but have notable effects and even can be clubbed for multiplex upshots. One such compound is the beta-carotene. Humans being unable to synthesize beta-carotene, the provitamin-A, depend on external sources as its supplement. Health benefits and dietary requirements of beta-carotene are interrelated. This orange-red colored pigment has been enormously examined for its capacity to alleviate several chronic diseases including various types of cancer, cystic fibrosis, as well as COVID-19. However, this class of phytoconstituents has witnessed a broad research gap due to several twin conclusions that have been reported.

There are various methods of synthesis of nanoparticles including physicochemical and biological methods. Biogenic mode of NP synthesis is widely used due to the non-toxic and sustainable nature of synthesis. A range of biological agents used for the production of NPs including bacteria, fungi, plants and its parts, as well as algae. Biogenic Ag-NPs find applications in diverse fields, such as antimicrobial properties, anticancer activities, and environmental remediation. Researchers have conducted a large number of studies employing Ag-NPs for their antimicrobial properties and found out that Ag-NPs are one of the most prominent antimicrobial activities as compared to any other NPs derived from other metals or their oxide. Ag-NPs have been found to be highly effective for environmental remediation strategies such as heavy metal removal and dye degradation from waste water. Thus, it can be concluded that biogenic Ag-NPs can be a sustainable alternative to the traditionally used antimicrobial agents which can also prevent the emergence of antimicrobial resistance. Biogenic Ag-NPs are also a cheap and highly effective alternative for dye degradation and removal of heavy metals from wastewater.

Hence, the present study is a significant step forward in utilization of microalgae for beta-carotene production as well as biogenic silver nanoparticle synthesis and its application.

FUTURE PERSPECTIVE

Till now, the main objective of work which was Isolation, Identification and Characterization of cement water has been achieved. Another aim of enhanced biomass production and culture condition optimization has been achieved along with algal strain, media type, efficient cell disruption technique, biomass type, extraction solvent, sonication ranges, light and salt stress have also been studied.

- Research should be aimed at contributing to the global scientific literature on beta-carotene's application in prevention and treatment of lifestyle diseases.
- More research is needed to improve the overall efficiency of the phyco-nano-remediation approach to deal with heavy metals and dyes pollution.
- More emphasis should be laid on the selective extraction of the target pigment from the solvent for further applications, for example on cancer cell lines and so on.
- The efficiency of algal strains for wastewater treatment, the application of residual biomass for nanoparticle synthesis, biochar preparation and its subsequent application should also be practiced.

REFERENCES

[1] D. A. Cooper, "Functions and Actions of Retinoids and Carotenoids: Building on the Vision of James Allen Olson Carotenoids in Health and Disease: Recent Scientific Evaluations, Research Recommendations and the Consumer 1," 2004. [Online]. Available: https://academic.oup.com/jn/article/134/1/221S/4688292.

[2] U. Gröber and M. F. Holick, "The coronavirus disease (COVID-19) – A supportive approach with selected micronutrients," *Int. J. Vitam. Nutr. Res.*, vol. 92, no. 1, pp. 13–34, Jan. 2022, doi: 10.1024/0300-9831/a000693.

[3] Y. Liu, Z. Hou, J. Yang, and Y. Gao, "Effects of antioxidants on the stability of β-Carotene in O/W emulsions stabilized by Gum Arabic," *J. Food Sci. Technol.*, vol. 52, no. 6, pp. 3300–3311, May 2014, doi: 10.1007/s13197-014-1380-0.

[4] R. Liang, Q. Huang, J. Ma, C. F. Shoemaker, and F. Zhong, "Effect of relative humidity on the store stability of spray-dried beta-carotene nanoemulsions," *Food Hydrocoll.*, vol. 33, no. 2, pp. 225–233, Dec. 2013, doi: 10.1016/j.foodhyd.2013.03.015.

[5] D. B. Rodriguez-Amaya, "Structures and Analysis of Carotenoid Molecules," in *Sub-Cellular Biochemistry*, vol. 79, Springer New York, 2016, pp. 71–108.

[6] S. Akram, M. Mushtaq, and A. Waheed, "β-Carotene: Beyond provitamin A," in *A Centum of Valuable Plant Bioactives*, Elsevier, 2021, pp. 1–31.

[7] G. Bartalucci, C. Delroy, S. Fisher, M. Helliwell, and S. Liaaen-Jensen, "13- *cis* -β,β-Carotene and 15- *cis* -β,β-carotene," *Acta Crystallogr. Sect. C Cryst. Struct. Commun.*, vol. 64, no. 3, pp. o128–o131, Mar. 2008, doi: 10.1107/S0108270108001583.

[8] L. Bogacz-Radomska and J. Harasym, "β-Carotene-properties and production methods," *Food Qual. Saf.*, vol. 2, no. 2, pp. 69–74, 2018, doi: 10.1093/fqsafe/fyy004.

[9] A. Eroglu and E. H. Harrison, "Carotenoid metabolism in mammals, including man: Formation, occurrence, and function of apocarotenoids," *J. Lipid Res.*, vol. 54, no. 7, pp. 1719–1730, 2013, doi: 10.1194/jlr.R039537.

[10] J. Huang, S. J. Weinstein, K. Yu, S. Männistö, and D. Albanes, "Serum Beta Carotene and Overall and Cause-Specific Mortality," *Circ. Res.*, vol. 123, no. 12, pp. 1339–1349, Dec. 2018, doi: 10.1161/CIRCRESAHA.118.313409.

[11] V. Mikkilä *et al.*, "Long-term dietary patterns and carotid artery intima media thickness: The Cardiovascular Risk in Young Finns Study," *Br. J. Nutr.*, vol. 102, no. 10, pp. 1507–

1512, Nov. 2009, doi: 10.1017/S000711450999064X.

[12] T. Marino *et al.*, "Natural beta-carotene: A microalgae derivate for nutraceutical applications," *Chem. Eng. Trans.*, vol. 79, no. August 2019, pp. 103–108, 2020, doi: 10.3303/CET2079018.

[13] Q.-H. Chen, B.-K. Wu, D. Pan, L.-X. Sang, and B. Chang, "Beta-carotene and its protective effect on gastric cancer," *World J. Clin. Cases*, vol. 9, no. 23, pp. 6591–6607, Aug. 2021, doi: 10.12998/wjcc.v9.i23.6591.

[14] T. Baysal, S. Ersus, and D. A. J. Starmans, "Supercritical CO_2 Extraction of β-Carotene and Lycopene from Tomato Paste Waste," *J. Agric. Food Chem.*, vol. 48, no. 11, pp. 5507–5511, Nov. 2000, doi: 10.1021/jf000311t.

[15] S. Dey and V. K. Rathod, "Ultrasound assisted extraction of β-carotene from Spirulina platensis," *Ultrason. Sonochem.*, vol. 20, no. 1, pp. 271–276, Jan. 2013, doi: 10.1016/j.ultsonch.2012.05.010.

[16] L. J. Yu and H. V. Rupasinghe, "Improvement of cloud stability, yield and β-carotene content of carrot juice by process modification," *Food Sci. Technol. Int.*, vol. 19, no. 5, pp. 399–406, Oct. 2013, doi: 10.1177/1082013212455342.

[17] M. Macedo, R. D. P. Robrigues, G. A. S. Pinto, and E. S. de Brito, "Influence of pectinolyttic and cellulotyc enzyme complexes on cashew bagasse maceration in order to obtain carotenoids," *J. Food Sci. Technol.*, Jun. 2014, doi: 10.1007/s13197-014-1411-x.

[18] Y. Zhang *et al.*, "A new regulatory mechanism controlling carotenogenesis in the fungus Mucor circinelloides as a target to generate β-carotene over-producing strains by genetic engineering," *Microb. Cell Fact.*, vol. 15, no. 1, p. 99, Dec. 2016, doi: 10.1186/s12934-016-0493-8.

[19] Y. Wu *et al.*, "Enhancing β-Carotene Production in Escherichia coli by Perturbing Central Carbon Metabolism and Improving the NADPH Supply," *Front. Bioeng. Biotechnol.*, vol. 8, p. 585, Jun. 2020, doi: 10.3389/fbioe.2020.00585.

[20] P. Farfán, S. Gómez, and A. Restrepo, "Dissection of the Mechanism of the Wittig Reaction," *J. Org. Chem.*, vol. 84, no. 22, pp. 14644–14658, Nov. 2019, doi: 10.1021/acs.joc.9b02224.

[21] R. Álvarez, B. Vaz, H. Gronemeyer, and Á. R. de Lera, "Functions, Therapeutic Applications, and Synthesis of Retinoids and Carotenoids," *Chem. Rev.*, vol. 114, no. 1,

pp. 1–125, Jan. 2014, doi: 10.1021/cr400126u.

[22] A. K. Biswas, J. Sahoo, and M. K. Chatli, “A simple UV-Vis spectrophotometric method for determination of β-carotene content in raw carrot, sweet potato and supplemented chicken meat nuggets,” *LWT - Food Sci. Technol.*, vol. 44, no. 8, pp. 1809–1813, 2011, doi: 10.1016/j.lwt.2011.03.017.

[23] A. I. Olives Barba, M. Cámara Hurtado, M. C. Sánchez Mata, V. Fernández Ruiz, and M. López Sáenz De Tejada, “Application of a UV-vis detection-HPLC method for a rapid determination of lycopene and β-carotene in vegetables,” *Food Chem.*, vol. 95, no. 2, pp. 328–336, 2006, doi: 10.1016/j.foodchem.2005.02.028.

[24] M. Ganea, C. Moisa, A. Cozma, and S. Bota, “Determination of carotenoids by thin layer chromatography,” *Analele Univ. din Oradea, Fasc. Protecția Mediu.*, vol. 26, pp. 247–252, 2016, [Online]. Available: http://protmed.uoradea.ro/facultate/publicatii/protectia_mediului/2016A/miscellaneous/04. Ganea Mariana.pdf%5Cnhttps://www.cabdirect.org/cabdirect/abstract/20173103202.

[25] D. B. Rodriguez-Amaya, “Structures and Analysis of Carotenoid Molecules,” in *Carotenoids in nature: biosynthesis, regulation, and function*, vol. 79, C. Stange, Ed. Cham: Springer International Publishing, 2016, pp. 71–108.

