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Biotechnology Carbon Capture and Storage by Microalgae to Enhance CO$_2$ Removal Efficiency in Closed-System Photobioreactor

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Additional information is available at the end of the chapter

Abstract

The global warming issue caused mainly by carbon dioxide (CO$_2$) has triggered various efforts to reduce excess amount of CO$_2$ emitted into the atmosphere. A viable option is to utilize biomass production potential of microalgal consortium in aquatic ecosystem that constantly requires CO$_2$ to perform photosynthesis. This study aims to provide scientific contributions to the development of environmental studies, particularly of using microalgal consortiums as carbon capture and storage (CCS) agent by engineering their culture conditions. A number of studies analyzing biological reduction of atmospheric CO$_2$ by using CO$_2$ absorption capability of terrestrial plants have been facing many difficulties. Compared to various terrestrial plants, microalgae are generally considered photosynthetically more efficient. Exploitation of microalgal capability has numerous advantages, including their faster regeneration time, ability to grow in less space than terrestrial plants, and because the cultivation of microalgae can be done on a small-scale or large-scale operation, under controlled conditions, and is independent to climatic changes. Taking into account long-term advantages, this study is a preliminary study which is expected to be able to provide information and feedback regarding integrated microalgal culture system that may lead to alternative solutions of eco-friendly and sustainable environmental management technology that are capable of mitigating environmental problems caused by CO$_2$ (as greenhouse gas) emissions. Hence, the results of this research could be implemented by building urban microalgal ponds in efforts to develop sustainable cities in terms of CO$_2$ emission reduction in urban areas.

Keywords: Microalgae, CO$_2$ removal, carbon fixation, biomitigation, carbon capture and storage, photobioreactor

1. Introduction

1.1. Carbon dioxide and global warming

Carbon dioxide (CO_2) is the most significant greenhouse gas that contributes to global warming. CO_2 emissions and other combustion products such as NO_x, SO_x, CH_4, and poly aromatic hydrocarbon (PAH) released from industrial, transportation, and residential activities have become environmental problems associated to uncertainty in annual climate prediction. Carbon dioxide is usually emitted freely from industrial processes in an uncontrolled way. In gas-controlled combustion units, CO_2 is a desirable by-product since it provides good indication of a complete combustion [1–3]. At an average concentration of 330 ppm in the atmosphere, CO_2 is harmless to humans because it is colorless, it is odorless, and it will not cause any chemical reactions in human body. At the same time, plants and microalgae will grow better in a CO_2-rich environment [4]. However, recently CO_2 concentration in the troposphere is getting serious attention as CO_2 is categorized as greenhouse gas that is believed to be the cause of global warming effects. Impacts of greenhouse gases are becoming more apparent mainly due to the increase of the earth's surface temperature [5].

CO_2 generated by the combustion of fossil fuels, as in the flue gas from power plants and exhaust gas from cement and steel manufacturing processes, can be captured and sequestered. Currently, the vast majority of large emission sources have CO_2 concentrations of less than 15%, although in some cases, substantially less [6]. However, a small portion (less than 2%) of the fossil fuel-based industrial sources has CO_2 concentrations of up to 95%. These high-concentration emission sources are potential candidates for implementation of CCS concept. However, some estimates predict the costs of non-biological CCS technology deployment to be economically attractive only after the year 2030, making implementation at a large scale unlikely in the near term [7].

1.2. Anthropogenic contribution

The major anthropogenic sources of CO_2 emissions over the last 20 years have resulted from fossil fuel burning, changes in land use, primarily deforestation [8–10] and other industrial processes like oil refineries; cement, lime, and steel production; and coal-fired power plants (13–15% of CO_2 concentration by volume) and natural gas power plant (8–10%). Globally, CO_2 emissions from fossil-fuel use in the year 2000 totaled about 23.5 Gt CO_2/year. Of this, close to 60% was attributed to stationary emission sources. However, not all of these sources are amenable to CO_2 capture and process [11].

Human activities are greatly increasing the atmospheric concentrations of carbon dioxide. The rate of increase in atmospheric CO_2 is reaching approximately 3 billion tons every year [12], mainly due to fossil fuel combustion and deforestation [8–10]. Various reports have mentioned that the atmospheric CO_2 concentrations have increased from around 280 ppm to 368 ppm over the past 200 years [13], contributing up to 50% to the global temperature increase known as greenhouse effects. The global warming causes sea level to rise and various climate anomalies linked to global warming, including floods and droughts [5, 14–15]. Considering

these high impacts, it is necessary to perform strategic activities which aim at reducing atmospheric CO$_2$ concentrations. A researcher [16] estimated that anthropogenic contribution to the carbon cycle in the form of CO$_2$ released into the atmosphere is approximately 9 Gigatons (Gt) per year. Approximately 7.6 Gt of this is from fossil fuels and 1.4 Gt from land-use change. As much as 55% of this carbon is absorbed by natural processes, and the rest up to 4 Gt are deposited in the atmosphere every year.

1.3. History of carbon dioxide biomitigation

Faced with issues of climate change, various efforts have been made by countries to find an appropriate solution. Despite the variety of strategies to reduce CO$_2$ emissions has been explored, so far there has been no single mitigation technology that can provide the ideal solution. Biological CCS technologies can be used to mitigate carbon emissions that would otherwise be released to the atmosphere. CCS technology incorporates three stages, the first of which is to collect CO$_2$ from specific emission sources such as industrial related sources and power plants by using certain techniques, the second stage is to transport the CO$_2$ to a suitable storage or processing location, and the third stage is to process the CO$_2$ and store it away to prevent it from being released into the atmosphere for a long period of time [6, 8].

