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THE INFLUENCE OF MEDIA COMPOSITION TO THE GROWTH OF *SCENEDESMUS* SP. MICROALGAE IN VARIED MEDIA

PENGARUH KOMPOSISI MEDIA TERHADAP PERTUMBUHAN MIKROALGA *SCENEDESMUS* SP. PADA BERBAGAI VARIASI MEDIA

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ABSTRAK

Media kultur merupakan salah satu faktor yang penting dalam pertumbuhan mikroalga. Media kultur mengandung nutrisi makro dan nutrisi mikro yang dibutuhkan untuk pertumbuhan mikroalga. Komposisi nutrisi yang lengkap dan konsentrasi nutrisi yang tepat menentukan produksi biomassa dan kandungan gizi mikroalga. Tujuan kegiatan ini adalah untuk mengetahui pengaruh variasi media terhadap pertumbuhan mikroalga Scenedesmus sp pada skala laboratorium. Pada skala laboratorium dilakukan uji variasi media pertumbuhan mikroalga. Media uji ini digunakan untuk melihat pertumbuhan mikroalga. Media-media pertumbuhan yang digunakan yaitu : media BBL sm, SS, MN, M4N, dan "Sederhana 2". Mikroalga yang digunakan adalah mikroalga Chlorophyceae mixed culture yang didominasi oleh Scenedesmus sp. Percobaan dilakukan secara batch dengan tiga kali pengulangan (triplo). Parameter yang diamati adalah pH, optical density (OD), kepadatan sel dan temperatur. Hasil penelitian menunjukkan bahwa dari 6 variasi media pertumbuhan yang digunakan pada penelitian ini ternyata media M4N adalah media yang paling baik untuk pertumbuhan mikroalga. Nilai kepadatan sel pada media M4N di akhir operasi mencapai 306,83 x 10^6 sel/mL dengan warna kultur hijau pekat yang masih bertahan hingga akhir operasi. Nilai koefisien pertumbuhan Scenedesmus sp. yang ditumbuhkan pada media M4N adalah 0,36. Hal tersebut kemungkinan diakibatkan komposisi nutrisi pada media M4N lebih lengkap dibandingkan media lainnya.
Kata Kunci: *mikroalga, media pertumbuhan*, *scenedesmus* sp.

ABSTRACT

Media culture is one of the important factors in the growth of microalgae. Culture medium contains macro and micro nutrition required for the growth of microalgae. Composition of nutrition and nutrition concentrations determine the production of microalgae biomass and nutrient content. The aim of this study is to evaluate the effect of medium composition variation to *Scenedesmus sp* growth in laboratory-scale in order to get the optimized composition of media for microalgae *Scenedesmus sp.* This study was conducted in a laboratory scale. The growth medium that were used in this study were: BBL sm, SS, MN, M4N, "Sederhana 2", and "Sederhana 3" medium. The microalgae that were used in this study were mixed culture Chlorophyceae microalgae which is dominated by *Scenedesmus sp.* The parameters observed were pH, optical density (OD), cell density and temperature. The results show that of the 6 variations of the growth medium used in this study M4N medium is the best medium for the growth of microalgae. At the end of the operation, cell density of *Scenedesmus sp*. from M4N medium was 306.83 x 10^6 cell/mL with a dark green color for the culture. The coefficient growth of *Scenedesmus sp.* which is grown in M4N medium

was 0,36. This is likely to be due to the composition of nutrients in the medium M4N which were more complete than other media.

Keywords: Microalgae, Growth Medium, *Scenedesmus* sp.

I. INTRODUCTION

The global energy crisis today must be handled soon. Continued exploitation of fossil fuel as unrenewable energy for the consumption of industry, transportation and households depletes it existence in nature. Many countries, including Indonesia, have been looking for alternative fuel resources which are categorized as renewable and environmentally friendly materials.

