Chapter 4
Cultivation of Microalgae: Effects of Nutrient Focus on Biofuels

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Abstract Several microalgae have potential to produce biofuels, carotenoids, polyunsaturated fatty acids, peptides, and phytosterols. Microalgae are capable of producing biofuels competently as another potential alternate as feedstock and may help to generate extra revenue, when its cultivation is handled scientifically at large-scale. The growth medium components, which is a major part of their cultivation, play a key role to improve its cellular components and mass accumulation. The medium components are varied according to the nature of microalgae, i.e., heterotrophic, autotrophic, and their nature of availability. In this chapter, nutritional factors, suitable compositions of media used for various microalgae cultivation, photosynthesis process, micronutrients requirements, and bioreactors for microalgae are discussed. For enhanced production of biofuels and bioactive compounds, optimized environmental conditions and nutritional factors for effective cultivation of microalgae have been revealed.

Keywords Microalgae · Photosynthesis · Lipid production · Cultivation

Nomenclature

ATP	Adenosine triphosphate
BBM	Bold basal medium
BG 11	Blue-green medium
E	Energy

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EDTA	Ethylenediamine tetraacetic acid
ESM	Enriched sea water medium
h	Plank's constant
K medium	Keller medium
$k_L a$	Mass transfer coefficient
MBM	Modified Bristol Medium
MDM	Modified Detmer's Medium
$NADPH_2$	Nicotinamide adenine dinucleotide
PBR	Photobioreactor
PU	Poly unsaturated fatty acids
TAP	Tris–acetate–phosphate medium
TG-FA	Triglyceride fatty acids
ν	Frequency

4.1 Introduction

Microalgae are photosynthesizing microorganisms; they may be prokaryotic or eukaryotic in nature, present in aquatic areas like ponds, lakes, streams, oceans, deep sea waters, soil, and on earth. They are characterized by rapid growth even at high pressures (deep seas), in acid environments, and in extreme conditions. The microalgae play a critical role in CO_2 sequestration, bioactive compounds, and biofuel production, involving in wastewater treatment, and needed a potential research for the enhancement of the end products (Guzzon et al. 2008; Kumar et al. 2009). Therefore, a critical research is needed to achieve full-scale and continuous process including (1) assess nutrient uptake, (2) improved algae growth, (3) wastewater utilization, (4) CO_2 sequestration efficiency, (5) improved algal harvesting, (6) enhanced extraction and purification, and (7) economic considerations (Pulz 2001; Carvalho and Malcata 2001).

4.2 Types of Microalgae

Microalgae are observed as single cells to colonies (a group of cells) and also as filamentous eukaryotic structures (chain like multiple cells), from prokaryotes to eukaryotes, and are classified according to biological evolution upon the time from cyanoprokaryotes to eukaryotes. Cyanoprokaryotes are with no distinct nucleus and chloroplasts (primitive of life), whereas eukaryotes are having well-defined cell wall, nucleus, and other cellular organelles. Further in algae vascular tissues, embryo and sex organs are absent like higher plants. Eukaryotic algae are classified into several divisions, i.e. *Chrysophyceae*, *Chlorophyceae*, *Euglenaphyceae*, *Cyanophyceae*, *Rhodophyceae*, *Pyrrophyceae*, *Phaeophyceae*, and *Xanthophyceae*. Further, general

Table 4.1 Major differences in characteristics present in various divisions of algae

Division	Cell wall	Pigment type	Storage type	Habitat	Type of algae
Chlorophyceae	Cellulose/pectose	Chlorophyll A and B	Starch	Marine and fresh water	Micro and macroalgae
Chrysophyceae	Cellulose with silicate	Chlorophyll A and C	Leucosin with oil drop	Marine and fresh water	Microalgae
Cyanophyceae	Mucopeptide with carbohydrates	Chlorophyll A and D	Starch and protein	Marine and fresh water	Microalgae
Euglenophyceae	No cell wall, cover proteinacious pellicle	Chlorophyll A and B	Paramylon	Fresh water	Microalgae
Phaeophyceae	Cellulose	Chlorophyll A and C	Laminarin	Marine water	Macroalgae
Pyrrophyceae	Stiff cellulose plates on outer wall	Chlorophyll A and C	Starch	Marine and fresh water	Microalgae
Rhodophyceae	Cellulose/ mucilagenous	Chlorophyll A and D	Floridean	Marine and fresh water	Micro and macroalgae
Xanthophyceae	Cellulose and hemicelluloses	Chlorophyll A	Leucosin	Marine and fresh water	Microalgae

classification depends on the pigments present, such as green (chlorophyll), blue (phycocyanin), yellow (xanthophylls), orange (carotene), red (phycoerythrin), and brown (fucoxanthin) algae (Frac et al. 2010). The major differences in characteristics and the classification of algae are summarized in Table 4.1.

4.3 Components Present in Algae

The major components of microalgae are very rich in lipids, carbohydrates, proteins, and also vitamins, pigments, etc. in various percentages of their dry weight, and these percentages may vary depending upon the variety of microalgae. Among them largest fractions of lipids and proteins are present, lipids are used for production of biofuels and edible oils (Delrue et al. 2012). In case of proteins and carbohydrates, they are used for the protein supplements and polymers manufacturing, respectively (Tables 4.2 and 4.3).

Table 4.2 Various components present in microalgae as percentages of dry weight

Name of the algae	Lipids	Proteins	Carbohydrates	References
Phaeodactylum tricornutum	16.1	34.8	16.8	Tibbetts et al. (2015)
Scenedesmus obliquus	12–14	48–56	10–17	González López et al. (2010)
Dunaliella sp.	14.36	34.17	14.57	Kent et al. (2015)
Chaetoceros calcitrans	23	40	37	Velasco et al. (2016)
Chaetoceros muelleri	31	59	10	Velasco et al. (2016)
Spirulina maxima	6–7	60–71	13–16	Becker (2007)
Nannochloropsis granulate	22	30	10	Kent et al. (2015)
Porphyridium cruentum	9–14	28–39	40–57	Brown (1991)
Tetraselmis chui	12	31–46	25	Tibbetts et al. (2015)
Tetraselmis sp.	15	36	24	Schwenzfeier et al. (2011)
Isochrysis galbana	11	27	34	da Silva Gorgônio et al. (2013)
Prymnesium sp.	22–38	28–45	25–33	Ricketts (1966)
Schizochytrium sp.	50–77	–	–	Chisti (2007)
Botryococcus braunii	25–34	39–40	19–31	Tibbetts et al. (2015)
Chlorella pyrenoidosa	02	57	26	Chisti (2007)
Aphanizomenon flos-aquae	03	62	23	FAO (2018)
Chaetoceros calcitrans	15	36	25	Milledge (2011)
Chlorella vulgaris	14–22	51–58	12–17	Wolkers et al. (2011)
Diacronema vlkianum	06	57	32	Bleakley and Hayes (2017)
Dunaliela bioculata	08	49	04	van Krimpen et al. (2013)
Euglena gracilis	22–38	39–61	14–18	van Krimpen et al. (2013)
Haematococcus pluvialis	15	48	27	Bleakley and Hayes (2017)
Prymnesium parvum	22–38	28–45	25–33	van Krimpen et al. (2013)
Scenedesmus dimorphus	16–40	8–18	21–52	van Krimpen et al. (2013)
Scenedesmus quadricauda	1.9	47	21–52	van Krimpen et al. (2013)
Spirogyra sp.	11–21	6–20	33–64	van Krimpen et al. (2013)
Arthrospira platensis	4–9	46–63	8–14	Milledge (2011)
Arthrospira maxima	6–7	60–71	13–16	Milledge (2011)
Synechococcus sp.	11	63	15	Christaki et al. (2011)
Tetraselmis maculate	03	52	15	van Krimpen et al. (2013)
A. Platensis F&M-C256	10.7	63.9	12.8	Niccolai et al. (2019)
Klamath	6.1	62.4	18.8	Niccolai et al. (2019)
N. spheaeroides F&M-C117	15.1	50.8	14.5	Niccolai et al. (2019)
C. sorokiniana F&M-M49	22.7	51.3	15.5	Niccolai et al. (2019)
C. sorokiniana IAM C-212	27.9	39.9	10.7	Niccolai et al. (2019)
C. vulgaris Allma	16.9	56.8	5.9	Niccolai et al. (2019)
T. suecica F&M-M33 (S)	22.4	18.3	36.8	Niccolai et al. (2019)
P. purpureum F&M-M46	13.1	34.2	17.0	Niccolai et al. (2019)
P. tricornutum F&M-M40	20.5	38.8	11	Niccolai et al. (2019)

(continued)

Table 4.2 (continued)

Name of the algae	Lipids	Proteins	Carbohydrates	References
Tisochrysis lutea F&M-M46	27.9	42.9	8.6	Niccolai et al. (2019)
N. oceanic F&M-M24	28.2	43.1	14.3	Niccolai et al. (2019)

4.4 Cultivation of Microalgae

Microalgae are generally unicellular autotrophs/heterotrophs which convert inorganic compounds in the presence of light energy into organic compounds. The earliest photoautotroph organisms (anoxygenic photosynthetic process) utilize light source to extract the protons and electrons from donors (Fe^{2+}, H_2S, etc.), and reduction of CO_2 ultimately produces organic compounds. Blue-green algae (cyanobacteria) are prokaryotic cells containing DNA in nucleoplasm and peripheral region (Chromoplast) covered with photosynthetic sheets in parallel and closed to cell surface. Eukaryotic algae (microalgae) have special organelle chloroplasts with alternative layers of thylakoids and stroma which is involved in photosynthetic process. Microalgae have the potential for CO_2 fixation, biofuel, and bioactive compounds production due their capability of photosynthesis which transforms CO_2 in to biomass and into several value-added products. So, microalgae cultivation is an option to reduce CO_2 release from the fossil fuel combustion.

During photosynthesis, light energy–controlled oxidoreduction reaction yielded by chlorophyll in which CO_2 and H_2O are transformed into oxygen and sugars in two stages, i.e., light and dark reactions. In thylakoid membranes, the light power is transformed into chemical force, facilitating a biochemical reductant $NADPH_2$ and ATP. In stroma, $NADPH_2$ and ATP are utilized for consecutive biochemical reduction of CO_2 to sugars (Fig. 4.1). Light energy is available as photons (quanta) and as a product of plank's constant and its frequency ($E = h\nu$). The light energy incident on the surface was measured as radian flux energy; the units are power per area (W m^{-2} or Jm^{-2} s^{-1}). In the process of photosynthesis, photon flux density is considered as the quantity of photons (quanta) that are accrued on the surface per unit time which is measured in μmol quanta m^{-2} s^{-1} or μEm^{-2} s^{-1}.

4.5 Nutritional Requirements of Algae Growth

Algae are categorized as autotrophs and heterotrophs and, depending upon energy and carbon sources, are categorized as photoautotrophs, chemoauthotrophs, chemoheterotrophs, phagocytosis. Further due to mixed characteristics, they are again subdivided into photoheterotrophs, auxotrophs, and mixotrophs. The autotrophic algae depend on light for the reduction of CO_2 and water by the release of O_2. Photo-autotrophs need only inorganic minerals for their growth, whereas some organic combinations are also required for growth (e.g., vitamins). Vonshak

Table 4.3 Various components present in microalgae as percentages of dry weight

Name of the algae	Lipids	Proteins	Carbohydrates	References
A. cylindrical	4–7	43–56	25–30	Becker (2007)
C. vulgaris	14–22	51–58	12–17	Becker (2007)
D. salina	6	57	32	Becker (2007)
E. gracilis	14–20	39–61	14–18	Becker (2007)
Arthrospira maxima	6–7	60–71	13–16	Becker (2007)
Scenedesmus obliquus	12–14	50–56	10–17	Becker (2007)
Poryphyridium cruentum	9–14	28–39	40–57	Becker (2007)
Spirogyra sp.	11–21	6–20	33–64	Becker (2007)
Spirulina platensis	4–9	46–63	8–14	Becker (2007)
Synechococcus sp.	11	63	15	Becker (2007)
Chlamydomonas reinhardtii	21	48	62	Becker (2007)
Chaetoceros calcitrans	16	34	6	Brown (1991)
Chaetoceros gracilis	7.2	12	4.7	Brown (1991)
Nitzschia closterium	13	26	9.8	Brown (1991)
P. tricornutum	14	30	8.4	Brown (1991)
Skeletonema costatum	10	25	4.6	Brown (1991)
Thalassiosira pseudonana	19	34	8.8	Brown (1991)
Dunaliella tertiolecta	15	20	12.2	Brown (1991)
N. atomus	21	30	23	Brown (1991)
Chlorella vulgaris	13	48	8	Brown (1991)
Chlorella pyrenoidosa	2	57	26	Brown (1991)
Chroomonas salina	12	29	9.1	Brown (1991)
Nannochloropsis oculata	18	36	7.8	Brown (1991)
Tetraselmis chui	17	31	12.1	Brown (1991)
Tetraselmis suecica	10	31	12	Brown (1991)
Isochrysis galbana	23	29	12.9	Brown (1991)
Pavlova lutheri	23	29	12.9	Brown (1991)
Pavlova salina	12	26	7.4	Brown (1991)
Arthrospira platensis	7	64	25	Brown (1991)
Arthrospira maxima	2	65	20	Brown (1991)
Chlamydomonas rheinhardtii	21	17	48	Hossain et al. (2019)
Chlorella sp.	19	22	56	Hossain et al. (2019)
Spirogyra sp.	16	55	20	Hossain et al. (2019)
Porphyridium cruentum	11	50	35	Hossain et al. (2019)
Spirulina platensis	8	12	60	Hossain et al. (2019)
Dunaliella salina	6	57	32	Hossain et al. (2019)
Bellerochea sp.	15	24	03	Hossain et al. (2019)
Chaetoceros sp.	18	18	02	Hossain et al. (2019)
Rhodomonas sp.	15	74	09	Hossain et al. (2019)
Scenedesmus sp.	12	56	18	Hossain et al. (2019)

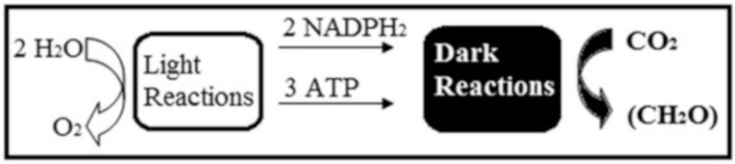

Fig. 4.1 Photosynthesis in microalgae with both light and dark reactions

(1986) summarized the nutritional necessities for the fabrication of algal recipes, i.e., (a) the total salt contents according to the habitat from where the algae originate, (b) the main ionic contents such as K^+, Mg^{2+}, Na^+, Ca^{2+}, $SO_4^{=}$, and Cl^-, (c) the nitrogen source, (d) carbon source either CO_2 or HCO_3^-, (e) pH, (f) minor elements and chelating agents, and (g) vitamins. Carbon (C), nitrogen (N), and phosphorus (P) are the three main essential nutrients for autotroph growth, and their supply is essential for the effective microalgae growth. The nutrient-rich supply could effectively impacted on the algal cultivation process for optimal biomass production.

