Chapter-2

Review of Literature

Increasing globalization leads to the rapid industrialization, in order to fulfil the requirement of food and feed which guzzle lots of freshwater and produce life threatening effluent. Among these industries, food and dairy industries are generating enormous amount of effluent or waste water & utilized a lot of fresh water in their processing. Globally, India has ranked one in the production and consumption of dairy products.[26] Indian dairy industry is individually contributing 5% of the Indian economy & providing employment for more than eight crore people. According to the estimation of NDDB, India, has near about 6.2% annual expansion rate in milk production. Which contributes approximately 23% of worldwide milk production. The production of milk was significantly increased in last few years, which was around 121.8 million tonnes in 2010-11 and this reached around 209.96 million tonnes in the 2020–2021 (NDDB, 2022).[26] The dairy sector consumes excess amount of fresh water for processing, 1 kg of milk (approximately, 8 to 35 litter). Because of having excess utilization of freshwater, and generating high amount (0.2 to 10 litter) of effluent or wastewater from the one litter of milk processing, it has a crucial impact on the environment. In the dairy industries, the production of cream, cheese, butter etc. and washing the equipment's are the main reasons of generating huge volume of wastewater. The dairy wastewater is the residual fluid which released, after the processing and packaging of dairy products.[9] This residue contains high concentration of milk components, viz. lactose sugar, casein protein, lipid molecules, and minerals which incredibly enhance, its (BOD) and (COD) parameters.[4]

2.1 Dairy Effluent:

Dairy industry primarily produces liquid effluent during the processing of different useful products from whole milk like buttermilk, condensed cream, sweet whey, curd, cheese, butter, and ice cream, etc. The distinguishing features of dairy effluent might be varying according to the formation of final products. Generally, dairy effluents containing high concentration of lipids such as triacylgycerides, phospholipids, unsaturated and saturated fatty acids, carbohydrates like; lactose, glucose and galactose etc.[27] The dairy waste water also contains two major soluble proteins casein and whey protein etc., resulting in high BOD and COD levels. Its high biological oxygen demands deplete the dissolved oxygen content and create anaerobic conditions very rapidly under the aquatic system. Dairy effluent contains various inorganic and organic compounds of N and P, available in higher concentration and responsible for the alkalinity of the dairy waste and other than it also contains trace amount of some mono-valent and bi-valent cations like Na, Mg, Ca, Fe, K, Ni, Mn etc. Use of detergents and chlorine in the dairy industry further deteriorates the quality of effluent.[28]

2.1.1 Types of Effluents or processing water:

Dairy effluents have developed hazardous impact on environmental resources. various types of effluents producing by different processing units of dairy industry. Which are varied in organic content (phosphorous and nitrogen), suspended solids, and in the presence of fat or grease molecules. Apart from this, the residual liquid having detergents and other cleaning agents, employed in washing purposes.[4,29] According to their origin and composition, dairy effluents can be categorized into the following three types;

i. Processing water

After the vaporization as well as the condensation process of milk in specialized containers, both produce processing water with droplets of whey or milk. Milk and whey drying create vapours that, following condensation, create the cleanest effluent. Processing waters typically don't contain contaminants and can be reused or released with storm water after just minimum pre-treatment.[4]

ii. Cleaning Wastewater

This effluent typically originates from washing machinery that comes into touch with milk or other dairy products, such as tanker washing, refrigeration points, floor washing, boiler houses, etc. spills of milk and whey, mistakes in operation, and broken equipment are all included. Cheese bits, cream, whey, fruit concentrates, water from starter cultures or fermentation units, yogurt, and stabilizers account for more than 85% of the total organic solid matter in residual fluid. These effluents must undergo additional treatment because they are produced in large amounts and are highly contaminated.[30]

iii. ***Sanitary Wastewater***

This type of wastewater produced in restrooms, showers, etc. and piped directly to sewage works which shares a similar composition to municipal wastewater. Prior to further aerobic treatment, this could be employed as a good nutrient source for anaerobic microorganism.

Dairy product processing units results a huge amount of chemically modified liquid waste which needs proper treatment before discharge.[4,16] These effluents could be effectively used as nutrient materials for microalgal growth and development for obtaining bioenergy molecules form these cultivations.[29]

2.2. Components of Dairy Effluent:

Depending on the processing of different milk products, components of dairy wastewater vary accordingly. The pH ranges between 4.0 –11.0, BOD and COD, ranges between 50–45,000 mg/L, and 90–94,000 mg/L, respectively.[17] The amount of total phosphorus and nitrogen concentrations ranges between 9–280 mg/L and 15–850 mg/L respectively. The quantity of inorganic salts (minerals) and heavy metals such as; Na, K, Mg, Ca, Cl, Zn, Mn, Ni, Co, Fe, and Mo can be considerably elevated in dairy effluents.[7] Eutrophication in revers or fresh water resources mainly caused by the higher concentration of phosphorus and nitrogen which destroy the stability of ecological balance. Its high BOD depletes the dissolved oxygen content and creates anaerobic conditions in the aquatic system. Moreover, dairy effluent contains various inorganic and organic compounds of nitrogen and phosphorus, present in higher concentration. Various mono-valent and bi-valent cations responsible for the alkalinity of the dairy waste, other than these, use of detergents and chlorine in the dairy industry, further deteriorates the quality of effluent.[17] Dairy effluents are biologically active because of the richness of organic matter and when these discharged either untreated or semi-treated into freshwater streams, leads to deficiency in oxygen level.[3] Hence, the proper treatment before the disposal of dairy waste effluent has become the major problem for the vitality of the dairy industry and environment.

2.3 Dairy Effluents Treatment Approaches:

There are different physico-chemical, and biological technologies, which can be used for the bioremediation of dairy effluent before being released into the natural habitat or surroundings.[13]

2.3.1 Physico-chemical treatment:

Several Physico-chemical methods are applied for the remediation of dairy effluent; these are as follows;

i. ***Chemical precipitation:*** It includes the addition of several chemical reagents such as calcium hydroxide (lime), sodium hydroxide etc. which separate the dissolved or suspended solids though the process of precipitation. Primarily, this process is used to settling down or eradicate the metallic ions (anions and cations), and fatty or greasy amalgams.

ii. ***Coagulation/Flocculation:*** These procedures applied directly to dairy effluent, in successive manner to remove dissolved, suspended, and colloidal materials from it. First, Coagulation which involves the use of coagulating reagents like; iron or aluminum salts to coagulate the suspended particles, present in dairy effluent by neutralizing or suppressing the attracting forces which stabilizing them. While in the process of flocculation, destabilized or unsettle particles aggregates with one another and producing the bigger one which could be easily isolated through gravitation forces.[14]

iii. ***Electrochemical process****:* such as electrocoagulation, electro flocculation,[15] and anodic oxidation etc. used for the eradication of suspended solids, metals and emulsified oils from wastewater. These worked at the principle of dissolution, aggregation and then flocculation. In electrolytic cell, metal electrodes used which hydrolyzed the water and oxidation of one substrate and reduction of another take place simultaneously. On applying electricity, negative ions continue to migrate towards anode and positive ions towards cathode. Hence, destabilization occur among the suspended particles or oily substances. Separation of these suspended particles the flocculated or emulsified oils

from the waste water at anode and gas micro bubbles released at cathode. Flocculated metal ions adhere on suspended particles and gas bubbles capture the flocculated particles and become larger in size. Large flocs are removed easily from the waste water surface. In electrocoagulation, commonly aluminum or iron electrodes are used as coagulants while potassium chloride (KCl) used as electrolyte.

iv. ***Adsorption:*** In this process silica-based adsorbents & activated carbon are used to adsorb broad range of organic molecules. This is the mass transfer process, in which the substance adheres or condensed at the solid surface through unbalanced forces such as Van der Waals forces. activated carbon is the widely used adsorbent to adsorb all kind of organic compounds.

v. ***Membrane processes:*** Similarly, microfiltration, nano-filtration, reverse osmosis, ultra-filtration, and dialysis etc. Membrane process is based on filtration process, for separating the colloidal substances from a fluid or effluent where membrane act as semipermeable membrane. Membrane technology is the non-thermal technique but higher in cost.[16]

There are various demerits regarding the use of physicochemical treatments such as its higher cost, generating secondary pollutants, because chemical reagents were used in these processes and high energy consumption in the form of valuable electricity. Next, they only perform partial treatment is another drawback.[3,4]

2.3.2 Biological Treatment:

In order to compensate for the negative aspects of physico-chemical approach, biological approaches are more suitable alternative for the remediation of dairy wastes. These are eco-friendly,

economical, cheap and highly efficient to eradicate the contaminants from dairy wastewaters.[3,4] Microorganisms such as bacteria, fungi, microalgae, etc. have been employed in biological treatment methods. Among these, the microalgae are well-known bioremediator because of their ability to grow photoautotropically and mixotrophically.[3,5,18] The DWW having huge load of phosphorus, nitrogen, and organic matter thus, the nutrient-rich dairy effluent proved to be a suitable medium for the flourishment of all kind of microalgae strains.[3,12,19]

2.4 Microalgae:

Microalgae are microscopic, unicellular, eukaryotic microorganisms which include green algae, blue-green algae, dinoflagellates, diatoms, etc. They can exist independently as a single colony or in a consortium as a group. Microalgae are a diversified group of microorganisms that have the capability to remove pollutants and produce lipids with high biomass production.[31] Microalgae represents promising biological system for remediating dairy effluent because of having metabolic flexibility, perform as photoautotrophic, mixotrophic, or heterotrophic metabolic activity. Moreover, the microalgae efficiently use solar energy for its photosynthetic activity and combine water with carbon dioxide in order to make their food autotrophically, further; it converts the environmental carbon to oxygen.[32] Micro algal biomass has been utilized for production of biological compounds like single cell-protein, bio-ethanol, biogas, hydrogen gas and lutein or astaxanthin,[23] and can also be used as bio fertilizers.[32] Among these microalgae, enormous diversity seen in their structure, mode of nutrition, stored food material and photosynthetic pigments.[33]

Table 2.1 shows the different microalgae, their photosynthetic pigments, and reserve food material with examples.

