CHAPTER 31

Exploring the dynamics of microalgal diversity in high-rate algal ponds

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31.1 Introduction

Pollution due to the presence of high concentration of nutrients in the surface of water of natural water bodies has become a global issue due to eutrophication and algal blooms. Bioremediation of wastewater is necessary for the reduction of chemical oxygen demand (COD), biological oxygen demand (BOD), and nutrient levels [60,65]. In addition to nutrients, chemicals such as pharmaceuticals, antibiotics, plastics, and heavy metals have shown to cause detrimental effects to aquatic and human beings [64,66]. When these sludges rich in organic waste are let into natural streams, the increase in turbidity decreases the clarity and visibility of water [79]. Groundwater tables also might get contaminated when the assimilation capacity of water for nutrients increases [144]. On the other hand, phosphate resources are depleting due to increase in world population and supply of nutrients for food. The deposits of rock phosphates for manufacture of fertilizers are becoming scarce. Hence, these nutrients which are lost in wastewater should be regained [2,120].

Conventional wastewater treatment plants used chemicals such as aluminum and iron for the removal of phosphorus. The water bodies were fitted with meshes of nylon or other material for the filtration of nutrients. But the phosphates of these salts are toxic to plants even in slight concentrations and hence cannot be used as fertilizers [40]. Later, biological methods of using microorganisms for removal of nutrients came into use. This method is eco-friendly and doesn't generate activated sludge which makes them efficient for the removal of nutrients [120].

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Wastewater can be used as an alternative source of nutrient to grow microalgae which can also aid bioremediation. The immense availability of nutrients in wastewater tends to accelerate microalgal growth. Microalgae require nitrogen (N) and phosphorus (P) for the synthesis of protein, lipids, and carbohydrates [36]. Table 31.1 depicts the various sources of wastewater used for the growth of microalgae, culture conditions, and their nutrient removal efficiencies. These organisms can inhabit all euphotic zones such as submerged under water, surface attachment, and live attached sediments [120]. The supply of oxygen by the microalgae matches with the oxygen demand of the wastewater. The oxygen produced can degrade the aromatic components present in wastewater [15].

Table 31.1: Sources of wastewater that favors microalgal growth and nutrient removal for biodiesel production.

Source of wastewater	Microalgal species	Mode of cultivation	Culture conditions	Nutrient removal efficiency	Yield	References
Piggery waste	*Chlorella vulgaris* JSC-6	Batch (heterotrophic cultivation) (fivefold dilution)	pH 7.5 Temperature 25 ± 2°C Light intensity 200 W m^{-2} CO_2 2.5% at a flow rate of 0.2 vvm	COD ≤76% TP ≤91%	Specific growth rate 3.96 g L^{-1} Carbohydrates 54.0%	[141]
	Chlorella zofingiensis	Semicontinuous	Temperature 21.5–34.5°C Light intensity 115–2046 µmol m^{-2} s^{-1} Photoperiod 12:12	COD 80%, TN 80% TP 92%	Biomass productivity 2.448 g L^{-1} d^{-1} at day 6 Lipid content 34.82% Lipid productivity 69.83 mg L^{-1} d^{-1}	[149]
	Scenedesmus spp.	Batch	pH 7.9–11 Temperature 22 ± 2°C Light intensity 12.8 mol m^{-2} Photoperiod 12:12	TN 21.2 ± 1.2 mg L^{-1} TP 3.5 ± 2.5 mg L^{-1} H_2S ≤3000 ppmv CO_2 assimilation 219 ± 4.8 mg L^{-1} d^{-1}	Biomass concentration 1.1 ± 0.2 g L^{-1} at day 9 Growth rate 141.8 ± 3.5 mg L^{-1} d^{-1}	[95]

(*continued*)

Source of wastewater	Microalgal species	Mode of cultivation	Culture conditions	Nutrient removal efficiency	Yield	References
Dairy waste	*Chlorella* sp./ *Scenedesmus* spp./*C. zofingiensis* (1:1:1)	Batch	pH 9.31 ± 0.10 Temperature 25 ± 1°C CO_2 5–6% for 7 days Light intensity 150 ± 5 µmol photons m^{-2} s^{-1}	COD removal 7.01–62.86% TP 91.16–95.96% TN 87.04–91.02%	Biomass concentration 5.31 ± 0.46 g L^{-1} Total lipid 19.03% ± 1.22% Lipid productivity 143.7 ± 3.3 g L^{-1} d^{-1}	[98]
	Chlamydomonas polypyrenoideum	Batch	pH 7.2 Temperature 28 ± 2°C Light intensity 10 W m^{-2} Photoperiod 12:12	Nitrate 90% Nitrite 74% TP 70% Chloride 61% Fluoride 58% Ammonia 90% (10^{th} day)	Biomass concentration 2.2 g L^{-1} (58%) Lipid 1.6 g L^{-1} (42%)	[57]
Municipal waste	*C. vulgaris*	Batch	pH 7.42–7.79 Temperature 25°C CO_2 2vol% at a flow rate of 3 mL min^{-1} Light intensity ~150 µmol m^{-2}·s^{-1}	TN 95% TP 98%	Biomass 2.91 ± 0.02 g L^{-1} Lipid 11.3 ± 0.1 g/100 gVS Carbohydrates 36.2 ± 0.3 g/100 gVS Protein 35.2 ± 1.4 g/100 gVS	[18]
	Chlorella kessleri	Batch		TN 96% TP 99%	Biomass 2.70 ± 0.08 g L^{-1} Lipid 7.4 ± 0.2 g/100 gVS Carbohydrates 44.6 ± 0.1 g/100 gVS Protein 36.7 ± 1.0 g/100 gVS	
Olive-oil mill waste	*Spirulina platensis*	Mixotrophic	pH 8.5 Temperature 30 ± 2°C Light intensity 10 klx Photoperiod 20:4	Removal of COD 28.77–66.88% Carbohydrate removal 54.30–91.15% Nitrate removal 87.38–95.61%	Biomass concentration 1696 mg L^{-1} Biomass productivity 4.4 mg L^{-1} h^{-1} Lipid 7.37–16.91%	[77]

(*continued*)

Table 31.1: (Cont'd)

Source of wastewater	Microalgal species	Mode of cultivation	Culture conditions	Nutrient removal efficiency	Yield	References
Poultry-litter waste	*Chlorella minutissima*	Batch (mixotrophic) (9% wastewater)	Temperature 25 ± 1°C Light intensity 75–80 µmol m^2 s^{-1} Photoperiod 12:12 h for 12 days	TN 53 mg L^{-1} TP 5.0 mg L^{-1}	Biomass productivity 69 mg L^{-1} d^{-1} Lipid 12.2% ± 0.4% Protein 40.9% ± 0.3% Carbohydrates 11.3% ± 0.8%	[111]
	Chlorella sorokiniana			TN 53 mg L^{-1} TP 5.2 mg L^{-1}	Biomass removal 63 mg L^{-1} d^{-1} Lipid 15.7% ± 2.2% Protein 39.1% ± 1.9% Carbohydrates 12.2% ± 1.1%	
	Scenedesmus bijuga			TN 44 mg L^{-1} TP 4.9 mg L^{-1}	Biomass removal 72 mg L^{-1} d^{-1} Lipid 10.0% ± 0.3% Protein 40.6% ± 0.2% Carbohydrates 10.2% ± 0.9%	
Pharmaceutical waste	*Microalgal consortium*	Batch (mixotrophic)	pH 7 Temperature 28 ± 2°C Light intensity 1500 lux Photoperiod 16:8	COD 74.48% Nitrate 77.6% TP ≤100%	Biomass productivity 2.8 g L^{-1} Lipid content 17.2%	[47]
Agricultural waste	*C. vulgaris*	Batch (1:10 dilution)	pH 7.65 Air/CO_2 (97/3, v/v) Light intensity 200 µmol m^2 s^{-1}	TN 7.8 mg L^{-1} d^{-1} (>99%) TP 0.28 mg L^{-1} d^{-1} (>94%)	Biomass growth rate 0.64 μd^{-1} Mean daily productivity 0.23 ± 0.04 g L^{-1} d^{-1}	[29]
	Neochloris oleoabundans			TN 6.9 mg L^{-1} d^{-1} (>99%) TP 0.3 mg L^{-1} d^{-1} (>94%)	Growth rate 0.27 μd^{-1} Mean daily productivity 0.20 ± 0.04 g L^{-1} d^{-1}	

(*continued*)

Source of wastewater	Microalgal species	Mode of cultivation	Culture conditions	Nutrient removal efficiency	Yield	References
	Scenedesmus obliquus			$NH4^+$-N elimination capacity 7.8 mg L^{-1} d^{-1} (>83%) PO_4^{3-}P elimination capacity 0.36 mg L^{-1} d^{-1} (>94%)	Growth rate 0.49 μd^{-1} Mean daily productivity 0.22 ± 0.03 g L^{-1} d^{-1}	
Textile/dye waste	*Chlorella pyrenoidosa*	Batch (unautoclaved)	pH 6–8.5 Temperature 28°C	Nitrate 81% ± 1% TP 36% ± 2.2% BOD 73% ± 1.6% Maximum color removal (methylene blue) after 30 min		[91]
Slaughter-house waste	*Chlamydomonas subcaudata, Anabaena* sp., *Nitzschia* sp.	Greenhouse conditions	Temperature 20 ± 6°C Light intensity 40,000 ± 7500 lux Photoperiod 13:11	COD 86% TP 91% TN 71–79%	Biomass production 12.7 g (VSS)/ m^2day Fatty acid 142 mg FFA/g biomass Biogas 142 mg FFA/g biomass	[49]
Brewery effluent	*S. obliquus*		Temperature 30 ± 3°C Light intensity 12,000 lux Photoperiod 12:12	COD 57.5% TN 20.8% TC 56.9%	Biomass 0.9 g L^{-1}	[78]

TP, Total phosphorus; *TN*, Total nitrogen; *COD*, Chemical oxygen demand.

High-rate algal ponds (HRAPs) are open, wide, and shallow ponds that are built in a raceway configuration for large-scale application of wastewater treatment. These photobioreactors treat organic wastes in wastewater and simultaneously promote the growth of microalgae by the uptake of nutrients in wastewater [37]. Paddle wheels were generally being used in HRAP for mixing purposes. Recent findings have shown that propeller mixers can also be energy efficient. HRAP doesn't require inoculation. The presence of high concentration of nutrients such as N and P automatically shifts the microalgal population dominance toward Chlorophytes and Cyanobacteria. Generally two mechanisms take place in an HRAP. Initially,

there is a rapid growth phase due to the presence of excess nutrients. The density of microalgae increases with time. Secondly, the depletion of nutrients creates a stress necessary for accumulation of lipids. The dense population also might affect the penetration of light due to self-shading [136].

