

Effectiveness of *Spirulina platensis* as a bioremediator candidate for vaname shrimp (*Litopenaeus vannamei*) wastewater

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Abstrak

Tujuan penelitian untuk mengetahui epektifitas *Spirulina platensis* dalam meremediasi limbah budidaya udang vaname. Metode yang digunakan dalam penelitian ini adalah Rancangan Acak Lengkap (RAL) Non-Faktorial dengan 4 perlakuan dan 3 ulangan yaitu (A) Kontrol; (B) 50 % limbah (1500 ml limbah + 1500 ml air) + pupuk teknis + inokulan *Spirulina*; (C) 75 % limbah (2.250 ml limbah + 750 ml air) pupuk teknis + inokulan *Spirulina*; (D) 100% limbah + pupuk teknis + inokulan *Spirulina*. Analisis data menggunakan ANOVA dengan tingkat kepercayaan 95%. Hasil penelitian menunjukkan bahwa pemanfaatan *Spirulina platensis* sebagai agen remediator limbah budidaya udang vaname memberikan pengaruh nyata terhadap penurunan kadar limbah amonia, fosfat, nitrat, dan kepadatan *Spirulina platensis* ($F_{hitung} > F_{tabel 0,05}$).

Kata kunci: Limbah; Remediasi; *Spirulina platensis*; Udang Vaname

Abstract

The purpose of this study was to determine the effectiveness of *Spirulina platensis* in the remediation of vannamei shrimp culture waste. The method used in this study was a Non-Factorial Completely Randomized Design (CRD) with 4 treatments and 3 replications, namely (A) Control; (B) 50% waste (1500 ml waste + 1500 ml water) + technical fertilizer + *Spirulina* inoculant; (C) 75% waste (2,250 ml of waste + 750 ml of water) technical fertilizer + *Spirulina* inoculant; (D) 100% waste + technical fertilizer + *Spirulina* inoculant. Data analysis used ANOVA with a 95% confidence level. The results showed that the use of *Spirulina platensis* as a remediation agent for vaname shrimp culture had a significant effect on reducing levels of waste ammonia, phosphate, nitrate, and density of *Spirulina platensis* ($F_{count} > F_{table 0.05}$).

Keywords: Bioremediator; Inoculant; Vanname shrimp; Wastewater

1. Introduction

Shrimp cultivation is one of the largest fish industries in Indonesia. Vaname shrimp (*Litopenaeus vannamei*) cultivation activities in Indonesia are increasing every year. In 2015 it even reached 1.45 x 10⁵ tons for export to various countries (BPS, 2017 in Febrinawati *et al.*, 2020). The high demand for this shrimp commodity further increases the economic value of the shrimp commodity and further increases the number of farmers who cultivate vaname shrimp, one of which is in Aceh Province, causing several problems in the waters. One of the problems that will arise from high aquaculture activities is the amount of waste that will be disposed of in the waters. If aquaculture waste is not managed properly, it will pollute the waters.

According to Gunadi and Hafsari Dewi (2008), one of the sources of pollution that need attention is aquaculture waste that is dumped directly into the waters. Aquaculture waste consists of solids and dissolved materials. Solid waste is generally in the form of feed residues, fish feces, and bacterial colonies,

while dissolved waste consists of ammonia, carbon dioxide, phosphorus, hydrogen sulfide, phosphate, and nitrogen. In particular, the management of dissolved waste can utilize microalgae to reduce waste to produce safer waste disposal. The content of N and P contained in the dissolved waste will be utilized by microalgae as nutrients for their growth. Dissolved waste will be safer to be disposed of in the environment after being used as a microalgae medium, while the biomass produced by microalgae can be focused on food or energy, so that the synergy between dissolved waste treatment and biomass production can work well (Hadiyanto and Azim, 2012 in Tinambunan *et al.*, 2017).

