The Ability of Spirulina sp. Microalgae as a Phytoremediation Agents in Liquid Waste of Handling Fish from Cemara Market, Medan

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Abstract

Contamination material from fish market activities, namely fish handling liquid waste, is a problem facing the city of Medan today. Reduce the level of liquid waste pollutants can be done biologically by using microalgae organisms. One of them is like microalgae Spirulina sp. This study aims to determine the ability of Spirulina sp. for reducing the levels of pollutants in liquid waste of handling fish. The research method was use a Completely Randomized Design (CRD) with five treatments and three replications. Addition of 100 mL of inoculant Spirulina sp. with a density of $1 \times 10^5$ ind/mL of liquid waste mixed with fresh water with to reach a volume of 1000 mL. Each liquid waste concentration of 30%, 60%, 80%, and control (0%) added 1 mL Walne fertilizer. The results of initial measurements of liquid waste for parameters BOD, COD, TSS, ammonia, and phosphate, respectively are 26.50 mg/L, 4400 mg/L, 894 mg/L, 1.10 mg/L, and 16.7 mg/L. Based on the results, the best reduction in BOD level occurred in the treatment of 30% (pA) of liquid waste is 17.64 mg/L with phytoremediation efficiency of 75.59%. The best reduction in COD levels occurred in the treatment of 30% (pA) of liquid waste is 1301.12 mg/L with phytoremediation efficiency of 79.19%. The best reduction in TSS levels occurred in the treatment of 60% (pB) of liquid waste is 411 mg/L with phytoremediation efficiency of 83.54%. The best reduction in ammonia levels was at 30% (pA) treatment, which was 0.38 mg/L with phytoremediation efficiency of 89.15%. While the best decrease in phosphate levels occurred in the control treatment (pK), which was 4.45 mg/L with phytoremediation efficiency of 97.35%.
1. Introduction

The development of fishery agroindustry in addition to having a positive impact that is as a foreign exchange earner, providing added value and employment, also has a negative impact in the form of waste disposal. Megalina (2016), states that the Aliarn River Basin in the city shows a high level of waste pollution, increased anthropogenic activity causes environmental quality to decline. Environmental pollution problems due to fishing activities need to be watched out for. In this case the simplest method of treatment needs to be chosen and does not cost much, is biological treatment. Phytoremediation is a plant mediated technique to absorb pollutants by destroying, deactivating or immobilizing pollutants into harmless forms (Abdel-Shafy and Mansour, 2018). Biological water management methods can be carried out using organisms that reduce organic waste (Ahammad et al., 2013). One of them is the type of microalgae Spirulina sp. (Simamora et al., 2017).

Microalgae Spirulina sp. has high adaptability so that it is able to grow in various growth conditions (Lutzu, 2011). This type of algae can be found in waters with an alkaline or slightly acidic pH (Ismaiel et al., 2016). The condition of alkaline pH provides an advantage in terms of cultivation, because it is relatively not easily polluted by other microalgae which generally live at lower or more acidic pH (Abdel-Raouf et al., 2012).

Growth of Spirulina sp. a part from being influenced by nutrient content, it is also influenced by environmental conditions (Fagiri et al., 2013). Environmental factors that support the growth of Spirulina sp. are temperature, dissolved oxygen, salinity and pH (Uebel et al., 2019). The data from the measurement of water quality during the study showed that the pH ranged from 7.7 to 8.9. The pH value is an important factor for the growth of Spirulina sp. Most blue-green algae grow well at pH 7 and are more resistant to alkaline than acidic conditions because they are able to utilize carbon dioxide available at low concentrations (Dejungkranonta et al., 2012). A good pH for Spirulina sp. growth ranges from 6-8 (Rajasekaran et al., 2016).

One alternative to be able to maintain water quality and reduce organic waste compounds effectively and efficiently is to use the phytoremediation method, namely the use of Spirulina sp. (Khalila et al., 2018), in the management of fish organic liquid waste. In this case, the researchers wanted to reduce the levels of pollutants from liquid waste in fish handling from Pasar Cemara, Medan, North Sumatra by using the microalgae Spirulina sp.