One of the basic materials that has potential to be developed is the biomass of microalgae Chlorophyceae aquatic species, which is a group cells of microalgae that contain quite a lot of algae oil. Microalgae are single-celled plant microorganisms, which multiplies very quickly with a relatively short life cycle. Microalgae are part of photosynthetic eukaryotes group with a simple cell structure from unicellular to multicellular forms. As long as there are water and sun, microalgae can be found in various environments, including land, ice, lakes, rivers, craters and seas. Microalgae have an ability to capture CO_2 which may convert the energy of sunlight into chemical energy (Deng et al. 2009). Microalgae production fields are smaller than the tall plants and produce more biomass per unit of time. Some species of microalgae are known to have oil content which is quite varied. They include *Botryococcus braunii* 25%-75%, *Chlorella* sp 28%-32%, *Spirulina platensis* 4%-16,6%, *Scenedesmus obliquus* 11%-55%, *Scenedesmus* sp. 19,6%-21,1% (Chisti 2007; Mata et al. 2010). *Scenedesmus* sp. contains 8-56% protein, 10% -52% carbohydrates, 2% -40% fat and 3%-6% *nucleic acid* (Kawaroe et al. 2010).

Scenedesmus is cosmopolitan microalgae. Most *Scenedesmus* sp.can live in aquatic environments such as freshwater and brackish water. *Scenedesmus* sp. colonies consisting of 2,4,8, or 16 cells arranged laterally (Graham et al. 2000). Media culture is one important factor in the growth of microalgae. The culture medium contains macro nutrients and micro nutrients required for the growth of microalgae. Complete nutrient and proper nutrient concentrations determine the production of microalgae biomass and nutrient content (Prihantini et al. 2007). The objective of this study is to evaluate the effect of medium composition variation to microalgae *Scenedesmus* sp growth in laboratory-scale and in order to identify the most optimized composition of media for microalgae growth.

II. METHODOLOGY

A. The source and Microalgae Species type.

Microalgae cultures used are mixed cultures dominated by *Scenedesmus* sp. obtained from the microalgae species collection which are available in biotechnology laboratory of Process Technology, PPPTMGB "LEMIGAS".

B. Media Growth

The growth media used in this study are media BBL sm, SS, MN, M4N, "Sederhana 2", and "Sederhana 3". Sterile distilled water was used as control without any addition of other fertilizers. Before use, the medium was sterilized using an autoclave at 121°C temperature for 15 minutes. The composition of the growth media conducted in this study are shown in Table 1.

C. Parameter Test and Analysis Laboratory

An observation process was carried out for 17 days. Parameters measured included pH, OD_{680}, temperature, and cell density. Measurement of pH was carried out using a pH meter, on the other hand OD was determined using a spectrophotometer at wavelength of 680 nm. The temperature was measured using a calibrated thermometer. The cell density was calculated based on a linear regression graph of the relationship between OD and cell density for microalgae *Scenedesmus* sp. The regression equation is: $y = 136.5 x - 0.799$, where y is the cell density, and x is OD.

D. Operating Conditions

This experiment was carried out in 500 ml Erlenmeyer and conducted in a batch with 3 repetitions. Comparison of microalgae seeding and growth medium was 1: 10. During the experiment, the erlenmeyer was aerated with filtered air and illuminated with artificial white light fluorescent continuously. Aeration was performed by an air pump, producing aeration which supplied CO_2 gas required for the growth of microalgae, stabilize

Table 1
The composition of growth media (in 1000 ml)

