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Utilization of Solid Waste from White Shrimp (*Litopenaeus vannamei*) Farm on the Growth and Chlorophyll Content in *Chlorella* sp.

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ABSTRACT: Solid waste from white shrimp farm contained the macro and micro nutrients that required for the growth of *Chlorella* sp. This microalgae usually used in the fisheries and pharmaceuticals, especially chlorophyll content. This study aims to assess dosage of solid waste from white shrimp farm that used to produce the highest cell density, the fastest of specific growth rate, and the highest chlorophyll content in the *Chlorella* sp. Waste samples obtained from white shrimp intensive farming. The method used in this study is the experimental method with five treatments (0, 2, 4, 6, and 8 g/l) and 3 replications. Data obtained from the results of subsequent studies analyzed statistically by completely randomized design (CRD) using statistical applications, SPSS version 16.0. The results utilization of a dose as much as 2 g/l resulted in the highest cell density (2,333,333 cells/ml), the fastest specific growth rate (0.7677), and the chlorophyll content is 89.0568 mg/m^3 (86.1568 mg/m^3 of chlorophyll a and 2.9000 mg/m^3 chlorophyll b).

Key words: Solid waste from white shrimp (*Litopenaeus vannamei*) farm, *Chlorella* sp., Cell Density, Specific Growth Rate, Chlorophyll

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INTRODUCTION

Solid waste shrimp farm containing 1.92% C organic; 0.54% N total; and 1.70% P. Nutrient levels contained in solid waste shrimp farm is sufficient for growth of *Chlorella* sp. because algae cells require 0.063 g N and 0.009 g P to produce 1 g cells new algae [1]. *Chlorella* sp. needs nitrogen in 0.14 to 0.7 g/l and phosphorus from 0.0075 to 0.3 g/l [2].

Chlorella sp. is one of the natural feed for shrimp larvae and fish that have high nutritional value. Microalgae contains 51-58% protein, 12-26% carbohydrate, 2-22% fat, and 4-5% of nucleic acids. In addition, Chlorella sp. also contains high chlorophyll pigments than other species, which the pigments are widely used in the pharmaceutical [3].

The purpose of this study is to assess the dose of solid waste white shrimp farm to produce high cell density, the fastest of specific growth rate, and the highest chlorophyll content in the *Chlorella* sp. Cell density and specific growth rate can be used as a benchmark for the growth of *Chlorella* sp. Chlorophyll is one of the essential elements which are owned by *Chlorella sp.* is widely used in the pharmaceutical.

MATERIAL AND METHODE

This study was conducted in November 2014 - March 2015 in the Laboratory of Soil Chemistry, Faculty of Agriculture, University of Brawijaya Malang and Laboratory of Chemistry, University of Muhammadiyah Malang for nutrient analysis of solid waste white shrimp *(L. vannamei)* farm. Identification of heterotrophic bacteria and nitrification performed on Fish Quarantine, Perak, Surabaya. Inorganic materials analysis conducted at the Laboratory of Aquatic Environment and Biotechnology, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang. The culture of *Chlorella* sp. conducted at the Workshop Laboratory, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang.

The method used in this study is the experimental method. This study consists of five treatments (0, 2, 4, 6, and 8 g/l) and 3 replications, conducted a pure culture of *Chlorella* sp. with Walne fertilizer of 1 ml/l as a comparison. Before being used as a culture medium *Chlorella* sp., solid waste white shrimp farm soaked for 24

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hours (without the light conditions and added by aeration) in accordance with the treatment given dose. Then, the solution was filtered to separate the solids with liquids, where liquid is used as a pure culture media *Chlorella* sp. Glass jars used as containers for pure culture of *Chlorella* sp., Then put liquids derived from solid waste white shrimp farm and also added seeds of *Chlorella* sp. The parameters observed in this study are the cell density [4], specific growth rate [4] and chlorophyll content [5]. The data obtained from the study will be analyzed statistically by completely randomized design (CRD) using statistical applications, SPSS version 16.0. For chlorophyll levels were analyzed descriptively among the best dose treatment (as seen from the cell density and the specific growth rate) with Walne fertilizer 1 ml/l to see the picture of levels of chlorophyll in two treatments.

RESULTS AND DISCUSSION

Cell Density of *Chlorella* sp.