The advantage of HRAP over the traditional waste-stabilizing ponds is that it is economically feasible in terms of nutrient recovery. At the same time, the microalgae grown can be used as fertilizers and other value-added products like protein-rich food (single cell protein) and biodiesel can be obtained from microalgal biomass [97]. The BOD and COD removal in an HRAP is a function of the photosynthetic efficiency, solar irradiation, heat combustion, and pond design parameters such as depth, hydraulic retention time (HRT), etc. [37]. The disadvantages include high cost associated with the harvest of microalgae [130] and low productivity due to hindrances such as light limitation, high dissolved oxygen level, and biomass loss due to grazing [82,112]. The effect of bacterial association with microalgae was neglected before as the pretreatment methods of wastewater involved autoclaving or filtration using microfilters for the removal of bacterial activities [149]. Cocultivation of microalgae with bacteria has shown to be beneficial in terms of nutrient exchange and removal of nutrients as well. Another upcoming approach is the coculturing of microalgae with yeast in wastewaters where both the organic nutrients (N and P) and organic carbon will be present at higher concentrations [118]. However, the species should be chosen carefully to increase productivity.

Abiotic stresses such as salinity, temperature, solar irradiation, and nutrient depletion affect the cellular mechanisms which are necessary for the production of nutraceuticals. Optimization of parameters such as mixing rate, mixing time, HRT, pH, CO_2 concentration becomes necessary for maintenance of yield [88]. This book chapter comprehends the various species of microalgae that are employed in an HRAP for biotechnological application, their composition, their interaction with other microorganisms such as bacteria in wastewater, and the various parameters that govern the productivity.

31.2 Nutritional composition of wastewater and its removal

The macronutrients present in the wastewater mainly include N and P. P is an essential element for all the living organisms. These nutrients are valuable and essential for the growth of plants and microorganisms (i.e., algae and bacteria). The high concentrations of P and N in wastewater originate from various sources such as municipal waste, domestic waste, human feces, urine, industrial waste, pharmaceutical waste, detergents, agricultural runoff containing fertilizers and pesticides, soil erosion infiltrates from animal husbandry, leakage from sewage drains, landfill leachates, etc. [61,63,69]. Proteins contribute to 60% of organic nitrogen in wastewater followed by urea. Also organic debris from wood, leaves, and grass decompose to release nutrients. Under dark fermentation conditions, anaerobic bacteria break down complex carbon compounds along with mineralization of N and P. The N:P ratio of wastewater

can vary depending on the source. These nonpoint sources of wastewater are very difficult to avoid and control.

Microalgae are known to be the best alternate for recovery of nutrients (N and P) from wastewater and the biomass can be used as a feedstock for biodiesel. Simultaneously, the high nutritional demands for growth of algae can be compensated by using wastewater as medium for culturing. Wastewater also contains other micronutrients essential for algal growth such as iron, copper, manganese, and zinc [38]. For these reasons, microalgae can be employed in HRAPs as suspension cultures for efficient nutrient recovery. The effect of nutrient availability was accessed by the elementary analysis of biomass (carbon 40%–60%, nitrogen 1%–10%, phosphorus 1%–10%). Table 31.2 represents the ultimate and proximate analysis of several microalgae. Microalgal species present in wastewater might vary depending on the source and the composition of nutrients present in them [85]. Nowadays, microalgal biofilms (attached algae-based system) are used in horizontal and vertical cultivation systems for recovery of nutrients [41,70].

Phycoremediation refers to the assimilation or disintegration of organic and inorganic compounds in wastewater such as carbon, nitrogen, phosphorus, metals, and other contaminants by microalgae and cyanobacteria. Among this, removal of P and N is of greater importance because of their involvement in eutrophication [99]. Some mechanisms by which the microalgae consume phosphates and nitrogenous compounds are discussed below.

31.2.1 Removal of phosphorus

The uptake of phosphates by microalgae mainly involves two mechanisms.

1. Assimilation of phosphate for the construction of organic cellular components such as phospholipids.
2. Excess or luxury uptake of phosphate as inorganic polyphosphate for storage.

Such inorganic polyphosphates can be soluble and insoluble. The soluble polyphosphates are involved in the metabolic activities whereas the insoluble polyphosphates are stored and used when the external P becomes limiting. Extracellular or cell wall–bound phosphatase enzymes convert these organic phosphates into orthophosphates when required. Microalgae have known to reduce their ribosome number to sustain the protein synthesis and compensate storage of polyphosphates during P limitation. Quick consumption of the stored polyphosphates occurs under high light conditions and leads to decrease in biomass phosphate concentration [107]. The P assimilation also depends on the rate of assimilation of carbon and HRT. The organic P is taken by microalgae such as *Chlorella* sp. and *Scenedesmus* sp. [9], whereas inorganic P is removed by *Nostoc muscorum, Oscillatoria limnetica,* and *Chlorella vulgaris* [119]. The phosphate removal mechanism of bacteria is mainly through overintake [71,122]. Precipitation of phosphorus as inorganic salts is the abiotic form of phosphate removal. The

Table 31.2: Ultimate and proximate analysis of various microalgae reported in the literature.

	Ultimate analysis							Proximate analysis				
Microalgae	C (%)	H (%)	N (%)	S (%)	O (%)	HHV (MJ kg^{-1})	LHV (MJ kg^{-1})	Volatile matter (%)	Ash (%)	Fixed carbon (%)	Moisture (%)	Reference
Chlorella vulgaris	47.84	6.41	9.01	1.46	25	23.2		55.37	10.28	34.35	5.2	[164]
Nannochloropsis oculata	49.72	7.35	5.02	0.64	37.27	18.25		71.52	12.33	8.26	7.89	[163]
Tetraselmis sp.	47.11	7.44	4.91	0.76	39.78	16.39		73.1	11.21	7.68	8.01	
Spirulina	53.6	7.3	9.2	0.5	29.4	21.2			7.6		7.8	[171]
Chlorella protothecoides	47.84	6.41	9.01	1.46	25	21.71	20.26	55.37	10.28	34.35		[172]
Scenedesmus sp.	32.1	4.8	5.3	0.5	22.1			59.7	35.2	2.1	2.9	[167]
Chlorogloeopsis fritschi	54.4	6.9	7.3	ND	31.4				7.6		6.8	[13]
Scenedesmus dimorphus	53.4	7.8	7.9	ND	31				11.8		1.6	
Spirulina platensis	55.7	6.8	11.2	0.8	26.4				7.6		7.8	
Porphyridium cruentum	51.3	7.6	8	ND	33.1	14.7			24.4		5.1	[12]
Nannochloropsis oceanica	47.77	7.16	6.11		24.02							[121]
Nannochloropsis gaditana	47.26	7.03	6.72	0.49	38.5			75.91	10.68	8.29	5.12	[73]
Scenedesmus almeriensis	41.9	6.7	5.9	0.8	44.7			67.9	19.4	9.7	2.9	[73]
Botryococcus braunii	77.04	12.4	1.23	0.18	9.86		35.58	99.15	0.7	0.15	1.64	[170]
Hapalosiphon sp.	47.94	7.44	6.45	0.58	37.58		14.75	74.29	13.98	11.73	3.97	
Dunaliella tertiolecta	39	5.37	1.99	0.62	53.02	14.24		54.48	13.54	27	4.98	[110]
Dunaliella salina	48.1	7.1	9.4	0.9	23.3	21.2		76.3	7.2	12.5	4	[39]
Chlamydomonas reinhardtii	52	7.4	10.7		29.8		23		14.3		5.1	[55]
Cyanidioschyzon merolae	48.13	5.14	9.99	1.24	35.5	18.11			10		53.73	[26]
Galdieria sulphuraria	42.4	3.9	9.41	1.36	42.93	16.4			9.4		67.35	

Tetraselmis suecica	43.32	7.27	5.75	3.11	40.55			67.92	12.2	12	7.88	[150]
Isochrysis	49.26	7.5	6.24	0.96	31.74	23.52		79.79	6.89	11.63	1.69	[153]
Scenedesmus obliquus	50.3	7.29	8.26	0.62	33.53	19.7	18.2	76.8	7.8	11	4.4	[161]
Phaeodactylum tricornutum	37.5	6.47	7.25	0.83	27.3				16.8			[165]
Chlorella kessleri	54.8		3.5									[160]
Chlorella sorokiniana	45.07	7.64	3.88		35.52	20.4		73.2	7.9	15.1		[162]
Chlorella pyrenoidosa	46.2	6.7	8.2	0.6	18.3	21.9			9		10	[166]
Pseudochoricystis ellipsoidea	61.3	9.1	2.1	NA	27	29.4			1		1.2	[174]
Scenedesmus acuminatus	46.49	6.48	5.93	1.39	30.37	20.58	19.26	81.63	9.34	9.03	3.34	[175]
Oscillatoria sp.	37.7	5.5	4.7	0.46	38.34	15.86		72.3	8.9	14.4	4.4	[169]
Scenedesmus quadricauda	20.74	0.88	1.04	0.5				68	0.68	22.12	9.2	[65]
Chlorococcum humicola	33.16	5.58	4.8	2.42	27.24	13.6		55.6	26.8	14.8	3.42	[168]
Scenedesmus acutus	42.47	6.5	6.77	0.52	24.38			66.54	19.36	9.09		[173]
Microcystis sp.	42.26	6.27	7.88	0.52	43.07	16.2		7.013	6.14	14.14	9.59	[145]
Desmodesmus sp.	75	8.8	6	10.2		35.8						[33]

C, Carbon; H, Hydrogen; N, Nitrogen; S, Sulfur; O, Oxygen; HHV, Higher heating value; LHV, Lower heating value.

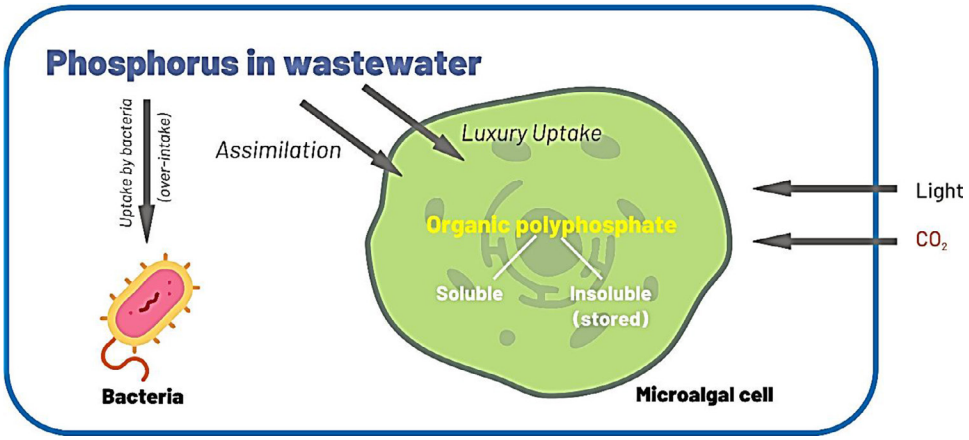

Fig. 31.1: Mechanism of phosphorus removal by microalgae–bacteria consortium in HRAP.

pictorial representation of the major phosphate removal mechanisms by microalgae and bacteria is given in Fig. 31.1. Other mechanisms include ion exchange and adsorption [16,124].

