We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Biotechnology Carbon Capture and Storage by Microalgae to Enhance CO₂ Removal Efficiency in Closed-System Photobioreactor

Dr. Astri Rinanti

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/62915>

Abstract

The global warming issue caused mainly by carbon dioxide (CO₂) has triggered various efforts to reduce excess amount of CO₂ emitted into the atmosphere. A viable option is to utilize biomass production potential of microalgal consortium in aquatic ecosystem that constantly requires CO₂ 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 CO₂ by using CO₂ 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 CO₂ (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 CO₂ emission reduction in urban areas.

Keywords: Microalgae, CO₂ removal, carbon fixation, biomitigation, carbon capture and storage, photobioreactor

1. Introduction

1.1. Carbon dioxide and global warming

Carbon dioxide (CO₂) is the most significant greenhouse gas that contributes to global warming. CO₂ emissions and other combustion products such as NO_x, SO_x, CH₄, 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, CO₂ 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, CO₂ 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 CO₂-rich environment [4]. However, recently CO₂ concentration in the troposphere is getting serious attention as CO₂ 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].

CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ concentration by volume) and natural gas power plant (8–10%). Globally, CO₂ emissions from fossil-fuel use in the year 2000 totaled about 23.5 Gt CO₂/year. Of this, close to 60% was attributed to stationary emission sources. However, not all of these sources are amenable to CO₂ capture and process [11].

Human activities are greatly increasing the atmospheric concentrations of carbon dioxide. The rate of increase in atmospheric CO₂ 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 CO₂ 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 CO₂ concentrations. A researcher [16] estimated that anthropogenic contribution to the carbon cycle in the form of CO₂ 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 CO₂ 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 CO₂ from specific emission sources such as industrial related sources and power plants by using certain techniques, the second stage is to transport the CO₂ to a suitable storage or processing location, and the third stage is to process the CO₂ 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 CO₂ emissions in the air are absorbed by microalgae, thus allowing photosynthesis process to occur. In microalgal cells, CO₂ molecules enter the Calvin-Benson cycle to form sugars. The mechanism in which CO₂ is bonded to the enzyme ribulose-1,5-bisphosphate (RuBP) or ribulose-1,5-bisphosphate carboxylase 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 CO₂ 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 CO₂. 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 CO₂. Growing algae to capture ambient CO₂ 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 CO₂ 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 CO₂ concentration and high CO₂ absorption rate. As a photosynthetic organism, microalgae are well adapted to capture ambient CO₂, although the limited CO₂ content is considered inefficient. High CO₂ concentration can hamper photosynthesis process and slow the growth of microalgae and take into account exhaust gas from combustion contains 5–15% CO₂. **Table 1** presents several types of microalgae that play a role in CO₂ bio-mitigation research in a laboratory scale.

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

NA = not available data.

Table 1 Some species of microalgae that play a role in CO₂ bio-mitigation.

There are many fundamental questions that still need to be answered regarding microalgal growth at elevated CO₂ concentrations. The most critical determination is the maximum amount of CO₂ sequestered from a given concentration of input gas. There was, however, a debate as to the actual amount of CO₂ that can be removed from the input stream. Data presented by [19] suggested that less than 5% of the CO₂ can be removed from a stream containing >1% CO₂ if the cells are only at a low density. However, [25] suggested that as much as 70% uptake from a 2% CO₂ stream could be captured in cyanobacteria. The pH of the media and the tolerance of the organism to high CO₂ concentration will play important roles in the amount taken up. Further results indicate that the maximum amount of CO₂ 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 CO₂ concentrations, there are important issues to consider when growing algae for the purpose of CO₂ 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 CO₂ from industrial and energy-related sources, transport to a storage location, and long-term isolation from the atmosphere. Capture of CO₂ can be applied to large point sources. The CO₂ 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 CO₂ from industrial and energy-related sources, transport to a storage location, and long-term isolation from the atmosphere. Capture of CO₂ can be applied to large point sources. The CO₂ 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 CO₂ from a source of carbon dioxide, such as power plant flue gas, into algal cultures to increase CO₂ capture efficiency and productivity could be a challenge [30, 31]. In this study, **the second stage** in the CCS concept is to transport the CO₂ for the purpose of microalgal photosynthesis, while **the third stage** is to store the CO₂ in microalgal biomass in forms useful to humans such as fuels, food additives, and medicines [32].

