



## THE UTILIZATION OF TOFU LIQUID WASTE AS LIQUID ORGANIC FERTILIZER OF *SPIRULINA SP* CULTIVATION

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### ABSTRACT

Tofu liquid waste contains high organic materials such as N 0,04% and P 0,0041% if waste directly discharged into the river will reduce water quality. The research was aimed to determine the use of tofu liquid waste as liquid organic fertilizer of *Spirulina sp* cultivation. The research was carried out from 28 December until 28 March. Waste obtained from Tofu Industry Lembang, West Bandung. Cultivation of *Spirulina sp* conducted over 16 days at Education Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University. The research method used is the experimental method, using a completely randomized design (CRD) by five treatments and three replications. Treatment in the form of commercial liquid organic fertilizer dose of 1 ml/l, unfermentation of tofu liquid waste dose of 30,5 ml/l and 37,5 ml/l, and then fermentation of tofu liquid waste dose of 46,5 ml/l and 53,5 ml/l. Observations made include the daily growth of *Spirulina sp*, specific growth constant, water quality parameters, namely pH, temperature, DO, Salinity, N, P, ammonia, nitrates and phosphates. The results of this research showed that unfermentation of tofu liquid waste dose of 30,5 ml/l in the cultivation of *Spirulina sp* can be alternative fertilizer to produces peak populations of  $1,36 \times 10$  filament/ml on the 7th day.

**Keywords:** Fertilizer dosage, Tofu liquid waste, Growth, *Spirulina sp*

### 1. INTRODUCTION

Characteristics of tofu liquid waste that is discharged directly into rivers without processing contains TSS 460 mg/l, BOD<sub>5</sub> 4000.12 mg/l, COD 6500.33 mg/l, Ammonia 32.33 mg /l, Nitrate 13.21 mg/l, pH 3.25 and DO 0.5 mg/l (Harahap 2015). According to the preliminary test results, tofu liquid waste contains N nutrients as much as 0.4% and P as much as 0.0041%. If equal, 1 ml of fertisim fertilizer (N15%) which is commercial fertilizer is equivalent to 37.5 ml of tofu liquid waste without fermentation. Utilization of tofu liquid waste for microalgae cultivation has been carried out on several types of microalgae, such as the provision of 6% tofu liquid waste without fermentation in aquaculture media to produce the best *Spirulina platensis* growth (Syaichurrozi and Jayanudin 2016a). And with the variety of synthetic nutrition 75%, it produces 91.27% *Spirulina platensis* protein content, higher than 50% and 100% synthetic nutrition variation (Syaichurrozi and Jayanudin 2016b). Giving 40% of tofu liquid waste in *Tetraselmis sp* culture produces the highest average population density that is 623.240 ind/ml (Muliadi *et al.* 2017).

Maulana *et al.* 2017 conducts further research on the utilization of fermented tofu liquid waste in advance to increase the nutrient content in tofu liquid waste, giving EM<sub>4</sub> concentration during fermentation of tofu liquid waste as much as 30 mg/l results in better growth of *Spirulina sp* brackish water and a peak population of  $1.406 \pm 39.32$  ind/ml on the 8th day with a pH value of 8.2 -9.5 and a salinity value of 20-23 g/l.

Utilization of tofu liquid waste as liquid organic fertilizer for the growth of microalgae, either without fermentation or by fermentation in advance, needs further research to find out the best treatment. Tofu liquid waste which still contains a lot of nutrients such as nitrogen from soy protein is assumed to be used as a source of liquid organic fertilizer for the growth of *Spirulina sp* that lives in brackish water, by looking at some microalgae research shows it is able to grow well in the medium of liquid tofu waste.

This research aims to utilize liquid waste from the tofu processing industry as liquid organic fertilizer in the cultivation of *Spi-*

*sp* and determine the appropriate concentration of waste to produce the highest productivity of *Spirulina* sp. It is expected to be used as one of the liquid organic fertilizers in *Spirulina* sp cultivation, providing solutions in reducing river water pollutant sources and reducing the impact of tofu liquid waste that is discharged directly without prior processing.

