

# The Utilization of *Gracilaria verrucosa* as shrimp ponds wastewater biofilter

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**Abstract.** *Gracilaria verrucosa* as biofilter can absorb and utilize inorganic nitrogen and phosphorus contained in pollutants for its growth. This research aims to know the ability of *G. verrucosa* as shrimp pond waste biofilter, to know the difference in the quality of shrimp pond wastewater before and after treatment, and to learn more about the absorption capacity of *G. verrucosa* capability against shrimp pond wastewater. The experiment was conducted on a laboratory scale using a Completely Randomized Design (CRD) consisting of four treatments with three replications using *G. verrucosa* of different weights which were 100 g, 150 g, 200 g, and controls. The parameters observed in the study were water quality parameters, seaweed biomass, and seaweed absorption. The results obtained were temperature 27.2–30.1°C, TSS 7–76 mg.L<sup>-1</sup>, pH 7.42–8.83, salinity 16–18 ppt, DO 1.7–5.3 mg.L<sup>-1</sup>, biomass 74–210.7 g, ammonia effectively decreased on 10<sup>th</sup> day by 90%, nitrate on 20<sup>th</sup> day was 22.2% and phosphate value on 30<sup>th</sup> day was 20.1%. *G. verrucosa* absorbed nitrogen (N) 0.08% and phosphorus (P) 0.35%. *G. verrucosa* is potential as a biofilter and can be used as a species candidate for IMTA system.

## 1 Introduction

Shrimp farming activity in the fishpond will produce a significant amount of organic and inorganic (dissolved nitrogen and phosphorus) materials [18] which produced from residual feed waste, feces, and residual metabolism activity. The waste can change sediment composition which triggers eutrophication, and also causes water hypoxic or anoxic condition [8, 16]. Aquaculture wastewater which has rich in nutrients is reported to cause blooming phytoplankton [12, 17]. If blooming occurs continuously, it will threat the biodiversity and disturb the ecosystem balance.

*Gracilaria* is a genus of red seaweed which has been utilized in the Integrated Multitrophic Aquaculture system (IMTA system) [1, 7, 17]. Furthermore, *Gracilaria* has high economic value as raw material for the nutraceutical and pharmaceutical industry. *Gracilaria verrucosa* is mostly cultivated by farmers in Indonesia, especially on the northern coast of Java Island[5].

*Gracilaria* can absorb and extract inorganic material such as dissolves nitrogen and phosphorus [14, 18] within its thallus cells for its growth. Therefore, *Gracilaria* is considered

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to increase the water quality of shrimp pond wastewater. This research is aimed to find out the ability of *G. verrucosa* as biofilter for shrimp pond wastewater to improve its water quality.

## 2 Methods

### 2.1 Location and Date of Research

This research was performed in January – April 2019 at the Center for Brackish Water Aquaculture (*Balai Besar Perikanan Budidaya Air Payau*) Jepara, Indonesia.

### 2.2 Water Quality Measurement

The water quality measurement included physical and chemical parameters. The physical parameter measured including temperature, Total Suspended Solid (TSS), and salinity. The water temperature was measured using a thermometer. Salinity was measured using a refractometer. The Total Suspended Solid (TSS) was measured using the photometric method.

The chemical parameter included dissolved Oxygen (DO), pH, nitrate, phosphate, and ammonia. pH was measured using pH meter and dissolved oxygen using the DO meter. The nitrate parameter measurement was done using method 2.6-Dimethylphenol, phosphate using Phosphomolybdenum Blue method, and ammonia using salicylate method. The measured water samples were collected from all research containers. The water quality observation using physical parameters was conducted every five days, and the observation for chemical parameters was conducted every ten days.

### 2.3 Absorption Measurement

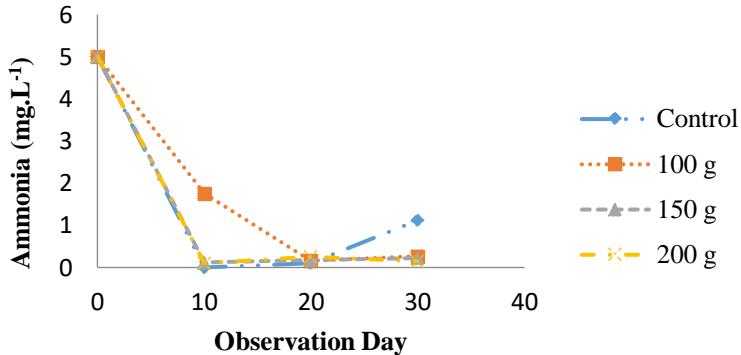
The absorption measurement of Nitrogen (N) and phosphorus (P) content in *G. verrucosa* seaweed was conducted on the 0<sup>th</sup> and 30<sup>th</sup> day. The measurement of N and P content in seaweed tissue was performed using AOAC 976.05.20<sup>th</sup> Ed. 2016 SNI 2803-2012 method.