[26] V. Shete and L. Quadro, “Mammalian metabolism of β-carotene: Gaps in knowledge,” *Nutrients*, vol. 5, no. 12, pp. 4849–4868, 2013, doi: 10.3390/nu5124849.

[27] E. Reboul, “Absorption of vitamin A and carotenoids by the enterocyte: Focus on transport proteins,” *Nutrients*, vol. 5, no. 9, pp. 3563–3581, 2013, doi: 10.3390/nu5093563.

[28] L. M. Renzi, B. R. Hammond, M. Dengler, and R. Roberts, “The relation between serum lipids and lutein and zeaxanthin in the serum and retina: Results from cross-sectional, case-control and case study designs,” *Lipids Health Dis.*, vol. 11, no. 1, p. 33, 2012, doi: 10.1186/1476-511X-11-33.

[29] W. Siems, C. Salerno, C. Crifò, O. Sommerburg, and I. Wiswedel, “β-Carotene Degradation Products – Formation, Toxicity and Prevention of Toxicity,” in *Forum of Nutrition*, vol. 61, no. February, 2009, pp. 75–86.

[30] D. Leštan, C. ling Luo, and X. dong Li, “The use of chelating agents in the remediation of metal-contaminated soils: A review,” *Environ. Pollut.*, vol. 153, no. 1, pp. 3–13, 2008,

doi: 10.1016/j.envpol.2007.11.015.

[31] S. Imani *et al.*, “Hg, Cd and Pb heavy metal bioremediation by Dunaliella alga,” *J. Med. Plants Res.*, vol. 5, no. 13, pp. 2275–2780, 2011.

[32] H. A. Aziz, M. N. Adlan, and K. S. Ariffin, “Heavy metals (Cd, Pb, Zn, Ni, Cu and Cr(III)) removal from water in Malaysia: Post treatment by high quality limestone,” *Bioresour. Technol.*, vol. 99, no. 6, pp. 1578–1583, 2008, doi: 10.1016/j.biortech.2007.04.007.

[33] T. N. V. K. V. Prasad, V. S. R. Kambala, and R. Naidu, “Phyconanotechnology: Synthesis of silver nanoparticles using brown marine algae Cystophora moniliformis and their characterisation,” *J. Appl. Phycol.*, vol. 25, no. 1, pp. 177–182, 2013, doi: 10.1007/s10811-012-9851-z.

[34] G. Singh, P. K. Babele, A. Kumar, A. Srivastava, R. P. Sinha, and M. B. Tyagi, “Synthesis of ZnO nanoparticles using the cell extract of the cyanobacterium, Anabaena strain L31 and its conjugation with UV-B absorbing compound shinorine,” *J. Photochem. Photobiol. B Biol.*, vol. 138, pp. 55–62, 2014, doi: 10.1016/j.jphotobiol.2014.04.030.

[35] A. Gour and N. K. Jain, “Advances in green synthesis of nanoparticles,” *Artif. Cells, Nanomedicine Biotechnol.*, vol. 47, no. 1, pp. 844–851, 2019, doi: 10.1080/21691401.2019.1577878.

[36] V. V. Pathak, D. P. Singh, R. Kothari, and A. K. Chopra, “Phycoremediation of textile wastewater by unicellular microalga Chlorella pyrenoidosa,” *Cell. Mol. Biol.*, vol. 60, no. 5, pp. 35–40, 2014, doi: 10.14715/cmb/2014.60.5.7.

[37] E. F. Aboelfetoh, R. A. El-Shenody, and M. M. Ghobara, “Eco-friendly synthesis of silver nanoparticles using green algae (Caulerpa serrulata): reaction optimization, catalytic and antibacterial activities,” *Environ. Monit. Assess.*, vol. 189, no. 7, 2017, doi: 10.1007/s10661-017-6033-0.

[38] P. Khanna, A. Kaur, and D. Goyal, “Algae-based metallic nanoparticles: Synthesis, characterization and applications,” *J. Microbiol. Methods*, vol. 163, no. September 2018, p. 105656, 2019, doi: 10.1016/j.mimet.2019.105656.

[39] M. K. Shukla, R. P. Singh, C. R. K. Reddy, and B. Jha, “Synthesis and characterization of agar-based silver nanoparticles and nanocomposite film with antibacterial applications,” *Bioresour. Technol.*, vol. 107, pp. 295–300, 2012, doi: 10.1016/j.biortech.2011.11.092.

[40] E. Parameswari, A. Lakshmanan, and T. Thilagavathi, “Phycoremediation of heavy metals in polluted water bodies,” *Electron. J. Environ. Agric. Food Chem.*, vol. 9, no. 4, pp. 808–814, 2010.

[41] P. Agarwal, R. Gupta, and N. Agarwal, “Advances in Synthesis and Applications of Microalgal Nanoparticles for Wastewater Treatment,” *J. Nanotechnol.*, vol. 2019, 2019, doi: 10.1155/2019/7392713.

[42] L. Wang, C. Hu, and L. Shao, “The antimicrobial activity of nanoparticles: present situation and prospects for the future,” *Int. J. Nanomedicine*, vol. Volume 12, pp. 1227–1249, Feb. 2017, doi: 10.2147/IJN.S121956.

[43] E. Sánchez-López *et al.*, “Metal-based nanoparticles as antimicrobial agents: An overview,” *Nanomaterials*, vol. 10, no. 2, pp. 1–39, 2020, doi: 10.3390/nano10020292.

[44] G. Meroni, J. F. S. Filipe, and P. A. Martino, “In vitro antibacterial activity of biological-derived silver nanoparticles: Preliminary data,” *Vet. Sci.*, vol. 7, no. 1, 2020, doi: 10.3390/vetsci7010012.

[45] R. Chaudhary, K. Nawaz, A. K. Khan, C. Hano, B. H. Abbasi, and S. Anjum, “An Overview of the Algae-Mediated Biosynthesis of Nanoparticles and Their Biomedical Applications,” *Biomolecules*, vol. 10, no. 11, p. 1498, Oct. 2020, doi: 10.3390/biom10111498.

[46] M. M. K. Peiris, S. S. N. Fernando, P. M. Jayaweera, N. D. H. Arachchi, and T. D. C. P. Guansekara, “Comparison of Antimicrobial Properties of Silver Nanoparticles Synthesized from Selected Bacteria,” *Indian J. Microbiol.*, vol. 58, no. 3, pp. 301–311, 2018, doi: 10.1007/s12088-018-0723-3.

[47] T. N. J. I. Edison, R. Atchudan, C. Kamal, and Y. R. Lee, “Caulerpa racemosa: a marine green alga for eco-friendly synthesis of silver nanoparticles and its catalytic degradation of methylene blue,” *Bioprocess Biosyst. Eng.*, vol. 39, no. 9, pp. 1401–1408, 2016, doi: 10.1007/s00449-016-1616-7.

[48] N. Aziz *et al.*, “Facile Algae-Derived Route to Biogenic Silver Nanoparticles: Synthesis, Antibacterial, and Photocatalytic Properties,” *Langmuir*, vol. 31, no. 42, pp. 11605–11612, 2015, doi: 10.1021/acs.langmuir.5b03081.

[49] H. M. El-Rafie, M. H. El-Rafie, and M. K. Zahran, “Green synthesis of silver nanoparticles using polysaccharides extracted from marine macro algae,” *Carbohydr*.

Polym., vol. 96, no. 2, pp. 403–410, 2013, doi: 10.1016/j.carbpol.2013.03.071.

[50] S. Azizi, F. Namvar, M. Mahdavi, M. Bin Ahmad, and R. Mohamad, "Biosynthesis of silver nanoparticles using brown marine macroalga, Sargassum muticum aqueous extract," *Materials (Basel).*, vol. 6, no. 12, pp. 5942–5950, 2013, doi: 10.3390/ma6125942.

[51] F. LewisOscar, S. Vismaya, M. Arunkumar, N. Thajuddin, D. Dhanasekaran, and C. Nithya, "Algal Nanoparticles: Synthesis and Biotechnological Potentials," in *Algae - Organisms for Imminent Biotechnology*, no. June, InTech, 2016.

[52] S. Qamer *et al.*, "Systematic Review on Biosynthesis of Silver Nanoparticles and Antibacterial Activities: Application and Theoretical Perspectives," *Molecules*, vol. 26, no. 16, p. 5057, Aug. 2021, doi: 10.3390/molecules26165057.

[53] Y. C. Yeh, T. H. Huang, S. C. Yang, C. C. Chen, and J. Y. Fang, "Nano-Based Drug Delivery or Targeting to Eradicate Bacteria for Infection Mitigation: A Review of Recent Advances," *Front. Chem.*, vol. 8, no. April, pp. 1–22, 2020, doi: 10.3389/fchem.2020.00286.

[54] R. Dhavale, S. Jadhav, and G. Sibi, "Microalgae mediated Silver nanoparticles (AG-NPS) synthesis and their biological activities," *J. Crit. Rev.*, vol. 7, no. 2, pp. 15–20, 2020, doi: 10.31838/jcr.07.02.04.

[55] J. Annamalai and T. Nallamuthu, "Green synthesis of silver nanoparticles: characterization and determination of antibacterial potency," *Appl. Nanosci.*, vol. 6, no. 2, pp. 259–265, 2016, doi: 10.1007/s13204-015-0426-6.

[56] V. N. Thekkudan *et al.*, "Review on nanoadsorbents: A solution for heavy metal removal from wastewater," *IET Nanobiotechnology*, vol. 11, no. 3, pp. 213–224, 2017, doi: 10.1049/iet-nbt.2015.0114.

[57] K. M. Al-Qahtani, "Cadmium removal from aqueous solution by green synthesis zero valent silver nanoparticles with Benjamina leaves extract," *Egypt. J. Aquat. Res.*, vol. 43, no. 4, pp. 269–274, 2017, doi: 10.1016/j.ejar.2017.10.003.

[58] J. Yang *et al.*, "Nanomaterials for the removal of heavy metals from wastewater," *Nanomaterials*, vol. 9, no. 3, 2019, doi: 10.3390/nano9030424.

[59] I. K. Attatsi and F. Nsiah, "Application of silver nanoparticles toward Co(II) and Pb(II) ions contaminant removal in groundwater," *Appl. Water Sci.*, vol. 10, no. 6, pp. 1–13, 2020, doi: 10.1007/s13201-020-01240-0.