31.2.2 Removal of nitrogen

Among the different forms of nitrogenous compounds present in wastewater, ammonium has been known as the most biologically accessible form of inorganic nitrogen that can be easily consumed by microalgae [29]. They exhibit chemotaxis toward ammonium and lose it during gametogenesis. This is affected by the circadian rhythm [6]. Nitrification and denitrification is considered as the major mechanism concerning the removal of total nitrogen content of wastewater. This mechanism is supported by the presence of high amount of dissolved organic carbon (DOC) and initial N:P ratio. Ammonia volatilization doesn't contribute much to the total Kjeldahl nitrogen (TKN) removal. The nitrification/denitrification mechanisms are used to convert ammonia into nitrate and then into nitrite [15,75,94]. Ammonium and nitrate are the main forms of nitrogen consumed by microalgae followed by urea and nitrite [76]. Fig. 31.2 portrays the nitrogen removal mechanism of microalgae and bacteria in HRAP. Compared to these, P is not frequently considered a growth-limiting factor. Each microalga prefers a specific form of nitrogen for its consumption. For example, *C. vulgaris* prefers ammonium and *Chlorella kessleri* prefers uptake of nitrate for its growth [69].

31.2.3 Carbon sequestration

Carbon dioxide fixation through microalgae is dependent on various physicochemical parameters such as pH, temperature, photoperiod, light intensity, CO_2 concentration and flue gas composition, and hydrodynamic parameters such as rate of mixing, flow, and CO_2 transfer. Microalgae can sequester carbon by the conversion of inorganic carbon (carbon dioxide) to organic carbon by carboxylation. The primary enzyme responsible to

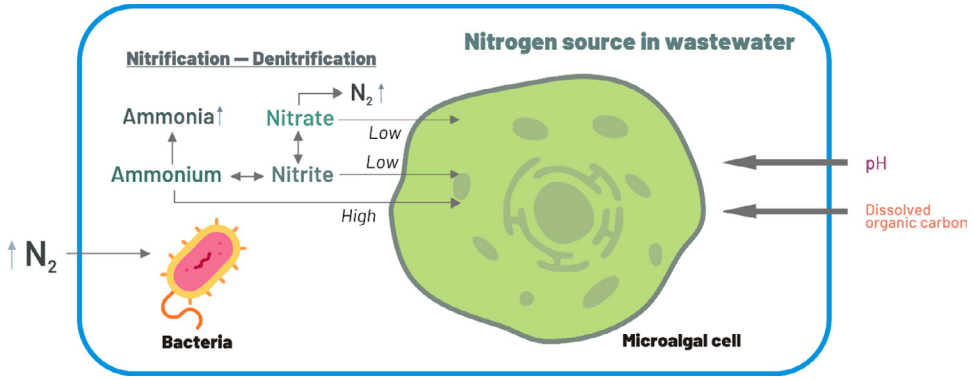

Fig. 31.2: Mechanism of nitrogen removal by microalgae–bacteria consortium in HRAP.

carboxylation is RuBisCo. But this mechanism is inactive at low concentrations of CO_2. Hence, cyanobacteria and eukaryotic algae have evolved an alternate mechanism known as CO_2-concentrating mechanism (CCM) which enhances CO_2 uptake inside carboxysome and pyrenoids. CCM involves in the transport of inorganic carbon into the intracellular system for sequestration inside the thylakoid lumen. In addition, very high concentrations of CO_2 resulted in the shift of electron flow and inhibition of CCM [106,124,125,134,137]. ClA5/CCM1 and LCR1 are some of the CCM regulators [156]. The carbon sequestration by microalgae and bacteria is briefly depicted in Fig. 31.3. Similarly, many mechanisms and shunts were evolved for adaptation of different microalgae. For example, 2-oxoglutarate genes in cyanobacteria were replaced by four different mechanisms as alternate for TCA cycle. Upregulation of existing carbon assimilation mechanisms was also noticed in green algae under high CO_2 conditions [156].

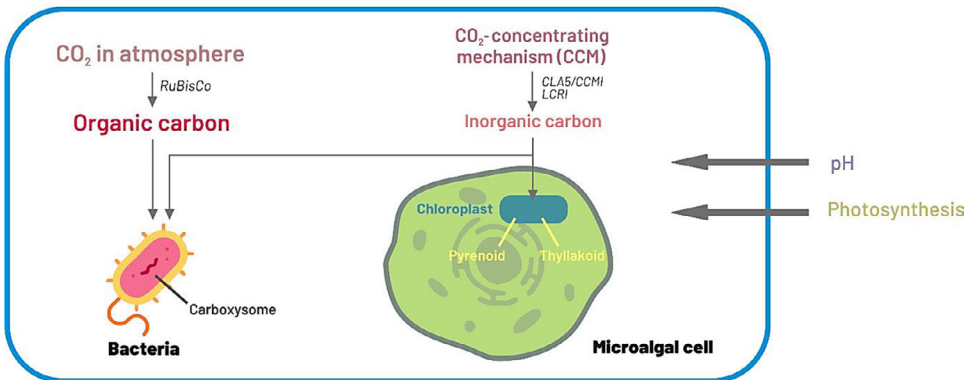

Fig. 31.3: Mechanism of carbon sequestration by microalgae–bacteria consortium in HRAP.

31.3 Microbial communities in wastewater

In the past decade, bacterial biomass was widely used for wastewater treatment. Many bacterial species have the ability to aerobically break down the organic matter in the effluent and also assimilate other hazardous contaminants for their metabolic activities [62]. Use of bacterial consortium for wastewater treatment was advantageous considering the fact that bacteria have a very short generation time and the genetic manipulations in bacteria for improving the efficiency were quite easy and feasible. The major drawback lay in the high operational cost involved in aeration of the sludge and removal of bacteria after the treatment. The applications associated with bacteria were also limited. In case of anaerobic treatments, the removal efficiency of nonorganic pollutants such as nutrients and pathogens was low [31,44].

In recent years, use of microalgae has been considered a feasible and alternate solution for sustainable treatment of wastewater. The supremacy over other conventional techniques is that microalgae can fix CO_2 in the atmosphere, alleviating global warming. Yet, the carbon dioxide concentration present in the atmosphere is only 0.04% which may not be a sufficient carbon source for efficient growth of microalgae. Microalgae can also be regarded as indicators of water quality as their composition and density varies with respect to the physicochemical conditions and environmental stresses. This technique requires simple reactor designs which in turn can minimize the operational cost. The microalgal biomass obtained after harvest can be further used as feedstock for biofuel and biofertilizer production. Also, microalgal metabolites have enormous biotechnological potential [69,82].

31.3.1 Microalgae as biological indicators of water quality

Water pollution causes striking changes in the biotic and abiotic communities of water. Some species are able to resist the changes taking place while others cannot or result in reduced actions. In this way, microalgae are considered "bioindicators" for the reason that they are quick responders of the anthropogenic changes pertaining to water quality. They are indicators of ecological health of a system. They can be employed for biologically monitoring the quality of water. They are well suited for assessment of water conditions because of their sensitive reactions to various ecosystem drivers such as changes in the nutrient composition, water hardness, transmission of light, light intensity, temperature and pH of water, heavy metal contamination, and the rate of flow and size of the water system [68,90,109]. The effect of these influential parameters on microalgae and its response in terms of biological indications is shown in Fig. 31.4.

Spoljaric et al. [116] used *C. kessleri* as an experimental model to study the level of reactive oxygen species. Similarly, delayed luminescence of freshwater microalgae *Selenastrum*

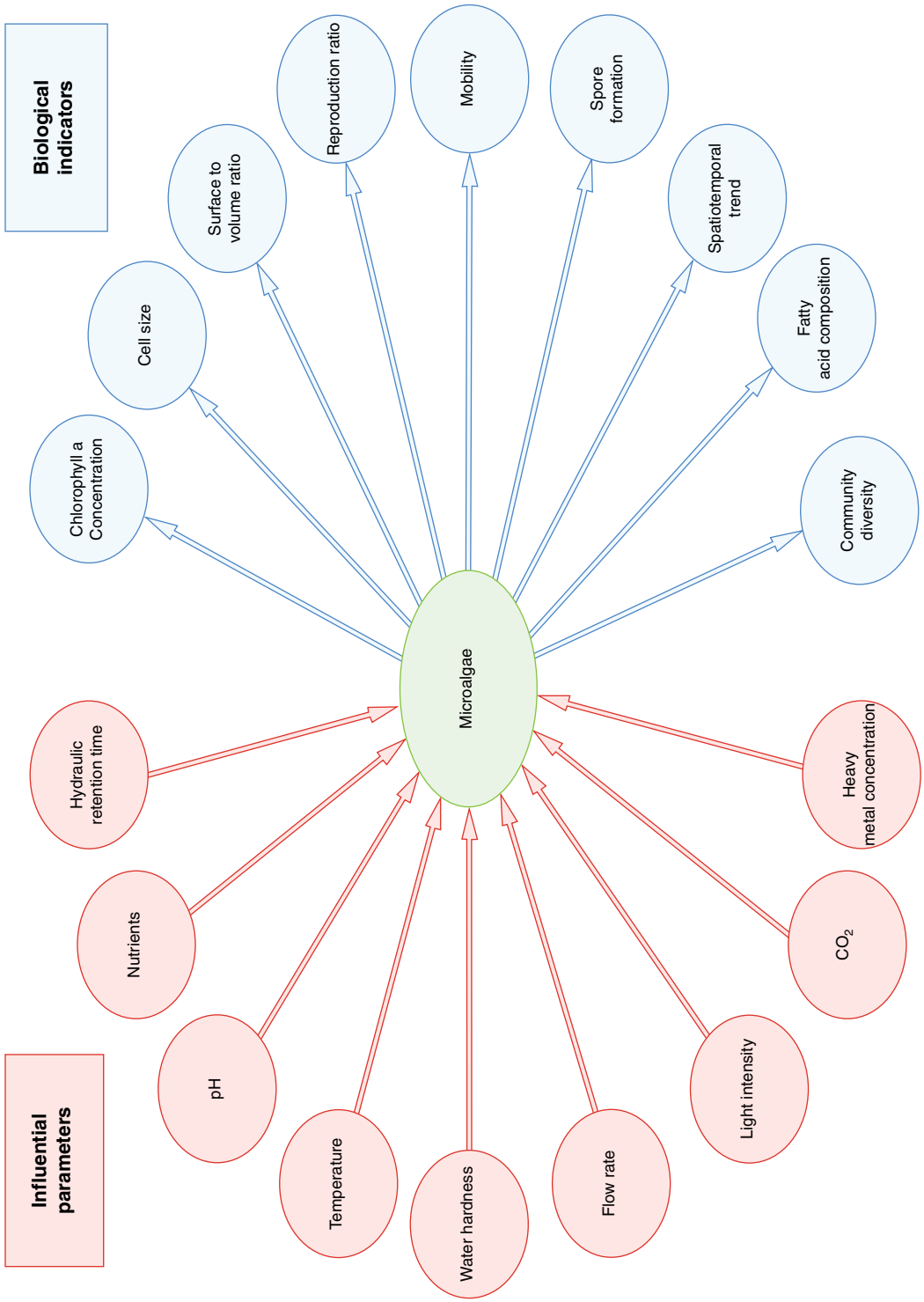

Fig. 31.4: The environmental influences on microalgae and their biological indication.

capricornutum was used to detect contamination by heavy metals such as cadmium, chromium, copper, and lead. By interactions taking place between the metals and cells, the microalgae can remove heavy metals by assimilating them for their metabolism. Higher concentrations can sometimes be too toxic to such organisms [109].

