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THE GROWTH OF *Ankistrodesmus* Sp IN RESPONSE TO CO_2 INDUCTION

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ABSTRACT

This research aims to know the effect of CO_2 induction to growth of fresh water microalgal cell of Ankistrodesmus sp. Microalgal culture was carried out by means of laboratory scale on Erlenmeyer tube containing Basal Bold Medium (BBM). The experiment design was completely randomized design in single factor with 3 levels: CO_2 induction, air induction and control (without treatment). The growth of Ankistrodesmus sp cell with air induction treatment was reach growth peak same as control at the 11^{th} day with cell density 1.063.166 cell.ml^{-1} and control 385.833 cell.ml^{-1}. Two peak of growth was occurred in CO_2 induction treatment, at the 9^{th} day with cell density 772.793 cell.ml^{-1} and the 13^{th} day with cell density 436.888 cell.ml^{-1}. The optimum growth of rate Ankistrodesmussp cell in CO_2 induction treatment was 1.59 cell division.day^{-1} at the 6.87^{th} day, in air induction 1.49 cell division.day^{-1} at the 7.69^{th} day and control 1.05 cell division.day^{-1} at the 3.96^{th} day. In the end observation, pH of culture medium in CO_2 induction treatment was reach 9.1, in air induction treatment 8.3 and control 7.6. The highest biomass of Ankistrodesmus sp cell was occurred in CO_2 induction treatment 2.4 g.l^{-1}, followed by air induction treatment 2.1 g.l^{-1} and control 1.6 g.l^{-1}. The highest chlorophyll consentration of Ankistrodesmus sp cell in CO_2 induction treatment 10.70 mg.l^{-1} followed by air induction teatment 10.57 mg.l^{-1} and control 7.84 mg.l^{-1}.

Keywords: Ankistrodesmus SP, CO_2, Growth

INTRODUCTION

Since a few decades ago, global warming has become a serious threat to human kind and the natural environment (Brennan and Owende, 2010). Increasing the temperature of the earth's surface can lead to extreme climate changes, rising sea levels, extinction of some species of organisms, the melting of icebergs in the poles and some other disaster. Rising global temperatures ca used by high levels of carbon dioxide gas (CO_2) in the atmosphere (Florides and Christodoulides, 2009). That way the seriousness of the threat of global warming, the Kyoto protocol in 1997 proposed the reduction of greenhouse gases about 5.2% of the emitted since 1990. Several mitigation measures

were attempted to carry out the CO_2 target of the proposal on the Kyoto protocol. Among the existing way of mitigation in biology, is the most promising. The benefits of CO_2mitigationinbiologyin addition to capturing CO_2 can also produce energy through photosynthesis (Li *et al.*, 2011).

Photo synthesis is performed by all plants, including microorganisms. However, plants are considered less efficient in capturing CO_2 for the slow growth rate. On the other hand, microalgae as photosynthetic microorganisms capable of capturing solar energy and more efficient CO_2 of about 10 to 50 times than in higher plants (Wang *et al.*, 2008).

There are more than 27,000 types of microalgae found in the earth and containing diverse chemicals that is worth selling (Hollar, 2012). Biomass resulting from the cultivation of microalgae has some uses (Sayre, 2010). 1) Sources of bio fuels (biodiesel and bio ethanol). 2) Nutritional supplements for human sin the form of tablets, capsules, powder and liquid, 3) Natural food colorings, 4) Natural food source for many species of fish, 5) Nutritional supplement for live stock in order to increase immunity and fertility, 6) Supplement ingredient cosmetics, 7) Source of valuable materials such as unsaturated fatty acids, ω-3 fatty acids, pigments and biochemical stable isotope, 8) Raw materials to form a "Biochar" through pyrolysis which can be used as biological fertilizers and carbon source, 9) Source of renew able hydrogen.