This study aims to analyze the effect of the amount of waste concentration in removing pollutants using the phytoremediation method with Spirulina sp. from fish handling waste, and determine the efficiency of pollutant removal using the phytoremediation method with Spirulina sp. from waste water from fish handling.

2. Materials and Methods

2.1 Time and Place of Research

This research was be carried out in October to November 2019. This research was conducted in Aquaculture Laboratory of Aquatic Resources Management Study Program, Faculty of Agriculture, University of North Sumatra. For water measurements were carried out at BTKLPP (Medan Center for Environmental Health and Disease Control Engineering) Class I Medan.

2.2 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, Aquadest, 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 inoculants Spirulina sp., density Spirulina sp. used is 1x10⁶ ind/mL. Then each of the five treatments with 3 replications. Spirulina sp. was culture for 7 days and given a light with 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, namely:

- Control treatment (pk): 0% liquid waste + 100% fresh water + Walne Fertilizer 1mL
- Treatment A (pA): 30% liquid waste + 70% fresh water
- Treatment B (pB): 60% liquid waste + 40% fresh water
- Treatment C (pC): 80% liquid waste + 20% fresh water
- Treatment D (pD): 100% liquid waste
2.4 Preparation of Containers

Waste containers and aquarium box are sterilized by means of tools washed with teepol and rinsed with fresh water until clean, then sprayed with 70% alcohol, and allowed to dry in the air. The aeration hose and stones are sterilize with fluid cleaner then rinsed with fresh water. Then immersed with 0.2% HCl for 24 hours and rinsed again with fresh water.

2.5 Liquid Waste Sampling

Liquid waste of handling fish used as a growing medium comes from Cemara market, Medan. Sample of liquid waste were taken from the liquid waste disposal control tub. It was put into plastic jerry cans with a volume of 20 liters. Then the liquid waste was analyzed in laboratory to find out the contents before entering microalgae for phytoremediation.

2.6 Observation of Physics and Chemistry Parameters

Measurement of water physical parameter is TSS and chemical parameters based on APHA (2005), are pH, Ammonia (NH3), BOD, COD and Phosphate (PO4).

Steps for measuring water quality for each parameter, TSS measurements were carried out before stocking inoculants and at the end of retention when Spirulina sp. enter the death phase using a TSS meter. Then, ammonia measurements were carried out before stocking the inoculant and at the end of the retention when Spirulina sp. enter the death phase using a spectrophotometer. BOD measurements were taken before stocking the inoculant and at the end of retention when Spirulina sp. entering the death phase using a titration tool with the iodometric method. Then, COD measurements were carried out before stocking the inoculant and at the end of retention when Spirulina sp. enter the death phase using a spectrophotometer. Then, phosphate measurements were carried out prior to inoculants dispersion using spectrophotometry with a wave length of 650 nm in the sample. The phytoremediation efficiency then is calculated by using the formula (Sudjarwo et al., 2014):

\[ Ef = \frac{Ci - Ce}{Ci} \times 100\% \]

Where:
- Ef = efficiency (%),
- Ci = initial concentration (mg/L),
- Ce = final concentration (mg/L).

2.7 Analysis Data

Data obtained in this research were water quality parameters. The data were then analyzed 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 The characteristics of liquid waste of handling fish

The characteristics of liquid waste of handling fish from Cemara market are known by measuring chemical and physical parameters (Table 1).

| No | Parameters                  | Result (mg/L) | Unit |
|----|-----------------------------|---------------|------|
| 1  | Total Suspended Solid (TSS) | 894           | mg/L |
| 2  | Biochemical Oxygen Demand (BOD) | 26.5          | mg/L |
| 3  | Chemical Oxygen Demand (COD) | 4400          | mg/L |
| 4  | Ammonia                     | 1.1           | mg/L |
| 5  | Phosphate                   | 16.7          | mg/L |

Based on physical and chemical measurements, it is known that Total Suspended Solids (TSS) 894 mg/L, Biochemical Oxygen Demand (BOD) 26.50 mg/L, Chemical Oxygen Demand (COD) 4400 mg/L, Ammonia 1.10 mg/L, and Phosphate 16.7 mg/L.

