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Article

Coupling Wastewater Treatment with Microalgae Biomass Production: Focusing on Biomass Generation and Treatment Efficiency

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Abstract. Combination of suitable algae species with wastewater condition is important to achieve high productivity of algae with remarkable removal of contaminants. However, the usage of algae in treating wastewater has not yet to show sufficient removal efficiency when the biomass productivity is extremely enhanced. This review aims to scrutinize and discuss: (1) several promising species for this coupling method; (2) main wastewater characteristics related to the microalgae biomass production and their removal efficiency; (3) metal occurrences and other biotic factors; and (4) constraint of microalgae biomass production and wastewater treatment process. Microalgae such as *Chlorella*, *Spirulina* and *Scenedesmus* are among the most utilized microalgae because of their utilities. Chemical oxygen demand (COD) total nitrogen (TN), and total phosphorous (TP) concentrations affect biomass yield of algae cultivation. Metals occurrences, light intensity and carbon dioxide availability play an important role in process of algae cultivation with diverse optimum levels of each factor. Sufficient but not excess concentration of N and P solely for building biomass and other metabolism activities, mixotrophic condition for algae to digest organic carbon, and heavy metals defense mechanisms are expected to address constraint of biomass generation demand and wastewater treatment efficiency.

Keywords: Algae species, biomass, wastewater content light intensity, carbon dioxide.

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1. Introduction

The coverage of microalgae's distribution and recognition of aquatic ecosystems has enable application based on this ability to extend in many ways [1]. Algae are cultivated in liquid containing nutrients with other factors needed for the growth. Some of them are cultivated in the wastewater for nutrient removal and production of microalgae biomass [2]. Microalgae biomass is further processed for many utilizations such as food stock, animal feed, fertilizer, and biofuel [3]. Among these utilizations, wastewater treatment along with biofuel production have emerged the algae as the commodity which within 10-15 years are expected to be the most promising feedstock for counteract the rise of fuel consumption [4]. This application has potential not only for water treatment process but also for producing microalgae biomass and conversion of CO_2 to O_2. However, there are still many factors that need to be enhanced as well as the drawback of this application before this utilization can be completely adapted in industrial scale.

Wastewater treatment focuses on processing wastewater to certain extent for safe disposal into environment [5]. The contaminants such as nutrients in the form of nitrogen (N), organic matter (OM), and phosphorous (P) which is known to be difficult to remove from the wastewater. Several advanced treatments have been reported to successfully remove these contaminants using chemical and physical based treatments, yet the cost of both technologies are still considered expensive. Moreover, the application of chemical and physical treatments is not able to produce any beneficial by-product.

The nutrients in wastewater is giving the chance for microalgae to grow as it is necessary to build biomass and support other metabolism processes of the algae cells [6]. In some of the traditional algae cultivation, artificial nutrients are commonly added into the water medium. This method is usually conducted in food-purposed algae biomass. However, for other applications algae free-contamination medium is not necessary since the nutrients shall be purchased. Cultivation of algae in wastewater can counteract high demands of nutrient for biomass generation. Moreover, the algae also can absorb the nutrient and other undesired contaminant in the water such as toxic in the wastewater [7]. The wastewater contents are able to be considered as nutrient for algae to grow.

Algae utilization in wastewater treatment and algal biomass generation has two main factors to consider. Algae species and wastewater characteristics need to be carefully considered in order to build effective and efficient coupling system. Algae niche and effective removal of substances in the wastewater needs to be deliberated. Niche of algae usually lies in lower than actual concentration of municipal and industrial wastewater characteristics. Most of recent studies were conducted to address either removal of wastewater nutrient or condition where algae can achieve high concentration in wastewater [8]. Nevertheless, it is important to understand and identify the optimum condition for algae cultivation and water treatment to run the coupling concept. It is expected that optimum condition of wastewater and the proper algae species are able to reach high density of biomass in short period while the wastewater characteristics can meet the requirements to be disposed. In this review, several most used algae species in wastewater are summarized and discussed in order to describe the difference between each species and strain. Wastewater characteristics, metal contamination, and other abiotic factors which affected the algae are further scrutinized to meet the niche of algae. The constrains between high biomass yield and optimum removal of the coupling are also addressed.

2. Microalgae Species for Biomass Generation

Microalgae can be described as photosynthetic microorganisms with the ability to survive and grow in lack of nutrient present because of their cellular morphology [1]. Microalgae can be found in the cool ecosystem with temperatures below the freezing point, absence of sunlight in some period every year and continuous exposure of light and UV in another period [9]. The ability to grow in different condition is also supported by the wide range of the species occurred in each condition. Microalgae can thus be classified by the environment that algae need to grow properly.

Green microalgae are usually referred as rich-oil biomass algae. Since microalgae are usually able to be isolated from the freshwater around the world, some studies have isolated the green microalgae near by the wastewater or other medium source before using that medium to cultivate that algae. Nevertheless, other advantages of algae biomass are not limited into the energy generation. Some other advantages of algae biomass include food [8] and cosmetics [10]. However, in these applications, wastewater is rarely used as the medium due to the safety and standard consideration.

Cultivation of biomass of algae for biodiesel production emphasizes on lipid content in the biomass. Production of biofuel such as biodiesel needs specific fatty acid content of the total oil for transesterification process in order to produce fatty acid methyl ester (FAME) product [11]. FAME is referred to be the biodiesel content which can be applied into the diesel engine of which the process to generate FAME involves alcohol displacement from ester group by another alcohol or usually called alcoholysis [12]. The oil content per total biomass products is a key to determine the proper algae species to use and its maximum biomass for biodiesel generation [13].

Numerous algae have been isolated and identified for many purposes including production of biomass in large scale. Specific purpose of microalgae and the medium used in the cultivation process are among the main consideration of screening process. For biofuel generation purpose, cell density, growth rate and tolerance of wide range environmental changes are usually occupied to screen the potential microalgae [3]. Moreover, metabolites of the algae including lipid content are crucial to be considered for obtaining the high amount of biomass. *Chlorella*, *Spirulina*, and *Scenedesmus* are among the most cultivated algae in order to obtain high biomass productivity with considerably high percentage of lipid (Table 1).

Table 1. Biomass yield with fatty acid percentage of several green microalgae.

