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The Impact of Gas from Coal Combustion toward the Degree of Acidity (pH), Biomass Productivity, and Chlorophyll A and B Content of *Chlorella emersonii*

Ramanisa Muliani Talib[1], Abdi Dharma[1], Khandra Fahmy[2] and Syafrizayanti[1]*

[1]*Department of Chemistry, Faculty of Mathematics and Natural Science, Chemistry Department, Andalas University, Limau Manis Complex (Kampus limau Manis), Padang, West Sumatera - 25163, Indonesia*
[2]*Department of Agricultural Technology, Faculty of Technology Department, Andalas University, LImau Manis Complex (Kampus limau Manis), Padang, West Sumatra - 25163, Indonesia*

**Corresponding author*

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A B S T R A C T

Coal and other industries produced waste in the form of CO_2, SOx and NOx gases. CO_2 gas from industrial waste is a causal factor of greenhouse emission which could lead toward global warming. Chemical or biological mitigation can be a solution to face this environmental damage problem which is due to excess CO_2. Biological mitigation is one of the efficient ways to fixate CO_2. Lately, people chose microalgae, due to its capability on CO_2 fixation, also could produce biomass, along with other metabolite content it has. *Chlorella* a kind of microalgae is a strain which could resist toward high CO_2 level, and could potentially produce plenty of biomass, Chlorophyll a and Chlorophyll b. This study aimed to determine the impact of CO_2 (with concentration of 0.0%, 0.8%, 1.5%, and 2.3%) toward pH of the medium, specific growth, then the content of Chlorophyll a and Chlorophyll b. The growth of microalgae is determined by measuring the cell density using UV-Vis Spectrophotometer at 680 nm. Chlorophyll a and Chlorophyll b determined using Spectrophotometer. The result showed that the 2.3% concentration of CO_2 resulting from combustion altered the pH of the medium, and increased toward a higher production of biomass, Chlorophyll a, and Chlorophyll b.

Introduction

Global warming is a serious environmental problem. Excessive CO_2 gas emission from industries activities is one of the leading causes of greenhouse emission. An example of the most contributing industry toward the CO_2 emission is steam power plants. As a fuel, coal is used to replace oil in various industries, for instance steam power plants and cement industries. The combustion from either one will result in exhaust gases in form of CO_2, NOx, and SOx, which could cause air pollution, then leading up to global warming. Till 2019, the amount of coal that has been used is around 21.01 tonnes.

Statistical Review of World Energy (2019) reported that the CO_2 gas emission produced till 2019 reached 5,568.1 million tonnes (BP, 2020). There are various available solutions to reduce this emission, and biological mitigation is an efficient one. Biological mitigation is done by microorganisms able to capture CO_2 to photosynthesize, then proceed to produce energy (Li *et al.*, 2011). Aside from photosynthesis, CO_2 gases could impact the cultivation of microalgae growth (Chiu *et al.*, 2011). Continuous effort could be done biologically by the microalgae.

Microalgae are microorganisms who have chlorophyll and are capable of growing bigger to capture CO_2 compared to vascular plants (Wang *et al.*, 2008). *Chlorella* a type of microalgae, is a microalgae strain which resistible toward high CO_2 content. *Chlorella vulgaris,* is another microalgae which tolerant toward CO_2, with maximum level reaching 40% (Hanagata *et al.*, 1992). Another kind of *Chlorella* who could resist toward high level of CO_2 is *C. emersonii* strains. These microalgae could tolerate CO_2 with concentration reaching up to 15% from cement industrial gas waste. This study aimed to know CO_2 tolerance toward CO_2 gas fixation resulted from industrial combustion, which later could benefit food industry. Aside from CO_2 fixation, hopefully, the process of providing CO_2 could also produce the best growth and metabolism from *C. emersonii* microalgae. Based on these explanations, a research was done regarding the impact of CO_2 gas from the smoke of coal combustion toward specific growth, shifting of PH in the medium, also the content of chlorophyll a and chlorophyll b of *C. emersonii* microalgae. Thereby, the cultivated *C. emersonii* strain with maximum CO_2 concentration could benefit the industry in terms of CO_2 fixation along with increasing chlorophyll a and chlorophyll b content which could be useful for pigment of food.

