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## *Chapter 2*

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# *Review of Literature*

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## **2. Literature Review**

### **2.1. Source for isolation of robust microalgae**

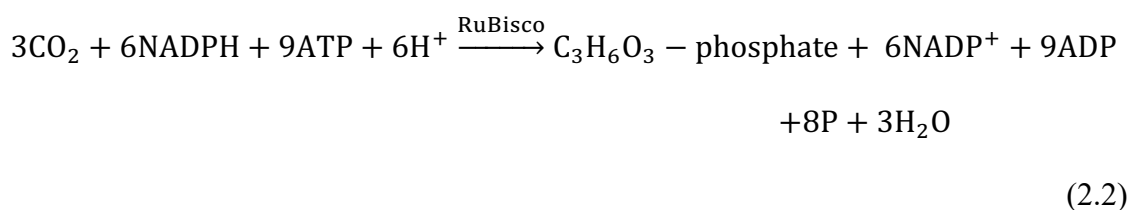
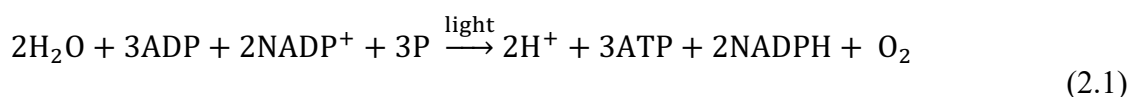
Natural water bodies, such as lakes, rivers, and ponds, have been primary sources for microalgal isolation. Studies have identified a multitude of microalgae species with high pollutant removal capacities, including nitrogen and phosphorus, heavy metals, and organic contaminants. These naturally occurring microalgae exhibit adaptability to specific environmental conditions, making them valuable candidates for bioremediation purposes. Wastewater treatment plants and sewage influents have also proven to be excellent sources for microalgal isolation. Microalgae thriving in such environments have developed mechanisms to efficiently assimilate nutrients and remove pollutants, contributing to the treatment of wastewater and reducing its environmental impact. Research efforts have extended to extreme environments, such as saline and hypersaline environments, where unique microalgal species have been discovered. These extremophiles exhibit resilience to harsh conditions and have shown potential for bioremediation and biomass production, even in saline wastewaters and brackish water bodies [57]. Additionally, industrial effluents and agricultural runoff have been explored as sources for microalgal isolation. Microalgae isolated from these polluted sources have displayed the ability to not only remove pollutants but also produce valuable biomass that can be used for bioenergy production, animal feed, and other value-added products. Microalgae from diverse habitats, such as coastal zones, mangroves, and wetlands, have been investigated for their potential in bioremediation and biomass production. These microalgae have adapted to fluctuating environmental conditions and have demonstrated efficient pollutant removal capabilities [32]. Furthermore, genetic engineering and strain selection have been employed to enhance the bioremediation and biomass production capabilities of isolated microalgae. By

manipulating the genetic makeup of microalgae, researchers have aimed to optimize their pollutant uptake efficiency and lipid productivity for biofuel applications.

## 2.2. Mechanism of pollutant removal by microalgae

### 2.2.1. Carbon Assimilation

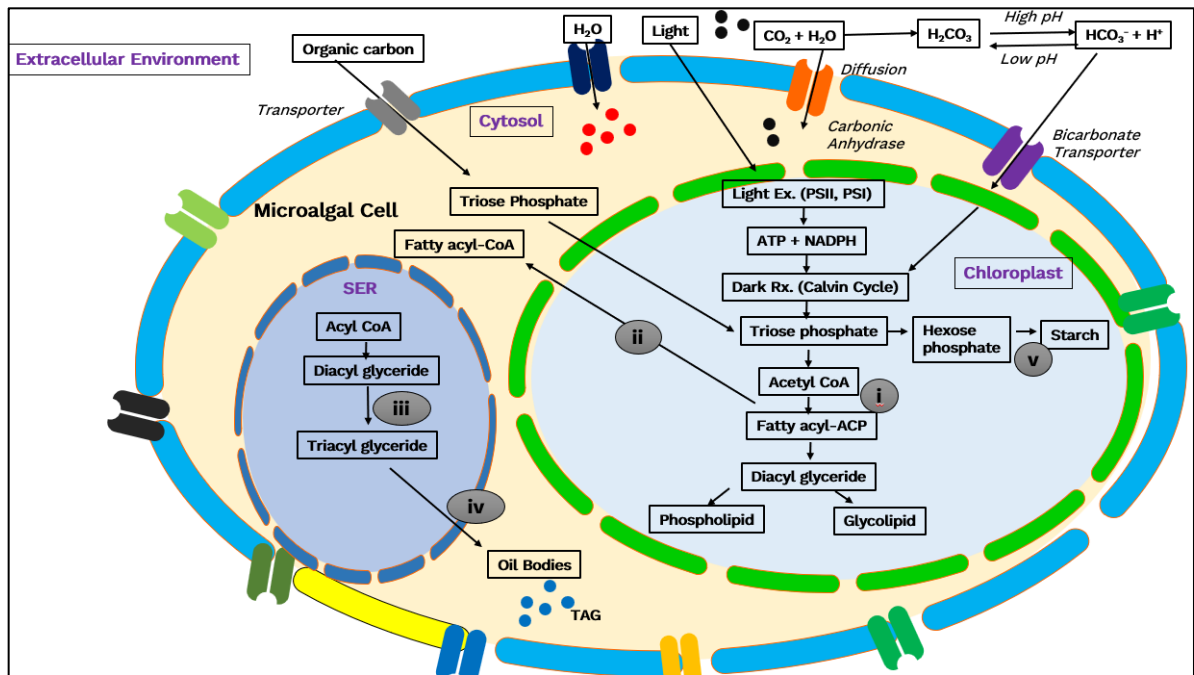
One of the major atmospheric pollutants that significantly affects the amount of greenhouse gases is carbon dioxide (CO<sub>2</sub>). Urban growth and industrial expansion are regarded as important sources of inorganic carbon [58]. One of the useful methods for CO<sub>2</sub> fixation is photosynthesis, which is mediated by microalgae [59], [60]. Nearly half of the carbon in microalgal biomass is found in its overall weight (in percent) [61]. Either autotrophic or heterotrophic microalgae use carbon. In both autotrophic and heterotrophic culture modes, the flow diagram of carbon assimilation and fatty acid biosynthesis is shown in Figure 2.1. In the autotrophic phase, CO<sub>2</sub> is fixed through a light-dark reaction (Calvin cycle), which simultaneously releases oxygen using light energy and water molecules [62], [63]. The diagrams are displayed in equations (2.1) and (2.2) [64]:



Microalgae use organic carbon sources when they are in heterotrophic mode.

**Figure 2.1** demonstrates how polysaccharides, precursor fatty acids, and other hydrocarbons are produced from the carbon fixed during photosynthesis. The converted triacylglyceride from synthesised fatty acids is subsequently transported to the smooth endoplasmic reticulum, where it buds out into oil bodies in the cytoplasm [65]. For the medium's pH to be maintained, CO<sub>2</sub> is also necessary [66]. Microalgae can also absorb

soluble carbonates to meet their carbon needs. Diffusion is how microalgal cells take up  $\text{CO}_2$  when the medium pH is low (5-7). The bicarbonate ( $\text{HCO}_3^-$ ) form of carbon is available in solutions with a high pH (greater than 7). Through active transport, it enters the cells thanks to the action of exogenous carbonic anhydrase [67]–[69].

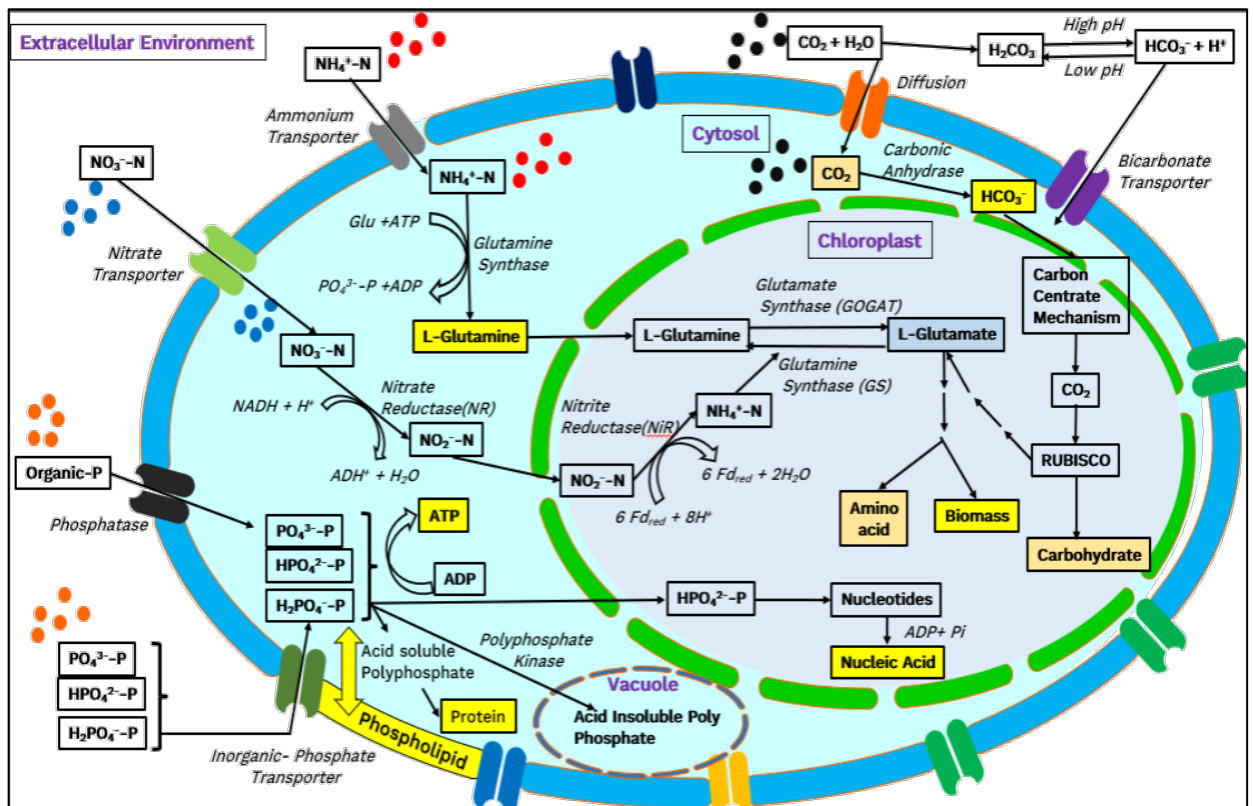


**Figure 2.1.** Flow chart for the production of lipids and the capture of carbon by microalgae. The autotrophic mode is represented by the green line, and the heterotrophic mode is represented by the blue dotted line. A number means: (i) Triacyl glyceride (TAG) biosynthesis enzymes, including acyl-CoA: DGAT (diacylglycerol acyltransferase); (ii) Acyl-CoA synthetases and fatty acid thioesterases; (iii) Pathway of Oil Body Formation; (iv) Starch Synthesis and ADP-Glucose Pyrophosphorylase.

