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The Effect of Aeration Rate on the Growth of Blue Green Microalgae in Buffalo Dung as Alternative Media

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The high demand of *Arthrospira platensis* as a veritable protein source encourages its mass production worldwide. Currently, mass production of *Arthrospira platensis* is hindered by the relatively high price of the growth media. Recently, it is discovered that *Arthrospira platensis* can be cultivated using buffalo dung as an alternative medium. Buffalo dung is an excellent source of nitrogen and phosphorus which are principal macronutrients for the growth of *Arthospira platensis*. In addition to nitrogen and phosphorus, carbon is also a macronutrient that is important to the growth of microalgae. The carbon source used by the microalgae is carbon dioxide, which is consumed through photosynthesis. Carbon dioxide can be derived directly from the atmosphere as atmospheric CO_2 existing as much as 0.04%-v/v in air, which can be provided directly using an aeration pump into the growth medium microalgae. During the aeration process, CO_2 mass transfer occurs from the gaseous phase into the liquid phase. This research aims to investigate the effect of the aeration rate on the growth of the blue-green microalgae *Arthrospira platensis* using buffalo dung media as an alternative medium. *Arthrospira platensis* will be cultivated on buffalo dung media using various aeration rates to determine the effect of aeration on the specific growth rate (μ). The air will also be pumped into the growth medium without *Arthrospira platensis* at the specific aeration rates to determine the mass transfer coefficient (k_La) that occurs from the air leading to growth medium. Analysis of mass transfer coefficient (k_La) of carbon dioxide will be conducted using the sulfite method. Variation of aeration that used in this research are 0.2 vvm; 0.4 vvm; 0.6 vvm; 1.2 vvm; 2.4 vvm that has mass transfer coefficient dan specific growth rate are 0.005 min^{-1} and 0.1987 day^{-1}; 0.009 min^{-1} and 0.2279 day^{-1}; 0.012 min^{-1} and 0.2044 day^{-1}; 0.034 min^{-1} and 0.1918 day^{-1}; 0.035 min^{-1} and μ in 2.4 vvm can't determine, respectively.

Key words: aeration rate, *Arthrospira platensis*, buffalo dung, mass transfer, sulfite method

Permintaan biomassa *Arthrospira platensis* semakin meningkat. Hal ini dilatarbelakangi oleh kandungan protein *Arthrospira platensis* yang cukup tinggi, sehingga mendorong produksi *Arthrospira platensis* semaksimal mungkin. Namun harga medium pertumbuhan yang digunakan relatif mahal. Dewasa ini, *Arthrospira platensis* diketahui dapat ditumbuhkan pada medium alternatif kotoran kerbau. Kototan kerbau dijadikan sebagai sumber nitrogen dan fosfor yang merupakan makronutrien oleh *Arthrospira platensis*. Selain nitrogen dan fosfor, sumber karbon juga merupakan makronutrien penting bagi pertumbuhan mikroalga. Sumber karbon yang digunakan oleh mikroalga adalah karbon dioksida. Karbon dioksida dibutuhkan dalam proses pertumbuhan *Arthrospira platensis*. Senyawa ini dapat berasal dari atmosfer berupa karbon dioksida atmosferik dengan menyalurkan udara melalui sistem aerasi ke dalam medium pertumbuhan mikroalga. Pada aerasi, terjadi perpindahan massa karbon dioksida dari fasa gas menuju fasa cair. Penelitian ini bertujuan untuk mengetahui pengaruh laju aerasi terhadap pertumbuhan *Arthrospira platensis* pada media alternatif kotoran kerbau. *Arthrospira platensis* ditumbuhkan pada medium kotoran kerbau dan dilakukan aerasi dengan laju alir tertentu untuk menentukan laju pertumbuhan spesifik. Analisis konsentrasi karbon dioksida menggunakan metode sulfit. Variasi laju alir yang digunakan adalah 0.2 vvm; 0.4 vvm; 0.6 vvm; 1.2 vvm; 2.4 vvm yang memiliki koefisien transfer volumetrik dan laju pertumbuhan spesifik secara berturut turut 0.005 $menit^{-1}$ and 0.1987 $hari^{-1}$; 0.009 $menit^{-1}$ and 0.2279 $hari^{-1}$; 0.012 $menit^{-1}$ and 0.2044 $hari^{-1}$; 0.034 $menit^{-1}$ and 0.1918 $hari^{-1}$; 0.035 $menit^{-1}$ and μ in 2.4 vvm tidak dapat dihitung.