Period and Place

This study was done in the Biochemistry Laboratory, Chemistry Department, Faculty of Mathematics and Natural Science, Andalas University.

Materials and Methods

UV-VIS Spectrophotometer (Thermo Scientific Genesyis 20), Compressor (Boston), Analytical Balance, Optical Microscope, Autoclave, Aerator, Centrifuge, Drop Pipette, Tube, CO_2 Sensor (COZIR-WX-5), and other glass apparatus. Microalgae from Biochemistry Laboratory of Andalas University. Coal acquired from Teluk Bayur. Bold's Basal Medium. Methanol.

Working Procedure

Morphological Assessment and Inoculation of *C. emersonii*

C. Emersonii microalgae culture acquired from microalgae culture stock in laboratory of Andalas University, Padang. Assessment was done using anoptical microscope (Olympus BX40), then cultivated using BBM (Bold Basal Medium).

Production Process, then Determining CO_2 Gas Concentration from Coal Combustion

Coal combusted inside the cylindrical tank, resulting in gas then vacuumed using a compressor. The concentration of CO_2 gas from the smoke of coal combustion is then determined using a CO_2 sensor (COZIR-WX-5) based on varied flow time (0-minute, 1 minute, 2.5 minute, and 4 minute) (Chiu *et al.*, 2011)

Microalgae Growth Rate

The density of microalgae cells was measured once in two days, till the culture reached stationary phase (26 days). The density was measured using the UV-Vis Spectrophotometer at 680 nm. Specific growth rate of microalgae then calculated using the following equation (Kawaroe *et al.*, 2009).

$$K = 3.22 \frac{\log \frac{N_t}{N_0}}{T_t - T_p}$$

Explanation

Nt : Density of the microalgae on t time

N0 : Early density of the microalgae, 3.22 is the constant

Tt : Observation time.

Determining Chlorophyll A and Chlorophyll B

The total of carotenoids is then determined using the Lichtenthaler method. 15 mg dried biomass dissolved into 5 mL methanol, then incubated for 30 minutes, and centrifuged at 49 °C for 10 minutes, 3500 rpm. The absorbent from the supernatant is then measured at 652 and 665 nm with UV-Vis Spectrophotometer. From the measured absorbance result then pigment content (chlorophyll a and chlorophyll b) calculated using the following equation (Pancha *et al.,* 2015)

Chlorophyll a (µg/mL) = 16,72 A665 – 9,16 A652

Chlorophyll b (µg/mL) = 34,09 A652 – 15,28 A665

Results and Discussion

C. emersonii Microalgae

The *C. emersonii* microalgae strain in this study was acquired from cultured microalgae in the Biochemistry Laboratory, Andalas University. Identification was aimed to figure out whether the used isolate was purely contained *C. emersonii* colony microalgae only, and wasn't contaminated by other kinds of microalgae colonies.

From the result, based on morphology, it can be identified that this type is classified as Chlorophyta group (green algae), *Chlorella* species. Microscopically, from the *Chlorella* cell characteristic can be seen that the cell form is circular with diameter 2 – 10 µm (Safi *et al.,* 2014). *C. emersonii* microalgae in this study has few characteristics, for instance in size around 5 – 7 mm, and has green pigment (chlorophyll).

CO_2 Gas Concentration from Coal Combustion

The CO_2 gas concentration varied based on the flow time of CO_2 gas resulting from coal combustion, which was measured using a CO_2 sensor (COZIR-WX-5). The amount of provided CO_2 gas from coal combustion smoke can be seen on Chart 4.1.

Duration of providing CO_2 gas from coal combustion smoke

The method of streaming CO_2 based on flow time was previously done by Chiu (2011), with the frequency for 12 hours each day (Chiu *et al.,* 2011). In our study, providing CO_2 was done by flowing it at various specified times.