4.5.1 Carbon

Carbon is an essential element as sole feedstock to form the carbon skeleton in the microalgae biomass growth and must available in the form of inorganic or organic source enriched medium. For high growth rate of microalgae, CO_2 or HCO_3^- are very important in the medium as it is maintained the pH like as buffer system in the liquid medium that is optimal for mass cultivation. The buffering system chemically expressed as CO_2 – H_2CO_3 –HCO_3^-– CO_3^{2-} is having greater importance in maintenance of the pH in cultivation of microalgae. On the other hand, glycylglycine and Tris(2-amino-2-[hydroxymethyl]-1–3-propanediol) are frequently preferred as buffering system. Chemically the buffering system involving photosynthesis process to produce CO_2 is as following:

$$2HCO_3^- \leftrightarrow CO_3^{2-} + H_2O + CO_2 \tag{4.1}$$

$$HCO_3^- \leftrightarrow CO_2 + OH^- \tag{4.2}$$

$$CO_3^{2-} + H_2O \leftrightarrow CO_2 + 2OH^- \tag{4.3}$$

The above reactions shows that OH^- accumulation leads to rise in pH during photosynthetic CO_2 fixation. This is natural phenomenon to rise in pH upto "11" and increased density of algal growth without additional supply of CO_2. One can control the pH rise to "11" by additionally sparging CO_2 into the growth medium; it ultimately leads to highly dense algal growth. Further, as discussed above, the carbon sources provide organic acids, alcohols, and sugars for mixotrophic algal cultivation.

4.5.2 Nitrogen

Nitrogen is an important element and occupies >10% of dry weight of microalgae and helps in biomass production. Nitrogen could be supplied as ammonia (NH_4^+), urea, and nitrate (NO_3^-) to the growth medium in mass cultures. Ammonia is useful in pH maintenance in case of additional supply of CO_2, but here the mechanism is releasing of protons (H^+). To achieve higher yields of microalgae biomass production, adequate supply of nitrogen is needed. Few cynobacterial species are capable of reducing the atmospheric elemental N_2 to NH_4^+ by nitrogenase enzyme. In the efficient production of biofuels by microalgae, in the microalgae medium ingredients few nutrients are rationalized or limited in quantity. In this regard, nitrogen is limited supply material (nitrogen starvation) in the medium leads to accumulation of lipids reaching to higher levels in microalgal biomass. In nitrogen starvation the microalgae synthesize the TG (triglyceride) and utilized to storage of energy and carbon. These stored TG-FA have undergone transesterification process and release methyl and ethyl esters. Nitrogen scarcity can lead to the accumulation of lipids, but it compromises the cell growth (Chen et al. 2017). To avoid it in the first stage of growth, rich supply of nitrogen is needed; in the later stage, nitrogen scarcity helps to build up lipids in biomass for biofuel production as good feedstock.

4.5.3 Phosphorus

This is a critical element for algae growth, energy transfer, and also in the biosynthesis of nucleic acids, adenosine triphosphate, phospholipids, etc. Algal biomass contains >1% of phosphorus, and it is easily bound with other ions (iron, CO_3^{2-}) which leads to precipitation, resulting in unavailability of phosphorus to algal uptake. In the growth medium, the preferred form of phosphorus is orthophosphate, and the uptake is energy dependent. Adenosine triphosphate is the preliminary product of photosynthesis of algae, so phosphorus is essential for achieving higher yields of microalgae production. Phosphorus limitation or scarcity has no superior effects on microalgae growth as nitrogen scarcity (Kamalanathan et al. 2015). Therefore, phosphorus supply is sufficiently needed (Chu et al. 2013) to attain effective density of microalgae for the biofuel production at the first stage, and then nitrogen scarcity is required at the second stage.

4.5.4 Macro- and Micronutrients

Essential nutrients required except phosphorus, nitrogen, and carbon are 30 elements in the form of minerals of high quantity (macronutrients) and in trace amounts (micronutrients) for microalgae growth. Macronutrients are Ca, Mg, S, K, Na, Fe,

etc. and micronutrients are enlisted as Cu, Mn, B, Zn, Co, Mo, V, Se, etc. Calcium is an important element in cell wall and membrane structures and performs as intracellular messenger that harmonizes response toward environmental changes. Magnesium as part of enzymes is directly related to photosynthesis and CO_2 fixation. Magnesium supplementation in the culture media could increase the photosynthesis activation, thereby increases the accumulation of lipids in biomass. Esakkimuthu et al. (2016) explained in their work on the starvation of calcium and phosphorus that decrease in lipid content in microalgae and they suggested as lower amount of calcium must be provided than the starvation. In modern cultivation of algae primarily provided all nutrients richly and later stages starvation of nitrogen, little amount of calcium could be preferred to achieve the higher yields of biomass and optimized production of biofuels. Salinity of the growth medium could be maintained by the addition of salts, i.e., NaCl, KCl, $MgCl_2$, $CaCl_2$, NaCl, and KCl are used as salinity modifiers to increase the external osmotic pressure of the cell which may affect the fluidity and permeability of the membrane. $MgCl_2$ and $CaCl_2$ are not modifying the salinity of the medium efficiently than the above salts but involving in other regulations. Sajjadi et al. (2018) revealed that the when high salinity in the medium, the osmo-regulation mechanism is started in the cell to equilibrate external salinity condition. This equilibration and osmoregulation could induce fatty acid metabolism which leads to accumulation of lipids. Silicon is also an important constituent which is involved in the formation of cell wall especially in diatoms. Cobalt is essential for the synthesis of vitamin B_{12}, and other minor nutrients are very important as they are cofactors with the enzymes and involved in the metabolism. Metal chelators (EDTA) are required to avoid phosphorus precipitation in the medium and avoid the unavailability of phosphorus which is essential for cellular structures and division of microalgal cells. So, the macro and micro inorganic nutrients are most necessary for microalgae-augmented biomass harvest and biofuel production. Several microalgae media available (55 types) in the literature are depicted in the Tables 4.4, 4.5, 4.6, 4.7, 4.8, and 4.9 along with their ingredients, quantities and compared with conventional medium. However, here the components, quantities, and suitability of the medium for microalgae cultivation are considered by diversity of algae and target of the product to be achieved.

4.5.5 Other Considerations

Along with the above conditions, many other parameters will influence the effective cultivation of microalgae. They are (1) temperature, (2) light, (3) pH, (4) CO_2, (5) salinity, (6) O_2, (7) mineral bioavailability, (8) carbon bioavailability and cellular characteristics, (9) growth inhibition, (10) cell density, and (11) cell fragility. Reactor characteristics affecting the microalgae growth are (1) rate of mixing, (2) gas bubble size, (3) gas bubble distribution, (4) fluid dynamics, (5) gas exchange capacity, (6) mass transfer, (7) dilution rate, (8) light intensity, (9) culture depth, and (10) maintenance of dark spots. Further, the cultural growth characteristics also

Table 4.4 Various culture media and their compositions (g L^{-1} or otherwise stated)

Name of the component	Name of the culture medium								
	Zarrouck's	BG 11	AW (g/kg)	MN	ASN III	Modified Allen's	Bristol's	Modified Zarrouck's	Bold's Basal BBM
$NaNO_3$	2.5	1.5	1.667	0.75	0.75	1.5	0.125	–	0.25
KNO_3	–	–	–	–	–	–	–	2.5	–
$K_2HPO_4 \cdot 3H_2O$	0.5	0.04	–	0.02	0.02	0.039	0.0375	0.5	0.075
KH_2PO_4	–	–	–	–	–	–	0.0875	–	0.175
$MgSO_4 \cdot 7H_2O$	0.2	0.075	–	0.038	3.5	0.075	0.0375	0.2	0.075
$MgCl_2 \cdot 4H_2O$	–	–	9.395	–	2.0	–	–	–	–
$CaCl_2 \cdot 2H_2O$	0.04	0.036	1.316	0.018	0.5	0.025	0.0125	0.04	0.084
$Ca(NO_3)_2 \cdot 4H_2O$	–	–	–	–	–	0.02	–	–	–
$SrCl_2 \cdot 6H_2O$	–	–	0.0214	–	–	–	–	–	–
$Na_2SiO_3 \cdot 9H_2O$	–	–	3.000	–	–	0.02	–	–	–
Citric acid	–	0.006	–	0.003	0.003	0.006	–	–	–
Fe-ammonium citrate	–	0.006	–	0.003	0.003	–	–	–	–
$FeCl_3$	–	–	0.016	–	–	0.002	0.001	–	–
$FeSO_4 \cdot 7H_2O$	0.01	–	–	–	–	–	–	0.01	0.00498
EDTA, 2Na–Mg salt	0.08	0.001	–	0.0005	0.0005	0.001	0.025	0.08	0.05
$NaHCO_3$	16.8	–	0.170	–	–	–	–	16.8	–
Na_2CO_3	–	0.02	–	0.01	0.02	0.02	–	–	–
NaCl	1.0	–	20.758	–	25.0	–	0.0125	1.0	0.025
KCl	–	–	0.587	–	0.5	–	–	–	–
K_2SO_4	1.0	–	–	–	–	–	–	1.0	–
Na_2SO_4	–	–	3.477	–	–	–	–	–	–

NaI	–	–	0.0027	–	–	–	–	–	–
KOH	–	–	–	–	–	–	0.0155	–	0.031
KBr	–	–	0.0845	–	–	–	–	–	–
H_3BO_4 (μg L^{-1})	2.86	2.84	0.0225	2.86	2.86	2.86	0.00571	–	11.42
$MnCl_2 \cdot 4H_2O$ (μg L^{-1})	1.81	1.81	–	1.81	1.81	1.81	0.00072	–	1.44
$MnSO_4 \cdot 4H_2O$	0.222	–	0.054	–	–	0.222	–	–	8.82
$ZnSO_4 \cdot 7H_2O$ (μg L^{-1})	–	0.222	0.0073	0.222	0.222	–	0.00442	–	–
$Na_2MoO_4 \cdot 2H_2O$ (μgL^{-1})	–	0.391	–	0.390	0.390	0.391	–	–	–
Vitamins	–	–	Thiamin, Vit. B_{12}	–	–	–	–	–	–
Sodium glyceryl PO_4	–	0.667	–	–	–	–	–	–	–
$CuSO_4 \cdot 5H_2O$ (μg L^{-1})	0.08	0.079	–	0.079	0.079	0.079	0.000079	–	1.57
$Co(NO_3)_2 \cdot 6H_2O$ (μgL^{-1})	–	0.0494	–	0.0494	0.0494	0.0494	0.000025	–	0.49
$CoSO_4 \cdot 7H_2O$	–	–	0.0016	–	–	–	–	–	–
MoO_3 (μg L^{-1})	0.01	–	–	–	–	–	0.000036	–	0.71
H_2SO_4	–	–	–	–	–	–	0.001	–	–
Adjust final pH	–	7.4	–	8.3	7.5	7.8	–	6.5	–
References	Grobbelaar (2004)	Grobbelaar (2004)	Paul et al. (1980)	Rippka et al. (1979)	Rippka et al. (1979)	Grobbelaar (2004)	Bold (1942)	Pai et al. (2008)	Grobbelaar (2004)

Table 4.5 Various culture media and their compositions (g L^{-1} or otherwise stated)