## 2. MATERIALS AND METHODS

This research was conducted from December - March at the Education Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University. The research method used was an experimental research method using a Completely Randomized Design (CRD) with five treatments and three replications. Treatment in the form of administering a dose of commercial liquid organic fertilizer 1ml/l (A), tofu liquid waste as much as 30.5 ml/l (B) and 37.5 ml/l (C), as well as giving tofu liquid waste that has been fermented as much as 46.5 ml/l (D) and 53.5 ml/l (E).

The research procedure included preparation of tofu liquid waste, taken as much as 20 ml as a sample to be analyzed for N and P in liquid waste. Tofu liquid waste that will be fermented as much as 3 liters, then added 60 ml of concentrated EM4 solution and 60 grams of brown sugar as a source of carbon and nitrogen for microorganisms in the fermentation process (1: 1). All ingredients are mixed until homogeneous and the container is tightly closed so as not to be contaminated by bacteria other than anaerobic bacteria. Cover the container was given a small hole and then inserted a hose connected to the jerry can contents of water as a gas channel that is produced from the fermentation process. The container was placed at room temperature for further fermentation, on the 14th day 20 ml of tofu liquid waste fertilizer samples were taken and the N and P of t were re-analyzed at the Animal Nutrition Laboratory, Faculty of Animal Husbandry and Central Laboratory of Padjadjaran University.

1 liter of culture media. Aquades that will be used as cultivation media are then stored in opaque and tightly closed containers, sterile jars are arranged on the observation table and filled with aquades, salt and fertilizer according to the specified treatment.

### 2.1 Calculating the Initial Stocking of *Spirulina* sp

*Spirulina* sp count was carried out. using the formula (Edhy et al. 2003 in Bangun *et al.* 2015) as follows:

$$V_1 \times N_1 = V_2 \times N_2$$

Information:

- $V_1$  = Volume of initial inoculum stocking(ml)
- $N_1$  = Stock density of *Spirulina* sp(filament/ml)
- $V_2$  = The desired medium volume(L)
- $N_2$  = The final density of *Spirulina* sp desired (filament/ml)

### 2.2 Inoculation of *Spirulina* sp

The population density of *Spirulina* sp was 52.740 filament/ml, then inoculated into the culture medium. The distance between the jars with a neon lamp of 10 cm with a long exposure time 24 hours per day and aeration carried out continuously during the cultivation time (Buwono and Nurhasanah 2018).

### 2.3 Parameters Observed

#### 2.3.1 Density of *Spirulina* sp

Media water samples were taken aseptically as much as 1 ml from each treatment with 3 replications. Calculations were carried out using 5 large Haemocytometer boxes on a microscope, magnification 10x10 times. Then it is entered into the Petroff-Hauser calculation formula to find out the amount of spirulina per ml of growing media, namely:

$$D = \frac{\sum_{\text{filament}}}{5(1 \times 10^{-4})}$$

Information:

- D : Density of *Spirulina* sp (filament/ml)
- $\sum_{\text{filamen}}$  : The number of *Spirulina* sp on 5 boxes
- 5 : number of boxes
- $1 \times 10^{-4}$  : volume of each box

#### 2.3.2 Specific Growth Constants

Calculate the specific growth constant (Fogg and Thake 1987) using the following formula:

$$k = \frac{\ln V_t - \ln V_0}{t}$$

Information:

- k : Specific growth constants

$\ln W_t$  : peak population  
 $\ln W_o$  : total initial population  
 $t$  : the time taken to reach the peak of the population

### 2.3.3 Water Quality Parameters

The parameters observed included testing the N and P content before the tofu wastewater was fermented and after fermentation. Measurements of temperature, pH, DO and salinity are carried out every day to determine the water quality of *Spirulina* sp. Cultivation media, then measurements of nitrate, phosphate and ammonia are carried out on day 0 and day 15.