[60] R. S. El-Tawil, S. T. El-Wakeel, A. E. Abdel-Ghany, H. A. M. Abuzeid, K. A. Selim, and A. M. Hashem, “An Overview of the Algae-Mediated Biosynthesis of Nanoparticle,” *Heliyon*, vol. 5, no. 9, pp. 1–9, 2019, doi: 10.1016/j.heliyon.2019.e02415.

[61] A. Patel *et al.*, “Integrating biometallurgical recovery of metals with biogenic synthesis of nanoparticles,” *Chemosphere*, vol. 263, 2021, doi: 10.1016/j.chemosphere.2020.128306.

[62] S. Husain, S. Afreen, Hemlata, D. Yasin, B. Afzal, and T. Fatma, “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*, vol. 162, no. May, pp. 77–82, 2019, doi: 10.1016/j.mimet.2019.05.011.

[63] P. Kumar, M. Govindaraju, S. Senthamilselvi, and K. Premkumar, “Photocatalytic degradation of methyl orange dye using silver (Ag) nanoparticles synthesized from Ulva lactuca,” *Colloids Surfaces B Biointerfaces*, vol. 103, pp. 658–661, Mar. 2013, doi: 10.1016/j.colsurfb.2012.11.022.

Annexure-I: Media Composition

BG11 Media (ATCC Medium 616)

S.no.	Component	Stock solution (gL^{-1} dH_2O)	Amount per liter of media
1	$NaNO_3$	-	1.5 g
2	$K_2HPO_4.3H_2O$	40.00	1 ml
3	$MgSO_4.7H_2O$	75.0	1 ml
4	$CaCl_2.2H_2O$	36.0	1 ml
5	Citric acid	6.0	1 ml
6	Ferric ammonium citrate	6.0	1 ml
7	$MgNaEDTA.H_2O$	1.0	1 ml
8	Na_2CO_3	20	1 ml
9	Trace metals*	-	1 ml

Preparation of Trace metals* solution components

Components	Amount per liter of double distilled water
H_3BO_3	2.86 g
$MnCl_2.4H_2O$	1.81 g
$ZnSO_4.7H_2O$	0.22 g

$Na_2MoO_4.2H_2O$	0.39 g
$CuSO_4.5H_2O$	0.08 g
$Co(NO_3)_2.6H_2O$	0.05 g

Preparation of Culture Solution

Add chemicals and stock solution as indicated above to 1000 ml of double distilled water. Adjust pH to 7.4 with NaOH, and autoclave.

Observation tables

Pigment estimation for algae under light stress:

Samples	**A450**	**A470**	**A505**	**A645**	**A662**
Day 0					
1 - Incubator	0.2854	0.181	0.0213	0.1301	0.4006
2 - Sunlight	0.2376	0.1489	0.0201	0.1193	0.3779
3 - Modified light stress	0.2736	0.1719	0.0305	0.1409	0.4315
Day 4					
1 - Incubator	0.4755	0.3016	0.0391	0.2107	0.5536
2 - Sunlight	0.2263	0.1624	0.059	0.1257	0.2762
3 - Modified light stress	0.2889	0.1777	0.0271	0.136	0.3752
Day 10					
1 - Incubator	0.6264	0.4001	0.0392	0.31	1.0031
2 - Sunlight	0.0206	0.0153	0.0148	0.0077	0.0146
3 - Modified light stress	0.2063	0.1323	0.0148	0.0844	0.226

Pigment estimation for algae under salt stress:

Samples	**A450**	**A470**	**A505**	**A645**	**A662**
Day 2					

0 M	0.01254	0.00904	0.00031	0.00296	0.00946
0.1 M	0.01995	0.01176	0.00024	0.00567	0.01611
0.2 M	0.028	0.02165	0.01261	0.01288	0.02006
0.4 M	0.01524	0.01125	0.00436	0.00529	0.01015
0.6 M	0.01196	0.00755	0.00236	0.004	0.01019
0.8 M	0.0175	0.01112	0.00381	0.00681	0.01658
1.0 M	0.01465	0.00943	0.00266	0.00522	0.01338
Day 4					
0 M	0.04345	0.03037	0.00352	0.01068	0.02891
0.1 M	0.06759	0.04754	0.02169	0.03165	0.05246
0.2 M	0.0493	0.0378	0.02411	0.02644	0.04064
0.4 M	0.03851	0.02901	0.01729	0.01975	0.03178
0.6 M	0.09752	0.08006	0.05496	0.06189	0.08804
0.8 M	0.10284	0.09321	0.07688	0.07377	0.08979
1.0 M	0.0107	0.00772	0.00059	0.00369	0.01148
Day 6					
0 M	0.08766	0.06209	0.03117	0.04414	0.08021
0.1 M	0.1265	0.09893	0.06434	0.07258	0.11056
0.2 M	0.0771	0.05447	0.02151	0.03685	0.07333
0.4 M	0.08604	0.06672	0.04136	0.04527	0.716
0.6 M	0.09351	0.0702	0.03127	0.04598	0.09129
0.8 M	0.07348	0.06093	0.03842	0.04337	0.06527
1.0 M	0.04945	0.04047	0.02147	0.02648	0.04577
Day 11					
0 M	0.1519	0.1053	0.0464	0.0727	0.1553
0.1 M	0.188	0.1385	0.0747	0.1021	0.1734
0.2 M	0.0918	0.0666	0.0265	0.0524	0.1208
0.4 M	0.0477	0.0353	0.0193	0.029	0.0582
0.6 M	0.2095	0.1808	0.1115	0.139	0.2215
0.8 M	0.1003	0.0889	0.0616	0.0719	0.1019

1.0 M	0.0789	0.0708	0.054	0.0606	0.0802
Day 19					
0 M	0.2382	0.1549	0.0199	0.1196	0.3835
0.1 M	0.2587	0.1695	0.0449	0.1438	0.3886
0.2 M	0.3779	0.2501	0.0724	0.2063	0.5503
0.4 M	0.1871	0.14	0.0688	0.1231	0.2614
0.6 M	0.1965	0.1275	0.0207	0.0932	0.2846
0.8 M	0.0783	0.0519	0.0101	0.0379	0.1074
1.0 M	0.094	0.0794	0.0486	0.0738	0.1288

Assessment of sonication time ranges

Sonication time	**450 nm**	**470 nm**	**505 nm**	**645 nm**	**662 nm**
0 min	0.0256	0.0229	0.0134	0.0232	0.0359
15 min	0.297	0.1836	0.0245	0.1561	0.4525
30 min	0.3642	0.232	0.0722	0.1722	0.5771
60 min	0.409	0.2953	0.0884	0.1871	0.6525
90 min	0.4419	0.3188	0.0433	0.1917	0.407
120 min	0.7239	0.559	0.1216	0.2231	0.5065
150 min	0.8922	0.6461	0.0766	0.2468	0.6549
180 min	1.0028	0.7807	0.1076	0.2207	0.5073

Assessment of wet biomass in culturing media variants for efficient beta-carotene extraction in Hexane: Ethanol (1:2)

Hexane: Ethanol (1:2)					
Samples	A450	A470	A505	A645	A662
GE in BG11					
Normal	0.4622	0.4226	0.0853	0.1796	0.3595
Sonicated	0.5305	0.4756	0.0841	0.1502	0.3709
Bead milling	0.4923	0.4411	0.0805	0.1354	0.3334
Heat treated	0.605	0.5628	0.0446	0.166	0.4377

GE in BG11 + Glucose					
Normal	0.30698	0.28637	0.075	0.085905	0.16927
Sonicated	0.31772	0.28889	0.0303	0.10426	0.25006
Heat treated	0.28872	0.27518	0.052845	0.09298	0.18244
Bead milling	0.35025	0.31486	0.035475	0.115505	0.27483
GE in RO spent water					
Normal	0.2764	0.2505	0.047	0.10105	0.2128
Bead milling	0.2737	0.2466	0.04445	0.09015	0.2012
Heat treated	0.303	0.2727	0.0274	0.06495	0.1586
Sonicated	0.2755	0.2487	0.05275	0.0936	0.1993

Assessment of dry biomass in culturing media variants for efficient beta-carotene extraction using Hexane: Ethanol (1:2)

GE Dry Biomass					
in BG11	0.77015	0.69525	0.01975	0.06942	0.05788
in BG11 + Glucose	0.19216	0.16523	0.00529	0.0177	0.1269
in RO water	1.3068	1.1621	0.0894	0.0901	0.1783

LIST OF PUBLICATIONS

1. Anand Raksha, Mohan Lalit, and Bharadvaja Navneeta (2022). Disease prevention and treatment using β-carotene: the ultimate pro-vitamin A. Revista Brasileira de farmacognosia. (Accepted)
2. Anand Raksha and Bharadvaja Navneeta (2022). Fungi: The Assorted Bio-Remediators ECS Trans. 107 13903. https://doi.org/10.1149/10701.13903ecst

Gmail lalit mohan <lalitmohan2405@gmail.com>

Fwd: RBFA-D-22-00183R1 - accepted - [EMID:fddc830ed5fab6b1]
1 message

Navneeta Bharadvaja <navneetab@dce.ac.in> 4 May 2022 at 06:39
To: Raksha Anand <r27anand@gmail.com>, lalit mohan <lalitmohan2405@gmail.com>

---------- Forwarded message ----------
From: **Revista Brasileira de Farmacognosia - Editorial Office** <em@editorialmanager.com>
Date: Wednesday, May 4, 2022
Subject: RBFA-D-22-00183R1 - accepted - [EMID:fddc830ed5fab6b1]
To: Navneeta Bharadvaja <navneetab@dce.ac.in>

CC: "Raksha Anand" r27anand@gmail.com "Lalit Mohan" lalitmohan2405@gmail.com

Dear Dr. Bharadvaja,

We are pleased to inform you that your submission Disease prevention and treatment using β-Carotene: The ultimate Provitamin A has been accepted for publication in
Revista Brasileira de Farmacognosia

---------- Forwarded message ----------
From: <ecs@confex.com>
Date: Monday, January 31, 2022
Subject: ECS Transactions: Manuscript #ICTSGS-2047 Decision Letter
To: navneetab@dce.ac.in

Dear Dr. Navneeta Bharadvaja,

I am pleased to inform you that your manuscript, "Fungi: The Assorted Bio-Remediators", has been reviewed and accepted for publication in the issue of "ECS Transactions" (ECST) from the First International Conference on Technologies for Smart Green Connected Society 2021. This issue is scheduled to be published in March 2022.

