

Growth Performance of Fresh Water Microalgae *Chlorella* sp. Exposed to Carbon Dioxide

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Abstract—It is generally recognized, that algae could be an interesting option for reducing CO₂ emissions. Based on light and CO₂, algae can be used for the production various economically interesting products. Current algae cultivation techniques, however, still present a number of limitations. Efficient feeding of CO₂, especially on a large scale, is one of them. Current methods for CO₂ feeding to algae cultures rely on the sparging pure CO₂ or directly from flue gas. The limiting factor in this system is the solubility of CO₂ in water, which demands a considerable amount of energy for an effective gas to liquid transfer and leads to losses to the atmosphere. Due to the current ineffective methods for CO₂ introduction into algae ponds very large surface areas would be required for enough ponds to capture a considerable amount of the CO₂. The purpose of this study is to assess technology to capture carbon dioxide (CO₂) emissions generated by industry by utilizing of microalgae *Chlorella* sp. The microalgae were cultivated in a bioreactor culture pond raceway type. The result is expected to be useful in mitigating the effects of greenhouse gases in reducing the CO₂ emissions. The research activities include: (1) Characterization of boiler flue gas, (2) Operation of culture pond, (3) Sampling and sample analysis. The results of this study showed that, the initial assessment absorption of the flue gas by microalgae using 1000 L raceway pond completed by heat exchanger were quite promising. The transfer of CO₂ into the pond culture system was run well. This identified from the success of cooling the boiler flue gas from the temperature of about 200 °C to below ambient temperature. Except for the temperature, the gas bubbles into the culture media were quite fine. Therefore, the contact between the gas and the media was well performed. Efficiency of CO₂ absorption by *Chlorella* sp reached 6.68 % with average CO₂ loading of 0.29 g/L/day.

Index Terms—*Chlorella* sp., CO₂ emission, heat exchange, microalgae, milk industry, raceway pond.

I. INTRODUCTION

Global warming is a phenomenon of global temperature increase from year to year due to the greenhouse effect caused by increased emissions of gases such as carbon dioxide (CO₂), methane (CH₄), dinitrooxida (N₂O) and CFC, so that solar energy trapped in the earth's atmosphere.

Increasing the amount of greenhouse gas emissions (GHG) in the atmosphere caused by human activities in various sectors, including energy. The use of fossil fuels such as oil, coal and natural gas in a variety of activities, for example in power generation, transportation and industry, will lead to the increasing amount of GHG emissions in the atmosphere.

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Although both produce GHG emissions, but the emissions resulting from the use of three types of fossil fuels is different. To produce 1 kWh of energy, power plants using coal emit about 940 grams of CO₂. While power plants that use petroleum and natural gas, resulting in the emission of approximately 798 and 581 grams of CO₂ [1].

Several alternatives have been studied in order to promote the reduction of emissions, sequestration and biological fixation of carbon dioxide emitted by stationary sources. One of them is the use of reforestation of photosynthetic organism with the capacity to absorb carbon dioxide from the atmosphere [2], but currently the alternative that has been more studied is the cultivation of microalgae. Microalgae are the main responsible for the biological uptake of atmospheric CO₂ in the oceans that cover ¾ of the globe surface, once that they are present in large numbers in the water column [3]. A portion of the CO₂ absorbed by microalgae is transferred to the deep ocean in a process known as "biological pump" [4]. This process, along with direct diffusion of CO₂ into the water, prevents that the buildup of "greenhouse effect" gases became even greater. Besides having the capacity to fix carbon dioxide from air, using it as a carbon source, microalgae have the capacity of cycling the organic matter and may offer during its growth a number of mineral elements, vitamins, lipids, pigments and proteins, presenting thus large industrial and commercial applicability [5].

Along with the increasing concentration of anthropogenic CO₂ in the atmosphere, a wide range of engineering effort has been made to capture and bury the atmospheric CO₂ through carbon capture and storage technology (CCS) from emission sources. One of the CCS technology that allows it to be applied in Indonesia is biosequestration, that capture and store atmospheric CO₂ by increasing the volume and quality of photosynthesis through microalgae bioreactor. Currently, experiments regarding the ability of microalgae to absorb CO₂ has been done to scale laboratory at the Agency for Assessment and Application of Technology (BPPT) to build a photobioreactor and microalgae culture ponds. The results showed that the absorption of CO₂ by microalgae *Chlorella* sp. can achieve more than 70%, the results are quite promising for the application in order to reduce CO₂ emissions from industry. To that end, there should be further engineered into account various important aspects that exist in the industry that technology is feasible biosequestration applies for a period of 1 to 2 years in order to support government action plan reduce 26% GHG emissions by 2020.