Table: 2.1. Types of Microalgae: Based on Their Pigment and Reserve Food

Class	Photosynthetic pigments	Reserve food material	Representative Genera
Chlorophyceae (Green algae)	Chlorophyll a, b, Carotene	Starch	*Chlorella sp., Heamatococcuspluvialis, Dunalialla salina*
Xanthophyceae (Heterokont or Yellow green algae)	Chlorophyll a, c, b-Carotene, Xanthophylls	Fats, leucosin	*Chlorochromonas, Chlorosuccus, Vaucheria, Tribonema, Botrydinum*
Chrysophyceae (Yellow-brown algae)	Chlorophyll a, b, Carotenoids	Oils and leucosin	*Ochromonas, Dinobryon, Mallormonas, Synura, Rhizochrysis*
Bacillariophyceae (Diatoms)	Cholorophyll a, c, Carotenes	Leucosin and Fats	*Cyclotella, Rutelaria, Corethron, Navicula, Bacillaria*
Cryptophyceae	Chlorophyll a, b-Carotenes, Xanthophylls	Starch	*Falcomonas, Rhinomonas, Plagioselis, Chilomonas, Cryptomonas*
Dinophyceae (Dinoflagellates)	Chlorophyll a, c Carotenoids, Xanthophylls	Starch and oil	*Dinophysis, Polykrikos, Gonyaulax, Gymnodinium*
Chloromonodiae (Raphidophytes)	Chlorophyll a, b Carotenes, Xanthophylls	Oils	*Vacuolaria*
Euglenophyceae (Phototrophic-euglenids)	Chlorophyll a, b	Fats and paramylon	*Euglena*
Phaeophyceae (Brown algae)	Chlorophyll a, Xanthophylls	Laminarin, mannitol and fats	*Pelagophycus, Laminaria, Pelvetia, Sargassum*
Rhodophyceae (Red algae)	Chlorophyll a, phycocyanin, phycoerythrin	Floridian starch or glucan	*Vacuolaria, Gomyostomum, Chattonella, Psammamonas*
Myxophyceae (Blue-green algae)	Chlorophyll a, b-carotene, Phycocyanin phycoerythrin c-	Glycoproteins, droplets of oil	*Chloroccus, Darmocarpa, Pleurocaspa, Oscillatoria, Stigonema*

2.4.1 Dual Role of Microalgae:

2.4.1.1 As A Bioremediator and Producer of Valuable Lipids:

Microalgae provide an efficacious way to solve the tertiary and quaternary treatments problems of dairy wastes due to having the capability to utilize phosphorus and nitrogen for their development purposes.[20] This type of bioremediation/ phycoremediation accompanied with the manufacturing of beneficial microalgae culture, which could be employed for numerous purposes. Earlier researches suggested that cultivation of microalgae for the production of lipid components from dairy waste, had provide better results than any other wastewater used as microalgae cultivation medium.[1,9,22] Microalgal strain which contain large number of fatty acids, like polar and non-polar lipids, and also flourishing in the native domain of dairy waste effluent have yet to be utilized for the manufacturing of bio-fuel.[24]

Many microalgae cultures displayed high growth on the diluted effluents from dairy. Moreover, the microalgae have the ability to utilize sunlight for its photosynthetic activity and combine water with carbon dioxide in order to make their food autotrophically, further; it converts the environmental carbon di oxide to oxygen. Due to this ability of microalgae, they have been used to remediate the dairy waste as the nutrient sources for their growth, and to reduce the concentrations of inorganic substances like; nitrogen, phosphorus and other organic substances. It has been reported that the microalgae are the better source for manufacturing of bio-fuel due to its elevated photorespiration activity, and rapidly growing biomass activity.[3,9,34] It has been documented that the microalgal species are the better source for manufacturing of bio-fuel due of its elevated photorespiration activity, and rapidly growing biomass activity.[35]

Microalgae *Chlorella protothecoides* displayed good growth in a pre-treated whey solution. Since the use of microalgae approach alone was not sufficient for satisfactory removal of pollutants,

therefore using hydrolysis and flocculation-struvite methods, the treated whey with 25:75 dilutions exhibited sufficient pollutants removal by designing biochemical approach. Using this approach, approximately up to 99% or 91–100% lessening of inorganic and organic contaminants was attained after nine days of growth of microalgae.[17] The similar research work, documented by Yong et al. 2019, they observed that freshwater chlorophyte microalgae *Chlorella* sp., UMACC344 was able to produce higher lipid amount in dairy waste, which could be utilized as potential biofuel.[36]

The microalgae strain which contains large amount of fatty acids, like polar and non-polar or neutral lipids, and also flourishing in the native domain of dairy waste effluent have yet to be used for bio-fuel production. Micro algal feedstock is one of the best medium for bio-fuel production and as the substitute of fossil fuel and also proved beneficial to eradicate greenhouse gas emissions.[37] Micro algal species collected from the dairy waste indicated great prospective for low-cost cultivation and producing lipid utilizing dairy waste, without modifying the procedure. It was reported that the *Chlorella* sp. showed good biomass production when grown on dairy effluent, reduced its COD, and nutrients.[20] 18S rDNA sequencing, was used for the identification purpose and sequences were analysed via BLAST representing the higher similarity with *Chlorella* sp. ArMoo29B (98% similarity). In another research, *C. pyrenoidos*, *A. ambigua* and *S. abundans* cultivated in dairy effluent, resulted enhanced microalgal biomass production along with good amount of lipid production. In this study, lipid composition were10.36%, 13.13%, and 16.93 % of DCW in *C. pyrenoidos*, *A. ambigua* and *S. abundans* respectively, after twenty-five days of inoculation in dairy effluent.[38] The microalgae reduced BOD and COD, trace elements and other nutrients, thereby remediated dairy waste water.[4] There is another research also showed that the higher amount of lipid production in *Scenedesmus sp*. when grown in dairy wastewater (507.81 ± 19.09 mg g−1) and also achieved high percentages of nutrient removal rate 97.07% and 88.41% for phosphorus and

nitrogen, respectively.[39] Another study also shown enhanced lipid accumulation 79.962 and 57.870 mg L^{-1} day^{-1}, respectively when cultivated in concentrated and artificial dairy effluent by *C. sorokiniana.* The fatty acid profile, revealed that it contains 40% saturated lipids while, 33% unsaturated lipids.[40]

Chlorococcum sp. RAP13 cultured in dairy effluent, produced great quantity of biological mass and lipids, particularly under heterotrophic cultural conditions. Lipid production and biomass increased expressively under heterotrophic cultural conditions. The 31% lipid accumulation, when cultivated mixotrophically, while, heterotrophically resulted 42% lipid production. Higher amount of lipid production by these micro algae makes it a suitable candidate for biodiesel production. Moreover, use of dairy effluent as a resource of energy biomolecules and organic carbon removal by micro algae from the effluent in terms of BOD and COD.[41]

Kothari et al. (2013) cultivated *Chlamydomonas polypyrenoideum* on dairy wastewater, which resulted in considerable lessening of nitrogen (90%) and phosphate (96%) on 10^{th} and 15^{th} day respectively. Biomass of microalgae after 10^{th} day treatment exhibited 42% (w/w) lipid contents, which was analogous to the bio-oil extracted from other natural resources as per FTIR results analysis.[42] These outcomes proposed the possible usage of microalgae for biodiesel production as well as phyco-remediation of the dairy wastewater.[8]

2.5 Lipid Producing Microalgal Strains:

Many microalgal strains have been examined for their lipids and hydrocarbons compositions, approx 25-50 % of dry biomass including components of lipids. Some microalgae such as species of *Nannochloropsis, Neochloris, Chlorella, Dunaliella, Crypthecodinium, Chlamydomonas, Cylindrotheca, Isochrysis, Nannochloris, Nitzschia, Schizochytrium,*

Phaeodactylum, *Porphyridium*, and *Tetraselmis* have been recognized for containing certain classes of polar and neutral lipids.[8]

Table 2.2. Some Lipid Containing Micro-Algal Strains

S.No.	Microalgae	Lipid Content %	References
1	*Schizochytrium sp.*	30–60%	[43]
2	*Botryococcus braunii*	25–45%	[44]
3	*Neochloris oleoabundans*	25–44%	[45]
4	*Nannochloropsis sp.*	31–68%	[46]
5	*Nitzschia sp.*	45–47%	[31]
6	*Chlorella sp.*	35-58%	[47]
7	*Scenedesmus sp.*	40-68%	[48]
8	*Chlococcum sp.*	40–67%	[49]
9	*Cylindrotheca sp.*	20 -35%	[50]
10	*Dunaliella sp.*	36-47%	[51]
11	*Chlamydomonas reinharditi*	55- 65%	[52]
12	*Phaeodactylum triconutum*	18- 57%	[53]
13	*Chlamydomonas sp.*	21-27%	[54]
14	*Pavlova salina*	12-30%	[54]
15	*Phorphyridium*	9-14%	[54]