Media BBL sm	Media SS	Media MN	Media M4N	Media "Sederhana 2"	Media "Sederhana 3"
$NaNO_3$- 50 g	Solution A - 9 ml :	Na_2EDTA-0,016 g	$CaCl_2$-0,01 g	Urea-0,1 g	Urea-0,3 g
$FeCl_3$- 1 g	$NaNO_3$- 10 g	$MgSO_4.7H_2O$-0,25 g	$MgSO_4.7H_2O$-2,5 g	TSP- 0,05 g	TSP- 0,1 g
Na_2-EDTA- 5 g	$Na_2HPO_4.12H_2O$-1 g	KNO_3-1 g	$FeSO_4.7H_2O$-0,003 g	ZA- 0,025 g	ZA- 0,3 g
Na_2HPO_4- 10 g	$NaHCO_3$- 16,8 g	$FeSO_4.7H_2O$-0,002 g	KNO_3-5 g	Na_2EDTA-0,0005 g	
Urea- 40 g		Trace element - 1ml :	KH_2PO_4-1,25 g	Trace element-1 ml :	
ZA- 30 g	Solution B-1 ml :		Solution A5-1 ml :		
Trace metal-0,5 ml :		H_3BO_3-2,86 g		$FeCl_3.6H_2O$-	
	Na_2-EDTA- 3 g	Na_2MoO_4-0,021 g	H_3BO_3- 2,86 g	H_3BO_3-	
$ZnCl_2$- 2,1 g/100 ml	$CuSO_4.5H_2O$ 0,0004 g	$CuSO_4$-1,8 g	$MnCl_2.4H_2O$-1,81 g	$ZnCl_2$-	
$CuSO_4.5H_2O$- 2 g/100 ml	$CoCl_2.6H_2O$-0,0008 g	$MnSO_4.4H_2O$-1,3 g	Na_2MoO_4- 0,21 g	$Na_2MoO_4.2H_2O$-	
$CoCl_2.6H_2O$-2 g/100 ml	$MnCl_2.4H_2O$- 0,27 g	$ZnSO_4.7H_2O$-0,22 g	$CuSO_4.5H_2O$-0,08 g	$CoCl_2.6H_2O$-	
$(NH_4)_6Mo_7O_{24}.4H_2O$-0,9 g/100 ml	$FeCl_3.6H_2O$- 0,24 g		$ZnSO_4.7H_2O$-0,22 g	$MnCl_2.4H_2O$-	
	$ZnCl_2$- 0,03 g		H_2SO_4 concentrated-1 drop	$CuSO_4.5H_2O$-	
	H_3BO_3- 3,44 g				

Source : Chang and Yang (2003) and Harrison and Berges(2005)

pH and homogenize the nutrients contained in the growth medium. The room temperature ranged between 24-26°C, while the culture temperature is 23-25°C. The initial OD of microalgae was 0.344. The configuration of the experiment equipment is exhibited in Figure 1.

E. Data Analysis

The quantitative data obtained was then transformed into a graph. Cell growth coefficient (k) calculated using a formula (Valderrama et al. 2002):

$$k = \frac{Log\left(\frac{N_t}{N_0}\right)}{t_t - t_0} \; x \; 3{,}22$$

where: N_0 = initial cell population density, N_t = the cell density at time t; T_0 = initial time, T_t = time of observation; 3,22 = a constant value.

Figure 1
Experiments configuration

III. RESULTS AND DISCUSSION

Growth of ***Scenedesmus*** sp.

1. Optical Density

Microalgae growth mainly depends on the nutrients that are given. Selection of the appropriate

