

## Identification Isolation And Harvesting Microalgae For Biofuels And Associated Value Added Products

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### Abstract

Microalgae are renewable, sustainable, and economic sources of biofuels, bioactive medicines, and food ingredients. Several microalgal species have been studied for their potential as value-added products with remarkable pharmacological and biological properties. *Oedogonium* and *Chlamydomonas* are microalgae belonging to the class Chlorophyceae. These microalgal species have rarely been studied. This project aims to promote an important metabolite, cellulose. This accounts for 22% of the dry weight of *Oedogonium* and 20% of the dry weight of *Chlamydomonas*. A low concentration of NaOH (1.75 %) and sodium hypochlorite (4.5 %) enabled the recovery of relatively pure cellulose, which was analyzed by Fourier transform infrared (FT-IR), X-ray diffraction (XRD), thermogravimetric analysis (TGA) and antimicrobial activity. Advances in the mass production of microalgae could help replace plant cellulose with cellulose from microalgae. Once the ease of technical extraction, yield, and sourcing of uncontaminated molecules with lignin is possible, this substitution will help protect the environment. The main challenges are to increase the growth rate of microalgae and product synthesis, dehydration of the algae culture for biomass production, pre-treatment of the biomass, and optimization of the fermentation process in the production of algae biofuels. This objective describes the benefits of microalgae for biofuels, various bioactive compounds, and value-added products, and discusses cultivation parameters.

**Keywords:** Identification, Biofuel production, Cellulose extraction, value added products.

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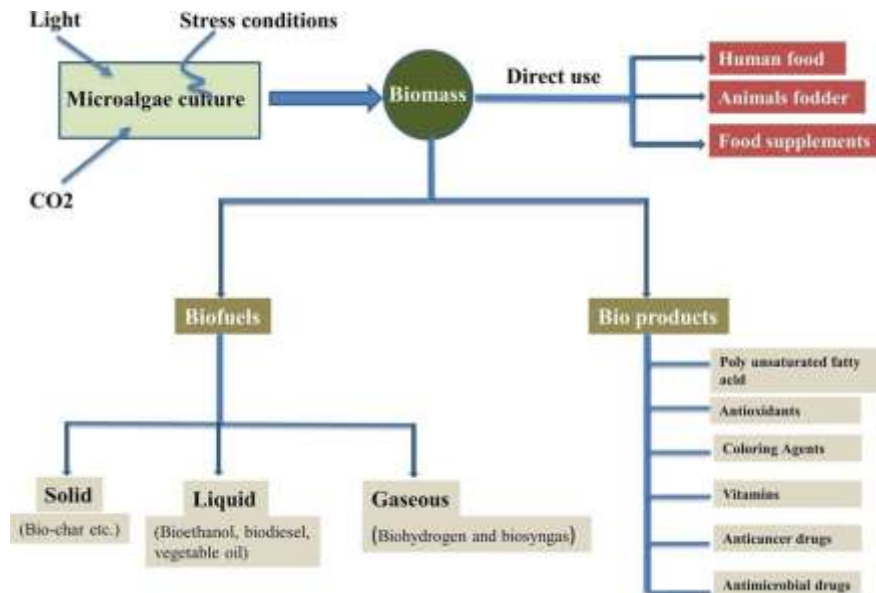
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### I. Introduction:-

Algae are roughly divided into Rhodophyta( red algae), Phaeophyta( brown algae), and Chlorophyta( green algae) and are subdivided into macroalgae and microalgae according to their size.

Microalgae can be a rich source of carbon composites that can be used in biofuels, food supplements, drugs and cosmetics. [1] They also have operations in wastewater treatment and in reducing CO<sub>2</sub> emigrations in the atmosphere. Microalgae produce a wide range of bioproducts, including polysaccharides, lipids, colors, proteins, vitamins, bioactive composites, and antioxidants.[2] Growth improvement ways and inheritable engineering can be used to ameliorate their eventuality as a unborn source of renewable bioproducts.



**Fig:1:** Microalage convert atmospheric co2 to carbohydrates , lipids, and other valuable bioproducts by using light. Microalgae biomass is a rich source for biofuels and bioactive compounds

Microalgae are fast- growing photosynthetic organisms that have the eventuality to convert 9- 10 of solar energy( average solar radiation) into biomass, with a theoretical yield of about 77g/ biomass/ m2/ day, original to about 280 tones ha/ time. [3]Algae have numerous desirable characteristics as energy directors

1. Algae are the most promising non-food source for biofuels.
2. Algae have a simple cellular structure.
3. Algae are rich in lipids( 40- 80 of dry weight) and produce 10- 259 times further lipids for biodiesel product than conventional crops grown on an area base.
4. They multiply snappily and have a high growth rate,e.g. doubling in 24 hours. They could be gathered further than formerly a time.
5. swab or wastewater could be used.
6. Carbon dioxide from the atmosphere is the carbon source for microalgae growth.
7. Algae biofuel contains no sulfur, is non-toxic, and is readily biodegradable.

virtually speaking, they are easy to grow, can grow with little or no attention, use water infelicitous for mortal consumption, and can prize nutrients painlessly.

Algal biomass contains three main factors Proteins, carbohydrates, and lipids. The chemical composition of colorful micro- and macroalgae is shown in Fig. 2. While the chance varies depending on the type of algae, adipose acids[4]( oil painting), which regard for over to 45 of the total mass, can be uprooted and

