Growth of *Spirulina* sp. microalgae in the media of fish waste water treatment from Cemara market, Medan, North Sumatera

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Abstract. *Spirulina* sp. is one of the potential microalgae as a source of protein because it contains 100% vegetable protein. Fisheries industry wastewater contains a lot of protein and fat, resulting in quite high values, nitrates and ammonia. Fish waste can be used as raw material for organic fertilizer microalgae that can grow in waste besides producing biomass and can reduce the burden of pollution. In this case, researchers want to know the growth rate of microalgae *Spirulina* sp. in the media of liquid waste resulting from fish handling from Cemara Market, Medan, North Sumatra. This research was conducted experimentally, the research design used was a Completely Randomized Design (CRD) consisting of five treatments with three replications with different waste concentrations with a volume of 1000 ml. Based on the Anova test results show that there is a real influence of the variation of treatment on the growth of *Spirulina* sp. with a significance value of 0.000 (p <0.05). Also supported by the results of Duncan’s further tests which showed that treatment A (30% waste) and treatment K (control) were significantly different from each other and also had a significant effect on treatment B (60% waste).

1. Introduction
*Spirulina* sp. is one of the potential microalgae as a source of protein because it contains 60-70% vegetable protein [1]. *Spirulina* sp. as a potential microalga caused by the following factors: *Spirulina* sp. characterized by the rapid reproduction and formation of biomass [2]. *Spirulina* sp. has a rich protein and peptide content not only from algae or plants. This compound can increase the adaptation of *Spirulina* sp. on waste media [3].

*Spirulina* sp. is a greenish-colored, bluish autotrophic living creature, with columned cells forming twisted filaments resembling spirals (helix) so that they are also called filamentous green blue algae (cyanobacterium). *Spirulina* sp. Body shape which resembles a thread is a series of cylindrical cells with a thin cell wall, 1-12 micrometres in diameter. *Spirulina* sp. filaments live independently and can move freely [4].

Waste is any substance generated from an industrial or domestic production process that has no economic value. Waste generated from fisheries activities is still quite high, which is around 20-30%. fish production which has reached 6.5 million tons per year. This means around 2 million tons are wasted as waste. Fisheries industry wastewater contains a lot of protein and fat, resulting in quite high values, nitrates, and ammonia. Fish waste can be used as raw material for organic fertilizer [5].
Spirulina sp. is one of the aquatic biological agents that can grow in alternative growth conditions with strong adaptability. Some research results state that Spirulina sp. able to be cultivated in liquid waste. Microalgae that can grow in waste besides producing biomass and can reduce the burden of pollution [6].

Cemara Market is located on Jalan Cemara, Pulo Brayan Darat II, Medan Timur District, Medan City. This market is a market engaged in the sale of fresh land and sea fisheries products that produce organic liquid waste, which can be used in increasing the growth of Spirulina sp. In this case, researchers want to know the growth rate of microalgae Spirulina sp. in the media of liquid waste resulting from fish handling from Cemara Market, Medan, North Sumatra.

2. Materials and methods

2.1. Time and place of research
This research will be carried out in October 2019 until November 2019. This research was conducted at the Aquaculture Laboratory of the Aquatic Resources Management Study Program at the Faculty of Agriculture, University of Sumatera Utara and water measurements were carried out at BTKLPP (Medan Center for Environmental Health and Disease Control Engineering) Class I Medan.

2.2. Tools and materials
The tools needed in this research are Transparent Bottles, Airstones, Aerated Hoses, Volume Pipettes, Drop Pipes, TL Lights 36 watts, Measuring Cups, Pumpkin, Glass Funnels, Culture Bottles, Microscopes, pH Meters, Thermometers, TDS Meters, filter paper, Sedwick Rafter, and Jerry cans, the materials used in this study are as follows: Pure culture Spirulina sp., Organic waste handling fish, Water, Aquades, 70% alcohol, Chlorine, Teepol, sodium thiosulfate, and Walne Fertilizer.

2.3. Experimental design
Culture of Spirulina sp. put into each of 15 transparent bottles containing media water with a total volume of each 1000 ml bottle plus 100 ml inoculum Spirulina sp., density Spirulina sp. used 1x10^5 sel/mL. Then each of the 5 treatments with 3 replications. Culture of Spirulina sp. cultured for 7 days and given a light with a 36 watt TL lamp. This research was conducted experimentally, the research design used was a Completely Randomized Design (CRD) consisting of five treatments with three replications with different waste concentrations with a volume of 1000 ml [7], namely:

- Control treatment: 0% organic waste handling fish + 100% fresh water + Walne Fertilizer 1 mL
- Treatment A: 30% organic waste handling fish + 70% fresh water
- Treatment B: 60% organic waste handling fish + 40% fresh water
- Treatment C: 80% organic waste handling fish + 20% fresh water
- Treatment D: 100% organic waste handling fish

2.4. Preparation of containers
Waste containers and aquaculture containers are sterilized by means of tools washed with teepol teepol (A multipurpose cleaner) and rinsed with fresh water until clean, then sprayed with 70% alcohol, and allowed to dry in the air.

