Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.5, No.1, 2015

The Chlorophyll Production and Hydrogen Produced Potency by Phytoplankton Chaetoceros Calcitrans, Chlorella Vulgaris, Dunaliella Salina, and Porphyridium Cruentum

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Abstract
The research about Determination of chlorophyll productivity and potency of hydrogen produced by phytoplankton *Chaetoceros calcitrans, Chlorella vulgaris, Dunaliella salina, and Porphyridium cruentum* have been done. It used sea water that was added by Conway medium and vitamin as culture media on the fourth species of phytoplankton test. Dry biomass of phytoplankton was extracted using acetone p.a. to take the chlorophyll. Analysis method was carried out by the sonication extraction for short and cheap in the lysis cell of phytoplankton and UV/VIS spectrophotometer to determine chlorophyll concentration through the Richards and Thompson equation. The result indicated that phytoplankton *C. vulgaris* produced the most amount of the chlorophyll and the potency of hydrogen are 72.541 µg/mL and 636,4 L/kg DW successively from the dry biomass 1,4187 g and the higher cell density 3.060 x 10^4 cell/mL. The amount of hydrogen indicated that *C. vulgaris* has potential as the source of renewable energy.
Keywords: *Chlorella vulgaris,* chlorophyll, energy, hydrogen, photosynthesis.

1. INTRODUCTION

Problems of energy demand in Indonesia is a serious problem in human life. Energy is an important component for human survival because almost all activities of human life depends on the availability of energy (Andewi and Hadi, 2011). Today and the next few years, people will experience an energy crisis, but the dependence on fossil energy sources is still a top priority because fossil energy source is capable of meeting the energy needs of humans on a large scale. While sources of alternative energy/ renewable energy can't meet the needs of large-scale human because of potential fluctuations and economic standards that have not been able to compete with conventional energy (Karyati et al., 2011).

One form of renewable energy today is of great concern in many countries, especially in developed countries is hydrogen (H_2). Hydrogen is projected by many countries will be future fuel more environmentally friendly and more efficient. Where the supply of energy produced is very clean because it only produces water vapor as emissions during the process (Muliawati, 2008).

Today, hydrogen is produced exclusively by electrolysis of water or steam reformation/methane gas. However, this process commercially more expensive than the production of hydrogen from natural gas (Muliawati, 2008). Hydrogen can also be produced through the process of photosynthesis by phytoplankton (Hemschemeier et al., 2009), which were analyzed in this research.

The process of oxygenic photosynthesis begins with the use of sunlight to the oxidation of water molecules and culminates with the conversion of inorganic minerals (eg carbon dioxide, nitrate, and sulfate) (Kundu et al., 2012). It is quite interesting because of the water as a fuel and abundant existence in the form of water, biomass or hydrocarbons, as well as nontoxic (Carlsson et al, 2007) and this is what will be the basic concept of this research.

Photobiological for hydrogen production by green algae provides a promising prospect as a source of energy for our future. The fact that the result is a clean fuel that is produced from light and water that occurs both as a resource that is abundant in nature. Algae that serve as biohidrogen not require fertile soil and don't affect fresh water resources, can be produced using sea water and waste water, and biodegradable, and relatively harmless to the environment if abundant (Kundu et al., 2012).

Chaetoceros calcitrans a diatom phytoplankton species, brownish yellow, has a size of 6-8 µm (Sumeru, 2008), and according to Boney (1983), cell moal composed of silicate and its structure is rather hard. *Chlorella vulgaris* is a phytoplankton dinoflagellates, cell moal composed of cellulose (Boney, 1983), and its chlorophyll is green (Anggreani, 2009). According Chinnasamy et al (2009), size ranges from 2-8 µm diameter. *Dunaliella salina* is a phytoplankton dinoflagellates cell moal composed of cellulose and strukutrnya rather soft (Boney,

1983), has two flagellates (Abusara et al, 2011), chlorophyll is green, and the diameter ranges from 9-11 µm (Fazeli et al, 2006) . Red microalgae *Porphyridium cruentum* is kind of dinoflagellates that cell moal composed of cellulose (Boney, 1983), diameter cell is around 4-9 µm and cell structure consists of a nucleus (core), chloroplasts, Golgi bodies, mitochondria, mucilage, starch, and vesicles (Lee, 1989). So, it seems clear that the reason for choosing the four phytoplankton above is because there are differences in terms of phytoplankton and chlorophyll color, type of cell moal, and the diameter of phytoplankton itself.