Efficiently capturing carbon dioxide from an elevated CO₂ 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 CO₂ 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 CO₂ concentrations, and at the same time able to efficiently mitigate large amounts of CO₂, is essential.

2.1. Biology of microalgae as photosynthetic organisms and CO₂ 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 CO₂ 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 CO₂ and water (which contains nutrients). The chemical reactions involved in photosynthesis are as follows:

Research studies that utilize the potential of microalgae as CO₂ absorber have been carried out in various countries, particularly in efforts toward adaptation and selection of microalgae species tolerant to high CO₂ concentrations and high CO₂ absorption rate. Researchers [33] and [19] have reported that the microalgal species *Chlorococcum littorale* could grow under CO₂ concentrations of over 20 *Chlorella* sp. were able to grow up even in aeration containing CO₂ up to 40%, at culture pH as low as 4 [36].

The saturation point of atmospheric CO₂ 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 CO₂ from the atmosphere, hence the dissolved CO₂ is abundant and the equilibrium of carbon ions will not shift toward the carbonate ions. As a result, at high CO₂ concentrations, the pH of the culture was relatively stable [37]. During photosynthesis, free CO₂ is the main inorganic carbon source to use in microalgal cultures. According to [38], absorption of dissolved CO₂ from the water due to photosynthesis will lower the concentration of dissolved CO₂. This reduction will increase the pH since the dissolved CO₂ exists in chemical equilibrium with bicarbonate ions (HCO₃⁻) and carbonate ions (CO₃²⁻) 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. CO₂ uptake rates of microalgal cells could be stimulated by increase in CO₂ concentration in the media, and therefore culture without additional CO₂ would exhibit low CO₂ 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 CO₂ 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 CO₂ 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 CO₂/l/day in *C. vulgaris* culture and 3.41 g CO₂/l/day in *A. convolutus* culture, respectively [40]. An observation using microscope has demonstrated that *Chlorella* cultures grown under high CO₂ concentrations tend to have bigger cell size. Thus, although they had lower cell density when grown under CO₂ 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 specificity 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 CO₂ and H₂O, is illustrated in **Figure 3**. The first step is the incorporation of CO₂ 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: $2\text{H}_2\text{O} + \text{CO}_2 + \text{energy} \rightarrow \text{CH}_2\text{O} + \text{H}_2\text{O} + \text{O}_2$

In oxygenic photosynthetic organisms, CO₂ 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 CO₂ [33], mass transfer of CO₂ 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 CO₂ and HCO₃⁻ interconversion. It is a major component in intracellular mobilization from pool HCO₃⁻ pool, by catalyzing CO₂ production for Rubisco enzyme [51]. The role of CCM is mainly to increase CO₂ concentration for Rubisco, which is the enzyme responsible for CO₂ initial fixation.

Photosynthetic microorganisms in water as eukaryote microalgae, cyanobacteria, and photosynthetic bacteria have an ability to utilize CO₂, facilitated by ribulose-1,5 bisphosphate carboxylase/oxygenase enzyme (Rubisco). This Rubisco enzyme serves to capture Ci in the form of CO₂ in darkness (light-independent) through photosynthesis reaction (Calvin cycle) [38, 98]. When pure CO₂ is dissolved, pH drops to below 7, therefore creating acidic condition.