Figure 1 illustrates the process by which industrial and transport CO$_2$ emissions in the air are absorbed by microalgae, thus allowing photosynthesis process to occur. In microalgal cells, CO$_2$ molecules enter the Calvin-Benson cycle to form sugars. The mechanism in which CO$_2$ is bonded to the enzyme ribulose-1,5-bisphosphate (RuBP) or ribulose-1,5-bisphosphate carbox-ylase oxygenase (RuBisCO) is called carbon fixation. Depending on the use of the accumulated biomass, products derived from this process are at best carbon neutral.
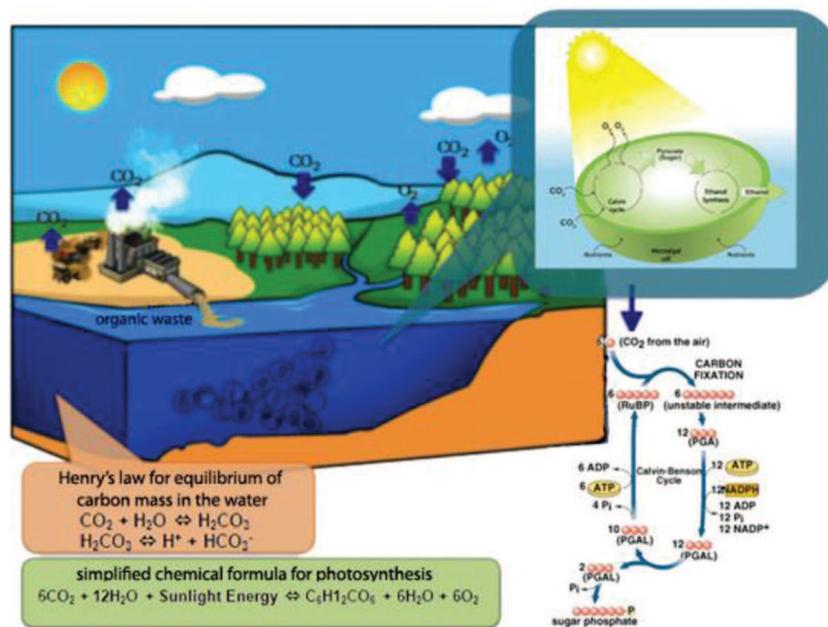
Figure 1. Illustration of atmospheric CO$_2$ absorption by microalgae in photosynthetic process.

Since microalgae are producers, they have the ability to continuously undertake photosynthesis. One of the primary requirements for photosynthesis is atmospheric CO_2. Microalgae are generally characterized by their relatively high photosynthetic efficiencies compared to various terrestrial plants and have been proposed as an alternative way of reducing carbon dioxide emissions into the atmosphere [15]. As photosynthetic organisms, microalgae are well adapted to capturing ambient CO_2. Growing algae to capture ambient CO_2 will remove carbon dioxide and sequester it in the form of biomass [17]. Thus, microalgae can be exploited as biological CCS agent.

Studies of microalgae as CO_2 catcher and absorber have been conducted in various countries around the world, particularly in terms of adaptation and selection of species that indicates tolerance of high CO_2 concentration and high CO_2 absorption rate. As a photosynthetic organism, microalgae are well adapted to capture ambient CO_2, although the limited CO_2 content is considered inefficient. High CO_2 concentration can hamper photosynthesis process and slow the growth of microalgae and take into account exhaust gas from combustion contains 5–15% CO_2. **Table 1** presents several types of microalgae that play a role in CO_2 bio-mitigation research in a laboratory scale.

Microalgae	Temperature (°C)	Max CO_2 concentration (%, v/v) that removed	P_{volume} (gL^{-1}day^{-1})	Carbon utilization efficiency (%)	Reference
Chlorella sp.	26	Air	0.682	-	[18]
Chlorella sp.	26	2	1.445	58	[18]
Chlorella sp.	26	5	0.899	27	[18]
Chlorella sp.	26	10	0.106	20	[18]
Chlorella sp.	26	15	0.099	16	[18]
Chlorella kessleri	30	18	0.087	-	[19]
Scenedesmus sp.	25	10	0.218	-	[20]
Chlorella vulgaris	25	10	0.105	-	[20]
Botryococcus braunii	25	10	0.027	-	[20]
Scenedesmus sp.	25	Flue gas	0.203	-	[20]
Botryococcus braunii	25	Flue gas	0.077	-	[20]
Chlorella vulgaris	25	Air	0.040	-	[21]
Chlorella vulgaris	25	Air	0.024	-	[21]
Haemotococcus pluvialis	20	16–34	0.076	-	[22]
Scenedesmus obliquus	-	Air	0.009	-	[23]
Scenedesmus obliquus	-	Air	0.016	-	[23]
Chlorella vulgaris	27	15	-	-	[24]
Scenedesmus obliquus	30	18	0.14	-	[19]
Spirulina sp.	30	12	0.22	-	[19]

NA = not available data.

Table 1 Some species of microalgae that play a role in CO_2 bio-mitigation.

There are many fundamental questions that still need to be answered regarding microalgal growth at elevated CO_2 concentrations. The most critical determination is the maximum amount of CO_2 sequestered from a given concentration of input gas. There was, however, a debate as to the actual amount of CO_2 that can be removed from the input stream. Data presented by [19] suggested that less than 5% of the CO_2 can be removed from a stream containing >1% CO_2 if the cells are only at a low density. However, [25] suggested that as much as 70% uptake from a 2% CO_2 stream could be captured in cyanobacteria. The pH of the media and the tolerance of the organism to high CO_2 concentration will play important roles in the amount taken up. Further results indicate that the maximum amount of CO_2 sequestered from a given concentration of input gas is all of it, while [26] reported that the determination of this limit depends on many factors including the design of the photobioreactor, bubble diameter, and cell density in the culture. The wide range of values necessitates further research into this key component of carbon capture. At both ambient and elevated CO_2 concentrations, there are important issues to consider when growing algae for the purpose of CO_2 capture and high productivity.