Robert Emerson and William Arnold perform key experiments in 1932 that provided the first evidence to link many chlorophyll molecules in the conversion of energy during photosynthesis. Chlorophyll contained in chloroplasts would absorb sunlight and will turn it into a form of chemical energy. The energy will be used to carry out the process of splitting water in plants to produce hydrogen gas and oxygen gas. Experiments were conducted on *Chlorella* cell (unicellular green algae) showed that only 1 molecule H_2 and 2 molecules O_2 produced for 2500 molecules chlorophyll (Berg et al., 2002) to make the process of water splitting. It's mean, the more chlorophyll contained in a microalgae, the more the process of photosynthesis that occurs, and the more the production of hydrogen produced. This process is the basis concept of this research was to determine the potential amount of hydrogen produced.

Chlorophyll-a and chlorophyll-b has a structure almost the same. The structure of chlorophyll-a is $C_{55}H_{72}O_5N_4Mg$ whereas chlorophyll-b is $C_{55}H_{70}O_6N_4Mg$, each with Mg as central atom. Difference between the two is located in the CH_3 group on the chlorophyll-a were substituted with HC = O on chlorophyll-b. Molecular weight for chlorophyll-a is 893 and chlorophyll-b is 907 (Riyono, 2007).

The principle of the method for the measurement of chlorophyll by spectrophotometry based on the maximum absorption by chlorophyll extract in acetone in the red region of the spectrum (wavelength 630-665 nm). Principles used in this method is based on the law of Lambert and Beer, the absorption at a certain wavelength of light is a function of the concentration of dissolved substances, absorption coefficient and the light path length cuvette. Maximum absorption for chlorophyll a, b, and c occur at 3 wavelengths, namely 665, 645, and 630 nm (trichromatic). Absorption at a wavelength of 665, 645, and 630 nm is reduced by absorption at a wavelength of 750 to the correction of turbidity. Extracted chlorophyll content can be calculated by Richards and Thompson (1952):

Klorofil a = 15,6 E_{665} – 2,0 E_{645} – 0,8 E_{630} µg/mL
Klorofil b = 25,4 E_{645} – 4,4 E_{665} – 10,3 E_{630} µg/mL
Klorofil c = 109 E_{630} – 12,5 E_{665} – 28,7 E_{645} µSPU/mL

where E is the absorption at the wavelength in question (eg E665 = absorption at 665 nm wave). Based on the formula, expressed in units of chlorophyll c µSPU (micro Specified Pigment Unit). To calculate the chlorophyll-c levels in seawater samples (in units of mg /L or mg/m3), then the above value multiplied by a factor (k) (Riyono, 2006):

$$k = \frac{Va}{Vs \, X \, d}$$

where; Va = volume of acetone extract (mL), Vs = volume of sea water samples were filtered (mL), d = width cuvette, path length (cm)

Based on the above description, then conducted research on "Determination of Chlorophyll Production and Potential Hydrogen produced by phytoplankton *Chaetoceros calcitrans*, *Chlorella vulgaris*, *Dunaliella salina*, and *Porphyridium cruentum*".

2. Materials

The materials used in this research are the phytoplankton *Chaetoceros calcitrans* cultures derived from Balai Riset Perikanan Budidaya Air Payau Maros, Chlorella vulgaris, Dunaliella salina, and Porphyridium cruentum from Balai Budidaya Air Jepara, sea water from the coast of Makassar , acetone p.a., alcohol, $FeCl_2.6H_2O$, $MnCl_2.4H_2O$, H_3BO_3, Na-EDTA, $NaH_2PO_4.2H_2O$, $NaNO_3$, $ZnCl_2$, $CoCl_2.6H_2O$, (NH_4) $6MoO_{24}.4H_2O$, $CuSO_4.5H_2O$, Vitamin B_{12}, Vitamin B_1, $Na_2SiO_3.5H_2O$, distilled water, filter paper, and aluminum foil.