### 2.2.2. Assimilation of nitrogen

One of the key ingredients for microalgal growth is nitrogen (N), which may be simply and abundantly obtained from wastewater [70]. It can be found in a wide range of biological macromolecules, such as proteins, enzymes, genetic material (DNA and RNA), and energy transfer molecules (ATP and ADP). Microalgae absorb inorganic nitrogen (such as  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and  $\text{NO}_2^-$ ) and transform it into organic nitrogen. Through the process of nitrogen

fixation, cyanobacteria also convert atmospheric nitrogen into ammonia [71], [72]. The process by which microalgae assimilate inorganic nitrogen is depicted in **Figure 2.2**.



**Figure 2.2.** The uptake of CO<sub>2</sub>, inorganic nitrogen, inorganic and organic phosphorus by microalgae is depicted schematically[57].

**Figure 2.2** makes it clear that two transport channels, each mediated by two steps of reduction, are used to carry out nitrate assimilation. At first, nitrate moves through the plasma membrane and into the cytosol. After that, nitrate is converted to nitrite in a reduction reaction that is performed by the cytosolic enzyme nitrate reductase (NR). Nicotinamide adenine dinucleotide, which is present in reduced form, gives up two electrons, which are then transferred to nitrate. The reduction process of nitrite to ammonium is then conducted by nitrite reductase (NiR), which is activated by the transfer of six electrons from a reduced form of ferredoxin (Fd). Finally, glutamate synthetase uses glutamate and adenosine triphosphate to catalyse the conversion of ammonium into the

amino acid glutamine [73]. Within the cell, all forms of inorganic N are first converted to ammonium N before combining with amino acids [66]. It has been noted that each *Chlorella vulgaris* cell reduced  $\text{NH}_4^+$  by an additional 70% when glutamate was added to the wastewater [74].

### 2.2.3. Assimilation of phosphorus

For their proliferation, microalgal cells also need phosphorus. It is essential for managing the freshwater biomass composition. It is a crucial component of the cell wall's DNA, RNA, ATP, proteins, amino acids, and lipids. Additionally, it can be found in cell membrane components, carbohydrates, and fatty acid metabolic intermediates. The photosynthetic process can be significantly impacted by the lack or depletion of this nutrient [75]. Microalgae actively transport orthophosphorus in the forms of  $\text{H}_2\text{PO}_4$  and  $\text{HPO}_4$  at the plasma membrane [76]. The following mechanisms: oxidative phosphorylation (ii), substrate-level phosphorylation (iii), and photophosphorylation (iii) combine  $\text{PO}_4^{3-}\text{-P}$  into the organic molecules during algal metabolism. These mechanisms involve energy input and ATP synthesis from ADP. The first process uses the electron transport system (ETS) found in mitochondria to obtain energy. The second process uses the oxidation of respiratory substrates as an energy source. The third process uses the conversion of light energy to produce ATP. Eq. (2.3) depicts the general phosphorylation reaction as follows [77]:



Microalgal cells use orthophosphate, which is produced when organic phosphate is converted to orthophosphate by the phosphatase enzyme found on the cell surface when inorganic phosphate is scarce. When there is an overabundance of phosphate, microalgal cells digest it and store it as polyphosphate (volatin) granules inside the cells. When the

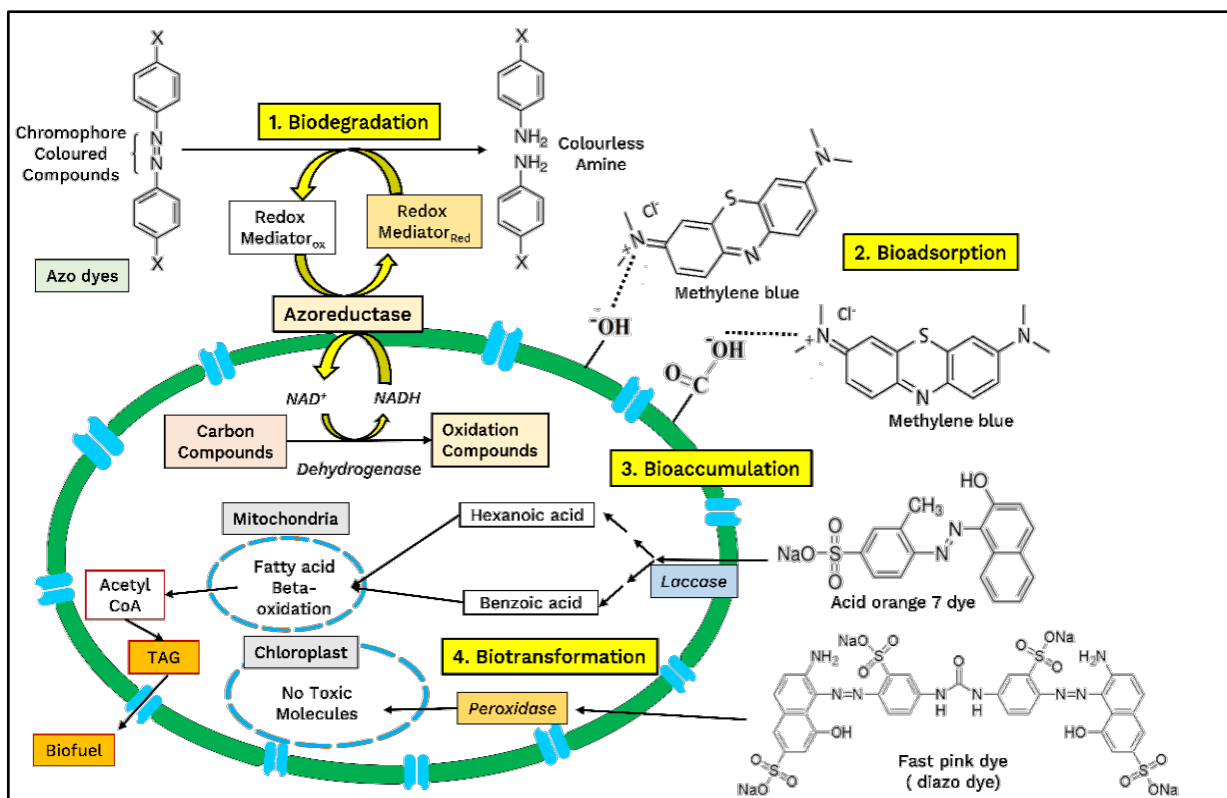
growth medium or environment lacks phosphate, these granules are used to maintain growth [78]–[80].

#### **2.2.4. Assimilation of dyes by microlage**

Color removal by microalgae has gained significant attention as a promising and eco-friendly approach for wastewater treatment. Microalgae possess various mechanisms that contribute to the reduction of color in wastewater, making them valuable agents in bioremediation processes.

The literature on color removal by microalgae through four mechanisms - biodegradation, bioadsorption, bioaccumulation, and biotransformation - provides comprehensive insights into the effectiveness and potential applications of microalgae in wastewater treatment and color removal. In Biodegradation as shown in figure 2.3, microalgae have been found to possess various enzymes, such as laccase, peroxidase, and azoreductase, which play a crucial role in the biodegradation of colored compounds present in wastewater. These enzymes facilitate the breakdown of complex-colored pollutants, such as azo dyes and aromatic compounds, into simpler and less harmful compounds through enzymatic reactions. The process of biodegradation involves the conversion of chromophoric groups within the colored molecules into colorless or less colored forms, resulting in a reduction in wastewater color. Studies have reported significant color removal efficiencies by various microalgal species via biodegradation mechanisms, making it a promising approach for color removal in wastewater treatment[81], [82]. In Bioadsorption mechanism as shown in figure 2.3, microalgae possess cell surfaces with a high density of functional groups, such as carboxyl, hydroxyl, and amino groups, which enable them to adsorb colored compounds through physical and chemical interactions. The adsorption capacity of microalgae has been extensively studied for various dyes and pigments, with encouraging results indicating high removal efficiency. The adsorption process involves

the attachment of colored molecules onto the microalgae cell surface, leading to the reduction of color in the wastewater. The advantage of bioadsorption is that it can occur quickly and does not require extensive metabolic activity, making it a potential option for efficient color removal [83], [84]. In Bioaccumulation as shown in **figure 2.3**, microalgae can accumulate colored compounds within their cells, converting them into simpler forms and storing them for later use. The process of bioaccumulation involves the uptake of colored pollutants by microalgae cells, followed by their transformation into less colored or colorless forms. This not only removes color from the wastewater but also offers the potential for resource recovery and bioproduct synthesis. Some microalgal species have shown a remarkable ability to accumulate colored compounds, making them promising candidates for bioremediation applications where color removal is required along with the production of value-added products[85], [86]. In Biotransformation as shown in figure 2.3, microalgae can also transform colored pollutants into non-toxic or less toxic compounds through metabolic pathways. This biotransformation process involves the enzymatic modification of colored molecules, resulting in the conversion of chromophores into non-colored or less colored forms. As a result, the wastewater is not only depigmented but also detoxified, reducing the potential environmental impact of the colored pollutants. Biotransformation is an important mechanism for color removal in wastewater treatment, as it offers a dual benefit of color reduction and pollutant detoxification[87].



**Figure 2.3.** Schematic diagram representing colour removal by microalgae via four mechanisms: Biodegradation (degradation into simpler compounds); Bio adsorption (adsorption on the cell surface); Bioaccumulation (conversion into simpler compounds and storage in cells for later use); Biotransformation (transformation into non-toxic compounds) [57].

The efficiency of color removal by microalgae is influenced by various factors, including the type of microalgal species, the concentration of colored pollutants, the duration of exposure, and environmental conditions such as light and nutrient availability. Researchers have explored different microalgal strains and optimized operational parameters to enhance color removal efficiency. Color removal by microalgae offers several advantages, including a sustainable and cost-effective approach to wastewater treatment. Additionally, the process can be integrated with other microalgal applications, such as biomass production and biofuel generation, promoting a circular economy and resource recovery. Overall, the potential of microalgae in color removal provides a

promising avenue for environmentally friendly and efficient wastewater treatment. Further research and technological advancements in this area will continue to unlock the full potential of microalgae as a versatile and valuable tool in bioremediation processes [57].

#### **2.2.5. Assimilation of metals by microalgae**

Microalgae possess a remarkable ability to uptake heavy metals from wastewater, leading to higher metal concentrations within their cells compared to the surrounding medium [88], [89]. This process involves two steps: rapid metal sorption at the cell surface and slower detoxification within the cell. Utilizing microalgae for metal bioremediation offers several advantages, such as faster metal uptake than other adsorption techniques, time and energy savings, rapid growth rates, and the capacity to bind up to 10% of their biomass with heavy metals [90]. Additionally, microalgae can be used in both batch and continuous processes, are environmentally friendly, recyclable, and highly effective in treating wastewater. A study by Dirbaz and Roosta (2018) focused on the biosorption capabilities of four microalgae species, including *Spirulina sp.*, *Parachlorella sp.*, *Nannochloropsis sp.*, and *Scenedesmus sp.*, for Cd<sup>2+</sup> ions in aqueous solutions. Among them, *Parachlorella sp.* demonstrated the highest biosorption capacity, being 1.5-3 times more efficient than other tested biosorbents, reaching 90.72 mg/g at 30 °C and pH 7. Further optimization of *Parachlorella's* biosorption process resulted in a maximum uptake of 96.20 mg/g at 35 °C and pH 7. Notably, increasing the agitation rate beyond 250 rpm decreased the uptake of heavy metals to less than half of the initial rate [91]. To enhance the metal uptake capacity of microalgal biomass, immobilization techniques were investigated by Ahmad, Bhat, and Buang (2018)[92]. They examined the sorption of Fe<sup>2+</sup>, Mn<sup>2+</sup>, and Zn<sup>2+</sup> ions using both free and immobilized *C. vulgaris* biomass trapped in calcium alginate beads. The immobilized biomass achieved maximum biosorption, with values of 129.83 mg/g for

Fe<sup>2+</sup>, 115.90 mg/g for Mn<sup>2+</sup>, and 105.29 mg/g for Zn<sup>2+</sup> at the optimal conditions of pH 6.0, biosorbent dosage of 0.4 g/L, and a contact time of 5 hours at 25 °C.