2. Biotechnology of Carbon Capture and Storage

CCS technology can be used to mitigate carbon emissions that would otherwise be released to the atmosphere. According to [27], the definition of CCS is as follows: a process consisting of the separation of CO_2 from industrial and energy-related sources, transport to a storage location, and long-term isolation from the atmosphere. Capture of CO_2 can be applied to large point sources. The CO_2 would then be compressed and transported for storage in geological formations, in the ocean, and in mineral carbonates, or for use in industrial processes. According to [6, 8, 27], CCS is a process consisting of the separation of CO_2 from industrial and energy-related sources, transport to a storage location, and long-term isolation from the atmosphere. Capture of CO_2 can be applied to large point sources. The CO_2 would then be compressed and transported for storage in geological formations, in the ocean, and in mineral carbonates, or for use in industrial processes.

Biological systems could potentially make a significant contribution to CCS technology, as they can be deployed in a sustainable and renewable manner [28]. Photosynthetic microbes are an attractive option as agents of biological CCS technology because they have the ability to capture sunlight and use that energy to store carbon in forms useful to humans, such as fuels, food additives, and medicines [4, 29]. Microalgae could be used as a biological capturing agent in CCS technology. However, there are some important limitations that need to be overcome. Transporting CO_2 from a source of carbon dioxide, such as power plant flue gas, into algal cultures to increase CO_2 capture efficiency and productivity could be a challenge [30, 31]. In this study, **the second stage** in the CCS concept is to transport the CO_2 for the purpose of microalgae photosynthesis, while **the third stage** is to store the CO_2 in microalgal biomass in forms useful to humans such as fuels, food additives, and medicines [32].

Efficiently capturing carbon dioxide from an elevated CO_2 source depends on many factors, but one of the most limiting factors at present is the ability of the microalgae to capture and

fix carbon at a proper concentration to avoid acidification of the medium and crash of the culture, relatively high temperatures of CO_2 exhaust gas, and the presence of NO_x and SO_x co-products [31], all of which could inhibit the growth of microalgae. Therefore, selecting microalgal strains tolerant to high CO_2 concentrations, and at the same time able to efficiently mitigate large amounts of CO_2, is essential.

2.1. Biology of microalgae as photosynthetic organisms and CO_2 absorbers

Microalgae are microorganisms that vary greatly in size, ranging from a few micrometers to several meters in length. Many of microalgal strains are single celled (unicellular) whose shapes may be spherical, rod shaped, spiral, and other shapes. Other microalgae exist as aggregates of single identical cells held together after cell division to create larger formation, while other microalgae may include various types of cells that perform specific functions or multicellular cells with enormous size and complex morphology [33].

Physiologically, microalgae are aerobic photosynthetic organisms, thus they all contain chlorophyll and other photosynthetic pigments. Microalgae are most commonly found in aquatic environments, mostly as phytoplankton that serve as food source for other organisms and are the primary producers of organic matters or an important source of oxygen and form the base of the aquatic food chain. As photosynthetic organisms, microalgae are producers of organic carbon compounds, analogous to those produced by terrestrial plants.

Researchers [34] explained that microalgae have a membrane-bound nucleus just like other eukaryotic organisms. Besides eukaryotic cells organelles, microalgal cells also contain starch granules, oil droplets, and vacuoles that are arranged in groups. Each microalgal cell contains one or more chloroplasts that may be ribbon- or disc-shaped like those found in plants. Within chloroplast matrix are found flat vesicles called thylakoids. The membrane of the thylakoid contains chlorophyll and other supplementary pigments as the photochemical reaction sites of photosynthesis. Just like chlorophyll-bearing protozoa, several microalgae possess flagella or cilia, thus they resemble protozoa. Furthermore, [34] also explained that microalgae possess three types of photosynthetic pigments, that is: chlorophylls, carotenoids, and phycobilins that are present in chloroplast. There are five types of chlorophyll called chlorophyll a, b, c, d, and e, which are all green in color. All microalgae contain chlorophyll a. A researcher [35] described that carotenoids are water-insoluble hydrocarbons, which are of two types, that is, carotenes and xanthophylls. Phycobilins or biliproteins are water-soluble protein complexes, which are classified into two types, that is, phycocyanin and phycoerythrin. The brownish color of microalgae results from the dominance of the carotenes and the xanthophylls, whereas the reddish color of microalgae results from the high content of phycobilins.

2.2. Carbon dioxide fixation in photosynthetic process

As stated previously, the second stage in the CCS concept means to transport the CO_2 to a suitable storage or processing location for the sake of microalgal photosynthesis, while the third stage means to store it, as microalgal biomass, in forms useful to humans such as fuels, food additives, and medicines. The occurrence of photosynthesis requires the presence of not

only chlorophyll but also CO$_2$ and water (which contains nutrients). The chemical reactions involved in photosynthesis are as follows:

$$(H_2O + NADP^+ \text{ light} \rightarrow NADPH + H^+ + O_2)\ 12x$$

$$(CO_2 + ATP + NADPH \rightarrow CH_2O + NADP^+ + ADP + P)\ 6x$$

$$H_2O + CO_2 \rightarrow C_6H_{12}O_6 + H_2O + O_2$$
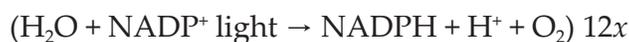
Research studies that utilize the potential of microalgae as CO$_2$ absorber have been carried out in various countries, particularly in efforts toward adaptation and selection of microalgae species tolerant to high CO$_2$ concentrations and high CO$_2$ absorption rate. Researchers [33] and [19] have reported that the microalgal species *Chlorococcum littorale* could grow under CO$_2$ concentrations of over 20 *Chlorella* sp. were able to grow up even in aeration containing CO$_2$ up to 40%, at culture pH as low as 4 [36].