Microalgae	Biomass (g/L)	Lipid content (%)	Ref
Chlorella sp.	1.7	13.7	[14]
Chlorella sp.	1.1	11	[13]
C. protothecoides	24.01	34	[15]
C. pyrenoidosa	0.73	59	[16]
C. sorokiniana	11	38	[17]
C. minutissima	0.97	37	[18]
Spirulina maxima	0.8	7.30	[19]
S. platensis	0.6	7.24	
S. platensis	0.8	13.70	[20]
Spirulina sp.	.3	60.13	[21]
Scenedesmus acutus	0.9	30.4	[22]
S. dimorphus	2.5	24.7	[23]
S. abundans	1.1	44	[24]
Desmodesmus spp.	-	58	[25]
Desmodesmus sp.	0.73	12.9	[26]
D. communis	1.23	19.0	[27]
Chlamydomonas sp.	4.15	19.4	[28]
C. reinhardtii	-	50	[29]
C. reinhardtii	0.73	18.8	[30]

2.1. *Chlorella*

Chlorella is one of the microalgae which is most likely to be found in the freshwater ecosystem since it moderately tolerates organic pollutants in the water [31]. However, it still manages to produce high amount of biomass with up to 59 % lipid content (Table 1). Showing the wide range of tolerance is also the part of consideration for using *Chlorella* as the inoculate alga. Utilization of these algae extends from freshwater to seawater environment [32] with promising yield of biomass. The advantages of these microalgae are the well-known strain and conditions that allow the better design of reactor and pick the suitable substrates.

Algae were also found in mixotropic condition (autotroph and heterotroph state) of which the consumption of organic matter in the wastewater is possible to reach noticeable amount. A study of Mu, Li [15] showed an incredible number of biomass (24.01 g L^{-1}) using sugarcane bagasse hydrolysate for mixotropic *C. protothecoides*. The sustainability of this mixotrophic alga is also potential be utilized in the wastewater treatment system since high carbon content is detected in wastewaters and it has been proven that mixotrophic culture can reach high removal of organic carbon, total nitrogen, and total phosphorous [33]. With consumption of organic matters up to 82.02 % in the medium, this study also proved that it is possible that this alga to apply in the open pond wastewater treatment. However, it is important to note that in the high initial concentration the removal rate the residual or recovered organic matter can occur above threshold level. This situation leads to the additional water processes in the system with even higher removal cost. Industrial species *C. sorokiniana* in the mixotrophic cultivation using wine waste as organic carbon source has been reported to improve specific growth rate (0.052 h^{-1}) and biomass 11 g L^{-1} [17]. The high yield using mixotropic culture is important and has emerged the possibility to reach certain condition where high yield and removal efficiency can be achieved.

Through wild strains and isolated *Chlorella* are simply cultivated for generating biomass of this species, some advanced approach was also made to obtain more productive alga. An effort using specific mutation process with N^+ beam implantation technique was conducted to design *C. pyrenoidosa* for obtaining higher lipid content from the alga [16]. It resulted an increase of the lipid content from the wild strain up to 32.4 %. Interestingly, the stability of *C. pyrenoidosa* after mutation proved that it is feasible to apply as potential strain for biodiesel production.

2.2. *Spirulina*

Genera of *Spirulina* is among the most studied algae because its usefulness to be pharmaceutical raw materials, animal feeding and food stock [34] [35]. Due to its high potential as food stock, most of this alga applications are oriented for biomass production in the artificial medium with controlled condition. However, some of the productions are still aimed to produce biomass non-food resources. It can be generated for bioethanol process with following fermentation using yeast [36] or biodiesel production through transesterification process [37]. Nonetheless, high biomass productivity is the most important parameter to achieve even though composition of the biomass determines the suitable process and utilization of the alga.

In comparison to carbohydrate and lipid, the production of protein should be increase as it is the expensive part of biomass and necessary for food supply. However, the application of protein utilization along with the residual biomass such as lipid and carbohydrate is still applicable and worth consideration [38]. Biomass utilization of *S. plantesis* can be in sequence from protein extraction to residual biomass as biofuel feedstock is possible. Although with emphasizing on protein yield up to 60.7 % by proper pH extraction time and biomass concentration, the biofuel potency is still available for about 8 % with proper techniques of extraction [39]. The potency of that residual biomass possibly achieves up to 8.9 % biofuel per biomass utilized by using proper chloroform and methanol [40]. Prates, Radmann [41] also reported 12.7% lipid utilization content of biomass form *Spirulina* sp. using the different treatment to the biomass.

For combination of food security and biodiesel production, protein and lipid in biomass require high portion where less carbohydrates. Protein (47.3%), carbohydrates (13.4%) and a high lipid content (32.7%) were achieved under modification in nitrogen availability and CO_2 supply [42]. Similar study of this genera was also achieved 46–63 % of protein content [43]. In contrary, the transesterification of this product used only 19.8% of the biomass with high amount of biomass production (4.86 g L^{-1}), which is less efficient and the quality of the generated biodiesel still needs to be enhanced [44]..

Spirulina has high amount of phycocyanin, blue substances with the ability to exhibit anti-inflammatory, antioxidant and anticancer properties and it is common to be added in the supplements and cosmetics [43]. As it is mentioned previously that *Spirulina* sp. can produce high amount of protein and phycocyanin is among the phycobiliproteins, the utilization of this algae for water treatment and cosmetic raw material production is possible. However, such active protein needs to be extracted from *Spirulina* sp before used in cosmetic and cosmeceutical products [45].

2.3. *Scenedesmus*

Scenedesmus is a genus of microalgae which gains more attention due to its ability to survive in low-nutrient environments. This alga is a prominent alga in the eutrophic and hypertropic waters [31]. It has been applied for many laboratory and pilot scale project for coupling wastewater treatments and biodiesel production [46] [47] . Many studies have explored the possibilities of this microalga with different characteristics of wastewater to produce biomass yield for biofuel since its ability to store high amount of lipid and carbohydrates [48]. However, different applications for this algae are rarely explored

Nutrient intake of this alga is the main key for the versatile applications. Optimum concentration still needs to be considered for better removal activity. Influent concentration tends to have great impact on the removal efficiency. A study of immobilized microalgae revealed that ammonium removal of *S. obliquus*. in wastewater was optimum at 50 mg L^{-1} rather than 70 and 30 mg L^{-1} [49].

Great removal of nutrients were found in the system that used *Scenedesmus* as the main organisms. Cultured *S. acutus* in wastewater reported to create higher biomass production than in enriched medium [22]. High removal efficiency as much as 98.5% of total nitrogen (TN) and total phosphorous (TP) and 96.6 ± 0.1% of ammonium was also reported [49] [46]. It has been also reported that this alga achieved 97% of total phosphorus and 90% of total nitrogen removal in direct application in domestic wastewater [50].

Specific development for wastewater treatment purpose and considerable amount of lipid in the biomass are the advantages of using *Scenedesmus* as the alga for coupling wastewater treatment and biofuel generation. However, it is important to design proper harvesting system and downstream processes for utilizing this algae. Undesired odor caused by this alga [31] that needs to be considered in the scaling up process.