2.6 Biosynthesis of lipids in microalgae:

The green microalgae generally synthesis two categories of lipids as free or neutral fatty acids and triacylglycerols. Neutral fatty acids are synthesized in chloroplast while triacylgylcerols are, in smooth endoplasmic reticulum.14–20 carbon chains fatty acids are utilized in the preparation of biofuel and long carbon chains of 20 or more carbons which employed in food industry.[55,56] Microalgae produce and store higher quantity of fat in them as triacylglycerides or oil droplets in

the various cell organelles. The conversion and accumulation of lipid content, varies according to cultural conditions and microalgal strains.[53,57,58] Biosynthesis of lipid in microalgae depends upon the production of carbon compounds by photosynthetic reaction. It includes light dependent and independent reactions. In light dependent reactions, oxygen, ATP and NAD(P)H are generated while in dark phase or Calvin cycle, a compound with three-carbon (glyceraldehyde-3-phosphate) is produced, which changes into acetyl Co enzyme A via glycolysis. This acetyl CoA is the main substrate for biogenesis of lipid and antecedent for proteins, nucleic acids, carotenoid and carbohydrates etc. The transformation of acetyl Co enzyme A to malonyl Co enzyme A is the foremost and confide path in synthesis of lipids.[59]

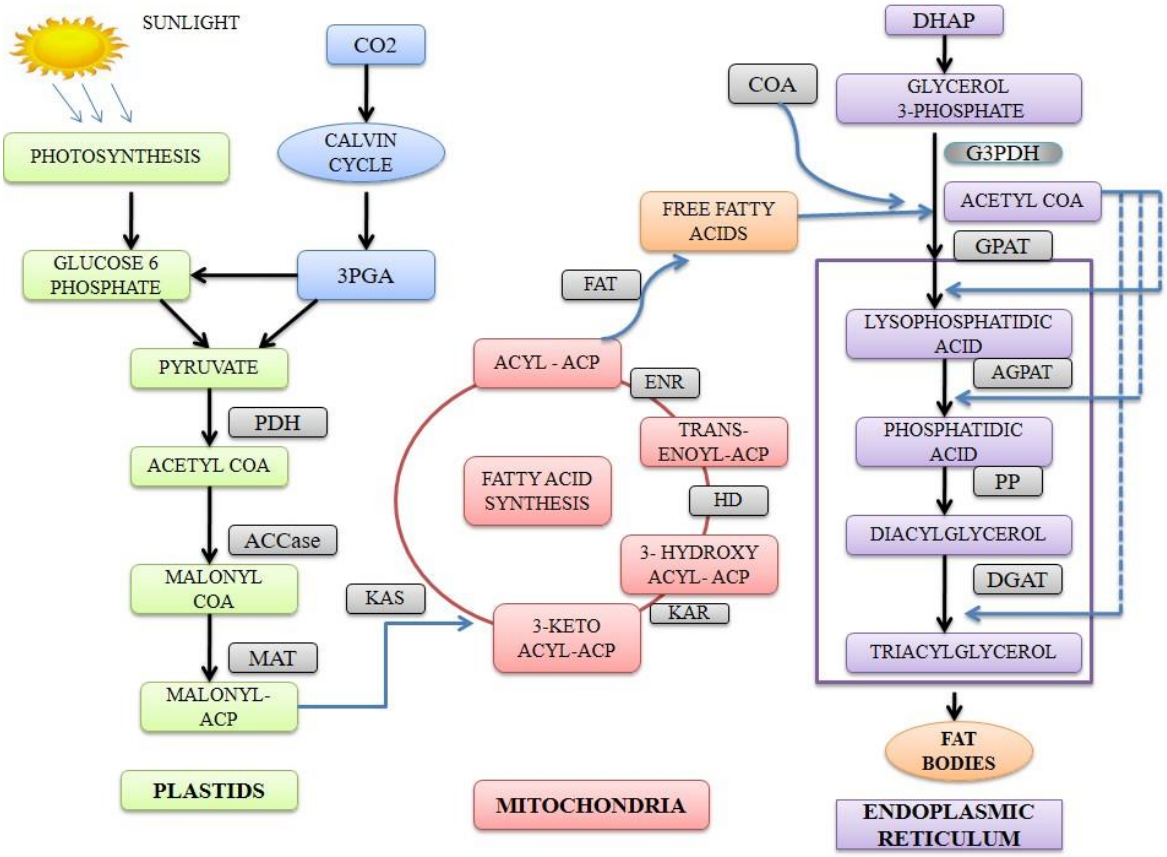

Figure 2.1. Biosynthesis of Lipids in Microalgae

ACP, acyl carrier protein; PDH, pyruvate dehydrogenase; CoA, coenzyme A; AGPAT, acylglycerophosphate acyltransferase; DGAT, diacylglycerol acyltransferase; ACCase, acetyl-CoA carboxylase; DHAP, dihydroxyacetone phosphate; FAT,fatty acyl transferase; ENR, enoyl-ACP reductase; MAT, malonyl-CoA; GPAT, glycerol-3-PO_4^{-3} acyltransferase; G3PDH, gycerol-3-PO_4^{-3} dehydrogenase; HD, 3-hydroxyacylACP dehydratase; KAS, 3-ketoacyl- ACP synthase; KAR, 3-ketoacyl- ACP reductase; ACP transacylase; PP, phosphatidic acid phosphatise; TAG, triacylglycerols

2.7 Major factors affecting microalgal growth:

Because of having many advantages such as non-arable land cultivation and capability to grow in waste water sources, Microalgae have become an auspicious feedstock for the generation of value-added compounds which used in the field of food, pharmacy & biofuels. But due to some limitations microalgae cultivation is a costly producer, even though it has become the most favorable choice for various industries such as nutraceuticals, pharmaceuticals, biofuel food & feed, biofuel etc. To make the process of microalgae cultivation viable and cost effective, for the accumulation of bio-suitable compounds should be enhanced. To optimize the cultivation conditions and abiotic & biotic growth factors which directly affect microalgal growth and accumulation of bio suitable value-added compounds (carbohydrate, protein, & lipids). This is the only technique to achieve the target of enhanced biomass and lipid yield. Microalgae growth directly affected with the supply of nutrients (macro and micronutrients), pH and salinity, temperature, light intensity, CO_2 concentration, and finally mixing.[60] The optimal environmental conditions are playing the key role for magnifying biomass and lipid productivity of microalgae.

The selection of micro algal strains is equally important, which used as a feedstock for several industrial applications.[61]

2.7.1. pH:

Microalgal growth is directly affected by the pH and salinity of the culture medium. Change in the pH of culture directly, affects the ion concentrations, which influence the microalgae culture; to promote or kill the microalgal growth. Hence, the pH has not only great impact on microalgal growth but also affect the amount of dissolved oxygen and carbon dioxide in the medium.[62] various factors viz. temperature, composition of buffer, levels of dissolved CO2, and metabolic activity of microalgae has straight impact on the pH of cultivation medium. The common range of pH for various microalgae species, falls between 6.0 to 9.0 i.e. slightly acidic to slightly basic in nature. But the levels of pH tolerance may vary according to species, which affect the growth and development of microalgae.[63] In photosynthetic reaction, during CO2 fixation, the hydroxide ions pile up in the cultivation medium, which gradually enhance the pH. pH usually rises due to the assimilation of carbon dioxide and no supply of CO_2 from outside, this can make the environment too basic (due to CO2 depletion) and sometimes it reaches up to 10- 11 or more. If, DO in the culture medium is low, it creates the acidic environment (accumulation of CO2). The chemical equilibrium turned towards the generation of CO2, when decrease the pH. Carbon dioxide is the most preferred sources of carbon by microalgae culture. The pH of cultivation medium also influences by the addition of nitrogen source into the medium. If ammonium ion (NH_4^+) is added in the growth media, pH enhances by reducing the value of available nitrogen for microalgae. Ammonium ion assimilation will be lowering the pH while assimilation of nitrate ion enhancing the pH levels. Phosphorus ion concentration, in cultivation medium also affected by raise the pH,

as it can enhance the phosphate precipitation with minerals as salts like; calcium phosphate, or aluminium phosphate, or iron phosphate etc. and reduction occur in the available phosphorus concentration in culture medium for microalga. Hence, phosphate assimilation increases the pH in the culture medium, reducing nutrient uptake and growth. The microalgae straightly affected by the change of pH, in culture media. This might be happening due to the inactivation of enzymes, which are highly pH sensitive. As microalga cell's cytoplasm is neutral to slightly basic in nature i.e. 7.0-8.5.[64] Consequently, adverse pH range can disturb the functioning of various cellular organelles, which may create a heavy fall down to the culture. There are several research studies which proves that the result of pH on microalgae dry cell weight and lipid production in culture media. Hence, production of biomass and cellular metabolism of microalgae mainly based on the pH.

