Growth kinetics of microalgae *Spirulina* sp. using vinasse media

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Abstract. *Spirulina* sp. is a type of microalgae that can accumulate functional metabolites in their biomass for use in various industries. However, due to the high production costs and low biomass content, there are still challenges in creating microalgae-based products. Therefore, it is necessary to utilize various nutrients in microalgae cultivation media to obtain biomass with high productivity. This research explores the use of vinasse as a cost-effective and highly nutritious cultivation medium obtained from vinasse to reduce harvest costs and develop the potential of a microalgae biorefinery that utilizes vinasse as a culture medium for *Spirulina* sp. strains. The aim of this research is to determine the ideal vinasse nutrition that can increase the number of *Spirulina* sp. cells, as well as considering the growth parameters of *Spirulina* sp. with the Gompertz and Richard Model. The parameter measured in this study was Optical Density (OD) which was measured every day during the 7 days observation period. The vinasse concentration used in this research was 4%. The results of this study indicate that the ideal amount of supplement for the growth of *Spirulina* sp. is a 0.04% supplement and the amount of biomass produced is 0.37 g/L.

1 Introduction

Microalgae is microorganisms that perform oxygenic photosynthesis found in freshwater conjointly marine situations, with extraordinary potential for biotechnology applications [1]. Microalgae can be used as sustainable renewable energy source that meets global energy needs [2]. In expansion to their tall development rate, microalgae too create and collect biomaterials that have commercial significance as pharmaceutical and nutraceutical items [3]. One of the primary by-products of microalgae biomass is lipids, which can subsequently be utilized as a biodiesel source [4]. Apart from being a feedstock for biodiesel, microalgae biomass is rich in protein [5].

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Spirulina sp. is a microalgae that can be used as a promising source of biomass, by-products, and bio-fuels because of its carbohydrates, and high fat content (its production is about 10 times that of palm oil) [6]. In addition, *Spirulina* sp. can also boost immunity [7]. Numerous microalgae species are able to develop viably in brackish water since they are effective at expelling N and P from wastewater and have the potential to play an imperative part in phyco-remediation. A few studies have appeared tall effectiveness within the expulsion of inorganic supplements, natural matter, overwhelming metals and colors from squander by microalgae [8].

In this context, the sugar-ethanol industry produces expansive amounts of fluid refining buildups commonly known as vinasse. The characteristics of vinasse depend on the crude materials and courses utilized for bioethanol generation. Vinasse is regularly acidic (pH 3.5–5), dull brown in color and with a tall natural substance related with a chemical oxygen demand (COD) esteem of 50 g/L to 150 g/L. Bioethanol generation from saccharide plants, starch plants, and cellulose materials can create huge sums of vinasse (on normal 1 L of ethanol yields 9–14 L of vinasse) [9].

In spite of the fact that vinasse can be utilized as a culture medium or as a cheap media supplement to deliver biomass, there are few reports of its utilize within the culture of photosynthetic microorganisms [10]. As of late, [11] assessed the generation of *Spirulina platensis* in a medium supplemented with vinasse, as well as analyzed the impact of the expansion of vinasse on biomass concentration and protein efficiency. In Brazil, [12] assessed the achievability of utilizing anaerobic stomach related squander handling sugarcane vinasse for the development and generation of lipids by *Chlorella vulgaris* and [13] assessed the possibility of creating *Scenedesmus* sp. utilizing sugarcane vinasse as an elective culture medium. The reason of this consider was to analyze the possibility of utilizing sugarcane vinasse as a supplement in culture media for the development of *Spirulina* sp., centering on the perfect supplement composition utilized and comparing the biomass gotten.

2 Material and Method

2.1 Cultivation of Spirulina sp.

Vinasse is collected directly and transported in bottles of 5 L. *Spirulina* sp. kindly donated by Nogotirto Algae Park, Yogyakarta, Indonesia. The strains are stored in 500 mL bottles under constant stirring and provided with fluorescent lamps in a 24-hour irradiation period and room temperature of 28-35 centigrade. To obtain inoculum, cells were grown in a 500 mL vial containing 250 mL of saline medium and 250 mL of *Spirulina* sp. The culture is gasified with filtered air pumped by an aerator. Water used in the cultivation process *Spirulina* sp. is fresh water that is sterilized to reduce the amount of microbial contaminants such as other microorganisms in it.

