



# Microalgal Cultivation: Cultivation Media and its Supplementary Compounds

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**Abstract:** Micro algal cultivation is emerging field in biological science especially algological domain. Before going mass cultivation these algal species to prepare mother culture under axenic species under suitable culture medium. Many researchers and algologists are trying their best combinations different organic, inorganic chemical with precious proportions to formulate suitable algal culture media. This review will be highlight the compilations of different culture media domains and their supplementary compositions how to transformation growing, mother culture, sub culture and harvest practices these fields. Most wildly cultivated micro algae are Spirulina, Chlorella, Chlamydomonas, Dunaliella, Botryococcus, Phaeodactylum, Thalassiosira, Pseudonana, Nannochloropsis, Oscillatoria and Isochrysis. This review focusing on four microalgal species such as Spirulina, Chlorella, Dunaliella, Chlamydomonas.

**Keywords:** Microalgae, Culture Media, Chemical Composition, Formulation.

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## **1. Introduction**

Liquid culture is often used to produce microalgae. A particular growth medium that contains the nutrients required for growth is supplemented with an algal inoculum until stationary phase is reached, at which point the entire culture is harvested (Richmond, 2004). Microalgal cultures have strain-specific ideal growth conditions, and the final cell density is determined by a variety of parameters likes light phase, intensity, temperature, nutritional quantity and quality, pH, carbon dioxide, salinity, biotic characteristics including cell fragility and density are the main abiotic elements that control algal growth. These elements have an impact on photosynthesis as well as the structure,

function, and activity of cellular metabolism, which leads to a dynamic cell composition (Andersen, 2005). Mass transfer, mixing, the size and distribution of gas bubbles are mechanical factors influencing the growth of microalgae (Schenk *et al.*, 2008)

A proper combination of important macronutrients and micronutrients is necessary for the growth of microalgae in culture media (Andersen, 2005). Supplemental CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> is frequently required for autotrophic development in order to meet the carbon requirements of high yield autotrophic algae production. Redfield ratio indicates that the phytoplankton's stoichiometric ratio is C: N: P=106:16:1, meaning that most media have a higher nitrogen content than carbon, and carbon can eventually become a limiting factor (Riebesell *et al.* 1993).

Phosphorus and nitrogen are two prime nutrients for algal productivity (Schenk *et al.* 2008). In algal media, ammonium, nitrate, or urea are usually used as nitrogen sources. Since the release of H<sup>+</sup> ions during active development can cause the culture pH to drop significantly, especially in dense cultures at high temperatures, extra attention must be paid to ammonium as the only source of nitrogen. The pH increases when nitrate is the only supply of nitrogen (Richmond, 2004). After urea is hydrolyzed by the enzymes urea amidolyase or urease, it can also be a useful source of nitrogen (Hodson *et al.* 1975). Phosphorus and nitrogen are two prime nutrients for algal productivity (Schenk *et al.* 2008). In algal media, ammonium, nitrate, or urea are usually used as nitrogen sources. Since the release of H<sup>+</sup> ions during active development can cause the culture pH to drop significantly, especially in dense cultures at high temperatures, extra attention must be paid to ammonium as the only source of nitrogen. The pH increases when nitrate is the only supply of nitrogen (Richmond, 2004). After urea is hydrolyzed by the enzymes urea amidolyase or urease, it can also be a useful source of nitrogen (Hodson *et al.* 1975).

Inorganic phosphates are the main source of phosphorus in algal growth media. Apart from C, N, and P, ionic forms of K, Ca, Mg, Cl, Mn, S, and Na are other important nutrients for algal growth and development. They play a significant role in photosynthesis's light reaction (Cl, Mn), cell metabolism (K, Na, Ca, S), and formation of chlorophyll (Mg). Important nutrients involved in redox processes are trace elements, such as Zn, Cu, Mo, and Mn (Lazar, 2003). Certain algae have a vitamin need (Croft *et al.* 2006). Thiamine, biotin, and vitamin B12 (cyanocobalamin) are the vitamins that are most frequently used. However, according to Provasoli and Carlucci (1974) a few algae require all three vitamins. A method must be developed in order to effectively create

growth media that are species-specific and manipulable for the large-scale cultivation of diverse strains of microalgae. These media need to take advantage of cell density unit volume and accumulation while reducing the expense and resources needed to give cells the vital mineral nutrients they requisite.

Microalgae are vital to aquaculture because they provide live food for zooplankton, all phases of bivalves, and the larval stages of numerous fish and crab species. Nevertheless, in hatcheries, late larval and juvenile fish and crustaceans are finally dependent on microalgae (Renaud *et al.*, 1991). Improved growth and survival of aquatic fish larvae have regularly been observed when different microalgae are added to the water during their early feeding time (Howell, 1979). Microalgae are utilized for space travel (Haldane, 1951) and as a human food supply in overpopulated regions (Burlew, 1953). Microalgae are said to provide a number of benefits. The protein output from algae may be significantly higher if they are grown in an environment that is suited for them (Spoehr and Milner, 1949). Seaweeds and a few other algae, such as *Porphyra*, *Ulva*, *Alaria*, and *Chlorella* are most frequently used in China and Japan. Algae have been shown to have fertilizing properties as well. Phosphorus and nitrogen-rich blue-green algae make great fertilizer. Bottom-mud from dried-up ponds is frequently used as manure in crop cultivation; the high concentration of blue-green algae in this manure is mainly responsible for its manurial value. A properly blended combination of seaweed and cyanophycée manures may serve as an ideal fertilizer and this can relieve the acute shortage of fertilizers in developing countries in the tropical region (Dash *et al.*, 2016).

## 2. Cultivation Media of different species

### 2.1. Cultivation Media of *Spirulina* species

*Spirulina* is a good candidate species for microalgae culture because it provides abundant nutrients and salts that promote microalgae growth. Algal growth is influenced by a number of physicochemical and biological parameters, the most important of which are the availability of light and carbon. Other factors that affect algal growth include temperature, pH, lightness, and nutrition. When microalgae combine with solar light during photosynthesis, they produce a large amount of inorganic carbon, which is then converted to glucose (Larsdotter, 2006). Furthermore, value-added products like proteins, lipids, and other secondary metabolites are created by microalgae.

The most commonly used culture in spirulina cultivation on a laboratory scale is Zarrouk's medium. The following is the guidelines for Zarrouk's

Medium (Zarrouk, 1966). One liter of Zarrouk's medium consists of (part A)  $\text{NaHCO}_3$  -16.80 g and  $\text{K}_2\text{HPO}_4$  -0.50 g; (part B)  $\text{NaNO}_3$  -2.50 g,  $\text{K}_2\text{SO}_4$  -1.00g,  $\text{NaCl}$  -1.00 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.20 g,  $\text{EDTA-Na}_2 \cdot 2\text{H}_2\text{O}$  -0.08 g,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  -0.04 g and  $\text{FeSO}_4 \cdot 2\text{H}_2\text{O}$  -0.01 g; trace elements mixture A (part C 10 mL/l): 1.00 mL, trace elements mixture B (part D 1.0 mL/l): 1.00 mL; part C mg/l :  $\text{H}_3\text{BO}_3$  -2.86,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  -1.810 g,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  -0.222 Mo $\text{O}_3$  -0.015, and  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  -0.074 (the used amount is 10 mL/l); part D mg/l:  $\text{NH}_4\text{VO}_3$  -22.9,  $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$  -47.8,  $\text{NaWO}_2$  -17.9,  $\text{Ti}_2(\text{SO}_4)_3 \cdot 6\text{H}_2\text{O}$ , and  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  -4.4 (the amount used was 1.0 mL/l). George's Medium is another well-known spirulina cultivation medium. Follow this guidance for preparation. Peptone (1.00 g),  $\text{KNO}_3$  -0.20 g,  $\text{K}_2\text{HPO}_4$  -0.02 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.02 g, and ferric citrate -0.035 g are the contents of one liter of George's medium (Atlas, 1997).

