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Utilization of Sago Liquid Waste Organic Fertilizer as a Culture Medium for *Chlorella* sp.

Pemanfaatan Pupuk Organik Limbah Cair Sagu sebagai Media Kultur *Chlorella* sp.

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ABSTRACT

Chlorella sp. is a microalga that can grow and develop in wastewater media, including sago liquid waste that is not utilized and becomes a pollution material. This study aims to determine the utilization of sago liquid waste organic fertilizer on the growth of *Chlorella sp*. This research was conducted in February 2023 at the Marine Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Riau University. The method used was the experimental method, by applying a complete randomized design (CRD) with four treatments and three replicates. The treatments were as follows: T0 (control), T1 (150 ml sago liquid waste), T2 (200 ml), and T3 (250 ml). The initial density of *Chlorella* sp. was 250 x10^3 cells/ml, and cell abundance observations were made for 14 days. The parameters observed were cell abundance, specific growth rate, and water quality (temperature and pH). The results showed that the utilization of sago liquid waste affected the abundance of *Chlorella* sp. 150 ml concentration and gave the best results on cell abundance of 233.33 x10^3 cells/ml with the peak population occurring on day 9. Water quality during the study was still in the normal range and can be tolerated for the growth of *Chlorella* sp, namely temperature ranging from 26-31^o C and pH 6.2-8.0.

Keywords: Liquid Waste, Cell Abundance, Microalgae

ABSTRAK

Chlorella sp. merupakan mikroalga yang dapat tumbuh dan berkembang pada media air limbah, termasuk limbah cair sagu yang tidak termanfaatkan dan menjadi bahan pencemaran. Penelitian ini bertujuan untuk mengetahui pemanfaatan pemberian pupuk organik limbah cair sagu terhadap pertumbuhan *Chlorella* sp. Penelitian ini dilaksanakn pada bulan Februari 2023 di Laboratorium Bioteknologi Kelautan, Fakultas Perikanan dan Kelautan, Universitas Riau. Metode yang digunakan adalah metode eksperimen, dengan menerapkan rancangan acak lengkap (RAL) dengan empat perlakuan dan tiga ulangan. Adapun perlakuannya sebagai berikut: T0 (kontrol), T1 (150 ml limbah cair sagu), T2 (200 ml), dan T3 (250 ml). Kepadatan awal sela *Chlorella* sp. sebanyak 250 x10^3 sel/ml, pengamatan kelimpahan sel dilakukan selama 14 hari. Parameter yang diamati, yaitu kelimpahan sel, laju pertumbuhan spesifik dan kualitas air (suhu dan pH). Hasil penelitian menunjukkan bahwa pemanfaatan limbah cair sagu memberikan pengaruh terhadap kelimpahan sel *Chlorella* sp. Konsentrasi 150 ml memberikan hasil terbaik terhadap kelimpahan sel yaitu 233,33 x10^3 sel/ml dengan puncak populasi terjadi pada hari ke-9. Kualitas air selama penelitian masih berada pada kisaran normal dan dapat ditoleransi bagi pertumbuhan *Chlorella* sp, yaitu suhu berkisar antara 26-31^{o}C dan pH 6,2-8,0.

Kata Kunci: Limbah Cair, Kelimpahan sel, Mikroalga

INTRODUCTION

Fish feed is one of the important needs that must be considered in determining the success of aquaculture. The availability of natural food in the waters must be in adequate quantities, timely and sustainable. The problem often faced by farmers is the high mortality rate in the larval phase (Rosyadi *et al.*, 2019). This is because at this stage the food reserves derived from the body (egg yolk) will be depleted, so it requires additional feed that is suitable for mouth openings and adequate nutrition (Priyadi *et al.*, 2010). The utilization of natural feed, such as microalgae, can be a promising alternative. According to Abd El-Hack *et al.* (2019), microalgae contain about

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50% crude protein, an amino acid profile similar to fishmeal, good PUFA fatty acids, and effective bioactive compounds. One type of microalgae that can be used is *Chlorella* sp.