The aeration hoses and stones are sterilized by being washed first with teepol (A multipurpose cleaner) which is then rinsed with fresh water. Then immersed with 0.2% HCl for 24 hours and rinsed again with clean water.

2.5. Waste water sampling
Wastewater used as a growing medium comes from organic waste handling fish in Cemara Market, Medan City, North Sumatra. Sampling of organic wastewater handling fish is only done once. Wastewater samples are taken from the liquid waste disposal control tub and put into plastic jerry cans with a volume of 20 litres. Then the waste is analysed at the Class I Laboratory BTKLPP Medan
(Center for Environmental Health and Disease Control) to find out the contents before entering microalgae for cultivation.

2.6. Cultivation of *Spirulina* sp.

In cultivation *Spirulina* sp. ini uses fresh water in a 5 liter aquarium container, which is first sterilized with chlorine and neutralized using sodium thiosulfate. The parameters maintained in stable conditions are temperature 25-27°C, and pH 7-8 because they affect the growth of *Spirulina* sp. While the fertilizer media used is the formula walne fertilizer, with administration of 1.2 ml/l.

2.7. Calculation of initial density and spread of inoculant *Spirulina* sp.

Calculation of initial density of *Spirulina* sp. conducted using sedgwick rafter with three replications to determine the density of inoculum cells to be used during culture. Inoculants were put into each culture aquarium as much as 100 ml. Calculation of *Spirulina* sp. Cell density contained in a square box that has a side of 1 mm counted as many as 50 boxes under a microscope with a magnification of 10 times as many as 3 replications, with the formula for calculating the density of plankton according to [6] is as follows:

$$N = \frac{C \times 1000}{A \times D \times F}$$

Information:
- **N** = density of inoculum cells *Spirulina* sp. (ind / ml)
- **C** = Number of inoculum cells *Spirulina* sp. Calculated (ind / ml)
- **A** = Field of View (A = \(\pi r^2\))
- **D** = Field of View Diameter
- **F** = Number of fields of view

The inoculum volume needed for inoculation can be calculated using the formula as follows:

$$N_1 = \frac{V_1 \times N_2}{V_2}$$

Information:
- **V1** = volume of inoculum cells *Spirulina* sp. used (ind ml)
- **N1** = density of inoculum cells *Spirulina* sp. Calculated (ind / ml)
- **V2** = volume of media to be used (ind / ml)
- **N2** = density of inoculum cells *Spirulina* sp. required (ind / ml)

2.8. Physical and chemical parameters of water

Measurement of water physical parameters are the steps for measuring water quality for each parameter:
1. Temperature measurements are carried out every 24 hours using a thermometer.
2. pH measurements are carried out every 24 hours using a pH meter.
3. DO measurements are carried out every 24 hours using a DO meter.

2.9. Biological parameters

Observations made on biological parameters are the calculation of phytoplankton density. Cells density are calculated once every 24 hours up to 216 hours.
2.10. Analysis data
Data obtained in this research were *Spirulina* sp density and water quality parameters. Both data were then analysed using the SPSS application for the ANOVA test at a level of 95%. If the test results are significantly different between treatments, then Duncan's further test is done at a level of 95%.

3. Results and discussion

3.1. Analysis of the characteristics of liquid waste from fish handling at Cemara Market, Medan City, North Sumatra
The characteristics of liquid waste in fish handling at Cemara Market, Medan City, were identified by measuring chemical and physical parameters. The characteristics of fish handling liquid waste at Cemara Market, Medan City are shown in table 1.

Table 1. Characteristics of liquid waste from fish handling at Cemara market.

| No | Parameters                  | Results | Unit   |
|----|-----------------------------|---------|--------|
| 1  | Temperature                 | 29.5    | °C     |
| 2  | pH                          | 6.5     | -      |
| 3  | Dissolved oxygen/DO         | 1.2     | mg/L   |
| 4  | Total Dissolved Solid/TDS   | 875     | mg/L   |
| 5  | Total Suspended Solid/TSS   | 894     | mg/L   |
| 6  | Amonia                      | 1.102   | mg/L   |
| 7  | Phosphate                   | 16.7    | mg/L   |

Table 1 shows some of the parameters for measuring the levels of liquid waste for fish handlers at Pasar Cemara, Medan City with a temperature value of 29.5 °C, dissolved oxygen (DO) with a value of 1.2 mg/L, ammonia with a value of 1.102 mg/L and phosphate with a value of 16.7 mg/L total suspended solid 894 mg/L, total dissolve solid with a value of 875 mg/L.