Name of the component	Name of the culture medium								
	Sorokin/ Krauss	George's	Amara and Steinbüchel	Wu's medium	Modified Chu's (μg/L)	Kessler and Cygan	MBM	M 11	Ben-Amotz and Avron
$NaNO_3$	–	–	1.5	–	–	–	–	0.1	–
KNO_3	1.25	0.20	–	–	400	81	–	–	0.505
$K_2HPO_4 \cdot 3H_2O$	–	0.02	0.5	0.3	80	47	–	0.01	0.014
KH_2PO_4	1.25	–	–	0.7	–	36	1.25	–	–
$MgSO_4 \cdot 7H_2O$	1.0	0.02	0.2	0.3	200	25	1	0.75	1.2
$MgCl_2 \cdot 4H_2O$	–	–	–	–	–	–	–	–	0.1
$CaCl_2 \cdot 2H_2O$	0.04	–	0.012	–	107	0.15	0.111	0.40	0.033
Citric acid	–	–	–	–	100	–	–	–	–
Fe-ammonium citrate	–	0.035	0.018	–	20	–	–	–	–
$FeSO_4 \cdot 2H_2O$	0.05	–	0.01	0.003	–	0.06	0.0498	0.01	–
EDTA, 2Na–Mg salt	0.5	–	0.08	–	–	0.08	0.5	0.01	–
$NaHCO_3$	–	–	9.214	–	–	–	–	–	1.7
Na_2CO_3	–	–	7.143	–	–	–	–	0.30	–
NaCl	–	–	1	–	–	47	–	–	117
K_2SO_4	–	–	0.571	–	–	–	–	–	–
Tris–HCl	–	–	–	–	–	–	–	–	6
H_3BO_3 (μg L^{-1})	114	–	–	–	5.72	0.005	0.1142	–	6
$MnCl_2 \cdot 4H_2O$ (μg L^{-1})	14	–	–	–	3.62	0.005	0.0142	–	–
$MnSO_4 \cdot H_2O$ (μg L^{-1})	–	–	–	–	–	–	–	–	–

$ZnSO_4 \cdot 7H_2O$ (μg L^{-1})	88	–	–	–	0.44	0.002	0.0882	–	–
$ZnCl_2$	–	–	–	–	–	–	–	–	14
$Na_2MoO_4 \cdot 2H_2O$ (μg L^{-1})	–	–	–	–	0.084	0.002	–	–	–
$CuSO_4 \cdot 5H_2O$ (μg L^{-1}1)	16	–	–	–	0.16	–	0.0157	–	–
$Co(NO_3)_2 \cdot 6H_2O$ (μg L^{-1})	5	–	–	–	–	–	0.0049	–	–
$CoCl_2 \cdot 6H_2O$ (μg L^{-1})	–	–	–	–	0.02	–	–	–	4.8
Vitamin B_1		–	–	0.01	–	–	–	–	–
MoO_3 (μg L^{-1})	7	–	–	–	–	–	–	–	–
Peptone	–	1	0.1	–	–	–	–	–	–
Glycine		–	–	0.1	–	–	–	–	–
Yeast extract	–	–	0.01	4	–	–	6	–	–
Glucose (mg L^{-1})	–	–	–	–	–	–	10	–	–
Glycerol	–	–	–	10	–	–	–	–	–
Adjust final pH	6.8	–	–	–	7.5	6.5	–	8.0	7.5
References	Grobbelaar (2004)	Atlas and Parks (1997)	Amro and Steinbüchel (2013)	Xiong et al. (2008)	Yamaguchi et al. (1987)	Kessler and Czygan (1970)	Xiong et al. (2008)	Yagi et al. (1979)	Grobbelaar (2004)

Table 4.6 Various culture media and their compositions (g L^{-1} or otherwise stated)

Name of the component	Name of the culture medium								
	Walne	Guillard	"F" medium	Fresh water WC	Half strength Chu's (μg/L)	TAP	Algal Assay Procedure (AAP) (mg/L)	Diatom medium (mg/L)	SOT
$NaNO_3$	100	84.2	75	0.08501	–	–	25.5	–	2.5
$K_2HPO_4 \cdot 3H_2O$	–	–	–	0.00871	0.005	0.108	1.04	–	0.5
$Na_2HPO_4 \cdot 3H_2O$	20	10	5	–	–	–	–	–	–
KH_2PO_4	–	–	–	–	–	0.056	–	12.4	–
$MgSO_4 \cdot 7H_2O$	–	–	–	0.03697	0.07	0.1	14.7	25	0.2
$MgCl_2 \cdot 4H_2O$	–	–	–	–	–	0.05	12.6	–	–
$CaCl_2 \cdot 2H_2O$	–	–	–	0.03676	–	0.05	4.41	–	0.004
$Ca(NO_3)_2 \cdot 4H_2O$	–	–	–	–	–	–	–	20	–
$Na_2SiO_3 \cdot 9H_2O$	30	50	30	0.02842	–	–	–	57	–
$FeCl_3 \cdot 6H_2O$	0.8	3.5	3.15	0.00315	0.001	–	1.6	–	–
$FeSO_4 \cdot 2H_2O$	–	2.9	–	–	–	4.99	–	2.25	0.001
EDTA, 2Na–Mg salt	45	–	4.36	0.00436	–	50	3	2.25	0.008
$NaHCO_3$	–	–	–	0.0126	–	–	–	15.9	16.8
NaCl	–	–	–	–	0.04	–	–	–	1
KOH	–	–	–	–	–	16	–	–	–
K_2SiO_3	–	–	–	–	0.02	–	–	–	–
NH_4Cl	–	–	–	–	–	0.4	–	–	–
$(NH_4)_2SO_4$	–	–	–	–	0.025	–	–	–	–

Tris–HCl	–	–	–	0.0005	–	2.42	–	–	–
H_3BO_3 ($\mu g\ L^{-1}$)	33.6	–	–	0.001	2.48	11.4	1.9	2.48	0.286
$MnCl_2 \cdot 4H_2O$ ($\mu g\ L^{-1}$)	0.4	0.36	0.180	0.00018	–	5.06	42	1.39	0.250
$MnSO_4 \cdot H_2O$ ($\mu g\ L^{-1}$)	–	–	–	–	1.47	–	–	–	–
$ZnSO_4 \cdot 7H_2O$ ($\mu g\ L^{-1}$)	–	–	0.022	0.000022	0.23	22	–	–	0.022
$ZnCl_2$	2.1	–	–	–	–	–	0.327	–	–
$Na_2MoO_4 \cdot 2H_2O$ ($\mu g\ L^{-1}$)	–	–	0.0063	0.000006	–	–	0.726	–	0.0021
$(NH_4)_6 MoO_7O_{24}$ ($\mu g\ L^{-1}$)	0.9	1.26	–	–	0.07	1.10	–	1.0	–
$CuSO_4 \cdot 5H_2O$ ($\mu g\ L^{-1}1$)	2	1.96	0.0098	0.00001	0.1	1.57	–	–	0.0079
$Co(NO_3)_2 \cdot 6H_2O$ ($\mu g\ L^{-1}$)	–	–	–	–	0.14	–	–	–	–
$CoCl_2 \cdot 6H_2O$ ($\mu g\ L^{-1}$)	2	2	0.010	0.00001	–	1.61	0.143	–	–
Hcl (mL)	10	–	–	–	–	–	–	–	–
Glacial acetic acid (mL)	–	–	–	–	–	1	–	–	–
Thiamine HCl	20	0.2	0.002	0.0001	50	–	–	0.04	–
Biotin	0.1	0.01	0.01	0.0005	2.5	–	–	0.04	–
Vitamin B_{12}	0.1	0.01	0.01	0.0005	2.5	–	–	0.04	–

(continued)

Table 4.6 (continued)

Name of the component	Name of the culture medium								
	Walne	Guillard	"F" medium	Fresh water WC	Half strength Chu's (μg/L)	TAP	Algal Assay Procedure (AAP) (mg/L)	Diatom medium (mg/L)	SOT
Vitamin B_1	0.2	–	–	–	–	–	–	–	–
Glycylglycine	–	–	–	0.0005	–	–	–	–	–
Glucose (mg L^{-1})	–	–	–	–	50	–	–	–	–
Adjust final pH	–	–	–	7–8	–	–	–	6.9	–
References	Vivi et al. (2012)	Vivi et al. (2012)	Guillard and Ryther (1962)	Guillard and Lorenzen 1972	Chu (1942)	Harris (1989)	ASTM (2012)	Barsanti and Gualtieri (2006)	Ogawa and Terui (1970)

Table 4.7 Various culture media and their compositions (g L^{-1} or otherwise stated)

Name of the component	Name of the culture medium									
	M4 (mg/L)	M7 (mg/L)	Combo (mg/L)	MNK (μg/L)	"K" medium (μg/L)	ESM medium (μg/L)	LO medium	MDM (mg/L)	Carefoot (mg/L)	F/2 (mg/L)
$NaNO_3$	0.0274	0.274	17.0	20	75	120	20	1000	247	75
$K_2HPO_4 \cdot 3H_2O$	0.0184	0.184	1.742	1	–	5	1	–	90	60
$Na_2HPO_4 \cdot 3H_2O$	–	–	–	0.28	–	–	0.28	–	–	–
KH_2PO_4	0.0143	0.143	–	–	–	–	–	250	23	–
$MgSO_4 \cdot 7H_2O$	123.3	123.3	36.97	–	–	–	–	250	–	–
$CaCl_2 \cdot 2H_2O$	293.8	293.8	36.76	–	–	–	–	100	11	–
$Na_2SiO_3 \cdot 9H_2O$	1	1	10	–	–	–	–	–	–	10
Na_2VO_4	–	–	0.0018	–	–	–	–	–	–	–
$Na_2Se\ O_3$	0.0022	0.0022	–	0.03	1.6	–	0.05	–	–	–
$H_2\ Se\ O_3$	0.0016	–	–	–	–	–	–	–	–	–
$FeCl_3 \cdot 6H_2O$	1	1	1	–	–	–	–	–	196	3.16
$FeSO_4 \cdot 7H_2O$	0.996	0.249	–	–	–	–	–	200	–	–
EDTA, 2Na–Mg salt	2.5	0.625	4.36	3.7	37,224	–	2.5	–	1000	4.4
Fe-EDTA	–	–	–	26	4927	259	30	–	–	–
Mn-EDTA	–	–	–	33	–	332	15	–	–	–
$NaHCO_3$	64.8	64.8	64.8	–	–	–	–	–	–	–
NaBr	0.016	0.004	–	–	–	–	–	–	–	–
KCl	5.8	5.8	5.96	–	–	–	–	100	15	–
LiCl	0.31	0.077	0.31	–	–	–	–	–	–	–
RbCl	0.07	0.018	0.07	–	–	–	–	–	–	–
$SrCl_2 \cdot 6H_2O$	0.15	0.038	0.15	–	–	–	–	–	–	–
KI	0.0033	0.0033	0.0033	–	–	–	–	–	–	–

(continued)

Table 4.7 (continued)

Name of the component	Name of the culture medium									
	M4 (mg/L)	M7 (mg/L)	Combo (mg/L)	MNK (µg/L)	"K" medium (µg/L)	ESM medium (µg/L)	LO medium	MDM (mg/L)	Carefoot (mg/L)	F/2 (mg/L)
H_3BO_3 (µg L^{-1})	2.86	0.715	24	–	–	–	–	286	–	–
$MnCl_2 \cdot 4H_2O$ (µg L^{-1})	–	0.090	0.18	9	178	–	15	–	36	0.18
$MnSO_4 \cdot H_2O$ (µg L^{-1})	–	–	–	–	–	–	–	250	47	–
$ZnSO_4 \cdot 7H_2O$ (µg L^{-1})	–	–	0.022	2.4	23	–	4	22.2	22	0.21
$ZnCl_2$	0.013	0.013	–	–	–	–	–	–	–	–
$Na_2MoO_4 \cdot 2H_2O$ (µg L^{-1})	–	0.016	0.022	0.7	7.3	–	1.5	2.1	2.5	0.07
Na_2 Glycerophosphate	–	–	–	–	2.2	–	–	–	–	–
$(NH_4)VO_3$	0.0006	0.0006	–	–	–	–	–	–	–	–
$CuSO_4 \cdot 5H_2O$ (µg L^{-1})	–	–	0.001	0.06	2.5	–	0.06	7.9	–	0.07
Cu $Cl_2 \cdot 2H_2O$	0.0165	0.0042	–	–	–	–	–	–	–	–
$CoSO_4 \cdot 7H_2O$	–	–	–	1.2	14	–	2	–	–	0.112
$CoCl_2 \cdot 6H_2O$ (µg L^{-1})	0.010	0.010	0.010	–	–	–	–	–	4	–
Thiamine HCl	0.075	0.075	0.1	20	101	100	40	–	20 (µg)	100 (µg)
Biotin	0.0008	0.0008	0.0005	0.15	0.5	1	0.3	–	0.2 (µg)	5 (µg)
Vitamin B_{12}	0.001	0.001	0.00055	0.15	0.5	1	0.3	–	0.2 (µg)	5 (µg)
Adjust final pH	–	–	–	–	–	–	–	8.0	7.5	–
References	Samel et al. (1999)	Samel et al. (1999)	Samel et al. (1999)	Noël et al. (2004)	Keller et al. (1987)	Watanabe et al. (2000)	Noël et al. (2004)	Watanabe et al. (2000)	Carefoot (1968)	Guillard and Ryther (1962)

Table 4.8 Various culture media and their compositions (g L^{-1} or otherwise stated)