Several researchers have used certain wastes as media for phytoplankton cultivation, such as Tinambunan *et al.*, (2017) researching the population growth of *Spirulina platensis* in liquid waste media, processed soy sauce, and zarrouk media. Febrinawati *et al.*, (2020) researched the utilization of vaname shrimp (*Litopenaeus vannamei*) culture waste as a culture medium for *Chaetoceros amami*. The advantages of *Spirulina platensis* are high tolerance to salinity, high protein, and is one of the algae whose selling price is expensive. Therefore, this study wanted to examine the utilization of vaname shrimp culture waste as a living medium for *Spirulina platensis*.

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Meanwhile, the purpose of this study was to determine the effectiveness of *Spirulina platensis* in remediating vaname shrimp culture waste.

2. Materials and Methods

The research method used in this study was a completely randomized design (CRD) with 4 treatments and 3 replications. The treatment in the study of the effectiveness of *Spirulina platensis* as a candidate for bioremediator of vaname shrimp wastewater. The treatment of this research in detail is as follows: 1) Treatment A (Control); 2) Treatment B (50% waste (1500 ml waste + 1500 ml water) + technical fertilizer + *Spirulina* inoculant); 3) Treatment C (75% waste (2250 ml waste + 750 ml water) technical fertilizer + *Spirulina* inoculant); 4) Treatment D (100% waste + technical fertilizer + *Spirulina* inoculants).

Tools used in this study were a jar, blower, pHmeter, refractometer, thermometer, microscope, dropper, cover glass, Sedgewick rafter, handtally counter, measuring cup, and beaker glass. The materials used in this study were seawater, waste, aquadest, spirulina inoculant, chlorine, ZA fertilizer, Urea, Sp-36, FeCl₃, and EDTA.

2.1. Research Procedures

2.1.1. Preparation of equipment sterilization

The equipment used in this study was first sterilized using distilled water and chlorine to remove mold and minimize contaminants that could hinder the productivity of *Spirulina platensis*.

2.1.2. Container preparation

The container used was a culture jar with a volume of 5 L as many as 12 pieces. The culture jars were washed using distilled water and chlorine, rinsed with running water, then dried and treated with labels, and placed in places that had been prepared according to the research design.

2.1.3. Preparation of culture media

If all treatment media have been prepared, then technical fertilizer is added. There are 5 types of fertilizers used, namely ZA, Urea, Sp-36, FeCl₃, and EDTA. The dose used for each fertilizer is 0.1mL/L, then aeration is given.

2.1.4. *Spirulina platensis* culture

Spirulina platensis inoculants obtained from Brackish Water Cultivation Center Ujung Batee were propagated for stock culture using technical fertilizers until the density reached 56,210 cells/ml. Then the results of the growth of spirulina are taken as much as 500 ml and put into 3,000 ml of water that has been given technical fertilizer according to the treatment. Furthermore, each treatment was lit using a fluorescent lamp with a power of 3 watts, 5 cm from the water surface for 12 hours/day, the length of time the test was carried out for 7 days according to the length of the spirulina life cycle (Tinambunan, *et al.*, 2017).

2.2. Data collection

2.2.1. Daily density

The daily density measurement of spirulina was carried out every day for all treatments and replications. To find out the density can be calculated using the formula:

$$\text{Density} = \frac{\text{Total number of spirulina} \times 1000}{3,14 \times 10}$$

2.2.2. Growth rate

The growth rate is calculated by a formula that refers to Becker, (1994) in Tinambunan *et al.*, (2017):

$$\text{Specific Growth Rate} = \frac{\text{Ln final density} - \text{Ln initial density}}{\text{time}} \times 100$$

2.2.3. Waste degradation rate

The measurement of waste degradation is carried out specifically on the parameters of ammonia, nitrate, and phosphate using the following formula:

$$\text{Waste Degradation Rate} = \frac{\text{last conc.} - \text{initial conc.}}{\text{last conc.}} \times 100$$

2.2.4. Data analysis

The research data such as the density of *Spirulina platensis* are presented in graphs and tables. The data referred to include population peaks, specific growth rates, and values of ammonia, phosphate, and nitrate. All of these data were analyzed using the variance test (ANOVA) with a 95% confidence level. Data analysis was carried out using SPSS software.