### 2.3.4 Data Analysis

Data were analyzed descriptively, comparing growth factors and growth time of *Spirulina* sp, to find out the best treatment.

## 3. RESULTS

### 3.1 Growth of *Spirulina* sp

The growth of *Spirulina* sp based on the observational data in Figure 1, the day after the inoculum stocking of *Spirulina* sp density decreased in each treatment, as well as treatment A which was a control treatment.

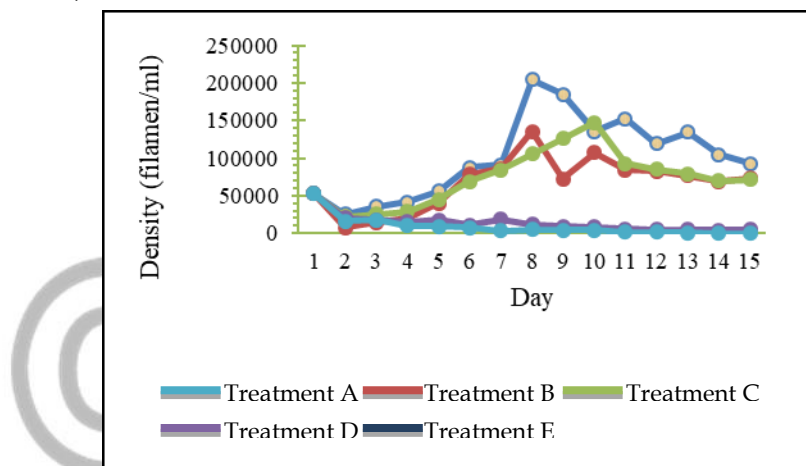


Figure 1. Daily Population of *Spirulina* sp

Then on the 2nd day of *Spirulina* sp cells treatment A, B and C had shown the ability to adapt to the culture media environment, but the cell growth that occurred was still very slow and few. In the treatment of D and E *Spirulina* sp inoculum cells cannot adapt to the culture media environment. because the adaptation phase is the phase of adjustment to the culture media (Zahidah et al. 2012).

*Spirulina* sp cell growth during cultivation is strongly influenced by the availability of nutrients, cell age and light intensity in growing media (Saveedra and Voltolina 2005). Then it is suspected that being one of the factors inhibiting the growth of *Spirulina* sp in the adaptation phase in each treatment is the equality of the inoculum used when stocking and the condition of culture media.

The lack of good quality of the inoculum bag used when inoculation affects cells in adapting to their environment, which can cause a decrease in cell population in the growing media during the adaptation phase as happened in this research. This is consistent with the statement of Fogg (1975) in Setyaningsih et al. (2012) because the age of the old inoculum requires quite a long time to reactivate cell growth. So in this research, it is suspected that the energy possessed by the *Spirulina* sp cell inoculum is focused on adjusting to the condition of the media and only a small number of cells can maintain survival in the adaptation phase. Based on research by Setyaningsih et al. (2012) a good inoculum to be spread around 4-6 days old so that cells can adapt to their environment.

Treatment A, which is a control treatment by administering commercial fertilizer with a dose of 1 ml / L, produced the highest average population of *Spirulina* sp on the 7th day of  $2.04 \times 10^5$  filament/ml (Figure 1). Treatment B with the highest dose of tofu wastewater 30.5 ml/l the highest population on the 7th day was the same as treatment A with a density of  $1.36 \times 10^5$  filament/ml and treatment C with tofu wastewater dose of 37.5 ml/l reached the population the highest was on the 9th day with an average density of  $1.47 \times 10^5$  filament/ml. Treatment A, B and C after the 7th day to the 9th day the density and the rate of regrowth decreased (Figure 1), this is because the availability of nutrients in the culture media needed *Spirulina* sp cells has been reduced, so that competition in getting greater nutrition and the intensity of incoming light becomes less with increasing population.