Name of the component	Name of the culture medium									
	AF-6 (mg/L)	CA (mg/L	C (mg/L)	HUT (mg/L)	D (mg/L)	MA (mg/L)	MW (mg/L)	P35 (mg/L)	URO (mg/L)	VT (mg/L)
$NaNO_3$	140	100	–	–	689	50	1.7	–	–	–
Urea	–	–	–	–	–	–	8.5	–	–	–
KNO_3	–	–	100	–	103	100	10	–	–	–
$KHCO_3$	–	–	–	–	–	–	9	–	–	–
NH_4NO_3	22	50	–	–	–	–	–	100	5	–
NH_4Cl	–	–	–	–	–	–	0.42	–	–	–
$K_2HPO_4 \cdot 3H_2O$	5	–	–	–	–	–	–	–	–	–
KH_2PO_4	10	–	–	20	–	–	–	–	–	–
$Na_2HPO_4 \cdot 3H_2O$	–	–	–	–	111	–	–	–	–	–
$MgSO_4 \cdot 7H_2O$	30	–	–	25	100	–	–	40	10	40
$MgCl_2 \cdot 6H_2O$	–	–	–	–	–	50	–	–	–	–
$CaCl_2 \cdot 2H_2O$	10	–	–	–	–	–	14	74	10	
$CaCO_3$	10	–	–	–	–	–	–	–	–	–
$Ca(NO_3)_2 \cdot 4H_2O$	–	20	150	–	–	50	100	–	–	117.8
$CaSO_4$	–	–	–	–	60	–	–	–	–	–
$Na_2\ SO_4$	–	–	–	–	–	40	–	–	–	–
$FeCl_3 \cdot 6H_2O$	–	196	196	–	0.5	0.5	196	196	196	196
Ferric citrate	2	–	–	–	–	–	–	–	–	–
Potassium citrate	–	–	–	40	–	–	–	–	–	–
Na_2 Acetate	–	–	–	400	–	–	–	1000	–	–
Citric acid	2	–	–	–	–	–	–		–	–
EDTA, 2Na–Mg salt	–	1000	1000	–	–	5	1000	1000	1000	1000
Fe-EDTA	–	1	–	–	–	–	–	–	0.5	–

(continued)

Table 4.8 (continued)

Name of the component	Name of the culture medium									
	AF-6 (mg/L)	CA (mg/L	C (mg/L)	HUT (mg/L)	D (mg/L)	MA (mg/L)	MW (mg/L)	P35 (mg/L)	URO (mg/L)	VT (mg/L)
HEPES	–	400	–	–	–	–	–	–	–	–
NaCl	–	–	–	–	8	–	–	–	1	–
KCl	–	–	–	–	–	–	–	50	–	50
NTA	–	–	–	–	100	–	–	–	–	–
KI	–	–	–	–	–	20	–	–	–	–
H_3BO_3 (mg L^{-1})	2860	–	–	–	500	–	–	–	–	–
$MnCl_2 \cdot 4H_2O$ (μg L^{-1})	–	36	36	–	–	5	36	36	36	36
$MnSO_4 \cdot H_2O$ (μg L^{-1})	2500	20	40	–	2280	15	–	–	–	–
$ZnSO_4 \cdot 7H_2O$ (μg L^{-1})	222	22	22	–	500		22	22	22	22
$ZnCl_2$	–	–	–	–	–	0.5	–	–	–	–
$Na_2MoO_4 \cdot 2H_2O$ (μg L^{-1})	21	2.5	2.5	–	25	0.8	2.5	2.5	2.5	2.5
β-Na_2 glycerophosphate	–	30	50	–	100	20	–	50	4	50
$CuSO_4 \cdot 5H_2O$ (μg L^{-1})	79	–	–	–	25	–	–	–	–	–
Cu $Cl_2 \cdot 2H_2O$	–	–	–	–	45	–	–	–	–	–
$CoCl_2 \cdot 6H_2O$ (μg L^{-1})	–	4	4	–	–	5	4	4	4	4
Polypeptone	–	–	–	600	–	–	–	–	–	–
Yeast extract	–	–	–	400	–	–	–	–	–	–
Glycylglycine	–	–	–	–	–	–	100	–	–	500

Bicine	–	–	–	–	–	500	–	–	–	–
Thiamine HCl ($\mu g\ L^{-1}$)	10	10	10	0.4	–	–	20	10	10	10
Biotin ($\mu g\ L^{-1}$)	2	0.1	0.1	–	–	–	0.2	0.1	0.1	0.1
Vitamin B_{12} ($\mu g\ L^{-1}$)	1	0.1	0.1	0.5	–	–	0.2	0.1	0.1	0.1
Vitamin B_6 ($\mu g\ L^{-1}$)	1	–	–	–	–	–	–	–	–	–
Tris (aminomethane) (mg)	–	–	500	–	–	–	–	500	–	–
Adjust final pH	6.6	–	7.5	6.4	–	8.6	7.2	8.0	7.5	7.5
References	Kato 1982	Ichimura and Watanabe (1974)	Ichimura (1979)	Hutner et al. (1966)	Castenholz 1969	Ichimura (1979)	Sako et al. (1984)	Ichimura (1979)	Kimura and Ishida (1985)	Robertson et al. (2001)

Table 4.9 Various culture media and their compositions (g L^{-1} or otherwise stated)

Name of the component	Name of the culture medium								
	W (mg/L)	M-ASP7 (mg/L)	STP (mg/L)	ASN (g/L)	PCR-S11 (mg/L)	Johnson's (g/L)	ESAW (g/L)	Allen (mg/L)	DY-III (mg/L)
$NaNO_3$	–	50	200	0.75	–	–	46.7 mg	–	20
Urea	17	–	–	–	–	–	–	–	–
KNO_3	10	–	–	–	–	1	–	–	–
$NaHCO_3$	–	–	–	–	–	0.043	0.174	–	–
NH_4SO_4	–	–	–	–	2.68	–	–	132	
NH_4Cl	–	–	–	–	–	–	–	–	2.68
$K_2HPO_4 \cdot 3H_2O$	–	–	10	0.02	–	–	–	–	–
KH_2PO_4	–	–	–	–	–	0.035	–	27.2	–
$NaH_2PO_4 \cdot 2H_2O$	–	20	–	–	–	–	3.09 mg	–	–
$Na_2HPO_4 \cdot 3H_2O$	–	–	–	–	14	–	–	–	–
$MgSO_4 \cdot 7H_2O$	15	9000	–	3.5	–	0.5	–	24.6	50
$MgCl_2 \cdot 6H_2O$	–	–	–	2	–	1.5	9.592		
$CaCl_2 \cdot 2H_2O$	–	300	–	0.5	–	0.2	1.344	7.4	75
$Ca(NO_3)_2 \cdot 4H_2O$	100	–	–	–	–	–	–	–	–
Na_2SO_4	–	–	–	–	–	–	3.55	–	–
$VSO_5 \cdot 5H_2O$	–	–	–	–	0.098	–	–	–	–
$Na_2WO_4 \cdot 2H_2O$	–	–	–	–	0.198	–	–	–	2
Na_2CO_3	–	–	–	0.02	–	–	–	–	–
$Na_2SiO_3 \cdot 9H_2O$	–	–	–	–	–	–	30 mg	–	14
Na_2SeO_3	–	–	–	–	–	–	0.173	–	2
$FeCl_3 \cdot 6H_2O$	196	63	–	–	–	2.44	1.77	–	1
NH_4 ferric citrate	–	–	–	0.003	–	–	–	–	–
NH_4VO_3	–	–	–		–	–	–	23	–
Citric acid	–	–	–	0.003	–	–	–	–	–

EDTA, 2Na–Mg salt	1000	1000	–	0.0005	8	1.89	3.09		8
Fe-EDTA	–	–	–	–	–	–	–	30.16 g	–
HEPES	–	–	–	–	200	–	–	–	–
NaCl	–	25	–	25	–	–	21.19	–	–
KCl	–	700	–	0.5	–	0.2	0.599	–	3
NTA	–	70	–	–	–	–	–	–	–
KBr	–	–	–	–	0.714	–	0.0863	–	–
NaF	–	–	–	–	–	–	0.0028	–	–
KI	–	–	–	–	0.498	–	–	–	–
$SrCl_2$	–	–	–	–	–	–	0.0218	–	–
H_3BO_3 (mg L^{-1})	–	1130	–	2.860	18.549	0.61	0.023	2.86 g	0.8
$MnCl_2 \cdot 4H_2O$ (μg L^{-1})	36	32	–	–	–	0.41	–	1.79 g	200
$MnSO_4 \cdot H_2O$ (μg L^{-1})	–	–	–	1.81	10.140	–	0.54	–	–
$ZnSO_4 \cdot 7H_2O$ (μg L^{-1})	–	46.6	–	0.222	1.725		0.073	220	40
$ZnCl_2$	–	–	–	–	–	0.041	–	–	–
$Na_2MoO_4 \cdot 2H_2O$ (μg L^{-1})	2.5	–	–	0.390	–	–	1.48 mg	–	20
$(NH_4)_2Mo_7O_{24} \cdot 4H_2O$	–	–	–	–	0.494	0.38	–	130	–
β-Na_2 glycerophosphate	20	–	–	–	–	–	–	–	–
$CuSO_4 \cdot 5H_2O$ (μg L^{-1})	–	–	–	0.079	0.749	–	–	79	–
$Cu\ Cl_2 \cdot 2H_2O$	–	–	–	–	–	0.041	–	–	–
$CoSO_4 \cdot 7H_2O$	–	0.93	–	–	–	–	0.016	–	–

(continued)

Table 4.9 (continued)

Name of the component	Name of the culture medium								
	W (mg/L)	M-ASP7 (mg/L)	STP (mg/L)	ASN (g/L)	PCR-S11 (mg/L)	Johnson's (g/L)	ESAW (g/L)	Allen (mg/L)	DY-III (mg/L)
$CoCl_2 \cdot 6H_2O$ ($\mu g\ L^{-1}$)	4	–	–	–	–	0.015	–		8
$Co(NO_3)_2 \cdot 6H_2O$	–	–	–	0.0494	0.873	–	–	–	–
$Cd(NO_3)_2 \cdot 6H_2O$	–	–	–	–	0.925	–	–	–	–
$NiCl_2 \cdot 6H_2O$	–	–	–	–	0.713	–	1.49 mg	–	–
$Cr(NO_3)_3 \cdot 9H_2O$	–	–	–	–	0.240	–	–	–	–
SeO_2	–	–	–	–	0.333	–	–	–	–
$VOCl_2$	–	–	–	–		0.041	–	–	–
$KAl(SO_4)_2 \cdot 12H_2O$	–	–	–	–	2.846	–	–	–	–
Sodium glutamate	–	–	500	–	–	–	–	–	–
Glucose	–	–	200	–	–	–	–	–	–
Glycine	–	–	100	–	–	–	–	–	–
D,L-Alanine	–	–	100	–	–	–	–	–	–
Tryptase	–	–	200	–	–	–	–	–	–
Yeast autolysate	–	–	200	–	–	–	–	–	–
Sucrose	–	–	1000	–	–	–	–	–	–
Soil extract	–	–	50	–	–	–	–	–	–
Glycylglycine	100	–	–	–	–	–	–	–	–
Thiamine B_1 ($\mu g\ L^{-1}$)	2	50	50	–	–	–	100 mg	–	100 mg
Biotin B_7 ($\mu g\ L^{-1}$)	0.2	0.1	0.1	–	–	–	1 mg	–	0.5

Vitamin B_{12} ($\mu g\ L^{-1}$)	0.2	1	1	–	10	–	2 mg	–	0.5
Nicotinic acid	–	10	10	–	–	–	–	–	–
Calcium pantothenate	–	10	10	–	–	–	–	–	–
p-Aminobenzoic acid	–	1	1	–	–	–	–	–	–
Inocitol	–	500	500	–	–	–	–	–	–
Folic acid	–	0.2	0.2	–	–	–	–	–	–
Thymine	–	300	300	–	–	–	–	–	–
Tris (aminomethane) (mg)	–	1000		–	–	2.45	–	–	–
Adjust final pH	7.5	8.0	7.5	7.5	7.5	7.5	8.2		6.5
References	Watanabe (1983)	Watanabe (1981)	Provasoli et al. (1957)	Laura and Paolo (2014)	Laura and Paolo (2014)	Laura and Paolo (2014)	Laura and Paolo (2014)	Allen (1959)	Laura and Paolo (2014)

influence the growth of microalgae, i.e., (1) toxic chemicals to microalgae, (2) pathogens to microalgae, (3) competitive growth of other algae, (4) management of photo inhibition, (5) management of photorespiration, and (6) yield frequency of microalgae.