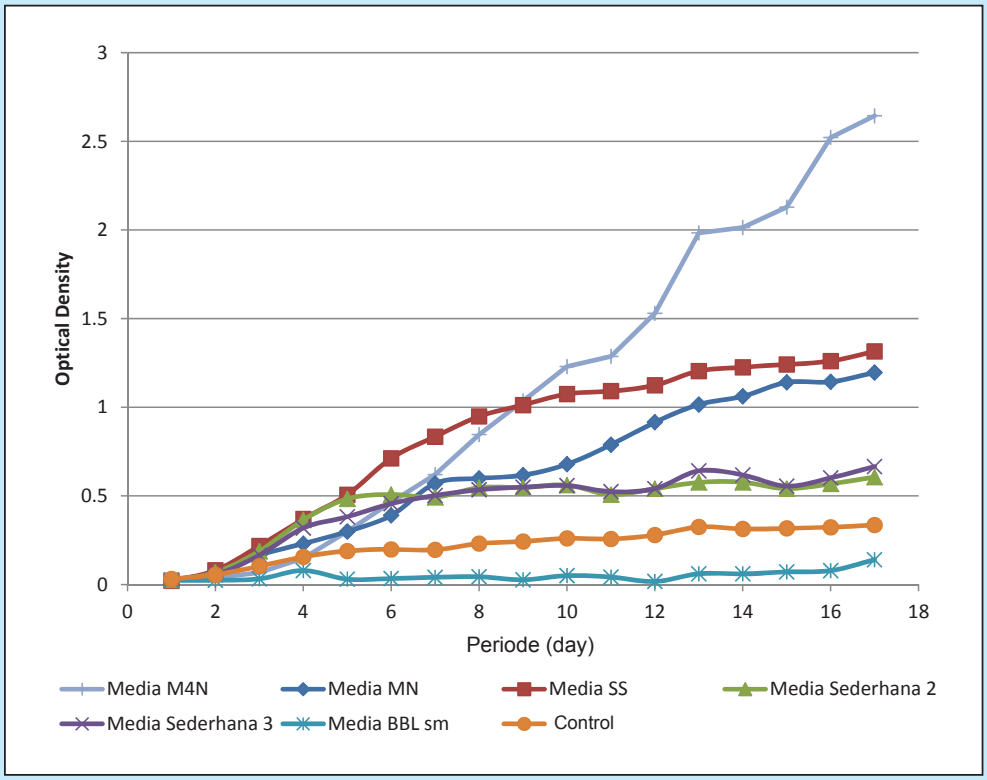

Figure 2
***Scenedesmus* sp. Growth at each variation of the growth media.**

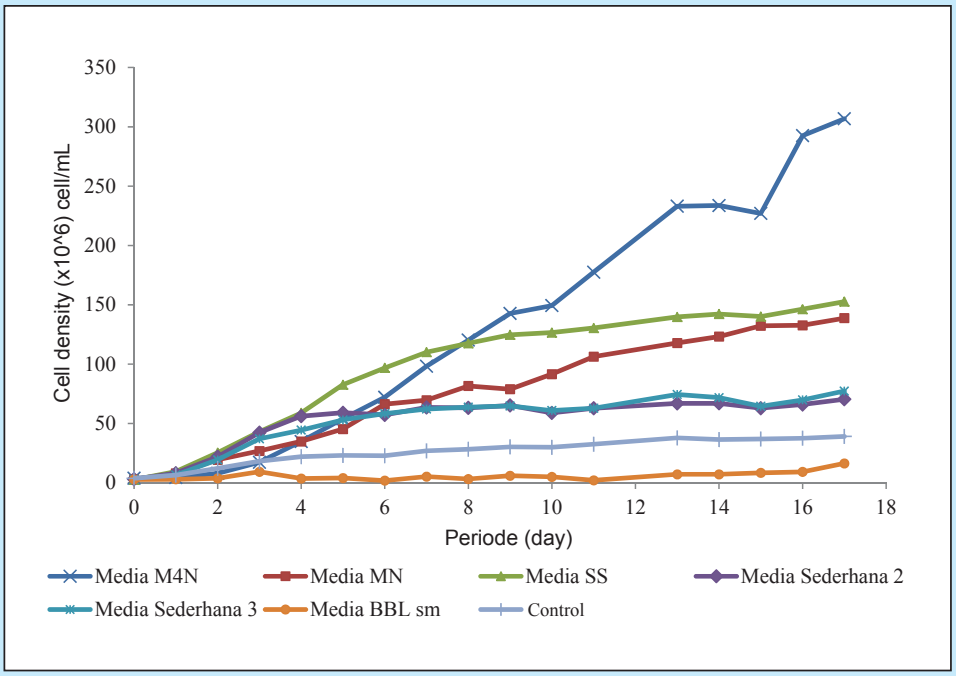

Figure 3
***Scenedesmus* sp. growth at each variation of the growth media**

nutrients for the growth of microalgae which consists of macro nutrients and micro nutrients will certainly be required. Complete composition and appropriate concentration will be crucial in the production of microalgae and the nutrient content of microalgae (Chrismadha and Nofdianto in Prihantini et al. 2007). Microalgae growth always shows a sigmoid curve. The sigmoid curve is a 'growth average' representing all organisms, where young organisms experience adaptation phase and then rapid accelerating growth to cope with their environment, followed by continuous steady growth. Towards maturity, the growth rates of organisms slow down until no growth occurs. At the beginning, microalgae need adaptation in all media types, but after 10 days microalgae with media M4N grow faster than the others. Fig.2. shows that each of nutrient media variation have similar trends, with the microalgae still on the exponential phase at the end of experiments, except for BBL sm media which shows steady growth from the beginning until the end of the experiments. Based on the experiments microalgae growth in 6 different nutrient medium, the highest growth is microalgae that used M4N media, followed by SS media second, then MN, "Sederhana 3" and "Sederhana 2" medium. The M4N media brought the best growth for *Scenedesmus*.sp because it contains nutrient that fit for *Scenedesmus*.sp growth. *Scenedesmus*.sp can absorb M4N culture media better than other nutrient media. Good nutrient absorption of *Scenedesmus*.sp is shown by optical density (see Fig 2, the optical density of microalgae measured). M4N media in the end of observation gave OD value: 2.64, SS media: 1.31, MN media: 1.19, "Sederhana 3" media: 0.66, "Sederhana 2" media: 0.60 and BBL sm media: 0.14