Kata kunci: *Arthrospira platensis*, kotoran kerbau, laju aerasi, metode sulfit, transfer massa

Arthrospira platensis or often called "Spirulina" is produced around 15,000 tons/year, especially in open pond systems (EUMOFA 2018). *Arthrospira platensis* has a high nutrient content such as proteins, essential amino acids, fatty acids, polysaccharides, carotenoids, vitamins, and iron (Hadiyanto and Azim 2012). Biomass production of *Arthrospira platensis* depends on various factors such as the composition of the medium, temperature, light intensity, pH of the medium, amount of inoculum, salt, and aeration rate (Pandey, Pathak and Tiwari 2010).

In general, *Arthrospira platensis* is grown on Zarrouk's medium, which is a synthetic medium and is

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relatively expensive. Only recently has it been found that buffalo dung can be used as an alternative medium for growing *Arthrospira platensis* microalgae. Buffalo manure is used as a source of nitrogen and phosphorus which is a macronutrient for *Arthrospira platensis* (Simangunsong and Sinaga 2018). However, the growth of microalgae is also influenced by carbon sources to produce biomass using light energy. Generally, carbon sources are available in inorganic forms, namely carbon dioxide gas (Tebbani *et al*. 2014).

Carbon dioxide is the main element that is important in the process of photosynthesis. Carbon dioxide is needed for the formation of carbohydrate compounds that will be converted into biomass. Microalgae biomass consists of about 50% carbon and almost entirely from carbon dioxide gas (Lam, Lee and Mohamed 2012).

In the process of cultivating *Arthrospira platensis*, aeration is carried out by flowing air into the microalgae growth medium. The air content is dominated by nitrogen and oxygen. In addition, air also contains 0.04% v/v of carbon dioxide gas needed by microalgae as a substrate in photosynthesis (Richmond 2003). Thus, aeration is an important parameter that needs to be considered in increasing *Arthrospira platensis* production. Aeration has advantages such as avoiding the deposition of microalgae, homogenizing the culture medium so that the cell can have adequate nutrients and light, avoiding temperature differences and facilitating the transfer of carbon dioxide gas from the air to the medium. Therefore, the phenomenon of carbon dioxide gas mass transfer needs to be reviewed.

The phenomenon of mass transfer of carbon dioxide is considered important to know in order to obtain the appropriate aeration rate so that microalgae biomass is obtained as much as possible. This is useful for increasing *Arthrospira platensis* production on a laboratory scale. So far there have been no studies on the effect of aeration rates on the growth of *Arthrospira platensis* blue-green microalgae on alternative media for buffalo dung.

MATERIALS AND METHODS

Strain and Culture Medium. *Arthrospira platensis* was produced form the culture collection of Algae, Institut Teknologi Del, Indonesia. The inoculum was grown in Zarrouk medium. The composition of inoculum on a g.L-1 basis was as follows: $Ca_2Cl_2.2H_2O$, 0.04; $FeSO_4$, 0.01; K_2SO_4, 1; K_2HPO_4, 0.5; $MgSO_4$, 0.2; Na_2-EDTA, 0.08; NaCl, 1; $NaHCO_3$, 16.8; $NaNO_3$, 2.5; $Na_2S_2O_5$, 0.05. Additionally, the medium contained trace metal solution, the composition on a $g.L^{-1}$ basis was as follows: H_3BO_3, 2.86; $MgCl_2$, 1.81; Na_2MoO_4, 0.01; $CuSO_4$, 0.07; $ZnSO_4$, 0.22. The buffalo medium contained $4g.L^{-1}$ buffalo dung. Additionally, the buffalo medium contained $NaHCO_3$ 8.5 mg and $Na_2S_2O_5$ 5 g to oxidize toxic compounds and keep pH in base condition.