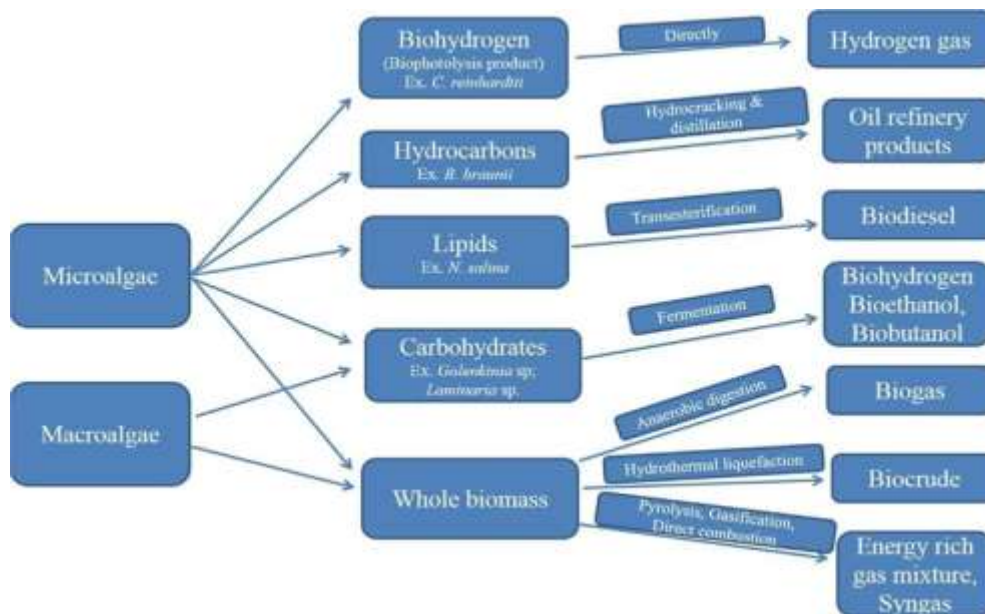


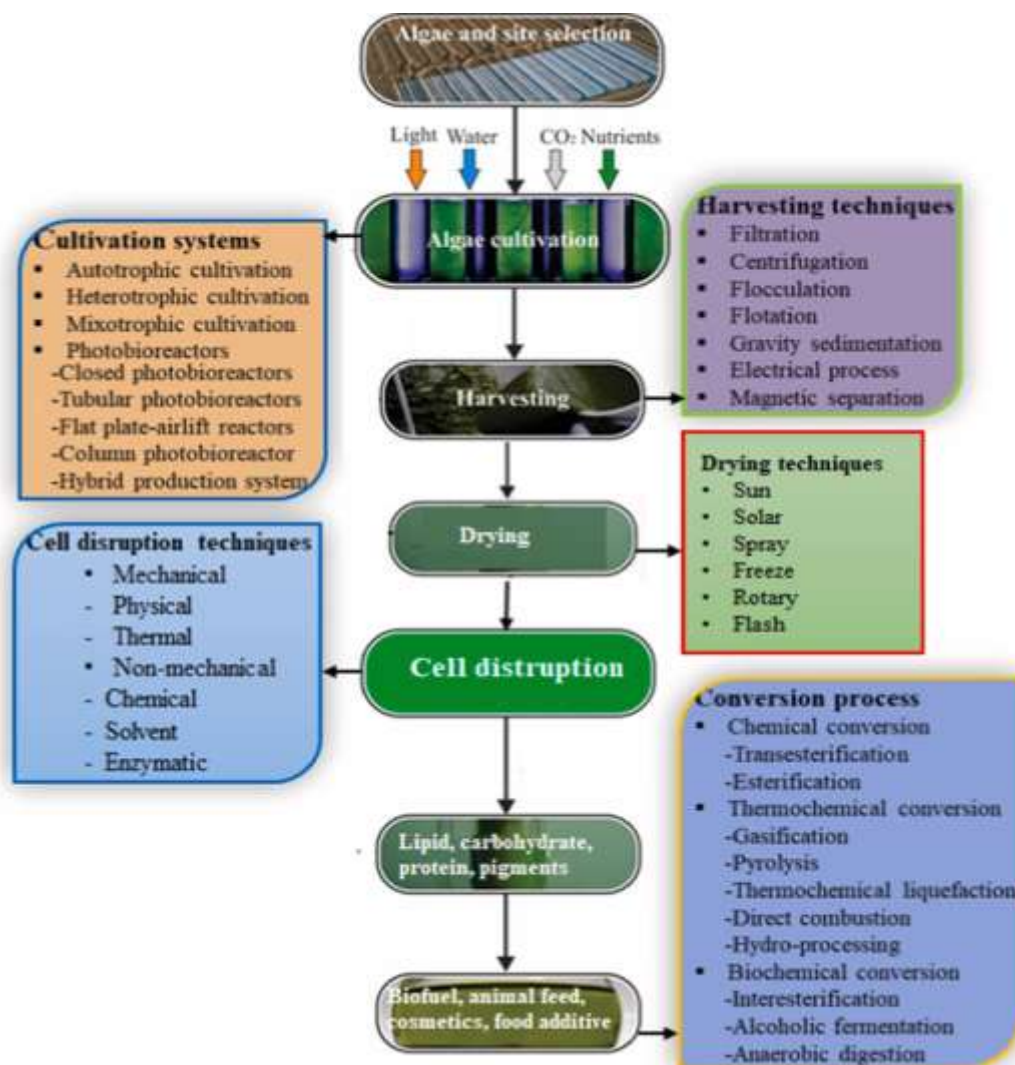
Fig. 2. Different routes of bioenergy generation from microalgae and macroalgae

Converted into biodiesel.

The microalgae species *Oedogonium* and *Chlamydomonas reinhardtii* have lately been described as belonging to two different strains.[5] To meet the global demand for cellulose( for the cloth, paper, plastics, makeup, chemical, medicinal, and cosmetics diligence) and vegetables, timbers are being cut down on a large scale and the terrain is being damaged.[6]

*Chlamydomonas* is a unicellular organism in the Chlorophyta group (the green algae). *Chlamydomonas* is certainly not a typical green algae but one could say that about any member of the group that includes filamentous forms (*Oedogonium*), sheet forming forms (*Ulva*), siphonaceous forms (*Caulerpa* and *Cladophora*), and multicellular forms (*Chara*) and even unicellular forms that are 1000 times bigger (*Acetabularia*) than *Chlamydomonas*.[7]

*Oedogonium* is a genus of filamentous, free-living green algae. It was first discovered in the fresh waters of Poland in 1860 by W. Hilse and later named by German scientist K. E. Hirn. The morphology of *Oedogonium* is unique, with an interior and exterior that function very differently from one another and change throughout its life cycle. These protists reside in freshwater ecosystems in both hemispheres and are both benthic and planktonic in nature.[6]