The dominant Ci species in acids are CO₂ and HCO₃⁻ [52]. Microalgae have developed various ways to ensure that Rubisco enzyme will accompany CO₂ 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 CO₂ gas diffusion within medium or through a carbonate conversion. Rubisco only reacts with CO₂, not with bicarbonate or carbonic ion. Therefore, another enzyme, CA, is needed to convert carbonic and bicarbonate ion into CO₂. This enzyme is intracellular or extracellular. CA is utilized to help photosynthesis process of carbonic compounds into biomass. CO₂ 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. CO₂ to HCO₃⁻ conversion in aquatic environment highly depends on pH, while basic environment affects HCO₃⁻ formation. Inside a cell, enzymatic interconversion takes place to transport and concentrate CO₂ 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 CO₂. 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, CO₂, 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 CO₂ removal efficiency in closed-system photobioreactor

In our previous study, controlling environmental parameters in closed-system photobioreactor could improve the ability of microalgae to remove CO₂. 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 (N₂) or as organic nitrogen compounds that have dissociated to inorganic salts such as nitrate (NO₃⁻), nitrite (NO₂⁻), and ammonium (NH₄⁺) [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: $\text{NH}_3 + \text{H}_2\text{O} \rightleftharpoons \text{NH}_4\text{OH} \rightleftharpoons \text{NH}_4^+ + \text{OH}^-$. 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 heterotrophically 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 photoautotrophic growth rate ($\mu_{\max}$) 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 $\mu_{\max}$ than when grown photoautotrophically, but the cost of the resulting fuel is increased because of the added cost of reduced carbon sources. Additionally, photomixotrophic 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 CO₂ 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 $\mu\text{E m}^{-2} \text{ s}^{-1}$) with a good estimation. For example, according to [84], saturation light intensity of *Chlorella* sp. and *Scenedesmus* sp. is around 200 $\mu\text{E m}^{-2} \text{ s}^{-1}$. 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 CO₂ 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 microalgae 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 $k_L a(\text{CO}_2)$ in general can demonstrate mass transfer condition occurring in the reactor. $k_L a(\text{CO}_2)$ value is a hydrodynamic parameters commonly used to assess bioreactor performance in microalgae cultivation process. This process requires an optimum $k_L a$ value; higher $k_L a$ value indicates a

better CO₂ 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 CO₂ from gas to liquid depends on many parameters. Physical parameters such as gas flow rate, CO₂ partial pressure, bubble diameter, and lifetime can have large influences on the rate of transfer [47, 95].

CO₂ transfer efficiency affects CO₂ 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 CO₂ gas bio fixation. Therefore, Sparger is installed in the lower part of bioreactor to transform gas with different CO₂ flow rate into a small bubble aiming to improve mass transfer. Sparger is also deliberately installed in the lower part to distribute CO₂. The formed bubbling can increase CO₂ mass transfer while removing O₂ produced during photosynthesis.

The water chemistry also influences the solubility of CO₂ and the transfer capacity. CO₂ can be dissolved in water according to Henry's law and reacts with water to form carbonic acid (H₂CO₃). The equilibrium shifts toward HCO₃⁻ (bicarbonate) as the pH increases to a neutral range [81, 96]. HCO₃⁻ is actively transported into microalgae while CO₂ 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 CO₂ and carbon uptake by the microalgae.

Author details

Dr. Astri Rinanti

Address all correspondence to: astririnanti@trisakti.ac.id, astri@fun-dering.com

Environmental Engineering Department, Trisakti University, Jakarta, Indonesia

References

- [1] Benemann JR. Greenhouse gas emissions and potential for mitigation from wastewater treatment processes. U.S. Dept. of Energy : Report to the Electric Power Research Institute. 2002.
- [2] Benemann JR. Utilization of carbon dioxide from fossil fuel-burning power plants with biological systems. *Energy Conversion Manage.* 2003; 34: 999–1004.
- [3] Skjanes K, Lindblad P, Muller J. BioCO₂ – A multidisciplinary, biological approach using solar energy to capture CO₂ while producing H₂ and high value products. *Biomol, Eng.* 2007; 24: 405–413.