Chlorella sp as a natural food in fish and shrimp larval stadia has several advantages, such as fast growth, contains protein and lipids, good adaptation (Nugraha *et al.*, 2020), easy to digest (Darosman *et al.*, 2019), easy to obtain, relatively cheap price, does not pollute the maintenance media (Buwono *et al.*, 2019), improves pigmentation (Sergejevova & Masojidek, 2012), immunostimulants (Zahran & Risha, 2014), increases growth and survival (Septian *et al.*, 2017). According to Canelli *et al.* (2020), *Chlorella* sp contains carbohydrates ranging from 8.5-20.4%, protein 59.6-65.6%, and fatty acids 8.4-11.2%. That it can grow and develop in wastewater media (Alagawany *et al.*, 2021). Mufidah *et al.* (2017) stated that *Chlorella* sp requires maintenance media containing macronutrients (C, N, P, S, Na, Mg, and Ca) and micronutrients (Fe, Cu, Mn, Zn, B, Mo, V, and Co).

Sago waste can be an alternative that can be used as a growth medium for *Chlorella* sp. because it contains macronutrients such as N around 1.2- 2.7%, P 0.3-0.6%, K) 0.5-2.5%, Ca 0.5-1.5%, Mg 0.2-0.5%, and S 0.1-0.3% of the dry weight of sago waste. Micronutrients such as Fe, Mn, Cu, Zn, B, and Mo (Singhal *in* Lestari, 2013). According to Restuhadi *et al.* (2017), 94% of sago liquid waste, or 3.8 million m^3 per year will become water pollution and not be utilized. The utilization of sago liquid waste to increase the growth and abundance of microalgae has been widely done, including *Chlorella* sp (Fernandiaz *et al.*, 2017), isolate LIPI11-2-AL002 (Cyanobacteria) (Sulilaningsih *et al.* 2014).

Based on this background, it is necessary to conduct research by utilizing sago liquid waste as an alternative fertilizer to increase the growth of *Chlorella* sp. This study aims to determine the utilization of sago liquid waste organic fertilizer on the growth of *Chlorella* sp.

MATERIALS AND METHOD

Time and place

The research was conducted in February 2023 at the Marine Biotechnology Laboratory, Faculty of Fisheries and Marine, Universitas Riau

Materials

The materials used in this study were *Chlorella* sp, sago liquid waste, clean water, and walne fertilizer. While the tools used are culture containers in the form of 1.5 L bottles, lights, erlenmeyer, an aerator, a hemocytometer, an Olympus CX21 microscope, a hand counter, a dropping pipette, tissue, a thermometer, and a pH indicator.

Experimental Design

The research method used is an experimental method, with the research design used is a completely randomized design (CRD), one factor with 4 treatments and 3 replications. The doses used in this study are based on the results of preliminary tests, as follows: T0: 1 ml/L walne fertilizer (control), T1: 150 ml Sago Liquid Waste (LCS), T2: 200 ml Sago Liquid Waste (LCS), T3: 250 ml Sago Liquid Waste (LCS)

Preparation of Sago Liquid Waste Containers and Fertilizers

Culture containers in the form of 1.5 L volume bottles as many as 12 containers were washed, dried, and sterilized. After cleaning, each container was filled with 1 L of water and added sago liquid waste according to the treatment (150 ml, 200 ml, and 250 ml). Next, the containers were arranged randomly under the lamp, and aeration was adjusted. Sago liquid waste was obtained from Sago Flour Mill Selatpanjang, Kepulauan Meranti Regency, Riau. Sago waste is precipitated so that it is separated from sago starch. Then the water is used as an organic liquid fertilizer in the culture of *Chlorella* sp.