### 2.4 Statistical Analysis

The research was performed using the experimental method by Complete Random Design. The data result of water quality parameter measurement (nitrate, phosphate, ammonia, biomass, nitrogen, and phosphorus) contents were analyzed using one-way ANOVA then followed by Tukey test analysis with the confidence level of 95%. The statistical analysis was conducted using SPSS program version 20.0.

### 3 Results

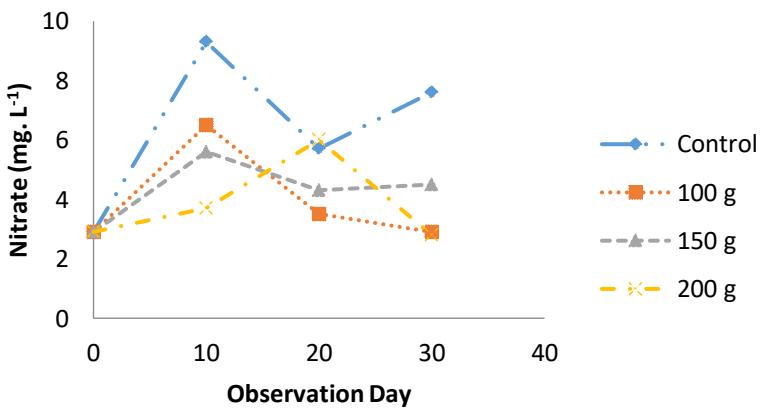
#### 3.1 Ammonia ( $\text{NH}_3$ )



**Fig 1.** The ammonia content of shrimp pond wastewater during treatment

The ammonia concentration on the 0<sup>th</sup> day was 5  $\text{mg.L}^{-1}$ , and the concentration on the 10<sup>th</sup> day was 0–1.76  $\text{mg.L}^{-1}$ . The control without *G. verrucosa* showed increasing of ammonia concentration after the 20<sup>th</sup> day. The ammonia concentration on the 20<sup>th</sup> day was 0.1–0.25  $\text{mg.L}^{-1}$ , and the 30<sup>th</sup> day was 0.14–1.12  $\text{mg.L}^{-1}$ . Meanwhile, the ammonia content on the 100 grams treatment on the 20<sup>th</sup> day and 200 grams on the 30<sup>th</sup> day were decreased because it absorbed the existing waste content.

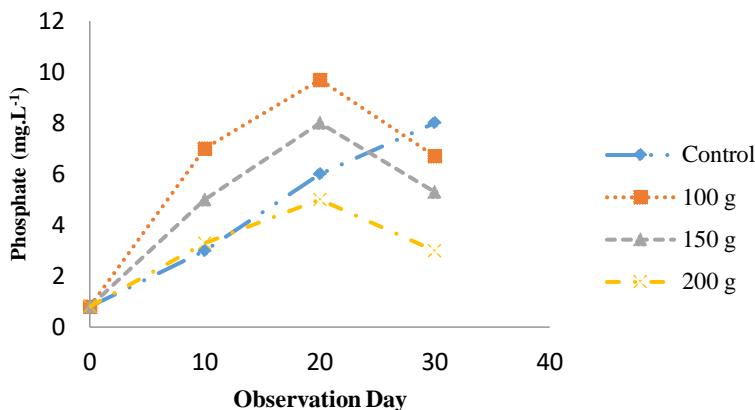
#### 3.2 Nitrate ( $\text{NO}_3$ )



**Fig 2.** The nitrate content of shrimp pond wastewater during treatment

Nitrate concentration on the 0<sup>th</sup> day was 2.9  $\text{mg.L}^{-1}$ , while on the 10<sup>th</sup> was 3.7–9.3  $\text{mg.L}^{-1}$ . Nitrate concentration on the day-20 was 3.5–6  $\text{mg.L}^{-1}$ , while on the 30<sup>th</sup> day was 2.8–7.6  $\text{mg.L}^{-1}$ . The nitrate absorbed by *G. verrucosa* occurred on the 20<sup>th</sup> day, therefore the nitrate concentration in fishpond wastewater also decreasing.