Amara and Steinbüchel (2013) are referred to as Amara and Steinbüchel (A-St) Medium, and their formula is comparable to George's Medium. There are four components in a 1X concentration.  $\text{NaHCO}_3$  -9.214 g,  $\text{NaCO}_3$  -7.143 g, and  $\text{K}_2\text{HPO}_4$  -0.5 g make up part A of an A-St medium. Part B includes  $\text{NaNO}_3$  -1.5 g,  $\text{K}_2\text{SO}_4$  -0.571 g,  $\text{NaCl}$  1 g,  $\text{MgSO}_4$  -0.2 g,  $\text{CaCl}_2$  -0.012 g,  $\text{FeSO}_4$  -0.01 g, and  $\text{EDTA-Na}_2$  -0.08 g. Part C includes ferric citrate 0.018 g, peptone 0.1 g, and yeast extract 0.01 g. Spirulina is growing in reasonably good condition in this medium.

Suitable culture Solutions for Various Planktonic Algae (Chu, 1942). Made immense contributions in variety of microalgae cultivation by developing suitable culture media as like they flourishing in their respective nature waters. In his paper summary he pointed out a few points as appropriate media for the growth of numerous plankton algae have been prepared by pure chemicals, the formulations being created on the results of series of trials and on the evaluates of fresh waters. In structure and grade of dilution they are comparable with ordinary natural waters, so that experimental results obtained with them should be applicable to natural conditions. Fourteen planktonic algae, isolated from various localities, have been maintained in these solutions in unialgal culture in a flourishing condition for more than two years and could no doubt be cultured indefinitely. The subsequent culture solutions have been used in the examination on microalgae cultivation, the quantities always being in grams per litre of distilled water. Cultivation: Solution-1(mg/L) -  $\text{NH}_4$ ,  $\text{NO}_3$  -0.025; K,  $\text{HPO}_4$  -0.001;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.025;  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  -0.025;  $\text{FeCl}_3$  -0.0005 (Botryococcus) Solution-2(mg/L):-  $\text{KNO}_3$  - 0.025;  $\text{K}_2\text{HPO}_4$  -0.001;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.025;  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  - 0.025;  $\text{FeCl}_3$  - 0.0005; Solution-3(mg/L) -  $(\text{NH}_4)_2\text{SO}_4$  0.025;  $\text{K}_2\text{HPO}_4$  0.001;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.025;  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  -0.025;  $\text{FeCl}_3$  -0.0005

Solution-4(mg/L) -  $(\text{NH}_4)_2\text{SO}_4$ - 0.025;  $\text{K}_2\text{HPO}_4$  -0,001;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.025;  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  -0.025;  $\text{FeCl}_3$  - 0.0005 (*Pediastrum*) cultivation: Solution- 5 (mg/L) - $(\text{NH}_4)_2\text{SO}_4$ -0.025;  $\text{K}_2\text{HPO}_4$  - 0,005;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.07;  $\text{NaCl}$ - 0.04 ;  $\text{K}_2\text{SiO}_3$ - 0.02;  $\text{CaCO}_3$  -0.001;  $\text{FeCl}_3$ - 0.0001. (*Pediastrum*) cultivation: Solution-6(mg/L) - $(\text{NH}_4)_2\text{Cl}$ -0.025;  $\text{K}_2\text{HPO}_4$  - 0,005;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.07;  $\text{NaCl}$ - 0.04 ;  $\text{K}_2\text{SiO}_3$ -0.02;  $\text{CaCO}_3$  -0.01;  $\text{FeCl}_3$ - 0.001. (*Staurastrum*) cultivation: Solution-7 (mg/L):  $\text{Ca}_2(\text{NO}_3)_2$  - 0.03;  $\text{K}_2\text{HPO}_4$  -0.005;  $\text{Mg}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$  0.07;  $\text{K}_2\text{SiO}_4$  -0.02;  $\text{Na}_2\text{CO}_3$  - 0.03;  $\text{FeCl}_3$ - 0.001. (*Pediastrum*) cultivation: Solution-8 (mg/L):  $\text{KNO}_3$ - 0.025;  $\text{K}_2\text{HPO}_4$  0.001;  $\text{Mg}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$  -0.025;  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  0.001;  $\text{K}_2\text{CO}_3$ -0.005; (or up to 0.05);  $\text{FeCl}_3$  0.0008. (*Asterionella*, *Nitzschia*, *Tabellaria*) cultivation: Solution-9 (mg/L):  $\text{Ca}_2(\text{NO}_3)_2$  - 0.02-0.04;  $\text{K}_2\text{HPO}_4$  -0.002;  $\text{Mg}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ - 0.01;  $\text{K}_2\text{SiO}_4$  -0.025;  $\text{CaCO}_3$ -0.01;  $\text{FeCl}_3$  0.001. (*Botryococcus*, *Staurastrum*, *Oscillatoria*, *Asterionella*, *Tabellaria*, *Nitzschia*) cultivation: Solution-10:  $\text{Ca}(\text{NO}_3)_2$  -0.04;  $\text{K}_2\text{HPO}_4$ - 0.01 or 0.005;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.025;  $\text{Na}_2\text{CO}_3$ - 0.02;  $\text{Na}_2\text{SiO}_3$ -0.025;  $\text{FeCl}_3$ - 0.0008. (*Staurastrum*, *Pediastrum*) cultivation: Solution-11:  $\text{KNO}_3$ -0.04;  $\text{K}_2\text{HPO}_4$ -0.001;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.025;  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  -0.001;  $\text{K}_2\text{CO}_3$  -0.05;  $\text{K}_2\text{SiO}_3$ -0.01;  $\text{FeCl}_3$  -0.001. (*Botryococcus*, *Staurastrum*, *Asterionella*, *Tabellaria*, *Nitzschia*) cultivation: Solution-12:  $\text{Ca}(\text{NO}_3)_2$ - 0.03;  $\text{K}_2\text{HPO}_4$  -0,005;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.075;  $\text{K}_2\text{SiO}_3$  -0.025;  $\text{KCl}$  -0.005;  $\text{Na}_2\text{CO}_3$  -0.02;  $\text{FeCl}_3$  -0.0005. (*Botryococcus*) cultivation: Solution-13:  $\text{KNO}_3$  -0.05;  $\text{K}_2\text{HPO}_4$ - 0.01;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.025;  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  -0.02;  $\text{FeCl}_3$  -0.001. (*Nitzschia*) cultivation: Solution-14:  $\text{Ca}(\text{NO}_3)_2$ -0.02-0.04;  $\text{K}_2\text{HPO}_4$  -0.002;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.04;  $\text{NaCl}$  -0.04;  $\text{K}_2\text{SiO}_3$  -0.025;  $\text{CaCO}_3$  -0.01;  $\text{NaHCO}_3$  -0.04;  $\text{FeCl}_3$ - 0.001. (*Nitzschia*) cultivation: Solution-15:  $(\text{NH}_4)_2\text{SO}_4$ -0.02-0.04;  $\text{K}_2\text{HPO}_4$  -0.002;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.04;  $\text{NaCl}$  -0.04;  $\text{K}_2\text{SiO}_3$  -0.025;  $\text{CaCO}_3$  -0.01;  $\text{NaHCO}_3$  -0.04;  $\text{FeCl}_3$ - 0.001. (*Fragilaria*, *Nitzschia*) cultivation: Solution-16:  $\text{Ca}(\text{NO}_3)_2$  0.05;  $\text{K}_2\text{HPO}_4$  - 0.001;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.04;  $\text{K}_2\text{SiO}_3$  -0.025;  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  -0.04;  $\text{Na}_2\text{CO}_3$  -0.02;  $\text{FeCl}_3$  -0.001.