**Fig. 3.** Schematic of microalgae biorefinery steps along with the list of involved technologies in each step. The 'biorefinery' concept is started with microalgae cultivation, harvesting, drying, cell disruption, fractionate extraction and conversion into biofuel and products.[8]

Cellulose conflation is carried out by membrane- bound cellulose synthase confines complexes( TCs) containing cellulose synthases( CesA). In the algal sphere, still, the TCs are more versatily organized,e.g. as trophies, single or multiple row.[9]The surface of cellulose fibres are covered with hydroxyl groups allowing facile functionalization to introduce the desired functionality and produce highly effective flocculants. Hydrolysis of this native cellulose using strong acids allows isolation of crystalline sections of the fibres, a fact known since the late 1940s.[10]. The crystals resulting from this hydrolysis are nano-sized rods called cellulose nanocrystals (cncs) with size and aspect ratio dependent on the source of cellulose used for the hydrolysis as well as hydrolysis time and temperature.

Cellulose carpeted with essence nanoparticles has attracted further attention with its antimicrobial exertion, with zinc essence having stronger antimicrobial exertion. Cellulose carpeted with zinc oxide has antimicrobial exertion.[11] The mechanical parcels of cellulose determine its use. These parcels depend on the spatial structure that's formed after birth by chemical treatment.[11]

The end of this study is to prize the main parietal emulsion of Oedogonium and Chlamydomonas( cellulose) at different attention of birth reagents. The quality of the cellulose is anatomized by Fourier transfigure infrared( FT- IR) andX-ray diffraction( XRD).

## II. Material and methods:

### Material

The chemicals used are Hexane, Methanol, Sodium Chlorite, HCL, Zinc Chloride, Sodium Carbonate, Sodium Hydroxide, Nutrient Agar, Nutrients Broth. All reagents were used

without further purification.[12]

*Extraction of Biofuel from Microalgae:*

The procedure for the extraction of biofuel is as follows: After the growth of the microalgae, the dried microalgae are crushed to release the oil from the cell wall. The samples were then placed in a round bottom flask where 20 ml of hexane and 15 ml of methanol were mixed with 50 ml of sodium hydroxide and placed in the flask. After the biomass had settled, the oil extraction took place

*Extraction of Cellulose from Microalgae:*

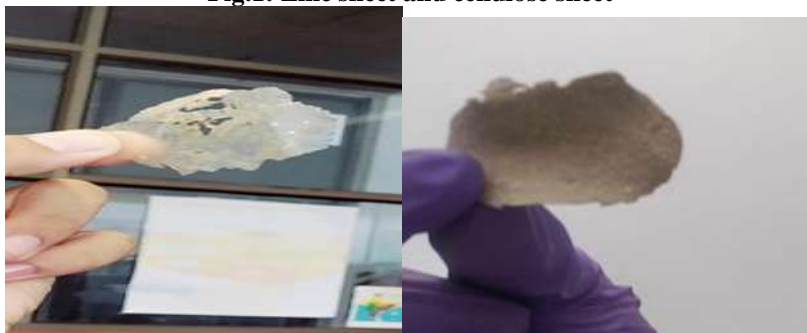
The procedure for extracting cellulose from microalgae is as follows: 30 g dried algal powder and 12 g NaClO<sub>2</sub> were mixed with 300 ml sodium acetate buffer (pH 4.9) at 60°C for 3 hours. The bleached algal mass was washed to neutrality using a centrifuge. The washed algal mass was treated with 260 ml of 0.5 M NaOH solution and kept at 60°C overnight. The resulting material was separated, washed with deionized water until neutral, and dried at room temperature. The dried product was suspended in 540 mL of 5% HCL solution overnight at room temperature.

*Production of Zinc Oxide*

0.2 g ZnCl<sub>2</sub>, 12 g Na<sub>2</sub>CO<sub>3</sub>, and 60 mL distilled water were stirred well for 45 minutes. To this 2.4 g of microalgal cellulose with 50 mL of distilled water was added. The suspension was stirred for 45 minutes and then transferred to a 100 mL Teflon-lined autoclave, which was heated to 135 °C for 14 hours and then cooled to room temperature. The liquid layer was decanted and the solid residue was washed with ethanol and hot water. The dispersion was filtered through a nylon philtre membrane (0.4 μm, Whatman) under vacuum filtration. Using a simple press, the collected mass was pressed into a flat sheet and dried overnight at 35 °C. After drying, the nylon support was removed with tweezers.

The extracted microalgal cellulose and the ZnO film applied to the algal cellulose were characterized using various analytical methods, e.g. Fourier transform infrared spectra (FT-IR), and Thermo gravimetric analysis (TGA).

**Fig.1: Zinc sheet and cellulose sheet**

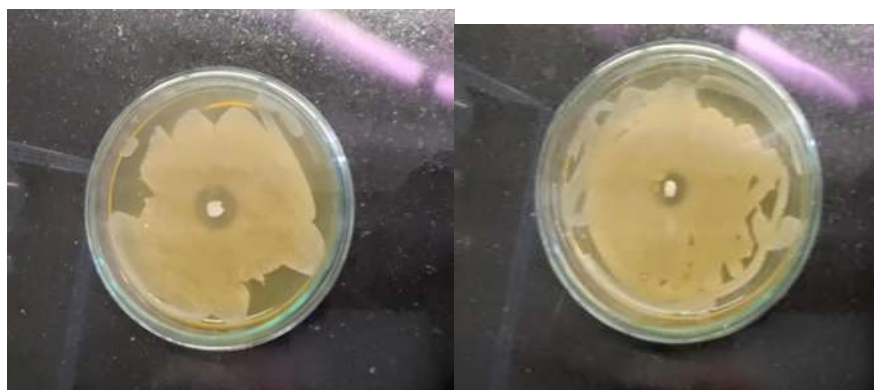


*Bacterial Cell Culturing Process:*

The antimicrobial property of the ZnO nanorod clusters deposited on cellulose sheet was evaluated using the following microorganisms; gram-positive *Streptococcus thermophilis* BAB 3369 and gram-negative *Escherichia coli* BAB 4455 respectively. The growth medium used for bacterial cells was Nutrient broth pH adjusted to 7.0. Further, to check for antimicrobial properties of the sheet, Nutrient Agar plates were prepared with a pH adjusted to 7.4. Nutrient broth and Nutrient agar were sterilized using an autoclave at 121°C and 15 lbs pressure for 30 minutes. After autoclaving the plates were prepared for the antimicrobial test with approximately 4 to 5 mm thickness of nutrient agar in Petridishes.