Raceway pond bioreactor given gear pedal/propeller for stirring process, driving electromotor, CO₂ gas intake pipe and pipe freshwater and nutrient intake. Pond is made of stainless steel allows the microalgae to absorb light and CO₂

on the surface to a depth at a certain limit [6]. The culture that is on the inside of the reactor and not get the light can be improved productivity through stirring [7]. In order for the whole microalgae cells in culture ponds can equally get light and absorb CO₂, thus increasing productivity, it is equipped with a stirring system. In this study used the pedal stirring system and circulation pump. Temperature carbon emissions come from industry above 100°C, for it needed a tool to convert temperature into ambient temperature for growth of microalgae.

The aim of this research is to apply technology of capture CO₂ emissions generated by industries that use combustion systems by utilizing microalgae *Chlorella* sp. are cultivated in a bioreactor culture pond raceway types.

II. MATERIALS AND METHODS

The research was carried out in milk processing industry (PT Indolakto) located in Sukabumi, West Java, Indonesia. Analysis of samples carried out in the Laboratory of Chemistry and Ecotoxicology Laboratory of Environmental Technology. GEOSTECH building 820, Puspiptek, Serpong.

A. Microalgae Collection, Isolation and Identification

Microalgae used in this study were collected using a phytoplankton net (20 µm mesh opening), in a mesocosmos located in the institute fresh water pond. After collection they were taken to the laboratory for isolation and culture.

For the microalgae isolation were utilized excavated blades with the aid of a stereoscope microscope, where the cells were isolated one by one by using glass capillary needles, being subsequently inserted and maintained in test tubes (5 ml) containing synthetic cultivation medium. After isolation, consecutive samplings of the biological material were carried out until achieve the complete purification of microalgae.

In parallel, the identification of the isolated microalgae was performed in test tubes using appropriate laminas and laminules with the assistance of a binocular optical microscope, according to morphological and morphometric characteristics for analysis achieved at the lowest possible taxonomic level based on literature [8]. These cultures were kept in BOD incubator in synthetic medium at 25 °C, 1klux light, 12h photoperiod and constant stirring.

B. Bioreactor Culture Ponds

Bioreactor culture ponds raceway type are ponds that were equipped with pedals/propeller for stirring process, driving electromotor, CO₂ gas intake pipe and pipe freshwater and nutrient intake. Microalgae culture ponds are made of stainless steel with a volume of 1,000 liters, the depth of water in the pool can be set 20 cm, 15 cm, and 10 cm. The depth of water used in the experiment have been 20 cm. Raceway ponds was equipped with a transparent lid made of plastic (mica) to minimize the risk of contamination of the culture of microalgae. The water used was fresh water.

C. Inoculation of Microalgae

Species of microalgae was used *Chlorella* sp. which has been cultivated in the medium Benneck and then cultured in an acclimatized before advance by using a medium

containing NPK 35 mg/L which would then be used in pond culture systems. Initial density used about 300,000 cells/ml, calculated microscopically using haemocytometer.

The next activity was upscaling gradually from laboratory scale to larger scale. Larger scale carried out in plastic bags volume of 20 liters, equipped aeration. The medium used for the multiplication of the microalgae was water contain 35 mg/L NPK fertilizer. The abundance of culture can reach approximately 6 x 10⁶ cells/ml of medium after 3-4 weeks that was the level of the density can be transferred to the pond culture volume of 1,000 L.

The pond was filled with water as much as 950 L who previously had undergone a process of filtration using ultra filtration process to minimize the contamination of the culture of microalgae. Then put microalgae cultures into pond culture as much as 50 liters, until the initial density of 300,000 cells/ml. The next step was the application of fertilizer 35 mg/L NPK for the growth of microalgae. Microalgae began to be treated by CO₂ emissions in the culture pond after 3 weeks of culture,.