The Impact of CO_2 gas from coal combustion smoke toward pH of microalgae media culture

The growing and metabolism of microalgae impacted by physical-chemical factors such as nutrition and pH. Environmental factors of culture media, as in factor of acidity level (pH) is one of the factors that need to be paid attention to, so the microalgae cell growing and metabolism process won't be disrupted. Changes in pH on microalgae growth generally affect mass, lipid and their primary metabolite content. Biomass content, chlorophyll, and amount lipids (Huang *et al.,* 2014).

Gases from coal combustion could contain various gases, such as CO_2, SOx and NOx. The impact of CO_2, SOx and NOx can be seen by the change of microalgae culture after being provided with smoke from coal combustion. As certain concentrations of CO_2 gas were provided to the culture media, the pH level of culture media decreased too.

Fig.1 Resulted from microscopic observation of microalgae culture (1000x magnification).

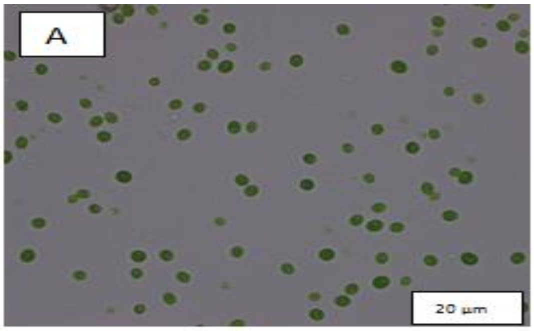


Chart.1 Frequency of Providing CO_2 Gas from Coal Combustion Smoke

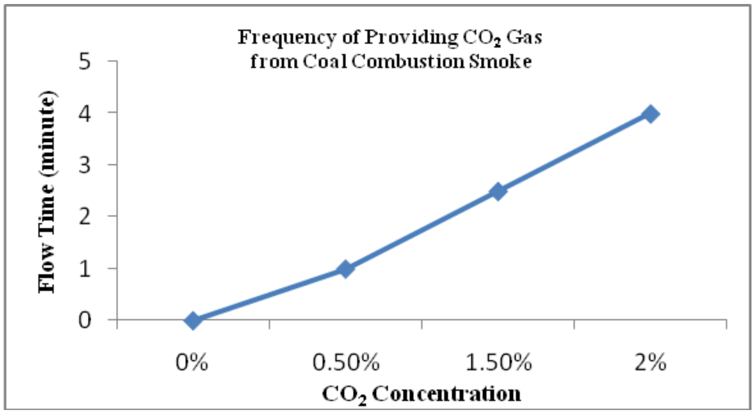


Figure 4.1 pH before providing CO_2

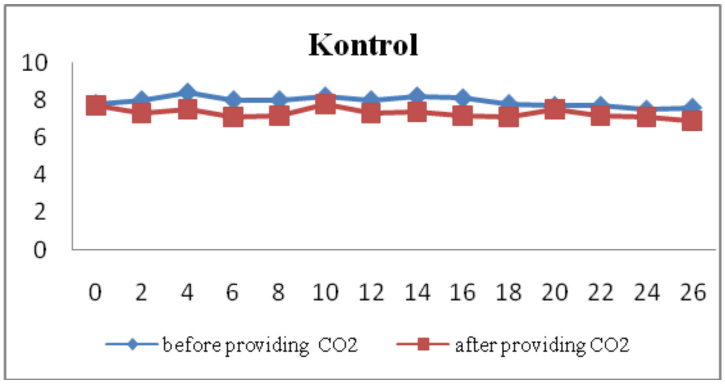


Chart.2 pH before providing CO_2

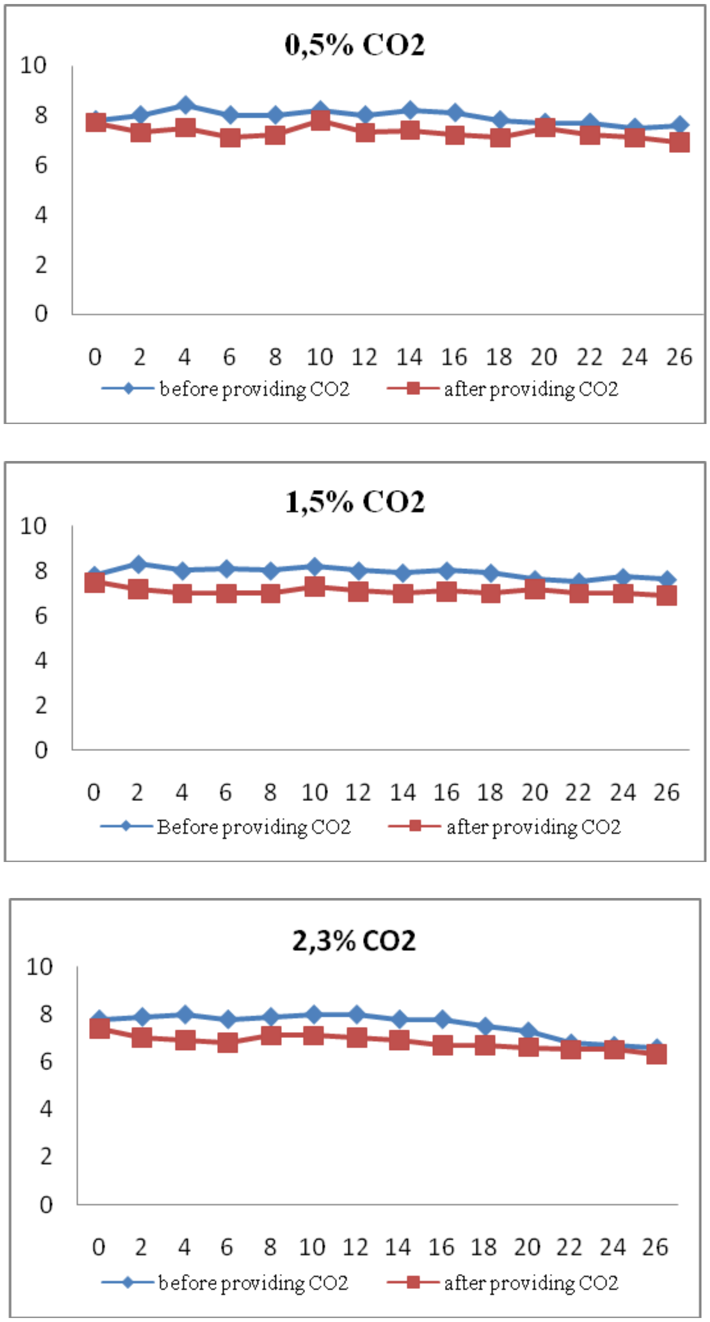


Chart.3 Various CO_2 Concentration toward Biomass Productivity

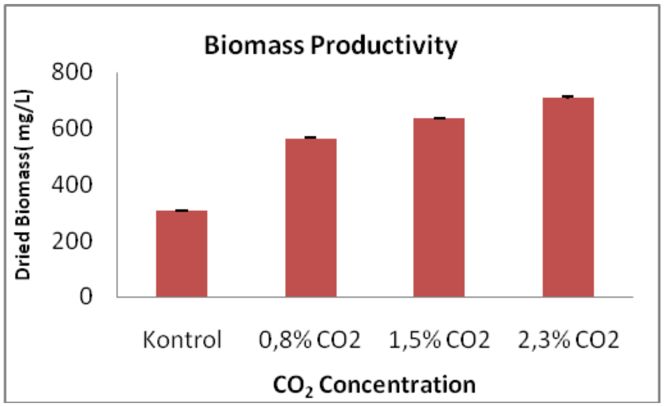
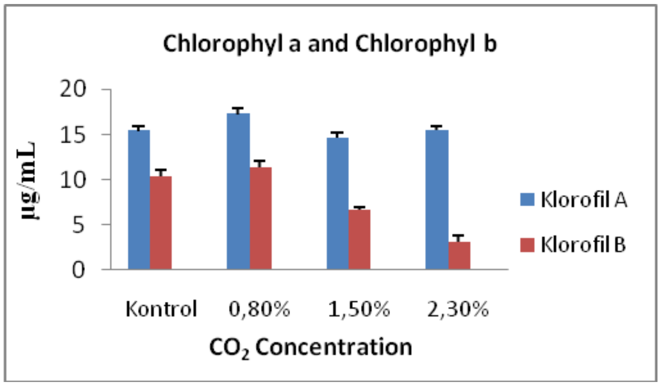