The interaction of heavy metals (HMs) with microalgae involves several processes, starting with the metal sorption at the cell wall. Metallic species react with carbohydrates, lipids, and proteins on the cell wall's external surface, forming coordination complexes with functional groups like sulphate, carboxyl, and amino groups. The pH of the medium plays a crucial role in determining the extent of protonation during this interaction [90], [93], [94]. The next step is the interaction with the plasma membrane, where metal transporters play a crucial role in transporting and detoxifying HMs. Group A transporters translocate metal ions into the cytoplasm, increasing their concentration, while Group B transporters decrease cytoplasmic metal ion concentration by providing metal ions for binding to metal-dependent proteins in organelles [95]. Physical adsorption is a reversible process where polyelectrolytes on the cell wall bind to metal ions through various interactions like electrostatic, Vander Waals forces, covalent bonding, etc. Microalgal organic acids perform two functions: chelating toxic metal ions to form metalloorganic molecules and leaching metal components from cell surfaces [96], [97], [98]. Precipitation occurs when the pH of the solution changes, blocking or exposing functional sites on the cell wall for metal ion binding. Metallothioneins are proteinaceous compounds that chelate HMs and help in metal detoxification and regulation [96]. The vacuole of microalgae plays a role in metal sequestration, as metal ions can bind with metallothioneins and be transported into the vacuole, forming electron-dense materials. Metal accumulation in chloroplasts and mitochondria has been observed, suggesting possible complex formation and translocation of HMs within these organelles [99].

## **2.3. Effect of Input parameters on microalgal growth**

### **2.3.1. Waste effluent Source**

Waste effluent can come from a variety of sources, including industrial wastewater, municipal wastewater, agricultural runoff, and stormwater runoff. Industrial wastewater is generated from industrial processes, such as manufacturing, mining, and oil and gas production [4]. Municipal wastewater is generated from homes, businesses, and institutions. Agricultural runoff is generated from agricultural activities, such as crop irrigation and livestock production. Stormwater runoff is generated from precipitation that runs off impervious surfaces, such as roads, roofs, and parking lots [100], [101]. According to the Central Pollution Control Board (CPCB), India generates approximately 68 billion liters of wastewater per day. Of this, only about 37% is treated. The remaining 63% is discharged into rivers, lakes, and other water bodies, where it can pollute the environment and pose a health risk to humans and animals. According to the World Bank, approximately 1.4 billion cubic meters of wastewater are discharged into the environment every day. Of this, only about 60% is treated. The remaining 40% is discharged into the environment, where it can pollute the environment and pose a health risk to humans and animals.

Wastewater can have both positive and negative effects on microalgal growth, depending on its composition and treatment status. The impact of wastewater on microalgal growth is influenced by various factors, including nutrient availability, organic matter content, presence of toxic substances, and the overall quality of the wastewater. As indicated in Table 2.1, different types of wastewaters have been employed to produce diverse microalgae species. One of the typical wastes generated by human daily activities is wastewater. Wastewater has different properties and compositions depending on where it is discharged from and how it is produced. Numerous organic and inorganic nutrients, such as nitrogen, phosphorus, and other micronutrients, are present in wastewater, which

favours the growth of microalgae. The growth, rate of pollutant removal, and biomass composition of microalgae are all influenced by the composition of the wastewater (lipid, protein, and carbohydrate). Based on the source of discharge, wastewater sources that are frequently treated by microalgae can be roughly divided into five categories: (i) industrial, (ii) residential, (iii) municipal, (iv) livestock, and (v) agricultural [101].

**Table 2.1.** Wastewater sources used for microalgae cultivation have corresponding removal efficiency and biomass concentrations.

Microalgae Species	Wastewater Source	Experimental Conditions	Removal Efficiency (%)		Biomass Conc. (g/L)	Reference
			NRE	PRE		
<i>Chlorella vulgaris</i> , <i>Scenedesmus obliquus</i> and <i>Ourococcus multisporus</i>	Municipal wastewater	500 mL serum bottles; IIL: 2%; 15% CO <sub>2</sub> ; 27 °C; 45-50 μmol photon m <sup>-2</sup> s <sup>-1</sup> ; 16h/8h; 150 rpm;	>99	>99	0.29-0.31	[102]
<i>Chlorella sorokiniana</i>	Potato processing industry	24 ± 2.7 °C; 6000 lux; 12 h;	82.7	58.0	0.789	[103]
<i>Chlorella sorokiniana</i>	Pig manure	24 ± 2.7 °C; 6000 lux; 12 h;	>95	80.7	0.564	[103]
Microalgae Consortia	carpet industry wastewater + municipal wastewater	6% CO <sub>2</sub> ; 15-25 °C; pH 7; 1 L Erlenmeyer Flasks; IIL: 0.1 g/L; 75-80	>96	>96	1.47	[104]



			photon m <sup>-2</sup> s <sup>-1</sup> ; IIL: 10%; 2-3% CO <sub>2</sub> ; 14:10 h: 23- 25 °C;				
<i>Desmodesmus</i> <i>sp.</i>	Facultative lagoon wastewater treatment plant	20 L open batch reactor; 12 h;	80	38	0.58	[110]	
<i>Chlorella</i> <i>sorokiniana</i>	Domestic wastewater	1 L Duran bottles; 0.2 vvm; 30 °C; 80 μmol photon m <sup>-2</sup> s <sup>-1</sup> ;	100		0.32	[111]	
<i>Chlamydomona</i> <i>s</i> <i>reinhardtii</i>	Industrial centrate	15 L Biocoil; 25 °C; 220 μmol photon m <sup>-2</sup> s <sup>-1</sup> ; Air flow rate: 1.8 L/min;	83	14.4 5	2	[112]	
<i>Chlorella</i> <i>pyrenoidosa</i>	Riboflavin manufacturin g unit effluent	83-278 μmol photon m <sup>-2</sup> s <sup>-1</sup> ; 24 h; 28 ± 2 °C;	78.7 6	94.7 8	1.25	[113]	
<i>Chlamydomona</i> <i>s sp.</i> TAI-2	Science industrial park	6 L glass columni-form flasks; 125 μmol photon m <sup>-2</sup> s <sup>-1</sup> ; 120 rpm; 5% CO <sub>2</sub> ;	100	33	1.5	[114]	

			Air flow rate:			
			165 mL/min;			
<i>Chlorella</i>	Municipal	2 L flasks;	100	0.832		[115]
<i>vulgaris</i>	wastewater	IIL: 0.1 g/L;				
		0.5 vvm;				
		2000-10000				
		lux;				
<i>Chlorella</i>	Soybean	500 mL	89.1	70.3	2-2.25	
<i>pyrenoidosa</i>	processing	conical				
	wastewater	flasks; IIL:	0.3 g/L; 40.5			
		$\mu\text{mol photon}$				
		$\text{m}^{-2} \text{s}^{-1}$ ; 14:10				
		h; $27 \pm 1 \text{ }^\circ\text{C}$ ;				

Conc.: Concentration; NRE: Nitrogen Removal Efficiency; PRE: Phosphorus Removal Efficiency; IIL: Initial Inoculum Level.

### 2.3.1.1. Industrial Wastewater

Depending on the type of industry, the properties and composition of industrial wastewater vary from one disposal site to another and contain a high concentration of decomposable and non-decomposable inorganic and organic compounds as well as growth-inhibiting constituents [58]. Numerous industrial wastewater types, such as the effluent from food processing units [116], ethanol biorefineries [117], pyropia processing plants [118], starch processing units and brewery industries [119], [120], tanneries and meat processing units [28], [121], soybean processing and vinegar production manufacturing divisions [122], [123], fertiliser production plants, palm oil mill effluent [30], textile industry effluent [124], petrochemical industry effluent [125], and gourmet powder factories [126], have been successfully used for the cultivation of microalgae.

#### **2.3.1.1.1. Textile Industry Effluents**

The textile effluent contains a variety of organic components as well as phosphate and nitrogen that can be used to grow microalgae. During the cultivation of *Chlorella sp. G-23* in various dilutions of textile effluent, the effects of pH and various sources of phosphorus and nitrogen were examined. The greatest  $\text{NH}_4^+\text{-N}$  RE values were 78.3% at a dilution rate of 0% and 84.4% at a dilution rate of 10% with aeration at pH 9. Peak RE (> 60%) for COD was attained at a 0% dilution rate with no aeration effect. The type of nitrogen source had no discernible effect on the growth of the microalgae [127]. In a different study, mixed microalgae consortia (*Chlorella* and *Scenedesmus sp.*) cleaned textile wastewater in a fed-batch reactor. Five cycles of the fed-batch reactor were run, with each cycle lasting 30 to 10 days less as it was repeated. As a result, microalgae in textile effluent gradually adapted. Throughout the process, RE of 70% of the total nitrogen and 95% of the total phosphorus were achieved [127]. Furthermore, Wu et al. (2017) demonstrated that *Chlorella sp.* could effectively grow using raw textile wastewater. With aeration at a 10% dilution rate,  $\text{NH}_4^+\text{-N}$  removal efficiency reached 84%, and COD removal efficiency with aeration at 0% exceeded 60% [128].

#### **2.3.1.1.2. Carpet Industry Effluents**

The carpet industry, a prominent sector generates a substantial quantity of hazardous pollutants encompassing organic and inorganic compounds, latex, dyes, as well as various non-biodegradable constituents. These industrial processes result in the discharge of significant volumes of wastewater characterized by a high degree of coloration. The rapid growth of these industries has led to a plethora of environmental challenges, thus warranting their classification as one of the most environmentally polluting sectors [129]–[132]. Carpet industry effluent is a wastewater stream that is generated during the processing of carpets. It contains high concentrations of organic matter, nutrients, and

pollutants, such as heavy metals and dyes. These pollutants can have a negative impact on the environment if they are not properly treated.

The carpet manufacturing process comprises three primary stages: extrusion, weaving, and tufting. In the extrusion phase, polypropylene and polyethylene granules, along with pigments, are introduced, yielding yarns as the final product. These yarns are then woven together in the weaving process, resulting in the production of carpets. The tufting process, responsible for creating the carpet's bottom layer or substrate, involves the addition of latex and filler materials, with the carpet's bottom layer being threaded into a fabric. This marks the concluding step in carpet production. The wastewater generated during these processes arises from various sources, including the mixing of washing tanks, machinery operations, and floor cleaning. It is noteworthy that a substantial volume of wastewater is generated throughout the entirety of the carpet manufacturing process. Addressing the removal of pollutants present in wastewater from the carpet industry is of paramount concern due to the typically slow biodegradation of these contaminants, which often requires several days or even weeks. It is essential to emphasize that there is no singular wastewater treatment process currently capable of effectively treating these wastewater streams for reuse[133]–[136].