D. Operation of CO₂ Emissions into the Pond

Microalgae are heterotrophic micro-organisms that resemble plants, thus requiring light and CO₂ in addition to nutrients. Determination of CO₂ intake is based on the concentration of the gas being sampled from the output (effluent) boiler chimney.

Supplies to distribute CO₂ into the pond was such that the gas supply was placed in the gas holder was made of plastic. CO₂ in a plastic bag was conditioned with a concentration of about 6%. Another device was the gas piping from plastic bags to pond culture. To suck CO₂, used compressors. Gas relief valve installed in the effluent gas,. The exit gas were sampled to measure its CO₂ levels.

E. Data Sampling

In the culture ponds already available pipe/port to perform sampling gases released by the system. Port was located on the side of the upper pond, ½ inch diameter. Sampling was done 2 times a day, at around 09.00 am, 12.00 pm and 15.00 pm. Parameters were the concentration of oxygen and carbon dioxide gas. Equipment used in the form of portable multi-gas detector brands Riken type RX-515. Temperature measurement was carried out around the pond culture and implementation of measurements are made every day at around 9:00 am, 12.00 pm and 15.00 pm.

Light intensity affects the activity of microalgae, particularly in terms of photosynthesis. The tool used is a digital instrument Light Meter LX-101A models. Implementation of measurements carried out every day at around 9.00 am, 12.00 pm and 15.00 pm.

The growth of microalgae as a result of the response to CO₂ emissions observed by microscopic every day of the number of cells per millimeter using haemocytometer.

III. RESULTS AND DISCUSSION

PT. Indolakto, Sukabumi using three (3) boilers are fired 'heavy oil'. This fuel must be stored at a temperature of about 38 °C and at the time would have to be heated further pumped

between the temperature of 66 °C-121 °C. Each boiler with a capacity of 5 tons of steam per hour. In operation every day, PT. Indolakto using two (2) pieces of the boiler, while the other is in stand-by position. The operating pressure of the boiler reaches 8 bar. The flue gas temperature reaches 230 °C. Exhaust gas discharged through the chimney (towers) as high as approximately 10 meters.

To study the utilization of CO₂ from flue boiler conducted in PT. Indolakto, Cicurug, not done desulfurization process. That was because the PT. Indolakto use gas from the State Gas Company. According to PGN, the gas has a calorific value of 900 - 1300 BTU/SCF or 7703-11127 Kcal/m³.

Before performing the assembly and installation of pond culture, prior to design a series of studies. After design was done, then done the manufacturing units of operations required to perform research utilization and absorption of CO₂ using microalgae culture. Emissions from the boiler has high temperature, which is about 210 °C. The series of processes added were: piping, compressor and scrubber for particulate and SO_x absorbing, heat exchangers, cooling pools, and condensate reservoir.

Exhaust gas piping by inserting the tip of a catheter into the chimney was made to resemble a funnel. So that the gas flow occurs, then used a modified compressor. Modifications made were utilizing the intake air inlet as CO₂ gas inlet. The gas then flows through a process of absorption of the content of particulates and sulfur compounds and accommodated in the gas bag equalization. In the gas bag is made of gas quality measurement. When it reached the desired quality of the gas by using an aerator, gas flowed into the gas reservoir was ready to put in a pond culture. Gas flows from the bottom of the pond through a distributor (sparger), amounting to 6 pieces.

The system of gas from the gas reservoir into an organized culture using a timer. An aerator was used to transport the gas into the pond culture. An agitation process (using the pedal) and the introduction of gas into the pond done simultaneously and regulated by a timer. With such a system, the gas flow will be in contact with the culture medium in a longer time.

A. Cultivation of Microalgae

The observation of microalgae *Chlorella* sp. initially showed poor growth response to seem yellowish green color. The results of the nutritional content analysis of the media showed deficiencies P. After the addition of P was contained in the fertilizer NPK (16:16:16) as much as 35 mg/l, the microalgae color turns to green, fresh and seemed to occur growth. The addition of nutrients to the growth of microalgae are needed every 7 days and the addition of P is required every 14 days. Graph microalgal growth in period I and II are presented in Fig. 1 and Fig. 2. It shows that the culture pond environment has supported the growth of microalgae. Microalgae *Chlorella* sp able to absorb CO₂ by 15-20% concentration [9], [10]. Microalgae *Chlorella* sp is a cosmopolitan microalgae who mostly live in aquatic environments both freshwater, marine and brackish which contains many nutrients, are also found in the soil and in a humid place. Cell *Chlorella* sp. have a high reproductive rate, each cell *Chlorella* sp. capable of developing into 10,000 cells within 24 hours [7].