Authors whose papers will be published in ECST are also urged to submit their papers to one of the Society's peer-reviewed journals: the Journal of The Electrochemical Society (JES) or the ECS Journal of Solid State Science and Technology (JSS). While the expectation is that six months is sufficient time to revise an ECST paper to meet the stricter standards of the journals, there is no deadline for submission. Submissions to the journals must be made using the online submission system. Click here for author instructions: http://ecsdl.org/site/ecs/manuscript_submissions.xhtml.

Thank you for contributing your work to ECST. If you have any questions or comments, please feel free to contact the ECST staff at ecst@electrochem.org.

Sincerely,

Prof. Sai Kiran Kiran Oruganti
Editor, First International Conference on Technologies for Smart Green Connected Society 2021
"ECS Transactions" Volume 106

INTERNATIONAL CONFERENCE ON TECHNOLOGIES FOR SMART GREEN CONNECTED SOCIETIES 2021

NOVEMBER 29-30, 2021|ONLINE| WORLDWIDE

RAKSHA ANAND

DELHI TECHNOLOGICAL UNIVERSITY

Presented a paper titled **Fungi: The assorted bio-remediators**
at the ICTSGS-1 conference led by Yamagata University Japan.
ICTSGS-1 IS ORGANIZED BY SPAST FOUNDATION AND ASSOCIATED PARTNER INSTITUTIONS.

SPAST FOUNDATION

Hidemitsu Furukawa,
YAMAGATA UNIVERSITY, JAPAN

山形大学 Yamagata University | SUSTAINABLE DEVELOPMENT GOALS
山形大学は持続可能な開発目標（SDGs）を支援しています

Ajit Khosla,
YAMAGATA UNIVERSITY, JAPAN

ACS Publications
Most Trusted. Most Cited. Most Read.

The Electrochemical Society
Advancing solid state & electrochemical science & technology

Fungi: The Assorted Bio-Remediators

ECS Transactions, 107 (1) 13903-13916 (2022)
10.1149/10701.13903ecst ©The Electrochemical Society

Fungi: The Assorted Bio-Remediator

Raksha Anand[a], and Navneeta Bharadvaja[a*]

[a] Plant Biotechnology Laboratory, Department of Biotechnology, Delhi Technological University, Shahbad Daulatpur, Main Bawana Road, Delhi, 110042

[*]Email ID: navneetab@dce.ac.in

The most widely used eukaryotic model organism fungi, with its simple yet specialized organismal structure, possess the biochemical ability to degrade pollutants, therefore being of economic importance. Its bioremediation capacity is attributed to either its chemical modification mechanisms or influential bioavailability, with effects of degradation on metals, metalloids as well as highly toxic radionuclides. Fungi form mycelial network extensions, their catabolic enzymes having low specificity, and can hence independently use pollutants as a substrate for growth. These features make fungi best suited for the process of bioremediation. However, fungi have been under-exploited for environmental bioremediation in spite of them being present as dominating creatures in the soil-living biomass and abundance in aqueous systems. Yeasts are very efficient in degrading wastes and can be easily engineered for targeted features. In this review, we will briefly discuss some of the potential "mycoremediation" techniques.

Introduction

The exponential increase in pollution associated with its harmful repercussions, attributed to the accelerated industrialization along with urbanization has been a matter of great concern. It's not only the air that has been polluted, but these contaminants have found their way into the soil and water as well, each type being polluted and plying its role in threatening the human health and native ecosystem(1). There have been research approaches carried out for discovering sustainable and affordable remediation methods and technology development (2). However, along with the development of cost-effective remediation techniques, the malpractices of untreated effluent release into the open as a general practice must be checked.

The current scenario of pollution with the rising pace has several adverse effects already like decreased domestic GDP, water-stress on population, crippling ecosystem, food security, biodiversity (3) and so on as declared by renowned bodies like WHO (4). The conventional methods of effluent treatment and removing contamination are quite effective, however, the expenses, toxic by-product formation and inefficiency on low concentrations of the highly toxic chemicals call for better in-situ process developments for remediation of such typical pollutants (5).

The involvement of microbes, be it in the form of biofilms or fungal engagement called myco-remediation, has been a major area of interest for researchers. Being an environment friendly, economical, and effective, the fungal use of remediation has been a majorly exploited strategy. There are various characteristics of fungi that have proved to be useful for application, like a high surface area to volume ratio, hyphal network, robust growth and proliferation, extracellular enzyme production capability like ligninolytes, fluctuating physicochemical conditions like temperature and pH adaptability, heavy metal resistance and so on (6–8). Along with their application in bioreactors (9), fungi can also be used for *in-situ* remediation from pharmaceutical drugs, dyes, and herbicidal chemicals. Studies have shown that the degradation of pollutants can be accelerated by regulated metabolite use along with the controlled biomass of fungi (10,11).

Although bioremediation has a very broad range, considering the variety, availability and metabolic potential of microbes has restricted it. Bacteria have been found as the most versatile and effective of all the micro-organisms attributing to a majority of the outcomes, only needing a pre-exposure to the pollutant for the stimulation of enzyme expression. Here, the concentration of pollutants acts as the limiting factor to the enzyme expression (12). Fungi potentially can control phytoremediation as they have the ability to increase the plants' uptake ability of heavy metals from the environment (13–16). The fungi have been found to degrade high molecular weighing PAHs and many other organic compounds when studies in the laboratory were made (17).

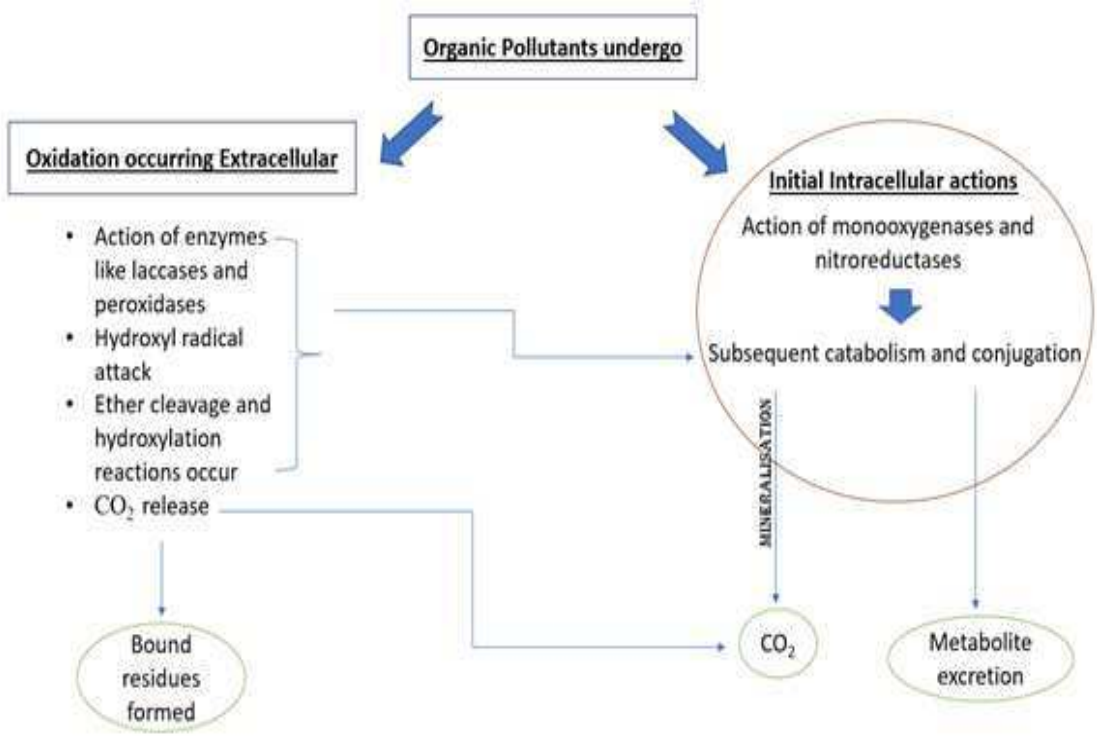


Figure 1: Overview of general mechanism on organic pollutants by fungi.

There is extensive growth of spatially evolved filamentous fungi to form thread-like hyphae. It maintains a highly polarized cellular organization internally, which

supports apical growth (18,19). Despite the diameters of these hyphae being microscopic (about 2–10 μm), the organisms belonging to the Kingdom Fungi have network extensions to over hectares (20). So, supported by this observation, fungi can also be regarded as "macro-organisms packaged in microscopic units" (21); devoted to the exhibition of unique lifestyles adapted to heterogeneous environments. The general work of fungi is to produce exoenzymes that supposedly digest organic matter to provide nutrition to the growing hyphae. These enzymes have a quite low specificity which makes their substrate compatible with varieties like AAs and their derivatives (22–24). These enzymes are called the XMEs (18).

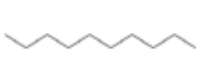
This review will consist of different fungi used specifically for remediation of pollutants, how the process can be accelerated and what is the mechanism for the degradation. There are also certain limitations to the current status and emphasis would be given to put them up. Fungi are used to degrade several pollutants ranging from simple to very harmful ones like Polycyclic Aromatic Hydrocarbons (PAHs), anti-fungal drugs, detergents, heavy metals and so on (3)

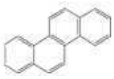
The Class of Pollutants

The Total Petroleum Hydrocarbons (TPH) have major components as nitrogen-sulfur-oxygen containing compounds, the alkanes, asphaltenes and the aromatic compounds consisting of the benzene rings along with the fusions and PAHs rings. These are the major pollutants having carcinogenic, toxic, and mutagenic properties causing environmental recalcitrance (25). The toxic metal contamination in soil with metals like As, Pb, Hg, Zn and so on which comes from the extraction of petroleum and refining (26). The quality of pollutants ranges from inorganic to organic leading to co-contamination, and these need to be treated differently (26). The microorganism that biodegrades petroleum has been found to have a negative growth effect by the co-contamination of toxic metals in the soil (27). The growth, metabolism as well as sulfur-nitrogen conversion has all been found altered by the toxic metals' presence (28,29).

