Factors Influencing CO2 Biofixation by Microalgae

Subjects: Biotechnology & Applied Microbiology
Contributor: Chih-Sheng Lin

The production of microalgal biomass is highly influenced by the suitability of microalgae strains, CO2, light, pH, culture system, temperature, and nutrients. The sources of CO2 and nutrients for microalgal cultivation can be flue gas and wastewater, respectively. Therefore, many studies have investigated whether flue gas and wastewater can be integrated with microalgal cultivations, to achieve not only CO2 reduction, but also CO2 reuse for microalgal biomass conversion to produce biofuels. Flue gas and wastewater can also be treated by microalgal cultivations to obtain environmentally friendly and health-friendly effects. In the process of microalgae cultivation, one single factor does not affect the growth of microalgae; it is often the interaction of multiple factors. Therefore, keeping the performance of long-term and stable microalgal cultivation will determine the microalgal growth, especially outdoor cultivation.

CO2 biofixation microalgae flue gas wastewater

1. Microalgal Strains

Many studies have indicated highly efficient ways to obtain CO_2-tolerant, alkali-tolerant, and/or thermotolerant microalgae with high CO_2 fixation efficiency. Microalgal strains could be obtained by screening the environment, by random mutagenesis or by genetic modification (**Table 1**). Improving the capacity of CO_2-tolerant microalgae was good for application in flue gas containing high concentrations of CO_2 to reduce the CO_2 poisoning effect and increase CO_2 fixation productivity [1]. The level of CO_2-tolerant microalgae is usually referred to as high, very high, and extremely high, according to ranges of 2–5, 5–20, and 20–100% CO_2-tolerant concentrations [2]. As shown in **Table 1**, these strains not only have the ability to withstand very high CO_2 concentrations, but also have better growth performances, to obtain higher CO_2 fixation efficiency. Flue gas from steel plants containing approximately 25% CO_2, 70–80 ppm nitrogen oxides (NO_x) and 80–90 ppm sulfur dioxide (SO_2) resulted in up to 90% NO_x and SO_2, along with 50% CO_2 removal efficiency by the cultivation of *Chlorella* sp. MTF-15 [3][4]. Because CO_2 is the main component in boiler flue gas with trace amounts of sulfur oxides (SO_x), the resulting biomass after CO_2 fixation may be used as an animal additive or feed without the concern of posing biosafety risks [5]. To improve the CO_2 fixation efficiency, the screening of alkali-tolerant microalgae has been investigated [6][7][8]. It is known that when the pH of water is above 6.3, dissolved CO_2, bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) are the dominant species [9]. Therefore, elevated CO_2 dissolution can be utilized in microalgae growth by increasing the pH of the culture medium. An alkali-tolerant *Chlorella* sp. AT1 was isolated and cultured in alkaline medium (pH = 11) with 10% CO_2 aeration [6]. *Chlorella sorokiniana* SLA-04, which was isolated from alkaline Soap Lake, could adapt to growth in extremely high-pH media (pH > 10) [7][8]. The high biomass productivities of *Chlorella sorokiniana* SLA-04 were obtained by scavenging CO_2 from only the atmosphere at high rates in pH > 10 medium during phototrophic cultivation. Excessive light intensity will cause the internal temperature of the cultivation system to rise, causing the growth of microalgae to be inhibited. Two effective thermotolerant mutants, M18, and M24 of *Chlorella pyrenoidosa* obtained by mutagen treatment, were capable of surviving at temperatures up to 47 °C, and showed optimal growth at 37 °C [10]. The research on screening specific algae strains in **Table 1** is mainly in Taiwan, including the characteristics of CO_2, alkali, and thermo-tolerance. However, in subtropical zones, the temperature of microalgal culture broth in PBRs can go up to about 40 °C by irradiation of sunlight [3], showing that the screening of thermotolerant strains is very important. The thermotolerance of *Chlorella* sp. M4, which was obtained by mutagenesis treatment from *Chlorella* sp. GD, was capable of overcoming high-temperature inhibition during outdoor culture due to high photosynthetic efficiency and biomass productivity at 40 °C with high-concentration CO_2 aeration [11]. Thermotolerant microalgal strains can also be screened from high-temperature zones, such as the effluent of steel-making, power generation plants, and hot springs [12]. Thermotolerant microalgae are excellent candidates for large-scale outdoor cultivation, especially in subtropical and tropical countries [13]. Dual CO_2 and thermotolerant *Chlorella* sp. strains 283 and 359 were isolated from their original strain of *Chlorella vulgaris* ESP-31 by *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (NTG) mutagenesis [14]. The microalgal strain grew well at 40 °C and had high biomass productivity, 0.73–0.89 g L^{-1} d^{-1}, for a 4-day culture.

Table 1. Growth performance and CO_2 fixation efficiency of microalgal *Chlorella* with different tolerant characteristics.

Tolerance Characteristics	Microalgae	Gas Aeration	Temp. (°C)	Maximum Biomass Conc. (g L^{-1})	Biomass Productivity (g L^{-1} d^{-1})	CO_2 Fixation Efficiency [1] (g L^{-1} d^{-1})	Country [2]	References
High-CO_2 tolerant	*Chlorella* sp. MTF-15	Flue gas [4]	26	2.52	0.515	0.942	TW	[4]
	Chlorella sp. AE20	10% CO_2	28	3.22	0.293	0.536	CN	[15]
		20% CO_2		3.13	0.285	0.522		
		30% CO_2		3.02	0.275	0.503		
	Chlorella vulgaris NIOCCV	5% CO_2	28	0.674	0.111	0.203	IN	[16]
		10% CO_2		1.58	0.265	0.485		
		20% CO_2		0.976	0.163	0.298		
High-CO_2 and CH_4 tolerant	*Chlorella* sp. MB-9	20% CO_2 and 80% CH_4	26	2.35	0.243	0.445	TW	[17]

Tolerance Characteristics	Microalgae	Gas Aeration	Temp. (°C)	Maximum Biomass Conc. (g L^{-1})	Biomass Productivity (g L^{-1} d^{-1})	CO_2 Fixation Efficiency [1] (g L^{-1} d^{-1})	Country [2]	References
CO_2 tolerant	*Chlorella* sp. GD	Boiler flue gas [3]	26	6.54	0.892	1.632	TW	[5]
High-CO_2 tolerant	*Chlorella* sp. LAMB 31	40% CO_2	26	~0.9	0.079	0.144	CN	[18]
High-CO_2 and thermotolerant	*Chlorella vulgaris* ESP-31, 283 and 359	Simulated flue gas (25% CO_2, 80–90 ppm SO_2, 90–100 ppm NO)	40	1.91 (283)/1.99 (359)	0.73 (283)/0.89 (359)	1.336 (283)/1.629 (359)	TW	[14]
Alkali-tolerant (pH 6–10)	*Chlorella* sp. AT1	10% CO_2	26	5.08	1.010	1.848	TW	[19]
Alkali-tolerant (pH > 10)	*Chlorella sorokiniana* SLA-04	Air	20	0.9	0.059	0.108	US	[7]
		Air	20–25	0.74	0.046	0.078		[8]
Thermotolerant	*Chlorella pyrenoidosa* M18	Air	37	4.65	0.931	1.702	IN	[10]
	Chlorella pyrenoidosa M24			4.11	0.822	1.504		
	Chlorella sp. M4	6% CO_2	40	4.2	1.05	1.922	TW	[11]
	Chlorella pyrenoidosa M18	Air	45	1.69	0.338	0.619	IN	[13]
	Chlorella sorokiniana	10% CO_2	37	1.16	0.232	0.425	IN	[12]
		15% CO_2		1.05	0.211	0.384		
		5% CO_2 and 80 ppm NO		1.27	0.254	0.465		

[1] CO_2 fixation efficiency (g L^{-1} d^{-1}) was calculated by 1.83-fold of biomass productivity. [2] Country abbreviation: Taiwan (TW), China (CN), and India (IN). [3,4] Concentration of CO_2 in the flue gas and boiler gas was 25% and 8%, respectively.