In general ,Ingredients of selective medium (BG11 medium) (g/L) for spirulina :  $\text{NaNO}_3$  -1.5;  $\text{K}_2\text{HPO}_4$  -0.04;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.075;  $\text{CaCl}_2 \cdot \text{H}_2\text{O}$  -0.036;  $\text{CaCl}_2 \cdot \text{H}_2\text{O}$  -0.036; Citric acid -0.006;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  -0.006;  $\text{Na}_2\text{CO}_3$  -0.001; EDTA -0.02; Distilled water 1000ml. Ingredients of selective medium (F/2 medium) (g/L) for spirulina :  $\text{NaNO}_3$ -75;  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ - 5;  $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$  -30;  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  -3.15;  $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$  -4.35;  $\text{CuSO}_4$  -9.8;  $\text{Na}_2\text{MO}_4 \cdot 2\text{H}_2\text{O}$  -6.3;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  -22;  $\text{COCl}_2 \cdot 6\text{H}_2\text{O}$  -10;  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  -18; Trace Biotin; Cyanocobalamin.

Agreeing Too many researchers' and scientists' perspectives on spirulina cultivation and their physico-chemical characterization, the ideal temperature range for spirulina growth is between 35° C and 38° C. Furthermore, spirulina needs a pH that is relatively high, which effectively prevents other algae from

growing in the culture media. To maintain the high pH and avoid fluctuations, the culture medium needs to contain high concentrations of sodium bicarbonate at all times. For many years, spirulina cultures have effectively used Zarrouk's medium, which is high in bicarbonate (Göksan, 2007). Zarrouk's medium has a high production cost, making commercial production impractical. From then on, numerous researchers attempted to grow spirulina using inexpensive resources like cow dung (Murugan, 2010), swine dung (Manikandavelu and Murugan, 2009), spent-wash (Murugan *et al.*, 2012), and so on. Various supplements were also added in an effort to increase biomass yield and produce bioproducts (Murugan, 2010). It was possible to obtain a culture medium for *S. maxima* growth that is just as good as the synthetic medium that has been documented in the literature. Specifically, sea water treated with  $\text{NaHCO}_3$  at pH 9.2 and 35° C for two hours, filtered to remove precipitates, and enriched with  $\text{K}_2\text{HPO}_4$ ,  $\text{NaNO}_3$ , and  $\text{FeSO}_4$  was used to cultivate spirulina. The best-known synthetic medium (Faucher *et al.*, 1979) and the 130-L culture open pond were used for the experiment, and (Tredici *et al.*, 1986) reported on the outdoor mass cultivation of *S. maxima* in sea water.

After adding  $\text{NaHCO}_3$  at a rate of 1 gram per liter to bring the pH of the seawater medium to 9–11, it was utilized to produce single-cell proteins (Teresa, 2000). Under laboratory conditions, the *Arthrospira spp.* autotrophic nutrition during the inoculum culture involves the use of Zarrouk's media, 150–200  $\mu\text{mol photon m}^{-2}\text{s}^{-1}$  lighting, a temperature range of 25–38 °C (minimum of 15 °C depending on *Arthrospira* strain), a pH range of 9.5–12, and salt tolerance up to 0.75 M sodium chloride (Grewe *et al.*, 2012). Therefore, the *Arthrospira* species' capacity to withstand highly alkaline conditions makes them suitable for cultivation in open systems (Grewe *et al.*, 2012). Plant supplements were used in an experiment with *S. platensis* cell weight ranges in different concentrations of papaya skin powder medium (PSPM), i.e., 0.004 g/L to 0.693 g/L in concentration of 0.3 g/L, 0.004 g/L to 0.578 g/L in concentration of 0.4 g/L, 0.004 g/L to 0.430 g/L in concentration of 0.5 g/L, and 0.004 g/L to 0.720 g/L in Kosaric Medium (KM). The cell's proliferation was observed in a range of mediums and concentrations. This fluctuation may result from varying medium concentrations and nutritional compositions (Tredici *et al.*, 1986). Tanticharoen *et al.* (1991) were found that the productivity of wastewater from the tapioca starch factory's stabilization ponds could be increased to 7–10  $\text{g/m}^2/\text{d}$  by adding nitrogen fertilizer and sodium bicarbonate ( $\text{NaHCO}_3$ ), which was higher than normal input. The biomass output rate in Chinese manufacturing facilities was found to be 7.0  $\text{g/m}^2/\text{d}$  (Li and Qi, 1997),

which was likewise significantly greater than the outcomes of the papaya skin powder medium. (Pulz, 1992) reported production values of 1.3 g/Ud, which is greater than the KM of the normal input and translates to 28 g/m<sup>2</sup>/d of lighted surface area and 120 g/m<sup>2</sup>/d of occupied land when achieved under natural illumination.

*S.platensis* was cultured in a tubular air-lift photobioreactor (Zhang *et al.*, 1992). They found that the batch-cultured *S.platensis* could reach a maximum cell dry weight of 2.4 g/L at a 12L culture volume, 5000 lux/m<sup>2</sup>/s, 30°C, and 200 KA/m. This was also higher than the medium containing papaya skin powder. *S.platensis* has been utilized as a model organism in many research over the past ten years on the outdoor cultivation of algal biomass as a source of chemicals and protein (Richmond, 1992).The original goal of developing a low-cost alternative protein source is still unmet, and production costs are still much higher than those of traditional protein sources despite substantial advancements in this field that have resulted in the construction of numerous commercial production facilities throughout the world. Therefore, it is indispensable to find a culture medium for *S. platensis* so that expensive inorganic media are not used.

Palacio Rodríguez *et al.* (2022) produced lipids using microalgae such as *Verrucodesmus verrucosus*, *Desmodesmus quadricauda*, *Neochloris oleoabundans*, and *Chlorella miniata*. These microalgae were selected due to their rapid growth and lipid accumulation on conventional culture media F/2 and in the foliar fertilizer Bayfolan forte (Becker, 1994; Richmond, 2013). Bayfolan forte culture media (BF) percentage (p/v): Zinc (Zn) 0.080; Zinc oxide (K<sub>2</sub>O) 6.000; Copper (Cu) 0.040; Molybdenum (Mo) 0.005; Thiamine hydrochloride 0.004; Phosphorus oxide (P<sub>2</sub>O<sub>5</sub>) 8.000; Boron (B) 0.036; Iron (Fe) 0.050. 11.470 total nitrogen; 6.000 potassium oxide; 0.230 sulfur; and 0.002 cobalt. Composition of the waste water, which includes the concentration of phosphate and nitrate compounds, as well as the Bayfolan forte culture media. N-[NH<sub>4</sub>] = P-[PO<sub>4</sub>-3] = 11.47 Wastewater 22.0 (N-[NH<sub>4</sub>]) 8.0 (P-[PO<sub>4</sub>-3]). Dayananda *et al.* (2007) conducted a study comparing several culture mediums, such as BG-11 and BBM, for the culture of *Botryococcus braunii*. According to the study's conclusions, BG-11 was the best medium for generating hydrocarbons and biomass. In the case of saltwater algae, microalgae monocultures are usually produced in a laboratory environment using altered Conway and f/2 media (Xin *et al.*, 2010). These media were created using the fundamental nutrients required for the growth of algae, which are comparable to the nutrients required in an environment similar to its natural habitat (Panahi *et al.*, 2019). The specific