The saturation point of atmospheric CO$_2$ concentration for the growth of microalgal culture varied from 2% to 5% v/v, which means that above this saturation point, the microalgal cells do not have the ability to absorb additional CO$_2$ from the atmosphere, hence the dissolved CO$_2$ is abundant and the equilibrium of carbon ions will not shift toward the carbonate ions. As a result, at high CO$_2$ concentrations, the pH of the culture was relatively stable [37]. During photosynthesis, free CO$_2$ is the main inorganic carbon source to use in microalgal cultures. According to [38], absorption of dissolved CO$_2$ from the water due to photosynthesis will lower the concentration of dissolved CO$_2$. This reduction will increase the pH since the dissolved CO$_2$ exists in chemical equilibrium with bicarbonate ions (HCO$_3^-$) and carbonate ions (CO$_3^{2-}$) in the water. Therefore, the rate of photosynthesis may be limited by a reduction in the amount of carbon, in this case carbon dioxide, changes in the forms of carbon in the water, and pH value. CO$_2$ uptake rates of microalgal cells could be stimulated by increase in CO$_2$ concentration in the media, and therefore culture without additional CO$_2$ would exhibit low CO$_2$ absorption rates, and although the pH of the culture seemed to increase, the pH never exceeded 8. In our previous study, [39] reported that the media pH varied from 7 to 8 which is considered sufficient for algal culture at laboratory scale.

Atmospheric CO$_2$ concentration also influences biomass concentration of microalgal culture in a hyperbolic curve response pattern. The accumulation of high biomass over a short time period is desirable and may be essential for making algal culture a viable option for contributing to the energy supply. The highest biomass concentration was achieved at CO$_2$ concentration of 10% v/v, which was equal to 2.05 g/l in *Chlorella vulgaris* culture and 2.95 g/l in *Ankistrodesmus convolutus* culture. These biomass concentrations correspond to absorptive capacity of 1.91 g CO$_2$/l/day in *C. vulgaris* culture and 3.41 g CO$_2$/l/day in *A. convolutus* culture, respectively [40]. An observation using microscope has demonstrated that *Chlorella* cultures grown under high CO$_2$ concentrations tend to have bigger cell size. Thus, although they had lower cell density when grown under CO$_2$ concentration of 10% v/v, they produced higher biomass weight. This can be understood as microalgal cells grown under adequate carbon

supply would have a chance to develop better than those grown under limited carbon supply. Therefore, in terms of biomass productivity, the most optimum CO_2 concentration for microalgal culture was found to be 10% v/v [41]. As a result, CO_2 as the primary carbon source for photosynthesis process in microalgae is adequately available, resulting in fast metabolism process and higher cell densities.

Carbon fixation competes with photorespiration because CO_2 and O_2 are both substrates for Rubisco [42, 43]. The oxygenase activity is not desirable as it leads to lose in carbon fixation. Analysis of the natural genetic variation in the kinetic properties of Rubisco from divergent photosynthetic organisms reveals that forms with higher specificity factors have lower maximum catalytic rates of carboxylation per active site, and vice versa (**Figure 2**). This inverse relationship implies that higher specificity factors would increase light-limited photosynthesis, while the associated decrease in catalytic rate would lower the light-saturated rate of photosynthesis. The CO_2 uptake by a crop canopy is determined by a dynamic combination of light-limited and light-saturated photosynthesis. Canopy simulations reveal that 10% more carbon could be assimilated by C3 crops if they were operating with a C4 Rubisco and this advantage would grow as atmospheric CO_2 levels continue to increase [43].
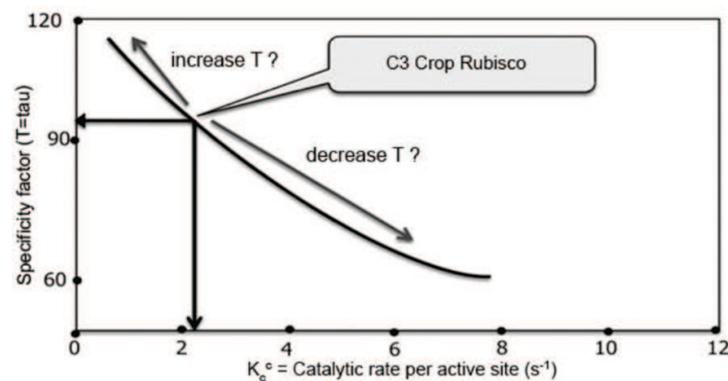
Figure 2. Relationship between Rubisco specifity and catalytic rate per active site [42].

2.3. Calvin-Benson cycle

Transformation of energy from one form to another through certain metabolic pathways corresponds to cellular functions. Enzymes are required for these transformation processes. The main energy production pathway is called cellular respiration, which can be aerobic or anaerobic. Photosynthesis is the major metabolic pathway in which energy is required under aerobic conditions.

Anaerobic pathway or fermentation pathway is a metabolic pathway that occurs in the absence of oxygen. Glycolysis is the first stage of anaerobic pathways. During glycolysis, glucose is broken down into two pyruvate molecules, yielding a net gain of two nicotinamide adenine dinucleotides (NADH) and two adenosine triphosphates (ATPs) [45]. However, the anaerobic reactions do not break down glucose completely to carbon dioxide and water, and the anaerobic pathways produce no more ATP beyond the yield from glycolysis. The final steps

serve only to generate NAD^+, a coenzyme that is essential for the anaerobic pathway's process [29].

The process of photosynthesis is divided into two parts, the first of which requires the presence of light, also called the "light reactions," that is, the transformation of solar energy that is captured by color pigments (chlorophyll, phycocyanin) into chemical energy in the form of ATP and NADH by releasing oxygen as a byproduct. These reactions take place in an inner membrane system that is called the thylakoid membrane system, and occur in three phases as follows:

1. Pigments absorb sunlight energy and give up electrons.

2. Electron and hydrogen transfers lead to ATP and NADPH formation.

3. The pigments that gave up electrons in the first place get electron replacements.

According to [46], systems that capture solar energy to produce energy molecules (including ATP) are called photosystems. In thylakoid membranes, there are two types of photosystems that excite electrons by two different electron transport systems as follows:

1. Photosystem I: The cyclic pathway of ATP formation.

2. Photosystem II: The non-cyclic pathway of ATP formation. In photosystem II photolysis process occurs, which is a series of reactions that dissociate water molecules into oxygen ions, hydrogen ions, and electrons. Electrons from photosystem II are passed to photosystem I.
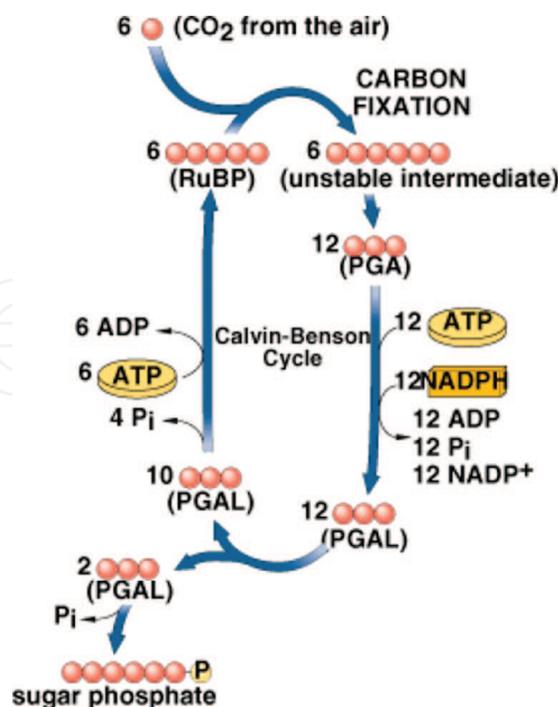

Figure 3. Calvin-Benson cycle [47].

During the first stage of photosynthesis, that is, the "light reactions," sugars have not yet been produced. Sugars are produced during the second stage [43].

The second stage, or the light-independent stage, as it can take place in the absence of light, provided there is sufficient ATP and NADPH to synthesize organic molecules from CO_2 and H_2O, is illustrated in **Figure 3**. The first step is the incorporation of CO_2 molecules into RuBP, catalyzed by Rubisco enzyme, commonly known as carbon fixation, followed by the next step, that is, entering the Calvin cycle, often referred to as the Calvin-Benson cycle, with the end product being organic groups, such as sugars [47, 48, 98].

Much research needs to be carried out to discuss the theoretical limits of photosynthetic efficiency in an effort to determine what can be done to reach these goals. Researcher [33] described that photosynthetic efficiency is the fraction of total solar radiation that is converted into chemical energy during photosynthesis, expressed as the following equation: $2H_2O + CO_2 + energy \rightarrow CH_2O + H_2O + O_2$

In oxygenic photosynthetic organisms, CO_2 is fixed in the Calvin cycle by Rubisco to increase the efficiency [49]. Substantial losses to photosynthetic efficiency lie between initial transfer reactions of photosynthesis and carbohydrate biosynthesis. Depending on the mechanism utilized to fix carbon and the amount of ATP and NADPH utilized, and assuming total incident radiation including infra-red, the maximal theoretical efficiency at this stage (including light capture and energy transduction) is between 8% and 13% before photorespiration and respiration [42].

Therefore, photosynthetic efficiency is affected by several factors, that is, light intensity, partial pressure of oxygen and CO_2 [33], mass transfer of CO_2 into liquid, temperature, and availability of nutrients [42]. Additionally, the amount of RuBisCO represents an intrinsic limit which determines the rate of carbon fixation (Bar-Even et al., 2010). However, in some cases, photosynthetic efficiency will be different in aquatic versus terrestrial species [13].

2.4. Carbon concentrating mechanisms

Carbon concentrating mechanism (CCM) acts as an enhancer to a higher microalgae growth, and therefore can be used to improve productivity in a photobioreactor [50]. The anhydrase carbonic enzyme expression is related with CCM induction. Carbonic anhydrase (CA) enzyme catalyzes CO_2 and HCO_3^- interconversion. It is a major component in intracellular mobilization from pool HCO_3^- *pool*, by catalyzing CO_2 production for Rubisco enzyme [51]. The role of CCM is mainly to increase CO_2 concentration for Rubisco, which is the enzyme responsible for CO_2 initial fixation.

Photosynthetic microorganisms in water as eukaryote microalgae, cyanobacteria, and photosynthetic bacteria have an ability to utilize CO_2, facilitated by ribulose-1,5 bisphosphate carboxylase/oxygenate enzyme (Rubisco). This Rubisco enzyme serves to capture Ci in the form of CO_2 in darkness (light-independent) through photosynthesis reaction (Calvin cycle) [38, 98]. When pure CO_2 is dissolved, pH drops to below 7, therefore creating acidic condition.

The dominant Ci species in acids are CO_2 and HCO_3^- [52]. Microalgae have developed various ways to ensure that Rubsico enzyme will accompany CO_2 in a certain CCM through a movement of such inorganic carbon across plasma membrane [30].

For microalgae, carbon dioxide is an important limiting factor that will affect growth and metabolism. An active, continuous supply of carbon dioxide by Rubisco in chloroplast is a requirement during photosynthesis. The carbon dioxide will enter through a CO_2 gas diffusion within medium or through a carbonate conversion. Rubisco only reacts with CO_2, not with bicarbonate or carbonic ion. Therefore, another enzyme, CA, is needed to convert carbonic and bicarbonate ion into CO_2. This enzyme is intracellular or extracellular. CA is utilized to help photosynthesis process of carbonic compounds into biomass. CO_2 in culture medium will reach saturation and will turn into carbonic compounds when it reacts with water. This carbonic compound will be transformed into biomass with the assistance of CA.

Concentration mechanism strategy depends on the forms of carbon in the process. CO_2 to HCO_3^- conversion in aquatic environment highly depends on pH, while basic environment affects HCO_3^- formation. Inside a cell, enzymatic interconversion takes place to transport and concentrate CO_2 in the carbon fixation process, particularly inside chloroplast pyrenoid of green microalgae cell or cyanobacteria carboxysome [98].