In the designing of the microalgae medium, the nutritional ingredients and their quantities are consider for the enhanced yields of specific metabolites. In few instances, it is essential to alter the quantities and amounts of one or more nourishments (Shin et al. 2018). So, one must know the definite features of each and every group of algae, their physiology, nourishment prerequisites, growth circumstances, and specific considerations to alter the composition of medium (Milano et al. 2016). Cuellar García et al. (2019) evaluated various culture mediums on two microalgae *S. obliquus* and *C. vulgaris* for biodiesel productivity. They used Bristol, Bold, and AAP (modified algal assay procedure) culture conditions and studied about which one is the best in favor of biomass growth for lipid accretion. The results have been revealed that the usage of Bold medium and AAP medium for *S. obliquus* and *C. vulgaris* respectively, showed higher production of biomass containing more lipids when compared with other media. BG 11 medium is suggested for freshwater and soil Cyanophyceae, diatom medium is recommended for freshwater *Bacillariophyceae* algae, and DY-III medium is the best for freshwater *Chrysophyceae* group of algae. Bold Basal Medium (BBM) is used broadly for the freshwater *Chlorophyceae, Xanthophyceae, Chrysophyceae*, *and Cyanophyceae*. AF-6 medium is useful for algae cultivation where slight acidic condition is required, i.e., Euglenophyceae, xanthophytes, dinoflagellate, green ciliate, volvocalean algae, and many cryptophytes. AK medium will be used for the cultivation of almost all varieties of marine algae. Both "F" medium and "G" medium are useful for marine algae cultivation in salt as well as brackish waters. Both "K" medium and "LO" medium are useful for marine microalgae, especially oligotrophic salt and brackish waters. Combo medium is used broadly for the cultivation of cryptophytes, cyanobacteria, diatoms, and green algae, whereas MNK medium is the best suitable for especially marine microalgae, *coccolithophores*.

Sueoka's medium is especially used for the cultivation of *Chlorophyceae* family algae. In the similar manner, MES and Jaworski's media are used broadly for pure water microalgae cultivation. Walne's culture medium is broadly used for marine microalgae principally set in commercial culturing. ASN-III, PCR-S11, and Chu-II media are most preferably recommended for the Cyanobacterial strain cultivation. *Botryococcus braunii* is a colonial microalgae cultivated in modified Chu 13 medium for the production of lipids (Yamaguchi et al. 1987). F/2 medium and ESAW medium are used for the broad spectrum for oligotrophic marine algae and coastal open ocean algae cultivation. CY-II, Zarrouk's, and Johnson's media were used to cultivate *Cyanophora paradoxa*, *Arthrospira* sp., and *Dunaliella* sp. (halophilic algae), respectively. *Chlorella vulgaris* is studied with Chu medium, and it is found that hydrocarbons (HC), free fatty acids, sterol esters (SE), acetone mobile polar lipids, aliphatic alcohols (ALC), and phospholipids (PL) yield are more. Debjani et al. (2012) experimented on different media, i.e., dry-grind ethanol thin stillage (TS), soy whey (SW), and modified basal medium (MBM) as media for the

cultivation of *C. vulgaris*. After 4 days of incubation, the biomass yield was 9.8, 6.3, and 8.0 g L^{-1} and the oil content was 43, 11, and 27% (w/w) in the media TS, SW, and MBM, respectively. The quality of fatty acid profile is also tested as linoleic and linolenic forms, the oil is richer and good quality when grown in TS and MBM media. Xiong et al. (2008) reported on the cultivation of *C. protothecoides* to achieve more dense biomass in the production of biodiesel using Wu's culture medium successfully.

As reported by Richmond and Becker (1986), the higher yields of algal biomass could be achieved when (a) providing nutrition-rich medium, (b) positive environmental parameters, and (c) turbulent flow mixing. The turbulent flow mixing enhances the diffusion rate of CO_2, exchange rate of nutrients in betwixt the algal cells, and fluid medium; further augmented light/dark periods lead to efficient photosynthesis and productivity (Grobbelaar 1994). Microalgae is small in size and available in liquid medium as suspension offers direct contact with nutrients present in medium. Upon the sparging of air due to higher surface area available on algae, CO_2 fixation and also solar energy transmission rate are high. The main advantage of microalgae cultivation is that the maximum annual biomass productivity is more, due to nutrients, solar light, adsorption of CO_2, and high surface area availability on algae. High surface area allows the easy exchange of gases, nutrients, and solar energy which leads to faster growth than other sources of biofuels.

4.6 Bioreactors for Microalgae Cultivation

Since microalgae could grow well at immense biodiversity, possibility to use waste water as nutritional medium, can sequester CO_2; producce O_2 and can produce high cell density are great features so that leads to much attention by scientists and considered as microalgae as feedstock for biofuels production. Accurate fabrication, design of reactors, and optimization of process by biotechnology of microalgae facilitate the achievement of augmented cell density mass cultures. The overall knowledge is required to scale up the operational parameters, i.e. (1) light energy, (2) mass transfer, (3) shear forces, and (4) rate of mixing. The above-mentioned variables are strongly interconnected and decide the yields and effectiveness of a particular reactor. Further, the augmentation in algae yield entails better microalgae culture and amplified CO_2 capture. In the scale-up process to industrialization, maintenance of controlled conditions in open ponds is the major issue (Fig. 4.2). To maintain the controlled conditions, closed photobioreactors are considered as alternative system against open ponds. Photobioreactor system has several advantages than open pond system except capital and operation costs.

Capital cost may be required for the manufacturing, installation, and supporting accessory systems. The operational cost due to the CO_2 addition, O_2 removal, maintenance and cleaning, etc. will increase the costs. The harvesting cost is low when compared with the open ponds, which compensates the initial costs. The other important factors, i.e., biomass quality, biomass concentration, production

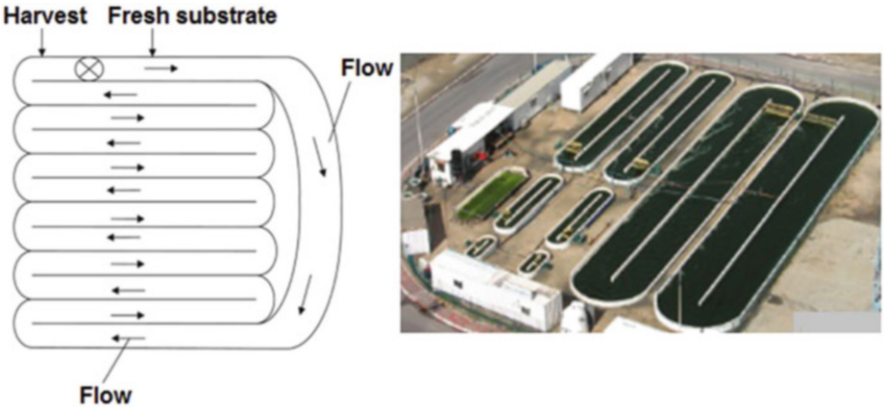

Fig. 4.2 Schematic diagram of raceway pond for the cultivation of microalgae. (Adapted from Seambiotic 2009)

flexibility, process controls, and reproducibility are more preferred in the PBRs than the open ponds. In case of open ponds, the requirement is huge space, water, and CO_2 loss and the contamination risk is also more.

4.6.1 Closed Reactor Designing for Microalgae Cultivation

Generally, the reactor is in cylindrical shape and fits with several accessories for proving specific temperature, pH, and mixing devices. Algae cultivation needs light source as energy which can be provided by a light source or sunlight for photosynthesis. Since algae cultivation is through a light source, these reactors are calling as photobioreactors (PBR). PBRs offer a protection (Fig. 4.3) from the contaminated species (bacteria, fungi, etc.) and make available closed environments to maintain desirable temperature, pH, mixing, etc. and ensure the specific algal strain cultivation. The below-mentioned variables are essential features that must be considered in the design of closed reactor system: (1) light source and orientation, (2) nutrition by culture medium, (3) water, (4) algae circulation (mixing), (5) pH, (6) temperature control, (7) CO_2 feed, (8) O_2 removal, (9) materials of construction, and (10) reactor maintenance. The parameters remains the same but there may be changes in their intensities as per the array of higher and lesser points for specific strain cultivation management.

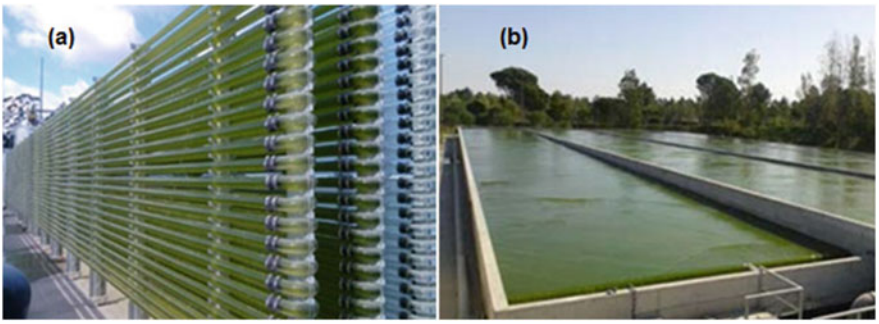

Fig. 4.3 Tubular photobioreactor (**a**), open pond (**b**) for microalgae cultivation. (Adapted from Li et al. 2008)

4.6.2 Classification of Photobioreactors (PBRs)

Photobioreactors are classified into various types depending upon the design of reactors, i.e., (a) tubular or flat, (b) horizontal, (c) inclined, (d) vertical or spiral, (e) manifold or serpentine, (f) hybrid, (g) floating, and (h) biofilm reactors.

Depending on the mode of operation, photobioreactors are categorized as (1) single-phase reactors and (2) air-mixed reactors. In single-phase reactors, gas exchange takes place, whereas in air-mixed reactors, both liquid and gas mass transfer occurs.

Based on the construction materials, again PBRs are classified into (a) glass or plastic and (b) rigid or flexible photobioreactors. Other than these, axenic PBRs are used for specific cultures and intended for sterilization before the operation of reactor.

4.6.2.1 Light

Light is an essential energy for photosynthesis; light must be radiated inside the reactor to avoid the dark zones (less-intensity light at some places in the reactor) or illuminate the fluorescent lights close to the outer surface of the reactor. When the light penetrates with high intensity and reaches to the cells with good frequency in a reactor then it is considered as an optimal reactor. Spatial dilution of illumination is a significant feature which reduces the mutual shading of cells in the culture medium. This may lead to higher growth rate, but the product component inside the cell is less. One can consider how to exploit the volume to surface area ratio and geometry of reactor for efficient light distribution in the designing process of PBR (Fig. 4.4).

The light gradient directly influences the productivity of cultured algae and is determined by the reactor dimensions and algal cell density. The cell density formation is a microalgae strain specific property, and it is essential to manage optimal conditions for the light penetration and light intensity to reach each and every cell in the reactor. The critical cell density is an operating parameter, which is

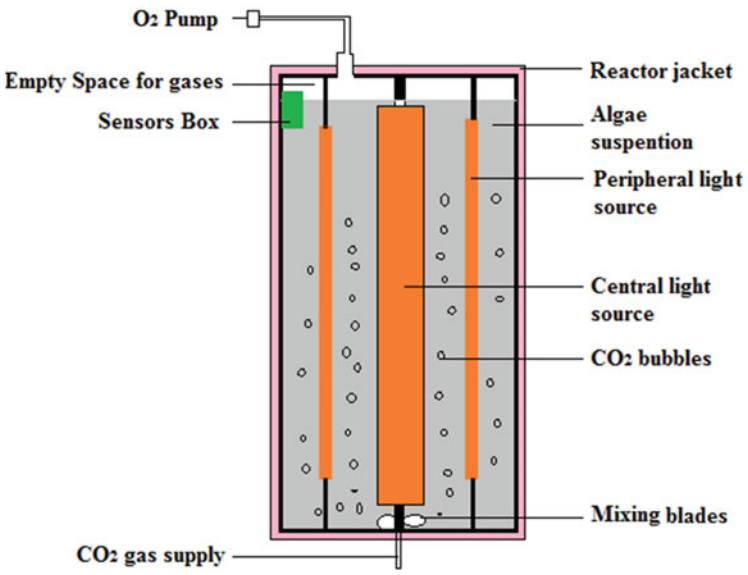

Fig. 4.4 The typical photobioreactor for microalgae production

nothing but the greatest cell mass without reciprocated shadows and one can take into consideration this point while designing the PBR. Naturally light/dark periods also influence the efficacy of photosynthesis of the microalgae. Janssen et al. (2001) explained the impact of illumination on photosynthetic effectiveness of *D. tertiolecta* and on short irradiance or dimness compared with continuous light. Photon flux of the illumination tenure and time of liquid duration in various radiance regions are two key parameters to attain the optimal dark period. The light regime of PBR is influenced by the extent of light/dark periods and illumination strength, and the light regime directly responsible for physiological responses of cells is called as photoacclimation. Zu and Richmond (2000) reported that the sudden flash of light is fatal for various algal species. Based on the photosynthetic efficiency and other data, the theoretical maximum fuel yield per area can be determined. LEDs are more prominently used as light source in algal cultivation, due to low cost, and can also use it as flash light to pulse the light whenever it is required to improve the productivity.

4.6.2.2 Mixing

Mixing is the critical parameter which enhances the rate of recurrence of the illumination and murk cycles and leads to improve the productivity and cellular contents. It provides the effective mass transfer from nutrients to the cell in the presence of light. In the designing of the bioreactor, effective mixing will be considered for effective availability of nutrients and light to the cell by avoiding

the diffusion barriers. Ugwu et al. (2008) scale out tubular reactors by setting up of static mixers with augmented tube diameter and confirmed that the enhanced light exploitation and microalgae mass improved yields. For optimal cell growth, the reactor maintains the capability to sustain the mass transfer coefficient (k_La), it build upon the category of bubbler, agitation rate, type of surfactants and also on temperature. Static mixers installation could reduce the bubble size which increases the surface area and leads to increase the contact area in between liquid and gas. Whereas fine spargers produce low liquid flow rate, and big bubble formation leads to low or poor mass transfer. Eriksen et al. (1998) explained a setting up of dual orifice with membrane-perforated sparger in a closed reactor, which resulted in efficient air and CO_2 supply separately and attained proper mixing. They achieved fivefolds supply of CO_2 to liquid phase as compared to conventional spargers. In the total cost of the reactor, usually the highest percentage is occupied by energy consumed and the mixing mechanism. In bubble column reactors, the sparger working efficiently mixing and formed small size bubbles thereby efficient CO_2 mass transfer takes place.