growth rates of several genera of algae, such as *Dunaliella sp.*, *Chlorella sp.*, *Chaetoceros sp.*, and *Tetraselmis sp.*, were increased in f/2 medium by 72.00 %, 40.36 %, 22.40 %, and 4.13 %, respectively (Lananan *et al.*, 2013). In contrast, *Pavlova sp.* and *Isochrysis sp.* flourished in Conway Medium by 16.39 % and 4.64 %, respectively. According to Elgohary *et al.* (2020), various attempts were made to cultivate in BG 11 growth medium in order to find the best culture media for *Spirulina* grown in studies conducted at Heliopolis University (Dineshkumar *et al.*, 2016):  $\text{NaNO}_3$  37.5 g/l,  $\text{K}_2\text{HPO}_4$  -1.0 g/l,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -1.875 g/l,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  -0.9 g/l, Citric acid 0.15 g/l, Ammonium ferric citrate green -0.15 g/l,  $\text{EDTANa}_2$  -0.025 g/l,  $\text{Na}_2\text{CO}_3$  -0.5 g/l,  $\text{H}_3\text{BO}_3$  -2.86 g/l,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  -1.81 g/l,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  -0.22 g/l,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  -0.39 g/l,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  -0.08 g/l,  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  -0.05 g/l are the ingredients of the medium recipe. *Spirulina* cultured in Heliopolis University studies using optimized cultivation media (CFTRI growth medium) (Salunke *et al.*, 2016).  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.20;  $\text{CaCl}_2$  -0.04;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  -0.015;  $\text{NaHCO}_3$  -4.5;  $\text{K}_2\text{HPO}_4$  -0.050;  $\text{NaNO}_3$  -1.5;  $\text{K}_2\text{SO}_4$  -1.00;  $\text{NaCl}$  -1.00. According to the researchers' findings, *Spirulina* favored Zarrouk medium over BG-11 and CFTRI mediums.

## 2.2. Cultivation Media of *Chlorella* Species

Members of the Chlorophyceae family, water-dwelling eukaryotic green microscopic algae *Chlorella sp.* are able to flourish in naturally occurring or artificially aerated liquid cultures that provide sufficient light, carbon dioxide, and other nutrients for their cells. The majority of these microalgae are photoautotrophic, but some species can also exist heterotrophically by decomposing organic sources, such as sugars, into biomass and lipid that can be used to produce biodiesel and other high-value products (Rosenberg *et al.*, 2008). Globally, scientists, researchers, and algologists have utilized a broad variety of media cultures; a few of these are examined here. (Muntalif and Rahkmadumila, 2020) growth performance of *C sp.* in nutrient media as a Walne medium was investigated:-Walne medium included :  $\text{FeCl}_3$  = -0.87 mg/L,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  = -0.36 mg/L,  $\text{Na}_2\text{EDTA}$  = -54.24 mg/L,  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  = -17.66 mg/L,  $\text{NaNO}_3$  = -100.264 mg/L,  $\text{ZnCl}_2$  = -0.021 mg/L,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  = 0.011 mg/L,  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  = 0.009 mg/L,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  = -0.02 mg/L, Vitamin B1 =  $10 \times 10^{-5}$  mg/L, and Vitamin B12 =  $5 \times 10^{-6}$  mg/L To culture *C.sp.*, 810 mL of Walne nutritional medium were placed in a 1-liter glass flask along with 10 mL of the inoculum. After that, the flask was sealed with room temperature air that was kept between 25 and 28°C. Additionally, 4000 lux of light intensity was supplied throughout the course of a 12-hour light/dark cycle. This injection



was given for four days, or until *C. sp.* started to reach the exponential phase.

Kumaran *et al.* (2016) were cultured *C.vulgaris* in Bold's Basal Medium (BBM), which contains the following ingredients in 10 mL per liter of culture medium, was used to maintain and cultivate it. (2) 1 mL per litre of culture medium containing the following chemicals: EDTA anhydrous- 50g/l, KOH- 31g/l,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ -4.98g/l,  $\text{H}_2\text{SO}_4$ -1m/l,  $\text{H}_3\text{BO}_3$ -11.4 g/l),  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  -8.82g/l,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ -1.44 g/l),  $\text{MoO}_3$ -0.71g/l,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ -1.57g/l),  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  -0.49g/l. The medium's pH was initially set to 6.8. The mother culture was maintained in a 100 mL Erlenmeyer flask with 50 mL of culture media, air aeration, and constant illumination with cool white fluorescent light (60–70  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ) at a temperature range 22 and 25°C (Lam and Lee, 2012). A modified GBI-I algal growth medium and a composition of inorganic algal growth media were used to cultivate other species of *Chlorella*, specifically *C. ellipsoidea* (Stanier and Van Niel, 1962). Major nutrients are  $\text{NaNO}_3$  (75.0 g/500 ml);  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (15.0 g/200 ml);  $\text{K}_2\text{HPO}_4$  (15.0 g/200 ml);  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (15.0 g/200 ml);  $\text{Na}_2\text{CO}_3$  (15.0 g/200 ml); EDTA (15.0 g/200 ml); Ferric ammonium citrate (15.0 g/200 ml); Citric acid (15.0/g200 ml b)  $\text{H}_3\text{BO}_3$  -2.680 g/L,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  -1.810 g/L, and  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  -0.390 g/L,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  -0.220 g/L;  $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$  -0.079 g/L;  $\text{CaSO}_4 \cdot 7\text{H}_2\text{O}$  -0.049g/L are trace elements.

Using raw materials including gram, lentil, and pulse powders as well as organic media for *C. sp.* cultivation (Mohshina *et al.*, 2017). Creation of a low-cost culture medium using *Vigna mungo* whole pulse powder, following Rahman's (2000) instructions. One kilogram of pulse powder was combined with thirty liters of tap water to create this medium. 15 g of urea was added to the mixture a week later. Following three weeks, the mixture of pulse powder was filtered through a thin marking cloth to remove any solid debris. A few days later, the clear supernatant was poured into a new bucket. After the medium was made clear by adding lime, its pH rose to roughly 10. The medium was then prepared for use as a *Chlorella* culture medium after one week of adding 0.325 ml of concentrated  $\text{H}_2\text{SO}_4$  to bring the pH down to 7.

*C.vulgaris* was cultivated in plant-based compost made from peat moss and animal-based compost made from goat dung (Kumaran *et al.*, 2016). Using a magnetic stirrer, 10 g of the compost was dissolved in 600 mL of tap water and swirled for a whole day. Subsequent to the stirring procedure, non-soluble particle materials were identified and filtered using filter paper. The pH of the organic fertilizer medium was then adjusted to predetermined values after a predetermined volume of the medium was added to a photo bioreactor along with 5 L of raw tap water. Next, a 1 L Erlenmeyer flask was filled with

*C. vulgaris* at a starting cell concentration of  $0.3 \times 10^6$  cells (about 10 mL from the seed culture). Compressed air was used to continually aerate the flask, and a Philip TL-D 36W/865 cool-white fluorescent lamp with a light intensity of  $60\text{--}70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  was used to illuminate it (Lam and Lee, 2012).