Tripathi et al. 2015 concluded that an optimal pH for *Scenedesmus sp*. was 8.0. They observed the impact of pH on the growth pattern of *Scenedesmus sp*. within the pH range between 7.0 to 10.0.[65] In another study, Munir et al. 2015 conducted experiment on 02 microalgae strains, *Oedogonium sp.* and *Spirogyra sp.* to examine the effect of pH between range of 6.5 - 9.0. They achieved higher growth rate, (biomass) by both species at pH 7.5. [66] In another study, Wu et al. 2016 also observed the impact of pH on the biomass of *Scenedesmus sp*. within the broad range of pH between 5 to 11 and concluded that, microalgae growth has not much influenced by varying pH values of 7, 9 and 11, in culture medium.[67] An another research conducted by Yang et al. 2016, also concluded that *scenedesmus obliquus* flourish very well at the pH 8.0 but decline when pH changes towards highly acidic or basic.[68]

In dairy waste water, the pH is varying between highly acidic to alkaline i.e. around (3.0 to 12.0). low pH values can be often arising in DWW because of the appearance of mineral acids such as hydrochloric and sulphuric acid etc. which reduces the pH. While high pH of effluent attributed

by salts like sodium carbonate, sodium chloride and detergents or cleaning agents. when the level of carbon dioxide reducing in culture by microalgae, gradually enhance the pH shifted toward the neutral. Bicarbonate ions are the major source of CO_2 in dairy waste which controls the pH. Rastogi et al. 2017, studied that when bicarbonate ions are in majority, the optimal range of pH falls between 6.0 to 10.0, for photosynthetic reaction.[69] The photosynthetic oxygen yield also affects the pH of culture medium. Chlorella sp. showed the maximum growth, when pH was maintained between 7.5 to 8.0. while in highly acidic (3.0 to 6.0) and highly alkaline (8.5 to 9.0) obtained reduced growth in both conditions.[20] In dairy wastewater *Tetraselmis sp.* grow well between the range 4.0 to 8.0. As microalgae have great tolerance toward the change in pH.[1] Hence, microalgae have the ability to survive in the wide range of pH by acclimatizing itself in adverse cultural conditions. But the most of microalgae prefer neutral to slightly alkaline pH to flourish in a better way. and all species of microalgae have the restricted optimal pH range[1].

2.7.2 Salinity:

During the cultivation of microalgae, salinity is considered another important factor, which need attention. Salinity creates a stress in microalgae culture which directly impact, the growth and lipid production of microalgal cells. It was reported that salinity enhances the lipid production while reducing the cell growth. This is the significant factor, in the selection of microalgal strain. Microalgae which grow very well in saline conditions and accumulated great amount of lipid, considered the best strain for cultivation.[70] Many microalgal strains are having exceptionally high tolerance for salinity. The impact of salinity on microalgae growth was studied and resulted that if salinity reduces, then enhances cell growth. A study on *Scenedesmus almeriensis* proved the higher

tolerance towards 0.1M concentration of sodium chloride, and resulted maximum biomass production, when compared with freshwater culture media.[71]

For measuring salinity, various salt (sodium chloride) concentrations was utilized viz. 0.05, 0.3, 0.6, 1.0, 2.0 and 3.0 M. in this study, *Scenedesmus sp.* showed the maximum biomass yield at the 0.05 M of salts concentration while resulted reduced growth at higher salt concentrations. It was concluded that low salt concentrations i.e., 0.0 Molar to 0.5 Molar were suitable for the flourishment of *scenedesmus sp.* [72] In another study conducted on *Nannochloropsis salina* was cultivated in various salt concentrations (ranges between 10 to 58 ppt). It accumulated higher biomass at 22 ppt and 34 ppt but nil growth at 58 ppt.[73] An another study conducted on *Mesotaeniumsp*. *Tetraedron* sp., *Desmodesmus armatu,* and *Scenedesmus quadricauda,* these four microalgae were cultured at different salt concentration range between 2.0 to 18.0 ppt. among these, *Desmodesmus armatus* showed maximum salinity tolerance by flourishing at 18 ppt. while on the other hand *Mesotaenium* sp., showed the least tolerance for salinity. It inhibited to grow when concentrate-on raise up to 11 ppt. Therefore, this study revealed that 2 ppt to 8 ppt. salinity range was the best for the growth of all the three microalgae i.e. *Mesotaenium* sp., *Tetraedron* sp., *Scenedesmus quadricauda.*[74]

In open pond culture, salinity trend to increase because of severe evaporation, in the medium. Some fresh water microalgae species, are very sensitive towards the high salt concentration in their growth medium. On the basis of salinity tolerance capacity, microalgae can be differentiating in to three groups; oligohaline, mesohaline, and polyhaline. Oligohaline; these types of microalgae, can easily grow with low salt concentration (0.5 to 5.0 g·kg−1) in their culture media. Mesohaline, are able to grow well in moderate salt concentration i.e. (5.0 to 18.0 g·kg−1), although polyhaline, have the tendency to flourish in higher salt concentrations ranges between 18.0 to 30.0 g·kg−1.[75] Growth rate ceased gradually, when maximum salinity was occupied in culture. It was observed that without

sodium chloride, growth not occurring in the culture while at higher salt concentration also inhibited the extension of microalgae. However, microalgae have the capability to maintain the cell constitution against the changes in external environment. In this phase, microalgae reduce the growth rate, in order to maintain cell functioning conveniently, without any alterations in the cellular constituents. This is the process called, homeostasis in microalgae. Still, there are some strains of microalgae that allow to make alterations in their cellular composition, against the adverse external conditions through acclimatization. Salinity is the key factor which is solely responsible to maintain cytoplasmic ion concentration or homeostasis in microalgae cells.

2.7.3 Temperature:

Temperature is considered a crucial environmental factor, which influence microalgae cell size, growth rate, biomass, biochemical composition (types of accumulated food) and nutrient necessity. The temperature of microalgae cultures raises due to the absorption of heat by radiation from the available source of light. It is mandatory to maintain the temperature of growth media, in order to achieve optimized growth of microalgae. Photosynthesis of microalgae affect the cellular division which directly relate with temperature. All the reactions are enzyme dependent and enzymes are activated by temperature and hence affect the biomass and lipid productivity. There are some studies, used Arrhenius theory to explain the relation between growth rate and temperature. Accordance with this theory, cell growth twice, at every 10 °C raise the temperature, until reached at optimum temperature. Cell growth will be continuing to decline immediately after reaches this point. The growth of microalgae declines due to excess heat absorption ceased the photosynthetic activity. The main reason behind the whole process is, the denaturation of proteins which ultimately inactivate the enzymes The microalgal biomass and lipid production increase with

increases the temperature, until reached an optimum limit. This ranges fall between 15–25 ºC for most of the microalgal species. When temperature increases beyond the optimum limit, leads to immediate reduction in culture. Microalgae culture are highly sensitive towards the light intensities and hence easily photo inhibited in higher light intensities at cold temperature. In cold temperate regions, microalgae creating operational challenges to operate open pond-based bioremediation system at low temperature. But microalgae are capable to tolerate high light intensities, at optimum temperature without getting photo inhibited. Hence, microalgae culture significantly affected by seasonal variations, which create differences in temperature during the cycle of day and night.

There are several documented studies which revealed the impact of temperature on microalgae growth and biomass production in culture medium. According to the study, conducted on *S. almeriensis* resulted that 35˚C, is the optimal temperature while cultures had no growth at the temperatures 45˚C.[76] Several researchers showed, temperature has a favourable effect on microalgal growth and their lipid production. In another research, evaluated that *Chlorella protothecoides* accumulated optimum lipid yield of 274.15 $mgL^{-1}d^{-1}$ at 28.63˚C and pH was maintained at 6.5.[77] In another study, four microalgal species (*P. subcapitata, Microcystis aeruginosa, Synechocystis salina* and *C. vulgaris*,) were used to study the impact of temperature on nutrients uptake and microalgae growth and. It was resulted that 25˚C was the optimum temperature for all the species.[19] According to the research conducted, on several species like; *Nannochloropsis, Neochloris, Chlorella, Spirogyra, Scenedesmus, Botrycoccus, Chlamydomonas, Haematococcus* etc. few species, of brown algae, blue-green, and red microalgae are having the capacity to grow in a broad range of temperature between 20˚C - 30˚C with a broad range of illuminance i.e.,33 - 400 μE·m−2 s−1.[78] This was also documented that, the cell metabolism disturbs at high temperature and cease the cell production due to enzyme inactivation.[79] The biochemical composition and nutrients

absorption in microalgal cells, are affected by the temperature alterations. Hence, microalgal strains must be selected according to temperature tolerance because this factor highly influenced the growth of microalgae.[80] In majority of cases, increasing temperature enhancing microalgae growth up to an optimum level, but declines with further increment in temperature. It was concluded that the temperature below 16 °C and above than 35 °C remarkably dangerous for microalgal culture. An analysis done by Adenan et al. 2013 that found *Chaetoceros calcitrans* and *Chlorella* sp. showed the highest biomass was obtained at temperatures of 25 °C by both microalge species (0.35 ± 0.04) and (0.27 ± 0.02) per day respectively.[81] Another study resulted that, three species of *Dunaliella salina* which were collected from saline soil, attained the maximum biomass at temperature 22 °C.[82]

There are some exceptional cases, some species of *Dunaliella* sp. were capable to survive at a broad range of temperature falls between 0 °C to 45 °C. Among this one strain, *Dunaliella antarctica* was reported to survive at below zero temperature, while another *Dunaliella* sp., grew well at temperatures 40 °C. Even though, it was not reducing the biomass but sequentially continue to enhance carotenoid production. Another experimental study revealed that *Tetraselmis subcordiformis* flourished at 20 °C temperature when it was cultured at various temperatures between 15^0C to 35°C.[79] Giannelli et al. 2015 examined, the development of *Haematococcus pluvalis*, in the laboratory environment with varying temperature range of 20 °C, to 30.5 °C. the maximum temperature for achieving optimum biomass productivity was 30.5°C.[83]