There has been a number of studies in investigating the use of microalgae for the treatment of carpet effluent[137]. In one study, researchers found that microalgae were able to remove up to 90% of the organic matter from carpet effluent. They also found that the microalgae were able to remove significant amounts of nutrients, such as nitrogen and phosphorus. Another study found that microalgae were able to remove up to 80% of the heavy metals from carpet effluent. They also found that the microalgae were able to remove some of the dyes from the carpet effluent. These studies suggest that microalgae can be a promising technology for the treatment of carpet effluent. They are able to remove a wide

range of pollutants from wastewater, and they can do so in a relatively efficient manner. In this study, a wastewater mixture of 85-90% carpet industry effluents and 10-15% municipal sewage was used to explore algal biomass and biodiesel production. A consortium of 15 native algal isolates achieved >96% nutrient removal in the treated wastewater. Biomass potential: 9.2-17.8 tons/ha/year with 6.82% lipid content. 63.9% of algal oil could be converted to biodiesel. Further investigations needed for economic viability [137].

There are a number of challenges that need to be addressed before microalgae can be widely used for the treatment of carpet effluent. One challenge is that microalgae can be sensitive to certain pollutants, such as heavy metals. This means that it is important to choose the right species of microalgae for the specific wastewater stream. Another challenge is that microalgae can grow rapidly, and this can lead to the formation of blooms. Blooms can be unsightly, and they can also produce toxins that can harm aquatic life. It is important to carefully control the growth of microalgae in wastewater treatment systems. Despite these challenges, microalgae have the potential to be a valuable tool for the treatment of carpet effluent. More research is needed to develop optimized treatment systems, but microalgae offer a promising alternative to traditional wastewater treatment methods.

#### **2.3.1.1.3. Petroleum industry effluents**

Petroleum wastewater is a major pollutant of the environment. It is generated from a variety of sources, including oil and gas production, refining, and transportation. Petroleum wastewater contains a wide range of pollutants, including organic matter, nutrients, heavy metals, and toxic compounds. These pollutants can have a negative impact on the environment, including harming aquatic life, contaminating drinking water, and contributing to climate change. Industrial wastewater is generally unsuitable for microalgae-based treatment due to its specific characteristics and properties, as highlighted

in previous studies. However, certain microalgae species show promise in treating toxic heavy metals through absorption and adsorption processes [138], [139]. Specifically, *Chlorella* and *Scenedesmus* have proven effective in treating olive oil and industrial wastewater [128]. While most industrial wastewater is not suitable for microalgae-based treatment, specific microalgae species, such as *Chlorella* and *Scenedesmus*, have shown promise in treating toxic heavy metals and effectively utilizing certain types of industrial wastewater for growth and pollutant removal. POME (Palm Oil Mill Effluent) is a type of wastewater produced by the palm oil industry and is a known pollutant discharged into rivers in Southeast Asia. The POME contains various contaminants, including TN (Total Nitrogen), NH<sub>3</sub>-N (Ammonia-Nitrogen), BOD (Biochemical Oxygen Demand), and COD (Chemical Oxygen Demand), with concentrations ranging from 180 to 1,400 mg L<sup>-1</sup> for TN, 4 to 80 mg L<sup>-1</sup> for NH<sub>3</sub>-N, 10,250 to 43,750 mg L<sup>-1</sup> for BOD, and 15,000 to 100,000 mg L<sup>-1</sup> for COD. The pH of POME typically falls within the range of 3.5 to 5.2 [140], [141]

#### **2.3.1.1.4. Tannery industry effluents**

Tannery wastewater is rich in nitrogenous chemicals and has a high carbon content, both of which are favourable for the autotrophic and heterotrophic growth of microalgae. *Scenedesmus sp.* was grown using various concentrations (20–100%) of tannery effluent at various light intensities (20–200 molphotons m<sup>-2</sup>s), at a constant temperature of 25 °C, and with aeration. At a light intensity of 102.5 mol photons m<sup>-2</sup> and a wastewater content of 88.4%, the highest REs of COD (80.33%), NH<sub>4</sub><sup>+</sup>-N (85.63%), and PO<sub>4</sub><sup>3-</sup>P (96.78%) were attained [142]. Microalgae demonstrated greater removal efficiency during the development of purple phototrophic bacteria (PPB) and microalgae in tannery wastewater. On five separate agro-industrial wastewater sources, microalgae and PPB were both cultured (poultry, pork, dairy, red meat, and sugar processing industries). Microalgae

showed higher RE (up to 91%  $\text{NH}_4^+\text{-N}$ , 73%  $\text{PO}_4^{3-}\text{-P}$ , and 91% COD), but PPB demonstrated moderate removal (up to 80%  $\text{NH}_4^+\text{-N}$ , 55%  $\text{PO}_4^{3-}\text{-P}$ , and 74% COD) [143]. Tao et al. (2017) conducted an experiment using *S. acuminatus* for the treatment of pulp and paper mill biosludge digestate. They observed a significant removal of  $\text{NH}_4\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$ , with both microalgae removing more than 99.99% of  $\text{NH}_4\text{-N}$  and more than 96.9% of  $\text{PO}_4^{3-}\text{-P}$ , achieving a biomass concentration of 2.9 g L<sup>-1</sup>. Additionally, *Chlorella* sp. was found to have a high tolerance to  $\text{CO}_2$  and effectively converted it into biomass [144].

### **2.3.1.2. Domestic Effluents**

Domestic wastewater is the wastewater that is released from homes and businesses. Institutional buildings in the public and private sectors are additional sources. Both organic (carbohydrates, fat, oil, protein, surfactants, etc.) and inorganic (nitrogen, phosphorus, sulphur, etc.) components can be found in DW's chemical makeup [145]. Numerous microalgal species have been successfully cultured in residential garbage, as indicated in Table 2.1. The impact of ultrasonic treatment for nutrients from non-sterile residential effluent was examined by Ren et al. in 2019. Mass transfer is accelerated and reaction time is decreased by ultrasonic vibrations. *Scenedesmus* sp. was grown by the authors in a bubble column reactor made of polymethyl methacrylate (600 ml working volume) for 7 days while being exposed to ultrasonic waves of various frequencies (ranging from 0 to 30 kHz), powers (ranging from 0 to 50 W), and exposure times (ranging from 0 to 50 min). The maximum TP and TN clearance rates were 97.7% and 96.8%, respectively, for the ideal ultrasonic therapy settings of 20 W of power, 18 kHz of frequency, and 10 minutes of treatment duration [146].

In a different study, microalgae were added to the sequencing batch biofilm reactor (SBBR) to create an algal-bacterial symbiosis (ABS) system, which enhanced the RE of

nutrients from DW. The SBBR's glass construction (4 L working volume) and ceramic carriers installed on its upper side make it ideal for the development of the ABS system as well as the improvement of algae. The ABS method increased the removal efficiency of TN and TP from 31.9% to 89.3% and 38.5% to 65.8%, respectively [147]. Immobilization strategies can also be used to improve nutrient removal effectiveness. Katam and Bhattacharyya (2019) compared the effectiveness of two systems for removing waste: Systems A and B are suspended co-culture systems, respectively. System A is a suspended activated sludge and immobilised mixed microalgal culture system. They used alginate polymers to immobilise the microalgal colonies. System A removed more nitrogen and phosphorus (91% and 93%, respectively) than did System B (58% and 80%) [44].

### **2.3.1.3. Municipal Waste effluents**

The potential and use of microalgae for the treatment of municipal wastewater have been thoroughly investigated. Municipal wastewater generation has increased as a result of urbanisation and population growth [148]. The percentage of home (80–95%) and industrial (5–20%) influents in municipal wastewater varies, generally depending on local activities. It contains inorganic components like ammonia and phosphates that promote the growth of microalgae, as well as micronutrients like copper and magnesium that are necessary for their development. *Chlorella sp.* was successfully grown in wastewater samples collected from four different locations at the municipal wastewater treatment facility [105]. Before primary treatment, after primary treatment, activated sludge chamber wastewater, centrate, and before primary treatment (wastewater generated during centrifugation of sludge) Removal efficiency (RE) was found to be between 78.3 and 82.4%, 83.2 and 90.6%, and 50.9 and 83.0%, respectively, for  $\text{PO}_4^{3-}\text{P}$ , COD, and  $\text{NH}_4^+\text{-N}$ . In a different investigation, heterotrophic and mixotrophic cultivation methods were used to cultivate *Chlorella sorokiniana* in influent going to the treatment plant and anaerobic

tank central. Under mixotrophic conditions, effective removal of  $\text{NH}_4^+\text{-N}$  (94.29%) and  $\text{PO}_4^{3-}\text{-P}$  (83.30%) was achieved using an anaerobic tank centrate [105]. Zhai et al. (2017) used the response surface methodology (RSM) technique to estimate the ideal circumstances for boosting the RE of substrate by *Spirulina platensis* from synthetically simulated municipal wastewater, which was confirmed by carrying out laboratory experiments. When the temperature was set at 25 °C with an aeration rate of 0.5 vvm and the daily illumination duration was set to 12 h, the ideal growth parameters fell in the range of light intensity of 3300-3400 lx and pH of 8.8-9. Under ideal circumstances, nitrogen removal efficiency was 81.51% and phosphorus removal efficiency was 80.52% [149].

Municipal wastewater was treated using photo-sequencing batch bioreactors with a mixed co-culture of bacteria and microalgae. Without the use of forced aeration, the RE of 98.2 percent for TKN and 87.5% for COD was measured to examine the combined impacts of microbial oxygen consumption and photosynthetic oxygenation [150]. *Chlorella zofingiensis* was grown in municipal wastewater with the help of pig biogas slurry to lower the cost of microalgae production on a larger scale. Various ratios of pig biogas slurry and municipal wastewater were used in batch trials. The findings showed that adding 8% of pig biogas slurry to municipal wastewater had a remarkable impact on microalgae growth and resulted in 93% TN and 90% TP elimination [151].

#### **2.3.1.4. Agriculture Waste effluents**

The main component of agricultural wastewater is an overabundance of nutrients that are discharged into water streams from agricultural lands. The widespread use of fertilisers, pesticides, and insecticides results in an accumulation of extra nutrients, which causes eutrophication and biodiversity loss [152]. *Chlorella sorokiniana*, a natural strain, was grown by Khalid et al. (2019) in agricultural wastewater that included a lot of nutrients (C: 2364 mg/L; N: 385 mg/L; P: 106 mg/L). More than 80% of the nutrient was digested by

the native strain, which had a growth rate and biomass productivity of 0.24/d and 100 mg/L/d, respectively [153].