3. Wastewater Characteristics for Cultivating algae

Application of wastewater as the medium for algae's growth has been adopted for decades since wastewater contains nutrition that algae need along with the inhibitory factors of the algae. Nutrients such as Nitrogen and Phosphorous are abundant in some kind of wastewaters as well as content of Chemical Oxygen Demand (COD). Some of the wastewaters also contain metals which can be in the trace components and some of the metals are also considered as toxic substances. Application of wastewater also needs to consider other operational parameters in wastewater treatment system such as light and Carbon dioxide (CO_2) (Fig. 1).

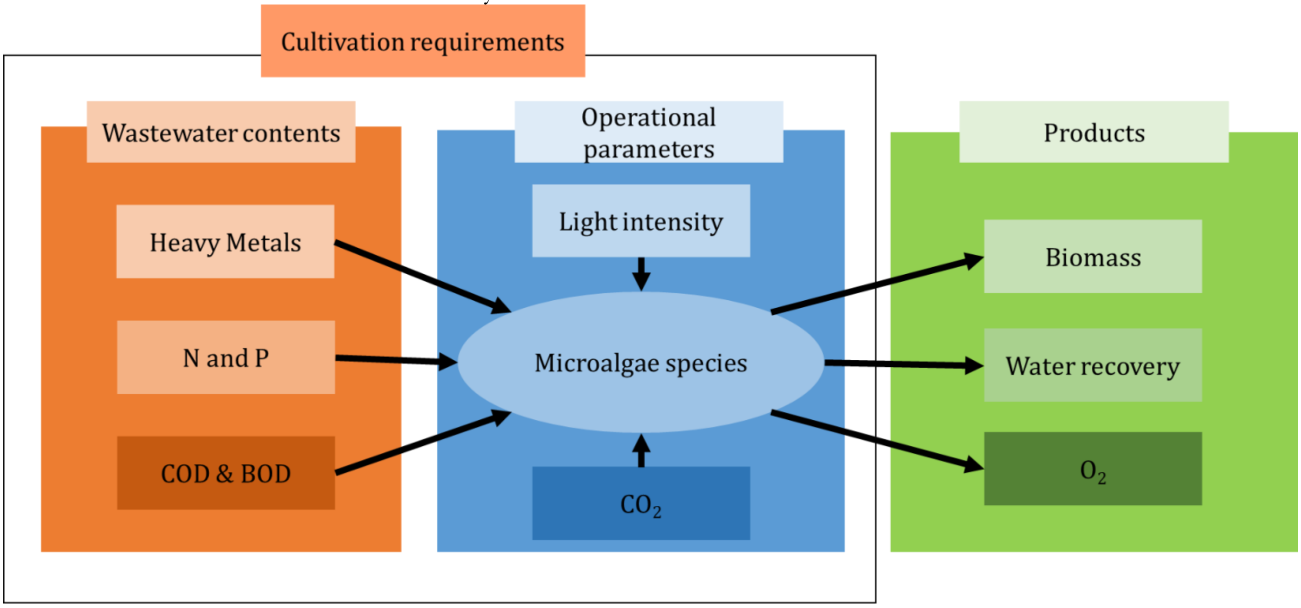

Fig. 1. Cultivation process and products of coupling wastewater treatment and generating algae biomass.

3.1. Chemical and Biological Oxygen Demands

As impact of algae in wastewater is widely studied, COD or chemical oxygen demand is found not to be the substance that can easily be removed nor consumed by the algae. Many studies have reported the removal of COD less than half of the initial concentration [51]. This is due to the fact that the source of carbon of the algae is mainly from carbon dioxide [52]. However, the occurrence of organic compound in the water or wastewater in very low concentration was reported to enhance the production of algae biomass [51].

A study from [53] revealed that the exact and highest condition of COD in the wastewater were not the most suitable concentration of algae to growth. Nonetheless, half of the COD from piggery wastewater displayed the optimum amount of COD for the algae to growth [53]. However, the least concentration also decreased the concertation of the algae by only a half of the highest concentration. High production of algae biomass were relatively found in the less than 2000 mg L^{-1} COD content (Table 2) which proves that the inhibition factor of COD in a very high concentration is significant for algae biomass.

Table 2. Chemical oxygen demand removal and biomass production using wastewater as growth medium.

Species	Wastewater	COD (mg L^{-1})	% removal	Biomass (g L^{-1})	pH	Temp (°C)	HRT (h)	Reactor	Ref
Scenedesmus sp.	Vinasse	27,100	36.2	4	7.0	25	30	Bioreactor	[54]
Chlorella vulgaris	Municipal wastewater	61.5	41	5.1[1]		25-26	216	PBR	[55]
Selenastrum gracile			53	4.3[1]					
Scenedesmus quadricauda			63	0.5[1]					
Indigenous algal population			45	1[1]					
S. obliquus	Piggery wastewater	3200	65.06	1.68	6.9	4	48	PBR	[53]
		2200	73.41	1.84					
		1600	75.29	2.18					
		1200	72.29	1.58					
		800	63.02	1.20					
		400	61.58	0.87					
C. vulgaris	synthetically-made municipal wastewater	490	96	2.20	8.8	25	48	PBR	[56]
C. vulgaris	Aquaculture wastewater	8.5	-	0.07	7.78	25	120	PBR	[57]
S. obliquus			-	0.06					
Chlorella kessleri	wastewater from the WWTP	70	-	2.70	7.5	25		PBR	[58]
Chlorella vulgaris	centrate from the WWTP			2.91					
Microspora willeana and other microalgae in less occurrence	Dairy manure	149	>99	4.98[2]	7-7.5	22	168	Pond	[59]
		79.7	>99	5.00[2]					
		128	>99	4.99[2]					
Chlorella sp.	Polluted water	1,200	51	-	-	-	168	Pond	[60]

[1] mg L^{-1} of chlorophyll a content. [2] g day^{-1} biomass generated from 1 m^3 pond.

As a part of COD, biological oxygen demand (BOD) reflects the biodegradable organic matters in the wastewater and often used as the parameters for biological wastewater treatment system. BOD can be removed drastically using microalgae culture as it was found by Henry et al that BOD was removed by *Chlorella spp.* as much as 92.8 % while the total COD was removed 59.5% in sewage effluent from sewage treatment plant [61]. Similarly, Usha et al applied pulp and paper mill effluent for cultivating *Scenedesmus* sp. and it resulted 75% and 82% removal of COD and BOD, respectively [62]. A study of synthetic wastewater by Das et al showed a relatively similar removal between BOD and COD (98.5% and 97.8%, respectively) [63]. It is important to note that in the study of Henry et al, BOD:COD ratio is lower (0.49) than in the study of Usha et al (0.98). Here, ratio of the BOD:COD are important to determine the total removal of the COD. However, ability of microalgae to remove COD does not always depend on the high ratio of BOD/COD. It is shown that even in 0.68 BOD/COD ratio, final concentration after treatment of BOD and COD were relatively identical [64]. Thus, it is important to consider both COD and BOD in the wastewater as one of the main parameters to be removed.