In a study conducted (Jahan *et al.*, 2014), *Chlorella sp.* was cultured in three different culture media: soil extract medium, pulse bran extract medium, and inorganic medium. The largest cell densities were discovered in the eutrophic-pond-water medium (Ara, 2010) during an experiment including the culture of *Chlorella sp.* in three different culture media like treated eutrophic-pond-water medium, inorganic medium, and organic medium. (Sultana, 2009) found that the organic medium had the highest cell densities while cultivating *Chlorella sp.* in three different culture media such as organic, inorganic, and eutrophic pond water media. (Mishu, 2008) was found that the largest cell densities were reached when green algae, *Scenedesmus sp.*, were cultivated in two different culture media, medium-I (organic) and medium-II (inorganic). The maximum cell density was discovered in an experiment conducted (Wongsnansilp *et al.*, 2007) on the culture of an alga, *Chlorosarcinopsis sp.* (Hossain, 1996) was conducted a study on *C. ellipsoidea* using five different media: Medium-I (Inorganic medium), Medium-II (Medium of whole pulse powder), Medium-III (Medium of pulsed bran), Medium-IV (Mixed medium = 50 % inorganic + 50 % Whole pulse powder medium), and Medium-V (Mixed medium = 50 % Inorganic + 50 % Pulse bran medium). It was discovered that the cell densities ranged from 0.08 to  $0.62 (\times 10^6)$  cells/ml in Medium -I, 0.02 to  $4.02 (\times 10^6)$  cells/ml in Medium-II, 0.18 to  $4.38 (\times 10^6)$  cells/ml in Medium-IV, and 0.07 to  $4.38 (\times 10^6)$  cells/ml in Medium V. (Dagon Manoel Ribeiro, 2019) was used four distinct culture media to cultivate *C. sorokiniana*. The NPK medium, for example, is made by diluting 1g of the chemical fertilizer N: P: K (20:5:20) for every 1L of distilled water (Sipaúba-Tavares *et al.*, 1999). Other examples of such media are the nitrogenated medium (Dragone *et al.*, 2011) and the mixed medium. One gram per liter of glucose and 50 % NPK and 50 % Bold Basal made up the mixed medium. Each media component was autoclaved for 15 minutes at  $121^\circ\text{C}$  to attain sterilization.

Agwa and Abu (2014) was carried out a study on the culture of *C. sp.* 1 ml of the flowered culture was aseptically injected into flasks holding 300 ml of poultry medium—a precisely defined synthetic medium made up of 0.066 milliliters of sodium silicate, 0.066 milliliters of potassium nitrate, 0.066 milliliters of monosodium phosphate, and 0.066 milliliters of EDTA. The pH was brought down to 7.5 before the autoclave was run for 15 minutes

at 121°C. 2.0 g/l NaHCO<sub>3</sub>, 0.05 g/l Urea, 1.0 g/l NaCl, and 1.50 g/l Gypsum (CaSO<sub>4</sub>·2H<sub>2</sub>O) were the contents of the Bangladesh- II medium. After adjusting the pH to 7.0–7.5, the mixture was autoclaved for 15 minutes at 121°C. These cultures were kept in a 500 ml conical flask at 28±2 °C (Golob *et al.*, 2009) *C. vulgaris* for use in microalgal culture and inoculum production. An aseptic inoculation was performed using the culture of *Candida vulgaris* derived from agar slants into several 200 mL Erlenmeyer flasks that held 150 mL of liquid Jaworski medium. The succeeding ingredients made up the medium in one liter distilled water): 36 mg of Na<sub>2</sub>HPO<sub>4</sub> ·12H<sub>2</sub>O-80 mg, NaNO<sub>3</sub>-12.4 mg, KH<sub>2</sub>PO<sub>4</sub>-20 mg, Ca (NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O-50 mg, MgSO<sub>4</sub>·7H<sub>2</sub>O-15.9 mg, NaHCO<sub>3</sub> -2.25 mg, EDTA-FeNa -2.25 mg, EDTANa<sub>2</sub> -2480 g, H<sub>3</sub>BO<sub>3</sub>-1390 g, MnCl<sub>2</sub>·4H<sub>2</sub>O-1000 g (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O -40 g, cyanocobalamin (B12)–40 g, thiamin HCl (B1) and biotin–40 g. The flasks were left in direct sunshine and at room temperature until the microalgae density was high enough for inoculation in subsequent studies.

*Chlorella sp.* were successfully cultured and maintained in microalgal cultures using this medium of (Guillard and Lorenzen, 1972). NaHCO<sub>3</sub> -12.6g/L; K<sub>2</sub>HPO<sub>4</sub> · 3H<sub>2</sub>O-11.4g/L; NaNO<sub>3</sub> -85g/L; Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O-21.2g/L; CaCl<sub>2</sub> · 2H<sub>2</sub>O-36.8g/L; MgSO<sub>4</sub> · 7H<sub>2</sub>O-37g/L; Vitamins: thiamin HCl-0.1 g/L and biotin 0.0005 g/L; micronutrients: Na<sub>2</sub>EDTA-4.36 g/L; FeCl<sub>3</sub>·6H<sub>2</sub>O-3.15 g/L; CuSO<sub>4</sub> · 5H<sub>2</sub>O-0.01 g/L; ZnSO<sub>4</sub>·7H<sub>2</sub>O-0.022 g/L; CoCl<sub>2</sub>·6H<sub>2</sub>O-0.01 g/L; MnCl<sub>2</sub>·4H<sub>2</sub>O-0.18 g/L; Na<sub>2</sub>MoO<sub>4</sub> · 2H<sub>2</sub>O-0.006 g/L; H<sub>3</sub>BO<sub>3</sub>-1g/L. Results from the growing of microalgae species on this medium are noteworthy. Comparable to WC with minor alterations is WCg Medium. NH<sub>4</sub>Cl<sub>2</sub>·-680 g/L; K<sub>2</sub>HPO<sub>4</sub> - 2.178 g/L; MgSO<sub>4</sub> · 7H<sub>2</sub>O - 9.243 g/L; CaCl<sub>2</sub>·2H<sub>2</sub>O-3.676 g/L; NaNO<sub>3</sub>-21.253 g/L; NaHCO<sub>3</sub>-3.150 g/L; H<sub>3</sub>BO<sub>3</sub>-1.500 g/L; NaEDTA · 2H<sub>2</sub>O -9.300 g/L; alkaline soil extract solution; FeCl<sub>3</sub>·6H<sub>2</sub>O- 3.15g/L; Na<sub>2</sub>EDTA · 2H<sub>2</sub>O- 4.36 g/L; CuSO<sub>4</sub> · 5H<sub>2</sub>O- 9.8 g/L; Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O- 6.3 g/L; ZnSO<sub>4</sub> · 7H<sub>2</sub>O- 22.0 g/L; CoCl<sub>2</sub>·6H<sub>2</sub>O- 10.0 g/L; MnCl<sub>2</sub>·4H<sub>2</sub>O- 180.0 g/L; Vitamins: Thiamine·HCl (vit.B1)-200 mg/l; biotin (vit.H)-1.0 g/L; cyanocobalamin (vit. B12) - 1.0 g/L. (Wright, 1964; Guillard, 1975).