#### **2.3.1.5. Livestock Waste effluents**

Animal feeding plants produce a large amount of wastewater that is contaminated with dung and manure. Larger-scale livestock processing has become more common over the past few decades, producing a lot of effluent in the process [154]. Nitrogen, organic phosphorus, and ammonium are frequently abundant in livestock effluent, delivering essential nutrients to enable the development of microalgae [155]–[157]. In a study, *Botryococcus braunii* was grown in a submerged membrane photobioreactor (SMPBR) to see if it could be used to treat the last stage of wastewater from livestock. To assess the effectiveness of nutrient removal, a semi-continuous photobioreactor was run in three phases based on the hydraulic retention time (HRT) (3, 4, and 5 days). The results showed that shorter HRT (3 days) offered greater TP (85%) and TN elimination efficiencies (96%) [158]. Five microalgal species were grown in undiluted cattle pasture wastewater: *C. vulgaris* (FACHB-1227), *Parachlorella Kesskeri* TY, *S. obliquus* (FACHB-417), *S. quadricauda* (FACHB-1468), and *Chlorococcum sp.* GD. The maximum nutrient removal efficiency was demonstrated by *C. vulgaris*, which was 98.69% for NO<sub>3</sub>-N, 81.16% for NH<sub>4</sub><sup>+</sup>-N, and 83.59% for NO<sub>2</sub>-N, TP, and COD, respectively [159].

Due to the high concentration of organic nitrogen and organic matter, piggery wastewater (PWW) is considered one of the most contaminated wastewaters due to its high BOD and COD values. When used for PWW treatment, microalgae can assimilate nutrients, organic matter, and emerging contaminants in a cost-efficient and efficient manner [160]. The effectiveness of algal-bacterial photobioreactors for treating PWW in both indoor and outdoor settings was assessed through comparative research. Four algal-bacterial photobioreactors (each 3L and without a lid) were used for the treatment of diluted

(10 and 20 times) PWW for four months and 26 days of HRT in both indoor and outdoor settings. The highest RE for TOC and TP was seen under indoor settings for 10 times dilutions (94.1% and 100%, respectively), whereas the highest RE for TN was achieved under outdoor conditions for 10 times dilutions (72.1%). In the aforementioned elimination, *Chlorella vulgaris* and proteobacteria were the two most prevalent species. The blending of PWW with other wastewaters is done to enhance the nutrient RE [161]. *Chlorella vulgaris* was grown for seven days in three different PWW-brewery wastewater mixtures: (i) PWW and wastewater from malt processing; (ii) PWW and wastewater from brewing (saccharifying and fermenting); and (iii) PWW and wastewater from packing. At pH 7.0 and a 1:5 mixing ratio, the mixture of PWW and packing processing wastewater displayed the greatest RE for ammonia (100%), TN (96%), TP (90%), and TOC (93%) [162]. A different study combined PWW with winery wastewater in the following ratios: 20:80, 50:50, 80:20, 100:0, and 0:100. The soil microalga *Chlorella sp.* MM3 was then added to the mixtures, and they grew for 10 days. The mixture of 20:80 demonstrated the highest removal efficiency, which was 100% for NH<sub>3</sub>-N, 96% for TN, 90% for TP, and 93% for COD, demonstrating its effectiveness as a phytoremediation strategy for the combination of piggery and winery effluent [163].

### **2.3.2. Pretreatment of wastewater**

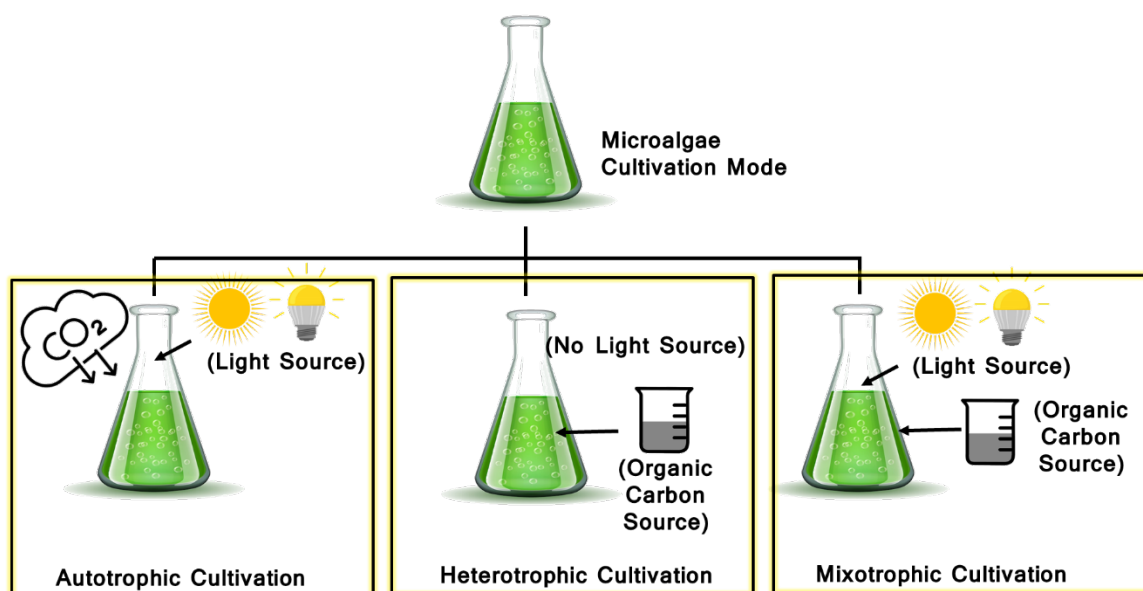
Wastewater is highly turbid and contains many kinds of particle materials, toxins, and bacterial and fungal contaminants. Additionally, wastewater contains foreign bacteria that compete with microalgae for nutrients and particle matter and turbidity that prevent proper light absorption. Pretreatment of wastewater is therefore required to lower the number of unwanted bacteria and particulates. Primary treatment techniques, such as aerobic and anaerobic treatment, autoclaving, dilution, acidogenic fermentation, magnetic treatment, ozonation, and chlorination, are pre-treatment techniques used before microalgae culture.

Gravity settling, centrifugation, and filtration are the three main types of treatment [164]. It has been found that in most situations, the primary pretreatment procedure is adequate to transform wastewater into a favorable environment for microalgae culture. When there is a high level of organic material in the wastewater, anaerobic and aerobic pretreatment techniques are used. While aerobic pretreatment produces a lot of carbon dioxide, anaerobic pretreatment produces biogas or other high-value products [116], [165]. To carry out the research on certain microalgae strains, autoclaving has been recommended as the most efficient way to reduce the microbial load. However, autoclaving consumes a lot of energy and may reduce the concentration of nutrients. For the pretreatment of wastewater, ozonation and chlorination have also been taken into consideration [105], [121], [117], [121]. Ozone pretreatment increases the biodegradability of the wastewater by microalgae while simultaneously reducing the microbial load. Additionally, ozone pretreatment reduces the turbidity of the effluent, improving light penetration [117]. Therefore, ozonation may be an appropriate technique for wastewater treatment for large-scale microalgae farming [166].

### **2.3.3. Cultivation Type**

Depending on the type of energy and carbon source used, microalgae are often grown in one of three ways: autotrophic, heterotrophic, or mixotrophic. Autotrophic is the cultivation method that is most frequently used among the three. However, compared to autotrophic mode, heterotrophic mode supports high biomass productivity. As long as carbon and an energy source are available, the growth rate is unaffected by light and remains constant [167]. When grown in a heterotrophic mode instead of an autotrophic mode, *Chlorella vulgaris*, *Chlorella protothecoides*, and *Chlorella sorokiniana* produced biomass at rates that were 4.8, 3.4, and 3.3 times faster, respectively [168]–[170]. Additionally, neither an autotrophic nor a heterotrophic mode of cultivation of *Coelastrum sp.* showed any

discernible differences in the nutrient RE [171]. Because of its simplicity, cost-effectiveness, and low maintenance requirements, the heterotrophic form of microalgae culture is therefore a better option than the autotrophic mode [172].



**Figure 2.4.** Schematic diagram representing cultivation mode of microalgae via four mechanisms: Autotrophic:((Light Source: Sunlight, Carbon Source: CO<sub>2</sub>) Heterotrophic:(Light Source: None, Carbon Source: Organic Compounds) Mixotrophic:(Light Source: Variable, Carbon Source: Mixed)

Autotrophic, mixotrophic, and heterotrophic are terms often used to describe different modes of cultivation or nutritional strategies in microorganisms, particularly in the context of algae and other single-celled organisms (Figure 2.4). These terms refer to how these organisms obtain their energy and nutrients [173], [174]. Autotrophic organisms can produce their own organic compounds using inorganic sources of carbon, such as carbon dioxide (CO<sub>2</sub>), and energy from sunlight through a process called photosynthesis. They convert light energy into chemical energy stored in organic molecules, like sugars. Algae are a common example of autotrophic organisms. In cultivation, autotrophic mode involves providing the necessary light, nutrients, and CO<sub>2</sub> to support photosynthesis. This is commonly done in large open ponds or closed bioreactors. This mode is used in various

applications such as biofuel production and wastewater treatment. Heterotrophic organisms are unable to perform photosynthesis and rely on consuming organic compounds as their carbon and energy sources. They obtain nutrients by breaking down complex organic molecules produced by other organisms. In cultivation, heterotrophic mode involves providing pre-formed organic nutrients like sugars, amino acids, and vitamins. This can be more efficient in terms of biomass production compared to autotrophic cultivation because the organisms do not need to allocate energy towards photosynthesis. Heterotrophic cultivation is often used in industrial settings to produce high-value compounds like enzymes, antibiotics, and other bioactive molecules. Mixotrophy is a combination of both autotrophy and heterotrophy. Mixotrophic organisms are capable of using both inorganic sources of carbon and organic compounds as energy sources. They can switch between photosynthesis and heterotrophic nutrition depending on environmental conditions. In cultivation, mixotrophic mode often involves providing a mix of light, organic nutrients, and sometimes even organic carbon sources. This mode is particularly advantageous when the availability of light or nutrients is limited. Mixotrophic cultivation can be used to enhance the growth rate and biomass production of certain microorganisms.

The choice of cultivation mode depends on factors such as the desired product, growth rate, resource availability, and the specific characteristics of the microorganisms being cultivated. Each mode has its own advantages and challenges, and the optimal approach varies based on the specific goals of the cultivation process. Due to its light independence, the mixotrophic mode has higher biomass productivity and nutrient RE than the autotrophic and heterotrophic modes. Both inorganic and organic carbon sources are used by mixotrophs, which also protect themselves from the photo-inhibitory effects of high light levels.