Use of microalgae for detecting the changes in the pollution levels is an economically viable alternate for monitoring the synergistic and antagonistic biological impacts of the system. These bioindicators are generally found in lakes, rivers, or streams which have been contaminated by leakages from sewage reservoirs which contain high amount of organic wastes. Microalgae assimilate phosphate, nitrate, and ammonium from sewage for their living. HRAPs also employ such organic pollution–tolerating microalgae for conversion of primary waste to secondary effluent. The N:P fluctuations caused by this wastewater determine the presence or absence of specific species of algae. For example, low inflow of freshwater will lead to a nutrient shift from P to N limitation, resulting in decreased algal growth (Table 31.3) [68].

Water samples from these water bodies can be collected and analyzed for the early warning signs of contamination such as density and diversity of algal species. The microalgal communities show variation in their chlorophyll a content, biovolume, surface area to volume ratio, cell size, reproduction rate, spore formation, eco-morphology, spatial and temporal trends, fatty acid composition, attachment to substratum, and mobility. Another important indicator is the variation in community structure (i.e., diversity). These changes can be assessed by traditional microscopic methods or fluorescence-based pigment analysis, photosynthetic activity, and size spectra analysis. The advantage of community structure assessment is the inclusion of heterotrophic species. But certain difficulties like complexity, high spatiotemporal variability, time consumption, lack of reference, boundary conditions and standardized methods also come along [72,117].

Table 31.3: The favorable culture conditions of various algal groups [68].

	Controlling factors					
Dominant algal groups	**Residence time**	**Flow**	**Salinity**	**Temperature**	**N:P**	**Si**
Cyanobacteria	High	Low	Hypersaline	High optimum	Low	Low
Dinoflagellates	High	Low	Marine/brackish	Warm		
Chlorophytes and cryptophytes	Low	High	Fresh/brackish	Cool	High	Low
Diatoms	Low	High	Marine/brackish and hypersaline		High	High

N, Nitrogen; P, Phosphorus; Si, Silicon.

Water pollution level and the diversity of algae can also be assessed numerically by the use of indices. They can be classified as (i) physicochemical indices which include parameters and (ii) biological indices which include biological formation. The indices are as follows.

a) Diversity index
b) Palmer's algal pollution index
c) Organic pollution index (OPI)

31.3.1.1 Diversity index

Diversity index provides information regarding the maximum and minimum diversity in a water system. Generally, high diversity exists in clean water and vice versa. In other words, high redundancy persists in polluted water. Maximum information is obtained when each individual organism belongs to different species in a community [157]. There are many diversity indices among which Shannon–Weaver index is being widely used.

According to Shannon–Weaver diversity index [59],

$$D = -\sum pi \log 2\ pi$$

where, $\bar{D}$ is Diversity index, *pi* is the No. of individuals in species / total no. of individuals in the sample.

The diversity index value declines with respect to polluted water. These values can be correlated with different degrees of pollution.

According to Trivedi's scale [158] for evaluation of pollution level, the classification of diversity index conditions (Table 31.4) are as follows:

Table 31.4: Classification of diversity index conditions to evaluate the pollution level.

$\bar{D}$	Condition
>4.0	Very clean water
3–4	Slightly polluted
2–3	Moderately polluted
<2.0	Heavily polluted

31.3.1.2 Palmer's algal pollution index

Palmer's algal pollution index provides numerical information on the level of tolerance of various algal species to organic pollution in a system. Palmer in 1969 prepared a list of 60 genera and 80 species which were identified tolerant to organic pollution by 165 authors.

Chlorophytes, blue-green algae, diatoms, and pigmented flagellates were well represented as high pollution–tolerating genera. Firstly, the organisms present in the system are identified. Then the score is calculated by summing up the numbers assigned to each genus. The pollution index (Table 31.5) for assessment of rate of organic pollution can be found from the total score [89].

Table 31.5: Pollution index for assessment of organic pollution [105].

Pollution index	Status of organic pollution
0–10	Lack of pollution
10–15	Moderate
15–20	Probable
>20	High

31.3.1.3 Organic pollution index

OPI is assessed based on the amount of oxygen present in a water body. It involves various parameters such as ammonia, COD, BOD, saturation of dissolved oxygen, and temperature. These parameters are measured on a time–time interval and consolidated for calculating the index value. The advantage of this technique is that a cluster of variable is taken into account instead of a single one. The quality index of the parameter ranges from 0 to 100 (dimensionless), where, 0 is the worst possible condition of organic pollution and 100 corresponds to completely natural environment not influenced by human inhabitation. But influence of organic pollution is unavoidable. The index can be calculated from the formula [159]:

$$OPI = e\left[\, 1/n \sum_{1}^{n} Ln\left(PQI\right)_n Wn \right]$$

Where, *OPI* is Organic pollution index,

$(PQI)_n$ is Quality index of the n^{th} parameter derived from parameter quality curves constructed according to target values

Wn is the Weightage factor for the n^{th} parameter. All the parameters have equal weightage (*Wn* is equal to 1/*n*).

These indices require the information of the algal populations present in the water body which in turn indicates the level of pollution of that body. Thus, algae play the role of biosensor for indication of the imbalance in the water quality.

31.3.2 Algal-bacterial interaction in wastewater

In natural water systems, microalgae coexist with other microorganisms such as bacteria and fungi. These organisms compete for resources and their cooperation for abatement of pollutants will determine the success of coexistence of the consortium. In addition, they harness the substances excreted from their partners for their metabolism. A similar technique has been exploited for wastewater treatment wherein microalgae-bacterial consortium is used for removal of organic matter and reduction of COD and BOD levels. In this synchronous survival, microalgae photosynthetically produce soluble oxygen required by bacteria for biodegradation of waste and survival. In return, bacteria support photoautotrophic growth, reproduction, and metabolism of microalgae by supplying carbon dioxide. They exchange nutrients coupled with detoxification of environmental pollutants. The decomposition leads to increase in pH of the medium which directs pathogen inactivation in algal-bacterial systems. With increase in concentration of these guilds, bioflocculation of algae and bacterial might occur, aiding efficient harvest of biomass [48,52,143]. For harnessing the potential of microalgae, the dynamics between algae and bacteria has to be deeply understood for prediction on the impact on the long-term performance. Though the interactions between them have not yet been profoundly clear, few aspects such as nutrient exchange, impact on their growth phase, physiological changes, and signal transduction mechanisms have been discussed below.

31.3.2.1 Overview of algal-bacterial interaction

The interaction between algae and bacteria coexisting in a system can be mutualistic, commensalistic, or parasitic depending on the nature of species, availability of nutrients, and other physical and chemical parameters of the system in which they are present. In other words, the existence of one species can range from being beneficiary to inhibitory to the other. In an algal system, inorganic carbon such as carbon dioxide can serve as a source of carbon for algal growth. But bacteria require organic carbon moieties for their survival [34,94,119]. During growth, algae secrete dissolved organic matter which gets deposited in the phycosphere, a mucus layer surrounding them. This nutrient-rich region inhabits various bacteria which feed on the organic matter. Additionally, the dead algal matter also serve a rich source of dissolved organic matter to the bacteria. Studies show that the abundance of bacteria on the decomposed algae is twice than present on the surface of live algae. The most common bacterial communities found associated with algae are proteobacteria, actinobacteria, bacteroidetes, firmicutes, verrucomicrobia, and cyanobacteria [24]. Their abundance can also be related to the nature of motility and chemoorganotrophy. Thus algae serve as a surface for attachment for the epiphytic bacteria. But excess accumulation can reduce the access of algae to light and nutrients. Algae are autophototrophs and hence produce soluble oxygen necessary for bacterial growth

by photosynthesis. They can also provide a secondary habitat for bacteria by protecting them from adverse environmental conditions. Polymeric compounds secreted by algae have shown to accelerate hydrolytic enzyme activities of bacteria. Contrarily, algal exotoxins can also act as antimicrobials which are detrimental to bacteria. These agonistic and antagonistic effects are briefed in Fig. 31.5. This aspect further suggests that healthy algae will be able to control the colonization of bacteria on their surfaces by mechanism that can alter the release of organic extrudes [16,58,93,131].

Bacteria, on the other hand can favor the growth of algae by producing certain vitamins and plant growth hormones such as auxin, abscisic acid, cytokinins, jasmonic acid, and polyamines [144]. But, excessive hydrolytic enzyme activities mediated by bacteria can cause modifications in the surface of algae and mitigate the production of dissolved organic matter. This nature also has a hand in the attachment and diversity of bacterial consortium. Sometimes, bacteria also secrete phycotoxins that can hinder algal growth (e.g., *Pseudomonas* and *Bdellovibrionaceae*) [131].

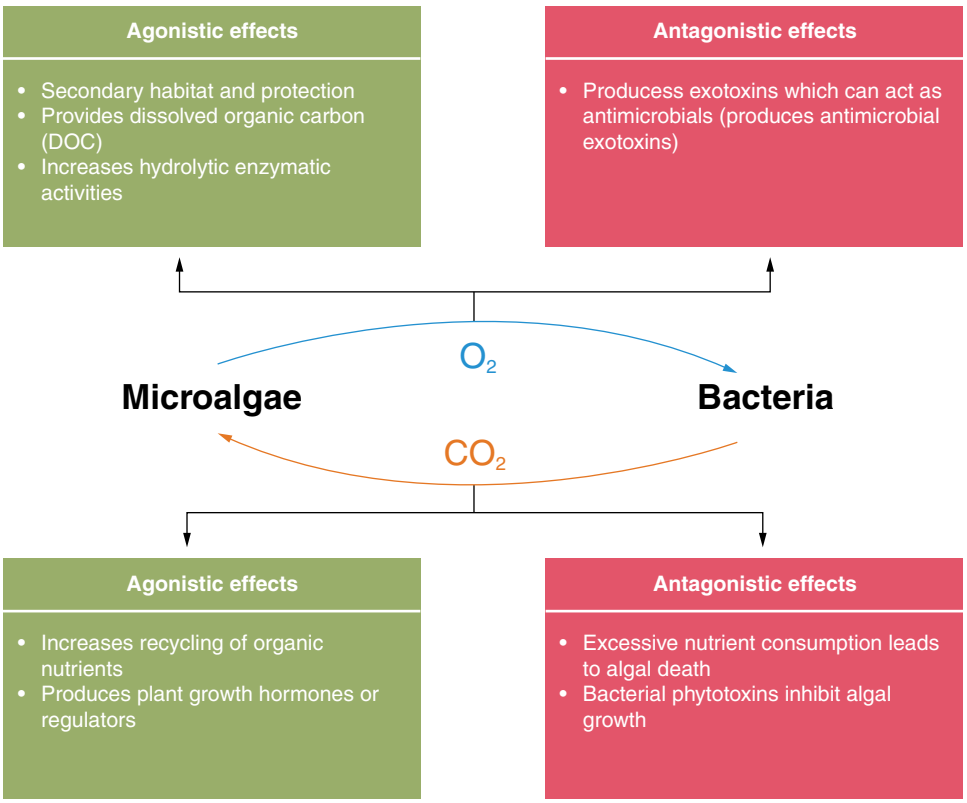

Fig. 31.5: The agonistic and antagonistic effects in an algal-bacterial consortium.