3.2. Nutrients

Wastewater containing nitrogen and phosphorous as the contaminants can trigger eutrophication in the natural water body and thus it is important for the removal system to eliminate those nutrients. The use of algae as the agent for nutrient removal in wastewater treatment has also been intensively studied over years [65]. Recently, coupling nutrient removal with biomass production obtain more attention for wastewater treatment development and mitigating energy crisis. Variety in nutrients concentration plays an important role since the difference of biomass yielded is often addressed by the slight different of nutrient concentration (Table 3).

Nitrogen is important in the forms of peptides, proteins, chlorophylls, energy transfer molecules (ADP, ATP), and nucleic acids (DNA, RNA) in cells where all the metabolism activities involve these molecules [66]. In nature, algae plays an important role for conversion of inorganic nutrient into organic form [49]. Nitrogen concentration in water body determines the algae growth since this nutrient is very important to synthesize biomass of algae. It was found that the 9.61×10^{-4} M of nitrogen in waterbody was the optimum concentration compared to both lower and higher concentrations [67]. Form of nitrogen also appears to be the determining factor for algae growth. It is found in *D. tertiolecta* that prefer nitrogen in the form of nitrate rather than ammonia [68]. Furthermore, for both nitrogen and phosphorous, the effect might be different in the limitation. Intake limitation of nitrogen in water can enhance the growth of algae in certain condition [69] while in phosphorous showed no significant impact on growth of the cultures [68].

Likewise, phosphate also plays an important role in metabolism of algae as it constructs many macromolecules in the cell of algae such as in lipids, proteins, and nucleic acid. In more detail, inorganic phosphate is the form that very important for algae growth and metabolism. [70] stated that phosphorus in the form of $H_2PO_4^-$ and HPO_4^{2-} are the most preferably form which algae prefer to use in the metabolisms. They also explained that these two forms of phosphorus are incorporated by phosphorylation to be organic compound. It is noteworthy that the cell energy in form of adenosine triphosphate and adenosine diphosphate contain phosphate group of which the dissimilation of each group generates energy to the cell. It is noteworthy that apart from the ATP and ADP energy source can come from oxidation of respiratory substrate and light in the photosynthesis case. These sources of energy, interestingly, are also used to transfer the phosphate from environment to the cell through plasma membrane. However, different from nitrogen, the phosphorus source of algae can be both organic and inorganic. Yet, some of the algae are likely to use organic phosphate in esters groups for growth substrate [71].

Concentration of TN varies from the source of the wastewater. High concentration of nitrogen can reduce optimum algae biomass as the inhibitory factor where insufficient concentration leads to reduction of algae productivity due to less nutrient for metabolism and cell generation [65]. However, high amount of nitrogen was acceptable for *Desmodesmus* sp. that produce 4 mg L^{-1} biomass in the wastewater with nitrogen up to 1,420 mg L^{-1} in batch reactor with 30 h HRT [54]. On the other hand, *Chlorella* sp. stil managed to produce 34.6 mg L^{-1} chlorophyll content in the occurrence of high phosphate concentration (392 g DW $m^{-2}day^{-1}$) [72]. Nonetheless, the removal of nutrient in both cases were less than 70 % in average which showed a lack of efficiency in the removal.

The trophic which manifests the carbon source utilization is important in the removal process. Mixotrophic algae usually obtain high yield with better nutrient removal efficiency [49] [73] [48]. Here, trophic condition plays an important role to determine the optimum removal. However, the price of effective carbon source is high and the cheap carbon source such as high COD wastewater tends to contain more nutrient which increases the total concentration to be removed. Thus, proper construction of system is crucial for this circumstance.

Combination of both COD and nutrients are important to support algae growth. However, it is rare to obtain all the parameters contained in the single wastewater source. Municipal wastewater usually contains moderate concentration of three parameters [3] [74] where it needs less COD and high amount of phosphorous and nitrogen. On the other hand, decrease of COD detected in the less nutrients. It is also found that high ration of Nitrogen and Phosphorous (N:P ratio) can reduce the possibility to obtain optimum concentration of both nutrients using dilution. For optimum uptake 5:1 until 12:1 of N:P ratio was described by [75]. Most of the municipal and industrial wastewaters have ratio between these values where the direct dilution using recycle water is possible to reach optimum concentration of both nitrogen and phosphorous [76]. Nevertheless, several wastewaters especially diluted digestate from the WWTP contain nitrogen in a very high concentration [74]. While nitrogen concentration shall be reduced, phosphorous concentration in wastewaters are mostly in the sufficient amount for supporting microalgae. Strong dilution is suggested in the condition of extremely high and concentrated substrates. Here, the dilution has two main advantages. Firstly, turbidity of wastewater can be reduced to increase the light penetration and secondly it helps reducing nitrogen content into optimum concentration for algae. In contrast, this situation can also induce phosphorus concentration to plunge and it becomes insufficient for biomass production.

Different strains respond differently to the change of nutrient content in the medium [74]. Thus, proper selection of algae strain from wild type can be the determining factors for optimum biomass generation [77]. Indigenous strains from wastewater ponds usually have been more subjected to the environmental stress and changes [78]. This kind of stains can ease the phase of acclimatization since the strains have been naturally adjusted to the wastewater condition. Another prospective path to generate synergistic combination between nutrient removal by the algae and biomass productivity is by utilizing genetically modified algae strains that the niche and characteristics are suitable with the treated wastewater [79]. Nevertheless, it is important to note that genetically modified algae have several drawbacks in lack of adjustment and adaptation in highly fluctuating concentration of nutrients in the wastewaters.

Table 3. Effect of nutrient in algae biomass yielded from wastewater.