Microalgae can capture CO_2 from the atmosphere, or from the gas industry(for example, the exit gas from power plants) an din the form of dissolved carbonate($Na_2CO_3$and$NaHCO_3$) (Chang and Yang.S.S., 2003). In general, the concentration of CO_2frompower plants is higher than in the atmosphere. Low concentrations of CO_2 in the atmosphere causes the slow growth of microalgae because of slow mass transfer of gas. Capture efficiency of CO2bymicroalgaeincreasedwith the flow of gas from power generation to 15% CO_2 (Amaro *et al.*, 2011). Green microalgae (Chlorophyta) such as thes pecies of *Ankistrodesmus* sp has a simple structure which is less than 2mmin diameter. Growth of micro algae requires three main factors: sunlight, nutrients and carbon dioxide(CO_2). Therefore in this study will be used induction gas carbon dioxide (CO_2) to the growth of the *Ankistrodesmus* sp. can be maximized. This research aims to know the effect of CO_2 induction to growth of fresh water microalgal cell of *Ankistrodesmus* sp.

MATERIALS AND METHODE

Microalgae *Ankistrodesmus* sp. was derived from the collection of the Laboratory Department of Biology, Faculty of Science and Technology, State Islamic University Sunan Gunung Djati Bandung, was the result of isolation from the fresh waters Cibiru Bandung. Research carried out at room Algae Culture Department of Biology State Islamic University Sunan Gunung Djati Bandung. Culture conditions: temperature 26°C, humidity 82%, light intensity 2970 Lux, and

photoperiode 24 hours of light. Experimental design used wascompletely randomize design consisting of 3 treatments that was, without treatment (control), air induction (aerators) and the induction of carbon dioxide (CO_2). The mediumwas used bold basal medium (BBM).

Initial density of cells inoculated *Ankistrodesmus*sp10.000 cells.ml^{-1} in 500ml Erlenmeyer tubes containing 200 ml of culture medium, initial pH 6.5 treatment medium was regulated by the Addition of solution of HCl 1% and KOH1%. Calculation of the number or density of cells was done periodically every 24hours for 14 days, using Haemacy to meter. For the growth rate calculated by the following equation (Chrismadha *et al*., 2006):

$$\mu = \frac{\text{Ln } (Xt / Xo)}{t}$$

Description: μ = The rate of growth (cell division.day^{-1}), Xt= cell density at time t, Xo = initial cell density, t = time (days).

Biomass measurements obtained by knowing the weight of the organic materials. Samples of 10mlfilteredwith filter paper what man GF/A and heated in an ovenat100^{o}C. Then the sample was dried at a temperature of 600^{o}Cis made of as hove ran hour. Micro algae weight organic materials obtained by subtracting the weight of sample shave beenheated100^{o}C with ash weight after it was created. Measurement of chlorophyll content of cultures performed onthe 14th dayusing a spectra photo meter at a wavelength of 663nm and 645nm (Hosikian et al., 2010) Measurement of pH of culture media are also performed daily using a digital H meter to see the status of the availability of CO_2 in the culture media for microalgae cells are cultured. The Mean value of all datas were compared by variances analysis (ANOVA) and then Duncan's Multiple Range Test (DMRT) for pair wise comparison was used at the 5% significance level (Gomez and Gomez, 1995).

RESULTS AND DISCUSSION

Culture of micro algae *Ankistrodesmus* sp. during 14days of observation showed a diversegrowth pattern foreach given treatment. The growth in the aeration treatment could reach the peak similar to the controls at the 11thday with the cell density was almost three times of 1,063,166 cells.ml^{-1}wereas the growth in CO_2 treatment reach the peak at the 9th day of 772,793 cells.ml^{-1} (Figure 1).