*Antimicrobial test:*

The sheet was cut into a round-shaped sheet (0.3 cm diameter) to evaluate its antimicrobial activity and the inoculum of respective bacterial strains was prepared in nutrient broth incubated at 35 °C for 24 h. Further, 100 μl of respective fresh bacterial suspension was spread individually throughout the plate in a clean laminar flow hood and the sheet was placed in the center of the plate.[13] These plates were incubated at 35 °C for 24 h. The zone inhibition method was used to assess the antimicrobial activity i.e. measurement of the area of non-viable bacterial cells around the sheet

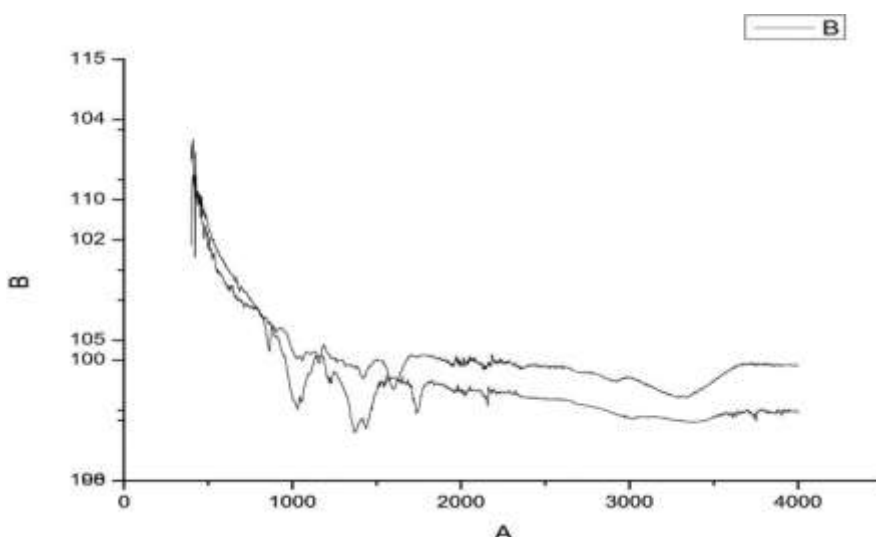


**Escherichia Coli** **Streptococcus Thermophilis**  
**Fig.2: Antimicrobial Activity Of Gram Positive And Gram Negative Bacteria:**

*Analysis of the cellulose by FT-IR:*

The analysis by FTIR of the extracted cellulose from 2% NaOH and 6% NaClO<sub>2</sub> (Figure 3) is carried out to identify polysaccharides and to determine their contaminants such as hemicelluloses, lipids, and proteins. The spectrum obtained (Figure 3) revealed a broad band in the 1000 to 4000 cm<sup>-1</sup> which corresponds to the (-OH). The peak occurred at 1283 and 1272 cm<sup>-1</sup>, which indicated the presence of hydroxyl groups in algae cellulose and carboxymethyl cellulose respectively. The peaks 1929/1857 and 2560 cm<sup>-1</sup> corresponded to the (-CH) groups stretching vibration in cellulose algae and carboxymethyl

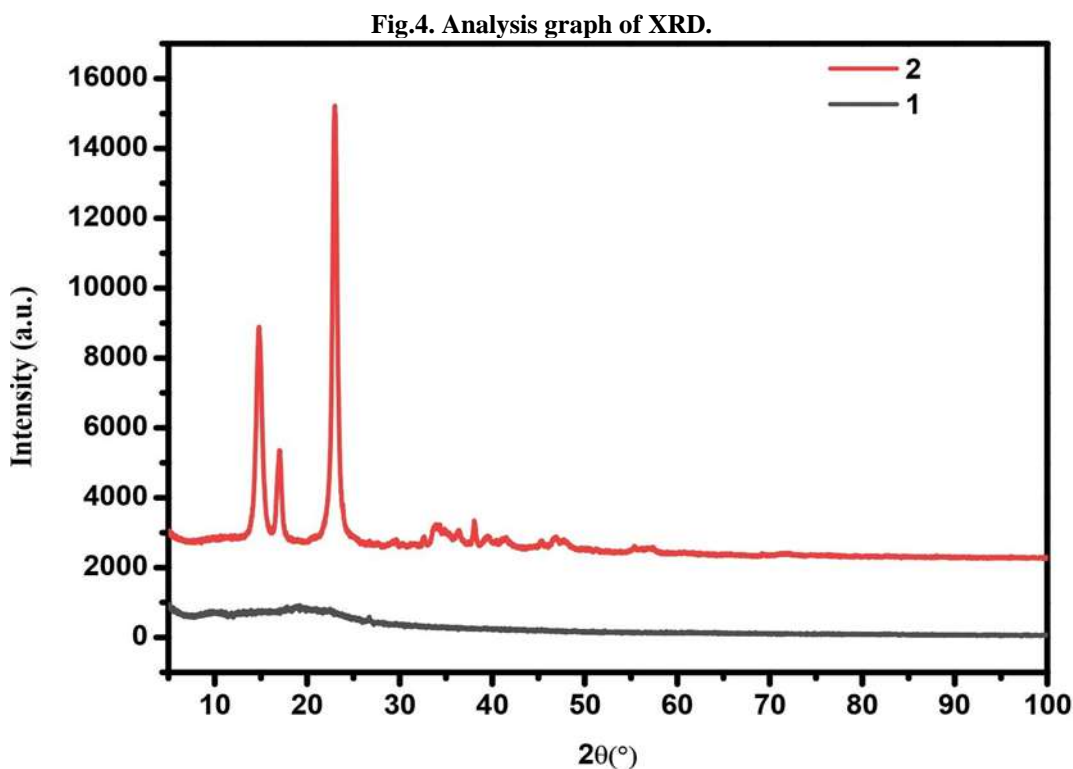
cellulose. The peak present at 2069 cm<sup>-1</sup> corresponding to the 950 to 1200 cm<sup>-1</sup> range is attributed to the C-O vibration of carbon C2, and also the 1632 cm<sup>-1</sup> band corresponded to the carbonyl groups. In addition, the FTIR absorption bands at 1458/1378 and 1412/1320 cm<sup>-1</sup> were assigned to O-H bending vibration. The occurrence of the peak at 1156 cm<sup>-1</sup>, located in 1170 to 1082 cm<sup>-1</sup> range corresponds to asymmetric C-O-C stretching vibration