3. Results and Discussion

3.1. Results

a. *Spirulina* daily density

Spirulina platensis daily density data during maintenance is presented in graphical form, it can be seen as follows.

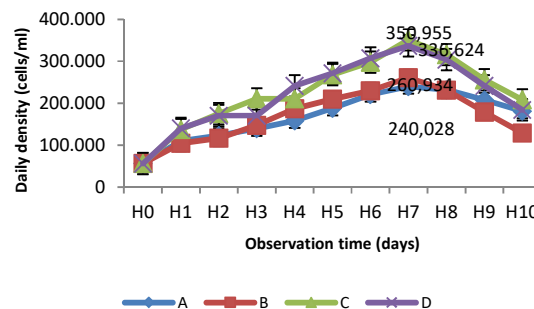


Figure 1. *Spirulina platensis* daily density chart.

Description: (A) Blue = Control;
(B) Red = 50% waste + technical fertilizer;
(C) Green = 75% waste + technical fertilizer;
(D) Purple = 100% waste + technical fertilizer.

Based on the results of research conducted providing media in the form of vaname shrimp culture waste can increase the density of *Spirulina platensis*. The highest density was found in treatment C which utilized 75% of waste with an average value of 685,617 cells/ml. Then the peak of the population occurred on the seventh day with the highest density reaching 350,955 cells/ml in treatment C. *Spirulina platensis* growth occurred in 4 phases, namely the lag phase (adaptation that occurred on day-0 to day two). The logarithmic (exponential) phase occurred from day to day. -3 to day 6, while the stationary phase occurred on day 7th. The declining growth phase occurred from day 8th to day 10th.

The results of the F ANOVA test showed that the utilization of vaname shrimp culture wastewater had a significant effect on the peak of the *Spirulina platensis* population with a value of $F_{\text{count}} 50,039 > F_{\text{table}} (0.05) 4.07$. Tukey test results showed that treatment C was significantly different from treatments A and B, but not significantly different from treatment D. The peak of the population that occurred on the 7th day is presented in the form of a graph that can be seen as follows.

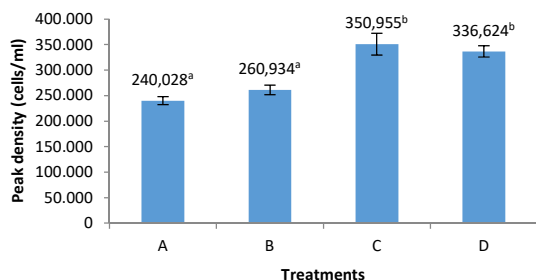


Figure 2. *Spirulina platensis* daily density chart.

Description: A = Control;
B = 50% waste + technical fertilizer;
C = 75% waste + technical fertilizer;
D = 100% waste + technical fertilizer.

b. *S. platensis* growth rate

The results of the average abundance of *Spirulina platensis* during culture can be seen in the following graph.

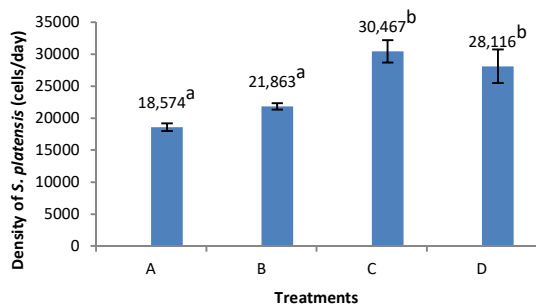


Figure 3. *S. platensis* growth rate chart.