3. Instruments

The tools used in this research are are the glass tools which are generally used in the laboratory, set philips 40 watt fluorescent lamps, jars made of glass, pots, hoses, stone aerator, aerator, salinometer, haemositometer, vacuum pumps , Buchner funnel, centrifuge, ultrasonic, Nikon type 102 microscope and spectrophotometer UV / Vis Shimadzu UV-2600 UV PROBE series.

4. Period and Place of Research

This research was conducted at the Laboratory of Inorganic Chemistry, Laboratory of Organic and Integrated Laboratory, Department of Chemistry, Faculty of Sciences at the University of Hasanuddin Makassar on June 2012 - February 2013.

5. Procedure

5.1. Preparations of Conway Media

one liter of stock A is boiled and added to 2 mL of stock B. Conway solution mixture was added to the sterile sea water containing no phytoplankton (1 mL per 1 L of sea water), then add 1 drop of stock C. For the Phytoplankton cell moal made of silica, added 1 mL of stock D.

5.2. Culturen of *Chaetoceros calcitrans, Chlorella vulgaris, Dunaliella salina,* and *Porphyridium cruentum*

Seawater is collected in a container and then sterilized and filtered using filter paper, and then measured salinity by using a salinometer. After that, sterile seawater added by medium Conway, then do the aeration for CO_2 conditioning, and added phytoplankton.

To obtain the appropriate salinity of sea water for phytoplankton species test done by dilution or concentration. How to obtain the desired density of phytoplankton used dilution formula:

$V_1 \times N_1 = V_2 \times N_2$

Where; V_1 = Volume needed, V_2 = Volume culture,

N_1= Density of stok phytoplankton cell & N_2= Density of culture phytoplankton cell

Measurements of phytoplankton cells using a microscope Haemositometer with observations. Observations of growth patterns observed in the conditions without and with exposure to metals Fe(II). After 3 days, the culture was transferred to jars made of glass with a volume of about 3 L. During the implementation of the culture, the physico-chemical parameters maintained.

5.3. Determination Growth Time of Phytoplankton Chaetoceros calcitrans, Chlorella vulgaris, Dunaliella *salina,* and *Porphyridium cruentum.*

Determination of phytoplankton growth pattern, done by counting the number of cells per milliliter of medium every 24 hours. Samples are taken with a sterile pipette, dropped about 0.1-0.5 mL at Haemositometer, then observed through a microscope. When the cell density is normal, the density calculation using the formula:

$$\text{amount}\frac{\text{cell}}{\text{mL}} = \frac{\textit{cell amount 4 dialog}}{\textit{blok amount } (=4)} x\ 10.000$$

If the cell density is too length, calculation using the formula:
Cell amount/mL =Amount cell on 4 part x 4 x 10.000

5.4. Determination Chlorophyll Concentration Use Spectrofotometer UV/Vis.

Once known optimum phytoplankton growth pattern test, the samples of each biomass of phytoplankton *Chaetoceros calcitrans, Chlorella vulgaris, Dunaliella salina*, and *Porphyridium cruentum* has dried, crushed with a porcelain dish and weighed the total dry biomass. Phytoplankton powder reconstituted with 5 mL of acetone p.a.. Then extracted using Ultrasonic for 60 minutes at a temperature of 50 ° C. After that, the solution centrifuged. The filtrate is separated, then the resulting filtrate was measured with a spectrophotometer UV/Vis wavelength waves at 665, 645, and 630 nm. Data analysis was calculated by the equation Richards and Thompson (1952):

Chlorofil a = $15,6\ E_{665} - 2,0\ E_{645} - 0,8\ E_{630}$ µg/mL

Chlorofil b = $25,4\ E_{645} - 4,4\ E_{665} - 10,3\ E_{630}$ µg/mL

5.5. Determination Potential Amount of Hydrogen produce by Phytoplankton *Chaetoceros calcitrans, Chlorella vulgaris, Dunaliella salina,* and *Porphyridium cruentum*

Analysing the potential amount of hydrogen generated is done by comparing the number of chlorophyll mole obtained with the number of hydrogen moles by photosynthesis reaction.

6. RESULTS AND DISCUSSION

6.1. The Growth Cell of Phytoplankton *Chaetoceros calcitrans, Chlorella vulgaris, Dunaliella salina,* dan *Porphyridium cruentum*

Observations of growth patterns of phytoplankton cells performed every fourth day for 18 days one time growth in the culture medium with the addition of sea water as well as vitamins Conway medium. The fourth chart growth patterns of phytoplankton cells is shown in Figure 1.