... ource for ... ems that ... ges flue gas into microalgal culture ponds, which might lead to rapid changes in the pH of the culture broth [4][20][21]. When microalgae cannot adapt to extreme culture conditions, death of the microalgae will occur. Therefore, it is necessary to screen microalgae for pH tolerance. In general, the main component of flue gas is CO_2, which presents a variety of CO_2 concentrations, depending on the fuel source and the design of the plant. *Chlorella* sp. MTF-15 was cultured with flue gas aeration from a hot stove (26% CO_2), coke oven (25% CO_2), or power plant (24% CO_2) at the China Steel Corporation, the largest steel plant in Taiwan. The biomass productivity of the microalgae cultured with flue gases from coke ovens, hot stoves, and power plants was 0.515, 0.314, and 0.342 g L^{-1} d^{-1}, respectively [4]. *Chlorella* sp. was cultured in medium, with a controlled pH of 6, by aerating with synthetic flue gas (30% CO_2) obtained from the African Oxygen Company in South Africa, and the maximum biomass concentration and biomass productivity were 3.42 g L^{-1} and 0.145 g L^{-1} d^{-1}, respectively [22]. When *Chlorella sorokiniana* was aerated with flue gas (16% CO_2) from the oil-producing industry of India, the maximum

CO_2 sequestration was 3.07 g L^{-1} [23]. The maximum biomass concentration and biomass productivity of *Chlorella* sp. KR-1 aerated with flue gas from a coal-burning power plant in Korea were 2.81 g L^{-1} and 0.561 g L^{-1} d^{-1}, respectively, and the CO_2 removal efficiency was approximately 13% [24]. The maximum specific growth rate and biomass concentration of *Chlorella fusca* LEB111 aerated flue gas (10% CO_2) from coal power plants in Brazil were 0.181 d^{-1} and 1.24 g L^{-1}, respectively [25]. The efficient biomitigation of CO_2 (12–15%), NO_x (0.01–0.08%), and SO_x (0.006–0.06%) of flue gas from a power plant was obtained by the cultivation of *Chlorella vulgaris* [26][27]. The biomass concentration and amounts of CO_2 sequestration of *Chlorella* sp. aerated with flue gas produced from the burning of coal were 1.92 g L^{-1} and 0.974 g L^{-1}, respectively [28]. When integrated with sewage and flue gas in microalgal cultivation, the biomass concentration and CO_2 removal efficiency of *Chlorella vulgaris* aerated with a coal-burning boiler (6% CO_2) in India were 1.72 g L^{-1} and 90%, respectively [29]. A microalga *Chlorella* sp. Cv could tolerate the full-simulated flue gas, 10% CO_2 + 200 ppm NO_x + 100 ppm SO_x. Under optimal conditions, the microalga could tolerate the simulated flue gas, and the maximum specific growth rate was 0.9824 d^{-1} [30]. It was proposed that the upregulation of several genes related to photosynthesis, oxidative phosphorylation, CO_2 fixation, sulfur metabolism, and nitrogen metabolism was beneficial for the evolved microalga strain to tolerate the simulated flue gas [21]. Countries with high dependence on coal, such as China and India, are also actively engaged in CO_2 carbon reduction research, using CO_2 from the exhaust gas in microalgal cultivation to achieve carbon reduction, and use the produced microalgae biomass as a feedstock of biofuels. It has the opportunity to achieve economic and environmental sustainability by integrating the CO_2 reutilization of exhaust gas and the effective development of biofuels.

Table 2. Growth, CO_2 fixation efficiency, and lipid productivity of the microalgae *Chlorella* cultures using flue gas.

Microalgae	Flue Gas Source	CO_2 (%)	Biomass Productivity (g L^{-1} d^{-1})	CO_2 Fixation Efficiency [1] (g L^{-1} d^{-1})	Lipid (%)	Lipid Productivity [2] (g L^{-1} d^{-1})	Country [3]	References
Chlorella sp. MTF-15	Coke oven	13	0.528	0.966	21.5	0.614	TW	[4]
		25	0.515	0.942	26.4	0.666		
	Hot stove	13	0.449	0.822	33.8	0.866		
		26	0.314	0.575	35.2	0.591		
	Power plant	12	0.423	0.774	36.3	0.792		
		24	0.342	0.626	41.6	0.633		
Chlorella sorokiniana	Industrial flue gas	16	0.231	0.423	21.1	0.049	IN	[23]
Chlorella sp. KR-1	Coal-fired flue gas	13	0.561	1.027	29.9	0.168	KR	[24]
Chlorella sp.	Coal	5	0.273	0.500	8.69	0.024	IN	[28]

Microalgae	Flue Gas Source	CO_2 (%)	Biomass Productivity (g L^{-1} d^{-1})	CO_2 Fixation Efficiency [1] (g L^{-1} d^{-1})	Lipid (%)	Lipid Productivity [2] (g L^{-1} d^{-1})	Country [3]	References
	burning							
Chlorella fusca LEB 111	Coal power plant	10	0.111	0.203	15.5	0.017	BR	[25]
Chlorella vulgaris	Coal burning boiler	6	0.312	0.571	23.2	0.074	IN	[29]
Chlorella sp. GD	Boiler flue gas	8	1.296	2.372	21.7	0.214	TW	[5]
Chlorella sp.	Flue gas	30	0.145	0.265	24.7	0.036	ZA	[22]
Chlorella sp. Cv	Simulated flue gas	15	0.53	0.969	ND	ND	CN	[21]
Chlorella vulgaris	Power plant	12	0.502	0.919	40.1	0.201	ES	[26]
Chlorella sp. C2	Power plant	3	0.314	0.575	31.5	0.099	CN	[31]

[1] CO_2 fixation efficiency (g L^{-1} d^{-1}) was calculated by 1.83-fold of biomass productivity. [2] Lipid productivity (g L^{-1} d^{-1}) = (biomass productivity × lipid content)/100. [3] Country abbreviation: Taiwan (TW), India (IN), Korea (KR), Brazil (BR), South Africa (ZA), China (CN), Spain (ES).

…esources globally will decrease by 40% by 2030. However, more than 80% of the world's wastewater is discharged into the environment without treatment. The management model for wastewater should be changed from "treatment and disposal" to "reuse, recycle, and resource recovery". Therefore, the use of wastewater for microalgae cultivation is a technological development trend [32][33][34]. The source of wastewater can be mainly divided into three categories: agricultural, municipal wastewater, and industrial wastewater. As illustrated in **Table 3**, the growth performance and biomass productivity of microalgae cultured in different types of wastewater were different because the contents of COD, total nitrogen (TN), total phosphorus (TP), and specific inorganic substances in wastewater were obviously different [35][36][5][37].