The preparation of salt media is carried out by adding 10 grams of NaCl to 1 liter of water so that salinity level of 10 ppm is obtained. Furthermore, the salt medium was sterilized by adding 0.03 grams of Calcium hypochlorite ($Ca(ClO)_2$) and precipitated for 30 minutes. Calcium hypochlorite functions to reduce contaminants contained in water. After 30 minutes, then 0.03 grams of Thiosulfate ($Na_2S_2O_3$) was added which functions to neutralize Calcium hypochlorite in salt medium.

Growth rate of *Spirulina* sp. is significantly affected by the source of supplements accessible within the development medium. Sources of supplements can be gotten from natural or inorganic (chemical) fertilizers. The composition of the fertilizer utilized in this ponder alludes to the standard composition that has been utilized in Nogotirto Algae Park.

The composition of the development medium for the mass development of the *Spirulina* sp. can be seen in Table 1.

Table 1. Composition of Microalgae Growth Medium *Spirulina* sp.

Composition	g/L	Sources of Nutrition
NaCl (sea salt)	5	Salt components
Urea Fertilizer ($CO(NH_2)_2$)	0.05	Source C, H, N
NPK Fertilizer	0.03	Source N, P, K
Ammonium Sulphate ($(NH_4)_{SO4}$)	0.15	Source S, N
Soda Ash Dense (Na2CO3)	0.075	Source C and Na

The vinasse used in this study is 4% v/v dilution vinasse in the cultivation process of the *Spirulina* sp. This study was carried out for 7 days in accordance with the results of observations and calculations of cell density that had been carried out in the pre-research stage for 3 cycles. Cultivation is carried out on a 500 ml bottle scale. The ratio of inoculum and culture medium used was 1:1. Observations made in the cultivation process include cell density.

2.2 Data Collection

In this study, the measurement of microalgae growth parameters includes measurement of temperature, pH, turbidity and salinity during the cultivation process. The measurement of microalgae growth parameters in this study aims to determine the optimal conditions of microalgae growth. Measurements are taken daily during the cultivation period using a digital measuring device.

Spirulina sp. cell counts were conducted through observations using a Yazumi Demonstrate XSZ-107BN magnifying glass and a Neubauer Moved Forward Haemocytometer for 7 days. Observations were made by taking a 1 ml sample, then dropping it into the Haemocytometer that had been installed under the objective lens of the magnifying glass with a magnification of 10x. The calculation of the number of microalgae cells in a large box with a width of 1 mm, a field of view of 1 mm^2, and a volume of 0.1 mm^3 ($1x10^4$mL) [14].

To conduct an initial investigation regarding the feasibility of *Spirulina* sp. culture, cultures were carried out in 5 different nutrient variations. The 7-day cycle ends with biomass harvesting. The biomass obtained is harvested by filtering. Furthermore, the filtered samples are then dried using an oven for 30 minutes at a temperature of 60°C so that the dry weight of the biomass *of Spirulina* sp. and weighed. Weighing is carried out until the weight is constant. The dry paper is cooled in a desiccant and re-weighed. The dry biomass weight is calculated from the subtraction between the ultimate weight and the introductory weight of the channel paper. The weight of biomass is measured in g/L units.

2.3 Data Analysis

The data analysis used in this study is in the form of empirical approaches. The empirical approach by making graphs using cultivation time data and analysis data, namely cell density, dry cell weight during the cultivation period at each nutrient concentration. Then a kinetic study was carried out using numerical simulations with the Richard Model (Equation 1) and Gompertz Model (Equation 2) on the most optimal medium result.