Over the years, these mechanisms must have been evolved to be benevolent for the sustenance of both the races. The interdependence of algae and bacteria can be categorized into the status of nutrient exchange between algal and bacterial community, the role of signal transduction and gene transfer between them, and the interlinkage between their growth phases.

31.3.2.2 Status of nutrient exchange between algal-bacterial communities

The algal and bacterial communities may vary with regard to the nutrient gradients and hydrodynamic pressure of the system. For example, the organic matter and transparent exopolymer particles excreted by algae can decide the type and activity of bacterial species that can co-culture with them. Diversely, the physiological state of the actively growing communities has the ability to alter the composition of nutrients and other physicochemical parameters of the system as per their convenience. The nutrient makeup of the system plays a key role such that it stimulates quantitative alterations such as the biomass growth and accumulation, chlorophyll intensity, abundance of coexisting species, and qualitative changes such as algal/bacterial community composition, their cell size, and morphology [93,131].

In a trophic system, the competition is mainly influenced by the nutrient availability between organisms. For instance, in eutrophic systems, high nutrient level results in less favorable production of dissolved organic matter leading to decreased accessibility to prokaryotic heterotrophs. This is a result of rapid settling of nutrients as particles. Also, the functionality couplings between the autotrophs and heterotrophs are weak leading to monospecific populations. But the algal productivity is high in such cases, whereas in oligotrophic environment, dissolved organic matter remains readily accessible for consumption by prokaryotic heterotrophs, which make both autotroph and heterotroph dominant. Yet, the algal productivity remains low [131].

Another key component required for the culturing microalgae is nitrogen. Algae can readily consume nitrogen in the form of ammonium [28]. In other cases, they require the help of their partners to fix nitrogen. Some cyanobacteria (e.g., *Azospirillum brasilense*) which inhabit algae (endosymbionts) have adapted the function of nitrogen fixation at the genomic level [112]. These cyanobacterial blooms are present when the nitrogen:phosphorus (N:P) levels are low, where phosphorus is the limiting factor for reproduction and growth. In conditions where N:P ratio is high, growth of green algae (Chlorophytes) is more prevalent. Few other bacteria can facilitate nitrogen assimilation by microalgae. Exhaust in ammonium concentrations results in the limitation of phosphorus removal by microalgae and COD by bacteria [75,155]. Certain mutant eukaryotic algae require an additional nutrient such as vitamin B_{12} for its growth (i.e., auxotroph). This can be synthesized by alga-associated heterotrophic bacteria. During the course of evolution, these bacteria have found to have obtained vitamin B_{12} genes in abundance [104].

31.3.2.3 The role of signal transduction and gene transfer between algal-bacterial communities

All components secreted by an organism may not be of nutritional importance to the other. Some chemicals have shown to assist in the activation or inhibition of gene expression and hence further physiological activities. Certain bacteria secrete chemicals that assist in the morphogenesis of algae [119]. In open water systems, bacterial species form biofilms with attachment to the algal blooms. Algae are able to control these structures by producing specific chemicals which have a negative impact on the bacterial quorum–sensing mechanism for formation of biofilms. In some cases, quorum-sensing molecules gain a role in the germination and growth of algae [58,104,131]. The volatile halogenated compounds secreted by certain marine algae are found to have antibacterial properties. Similarly, bacteria also are able to secrete algicidal metabolites [58].

Along the course of evolution, it is noted that genetic materials have been transferred independently from one organism to the nuclear or organelle genome of other organisms through horizontal gene transfer. These genes are of ecological significance through which the organisms gain potential to survive in challenging environments. For example, there are evidences for the presence of vitamin B genes, which is suspected to be transferred from bacteria through phycosphere. Thus, these bacterial populations associated with algae play an indispensable role in algal adaptation and survival [58,119].

31.3.2.4 The interlinkage between algal-bacterial growth phases

In natural water bodies, the growth and survival of every organism is interdependent. The metabolite produced by one organism can be essential to the other at a particular time, whereas, the same can be detrimental at the other. In case of algal-bacterial system, they maintain a specific biomass ratio for a sustainable survival [115].

During the lag phase of algal growth, they require supplements in addition to nutrients which are provided by the bacterial consortium dwelling with them, whereas, during the exponential phase, the rate of metabolite production is high in addition to nutrient consumption. These metabolites act as antimicrobials which limit bacterial population. This situation reverses during stationary phase, where the rate of algal growth decreases and bacteria increases. But these conditions are suitable only for bacteria which inhabit or depend on algae for their growth. Free-living bacteria are almost abundant in all phases of growth. Hence, for algal-bacterial communities, the biological and chemical cycles, energy fluxes, and food web dynamics are all interlinked [131,143].

31.3.2.5 Biofilms

Microalgal biofilms are formed by the secretion of extracellular polymeric substances (EPS) by microalgae. EPS traps both photoautotrophs and chemotrophs. The organic carbon present in EPS enhances the growth of heterotrophs and its metabolism. EPS is also involved in the removal of nutrients and contaminants by adsorption. The secretion of EPS is influenced by

various stimuli such as N:P ratio, salinity, light intensity, and temperature. For example, the presence of certain heavy metals can promote EPS production. Hence it can also be regarded as a biological indicator of heavy metal contamination [46]. Some modifications in EPS secreted help the organism to survive under extreme conditions. For example, secretion of sulfated EPS by *Arthrospira platensis* [88]. However, hydrolysis of EPS or certain metabolic activity taking place in biofilms can release soluble COD or DOC into the system. Use of biofilms in HRAP has advantages like low energy requirements as mixing is not necessary and harvesting of biomass is easy. HRAP can be operated at low HRT and the effluent need not be treated before discharge [15].

31.4 Effect of influencing factors on algal community dynamics

For successful wastewater treatment, proliferation of microalgae and removal of nutrients, the HRAPs have to be maintained under optimum conditions year-round. The factors which have positive or negative impact on the algal growth such as pond temperature, pH, conductivity, dissolved oxygen, daily inflow rate, retention time, organic matter, nutrient level, chlorophyll a content, and photosynthetically active radiations (PARs) have to be clearly understood for increasing the efficiency [7,84,127]. Some of the parameters which have a significant effect on microalgal production are discussed below.

31.4.1 Climatic and seasonal variation

Solar energy is essential for the culturing of microalgae. They depend on light and temperature for photosynthesis and accumulation of lipids. The nutrient removal and wastewater treatment are also indirectly dependent on the availability of light. The intensity of light and hence temperature fluctuates during the seasons from summer to winter. Rainfall during monsoon can also play an important role [30,123].

31.4.1.1 Influence of natural sunlight

The light intensity available from natural sunlight collates temperature and PARs which vary with seasonal timescales. These parameters have a major role on the photosynthetic potential and the production of chlorophyll a by the microalgal cells [123]. The magnitude of mean daylight received by a microalgal cell in summer is thrice than experienced during winter. Amount of light directly corresponds to the chlorophyll content via photosynthesis. Hence the amount of chlorophyll a also varies seasonally. It is high in summer and relatively low in autumn and winter [125]. High biomass yield during summer also contributes to the chlorophyll a content. Contrarily, the light conversion efficiency was low in early summer and higher in winter. This is because the proportional elevation in the electron transport rate from winter to summer was lower than that of the mean light intensity [22,123]. The impact of climatic variations on the biomass productivity is represented in Fig. 31.6.

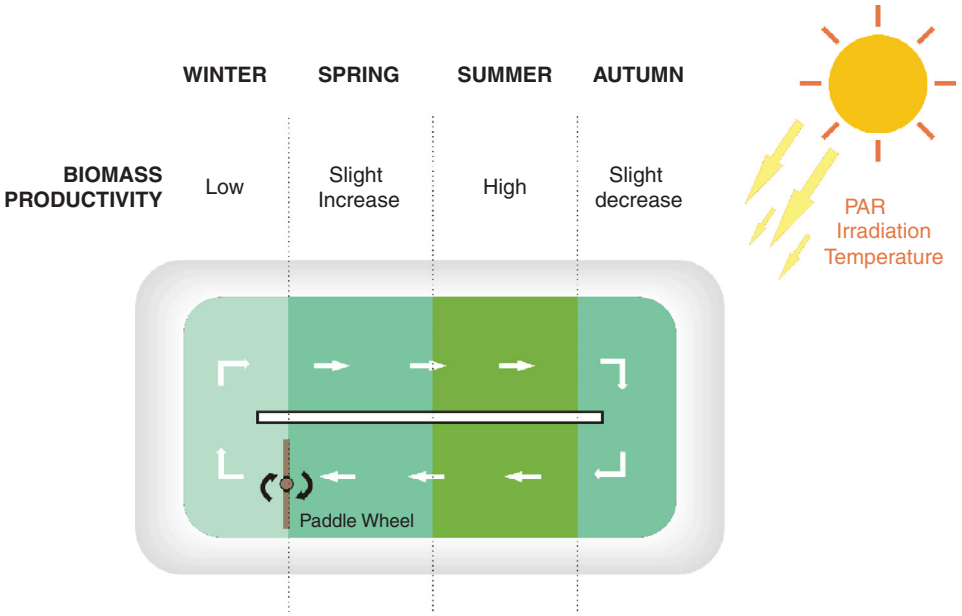

Fig. 31.6: Effect of climatic variations on the microalgal biomass productivity.

However, only an optimum intensity of light will help biomass growth. Increase in the rate of irradiance will have an adverse effect on the biomass as well as chlorophyll content. This is the reason for decrease in chlorophyll content during very high temperatures in summer. As an effect of this, photoprotective carotenoids tend to increase in microalgae whose function is to dissipate excess energy [88].

Sometimes, high chlorophyll content will also hinder the light absorption capacity of the biomass. This is due to the package effect. This effect mainly depends on the cell size and pigment content of the microbial cell. Accumulation of pigments will lead to self-shading from light and prevent pigments from receiving light [123,125]. Hence, high packaging effect will result in reduced light conversion efficiency. This is why the cells are less efficient in photosynthesis and capturing of light during late summer [46,84].

PAR has a positive role on the growth rate of microalgae. This is because microalgal species have the ability to capture specific wavelengths of PAR ranging from 400 to 700 nm [15]. Beyond the optimum range, it causes photoinhibition. Temperature also has a role in photoinhibition. Decrease in incidence of solar irradiation is known to increase unsaturated fatty acid content of microalgal species. Also, species which were subjected to unblocked solar irradiation are known to accumulate more saturated oil. On the other hand, decrease in PAR will lead to growth limitation. Other factors known to affect lipid accumulation are sub-PAR, chlorophyll a and volatile suspended solids [84].

