

## Growth Kinetic Study of Blue-green Microalgae *Arthrospira platensis* Using Buffalo Manure as Alternative Media

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North Sumatera is one of the provinces in Indonesia with the highest buffalo population, which is responsible for the high accumulation of buffalo manure that can cause environmental and aesthetic problems if left untreated. One of the possible alternatives for solving this issue is by implementing buffalo manure as growth media for microorganisms, e.g. microalgae. In this research, buffalo manure was used as alternative media for *Arthrospira platensis* cultivation. Buffalo manure was taken from Sitoluama Village, Laguboti, Toba Regency of North Sumatra Province. Research steps included media and culture preparation, cultivation, sampling, sample analysis, and verification of constructed models and validation. Buffalo manure concentration in media is varied from 1 g.L<sup>-1</sup> to 8 g.L<sup>-1</sup> which is analogous to nitrogen content of 0.002 mg.L<sup>-1</sup> to 0.018 mg.L<sup>-1</sup>. Growth data was used for growth kinetic modelling, which was most satisfactory for Monod model ( $\mu_{max} = 0.5915 \text{ day}^{-1}$ ,  $K_s = 0.421 \text{ g.L}^{-1}$ ).

Key words: alternative media, *Arthrospira platensis*, buffalo manure, growth kinetics

Sumatera Utara merupakan salah satu propinsi di Indonesia dengan populasi kerbau terbesar, yang menyebabkan tingginya akumulasi kotoran kerbau yang dapat menyebabkan masalah lingkungan dan estetika bila tidak ditangani. Salah satu alternatif solusi untuk masalah ini adalah dengan memanfaatkan kotoran kerbau sebagai media pertumbuhan untuk mikroorganisme, misalnya mikroalga. Dalam penelitian ini, kotoran kerbau digunakan sebagai media alternatif untuk kultur *Arthrospira platensis*. Kotoran kerbau diambil dari Desa Sitoluama, Laguboti, Kabupaten Toba, Propinsi Sumatera Utara. Tahapan penelitian meliputi persiapan media, persiapan kultur, kultivasi, pengambilan sampel, analisis sampel, serta verifikasi dan validasi model kinetika. Konsentrasi kotoran kerbau dalam media bervariasi dari 1 g.L<sup>-1</sup> hingga 8 g.L<sup>-1</sup> yang analog dengan kandungan nitrogen 0,002 mg.L<sup>-1</sup> hingga 0,018 mg.L<sup>-1</sup>. Data pertumbuhan digunakan untuk menentukan model kinetika pertumbuhan, yang menunjukkan kesesuaian tertinggi dengan model Monod ( $\mu_{max} = 0,5915 \text{ hari}^{-1}$ ,  $K_s = 0,421 \text{ g.L}^{-1}$ ).

Kata kunci: *Arthrospira platensis*, kinetika pertumbuhan, kotoran kerbau, media alternatif

Per 2016, North Sumatera was the province in Indonesia with third-highest buffalo population, which equals 116,403 (Badan Pusat Statistik 2016). Toba district in North Sumatera was no exception, with the total buffalo population of 3,681 in the same year (Badan Pusat Statistik Kabupaten Toba Samosir 2013). The high population of buffalo corresponds to the high buffalo manure production, which is still under-utilized despite its possibilities for use as organic fertilizer and feedstock for biogas production (Karttek *et al.* 2014). Mostly Toba district people use buffalo manure as organic fertilizer due to its three highest content needed for plants growth, those are nitrogen (2 g/kg), phosphor (2 g/kg), and potassium (10.1 g/kg)

(Irshad *et al.* 2013; Ghimire *et al.* 2017). This has been causing the accumulation of manure in many places, especially in rural areas. It can cause terrible odour, carries out diseases, and even worse, river pollution and eutrophication in lakes when the manure is leached to water bodies.