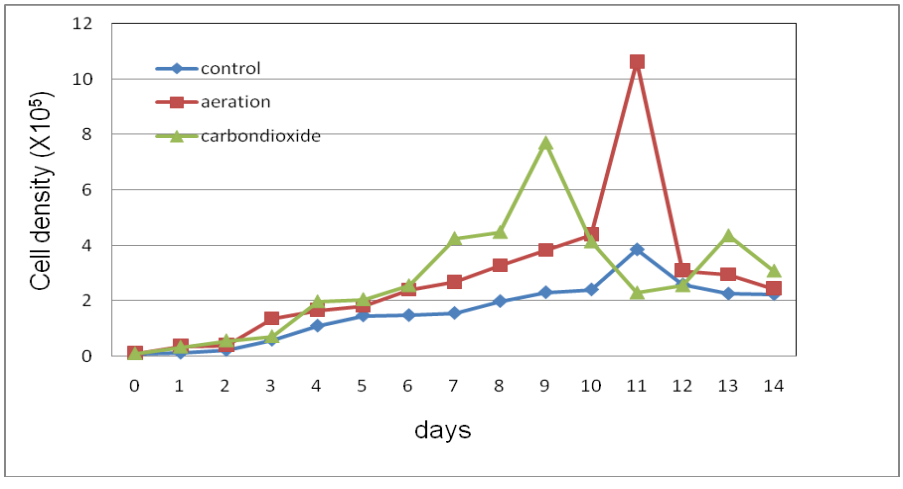
In the treatment of CO_2 induction, the two of growth peak of microalgae *Ankistrodesmus* sp. On BBM occuredon the 9th day and the 13thday respectively. Were as the control and treatmentof air induction, only had one peak of growth on the 11thday. This was probablydue to that induction of CO_2 could provide microalgae rapidly to carry out photosynthesis process, as nutrient sin the medium stillavailable andutilize for subsequent growth until it reached a second peak of growth. Cell density at the peak of growth for CO_2 induction treatment was lower than the air induction

treatment butit was higher than the control. Uunder the microscope view, it was found large cells of *Ankistrodesmus* sp. hadbeen growing on CO_2 induction.

The growth of microalgaeof *Ankistrodesmus* sp did not exhibit a phase of adaptation. This happeneddue to adaptation phase lasted less than 24 hours there fore it was not clearly observed. From the first day of the average density of the cell had begun improvement. Moreover, the absence of adaptation phasewas also due tothe utilization of medium in this experiment was similar to the maintenance medium. According to Pittman *et al.* (2011), a number of microalgae cell on the environmental conditions of culture media was similar to the previous culture of maintenance led to a phase of adaptation was not visible, or the cells had moved into the exponential phase.

At the exponential phase,the cell density in creased which cause rapid the multiplication process was still occured. Exponential phasewas likely occuredbecause the content of nutrients in the BBMwas stillavailable insignificant amounts, led to an increase in the growth and cell division.After reaching the peak of growth, cell density began decline, and then enterto stationary phase. The sstationary phase culture of microalgae associated with a reduced amount of nutrient sin the medium and accumulation of toxic metabolic was decompounds. In addition, the decrease occurred due to the reduced intensity of light received by microalgae cellswhich were caused by the phenomenon of the formation of shadows(self-shading) by microalgae cell sin the culture (Costa and Morais, 2011).

Figure-1. Growth of microalgae *Ankistrodesmus* sp. for 14daysonBold Basal Medium(BBM)



The growth patterns of *Ankistrodesmus* sp. for 14 days in culture of BBM with various treatments in accordance with the rate of growth in the number of cell divisions per day, form a hyperbolic curve with a quadratic regression equation (Figure 2, 3 and 4). Thiswas consistent with observations by (Mata *et al.*, 2010),which reported a growth rate of microalgae cultures that make up the hyperbolic curve.