### 2.3.4. Culture Type

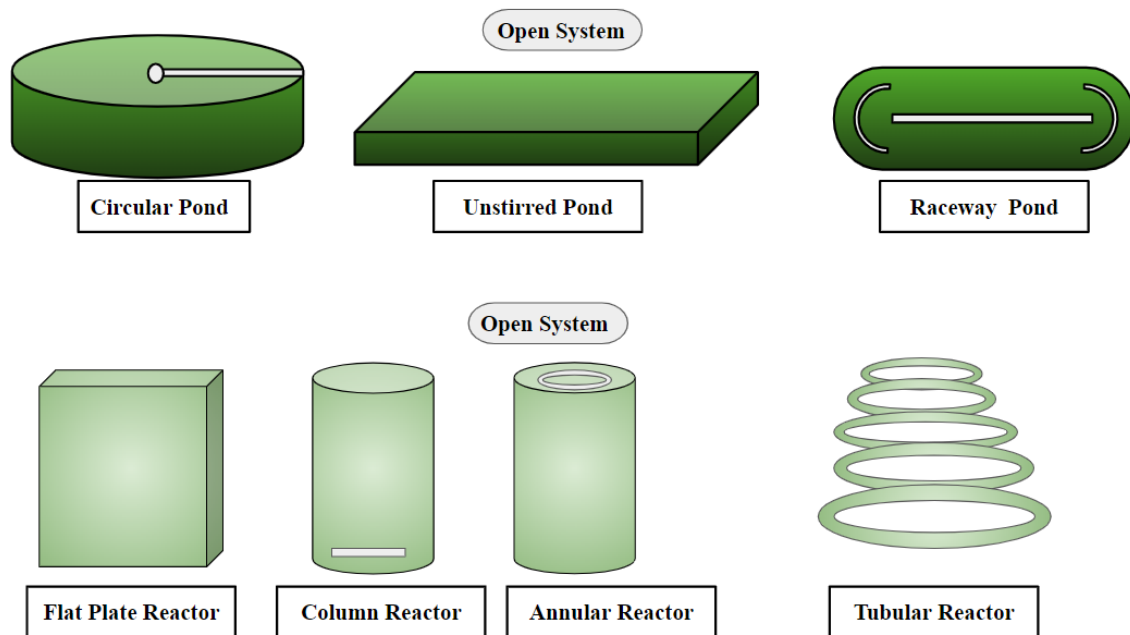
As was previously said, microalgae are either grown in monoculture or in co-culture with other species of microalgae, bacteria, and fungi [175]. Research on the use of bacterial-microalgae consortiums for wastewater treatment is expanding quickly [176]. Bacteria break down the organic nutrients in the medium, whereas microalgae eat a major part of the inorganic elements like nitrogen and phosphorus. Additionally, because microalgae absorb CO<sub>2</sub> produced by bacterial respiration during the photosynthesis process while producing O<sub>2</sub>, which is then used by bacteria, microalgae and bacteria mutually support one another [177]. As a result, microalgae can take on the role of the mechanical aeration device used in activated sludge, considerably reducing energy consumption and operating expenses [178].

The co-cultivation strategy, which involves cultivating multiple species of microalgae simultaneously, presents a viable approach to address this issue. By cultivating different microalgal species within the same environment, the effectiveness of treating toxic effluents (TxE) can be enhanced. Previous studies have explored the co-cultivation of microalgae alongside bacteria as a method for TE remediation [40], [41]. For example, Mubashar et al. (2020) successfully co-cultivated *Enterobacter* sp. MN17 and *Chlorella vulgaris* to treat TE, resulting in over 70% removal of chemical oxygen demand (COD) and color from the effluent [41]. However, it's worth noting that bacteria tend to exhibit a faster growth rate compared to microalgae. This rapid bacterial growth can lead to accelerated nutrient consumption, thereby influencing the composition of biomass and metabolites [43]. Additionally, the separation of microalgal cells from a consortium of microalgae and bacteria is a complex and challenging process [42]. In the context of co-cultivation involving microalgae, different species with diverse characteristics and metabolic systems coexist in a symbiotic manner. This symbiosis enables them to adapt to the challenging

conditions presented by TE and other pollutants. These cells, whether belonging to one or more species, communicate through mechanisms such as quorum sensing. Through quorum sensing, they release secondary metabolites that regulate mutual interactions, including defense against invading pathogens [43].

### **2.3.5. Reactor Type**

In general, either an open system (raceway ponds) or a closed system is used for microalgae cultivation (flasks, PBR) (**Figure 2.5**) [179]. Ponds' key benefit is their ease of construction and operation, which results in inexpensive production and operation costs [180]. They are, however, insufficient for monoculture production because of their great susceptibility to bacterial and fungal contamination [180]. PBRs are illuminated culture tanks that allow for higher biomass concentration and better control over predictive parameters including CO<sub>2</sub> level, temperature, pH, light intensity, etc. [179]. Flasks (for low volume cultivation), fluidized bed reactors [30], flat-panel PBRs [181], tubular PBRs [116], helical-type PBRs [179], stirred tank reactors [175], and other PBRs have all been utilised for microalgae cultivation [182]. PBRs have several drawbacks, including the following: (a) they cannot be used for large-scale microalgae culture, especially for inexpensive goods; (b) they have a complex construction design and a high cost of operation and maintenance [179].



**Figure 2.5.** Schematic diagram representing Reactor type of microalgae: Open type and Close type.

### 2.3.6. CO<sub>2</sub> content

All types of microalgae and cyanobacteria can absorb inorganic carbon, whether it comes from flue gas emissions, atmospheric CO<sub>2</sub>, or soluble carbonates. When aerating a microalgae culture, a specific amount of CO<sub>2</sub> should be employed [175], [183]. The growth of microalgae is adversely affected by any deviation from the ideal level [184]. Carbonic anhydrase's activity declines when CO<sub>2</sub> concentrations rise over 5% (v/v), increasing its sensitivity to O<sub>2</sub> and decreasing its affinity for CO<sub>2</sub> [185], [186]. Goncalves et al. (2016) used synthetic simulated wastewater to cultivate *Chlorella vulgaris* and *Pseudokirchneriella subcapitata* at various CO<sub>2</sub> concentrations (air, 3, 5, 7, 9, 10 percent (v/v)). Up to 5% CO<sub>2</sub> (v/v), both biomass concentration and nutrient RE increased, but afterwards they both started to decline. The optimal CO<sub>2</sub> (v/v) concentration for both species was discovered to be 5.35%, and at that concentration, nearly 100% RE was produced [187].

### 2.3.7. Temperature

Most microalgae strains do best at temperatures between 25 and 30 °C [182]; others can tolerate temperatures as high as 35 degrees [188] or as low as 15 degrees [189]. Algal photorespiration rates rise at high temperatures, whereas photosynthesis and growth rates fall at low temperatures [190], [191]. The solubility of gases in the medium and the activity of intracellular enzymes are both influenced by temperature [192], [193]. Therefore, equipment for heating or cooling is required to maintain the ideal temperature. Chu et al. (2015) anticipated the impact of seasonal temperature fluctuations by cultivating *Chlorella pyrenoidosa* in starch manufacturing wastewater outdoors. Summer (30.2 °C) had the highest nutrient RE with a maximum biomass concentration of 2.26 g/l, while winter (9.3 °C) had the lowest nutrient RE and biomass concentration (1.16 g/l) [189].

### 2.3.8. pH

Each type of microalga has a specific pH range in which it can develop. But most of them are active in the neutral pH range (6.5–7.5). CO<sub>2</sub> and other nutrients' solubility, as well as their shape and transformation in the medium, are all influenced by pH [194]. Microalgae directly absorb CO<sub>2</sub> through diffusion at low pH levels. Bicarbonate is the primary form of inorganic carbon in media with high pH levels, and microalgae consume it by using the carbonic anhydrase enzyme [68]. By utilizing chemical buffers or CO<sub>2</sub> purging at various concentrations, numerous studies have been undertaken that have documented the impact of pH on biomass production and nutrient RE [195]–[197].

### 2.3.9. Initial Inoculum Level

One of the important parameters during the early stages of culture is the initial inoculum level. Due to the high inoculum level, microalgae species are easily adapted to the challenging wastewater conditions, such as the presence of hazardous compounds. As the lag phase duration is shortened, a high inoculum level enables a high growth rate and high

RE in a shorter amount of time [198]. It should be remembered that mutual shadowing between cells may limit light penetration at very high inoculum densities [199]. In comparison to inoculum levels above or below 30% (v/v), a high inoculum level of 30% (v/v) supported *Chlorella sorokiniana* acclimation during growth in tannery effluent containing hazardous contaminants and a relatively high biomass concentration (1.06 g/l). This resulted in improved nutrient RE [31].

#### **2.3.10. Light Intensity & Photoperiod**

The luminous intensity, which provides the energy needed for microalgal growth during autotrophic and mixotrophic cultivation, is crucial to biomass productivity and nutrient removal. It encourages the conversion of the medium's inorganic nutrients into organic microalgae biomass [200]. Low biomass productivity is caused by low light intensity, and high biomass productivity is caused by photoinhibition or photo-oxidation because of the creation of hydrogen peroxide [201], [202]. Therefore, for maximal biomass output and high nutrient RE, microalgae species require optimal light intensity [203]. According to Chu et al. (2015), *Chlorella pyrenoidosa* was grown with high intensity during the summer and produced high biomass productivity and nutrient RE. In contrast, low light intensity throughout the winter led to reduced biomass productivity and nutrient RE [189]. The growth of the microalgae is influenced by both photoperiod and light intensity. The productivity of biomass is increased by increasing light intensity and photoperiod; however, photoinhibition is generated by the high light intensity and extended photoperiod [204].

#### **2.3.11. Initial Nutrient Concentration**

For microalgae to produce a high amount of biomass, the N/P ratio must be at its optimum level. A high concentration of nutrients, especially ammonia, can nevertheless be harmful for the development of microalgae. Therefore, if the initial concentration of nutrients in the

wastewater medium is large, appropriate dilution is required to improve the N/P ratio. Ge et al. (2016) grew *C. vulgaris* in centrate wastewater with varying nutrient concentrations and noticed that biomass productivity and nutrient RE improved as nutrient concentrations increased. However, both began to decline after reaching their maximum values as nutrient concentrations continued to rise [205]. Choosing an organic carbon source is crucial when cultivating in heterotrophic and mixotrophic conditions. According to reports, during mixotrophic cultivation, glucose and acetate allow for high biomass productivity [206].

#### **2.4. RSM (Response Surface Methodology) and Artificial neural network (ANN)**

Response Surface Methodology (RSM) stands out as a distinct mathematical technique for analyzing scenarios where outcomes are influenced by multiple independent factors. Response surface methodology (RSM) is a statistical technique that can be used to optimize the growth of microalgae for bioremediation applications. RSM can be used to identify the optimal levels of the factors that influence microalgae growth, such as nutrient concentration, light intensity, and temperature. Its principal objective revolves around pinpointing the optimal operational state of a process or the region where all performance indicators align favorably. The use of RSM to optimize microalgae growth in bioremediation has been demonstrated in a number of studies. For example, Sultana et al. 20220 investigates microalgae, specifically *Chlorella kessleri*, as a biosorbent for removing heavy metal ions from wastewater. They optimized conditions (pH 6.34, temperature 27.71 °C, biomass 1.5 g L<sup>-1</sup>) using response surface methodology (RSM) and crow search algorithm (CSA), achieving a 99.54% lead (II) removal efficiency. Experimental validation closely matched predictions (97.1%)[207]. In one study designs a micro-photobioreactor for bio-hydrogen production, optimizing variables using response surface methodology (RSM) [128]. Significant impact of run time on hydrogen production was found, with slight effects from sulfur and biomass concentrations. Optimal conditions were 0.75% sulfur,

101.96 h run time, and 53.31 g/L biomass, resulting in maximum bio-hydrogen production (66.32 mL g-VS<sup>-1</sup>). RSM effectively predicted bio-hydrogen production in photobioreactors [208]. These studies demonstrate that RSM can be used to optimize microalgae growth in bioremediation with a high degree of accuracy. This makes RSM a promising tool for developing more efficient and cost-effective bioremediation systems. RSM operates by harnessing quantitative data from relevant experiments, enabling the simultaneous solution of multivariate equations. In another study explored biomass production of ten microalgae species on different media with varying CO<sub>2</sub> and nitrogen levels, aiming to promote microalgae-based CO<sub>2</sub> sequestration (MSCO<sub>2</sub>). Feasibility was found across diverse CO<sub>2</sub> and N concentrations, suggesting MSCO<sub>2</sub> could become a profitable industry, aiding in global warming mitigation and transitional fossil fuel use [209]. A prominent feature of RSM is its capacity to curtail the resources expended on experimental trials. Moreover, it stands as an attractive alternative to experimental design due to its ability to swiftly and accurately anticipate study outcomes [208].