**Fig. 3: Analysis graph of FT-IR of Cellulose sheet and zinc sheet of microalgae:**

*Analysis of cellulose by XRD:*

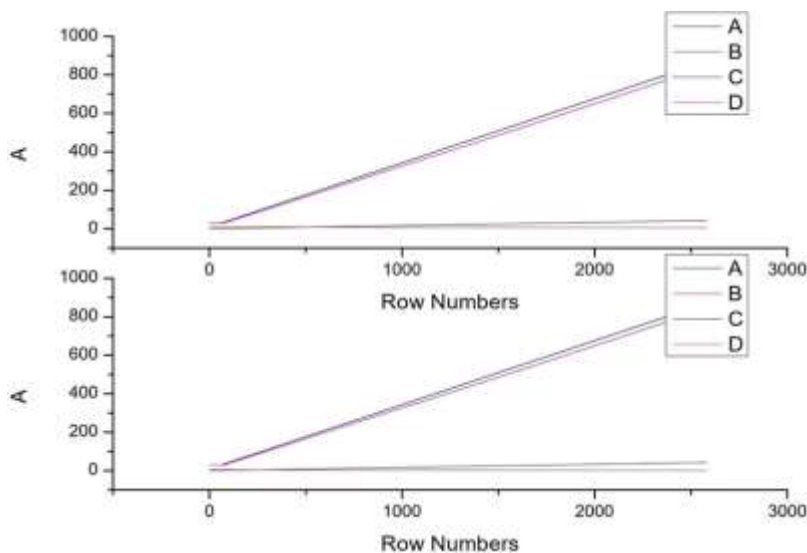
In order to identify the crystalline and amorphous areas of celluloses obtained in this work, a diffractometry X-Ray was carried out. Cellulose samples were obtained at different concentrations of NaOH (2 and 4%), followed by bleaching with 6% sodium hypochlorite, and another obtained after treatment with 2 and 4% NaOH, followed by 10% hypochlorite bleach was analyzed (Figure 4) The XRD confirmed the hypothesis that the variable of NaOH concentration of 2 to 4% shows an amorphous structure, which becomes increasingly abundant to a maximum concentration of 10% sodium hypochlorite



*Analysis of cellulose by TGA:*

Thermograms analysis or TGA have been presented in Fig.5 is algal cellulose that of ZnO nanorod clusters deposited cellulose. In both cases, weight loss was observed mainly in three steps.

1. Weight loss started around 100 degrees Celsius which could be due to moisture loss. The major thermal degradation of cellulose was observed in the second stage at 200 degree Celsius to 360 degrees Celsius. In the case of ZnO-deposited cellulose, moisture loss was observed less, and decomposition was delayed by around 50 degrees Celsius showing stability after 250 degrees Celsius.
2. The maximum mass loss of algal cellulose and ZnO-deposited cellulose was found to be 45.46% and 56.65% respectively.
3. The total mass loss of algal cellulose and ZnO-deposited cellulose were 86.3% and 75% respectively. It showed that metallic nanorod clusters deposited in cellulose were increased thermostability compare to cellulose.



**Fig.5: Analysis graph of TGA of Cellulose sheet and zinc sheet of microalgae:**

*a-Time, b- subtracted weight, c- programme temperature, d-sample temperature, e-approx gas flow*

### **III. Extraction of essential products from microalgae.**

#### *Light:*

Light duration and intensity directly affect microalgal photosynthesis and influence the microalgal biochemical composition and biomass yield when modeling algal culture systems outdoors or indoors, growth rate and biomass productivity are predicted as a function of light. [15] Light intensity varies within the culture and decreases with culture depth; this should be taken into account when modeling bioreactors or open pond systems. Algal species differ in their light requirements for maximum growth and biomass build-up. At the compensation point, where photosynthetic CO<sub>2</sub> uptake exactly equals CO<sub>2</sub> release through respiration, net growth is zero. Higher light intensities increase the photosynthetic rate up to a certain maximum value, after which it levels off until the photosynthetic rate is balanced by photorespiration and photoinhibition. [15] The growth rate and productivity of the biomass decrease with decreasing light duration. Most studies have shown that 16 hours light/8 hours' dark is best for algal growth. LED Lights are a good choice for this purpose, although fluorescent tubes can also be used. The light thus directly affects the final biomass yield and the synthesis and accumulation of carbohydrates in the algal cells. Experimentally demonstrated that a light intensity of 100  $\mu\text{mol}/\text{m}^2/\text{s}$  is optimal for some microalgal species.

#### *Temperature*

Increasing the temperature to the optimal range increases algal growth exponentially, but increasing or decreasing the temperature beyond the optimal point retards or even stops algal growth and activity. [16] The optimal temperature range for most algal species is 20-30 °C, although thermophilic algae such as *Anacystis nidulans* and *Chaetoceros* can withstand temperatures up to 40 °C, and algae growing in hot springs can reach 80 °C. Low temperatures impair photosynthesis by reducing the activity of carbon assimilation, while excessively high temperatures reduce photosynthesis by inactivating photosynthetic proteins and disturbing the energy balance in the cell. Higher temperatures also reduce cell size and respiration. [16] The main effect of temperature on photosynthesis is due to a decrease in the activity of ribulose-1,5-bisphosphate (Rubisco), an enzyme with dual functions. It can act as an oxygenase or as a carboxylase, depending on how much O<sub>2</sub> and CO<sub>2</sub> are present in the chloroplasts.