Despite the problems it poses, buffalo manure possesses high organic matter content, which makes it a sound option as an alternative growth medium for several species of microalgae, especially *Arthrospira platensis*. *Arthrospira* has a high market value due to its potential as food supplement or feed due to its high protein content of 50-70% dry weight (Michael, Kyewalyanga, and Lugomela 2019). Commonly, *A. platensis* grows very well in synthetic media such as Zarrouk's media, but due to its considerable material cost, many researchers were driven to discover

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alternative media, such as tofu liquid waste (Syaichurrozi and Jayanudin 2016), tapioca liquid waste (Insan *et al.* 2018), chicken manure (Yilmaz, Sezgin, and Duru 2015) and cow manure (Astiani, Dewiyanti, and Mellisa 2016). There are other microalgae biomass productions that also use waste as alternative growth media, such as aquaculture wastewater for *Chlorella minutissima* biomass production (Hawrot-Paw *et al.* 2019), aquaculture biological waste for *Ankistrodesmus gracilis* (Sipauba-Tavares, Florencio, and Scardoeli-Truzzi 2018), and diluted composting fluids for *Selenastrum* sp. (Marika *et al.* 2017). Up to this research, there has been no prior research that discusses the kinetic study of the growth of *A. platensis* on alternative buffalo manure media. The discovery of the aforementioned kinetic parameters would be fundamental in developing a closed-nutrient cycle between buffalo farming and *A. platensis* aquaculture.

## MATERIALS AND METHODS

**Culture Preparation.** *Arthrospira platensis* was cultivated from stock culture into Zarrouk's media (Michael *et al.* 2018) for 9 days. During cultivation, culture was provided with 3000-4500 lux light using LED lamps and aeration.

**Medium Preparation.** Buffalo manure was collected directly from the cage, not from the manure shelter. All of the buffalo manure can be utilized to reduce the overflow of manure waste according to the purpose of this research and utilize it as substrate in microalgae growth media. Buffalo manure was sun-dried for 1-2 days, cooled and sieved twice using 60 fine mesh. 100 g of this manure were dissolved in 1 L sterilized distilled water and left for 3 days, with addition of 8.5 mg.L<sup>-1</sup> sodium metabisulfite on the first day and about 5 g.L<sup>-1</sup> sodium bicarbonate on the second day. This mixture was furthermore filtered using filter paper and taken 10-80 mL, each to be diluted with distilled water to 720 mL.

**Variation.** The concentration of buffalo manure was varied between 1-8 g.L<sup>-1</sup>. This variation aimed to collect sufficient data for fitting to various growth kinetic models.

**Cultivation.** Cultivation of *A. platensis* was carried out in 1 L Erlenmeyer flasks with total liquid volume of 800 mL, consisting of 720 mL prepared buffalo manure medium and 80 mL prepared microalgae culture (10% v/v inoculum with microalgae concentration of 2 g.L<sup>-1</sup>). Each flasks were aerated continuously in order to

prevent carbon dioxide acting as limiting substrate. In order to ensure the photoautotrophic growth of *A. platensis*, light was provided by a similar fashion as during culture preparation. These experiments were conducted in two repetitions.

**Sampling and Sample Analysis.** Liquid samples of the cultures were taken on daily basis for 10–11 days. Each sampling was carried out twice. Taken samples were examined for biomass concentration and nitrogen content measurement. Biomass concentration was determined by measuring the sample absorbance in 560 nm wavelength and calculating the biomass concentration using the standard curve, which was previously prepared by measuring the absorbance values of liquids with known biomass concentrations. Biomass cells were harvested from the sample by centrifugation in 3000 rpm for 15 minutes, followed by decantation, drying in 60°C oven and dehydration using desiccator.

**Data Interpretation.** Biomass cell concentration data obtained through sample analysis was used to determine the specific growth rate ( $\mu$ ) achieved in the logarithmic phase, which follows the following first-order rate form:

$$\ln \frac{X}{X_0} = \mu t \quad \text{Equation (1)}$$

$X$  = cell concentration over time (g.L<sup>-1</sup>)

$X_0$  = initial cell concentration (g.L<sup>-1</sup>)

$t$  = cultivation time (day)

$\mu$  = specific cell growth rate (day<sup>-1</sup>)

Specific growth rates of *A. platensis* in varied buffalo manure concentrations were fitted to five conventional growth kinetic models:

$$\text{Monod: } \frac{1}{\mu} = \frac{K_s}{\mu_{max} S} + \frac{1}{\mu_{max}}$$

$$\text{Contois: } \frac{1}{\mu} = \left[ \frac{K_s}{\mu_{max}} \right] \left[ \frac{X}{S} \right] + \frac{1}{\mu_{max}}$$

$$\text{Verhulst: } \mu = \mu_m - \left[ \frac{\mu_{max}}{X_{max}} \right] X$$

$$\text{Tessier: } \ln \mu = \left[ \frac{1}{K_s} \right] S + \ln \mu_m$$

$K_s$  = saturation constant (g.L<sup>-1</sup>)