2.7.4 Light Intensity:

Microalgae are photoautotrophs as they utilize sunlight for their food formation. They are highly efficient to absorb sunlight in the form of photon or packets and accumulated kinetic energy molecules such as ATP, FAD, NADPH etc. which utilized by the cell for generating stored material

in the dark. While they can also flourish in the dark by consuming organic nutrients from wastewater. water is hydrolysed to produce oxygen, in the light reaction, and during the period of dark reaction, CO_2 is absorbed by the cell via Calvin cycle. the microalgae accumulated food in the form of carbohydrates, lipids and proteins.[69] Microalgae growth and development is highly influenced by several light conditions. These are limitation of light, saturation of light, and inhibition of light. During the light limitation, microalgae growth rises with rising the light intensity. While, light saturation, is the situation when photosynthesis reaction decreases or inhibiting because of the excess absorption of photon due to higher electron turnover. In photo-inhibition, intensity of light again increased, which completely destroy the photosynthetic apparatus.[84] Moreover, many experimental studies documented that the irradiation or light intensity have a remarkable impression on microalgae biomass yield. Microalgae growth rate directly affected by the light intensity. According to the diversified microalgae, adequate light with correct wavelengths and intensity, is the major requirement for photosynthesis. During photosynthesis, microalgae require sun energy for producing ATP and NADPH, in the photochemical phase. Insufficient light creating photo oxidation while excess light intensity result photo inhibition which reducing the photosynthetic activity. Several studies revealed sunlight is the most limiting component which directly affects microalgae growth rate. Microalgae decrease the light availability for themselves, in dense cultures, due to self-shading. This is avoiding by proper mixing to provide light uniformly, to all the microalgal cells. The CO2 is first source of carbon which turned in carbohydrates by cellular organization of microalgae. This is the prime requirement for microalgae growth and survival. Hence, adequate amount of light supply is the absolute need for optimized microalgae cultivation system.[85] The light absorbed by the microalgal culture is directly associated with the carbon capturing by the cultures affect the growth. The photosynthetic rate determines the biomass of

microalgae; the irradiance is a direct factor which exposed culture internally. As light intensity raises, photosynthetic rate in microalgae also rises until it reaches up to the saturation point.[86] The excess light intensity cause photo-inhibition. above than this saturation point and culture is called photo inhibited. This type of irradiance, is termed as inhibition irradiance. The rate of photosynthesis is termed as saturated at the stretch of 100 to 500 μE·m−2 ·s−1 intensity, while between 50 - 100 μE·m−2 ·s−1 (average intensity) attained highest biomass productivity by many microalgae species.[87] The photo inhibition occurs at the light intensities above than 1000 μE·m−2 ·s−1 in several microalgae, while few are very sensitive, at lower intensities at 300 μE·m−2 ·s−1 or below this level, are called photo inhibited. The light intensity is the quantity of radiation entering a position from everywhere, at all wavelength. Although, microalgae only utilized Photo Synthetically Active Radiation (PAR), to perform photosynthesis, which range between 400 to 700 nm. The light source may be sun, LED light etc. However, the irradiance is not homogeneous inside the culture of microalgae, because of mutual shading. Hence, the outer surface of culture can be received high intensities, while complete dark inside the culture without exposer of light.[88] One more research, explored the effect of radiance on microalgae growth and absorption of nutrients by using four microalgal species namely; *P. subcapitata*, *Microcystis aeruginosa, C. vulgaris*, and *Synechocystis salina*. It was concluded that the maximum daily exposed irradiation was 208 μE·m−2 ·s−1 suitable for growth and lipid production to all microalgae.[89] It has been also documented that microalgae growth not only dependence on the intensity of light but also depends on photoperiod and its wavelength.[90] Microalgae required light source for their growth and other metabolic activities. The culture parameter such as source of light, its intensity, wavelength, photoperiod, and quantity directly impact the microalgae growth. Various artificial light resources can be utilized in microalgal culture, like; halogen lamps, light-emitting diodes, fluorescent lamps

etc. These light resources differing from one another in term of their cost, energy consumption, spectrum. By comparing all these features, LEDs and fluorescent lamps are frequently utilized light resources for microalgal cultivation. While LEDs has better control of light than the fluorescent lamps, which can be more beneficial for higher biomass production.[91] An enough intensity of natural light is not reaching at depth in the culture so that artificial lights such as LEDs are suggested to place internally in culture medium for improving the transportation of light and photons distribution there. In microalgae cultivation tanks, the stirring system are responsible for generating the light/dark (L/D) cycles. The biomass production and photosynthetic conversion in microalgae, depends on these light/dark (L/D) cycles.[92] In this research, it was also documented that photosynthetic activity and growth rate was enhanced up to 40% in *Chlamydomonas reinhardtii*, while using L/D cycles of lesser than 20 s. The difference in the wavelength of light has also create remarkable impact on microalgal growth. The blue light influences the gene expression in microalgae by triggering the metabolic pathways towards higher nutrient uptake, resulted larger cells but lower growth rates. While, red light can minimize nutrient adsorption but enhances the growth rates with smaller cells. On another array, chlorophytes cannot utilize yellow and green light efficiently because of the absence of phycobilins.[91]

An experimental study conducted on *Odontella aurita* under different irradiance capacities, 150 and 300 µmol $m^{-2}s^{-1}$ resulted that the strain was easily cultivated under 150 µmol $m^{-2}s^{-1}$, while, at early stages it was able to grew faster under high light radiation i.e.,300 µmol $m^{-2}s^{-1}$. The reason behind this was low cell density at initial phases which make allow microalgae to absorb an extra radiance under high light intensities.[93] In another research on *Neochloris oleoabudans,* showed enhanced biomass between 1.2 g L^{-1} to 1.7 g L^{-1} on increasing irradiance range between 50 to 200 µmol m^{-2} s^{-1}.[94] Another research work on *Isochrysis sp.* under various illuminations 1,458 µmol m^{-}

^{2} s^{-1}, 1,073 µmol m^{-2} s^{-1}, 840 µmol m^{-2} s^{-1}, 423 µmol m^{-2} s^{-1} and 0 µmol m^{-2} s^{-1} declared that an optimum growth rate was observed in 1,073 µmol m^{-2} s^{-1} irradiance. It was reported that alterations in dark and light cycles are responsible for maximum carbon fixation and growth in microalgae culture. Extreme exposure to sunlight could injured the microalgae cells whereas, insufficiency of sunlight also has negative effect on microalgae growth.[95]

A study on four microalgae species; *Synechocystis salina, Pseudokirchneriella subcapitata, Microcystis aeruginosa,* and *Chlorella vulgaris*, were conducted with various light intensities 36, 60, 120 and 180 µmol $m^{-2}s^{-1}$and under varying ratio of light: dark regime (10:14, 14:10 and 24:0). It was concluded that maximum biomass productivity achieved at continuous irradiance of 180 µmol $m^{2}s^{-1}$ for 24 h by all the species.[96] Another work, by using light emitting diode lights (blue, warm white, natural white, and red,) at several illuminance ranges from 50 µmol m^{-2} s^{-1}, 80 µmol m^{-2} s^{-1} and 110 µmol m^{-2} s^{-1} resulted that of *Chlorella vulgaris* produced maximum biomass with enhanced photosynthetic rate in warm white light of 380-760 nm wavelength of 80 µmol m^{-2} s^{-1} illuminance.[97] Teo et al. 2014 in another study, to see the effect of irradiance on *Tetraselmis* sp., and *Nannochloropsis* sp. Both species were grown under blue light of 420-470 nm wavelength and red light of 660 nm wavelength with 100 µmol m^{-2} s^{-1} illuminance along with 24:0 light-dark regime. It was resulted that both strain flourish well under blue light.[98] Another study was carried on *Pyropia haitanesis* under various lights such as (blue, red, fluorescent and green) with 100 µmol m^{-2} s^{-1} intensity, along with 12/12hour, light-dark regime resulted, maximum biomass obtained under fluorescent light.[82]

2.7.5 Macronutrient's concentration (Carbon, Nitrogen & Phosphorus):

The microalgae cultivation is directly depending on the amount of nutrient uptake which is explained using Michaelis-Mentis reaction as mentioned in the following equation;

$$\mu=\mu_{max}[S/S+K]$$

where μ = growth rate of microalgae, μ_{max} = optimum growth rate of microalgae,

S = concentration of nutrients, and K = half saturation constant.[99]

The biochemical constitution of microalgae, related on the Concentration of nutrient variations in culture medium. The macronutrients or the most essential nutrient required for photoautotrophic growth of microalgae are carbon (C), phosphorus (P) and nitrogen (N). Microalgae can store huge amount of energy molecules such as; carbohydrates, proteins and oils. Among these lipids or oils are highly concentrated form of energy, hence lipid is the best suited storage form for long-time. microalgae, responding towards the high nutrient concentrations by growing rapidly and storing carbohydrates, proteins and lipids. Carbon is the key nutrient of microalgae for getting enhanced lipids. Microalgae are capable to assimilate inorganic carbon (CO_2) and producing carbohydrate and lipids, during photosynthesis. The average stoichiometric formula of algae is $C_{106}H_{181}O_{45}N_{16}P$. Microalgae contain more than fifty percent of carbon content in it constitutes composition. According to the above-mentioned formula of microalgal biomass, this is appropriate to speak that approximately 50% of microalgal biochemical composition consist of carbon (C). Carbon is required in huge quantity, being the main element of all organic compounds, which produced by the microalgae, like; primary metabolites (carbohydrates, lipids, nucleic acids, and proteins), and other bioactive compounds (secondary metabolites). Microalgae can assimilate inorganic carbon through diffusion processes at the pH between 5.0 to 7.0 and at pH > 7.0 by active transport.[19] The supply of bicarbonates (HCO_3^-) and carbon dioxide are playing crucial role in

attaining optimum photosynthetic rate. In mixotrophic conditions, microalgae are having the capacity to utilize organic compounds like; acids, sugars, and alcohols. Microalgae culture need proper aeration to balance its carbon di oxide and oxygen level. In environment, the level of CO_2 in air is extremely less i.e. (0.033 %) which is not sufficient for maximum growth. Hence, to enhance the microalgae biomass, culture should provide 1 to 5 % CO_2 along with air. During heterotrophic mode of nutrition, microalgae can also utilize some organic source of carbon sources like; glycerol (3C) glucose (6C).