2. Cells Density

Culture medium is an important factor for microalgae growth. A better media produces a better growth of microalgae. Good growth of microalgae can be identified from total cells density. Figure 3 shows that, in general, algae have grown since the first days of observation. During the first 4 days of operation, the value of the cell density of M4N media is lower compared to media MN, SS, "Sederhana 2" and " Sederhana 3". From day 5 to day 7 the M4N media cell density value is still smaller than the media SS. From day 9 until the end of the operation, M4N media become the highest cell density values with dark green color media. In general, the exponential phase of the growth of microalgae in M4N media, MN, SS and "Sederhana 3", were longer than the media "Sederhana 2" (4 days) and BBLsm (1 day). It is in line with research conducted by Mandal and Mallick (2009) who used *Scenedesmus obliquus* grown on N 11 medium and found that the species is able to grow in exponential phase of up to 18 days.

The color of microalgae culture coming from the primary pigment colors contained in the cytoplasm of chlorophyll. Figure 4 shows that the observation day 0 (time of inoculation), mixed culture dominated by Scenedesmus sp grown in media M4N, MN, SS, "Sederhana 2", "3 Sederhana ", BBLsm and distilled water looks clear. The condition is caused by the number of inoculum cell which is not proportional to the total volume of media. Besides that, the ratio between the volume of media with chlorophyll content cannot provide color on the culture.

On day 1 observations, the color of the culture grown on media M4N, MN, SS, "Sederhana 2" and "Sederhana 3" started to appear a pale green color, while the media BBLsm only has a slight change of green color and is not much different than on day 0. On the 3rd day of observation, the color of the culture in the media MN, SS and "Sederhana 2" looks like green apple. Shades of green in the culture, besides showing an increase in the population of cells, also indicates the levels of chlorophyll contained in cells. But according to Prihantini et al. (2007), high levels of chlorophyll are not always followed by high cell density as well. On day 15, the media MN (2 erlenmeyers), SS, "Sederhana 2" and "Sederhana 3" culture begins to change color to yellow (at "Sederhana 3" the yellow color is not too flashy) caused by Mg elements deficiency that are useful for the formation of chlorophyll. Among the MN, SS, "Sederhana 2" and "Sederhana 3" media, visually the color of culture media "Sederhana 3" still endure until the end of the operation, with a light green color. The color conditions of microalgae culture media at day-0 and day-17 as the last experiment day can be see in Figure 4.

The growing cell will cause increased demand for nutrients, while the amount of nutrient inventory in culture media was constant. This condition would lead to a decrease in population which indicates the culture medium is in a limited state, both in terms of the volume and nutritional content. After the nutrients N, P and Fe decreased, cells multiplication declined and media color visually turned to yellow at the end of the experiment. SS Media is a media culture that had the most rapid growth of algae in the first week. This rapid grow can happen because the