De Camargo *et al.*, (2015) were able to successfully cultivate *C. vulgaris* in synthetic medium and determine the chemical composition of the hydroponic solution. This was done by adapting (Hoagland and Arnon, 1950) and preparing it for dual purposes in dechlorinated water for greenhouse cultures and distilled water for laboratory cultures. (NH<sub>4</sub>) H<sub>2</sub>PO<sub>4</sub>–6.95; Fe-EDDHMA (C<sub>18</sub>H<sub>16</sub>N<sub>2</sub>O<sub>6</sub>FeNa) –2.3 g/L; Micronutrients (KSC Mix®) – 6.15 g/L; Ca (NO<sub>3</sub>)<sub>2</sub>- 3.9 g/L; KNO<sub>3</sub>–3.96 g/L; MgSO<sub>4</sub> – 2.82 g/L. They created a new algal culture

medium for their compositions and *C. vulgaris*. Chemical makeup of the culture medium changed and adapted from (Afnor, 1980) concentration (mol L<sup>-1</sup>) \*NaNO<sub>3</sub>-9.4 10<sup>-4</sup>; \*NH<sub>4</sub>NO<sub>3</sub>-1 x 10<sup>-3</sup>; Ca (NO<sub>3</sub>)<sub>2</sub>. 4 H<sub>2</sub>O-3.4 x 10<sup>-4</sup>; MgSO<sub>4</sub>. H<sub>2</sub>O-2.4 x 10<sup>-4</sup>; K<sub>2</sub>HPO<sub>4</sub>-4.6 x 10<sup>-4</sup>; CuSO<sub>4</sub>.5 H<sub>2</sub>O-1.2 x 10<sup>-7</sup>; (NH<sub>2</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>.4 H<sub>2</sub>O-4.8 x 10<sup>-8</sup>; ZnSO<sub>4</sub>. 7H<sub>2</sub>O-2 x 10<sup>-7</sup>; CoCl<sub>2</sub>. 6H<sub>2</sub>O-2.52 x 10<sup>-7</sup>; Mn (SO<sub>4</sub>).H<sub>2</sub>O -2.4 x 10<sup>-7</sup>; H<sub>3</sub>BO<sub>3</sub>-9.8 x 10<sup>-7</sup>; FeCl<sub>3</sub>. 6 H<sub>2</sub>O-6.28 x 10<sup>-6</sup>; FeSO<sub>4</sub>.7 H<sub>2</sub>O-4.48 x 10<sup>-6</sup>; C<sub>6</sub>H<sub>5</sub>FeO<sub>7</sub>.5H<sub>2</sub>O-5.8 x 10<sup>-6</sup>; NaHCO<sub>3</sub>-3.58 x 10<sup>-4</sup> (\*Components not included in original medium).

### 2.3. Cultivation Media of *Dunaliella* Species

*Dunaliella* is a halophilic microalgae with biflagellate, pear-shaped cells that is a member of the Chlorophyceae family. Algal cells have a thin, elastic plasma membrane and no hard cell wall. This algae provides carotenoids for animal and human nutrition naturally. Facilities that cultivate microalgae usually use seawater that has been supplemented with nutrients, particularly phosphate, nitrate, and carbon (Fu *et al.*, 2012). The concentration of biomass production and the nutrient content of microalgae can be impacted by *D. salina*'s requirement for a full nutrient composition. Controlling the amount of macro and micronutrients in the cultivation environment is one area of research being done to promote the growth of microalgae (Harrison, 2005). The uses of *D. salina* were very varied, ranging from its marketing as a health food in affluent nations (Chang *et al.*, 2011). Stressful situations like high light intensity, temperature, and salt concentration induce *D. salina* to accumulate several secondary metabolites as lutein, zeaxanthin, β-carotene, and lycopene (Lamers, 2012.). The most promising options for value-added products across a broad spectrum are *D. salina*. Therefore, many researchers and scientists throughout the world are attempting to cultivate this species by using various synthetic and organic chemicals with various forms to create culture mediums that are appropriate for laboratory cultivation as well as outdoor (Duc *et al.* 2013).

According to (Chitlaru and Pick, 1989) *D. salina* and medium *D. salina* were produced and maintained in an artificial 1.5 M NaCl medium. In summary, the medium included 1.5 M NaCl; 0.4 M Tris-HCl, 5 mM KNO<sub>3</sub>, 5 mM MgSO<sub>4</sub>, 0.3 mM CaCl<sub>2</sub>, 0.2 mM KH<sub>2</sub>PO<sub>4</sub>, 1.5 μM FeCl<sub>3</sub> in 0.185 mM H<sub>3</sub>BO<sub>3</sub>, 7 μM MnCl<sub>2</sub>, 0.8 μM ZnCl<sub>2</sub>, 0.2 nM CuCl<sub>2</sub>, 0.2 μM Na<sub>2</sub>MoO<sub>4</sub>, 20 nM CoCl<sub>2</sub>, 50 mM NaHCO<sub>3</sub>. *D. salina* is most suited for this media. *D. viridis* was cultivated in artificial medium (Mary *et al.*, 1968). Expansion of cultures. In a synthetic medium, *D. viridis* was cultivated with the following ingredients (per liter): MgCl<sub>2</sub>-6H<sub>2</sub>O-1.5

g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  -0.5 g; KCl- 0.2 g;  $\text{CaCl}_2$ - 0.2 g;  $\text{KNO}_3$ , -1.0 g;  $\text{NaHCO}_3$ -0.043 g; tris(hydroxymethyl)amino methane (Tris), 2.45 g (pH adjusted to 7.5 with HCl);  $\text{KH}_2\text{PO}_4$  -0.035 g; ethylenediaminetetraacetate, 1.89 mg;  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  -2.44 mg;  $\text{ZnCl}_2$  - 0.041 mg;  $\text{H}_3\text{BO}_3$  -0.61 mg;  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  -0.015 mg;  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  -0.041 mg;  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  -0.41 mg;  $(\text{NH}_4)\text{SMo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ -0.38 mg; and  $\text{VOCl}_2$  -0.041 mg. With every experiment, the amount of sodium chloride in the medium is indicated. After being autoclaved independently, the potassium phosphate solution was aseptically added to the sterile medium. The cultures were cultured for seven to ten days at 25 °C on a rotating shaker in 2,000 ml Erlenmeyer flasks with 500 ml of media.

Zhang *et al.* (2000) solid medium preparation was made in order to cultivate *Dunaliella sp*-The makeup of the medium is as follows: 1-M glycerol, 0.25 M NaCl, 10 mM  $\text{NaHCO}_3$ , 5 mM  $\text{MgSO}_4$ , 0.3 M  $\text{CaCl}_2$ , 0.2 M  $\text{KH}_2\text{PO}_4$ , 0.2 M  $\text{H}_3\text{BO}_3$ , 7  $\mu\text{M}$   $\text{MnCl}_2$ , 6  $\mu\text{M}$   $\text{Na}_2$  EDTA, 1.5  $\mu\text{M}$   $\text{FeCl}_3$ , 0.8  $\mu\text{M}$   $\text{ZnCl}_2$ , 20 nM  $\text{CoCl}_2$  and 0.2 nM  $\text{CuCl}_2$ . Furthermore, Solid Gel Medium contains 0.1 % phytagel as a gelling agent. The  $\text{NaHCO}_3$  solution is autoclaved separately and then added to the other autoclaved components to reach the final concentration; pH modification is not required in order to prevent precipitation during autoclaving. Once the melted media has cooled, the plates are poured and can be kept at room temperature, unwrapped, for a day or two before being used.