Solid waste white shrimp farm contain of nutrients and required for the growth of *Chlorella* sp. Doses of solid waste white shrimp farms are used as a medium for the growth of *Chlorella* sp. must be adapted to the needs of these microalgae. The following will be presented on the cell density of *Chlorella* sp. were cultured with various doses of solid waste white shrimp farms (Table 1 and Figure 1).

Table 1. The peak population of *Chlorella* sp. were cultured with various doses of solid waste white shrimp farm

Treatments	Replication			Total (cell/ml)	Average (sel/ml)	STD
	1	2	3			
A	470.000	418.000	398.000	1.286.000	428.667	37.166,293
B	2.100.000	2.440.000	2.460.000	7.000.000	2.333.333	202.319,879
C	760.000	720.000	860.000	2.340.000	780.000	72.111,026
D	600.000	700.000	660.000	1.960.000	653.333	50.332,230
E	500.000	440.000	540.000	1.480.000	493.333	50.332,230
W	1.180.000	1.060.000	1.100.000	3.340.000	1.113.333	61.101,01

Specification: A: Dose of waste 0 g/l; B: Dose of waste 2 g/l; C: Dose of waste 4 g/l; D : Dose of waste 6 g/l; E: Dose of waste 8 g/l; W: Dose of Walne fertilizers 1 ml/l; STD: Standard deviation

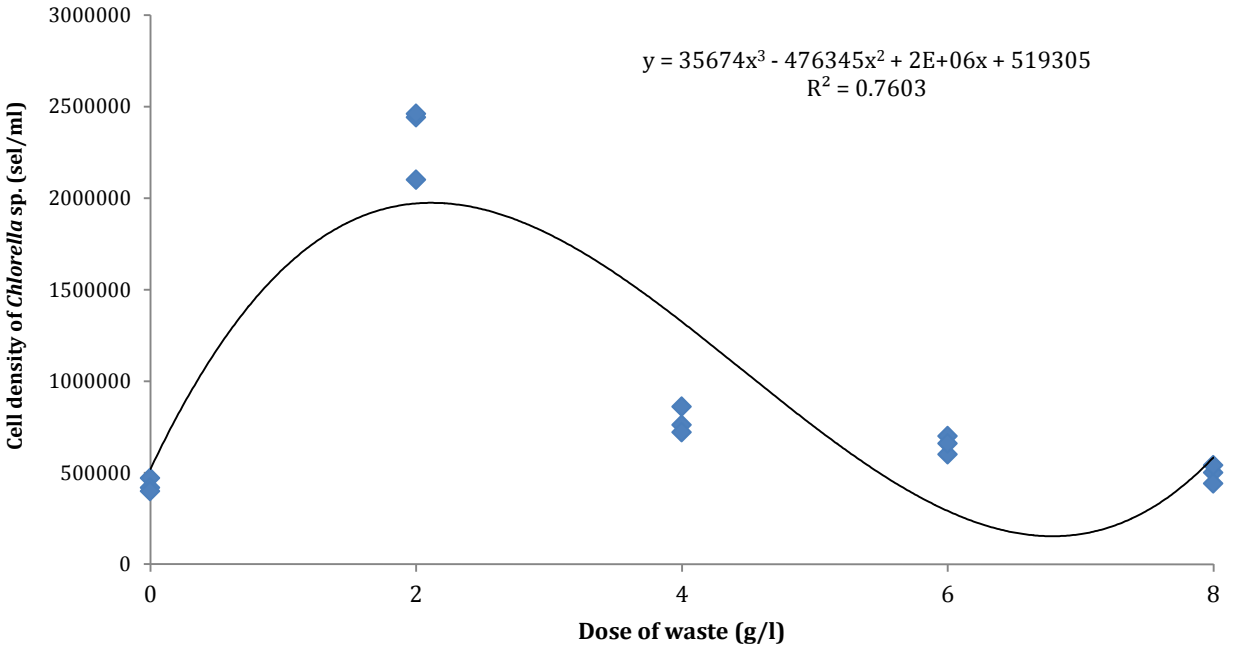

Figure 1. The regression curve of cell density of *Chlorella* sp. at different dose of solid waste white shrimp farm

Based on Table 1 it can be seen that treatment of B produce the highest cells density of *Chlorella* sp., is 2,333,333 cells/ml. This results shows that the used of solid waste white shrimp farm as much as 2 g/l give the maximum results for this phytoplankton growth. The higher dose of solid waste that is used for the culture of *Chlorella sp.*, the microalgae growth is declining. This is due to the higher dose of solid waste given the culture of *Chlorella* sp., it will increase the amount of organic material. Organic matters that are toxic algae causes cell death by disrupting the cell's metabolism by increasing maintenance time [6].