Algae Species	**Wastewater**	**Total Nitrogen (mg L^{-1})**	**Total Phosphorous (mg L^{-1})**	**Biomass (g L^{-1})**	**pH**	**Temp (°C)**	**HRT (h)**	**Reactor**	**Ref**
Desmodesmuss sp.	Vinasse	1,420	2.61	4	7.0	25	30	Bioreactor	[54]
C. vulgaris *Selenastrum gracile* *S. quadricauda* Indigenous algal population	Municipal wastewater	32.2	3.90	5.1[1] 4.3[1] 0.5[1] 1[1]		25-26	216	PBR	[55]
S. obliquus	Piggery wastewater	120.69	129.22	241.67	6.43±0.09	25±1	12	PBR	[53]
C. vulgaris	synthetically-made municipal wastewater	50	10	2.2	8.8	25	48	PBR	[56]
C. vulgaris *S. obliquus*	Aquaculture wastewater	6.81	0.42	0.07 0.06	6.8-7.2	25±2	24	PBR	[57]
C. kessleri *C. vulgaris*	wastewater from the WWTP	140	5.76	2.70 2.91	7.5 7.5	25 25	240 240	PBR	[58]
Auxenochlorella protothecoides	Concentrated municipal wastewater	134	212	1.16	6.31	25±2	192	Bioreactor	[80]
Algae consortium	Domestic wastewater	50.0	50.0	0.43	7.23±0.29	16-23	96	Pond	[81]
Lygnbya sp. and *Spirogyra* sp.	Rivers	1.29 1.03 1.05	0.23 0.14 0.11	16.3[2] 3.6[2] 3.8[2]	-	5-30	144	Pond	[82]
Chlorella sp.	Centrate of primary and secondary effluents	275	392	34.6[2]	7.0~7.5	25	39.5	PBR	[72]
Chlorella sp.	Anaerobically digested manure	200	2.5	6.83[2]	8.5	18±2	720	Pond	[83]

[1] mg L^{-1} of chlorophyll a content. [2] g DW m^{-2}day^{-1}

3.3. Metals

Some metals are important parts of cell metabolism. Copper, iron, zinc and manganese are found as the cofactor of several enzymes in the chloroplast and mitochondria [84]. Excess occurrence of metals (especially heavy metals) may lead to harmful effects for the algae. Occurrence of metals in proper concentration induces the uptake through transporter protein in the membranes of algae [85]. Metals toxicity inside the cell is related to the production of the reactive oxygen species (ROS) in the intracellular compartment and its matrix. As a consequence, unbalanced cellular redox status may occur inside the cell along with the reduction of antioxidant concentration [86]. Monteiro [85] also proposed several toxic mechanisms that affect the structure of the microalgae. The first effect is replacement the substantial metals for the microalgae. This mechanism may lead to the disruption of some macromolecules inside the cells. The second effect is the interruption the pathway of important metabolism inside the cells. This mechanism may create insufficient metabolites availability as building blocks for the cells. The third effect is the removing or weakening the bonds between cell membrane and proteins. This mechanism can easily create the cell rupture. Lastly, reducing the photosyntate of autotrophic and mixotrophic microalgae can create lower biomass for mitotic activity and biomass generation.

To counteract the damage inside the cell, metals are stored or converted into less harmful state by changing the oxidation state to create possible condition for enzyme to convert the metals [85] (Fig. 2). Removal of metal especially heavy metals mostly relied on the immobilized microalgae and only some of the treatments occupied microalgae in the unattached and live biomass [87]. Copper (Cu), Zinc (Zn), Lead (Pb), Cadmium (Cd), and Manganese (Mn) are the most common metals to be treated by algae (Table 4). *Cladophora glomerata*, *Oedogonium westii*, *Vaucheria debaryana* and *Zygnema insigne* were also reported to remove several heavy metals in industrial wastewater such as cadmium, chromium, lead and nickel with considerable bioaccumulation of *Cladophora glomerata* (80.3%), *O. westii* (63.3 %), *V. debaryana* (92.1 %) and *Z. insigne* (93.0 %) [88]. The maximum removal of copper (85%) was reported by using batch system while in the sodium alginate algae beads algae beads the removal was up to 95.4% using *Spirulina* sp. [89]. The order of the removal is also different in different heavy metals [90]. Chromium has been reported to be among the most pertinent heavy metals to be removed and accumulated by the algae [91] [92]. These removals also included reductions of electrical conductivity, biological oxygen demand, chemical oxygen demand, total dissolved solids and nitrate of the wastewater with an increase in dissolved oxygen.

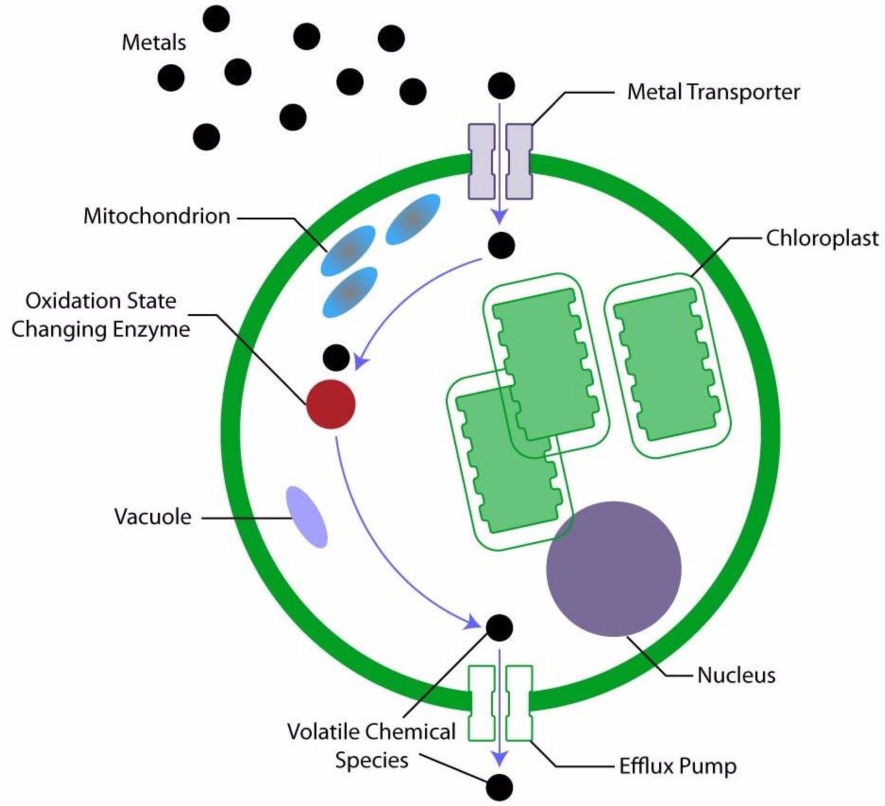

Fig. 2. Fate of metals in the algae cell.

Heavy metals in the water also cause various effects to the algae. Combinations of heavy metals were found to cause additive effects for *Chlorella sp.* while the other combinations occurred as synergistic effects for the alga by [93]. Copper and zinc were also reported to decrease the cell density in a culture of *Chlorella* sp. where copper decreased amount of the algae by decreasing the pigment of the alga and zinc eliminated the alga growth after 5-day incubation [94]. These effects vary between the algae and the contamination level of heavy metals and thus consideration of the production of biomass in the detrimental excess heavy metals shall be considered.