Based on the hyperbolic curve showed the maximum of growth rate which obtained CO_2induction was 1.59cell division.day^{-1} on the 6.87th day, while in the air induction was 1.49 cell division.day^{-1} on the 7.69th day, and the control was 1.05 cell division. day^{-1} on the 3.96th day. From the curve of growth rate could be seen that the highest of *Ankistrodesmus* sp. growth per day with CO_2inductionwas1.59cell division.day^{-1}and no far from the treatment of the air induction was 1.49 cell division. day^{-1}. As reported by (Mutanda *et al.*, 2011), microalgae cells couldmultiplicate upto 3 timesper day. If cell of microalgaeobtained adequate nutrition, theywould grow well especially when supplied with CO_2 into the mediumwhich was associated with photosynthesis reaction. Induction of air through the aerator and the induction of CO_2 through a CO_2 gas cylinder wouldcreate a stirring in the medium so that each cellof microalgae wouldgetan adequate nutrition for the growth.

Figure-2. The growth rate of microalgae *Ankistrodesmus* sp. Without treatment (control) for 14dayson Bold Basal Medium (BBM). Regression equation: y= -0.10+0.28x -0.02x^2

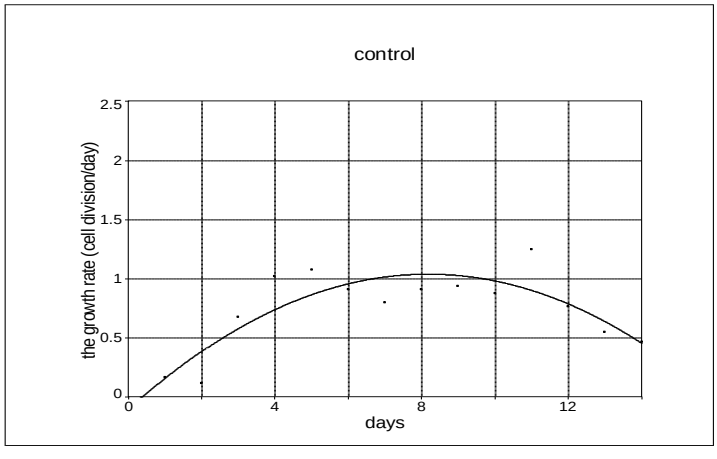


Figure-3. The growth rate of microalgae *Ankistrodesmus* sp. With CO2 air induction for 14 days on Bold Basal Medium (BBM). Regression equationy= 0.72+0.23x - 0.02x^2

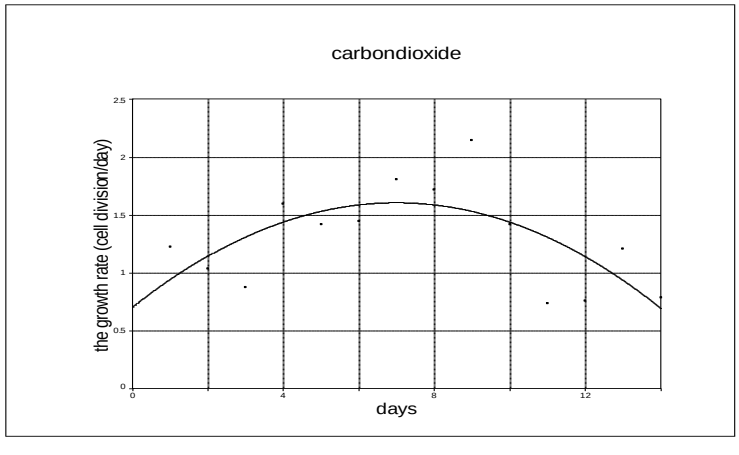


Figure-4. The growth rate of microalgae *Ankistrodesmus* sp. With Aeration induction for 14 days on Bold Basal Medium (BBM). Regression equation$y=0.71+0.26x - 0.02x^2$