The specialty of this type of reactor with no moving parts is that it provides elevated diffusion area volume ratio, excellent mass and heat transmittance, excellent release of O_2, and also small capital cost. This was scaled up by perforated plates that are set inside the reactor, resulting in break-up of coalesced particles and are redistributed. An airlift reactor is also one type of bubble column reactors; it has internal draft tube which enhances the mixing and mass transfer coefficient. In Fig. 4.5, the left side designated three zones: air-riser up, bubble down-comer, and disentanglement zones in air lift reactor.

Air bubbles are sprinkled by sparger through the bottom of the reactor, maybe inside or outside of the draft tube. The air bubbles go up (rise) and lead to fluid flow vertically, due to the opposite action against this, and the fluid flow will be downwards causing down flow. Upon the continuous supply of air bubbles, there is an effective movement that leads to improved mixing. Further, this movement facilitates in uniform distribution in one direction with same velocity, thereby no or less coalescence, and no fusion of bubbles ensuing greater kL_a (the volumetric mass transfer coefficient) than bubble-column reactors. The above positive parameters result in greater mass yields in airlift reactors than other stirred reactors. Several researchers are examined on improved mixing and mass transfer in flat-panel reactors (Wondraczek et al. 2013; Hoekman et al. 2012; Posten and Schaub 2009; Tredici and Materassi 1992).

4.6.2.3 Water Consumption

Water utilization is an important parameter as it is precious along with reactor options. Algae are able to grow in various water sources, i.e., fresh drinking water, brackish water (saline), and also in waste water (Wogan et al. 2008) effluents. Based upon the type of water (pure or saline), strains of microalgae are classically divided into two categories: freshwater algae and salt water algae. The overall productivity

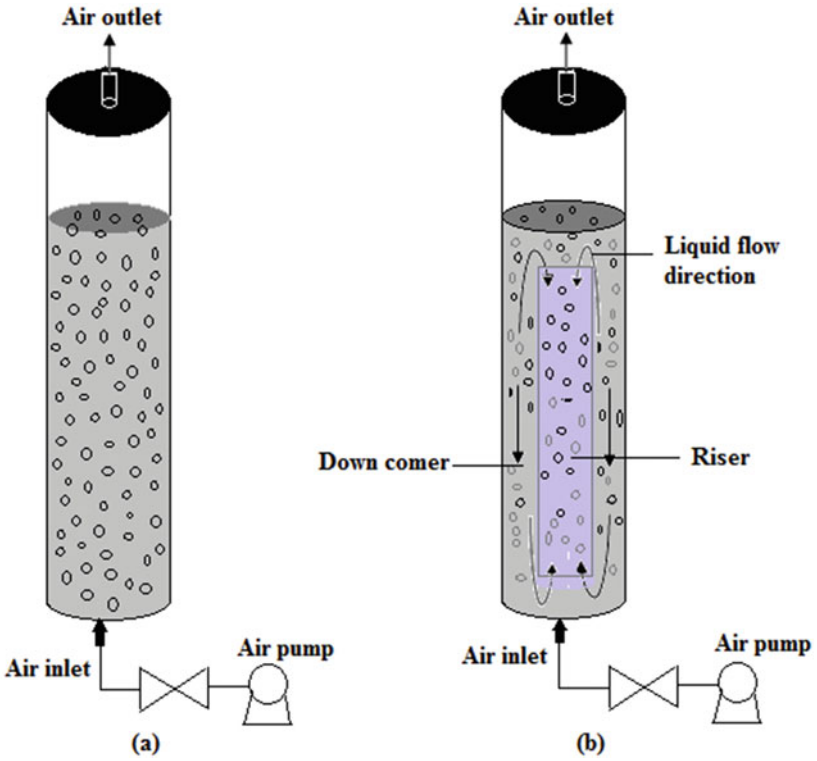

Fig. 4.5 Description of the bubble column PBR (**a**) and airlift PBR (**b**)

and individual content (lipids, carbohydrates, etc.) productivity in each strain are influenced by the level of salinity. Abu-Rezq et al. (1999) reported on the saline conditions of high-quality marine algae for the production, i.e., *Nannochloropsis* salinity range was 20–40, *Tetraselmis* 20–35, and *Isochrysis* 25–35 (Rao et al. 2007).

Waste water having rich nutrients (nitrogen and phosphorus) with high concentrations may be treated further when used as medium for algae cultivation. In open ponds, due to evaporation of water, high concentration of nutrients will be formed; high concentrations of these nutrients causing for growing of unwanted algae and may be undergone eutrification. If it occurs in the salt water ponds, where the salinity of the pond reaches the extreme and above-tolerance levels. In PBRs, by minimizing the evaporation/loss of water and minimizing these extreme concentrations of nutrients in waste water, the unwanted algae growth can be avoided, and these nutrients can be utilize efficiently for the particular strain of algae growth (Aslan and Kapdan 2006). One of the huge costs-saving approaches in algae cultivation is usage of waste water as medium. Chinnasamy et al. (2010) used corporate industry waste water as medium for the cultivation of algae in vertical tank reactors, polybags, and also raceway ponds for mass cultivation of algal consortium. They

attained huge proteins and lesser carbohydrates in algal consortium. Yang (2011) reported the same as above results that the overall biomass productivity in poybags is more than the vertical tank reactor and raceway. Attractively, with diverse positioning, the polybag reactor can attain the highest yields in commercial systems (large scale) and diminish running costs.

4.6.2.4 CO_2 Consumption

Carbon is essentially required for photosynthesis, i.e., organic and inorganic carbon (CO_2), among them inorganic carbon is the best in commercial aspect. For photosynthesis of algae, sufficient CO_2 is essential along with light, nutrients, water, and high partial pressures (pCO_2). Several studies revealed that excess partial pressure of CO_2 is detrimental for microalgae. Lee and Tay (1991) reported that exposure of CO_2 with high partial pressures on *Chlorella pyrenoidosa* leads to declining the rate of growth. They suggested that the supply of CO_2 is through a gas permeable membrane to provide controlled release for the entire culture and prevent high partial pressures of CO_2. Even though the laboratories routinely aerate 5–15% of CO_2, the available concentration from 1 to 5% is often enough for maximum growth (Suh and Lee 2003). Flue gas is the best for carbon source as it reduces the greenhouse gas emissions and also low cost. Thermal power stations are good sources for exhaust gas which exhibit up to 13% of carbon dioxide, and it is of lower cost compared to pure CO_2 supply. In the photobioreactor, infrared analyzer is used to measure the CO_2 concentration in the gas phase through which the flow of flue gas is regulated. Schenk et al. (2008) reported that pCO_2 is 0.15 kPa is the minimum requirement to avoid the limitation for CO_2 kinetics, and the required CO_2/g dry biomass is 1.7–1.8 g (Chisti 2007). Doucha et al. (2005) explained that even flue gas contains CO and NOx along with CO_2; however they may not interfere in the photosynthetic efficiencies even with low mass transfer of CO_2, and interestingly the productivity was similar to pure CO_2 supply. For further decreasing the cost of CO_2 in biofuel production by microalgae, Carvalho et al. (2006) suggested an option that is the use of hollow fiber membranes for interrupted supply of CO_2 and enhanced mass transfer.

4.6.2.5 O_2 Removal

In algal photosynthesis, oxygen is delivered as byproduct, the maximum allowable is as 10 g O_2 m^3 min^{-1} in a typical tubular reactor. Likewise, in a photobioreactor, the dissolved oxygen when excess than the minimum allowable levels leads to photo-oxidative damage in the presence of intensive irradiation ultimately causing to the drastically reduced productivity. In general, O_2 concentration should be 400% of air saturation. In case of open ponds, oxygen is evolved from the medium easily, but in photobioreactors, the oxygen pressure is built inside, so periodically oxygen should be pumped out of the reactor. In tubular reactors, it is very difficult to do it, which

limits the scale-up process. Molina et al. (2011) designed the airlift zone by algae culture frequently returned in the tubular reactor where oxygen was stripped by air. In the airlift zone, gas–liquid separator prevents recirculation air bubbles to horizontal loop. The fluid travel time through the extent of the degasser should be the same as oxygen bubbling rising time.

4.6.2.6 Nutrient Supply

Nitrogen and phosphorus are the main nourishments to manage the growth rate and yield of lipid in algae. The essential other nutrients required for microalgae are carbon, oxygen, hydrogen, sulfur, chlorine, sodium, calcium, potassium, and magnesium. The micronutrients are iron, silicon, cobalt, nickel, copper, manganese, molybdenum, boron, vanadium, and selenium. In N-8 medium (common medium) containing deficient quantities of Mg, S, Fe and N which results huge cell mass. Even in separate supplementation of these elements not showed better yields, but the four elements reasonable boosting reported enhanced performance. Chisti (2007) explained the need for the excess nutrients like phosphorus which reacts with metal ions. Applying stress condition by providing limited nutrients can enhance the lipid percentages with biomass. But the stress application may limit the rate of growth and decrease the overall lipid production. Rodolfi et al. (2009) illustrated three types of nutrient supply: (1) nutrient-sufficient, (2) nutrient-limited, and (3) nutrient-deficient. They applied the above three condition on few strains, among them *Nannochloropsis* showed exceptional results, lipid production was enhanced, when applied deficient scenario (N-deficient environment) after grown formerly with nutrient sufficient medium. Xinxin et al. (2019) studied the consequences of N-limitation on fatty acids and lipid quantity on *P. tricornutum*, *Isochrysis galbana*, *Rhodomonas baltica*, and *N. oceanica*, and higher yields are achieved. Various media are tabulated and their compositions with quantities are discussed in detailed in the cultivation section.

4.6.2.7 Temperature

Temperature is one of the critical parameters which play a vital role for large-scale culturing of microalgae. Because the daily variations may be seen upon the presence of illumination and at dark cycles, which leads to the reduction in biomass production and algal lipid capacity. Microalgae showed decline in cell volume with an enhanced temperature (upon extra illumination), and the maximum growth occurred at the temperature range was noted as 20–30 °C. Venkata Subhash et al. (2014) revealed that several algae species tolerate the temperatures up till 15 °C lesser than their optimistic point, with lower growing rate. But the temperature is fewer degrees

more than the tolerable point causing to death of microalgae as it is an environmental parameter (Huang et al. 2010).

Zhu et al. (2013) critically explained that temperature choice may vary from species to species. For example, *Scenedesmus* and *Chlorella* were adapted in the temperature range of 5–35 °C; the best range of temperature is 25–30 °C. Kurpan et al. (2015) experimented on *Isochrysis galbana*, *N. oceanica*, and *P. tricornutum* for biodiesel and PUFAs. *Isochrysis galbana* and *Phaecodactylum tricornutum* yielded high TAG at 20 °C and 30 °C, respectively, and *Nannochloropsis oceanic* yield was noted as very less. They stated that, if an ideal temperature was not maintained during the course of cultivation, the biochemical pathways are changed, which leads to improper build-up of lipids. Singh and Singh (2015) reported an optimum temperature of 20–30 °C for the algae, i.e. *Nannochloropsis*, *Chlorella*, *Scenedesmus*, *Botryococcus*, *Spirogyra*, *Neochloris*, *Chlamydomonas*, *Haematococcus*, and *Ulva* species. The studies in the literature report that the optimum growth temperature depends on algal species and geographical origin, where an optimum growth can be attained (Goldman and Carpenter 1974). When the tolerable temperatures exceeds, the response of microalgae against temperature variation could affect (1) nutritional necessities, (2) nature of metabolism and rate, and (3) cell composition (Richmond 1999). Torzillo et al. (1991b) examined the temperature consequences on *S. platensis* at outdoor environments at different temperatures, i.e., 25 and 35 °C (from May to September). They reported that there is 14% increase in biomass productivity at 35 °C, (1) when the temperature decreases in the period and the average biomass productivity is decreased, and (2) during night, there is a loss of biomass significantly at 25 °C (Torzillo et al. 1991a).

According to geographical regions, microalgae cultivation in outdoors (open ponds and race ponds) may be exposed to extreme temperatures. Even though microalgae competent grows at various temperatures, favorable growth is restricted with a slight range of temperature in particular to each strain. Abu-Rezq et al. (1999) reported the best range of temperatures for *Isochrysis*, *Nannochloropsis*, and *Tetraselmis* were 24–26 °C, 19–21 °C, and 19–21 °C, respectively. Daily and seasonal temperature fluctuations also obstruct the algae growth. In the absence of controlling units in PBRs can result high temperatures, then evaporate cooling method is frequently adapted to reduce that much magnitude from required temperatures. Further at low temperatures especially at night times owing respiration could loss the biomass. So, maintenance of temperature is required for attaining optimum growth in large scale PBRs and in open ponds.