$\mu_{max}$  = maximum specific growth rate (day<sup>-1</sup>)

$X_{max}$  = maximum cell concentration (g.L<sup>-1</sup>)

## RESULTS

Growth curves of *A. platensis* cultivated in various buffalo manure concentrations are shown in Figure 1

Table 1 Parameter Kinetics for Several Growth Kinetic Model

Kinetics Model	Kinetic Parameters	$R^2$
Monod	$\mu_{max} = 0.5915 \text{ day}^{-1}$ $K_s = 0.421 \text{ g.L}^{-1}$	0.9775
Contois	$\mu_{max} = 0.6695 \text{ day}^{-1}$ $K_S = 5.143 \text{ g.L}^{-1}$	0.4602
Tessier	$\mu_{max} = 0.417 \text{ day}^{-1}$ $K_S = 16.92 \text{ g.L}^{-1}$	0.7227
Verhulst	$\mu_{max} = 0.3924 \text{ day}^{-1}$ $X_{max} = 0.2317 \text{ g.L}^{-1}$	0.6866

Table 2 Comparison of  $\mu_{max}$  for *A. platensis*

$\mu_{max} (\text{day}^{-1})$	Medium	Reference
0.4	Zarrouk's medium	Wang, Fu and Liu (2006)
0.22	Modified Bristol's medium	Sydney <i>et al.</i> (2010)
0.4	Swine manure	Mezzomoet <i>et al.</i> (2008)
0.5915	Buffalo manure	Current research

and Figure 2, which exhibit no correlation between buffalo manure concentration and final biomass concentration. Highest biomass concentration was obtained in  $8 \text{ g.L}^{-1}$ . Specific growth rates of *A. platensis* cultivated in various buffalo manure concentrations are shown in Figure 3. By linear regression, kinetic parameters of various growth kinetic models of *A. platensis* were determined and can be seen at Table 1. Whereas the comparison of  $\mu_{max}$  for *A. platensis* from different researches is stated in Table 2.

## DISCUSSION

Figure 1 until figure 8 show that lag phase of *A. platensis* cell growth is very short and almost unseen. This condition is caused by the phase of inoculum when it was inoculated to buffalo manure alternative media is in the exponential phase. It is supported by Maier, Pepper and Gerba (2009), that if the inoculum condition is in the stationary phase then the lag phase will be clearly seen, because cells need time for adaptation in a new medium condition that physiologically the cell phase will move from stationary phase to exponential phase. Other parameters that affect lag phase of *A. platensis* are pH, light intensity, and temperature. Condition of pH, light intensity, and temperature of inoculum medium and buffalo manure alternative media are the same, those are pH 10, light intensity 3-4.5 Klux, and temperature 25-27°C.

In each concentration of buffalo manure media, in general exponential phase is started at day-0, day-1, or day-2. This condition is proven that lag phase of *A. platensis* growth occurred in a short period. The research conducted by Syaichurozzi and Jayanudin (2016) about *A. platensis* growth in liquid tofu waste

media and the research conducted by Astiani, Dewiyanti and Mellisa (2016) about utilization of bovine, birds, and buffalo manure as *A. platensis* growth media, also give the results that lag phase took place in a very short period that was in day-0. Exponential phase is occurred until day-6 or day-7, and after that *A. platensis* cells enter deceleration phase until death phase at day-10.

Substrate concentration in buffalo manure media affects specific growth rate of *A. platensis* cells, as seen on Figure 9. To calculate *A. platensis* specific growth rate, cell concentration data from each points of sampling time of both repetition experiments was averaged, and specific growth rate value of *A. platensis* cell in the batch system is determined by the equation (1). Figure 9 shows the increasing specific growth rate of *A. platensis* cells in buffalo manure concentration of  $1 \text{ g.L}^{-1}$  until  $4 \text{ g.L}^{-1}$ , and then the specific growth rate is decrease insignificantly at concentration  $5 \text{ g.L}^{-1}$ . The higher the buffalo manure concentration in the media the higher the specific growth rate of *A. platensis* to the limit concentration of  $5 \text{ g.L}^{-1}$ . The decreasing specific growth rate is due to the color of media that was getting darker in the concentration of  $5 \text{ g.L}^{-1}$ .