The Gomperzt Model assumes exponential curve-like microbial growth [15]. This model can determine the maximum cell production rate (r_m) and *lag* time (t_L) as shown in Equation 1.

$$X = X_0 + X_{max} \cdot \exp\left[-\exp\left(\left(\frac{r_m \cdot \exp(1)}{X_{max}}\right)(t_L - t) + 1\right)\right] \quad (1)$$

The dynamics of microbial growth are generally also often described by the Richard Model. Equation 2 illustrates how this model represents the maximum growth rate in the logarithmic, stagnant, and dead phases.

$$X = \frac{X_{max}}{\left(1+k*\{exp[r*(t-t_{max})]\}^{\frac{1}{k}}\right)} \quad (2)$$

3 Result and Discussion

Cell density determines the growth rate of *Spirulina* sp. The development phase of *Spirulina* sp. during the cultivation period begins with the adaptation phase, continues through the exponential phase (log phase), log phase decline, stationary phase, and ends with the death phase. During cultivation, *Spirulina* sp. requires light, CO_2 and nutrients such as nitrogen, phosphorus and potassium which play a role in the metabolism process. In this study, *Spirulina* sp. was cultured in a salt medium containing nutrients in the form of technical inorganic fertilizers and vinasse was added as a nutrient additive with different concentrations in each medium. The density of *Spirulina* sp. cells was measured for seven days. The results showed that differences in nutrient concentrations resulted in differences in the number of *Spirulina* sp. cell densities. Figure 1 shows the results of observations of *Spirulina* sp. cell density

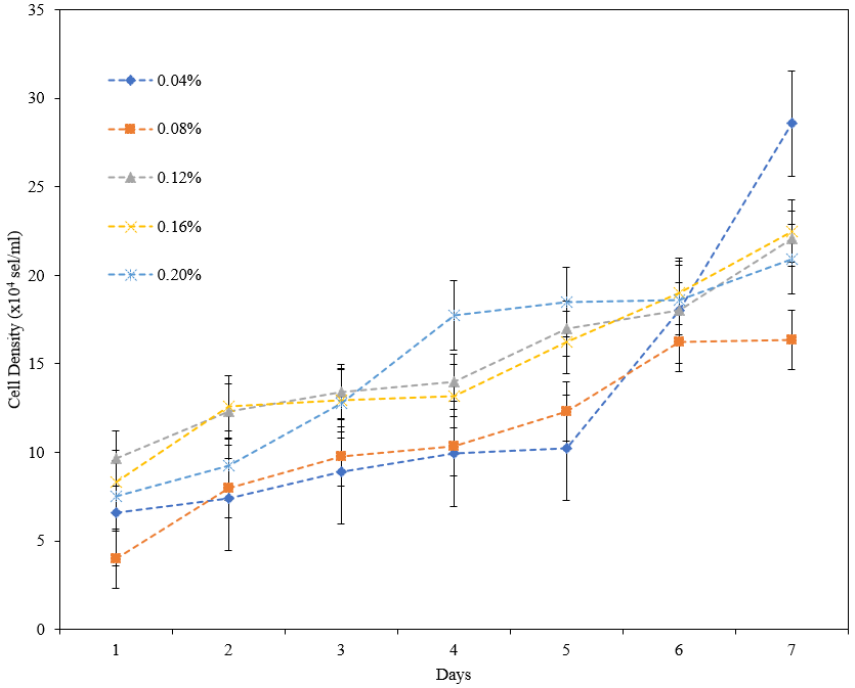

Fig. 1. Cell density of *Spirulina* sp. in various doses of technical fertilizers

Figure 1 illustrates how growth patterns at different fertilizer doses can be classified into three phases: the lag phase (adaptation), the exponential phase (log phase), and the log phase decrease. By the second day, it has increased but is not significant. This indicates that at the beginning of the growth period, the cells. *Spirulina* sp. undergoes an adaptation phase. During the adaptation phase, the process of absorption of nutrients into *Spirulina* sp. cells occurs. The ability of microalgae to adapt to their intracellular conditions is influenced by organic and inorganic compounds that function as nutrients [16]. Well-adapted *Spirulina* sp. cells will experience increased cell division activity as the cultivation process progresses, and will accelerate growth and increase population density. This phase is also referred to as the exponential phase, in which *Spirulina* sp. cells synthesize proteins for cell division. When *Spirulina* sp. grows well and the nitrogen availability is good, the microalgae will utilize the protein for the cell division process, and whenever the nitrogen content of the medium drops, the microalgae accumulate the result of photosynthesis in the form of lipids.