4.6.2.8 pH

pH is an important parameter that influences the microalgae metabolism in lipid production. It influences the fluid chemistry like accessibility of nourishments,

organic acids and CO_2. Further in marine environment react with water forms (H_2CO_3) carbonic acid, readily dissociate into bicarbonate (HCO_3^-) and again detached into carbonate ion (CO_3^-) and protons (H^+) leads to decrease pH ultimately damage the algae growth (Bautista-Chamizo et al. 2018). Bicarbonate (HCO_3^-) is utilized by microalgae through active transport leads to catalytic conversion of cation exchange and formed as CO_2 and OH^- (Seyed et al. 2018). Bicarbonate (HCO_3^-) is utilized by microalgae through active transport, catalytic conversion of cation exchange forms of CO_2 and OH^- (Seyed et al. 2018). Each and every algal strain has a lesser pH array, so commercial pH regulators must be preferred in reactors to attain the maximum growth. Xinxin et al. (2019) studied the consequences of N-limitation on profile of fatty acids and lipid quantity on *P. tricornutum*, *Isochrysis galbana*, *Rhodomonas baltica*, and *N. oceanica* and maintained the optimal pH as 8.5. Sharma et al. (2018) studied on *Lyngbya confervoides*, *Chroococcus turgidus*, *Nostoc commune*, *Chlorella* sp., *Skeletonema costatum*, and *Chaetoceros calcitrans* and reported that the suitable pH is the neutral pH. They also stated that the best pH for *N. oculata* and *Chlorella sorokiniana* was noted as 8.5 and 8, respectively. For lipid production by *Scenedesmus obliquus,* it is noted as neutral pH (Breuer et al. (2013), for *chlorella sorokiniana* optimum pH was 6 (Qiu et al. 2017). Liao et al. (2018) stated that lower pH leads to decreased growth rate, and this effect is reversible and at high intensity. Ramanna et al. (2017) explained about pH as it is specific to strain, set the pH prior to illumination flux to keep away from photo-inhibition which causes irreversible damage to essential proteins.

4.6.3 Other Considerations

When designing a photobioreactor configuration, it is essential to judge the whole process of the production (Table 4.10). Our aim is the highly dense biomass production, in the biomass lipid content and oil downstream processing with good quality. The downstream processing and reactor design integration are very important that impact on the product excellence and cost. Chisti (2008) recommended that the genetic engineering may improve microalgae biomass yield and lipid quantity in cellular level and may have the supreme enhancement in the economics of biofuels. Genetic engineering could improve fuel production in different ways, i.e., upgrading in photosynthesis, enhanced biomass yield, augmented lipid quantity, and better temperature tolerance of algae. In addition, genetic engineering could increase tolerable levels of algal cells to light saturation, photo-inhibition, and photo-oxidation. Geography is important for area selection, assessing the viability of biofuel production, as few regions of the globe are best than other places. In USA, southwest locations are good; Texas is well suitable for large-scale production.

Table 4.10 Various reactor configurations for microalgae with biomass productivity

Reactor type	Microalgae species	CO_2 feed gas (%)	[a]Specific growth rate (/h) or [b]Biomass productivity ($g/m^3/h$)	References
Open pond reactors	*N. saline*	5	1.25[b]	Matsumoto et al. (1995)
	Chlorella sp.	6.8	–	Doucha et al. (2005)
	Nannochloropsis saline	15	4.1[b]	Doucha et al. (2005)
Batch reactors	*Chlamydomonas reinhardtii*	30[b]	0.06 ± 0.01[a]	Yang and Gao (2003)
	Spirulina platensis	0.03	0.0082 ± 0.002[a]	Kumar et al. (2009)
	Chlorella pyrenoidosa	100[b]	0.09 ± 0.09[a]	Yang and Gao (2003)
	Scenedesmus obliquus	60[b]	0.06 ± 0.04[a]	Yang and Gao (2003)
	Chlorogloeopsis sp.	5	0.0007 ± 0.0060[a]	Kodama et al. (1993)
Bioreactors	*Porphyridium* sp.	2–3	–	Nakamura (2004)
	Euglena gracilis	11	4.8[b]	Chae et al. (2006)
	Chlorella vulgaris	1	–	Fan et al. (2007)
Membrane reactors	*Nannochloropsis* sp.	1	4.2–5.8[b]	Carvalho and Malcata (2001)
	Spirulina platensis	2–15	3–17.8[b]	Kumar et al. (2009)
	Chlorella vulgaris	1	4[b]	Ferreira et al. (1998)
	Chlorella vulgaris	1	–	Fan et al. (2007)
	Chlorella vulgaris	0.045	–	Fan et al. (2008)

[a]Specific growth rate is cell density improvement upon the time (hour)
[b]Biomass productivity is biomass weight in reactor per hour. If the growth rate or biomass productivity is increased the lipid content also will be increased. That is the reason researchers are measuring the growth rate from the reactor by taking sampling

4.7 Conclusions

This chapter discusses the various essential elements required for the cultivation of the microalgae. The basics of the microalgae classification, components present, and cultivation requirements have been explained. A review on the components (from carbohydrates to bioactive compounds) along with the compositions, cultivation, media, and various media used for the cultivation of microalgae with metabolic

pathways are consolidated in the tables and figures. In this chapter, around 55 media compositions are tabulated and their specialties and applications for the microalgae as feedstock are explained.

References

Abu-Rezq TS, Al-Musallam L, Al-Shimmari J, Dias P (1999) Optimum production conditions for different high-quality marine algae. Hydrobiologia 403:97–107

Allen MB (1959) Studies with *Cyanidium caldarium*, an anomalously pigmented chlorophyte. Arch Mikrobiol 32:270–277

Amro AA, Steinbüchel A (2013) New medium for pharmaceutical grade *Arthrospira*. Int J Bacteriol 2013:203432. https://doi.org/10.1155/2013/203432

Aslan S, Kapdan IK (2006) Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Eco Eng 28(1):64–70

ASTM (2012) Standard guide for conducting static toxicity tests with microalgae. E1218-04. In: Annual book of ASTM Standards, vol 11.06, West Conshohocken, PA, USA

Atlas RM, Parks LC (1997) Handbook of microbiological media, 2nd edn. CRC Press, Boca Raton

Barsanti L, Gualtieri P (2006) Algae—anatomy, biochemistry, and biotechnology. CRC Press, Taylor and Francis Group, Boca Raton, pp 215–235

Bautista-Chamizo E, Borrero-Santiago AR, De Orte MR, DelValls Á, Riba I (2018) Effects of CO_2 enrichment on two microalgae species: a toxicity approach using consecutive generations. Chemosphere 213:84–91

Becker EW (2007) Microalgae as a source of protein. Biotechnol Adv 25:207–210

Bleakley S, Hayes M (2017) Algal proteins: extraction, application, and challenges concerning production. Foods 6:34

Bold HC (1942) Cultivation of algae. Bot Rev 8:69–138

Breuer G, Lamers PP, Martens DE, Draaisma RB, Wijffels RH (2013) Effect of light intensity, pH, and temperature on triacylglycerol (TAG) accumulation induced by nitrogen starvation in *Scenedesmus obliquus*. Bioresour Technol 143:1–9

Brown MR (1991) The amino-acid and sugar composition of 16 species of microalgae used in mariculture. J Exp Mar Biol Ecol 145:79–99

Carefoot JR (1968) Culture and heterotrophy of the freshwater dinoflagellate, Peridinium cinctum fa. ovoplanum Lindeman. J Phycol 4:129–131

Carvalho AP, Malcata FX (2001) Transfer of carbon dioxide within cultures of microalgae: plain bubbling versus hollow-fiber modules. Biotechnol Prog 17:265–272

Carvalho AP, Meireles LA, Malcata FX (2006) Microalgal reactors: a review of enclosed system designs and performances. Biotechnol Prog 22(6):1490–1506

Castenholz RW (1969) Thermophilic bluegreen algae and the thermal environment. Bacteriol Rev 33:476–504

Chae SR et al (2006) Single cell protein production of Euglena gracilis and carbon dioxide fixation in an innovative photobioreactor. Bioresour Technol 97:322–329

Chen B, Wan C, Mehmood MA, Chang J-S, Bai F, Zhao X (2017) Manipulating environmental stresses and stress tolerance of microalgae for enhanced production of lipids and value-added products—a review (Part 2). Bioresour Technol 244:1198–1206

Chinnasamy S, Bhatnagar A, Hunt RW, Das KC (2010) Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresour Technol 101:3097–3105

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25:294–306

Chisti Y (2008) Biodiesel from microalgae beats bioethanol. Trends Biotechnol 26:126–131

Christaki E, Florou-Paneri P, Bonos E (2011) Microalgae: a novel ingredient in nutrition. Int J Food Sci Nutr 62:794–799

Chu SP (1942) The influence of the mineral composition of the medium on the growth of planktonic algae. J Ecol 30:284–325
Chu F-F, Chu P-N, Cai P-J, Li W-W, Lam PKS, Zeng RJ (2013) Phosphorus plays an important role in enhancing biodiesel productivity of *Chlorella vulgaris* under nitrogen deficiency. Bioresour Technol 134:341–346
Cuellar García DJ, Rangel-Basto YA, Barajas-Solano AF, Muñoz-Peñalosa YA, Urbina-Suarez NA (2019) Towards the production of microalgae biofuels: the effect of the culture medium on lipid deposition. J Biotechnol Comput Biol Bionanotechnol Biotechnol 100(3):273–278
da Silva Gorgônio CM, Aranda DAG, Couri S (2013) Morphological and chemical aspects of *Chlorella pyrenoidosa*, *Dunaliella tertiolecta*, *Isochrysis galbana* and *Tetraselmis gracilis* microalgae. Nat Sci 5:783
Debjani M, van Leeuwen J, Buddhi L (2012) Heterotrophic/mixotrophic cultivation of oleaginous *Chlorella vulgaris* on industrial co-products. Algal Res 1:40–48
Delrue F, Setier PA, Sahut C, Cournac L, Roubaud A, Peltier G, Froment AK (2012) An economic, sustainability, and energetic model of biodiesel production from microalgae. Bioresour Technol 111:191–200
Doucha J, Straka F, Livansky K (2005) Utilization of flue gas for cultivation of microalgae (*Chlorella* sp.) in an outdoor open thin-layer photobioreactor. J Appl Phycol 17(5):403–412
Eriksen NT, Poulsen BR, Iversen JJL (1998) Dual sparging laboratory-scale photobioreactor for continuous production of microalgae. J Appl Phycol 10(4):377–382
Esakkimuthu S, Krishnamurthy V, Govindarajan R, Swaminathan K (2016) Augmentation and starvation of calcium, magnesium, phosphate on lipid production of *Scenedesmus obliquus*. Biomass Bioenergy 88:126–134
Fan L et al (2007) Optimization of carbon dioxide fixation by *Chlorella vulgaris* cultivated in a membrane photobioreactor. Chem Eng Technol 30:1094–1099
Fan L et al (2008) Evaluation of a membrane-sparged helical tubular photobioreactor for carbon dioxide biofixation by *Chlorella vulgaris*. J Memb Sci 325:336–345
FAO (2018) News article: world's future multiple food security "in jeopardy" due to challenges, report warns item/471169/icode/. http://www.fao.org/news/story/en/
Ferreira BS et al (1998) Microporous hollow fibres for carbon dioxide absorption: mass transfer model fitting and the supplying of carbon dioxide to microalgal cultures. Chem Technol Biotechnol 71:61–70
Frac M, Jezierska TS, Jerzy T (2010) Microalgae for biofuels production and environmental applications: a review. Afr J Biotechnol 9(54):9227–9236
Goldman JC, Carpenter EJ (1974) A kinetic approach to the effect of temperature on algal growth. Limnol Oceanogr 5:756–766
González López CV, García MDCC, Fernández FGA, Bustos CS, Chisti Y, Sevilla JMF (2010) Protein measurements of microalgal and cyanobacterial biomass. Bioresour Technol 101:7587–7591
Grobbelaar JU (1994) Turbulence in mass algal cultures and the role of light/dark fluctuations. J Appl Phycol 6:331–335
Grobbelaar JU (2004) Algal nutrition: mineral nutrition. In: Richmond A (ed) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Publishing, Oxford, pp 97–115
Guillard RRL, Lorenzen CJ (1972) Yellow-green algae with Chlorophyllide C. J Phycol 8:10–14
Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. *I. Cyclotella* nana *Hustedt* and *Detonula confervacea Cleve*. Can J Microbiol 8:229–239
Guzzon A et al (2008) Cultured phototrophic biofilms for phosphorus removal in wastewater treatment. Water Res 42:4357–4367
Harris EH (1989) The *Chlamydomonas* sourcebook. Academic Press Inc., San Diego
Hoekman SK, Broch A, Robbins C, Ceniceros E, Natarajan M (2012) Review of biodiesel composition, properties, and specifications. Renew Sustain Energy Rev 16:143–169
Hossain N, Mahlia TMI, Saidur R (2019) Latest development in microalgae-biofuel production with nano-additives. Biotechnol Biofuels 12:125–141

Huang G, Chen F, Wei D, Zhang X, Chen G (2010) Biodiesel production by microalgal biotechnology. Appl Energy 87:38–46

Hutner SH, Zahalsky AC, Aaronson S, Baker H, Frank O (1966) Culture media for *Euglena gracilis*. In: Prescott DM (ed) Methods in cell physiology, vol 2. Academic Press, New York, pp 217–228

Ichimura T (1979) 2. Isolation and culture methods of algae. 2.5.B. Freshwater algae. In: Nishizawa K, Chihara M (eds) Methods in phycological studies. Kyoritsu Shuppan, Tokyo, p 294305