Figure 3 shows the highest growth rate of *Spirulina platensis* in treatment C with an average of 30.467 cells/day. The highest growth rate was followed by treatments D, B, and A with the number of cells at 28,116 cells/day, 21,863 cells/day, and 18,574 cells/day. The results of statistical analysis using the F test (ANOVA) showed that the use of vaname shrimp culture wastewater as a culture medium for *Spirulina platensis* had a significant effect on increasing the population of *Spirulina platensis* with a value of $F_{\text{count}} 34.364 > F_{\text{table}} (0.05) 4.07$. Tukey's test showed that treatments A and B were significantly different from Treatments C and D. Treatment A was not significantly different from Treatment B, and Treatment C was not significantly different from Treatment D.

c. Ammonia, nitrate, and phosphate absorption

The highest wastewater absorption efficiency was found in treatment B for the ammonia parameter with an efficiency percentage of 99% with a final value of 0.01mg/L. Meanwhile, the highest efficiency for the absorption of nitrate was found in treatment C with a percentage of 95% with a final value of 0.01 mg/L. As for the phosphate parameter, the best efficiency of absorption of phosphate wastewater was also found in treatment C with a percentage value of 85% with a final value of 0.01mg/L. The results of the one-way ANOVA statistical test showed that the utilization of vaname shrimp culture wastewater as a living medium for spirulina with different concentrations had a significant effect with the value of $F_{\text{count}} 4516.960 > F_{\text{table}} (0.05) 4.07$. Tukey's test results showed that treatment A was significantly different from all treatments.

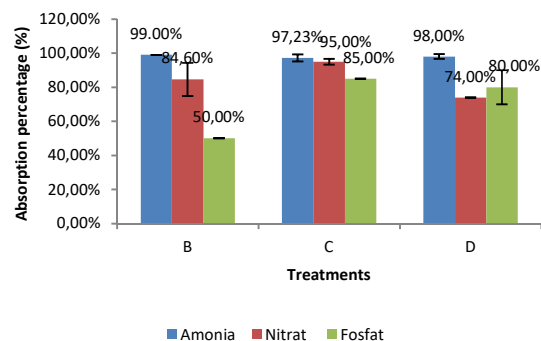


Figure 4. Ammonia, nitrate, and phosphate absorption percentage.

Vannamei shrimp culture wastewater generally contains ammonia, nitrate, and phosphate. So that it becomes the main water quality parameter that must be measured in this study as presented in the following graph (Fig. 4).

d. Water quality parameters

Water quality parameters measured during the study include temperature, pH, and salinity can be seen in the following table.

Table 1
Water quality data.

No	Treatments	Water quality parameters		
		Temperature (°C)	pH	Salinity (ppt)
1	A	26 – 27	7.10 – 7.15	18
2	B	26 – 28	7.10 – 7.2	18
3	C	26 – 28	7.11 – 7.2	18
4	D	26 – 27	7.10 – 7.2	18

The results of the average measurement of water quality during the culturing of *Spirulina platensis* for all treatments did not have a significant difference as shown in table 1. This shows that the culture of *Spirulina platensis* using vaname shrimp culture waste media can be utilized.

3.2. Discussion

a. *Spirulina* daily density

The results showed that the highest population was found in treatment C with vaname shrimp culture waste media as much as 75% with an average value of 685,617 cells/ml, due to treatment C containing sufficient nutrients, to support the growth of *Spirulina platensis*. One of the nutritional content that supports the abundance of *Spirulina platensis* is nitrate and phosphate. This statement is in line with the statement of Astiani *et al.*, (2016) in Buono *et al.*, (2018) that *Spirulina* growth is influenced by nutrient availability. This statement is proven by the results of research from Jalal (2011) in Lesmana *et al.*, (2019), namely *Spirulina* sp. can absorb nitrate sourced from domestic waste from a concentration of 9.5 mg/L to 1.5 mg/L a decrease of (84.2%) and phosphate from a concentration of 58.98 mg/L to 34.32 mg/L a decrease of (41,8%).