The exponential phase is the best phase to harvest *Spirulina* sp. because optimal growing conditions are achieved in this phase. Nutrients were given to *Spirulina* sp. which was cultured in various concentrations, namely 0.04%, 0.08%, 0.12%, 0.16% and 0.2%. Based on the observations obtained, there was an increase in cells in *Spirulina* sp.

In the addition of 0.04% nutrients, the highest total cell density is 28.58×10^4 cells/ml. These results show that with a nutrient concentration of 0.04%, *Spirulina* sp. can utilize nutrients along with vinasse by breaking them down into compounds used for growth and metabolism, so that it can increase its growth rate.

The growth of cultured *Spirulina* sp.cells is determined by dry cell weight or biomass [17]. Biomass is produced from the process of assimilation of various nutrients through the process of photosynthesis. When nutrient uptake and vinasse are high, a logarithmic phase is achieved and followed by biomass harvesting [18]. Figure 2 presents a comparison of the development of *Spirulina* sp. on distinctive supplements. After 7 days, the most elevated development created 0.37 g/L of biomass in a medium fed with 0.04% nutrients. Meanwhile, at the highest dose of 0.2%, the weight of biomass is only 0.19 g/L.

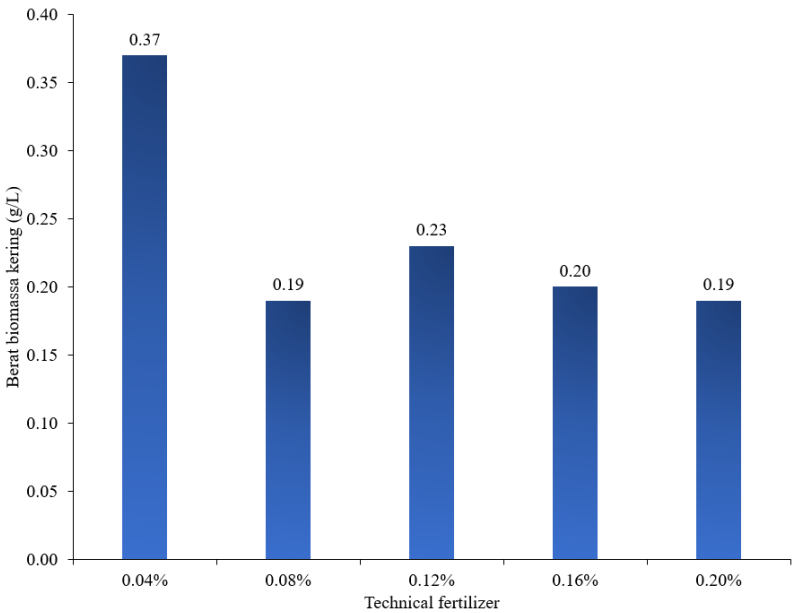

Fig. 2. Comparison of dry biomass at different doses of technical fertilizers

Based on observations,the optimal point of the best vinasse concentration that can produce the highest cell density and biomass is the addition of 0.04% of the total culture medium. The growth kinetics study of *Spirulina* sp. was carried out on the most optimal media. The growth kinetics of *Spirulina* sp. were determined using two mathematical models, namely Gompertz and Richard Model.

The results of the kinetic simulation of *Spirulina* sp. growth cultured on medium with the addition of vinasse modeled by Gompertz and Richard's equation are shown in Figure 3. The chart of the relationship between nutrient concentration information and *Spirulina* sp. cell density results information during cultivation.