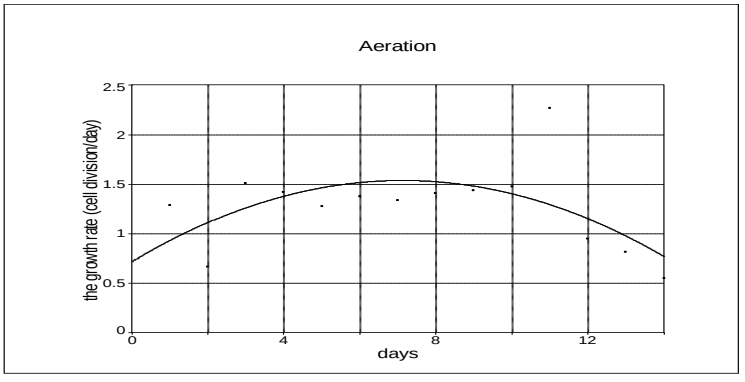
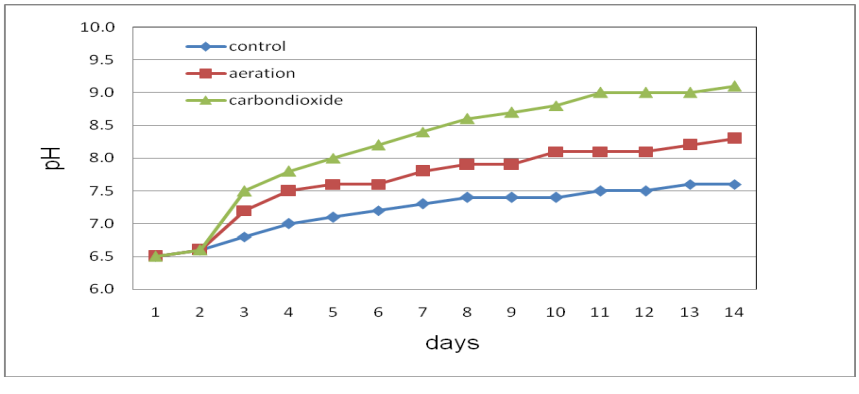


Figure 5 showed that the development of pH in culture medium increased both in controls and air induction as wellCO_2induction. Increasing the pH in the treatment of CO_2 induction was quite sharp reached 9.1 at the end of the observation, while in the air induction and control, the pH rise up to 8.3 and 7.6 respectively. Increasing the pH of the culture medium of *Ankistrodesmu* ssp. From the initial pH 6. 5 showed that all of microalgal cells using theCO_2which contained in the growth medium. Although the control and treatment of air inductionwas notsuppliedwithCO_2 gas, but microalgae cells still could obtained and utilize the existingCO_2in mediumfor growing well.

$CO_2$was used by microalgae as a source of carbon because of the nature of photo autotrophic life. $CO_2$was the main inorganic carbon sources for microalgae culture. Another source of organic carbon in the medium according to Singh *et al.* (2011), canobtain from carbonations (CO_3^{2-}) and bicarbonate ions (HCO_3^-). The use of CO_2 and bicarbonate ions by microalgae would decrease the concentration of CO_2, so it could increase the pH in the growth medium. The highest increased of pH occurred in the *Ankistrodesmus* sp culture medium that obtained CO_2 induction. It was possible, with the supply of CO_2 to the medium, the more microalgae cells to carry out photosynthesis which would increase the cell biomass of microalgae and pH in the medium wouldcontinue to increase.