#### 2.4. Cultivation Media of *Chlamydomonas species*

*Chlamydomonas sp* is a green alga that lives in soil and is around 10  $\mu\text{m}$  in diameter. It has two anterior flagella, many mitochondria, and a chloroplast. Investigation on *Chlamydomonas* has been conducted more recently for the production of biofuels and for bioremediation (Vílchez *et al.* 2001; Kosourov *et al.*, 2007). Researchers have termed *Chlamydomonas reinhardtii* the “green yeast” because, among the several species of *Chlamydomonas*, it has shown to be such an effective model for deconstructing basic biological processes (Goodenough, 1992 ; Rochaix, 1995). This green alga has a single cell and is found in settings with temperate soil. Ehrenberg and Dangeard (1888) described the genus *Chlamydomonas* and the species *C. reinhardtii*, respectively (Elizabeth Harris, 2009). Early in the 20th century, *Chlamydomonas* was determined to be a good subject for genetic research (Elizabeth Harris, 2001), but *C. reinhardtii* was initially developed as a model organism in the 1950s, when the first mutations were produced (Elizabeth Harris, 2009). Because *C. reinhardtii* grows vegetative as a haploid, it is a great species to work with in the lab because mutant traits can be displayed right away. *C. reinhardtii* can double in population every eight

hours under ideal conditions because to its rapid growth (Elizabeth Harris, 2001). Even light-sensitive photosynthesis mutants have been discovered thanks to *Chlamydomonas*' ability to thrive in the dark on an acetate-containing medium while maintaining a functional photosynthetic apparatus (Levine, 1969; Sprouter and Mets, 1980).

Genetically modified *Chlamydomonas sp.* can produce oil instead of starch, making it a promising species for biofuel production (Spalding, 2008). It appears that maintaining the activity of the CO<sub>2</sub> absorption genes increases culture biomass by around 50 % compared to wild type cells in the presence of high CO<sub>2</sub> concentrations. Starch is the primary chemical energy storage material in chlorophyceae. It has a functioning photosynthetic machinery and grows in the dark on an organic carbon source (Elizabeth Harris, 2001). In recent times, *Chlamydomonas* studies have been conducted for the production of biofuels and bioremediation (Vílchez *et al.*, 2001; Kosourov *et al.*, 2007). Zheng *et al.* (2022) the cultivation conditions of the *C. reinhardtii* algae strain. The cultures were kept in a medium called Tris-acetic acid-phosphate (TAP), which comprised 2.42 g of tris powder, 0.38 g of NH<sub>4</sub>Cl, 0.10 g of MgSO<sub>4</sub>·7H<sub>2</sub>O, 57.00 mg of CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.11g of K<sub>2</sub>HPO<sub>4</sub>, 54.00 mg of KH<sub>2</sub>PO<sub>4</sub>, 50.00 mg of EDTANa<sub>2</sub>, 22.00 mg of ZnSO<sub>4</sub>·7H<sub>2</sub>O, 11.40 mg of H<sub>3</sub>BO<sub>3</sub>, and 5.06 mg of 1 mL of glacial acetic acid, 1.61 mg of CoCl<sub>2</sub>·6H<sub>2</sub>O, 1.57 mg of CuSO<sub>4</sub>·5H<sub>2</sub>O, 1.10 mg of (NH<sub>4</sub>)<sub>2</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O, 4.99 mg of FeSO<sub>4</sub>·7H<sub>2</sub>O, and MnCl<sub>2</sub>·4H<sub>2</sub>O (Gorman & Levine, 1965). In a 250 mL Erlenmeyer flask with a 200 mL working volume, 5 % (v/v) of logarithmic phase algal cells (0.03 g/L) were used as the starting inoculum. *C. reinhardtii* was grown in a shaker with a 14/10 h light/dark cycle and a temperature of 25 °C and 50 mE/m<sup>2</sup>/s of light intensity (ZhouFei and Deng, 2012) was measurement of biomass, culture conditions, and microalgal strain. In this investigation, they used the microalgae strain *C. reinhardtii* CC124 (137°C) that was maintained (Randolph-Anderson *et al.*, 1998). A 2 mL centrifuge tube was filled with inoculated cells grown on an agar plate and 1 mL of dd H<sub>2</sub>O. The mixture was well mixed by gently pipetting and the solutions were added to 100 mL Erlenmeyer flasks that held 50 mL each of HSM (Randolph-Anderson *et al.*, 1998) and HSM-N (N-deficient HSM) medium.

All cultures were maintained in an incubator shaker with 230 rpm at 24°C and were exposed to continuous illumination at a light intensity of 100 μmol/m<sup>2</sup>/s<sup>-1</sup>. The HSM medium is composed of NH<sub>4</sub>Cl -0.500 g/l, MgSO<sub>4</sub>·7H<sub>2</sub>O -0.020 g/l, CaCl<sub>2</sub>·2H<sub>2</sub>O- 0.010 g/l, K<sub>2</sub>HPO<sub>4</sub> -1.440 g/l, KH<sub>2</sub>PO<sub>4</sub> -0.720 g/l, C<sub>2</sub>H<sub>3</sub>NaO<sub>2</sub> -2.000 g/l, H<sub>3</sub>BO<sub>3</sub> -0.001 g/l, MnCl<sub>2</sub>·4H<sub>2</sub>O -0.005g/l, ZnSO<sub>4</sub>·7H<sub>2</sub>O-0.022 g/l, FeSO<sub>4</sub>·7H<sub>2</sub>O-0.005 g/l, CoCl<sub>2</sub>·6H<sub>2</sub>O-0.002 g/l, Na<sub>2</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O- 0.002 g/l, and