B. Analysis and Interpretation of Culture Pond Performance

Gas is sucked from the boiler chimney using piping (galvanized) ½ inch diameter. Gas discharge into the pond is about 2,091 L/min. The average concentration of CO₂ that goes into the pond is 5.2-5.9%. By setting a timer, then made a two-stage intake amount of gas that goes into a pond that each value of 0.21 g/L/day in the first period and 0.3 g/L/day in the second period. Conditions of gas into the pond can be seen in Table 1. It can be calculated that the CO₂ absorption efficiency 96.90% for the first period (days 1-30) (Fig. 1) and 98.87% for the second period (days 1-49) (Fig. 2).

Raising *Chlorella* sp. culture depends on the match between the types of microalgae are cultivated and some environmental factors, one thing to note is that the mixing factor does not interfere with the metabolism of microalgae cells [11].

Utilization of microalgae culture in the industry for the absorption of CO₂ emissions is necessary to control the photobioreactor. Efforts to use algae as a carbon sink requires knowledge about the types of suitable and optimum environmental conditions to encourage maximum growth [12], [13].

TABLE I: THE AVERAGE CAPACITY OF CO₂ ABSORPTION

Period	CO ₂ Input	CO ₂ Output	CO ₂ Absorption			Biomassa (max)
	CO ₂ Concentration (% vol)	CO ₂ Concentration (% vol)	CO ₂ Concentration (% vol)	g/L/day	Efficiency (%)	x 10 ⁵ (cells/ml)
I Days 1-30	5.2	0.3	6.11	0.21	96.90	156.2
II Days 1-49	5.9	0.5	6.68	0.30	98.87	890.2

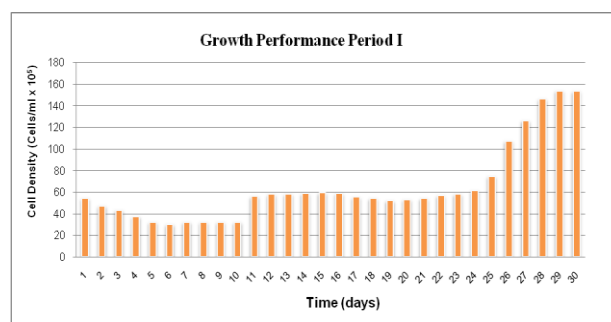


Fig. 1. Microalgal growth *Chlorella* sp in the first period giving CO₂ emissions.

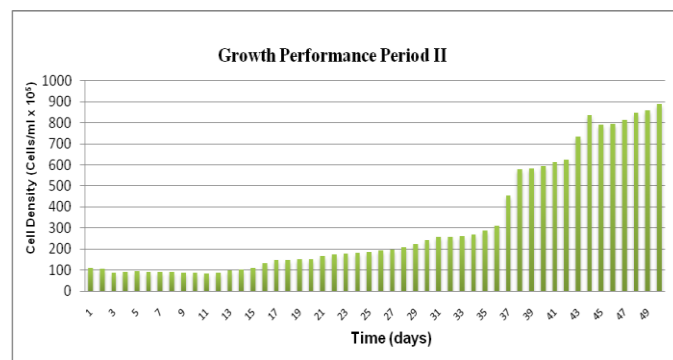


Fig. 2. Microalgal growth *Chlorella* sp in the second period giving CO₂ emissions.

C. Environmental Conditions and the Relationship of CO₂ Absorption

Recording the results of the temperature around the pond culture at 27°C on a measurement was around 09.00 am and 34°C on the measurement around 15.00 pm. If the temperature increases, the humidity decreases, until the recorded minimum humidity was 45% while the maximum was 70%. Microalgal growth was not seem affected by pH value and debit of CO₂ fluctuations around the pond (Fig. 3), because it was still in the range appropriate for metabolic activities.