Light is a heterogeneous source comprising of different wavelengths. In an open pond of diverse microalgal species, each species will have different light-harvesting structures or pigments. For each pigment, there is a corresponding light wavelength range that can be captured. For example, though chlorophyll a is the foremost light-harvesting pigment, certain algae have accessory pigments such as phycocyanin in Cyanophyta and β-carotene in diatoms which capture a small portion of the PAR spectrum. In these cases, where each pigment has its own demanding wavelength, complete utilization of the resource is achieved, in addition to increased algal growth [67,84].

31.4.1.2 Influence of photoperiod

Though sunlight is proven to be an essential source of algal productivity, it is limited due to the photoperiod effect that exists in an open system. Photoperiod might affect the cell size, microalgal growth rate, nutrient removal, algal/bacterial dynamics, and lipid accumulation [84].

The removal of nutrients such as nitrogen and phosphate is positively related to the length of light cycles, whereas the carbon removal relies on dark cycles. It was also related to the ratio of algae and bacteria in the consortium. With prolonged dark cycles, bacterial populations increased and played a major role in the assimilation of phosphorus and removal of COD. In these conditions, the carbon dioxide assimilated by microalgae is stored as a polysaccharide reserve by the activated dark reaction of Calvin cycle. For examples, under light stress conditions, accumulation of carbohydrates was seen in *Chlorella sorokiniana* [21,49].

It can be found from the literature that a high yield in microalgal biomass in terms of dry weight and chlorophyll a content can be obtained in a 12h:12h dark-light cycle. The chlorophyll a to chlorophyll b ratio also increases due to alternating light and dark cycles. Thus, maintenance of a metabolic balance in an algal/bacterial consortium with a photoperiod control can be an alternative solution for nutrient removal without addition of carbon sources [67].

31.4.1.3 Influence on nutrient removal

Photosynthesis is found to be the major driving force for the uptake of nutrients and culturing of microalgae. The removal of nitrogen and phosphate is effective during summer and decreased in autumn and winter. During summer, the nitrogen removal is mainly due to assimilation by microalgae and partially by nitrification. The removal of nitrogen was steady in autumn [49,127]. The reduction in the nutrient removal and microalgal productivity during monsoon is mainly due to the dilution of water and the resulting cell dispersion along the column of the pond. However, the respiration of the community increased post-monsoon [133,149].

The decline in the nutrient removal efficiency (NRE) can be due to low seasonal growth during winter. There is a lag or slow rise in NRE in the early spring due to the seasonal

shift from winter to spring and increased during late spring and early summer [140]. This can also be due to the carbon limitation as HRAPs generally have a carbon to nitrogen (C/N) ratio half that of the carbon required to remove all the nitrogen present. The C/N ratio of microalgae ranges from 5 to 20 [76]. High pH values (>9) can be an indication of low carbon levels [125].

Temperature plays an important role in the availability of nutrients to microalgae. It varies with seasonal settling and salt dissolution. In fact, the concentration and composition of chemicals present in wastewater vary throughout the day. High temperatures lead to settling of salts and decrease in water hardness. For example, nitrate load is very low in summer and high in winter [135]. Combination of high temperature and pH during summer leads to stripping of ammonia but it is not regarded as a main mechanism for removal of ammonium [124]. In addition, when the wastewater is rich in nitrogen, phosphorus becomes limiting for algal growth. On the other hand, the amount of total dissolved solids was found to be lower in fall and winter than spring and summer. When nutrients are concerned, both external nutrients and internal biomass nutrients should be taken into account [125].

Light is found to have a null or negative impact on the phosphorus concentration of biomass. Thus, when light intensity increases, external phosphorus concentration declines and the cells become rich in carbon. But internal phosphorus concentration is independent of external phosphorus concentration, whereas in cold regions, the removal of phosphorus is aided by the increase in biomass phosphorus concentration. Facultative waste stabilization ponds are generally preferred for wastewater treatment in cold countries and they solely rely on the microalgae for aeration purposes. The initial concentration of phosphate present in the wastewater doesn't contribute to the growth rate of microalgae. When initial phosphate concentration combines with PAR, a positive effect on the biomass phosphate concentration is observed. But interaction with temperature has shown to possess a negative impact on the biomass phosphate concentration [11,107,123,125].

Increase in solar irradiations during summer seasons resulted in the decrease of DOC. Higher DOC levels were recorded in seasons having considerably low temperatures. During such conditions, bacterial activities were limited and microalgal activities were found to be more acclimatized to the environment. The organic matter already present in the wastewater is shown to be necessary for the growth of microalgae during initial stages [45,127]. Thus when optimum environmental conditions are maintained in an HRAP, efficient removal of carbon, phosphorus, and nitrogen can be obtained.

31.4.1.4 Influence on community structure

Light utilization is known to be a key mechanism for the maintenance of niche complementarity in microalgal community. According to the "diversity–productivity" theory, diverse communities store high solar energy. Some of the factors which contribute to the community

diversity in a water system include solar irradiance, temperature, nutrient concentration, loading rate, and retention time [113]. An increase in water temperature directly corresponded to the species richness [27]. The ability of the microalgal species to remain in suspension is also a significant factor when ponds with shallow operational depths are dealt with [4]. The main algal groups which were reported to be dominant in a community throughout a year in spite of seasonal variations are Chlorophytes and Cyanophytes [11,87,102,153]. Other groups include Bacillariophyta, Xanthophyta, and Euglenophyta [118]. The sustenance of these groups among a community mainly depended on their fast-growing capacity. Cyanophytes remain dominant during late summer and fall, and slightly become recessive during winter. They are low lipid–yielding groups of algae [11,100]. Diatoms on the other hand sustain harsh stresses such as fluctuating light conditions and phosphate limitation. They are high lipid–yielding cultures [68,117,124,125].

The presence of microalgal species such as *Chlorella, Chlamydomonas, Scenedesmus, Ankistrodesmus, Phormidium, Nostoc, Achnanthes, Micractinium, Mucidosphaerium, Nitzschia,* and *Desmodesmus* was also recorded in HRAPs [4,22,51,101]. Among them *Chlorella* and *Scenedesmus* are found to be dominant throughout the year and in high abundance during spring and summer. They are fast-growing species and combine with nitrifying and denitrifying bacteria for the removal of ammonia [35,43,129,143].

Bacterial species are also known to exist in harmony with microalgal species in a consortium. Some of the dominant phyla of bacteria comprise of proteobacteria, bacteroidetes, actinobacteria, and cyanobacteria. Since photosynthesis acts an indirect driver of wastewater treatment, it is known that, along with microalgae, cyanobacteria are found to contribute to the wastewater remediation [22,25,30,72]. Other dominant photoautotrophs include leptolyngbya [22,119]. Some polynucleobacter cosmopolitans also contain photosynthetic gene clusters for anoxygenic photosynthesis [76]. Microalgal-bacterial consortiums have the ability to transform the nitrogen present in proteins to ammonium which is the most accessible form of assimilation of inorganic nitrogen. However, the growth rate of microalgae is much slower than bacteria. Hence an extended HRT will prevent washout effects [67].

Exposure to natural sunlight enhances the formation of algal-bacterial granules. Filamentous bacteria, waterborne algae, and the secreted exopolymeric substances combine to form granules. The algal-bacterial symbiosis occurred on the outer layer of the granules due to availability of sunlight on the surface. This granulation process enhances settling and harvest of microalgal biomass. It can also be noted that no biofilm formation was found during granulation as it would prevent the consortium from exposure to sunlight in summer [3]. In addition to microalgae, bacterial growth also seems to be higher at warm temperatures [115]. Considering the difficulties in the separation of algae and bacteria, the total yield is sometimes overestimated during summer. However, when microalgal densities stagnated during low temperatures, bacterial biomass was higher. The organic carbon

secreted by algae is said to increase the growth of bacteria [125,148]. Hence, an optimum combination of moderate solar irradiations, temperature, organic loading rate, and photoperiod is required for the maintenance of microalgal dynamics necessary for high biomass yield and biodiesel production.

31.4.1.5 Other influential parameters

The physical parameters which have a negative impact on photosynthesis, microalgal productivity, and nutrient removal include light, pH, temperature, and turbulence [101]. The intensity of light imposed in an HRAP can be modified by the depth and dimensions of the pond, mixing/turbulence patterns, and biomass concentrations. The pond depth and biomass concentration mainly governs the light attenuation througout the water column, whereas the mixing/turbulence pattern governs the frequency of photoperiods. This pattern also affects the nutrient uptake of microalgae by modifying the thickness of the cell boundary layer. The operational design of the HRAP also plays an important role [112]. pH has an effect on ammonia volatalization. An increase in pH corresponds to decrease in the ratio of ammonium ion to ammonia. High pH affects the inorganic carbon species, rate of nutrient removal, photosynthetic capacity, and inhibits the growth rate of aerobic bacteria. It also leads to chemical precipitation of phosphate [115].

The chemical parameters include dissolved oxygen, nutrients, and salinity. High dissolved oxygen along with high pH condition favors the enhanced microalgal productivity and photosynthetic rate. When salinity is concerned, the nutrient requirements and composition of freshwater microalgae are comparatively less rigid than marine microalgae [68,138,139].

The biological parameters which have negative effects on the growth rate of microalgae cover competition between species, grazing by zooplanktons and invertebrates, and presence of parasitic fungi, infective bacteria, and viruses. The changes mediated by zooplanktons are season dependent [84].

31.4.2 Chemical composition of wastewater

The chemical composition in the wastewater includes those components whose presence in a high concentration is a mishap and causes detrimental effects to the organisms present in it. The components include pharmaceuticals, metals, disinfectants, pesticides, biocides, plastics, resin, petroleum products, etc. These chemicals when assimilated by microalgae can sometimes be carried to human consumers [80,126].

Diversely, some contaminants can also increase the growth of microorganisms and their nutrient consumption. For example, presence of magnesium sulfate has a positive effect on the accumulation of lipids in *Scenedesmus dimorphus* as magnesium is the primary component

for chlorophyll production. High levels of magnesium can also maximize the growth rate of microalgae. But the presence of magnesium sulfate along with potassium phosphate has negative effects on lipid accumulation [108]. Similarly presence of chemicals such as dodecyltrimethylammonium bromide (DTAB) and didodecyldimethylammonium bromide (DDAB) caused inhibition of growth in *Scenedesmus obliquus* [56].

The presence of antibiotics in wastewater remains a major problem. They destroy the algal-bacterial symbiosis. High concentrations of antibiotics can make organisms resistant to the antibiotic which might increase the risk of human health. But antibiotics like tetracycline have been known to be removed by microalgae by adsorption and photodegradation [86]. Other pharmaceuticals such as sodium azide and brefeldin A have known to induce lipid formation. In high concentrations, sodium azide can inhibit photosynthesis, respiration and causes retardation of microalgal growth [142,151].