3.2. Biological parameter analysis of growth variations in *Spirulina* sp.
The measurement results of the growth parameters of microalgae *Spirulina* sp. given the handling of liquid organic fish waste in the Cemara Medan market with different amounts, namely, pK (Control), pA (30% waste), pB (60% waste), pC (80% waste) and pD (100% waste) treatment during 9 days can be seen in the following Figure.

![Figure 1](image-url)

**Figure 1.** Daily growth chart of *Spirulina* sp. in different treatments.

Figure 1 shows the daily growth of *Spirulina* sp. among treatments in the highest population in the study of microalgae growth *Spirulina* sp. obtained in the treatment K (control), namely the addition of...
1 ml/L Walne fertilizer with a density of 35828.03 ± 2206.43 Ind/ml and the lowest is the PD treatment with a density of 11464.97 ± 735.48 Ind/ml. Based on the analysis of variance shows that there is a real influence on the variation of treatment on the growth of *Spirulina* sp. with a significance value of 0.000 (p <0.05). Where in [8], the stationary phase is called the peak phase of the population. the stationary phase is referred to as the peak phase of the population. in treatment K (control), namely the addition of 1 ml/ L Walne fertilizer with a density of 35828.03 ± 2206.43 Ind/ml and the lowest is a pD treatment with a density of 11464.97 ± 735.48 Ind/ml.

**Table 2.** Significant values of growth of *Spirulina* sp. in different treatments.

| Observation Parameters | pA          | pB          | pC          | pD          | pK          |
|------------------------|-------------|-------------|-------------|-------------|-------------|
| Biomass *Spirulina* sp. (Ind/ml) | 28662.42±  | 15339.70±  | 12526.53±  | 11464.97±  | 35828.03±  |
|                        | 1746.76b    | 920.88a    | 781.90a    | 735.48a    | 2206.43c   |

The results of the significance of the growth of *Spirulina* sp. table 2 shows that there are 3 different letter motifs that have shown significant differences, treatment A (30% waste) behaves b and treatment K (control) is significantly different from each other and has a significant effect on treatment B (60% organic liquid waste), treatment C (80% organic liquid waste) and treatment D (100% organic liquid waste). However, treatment D (100% organic liquid waste) was not significantly different from treatment C (80% organic liquid waste) and treatment B (60% organic liquid waste). This is due to the large number of organic compounds from organic waste in each treatment, the more the waste content, the greater the turbidity which causes obstruction of light from entering the media. This is according to research conducted by [8], that the addition of solid organic and inorganic into the waters will increase the turbidity which inhibit the penetration of light. Reduced light penetration will affect the photosynthesis process carried out by phytoplankton.

The lag phase is the phase in which *Spirulina* sp. began to adapt to his new environment. The lag phase occurs from day 0 to day 1 (figure 1). Where the highest population is in the pK treatment with a value of 9979 Ind / ml and the lowest in the pD treatment is 6635 Ind / ml. In this phase *Spirulina* sp. has not experienced an increase in growth. This is due to *Spirulina* sp. unable to adapt to the environment optimally. *Spirulina* sp. will adjust to the content of organic matter contained in *Spirulina* sp. According to [7], States that in the Lag phase cell adjustments occur to the new environment. At the time of adaptation, the cell has an enzyme or coenzyme deficiency, so it must be synthesized first for the subsequent biochemical activity of the cell. The length of the adaptation phase is influenced by several factors namely the media, the growth environment, and the number of inoculants.

The exponential or logarithmic phase is a phase of a significant increase in population density over a period, at this time cell division activities occur causing an increase in *Spirulina* sp. The exponential phase occurs from day 2 to day 5 (figure 1). The exponential phase average in treatment K was 19559 Ind / ml, treatment A was 18047 Ind / ml, treatment B was 11837 Ind / ml, treatment C was 9501 Ind / ml, and treatment D was 9103 Ind / ml. This is in accordance with [9], which states that after the lag phase, culture algae will experience rapid growth, or the so-called exponential growth phase. This is characterized by the addition of a very rapid number of cells through algal cell division.