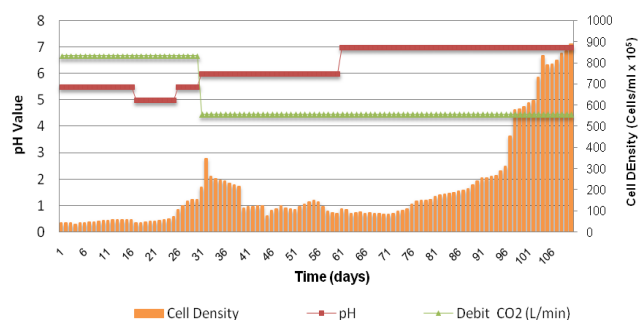


Fig. 3. Effect of pH value and Debit of CO₂ (L/min) to microalgal growth (x 10⁵ cells/ml).

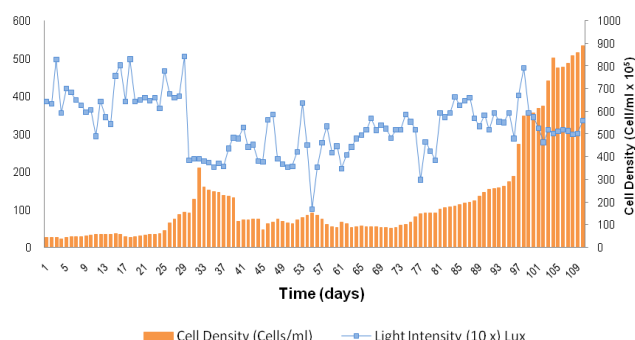


Fig. 4. Effect of light intensity to microalgal growth (x 10⁵ cells/ml).

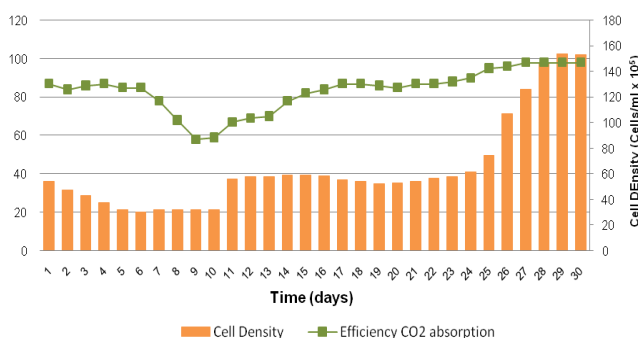


Fig. 5. The efficiency of CO₂ absorption by microalgae.

Measurements showed that the light intensity at 09.00 am about 50,000 lux, at 15.00 pm was reach 80,000 lux if sunny weather conditions. The range of light intensity was very good for the growth of microalgae (Fig. 4).

CO₂ absorption by microalgae showed the range 89% to 100%. High absorption occurs primarily in the sunny weather conditions and low absorption occurs in cloudy weather. Thus microalgae *Chlorella* sp. able to absorb CO₂ up to 98.87% of CO₂ absorption dynamics was not showed a decrease with increasing CO₂ and microalgal growth tends to increase (Fig. 5).

Microalgae *Chlorella* sp. able to absorb CO₂ at a

concentration of 15-20% [12], [13]. In this study, the CO₂ concentration used 5.2-5.9%. Thus, there is still possibility of giving the CO₂ concentration increased to 20%. In a given gas composition with a concentration of CO gas contained more than 1000 ppm, but the growth of microalgae was not indicate a problem. The study uses microalgae for the absorption of the industrial CO₂ emissions is mitigation impacts of air pollution [14]. The system was equipped with a culture pond heat exchangers demonstrated the ability to lower the temperature of the exhaust gas until it reaches a temperature that can be adapted by microalgae [15].

IV. CONCLUSION

The addition of nutrients to the growth of microalgae are needed every 7 days and the addition of P is required every 14 days.

Based on the microalgal growth and graphic element content of anions in the media pond appears that the growth of microalgae culture greatly influenced by the availability of nutrients in pond culture media.

Microalgae *Chlorella* sp able to absorb 21-29% concentration of CO₂

The efficiency of CO₂ absorption by microalgae *Chlorella* sp was 98.87% with CO₂ giving an average of 160.32 g/day.

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