In a parallel vein, Artificial Neural Networks (ANN) serve as another modeling methodology adept at swiftly and accurately analyzing diverse input parameters, thereby facilitating predictions grounded in these parameters[210], [211]. Artificial neural networks (ANNs) have been used to predict microalgae growth in bioremediation applications with promising results. ANNs are able to learn complex relationships between input and output variables, making them well-suited for predicting the growth of microalgae, which is influenced by a variety of factors. The significance of ANN extends to its aptitude for comprehending nonlinear relationships within the intricate network of parameters introduced into a biological system[212]. A significant advantage inherent to ANN lies in its capacity to simulate intricate systems without necessitating intricate physical equations, predetermined boundary conditions, or preliminary assumptions about the nature of fitting

functions or data distributions. Moreover, ANNs can be trained with experimental data without mandating an exhaustive grasp of the biological process under study. When contrasted with other mathematical models, the error tolerance of ANN remains elevated due to its prowess in constructing models even from imperfect data[213]. Nevertheless, it is acknowledged that the computational demands and time investment inherent to ANN models for predictive tasks surpass those entailed by RSM models[214].

One study explores factors affecting *Chlorella vulgaris* microalgae growth and CO<sub>2</sub> capture. Using AI models (BRT, ANN, SVR) with Bayesian optimization, optimal conditions are found. SVR outperforms others for CO<sub>2</sub> prediction, achieving 0.911 R<sup>2</sup> and low errors. It surpasses Box-Behnken design by 17.16%. Crow search algorithm combined with SVR determines ideal conditions (40°C, 1:1 N/P, 12/12 h light-dark) for max biomass (0.0979 g L<sup>-1</sup>d<sup>-1</sup>) and CO<sub>2</sub> capture (0.1408 g L<sup>-1</sup>d<sup>-1</sup>)[215]. In one study A feed-forward back-propagation neural network (FBN) predicts *Karlodinium veneficum* microalgae growth in customizable nutrient concentrations. A 3-layer FBN with 27-25 nodes captures complex nutrient interactions, trained on over 420 experiments. Microelements and vitamins notably influence growth more than macronutrients. FBN forecasts cell concentrations even with untested conditions, proving its effectiveness in relevant culture conditions [216]. In another study, an artificial neural network model is developed to characterize microalgae cultures efficiently. FlowCam captures cell images for model input. The model distinguishes 6 microalgae genera with up to 97.27% accuracy. Complemented by a classification threshold, it ensures precise classification and quality assurance. This Deep Learning approach proves effective for monitoring large-scale microalgae production, offering accurate characterization across various genera[217]. Microalgae-based wastewater treatment addresses water scarcity and promotes resource recovery. AI/ML algorithms optimize microalgae cultivation for efficient operation and

biorefinery. This review explores AI's potential in microalgae wastewater treatment and outlines future directions. Valuable for researchers in the digital industrial era[218]. These studies demonstrate that ANNs can be used to predict microalgae growth in bioremediation with a high degree of accuracy. This makes ANNs a promising tool for optimizing microalgae-based bioremediation systems. An enhanced chlorophyll dynamics prediction model using artificial neural networks (ANN) has been developed for proactive algal bloom prevention. By focusing on changes in chlorophyll values rather than just the base value, this optimized approach improves accuracy while reducing monitoring costs. Tested in a case study, the optimized model outperforms the traditional version. The success is explained through the application of non-stationary time series analysis. This advancement offers a valuable strategy for controlling algal blooms[211].

In addition to the studies mentioned above, there have been a number of other studies that have used ANNs to predict microalgae growth in bioremediation. These studies have shown that ANNs can be used to predict the growth of microalgae in a variety of different conditions, including different wastewater types, different nutrient concentrations, and different temperature and light conditions. The use of ANNs to predict microalgae growth in bioremediation is still in its early stages, but the results of the studies that have been conducted so far are very promising. ANNs have the potential to be a powerful tool for optimizing microalgae-based bioremediation systems, and they could lead to the development of more efficient and cost-effective ways to clean up polluted water [218].

### **2.5. Genetic Algorithm Optimization (GA)**

Genetic algorithms (GAs) are a type of heuristic optimization algorithm that are inspired by the process of natural selection. GAs work by iteratively generating a population of solutions to a problem, and then selecting the best solutions to create a new population of solutions. This process is repeated until a solution that meets the desired criteria is found.

GAs have been used to optimize microalgae growth for bioremediation in several studies[218]. In one study, Genetic algorithm optimizes medium for toxic microalga *Protoceratium reticulatum*. 26 components boost cell concentration 60% vs. L1 control. Yessotoxin titer improved 40% vs. control medium[219]. In one study, predictive models for *Chlorella kessleri* microalgae's efficiency in removing inorganic Nitrogen (N) and Phosphorus (P) from municipal wastewater. It explores the impact of operational factors like temperature, light-dark cycle, and N:P ratio. Using response surface methodology (RSM), artificial neural network (ANN), and support vector regression (SVR) with real experimental data from Box-Behnken Design (BBD), models are constructed. SVR outperforms ANN and RSM for simultaneous N and P removal prediction. Bayesian optimization refines MLP-ANN and SVR models. Extra simulated data supports SVR's prediction capability across conditions. SVR models are hybridized with genetic algorithm (GA) to achieve >93% nutrient removal efficiency at optimal conditions: 29.3 °C, 24/0 h/h light-dark, and 6:1 N:P ratio[220]. In another study, author develops an optimized process integrating flue gas CO<sub>2</sub> capture and wastewater use for cleaner microalgal biomass production. A combination of artificial neural network (ANN) and genetic algorithm (GA) predicts optimal conditions. *Scenedesmus* sp. growth is enhanced using domestic wastewater and coal-fired flue gas. A 4-12-1 ANN topology is optimal, predicting conditions like light intensity, photoperiod, temperature, and initial pH. The optimized parameters boost biomass productivity by 57%, CO<sub>2</sub> sequestration by 578.1 ± 23.1 mg L<sup>-1</sup> d<sup>-1</sup>, and COD reduction by 95.9 ± 2.4%. Biomass has 34.6% lipid content and 106.4 mg L<sup>-1</sup> d<sup>-1</sup> productivity, meeting biodiesel standards[221].

Following the formulation of RSM and ANN models, a synergistic approach emerges through their amalgamation with Genetic Algorithms (GA), an artificial intelligence-based methodology. This integration aims to deduce the globally optimal values of process

parameters. Genetic Algorithms offer a pragmatic and efficient means of search. Grounded in the principles of evolution, GA hinges on selecting the fittest entities and has substantiated its utility in multiple investigations as a potent optimization tool (Lianda and Amri, 2016). The RSM-GA and ANN-GA hybrid methodologies represent computational strategies that unite RSM or ANN models with GA to address challenges within optimization and modeling endeavours. In the RSM-GA synergy, RSM is employed to formulate a mathematical approximation of the response surface, which is subsequently enhanced through GA-driven optimization to enhance efficiency. Within the ANN-GA convergence, ANN assumes the role of the foundational model, capturing intricate relationships, while GA optimizes the parameters or architecture of the ANN. Both approaches harness the extensive optimization capabilities of GA and the versatile modeling potential of RSM or ANN. However, it is acknowledged that these approaches might grapple with amplified computational intricacies, necessitate meticulous parameter calibration, and exhibit limitations in terms of interpretability. In spite of these complexities, the RSM-GA and ANN-GA hybrid methodologies present robust solutions for formidable optimization and modeling challenges (Lianda and Amri, 2016). These studies demonstrate that GAs can be used to optimize microalgae growth for bioremediation with a high degree of accuracy. This makes GAs a promising tool for developing more efficient and cost-effective bioremediation systems [220], [221].

## **2.6. Co-Cultivation of Two Microalgae**

Co-cultivation of microalgae, a novel and promising approach, has garnered significant attention in recent years due to its potential to address two critical challenges: biomass production and bioremediation. This comprehensive review delves into the current state of research surrounding the co-cultivation of microalgae, with a particular focus on its applications in these two domains. By examining an array of studies and experiments, this

review aims to shed light on the advantages, challenges, and key findings associated with co-cultivating different microalgal strains in a shared environment. The cultivation of more than one microalgal species in the same culture will increase TE treatment efficiency. One notable advantage of the microalgae-microalgae co-culture approach is the development of a dense culture with a significantly faster growth rate compared to monocultures. Consequently, increased interactions among microorganisms lead to elevated EPS (extracellular polymeric substances) production as a metabolic strategy for adaptation, particularly in adverse conditions like nutrient scarcity[222]–[224]. However, certain considerations should be taken into account when conducting co-culture experiments. Excessive EPS accumulation may hinder mass transfer, impeding nutrient uptake and the availability of dissolved CO<sub>2</sub> to microorganisms. Thus, careful selection of microalgal-microalgal consortia is essential, especially for large-scale applications. In addition to enhancing biomass and lipid production, this co-culture strategy aids in bioremediation of various waste materials and facilitates efficient biomass harvesting through bio-flocculation[225]–[232].

As per previous literature, authors have co-cultivated microalgae and bacteria to treat TE [40], [41]. Mubashar et al. (2020) co-cultivated *Enterobacter* sp. MN17 and *Chlorella vulgaris* for TE treatment, resulting in more than 70% removal of COD and colour [41]. But the growth rate of bacteria is much faster than that of microalgae; it will consume nutrients at a quicker rate and alter biomass and metabolite composition [43]. Also, separating microalgal cells from the microalgae-bacteria consortium is very difficult and requires complex procedures [42]. This enhanced lipid production in the binary culture is attributed to the synergistic effects between the two microalgal strains, consistent with findings in prior studies. For instance, Rashid et al. observed that co-culturing *Chlorella* sp. with *Ettlia* sp. resulted in the highest biomass yield of  $740 \pm 0.06 \text{ mg L}^{-1} \text{ d}^{-1}$  and a lipid

yield of  $180.8 \pm 14 \text{ mg g}^{-1}$  [47]. In the algae co-cultivation system, species with different properties/metabolic systems grow in symbiosis, adapting to the harsh environment of TE and other effluents. Two or more different cells connect through quorum sensing, in which they release secondary metabolites to regulate mutual interactions, including invasive pathogens [43]. Thus, microalgae co-cultivation for TE treatment can be a suitable approach for increasing assimilation efficiency, preventing culture crashes, and increasing biomass productivity. Therefore, based on various studies exploring the effects of microalgae-microalgae binary cultivation on biomass and lipid productivity, it is apparent that while it enhances overall biomass and lipid yield, further research is needed to elucidate the precise mechanisms behind this symbiosis for enhanced productivity. Consequently, it can be inferred that the co-culture approach not only contributes to bioenergy production but also proven effective in wastewater treatment, provided a judicious selection of consortia is made to ensure positive outcomes [81], [82], [84], [86], [87], [233]–[236].