#### *Nutrients:*

The amounts of macronutrients such as nitrogen and phosphorus may vary among different microalgal species. It was reported that the growth of *Chlorella* decreased when the concentrations of nitrogen and phosphorus decreased from 31.8 and 10.8 mg/l, respectively [17]. The amount of available nitrogen in the culture directly affects cell growth. Nitrogen limitation in microalgal culture can reduce biomass growth and productivity, although it increases the production of carbohydrates and lipids. 0.5 g/l nitrogen has been shown to be the optimal concentration for *Chlorella vulgaris*, at which it produces 3.46 g/l biomass. [17] The micronutrients Mo, K, Co, Fe, Mg, Mn, B, and Zn are only needed in traces, but have a strong influence on the growth of the microalgae, as they affect many enzymatic activities in the algal cells. Normally, inorganic nitrogen and phosphorus are taken up as nitrates and phosphates. Urea is also a suitable source and a cost-effective alternative to other inorganic nitrogen sources. For commercial production of microalgal biomass, the culture must grow rapidly; therefore, providing the right nutrients is very important to accelerate algal growth. Some highly limiting substances can be used as growth promoters for microalgae.

#### *Mixing:*

Mixing and aeration ensure an even distribution of nutrients, air and CO<sub>2</sub> in the microalgae culture. They also allow light to penetrate and evenly distribute the culture and prevent the biomass from settling and clumping. [18] Therefore, microalgae cultures need to be constantly mixed so that all cells remain in suspension and have free access to light.

#### *pH and salinity:*

Microalgae species have different pH requirements. Most grow well in a pH range of 6 to 8.76. Different sources of growth media have different pH values. Most algal species are pH sensitive and few can tolerate a pH range as wide as that tolerated by *C. vulgaris*. *C. vulgaris* can grow in a wide pH range, [18] [19] but maximum growth rate and biomass productivity is reported at pH 9-10. Increasing the pH increases the salinity of the culture medium, which is very harmful to the algal cells.

### **IV. Results and Discussion:**

The microscopic image (Fig. 1) shows the surface view of the cell arrangement in the middle of the thallus, indicating the microalgae *Oedogonium* and *Chlamydomonas Reinhardt*. The yield of extracted cellulose from *Chlamydomonas Reinhardt* and *Oedogonium* was 13.8  $\pm$  0.7 %. The thickness of the leaves was about 0.7



mm.

#### *FT-IR analysis*

Fig.3 shows the FT-IR spectrum of Oedogonium and Chlamydomonas Reinhardt cellulose. In general, the broad peak at 1440 cm<sup>-1</sup> corresponds mainly to the O-H stretching, and the band at 14434 cm<sup>-1</sup> shows the O-H bending of the adsorbed water. The bands at 3916 cm<sup>-1</sup> and 3371 cm<sup>-1</sup> were assigned to the C-H vibration and O-H bending, respectively, and the C-H bending at 3423 cm<sup>-1</sup>. [17] The bands at 3159 cm<sup>-1</sup> and 3355 cm<sup>-1</sup> correspond to the glycosidic bonds of C-O-C and C-OH showed the presence of carbohydrates. Fig. 3 shows that the intensity of O-H stretching at 1347 cm<sup>-1</sup> and O-H bending at 3634 cm<sup>-1</sup> decreased compared to algal cellulose because the hydroxyl group of cellulose interacts with Zn<sup>+2</sup>, reducing the OH groups in cellulose.

#### *X-rd analysis*

Fig. 4 shows the XRD profile of microalgal cellulose in the range from 12° to 20°. The main peak at 2θ = 21.3°, which is related to the native and predominant crystalline structure of cellulose I, and the low diffraction intensity at 2θ = 14° show an amorphous background. In general, the intra- and intermolecular hydrogen bonds influence the crystalline structure of cellulose. The sample shows peaks at 14°, 24.6°, and 32.7° indicating the typical cellulose I structure and crystalline assignments of 110, 200, and 004 planes, respectively. Fig.4 shows the XRD phase of the Blanche k aluminum plate used as the sample holder. It shows the peak at 2θ= 42.07°, 48.0°, and 71.56° which we can minimize. Fig.3 shows the XRD phase of the deposited ZnO nanorod clusters on cellulose, which shows that the particles have highly crystalline hexagonal wurtzite structures[18] [19]. The XRD peaks of the ZnO nanorod clusters showed peaks at 2θ values of 31.4° (100), 34.2° (002), 38.0° (101), 47.2° (102), 54.19° (110), 60.3° (103), 63.9° (200), 65.8° (112), 66.6° (201) and 74.5° (004) with the respective diffraction plane. The crystallinity (Xd) of the microalgal cellulose was 80% and that of the cellulose deposited with ZnO was 65%.

#### *Thermal Stability Thermograms*

(TGA/DTG) have been presented in Fig.5. is the TGA of microalgae cellulose and Fig.4 is that of ZnO nanorod clusters deposited cellulose. In both cases, weight loss was observed mainly in three steps. In the first step, weight loss started around 100 C which could be due to moisture loss. The major thermal degradation of cellulose was observed in the second stage at 200 C to 360 C. In the case of ZnO-deposited cellulose, moisture loss was less and decomposition was delayed by around 45 C showing thermal stability after 245 C. In the second stage, the maximum mass loss of microalgae cellulose and ZnO-deposited cellulose was found to be 40.64% and 45.56% respectively. The material decomposes on heating beyond 350 C. It showed that metallic nanorod clusters deposited in cellulose increased thermostability compared to cellulose.[19] [20]

#### *Antibacterial activity*

The antimicrobial activity of the extracted algal cellulose proved negative against Gram-positive (Streptococcus thermophiles) and Gram-negative bacteria (Escherichia coli). The microalgal cellulose coated with ZnO nanorod clusters showed good antibacterial activity. Fig. 2 shows that the maximum zone of inhibition for the antibacterial activity of this film was observed for Gram-positive strains (Streptococcus thermophiles 9.0 mm) and Gram-negative strains (Escherichia coli was 11 mm). Fig. 2 shows the result of the quantitative analysis using the mean colony-forming unit method for the antimicrobial ratio. [19]It was found for gram-positive strains (Streptococcus thermophiles 97.9 %) and gram-negative strains (Escherichia coli 99.3 %).The shape, size, and concentration of synthesized ZnO nanoparticles play an important role in antibacterial activity.