Na<sub>2</sub>EDTA-0.050 g/l. The HSM-N (N deficiency) medium is composed of NaCl -0.547 g/l, MgSO<sub>4</sub>.7H<sub>2</sub>O -0.020 g/l, CaCl<sub>2</sub>.2H<sub>2</sub>O -0.011 g/l, K<sub>2</sub>HPO<sub>4</sub> -1.440 g/l, KH<sub>2</sub>PO<sub>4</sub> -0.720 g/l, CH<sub>2</sub>COONa.3H<sub>2</sub>O -2.000 g/l, H<sub>3</sub>BO<sub>3</sub> -0.001 g/l, MnCl<sub>2</sub>.4H<sub>2</sub>O -0.005 g/l, ZnSO<sub>4</sub>.7H<sub>2</sub>O -0.022 g/l, FeSO<sub>4</sub>.7H<sub>2</sub>O-0.005 g/l, CoCl<sub>2</sub>.6H<sub>2</sub>O-0.002 g/l, Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O-0.002 g/l, and Na<sub>2</sub>EDTA-0.050 g/l. It is employed in the production of *Chlorella sp.* biofuel. Algal cell proliferation and the amount and makeup of fatty acids are influenced by the medium's composition (Fabregas *et al.*, 1989; Hikfors, 1986). The composition of the medium affects how the pH and salinity alter. By maximizing the composition of the medium, microalgae strains' weight of biomass and oil content can be significantly increased. According to Yang *et al.* (2008) the biomass weight of the *C. pyrenoidosa* No. 2 strain increased from 3.73 to 6.56 g/L and the oil content increased by 19.75 %, from 40.15 to 59.90 %, as a result of optimizing the medium's composition. The first publication of Sager and Granick's medium compositions was (Sager, 1953). This recipe is a minor alteration of *C. reinhardi's* "medium I," which was prepared in Dr. Joel Rosenbaum's laboratory at Yale for nutritional studies. Create the individual stock solutions listed below: 1. Elements of trace CoCl<sub>2</sub>.6H<sub>2</sub>O-0.20 g/l; Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O-0.20 g/l; CuSO<sub>4</sub>-0.04 g/l; H<sub>3</sub>BO<sub>3</sub>-1.0 g/l; ZnSO<sub>4</sub>.7H<sub>2</sub>O-1.0 g/l; MnSO<sub>4</sub>.4H<sub>2</sub>O-0.30 g/l: 2. Na-citrate, 500 g/l of 2H<sub>2</sub>O: 3. 10g of ferrous chloride (FeCl<sub>3</sub>.6H<sub>2</sub>O):4. 53 grams of calcium chloride (CaCl<sub>2</sub>.2H<sub>2</sub>O): 5. 300g/l of magnesium sulfate (MgSO<sub>4</sub>.7H<sub>2</sub>O): 6. Ammonium chloride (NH<sub>4</sub>Cl-600 g/l or NH<sub>4</sub>NO<sub>3</sub>) salt (450 g/l):7.Monobasic potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>.7H<sub>2</sub>O-200 g/l); dibasic potassium phosphate (K<sub>2</sub>HPO<sub>4</sub>.7H<sub>2</sub>O-200 g). Combine 0.5 ml of solutions 7 and 8 with 1.0 ml of each of solutions 1 through 6 to create the final medium. The pH in the end should be roughly 6.9. In order to prepare nitrate medium for testing nit1 transformants, remove solution 6 and replace it with 4.0 milliliters of a KNO<sub>3</sub> 1 M solution. Add 2.5 ml of a 600 g/l sodium acetate hydrate (NaOAc.3H<sub>2</sub>O) solution to cultivate non-photosynthetic strains. To boost the buffering capacity, increase the amount of solutions 7 and 8 to 1.0 ml each. The TAP and Tris-minimal medium formulation was developed in 1965 by Gorman and Levine. Use 1 L of TAP medium and adjust the final pH to 7.0. 1M Tris base, such as Trizma Solution A: 1.0 ml Phosphate Buffer II (20 ml) Hutner's trace elements, 10.0 ml Glacial acetic acid, 1.0 ml One milliliter. Remove the acetic acid from the Tris-minimal medium and use HCl Phosphate Buffer II to titrate the final solution to pH 7.0 (for 100 ml). Solution A: KH<sub>2</sub>PO<sub>4</sub> -5.6 g; K<sub>2</sub>HPO<sub>4</sub> -10.8 g (for 500 ml) NH<sub>4</sub>Cl -20 g CaCl<sub>2</sub>.2H<sub>2</sub>O -2.5 g MgSO<sub>4</sub>.7H<sub>2</sub>O. (Sueoka, 1960): Sueoka's high salt medium also referred to as HS or HSM, this requires less preparation time than TAP. Create

the ensuing stock fixes:  $\text{NH}_4\text{Cl}$ -100.0 g/l;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -4.0 g/l;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ -2.0 g/l; salts solution.2.Solution of phosphate;  $\text{KH}_2\text{PO}_4$ -144.0 g/l;  $\text{K}_2\text{HPO}_4$  -288.0 g/l. (Hutner *et al.*, 1950).The Sueoka high salt medium and TAP both employ this blend. To find out more about how well this trace element solution satisfies *C. reinhardtii* nutritional needs (Merchant *et al.*, 2006) .Dissolve each ingredient in the designated volume of water for a final mix of one liter. In order to prevent oxidation, dissolve the EDTA in boiling water and prepare the  $\text{FeSO}_4$  last. The compound EDTA disodium salt should be prepared as follows: 50 g/250 ml;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ -22 g/100 ml;  $\text{H}_3\text{BO}_3$ -11.4 g/200 ml;  $\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$ -5.06 g/50 ml;  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ -1.61 g/50 ml;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ -1.57 g/50 ml;  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ -1.10 g/50 ml;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ -4.99 g/50 ml.

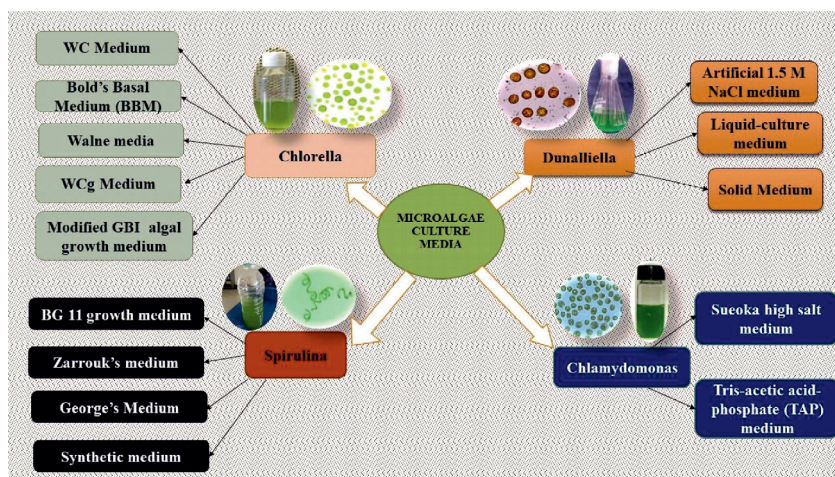


Fig. 1 : Graphical abstract of microalgae culture media

### 3. Conclusion

Recent years have seen a significant increase in interest in microalgae around the world because of their wide range of potential applications in the food, pharmaceutical, and related industries. Microalgae are inexpensive, sustainable, and renewable sources of food components and bioactive compounds. The growing of microalgae under aseptic or artificial circumstances using appropriate culture medium is an artistic endeavor; furthermore, the meticulous growth of inorganic and organic materials in a delicate proportion is a laborious operation. Important commonly used culture media for various microalgal growing techniques by various algae experts, researchers, scientists, etc. were emphasized in this review. The utilization of food products and related businesses will become less appealing as successful microalgae culture media



and culturing technologies require greater biomass production. The algal cells go through several growth phases as they develop. The requirements of various microalgal species for growing media can differ. While vital nutrients, an organic or inorganic carbon source, nitrogen, and phosphorus are the building blocks and are categorized as macronutrients necessary for algal growth, the main requirements are the same for nearly all species. A variety of physical factors, such as light, temperature, pH, aeration, carbon dioxide, and so on, are critical to the optimal development of microalgae in both fresh and saltwater in addition to culture media. In microalgae production, mixing and aerating ensure that nutrients, air, and CO<sub>2</sub> are distributed uniformly.

Additionally, they keep the biomass from settling and aggregating and allow light to enter the culture and distribute evenly throughout it. In the event that all other conditions are satisfied but mixing is not done, biomass productivity will be greatly reduced. Therefore, to maintain all of the cells in suspension and unrestricted access to light, microalgae cultures need to be constantly mixed. Even though it assists newcomers to this field in gaining a general understanding of various culture media, their compositions and specifications, specific specificity, etc., this review works are compiled different culture media with specific Microalgal stains by various algologists, researchers, and scientists.

### Conflict of Interest

The authors declared that there is no potential conflict of interest.

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