Treatment D (fermented tofu liquid waste at a dose of 46.5 ml/l) and E (fermented tofu liquid waste at a dose of 53.5 ml/l), after inoculation activity *Spirulina* sp density continued to decline and cell growth did not occur, even on the day to -6 for treatment D, the cell population is only  $1.89 \times 10^4$  filament/ml. This value cannot be said to be the peak of the cell population, smaller than the initial stocking value, as well as in treatment E the *Spirulina* sp cell population continued to decline and died on the 13th day (Figure 1). The use of fermented tofu liquid fertilizer E makes the media more turbid while the light intensity used is 930 lux, then the media

grows differently from the previous environment which was given walne fertilizer.

Determination of the dosage of tofu liquid waste given is adjusted to the results of equalizing the nutritional content of commercial fertilizers. Fertisim Fertilizer which is a commercial fertilizer in the control treatment has an N content of 15% and P as much as 18%, while tofu liquid waste without fermentation and fermentation (N 0.28%, P 0.032%) for 14 days has N and P content that is still below the value of commercial fertilizer.

The condition of brown fertilizer and high doses, the cultivation media water becomes turbid, the light received by the cultivation media is reduced and the photosynthesis process of *Spirulina* sp cells in treatments D and E does not work well.

The low value of N and P after fermentation is likely to be influenced by the incompleteness of N elements during the fermentation process, as well as the need for other additives to increase the N and P content in tofu liquid waste fertilizers.

### 3.2 Growth Specifics of *Spirulina* sp.

The highest specific growth constant value was produced by treatment A, namely the application of 1ml/L commercial fertilizer with a value of 10.98, then followed by treatment C (tofu liquid waste 30.5 ml/L) of 10.74 and B (tofu liquid waste 30.5 ml/L) of 10.36. *Spirulina* sp cells in the treatment Treatment D (fermented tofu liquid waste dose 46.5 ml/L) and E (fermented tofu liquid waste dose 53.5 ml/L) did not show growth so the value of growth constant could not be determined.

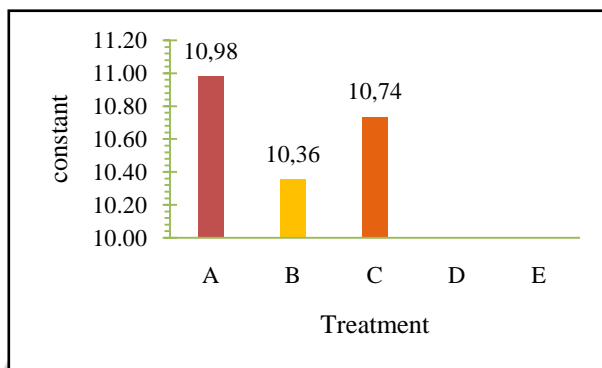


Figure 2. Growth Specifics of *Spirulina* sp

The value of growth constants in treatments A, B and C is quite large (Figure 2), this shows that *Spirulina* sp cells in culture media need a long time to double the number of cells. Ammonia content in culture media still exists at the tolerance level for *Spirulina* sp cell growth, as well as in the range of nitrate concentrations (0.683 mg/L-0.877 mg/L) and phosphate (0.217 mg/L-0.367 mg/L) in accordance with growth needs optimal for phytoplankton ranges from 0.9 to 3.5 mg/L for nitrate and phosphate from 0.09 to 1.80 mg/L (Mackentum 1969 in Asriyana and Yuliana 2012). Then other factors inhibit cell growth.