Photo Bioreactor Design and Operation. The photobioreactor was a glass bubble column. The operating volume of the reactor was 0.8 L. The air flow rate was adjusted using a gas flow meter. The air was sparged through a cross-shaped pipe sparger via 1 mm holes. The light intensity of 3000-4500 lux mantained during the operation. All photobioreactor were run at room temperature (24 °C). A 10% inoculum (9-day-old culture grown under similar condition) was used to inoculate in buffalo medium.

To highlight the suitable aeration rate, the experiment was performed in the bubble column photo bioreactor with variation continuous flow rate. The cultivation study were conducted at 5 different flow rates (0.2, 0.4, 0.6, 1.2, and 2.4 vvm) in a cycle 24 h.

Analytical Methods. UV/Vis Spectrophotometer was employed to determine the absorbance of culture at 560 nm. The sample was taken 2 mL each day to known the absorbance of biomass. The biomass concentration was determined by recorded the absorbance of variation biomass dilution that diluted with medium. The biomass then filtered cells with Whatman GF/C filters (1.2 µm). The filtered cells were dried at 70 °C at oven until a steady weight was recorded for dry weight measurement.

The specific growth rate was calculated using a graphical method. The specific growth rate determined by the natural logarithm value of the biomass concentration (corresponding to the logarithm phase) of the algae was plotted against time, and the slope was used to derive the specific growth rate.

The mass transfer coefficient volumetric (k_La) of carbon dioxide analysis by sulphite method. Buffalo medium added with excess sulphite then aerated at certain aeration rate. The sample taken every 15 minutes. The sample mixed KIO_3, KI, and H_2SO_4 then titrated with $Na_2S_2O_3$. The quantity of sulphite is equivalent to the quantity of reduced iodine and since the quantity of sodium thiosulfate used in the titration is equivalent to the quantity of remaining iodine. The difference between the total iodine and the volume of

sodium thiosulfate is a measure of the sodium sulphite concentration. The oxygen dissolved determined form the sodium sulphite concentration that reduces. The natural logarithm of difference bulk liquid (Henry law) and oxygen dissolved was plotted against time. The slope was used to derive the mass transfer coefficient volumetric**.**

RESULTS

Alternative media with a concentration 4 $g.L^{-1}$ of buffalo dung given different variations in aeration rate (0.2, 0.4, 0.6, 1.2, and 2.4 vvm) which has CO_2 volumetric mass transfer coefficient of 0.0054; 0.0095; 0.0121; 0.034; and 0.0349 min^{-1}. The graph of the relationship between the aeration rate and the volumetric mass transfer coefficient of CO_2 (k_La) is shown in Figure 1.

Figure 1 shows that the value of the CO_2 volumetric mass transfer coefficient (k_La) in the photobioreactor will increase with increasing air aeration rate. Low air aeration rates will form smaller and slower bubbles so that the flow regime in the photobioreactor will form a laminar flow. This phenomena results in a low value volumetric mass transfer coefficient of CO_2 (k_La).

Arthrospira platensis was grown in five (5) variations in the aeration flow rate on alternative buffalo dung mediums namely 0.2, 0.4, 0.6, 1.2, and 2.4 vvm. The *Arthrospira platensis* growth curve at various aeration flow rates of 0.2, 0.4, 0.6, and 1.2 vvm is stated in Figure 2.

Figure 2 shows that the lag phase experienced by *Arthrospira platensis* is very short. This is caused by the inoculum in alternative media buffalo manure already in the exponential phase. If the initial culture conditions have reached the stationary phase, then the lag phase in the new medium will last long because the cell will adjust to the new conditions of the media (Maier *et al*. 2009).