Measured parameters

The number of microalga cells can be calculated with the cell abundance formula according to Armanda (2013) as follows:

$$\text{Microalgae cell (cell/ml)} = \frac{\text{the number of cells in the field of view}}{\text{Number of the field of view}} \text{ x } 25 \text{ x } 10^4$$

The specific growth rate is the speed of growth in the population in a certain unit of time, the specific growth rate with the formula (Wood et al, 2005).

$$\mu = \frac{\text{Ln X2- Ln X1}}{\text{t2-t1}}$$

Description:

μ : Specific growth rate (/day) $^{-1}$
X1 : Initial cell density (cells/ ml)
X2 : Final cell density (cells/ ml)
t1 : Initial sampling time (days)
t2 : End of sampling time (days)

Data Analysis

Data obtained during the study, such as the abundance of Chlorella sp cells and specific growth rates were tabulated in tabular form and analyzed statistically using SPSS version 26 software. data on abundance and growth rate were analyzed using ANOVA. Meanwhile, water quality data were analyzed descriptively

RESULT AND DISCUSSION

Cell Abundance and Specific Growth Rate of *Chlorella* sp.

Data obtained during the study on the abundance of *Chlorella* sp. in each treatment are presented in Table 1.

Table 1. Average abundance of *Chlorella* sp. cultured using sago liquid waste fertilizer with different doses

Observation time (days)	The abundance of *Chlorella* sp (cell/ml)			
	T0	T1	T2	T3
1	266.667±57.735	283.333±28.868	283.333±57.735	283.333±28.868
2	300.000±50.000	350.000±50.000	333.333±76.376	316.667±125.630
3	350.000±50.000	450.000±50.000	400.000±86.602	383.333±57.735
4	416.667±28.868	600.000±123.288	500.000±50.000	466.667±76.376
5	533.333±57.735^{a}	800.000±50.000^{c}	650.000±50.000^{b}	616.667±28.868^{ab}
6	650.000±50.000^{a}	1.066.667±28.868^{c}	866.667±125.830^{b}	800.000±50.000^{b}
7	816.667±76.376^{a}	1.483.333±28.868^{d}	1.233.333±104.083^{c}	1.000.000±50.000^{b}
8	1.116.667±76.376^{a}	2.066.667±28.868^{d}	1.866.667±76.376^{c}	1.600.000±50.000^{b}
9	1.350.000±50.000^{a}	2.333.333±57.735^{d}	2.200.000±50.000^{c}	2.100.000±50.000^{b}
10	1.466.667±104.083^{a}	2.133.333±28.868^{b}	2.083.333±28.868^{b}	2.033.333±28.868^{b}
11	1.483.333±76.376^{a}	1.900.000±50.000^{c}	1.750.000±50.000^{b}	1.683.333±28.868^{b}
12	1.316.667±28.868^{a}	1.550.000±50.000^{c}	1.373.000±28.868^{b}	1.283.333±28.868^{a}
13	1.100.000±86.603^{a}	1.300.000±50.000^{b}	1.066.667±76.376^{a}	966.667±76.376^{a}
14	700.000±50.000^{a}	850.000±50.000^{b}	800.000±50.000^{ab}	750.000±50.000^{ab}

Notes: Mean values in the same column followed by different letters indicate significantly different results ($p<0.05$).

The highest abundance of *Chlorella* sp. given sago liquid waste occurred on day 9, which ranged from 210.00-233.33 x 10^4 cells/ml. The addition of sago liquid waste (LCS) of as much as 150 ml produced the highest abundance on day 9, which was 233.33x10^4 cells/ml. While the lowest was at 250 ml LCS concentration, which was 210 x 10^4 cells/ml. Furthermore, in the control treatment, the peak population occurred on day 11 with an abundance of *Chlorella* sp of 148.33 x 10^4 cells/ml. Based on the analysis of variance (ANOVA) test shows that the abundance of *Chlorella* sp. cells cultured using organic fertilizer liquid waste sago (LCS) gives a real effect (p <0.05).