According Martossudarmo and Wulani (1990), in Budidaya (2009), phytoplankton growth is generally characterized by four separate stages , namely the stage adaptation, exponential phase, stationary phase , and death phase . Based on the graph shown in Figure 1, it can be seen that the time needed by phytoplankton growth *C. calcitrans, C. vulgaris, D. salina*, and *P. cruentum* to adapt to the culture medium (sea water is added to the medium Conway and vitamins) is quite short, ie two days . This can be seen in the chart cell density, where the

density of cells on the first day to the second day have not shown a significant amount of cell growth, it is due to very small number of cells fragmentation process. Significant growth began to take place on the third day, which means the cell fragmentation process that occurs from optimal . Significant growth process occurred until 11th day for *C. calcitrans* with the cell density around 1238 x 10^4 mL/cell , and 14th day for *C. vulgaris* with the highest cell density amounted to 3060 x 10^4 mL/cell and *D. salina* with the highest cell density of 515 x 10^4 mL/cell , and day 16 for *P. cruentum* with the highest cell density amounted to 2387 x 10^4 mL/cell. After reached the top of the cell fragmentation process, then the process of cell fragmentation does not occur again, which means that the rate of growth was balanced by mortality. This stage is called the stationary phase . Stationary phase began on day 12 for *C. calcitrans*, day 15 for *C. vulgaris* and *D. salina*, and day 17 for *P. cruentum*. Stationary phase of growth occurs due to the amount of phytoplankton cells in the culture medium increased, but the amount of nutrient content in the culture medium decreased. The next four stages of phytoplankton experiencing death, the rate of decrease in cell number due to cell death is higher than the rate of cell growth so that the population density decreases. According Rusyani (2001), a decrease in cell number due to both nutrient content and media culture are in limited quantities. In early cultures, nutrient content is high , which is utilized by each phytoplankton for growth process. Increase in cell number stops at the cusp of the population, at that point nutrient needs become greater , while the nutrient content in the media decrease because does not the addition of nutrients . In addition, there is competition for places to live because the greater the number of cells in a fixed volume . According to Fogg (1975) in Utomo et al (2005), the shadow population of cells (self shading) also cause a reduction in the intensity of the light is absorbed so can result in death. The third factor that causes the death of an individual and at the same time minimize the number of cells that grow, so after experiencing the peak will decrease the number of cells .

The usung of Conway medium and vitamins as phytoplankton culture medium for fourth with initial density of 10 x 104 cells/mL medium for *C. calcitrans*, *C. vulgaris*, and *P. cruentum* and 20 x 104 cells/mL medium for *D. salina*. *C. calcitrans* increased as much as 28 times the initial density culture for 14 days, *C. vulgaris* as much as 69.5 times and *D. salina* as much as 10.5 times the initial density culture for 15 days, as well as *P. cruentum* 85 times the initial density culture for 17 days.

According to Garofalo (2010) in Mayasari (2012), nutrient needs are highly correlated with morphological characteristics of phytoplankton, in this case the cell size and rate of movement of cells, ie 6-8 µm for *C. calcitrans* (Sumeru, 2008), 2-8 µm for *C. vulgaris* (Chinnasamy et al, 2009), 9-11 µm for *D. salina* (Abusara et al, 2011), and 4-9 µm for *P. cruentum* (Lee, 1989).

Figure 1 shows the pattern of the four phytoplankton growth in the medium condition Conway and vitamins which is the amount contained the same nutrients. From the figure it can be seen that the amount of cell growth of phytoplankton in the fourth highest to the lowest in a row is *C. vulgaris*, *P. cruentum*, *C. calcitrans*, and *D. salina*. This is because the morphological characteristics of *C. vulgaris*. *C. vulgaris* has the smallest cell size when compared with *P. cruentum, C. calcitrans*, and *D. salina*, which led to the greater surface area of the cell so that the entry of nutrients into the cell tissue occurs sooner. Moreover, according Mayasari (2012), *C. vulgaris* does not have a locomotor flagella, unlike the three other phytoplankton, so the nutrients are only used for the growth and movement of cells only, not for movement.