Figure 1 shows the regression curve those results in the regression equation and the coefficient of determination. The regression equation obtained is $y = 35674x^3 - 476345x^2 + 2E + 06x + 519305$ with $R^2 = 0.7603$ and r = 0.872. The correlation coefficient (r) states the degree of influence between independent variable and dependent variable dose treatment means solid waste affects the shrimp ponds for 87.20% of the cell density of *Chlorella sp.*

Walne fertilizer, Guillard's f/2, and Erdscheiber are common medium used to grow phytoplankton types of Chlorophyceae, including *Chlorella* sp. [7]. Several alternative media used for culturing *Chlorella* sp. is soil extract that results in cell density 5.6666×10^7 cells/ml [8], liquid fertilizer of waste lemuru (*Sardinella* sp.) that produce the cell density 3.5×10^6 cells/ml [2], waste seaweed *Gracilaria* sp., which results in cell density 2.64251×10^5 cells/ml [9], tilapia fish farming waste [10], and waste food [11]. Solid waste white shrimp farms (*L. vannamei*) is also capable of functioning as a medium for the growth of *Chlorella* sp. and the best dosage that can produce the highest cell density was 2 g/l, the average density of the resulting cells is 2.333333×10^6 cells/ml.

Specific Growth Rate of *Chlorella* sp.

The successful culture of microalgae can be seen from the cell density and specific growth rate of the microalgae. Specific growth rate of phytoplankton describe the growth rate per unit of time that can be used as a reference to illustrate the carrying capacity of the media on the growth of phytoplankton. The specific growth rate of *Chlorella* sp. experience changes during the culture progresses, as shown in Table 2 and Figure 2.

Table 2. Specific growth rate of *Chlorella* sp. were cultured with various doses of solid waste white shrimp farm

Treatments	Replication			Total	Average	STD
	1	2	3			
A	0,5135	0,4929	0,4991	1,5055	0,5018	0,0106
B	0,7567	0,7573	0,7890	2,3030	0,7677	0,0185
C	0,5817	0,5744	0,6006	1,7567	0,5856	0,0135
D	0,5422	0,5861	0,5654	1,6937	0,5646	0,0220
E	0,5353	0,5407	0,5530	1,6290	0,5430	0,0091
W	0,6643	0,6660	0,6589	1,9892	0,6631	0,0037

Specification: A: Dose of waste 0 g/l; B: Dose of waste 2 g/l; C: Dose of waste 4 g/l; D : Dose of waste 6 g/l; E: Dose of waste 8 g/l; W: Dose of Walne fertilizers 1 ml/l; STD: Standard deviation