Generally, the presence of sulfate in optimum concentration helps growth of microalgae and deprivation lead to inhibition in *Chlorococcum* sp. due to accumulation of reactive oxygen species (ROS) [74]. But in *Chlamydomonas reinhardtii*, the amount of triacylglycerides tend to increase under decreased sulfate concentrations. Minuscules of copper and cadmium are also known to increase lipid percentage in *Chlorella minutissima* [147]. Presence of lignocellulosic components in wastewater has shown to be a good substrate for cultivation of heterotrophic bacteria [152].

31.4.3 Operational and culture conditions

For maintenance of a microalgal culture, especially in an open system, optimization of parameters is a vital task. The changes occurring in a single variable can drastically alter other variables resulting in the compromise of biomass yield, lipid accumulation, and nutrient removal. Though microalgal culturing in HRAP seems feasible in terms of economical assessment, the environmental alterations which have a negative effect on the production are highly unavoidable. Scale-up of the process requires efforts to minimize these impacts. For example, due to the phototrophic nature of microalgae, high concentration of biomass led to light limitation. Hence during scale-up, the HRAP should be designed in such a way that the phototrophic process occurs on a surface basis rather than volume basis as practiced in chemotrophic processes [8,83]. The influential parameters which are considered to be the most essential for optimization concerning productivity are discussed below.

31.4.3.1 Influence of carbon dioxide

Carbon augmentation in the form of CO_2 has a part in maintaining the balance between biochemical and physiological reactions. The CO_2 present in the atmosphere may not be sufficient enough to enhance the growth of microalgae. Deficiency in carbon might lead to inhibition of growth and subsequent nutrient removal. Hence external supply of carbon dioxide

becomes necessary in an HRAP. CO_2 addition has a positive effect on biomass growth and parameters such as light intensity, pH, HRT, nutrient recovery, mixing, and photosynthesis [102,125]. Supplementation of CO_2 slightly shifts the pH to acidic conditions. On the other hand, CO_2 has been known to prevent pH inhibition of algal growth [129]. CO_2 sparging is considered essential for increasing the biomass yield, biovolume, photosynthesis, nutrient reduction, light adsorption capacity, and reduction of the packaging effect. Enhanced concentration of CO_2-elevated lipid yields in *Dunaliella tertiolecta, C. reinhardtii, S. obliquus,* and *C. minutissima*. In addition to this, sufficient aeration increases the agitation and promotes uniform distribution of light among the colonies [156].

31.4.3.2 Influence of nitrogen

Nitrogen in wastewater can exist in various forms depending on pH which include ammonium, organically bound nitrogen, nitrate, and nitrite ions. Inter- and intraspecific variations in microalgae occur depending on their nitrogen request. Optimal nitrogen levels can vary with every species. So far, ammonium is known as the best source of nitrogen for the growth of microalgae [29]. Unionized ammonia and nitrite are considered to be more toxic to aquatic organisms compared to nitrate. Hence consumption of such ammonium and other ions by microalgae proves to be an advantage in HRAP [32]. Contrarily, presence of high ammonium levels in microalgal ponds can control grazing by zooplanktons [93]. Nitrogen limitation stresses can also be beneficial in terms of carbon and lipid accumulation. The carbon sequestered during photosynthesis is utilized for the production and storage of carbohydrates rather than protein synthesis. Microalgae accumulate lipid when the nitrogen concentration is below 2.5 mg/L. Synthesis of triacylglycerides increases as a result of nitrogen deprivation while decreasing the content of polar lipids [49,134]. This mechanism makes use of the assimilated P for the biosynthesis of enzymes involved in lipid production [20]. On the other hand, a rise in nitrogen concentration contributes to the increase in protein composition rather than lipid, meanwhile maintaining a stable fatty acid composition [92]. Excessive nitrogen can lead to transfer of nitrogen to atmosphere which will have no use as a nutrient for biomass production [53]. Hence an optimum concentration of the nitrogen source has to be provided for sustenance in the production. Variation in concentration can be employed for the increase in yield of the desired product.

31.4.3.3 Influence of salinity

Atypical salinity levels can be a matter of concern when high biomass productivity and lipid content are aimed. High salinity condition is a form of stress to the microalgal cells which induces accumulation of lipids and high value-added products. It lowers the photosynthetic efficiency of the microalgal cells. Reactive oxygen species produced during stress alters the signal transduction mechanism from production of energy-storing compounds (starch) to lipids [20]. Secretion of EPS also tends to increase under stress in *Dunaliella salina*. EPS

composition varies with increase in salt concentration which leads to the reduction in the protein content of cells [88]. Under situations of salt tolerance, microalgae are dependent on the uptake of K^+ ions and ejection of Na^+ ions through sodium–potassium pumps. Salt concentration greater than 1% is considered detrimental to biologic ecosystem. Also, high concentrations exert high osmotic pressure on the microalgal cell causing dehydration by sucking water out of the cells [140]. Hence salinity also proves to be a significant variable of concern.

31.4.3.4 Influence of dilution rate

Though other operational conditions have no remarkable effect on dilution rate, the sequel of dilution rate on other parameters should be keenly noted. Increase in dilution rate decreases biomass concentration as the nutrients tend to replenish at a higher rate. This condition is not applicable to the algal-bacterial system where microalgae are in the exponential phase of growth [103]. They produce exotoxins (antimicrobials) which reduce the bacterial log phases [131]. Though high dilution of wastewater is considered as a stress due to low nutrient concentration, lipid accumulation seems to increase up to 30%. The composition of carbohydrates, proteins, and ash contents is equally maintained [152]. Large surface areas combined with highly diluted microalgal cultures hinder the gas–liquid mass transfer rate of the cultivation system. Diversely, stress due to low dilution rates is proportional to accumulation of high carbohydrate fractions [83]. But on a real-time basis, high dilution rates become economically feasible. One of the reasons can be the increase in NRE by microalgal consortium with increase in dilution rates [124].

31.4.3.5 Influence of pH

pH is a significant parameter as its imbalance can lead to drastic effects in the microalgal growth. Each microalga has its own optimal pH. The control of pH becomes crucial in an algal culturing system. Generally, pH values ranging from 7 to 9 are usually favorable for microalgae cultivation. At around neutral pH, nutrient removal by chemical precipitation was not significant. Assimilation was the main mechanism of nutrient removal at this pH range. But chemical precipitation increased with an increase in pH [16]. Heavy metal precipitation was enhanced at high pH during photosynthesis [144]. On the other hand, an increase in pH decreased bacterial biomass. Carbon dioxide augmentation can support the growth of bacteria in an algal-bacterial system. But with increase in nutrient removal, pH shifted from neutral to acidic in an algal-bacterial consortium. At acidic pH of 5.5–6, death of *Anabaena spiroides* and *Microcystis aeruginosa* occurred. Also, acidic pH decreased the chlorophyll a content of microalgae. Hence a pH above 7 has to be maintained for significant algal growth [71,144].

31.4.3.6 Influence of light intensity

Chlorophytes or green microalgae have chlorophyll a pigments which absorb light energy essential for the growth of microalgae. Chlorophyll a pigment selectively receives light in

the wavelength range of 450–475 nm which corresponds to blue light (short wavelength) and 630–675 corresponding to red light (long wavelength). It is found that multiple wavelengths are preferred by various microalgae for removal of N and P in an HRAP. High removal rate of nutrients and biomass production were observed when mixed red and blue lights were used instead of single wavelengths of light. Red and blue light energies were found to be more suitable for microalgal growth. They have to be provided in a suitable mixed ratio for optimum growth. It was found from the literature that the removal of ammonium and COD was highest at a light intensity of 6000–7000 lux. However, high intensities and radiation lead to photoinhibition. Hence, it was concluded that the intensity of light was more essential for algal growth than the period of illumination [5,128,154]. But this mechanism cannot be correlated for the phosphorus removal. Cultivation of heterotrophic organisms instead of only autotrophic can be effective to overcome the light limitation and can increase algal growth [134]. One main factor affecting the light intensity is the color of water. When the wastewater comprised organic compounds such as humic acid, urobilin, and fulvic acid, it hindered transmittance of light [122]. In case of longer dark cycles, the capacity of microalgae to store energy compounds (carbohydrates) can be increased by limiting the availability of essential nutrients [83].

31.4.3.7 Influence of contamination

Contamination in an HRAP indicates the presence of chemicals, bacteria, and fungi which are detrimental to the production of microalgae. This remains a major bottleneck in the microalgae cultivation in open ponds. The emerging contaminants in an HRAP include ketones, naphthalene, phenols, ethers, acetonitrile, tributyltin, and grazing of microalgae by herbivores and zooplanktons [79]. The biouptake of contaminant by microalgae is by the transport of contaminant molecules inside the cell by passive diffusion, passive-facilitated diffusion or active diffusion, and then binding to the intracellular proteins [126]. Competing microalgae are considered the most difficult form of contamination to control because of the similarities in the physical and biological properties. The desired characteristics of the microalgae of interest can be prone to suppression if the growth rate of the competent organism is high under axenic conditions. This drawback can be overcome in a closed system by maintaining a controlled environment which will impose a physical barrier between the desired and competent species [83]. For open systems, the choice of microalgae can depend on those organisms which have the capacity to withstand extreme environmental conditions such as acidic pH, high temperature, etc., and serve the purpose of high yield and lipid content. For example, in conditions of high salinity and irradiation, β-carotene produced intracellularly by *D. salina* can act as a photoprotector. Similarly, herbicide-tolerant or herbicide-resistant microalgae can be employed. Under such extremophilic conditions, only a few microalgae can survive and hence contamination can be controlled [96].

Measures have to be taken to minimize the shortcomings occurring due to environmental stresses. Initial seeding of microalgae can decrease the lag phase and contamination and simultaneously increase the biomass concentration. High oxygen saturation levels also might contribute to the inactivation of coliforms. The ratio of HRT and solids retention time (SRT) could influence the dynamics of nutrition and biological composition of biomass [146]. The characteristics of the pond should be given enough importance so as to increase the light availability. The operational depth of an HRAP can be within 0.30–0.35 m [8]. Greater reduction in the pond death is also disadvantageous regarding the rate of evaporation and temperature buffer capacity [38]. Under well-mixed conditions, the photoinhibition effect due to increase in the exposure to radiation can be minimized. In addition, optimum mixing can enhance the mass transfer rate of gases supporting respiration as highly abundant species are mostly nonmotile. Similar variations can be made to control the conditions in an HRAP so as to increase productivity [93].

31.5 Bioassimilation of heavy metals by microalgae

Heavy metals are those compounds which cannot be degraded by the metabolic activities of microorganisms that are present in wastewater. Certain metals ions such as Zn^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+}, Ni^{2+}, and Mo^{2+} are required in trace amounts (micronutrients) for the growth of microbes, whereas ions like Cd^{2+}, Al^{3+}, Ti^{3+}, Au^{3+}, Sn^{2+}, Sr^{2+}, Pb^{2+}, and Hg^{2+} do not have an actual biological function and hence start accumulating in the tissues of aquatic organisms which can later be passed onto humans (e.g., accumulation of mercury in fish tissues). Such heavy metals can be treated in an HRAP by using various species of microalgae [42,100].