In the study seen a decline in growth (declination phase) starting on day 8. Declination phase is the phase in which the live competition of *Spirulina* sp. due to nutrients contained in the culture media has been exhausted so it cannot be used anymore and has decreased. This is in accordance with the statement of [9], that the declination phase or death phase is the phase of cell death. Morphologically, in this phase, algal cells die more than divisions.

### 3.3. Temperature

The measurement results of the temperature parameters of microalgae *Spirulina* sp. for 9 days after adding liquid waste from the handling of different fish.
In figure 6 shows that the temperature at each treatment both K (control), pA (30% waste), pB (60% waste), pC (80% waste) and pD (100% waste) is 27-30°C. In pD treatment (100% waste) the temperature is always above the other treatments. Figure 2 shows that water temperature is a physical factor affecting algal culture in the laboratory. In each treatment both K (control), pA (30% waste), pB (60% waste), pC (80% waste) and pD (100% waste) treatment are 27-30°C. The temperature at the culture place is a closed environment in the laboratory so that the temperature in the environment is relatively stable. At this temperature the *Spirulina* sp that is cultured can live well. This refers to [10], that environmental conditions will affect the metabolic and reproductive processes of algae cells. Good temperature for algae culture in the laboratory is in the range of 20°C - 30°C.

### 3.4. Acidity (pH)

Measurement results of the acidity (pH) parameter of the microalgae *Spirulina* sp. before and after the addition of liquid waste from the handling of fish with a dose that is, treatment K (control), pA (30% waste), pB (60% waste), pC (80% waste) and pD (100% waste) for 9 days can see in the following.

Figure 3 shows the average pH for each treatment K (control), treatment A (30% waste), treatment B (60% waste), and treatment C (80% waste) is 6.83-8.31. Whereas in treatment D (100% of waste) it can be seen that the pH tends to be lower than other treatments that is equal to 6.57-7.50.
The average pH value in all *Spirulina* sp. culture media using liquid waste from the handling of fish handling fish decreased at the beginning of cultivation and then increased (figure 3). Media pH value is low from 6.5 - 7 on day 1. Furthermore, the pH increased until the end of cultivation to a value of 7.6. This is due to the activity of the decomposing bacteria found in the liquid waste from the handling of fish handling so that it produces CO\(_2\) gas which makes the media become acidic. At the time of increasing CO\(_2\) gas will increase the population of *Spirulina* sp. Because CO\(_2\) is a source of carbon for photosynthesis. This is in accordance with [11], who stated that it was caused by the activity of oxidation bacteria contained in liquid waste resulting from fish handling. Oxidation bacteria that emit organic matter will produce CO\(_2\) gas, so it causes the media to become acidic (low pH). And when the population of *Spirulina* sp. increases, CO\(_2\) gas is used as a carbon source for photosynthesis so that the pH gradually increases.

3.5. Dissolved Oxygen (DO)

The measurement results of the Dissolved Oxygen (DO) parameter of Microalgae *Spirulina* sp. added by the liquid waste from the handling of fish with a different dose that is, treatment K (control), pA (30% waste), pB (60% waste), pC (80% waste) and pD (100% waste) for 9 days can be seen as follows.

![Figure 4. Daily dissolved oxygen (DO) chart of *Spirulina* sp. in different treatments.](image)

Figure 4 shows that the average DO value at each pK treatment (control), pA treatment (30% waste), pB treatment (60% waste), pC treatment (80% waste), and pD treatment (100% waste) experienced enhancement. In treatment D (100% of waste) it can be seen that DO tends to be lower than other treatments that is equal to 1.20 - 4.13 mg/L.

Wastewater treatment treated with microalgae *Spirulina* sp. affect the amount of DO at all concentrations. The final DO value in all treatments has increased from the initial DO before the cultivation of microalgae *Spirulina* sp. The higher the concentration of liquid wastewater from the handling of fish management fish, the lower the DO content. According to [12], the low dissolved oxygen condition allows anaerobic bacterial activity in water bodies. Dissolved oxygen is influenced by several things, including vegetation cover, BOD (Biological Oxygen Demand), phytoplankton development, body size of water, and the presence of wind currents. Water plants effectively increase the value of oxygen in water through the process of photosynthesis.

4. Conclusions

Based on the Anova test results, it was found that the significant effect of treatment variations on the growth of *Spirulina* sp. with a significance value of 0.000 (p <0.05). This is also supported by the results of Duncan's continued test which showed that treatment A (30% waste) and treatment K (control) were significantly different from each other and had a significant effect on treatment B (60% waste).
waste). So the treatment of culturing spirulina sp with walne fertilizer is the best but when treated with waste, treatment A (30% waste) is the best treatment with other wastes.

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