3.2 Total Suspended Solid(TSS)

TSS is one of the important factors in decreasing water quality, causing changes in physics, chemistry, and biology. Physical changes include the addition of solids both organic and inorganic into the water, there by increasing turbidity, which in turn will inhibit the penetration of sunlight into water bodies. The reduced penetration of sunlight will affect the photosynthesis process carried out by phytoplankton and other aquatic plants. The amount of TSS that is in the waters can reduce the availability of dissolved oxygen (Hadiyanto et al., 2013) evaluate water quality, as well as determine the efficiency of handling units (Rinawati et al., 2016).

The measurement results of the parameters of TSS with microalgae of Spirulina sp. before and after being given liquid waste of handling fish with different amounts, namely, K (control), pA (30% waste), pB (60% waste), pC (80% waste) and pD (100% waste) as follows in Table 2.
Table 2. TSS phytoremediation efficiency values on Spirulina sp. in different treatments

| Parameter                      | Treatments (waste) |
|--------------------------------|---------------------|
|                                | pK (0%) | pA (30%) | pB (60%) | pC (80%) | pD (100%) |
| Before                         | 62±1.53  | 331.67±0.65 | 492.00±4.04 | 768.00±5.20 | 894±0.58  |
| After                          | 22±3.51  | 69±1.73   | 81.00±4.62  | 149.00±2.89 | 272.67±7.17 |
| Decreased TSS Levels (mg/L)    | 40      | 262.67    | 411       | 619      | 621.33    |
| Phyto remediation Efficiency (%)| 64.67a  | 79.19b    | 83.54b    | 80.59b   | 69.50a    |

Description: The numbers followed by different letters, show significantly different (p<0.05).

3.3 Biochemical Oxygen Demand (BOD)

BOD indicates the amount of oxygen needed by decomposers (bacteria) to decompose organic matter into inorganic materials (aerobic decomposition) over a certain period of time, so BOD shows the level of oxygen demand for the biological decomposition process. High and low BOD is determined by temperature, plankton density, the presence of microbes and the type and presence of organic matter contained in the waters (Asmar, 2005). Measurement of the level of Biological Oxygen Demand (BOD) in waste water is used to determine the amount of dissolved O2 needed by aerobic microorganisms to oxidize organic material in a waste sample.

The measurement results of the parameters Biochemical Oxygen Demand (BOD) Microalgae Spirulina sp. before and after the fish handler’s liquid organic waste, namely, K treatment (control), pA (30% waste), pB (60% waste), pC (80% waste) and pD (100% waste) follows in Table 3.

Table 3 shows that the concentration of Spirulina sp. with different levels of waste content can reduce the value of the BOD content contained in fish handling liquid waste. The BOD level decreases along with the less variation of fish handling liquid waste in each treatment. Table 3 shows the phytoremediation efficiency of BOD pA (30% liquid waste) of 75.59% greater than treatment control of 52.11%, pB (60% liquid waste) of 60.44%, pC (80% liquid waste) of 26.78%, and pD (100% waste) 9.74%.

Liquid waste of handling fish is not too large in the cultivation medium can increase phytoremediation efficiency by Spirulina sp. because it does not affect the penetration of light into the media so that it further improves the phytoremediation process. Photosynthesis of microalgae produces O2 which plays a role for respiration in the growth...
of waste oxidizing bacteria, conversely oxidation reactions or decomposition of waste carried out by bacteria produce CO\(_2\), which can support the growth of microalgae, this will also speed up the process of decomposition of waste carried out. BOD according to Cahyanto et al. (2018), BOD is the amount of dissolved oxygen needed by decomposing bacteria to decompose organic pollutants in water. The greater the BOD concentration of a waters, shows the concentration of organic matter in the water is also high organic material must be degraded by microorganisms (Olguín et al., 2001).