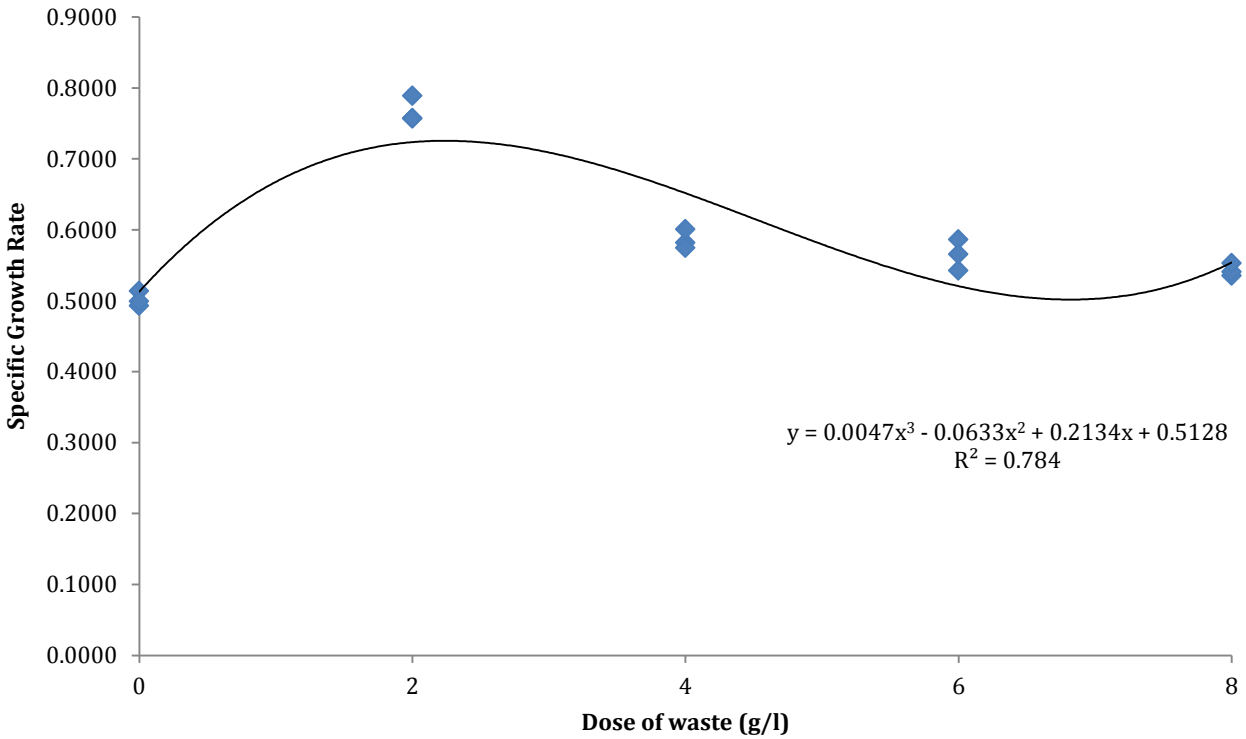

Figure 2. The regression curve of specific growth rate of *Chlorella* sp. at different dose of solid waste white shrimp farm

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Based on Table 2 it can be seen that the highest specific growth rate was obtained in the culture of *Chlorella* sp. with 2 g/l of solid waste white shrimp farm by 0.7677; while the lowest value was obtained from the culture of *Chlorella* sp. with 0 g/l of solid waste white shrimp pond or sea water only, 0.5018. Nutrient content contained in solid waste white shrimp (*L. vannamei*) farm has nutrient needs *Chlorella* sp. so it can support the growth and specific growth rate of the microalgae.

Figure 2 shows the regression equation between dose and R^2 solid waste white shrimp farm with a specific growth rate of *Chlorella* sp. the regression equation obtained is $y = 0{,}0047x^3 - 0{,}0633x^2 + 0{,}2134x + 0.5128$ with $R^2 = 0.784$ and $r = 0.885$. Value r stated that the dosing of solid waste white shrimp (*L. vannamei*) farms affect 88.50% of the specific growth rate of *Chlorella sp.*

Treatment B (dosage waste 2 g/l) resulted in specific growth rate (k) *Chlorella* sp. higher than other treatments. According to Suminto and Hirayama [12], a larger k value means that phytoplankton cell division process becomes faster so that an increase in cells per unit time will be greater. Solid waste white shrimp farms contain of nutrients required for the growth *of Chlorella* sp. which influenced the high specific growth rate. When used with an appropriate dose to needs of *Chlorella* sp., the waste can be used as an alternative medium for growing the phytoplankton.

Chlorophyll content of *Chlorella* sp.

Chlorophyll is a type of photosynthetic pigments possessed by plants, which are able to absorb red, green, and blue, and green colors to reflect that because plant has its distinctive color. Chlorophyll a is the type of pigment that is present in all organisms autotrof. Chlorophyll b is a pigment found in Chlorophyta and land plants. Chlorophyll c is the pigment contained in Phaeophyta and diatom Bacillariophyta. Chlorophyll d is a pigment contained in Rhodophyta. Chlorophyll content contained in phytoplankton varies, depending on the type of media used to cultivate this phytoplankton. Solid waste white shrimp farm and fertilizers Walne can be used to grow *Chlorella* sp. Chlorophyll content resulting from both the media is certainly different, as shown in Figure 3.