Depending on the concentration and speciation of metal, the contamination can exhibit toxic effects, either intrinsic or extrinsic, on the organism's ability (30–32). The metal toxicity ranges from enzyme inhibition, cell and organellar membrane disruption, altered homeostasis to cell response mechanism and essential metals substitution or displacement (29). The hydrophobic petroleum hydrocarbons reduce toxic metal's bioavailability to the bioremediating system by attaching it to the soil matrix (33). The bacterial system has been much tested for its efficacy however, the fungal bioremediation capacity of soil contaminants like toxic metals and petroleum hydrocarbons needs to be exploited.

Table 1. Different fungal species involved in the degradation of Petroleum hydrocarbons.

Species	Hydrocarbons	Formula	Structure	Efficiency Of Removal	Reference
Penicillium sp.	Decane	$C_{10}H_{22}$		49.0%	(24)

Aspergillus sp.	N-hexadecane	$C_{16}H_{34}$		86.3%	(34)
Pleurotus ostreatus	Anthracene	$C_{14}H_{10}$		60.0%	(35)
Irpex lacteus	Anthracene	$C_{14}H_{10}$		56.0%	(35)
Polyporus sp.	Chrysene	$C_{18}H_{12}$		65.0%	(36)

The Remediator

Fungi are ubiquitous organisms, chemo-organo-heterotrophic in nature (37) which have independently evolved as multicellular eukaryotes (38). The fungal mycelia colonize the soil, enmeshing and forming soil aggregates. This facilitates contaminant bioavailability improving the soil structure (18) performing better when compared to the bacterial mode of action. Thus, filamentous bacteria show effective results in contrast to bacteria in the matter of translocation or transport of essential nutrients and the pollutant itself to a significant distance, which is an advantage of filamentous fungi (18,39–41)(41,42) There have been studies stating that the mycelia of fungi are the facilitator "highways" in soil over a distance for the bioremediation through pollutant degrading bacteria (43–45).

Table 2. Major enzymes active in fungal remediation

Enzyme (Commission number)	Taxa of Fungi	Occurrence and localization	Mechanism of Reaction	Remark	References
Laccases (EC 1.10.3.2)	Basidiomycota and Ascomycota	Extra-cellular	• Organic compounds undergoing O_2 dependent one e^- oxidation	• Direct oxidation of pollutants • Mostly active in acidic pH and rarely works in neutral or basic environment	(46,47)
Tyrosinases (EC 1.14.18.1)	Mucoromycotina, Basidiomycota and Ascomycota	Mostly intra-cellular but sometimes even extra-cellular	• cresolase activity (O_2 dependent monophenol's hydroxylation) producing *o*-diphenols • catecholase activity (Oxidation of the above produced *o*-diphenols) producing catechols	• pH independent - activity in alkaline as well as acidic pH • Oxidation of various phenols – even the chlorinated ones.	(48,49)
Lignin peroxidases (EC 1.11.1.14)	Basidiomycota	Extra-cellular	• Hydrogen peroxide-dependent; one-	• Rapid inactivation during phenol oxidation	(50,51)

			e^- oxidation of aromatic compounds	• Direct oxidation of high redox potential aromatics • Favourable acidic pH • Extended range of substrates, all from dyes and phenols	
Dye-deoxidising peroxidases (EC 1.11.1.x)	Basidiomycota	Extra-cellular	• H_2O_2-dependent one-electron oxidation of organic compounds • Additional hydrolysing activity	• Works in acidic pH • Stable at high temperature and pressure. • Oxidation of dyes like anthraquinone with high redox potential, which are not oxidised by usual peroxidases	(50)
Nitroreductases	Ascomycota, Mucoromycotina and Basidiomycota	Cell-bound	• NAD(P)H-dependent reduction nitroaromatics → hydroxylamine and amino(nitro) compounds • Similarly, reduction of N-groups of heterocycles	• Common in fungi • Reduces TNT	(52–54)
Reductive dehalogenases	Basidiomycota and Ascomycota perhaps (PCDD degradation)	Cell-bound	• The membrane-bound glutathione S-transferase produces glutathionyl. • It conjugates with concomitant chlorine removal. • This soluble conjugate reductase releases dechlorinated compounds	• Two-component system • Probable for reductive dechlorination of chlorocatechols • Reductive dechlorination of cholorohydroquinones from herbicides by Basidiomycota	(55,56)
Miscellaneous transferases	Ascomycota, Mucoromycotina and Basidiomycota	Cell-bound	• Glucoside or Xyloside formation • Sulphate or methyl conjugation from hydroxylated compounds	• Phase II enzymes prominently metabolise PAH • Also acts on other pollutants • Common in fungi	(57)

Depending on the physiological or genetic adaptation, intrinsic structural or biochemical property, morphological changes, and many other parameters like bioavailability of metals, speciation and toxicity, the Fungi have developed survival and growth capacity (30,58,59). There has been recognition of species that have been found

to have action on a particular class of pollutants. The filamentous kind of fungi belonging to *Aspergillus* and *Penicillium* spp. degrade aliphatic, poly-cyclic aromatic hydrocarbons; and chlorophenols, the organic components serving as energy sources (18,60,61). The *Neurospora crassa,* are ureolytic fungi which have the ability of immobilizing metals as these when incubated in media with urea supplements, carbonates and/or oxides of toxic metals are precipitated (62–66). When this urea-supplemented media was mixed with heavy oil and ions like Ca^{2+}, there was aggregated precipitate of minerals towards the heavy oil edge, that might be an additional acting source of energy as well as carbon, during the process of biomineralization. The fungi have the ability to primarily decompose the plant biomass and organic matter present in the soil. Their chemical structure wood's lignin polymers can be compared with Polycyclic Aromatic Hydrocarbons (PAHs) aromatic structures (67,68). Thus, fungi like *Phanerochaete chrysosporium* which are lignin-degrading organisms that can attack a wider range of organisms were investigated for PAHs and other similar compounds like aromatic hydrocarbon degradation (69).

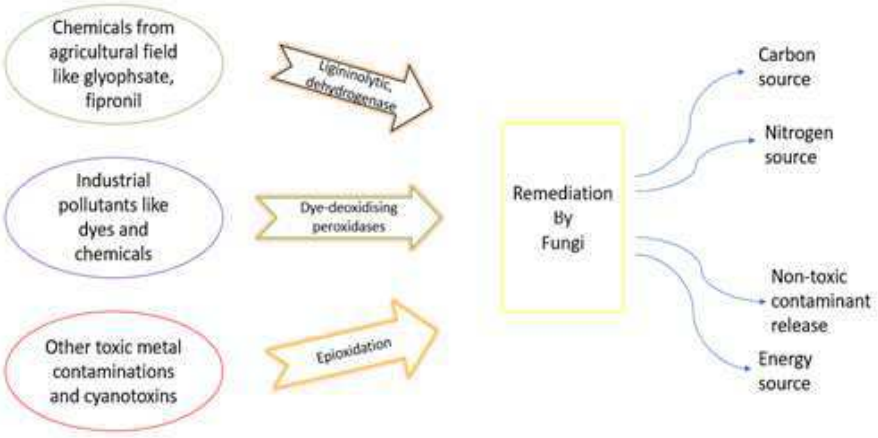


Figure 2: Kinds of mycoremediation

Some fungi can convert highly carcinogenic benzo(α)pyrene, which are high molecular-mass PAHs, by the non-specific mechanism of detoxification into products water-soluble in nature (18). Fungi obtained from soils of petrol stations, like *Fusarium solani* and *Hypocrea lixii*, have the ability to degrade pyrene up to 60% and show tolerance to metals, copper and zinc by accumulation (70).

The Iron (Fe (III)) co-ordinating fungal siderophores in co-contaminated soil not only binding to Fe (III) but also to other metals like Pb, Ni, Zn, Th (IV), Cu, Cd, U(IV), and Pu (IV) (71). They also facilitate petroleum hydrocarbons biodegradation by providing Fe to the degrading microorganisms in habitats having Fe-limitations. Sometimes, the co-contaminating PAHs interact with lipophilic components of the fungal cell membrane which alters its permeability. This results in toxic metal penetrating inside the cells, leading to remolded cellular functioning. Shen et al. 2005 investigated the effects on fungal growth imposed by Cd and phenanthrene (Phe) (72). Certain fungi have

shown strongly inhibited growth in Cd and Phe containing soil when compared with soil containing only Cd.

Factors Affecting Fungal Bioremediation Efficiency

Reduced bioavailability, heavy metal toxicity, soil characteristics, present metabolites and enzymes all play a role when myco-remediation is taken into account. Other physicochemical factors include pH, temperature, and mineral content of the soil, metal speciation and so on which ultimately hamper the transportation of pollutants and bioavailability (32,73).

Soil Characteristics

The solution-phase metal-ions concentration is significantly reduced by clay minerals and organic matter. As reported, the mineral-dominated soil with 0.01-mg L^{-1} Cd^{2+} inhibits trichloroaniline (TCA) dichlorination, while about 0.2-mg L^{-1} of Cd^{2+} necessarily from an organic-dominated soil correlates with organic material metal-binding capacity (74). The high cation exchange capacities (CECs) of clay minerals like montmorillonite efficiently reduces metal bioavailability and toxicity (29). Also, the pre-existing metal of the soil reacts with organic pollutants, affecting its speciation, bioavailability as well as toxicity (75).

Another crucial factor is pH, that determines hydrocarbons of petroleum biodegradation along with toxic metal biotransformation. Any change in pH alters fungal as well as bacterial community structure, also manipulating enzyme activities and metal speciation. Simulations of such altered pH effects on metal ion speciation can be done using software like Geochemists' workbench (GWB) (76). These are geo-chemical modelling software, more examples like MINEQL+ (77,78), and PHREEQC (79,80).