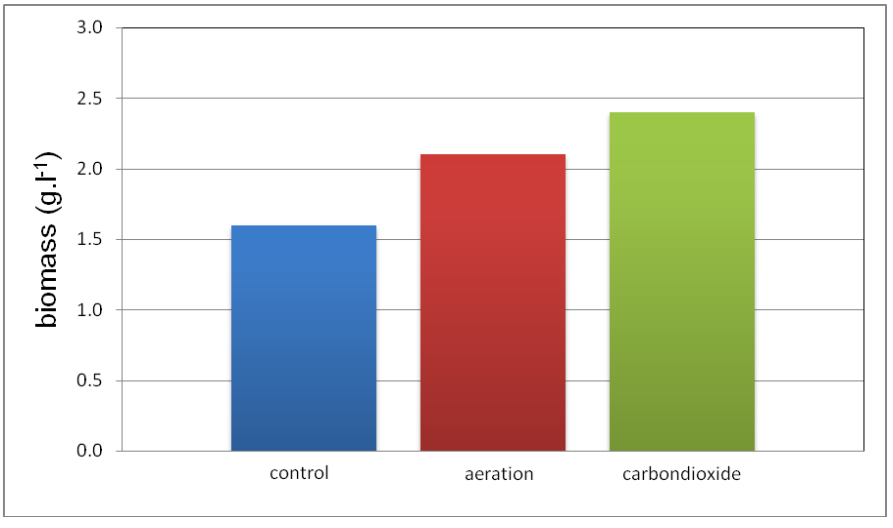
Figure-5. The development of pH in mediumof *Ankistrodesmus* sp. culture for 14days



Biomassimplied the organic matter content of microalgae cells would markedly increased when the medium induced by CO_2 gas (Figure 6). CO_2 was the sole source of carbon which was necessary for the growth of microalgae. As described by Khoo *et al.* (2011),in the growth of microalgae required sunlight, nutrients and CO_2. The presence of CO_2 in the medium would be utilized by the microalgae cells to carry out photosynthesis. According to (Widjaja *et al.*, 2009), the photosynthesis process would produce simple carbohydrates that could be converted into other organic compounds. Formation of organic compounds would increase the biomass of microalgae.

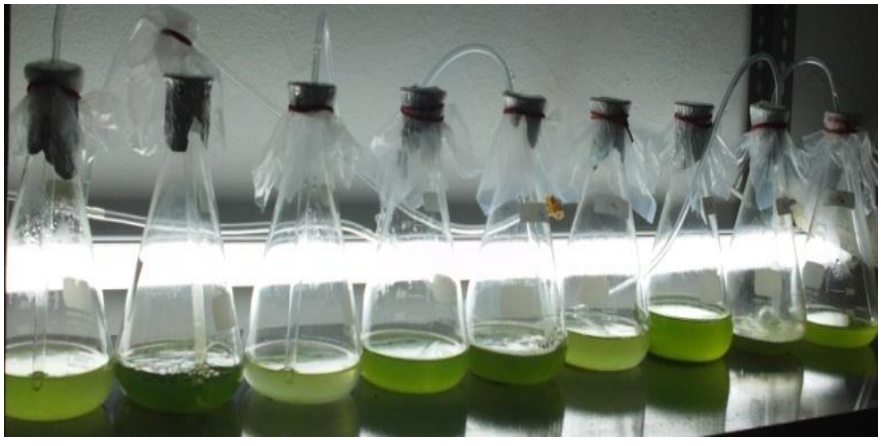
Nutrient content in the BBM was sufficient to support the growth of microalgae cells. Macro nutrients such as Mg, Ca, K, and P was used by microalgal cells as components of the cell, while the micro nutrients such as Fe, Zn, Mn, and Cu was required by microalgal cells either as an enzyme cofactor, and as a component for chlorophyll formation (Demirbas, 2011).

Figure-6. Biomass of *Ankistrodesmus* sp. at the end of the 14th day of observation



Increased biomass of microalgae cells could also be seen from the color change of culture medium(Figure 7). The color of micro algae culture mediumdue to chlorophyll asthe main pigment contained in the cell cytoplasm. At the beginning of the experiment, cultured microalgae grown in media treatment of pale white. This color of medium appeared which cause by theamount of microalgae cells were not proportional to the volume of media. The color of culture medium on the third day was like green apples, on the 7th day was green,on the 10th day was dark green and on the 14th daywas the final day of microalgae culture medium changed to yellow like copper. Changes in culture mediumof green color ranging from light green to green copper (yellow like copper) showed that the cell population increases with age of culture.