3.1. Agriculture Wastewater

The main source of agricultural wastewater was large livestock and poultry operations, and the main components in this wastewater were ammonium and organic nitrogen, which are good for microalgal growth. Piggery wastewater is commonly used in microalgal cultivation because this wastewater is rich in nutrient sources [38][39][40][41][42]. Additionally, aquaculture is a fast-growing industry because it has significantly increased the global demand for fish and seafood. Novel aquaculture systems incorporating wastewater treatment and effluent reuse have been rapidly developed for compliant wastewater discharge. Although the nutrient content of aquaculture wastewater is significantly lower than that of piggery wastewater, the content of pathogenic microorganisms and heavy metals contained in aquaculture wastewater is relatively low [43][44]. Therefore, aquaculture wastewater can be used as a large amount of water needed for microalgal cultivation, and the resulting microalgae biomass can be applied not only to a feedstock of biofuels, but also to animal additives or feed, which is a more minimal biosafety issue [5]. In Taiwan, most livestock wastewater is produced from pig farming. Therefore, it can be seen that the state has actively invested in research on the treatment of piggery wastewater. The raw piggery wastewater without pretreatment could also be applied in microalgal cultivation. The produced microalgal biomass has about 20% lipids and is suitable for use as a feedstock of biodiesel [35][5][39][45].

3.2. Municipal Wastewater

At present, a large amount of municipal wastewater is being produced due to an increase in urban population growth. The composition of municipal wastewater varies greatly because of the substances from various families, businesses, and institutions. For example, the COD and TN in a municipal sludge digestate were 2175 mg L^{-1} and 840 mg L^{-1}, and 164 mg L^{-1} and 43.2 mg L^{-1} [46], in municipalities with reserve osmosis concentrate [47], respectively. Generally, the COD, TN, and TP utilization efficiencies of municipal wastewater in microalgal *Chlorella* cultivation were approximately 85–100%, 80–100%, and 90–100%, respectively (**Table 3**). However, growth and biomass productivity are low because municipal wastewater lacks nutrients for microalgae utilization [48][49][50]. Research on the reutilization of municipal wastewater in microalgae cultivation is commonly seen in many countries, such as United Kingdom (GB), USA (US), Australia (AU), etc. Due to the difference in the compositions of wastewater, to apply the technology of microalgal cultivation to cities, the culture process needs to be modified depending on the region to achieve stable growth of microalgae, and further, to achieve the dual advantages of wastewater purification and CO_2 reduction.

3.3. Industrial Wastewater

Some small- and medium-sized enterprises and informal industries often discharge wastewater into municipal pipelines or directly discharge it into the environment. Compared with the hazards caused by agricultural and municipal wastewater, industrial wastewater could be more harmful to water resources and the environment due to the contents of toxic heavy metal components. There are also studies on diluting the wastewater to reduce the sensitivity of the microalgal strain towards the toxicity of wastewater, and increase the wastewater utilization effectivity to obtain the microalgal growth [35][51]. However, wastewater from food processing is usually regarded as a safety resource and is suitable for the production of microalgal biomass for feed or food uses [52]. Because the sources of industrial wastewater were obviously different, the ranges of COD, TN, and TP utilization efficiencies of industrial wastewater in microalgal *Chlorella* cultivation were approximately 25–95%, 30–100%, and 50–100%, respectively [46][53][54][55] (**Table 3**). The COD, TN, and TP contents of the food industry wastewater is relatively rich, which is very suitable for use as nutrient sources for microalgae cultivation. Therefore, the better growth of microalgae can be obtained. However, the problem of bacterial contamination is more likely to occur because of the higher nutrient contents. This will affect the long-term stable performance of the microalgal cultivation technology.

Table 3. Biomass and lipid production and productivity of the microalgae *Chlorella* cultures using wastewater.

Wastewater Source	Microalgae	COD [1] (mg L^{-1})	TN [1] (mg L^{-1})	TP [1] (mg L^{-1})	Biomass Productivity (g L^{-1} d^{-1})	CO_2 Fixation Efficiency [2] (g L^{-1} d^{-1})	Lipid (%)	Lipid Productivity [3] (g L^{-1} d^{-1})	Country [4]	References
Agricultural wastewater										

Wastewater Source	Microalgae	COD [1] (mg L^{-1})	TN [1] (mg L^{-1})	TP [1] (mg L^{-1})	Biomass Productivity (g L^{-1} d^{-1})	CO_2 Fixation Efficiency [2] (g L^{-1} d^{-1})	Lipid (%)	Lipid Productivity [3] (g L^{-1} d^{-1})	Country [4]	References
Raw dairy	*Chlorella* sp.	2593	283	116	0.261	0.478	-	-	CN	[38]
Anaerobically treated piggery	*Chlorella vulgaris* CY5	377	287	28	0.281	0.514	19.6	0.055	TW	[39]
Piggery	*Chlorella* sp. GD	490	550	20	0.681	1.246	21.8	0.148	TW	[35]
Aquaculture		121	234	15	1.296	2.372	21.3	0.276	TW	[5]
Swine	*Chlorella vulgaris* UTEX-265	1481	307	4.3	0.247	0.452	27.1	0.067	KR	[40]
Piggery	*Chlorella sorokiniana* AK-1	1500–4500	500–700	150–250	0.55	1.006	-	-	TW	[45]
Livestock waste	*Chlorella* sp.	2000	222	103	0.289	0.529	36.3	0.105	CN	[41]
Municipal wastewater										
Centrate	*Chlorella sorokiniana* UTEX1230	-	53	9.4	0.083	0.152	9.4	0.008	GB	[48]
Domestic	*Chlorella vulgaris*	142	56	9	0.054	0.099	21.5	0.012	US	[56]
	Chlorella minutissima				0.049	0.090	22.9	0.011		
Municipal	*Chlorella vulgaris* SAG 211-11b	2175	840	10	0.144	0.264	23	0.033	FI	[46]
Secondary	*Chlorella vulgaris* UTEX 26	131	112	35	0.078	0.143	8.7	0.021	MX	[49]
	Chlorella vulgaris CICESE				0.105	0.192	20.2	0.025		
Centrate	*Chlorella vulgaris*	513	803	32	0.071	0.130	29.6	0.021	CN	[50]
Municipal (osmosis concentrate)	*Chlorella vulgaris*	164	43.2	13.1	0.32	0.585	-	-	AU	[47]
Industrial wastewater										
Meat processing	*Chlorella* sp. UM6151	2100	212	54	0.171	0.313	17.5	0.029	US	[52]

4. Light

Because of photosynthesis for microalgal growth, light is the most important parameter in microalgal cultivation. Lighting in microalgal cultivation contains two main factors: light intensity and the wavelength of light. In general, the growth rate of microalgae can be greatly increased along with an increase in light intensity; however, when the light intensity exceeds the saturation light that can be tolerated by microalgae, the growth rate of microalgae will be significantly decreased [57]. Therefore, to achieve the maximum growth rate of microalgae, the light intensity is usually controlled to “light saturation”. Because microalgae itself will block light from passing, the light intensity decreases sharply with distance through the surface, causing a decrease in the growth rate of microalgae [58].