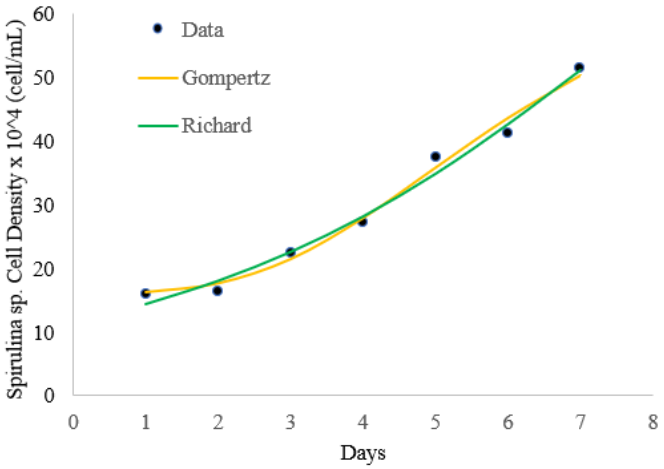

Fig. 3. Cell density *of Spirulina* sp. with Gompertz and Richard Model

Figure 3 appears that between the comes about of the recreation information and the exploratory information shape a bend that characterizes the development design where the information dispersion is still around the dispersion of the exploratory information, but there's a deviation between the test information and the simulation information within the two models. Figure 3 illustrates how the Gompertz and Richard Model provide simulation results that are equally superior in terms of the kinetics of the growth rate *of Spirulina* sp. Richard's model can describe exponential, linear, slowing and death phases [19]. While the Gompertz Model can describe it with higher accuracy in the cultivation of *Spirulina* sp. [20].

The simulation graph shows that there is a slight difference between the simulation results and the experimental data, or it can be said that the simulation data produces a value that is close to the value of the experimental data. The characteristics derived from kinetic simulation and mathematical modeling of *Spirulina* sp. growth rate are presented in Table 2. The simulation parameters include maximum cell production rate (r_m), time lag (t_L), and determination coefficient (*R2*).

Table 2. Kinetic Parameters of Simulation Results

Parameters	**Type**	
	Gompertz	**Richard**
Rm	8.1087	10.2780
tL,	2.5532	1.0664
R2	0,9874	0,9856

Table 2 explains that the parameter values generated by each model give different results. The exponential phase is the point at which the growth rate reaches its maximum, so

here the maximum density is reached. In the Gompertz Model, the r_m value obtained is 8.1087. Meanwhile, in the Richard Model, the r_m value obtained is 10.2780. The obtained r_m values describe the maximum cell production when in the exponential phase. The lag time (t_L) indicates how long it takes *Spirulina* sp. to adapt to the new growing medium. The t_L value obtained is 2.5532 days in the Gompertz Model and 1.0664 days in the Richard Model.

The t_L time tends to be long due to the presence of organic ions in the vinasse absorbed by *Spirulina* sp., as well as the presence of contaminants in the vinasse. Based on the analysis of *R2* in each model in this study, it can be seen that the highest *R2* value was obtained in the Gompertz Model. A high *R2* value indicates that this model is able to adequately validate the experimental data. The mathematical approach in this study shows that the growth curve modeled using the Gompertz Model produces the best simulation compared to the Richard Model. The Gompertz equation can be used when the *Spirulina* sp. undergoes a lag phase or adjustment time before finally undergoing cell division.

4 Conclusion

This study observed the effect of nutritional differences on the kinetics of the growth rate and production of biomass *Spirulina* sp. The results showed that the optimal conditions for cell density, growth rate and dry biomass yield of *Spirulina* sp. were at the addition of 0.04% nutrients with vinasse. The highest biomass concentration produced was 0.37 g/L on day 7. This study proves that sugarcane vinasse can be used as an alternative supplement to *Spirulina* sp. culture, so that vinasse can be a promising strategy to produce high biomass with efficient fertilizer substitutes.