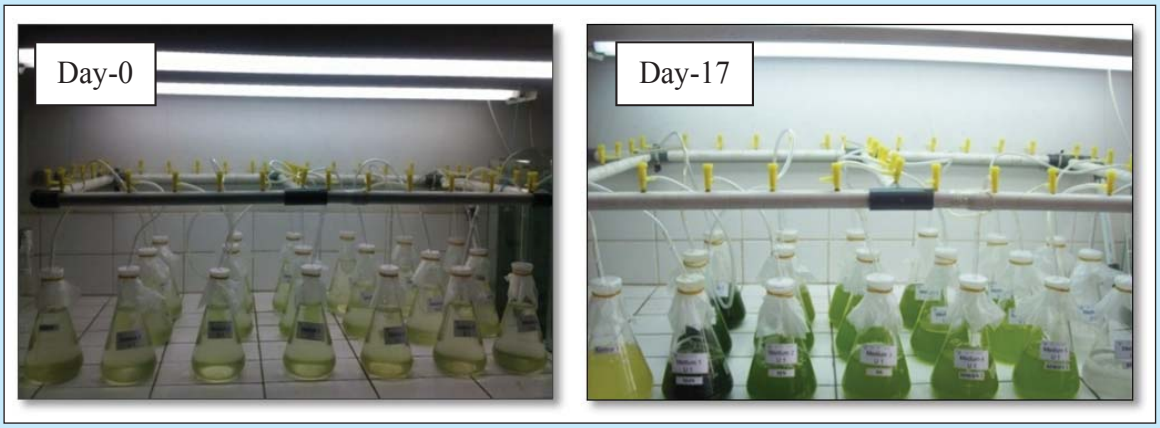

Figure 4
Microalgae color at the beginning and end of the experiment

composition of the nutrient content contained in SS media was better than other media with good acidity. These conditions also happen in media "Sederhana 2" and " Sederhana 3" which gave nearly the same growth pattern.

Culture media with M4N media were the best media for *Scenedesmus* sp, with the cells density up to 306.83 x 10^6 sel/mL with dark green culture color and remaining until the end of the experiment. Dark green color microalgae that endure in M4N media could be caused by Mg element content in M4N which is the highest among other media, thus, chlorophyll synthesis of microalgae in media M4N is also high.

3. Coefficient of growth (k)

During 17 days of experiment, microalgae growth indicates the highest cells density is in M4N media. On these media, *Scenedesmus* sp. was growing faster than other growth media. At the end of the experiment, the growth coefficient microalgae grown on media M4N was 0.36 followed by SS media, which is 0.34 (Table 4). Although the difference in the coefficient of microalgae growth in media M4N and SS are slight, the density of cells in media M4N was relatively much higher than SS media.

The coefficient of growth in media "Sederhana 2" was higher only 0.01 of differences compared to media "Sederhana 3", and the relative value of the cell density is also not much different. But the media "Sederhana 3" is better in growing *Scenedesmus* sp. compared to media "sederhana 2". On the other hand, media BBL sm has the smallest coefficient value of growth compared to other media and even smaller than the control. This indicates that BBL sm media is not suitable for *Scenedesmus* sp to grow. The high growth coefficient of *Scenedesmus* sp. can be caused by the composition of the medium used. That means the composition of nutrients in the media M4N were more complete than other media. According to Hu and Gao (2006), the availability of nutrients will generate cells growth and high cells density values that influence the speed of growth.

Table 2
Value of growth coefficient (k) at each variation of media

Media	Cells Density (x 10^6) sel/mL	K
M4N	306,83	0,36
MN	138,80	0,32
SS	152,67	0,34
Sederhana 2	70,41	0,26
Sederhana 3	77,32	0,27
BBL sm	16,35	0,15
Kontrol	39,06	0,19

B. pH conditions Culture

According to Dori (2011), the pH value is a factor that controls and determines the biological ability of microalgae in utilizing nutrients. The average pH for most species of microalgae cultivation is between 7-9, with an average optimum pH range between 7.8-8.5 (Fachrullah, 2011). Figure 5 illustrates that

the pH value until the 8th day of experiments on each media tended to fluctuate. The highest pH values found in the culture was using SS media, which is 10.53 on the 3rd day of experiments. High pH values will reduce the photosynthetic activity of microalgae. Photosynthesis is the process of taking the CO_2 dissolved in water, and the result is a decrease of CO_2 dissolved in water. And CO_2 reduction will increase the pH. Based on the density of the cells in Figure-3, culture microalgae that used media SS was still in good condition although it had the highest pH value among the others. It is likely the culture buffer ability of the culture media can be overcome at pH fluctuations that had reached 10.53.