The provision of organic fertilizers such as sago liquid waste (LCS) can affect the growth of *Chlorella* sp., which is marked by an increase in abundance every day. The provision of sago liquid waste (LCS) reached the peak population on day 9. While in the control media, the peak population occurred on the 11th day. More clearly can be seen in Figure 1

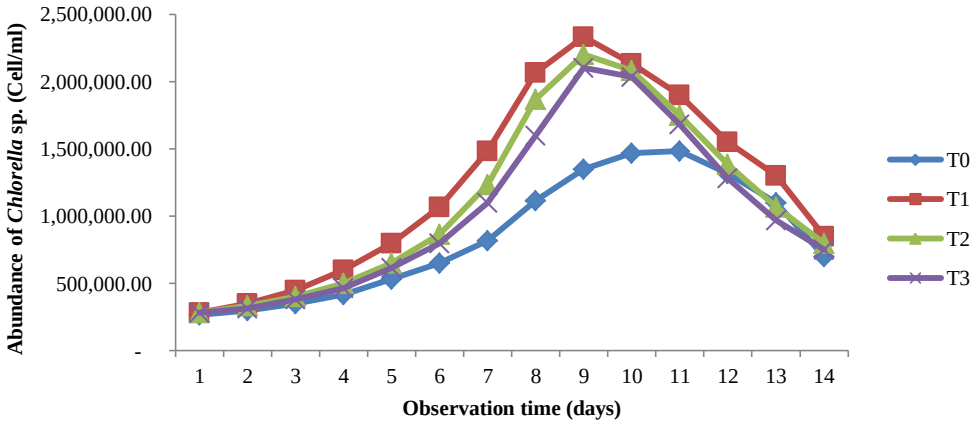

Figure 1. Cell abundance of *Chlorella* sp. with different concentrations

T1 (LCS 150 ml/L water) was able to produce the highest abundance of *Chlorella* sp. cells, namely 233.33 x 10^4 cells/ml. This is due to the sago liquid waste (LCS) given in sufficient quantities so that *Chlorella* sp. can utilize nutrients well and effectively. Sylvester *et al.* (2002) stated that nutrients that function as raw materials will be absorbed into cells and utilized through the metabolic process. Nutrient serves as raw material, without the nutrient biosynthesis process does not run.

At higher concentrations of sago liquid waste (LCS), namely 200 ml (T2) and 250 ml (T3), produced a lower abundance of Chlorella sp., namely 220.00 x10^4 cells/ml and 210.00 x10^4 cells/ml. This is thought to be due to the concentration of sago liquid waste (LCS) given exceeding the nutritional needs of cells so that the effectiveness of nutrient utilization is lower and inhibits cell growth *Chlorella* sp. According to Hastuti & Handajani (2001), nutrients given to the culture medium in excessive amounts, can be toxic and inhibit growth. Munir *et al*. (2017) state that the higher the concentration given does not increase the growth rate but inhibits the growth rate of microalgae because it interferes with the absorption of nutrients that are toxic to the growth of *Chlorella* sp. microalgae *Chlorella* sp. will grow well if the nutrients provided meet their growth needs. The concentration of nutrients will affect cell growth because excess nutrients can be toxic to aquatic organisms (Indriana *et al.*, 2020).

The control media (T0) produced an abundance of *Chlorella* sp at 148.33 x 104 cells/ml lower than the other treatments. This is due to the provision of insufficient fertilizer to meet the needs of *Chlorella* sp. Prihantini *in* Putra *et al.* (2023) stated that if the microalgae culture media lacks nutrients, it will affect the photosynthesis process and cell growth. In addition, it also results in competition in obtaining existing nutrients.