References

1. D. B. Stengel, S. Connan, & Z. A. Popper. Algal chemodiversity and bioactivity: Sources of natural variability and implications for commercial application. Biotechnology Advances **29,** 483–501 (2011).
2. E. Habibah, et al. Potential of natural sunlight for microalgae cultivation in Yogyakarta. in IOP Conference Series: Earth and Environmental Science **963,** (IOP Publishing Ltd, 2022).
3. M. A. Borowitzka. High-value products from microalgae-their development and commercialisation. Journal of Applied Phycology **25,** 743–756 (2013).
4. F. Cahyani, A. Ardi, T. T. Samudra, A. Budiman, & E. A. Suyono. Biomass productivity and lipid content between natural mixed culture consortia and artificial mixed culture. in AIP Conference Proceedings **2260,** (American Institute of Physics Inc., 2020).
5. D. R. Prasetya, E. Baliarti, L. M. Yusiati, N. Dewayanto & A. Budiman. Potential of Spirulina platensis as a Feed Protein Supplement for Sheep Ration. in AIP Conference Proceedings **2628,** (American Institute of Physics Inc., 2023).
6. N. E. Wahyuni, et al. Variation of Biomass and Lipid Content of a Mixed Culture of Glagah Isolate and Arthrospira maxima in Thin-Layer Photobioreactor Using Three Different Media (BBM, Farmpion and TEAM). in AIP Conference Proceedings **2260,** (American Institute of Physics Inc., 2020).
7. Z. Afililla et al. Inability of polysaccharides of Spirulina platensis to protect hepatocyte cells line on Toxoplasma gondii infection in vitro. Journal of Global Pharma Technology **12,** 654–659 (2020).

8. J. C. M. Pires, M. C. M. Alvim-Ferraz, F. G. Martins & M. Simões. Wastewater treatment to enhance the economic viability of microalgae culture. Environmental Science and Pollution Research **20,** 5096–5105 (2013).
9. E. España-Gamboa, et al. Vinasses: Characterization and treatments. Waste Management and Research **29,** 1235–1250 (2011).
10. R. S. Raquel, Q. F. Ofélia, L. M. José, M. C. Ricardo. Cultivation of Spirulina maxima in medium supplemented with sugarcane vinasse. Bioresource Technology **204,** 34-48 (2016).
11. M. Coca, V. M. Barrocal, S. Lucas, G. González-Benito & M. T. García-Cubero. Protein production in Spirulina platensis biomass using beet vinasse-supplemented culture media. Food and Bioproducts Processing **94,** 306–312 (2015).
12. S. S. I. Marques, I. A. Nascimento, P. F. De Almeida & F. A. Chinalia. Growth of Chlorella vulgaris on sugarcane vinasse: The effect of anaerobic digestion pretreatment. Applied Biochemistry and Biotechnology **171,** 1933–1943 (2013).
13. N. N. V. Ramirez, M. Farenzena & J. O. Trierweiler. Growth of microalgae *Scenedesmus* sp. in ethanol vinasse. Brazilian Archives of Biology and Technology **57,** 630–635 (2014).
14. BTI. Algae to Energy - Using and Re-using a Hemocytometer to Count Algae Cells. BTI Curriculum Development Projects in Plant Biology. (2015)
15. N. Phukoetphim, A. Salakkam, P. Laopaiboon, & L. Laopaiboon. Kinetic models for batch ethanol production from sweet sorghum juice under normal and high gravity fermentations: Logistic and modified Gompertz models. Journal of Biotechnology **243,** 69–75 (2017).
16. J. Prayitno. Biomass growth and harvesting patterns in microalgae photobioreactors for carbon capture. Journal of Environmental Technology **17**, 45-52 (2016).
17. W. Basuki. Bioconversion of Glycerol to Docosahexaenoic Acid by Thraustochytrium WB-02 an Indigenous Indonesian Microalga Strain. Makara Journal of Science **23,** 97–102 (2019).
18. K. Kusmiati, W. Rahmawati, N. W. S. Agustini. Effect of Mg^{2+} and Fe^{2+} Concentrations in Culture Medium on CGF Formation from Microalgae Chlorella Pyrenoidosa Ink and Analysis of Amino Acids by Liquid Chromatography-Mass Spectrometry. Makara Journal of Science **19,** (2015).
19. Y. Fujihara. *et al.* Spirulina platensis and its ingredient biopterin glucoside improved insulin sensitivity in non-alcoholic steatohepatitis model. Journal of Clinical Biochemistry and Nutrition **69,** 151–157 (2021).
20. V. Vásquez, P. Artega, K. Chanamé, & A. Esquivel. Mathematical modeling and artificial neural networks of the growth of *Spirulina* sp. in photobioreactor with fluorescent light source and solid state illumination. Scientia Agropecuaria **4**, 199–209 (2013).