Wastewater Source	Microalgae	COD [1] (mg L^{-1})	TN [1] (mg L^{-1})	TP [1] (mg L^{-1})	Biomass Productivity (g L^{-1} d^{-1})	CO_2 Fixation Efficiency [2] (g L^{-1} d^{-1})	Lipid (%)	Lipid Productivity [3] (g L^{-1} d^{-1})	Country [4]	References
Food	*Chlorella vulgaris*	341	-	-	0.207	0.379	31	0.064	CN	[53]
Pulp and paper	*Chlorella vulgaris* SAG 211-11b	905	350	28	0.208	0.381	21.7	0.045	FI	[46]
Alcohol and starch processing	*Chlorella pyrenoidosa*	3599	334	39	0.376	0.688	19.7	0.074	CN	[54]
Tofu whey	*Chlorella pyrenoidosa* FACHB-9	-	592	49	0.283	0.518	17.5	0.049	CN	[55]

... strain in ... raceway ... [59] ... -emitting ... intensity ... [60][61] ... re phase ... of light, a ... [62] ... e for the autotrophic cultivation of *Chlorella vulgaris* was investigated, and the results showed that red LED light (630–665 nm) resulted in small cells with active divisions, while blue light (430–465 nm) LED illumination led to a significant increase in cell size [63]. The mixed LED light wavelength with red and blue LED light (e.g., red:blue is 5:5) also affects and enhances microalgal growth, including *Scenedesmus obliquuus*, *Neochloris oleoabundans,* and *Chlorella vulgaris* [64].

[1] COD, TN, and TP: chemical oxygen demand, total nitrogen, and total phosphorus of wastewater. [2] CO_2 fixation efficiency (g L^{-1} d^{-1}) was calculated by 1.83-fold of biomass productivity. [3] Lipid productivity (g L^{-1} d^{-1}) = (biomass productivity × lipid content)/100. [4] Country abbreviation: China (CN), Taiwan (TW), Korea (KR), United Kingdom (GB), USA (US), Finland (FI), Mexico (MX), Australia (AU). -: Data not shown.

5. pH

The pH of culture broth affects the enzyme activity related to the metabolism of microalgae and the ion absorption efficiency of microalgae, which in turn affects the growth and carbon fixation efficiency of microalgae [6][65]. The optimal pH for growth varies among microalgal species, and in general, the optimum pH is neutral for most microalgae [66]. Flue gas usually contains high concentrations of CO_2, NO_x, and SO_2 [4]. When microalgae were directly aerated with flue gas containing 10–30% CO_2, the pH of the culture broth might be reduced to 5.5 [67]. When the microalgae were aerated with flue gas containing SO_2 at 100 to 250 ppm, the pH of the culture broth decreased to pH 2.5 to 3.5 to generate bisulfite (HSO_3^-), sulfite (SO_3^{2-}), and sulfate (SO_4^{2-}) [67]. If the flue gas is directly aerated into the culture broth of microalgae without dilution, the excess CO_2 of flue gas will be discharged back to the atmosphere. To reduce the CO_2 discharged back to the atmosphere, the CO_2 captured from flue gas aerated into alkaline medium is easily converted into HCO_3^-, which is dissolved in water and used for microalgal growth. The solubility of CO_2 in water is low, but the CO_2 content in the culture broth can be increased under alkaline conditions to further increase the CO_2 utilization efficiency of microalgae [68]. In addition, gradually increasing the pH in a microalgal culture is desirable for reducing microbial diversity and is good for outdoor cultivation of microalgae [69].

6. Temperature

The optimal temperature range for microalgae growth is generally 15–26 °C [70]. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity may be a primary site of damage by elevated temperature to cause a decrease in photosynthesis efficiency [71]. In contrast, there was not only a decrease in the metabolic rate of microalgae, but also a decrease in CO_2 solubility in culture broth. Therefore, the optimal temperature for growth varies among microalgal species. The temperature of the flue gas will generally be as high as 120 °C or even higher [4]. Flue gas usually needs cooling to be aerated into the culture broth because the temperature of flue gas is too high. If the thermal-tolerant potential of microalgae is good, the cost of flue gas cooling can be reduced. In addition, when sunlight is used outdoors as a light source, the temperature of the culture broth easily changes with the surrounding environment. Béchet et al. [72] indicated that 18,000 GJ year^{-1} ha^{-1} of heat energy must be removed to maintain the broth temperature of column PBRs at or below 25 °C. Considering the cost of temperature control, thermotolerant microalgal strains are needed, especially in large-scale outdoor cultivation. When *Chlorella sorokiniana* was cultivated in outdoor 51-L column PBRs, the culture broth temperature reached 41 °C without growth inhibition [73], and similar results showed better growth performance under uncontrolled temperature in outdoor conditions [11][13].

7. Microalgal Cultivation System

Open (raceway) ponds and PBRs of microalgal cultivation systems are usually—and primarily—adopted. Studies on CO_2 fixation by microalgae used in open ponds and PBRs are outlined in **Table 4**. It has been reported that microalgal biomass production produced from open ponds is more efficient than 90% of worldwide biomass production [74]. The most prominent features of open ponds include simple construction, low cost and easy operation [75][76]. However, disadvantages of open ponds are also obvious, such as a large footprint, difficulties in operation control, unstable culture conditions, high evaporation loss, easy contamination, and the decay of light intensity with medium depth. Compared with open ponds, PBRs have many advantages, such as the most efficient mixing, the best growth conditions, high volumetric mass transfer rates, low risk of contamination, lowest losses of CO_2, low shear stress and relatively low energy consumption [77][37][78]. However, the limitations of PBRs are construction cost and scale-up [79]. Overcoming the above shortcomings of cultivation systems is a future research direction for developing advanced cultivation systems. The two cultivation systems still have many challenges in practical operation [80]. Closed cultivation systems, e.g., PBRs, are still not widely applied in industry because the operation cost and construction costs of the systems are too high despite the high microalgal biomass productivity [81]. To solve the limitations of large-scale outdoor microalgae cultivation systems, from an engineering perspective, how to increase the efficiency of gas aeration and mixing should be considered. Low cost and energy consumption can both be achieved by the design of air mixing with flue gas CO_2 aeration to improve microalgal growth by sufficient CO_2 utilization. Therefore, outdoor large-scale microalgae cultivation systems can become closer to the industrialization process and commercial application by improving the efficiency of gas aeration and mixing. Suitable microalgal cultivation systems usually depend on factors such as cost, CO_2 capture source, nutrient sources, and the type of target products. At present, most studies on CO_2 fixation by microalgae are used in open ponds or PBRs, and few studies have integrated both microalgal cultivation systems to enhance biomass productivity [59][82]. In our previous study [59], an efficient PBRs/raceway circulating (PsRC) system integrated with the advantages of PBRs and paddlewheel-driven raceway ponds had great potential for the mass cultivation of microalgae. The total amount of CO_2 fixation of the PsRC system was approximately 1.2 kg d^{-1} with 50% CO_2 utilization efficiency, as simultaneous microalgal biomass production and CO_2 fixation occurred by cultivating alkali-tolerant *Chlorella* sp. AT1 with alkaline-CO_2 capturing operation in the PsRC system. Long-term cultivation for 40 days in a novel membrane photobioreactor, the steadily growth of *Chlorella vulgaris* were obtained and the maximum removal efficiency of CO_2 was 80%. Because the self-forming dynamic membrane from microalgae was easy to harvest, the potential of achieving a sustainable CO_2 fixation technology [83]. To investigate the carbon fixation effectivity of microalgae in outdoor cultivation, many studies have used the design of the cultivation system to scale up to pilot scale and industrial scale. The pilot scale is mainly used for research, because the expansion of the outdoor cultivation system may increase the cost of construction, the risk of microorganism pollution, and the release large amounts of CO_2. In **Table 4**, the research in China and Taiwan has reached a ton scale, and it can be combined with waste gas for microalgae cultivation. The cultivation system combination the strategy of an increase of the CO_2 content in the water for the microalgal growth and enhance the CO_2 carbon fixation efficiency. One is to couple with spraying absorption tower to increase the CO_2 content in the water [84], another is to use alkali-tolerant mutant strain combined with alkaline-CO_2 capturing medium [59].