However, when compared growth between *C. calcitrans, D. salina*, and *P. cruentum*, then *P. cruentum* cells had a growth rate higher than *C. calcitrans* and *D. salina*. When compared again between *C. calcitrans* and *D. salina*, *C. calcitrans* has a higher growth rate than *D. salina*. This occurs because the cell size of the phytoplankton. The smaller size of phytoplankton cells, the greater the surface area of the cell, so that the process of entry of nutrients into the cell tissue occurs sooner. Cell size order from the smallest to the biggest in a row is *P. cruentum* 4-9 µm, *C. calcitrans* 6-8 µm, followed by *D. salina* 9-11 µm.

6.2. Determination The Amount of Chlorophyll Phytoplankton *Chaetoceros calcitrans, Chlorella vulgaris, Dunaliella salina,* and *Porphyridium cruentum*

Determination of the amount of phytoplankton chlorophyll done by solvent extraction method using acetone p.a., wherein the dry biomass of each phytoplankton weighed prior to extraction. Extraction process performed by the method sonifikasi. This is done to shorten the time and amount of extract produced far more, but the volume of solvent used is much less when compared to other extraction methods. This method utilizing ultrasonic waves that can destroy the cell so as to accelerate the process of mass transfer of compounds from the cell to the solvent. Chlorophyll amount of data on each species of phytoplankton can be seen in Table 1. The Table 1 showed that the four species of phytoplankton were cultured with media Conway with the addition of vitamins in volume 500 mL, has different of chlorophyll content. According Madja (1997) in Kurniawan et al (2010), species differences in plants cause chlorophyll biosynthetic capacity is not the same between the species with other species. This is because the chlorophyll biosynthesis performed by specific genes in the chromosome. According to Wang et al (1974) and Suzuki et al (1997), these genes encode enzymes that will have a role in the biosynthesis pathway tetrapirol (core porpirin) as the central structure of chlorophyll.

Table 1 shows the highest amount of chlorophyll content in *C. vulgaris* with the total amount of chlorophyll as many as 72.541 mg/mL of 1,4187 g of dry biomass and the amount of the highest cell density of 3060 x 104 cells/mL, then *D. salina* as many as 59.258 mg / mL of 1,1778 g of dry biomass and the amount of the highest cell density of 515 x 104 cells/mL, *P. cruentum* as many as 29.442 mg/mL of 2,3965 g of dry biomass and the amount of the highest cell density of 2387 x 104 cells/mL, and *C. calcitrans* as many as 15.558 mg/mL of 0,9548 g of dry biomass and the amount of the highest cell density of 1238 x 104 cells/mL.

According Herlinah (2010), *C. calcitrans* has a more dominant carotenoid content when compared with the pigment chlorophyll. According Yudha (2008), the phytoplankton pigment related to the pigment contained in phytoplankton, is what causes the cell *C. calcitrans* golden yellow to brown as the pigment content karotenoidnya more dominant. Cell *D. salina* has a yellowish green color, according Borowitzka and Borowitzka (1998) in Yudha (2008), *Dunaliella* containing pigment chlorophyll a and b and carotenoid pigments are generally in the form of β-carotene. According to Bellinger and Sigee (2010), the green color is caused *Chlorella* cells contain the pigment chlorophyll a and b in large numbers, as well as carotene and xantofil. Whereas for *P. cruentum*, has fikoeretrin and phycocyanin pigment content which is much higher than the chlorophyll content (Kusmiyati and Agustini, 2007). Fikoeretrin and phycocyanin is what causes the cells of *P. cruentum* red.