Co-contaminated soil bioremediation is also influenced by temperature as it affects the pollutant's chemistry along with fungal biodiversity (32). At a lower temperature, there is increased viscosity of petroleum, with reduced volatility resulting in retarded biodegradation. Generally, the degradation rates for hydrocarbon pollutants are highest around temperatures of 30–40 °C in the soil environment (81). When the temperature is high, the PAHs' solubility increases along with metal ion toxicity. This improves the bioavailability; however, such high temperatures are influential to the structure of the microbial community as well as its activity. It was evidently confirmed upon the comparison of the effects of temperatures between 20 °C and 40 °C for metal removal, the optimum temperature for *Beauveria bassiana* was found to be 30°C which occurred due to an increase in biomass production that ultimately provided more sites for metal-binding (82).

Metabolites and Enzymes

A variety of excreted substances and extracellular metabolites have several upshots that transform interactions between fungi and different pollutants in the environment (69,83). By secreting enzymes like laccases and tyrosinases, fungi degrade

petroleum hydrocarbons, and metal speciation is managed by a variety of other excreted metabolites like organic acids, amino acids. Other enzymes secreted by fungi are reductive dehalogenases, manganese peroxidases and cytochrome P450 monooxygenases, whereas extrinsically secreted metabolites are siderophores, extracellular proteins and so on (84). The degradation of Petroleum Hydro-Carbons (PHCs) is a sequential process involving diverse enzymes and depends on several factors like the chemical structure otherwise imparting stability to the pollutant, along with the fungal growth in the polluted medium.

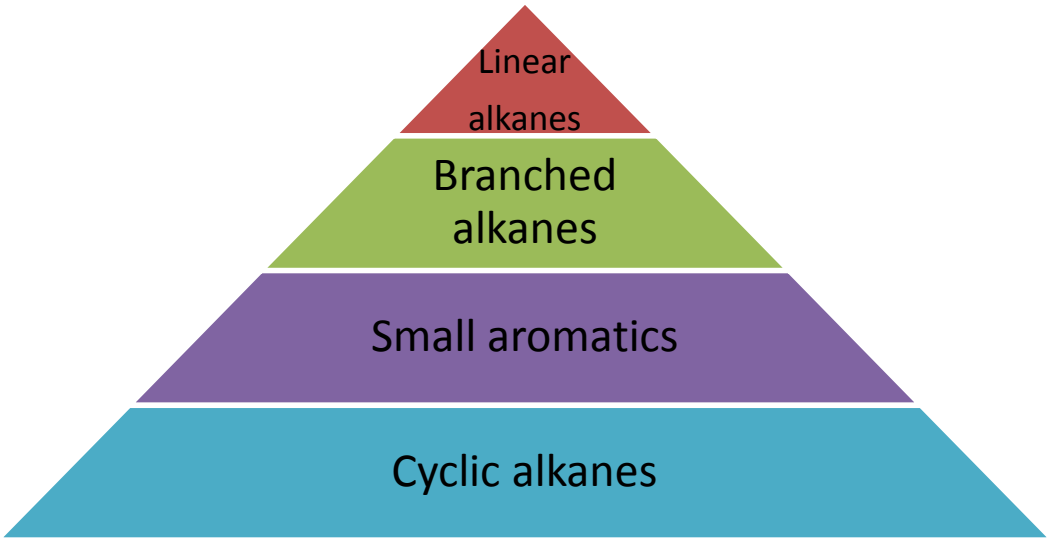


Figure 3: The bio-degradation efficiency for varied classes of pollutants in different and can be ranked as shown in the figure; tip resembling the easily degraded and the base showing the more stable pollutant category.

The PHCs of co-contaminated soil has been found to be the C- and energy source for certain species of fungi. Apart from this, toxic metals also exert significant effects on fungal activity. In spite of the metal species toxicity potential, there are numerous fungi species that can flourish with composition shift, in contaminated conditions (31).

The fungal mechanisms that lead to tolerance and resistance are the actual reason behind the survival of these species in a toxic environment (31,54). The toxic metal mobility is influenced by various properties possessed by fungi, along with mechanisms like production of proteins with metal affinity, precipitation of organics and inorganics, transport, and compartmentalization (30,31). The transformation of minerals and metals is highly dependent on fungal secretions. This play role in the mobilization as well as immobilization of metal species (31,86). In addition to this, the melanin and the phenolic polymers from fungi have the potential to bind metals with O-containing groups (87). Formation of the complex by fungal surface can be linked to the metal ion coordination, releasing proton, with oxygen donor atoms (88).

Metal immobilization is relevant particularly to approaches in bioremediation. The fungi mediate metal precipitation by forming insoluble oxalates, oxides, phosphates,

and carbonates (86,89–91). As an example, phosphate is liberated from the hydrolysis of organic or inorganic phosphate, which is an efficient metal immobilization method for metals like Zn, Pb, La, and U. The metal precipitates were found over and around the hyphae (92,93). *Neurospora crassa*, *Pestalotiopsis sp*., and other Urease-positive fungi like *Myrothecium gramineum* promisingly immobilize toxic metals through their involved mechanism of urea degradation.

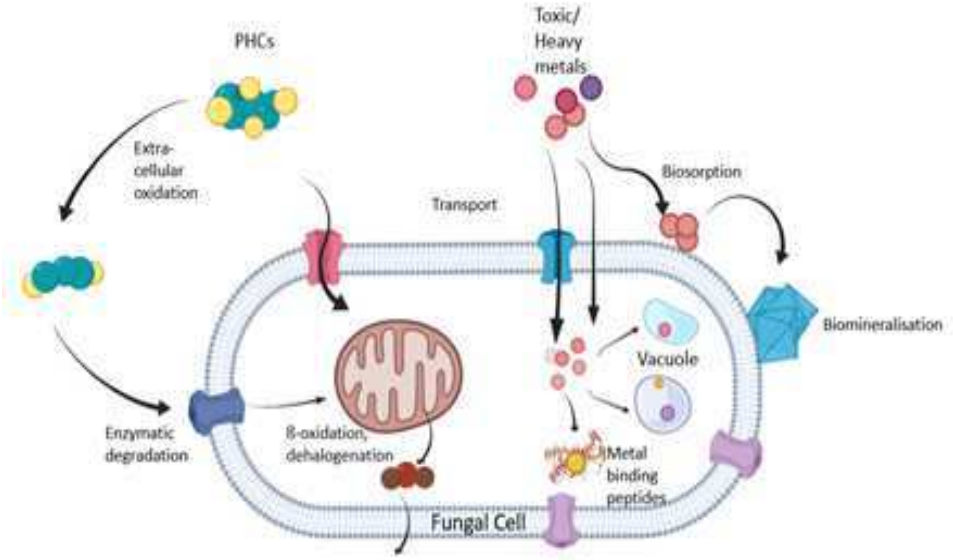


Figure 4: Fungal interaction with petroleum hydrocarbons (PHCs) schematic diagram.

The cell membranes of fungi are permeable not only to PHCs, but also many other simple organic compounds produced upon oxidation by extracellular enzymes. Further, they undergo a range of metabolisms including hydrolysis, removal of the halogen group, as well as β-oxidation. Later, they enter into the citric acid cycle. As per their fate, these heavy/toxic metals can either undergo (i) biosorption by accumulation on cell surface; which result in the nucleation of biomineral followed by its precipitation; or (ii) intracellular accumulation of other minerals followed by their localization into organelles, either due to active transport or cross-membrane diffusion mechanism (84,85).

Fungi can actively hydrolyze urea from the medium and produce ammonia along with free carbonate. These carbonate ions responsibly precipitate metals as carbonates, like $BaCO_3$, $CoCO_3$, $Cu_2(OH)_2CO_3$, $La_2(CO_3)_3$, and $CdCO_3$, $NiCO_3$ (64,76,90,94). A variety of oxalates of metals like Cd, Co, Cu, Mg, Sr can be produced by the interaction of fungi with metals and their minerals (86). Other important factors in the immobilization of toxic metals in played by a variety of extracellular proteins, amino acids, and polysaccharides. The removal of nickel present extracellularly in the form of precipitate was associated with extracellular protein removal (76). Demonstrations have shown that the templates for mineral formation can be extracellular protein, which also influences the resultant biominerals' size (63,76).

Conclusion and Future perspectives

Currently, the petroleum hydrocarbon and toxic metals contaminated soil's bioremediation, referred to as co-contaminated soil, is a mostly step-wise treatment involving removal followed by detoxification. There is an efficiency of degradation for both single and composite kinds of pollutants. There has been very little research on the fungal metabolism as a response to pollutant complex and its stress. The contrasting fungal metabolism, simulated synthesis of enzymes upon induction, extracellular release of metabolites reflects the fungal responses to organic pollutant from petroleum. This also affects the transformation of toxic metals and their migration (84).

This convoluted situation due to the effect of variables, and limitations put up by the unavailability of suitable analytical techniques is a major target for the upcoming researchers. Toxic metals are spatially distributed in the soil, accompanied by various fungi-mediated migration and transformation mechanisms depending on the methods of sampling and analysis adopted. The main bottleneck limiting the understanding of soil heterogeneity is the sampling technology taken up for vertical and horizontal dimensions (84).

The conventional techniques of sample collection and handling are also similar however there have been deviations found in the samples collected that might be the result of different reasons like contamination while sample picking, changed environmental conditions like pH, temperature, humidity, dissolved oxygen and so on. So, new technologies like microbial proteomics and metabolomics (95) must be applied in the field of study to overcome such issues. This will lead to the discovery of fungal responses to pollutants on a molecular level (96,97).

Several other new methods for getting a high-resolution toxic metal characteristic of spatial distribution, Gradient Diffusion Film Technology (DGT) which is performed in combination with LA-ICP-MS. This also reveals reaction processes interface for soil-metals-fungal interactions and can be applied for the analysis of vigorous alterations in the physicochemical state of the toxic metal at the interfaces. Hence, contributions to developing new strategies for fungi-assisted bioremediation of toxic-co-contaminated soil must be made on an interdisciplinary level.

Acknowledgments

The authors would like to thank the Department of Biotechnology, Delhi Technological University for their constant support.

References

1. M. A. I. Khan, B. Biswas, E. Smith, R. Naidu, and M. Megharaj, *Chemosphere*, **212**, 755–767 (2018).
2. Z. Y. Dong, W. H. Huang, D. F. Xing, and H. F. Zhang, *Journal of Hazardous Materials*, **260**, 399–408 (2013).
3. N. Akhtar and M. A. ul Mannan, *Biotechnology Reports*, **26**, e00452 (2020).