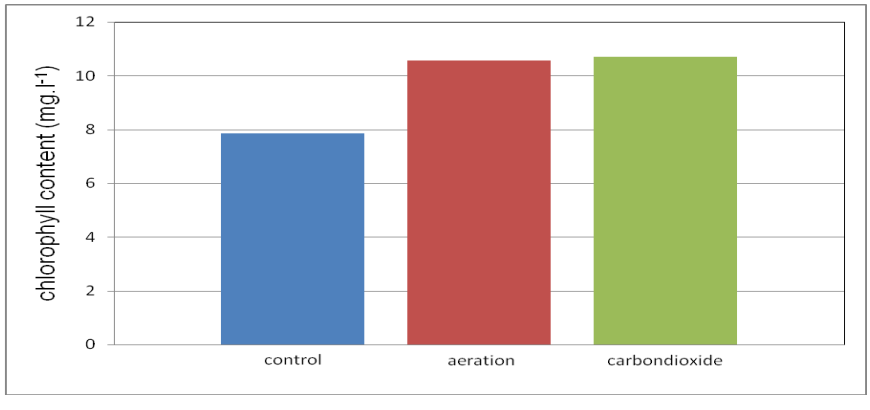
Figure-7. The 7th day of culture of *Ankistrodesmus* sp.



The green color indicatedan increase of population of culture cells, also indicated thecontaining of chlorophyllin microalgal cells. Chlorophyll content of micro algae *Ankistrodesmus* sp. cells were measured at the end day of the experimentorthe 14th day culture. In Figure 8.showed that microalgal cell treated by induction ofCO_2 has the highest chlorophyll content of 10.70mg.l^{-1} and statistically datawas not significantly different from the chlorophyll content of microalgae cells were treated air induction of 10.57mg.l^{-1}, while the control have the lowest chlorophyll content of7.84mg.l^{-1}.

Ankistrodesmus sp. cell densities were higher in cultures treated the air induction than the induction of CO_2 (Figure 1.) which was not associated with thecontent of chlorophyll. High content of chlorophyll which occured in *Ankistrodesmus* sp. cell cultures treated induction ofCO_2.This could occur due to high cell densities on *Ankistrodesmus* sp. Culture could inhibit the penetration of light into the column so that the culture would be reduced the process of photosynthesis and chlorophyll synthesis.

Figure-8.Chlorophyll content of *Ankistrodesmus* sp at the end of the observation ofthe 14th day culture



CONCLUSIONS

Ankistrodesmus sp cell growth for 14 days in culture medium treated air induction reached peak growth similar to the controls at the 11th day culture, but the highest cell density of 1,063,166

cells.ml^{-1}, whereas in the control of 385,833 cells.ml^{-1} . In the culture medium induced with CO_2 reached the first growth peak at the 9thday with a cell density of 772,793 cell.ml^{-1} and the second growth peak at the 13th day with a cell density of 436,888 cells.ml^{-1}. The growth rate of cell of *Ankistrodesmus* sp. cultures with induction of CO_2 was1.59 cell division.day^{-1}on the 6.87th day, while in the air induction treatment was 1.49 cell division.day-1 on the 7.69th day, and on the control was 1.05cell division.day^{-1} on the 3.96th day. At the end of the observation, the pH of the culture medium treated CO_2 induction reached was 9.1while in the air induction treatment and control reachedwas 8.3and7.6respectively.

The highest biomass of *Ankistrodesmus* sp cells in culture that had achieved the CO_2 induction treatment was2.4g.l^{-1}followed air induction treatmentand control were 2.1g.l^{-1} and1.6g.l^{-1} respectively.The highest chlorophyll content of *Ankistrodesmus* sp. cells achieved in culture treated CO_2 induction was 10.7 mg.l^{-1} followed air induction treatment and controlwere 10.57 mg.l^{-1} and 7.84 mg.l^{-1} respectively.

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