According to Mauretsa *et al.*, (2019), stating that the growth of *Spirulina platensis* is strongly influenced by the nutrient content in the culture media. The higher the N, P, and K elements contained in the culture medium, the higher the microalgae production to a certain extent. Then Widianingsih (2008) Nitrates and phosphates are limiting factors for microalgae in general, the lack of nutrients in microalgae affects the decrease in protein content, photosynthetic pigments, and the content of carbohydrate and fat products.

Jalal *et al.*, (2011) stated that *Spirulina platensis* itself can absorb nitrate sourced from domestic waste from a concentration of 9.5 mg/L to 1.5 mg/L a decrease of (84.2%), and

phosphate from a concentration of 58.98 mg/L. L to 34.32 mg/L decreased by (41.8%). According to Kawaroe *et al.*, (2010) in Khanza (2019), the growth pattern of microalgae in the cultivation system is divided into 5 stages, namely: the adaptation phase (lag phase), the exponential phase (log phase), the stationary phase, and the declining growth phase.) and the death phase.

The life phase of *Spirulina platensis* starts from the initial stocking, which is day zero. From day 0 to day 2, *Spirulina platensis* was in the lag phase. Following the statement of Kawaroe *et al.*, (2010) in Khanza (2019), this phase is a phase where there is an increase in the abundance of microalgae in small quantities. According to Widyanto *et al.*, (2014) in Muliani *et al.*, (2018) which states that the adaptation phase of microalgae will be faster if the inoculated cells come from cultures that are in the exponential phase. This is supported by the opinion of Fogg and Thake (1987) in Muliani *et al.*, (2018) who state that the length of the lag phase depends on the number and age of inoculants and the culture media used.

The log phase (exponential) occurred from day 3 to day 6 marked by the increase in *Spirulina platensis* cells. Following the statement of Hadiyanto and Azim (2012), the exponential phase indicates a state of balanced microalgae growth between food intake and an increase in microalgae. According to Mauretsa *et al.*, (2019), adequate nutrition can facilitate *Spirulina platensis* in breeding to achieve maximum growth. According to Utomo and Winarti (2005), the population was increasing because algal cells were actively proliferating and there was the formation of proteins and components that make up the plasma cells needed for growth.

The stationary phase occurred on the seventh day, marked by the density of *Spirulina platensis* reaching the peak of the population. This is following the opinion of Muliani *et al.*, (2018) who said that the stationary phase was characterized by relatively the same growth and death rates, in this phase, the abundance of *Spirulina platensis* reached the peak of the population. The declining growth phase occurs after the seventh day or after the highest density peak. This is under the opinion of Utomo (2005) in Muliani *et al.*, (2018) who states that the increased mortality rate is caused by a decrease in the number of nutrients at a level that is no longer able to support continued growth. According to Mauretsa *et al.*, (2019), the growth of *Spirulina platensis* decreased or died on the eighth day. If there is a population decline due to a lack of nutrition. Giving excess nutrients will also result in stunted growth of *Spirulina platensis* because it will cause toxins to inhibit growth.

b. *S. platensis* growth rate

The difference in the growth rate in each treatment indicated that the cell division process in each treatment was different. The higher the growth rate, the faster the microalgae will multiply. Based on Figure 4, the best growth rate was shown in treatment C with an average of 30.467 cells/day. this is because treatment C contains sufficient nutrients, so *Spirulina platensis* self-multiplication is faster. One of the nutritional content that supports the self-multiplication of *Spirulina platensis* is nitrate and phosphate. The highest growth rate was followed by treatments D, B, and A with the number of cells at 28,116 cells/day, 21,863 cells/day, and 18,574 cells/day.