3. Microalgal cultivation

The key to the success of culture techniques depends on the suitability between microalgal species being cultured and several environmental factors. Researcher [53] described factors affecting microalgal growth (cultivation) as growth factors. The growth factors are further classified into resource factors and supporting factors (non-resources factors). The resource factors involve resources that are directly utilized by algal cells for their growth, such as nutrient elements, sunlight, and CO_2. While the supporting factors consist of environmental factors affecting metabolism process in microalgal cells, such as temperature and acidity level (pH). Influences of the resource factors on microalgal growth are commonly illustrated by a hyperbolic function that describes saturation phenomenon, in which increasing the availability of resource factors will not be able to increase the growth of microalgae anymore. The saturation phenomenon is further used in microalgal culture assessments in determining optimum conditions to achieve the most efficient productivity level.

Nitrogen and phosphorus are parts of the resource factors. Numerous studies have been conducted to assess the optimum concentrations of both elements to culture microalgae based on their uses. Many previous studies have reported decreased algal cell viability as the result of nutrient deficiencies of various nutrient elements. This is due to the loss of cell's ability to construct functional structures associated with the limited nutrients. Researchers have [54] underlined the importance of biomass productivity to assess economic feasibility of algal culture, while [55] reported the necessity of attaining biomass productivity in line with production of unsaturated fatty acids in algal culture.

3.1. Promising photobioreactor as a closed cultivation system

Researchers [56] suggested that the best result obtained from the microalgal cultivation in terms of productivity depends greatly on the choice of culture systems. Microalgae can be cultured using a wide variety of systems ranging from controlled indoor systems such as closed laboratory to less predictable systems, such as tanks and pools. There are two microalgal culture systems: (1) open ponds and (2) closed photobioreactors [57]. In laboratories, there are three culture techniques that are most routinely adopted including static, semi-continuous, and continuous cultures [58]. Advantage of indoor culture is its high degree of control over environmental factors such as temperature, light intensity, contamination, and nutrition; however, it requires higher cost than outdoor culture. Outdoor culture is less expensive, but it is difficult to control environmental conditions for optimum growth of microalgae, and it is readily contaminated.

The major limiting factor for both open-pond and enclosed photobioreactor operation is water usage. Typically, sites considered the best for algal production have warm temperatures and high average irradiance throughout the year. In locations with these properties, evaporation from open ponds, and gradual heating of photobioreactors become a problem. The solution to both of these problems is to use more water, either to replace the water lost through evaporation or to evaporatively cool the photobioreactor. Total water usage for production processes inflates dramatically, and sometimes the cost and availability of water become necessary to ensure continuous process. Open pond design needs to become more resistant to contamination, and resistant to evaporation. Additionally, a low-cost gas delivery technique needs to be designed if microalgae are to ever capture carbon from power plants.

Laboratory-scale photobioreactors are mainly equipped with fluorescent light or other light source distributions, as performed to other types of bioreactor, for example, bubble column [59, 60], airlift column [61], stirred tank [62], helical tubular [63], conical [64], and torus [65].

Central to microalgae-based carbon capture are photosynthesis processes, where such processes are supposed to take place, how to improve capture efficiency, and how to easily maintain the system. Therefore, photobioreactor needs to be designed as a reaction vessel.

Researchers [26] state that photobioreactor is a device used to provide an optimum condition for microalgae to perform the process of photosynthesis. This is because it is designed to adapt with available lighting, temperature, pH, CO_2, and nutrition. In term of microalgae productivity, the photobioreactor diameter is a critical design element. Researchers [66] state that light intensity directly depends on photobioreactor diameter, while [67] proved that an increase of vertical photobioreactor of 13 ft (3.96 m) does not affect biomass. The photobioreactor specifically designed for this research is made from transparent glass with tube diameter of 15 cm to receive adequate lighting for a high density of microalgae culture. Therefore, photosynthesis efficiency in artificial environment is higher compared to natural environment.

Every photobioreactor known at present has its own advantages and limitations (**Table 2**). However, regardless the selected reactor design, there are similar technical requirements to a maximization of microalgae growth. When designing a photobioreactor, the main objective

remains to maximize specific growth rate (μ), defined as "an increase of cell mass in culture per time unit per cell mass unit."

Photobioreactor system	Advantages	Disadvantages
Vertical column	A high mass transfer, good mixing with a low shear stress force, potential for multi-scale application, easy to sterilize, ready to use, appropriate for algae immobilization, reducing photo-inhibition and photo-oxidation	Small surface area exposed by light, complex material for its construction, shear stress in microalgae culture, reduced surface area exposed by light during scale up
Plate type	Large surface area exposed by light, appropriate for outdoor culture, appropriate for algae immobilization good lighting path, high biomass productivity, relatively cheap, easy to clean, ready to use, small accumulation of oxygen	Scale u requires many spare parts and supporting material, difficult to control culture temperature, the presence of growth in wall area, possible hydrodynamic stress in some algae species
Tubular	Large surface area exposed by light, appropriate for outdoor culture, high biomass productivity, relatively cheap	The presence of pH, dissolved oxygen, and CO_2 gradients along the pipes, scaling, the presence of growth in wall area, vast land requirement

Table 2. Advantages and limitations of microalgae photobioreactor systems [26].

3.2. Enhancing CO_2 removal efficiency in closed-system photobioreactor

In our previous study, controlling environmental parameters in closed-system photobioreator could improve the ability of microalgae to remove CO_2. Important parameters will be discussed below.

3.2.1. Nitrogen and phosphorus requirement

Nutrients required by microalgae include macronutrients and micronutrients. Elements that belong to macronutrients are C, H, N, P, K, S, Mg, and Ca, while micronutrients include Fe, Cu, Mn, Co, Mo, Bo, Vn, and Si. Specifically, Mn, Fe, Zn, and Vn are required for photosynthesis. Mo, Bo, Co, and Fe for nitrogen metabolism, and Mn, Co, and Cu for other metabolic functions. Of these nutrients, N and P frequently become limiting factor for microalgal growth [68]. Micronutrients act in enzyme systems, oxidation and reduction process in microalgal metabolism, and chlorophyll production. Micronutrients are required to perform various functions during microalgal growth. The most general effect of nutrient deficiency on microalgae is a decrease in protein and photosynthetic pigments and an increase in concentration of carbohydrate and lipid [69, 94].