The value of specific growth rate in each variation concentration of substrate is used to determine growth kinetic models such as Monod, Contois, Tessier, and Verhulst (Table 1). Parameters determined based on those kinetic models are  $\mu_{max}$  to know the value of maximum growth rate of *A. platensis* and  $K_s$  to know the rate of *A. platensis* cells to reach the saturation point. In buffalo manure concentration more than  $5 \text{ g.L}^{-1}$  the media condition is more murk, therefore in the determination of growth kinetic model of *A. platensis*, the specific growth rates of *A. platensis* in concentration media more than  $5 \text{ g.L}^{-1}$  was not included. There was a

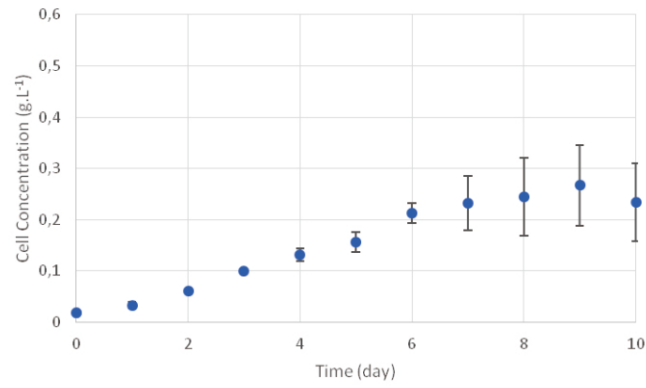


Fig 1 *Arhtospira platensis* growth curves in buffalo manure concentration 1 g.L<sup>-1</sup>.

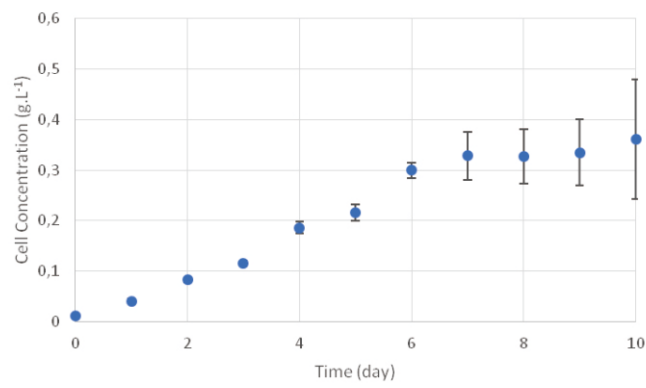


Fig 2 *Arhtospira platensis* growth curves in buffalo manure concentration 2 g.L<sup>-1</sup>.

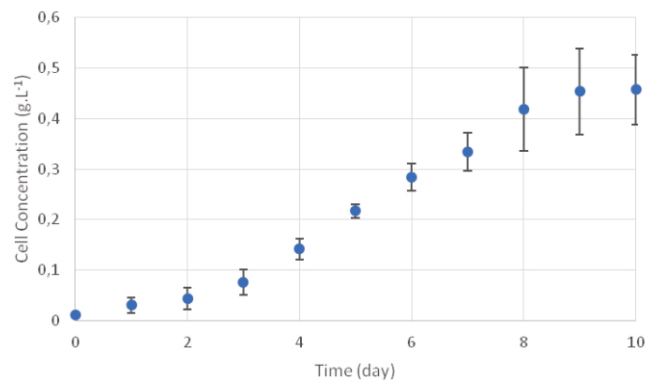


Fig 3 *Arhtospira platensis* growth curves in buffalo manure concentration 3 g.L<sup>-1</sup>.

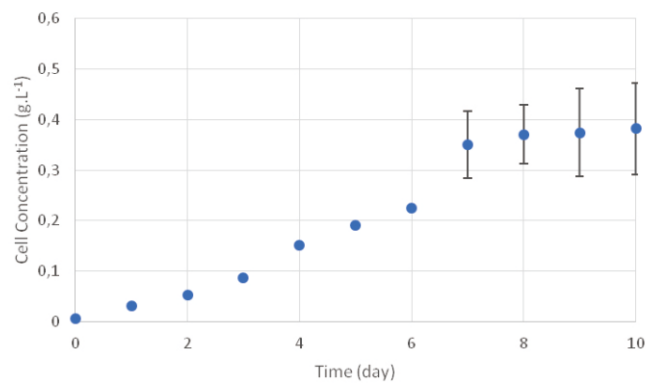


Fig 4 *Arhtospira platensis* growth curves in buffalo manure concentration 4 g.L<sup>-1</sup>.