4. https://www.who.int/news-room/fact-sheets/detail/drinking-water.
5. I. Khan et al., *Environmental Monitoring and Assessment*, **191** (2019).
6. M. Singh et al., *Journal of Applied Microbiology*, **119**, 1278–1290 (2015).
7. M. Kapahi and S. Sachdeva, *Bioresources and Bioprocessing*, **4** (2017).
8. S. Bhattacharya, A. Das, G. Mangai, K. Vignesh, and J. Sangeetha, *Brazilian Journal of Microbiology*, **42**, 1526–1536 (2011).
9. M. S. Aragão et al., *Chemosphere*, **244**, 125432 (2020) https://doi.org/10.1016/j.chemosphere.2019.125432.
10. S. Rodríguez Couto, A. Rodríguez, R. R. M. Paterson, N. Lima, and J. A. Teixeira, *Letters in Applied Microbiology*, **42**, 612–616 (2006).
11. M. Tekere, *Biotechnology and Bioengineering*, 1–19 (2019).
12. C. J. Rhodes, *Chemical Speciation and Bioavailability*, **26**, 196–198 (2014).
13. J. Silber, A. Kramer, A. Labes, and D. Tasdemir, *Marine Drugs*, **14** (2016).
14. J. C. Nielsen and J. Nielsen, *Synthetic and Systems Biotechnology*, **2**, 5–12 (2017).
15. T. Degenkolb and A. Vilcinskas, *Applied Microbiology and Biotechnology*, **100**, 3813–3824 (2016).
16. Y. Yuan et al., *PLoS ONE*, **12** (2017).
17. D. Ghosal, S. Ghosh, T. K. Dutta, and Y. Ahn, *Frontiers in Microbiology*, **7** (2016).
18. N. L. Glass, C. Rasmussen, M. G. Roca, and N. D. Read, *Trends in Microbiology*, **12**, 135–141 (2004).
19. B. A. Ferguson, T. A. Dreisbach, C. G. Parks, G. M. Filip, and C. L. Schmitt, *Canadian Journal of Forest Research*, **33**, 612–623 (2003).
20. M. F. Allen, *Vadose Zone Journal*, **6**, 291–297 (2007).
21. L. R. Martins;, F. H. Lyra;, M. M. H. Rugani;, and J. A. Takahashi, *Journal of Environmental Engineering*, **142** https://ascelibrary.org/doi/pdf/10.1061/%28ASCE%29EE.1943-7870.0000998.
22. C. Zhao et al., *Environmental Science and Pollution Research*, **23**, 24846–24856 (2016) http://dx.doi.org/10.1007/s11356-016-7722-x.
23. M. Govarthanan, S. Fuzisawa, T. Hosogai, and Y. C. Chang, *RSC Advances*, **7**, 20716–20723 (2017) http://dx.doi.org/10.1039/C6RA28687A.
24. H. Harms, D. Schlosser, and L. Y. Wick, *Nature Reviews Microbiology*, **9**, 177–192 (2011) http://dx.doi.org/10.1038/nrmicro2519.
25. J. Czarny et al., *Journal of Hazardous Materials*, **383** (2020).
26. A. A. Adeniyi and J. A. Afolabi, *Environment International*, **28**, 79–82 (2002).
27. B. Biswas, B. Sarkar, A. Mandal, and R. Naidu, *Journal of Hazardous Materials*, **298**, 129–137 (2015) http://dx.doi.org/10.1016/j.jhazmat.2015.05.009.
28. S. A. M. Abd El-Azeem et al., *Environmental Earth Sciences*, **70**, 3411–3420 (2013).
29. T. R. Sandrin and R. M. Maier, *Environmental Health Perspectives*, **111**, 1093–1101 (2003).
30. G. M. Gadd, in *The Genus Aspergillus*,, p. 361–374, Springer, Boston, MA (1994) https://link.springer.com/chapter/10.1007/978-1-4899-0981-7_28.
31. G. M. Gadd, *Mycological Research*, **111**, 3–49 (2007).
32. D. E. N. Rangel, R. D. Finlay, J. E. Hallsworth, E. Dadachova, and G. M. Gadd, *Fungal Biology*, **122**, 602–612 (2018).
33. C. C. Lai, Y. C. Huang, Y. H. Wei, and J. S. Chang, *Journal of Hazardous Materials*, **167**, 609–614 (2009).
34. A. B. Al-Hawash et al., *Ecotoxicology and Environmental Safety*, **164**, 398–408 (2018) https://doi.org/10.1016/j.ecoenv.2018.08.049.
35. T. Drevinskas et al., *Analytical and Bioanalytical Chemistry*, **408**, 1043–1053 (2016).

36. T. Hadibarata, S. Tachibana, and K. Itoh, *Journal of Hazardous Materials*, **164**, 911–917 (2009).
37. G. M. Gadd, *Nature Microbiology*, **2**, 1–9 (2017) http://dx.doi.org/10.1038/nmicrobiol.2016.275.
38. J. E. Stajich et al., *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 11889–11894 (2010).
39. G. P. Boswell, H. Jacobs, F. A. Davidson, G. M. Gadd, and K. Ritz, *Bulletin of Mathematical Biology*, **65**, 447–477 (2003).
40. S. Furuno et al., *Environmental Science and Technology*, **46**, 5463–5470 (2012).
41. H. Jacobs, G. P. Boswell, K. Ritz, F. A. Davidson, and G. M. Gadd, *FEMS Microbiology Ecology*, **40**, 65–71 (2002).
42. A. Worrich, L. Y. Wick, and T. Banitz, *Advances in Applied Microbiology*, **104**, 93–133 (2018).
43. T. Banitz et al., *Environmental Microbiology Reports*, **5**, 211–218 (2013).
44. S. Kohlmeier et al., *Environmental Science and Technology*, **39**, 4640–4646 (2005).
45. L. Y. Wick et al., *Environmental Science and Technology*, **41**, 500–505 (2007).
46. K. S. Hofmockel, D. R. Zak, and C. B. Blackwood, *Ecosystems (New York, N.y.)*, **10**, 215–242 (2007).
47. J. A. Majeau, S. K. Brar, and R. D. Tyagi, *Bioresource Technology*, **101**, 2331–2350 (2010).
48. S. Halaouli, M. Asther, J. C. Sigoillot, M. Hamdi, and A. Lomascolo, *Journal of Applied Microbiology*, **100**, 219–232 (2006).
49. R. Ullrich and M. Hofrichter, *Cellular and Molecular Life Sciences*, **64**, 271–293 (2007).
50. M. Hofrichter, R. Ullrich, M. J. Pecyna, C. Liers, and T. Lundell, *Applied Microbiology and Biotechnology*, **87**, 871–897 (2010).
51. F. J. Ruiz-Dueñas et al., *Journal of Experimental Botany*, **60**, 441–452 (2009).
52. A. Esteve-nu and J. L. Ramos, **65**, 335–352 (2001).
53. F. H. Crocker, K. J. Indest, and H. L. Fredrickson, *Applied Microbiology and Biotechnology*, **73**, 274–290 (2006).
54. B. Octahydro- and D. Fournier, **38**, 4130–4133 (2004).
55. K. Nakamiya et al., *FEMS Microbiology Letters*, **248**, 17–22 (2005).
56. G. V. B. Reddy and M. H. Gold, *Biochemical and Biophysical Research Communications*, **257**, 901–905 (1999).
57. K. Hundt et al., *Applied and Environmental Microbiology*, **66**, 4157–4160 (2000).
58. G. M. Gadd, *Microbiology*, **156**, 609–643 (2010).
59. T. S. Sullivan and G. M. Gadd, thesis, https://discovery.dundee.ac.uk/en/publications/metal-bioavailability-and-the-soil-microbiome.
60. M. Hofrichter, F. Bublitz, and W. Fritsche, *Journal of Basic Microbiology*, **34**, 163–172 (1994).
61. C. Pinedo-Rivilla, J. Aleu, and I. Collado, *Current Organic Chemistry*, **13**, 1194–1214 (2009).
62. Q. Li, L. Csetenyi, G. I. Paton, and G. M. Gadd, *Environmental Microbiology*, **17**, 3082–3097 (2015).
63. Q. Li and G. M. Gadd, *Applied Microbiology and Biotechnology*, **101**, 7397–7407 (2017).
64. Q. Li and G. M. Gadd, *Microbial Biotechnology*, **10**, 1131–1136 (2017).