Figure 3 shows the specific growth rate of *Arthrospira platensis* increased at a flow rate of 0.2 and 0.4 vvm. An increase in the specific growth rate of 0.2 vvm towards 0.4 vvm occurs significantly. The greater the aeration rate given to *Arthrospira platensis* culture on alternative buffalo dung media, the higher the specific growth rate but only up to 0.4 vvm, above 0.4 vvm the specific growth rate will decrease. Increasing the aeration rate which results in an increase in the specific growth rate in *Arthrospira platensis* due to the equilibrium between the negative effects caused by shear stress and the positive effect caused by an increase in aeration rate and CO_2 mass transfer and the positive influence is more dominant (Garcia 2017). Shear stress is a force that causes the deformation of a material by producing slippage along a parallel plane.

DISCUSSION

CO_2 absorption into the medium is greater as the value of the CO_2 volumetric mass transfer coefficient (k_La) increases. The greater the surface area of the gas and liquid that are in contact with each other, the absorption of CO_2 will be greater because the surface of the gas and liquid that are in contact with each other will increase the chance of CO_2 to diffuse into the medium. In the study, the value of (k_La) in buffalo dung medium was 0.0054 min^{-1} to 0.0349 min^{-1} (0.2 vvm to 2.4 vvm) which showed that the value of (k_La) gets lower with the flow rate increasing.

The growth of *Arthrospira platensis* in several variations of the aeration rate experiences an exponential phase until on the 9th day or the 10th day. Previous studies conducted grew *Arthrospira platensis* on alternative buffalo dung media and the results showed that the stationary phase had occurred on the 9^{th} day.

Growth at the aeration rate of 2.4 vvm does not produce a stationary phase until the 20^{th} day until the remaining 100 mL medium is obtained and cannot be re-aerated. The aeration rate of 2.4 vvm is considered very unsuitable for use in bubble reactors on a lab scale of 800 mL with one aeration hole without condenser. The condenser is needed in condensing the evaporated medium during the aeration process. The evaporated medium will then be condensed into a liquid form again by the condenser.

An aeration rate that is too high causes a decrease in the amount of medium due to the high rate of evaporation (Simangunsong and Sinaga 2018). There are two phenomena that contribute when there is an increase in evaporation of the liquid. First, evaporation is a function of the rate at which water vapour is released from water. When air bubbles burst on the surface of the liquid, the burst bubbles will add momentum to the air which will then increase the rate at which moist air will be released from the surface of the liquid. Second, when air is injected into water, bubbles will form and steam will diffuse from water into the air (Helfer, Lemckert and Zhang 2012). Thus, increasing the aeration flow rate will increase momentum which will push moist air out.

The effect of large bubbles on the rate of

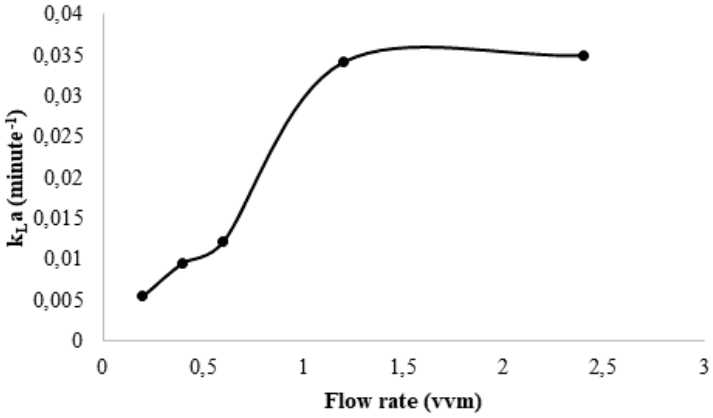

Fig 1 Relationship of flow rate with CO_2 volumetric mass transfer coefficient (k_La).