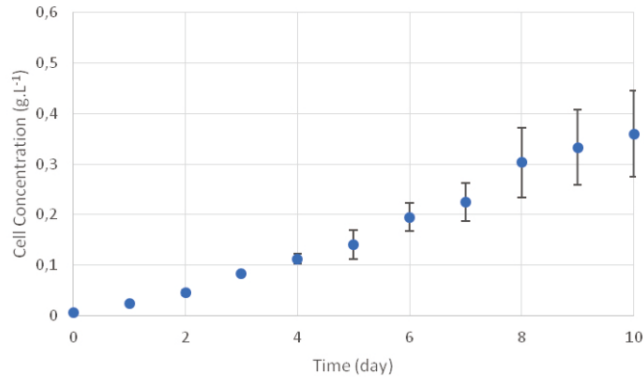


Fig 5 *Arhtospira platensis* growth curves in buffalo manure concentration 5 g.L<sup>-1</sup>.

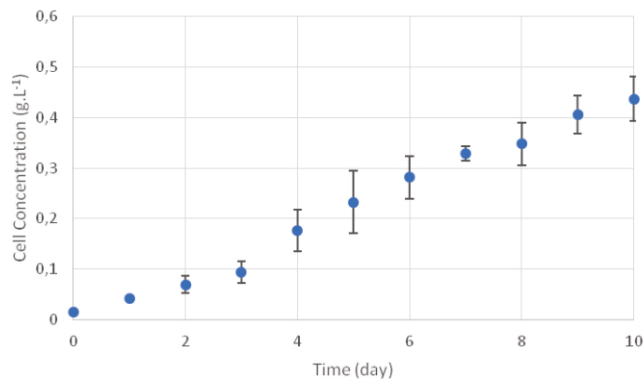


Fig 6 *Arhtospira platensis* growth curves in buffalo manure concentration 6 g.L<sup>-1</sup>.

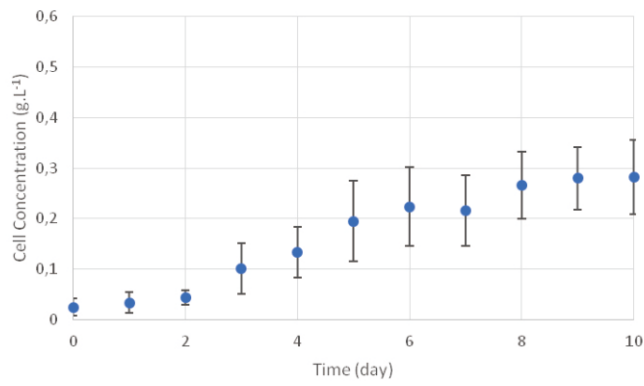


Fig 7 *Arhtospira platensis* growth curves in buffalo manure concentration 7 g.L<sup>-1</sup>.

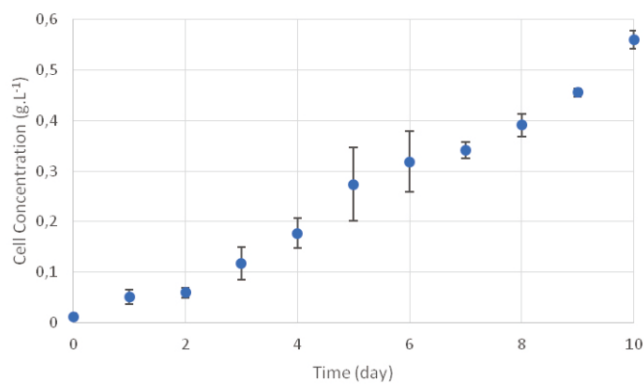


Fig 8 *Arhtospira platensis* growth curves in buffalo manure concentration 8 g.L<sup>-1</sup>.

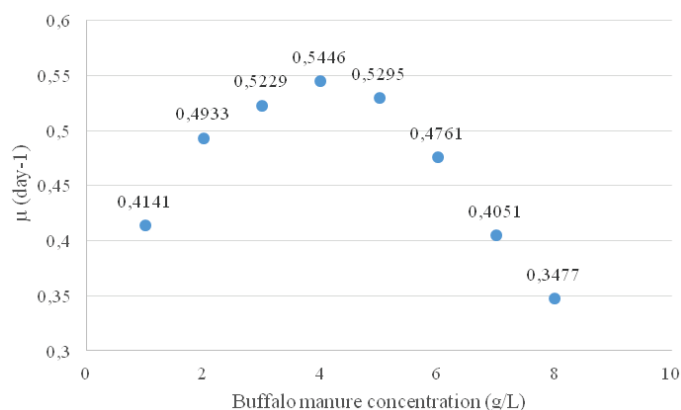


Fig 9 *Arhospira platensis* growth rates at various buffalo manure concentrations media

growth inhibition in murky media because the light was obstructed therefore the photosynthesis process of *A. platensis* cell was inhibit (Cheunbarn and Peerapornpisal 2010).