- [4] Bhaya D, Schwarz R, Grossman AR. Molecular responses to environmental stress, in Whitton BA and Potts M. (ed). The Ecology of Cyanobacteria: Their Diversity in Time and Space. USA: Kluwer Academic Publisher. 2000. 397–442.
- [5] Wang B, Li Y, Wu N, Lan CQ. CO₂ Bio-mitigation using microalgae. Applied Microbiology and Biotechnology. 2008; 79: 707–718.
- [6] Haszeldine SR. Carbon capture and storage: How green can black be? Science. 2009; 32: 1647–1651.
- [7] Herzog H. A Research Program for Promising Retrofit Technologies. Prepared for the MIT Symposium on Retro-Fitting of Coal-Fired Power Plants for Carbon Capture (2009). [http://web.mit.edu/mitei/docs/reports/herzog_promising.pdf]
- [8] Rochelle GT. Amine scrubbing for CO₂ capture. Science. 2009; 325: 1652–1653.
- [9] Song D, Fu J, Shi D. Exploitation of oil-bearing microalgae for biodiesel. Chinese Journal of Biotechnology. 2008; 24: 341–348.
- [10] Kondili, Kaldellis JK. Biofuel implementation in east Europe: Current status and future prospects. Renewable and Sustainable Energy Review. 2007; 11: 2137–2151.
- [11] Hamilton MR, Herzog HJ, Parsons JE. Cost and U.S. public policy for new coal power plants with carbon capture and sequestration. Energy Procedia. 2009; 1: 4498–4494.
- [12] Scharlemann J, Laurance W. How green are biofuels? Science. 2008; 319(5859): 43–44.
- [13] Karube I, Takeuchi T., Barnes DJ. Biotechnological reduction of CO₂ emission. Advances in Biochemical Engineering/Biotechnology. 1992; 46: 63–79.
- [14] Gutierrez R, Gutierrez-Sanchez R, Nafidi A. Trend analysis using nonhomogeneous stochastic diffusion processes: Emission of CO₂; Kyoto protocol in Spain. Stochastic Environmental Research and Risk Assessment. 2008; 22: 57–66.
- [15] Pedroni P, Davison J, Beckert H, Bergman P, Benemann J. A proposal to establish an international network on biofixation of CO₂ and greenhouse gas abatement with microalgae. Journal of Energy and Environmental Research. 2004; 1(1), 1–20.
- [16] Lal R. Sequestration of atmospheric CO₂ in global carbon pools. Energy and Environmental Science. 2008; 1: 86–100.
- [17] Zhu XG, Long SP, dan Ort DR. What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? Current Opinion in Biotechnology. 2008. 19: 153–159.
- [18] Chiu SY, Kao CY, Chen CH, Kuan TC, Ong SC, Lin CS. Reduction of CO₂ by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor. Bioresource Technology. 2008; 3389–3396.

- [19] De Moraes MG, Costa JAV. Biofixation of carbondioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor, Journal of Biotechnology. 2007; 129: 439–445.
- [20] Yoo C, Jun SY, Lee JY, Ahn CY. Oh HM. Selection of microalgae for lipid production under high levels carbon dioxide. Journal of Bioresource Technology. 2010; 101: S71–S74.
- [21] Scragg AH, Illman AM, Carden A, Shales S. Growth of microalgae with increased caloric values in a tubular bioreactor. Biomass and Bioenergy. 2002; 23: 67–73.
- [22] Huntley ME, Redalje DG. CO₂ mitigation and renewable oil from photosynthetic microbes, in a new appraisal. Mitigation and adaptation strategies for global change. 2007; 12: 573–608.
- [23] Gomez Villa H, Voltolina D, Nieves M, Pina P. Biomass production and nutrient budget in outdoor cultures of *Scenedesmus obliquus* (Chlorophyceae) in artificial wastewater, under the winter and summer conditions of Mazatlán, Sinaloa, Mexico, Vie et milieu, 2005; 55(2): 121–126.
- [24] Yun YS, Lee SB, Park JM, Lee CI, Yang JW. Carbon dioxide fixation by algal cultivation using wastewater nutrients. Journal of Chemical Technology and Biotechnology. 1997; 69(4): 451–455.
- [25] Maeda K. CO₂ fixation from the flue gas on coal-fired thermal power plant by microalgae. Fuel and Energy Abstracts. 1996; 37(3): 217.
- [26] Ugwu CU, Aoagi H, Uchiyama H. Photobioreactors for mass cultivation of algae. Bioresource Technology. 2008; 99(10): 4021–4028.
- [27] IPCC. Summary for Policymakers, in Climate Change: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Metz B, Davidson O, Bosch P, Dave R, Meyer L (ed). New York: Cambridge University Press. 2007. www.ipcc.ch
- [28] Papazi A, Makridis P, Divanach P, Kotzabasis K. Bioenergetic changes in the microalgal photosynthetic apparatus by extremely high CO₂ concentrations induce an intense biomass production. Physiology Plantarum 2008; 132: 338–349.
- [29] Buchana BB, Gruissam W, Jones RL. Biochemistry and Molecular Biology of Plants. USA, Rockville: American Society of Plant Physiologists. 2000.
- [30] Beardall J, Raven JA. The potential effects of global climate change on microalgal photosynthesis, growth and ecology. Phycologia. 2004; 43(1): 26–40.
- [31] Chang EH, SS Yang. Some characteristics of microalgae isolated in Taiwan for biofixation of carbondioxide. Botanical Bulletin of Academia Sinica. 2003; 44: 43–52.