Table 4. Biomass productivity and CO_2 fixation efficiency of microalgae *Chlorella* in different cultivation systems.

Microalgae	Cultivation System	Cultivation Scale (L)	CO_2 (%)	Maximum Biomass Conc. (g L^{-1})	Biomass Productivity (g L^{-1} d^{-1})	CO_2 Fixation Efficiency [1] (g L^{-1} d^{-1})	Country [2]	References
Chlorella sp. MTF-15	Column-type PBR	1	12.5 (1/2 flue gas)	2.855	0.528	0.966	TW	[4]
		1200		1.555	0.197	0.361		
Chlorella vulgaris	Porous air-lift PBR	16	0.03 (air)	0.095	0.004	0.174	HK	[85]
	Loop air-lift PBR			0.126	0.007	0.231		
	Bubbling PBR			0.783	0.054	1.433		
Chlorella sp. GD	Column-type PBR	1	2	4.813	0.870	1.592	TW	[35]
			8 (boiler flue gas)	4.921	1.296	2.333		[5]
Chlorella vulgaris	Plastic bottle	15	4	3.151	0.378	0.711	PL	[86]
Chlorella vulgaris	Flat-plate PBR	1.6	5	2.303	0.551	1.008	CN	[57]
Chlorella vulgaris	Bubble column PBR	56	0.03 (air)	0.962	0.043	0.079	MY	[87]
Chlorella pyrenoidosa	Open raceway pond	8000	99.5	0.927	0.114	0.214	CN	[84]
Chlorella vulgaris	Coiled tubular tree PBR	1.2	0.03 (air)	0.552	0.084	0.153	CA	[88]
Chlorella	Flat panel PBR	90	5	1.913	0.091	0.167	US	[89]

Microalgae	Cultivation System	Cultivation Scale (L)	CO_2 (%)	Maximum Biomass Conc. (g L^{-1})	Biomass Productivity (g L^{-1} d^{-1})	CO_2 Fixation Efficiency [1] (g L^{-1} d^{-1})	Country [2]	References
sorokiniana								
Chlorella vulgaris	Pilot-scale PBR	150	Without aeration	2.211	0.198	0.362	CN	[90]
Chlorella sp. AT1	Column-type PBR	1	10	7.372	1.011	1.851	TW	[19]
Chlorella sp. AT1	PBRs/Raceway circulating system	288	2	2.561	0.321	0.588	TW	[59]
		528		1.963	0.237	0.434		
		1008		1.052	0.107	0.195		
		3600		1.686	0.150	0.275		
		6600		1.257	0.109	0.199		
Chlorella sp. HS2	Flat panel PBR	2	1	3.811	0.543	1.021	KR	[91]
Chlorella vulgaris UTEX 26	Raceway	1100	0.03 (air)	0.25	20–26 (g m^{-2} d^{-1} for 65 days culture)	-	MX	[75]
Chlorella pyrenoidosa PY-ZU1	Pond-tubular hybrid PBR	<5 (a model system)	15	2.3	0.770	1.409	CN	[82]
Chlorella vulgaris	Raceway with computational fluid dynamics	20	50 (mix with air and pure CO_2 gas)	5.2	11.89 (g m^{-2} d^{-1}, 14 cm depth of raceway)	-	TW	[76]
Chlorella vulgaris CCAP 211/11B	Membrane photobioreactor	40	15	1.01	0.166	0.704	IT	[83]

[1] CO_2 fixation efficiency (g L^{-1} d^{-1}) was calculated by 1.83-fold of biomass productivity. [2] Country abbreviation: Taiwan (TW), Hong Kong (HK), Poland (PL), China (CN), Malaysia (MY), USA (US), Korea (KR), Mexico (MX), Italy (IT). -: Data not shown.

...sch, A.; ...O2 ...sms and implications for biotechnology and bioremediation. Biotechnol. Lett. 2013, 35, 1745–1752.

3. Ong, S.-C.; Kao, C.-Y.; Chiu, S.-Y.; Tsai, M.-T.; Lin, C.-S. Characterization of the thermal-tolerant mutants of Chlorella sp. with high growth rate and application in outdoor photobioreactor cultivation. Bioresour. Technol. 2010, 101, 2880–2883.

4. Kao, C.-Y.; Chen, T.-Y.; Chang, Y.-B.; Chiu, T.-W.; Lin, H.-Y.; Chen, C.-D.; Chang, J.-S.; Lin, C.-S. Utilization of carbon dioxide in industrial flue gases for the cultivation of microalga Chlorella sp. Bioresour. Technol. 2014, 166, 485–493.

5. Kuo, C.-M.; Jian, J.-F.; Lin, T.-H.; Chang, Y.-B.; Wan, X.-H.; Lai, J.-T.; Chang, J.-S.; Lin, C.-S. Simultaneous microalgal biomass production and CO2 fixation by cultivating Chlorella sp. GD with aquaculture wastewater and boiler flue gas. Bioresour. Technol. 2016, 221, 241–250.

6. Kuo, C.-M.; Lin, T.-H.; Yang, Y.-C.; Zhang, W.-X.; Lai, J.-T.; Wu, H.-T.; Chang, J.-S.; Lin, C.-S. Ability of an alkali-tolerant mutant strain of the microalga Chlorella sp. AT1 to capture carbon

dioxide for increasing carbon dioxide utilization efficiency. Bioresour. Technol. 2017, 244, 243–251.

7. Vadlamani, A.; Viamajala, S.; Pendyala, B.; Varanasi, S. Cultivation of Microalgae at Extreme Alkaline pH Conditions: A Novel Approach for Biofuel Production. ACS Sustain. Chem. Eng. 2017, 5, 7284–7294.
8. Vadlamani, A.; Pendyala, B.; Viamajala, S.; Varanasi, S. High Productivity Cultivation of Microalgae without Concentrated CO2 Input. ACS Sustain. Chem. Eng. 2019, 7, 1933–1943.
9. Santos, A.; Lamers, P.; Janssen, M.; Wijffels, R. Biomass and lipid productivity of Neochloris oleoabundans under alkaline–saline conditions. Algal Res. 2013, 2, 204–211.
10. Sachdeva, N.; Gupta, R.P.; Mathur, A.S.; Tuli, D.K. Enhanced lipid production in thermo-tolerant mutants of Chlorella pyrenoidosa NCIM 2738. Bioresour. Technol. 2016, 221, 576–587.
11. Lee, T.-M.; Tseng, Y.-F.; Cheng, C.-L.; Chen, Y.-C.; Lin, C.-S.; Su, H.-Y.; Chow, T.-J.; Chen, C.-Y.; Chang, J.-S. Characterization of a heat-tolerant Chlorella sp. GD mutant with enhanced photosynthetic CO2 fixation efficiency and its implication as lactic acid fermentation feedstock. Biotechnol. Biofuels 2017, 10, 214.
12. Varshney, P.; Beardall, J.; Bhattacharya, S.; Wangikar, P.P. Isolation and biochemical characterisation of two thermophilic green algal species- Asterarcys quadricellulare and Chlorella sorokiniana, which are tolerant to high levels of carbon dioxide and nitric oxide. Algal Res. 2018, 30, 28–37.
13. Mehta, P.; Rani, R.; Gupta, R.; Mathur, A.S.; Puri, S.K. Biomass and lipid production of a novel freshwater thermo-tolerant mutant strain of Chlorella pyrenoidosa NCIM 2738 in seawater salinity recycled medium. Algal Res. 2018, 36, 88–95.
14. Chou, H.-H.; Su, H.-Y.; Song, X.-D.; Chow, T.-J.; Chen, C.-Y.; Chang, J.-S.; Lee, T.-M. Isolation and characterization of Chlorella sp. mutants with enhanced thermo- and CO2 tolerances for CO2 sequestration and utilization of flue gases. Biotechnol. Biofuels 2019, 12, 251.
15. Li, D.; Wang, L.; Zhao, Q.; Wei, W.; Sun, Y. Improving high carbon dioxide tolerance and carbon dioxide fixation capability of Chlorella sp. by adaptive laboratory evolution. Bioresour. Technol. 2015, 185, 269–275.
16. Jain, D.; Ghonse, S.S.; Trivedi, T.; Fernandes, G.L.; Menezes, L.D.; Damare, S.R.; Mamatha, S.; Kumar, S.; Gupta, V. CO2 fixation and production of biodiesel by Chlorella vulgaris NIOCCV under mixotrophic cultivation. Bioresour. Technol. 2019, 273, 672–676.
17. Kao, C.-Y.; Chiu, S.-Y.; Huang, T.-T.; Dai, L.; Hsu, L.-K.; Lin, C.-S. Ability of a mutant strain of the microalga Chlorella sp. to capture carbon dioxide for biogas upgrading. Appl. Energy 2012, 93, 176–183.