Lineweaver-Burk linearization is determined by comparing the value and substrate concentration at exponential phase. The equation obtained is used to determine the Monod kinetic parameters, those are  $\mu_{max}$  and  $K_s$ . Based on the equation, gradient value of 0.7124 indicates  $K_s/\mu_{max}$  value and intercept value of 1.6905 is equal to  $1/\mu_{max}$ , therefore it is obtained the  $\mu_{max}$  value of  $0.5915 \text{ day}^{-1}$  and  $K_s$  value of  $0.421 \text{ g.L}^{-1}$ . Contois kinetic model is different from Monod's due to both substrate and cell concentration are independent variables. In general, Contois model is used to determine the correlation of microbe cell growth rate and the concentration of substrate and cell mass (Abdullah *et al.* 2016). According to the linearization result for Contois model, it is obtained  $\mu_{max}$  value of  $0.6695 \text{ day}^{-1}$  and  $K_s$  value of  $5.143 \text{ g.L}^{-1}$ . Based on linearization for Tessier model, gradient value of 0.0591 indicated  $1/K_s$  value and intercept value of 0.8732 is equal to  $\ln \mu_{max}$ , therefore it is obtained the  $\mu_{max}$  value of  $0.417 \text{ day}^{-1}$  and  $K_s$  value of  $16.92 \text{ g.L}^{-1}$ . Different from Monod, Contois, and Tessier kinetic models, Verhulst kinetic model involves kinetic parameters of  $\mu_{max}$  and  $X_{max}$ , whereas  $X_{max}$  constitutes maximum dry cell weight. According to linearization for Verhulst model, it is obtained gradient value of 0.5905 indicates  $\mu_{max}/X_{max}$  value and intercept value of 0.3924 that is equal to  $\mu_{max}$  value, therefore the  $X_{max}$  value is  $0.2317 \text{ g.L}^{-1}$  and  $\mu_{max}$  value is  $0.3924 \text{ day}^{-1}$ . The most suitable growth kinetic model of *A. platensis* is chosen based on the correlation coefficient ( $R^2$ ) that is close to one. Correlation coefficient is an important value to describe the data accuracy and to consider the accuracy of regression model (Abdullah *et al.* 2016). Based on data in Table 1, Monod kinetic model is the most suitable growth kinetic model for *A. platensis* because it

has the highest correlation coefficient (0.9775) compared to the other kinetic models.

The  $\mu_{max}$  value of Monod model obtained from current research ( $0.5915 \text{ day}^{-1}$ ) is higher compared to  $\mu_{max}$  value obtained by Wang, Fu and Liu (2007) that observed the effects of light intensity variation to *A. platensis* growth rate,  $\mu_{max}$  value obtained by Sydney *et al.* (2010) about *A. platensis* strain LEB-52 growth in modified Bristol media, and  $\mu_{max}$  value obtained by Mezzomo *et al.* (2010) about the cultivation of *A. platensis* in swine manure, as shown in Table 2.

Due to the existence of inhibition in higher buffalo manure concentrations, it is obvious that the growth of *A. platensis* in this medium would not satisfy any conventional growth kinetic models. However, growth rates in concentration up to  $4 \text{ g.L}^{-1}$  was deemed normal enough for fitting to conventional models.

By statistic comparison, it was concluded that Monod model fits the experimental growth rates significantly better than other models. Monod model is the most fundamental model for biomass growth rate and only suitable for low substrate concentration. However, it is frequently found that this model is relatively representative in many situations, which has been proved in this study. This research confirmed the possibility of using buffalo manure as an alternative growth medium for *A. platensis*. In buffalo manure concentrations lower than  $5 \text{ g.L}^{-1}$ , the growth followed the Monod model while in higher concentrations, growth inhibition was detected. It is highly advised that further studies investigate more on the effect of other limiting substrate, such as carbon dioxide.

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