Chlorella sp. consists of the adaptation phase, exponential, stationary phase, and death phase. Each phase affects the value of the cell growth rate of *Chlorella* sp. The adaptation phase in this study lasted an average of 2 days, namely the observation of days 0 to 2, and the cell density continued to increase (Figure 2). Dahril *et al.* (2020) stated that the length of time in the adaptation phase depends on the amount and age of the inoculum, as well as the media used. The adaptability of microalgae is influenced by nutrients in the media which can be a limiting factor for cell growth. If one of the nutrients is not available or the amount is too large, it can inhibit the reduction of organic compounds and the growth of the microalgae (Rini, 2012).

The highest growth rate occurred on day 7, in line with the research of Tamalonggehe *et al.* (2020), the highest microalgae growth occurred on day 7. According to Balaira *et al*. (2017), the highest microalgae growth phase occurred on day 9. The difference in the highest growth phase can occur due to microalgae experiencing varying adjustments to the growth medium. Jeheskiel *et al*. (2022), in the adaptation process cells, recover the enzyme and substrate concentrations needed for growth, as well as the entry of nutrients into microalgae cells through the diffusion process as a result of differences in the concentration of culture media with body fluids.

The exponential phase is a phase of cell division where this phase is evidenced by the occurrence of very rapid growth. According to Wibowo *et al.* (2017), the exponential phase begins with cell division with a growth rate that occurs continuously, the exponential phase occurs until the cell density reaches the maximum density. In this study, there was an increase in cell density from day 1 until the peak population on day 9, which was marked by a change in the color of the culture medium to green. The increase in growth rate values from the adaptation phase to the exponential phase occurs because the cell density continues to increase from day to day.

Cell growth is directly proportional to the specific growth rate because optimal cell growth will produce an optimal specific growth rate (Sigalingging *et al.*, 2019).

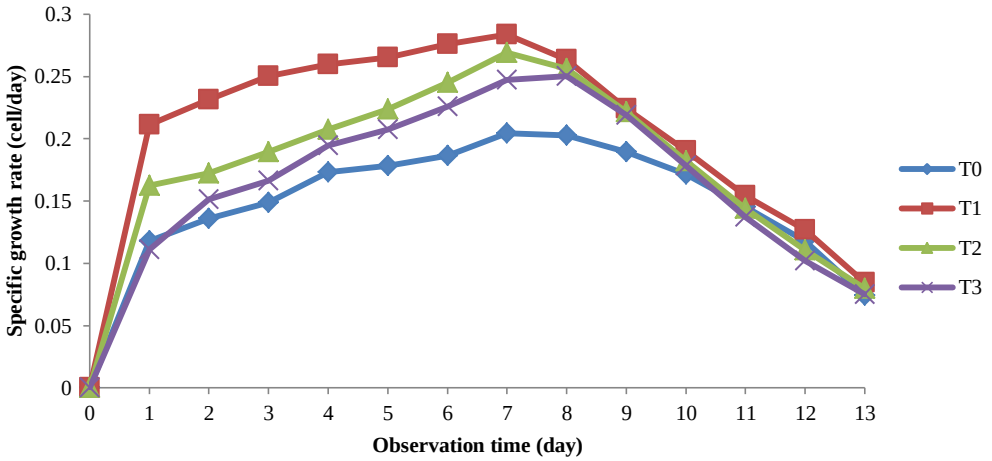

Figure 2. The specific growth rate of *Chlorella* sp

The high growth rate in the T1 treatment is thought to be due to the dose of sago liquid waste fertilizer following the needs so that it can be utilized effectively. Dianita *et al.* (2020) stated that the early growth of high specific growth rate values indicates that microalgae have a fast and short adaptation to the new culture environment. Environmental factors can support the growth of Chlorella sp. Nutrients have a special function in Chlorella and are reflected in its growth without ignoring the influence of environmental conditions (Nuraini *et al.*, 2020). Nutrients contained in sago liquid waste fertilizer including N, P, and K are very important in the photosynthesis process. In this phase, there is a high increase in cell density because the metabolite compounds and enzymes needed are available and absorbed optimally (Meria *et al.*, 2021).