Heavy metals removal by biosorption process can be done by application of algae to effectively eradicate the pollutant through accumulation in biomass generation. However high dose of metals can cause an increase of ROS inside the alga cells and consequently it creates damage in cell structures. The mechanism of heavy metal removal in moderate dose is related to the high affinity and larger surface area of the algae. The mechanisms can vary between each alga since the surface, morphology, cell wall, membrane structure, and composition are different and specific for each alga [95]. Thus, it is important to choose proper algae strain for generating biomass and lipid with sufficient biosorption of metal. Generally, absorption, precipitation, oxidation, and reduction of metals in the water by microbial are the mechanisms that required in order to perform metals removal. By the all mechanisms, algae are more advantageous than other microbes in this application since algae requires less carbon source for metabolisms and for building biomass. As the biomass can be generated autotrophically, sufficient amount of biomass is generated in minimum organic carbon requirement and sufficient amount of biomass to remove metals from the wastewaters is achieved in modest solution [96]. Nevertheless, high concentration of metals can reduce algae biosorption since excess amount of metal occurrence can cause lethal effect to algae. Here, capacity of metal accumulation in cells in regards with initial dose is crucial to build successful system. Algae resistance of metal plays important role here to keep the growth state of the algae by generating biomass while the amount of metals that can be absorbed and further it increases along with the growth of the biomass. Apart from selection of natural strains in the wastewater containing high amount of metals, there is always an opportunity for genetically modified algae in this system. Metals removal mechanisms involves many proteins and genes that regulating metal absorption, detoxification, and tolerance mechanisms [97]. By expressing certain peptides for metals ion captures, enhancing capacity of removal as well as the resistance of metals are possible to reach [98].

Although lipid accumulation is also triggered under environmental stress, excess metals is potentially able to reduce the total lipid generated from the biomass. Excess intake of metals was reported to enhance oxidation of lipid [86]. In the final process of cultivation, even though high alage biomass can be generated, low lipid content to convert into biofuel is still great bottleneck of this system. A very high dose metals can also lead to the failure of the system since all the algae are potentially fail to grow for treating metals in the wastewater. Wastewater with moderate dose of metal is recommended to utilize in this system because the algae can still maintain the growth with sufficient removal of metal from the wastewater.

Table 4. Metals removal and accumulation by algae.

Species	Elements	Metal accumulation (mg g^{-1})	Initial concentration (ppm)	% removal	Ref
Chlorella spp.	Cu	15×10^{-3}–2.6	79–1,789	-	[99]
	Zn	1×10^{-3}–0.18	23-11,625	-	
	Pb	2×10^{-3}–0.02	10-6,887	-	
C. minutissima	Zn	9.17	2[1]	62.05 ± 0.04	[100]
	Mn	4.04	2[1]	83.68 ± 0.14	
	Cd	4.27	2[1]	74.34 ± 0.03	
	Cu	1.40	2[1]	83.60 ± 0.09	
Alginate-*Spirulina*[2]	Pb(II)	12.9	100	-	[101]
	Cd(II)	4.5	100	-	
	Cu(II)	4.1	100	-	
Chitosan-*Spirulina*[2]	Pb(II)	4.8	100	-	
	Cd(II)	3.6	100	-	
	Cu(II)	2.7	100	-	
Arthrospira	Ce	18.1	80–800	-	[102]
		38.2		-	
S. platensis	Pb	-	100	91	[103]

[1] concentration in mM. [2] immobilized state of algae.

4. Operational Parameters

4.1. Light

Utilization of light by the algae determines the biomass yield because the different time of light intensity can create variety of metabolisms by which biomass is synthesized in several pathways [104]. Algae cells respond the light based on its intensity. [3] described the dynamic of algae photosynthesis based on the intensity level. At the low light intensity, photosynthesis increases with the higher intensity. When the light intensity reaches the saturation level and exceeds the demands of photon in photosystem II in the chloroplast organelle, the photosynthesis rate does not increase in this stage. Higher amount of light is able to decrease the photosynthesis rate due to damage to the photosynthetic apparatus.

Light penetration plays a crucial effect to the cultivation. Culture of *C. sorokiniana* with high density has less gross productivity than that with less density [105]. Study of the light amount exposed into *C. vulgaris* cultivation pointed out that 360 μmol m^{-2} s^{-1} of photon could be the most suitable for the algae to growth where high and low photon decreased the biomass yield of the algae [106]. Conversely, [107] reported that the condition above 150 μmol m^{-2} s^{-1} was observed as the start of inhibition factor for algae to grow and 80 μmol m^{-2} s^{-1} of light was proper for *C. vulgaris* to reach optimum yield production. Nonetheless, the low photon concentration was compensated with rich molasses under mixotrophic condition. It is important to note that the algae in the heterotroph condition produced higher biomass and nutrient removal than the mixotropic or autotroph condition [108]. Also, the low light intensity was only suggested for mixotropic algae. For application in biofilm, [109] emphasized a slightly higher standard of light intensity by using 120 μmol m^{-2} s^{-1} as the optimum condition of algae growth.

Aside from the previously mentioned, strain selection is also an important factor for designing the proper light intensity for the algae. 420 μmol photons m^{-2} s^{-1} was reported to be the optimum light intensity for *C. protothecoides* in the normal system while relatively moderate light intensity of 100 μmol m^{-2} s^{-1} was reported to be the optimum condition for *A. platensis* [110]. It is also noteworthy that wastewater nutrient removal is also affected by the light intensity. Thus, selection of strain also needs to consider the light availability where the strain will be cultivated.

The purpose of wastewater treatment system also needs to be considered for light adjustment. Low light intensity can also enhance the removal of COD from the water. *C. kessleri* and *C. protothecoide* have been reported to have different optimum light intensity for TN, TP, and COD removal [111]. TN and TP are able to be removed effectively by those algae in the range of 30 -120 μmol photons m^{-2} s^{-1} where the COD is effectively removed in the absence of light. This condition is strongly related with the trophic condition of the cultivation system. Heterotroph algae growth was proven to produce higher biomass with the absence of light than that in the autotroph condition with sufficient amount of N [78]. Thus, light intensity setting needs to consider the wastewater contents as well.

4.2. Carbon Dioxide

Similar with the light intensity, carbon dioxide (CO_2) concentration is strongly affected by the cultivation condition. It is used in the photosynthesis as most of the algae are photoautotrophic [112]. The CO_2 sequestration capacity of microalgae is ten times higher than that from terrestrial plant [113] and thus, algae cultivation has been emerging as the promising negative CO_2 emission. Green microalgae are expected to be applied in the system where not only nutrient removal and biomass as biodiesel are obtained as the advantages but also the carbon sequestration is expected to be applicable to absorb the gas. Application of CO_2 in the algae cultivation can run not only carbon sequestration process but also enhance the other advantage such as metals bioremediation [114].