- [32] Hsueh HT, Chu H, Yu ST. A batch study on the bio-fixation of carbon dioxide in the absorbed solution from a chemical wet scrubber by hot spring and marine algae. *Chemosphere*. 2007; 66(5): 878–886.
- [33] Ono E, Cuello JL. Mini Review: Selection of Optimal Microalgae Species for CO₂ Sequestration. USA: Department of Agricultural and Biosystem Engineering. 2009.
- [34] Graham LE, Wilcox LW. *Algae*. Upper Saddle River, NJ: Prentice-Hall, Inc. 2000. pp1356
- [35] Othman N, Manan ZA, Wan Alwi SR, Sarmidi MRA. Review of extraction technology for carotenoids and vitamin e recovery from palm oil. *Journal of Applied Sciences*. 2010; 10 (12): 1187–1191.
- [36] Reinehr CO, Costa JAV. Repeated batch cultivation of the microalga *Spirulina platensis*. *World Journal of Microbiology & Biotechnology*. 2006; 22: 937–943.
- [37] Kodama M, Ikemoto H, Miyachi S. A new species of highly CO₂-tolerant fast growing marine microalga suitable for high density culture. *Journal of Marine Biotechnology*. 1993; 1: 21–25.
- [38] Jacob-Lopes E, Lacerda L, Franco T. Biomass production and carbon dioxide fixation by aphanothece *Microscopica nageli* in a bubble column photobioreactor. *Biochemical Engineering Journal*. 2008; 40: 27–34.
- [39] Rinanti A, Kardenia E, Astuti DI, Dewi K. Improvement of carbon dioxide removal through artificial light intensity and temperature by constructed green microalgae consortium in a vertical bubble column photobioreactor. *Malaysian Journal of Microbiology*. 2014; 10(1): 29–37.
- [40] Carlozzi P. Dilution of solar radiation trough “culture” lamination in photobioreactor rows facing south-north: A way to improve the efficiency of light utilization (*Arthrospira platensis*). *Biotechnology and Bioengineering*. 2003; 81: 305–315.
- [41] Rinanti A, Kardenia E, Astut, DI, Dewi K. Carbon dioxide fixation by co-culture green microalgae through overall volumetric mass transfer coefficient ($K_L a$) of carbon dioxide in closed system. *Asian Journal of Microbiology, Biotechnology and Environmental Science*. 2014b; 16(2): 253–258.
- [42] Zhu X-G, Long SP, Ort DR. Towards more efficient crop photosynthesis. *Annual Reviews of Plant Biology*. 2010; 61: 235–261.
- [43] Carrieri D, Ananyev G, Brown T, Dismukes GC. In vivo bicarbonate requirement for water oxidation by photosystem ii in the hypercarbonate-requiring cyanobacterium *Arthrospira maxima*. *Journal of Inorganic Biochemistry*. 2007; 101: 1865–1874
- [44] Vance P, Spalding MH. Growth, photosynthesis, and gene expression in *Chlamydomonas* over a range of CO₂ concentrations and CO₂/O₂ ratios: CO₂ regulates multiple acclimation states. *Canadian Journal of Botany*. 2005; 83: 796–809