This is under the statement of Suminto and Chilmawati (2008) in Lesmana *et al.*, (2019), in their research stating that a greater value of the growth rate constant means that the process of algal cell division becomes faster, so that the number of cells per unit time will increase. greater than the increase in time itself. The speed of the algal cell division process is also influenced by the nutrient content available in the microalgae

growing media. Nutrients play an active role in the process of microalgae cell division because they are a source of microalgae nutrition. This statement is in line with the opinion of Chilmawati (2008) in Lesmana *et al.*, (2019) the study, namely, the treatment that had the highest growth rate in her research was due to the macro and micronutrients contained in the growing media in sufficient quantities.

c. Ammonia, nitrate, and phosphate absorption

Reduction of ammonia, nitrate, and phosphate levels from waste because these organic materials are utilized by *Spirulina platensis* for its growth. In this study, the parameters of ammonia, nitrate, and phosphate were used to see how much of these three things were utilized by *Spirulina* for growth.

The highest efficiency for ammonia was found in treatment B with a percentage of 99% the final value was 0.01 mg/L. The highest efficiency for nitrate was found in treatment C with a percentage of 95% the final value was 0.01 mg/L. For phosphate, the highest percentage was found in treatment C with a percentage value of 85%, the final value was 0.01 mg/L.

The high value of reducing ammonia in the treatment was in line with the large population of *Spirulina*. that grow and develop during the treatment. It is suspected that ammonia will be converted by good bacteria into nitrite and then into nitrate which can be utilized by phytoplankton for growth. Following Mawaddah *et al.*, (2016), ammonia is the main source of nitrogen compounds that can be used by microalgae for their metabolic processes, while the use of nitrite is limited by its toxicity.

The reduction in nitrate and phosphate levels from the waste was caused by *Spirulina* sp. utilizing these elements as nutrients (Jalal *et al.*, 2011). Based on research by Suminto (2009), the nitrogen content is very influential on the abundance of *Spirulina platensis* cells, the abundance of *Spirulina* cells.

According to Buwono *et al.*, (2018), phosphate is often considered a limiting factor, which is based on the fact that phosphate is indispensable in the transfer of P energy within the cells of organisms. Phosphate in very small amounts will cause nutrient deficiency which can suppress the growth of phytoplankton. The high absorption rate of phosphate levels in treatment C was followed by a high population of *Spirulina* microalgae. Phosphate elements in water are used by microalgae to continue to grow and grow. This is following Handajani (2006), which states that *Spirulina* sp. is one of the cosmopolitan microalgae that can be cultivated in different media. *Spirulina* growth requires the availability of nutrients N and P which can come from chemicals or solutions of decay or waste for its growth.

d. Water quality parameters

The results of water quality measurements during the study showed that the water quality parameters obtained were still optimal for the growth of *Spirulina platensis*. In this study, the average temperature ranges from 26 to 28 °C. This is under the statement of Wulandarai (2011) in Muliani *et al.*, (2018) who say that temperature is a factor that determines the growth of microalgae. *Spirulina* sp belongs to mesophilic microalgae, which can grow at a temperature of 20-40 °C with an optimum growth temperature of 25-33 °C.

pH in the study ranged from 7.10 to 7.2. This is under the statement by Amanantin (2013); and Ana *et al.*, (2019), which is a good pH for the growth of *Spirulina* sp. ranges from 6-to 8. The salinity of each treatment was 18 ppt. this range is still optimal for the growth of *Spirulina platensis*. This is under the statement of Utomo and Winarti (2005) in Muliani *et al.*, (2018) which said that salinity affects organisms in maintaining osmosis with their environment. *Spirulina* sp is euryhaline with a salinity range between 15-30 ppt.

4. Conclusion

Modification of *Spirulina platensis* media by utilizing vaname shrimp culture waste affects the density and growth rate of *Spirulina*. Vaname shrimp culture waste contains ammonia, nitrate, and phosphate which can be utilized by *Spirulina* as nutrients for growth. The results also showed that *Spirulina platensis* can be used to reduce nitrate content by 0%, 85%, 95%, and 74% respectively. Reduces phosphate content by 0%, 50%, 85% and 80% respectively. Water quality in *Spirulina* culture with the use of vaname shrimp culture waste is still within optimal limits.

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