The heavy metal removal by microalgae can occur by two mechanisms [16]:

1. Adsorption on the surface of microalgae by means of ion exchange, physical adsorption, or complexation.
2. Bioaccumulation by actively taking in the metal ions for their metabolism.

These mechanisms can be explained in terms of kinetic adsorption patterns as [16]:

a. Rapid but passive removal of metal ions by adsorption on the surface.
b. Slow but active intake of ions for metabolism.

The zeta potential value shows that the surface of microalgae is electronegative. This suggests that at high pH algae tend to be dispersed, increasing the surface to volume ratio. Hence the biosorption of heavy metals can take place by binding of metal ions to the surface of microalgae by electrostatic attraction [72]. For example, *Scenedesmus* sp. has the ability to assimilate heavy metals such as cadmium, mercury, lead, and arsenic [81]. Abinandan et al. [1] used two acid-tolerant microalgae, *Desmodesmus* sp. *MAS1,* and *Heterochlorella* sp. *MAS3* for the removal of

copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) at pH 3.5. The metal removal efficiency reached a maximum of 86% and the accumulation of metal ions by microalgae was as high as 99%. While treating the wastewater containing high concentration of heavy metals with microalgae, the application of the biomass should be carefully chosen.

31.6 Consequences and challenges associated with algal community change

HRAPs are advantageous and profitable for the production biodiesel, biogas, and value-added products. On an approximate 168 L of methane was generated out of 1 kg of biomass which might have an impact on the energy economy [17]. The harvesting of microalgae is a major challenge due to the small size, low density, and poor settleability of microalgae. Even after harvesting, the challenge of complete dewatering persists [16,46,82]. Bioflocculation of microalgae with bacteria seems to be promising to improve settling [132]. Yet, the inoculation ratio is diverse and varies with species. Also, its application in a pilot scale is lacking as the dynamics of algae and bacteria has a profound impact on the long-term performance of the system [54]. Sometimes, presence of significant quantum of bacteria, fungi, and other forms in high strength wastewaters can be detrimental to the growth of microalgae. Though microalgae have the ability to remove pathogenic organisms, the removal efficiency is highly species dependent [42]. Another major constraint is the cost associated with the construction of the pond. Yuan et al. [149] built the HRAP with 100,000 USD per hectare. Additional operational costs such as sterilization, energy input, and maintenance will also contribute to a large proportion of the total cost. The nutrient recycling by microalgae can increase the phosphate consumption economy [120]. Very high wastewater concentrations are not suitable for algal growth. They have to be diluted with fresh water to optimum concentrations for efficient nutrient removal and algal growth [42]. This can be due to the presence of emerging chemical contaminants such as pharmaceuticals. Surface modification of microalgae by physical or chemical pretreatment can lead to enhanced adsorption of chemicals in wastewater [126]. The efficient extraction of algal oil for the production of biodiesel remains a critical challenge [114].

Engineering solutions for careful real-time monitoring of the wastewater characteristics may be required for proper selection of the microalgal strain. Such monitoring will also be useful in case of specific chemicals that are necessary for harvesting purposes. Probes can be designed for identification of species for use as consortium in a heterotrophic or mixotrophic environment. This will reduce the pressure caused by predators [19,22].

31.7 Applications of microalgae

Microalgae like *Chlorella, Spirulina, Dunaliella, Nannochloropsis, and Haematococcus* are commercially grown for the production nutritional dietary supplements, cosmetic products, and feedstock for animals [23]. Microalgae are characterized for the presence high content

of valuable components like proteins, essential amino acids, unsaturated fatty acids (e.g., eicosapentaenoic acid, docosahexaenoic acid, etc.) and vitamins which help in the prevention of various human diseases [16]. Among the essential amino acids which the algae produced, lysine was present in the highest composition. Lysine is a demanding amino acid in the animal industry [152]. In addition, microalgae (cyanobacteria) have the capacity to accumulate biopolymers such as polyhydroxybutyrates (PHBs) [10]. Some of the applications of microalgae are presented in Fig. 31.7. The lipids produced serve as substrate for biodiesel production. In addition, the carbohydrates present in microalgae are used for the production of bioethanol and biobutanol [50].

Supercritical carbon dioxide extraction (SCO_2) is a green process by which nonpolar lipids (triacylglycerides) can be selectively extracted without solvents. High yield of biogas such as methane was also obtained from the biologically degraded lipid exhausted biomass [49]. The yield and composition of algal oil might vary based on the difference in the nutrition composition in wastewater because of the variation in lipid-accumulating potential. Algal oils

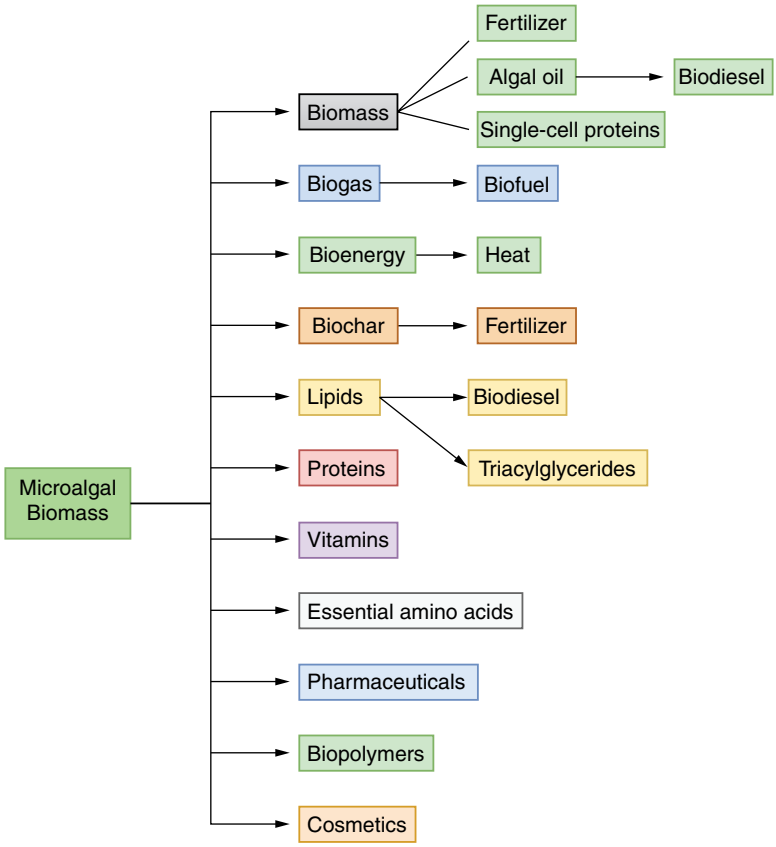

Fig. 31.7: Various applications of microalgae.

serve as a rich source for omega-3 and omega-6 fatty acids which have therapeutic benefits to humans [88]. Sometimes, there is an increase only in the internal cellular nutrient level (bio-volume) rather than increase in the number of algal cells [23]. The microalgae should be chosen based on our product of interest (e.g., in case of biodiesel product, microalgae with high lipid content has to be chosen). Table 31.6 describes the biological content of several microalgae. Hence, the species should be selected depending on the demands like high lipid content or high biomass.

Microalgae can be directly used as slow-release fertilizers. Dried microalgal biomass used as biofertilizer in rice production gave results similar to that of the commercial fertilizer. Biochar form of the microalgae is also known to retain the nutrients in low-quality soils. The nutrient composition of the microalgal biochar might vary depending on the species of algae from which it is produced. Hence depending on the type of nutrient essential for the soil, the desired microalgae can be culture and charred. For example, if the microalgae are rich in phosphorus, the converted biochar can be used for enrichment of soil with phosphorus [130].

Recent studies have shown the use of algal-bacterial system for producing self-sustained electric power called microbial solar cells (MSCs). In this setup, when the microbial fuel

Table 31.6: The percentage of carbohydrate, protein, and lipid of various microalgae.

Microalgae	Carbohydrates (%)	Protein (%)	Lipids (%)	Reference
Tetraselmis suecica	17.41 ± 2.50	52.33 ± 4.19	23.11 ± 2.13	[150]
Cyanidioschyzon merolae	37.85	47.8 ± 1.6	4.35 ± 0.91	[26]
Galdieria sulphuraria	42.29	45.1 ± 1.1	3.21 ± 0.55	
Isochrysis	42.9	35.9	15.20	[153]
Chlorella sorokiniana	10–16	44.6	10.7	[145]
Chlorella sp.	9.42	42.7	2.5	
Microcystis sp.	20.19	59.93	5.22	
Nannochloropsis sp.		14.3	21.7	
Scenedesmus sp.	29.3	36.4	19.5	
Spirulina platensis	30.21	48.36	13.3	
Nannochloropsis oculata	8	57	32	[14]
Chlorogloeopsis fritschi	44	50	7	
Pseudochoricystis ellipsoidea	7	25	67	
Desmodesmus sp.	13–20	38–44	10–14	[33]
Chlamydomonas reinhardtii		47.4	18.1	[55]
Chlorella vulgaris	13.2	47.4	15.6	[39]
Dunaliella salina	11.9	58.8	10.5	
Scenedesmus dimorphus	16	43	18 ± 1.6	[13]
Porphyridium cruentum	40	43	8	[12]
Dunaliella tertiolecta	21.69	61.32	2.89	[110]
Scenedesmus almeriensis	25.2	44.2	24.6	[73]
Nannochloropsis gaditana (NG)	25.1	40.5	26.3	
Nannochloropsis oceanica	37.3	38.2	9.6	[121]

cells (MFCs) were irradiated, microalgae were found to grow on the upper aqueous layer. These deposits were used as a substrate for the heterotrophic bacteria to generate electricity. When these MSCs were inoculated with upstream hot spring green phototrophic mats, light-dependent current was generated leading to deposition of microalgal biofilms on the surface of anode with bacteria at the base. The decomposition of photosynthetic algal products by the bacteria under anaerobic conditions produced electrons at the anode, generating electricity [38,58,119].

31.8 Summary

The integration of wastewater treatment and microalgal cultivation in HRAPs has been accelerated in the recent decade. Microalgae have been employed for numerous applications such as production of biodiesel, biogas, biofertilizer, and metabolites of pharmaceutical importance. However, for application in pilot scale, a quantum of knowledge about the factors influencing the microalgal biomass, the nutrient uptake efficiency, and heavy metal removal is essential. Understanding the effect of environmental changes such as climatic conditions and symbiotic relationship with other coexisting microorganisms on the microalgal community is indispensable. This book chapter highlighted the need to apprehend the internal and external factors that would have a positive or negative effect on the cultivation of microalgae. The interaction between microalgae and bacteria in open systems was detailed. The role of microalgae as a bioindicator and its capacity to remove nutrients and various chemical compounds was also elucidated. The comprehension of algal diversity on high rate algal ponds and the integrated approaches would make the technology economically feasible.

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