### **V. Value-added products of microalgae:**

According to one estimate, about 5000 tons of dry algal biomass processed into bioproducts generates \$1.25 × 10<sup>9</sup> annually. Microalgae produce a wide range of other commercially important and valuable products. They produce vitamins, which increases their importance as a food source for humans and animals. They also produce various types of medicinally important polysaccharides. Several species produce bioactive and commercially important pigments, such as chlorophyll, β-carotene and other carotenoids, phycobiliproteins, and astaxanthin. [20]These pigments play an important role in the treatment of tumorigenesis, neuronal disorders and optical diseases. The high content of carbohydrates in algal cells makes them an important food source. Microalgae also produce and accumulate large amounts of lipids, which vary among species and are influenced by various factors. Therefore, a new area of research is the extraction and identification of substances from microalgae and the determination of their biological and medicinal activities. Polyunsaturated fatty acids produced by microalgae are important commercial products with high therapeutic value for heart disease, asthma and arthritis. Many important microalgal products, such as eicosapentaenoic acid and docosahexaenoic

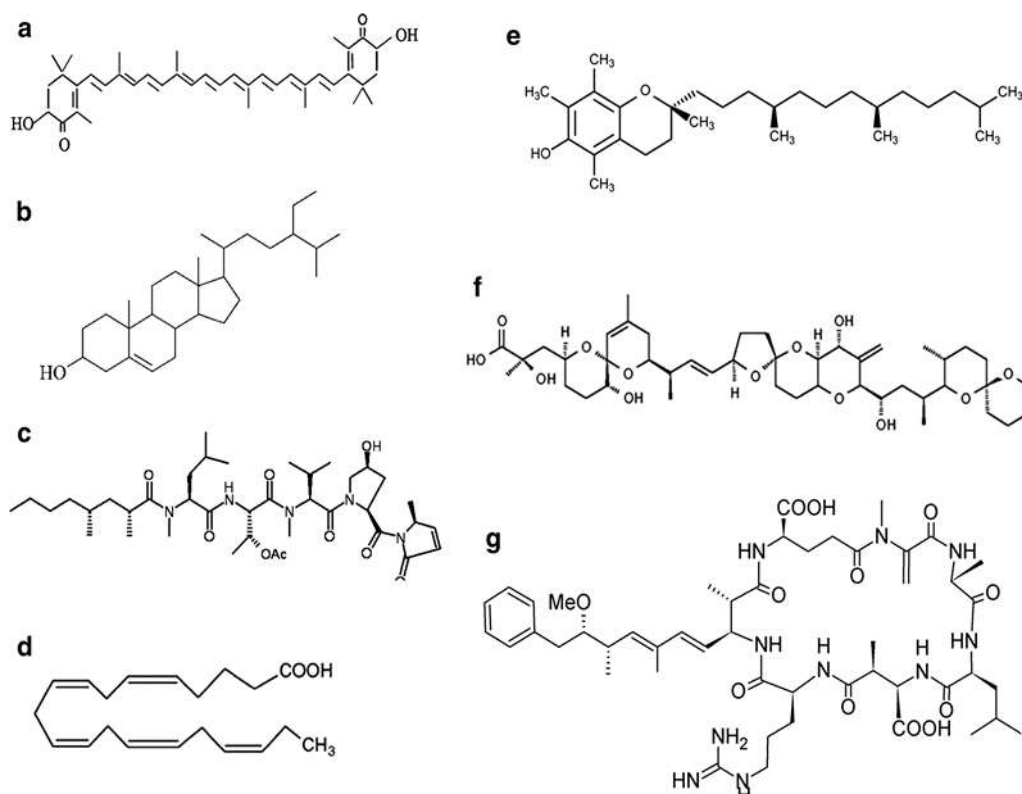
acid (DHA), have been commercialized by various biotechnology companies. These compounds are used as antioxidants in dietary supplements and foods.

#### Stress conditions.

Astaxanthin is multifunctional carotenoids normally extracted from *Haematococcus Pluvialis*. [20] This microalga synthesizes astaxanthin in response to environmental stress. The synthesis of microcystins and other metabolites by *M. aeruginosa* is highly stimulated and influenced by light intensity, temperature, pH, and nutrients such as carbon, nitrogen, and phosphorus.

#### Carotenoids

Among the most important are phycobiliproteins, phycocyanin, phycoerythrin,  $\beta$ -carotene, lutein, and astaxanthin. Phycobiliprotein pigments are used as fluorescent agents in microscopy. Phycocyanin and other pigments from red algae have antioxidant and anti-inflammatory effects and are therefore used in food and cosmetics. [21] The microalga *Dunaliella salina* produces the carotenoid pigment  $\beta$ -carotene in quantities that account for about 10-14% of its dry mass. Due to its relationship with vitamin A,  $\beta$ -carotene plays an important role in vision and the immune system. Another important carotenoid pigment is astaxanthin, which sells on the market for US\$ 2500/kg. The microalga *Haematococcus pluvialis* produces 4-5% astaxanthin per dry biomass. The dried biomass of *Haematococcus pluvialis* has been marketed as an astaxanthin-rich source.  $\beta$ -Carotene protects membrane lipids from peroxidation, which has been linked to many serious and fatal diseases such as cancer, cardiovascular disease, Parkinson's disease, and atherosclerosis. *Tecis* and *Trans* forms of  $\beta$ -carotene are different isomers that confer anti-cancer activity. [21]