Nitrogen and phosphorus are the main inorganic nutrients required by microalgae to grow and reproduce. Nitrogen in waters is present as molecular nitrogen (N_2) or as organic nitrogen compounds that have dissociated to inorganic salts such as nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) [34]. Dissociation of protein and other nitrogenous compounds can result

in pH level increase. Generally, when utilizing nitrogen, microalgae have tendency to gradually and sequentially take up ammonium, nitrate, and nitrite [70].

Microalgae commonly use nitrate as their primary source of nitrogen. Nitrogenous compounds are greatly influenced by the amount of oxygen dissolved in the water. In presence of low oxygen, nitrogen is converted into ammonia (NH_3), whereas high oxygen content encourages conversion of nitrogen into nitrate (NO_3^-). However, under unfavorable environmental conditions, ammonium, or urea may serve as nitrogen sources [71, 72]. Ammonium is generated through dissociation process of ammonium hydroxide. Ammonium hydroxide is a solution of ammonia in water. Researchers [70] reported that the ammonium formation reaction is as follows: $NH_3 + H_2O \Leftrightarrow NH_4OH \Leftrightarrow NH_4^+ + OH^-$. As the forward reaction proceeds, the concentration of ammonium increases, and the medium pH becomes alkaline. Components of organic nitrogen include amino acid (constituent of protein), nucleic acid, enzyme and energy carriers such as chlorophyll, adenosine diphosphate (ADP), and ATP. Nitrogen is required by *Arthrospira* sp. during amino acid formation, cellular growth, and gas vacuole regulation.

Absorbed phosphorus constitutes part of the cell's structural component and contributes in cellular energy conversion processes. Commonly, phosphorus is absorbed by *Arthrospira* sp. in the form of phosphate (PO_4^{2-}). Phosphate is required to synthesize nucleotides, phospholipids, and sugar phosphates [70, 94]. The ratio of N to P in a water body also results in the growth of microalgae with different strain compositions. In a laboratory algal culture, the ratio of nitrogen to phosphorus equal to 30 : 1 was found to be more favorable for diatoms, the ratio of N to P equal to 20 : 1 is more favorable for chlorophyceae, while the ratio of N to P equal to 1 : 1 was more favorable for dinoflagellata [70]. Phosphate uptake requirement is higher when the nitrogen is present as nitrate than ammonium salts. The lowest phosphate concentration for optimal microalgal growth ranges from 0.018 to 0.090 ppm P-PO_4, and the highest concentration ranges from 8.90 to 17.8 ppm P-PO_4 when the nitrogen is present as nitrate and 1.78 ppm P-PO_4 when N occurs as ammonium. For optimum growth of phytoplankton, NO_3^-N should be between 0.9 and 3.5 mg/l, while phosphate should range from 0.09 to 1.80 mg/l [73].

In laboratories, microalgae are cultured in artificial medium containing macronutrients and micronutrients. Compared to heterotrophic organisms, photosynthetic organisms require substantially more metal ions as they act as redox active cofactors in photosynthetic electron transfer. Additionally, many algae are autotrophic for certain vitamins such as vitamin B12 which they must obtain from the environment. Potassium is a nutrient that is required as a cofactor for several enzymes and is involved in protein synthesis and osmotic regulation. Sulfur is an essential constituent of some amino acids, vitamins, and sulfolipids, and is essential for the growth of *Arthrospira* sp. [74]. Sodium plays a role in cellular osmoregulation. However, excessive levels of sodium may cause a reduction in photosynthetic pigments [75, 76]. Micronutrients such as Mg, Ca, Fe, EDTA, Cu, Mn, Mo, B, Co, and Zn are essential nutrients that are required in very small amounts. However, a lack of these essential nutrients may inhibit the growth of phototrophic organisms as the metabolism was disturbed [77]. Magnesium is important for the synthesis of accessory photosynthetic pigments due to its position

as the center of the molecular structure of chlorophyll. Additionally, magnesium has a key function in the aggregation of ribosomes into functional units and for the formation of catalase. Calcium is required in cellular membrane activities and acts as a catalyst in enzymatic reactions. Iron and the other metals act as cofactors [74].

3.2.2. Light intensity and temperature in photobioreactor

The development of micro-communities of algae is a function of factors regulating the growth of their components. Each species of microalgae has its own unique temperature and light intensity requirements for its maximum growth [78]. Light intensity plays a significant role in electronic excitation in photosystems, thus allowing photosynthesis to occur [79]. Algae can grow in the absence or presence of light. In the absence of light, microalgae can grow hetero-tropically using limited carbon, such as glucose, as substrate. In this mode of growth, the growth rate is much higher than it can be when microalgae grow in the presence of light and photosynthetically or photoautotrophically. Under optimal conditions, the maximum photo-autotrophic growth rate (μ_{max}) is only half of that of heterotrophic bacteria because of major differences in the allocation of cellular resources [80].

During photoautotrophic growth, as much as 30% of the total cellular protein is allocated to the processes of photosynthesis and carbon fixation. Typically, RuBisCO accounts for 10% of total protein content of these cells and the apoproteins in the photosynthetic organelles accounts for up to 20% [79]. Microalgae grown photomixotrophically, where they use not only endogenous but also exogenous carbohydrates as an energy source, show a higher μ_{max} than when grown photoautotrophically, but the cost of the resulting fuel is increased because of the added cost of reduced carbon sources. Additionally, photomixo-trophic growth has many implications for greenhouse gas emissions depending on how the feedstock anticipates the availability of the reduced carbon [54], and how the feedstock was obtained and processed [81].