18. Li, J.; Tang, X.; Pan, K.; Zhu, B.; Li, Y.; Ma, X.; Zhao, Y. The regulating mechanisms of CO2 fixation and carbon allocations of two Chlorella sp. strains in response to high CO2 levels. Chemosphere 2020, 247, 125814.
19. Kuo, C.M.; Chang, Y.B.; Lin, T.H.; Yang, Y.C.; Zhang, W.X.; Lai, J.T.; Wu, H.T.; Lin, C.S. Effects of a protein-enriched crude lysate from Chlorella sp. against acetaminophen-induced liver injury. IOSR-JBB 2017, 3, 82–88.
20. Chiu, S.-Y.; Kao, C.-Y.; Huang, T.-T.; Lin, C.-J.; Ong, S.-C.; Chen, C.-D.; Chang, J.-S.; Lin, C.-S. Microalgal biomass production and on-site bioremediation of carbon dioxide, nitrogen oxide and sulfur dioxide from flue gas using Chlorella sp. cultures. Bioresour. Technol. 2011, 102, 9135–9142.
21. Cheng, D.; Li, X.; Yuan, Y.; Zhao, Q. Kinetic model for effects of simulated flue gas onto growth profiles of Chlorella sp. AE10 and Chlorella sp. Cv. Biotechnol. Appl. Biochem. 2020, 67, 783–789.
22. Bhola, V.; Swalaha, F.M.; Nasr, M.; Bux, F. Fuzzy intelligence for investigating the correlation between growth performance and metabolic yields of a Chlorella sp. exposed to various flue gas schemes. Bioresour. Technol. 2017, 243, 1078–1086.
23. Kumar, K.; Banerjee, D.; Das, D. Carbon dioxide sequestration from industrial flue gas by Chlorella sorokiniana. Bioresour. Technol. 2014, 152, 225–233.
24. Praveenkumar, R.; Kim, B.; Choi, E.; Lee, K.; Park, J.-Y.; Lee, J.-S.; Lee, Y.-C.; Oh, Y.-K. Improved biomass and lipid production in a mixotrophic culture of Chlorella sp. KR-1 with addition of coal-fired flue-gas. Bioresour. Technol. 2014, 171, 500–505.
25. Duarte, J.H.; Fanka, L.S.; Costa, J.A.V. Utilization of simulated flue gas containing CO2, SO2, NO and ash for Chlorella fusca cultivation. Bioresour. Technol. 2016, 214, 159–165.
26. García-Cubero, R.; Moreno-Fernández, J.; García-González, M. Potential of Chlorella vulgaris to Abate Flue Gas. Waste Biomass-Valorization 2017, 9, 2015–2019.
27. Hanifzade, M.M.; Nabati, Z.; Tavakoli, O.; Sarrafzadeh, M.H. Waste to Energy from Flue Gas of Industrial Plants to Biodiesel: Effect of CO2 on Microalgae Growth. Int. J. Waste Resour. 2017, 7, 2.
28. Yadav, G.; Karemore, A.; Dash, S.K.; Sen, R. Performance evaluation of a green process for microalgal CO2 sequestration in closed photobioreactor using flue gas generated in-situ. Bioresour. Technol. 2015, 191, 399–406.
29. Kandimalla, P.; Desi, S.; Vurimindi, H. Mixotrophic cultivation of microalgae using industrial flue gases for biodiesel production. Environ. Sci. Pollut. Res. 2015, 23, 9345–9354.

30. Cheng, D.; Li, X.; Yuan, Y.; Yang, C.; Tang, T.; Zhao, Q.; Sun, Y. Adaptive evolution and carbon dioxide fixation of Chlorella sp. in simulated flue gas. Sci. Total Environ. 2019, 650, 2931–2938.
31. Chen, H.; Wang, J.; Zheng, Y.; Zhan, J.; He, C.; Wang, Q. Algal biofuel production coupled bioremediation of biomass power plant wastes based on Chlorella sp. C2 cultivation. Appl. Energy 2018, 211, 296–305.
32. Shahid, A.; Malik, S.; Zhu, H.; Xu, J.; Nawaz, M.Z.; Nawaz, S.; Alam, A.; Mehmood, M.A. Cultivating microalgae in wastewater for biomass production, pollutant removal, and atmospheric carbon mitigation; a review. Sci. Total Environ. 2020, 704, 135303.
33. Moreno-Garcia, L.; Gariépy, Y.; Bourdeau, N.; Barnabé, S.; Raghavan, G. Optimization of the proportions of four wastewaters in a blend for the cultivation of microalgae using a mixture design. Bioresour. Technol. 2019, 283, 168–173.
34. Hussain, F.; Shah, S.Z.; Ahmad, H.; Abubshait, S.A.; Abubshait, H.A.; Laref, A.; Manikandan, A.; Kusuma, H.S.; Iqbal, M. Microalgae an ecofriendly and sustainable wastewater treatment option: Biomass application in biofuel and bio-fertilizer production. A review. Renew. Sustain. Energy Rev. 2021, 137, 110603.
35. Kuo, C.-M.; Chen, T.-Y.; Lin, T.-H.; Kao, C.-Y.; Lai, J.-T.; Chang, J.-S.; Lin, C.-S. Cultivation of Chlorella sp. GD using piggery wastewater for biomass and lipid production. Bioresour. Technol. 2015, 194, 326–333.
36. Chiu, S.-Y.; Kao, C.-Y.; Chen, T.-Y.; Chang, Y.-B.; Kuo, C.-M.; Lin, C.-S. Cultivation of microalgal Chlorella for biomass and lipid production using wastewater as nutrient resource. Bioresour. Technol. 2015, 184, 179–189.
37. Salama, E.-S.; Kurade, M.; Abou-Shanab, R.A.; El-Dalatony, M.M.; Yang, I.-S.; Min, B.; Jeon, B.-H. Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation. Renew. Sustain. Energy Rev. 2017, 79, 1189–1211.
38. Lu, W.; Wang, Z.; Wang, X.; Yuan, Z. Cultivation of Chlorella sp. using raw dairy wastewater for nutrient removal and biodiesel production: Characteristics comparison of indoor bench-scale and outdoor pilot-scale cultures. Bioresour. Technol. 2015, 192, 382–388.
39. Marjakangas, J.M.; Chen, C.-Y.; Lakaniemi, A.-M.; Puhakka, J.A.; Whang, L.-M.; Chang, J.-S. Selecting an indigenous microalgal strain for lipid production in anaerobically treated piggery wastewater. Bioresour. Technol. 2015, 191, 369–376.
40. Nam, K.; Lee, H.; Heo, S.-W.; Chang, Y.K.; Han, J.-I. Cultivation of Chlorella vulgaris with swine wastewater and potential for algal biodiesel production. Environ. Boil. Fishes 2017, 29, 1171–1178.
41. Zhu, L.-D.; Li, Z.-H.; Guo, D.-B.; Huang, F.; Nugroho, Y.; Xia, K. Cultivation of Chlorella sp. with livestock waste compost for lipid production. Bioresour. Technol. 2017, 223, 296–300.