65. Q. Li, L. Csetenyi, and G. M. Gadd, *Environmental Science and Technology*, **48**, 14409–14416 (2014).
66. Q. Li, D. Liu, Z. Jia, L. Csetenyi, and G. M. Gadd, *Current Biology*, **26**, 950–955 (2016) http://dx.doi.org/10.1016/j.cub.2016.01.068.
67. A. K. Haritash and C. P. Kaushik, *Journal of Hazardous Materials*, **169**, 1–15 (2009).
68. R. Vanholme, B. Demedts, K. Morreel, J. Ralph, and W. Boerjan, *Plant Physiology*, **153**, 895–905 (2010).
69. G. M. Gadd, *Mycologist*, **18**, 60–70 (2004).
70. J. W. Hong, J. Y. Park, and G. M. Gadd, *Journal of Applied Microbiology*, **108**, 2030–2040 (2010).
71. E. Ahmed and S. J. M. Holmström, *Microbial Biotechnology*, **7**, 196–208 (2014).
72. G. Shen, L. Cao, Y. Lu, and J. Hong, *Environmental Science and Pollution Research*, **12**, 259–263 (2005).
73. S. H. Liu et al., *Bioresource Technology*, **224**, 25–33 (2017) http://dx.doi.org/10.1016/j.biortech.2016.11.095.
74. H. Zhang et al., *Journal of Hazardous Materials*, **320**, 265–277 (2016) http://dx.doi.org/10.1016/j.jhazmat.2016.07.065.
75. A. Ceci, F. Pinzari, F. Russo, A. M. Persiani, and G. M. Gadd, *Applied Microbiology and Biotechnology*, **103**, 53–68 (2019).
76. Q. Li et al., *Environmental Science: Nano*, **6**, 1866–1875 (2019).
77. B. Cloutier-Hurteau, S. Sauvé, and F. Courchesne, *Environmental Science and Technology*, **41**, 8104–8110 (2007).
78. S. Kocaoba, *Separation Science and Technology (Philadelphia)*, **55**, 896–906 (2020) https://doi.org/10.1080/01496395.2019.1579841.
79. A. Ceci et al., *Environmental Microbiology*, **17**, 2018–2034 (2015).
80. X. Liang, M. Kierans, A. Ceci, S. Hillier, and G. M. Gadd, *Environmental Microbiology*, **18**, 219–231 (2016).
81. N. Das and P. Chandran, *Biotechnology Research International*, **2011**, 1–13 (2011).
82. D. Gola et al., *Bioresource Technology*, **218**, 388–396 (2016) http://dx.doi.org/10.1016/j.biortech.2016.06.096.
83. J. Kirtzel et al., *Environmental Microbiology*, **22**, 1535–1546 (2020).
84. Q. Li, J. Liu, and G. M. Gadd, *Applied Microbiology and Biotechnology*, **104**, 8999–9008 (2020).
85. A. Guermouche M'rassi, F. Bensalah, J. Gury, and R. Duran, *Environmental Science and Pollution Research*, **22**, 15332–15346 (2015).
86. G. M. Gadd et al., *Fungal Biology Reviews*, **28**, 36–55 (2014) http://dx.doi.org/10.1016/j.fbr.2014.05.001.
87. M. Fomina and G. M. Gadd, *Bioresource Technology*, **160**, 3–14 (2014) http://dx.doi.org/10.1016/j.biortech.2013.12.102.
88. G. M. Gadd, *Journal of Chemical Technology and Biotechnology*, **84**, 13–28 (2009).
89. M. Fomina et al., *Current Biology*, **18**, 375–377 (2008).
90. X. Liang and G. M. Gadd, *Microbial Biotechnology*, **10**, 1199–1205 (2017).
91. B. Suyamud, J. Ferrier, L. Csetenyi, D. Inthorn, and G. M. Gadd, *Environmental Microbiology*, **22**, 1588–1602 (2020).
92. T. Ezawa and K. Saito, *New Phytologist*, **220**, 1116–1121 (2018).
93. X. Liang, L. Csetenyi, and G. M. Gadd, *Applied Microbiology and Biotechnology*, **100**, 5141–5151 (2016).
94. D. Rautaray, A. Ahmad, and M. Sastry, *Journal of Materials Chemistry*, **14**, 2333–2340 (2004).

95. N. Dombrowski et al., *Nature Microbiology*, **1**, 1–7 (2016) http://dx.doi.org/10.1038/nmicrobiol.2016.57.
96. S. Aydin et al., *Fungal Biology Reviews*, **31**, 61–72 (2017).
97. S. Wang, Z. Wang, L. Zhou, X. Shi, and G. Xu, *Analytical Chemistry*, **89**, 12902–12908 (2017).

PAPER NAME

RakshaAnand_Thesis.docx

WORD COUNT

10998 Words

CHARACTER COUNT

64262 Characters

PAGE COUNT

53 Pages

FILE SIZE

4.4MB

SUBMISSION DATE

May 5, 2022 11:23 AM GMT+5:30

REPORT DATE

May 5, 2022 11:26 AM GMT+5:30

10% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

- 6% Internet database
- 5% Publications database
- Crossref database
- Crossref Posted Content database
- 6% Submitted Works database

Excluded from Similarity Report

- Bibliographic material
- Cited material

10% Overall Similarity

Top sources found in the following databases:

- 6% Internet database
- Crossref database
- 6% Submitted Works database
- 5% Publications database
- Crossref Posted Content database

TOP SOURCES

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

1	**marinebiotechnology.org** Internet	1%
2	**researchgate.net** Internet	<1%
3	**jalgalbiomass.com** Internet	<1%
4	**link.springer.com** Internet	<1%
5	**mdpi.com** Internet	<1%
6	**Manipal University on 2022-03-17** Submitted works	<1%
7	**University of Strathclyde on 2021-08-25** Submitted works	<1%
8	**"Bioprospecting Algae for Nanosized Materials", Springer Science and ...** Crossref	<1%

9	Higher Education Commission Pakistan on 2018-01-16 Submitted works	<1%
10	cs.northwestern.edu Internet	<1%
11	University of Brighton on 2010-04-30 Submitted works	<1%
12	hdl.handle.net Internet	<1%
13	worldwidescience.org Internet	<1%
14	Khalifa University of Science Technology and Research on 2021-05-15 Submitted works	<1%
15	Royal Holloway and Bedford New College on 2021-04-13 Submitted works	<1%
16	A.K. Biswas, J. Sahoo, M.K. Chatli. "A simple UV-Vis spectrophotometri... Crossref	<1%
17	"Nanotechnology for Food, Agriculture, and Environment", Springer Sci... Crossref	<1%
18	Higher Education Commission Pakistan on 2014-04-30 Submitted works	<1%
19	Thomas, Roshmi, Anju Janardhanan, Rintu T. Varghese, E.V. Soniya, Jy... Crossref	<1%
20	University of Sheffield on 2019-03-04 Submitted works	<1%

21	"Carotenoids and Human Health", Springer Science and Business Medi... Crossref	<1%
22	Shete, Varsha, and Loredana Quadro. "Mammalian Metabolism of β-Ca... Crossref	<1%
23	Shixi Zhang, Junhu Dai, Quansheng Ge. "Responses of Autumn Phenol... Crossref	<1%
24	docplayer.net Internet	<1%
25	"Microbial Carotenoids", Springer Science and Business Media LLC, ... Crossref	<1%
26	Siddhardha Busi, Jobina Rajkumari. "Microbially synthesized nanoparti... Crossref	<1%
27	ia902604.us.archive.org Internet	<1%
28	Jianjun Yang. "Transformation of Lead Solid Fraction in the Rhizospher... Crossref	<1%
29	SASTRA University on 2014-07-08 Submitted works	<1%
30	The University of Manchester on 2010-05-04 Submitted works	<1%
31	University of Sheffield on 2014-11-27 Submitted works	<1%
32	nih.go.kr Internet	<1%

CANDIDATE'S DECLARATION

I, Raksha Anand, hereby certify that the work which is being presented in the research work entitled "**Bioprospecting Microalgae for β-Carotene and Synthesis of Nanoparticles for Environmental Applications - A Zero Waste Approach**" in fulfillment of the requirement for the award of Degree of Masters of Sciences in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own work, carried during 4th semester of MSc. course, under the supervision of Dr. Navneeta Bharadvaja. The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other University. The work has been published and communicated in various journals under my name with the guide.

Raksha Anand.

Place: Delhi
Date: 06/05/2022

CERTIFICATE

This is to certify that the Project dissertation titled "**Bioprospecting Microalgae for β-Carotene and Synthesis of Nanoparticles for Environmental Applications - A Zero Waste Approach**" which is submitted by Raksha Anand, 2K20/MSCBIO/24, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Sciences, is a record for the project work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Dr Navneeta Bharadvaja
In-charge, Plant Biotechnology Laboratory
Department of Biotechnology
Delhi Technological University
Delhi, India 110042

06/05/2022

Prof Pravir Kumar
Head of the Department
Department of Biotechnology
Delhi Technological University
Delhi, India 110042

ACKNOWLEDGMENT

A formal statement of acknowledgment will hardly meet the needs of justice in the matter of expression of deeply felt sincere and allegiant gratitude to all who encouraged and helped me in many ways throughout the dissertation of Master of Sciences. I would like to thank the Head of the Department, Prof Pravir Kumar for providing me with this opportunity. It is my privilege to express my profound sense of gratitude and indebtedness to my supervisor Dr. Navneeta Bharadvaja, Assistant Professor in the Department of Biotechnology, Delhi Technological University for her valuable guidance and consistent encouragement during the progress of the project work. The dissertation wouldn't be completed without her insightful suggestions and support. A special thanks to my senior, Mr. Lakhan Kumar for his constant support and immense faith in me. I have completed this project because of his guidance, inspiration, and motivation provided by him. I am equally grateful and wish to express my wholehearted thanks to the technical staff Mr. CB Singh and Mr. Jitendra K Singh, and Mr. Sandeep for their kind support. I would also like to thank other lab members Mr. Sidharth Sharma, Ms. Harshita Singh, Ms. Anuradha and Mr. Lalit Mohan, Ms. Neha Nanda, Mr. Shaubhik Anand and Mr. Vijay for their support and help in the course of my research work. I prevailed enough to experience a sustained enthusiasm and involved interest from their side. This fueled my enthusiasm even further and encouraged me to boldly step into what was totally dark and unexplored expanse for me. On a personal note, I wish to express my gratitude and affection to my family for their constant love and support.

Raksha Anand.

Raksha Anand
2K20/MSCBIO/24

turnitin

Similarity Report ID: oid:27535:16537174

PAPER NAME

RakshaAnand_Thesis.docx

WORD COUNT

10998 Words

CHARACTER COUNT

64262 Characters

PAGE COUNT

53 Pages

FILE SIZE

4.4MB

SUBMISSION DATE

May 5, 2022 11:23 AM GMT+5:30

REPORT DATE

May 5, 2022 11:26 AM GMT+5:30

10% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

- 6% Internet database
- Crossref database
- 6% Submitted Works database
- 5% Publications database
- Crossref Posted Content database

Excluded from Similarity Report

- Bibliographic material
- Cited material

Summary

21	"Carotenoids and Human Health", Springer Science and Business Medi... Crossref	<1%
22	Shete, Varsha, and Loredana Quadro. "Mammalian Metabolism of β-Ca... Crossref	<1%
23	Shixi Zhang, Junhu Dai, Quansheng Ge. "Responses of Autumn Phenol... Crossref	<1%
24	docplayer.net Internet	<1%
25	"Microbial Carotenoids", Springer Science and Business Media LLC, ... Crossref	<1%
26	Siddhardha Busi, Jobina Rajkumari. "Microbially synthesized nanoparti... Crossref	<1%
27	ia902604.us.archive.org Internet	<1%
28	Jianjun Yang. "Transformation of Lead Solid Fraction in the Rhizospher... Crossref	<1%
29	SASTRA University on 2014-07-08 Submitted works	<1%
30	The University of Manchester on 2010-05-04 Submitted works	<1%
31	University of Sheffield on 2014-11-27 Submitted works	<1%
32	nih.go.kr Internet	<1%

Raksha Anand.

Sources overview