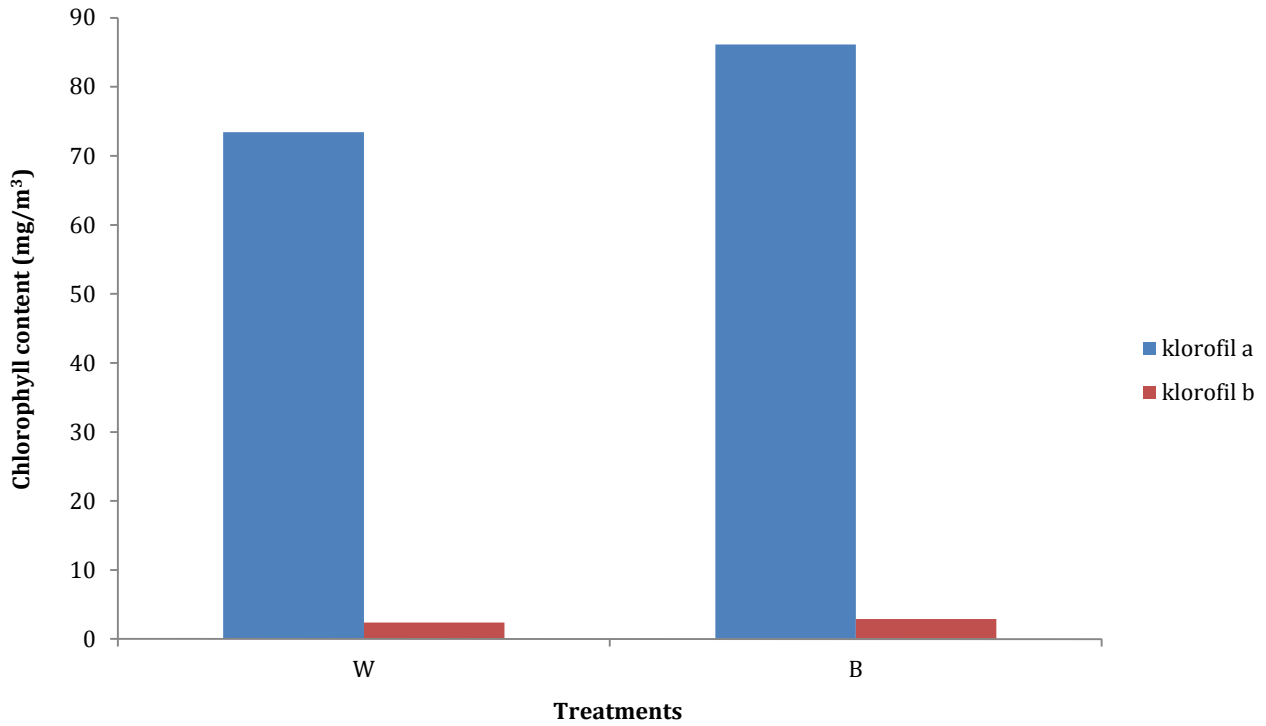

Figure 3. Chlorophyll content of *Chlorella* sp. in a different culture media
Specification: W: Walne fertilizer 1 ml/l; B: Dose of waste 2 g/l

Based on the figure above it can be seen that *Chlorella* sp. cultured with 2 g/l of solid waste white shrimp farm produce high levels of chlorophyll a and b are higher than *Chlorella* sp. cultured with Walne fertilizer. Levels of chlorophyll a and b Chlorella sp. cultured with 2 g/l of solid waste shrimp farms, respectively, was 86.1568 mg/m^3 and 2.9000 mg/m^3. Levels of chlorophyll a and b *Chlorella* sp. cultured with fertilizer Walne, respectively, was 73.4168 mg/m^3 and 2.3728 mg/m^3.

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The content of chlorophyll in *Chlorella* sp. comparable with the growth of the number of cells [13]. The higher density of algae cells, the levels of chlorophyll will be higher as well. This is consistent with the results obtained, in which *Chlorella* sp. cultured with 2 g/l of solid waste white shrimp farms generate higher cell density than *Chlorella* sp. cultured with Walne fertilizer. Cell density *Chlorella* sp. cultured with 2 g/l of solid waste white shrimp farm is 2,333,333 cells/ml while the cell density *Chlorella* sp. cultured with Walne fertilizer is 1,113,333 cells/ml.

CONCLUSION

Utilization of waste with dose 2 g/l resulted in the growth of *Chlorella* sp. The best that can be seen from the highest cell density (2,333,333 cells/ml), the fastest of specific growth rate (0.7677), and the highest chlorophyll content (89.0568 mg/m^3 consisting of 86.1568 mg/m^3 of chlorophyll a and 2.9000 mg/m^3 of chlorophyll b).

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