42. Cheng, D.; Ngo, H.; Guo, W.; Chang, S.; Nguyen, D.D.; Kumar, S. Microalgae biomass from swine wastewater and its conversion to bioenergy. Bioresour. Technol. 2019, 275, 109–122.

43. Toze, S. Reuse of effluent water—benefits and risks. Agric. Water Manag. 2006, 80, 147–159.

44. Abdel-Raouf, N.; Al-Homaidan, A.A.; Ibraheem, I.B.M. Microalgae and wastewater treatment. Saudi J. Biol. Sci. 2012, 19, 257–275.

45. Chen, C.-Y.; Kuo, E.-W.; Nagarajan, D.; Dong, C.-D.; Lee, D.-J.; Varjani, S.; Lam, S.S.; Chang, J.-S. Semi-batch cultivation of Chlorella sorokiniana AK-1 with dual carriers for the effective treatment of full strength piggery wastewater treatment. Bioresour. Technol. 2021, 326, 124773.

46. Tao, R.; Kinnunen, V.; Praveenkumar, R.; Lakaniemi, A.-M.; Rintala, J. Comparison of Scenedesmus acuminatus and Chlorella vulgaris cultivation in liquid digestates from anaerobic digestion of pulp and paper industry and municipal wastewater treatment sludge. Environ. Boil. Fishes 2017, 29, 2845–2856.

47. Mohseni, A.; Kube, M.; Fan, L.; Roddick, F.A. Potential of Chlorella vulgaris and Nannochloropsis salina for nutrient and organic matter removal from municipal wastewater reverse osmosis concentrate. Environ. Sci. Pollut. Res. 2020, 27, 26905–26914.

48. Lizzul, A.; Hellier, P.; Purton, S.; Baganz, F.; Ladommatos, N.; Campos, L. Combined remediation and lipid production using Chlorella sorokiniana grown on wastewater and exhaust gases. Bioresour. Technol. 2014, 151, 12–18.

49. Fernández-Linares, L.C.; Barajas, C.G.; Páramo, E.D.; Corona, J.A.B. Assessment of Chlorella vulgaris and indigenous microalgae biomass with treated wastewater as growth culture medium. Bioresour. Technol. 2017, 244, 400–406.

50. Ge, S.; Qiu, S.; Tremblay, D.; Viner, K.; Champagne, P.; Jessop, P.G. Centrate wastewater treatment with Chlorella vulgaris: Simultaneous enhancement of nutrient removal, biomass and lipid production. Chem. Eng. J. 2018, 342, 310–320.

51. Bibi, R.; Ditta, A.; Hussain, A.; Noureen, S.; Khalid, A.; Aziz, I. Production of algal biomass using different dilutions of textile effluent wastewater. Sci. Lett. 2016, 4, 71–77.

52. Lu, Q.; Zhou, W.; Min, M.; Ma, X.; Chandra, C.; Doan, Y.; Ma, Y.; Zheng, H.; Cheng, S.; Griffith, R.; et al. Growing Chlorella sp. on meat processing wastewater for nutrient removal and biomass production. Bioresour. Technol. 2015, 198, 189–197.

53. Hou, Q.; Pei, H.; Hu, W.; Jiang, L.; Yu, Z. Mutual facilitations of food waste treatment, microbial fuel cell bioelectricity generation and Chlorella vulgaris lipid production. Bioresour. Technol. 2016, 203, 50–55.

54. Tan, X.-B.; Zhao, X.-C.; Zhang, Y.-L.; Zhou, Y.-Y.; Yang, L.-B.; Zhang, W.-W. Enhanced lipid and biomass production using alcohol wastewater as carbon source for Chlorella pyrenoidosa

cultivation in anaerobically digested starch wastewater in outdoors. Bioresour. Technol. 2018, 247, 784–793.

55. Wang, S.-K.; Wang, X.; Miao, J.; Tian, Y.-T. Tofu whey wastewater is a promising basal medium for microalgae culture. Bioresour. Technol. 2018, 253, 79–84.

56. Limayem, A.; Gonzalez, F.; Micciche, A.; Haller, E.; Nayak, B.; Mohapatra, S. Molecular identification and nanoremediation of microbial contaminants in algal systems using untreated wastewater. J. Environ. Sci. Health Part B 2016, 51, 868–872.

57. Chang, H.-X.; Huang, Y.; Fu, Q.; Liao, Q.; Zhu, X. Kinetic characteristics and modeling of microalgae Chlorella vulgaris growth and CO2 biofixation considering the coupled effects of light intensity and dissolved inorganic carbon. Bioresour. Technol. 2016, 206, 231–238.

58. Ogbonna, J.C.; Tanaka, H. Light requirement and photosynthetic cell cultivation—Development of processes for efficient light utilization in photobioreactors. Environ. Boil. Fishes 2000, 12, 207–218.

59. Kuo, C.-M.; Jian, J.-F.; Sun, Y.-L.; Lin, T.-H.; Yang, Y.-C.; Zhang, W.-X.; Chang, H.-F.; Lai, J.-T.; Chang, J.-S.; Lin, C.-S. An efficient Photobioreactors/Raceway circulating system combined with alkaline-CO2 capturing medium for microalgal cultivation. Bioresour. Technol. 2018, 266, 398–406.

60. Zhao, Y.; Wang, J.; Zhang, H.; Yan, C.; Zhang, Y. Effects of various LED light wavelengths and intensities on microalgae-based simultaneous biogas upgrading and digestate nutrient reduction process. Bioresour. Technol. 2013, 136, 461–468.

61. Ma, R.; Thomas-Hall, S.R.; Chua, E.T.; Eltanahy, E.; Netzel, M.E.; Netzel, G.; Lu, Y.; Schenk, P.M. LED power efficiency of biomass, fatty acid, and carotenoid production in Nannochloropsis microalgae. Bioresour. Technol. 2018, 252, 118–126.

62. Yan, C.; Muñoz, R.; Zhu, L.; Wang, Y. The effects of various LED (light emitting diode) lighting strategies on simultaneous biogas upgrading and biogas slurry nutrient reduction by using of microalgae Chlorella sp. Energy 2016, 106, 554–561.

63. Kim, D.G.; Lee, C.; Park, S.-M.; Choi, Y.-E. Manipulation of light wavelength at appropriate growth stage to enhance biomass productivity and fatty acid methyl ester yield using Chlorella vulgaris. Bioresour. Technol. 2014, 159, 240–248.

64. Zhao, Y.; Sun, S.; Hu, C.; Zhang, H.; Xu, J.; Ping, L. Performance of three microalgal strains in biogas slurry purification and biogas upgrade in response to various mixed light-emitting diode light wavelengths. Bioresour. Technol. 2015, 187, 338–345.