Design of the system is very important in this parameters. Different diffuser can cause different production of biomass as the result of the gas dispersion and air bubble size which related to the CO_2 dilution factors. The gas introduced into the system is usually affected by two factors, diffuser type and flow rate of the gas. Sintered stone, porous curtain, perforated ring, and porous wood diffusers was proven to generate different *Spirulina* biomass concentration with 0.05 vvm concentration of CO_2 [115]. Higher concentration of CO_2 introduced into the system could lower the efficiency of uptake rate of gas from the system to the biomass.

Application in the flue gas of industrial system has been reported in several study. *Spirulina* sp. was reported to achieve 24% reduction in flue gas CO_2 in autotroph condition [116]. In that study, approximately 35% growth rate increase was recorded when CO_2 supplied as the only carbon source in the artificial medium. Nevertheless, the low carbon accumulation was still the main bottleneck of this technology. In that particular study, the carbon fixation was only 7.5 %. It is important to note that to completely reduce high concentration CO_2 in the flue gas, further study in the efficiency shall be made since the feasibility of this technology for high demand treatment gas needs large volume of reactors.

Practical approach has been made by [117] who applied1 L min^{-1} (vvm) of CO_2 from flue gas in the industry into the tank of *Spirulina*. The usage efficiency of CO_2 has been found to be 75 %. Although the usage was low and it remained roughly 25% of the CO_2, the system has given a breakthrough that this scheme can be applied in the near future as the industrial carbon capture, utilization and storage. Another study with the same concentration of CO_2 was conducted by Uggetti, Sialve [118]. It has been found that 66-100 % increase of biomass was detected after additional CO_2 with the increase of COD and NH_4^+-N removal. Increase of this biomass along with injection of CO_2 indicates recognition of high tolerance of CO_2 in the system. It is noteworthy that excess CO_2 injection can increase the bicarbonate ion where it can lower physiological pH and eventually inhibit the growth of algae [113]. However, an increase of bicarbonate was still reported to increase the biomass production [119].

In contrary, an increase of CO_2 has also been made to enhance heavy metals bioremediation using mixed culture algae. The treatment of flue gas however still succeeded proper removal of heavy metals with additional CO_2 that reached 30 % [114]. Combining the aim of heavy metal removal with carbon sequestration possibly addressing the lack of removal in either treatment purpose. However, to achieve sufficient removal activity the improvement is a must for industrial application since the flow rate of flue gas is very high and with current technology it is difficult not to occupy large space for cultivation.

4.3. Reactor Designs

To achieve sufficient amount of algal biomass production and nutrients removal in wastewater, reactor as the place for cultivating the microalgae plays an important role to determine final products of this coupling method. The models and designs of photobioreactors that applied in the laboratory and pilot scales nowadays can be divided into two major groups which are open pond and closed photobioreactor [120]. Even though many development and variation of the designs, scales, and models, these two group define almost all of the current developed technologies in microalgae reactor using wastewater as a substrate.

The Open pond reactors is reflected as a pond in exposed area to the natural environment with sunlight as major source of light [121]. In the other hand, closed photobioreactors (PBRs) have many advantages where most of the factors can be controlled inside isolated environment [122]. These characteristics affect the consumption of nutrient, production of biomass, and effluent quality after cultivation. Application of wastewater in the algal culture has more room for application in both open pond reactor and closed photobioreactors. However, many considerations such as location and contamination are important regarding the proper application of each groups. Location can be very important cultivation process. In open pond system, location can determine the temperature, light intensity, additional agitation for wind drive while the PBRs are more flexible in terms of location [123-125] but several PBRs which utilize sunlight as the main source of light might be affected in the shading of location [123, 126]. Moreover, location in the feasibility of this coupling idea must be near by the source of wastewater to reduce the transfer cost. As most of the studies already proved that open pond can be very susceptible for contamination [127, 128], different approach is needed for wastewater as an influent medium. In contrast, PBRs system cause material exchange limited from the gasses injection and/or feeds water or medium which creates low risk of contamination yet the possibility to be contaminated still occur [129].

Application of wastewater in PBRs is relatively common to study the microalgae cultivation in wastewater in order to remove the nutrients and yield algal biomass (Table 2 and 3). Yet, further development from the data obtained in PBRs takes place in open pond [62]. In the open pond, location and contamination affect the holistic process. However, application of open pond as the model for microalgae cultivation is more feasible due to its lower operational and capital costs [120]. Thus, economical factors shall be put as one of the main consideration for the reactor design since one of the obstacle to put this coupling concept into full industrial scale production is the profitability [130].

5. Constrain between High Biomass Productivity and Removal Efficiency

Hundreds of algae species have been tested to grow in many substrates for various purposes. The studies of several wastewaters were ranging from dairy manure [14], municipal wastewater [22], chicken [131], piggery and brewery wastewater [132]. The usage of the wastewater was preferably easier than that using other nutrients sources. However, some of the studies tried to analyze and modify the condition in order to obtain more lipid content and biomass such as varying the salinity [23], nutrient starvation [23, 133] as well as the occurrence of metal as a trace elements in the environments [23, 26, 133, 134]. These particular factors are important to algae for growth. It might show the increase of growth rate if the light is properly penetrating the water as [133] found that the light was significantly affecting the rate of biomass in the end of the cultivation period. This fundamental finding is crucial since there are numerous that stated the removal of nutrient is as important as the growth of algae productivity.

The application of waste or wastewater to cultivate *Spirulina* is not a breakthrough study. A considerably high amount of biomass (1.47 g/L) was achieved under optimum condition by using cow effluent [135]. High removal efficiency (>90%) of TP and COD by *Spirulina* was also reported in the saline environment but with low biomass production 0.76 g L^{-1} [136]. A similar study using synthetic municipal wastewater, showed the TN and TP removal of 92.58% and 94.13%, respectively with biomass production up to 262.50 mg/L [137]. A shortcoming of this finding was probably simply the separation of the *Spirulina* which difficult considering the small size of this algae. However, it has many advantages such as nutrient recovery and wastewater treatment alternative. The result of high biomass accumulation can also be the provider for cheap and supplementary food for aquaculture animals [35].

The lack of nutrient removal efficiency can mean there must be another treatment process following the algae cultivation and the objective to obtain nutrient removal activity from the system is considerably unsuccessful. However, removals of ammonia, total nitrogen, total phosphorus, and COD that achieved were still lower than the system specified only for removal of these particular contaminants [14]. Thus, in high concentration of the contaminant in wastewater, high dilution factor where the algae can grow rapidly with higher efficiency of removal is preferable solution. Furthermore, the notable usage of organic and inorganic carbon was important since it indicated the ability for this alga to use organic carbon beside CO_2 as carbon source for synthesizing the biomass [138].