Based on the theory put forward by researchers above, it seems clear why *C. vulgaris* and *D. salina* contain the pigment chlorophyll more when compared with *C. calcitrans* and *P. cruentum*, the chlorophyll pigment content is more dominant when compared to *C. calcitrans* and *P. cruentum* that contain less pigment chlorophyll. This was confirmed by Kurniawan et al (2010), which states that some aquatic plants that have a low content of chlorophyll pigment have compound other more abundant plant to assist in carrying out the process of photosynthesis . However, when compared between *C. vulgaris* and *D. salina*, pigment chlorophyll content of *C. vulgaris* more than *D. salina*. This is because the morphological properties of the phytoplankton. Cell size or the size of the leaves on phytoplankton affect the intensity of light that can be absorbed by phytoplankton that would affect the amount of chlorophyll contained in the phytoplankton. According Djukri and Purwoko (2003) in Kurniawan et al (2010), the surface area of the leaves or the cell will render efficient capture light energy for photosynthesis normally at low light intensity conditions. Leaf morphology or larger phytoplankton cells in *D. salina*, allowing optimal light capture, but in *C. vulgaris* which has leaf morphology or smaller phytoplankton cells with higher numbers , are able to optimize the absorption of light at the surface of the leaf or whole cell, so that high total chlorophyll content. Morphology thus allowing light absorption can occur in all the cells making up the body, so that the entire cell will be able to synthesize chlorophyll. Another thing that causes the total amount of chlorophyll *C. vulgaris* which is abundant because of the shadow population of cells (self shading) because abundant amount of cell density in culture medium leads to reduced intensity of the light is absorbed, so according Irwanto (2009) in Kurniawan et al (2010) which states that the result of the limited light because it was blocked by the shadow of its own cell population , will spur the formation of phytoplankton chlorophyll to capture light render efficient, so abundant chlorophyll content . Moreover, according to Bothwell (1989) in Rahman (2010), an increase in chlorophyll production will only occur if the level of nutrients in the environment is very low and the increase will be moderate chlorophyll when higher levels of nutrients in the environment. This can be attributed to the number density of phytoplankton cells in *C. vulgaris* which has the most number of high cell growth compared with the three other phytoplankton. Due to the amount of cell growth *C. vulgaris* is the most high, causing nutrient needs are limited in the medium with fixed volume would be higher as well, which resulted in the amount of nutrients in the medium will continue to experience a drastic reduction caused by the absorption of nutrients into the cell, so that it will spur an increase in the amount of chlorophyll in phytoplankton is caused by the lower amount of nutrients in the culture medium

6.3. Determination Amount of potency Hydrogen Phytoplankton *Chaetoceros calcitrans, Chlorella vulgaris, Dunaliella salina,* and *Porphyridium cruentum*.

the number of potential hydrogen and energy produced by dry biomass used for each species of phytoplankton can be seen in Table 2. ranging from the most to the least in a row is *C. vulgaris, D. salina, C. calcitrans*, and then *P. cruentum*. The resulting number of potential hydrogen contained in most phytoplankton *C. vulgaris* with the amount of hydrogen produced as much as 636.4 L/kg DW and energy generated at 1641.9 Kcal/kg DW, and *D. salina* as much as 626.2 L/kg DW and energy produced as much as 1615.5 Kcal/kg DW, *C. calcitrans* as much as 202.8 L/kg DW energy generated at 523.2 Kcal/kg DW, and *P. cruentum* as much as 152.9 L/kg DW and the resulting energy of 394.5 kcal/kg DW.

Based on Table 2, it appears that the higher total chlorophyll contained in the phytoplankton species, the potential of the hydrogen produced more and more so that the energy generated greater. This occurs because chlorophyll is the pigment that helps in the process of photosynthesis, which is the pigment that will absorb the energy from the light and the light will be used to break water molecules (water thermolysis) to produce hydrogen gas. Therefore, the more chlorophyll contained in phytoplankton, the more often the process of

photosynthesis occurs and the more hydrogen gas is produce.

CONCLUTION

Phytoplankton *Chlorella vulgaris* has potential to produce hydrogen total chlorophyll and most consecutive 72 541 mg/mL and 636.4 L/kg of dry biomass weighing 1.4187 g with the highest cell density of 3060 x 104 cells/mL.

REFERENCES

Abusara, N.F., Emeish, S., and Sallal, A.K.J., 2011, The Effect of Certain Environmental Factors on Growth and β-carotene Production by *Dunaliella sp*. Isolated from Dead Sea, *Jordan Journal of Biological Science*, **4** (1): 29-36.

Andewi, N.M.A.Y. dan Hadi, W., 2011, *Produksi Gas Hidrogen melalui Proses Elektrolisis Air sebagai Sumber Energi,* skripsi tidak diterbitkan, Jurusan Teknik Lingkungan, Fakultas Teknik Sipil dan Perencanaan, Institut Teknik Sepuluh November, Surabaya.

Anggreani, N., 2009, *Penentuan Parameter Pencemaran berdasarkan Keragaman Jumlah Fitoplankton Cholerra sp. di Perairan*, skripsi tidak diterbitkan, Jurusan Teknik kimia, Fakultas Teknik, Universitas Indonesia, Jakarta.