65. Papone, T.; Kookkhunthod, S.; Paungbut, M.; Leesing, R. Producing of Microbial Oil by Mixed Culture of Microalgae and Oleaginous Yeast Using Sugarcane Molasses as Carbon Substrate. J. Clean Energy Technol. 2015, 4, 253–256.

66. Sakarika, M.; Kornaros, M. Effect of pH on growth and lipid accumulation kinetics of the microalga Chlorella vulgaris grown heterotrophically under sulfur limitation. Bioresour. Technol. 2016, 219, 694–701.

67. Zhao, B.; Su, Y. Process effect of microalgal-carbon dioxide fixation and biomass production: A review. Renew. Sustain. Energy Rev. 2014, 31, 121–132.

68. Liu, G.; Qiao, L.; Zhang, H.; Zhao, D.; Su, X. The effects of illumination factors on the growth and HCO3− fixation of microalgae in an experiment culture system. Energy 2014, 78, 40–47.

69. Bell, T.A.S.; Prithiviraj, B.; Wahlen, B.; Fields, M.W.; Peyton, B.M. A Lipid-Accumulating Alga Maintains Growth in Outdoor, Alkaliphilic Raceway Pond with Mixed Microbial Communities. Front. Microbiol. 2016, 6, 1480.

70. Kumar, A.; Ergas, S.; Yuan, X.; Sahu, A.; Zhang, Q.; Dewulf, J.; Malcata, F.X.; van Langenhove, H. Enhanced CO2 fixation and biofuel production via microalgae: Recent developments and future directions. Trends Biotechnol. 2010, 28, 371–380.

71. LILLEY, R.M.; Ralph, P.J.; Larkum, A.W.D. The determination of activity of the enzyme Rubisco in cell extracts of the dinoflagellate alga Symbiodinium sp. by manganese chemiluminescence and its response to short-term thermal stress of the alga. Plant Cell Environ. 2010, 33, 995–1004.

72. Béchet, Q.; Shilton, A.; Fringer, O.B.; Muñoz, R.; Guieysse, B. Mechanistic Modeling of Broth Temperature in Outdoor Photobioreactors. Environ. Sci. Technol. 2010, 44, 2197–2203.

73. Béchet, Q.; Muñoz, R.; Shilton, A.; Guieysse, B. Outdoor cultivation of temperature-tolerantChlorella sorokinianain a column photobioreactor under low power-input. Biotechnol. Bioeng. 2013, 110, 118–126.

74. Shuba, E.S.; Kifle, D. Microalgae to biofuels: ‘Promising’ alternative and renewable energy, review. Renew. Sust. Energ. Rev. 2018, 81, 743–755.

75. Ramírez-López, C.; Perales-Vela, H.V.; Fernández-Linares, L. Biomass and lipid production from Chlorella vulgaris UTEX 26 cultivated in 2 m3 raceway ponds under semicontinuous mode during the spring season. Bioresour. Technol. 2019, 274, 252–260.

76. Kusmayadi, A.; Philippidis, G.P.; Yen, H.-W. Application of computational fluid dynamics to raceways combining paddlewheel and CO2 spargers to enhance microalgae growth. J. Biosci. Bioeng. 2020, 129, 93–98.

77. Lim, Y.A.; Chong, M.N.; Foo, S.C.; Ilankoon, I. Analysis of direct and indirect quantification methods of CO2 fixation via microalgae cultivation in photobioreactors: A critical review. Renew. Sustain. Energy Rev. 2021, 137, 110579.

78. López-Rosales, L.; Sánchez-Mirón, A.; Contreras-Gómez, A.; García-Camacho, F.; Battaglia, F.; Zhao, L.; Molina-Grima, E. Characterization of bubble column photobioreactors for shear-

sensitive microalgae culture. Bioresour. Technol. 2019, 275, 1–9.

79. Lam, M.K.; Khoo, C.G.; Lee, K.T. Chapter 19-Scale-up and commercialization of algal cultivation and biofuels production. In Biofuels from Algae, 2nd ed.; Pandey, A., Chang, J.S., Soccol, C.R., Lee, D.J., Chisti, Y., Eds.; Elsevier: Amsterdam, The Netherlands, 2019; pp. 475–506.
80. Barceló-Villalobos, M.; Olmo, P.F.-D.; Guzman, J.L.; Sevilla, J.M.F.; Fernández, F.A. Evaluation of photosynthetic light integration by microalgae in a pilot-scale raceway reactor. Bioresour. Technol. 2019, 280, 404–411.
81. Rastogi, R.P.; Pandey, A.; Larroche, C.; Madamwar, D. Algal Green Energy—R&D and technological perspectives for biodiesel production. Renew. Sustain. Energy Rev. 2018, 82, 2946–2969.
82. Xu, J.; Cheng, J.; Xin, K.; Xu, J.; Yang, W. Strengthening flash light effect with a pond-tubular hybrid photobioreactor to improve microalgal biomass yield. Bioresour. Technol. 2020, 318, 124079.
83. Senatore, V.; Buonerba, A.; Zarra, T.; Oliva, G.; Belgiorno, V.; Boguniewicz-Zablocka, J.; Naddeo, V. Innovative membrane photobioreactor for sustainable CO2 capture and utilization. Chemosphere 2021, 273, 129682.
84. Zhang, C.-D.; Li, W.; Shi, Y.-H.; Li, Y.-G.; Huang, J.-K.; Li, H.-X. A new technology of CO2 supplementary for microalgae cultivation on large scale–A spraying absorption tower coupled with an outdoor open runway pond. Bioresour. Technol. 2016, 209, 351–359.
85. Wong, Y.K.; Ho, K.C.; Tsang, Y.F.; Wang, L.; Yung, K.K.L. Cultivation of Chlorella vulgaris in Column Photobioreactor for Biomass Production and Lipid Accumulation. Water Environ. Res. 2016, 88, 40–46.
86. Adamczyk, M.; Lasek, J.; Skawińska, A. CO2 Biofixation and Growth Kinetics of Chlorella vulgaris and Nannochloropsis gaditana. Appl. Biochem. Biotechnol. 2016, 179, 1248–1261.
87. Khoo, C.G.; Lam, M.K.; Lee, K.T. Pilot-scale semi-continuous cultivation of microalgae Chlorella vulgaris in bubble column photobioreactor (BC-PBR): Hydrodynamics and gas–liquid mass transfer study. Algal Res. 2016, 15, 65–76.
88. Gong, M.; Bassi, A. Investigation of Chlorella vulgaris UTEX 265 Cultivation under Light and Low Temperature Stressed Conditions for Lutein Production in Flasks and the Coiled Tree Photo-Bioreactor (CTPBR). Appl. Biochem. Biotechnol. 2017, 183, 652–671.
89. Qiu, R.; Gao, S.; Lopez, P.A.; Ogden, K.L. Effects of pH on cell growth, lipid production and CO2 addition of microalgae Chlorella sorokiniana. Algal Res. 2017, 28, 192–199.
90. Ren, H.; Tuo, J.; Addy, M.M.; Zhang, R.; Lu, Q.; Anderson, E.; Chen, P.; Ruan, R. Cultivation of Chlorella vulgaris in a pilot-scale photobioreactor using real centrate wastewater with waste

glycerol for improving microalgae biomass production and wastewater nutrients removal. Bioresour. Technol. 2017, 245, 1130–1138.

91. Nayak, M.; Suh, W.I.; Lee, B.; Chang, Y.K. Enhanced carbon utilization efficiency and FAME production of Chlorella sp. HS2 through combined supplementation of bicarbonate and carbon dioxide. Energy Convers. Manag. 2018, 156, 45–52.