Photosynthetic rates may increase when microalgae are cultured in a photobioreactor. For the CO_2 fixation and biomass production, optimum light intensity is necessary. Below the optimum light intensity, light becomes the limiting factor for the microalgae productivity, while exposure of cells to long period with high light intensity causes photoinhibition [82]. Researchers [83] also described phenomenon of photoinhibition. Under prolonged irradiation at a supraoptimal level, photosynthetic rates usually decline from their light-saturated values. A further rise in light intensity to above 8000 lux did not make much difference to either the growth rate or the dry weight of the microalgae, suggesting that a light saturation point had been reached. Saturation light intensity roughly varies from 30 to 45 W/m2 (140–210 µE m^{-2} s $^{-1}$) with a good estimation. For example, according to [84], saturation light intensity of *Chlorella* sp. and *Scendesmus* sp. is around 200 µE m^{-2} s^{-1}. The ratio of light to dark (or low-intensity light) periods in a cycle is crucial for microalgae productivity [85].

Some experts suggested that while durations of daily light/dark cycles are considered long enough to allow algal cells to adapt to the light/dark cycles, the cycle durations of intermittent illumination caused by stirring the culture are too short to adapt, thus microalgal cells may otherwise adapt to an average light intensity in the reactor [86]. A biomass/chlorophyll ratio which tends to decrease as the atmospheric CO_2 level increases indicates excessive chlorophyll

synthesis and shows the importance of high light intensity to stimulate CO_2 absorption rate and enhance productivity rate of microalgal culture [87]. Some other experts suggested that the increase in photosynthetic efficiency might be associated with time synchronization between photosynthetic dark-light reactions in the cultures. Dark fractions of intermittent illumination are long enough to permit microalgal cells to accomplish the dark reaction process prior to receiving light energy for activation of the next photosynthetic process [53]. Dark period of 10–12 h is the optimum photoperiod for the growth of diatoms. Increase in light intensity of 5000–12,000 lux can enhance growth of diatoms, but the growth decreases when the light intensity exceeds 12,000 lux [80]. Light intensity of 4000–5000 lux is an optimum light intensity range for auxospore formation [39].

When optimum light intensity is maintained during the process, *Arthrospira* will attain optimum level of biomass and optimum growth rate. However, light intensity may also result in photoinhibition and photooxidation [88]. Researchers [78] defined photoinhibition as the decrease in photosynthetic capacity caused by excessive photon flux densities (PFDs), which leads to damage to photosynthetic pigments. The damage occurs when electron energy produces superoxide dismutases which play a role as free radicals in the cells. On the contrary, when the light intensity is not optimum, the growth of *Arthrospira* will become less optimum as the energy produced via the photosynthetic process is limited due to low electron excitation.

Temperature is the most important limiting factor, after light, for culturing algae in both close and open outdoor systems that regulate cellular, morphological, and physiological responses of microalgae [40, 89, 90, 91]. Higher temperatures generally accelerate the metabolic rates of microalgae, whereas low temperatures lead to inhibition of microalgae growth [85]. The optimum growth temperature of most microalgae is in the range of 20–30°C [5]. When the temperature is much lower or much higher than the optimum, specific growth rate of micro-algae is reduced [92, 93]. Temperature is not a limiting factor for microalgae in natural waterbody as long as many species can grow in appropriate environmental conditions. However, temperature greatly affects speed of growth and reproduction.

In our previous study, controlling environmental parameters in closed-system photobioreactor could improve the ability of microalgae to remove CO_2. CO_2 removal efficiency was highest when microalgae consortium cultivated in 4000 lux light intensity, periods of light/dark (16/8), and temperature 30°C. Microalgae consortium demonstrated optimum capacity to remove CO_2 at 10% CO_2 supplied. This was evidenced by dry weight of biomass which was 2.5 times higher, CO_2 removal efficiency above 2.5 times higher and the CO_2 utilization efficiency over 5 times higher. In addition, carbon transfer rate also increased. All results were compared with initial condition (2500 lux, light/dark (24/0) and 25°C [39, 41, 94].

3.2.3. Mass transfer

Carbon dioxide mass transfer is one among hydrodynamics variables related to microalgae growth and carbon dioxide reduction effectiveness. CO_2 mass transfer coefficient or $k_La(CO_2)$ in general can demonstrate mass transfer condition occurring in the reactor. $k_La (CO_2)$ value is a hydrodynamic parameters commonly used to assess bioreactor performance in microalgae cultivation process. This process requires an optimum k_La value; higher k_La value indicates a

better CO_2 mass transfer within microalgae culture. Mass transfer of carbon dioxide from air into the media can be growth limiting in dense microalgal cultures. Transfer of CO_2 from gas to liquid depends on many parameters. Physical parameters such as gas flow rate, CO_2 partial pressure, bubble diameter, and lifetime can have large influences on the rate of transfer [47, 95].

CO_2 transfer efficiency affects CO_2 bio fixation in regard to improving microalgae productivity in a photobioreactor culture system. Mass transfer rate of gas mass transfer in a photobioreactor serves as one of parameters determining microalgae growth and CO_2 gas bio fixation. Therefore, Sparger is installed in the lower part of bioreactor to transform gas with different CO_2 flow rate into a small bubble aiming to improve mass transfer. Sparger is also deliberately installed in the lower part to distribute CO_2. The formed bubbling can increase CO_2 mass transfer while removing O_2 produced during photosynthesis.

The water chemistry also influences the solubility of CO_2 and the transfer capacity. CO_2 can be dissolved in water according to Henry's law and reacts with water to form carbonic acid (H_2CO_3). The equilibrium shifts toward HCO_3^- (bicarbonate) as the pH increases to a neutral range [81, 96]. HCO_3^- is actively transported into microalgae while CO_2 enters the cell by passive diffusion [97]. The pH of the media plays a major role in mass transfer and can drastically alter growth dynamics of the microalgae. Controlling pH by the addition of buffering agents can affect mass transfer of CO_2 and carbon uptake by the microalgae.

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