### 3.4 Chemical Oxygen Demand (COD)

Measurement the level of Chemical Oxygen Demand (COD) in liquid waste is used to determine how much the level of pollution in water bodies by organic matter. COD is a description of the total oxygen needed to chemically oxidize organic material, both biodegradable and non-biodegradable, into CO\(_2\) and H\(_2\)O. The existence of organic material can come from nature or human activities through households and industry COD value in uncontaminated waters is usually less than 20 mg/l, whereas in polluted waters can be more than 200 mg/l (Wijaya, 2009).

The measurement results for the range of changes in the value of the Chemical Oxygen Demand (COD) Microalgae Spirulina sp. before and after being given liquid waste of handling fish from Pasar Cemara, Medan with different amounts, namely, K (control), pA (30% waste), pB (60% waste), pC (80% waste) and pD (100% waste) as follows in Table 4.

### Table 3. BOD phytoremediation efficiency values on Spirulina sp. in different treatments

| Parameter                          | Treatments (waste) |
|------------------------------------|--------------------|
|                                   | pK (mg/L)         | pA (mg/L) | pB (mg/L) | pC (mg/L) | pD (mg/L) |
| Before                             | 4.74±0.04         | 23.33±0.73 | 17.83±0.81 | 26.33±0.44 | 27.17±0.44 |
| After                              | 2.27±0.14         | 5.69±0.18  | 10.15±2.05 | 19.28±0.33 | 24.52±0.40 |
| Decreased BOD Levels               | 2.47              | 17.64      | 7.68       | 7.05       | 2.65       |
| Phytoremediation Efficiency        | 52.11%c           | 75.59%c    | 60.44%c    | 26.78%c    | 9.74%c     |

Description: The numbers followed by different letters, show significantly different (p <0.05).

### Table 4. COD phytoremediation efficiency values on Spirulina sp. in different treatments

| Parameter                          | Treatments (waste) |
|------------------------------------|--------------------|
|                                   | pK (mg/L)         | pA (mg/L) | pB (mg/L) | pC (mg/L) | pD (mg/L) |
| Before                             | 54.2±1.89         | 1560.6±36.55 | 2522.3±11.86 | 3007.6±0.33 | 4421±222.52 |
| After                              | 20.7±2.39         | 259.5±15.15  | 1469.1±57.07 | 2285.2±316.64 | 3591.5±59.79 |
| Decreased COD Levels               | 33.5              | 1301.12     | 1053.26     | 722.48     | 829.54     |
| Phytoremediation Efficiency        | 61.70%c           | 79.19%c     | 41.77%c     | 24.02%c    | 18.85%c    |

Description: The numbers followed by different letters, show significantly different (p <0.05).

Duncan’s follow up test in Table 3 shows that all the treatments, both pK (control), pA (30% waste), pB (60% waste), pC (80% waste) and pD (100% waste) are significantly different from each other, this because the decrease in BOD is in line with the decrease in the content of organic matter in organic waste water handling fish, oxygen produced from the metabolism of microalgae Spirulina sp. that is photosynthesis, this increased oxygen content in wastewater will increase the process of decomposition of aerobic bacteria so that it can suppress high levels of BOD in water. According to Cahyanto et al. (2018), BOD value is influenced by the presence of plants that cover the surface of wastewater. The existence of these plants absorbs organic substances found in wastewater. The more photosynthetic organisms, the more organic material is absorbed and the organic material must be degraded by microorganisms (Olguín et al., 2001).
The data show a significantly different treatment at 30% pA concentration compared to other liquid waste concentrations. The decrease in pollutants shows the value of phytoremediation efficiency of COD in the treatment of pA (30% waste) 79.19% greater than pK (control) 61.70%, pB (60% waste) 41.77%, pC (80% waste) 24.02% and pD (100% waste) 18.85%.