The light source used during cultivation is a 36 watt TL lamp with a light output of 2500 lm, but the space used as a place of cultivation is large enough so that when re-checking the light intensity only 930 Lux. Based on the research results of Indrastuti et al. (2014) in *Spirulina platensis*, spirulina cells can grow with light intensity tolerance ranging from 450 lux - 9500 lux. So naturally *Spirulina* sp cells in treatments A, B and C can still grow, even though the growth is not optimal. Then according to the research results of Utomo et al. (2015) optimal light for algal growth on a laboratory scale of around 1500-3000 Lux to meet the photosynthesis process. So it can be assumed that the occurrence of *Spirulina* sp cell growth in treatments D and E with a more turbid color of cultivation media, other than because the quality of the inoculum used is not good, less optimal light intensity is one of the factors in the low growth of *Spirulina* sp. Likewise with treatments A, B and C. This is consistent with the results of Firdaus and Fauzan's research (2015) that the difference in light intensity significantly influences aspects of cell production represented by cell density, specific growth rates, doubling time and harvesting biomass.

### 3.3 Water Quality Parameters

Organic matter which is degraded to ammonia will then be ionized into ammonium which can be tolerated by green algae to the level of 151,165 mg/L (Indaryanto and Saifullah 2015). So *Spirulina* sp which is a blue-green algae can still adapt to the culture media with ammonia concentration as in Table 1.

Table 1. Percentage reduction in ammonia concentration

Treatment	Day to		Decrease	Percentage (%)
	0	15		
A	2,853	1,462	1,391	48,762
B	0,007	0,004	0,002	35,000
C	0,009	0,005	0,004	42,857
D	0,058	0,045	0,013	22,857

<b>E</b>	0,107	0,082	0,025	23,292
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Unit: mg/L

Likewise with the content of nitrate and phosphate in the growing media has met the optimal growth needs for phytoplankton (Tables 2 and 3), so that it can be utilized for the growth and reproduction of *Spirulina* sp.

Table 2. Percentage reduction in nitrate concentration

Treatment	Days to-		Decrease	Percentage (%)
	0	15		
<b>A</b>	0,877	0,257	0,619	70,646
<b>B</b>	0,746	0,287	0,459	61,573
<b>C</b>	0,838	0,265	0,573	68,417
<b>D</b>	0,710	0,444	0,266	37,482
<b>E</b>	0,683	0,403	0,280	41,024

Unit: mg/L

Table 3. Percentage reduction in phosphate concentration

Treatment	Days to-		Decrease	Percentage (%)
	0	15		
<b>A</b>	0,231	0,150	0,080	34,827
<b>B</b>	0,261	0,174	0,087	33,461
<b>C</b>	0,217	0,170	0,047	21,692
<b>D</b>	0,367	0,339	0,028	7,636
<b>E</b>	0,361	0,272	0,089	24,746

Unit: mg/L

*Spirulina* sp at each replication on average was able to reduce the concentration of ammonia with a percentage decrease in the range of 22.85% - 48.77%, then the concentration of nitrate in the growing media percentage decreased in the range of 37.48% - 70.64% and the percentage reduction in phosphate concentration in the growing media during the cultivation period is 7.63% - 34.82%.

Percentage decrease in treatment A is the highest value in the use of nitrate, seeing that the cell population even in treatment A shows the highest value. This is very reasonable because treatment A is given commercial fertilizer which is a control treatment. The D and E treatments have a smaller reduction percentage. This is because the growth of *Spirulina* sp cells (Table 1) continues to decline, nutrients contained in the growing media are not optimally utilized for cell growth. The ability of *Spirulina* sp in reducing the concentration of ammonia, nitrate and phosphate in the culture medium is lower.

Treatment A and Treatment C have the same N concentrations based on the results of the statement, but the cell population between the two is different. This is because tofu liquid waste has a higher turbidity characteristic. Solid substances suspended in the growth media *Spirulina* sp cause water to become turbid, as well as liquid organic fertilizer from fermented tofu liquid waste which is more turbid. The process of photosynthesis will be disrupted because the intensity of the incoming light is blocked by suspended solids.