Consecutive treatment between primary treatment system as demonstrated in the process combining anaerobic digestion and algae cultivation is promising for removal of nutrient and algae cultivation [14]. Nevertheless, the following process after primary process is only expected to remove excess nutrient that cannot be removed in the prior treatment. Therefore, the occurrence of high COD is assumed to be lower than the initial concentration of influent of most of the primary wastewater treatment system.

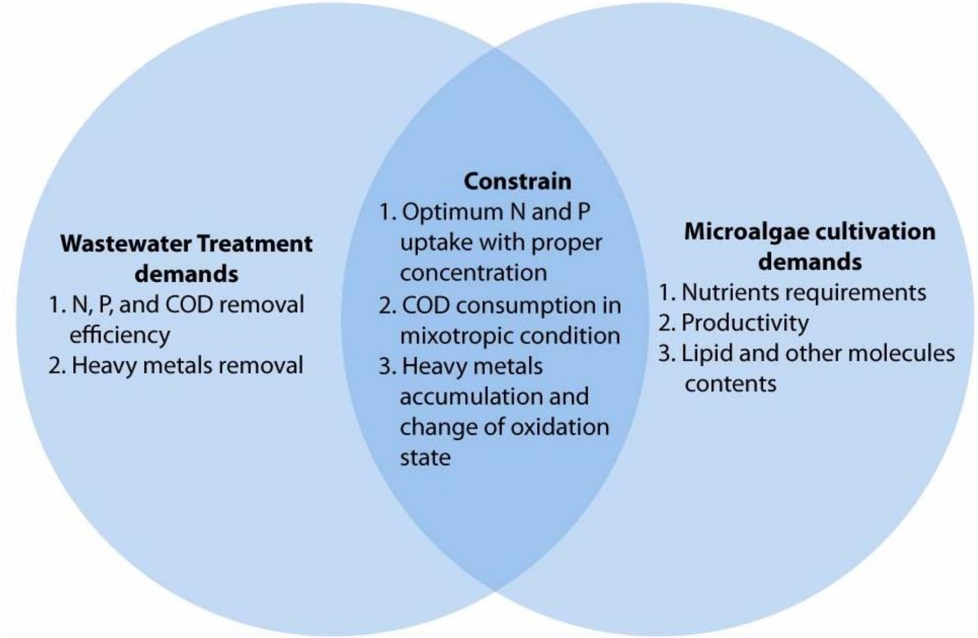

Fig. 3. Constrain between wastewater treatment demands and microalgae cultivation demands.

Cultivating algae in wastewater contaminated with heavy metals or other toxic substances cannot be adapted for generating feed for animals or food source. In terms of re-utilization of the alga, there must be such simplification and pre-treatment regarding the water that is utilized for growth medium. Monosodium glutamate wastewater was proposed to be able to apply as a medium for *Spirulina* sp. using concentration of 25% CW for protein production and 50% for lipid and carbohydrate production since the wastewater was claimed as the wastewater with high amount of nutrient and organic compound yet the contaminants are not poisonous nor dangerous [139].

Light period and intensity also obtain great part of installation for algae cultivation. It is also important to pay attention in the combined system of wastewater treatment and biomass production. Several reactors are usually relying on artificial light which continuously or periodically produced light for the algae. Standing in contrast, algae which cultivated outdoor usually depends on the sunlight as the photon source. In tropical areas, the photoperiodic cycle is usually stable through the year. However, in certain places such as in Europe the light phase in the day time can change in the spring/fall to the midsummer. Since this phenomenon can cause bias in the system and unstable production of biomass, geographical factor is important for sunlight dependent algae cultivation system while in the bioreactor with light feeding system, capital and operational cost are still the bottlenecks. Nonetheless, the acclimatization process of algae is able to address the light factors drawback. The ability for modulation of light harvesting capacity in order to optimizing light suggests the production of algae can be managed in low light conditions [3]. However, growth rate and productivity of algae are strictly related to light factor and alteration or limitation of light intensity definitely affects the algae yield.

Apart from water treatment to remove undesired substances point of view, the production of high biomass yielded from the system shall be focused. Taking *Chlorella* sp. as the most cultivated microalgae for biodiesel, the percentage of the lipid content was relatively wide which around 13.2-60 % of the total biomass (Table 1). The utilization of this alga should be considered. This range of percentage is necessary to be noticed for further decision of algae cultivation. It is important to note that wide range of this percentage can create gap in the production system and capital cost calculation for the system to be built. To each high biomass of this alga, it is also necessary to create the condition based on the wastewater characteristics. Optimum biomass concentration up to 11 g L^{-1} is still possible to reach by using wastewater alongside with effective removal of undesired substances [17]. To obtain such concentration, proper water recovery, sufficient but not excess concentration of N and P solely for building biomass and other metabolism activities, mixotrophic condition for algae to digest organic carbon, and heavy metals defense mechanisms are expected to address both importance (Fig. 3).

6. Conclusion and Further Possibilities

Green microalgae have emerged the importance of its application not only for wastewater remediation but also for biomass orientation. Many aspects need to be reconsider for the algae until it can be adopted in practical application. To meet the demand of single stage treatment using algae cultivation, the alteration in removal efficiency in organic matters and nutrients are important to achieve at the beginning. As for the biomass yield, several species with certain systems applied were able to produce high biomass productivity, so that it can ease the selection and decision for proper species in specific wastewater. However, organic matters, nutrient and contaminants such as metals of wastewater still able to interfere the biomass production when the amount exceeds the limits of algae. Furthermore, factors such light and inorganic carbon source are potential to interfere the optimum condition. Design of the reactors where the algae cultivates is also important to be suitable to reach the goal of removal contaminants as well as yielding microalgal biomass. Eventually, intersection between wastewater treatment importance and biomass generation for biofuel shall be pictured carefully to place direction of development in the future.

The application of the wastewater as the source of microalgae nutrient and the cultivation as wastewater treatments are the ideal coupling idea for addressing energy demand and environmental pollution problems. Nevertheless, application is still limited to the pilot scale and other large scale experimental reactors. Apart from the composition of wastewater, suitable strains and cultivation conditions, to meet reasonable capital and operational costs, this concept is still limited to be performed. Here, challenge of full scale application in industrial level is facing several problems regarding the profitability such as land requirement, cost of harvesting and post-harvest processes, as well as limited capacity in large and high removal demand of metropolitan city. Here, integrated studies regarding this idea with combination of improvement in each aspect are important. In the future, to approach the industrialization goals of this idea, constrains such as demands of suitable microorganism and wastewater condition still need to be improved. Valuable product from the coupling requires to be emphasized by obtaining suitable steps for post-cultivation process.

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Mohamad Padri, photograph and biography not available at the time of publication.

Nittaya Boontian, photograph and biography not available at the time of publication.

Chatlada Piasai, photograph and biography not available at the time of publication.

Thunchanok Phorndon, photograph and biography not available at the time of publication.