Nitrogen is the next essential macronutrients for promoting microalgae growth and regulating metabolic activities. lipid and fatty acid yield directly affected by amount of phosphorus and nitrogen in microalgae culture. Microalgae having the capacity to assimilate nitrogen in various forms viz. nitrate (NO_3^{-1}), nitrite (NO_2^{-1}), urea (NH_3) and ammonium (NH_4^+). Microalgae can absorb high amount of nitrogen and phosphorus (up to 40–60% of their dry weight) for synthesis of various proteins or enzymes and nucleic acids (DNA and RNA).[63] Usually, the amount of nitrogen in culture media strongly impact the biomass and biochemical constituents of microalgae. However, nitrogen depletion in microalgae culture create reduction in biomass while expansion in lipid production. When compared with ammonium ion, NO_3^- is the most utilized type of nitrogen in microalgae cultivation, due to very stable nature, even towards the pH shift. In addition, ammonium ion (NH_4^+) in higher quantity, more than 25 µM are very harmful for microalgae culture. Hence, NO_3^- is the most favorable form of nitrogen for microalgae cultivation. Yang et al. 2018 concluded that nitrogen insufficiency in *Chlamydomonas reinhardtii* culture media, reduced biomass accumulation up to 31.7%, with simultaneously rise in lipid yield up to 93%. They coupled nitrogen deficiency with the increase lipid yield up to 113.46 ± 1.78 mg/L[52]. Hence, resulted that higher nitrogen concentration favors optimum biomass yield, while lower nitrogen concentration

enhances higher lipid production.[100] Microalgae might be capable to produce some alterations in their lipid metabolic passage, in nitrogen-deficient culture and accumulating triacylglycerides (TAGs) largely. These TAGs are stored in microalgae cytoplasm as energy source. In nitrogen deprived conditions, the thylakoid membrane declines and acyl hydrolase enzyme activates which promote hydrolysis of phospholipids. Therefore, an enhanced intracellular fatty Acyl-CoA.[101] Moreover, nitrogen deprivation in microalgae cultivation, is an effective way to produce optimum lipid. Nitrogen-deprived culture can generate two-fold lipids when compared with nitrogen-sufficient culture.[102]

Nitrogen is advised to be the structural units for proteins, amino acids and nucleic acids while phosphorus is the major constituent of phospholipids and nucleic acids. If these macronutrients are available in adequate amount, then leads to alter the metabolic pathways.[103] It was documented, if the ratio of nitrogen and phosphorus beyond 16, at this point, P assumed to be the controlling factor and N concentration requires to be directed to enhance the growth state of microalgae.[103]

Phosphorus is another important nutrient which highly involves in microalgae cultivation (biomass and lipid yield). It is also the key component in several metabolic reactions like; photosynthesis, energy transfer, and signal transduction etc.[52] Phosphorous is very necessary for the synthesis of cellular constituents like; energy molecules (phospholipids, and ATP), nucleic acids (DNA, RNA). Phosphorus can be absorbing by the microalgae for their growth and development as polyphosphate or orthophosphate.[104] Polyphosphate form of phosphorus provide protection against metal toxicity in microalgae culture as this can bind with toxic heavy metals and converted them into a detoxified form of complex. An experimental study resulted that *C. reinhardtii* was easily assimilated copper (Cu) and cadmium (Cd) and succeed to viable with metallic toxicity. While the culture was supply with highly rich polyphosphate components in to it.[105] Still, few

microalgal strain could succeed to accumulate high phosphorus amount as they need for their growth. Microalge can be able to converted this phosphorus into ATP under scarcity of nutrients. An energy form, is required for the assimilation of polyphosphate into the microalgae and conversion of phosphorus to polyphosphate. Photosynthetic process and respiration are the main source of ATP production.[106]

The optimum level of phosphorus was required by microalgae culture for achieving enhanced biomass. It was estimated that phosphorus concentration about 0.2 mg L^{-1} is the most favored value, by many microalgae species. While lower than 0.045 mg L^{-1}, or higher than 1.65 mg L^{-1}, inhibits microalgae development.[107]

Another research, which conducted on and *Nannochloropsis oculata* and *Chlorella vulgaris* revealed that on decreasing the nitrogen content, the lipid yields enhanced. While the microalgae biomass was unaffected.[108] In another study, it was concluded that nitrogen deprivation promoted to store a high number of carotenoids and astaxanthin by *Dunaliella* sp. Hence, phosphorus and nitrogen were suggested to be controlling nutrient for microalgal growth and for the synthesis of valuable byproducts.[103] P scarcity in *Scenedesmus* sp. (between 2.0 mg L^{-1} to 0.1 mg L^{-1}) create enhance lipid concentration from 23% to 53%.[103] In another experimental study, *Nannochloropsis salina* resulted extreme rise in lipid content i.e., from 34.6% to 59.3% by 75% decrease nitrogen in the medium. While, biomass significantly decreased.[109]

In dark reaction, carbon di oxide (inorganic form of carbon) is absorbed by the microalgae from the culture media and produce 3-phosphoglyceraldehyde. After that metabolic pathway, move towards the synthesis of biomolecules, which stands on the ratio of C: N. At the beginning phase, microalgae exceed the internal C: N ratio due to elevated photosynthesis hence, producing various

proteins and amino acids. Although, as biomass increases, the available N in the culture medium is utilized and move the biosynthesis pathway from proteins to lipids.

2.7.6 Micronutrient's Concentration:

Along with some macronutrients, microalgae also need adequate mount of mono and bi valent ions including Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, Cl^{-} and $SO4^{2-}$, and few trace metal ions of (Mn, Zn, Fe, Co, Mo and Cu) also required for proper progression. Trace metals viz. Fe, Mn, Co, Zn, Ni and Cu are needed by microalgae for their metabolic reactions. If they are not available in appropriate amount, then growth of microalgae may be slow or inhibited. An experimental work, concluded about the biomass of *Nannochloropsis oculata* enhanced by the supply of few micronutrients such as Mn^{2+}, Fe^{3+}, Co^{2+}, Zn^{2+}, Cu^{2+}, and EDTA (ethylene diamine tetra acetic acid). The photosynthetic rate of microalgae enhancing by rising the carbon di oxide uptake, when trace elements were added into the culture medium.[110] The pH is directly influenced by ions concentrations, in the medium because these metals can bind to one another, precipitate out by making various salt combinations in medium, sometimes create toxicity. Adding EDTA (ethylene di amine tetra acetic acid) in to cultivation medium, balanced the pH, so that keep metal ions in dissolved conditions, or make ions available to microalgae. The ions are able to make the chelated compounds with EDTA, when free ones decreasing in the media, ions are gradually released from these compounds and helped to maintain pH while the free ions absorbed by the microalgae. This combination act as a "metal ion buffer", which maintaining the appropriate micronutrient ions concentration in growth medium.[111] A study resulted that lower Fe concentrations affect the growth rate of *Chlorella vulgaris* many times.[112] Manganese depletion also resulted arrested growth pattern.[113] The depreciation of growth under nitrogen and manganese starvation in *N. oceanica* showing arrested cell cycle. Mn, depletion

in *Chlorella vulgaris* revealed similar result as in *Nannochloropsis oceanica.* Manganese resulted up to 20% reduction in growth rate.[114] Manganese concentration directly impact the growth of *N. oceanica* because it is the constituent of various proteins, metalloenzymes, and vitamins which regulate the all microalgal metabolic pathways. The findings of one research are also agreed with familiar results of N and Mn starvation on microalgae growth rate, again confirmed that the biomass of *N. oceanica* was negatively impacted by these two constituents.[115] Hence, all the microalgae culture adversely affected by the deprivation of these micronutrients.

2.7.7 Mixing:

The light and temperature are two crucial factors which impact directly microalgae growth. These are interdependent on each other, however, controlling both can be costly and difficult. Mixing is one of the simplest and potential way to make sure consistent dispersal of light and temperature into the whole depth of culture. Shading is the major problem which inhibits the light to reaching all microalgae cells. Mixing or blending is the only cost-effective solution, which remove this problem completely. Gas mixing is the low cost but effective way to provide, Proper mixing or blending to the microalgae culture.[107] Another research showed that the impact of proper blending on *Spirulina platensis* by three methods (blending with bubbling air into the column, blending with a magnetic stirrer inside the column, and continuous stirring via circulating pump) revealed that the biomass of *Spirulina platensis* was maximum when mixing done with a bubble column. Although, analogous value was obtained for stirring and blending too (0.0122 h^{-1}, 0.009 h^{-1}, and 0.010 h^{-1}) respectively.[116] One more work examined the impact of blending on *Desmodesmus communis* resulted that the biomass production significantly enhanced when comparison between mixed and un-mixed cultures.[117] A study on *Scenedesmus obliquus* employing three methods;

aeration, stirring, and amalgamation of both resulted that increased biomass yield with mixing method as compared to the others.[118] Hence, many studies exposed that mixing, carbon dioxide consumption, and mass transfer could be the decisive element that control growth of the microalgae culture. stirring tends to enhanced if the flow rate of gas is hiked, consequentially the availability of CO_2 rises and hence, the growth also increased. Biomass yield, and CO_2 assimilation were controlled either via, CO_2 levels in the liquefied phase or by CO_2 transmit from the gaseous phase. However, additional experimental work towards this region should be performed to provide a finer understanding regarding the impact of mixing through dissolved gases on microalgae growth and development.