The growth rate decreased starting on day 10 until day 14. This is because the amount of nutrients in the culture medium has decreased, but *Chlorella* sp. cells can still divide, but not as much as in the exponential phase. According to Mahdi *et al.* (2012), nutrients in microalgae culture media will decrease as microalgae growth increases. Conversely, nutrient concentrations that are too high can be toxic in the culture medium, which can inhibit growth and low nutrient utilization efficiency (Dianita *et al.*, 2020; Meria *et al.*, 2021).

Population density results in increased cell competition for nutrients that have decreased, causing the growth rate to also decrease (Ginting *et al.*, 2022). In addition, the decrease in growth rate is not only because the cells begin to experience nutrient deficiencies, but also due to the formation of shadows from the microalgae cells themselves (*self-shading*). The formation of shadows from *Chlorella* sp cells goes hand in hand with increasing cell density. The denser the number of cells causes light penetration in the culture medium to be increasingly obstructed, resulting in parts of the culture medium that do not receive enough light (Nuraini *et al.*, 2020). Sudhakar *et al.* (2011) stated that light is needed by *Chlorella* sp in the process of photosynthesis and has a certain limit or range. Generally, large light intensity is more effective for carrying out the photosynthesis process, but very high light levels can reduce the rate of the photosynthesis process. Biolita & Harmadi (2017) added that if the light absorbed by *Chlorella* sp is reduced, it causes photosynthesis to run slowly, resulting in decreased cell growth.

The next phase is the death phase characterized by a drastic decrease in cell density. The death phase in microalgae usually occurs when the age of the culture has been more than a week (Novianti *et al.*, 2017). *Chlorella* sp cell abundance decreases due to the absence of new nutrient additions from outside the media and nutrients in the culture media are reduced until the period is unable to maintain phytoplankton growth (Taradifa *et al.*, 2022). According to Meritasari *et al.* (2012), cell death can occur due to changes in water quality, unfavorable environmental conditions, long cultivation age, and decreased nutrients in the culture medium.

Water Quality

Water quality is one of the important factors in the culture process of *Chlorella* sp. In this study, water quality was measured at the beginning and end of the study. The measured water quality parameters are

temperature and pH. Observation data of water quality parameters during the study are presented in Table 2.

Table 2. Water quality parameters

No	Parameters	Treatment			
		T0	T1	T2	T3
1	Temperature (^oC)	26 - 29	26 - 31	26-30	26-30
2	pH	6,2-8,0	6,5-7,8	6,6-7,7	6,4-7,6

The results of temperature measurements on *Chlorella* sp. culture media ranged from 27-31^o C. This temperature range is still in a good range for the growth of microalga. The temperature range that can still be tolerated for the growth of *Chlorella* sp is 25-30^o C (Mufidah *et al.*, 2017), 20-35^o C (Mookiah *et al.*, 2020). According to Boroh *et al*. (2019), temperature affects a stage of an organism's life cycle and is a limiting factor in the spread of a species. In maintaining the survival and reproduction of ecological changes in temperature cause differences in the composition and abundance of *Chlorella* sp.

The degree of acidity (pH) has an important role in the growth of the cell population of *Chlorella* sp. The pH of the media water plays a role in shaping oxygen concentration and the balance between bicarbonate and carbonate. *Chlorella* sp. photosynthesizes in the pH range of 7 - 8 (Boroh *et al.*, 2019). In this study, the pH obtained ranged from 6.2 to 8. This pH range can still be tolerated for the growth of *Chlorella* sp. According to Mookiah *et al*. (2020), *Chlorella* sp. can grow on media that has a pH ranging from 4-8.

CONCLUSION

Based on the results of the study, it can be concluded that the utilization of sago waste as a culture medium with a concentration of 150 ml can increase the abundance and specific growth rate of *Chlorella* sp.

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