In the culture media using BBL sm media, the pH value until the 6th day tended to be more acidic than other growth media. Even on day1 of experiments, the pH value is less than 5. The value of pH below 5 means only free CO_2 has the role in the system, between pH 7-9 bicarbonate are the most significant elements and above pH 9.5, carbonate will be important. Based on Boyd (1990) in Kawaroe et al. (2010), carbonate equilibrium will act as a buffer pH. In the alkaline condition, bicarbonate ion will form carbonate ions and release hydrogen ions which are acidic, that neutralized the system. Instead if the state is too acidic, carbonate ions will through a hydrolysis and become bicarbonate ions and release hydrogen ions alkaline oxides, so that the situation returns to neutral. Neutral pH conditions occurred on day 9, and the pH value reached 6.93. Although neutral pH values can be achieved, the microalgae growth in BBL sm media is smaller than the other media.

IV. CONCLUSION

The results show that among six variations of the growth media used in this study, M4N media is excellent for the growth of microalgae *Scenedesmus* sp. The value of the cell density of microalgae grown on M4N media reached up to 306.83 x 10^6 cells/mL with a dark green color culture that remained until the end of the experiment. The growth coefficient of *Scendesmus* sp. in M4N media is 0.36. This is likely because the composition of nutrients in M4N media were more appropriate for microalgae *Scenedesmus* sp than other media. Although M4N media is a good medium for the growth of microalgae *Scendesmus* sp.,further study is still needed to obtain the most economic use of the media for mass production.

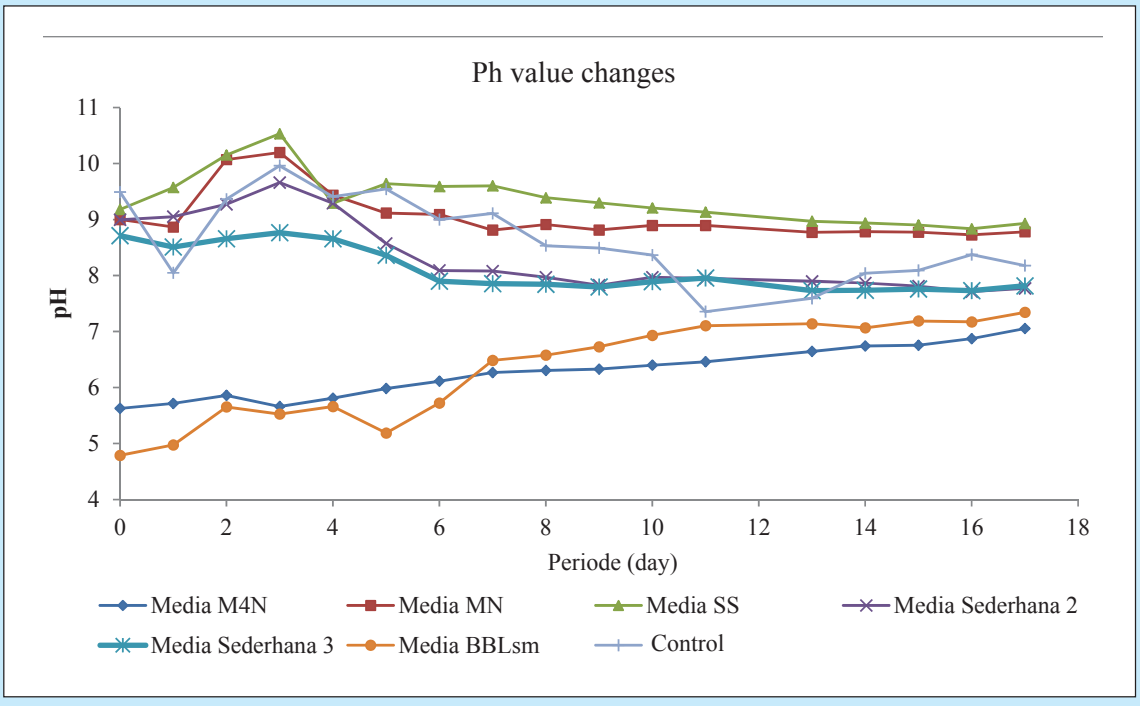

Figure 5
pH fluctuation in the culture of each variation of the growth media.

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