2.7.8 Screening and Selection of Microalgae Species:

The Screening of Microalgae species included various steps. The introductory step in this domain is the isolation, which is generally performed to attain pure cultures (single species of microalgae). Micropipette and microscope are the two main equipment which involves in this step. Currently, the isolation of single microalgae culture is the most commonly utilized technique, due to its economic and a wide range of species applicability. This technique is used with the association with an advance technology, flow cytometry. Flow cytometry is based on the concept of fluorescence for cell separation. The desired microalgae species are isolated from the assortment of microalgae strains available in wastewater. An innovative approach using bioinformatics give an entrance for inventing new microalgae strains. The steps usually included DNA/RNA isolation and purification, amplification through PCR technique, and gene sequencing.[119] By applying the above discussed steps, isolation and identification of microalgae strains i.e., first level of screening was done. After completing the first level, continue to entering into the next level of screening, which help to choose a certain growth medium that should be utilized for producing enhanced microalgae

biomass. The commonly used media for fresh water microalgae, are BBM medium and BG11 medium while, marine microalgae strains used F/2 media. After selecting the suitable growth medium, microalga species move forward the next level, which evaluate the growth and development of microalgae strain in the deprivation of various elements like; carbon dioxide, oxygen, and constant pH level. Those microalgae Strains that flourishingly surpass all screening levels, are considered to be potential strains for commercialization. Hence, for future prospects profit-oriented implementation of microalgae cultivation, with biological-prospecting will be determining.[80]

2.8 A Source of Value-Added Lipid By-products:

A great advantage of microalgae, its capability to flourish on wastewater streams by utilizing the nutrients present there. Therefore, it is environmentally safe with extremely low freshwater utilization. Apart from this, microalgae can also be able to eradicate carbon dioxide and flue gasses from the environment which discharged by several industries. consequently, microalgae have become a sustainable raw material for the bio-refinery manufacturing for future fuels.[9] Microalgae contain severe therapeutic attributes in their composition, which makes them suitable candidates for synthesizing biochemical for pharmaceuticals and nutraceuticals. Assembly-line production of microalgae, for biofuel production was evaluated to provide 20 times higher amount of biodiesel than any traditional bio-diesel crops. Therefore, the microalgal biomass, could be used as a potential raw material for obtaining valuable byproducts. Although, the success in this domain, depends on the variety of conditions viz. understanding of microalgal strain and their behavior under specific environmental factors and desirable final products The following characteristics make microalgae an attractive resource among researchers. First, microalgae can be able to produce

some various valuable byproducts such as lipids, proteins, carbohydrates, antioxidants etc. and their amount would be increased under stressed situations.[120] Next, these microalgae have the capability to synthesized byproducts after the insertion of stable isotopes (13C and 15N) into them. Third, very few species of microalgae have been recognized till date, creating them a curious regime to be explored. Although, the downstream processing utilized in manufacturing these valuable byproducts. The most important way for decreasing the processing value, is to generate several byproducts in every cycle (Bio refinery concept).[120] Microalgal dry cell mass has been successfully utilized for a variety of valuable byproducts.

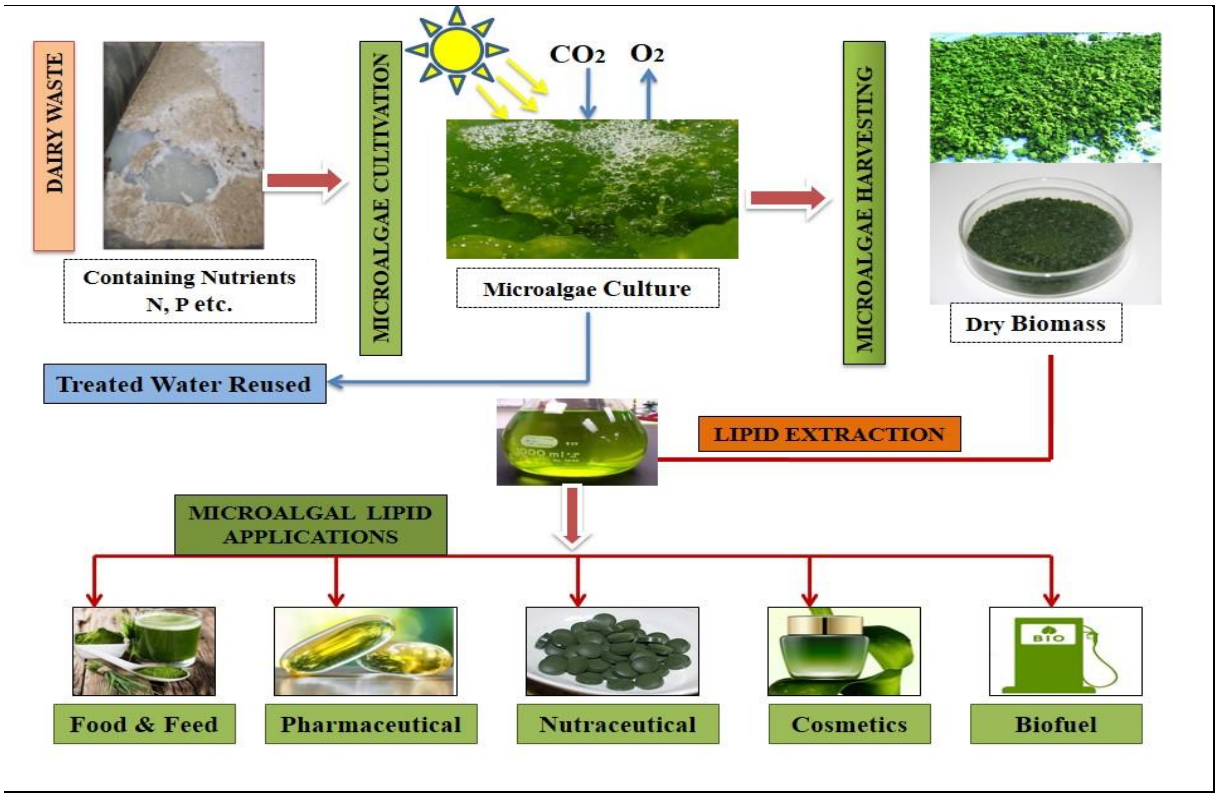

Figure no 2.2. Production &Applications of Microalgal Lipids

2.9 Applications of Microalgal Lipids:

The most prevailing experimental study, neighboring the microalgae, is targeting the biosynthesis of lipids, which utilized for the manufacturing of value-added compounds. Microalgae are having abundant amount of lipids, high density lipoproteins, oxylipins, carotenoids, proteins and carbohydrates etc.[121] Many micro algal strains are capable to synthesize free fatty acids or long chain fatty acids, with yields between 25 to 75% of their total lipids. Many fresh water microalgae such as *Scenedesmus sp.* and *Chlorella sp.* etc. and seawater microalgae *Thraustochytriacea sp.* and *Crythecodiniacea sp.* are currently utilized for the manufacturing of PUFAs.

Some microalgae strains have an incredible adaptability to survive in extreme environmental conditions, and also have the huge potential for producing several advantageous by-products. Nowadays, the micro algal species are considered as lipid bio factories because of producing various kinds of lipids with remarkable applications in several fields like; food and feed manufacturing industries, biofuel production, cosmetics, and pharmaceuticals etc.[23,122]

2.9.1 In Food and Nutrition Filed:

The most common species of microalgae which are exploited in lipids production are *Schizochytrium sp., Scenedesmus sp., Arthrospira platensis, Ulkenia, Haematococcus pluvialis, Isochrysis galbana, Chlorella vulgaris, Chlorella pyrenoidosa, Chlorella ellipsoidea, Tetraselmis sp Dunaliella salina and Crypthecodinium.*[3,36,47,123]

Polyunsaturated fatty acids (PUFAs) have been known for its contribution in the field of human health benefits. Earlier, the major sources of polyunsaturated fatty acids were fish oil. But in the current scenario, the profit-oriented production of PUFA from fish or other marine sources, are declined due to some limitations (fish population and diversity threatening and environmental hazards). Recently microalgae investigated for the biosynthesis of oils or fatty acids which contain

high amount of PUFAs like; EPA and DHA.[124] Various strains of microalgae are able to amalgamate various series of the PUFAs like1-6 series, which contains arachidonic acid and linoleic acid, along with the PUFAs 1-3 series, contain EPA, ALA and DHA.[120] These lipids are having one or more double bond in their carbon skeleton. They have important ingredients for food and feed supplements, with many healthful benefits.