**Fig. 3** Some value-added compounds produced by microalgae. **a** Astaxanthin, a strong antioxidant produced by the microalga *Haematococcus pluvialis*. **b**  $\beta$ -Sitosterol, produced by various genera of micro algae. Different species of *Glaucocystophyte* produce  $\beta$ -sitosterol. **c** Microcolin-A, an immunosuppressive agent produced by the microalga *Lyngbya majuscula*. **d** Docosahexaenoic acid DHA produced by the engineered strain of diatom *Phaeodactylum tricornutum*. **e** Structure of vitamin E (tocopherols), the microalga *Haslea ostrearia* naturally produce vitamin E. **f** Chemical structure of okadaic acid, an anti-fungal agent produced by some species of dinoflagellates. **g** Chemical structure of microcystin-LR, produced by the blooming *Microcystis aeruginosa*

#### Sterols

Sterols produced by plants are called phytosterols. Microalgal sterols have some health-promoting effects, such as cholesterol reduction, anti-cancer, anti-inflammatory, and neurological disorders such as

Parkinson's disease. Phytosterols are used in pharmaceutical formulations for health benefits and in dietary supplements as ingredients. Some microalgae species, such as those of the genera *Pavlova* and *Thalassiosira*, are rich in sterols.[22]. The microalga *Chaetoceros* has been reported to produce 27.7 µg sterols per gram dry weight. 40 different sterols have been identified in 100 different diatom species. Recently, the microalgae *Nannochloropsis* sp, *Pavlova lutheri*, and *Tetraselmis* sp were studied to determine whether they produce sterols with a net yield of 0.5-- 2.7%/dry weight. *Euglena gracilis* produced a mixture of sterols, 0.68-3.24 mg/g dry biomass. The major sterols found in Glaucocystophyte are sitosterol, campesterol, and stigmasterol. 24-Ethylcholesterol is produced mainly by cyanobacteria. Dinoflagellates mainly produce 4 $\alpha$ -methyl sterols and 24-propylidene cholesterol is mainly produced by Pelagophyceae.

#### *Proteins and enzymes:*

Microalgae proteins have a comparatively high nutritional value. Microalgae produce 2.5-7.5 tons/Ha/year of proteins. The green microalgae *Chlorella* is a rich source of various types of proteins that have been commercialized. Another protein-rich microalgae is *Arthrospira*. [23]Proteins from microalgae or plants can lower cholesterol levels by activating cholecystokinin. They also have important enzymatic effects. *Lynghya majuscula* produces microcolin-A, an immunosuppressive agent. *Nostoc* produces the protein cyanovirin, known for its antiviral activity against HIV and influenza viruses. *Anabaena* and *Porphyridium* produce the enzyme SOD (superoxide dismutase), which protects against oxidative damage, while *Isochrysis galbana* produces the vital enzyme carbonic anhydrase, which plays a crucial role in the conversion of CO<sub>2</sub> to carbonic acid and bicarbonate.[23] *M. aeruginosa* produces a variety of amino acids, including proline, serine, glycine and valine.

#### *Polyunsaturated fatty*

DHA linoleic acid, eicosapentaenoic acid, arachidonic acid and gamma-linolenic acid have been shown to lower cholesterol, delay aging, protect membrane integrity and prevent cardiovascular disease. Many microalgae species (e.g. *Porphyridium cruentum*, *Arthrospira platensis*, *Odontella*, *I. galbana*) have been studied for their ability to synthesize these valuable fatty acids. *Pavlova lutheri* produces polyunsaturated fatty acids in large quantities, while *A. platensis* produces and accumulates stigmasterol, sitosterol, and  $\gamma$ -linolenic acid. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the medically important omega-3 polyunsaturated fatty acids, which are crucial in inflammatory diseases, heart problems, arthritis, asthma, and headaches, etc. Recently, *Phaeodactylum tricorutum* has attracted attention as a potential source for the production of EPA and DHA. [24]The diatom *P. tricorutum* has been genetically sequenced and modified to produce increased omega-3 polyunsaturated fatty acids such as EPA and DHA. It was reported that the genetically modified strain of *P. tricorutum* produced a maximum yield of 36.5 and 23.6% of DHA and EPA per total fatty acid of biomass, indicating that it's suitable for the production of EPA and DHA on a commercial scale.

#### *Vitamins:*

Microalgae are also rich sources of various vitamins. *Haslea ostrearia* is a rich source of vitamin E (tocopherols). *P. cruentum* produces large amounts of vitamins E and C, as well as  $\beta$ -carotene (vitamin A). [25]The microalga *D. salina* easily produces vitamins A and E, pyridoxine, nicotinic acid, thiamine, riboflavin and biotin.

#### *Toxic metabolites*

Most microalgae species, especially cyanobacteria, produce a variety of toxic substances commonly referred to as cyanotoxins. The bloom is also responsible for shellfish poisoning due to the presence of toxins. Several types of microcystins have been identified. The microcystins are hepatocyclic peptides with a C<sub>20</sub> amino acid chain that determines the degree of toxicity.

Cyanotoxins are of great interest as environmental hazards and because of their chemical toxicity. They're classified according to their effects on vertebrates as neurotoxins, hepatotoxins, and dermatotoxins, and according to their chemical structure as cyclic peptides, alkaloids, or lipopolysaccharides.[26] These toxic substances also have important antibacterial and antifungal activities.

## **VI. Conclusion:**

The identification and isolation of microalgae was done on a laboratory scale. Gujarat Energy Research and Management Institute is the place where research work done. Extraction of biofuel is still the minimum quantity to fulfil the demand of fuels in third generation. As per the Z.H. Yin et.al. Research, more further research is needed for biofuel production on large scale. The protein extraction from *Oedogonium* and *Chlamydomonas reinhardtii* species was given a useful results for future prospects. The analysis of FT-IR and

XRD shown the crystalline cellulose in graph which results that microalgae have an  $\beta$ - Cellulose. The  $\beta$ Cellulose was need futher research for high results of proteins extraction which are going to used as value added products from microalgae.

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