Aquatic organisms such as *Spirulina* sp do need phosphorus, but their presence is very limited. The amount of phosphorus must be smaller than the amount of nitrogen. Based on Tables 1 and 2 above, it can be concluded that the reduction of nitrate concentration is greater than the decrease in phosphate concentration, which means that *Spirulina* sp cells need more element N than P.

The temperature of the media in each treatment during cultivation ranged from 25.0 to 26.4°C. Based on the research results of Utomo et al. (2005) the optimum temperature for phytoplankton growth is in the range of 25-35°C, the temperature at each treatment is the optimum temperature for cell growth. Likewise, the DO values for all treatments at each replicate (Table 4) averaged a range of 5.0 - 6.9 mg/l, according to the optimum range for growth of *Spirulina* sp based on the research results of Astiani et al. (2016) which is 4.70 - 6.12 mg/l.

Table 4. Water Quality Parameters Range

T	Day to-	Parameters			
		Temperature (°C)	DO (mg/l)	pH	Salinity (ppt)
<b>A</b>	0	25,1 - 26,0	5,0 - 6,8	7,0 - 7,7	6 - 8
	15	25,2 - 25,3	7,3 - 7,4	7,5 - 7,7	5 - 9
<b>B</b>	0	25,2 - 26,4	5,8 - 6,8	7,2 - 7,9	5 - 7

T	Day to-	Parameters			
		Temperature (°C)	DO (mg/l)	pH	Salinity (ppt)
T	15	25,1 - 25,8	6,5 - 6,8	7,52 - 7,55	6 - 8
	0	25,9 - 26,3	6,4 - 6,6	7,2 - 7,9	5 - 8
C	15	25,1 - 25,4	6,9 - 7,3	7,52 - 7,57	9 - 10
	0	25,0 - 25,9	6,0 - 6,5	6,6 - 6,8	5 - 7
D	15	24,5 - 25,2	6,6 - 6,9	7,4 - 7,7	10 - 11
	0	25,0 - 26,3	6,2 - 6,7	4,8 - 5,8	5 - 9
E	15	25,0 - 25,4	6,1 - 6,8	7,6 - 7,7	7 - 10
	<b>Optimum range</b>	25,0 - 35,0	4,7 - 6,1	7,5 - 8,7	8 - 12

The pH value on the 0th day for treatments A, B and C ranged from 7.0 to 7.9. As for the treatment of D and E, although dilution with aquades has been carried out as a growth medium it has a lower pH range value, from 4.8 to 6.8 mg/L. It is suspected that due to the effect of giving a dose of fermented fertilizer that was too acidic (4,6), the concentration of hydrogen ions in the D and E growth media was higher. However, the daily pH value of each treatment (Table 4) still averages in the range of tolerance for phytoplankton growth which is 6.5 - 8.0 (Pescod 1973 in Asriyana and Yuliana 2012). And the pH value of the media increases after administration of aeration during cultivation and the photosynthesis process that utilizes CO<sub>2</sub> by *Spirulina* sp cells so that hydrogen ions in the culture medium are estimated to decrease.

The salinity value in the growing media is adjusted to the initial conditions of the inoculum (8 g/l) with the amount of salt given based on the calculation results. So when the salinity value decreases because aeration and photosynthesis process (Isnadina and Hermiana 2013), to maintain cell growth the salt solution is added back to the growing medium until the salinity value is suitable for *Spirulina* sp cell growth in the range of 8-12 g/l that has been recommended. So that the salinity value on the 15th day in each treatment is still in the range of 5-11 g/l (Table 4).

#### 4. CONCLUSIONS

Research results can be concluded that tofu liquid waste can be used as an alternative fertilizer. Cell growth that was given commercial fertilizer as much as 1 ml/l resulted in a population growth of *spirulina* sp of  $2,04 \times 10^5$  filament/ml, which can be equalized with the administration of tofu liquid waste without fermentation as much as 30,5 ml/l in the culture medium resulting in *Spirulina* sp population growth of  $1,36 \times 10^5$  filament/ml, each on the 7<sup>th</sup> day.

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