Chart.4 Content Chlorophyll a and b with CO_2 variant



The more CO_2 streamed into the microalgae culture, will decrease the acidity level (pH) of microalgae culture media. This change of acidity level (pH) using 2.3% CO_2 concentration (Figure 4.2).

Based on the measurement of pH level from various CO_2 concentrations, respectively 0.0%, 0.8%, 1.5%, and 2.3%, the decreasing acidity level (pH) can be seen after providing CO_2 gas from coal combustion smoke. This change in pH resulted into an acid environmentally medium. The change of pH level in culture media in 2.3% CO_2 gas concentration reached down to 6.3. Change of pH concurred in optimum pH range to grow *Chlorella* sp.

Even though there is decreasing pH level in culture media, *C. emersonii* microalgae still tried to balance their environment condition, so the cells could still regenerate.

Microalgae biomass productivity with varied CO_2

The increasing of CO_2 in culture media with specified concentration could increase the microalgae biomass productivity. The total biomass productivity (g/L) measured from the cultivation for 26 days. Providing varied CO_2 concentration from coal combustion smoke impacted the productivity of resulting biomass.

The longer flow time of streaming CO_2 gas from coal combustion, the more CO_2 concentration resulted inside the culture, which resulted in the increasing of biomass productivity (Figure 4.2).

The biomass productivity from *C. emersonii* microalgae when cultured using plain air resulted as 0.041 g/L (Cuellar-Bermudez *et al.*, 2015). A few studies regarding the providing of CO_2 gas from coal combustion smoke toward *Chlorella* microalgae has been done by various researchers. Yadav (2015) and Prevenkumar (2014) have done few studies, providing CO_2 gas from coal combustion smoke toward *Chlorella* s.p. and *Chlorella* s.p. KR-11 provided CO_2 concentration around 2 – 10% and 13.3%.

The resulting biomass is 0.015 – 1.4 g/L and 0.561 g/L (Yadav *et al.,* 2015), (Praveenkumar *et al.*, 2014). Same with previous studies, in our study, we also cultivated *C. emersonii* microalgae with 2.3% CO_2 concentration from coal combustion smoke, which resulted in the increase of the result compared to the control one, which is 704.6 mg/L.

Chlorophyll a and Chlorophyll b content with varied CO_2 concentration

The analysis toward Chlorophyll a and Chlorophyll b content was done using UV-Vis Spectrophotometer. The highest Chlorophyll a and Chlorophyll b pigment found at the 0.8% CO_2 concentration, which is 21,25 µg/ml, and 16,32 µg/ml.

According to Richmond (2004), when the photosynthesis process happens, chlorophyll will capture specific light, which is blue light (450 – 475 nm) and red light (630 – 675 nm).

Red light could increase chlorophyll pigment compared to white light (richmond *et al.,*). In our study, the CO_2 concentration from coal combustion smoke varied at 0.0%, 0.8%, 1.5%, and 2.3%, respectively.

The resulted chlorophyll a content, was 15.40664, 17.2538, 14.60036 and 15.4148 µg/mL, in the other side acquired chlorophyll b was 10.4046, 11.4170, 6.6921 dan 3.1444 µg/mL. The highest chlorophyll a and chlorophyll b based on the impact of CO_2 concentration from coal combustion smoke was at 0.8%.

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