2.9.2 In cosmetic industry:

Microalgal PUFAs have been considered very useful and safe in the preparation of various cosmetic products, because of having antioxidant and anti-inflammatory properties. Fatty acyls including fatty acids, fatty alcohols, esters, carotenoids, and eicosanoids etc. are the needed oily raw materials used as emulsifiers in cosmetics. In the cosmetic field, the fatty acyls used as the major bio-based oil surfactants.[125]

The microalgal lipids which are used as biosurfactants possess amphiphilic nature because of the presence of both hydrophilic and hydrophobic components within the chain. The main function of these biosurfactants, are reducing the interfacial tension which make easier solubilization of hydrophobic molecules in water and its lower micelle concentrations, allowing it to work at a very small concentration when compared with any synthetically prepared surfactant.[126] They are also having antibacterial, antifungal, anti-tumor bioactivities and very less eco-toxicity. Many lipids like; stearic acid, palmitic acid, lauric acid, and myristic acid, are playing a pivotal role in the preservation of normal skin functions and resulting, smooth, soft and brighter skin.[127]

2.9.3 In Pharmaceuticals:

Many micro-algal species produce high-value lipids which are utilized for pharmaceutical purposes. Production of these high value lipids or fats compounds (bioactive molecules) from microalgae has been suggested as an emerging area. The microalgae strains exploited in production of lipid bioactive compounds (carotenoids), include *Chlamydomonas* sp., *Spirulina* sp., *Chlorella* sp., *Dunaliella, Nannochloropsis, Nostoc, Crypthecodinium* and *Haematococcus etc*. Microalgal produced various kind of carotenoids such as astazanthin, lutein, zeaxanthin, etc.[128] The green microalgae species commonly contain beta carotene which can derived other forms of it by the action of various enzymes like hydroxylase and ketolase.[129]

Microalgae represent a good source of natural carotenoids which having anti cancerous, antioxidental properties, these are highly used in food, cosmetics, nutraceuticals and also in pharmaceutical industries. *Nostoc sp.* has the capability to produce polyunsaturated and EFA (ω 3 and ω 6 fatty acid series) while, *Chlamydomonas reinhardtii* is able to produce glycerol, when grown in sulphur deprived medium, which is widely utilized in pharmaceutical industries. *Chlorella* species containing high amount of α-tocopherol (vitamin E) and this can help in preventing macular degeneration and the occurrence of certain cancers. *Nannochloropsis gaditana* known to produce good amount of eicosapentaneoic acid (EPA) and *Crypthecodinium cohnii* for producing DHA.[130] Long chain fatty acids DHA and EPA are the most valuable ones as having very useful effects in the cure and inhibition of many diseases like; inflammation, thrombosis, arthritis, hypertension, atherosclerosis, and risk of chronic disease.[131] DHA promotes eye health by enhancing the performance of retina and it also contribute in the brain functioning. EPA is showing the significant hypolipidemic activity, and helps in reducing triglyceride volume and rising high-density lipoprotein cholesterol measures. Hence it is very useful in preventing cardiovascular disease, and

reducing the symptoms of depression. It also helps in the maintenance of normal growth and development of bone and hair, relaxation and contraction of muscles.[130, 131]

PUFAs also serve as precursors of eicosanoids and docosanoids which regulate gene expressions and biosynthesis of steroidal hormones that can maintain the good health of reproductive system. These are utilized in promoting the vasoconstrictive and aggregative activity of platelets in the process of coagulation. PUFAs are able to make some changes in their composition in the plasma membrane so that they can affect the fluidity and signaling process of the cell membrane. Polyunsaturated essential fatty acids having inhibitory impacts on antibody production, lymphocyte proliferation and it also suppress to the activation of programmed cell death. While Polyunsaturated fatty acids (omega 6 fatty acids) help in regulating metabolism process and showed an anti-proliferative effect on epithelial cells thus prevent skin cancer and improved glycogenesis in diabetic mice.[132] Due to having above mentioned characteristic features, all these lipids are very important for pharmaceutical formulation.[133]

2.9.4 As Biofuel:

The microalgal biomass has the potential to synthesize various kind of biofuel such as biodiesel by trans esterification of the fatty acids, biogas by gasification or anaerobic digestion, bioethanol by direct fermentation of carbohydrate fraction of microalgae, methane gas by anaerobic fermentation of microalgal biomass. Microalgal lipids are the alternate resources which can be used to decrease the consumption of fossil fuels.[134] Microalgae cultivation gives much higher yields than any other conventional bioenergy crops. Some microalgal species eg. *Scenedesmus sp, Schizochytrium* sp., *Nitzschia* sp., and *Botyococcus braunii* which contain higher amount of lipid approximately 40 to 60 %, therefore, are an excellent source of biofuel.[135, 136]

2.9.4.1 Biodiesel:

Nowadays, microalgae are utilized as a promising source for synthesis of biodiesel to overcome the ever exhausting and often increasing price of fossil fuels. These diverse microbes have the capability to survive in adverse environmental situations. They can produce high oil content (approx. 50 to 80% of their dry weight) than any other traditional crops.[137, 56]

Microalgal biomass contain good amount of triglycerols which is the main constituent of the biodiesel. Hence, microalgae are supposed to be the great source of future biodiesel production. There are various procedures, for changing microalgal biomass (lipids) into biofuels. These are Trans esterification (chemical reaction), biochemical conversion, direct combustion and thermo-chemical conversion. [138,139] Among them, transesterification is widely used process for the conversion of microalgal lipids into biodiesel. The microalgae biodiesel contains high caloric value and lower density and viscosity differentiate with the biodiesel collected from other feedstock sources.[140] Microalgae biodiesel also help to reduce the level of carbon monoxide and other pollutants suspension in the atmosphere, as it does not contain aromatics hydrocarbons and sulfates.[23] Moreover, exploitation of microalgae for biodiesel is better option compared to usage of traditional food cops (oleaginous crops) and animal lipids for the synthesis of biofuel, since food supply could be affected severely.

Therefore, microalgae are proposed to be a better choice for generating biodiesel in a sustainable way because they possess capacity to flourish very well on diverse industrial waste effluent and they can adapt themselves according to adverse environmental conditions. Many studies have been carried out by using *Scenedesmus sp.* and *Chlorella sorokiniana*, for increasing

the target number of fatty acids under a variety of culture conditions that could be used as a potential feedstock for developing good quality of biodiesel, under a variety of stress conditions.[133]

2.9.4.2. Other Fuel Applications (Biohydrogen):

The first green microalgae, *Scenedesmus obliquus* which was noted to produce hydrogen.[141] After that, several microalgal strains like; *Chlorella sorokiniana*, *Chlamydomonas reinhardtii*, *Chlorococcum littorale, Platymonas subcordiformis* and *Chlorella fusca* etc. were used to produce hydrogen. Microalgae produce biohydrogen by employing various processes like photosynthesis, photo-fermentation indirect and direct biophotolysis, and dark fermentation.[142, 143] It was reported that microalgae generate low amount of hydrogen in dark but high amount of hydrogen produced in presence of sunlight. The hydrogen production in microalgae depend on the enzyme hydrogenase and as well as the availability of Sulphur containing components in the growing media.[144] It was documented that *Chlamydomonas reinhardtii* has high amount of hydrogenase enzyme around 10 to 100-fold, compared to other microalgal strains.[145] But the commercial production of biohydrogen from this strain could not outstretch due to some technical issues. The studies on marine alga, *Platymonas subcordiformis* and on the fresh water green microalga *Chlorella sorokiniana* & *Chlamydomonas reinhardtii* demonstrated that they produced a high amount of biohydrogen when cultivated in sulphur deprived medium.[146, 147]

Ali et al. 2011 examined that 03 species of *Chlorella*, (*Chlorella ellipsoidea* TISTR 8260, *Chlorella vulgaris* TISTR 8680, and *Chlorella* sp. TISTR 8262) had been used to find out their potentiality regarding bio-hydrogen production. All three species were cultured under anaerobic conditions and with BG-11 medium under sulfur-deprived condition. They observed that *Chlorella* strain, TISTR 8262 had the maximum yield (13.03%.) of biohydrogen, followed by *Chlorella*

ellipsoidea TISTR 8260 (3.05%). While, *Chlorella vulgaris* TISTR8680 did not generate hydrogen.[148] To attain profitable achievement in this field, genetically modified strains should be used which can be survive under adverse environmental conditions like sulfur- improvised state.[132] The overall target of microalgal cultivation is the conversion of sun-energy into lipid and other organic compounds. There are very few researches which provided the whole characterization of lipids of a particular micro alga. Due to the huge differentiation among the species of microalgae and their culture conditions. Frequently, it is laborious to compare the outcomes for a comprehensive biochemical profiling of bio molecules such as lipid, protein, and carbohydrates etc. present in all microalgae species. Because of this reason, there is still a need for complete characterization of several bio molecules within single microalgae and helps in demonstrating the suitable analytical method which can be used for further use. The complete characterization of fatty acids or oils in microalgae is also crucial for the selection of good species, so that the production of oils can be utilized as bio-fuels and other high-value compounds in the field of food,

Algal transgenomics offers a new approach for tolerance to environmental factors and higher biomass production.[149] For yielding, optimum lipid productivity in the microalgal cells, Strategies to acquire this goal consisting; genetic engineering of well characterised strains screening and selection for novel species, and the cultivation conditions. various techniques are used to improve light supply including operating the reactor design, the use of optics to transferring light to the all corner of the reactor, flashes, and using mixed cultures to used various wavelengths of light, enhancing the efficiency of photosynthesis by carbon capturing. The genes and Key enzymes which are responsible for the regulation of lipid synthesis metabolic pathways are currently being explored and manipulated to improve the productivity rate. While the lipid composition of cells can be increased by manipulation of the nutrient supply.[150]

Microalgal culture has the Potential to contributing in bioremediation of environment and also in the formation of valuable by-products like; polyunsaturated fatty acids, ω-3 fatty acids, B-carotenoids etc. microalgae could also be altered to synthesize other types of fuels e.g., bio hydrogen, bioethanol and biobutanol. These bioproducts has huge applicability in the sphere of biofuel, food and nutrition, pharmaceuticals, nutraceuticals, cosmetics, aqua feed and animal feed. Microalgae biomass could be modified energy containing fuels through thermal processes. In future with continued progress being made in the field of systems biology metabolomics will play an important role as a holistic approach in generation of bio-fuels which produce lower emissions, for green energy production and thereby maintaining environmental sustainability.[150]