- [45] Atsumi S, Hanai T, Liao JC. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. *Nature*. 2008; 451: 86–89
- [46] Wen X, Gong H, Lu C. Heat stress induces an inhibition of excitation energy transfer from phycobilisomes to photosystem II but not to photosystem I in a cyanobacterium *Arthrospira (Spirulina) platensis*. *Plant Physiology and Biochemistry*. 2005; 43: 389–395.
- [47] Bar-Even A, Noor E, Lewis NE, Milo R. Design and analysis of synthetic carbon fixation pathways. *Proceedings of the National Academy of Sciences, USA*. 2010; 107: 8889–8894.
- [48] Badger MR, Spalding MH. *Photosynthesis: Physiology and Metabolism*. Switzerland: Springer; 2000. Pp. 369–397.
- [49] Laws EA, Terry KL, Wickman J, Chalup MS. A simple algal production system designed to utilize the flashing light effect. *Biotechnology and Bioengineering*. 2004; 25(10): 2319–2335.
- [50] Ramanan R, Kannan K, Deshkar A, Yadav R, Chakrabarti T. Enhanced algal CO₂ sequestration through calcite deposition by *Chlorella* sp. and *Spirulina platensis* in a mini-raceway pond. *Bioresource Technology*. 2010; 101: 2616–2622.
- [51] Matsuda Y, Colman B. Induction of CO₂ and bicarbonate transport in the green alga *Chlorella ellipsoidea*: Evidence for induction in response to external CO₂ concentration. *Plant Physiology*. 1995; 108: 253–260.
- [52] Ota M, Kato Y, Watanabe H, Watanabe M, Sato Y, Smith RL. Fatty acid production from a highly CO₂ tolerant alga, *Chlorococcum littorale*, in the presence of inorganic carbon and nitrate. *Bioresources Technology*. 2009; 100: 5237–5242.
- [53] Goldman JC. Outdoor algal mass culture II. Photosynthetic yield limitation. *Water Research*. 1999; 13: 119–136.
- [54] Otto Pulz, Wolfgang Gross. Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology*. 2004; 65(6): 635–648.
- [55] Li Y, Horsman M, Wu N, Lan CQ, Dubois-Calero N. Biofuels from Microalgae. *Biotechnology Progress*. 2008; 24: 815–820.
- [56] Del Campo JA, Garcia-Gonzalez M, Guerrero MG. Outdoor cultivation of microalgae for carotenoid production: Current state and perspectives. *Applied Microbiology and Biotechnology*. 2007; 74: 1163–1174.
- [57] Borowitzka MA. Commercial production of microalgae in ponds, tanks, tubes and fermenters. *Journal of Biotechnology*. 1999; 70: 313–321.
- [58] Barsanti L, Gualtieri P. *Algae: Anatomy, Biochemistry and Biotechnology*. USA: CRC Press. 2006