Ichimura T, Watanabe M (1974) The *Closterium calosporum* complex from the Ryukyu Islands—variation and taxonomical problems. Mem Natn Sci Mus Tokyo 7(89102):13–14

Janssen M, Slenders P, Tramper J, Mur LR, Wijffels RH (2001) Photosynthetic efficiency of *Dunaliella tertiolecta* under short light/dark cycles. Enzyme Microbial Technol 29:298–305

Kamalanathan M, Gleadow R, Beardall J (2015) Impacts of phosphorus availability on lipid production by *Chlamydomonas reinhardtii*. Algal Res 12:191–196

Kato S (1982) Laboratory culture and morphology of *Colacium vesiculosum* Ehrb. (Euglenophyceae). Jpn J Phycol 30:63–67

Keller MD, Selvin RC, Claus W, Guillard RRL (1987) Media for the culture of oceanic ultraphytoplankton. J Phycol 23:633–638

Kent M, Welladsen HM, Mangott A, Li Y (2015) Nutritional evaluation of Australian microalgae as potential human health supplements. PLoS One 10:0118985

Kessler E, Czygan FC (1970) Physiologische und biochemische Beiträge zur Taxonomie der Gattung Chlorella. Arch Mikrobiol 70:211–216

Kimura B, Ishida Y (1985) Photophagotrophy in *Uroglena americana*, Chrysophyceae. Jpn J Limnol 46:315–318

Kodama M et al (1993) A new species of highly CO_2-tolerant fast growing marine microalga suitable for high-density culture. J Marine Biotechnol 1:21–25

Kumar A et al (2009) Hollow fiber membrane photo-bioreactor for CO_2 sequestration from combustion gas coupled with wastewater treatment: a process engineering approach. J Chem Technol Biotechnol 85:387–394

Kurpan NDP, Silva AF, Araújo OQF, Chaloub RM (2015) Impact of temperature and light intensity on triacylglycerol accumulation in marine microalgae. Biomass Bioenergy 72:280–287

Laura B, Paolo G (2014) Alga: anatomy, biochemistry and biotechnology, 2nd edn. CRC Press, Boca Raton, pp 246–250

Lee YK, Tay HS (1991) High CO_2 partial pressure depresses productivity and bioenergetic growth yield of *Chlorella pyrenoidosa* culture. J Appl Phycol 3:95–101

Li Q, Du W, Liu D (2008) Perspectives of microbial oils for biodiesel production. App Microbiol Biotechnol 80:749–756

Liao Q, Chang H-X, Fu Q, Huang Y, Xia A, Zhu X et al (2018) Physiological-phased kinetic characteristics of microalgae *Chlorella vulgaris* growth and lipid synthesis considering synergistic effects of light, carbon and nutrients. Bioresour Technol 250:583–590

Matsumoto H et al (1995) Carbon dioxide fixation by microalgae photosynthesis using actual flue gas discharged from a boiler. Appl Biochem Biotechnol 51(52):681–692

Milano J, Ong HC, Masjuki HH, Chong WT, Lam MK, Loh PK, Vellayan V (2016) Microalgae biofuels as an alternative to fossil fuel for power generation. Renew Sust Energ Rev 58:180–197

Milledge JJ (2011) Commercial application of microalgae other than as biofuels: a brief review. Rev Environ Sci Biotechnol 10(1):31–41. https://doi.org/10.1007/s11157-010-9214-7

Molina E, Fernandez J, Acien FG, Chisti Y (2011) Tubular photobioreactor design for algal cultures. J Biotechnol 92(2):113–131

Nakamura T (2004) Local and chemical distribution of phlorotannins in brown algae. J Appl Phycol 16:291–296

Niccolai A, Chini ZG, Rodolfi L, Biondi N, Tredici MR (2019) Microalgae of interest as food source: biochemical composition and digestibility. Algal Res 42:101617

Noël MH, Kawachi M, Inouye I (2004) Induced dimorphic life cycle of a coccolithophorid, *Calyptrosphaera sphaeroidea* (Prymnesiophyceae, Haptophyta). J Phycol 40:112–129
Ogawa T, Terui G (1970) Studies on the growth of *Spirulina platensis*. (I) On the pure culture of *Spirulina platensis*. J Ferment Technol 48:361–367
Pai PR, Manasa A, Kalaivani T, Ajeesh CPM, Rajasekaran C, Prasad BN (2008) Simplified cost effective media variants for the rapid culture of *Spirulina platensis*. In: Prasad BN, Mathew L (eds) Recent advances in biotechnology. Excel India Publishers, New Delhi, pp 1–129
Paul JH, Rosemary EW, Taylor FJR (1980) A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. J Phycol 16:28–35
Posten C, Schaub G (2009) Microalgae and terrestrial biomass as source for fuels—a process view. J Biotechnol 142:64–69
Provasoli L, McLaughlin JJA, Droop MR (1957) The development of artificial media for marine algae. Arch Mikrobiol 25:392–428
Pulz O (2001) Photobioreactors: production systems for phototrophic microorganisms. Appl Microbiol Biotechnol 57:287–293
Qiu R, Gao S, Lopez PA, Ogden KL (2017) Effects of pH on cell growth, lipid production and CO_2 addition of microalgae *Chlorella sorokiniana*. Algal Res 28:192–199
Ramanna L, Rawat I, Bux F (2017) Light enhancement strategies improve microalgal biomass productivity. Renew Sust Energ Rev 80:765–773
Rao AR, Dayananda C, Sarada R, Shamala TR, Ravishankar GA (2007) Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. Bioresour Technol 98(3):560–564
Richmond A (1999) Physiological principles and modes of cultivation in mass production of photoautotrophic microalgae. In: Cohen Z (ed) Chemicals from microalgae. Taylor & Francis, London, pp 353–386
Richmond A, Becker EW (1986) Technological aspects of mass cultivation—a general outline. In: Richmond A (ed) Handbook of microalgal mass culture. CRC Press, Boca Raton, pp 245–263
Ricketts TR (1966) On the chemical composition of some unicellular algae. Phytochemistry 5:67
Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Genl Microbiol 111:1–61
Robertson BR, Tezuka N, Watanabe MM (2001) Phylogenetic analyses of *Synechococcus* strains (cyanobacteria) using sequences of 16S rDNA and part of the phycocyanin operon reveal multiple evolutionary lines and reflect phycobilin content. Int J Syst Evol Microbiol 51:861–871
Rodolfi L, Zittelli GC, Bassi N, Padovani G, Biondi N, Bonini G, Tredici MR (2009) Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng 102(1):100–112
Sajjadi B, Chen W-Y, Raman AAA, Ibrahim S (2018) Microalgae lipid and biomass for biofuel production: a comprehensive review on lipid enhancement strategies and their effects on fatty acid composition. Renew Sust Energ Rev 97:200–232
Sako Y, Ishida Y, Kadota H, Hata Y (1984) Sexual reproduction and cyst formation in the freshwater dinoflagellate *Peridinium cunningtonii*. Bull Jpn Soc Sci Fish 50:743–750
Samel A, Ziegenfuss M, Goulden CE, Banks S, Baer KN (1999) Culturing and bioassay testing of Daphnia magna using Elendt M4, Elendt M7 and Combo media. Ecotoxicol Environ Saf 43:103–110
Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, Kruse O, Hankamer B (2008) Second generation biofuels: high efficiency microalgae for biodiesel production. Bioenergy Res 1:20–43
Schwenzfeier A, Wierenga PA, Gruppen H (2011) Isolation and characterization of soluble protein from the green microalgae Tetraselmis sp. Bioresour Technol 102:9121–9127
Seambiotic (2009). http://www.seambiotic.com. Accessed 6 Jul 2009
Seyed HN, Shang H, Scott JA (2018) Biosequestration of industrial off-gas CO_2 for enhanced lipid productivity in open microalgae cultivation systems. Renew Sust Energ Rev 92:458–469
Sharma J, Kumar SS, Bishnoi NR, Pugazhendhi A (2018) Enhancement of lipid production from algal biomass through various growth parameters. J Mol Liq 269:712–720

Shin YS, Choi HI, Choi JW, Lee JS, Sung YJ, Sim SJ (2018) Multilateral approach on enhancing economic viability of lipid production from microalgae: a review. Bioresour Technol 258:335–344

Singh SP, Singh P (2015) Effect of temperature and light on the growth of algae species: a review. Renew Sust Energ Rev 50:431–444

Suh IS, Lee CG (2003) Photobioreactor engineering: design and performance. Biotechnol Bioprocess Eng 8(6):313–321

Tibbetts SM, Milley JE, Lall SP (2015) Chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors. J Appl Phycol 27:1109–1119

Torzillo G, Sacchi A, Materassi R, Richmond A (1991a) Effect of temperature on yield and night biomass loss in *Spirulina platensis* grown outdoors in tubular photobioreactors. J Appl Phycol 3:103–109

Torzillo G, Sacchi A, Materassi R (1991b) Temperature as an important factor affecting productivity and night biomas loss in *Spirulina platensis* grown outdoors in tubular photobioreactors. Bioresour Technol 38:95–100

Tredici MR, Materassi R (1992) From open ponds to vertical alveolar panels: the Italian experience in the development of reactors for the mass cultivation of phototrophic microorganisms. J Appl Phycol 4:221–231

Ugwu CU, Aoyagi H, Uchiyama H (2008) Photobioreactors for mass cultivation of algae. Bioresour Technol 99(10):4021–4028

van Krimpen MM, Bikker P, Van der Meer IM, van der Peet-Schwering CMC, Vereijken JM (2013) Cultivation, processing and nutritional aspects for pigs and poultry of European protein sources as alternatives for imported soybean products, Wageningen. http://www.livestockresearch.wur.nl. Accessed 26 Nov 2018

Velasco LA, Carrera S, Barros J (2016) Isolation, culture and evaluation of *Chaetoceros muelleri* from the Caribbean as food for the native scallops, *Argopecten nucleus* and *Nodipecten nodosus*. Lat Am J Aquat Res 44:557–568

Venkata Subhash G, Rohit MV, Devi MP, Swamy YV, Venkata MS (2014) Temperature induced stress influence on biodiesel productivity during mixotrophic microalgae cultivation with wastewater. Bioresour Technol 169:789–793

Vivi E, Sarjito, Johannes H, Budi P (2012) Effect of using Guillard and Walne technical culture media on growth and fatty acid profiles of microalgae *Skeletonema* sp. in mass culture. J Coast Dev 16:50–56

Vonshak A (1986) Laboratory techniques for the cultivation of microalgae. In: Richmond A (ed) Handbook of microalgal mass culture. CRC Press, Boca Raton, pp 117–145

Watanabe S (1981) Observations on *Urnella terrestris* Playfair (Chlorophyceae, Chlorococcales) in culture. Phycologia 20:12–15

Watanabe MM (1983) Growth characteristics of freshwater red tide alga, Peridinium based on axenic culture. Establishment of synthetic culture medium. Res Data Natl Inst Environ Stud No 24:111–121

Watanabe MM, Kawachi M, Hiroki M, Kasai F (2000) NIES collection list of strains. In: Microalgae and protozoa. Microbial culture collections, 6th edn. National Institute for Environmental Studies, Tsukuba, p 159

Wogan DM, Silva AKD, Webber ME, Stautberg E (2008) Algae: pond powered biofuels; ATI clean energy incubator. The University of Texas at Austin, Austin, pp 1–23

Wolkers H, Barbosa M, Kleinegris D, Bosma R, Wijffels RH (2011) Microalgae: the green gold of the future?, Wageningen. www.groenegrondstoffen.nl

Wondraczek L, Batentschuk M, Markus AS, Borchardt R, Scheiner S, Seemann B, Schweizer P, Christoph JB (2013) Solar spectral conversion for improving the photosynthetic activity in algae reactors. Nat Commun 4:2047

Xinxin W, Hilde KF, Keshuai L, Matilde SC, Olav V, Kjell IR (2019) Influence of nitrogen limitation on lipid accumulation and EPA and DHA content in four marine microalgae for possible use in aquafeed. Front Mar Sci 6:1–10

Xiong W, Li XF, Xiang JY, Wu QY (2008) High-density fermentation of microalga *Chlorella protothecoides* in bioreactor for microbio-diesel production. Appl Microbiol Biotechnol 78:29–36

Yagi O, Okada M, Sudo R (1979) Cultivation of *Microcystis* and red-tide organisms. Res Rep Natl Inst Environ Stud 6:223–229

Yamaguchi K, Nakano H, Murakami M, Konosu S, Nakayama O, Kanda M, Nakamura A, Iwamoto H (1987) Lipid composition of a green alga, *Botryococcus braunii*. Agric Biol Chem 51 (2):493–498

Yang AD (2011) Modeling and evaluation of CO_2 supply and utilization in algal ponds. Ind Eng Chem Res 50:11181–11192

Yang Y, Gao K (2003) Effects of CO_2 concentrations on the freshwater microalgae *Chlamydomonas reinhardtii*, *Chlorella pyrenoidosa* and *Scenedesmus obliquus* (Chlorophyta). J Appl Phycol 15:379–389

Zhu J, Rong J, Zong B (2013) Factors in mass cultivation of microalgae for biodiesel. Chin J Catal 34:80–100

Zu N, Richmond A (2000) Light-path length and population density in photoacclimation of *Nannochloropsis* sp. (Eustigmatophyceae). J Appl Phycol 12:349–354