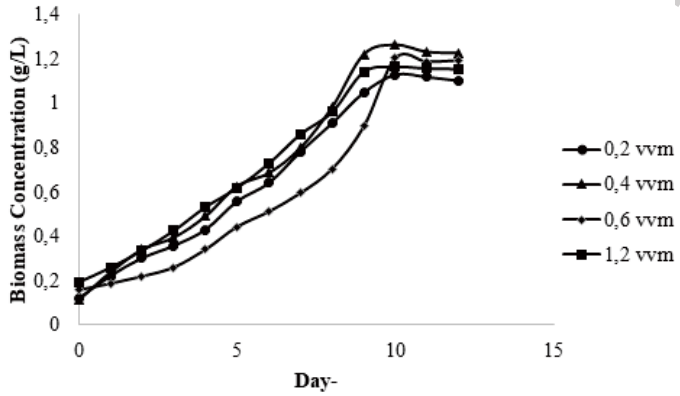

Fig 2 *Arthrospira platensis* growth curve at various aeration rates of 0.2, 0.4, 0.6, and 1.2 vvm.

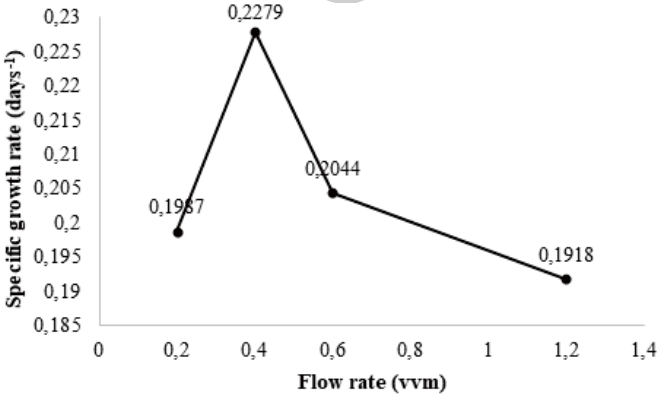

Fig 3 Spesific growth rate of *Arthrospira platensis* at various flow rates in buffalo dung media.

evaporation conclude that the greater the rate of aeration in the media, the greater the evaporation of the liquid that will be caused a decrease in the amount of water (Garcia 2017). Thus, the aeration rate that is too high makes the measurement of cell concentration be inaccurate, this is because evaporation will reduce the amount of media volume which results in measurements of cell concentration will always increase.

The decrease of specific growth rate is caused by excess shear stress which occurs due to an overly high flow rate which causes the cell to experience a decrease in color and a decrease in the value of cell absorbance (Converti *et al*. 2006). The cell will experience stress at a flow rate that is too high and then lose the color of *Arthrospira platensis* cells which indicates cell death. The shear stress can cause a decrease in the rate of cell growth, decreased cell productivity, damage to cells and lysis in cells (Rodriguez *et al*. 2011).

The highest specific growth rate (μ) in this study was 0.2279 days^{-1} at a flow rate of 0.4 vvm. The value of the specific growth rate (μ) is higher than the value of the specific growth rate (μ) (1.2 vvm) growth medium is in the form of Bicarbonate-enriched SOT medium

with a type of photo bioreactor glass bubble column with a volume of 20 L (Ronda *et al*. 2012). The specific growth rate (μ) of the buffalo dung medium was lower than the Zarrouk medium in the 8 L bubble bubble photo reactor which was an aeration rate of 0.5 vvm of 0.3 $days^{-1}$ (Oncel and Akpolat 2006). With mathematical models, the flow rate of 0.36 vvm or less than 0.36 vvm is the aeration rate suitable for the growth of *Arthrospira platensis* in airlift-circulated tubular PBR (Converti *et al*. 2006). The results of the research stated above provide information that the aeration rate, type of photo bioreactor, and type of medium influence the value of the specific growth rate (μ) of *Arthrospira platensis*.

In conclusion, The relation between CO_2 that flowed into the growth medium, the mass transfer coefficient of volumetric and specific growth rate has been described. The results showed that the flow rate significantly affects the operational conditions of photo bioreactor that are observed through microalgae growth rate, and the mass transfer coefficient of volumetric. Aeration flow rate contains CO_2 that supplied to the system is inversely proportional to the specific growth rate from 0.4 vvm. Flow rate is too high causes sheer stress on the cells. The aeration rate condition is 0.4 vvm also gives that the value of the mass transfer coefficient of volumetric is maximum for the growth of *Arthrospira platensis*.

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