Berg, J.M., Tymoczko, J.L., and Stryer L., 2002, *Biochemistry 5th edition*, New York, WH Freeman.

Boney, A.D., 1983, *Phytoplankton*, Edwar Arnold (Publishers) Limited, London.

Budidaya P., 2009, *Budidaya Pakan Alami (Fytoplankton, Zooplankton, dan Benthos)*, (online), (http://ardivedca.blogspot.com/, 25 Mei 2012, at 22.45 wita).

Carlsson, A.S., Bellen, J.B.V., Moller, R., and Clayton, D., 2007, *Micro and Macro Algae: Utility For Industrial Applications*, EPOBIO Project, USA.

Fazeli, M.R., Tofighi, H., Samadi, N., Jamalifar, H., and Fazeli, A., 2006, *Carotenoid Accumulation by Dunaliella tertiolecta (Lake Urmia Isolate) and Dunaliella salina (CCAP 19/18 & WT) under Stress Condition*, Department of Drug and Food and Pharmaceutical Science Research Center, Iran.

Hemschemeier, A., Melis, A., and Happe, T., 2009, Analytical approaches to photobiological hydrogen production in unicellular green alga, *Photosynth res,* **102** (2009): 523-540.

Herlinah, 2010, *Karakteristik Genetik Berbagai Spesies Chaetoceros Serta Analisis Pemanfaatannya pada Perbenihan Udang Windu (Panaeus monodon)*, laporan tidak diterbitkan, Dewan Riset Nasional Kementrian Negara Riset dan Teknologi, Jakarta.

Karyati, Y., Rahmawati, I., Rachmawaty, R., Addarojah, Z., 2011, *Manajemen dan Konservatif Energi Analisa Biohidrogen*, skripsi tidak diterbitkan, Jurusan Teknik Kimia, Fakultas Teknik, Universitas Diponegoro, Semarang.

Kundu, K., Kulshrestha, M., Dhar, N., and Roy, A., 2012, Production of Hidrogen as a Potential Source of Renewable Energy from Green Algae, *2012 IACSIT Coimbatore Conferences,* **28**: 57-62

Kurniawan, M., Izzati, M., dan Nurchayati, Y., 2010, Kandungan Klorofil, Karotenoid, dan Vitamin C pada Berbagai Spesies Tumbuhan Akuatik, *Buletin Anatomi dan Fisiologi*, **18** (1): 31-37.

Kusmiyati dan Agustini, N.W.S, 2007, Uji Aktivitas Senyawa Antibakteri dari Mikroalga *Porphyridium cruentum, Jurnal Biodiversitas* **8** (1): 48-53.

Lee, E.R., 1989, *Phycology,* Second edition, Cambridge, Cambridge University Press.

Mayasari, E., 2012, *Efek Penambahan Fe^{2+} dan Mn^{2+} Terhadap Produktifitas β-Karoten oleh Fitoplankton Dunaliella salina, Isocrysis galbana, dan Chlorella vulgaris*, thesis tidak diterbitkan, Program Magister Ilmu Kimia, Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Hasanuddin, Makassar.

Muliawati, N., 2008, *Hidrogen sebagai Sel Bahan Bakar Sumber Energi Masa Depan*, skripsi tidak diterbitkan, Jurusan Teknik Kimia, Fakultas Teknik, Universitas Lampung, Lampung.

Rahman, A., 2010, Nutrien Pembatas di East River; Korea, *Bioscientiae*, **7** (2): 1-16.

Riyono, S.H., 2006, Beberapa Metode Pengukuran Klorofil Fitoplankton Laut, *Oseana*, **31** (3): 33-44.

Riyono, S.H., 2007, Beberapa Sifat Umum dari Klorofil Fitoplankton, *Oseana,* **32** (1): 23-31.

Rusyani, E., 2001, *Pengaruh Dosis Zeolit yang Berbeda terhadap Pertumbuhan Isochrysis galbana Klon Tahiti Skala Laboratorium dalam Media Komersial*, skripsi tidak diterbitkan, Progran Studi Budidaya Perairan, Fakultas Perikanan dan Kelautan, Institut Pertanian Bogor, Bogor.