- [59] Degen J, Uebele A, Retze A, Schmid-Staiger U, Trösch W. A novel airlift photobioreactor with baffles for improved light utilization through the flashing light effect. *Journal of Biotechnology*. 2001; 92: 89–94.
- [60] Ogbonna JC, Tanaka H. Light requirement and photosynthetic cell cultivation – Development of process for efficient light utilization in photobioreactors. *Journal of Applied Phycology*. 2000; 12: 207–218.
- [61] Kaewpintong K, Shotipruk A, Powtongsook S, Pavasant P. Photoautotrophic high-density cultivation of vegetative cells of *Haematococcus pluvialis* in airlift bioreactor. *Bioresource Technology*. 2007; 98: 288–295.
- [62] Ogbonna JC, Soejima T, Tanaka H. An integrated and artificial light system for internal illumination of photobioreactors. *Journal of Biotechnology*. 1999; 70: 289–297.
- [63] Hall DO, Acien Fernández FG, Cañizares Guerrero E, Krishna Rao K, Molina Grima E. Outdoor helical tubular photobioreactors for microalgal production: modeling of fluid-dynamics and mass transfer and assessment of biomass productivity. *Biotechnology and Bioengineering*. 2003; 82(1): 62–73.
- [64] Watanabe Y, Saiki H. Development of a photobioreactor incorporating *Chlorella* sp. for removal of CO₂ in stack gas. *Energy Conversion and Management*. 1997; 38: 499–503.
- [65] Pruvost J, Van Vooren G, Cogne G, Legrand J. Investigation of biomass and lipids production with *Neochloris oleoabundans* in photobioreactor. *Bioresource Technology*. 2009; 100: 5988–5995.
- [66] Hulatt CJ, Thomas DN. Productivity, carbon dioxide uptake and net energy balance of microalgal bubble column photobioreactors. *Bioresource Technology*. 2011; 102: 5775–5787 (id:1891).
- [67] Miron AS, Garcia MCC, Camacho FG, Grima EM, Chisti Y. Growth and biochemical characterization of microalgal biomass produced in bubble column and airlift photobioreactors: studies in fed-batch culture. *Enzyme and Microbial Technology*. 2002; 31:1015–1023.
- [68] Westerhoff P. Growth parameters of microalgae tolerant to high levels of carbon dioxide in batch and continuous-flow photobioreactors. *Environmental Technology Journal*. 2010; 31: 523–532.
- [69] Cuaresma M, Casal C, Forján E, Vilchez CV. Productivity and selective accumulation of carotenoids of the novel extremophile microalgae *Chlamydomonas acidophila* grown with different carbon sources in batch systems. *Journal of Industrial Microbiol Biotechnology*. Society for Industrial Microbiology. Switzerland: Springer. 2010.
- [70] Burkhard S, Zondervan I, Riebesell U. Effect of CO₂ concentration on C:N:P ratio in marine phytoplankton: A species comparisons. *Association for the Sciences of Limnology and Oceanography*. 1999; 44 (3): 683–690.

- [71] Sassano CEN, Gioielli LA, Almeida KA, Sato S, Perego P, Converti A, Carvalho JCM. Cultivation of *Arthrospira (Spirulina) platensis* by Continuous process using ammonium chloride as nitrogen source. *Biomass and Bioenergy*. 2007; 31: 593–598.
- [72] Soletto D, Binaghi L, Lodi A, Carvalho JCM, Converti A. Batch and fed-batch cultivations of *Arthrospira (Spirulina) platensis* using ammonium sulphate and urea nitrogen sources. *Aquaculture*. 2005; 243: 217–224.
- [73] Gonzales LE, Canizares RO, Baena S. Efficiency of ammonia and phosphorus removal from a colombian agroindustrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*. *Bioresource Technology*. 1997; 60: 259–262.
- [74] Raoof B, Kaushik BD, Prasanna R. Formulation of low-cost medium for mass production of *Arthrospira (Spirulina)*. *Biomass and Bioenergy*. 2006; 30: 537–542.
- [75] Dhiab RB, Ouada HB, Bousetta H, Franck F, Elabed AA, Brouers M. Growth, fluorescence, photosynthetic O₂ production and pigment content of salt adapted cultures of *Arthrospira platensis*. *Journal of Applied Phycology*. 2007; 19: 293–301.
- [76] Verma K, Mohanty P. Alteration in the structure of phycobilisomes of the cyanobacterium *Arthrospira (Spirulina) platensis* in response to enhanced Na⁺ level. *World Journal of Microbiology and Biotechnology*. 2000; 16: 795–798.
- [77] Moroney JV, Somanchi A. How do algae concentrate CO₂ to increase the efficiency of photosynthetic carbon fixation? *Plant Physiology*. 1999; 119: 9–16.
- [78] Vonshak A, Tomaselli L. *Arthrospira (Spirulina)*: Systematics and Ecophysiology, in Whitton BA and Potts M (ed). *The Ecology of Cyanobacteria: Their Diversity in Time and Space*. USA: Kluwer Academic Publisher. 2000.
- [79] Beardall J, Raven JA. Limits to Phototrophic Growth in Dense Culture: CO₂ Supply and Light, in Brorowitzka MA and Moheimani NR (ed). *Algae for Biofuels and Energy*. London: Springer; 2013. pp 91–97.
- [80] Raven JA. Contributions of anoxygenic and oxygenic phototrophy and chemolithotrophy to carbon and oxygen fluxes in aquatic environments. *Aquatic Microbial Ecology*. 2009; 56: 177–192.
- [81] Jacob-Lopes E, Franco TT. Microalgae-based Systems for Carbon dioxide Sequestration and Industrial Biorefineries, in Momba M and Bux F (ed). *Biomass*. www.sciyo.com; 2010. ISBN 978-953-307-113-8, Croatia: Intechopen; 2010. pp. 202.
- [82] Rubio FC, Camacho FG, Sevilla JMF, Chisti Y, Grima EM, 2003. A mechanistic model of photosynthesis in microalgae. *Biotechnology and Bioengineering*. 81 (4), 459–473.
- [83] Masojídek J, Kopecký J, Gianelli L, Torzillo G. Productivity correlated to photochemical performance of *Chlorella* mass cultures grown outdoors in thin-layer cascades. *Journal of Industrial Microbiology and Biotechnology*. 2011; 38: 307–317.