In Table 4 shows the less waste, the greater the phytoremediation efficiency produced. This is because the greater the level of waste will increase the organic material in it so that it affects the penetration of light into the media. Large light penetration will increase the metabolic activity of Spirulina sp. There by increasing the absorption of COD pollutants. In accordance with Cahyanto et al. (2018), Spirulina sp. has a high phytoremediation ability (efficiency above 75%) at a wastewater concentration of 25%. Decreasing the concentration of organic wastewater optimizes the absorption of pollutants and can be safely disposed of in water bodies (Pacheco et al., 2015).

Duncan’s further test in Table 4 shows the treatment of pA (30% of waste) has a significant influence on the treatment of pK (control), pB (60% of waste), pC (80% of waste), and pD (100% of waste), this is because a decrease in the value of COD causes oxygen to be higher. This is caused by the presence of microalgae Spirulina sp. which absorbs organic material from liquid waste, so that oxygen consumption by microbes to degrade organic matter becomes less. According to Siregar et al. (2017), the high value of COD indicates the high organic matter in the research media. The contribution of organic matter from dead plants is not balanced by the utilization of nutrients resulting from the decomposition of organic material caused by the small number of plants in the treatment.

3.5 Ammonia

Ammonia (NH₃) and its salts are soluble in water. The source of ammonia in waters is the breakdown of organic nitrogen (protein and urea) and inorganic nitrogen present in the soil and water, which comes from the decomposition of organic matter (dead aquatic plants and biota) by microbes and fungi (Effendi, 2003).

The measurement results of ammonia microalgae Spirulina sp. before and after being given liquid waste of handling fish from Cemara market, Medan with different amounts, namely, K (control), pA (30% waste), pB (60% waste), pC (80% waste) and pD (100% waste) as follows in Table 5.

Table 5 shows the value of phytoremediation efficiency of ammonia in the treatment of greater pA (30% waste) 89.15% than pK (control) 32.22%, pB (60% waste) 59.45%, pC (80% waste) 63.37% and pD (100% waste) 69.39% and have met the quality standards according to the Ministry of Environment. No. 5 of 2014 which is 5 mg/L. This is due to the population of Spirulina sp. higher than pB, pC and pD so that the microalgae absorb more ammonia in treatment A compared to other treatments.

At least the ammonia material used by Spirulina sp. for phytoremediation and degradation by microbes also causes ammonia in treatment A to be less than other treatments. According to Effendi (2003), the source of ammonia in waters is the breakdown of organic nitrogen (protein and urea) and inorganic nitrogen found in soil and water, which comes from the decomposition of organic matter.

Duncan’s further test in Table 5 shows the treatment of pA (30% waste) has a significant effect on the treatment of pK (control), pB (60% waste), pC (80% waste) and pD (100% waste), this is because the high value of ammonia reduction in treatment is in line with the large population of Spirulina sp. which grows and develops in the treatment. Because ammonia contains nitrogen compounds that are needed by microalgae for growth. In accordance with Elystia et al. (2019), ammonia is the main source of nitrogen compounds other than nitrites that can be used by microalgae for their metabolic processes, while the use of nitrites is limited by their toxicity.

Table 5. Ammonia phytoremediation efficiency values on Spirulina sp. in different treatments

| Parameter                  | pK (mg/L)     | pA (mg/L)     | pB (mg/L)     | pC (mg/L)     | pD (mg/L)     |
|----------------------------|---------------|---------------|---------------|---------------|---------------|
| Before                     | 3.2±0.07      | 0.43±0.02     | 0.61±0.02     | 0.85±0.03     | 1.05±0.08     |
| After                      | 2.17±0.23     | 0.05±0.01     | 0.25±0.03     | 0.31±0.01     | 0.32±0.03     |
| Decreased Ammonia Levels   | 1.03          | 0.38          | 0.36          | 0.54          | 0.73          |
| Phyto remediation Efficiency| 32.22%       | 89.15%       | 59.45%       | 63.37%       | 69.39%        |