Sumeru, S., 2008, Artikel Budidaya Perikanan, (online), (http://www./2008_10_01_arcive.html, diakses tanggal 25 Mei 2012, pukul 01.30 wita).

Suzuki, J.Y., Bollivar, D.W., dan Bauer, C.E., 1997, Genetic Analysis of Chlorophyll Biosynthesis, *Annual Review of Genetics,* (31): 61-89.

Utomo, N.B.P., Winarti, dan Erlina, A., 2005, Pertumbuhan *Spriluna plantesis* yang Dikultur dengan Pupuk Inorganik (Urea, TSP, dan ZA) dan Kotoran Ayam, *Jurnal Akuakultur Indonesia,* **4** (1): 41-48.

Wang, W.Y., Wang, W.L., Boynton, J.E., dan Gillham, N.W., 1974, Genetic Control of Chlorophyll

Biosynthesis in *Chlamydomonas, The Journal of Cell Biology*, (63): 806-823.

Yudha, A.P., 2008, *Senyawa Antibakteri dari Mikroalga Dunaliella sp. Pada Umur Panen yang Berbeda*, skripsi tidak diterbitkan, Program Studi Teknologi Perikanan Fakultas Perikanan dan Ilmu Kelautan, Institut Pertanian Bogor, Bogor.

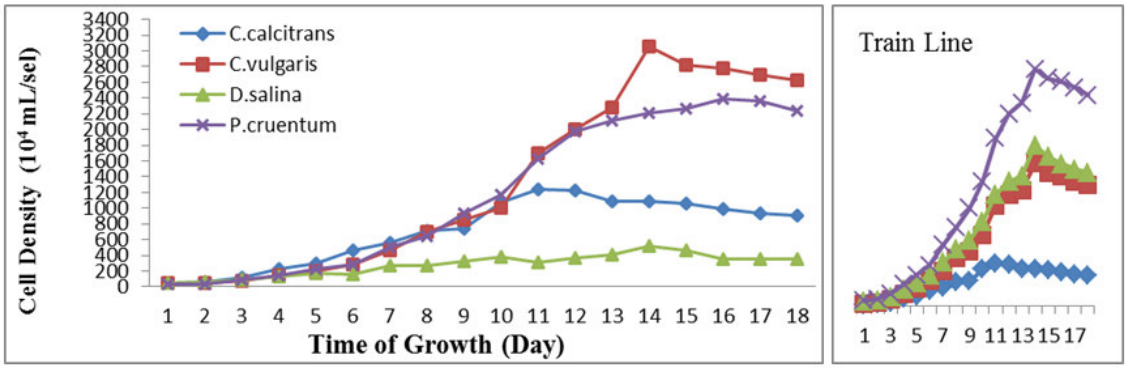

Figure 1. Growth Pattern of *C. calcitrans, C. vulgaris, D. salina, and P. cruentum* Phytoplanktons

Table 1. The Amount of cell density higher and Chlorophyll concentration from each spesies phytoplankton on volume 500 mL media

NO	Phytoplankton Species	Cell Density Higher (10^4 sel/mL)	Dry Biomass (g)	Amount Chlorophyll a (µg/mL)	Amount Chlorophyll b (µg/mL)	Total Klorofil (µg/mL)
1	*Chaetoceros calcitrans*	1238	0,9548	11.782	3.776	15.558
2	*Chlorella vulgaris*	3060	1,4187	56.859	15.682	**72.541**
3	*Dunaliella salina*	515	1,1778	40.342	18.916	59.258
4	*Porphyridium cruentum*	2387	2,3965	25.432	4.010	29.442

Table 2. The Number of Hydrogen Potency and energy produced from each dry mass phytoplankton, cultured in the same conditions.

No	Phytoplankton Species	Dry Mass (g)	Dencity Cell (10^4 cell/mL)	Amount Chlorophyll (µg/mL)	Potency H_2 produced (L/kg DW)	Energy (Kcal/kg DW)
1	*C. calcitrans*	0,9548	1238	15.558	202,8	523,2
2	*C. vulgaris*	1,4187	3060	**72.541**	**636,4**	**1641,9**
3	*D. salina*	1,1778	515	59.258	626,2	1615,5
4	*P. cruentum*	2,3965	2387	29.442	152,9	394,5

The Table 2 shows the potential of the hydrogen produced by each phytoplankton

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