- [84] Hanagata N, Takeuchi T, Fukuju Y, Barnes DJ, Karube I. Tolerance of microalgae to high CO₂ and high-temperature. *Phytochemistry*. 1992; 31: 3345–3348.
- [85] Muñoz R, Guieysse B. Algal-bacterial processes for the treatment of hazardous contaminants: A review. *Water Research*. 2006; 40: 2799–2815.
- [86] Feuga AM, Guedes RL, Herve A, Durand P. Comparison of artificial light photobioreactors and other production systems using *Porphyridium cruetum*. *Journal of Applied Phycology*. 1998; 10: 83–90.
- [87] Chaumont D. Biotechnology of algal biomass production: A review of systems for outdoor mass culture. *Journal of Applied Phycology*. 1993; 5: 593–604.
- [88] Bayless D, Vis M, Kremer G, Prudich M, Cooksey K, Muhs J. Enhanced practical photosynthetic CO₂ mitigation. Technical report. Ohio University. 2001.
- [89] Vonshak A, Torzillo G, Masojidek J, Boussiba S. Sub-optimal morning temperature induces photoinhibition in dense outdoor cultures of the algal *Monodus subterraneus* (Eustigmatophyta). *Plant, Cell and Environment*. 2001. 24: 1113–1118.
- [90] Moheimani NR. The culture of Coccolithophorid algae for carbon dioxide bioremediation. [PhD thesis]. Perth Australia: Murdoch University; 2005.
- [91] Chisti Y. Biodiesel from microalgae. *Biotechnology Advances*. 2007; 25: 294–306.
- [92] Madigan MT, Martinko JM, Stahl D, Clark DP. Other Foodborne Infectious Diseases. In : Brock (ed) *Biology of Microorganism*. 13th ed. San Fransisco: Benjamin Cummings; 2012. pp. 1043.
- [93] Thébault JM, Rabouille S. Comparison between two mathematical formulations of the phytoplankton specific growth rate and temperature, in two stimulation models (ASTER & YOYO). *Ecological Modeling*. 2003; 163: 145–151.
- [94] Rinanti A, Kardena E, Astuti DI, Dewi K. Screening of potential photosynthetic microalgae from wastewater treatment plant for carbon dioxide capture and storage (CCS) agent. *Asian Transactions on Science and Technology*. 2013; 3(1): 1–8.
- [95] Barbosa MJ, Janssen M, Ham N, Tramper J, Wijffels RH. Microalgae cultivation in air-lift reactors: modelling biomass yield and growth rate as a function of mixing frequency. *Biotechnology and Bioengineering*. 2003; 82: 170–179.
- [96] Kargi F, Uygur A. Effect of carbon source on biological nutrient removal in a sequencing batch reactor. *Bioresource Technology*. 2003; 89: 89–93.
- [97] Thauer RK. A fifth pathway of carbon fixation. *Science*. 2007; 318: 1732–1733