## **2.7. Microalgal Harvesting optimization**

Microalgae are a promising source of biomass for a variety of applications, including biofuels, bioproducts, and bioremediation. However, the harvesting of microalgae is a major challenge due to their small size, low concentration in culture, and negative surface charge. A number of different harvesting techniques have been developed for microalgae, including centrifugation, gravity sedimentation, screening, filtration, air flotation, and flocculation. However, no single technique is ideal for all algal species or applications [237]. Centrifugation is a high-efficiency harvesting method, but it is also energy-intensive and expensive. Gravity sedimentation is a low-cost harvesting method, but it is slow and inefficient. Screening and filtration are size-exclusion methods that can be effective for harvesting microalgae, but they can be clogging and difficult to scale up. Air flotation is a promising harvesting method for microalgae, but

it is sensitive to the surface hydrophobicity of the algal cells [238]. Flocculation is a promising harvesting technique for microalgae that is simple, efficient, and scalable. Flocculation involves the aggregation of microalgae cells into flocs through the addition of flocculants. The flocs can then be separated from the liquid by gravity settling or other conventional separation methods. The development of a high-efficiency and cost-effective harvesting process is key to the commercialization of microalgae-based technologies [239]–[241]. Flocculation is a promising harvesting technique that has the potential to meet these requirements. In addition to the challenges mentioned above, the harvesting of microalgae is also affected by a number of other factors, including: The morphological and physiological characteristics of the algal cells, which can vary significantly depending on the culture conditions. The presence of impurities in the culture medium, which can interfere with the harvesting process. The cost of the harvesting equipment and chemicals are also critical parameters. The development of a cost-effective and efficient harvesting process for microalgae is a critical research area. Comparison of most common techniques used for microalgae harvesting is shown in **Table 2.2**. This will be essential for the commercialization of microalgae-based technologies and the realization of their full potential[241]–[243].

**Table 2.2.** Comparison of five flocculation techniques for algae harvesting.

Technique	Mechanism	Advantages	Disadvantages	References
Auto-flocculation	Spontaneous aggregation and sedimentation under stress conditions	Cheap Eco-friendly No chemical flocculant is required	Limited to certain algal species Time-consuming Low efficiency	[237]
Bio-flocculation	Co-pelletization of target algal species using bio-flocculants (fungi, bacteria, yeast, algae and their extracellular polymers)	Renewable No chemical flocculant is required	Species-specific Biomass contamination Environmental concern due to flocculant release	[237], [244], [245]
Chemical flocculation	Charge neutralization, bridging, and sweeping of algal cells with charged chemicals	Fast Effective Scalable	Biomass contamination Environmental concern due to flocculant release Efficiency is sensitive to culture conditions	[237], [246] – [249]
Particle-based flocculation	Charge neutralization and electrostatic bridging with functional nano/macro-particles	Rapid (e.g., magnetic separation) Multi-functionalities for post-processing Reusability of flocculant	Expensive manufacturing Limited to laboratory-scale studies	[237]

Electrochemical flocculation	Floc formation using metal ions and charge neutralization by passing direct electrical current through electrodes	Fast Suitable for almost all types of algae No chemical is required	Fouling and short life-time of electrodes Biomass contamination by metal ions Electrical energy demand	[237], [250]
Dual Flocculation	Charge neutralization, bridging, and sweeping of algal cells with charged chemicals	Fast Effective Scalable	Less expensive Less contamination	[251]

Flocculation is a promising harvesting technique for microalgae that involves the aggregation of microalgae cells into flocs through the addition of flocculants. Flocculation is simple, efficient, and scalable, and has been extensively investigated as a harvesting strategy for various algal species. The most common flocculants used for microalgae harvesting are inorganic metal salts, natural organic flocculants, synthetic organic flocculants, metal cations released from electrodes, and nanoparticles[50], [51], [252]. However, each of these flocculants has its own limitations, which can hinder their commercialization for microalgae harvesting. Inorganic metal salts are effective flocculants, but they can be toxic to microalgae and can also contaminate the harvested biomass. For example, aluminum sulfate and ferric sulfate are commonly used inorganic metal salts for flocculation, but they have been shown to be toxic to some microalgae species. Additionally, these salts can leave residues on the harvested biomass, which can make it difficult to use for some applications. Natural organic flocculants are less toxic than inorganic metal salts, but they have a short shelf-life and can be expensive to produce[237],

[253]–[257]. For example, chitosan and alginate are commonly used natural organic flocculants, but they can degrade over time and become less effective. Additionally, these flocculants can be expensive to produce, which can make them cost-prohibitive for large-scale microalgae harvesting[256], [257]. Synthetic organic flocculants are more effective than natural organic flocculants, but they are also more toxic and can have adverse effects on the harvested biomass. For example, cationic polymers are commonly used synthetic organic flocculants, but they have been shown to have negative effects on the cell viability of some microalgae species[258].

Additionally, these flocculants can be difficult to remove from the harvested biomass, which can make it difficult to use for some applications. Metal cations released from electrodes are a promising new type of flocculant, but they are still in the early stages of development and their scalability is not yet clear. For example, zinc and copper cations have been shown to be effective at flocculating microalgae cells, but it is not yet known how these flocculants would perform at large-scale. Additionally, the use of electrodes for flocculation can be expensive, which can make this approach cost-prohibitive for large-scale applications[249], [259]. Nanoparticles are also a promising new type of flocculant, but they are expensive and are mostly limited to laboratory-scale studies. For example, gold nanoparticles have been shown to be effective at flocculating microalgae cells, but they are prohibitively expensive for large-scale applications. Additionally, the use of nanoparticles for flocculation can raise concerns about their environmental impact. The ideal flocculant for microalgae harvesting would be a low-cost, non-toxic, and environmentally friendly compound that is effective at a wide range of algal concentrations and that can be easily scaled up for commercial production. The development of such a flocculant would be a significant breakthrough for the commercialization of microalgae-based technologies[237], [260], [261].

Chemical flocculants available for microalgal harvesting fall into three main categories: (i) inorganic flocculants like iron and aluminum salts, (ii) synthetic polymers such as polyacrylamide and polyelectrolytes, and (iii) natural organic polymers like chitosan and cationic starch[248], [250]. Synthetic polymers offer high harvesting efficiency at low doses, but their cost is a drawback. Inorganic flocculants like ferric chloride and aluminum sulfate are more economical but require larger amounts. Their use might lead to contamination and discoloration of microalgal biomass, affecting its application for biofuel and pigment extraction. The challenges with biomass quality can be overcome by employing natural polymers like chitosan, known for its benefits such as being natural, biodegradable, and non-toxic. Chitosan's non-toxic presence in culture media post-harvest enhances media reusability, potentially reducing costs. However, chitosan's relatively high cost, ranging from 20 to 50 USD/kg depending on purity, limits its large-scale adoption[245], [251]. Inorganic salts neutralize microalgal cell charges for flocculation, while chitosan employs bridging to aggregate microalgal biomass. Combining these mechanisms is thought to enhance flocculation efficiency[247].

One study examines *Chlorella zofingiensis* algae flocculation efficiency. Factors include algae concentration, ferric chloride dose, and pH. Two critical conditions for effective flocculation are identified: minimum ferric chloride concentration and negative algae surface charge. Flocculation mechanisms shift from hydroxide precipitate bridging to sweep flocculation at higher algae concentrations. These findings offer insights into designing efficient flocculation systems for other freshwater algae[262]. Microalgae harvesting is challenging due to their small size. Cationic starch is tested as a flocculant, effective for freshwater microalgae but not marine types. The needed dose increases with initial biomass. Greenfloc 120 is an efficient, non-toxic flocculant. Ratios of 0.1 for *Parachlorella* and 0.03 for *Scenedesmus* achieve 80% biomass flocculation. Greenfloc 120

works across pH 5 to 10. It is a cost-effective option for microalgae harvesting [263]. In another study, *Chlorella vulgaris* flocculation efficiency for harvesting was studied using single flocculants (inorganic salts, synthetic polymer, chitosan) and dual flocculants (inorganic salts + chitosan). Synthetic polymer achieved >90% removal with low doses (20-40 mg polymer/L) via bridging and charge neutralization. Inorganic salts and chitosan had <90% efficiency even with high doses (160-200 mg/L).

**Table 2.3.** Summary of literature on the flocculation of *Chlorella* genus using aluminium sulphate and ferric chloride.

Microalgae species	Dry biomass (g/L)	Flocculant	Optimal dose (mg/g dry biomass)	Efficiency (%)	References
<i>Chlorella vulgaris</i>	0.36	• Aluminium sulphate	504	77	[251]
		• Ferric chloride	448	86	
<i>Chlorella vulgaris</i>	1.2	• Aluminium sulphate	2083	>90	[264]
		• Chitosan	208		
<i>Chlorella</i> sp.	0.12	• Aluminium sulphate	1266	>90	[265]
		• Ferric chloride	1191		
<i>Chlorella vulgaris</i> (freshwater)	1.0	• Aluminium sulphate	350	>95	[266]
		• Ferric chloride	300		
<i>Chlorella vulgaris</i>	0.25	• Aluminium sulphate	600	>95	[267]

Dual flocculation involving a combination of inorganic salts and chitosan has been found to elicit synergistic effects in the context of microalgae flocculation for harvesting. Summary of literature on the flocculation of *Chlorella* genus using aluminium sulphate and ferric chloride is shown in **Table 2.3**. This means that when both inorganic salts (such as ferric chloride or aluminium sulphate) and chitosan are used together as flocculants, their combined action results in a higher flocculation efficiency than what would be expected from the sum of their individual effects. For instance, in the case of ferric chloride/chitosan dual flocculation, the improvement in flocculation efficiency was measured at 57%. Similarly, when aluminium sulphate was paired with chitosan, there was a 24% enhancement in flocculation efficiency[251]. This synergistic enhancement is attributed to the unique roles that each component plays. The inorganic salts, such as ferric chloride or aluminium sulphate, are effective at charge neutralization. They can neutralize the electrostatic repulsion between the negatively charged microalgal cells, facilitating their aggregation. On the other hand, chitosan acts as a bridging agent. It forms links between microalgal cells, creating larger and more cohesive aggregates. When used alone, inorganic salts or chitosan might have limitations in achieving high flocculation efficiency due to their specific mechanisms. However, when used in combination, they complement each other's effects, resulting in a more effective and efficient flocculation process. In essence, the dual flocculation strategy capitalizes on the strengths of both charge neutralization and bridging mechanisms, allowing for better aggregation of microalgal cells. This cooperative action leads to a higher overall flocculation efficiency, making the harvesting process more successful and cost-effective [251], [258].