Description: The numbers followed by different letters, show significantly different (p <0.05).
3.6 Phosphate

Phosphate is one of the mineral elements which is an essential element in feed. Phosphate with sulfur is a mineral contained in organic compounds which acts as a constituent of various proteins. The use of these elements affects the manufacture of fish feed. The mineral element is known as inorganic material or ash content which functions as a builder and regulator. Phosphate is often considered a limiting factor, which is based on the fact that phosphate is indispensable in the transfer of P energy in organism cells. Very small amounts of phosphate will cause nutrient deficiencies that can suppress the growth of phytobenthos, and ultimately reduce productivity in a water (Baharsyah, 2014).

The measurement results of phosphate microalgae *Spirulina* sp. before and after being given liquid waste of handling fish from Cemara market, Medan with different amounts, namely, K (control), pA (30% waste), pB (60% waste), pC (80% waste) and pD (100% waste) as follows in Table 6.

![Image](Image38x308 to 541x467)

**Table 6.** Phosphate phytoremediation efficiency values on *Spirulina* sp. in different treatments

| Parameter                        | Treatments (waste) (mg/L) |
|----------------------------------|--------------------------|
|                                  | pK   | pA   | pB   | pC   | pD   |
| Before                           | 4.58±0.19 | 20.23±3.78 | 21.4±2.46 | 22.4±4.35 | 22.67±4.84 |
| After                            | 0.13±0.06 | 11.2±2.11 | 12.49±1.54 | 15.74±3.28  | 16.5±3.54 |
| Decreased Phosphate Levels       | 4.45 | 9.03 | 8.91 | 6.66 | 6.17 |
| Phytoremediation Efficiency      | 97.35%a | 44.70%b | 41.51%b | 30.27%a | 27.23%a |

Description: The numbers followed by different letters, show significantly different (p <0.05).

Duncan’s further test in Table 6 shows the pK (control) treatment is significantly different from the treatment of pA (30% waste), pB (60% waste), pC (80% waste) and pD treatment (100% waste), this is because the high absorption rate of phosphate in treatment K was 97.25% because it was followed by high population of microalgae *Spirulina* sp. The element of phosphate in water is used by microalgae to continue to grow and grow. This is in accordance with Handajani (2006), *Spirulina* sp. is one of the microalgae that is cosmalsite which can be cultivated in different mediums. *Spirulina* sp. growth requires the availability of nutrients N and P which can be derived from chemicals or decomposed solutions or waste for growth.

4. Conclusion

Based on the results, it can be concluded that the addition of *Spirulina* sp. at a volume of 100 ml with a density were 1x10³ ind/mL with a variation in the levels of liquid waste of handling fish can reduce the content of these pollutants. Different levels of liquid waste have a significant effect on the value of TSS, BOD, COD, ammonia, and phosphate in reducing the load of the waste pollutant. The best reduction in BOD level occurred in the treatment of 30% (pA) of liquid waste is 17.64 mg/L with phytoremediation efficiency of 75.59%. The best reduction in COD levels occurred in the treatment of 30% (pA) of liquid waste is 1301.12 mg/L with phytoremediation efficiency of 79.19%. The best reduction in TSS levels occurred in the treatment of 60% (pB) of liquid waste is 411 mg/L with phytoremediation efficiency of 83.54%. The best reduction in ammonia levels was at 30% (pA) treatment, which was 0.38 mg/L with phytoremediation efficiency of 89.15%. While the best decrease in phosphate levels occurred in the control treatment (pK), which was 4.45 mg/L with phytoremediation efficiency of 97.35%.
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Authors’ Contributions

AFD devised the main conceptual ideas for this research, make an analysis for results in this article, and draft the manuscript. ESH collected sample and data, did experiment on a laboratory, make an analysis for data results. EY, IES, and RFS discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests

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