### 3.3. Phosphate ( $\text{PO}_4$ )



**Fig 3.** The phosphate content of shrimp pond wastewater during treatment

Phosphate concentration on day 0 was  $0.8 \text{ mg.L}^{-1}$ , 10<sup>th</sup> day was  $3-7 \text{ mg.L}^{-1}$  and the 20<sup>th</sup> day was  $5-9.7 \text{ mg.L}^{-1}$ . The increase of phosphate on the 10<sup>th</sup> and 20<sup>th</sup> day occurred on all treatments. The phosphate concentration on the 20<sup>th</sup> was  $5-9.7 \text{ mg.L}^{-1}$ , while the 30<sup>th</sup> day was  $3-8 \text{ mg.L}^{-1}$ . Phosphate concentration on the 10<sup>th</sup> to 20<sup>th</sup> day increased due to the waste decomposition process.

### 3.4 *G. verrucosa* Biomass

**Table 1.** *G. verrucosa* biomass as biofilter during treatment

Treatment of <i>G. verrucosa</i>	Biomass (gram)			
	H-0	H-10	H-20	H-30
A (100 gram)	100	105 <sup>c</sup>	92.7 <sup>c</sup>	74 <sup>a</sup>
B (150 gram)	150	170.7 <sup>b</sup>	155.7 <sup>c</sup>	150.3 <sup>b</sup>
C (200 gram)	200	210.7 <sup>a</sup>	186.3 <sup>a</sup>	188.3 <sup>b</sup>
Average	150	162.1	144.9	137.5

\*) The values in the column followed by the same letters indicate no real difference at the level of 95% confidence.

The biomass of *G. verrucosa* experienced increasing on the 10<sup>th</sup> day. After that, the biomass tended to reduce on the 20<sup>th</sup> and 30<sup>th</sup> day since waste nutrient content decreased.

### 3.5. Absorption

**Table 2.** N content in *G. verrucosa* tissue during treatment

Treatment of <i>G. verrucosa</i>	Total N content (%)	
	H-0	H-30
A (100 g)	0.14	0.18 <sup>b</sup>
B (150 g)	0.14	0.22 <sup>b</sup>
C (200 g)	0.14	0.18 <sup>a</sup>
Average	0.14	0.19

\*) The values in the column followed by the same letters indicate no real difference at the level of 95% confidence.

The nitrogen (N) content in *G. verrucosa* tissue increased at the end of the research from 0.14% to 0.18–0.22%. The statistical test results in Table 2 indicated a significant difference for the nitrogen (N) content of the 30<sup>th</sup> day on the treatment of 200 grams during the research. A significant difference showed in the 30<sup>th</sup>-day nitrogen (N) content at 200-gram treatments during the study period

**Table 3.** P content in *G. verrucosa* tissue during treatment

Treatment of <i>G. verrucosa</i>	Total P content (%)	
	H-0	H-30
A (100 gram)	0.72	0.99
B (150 gram)	0.72	1.07
C (200 gram)	0.72	0.95
Average	0.72	0.92

\*) The values in the column followed by the same letters indicate no real difference at the level of 95% confidence.

The phosphorus content (P) in *G. verrucosa* tissue increased at the end of the research, from 0.72% to 0.95–1.07%. The statistical test in Table 2 does not indicate a significant difference in phosphorus (P) content during the research period. There was no significant difference in phosphorous (P) content during the study period.

### 3.6 Supporting Parameters

**Table 4.** Supporting parameters

Supporting Parameters	Value
pH	7.42–8.83
DO (mg/L)	1.7–5.3
Temperature (°C)	27.2–30.1
Salinity (ppt)	16–18
TSS (mg/L)	7–76

## 4 Discussions

### 4.1 Ammonia ( $\text{NH}_3$ )

The decreasing ammonia on the 10<sup>th</sup> day occurred on all treatment. It is also reported by Silviana *et al.* [15] that the initial ammonia concentration in the IMTA system is sharply increased but decreased drastically to stable on the 10<sup>th</sup> day. The decreasing of ammonia concentration on this treatment revealed that *G. verrucosa* starts absorbing the ammonia in wastewater after the 10<sup>th</sup> day through ammonification which is extracted for its growth. If the pH is 7 or less than that, it means that ammonia undergoes ionization.

The pH during the experiment was approximately 7.42–8.83. When pH increased, a higher amount of ammonium will be absorbed by *G. verrucosa*. As a result, it will be decreased faster than nitrate and phosphate because of the ammonification process. *G. verrucosa* is proven to reduce ammonia concentration found in shrimp fishpond wastewater compared with control until at the end of the experiment. The plant weight on the 30<sup>th</sup> day in treatment showed that there were significant differences with controls.

### 4.2. Nitrate ( $\text{NO}_3$ )

Nitrate is the primary nitrogen form that exists in the natural water will quickly dissolve and is required for the algae growth. The increasing on the 10<sup>th</sup> day on all treatments occurred as a result of physiological adaptation within the *G. verrucosa* to tolerate the high concentration of wastewater so that the nitrate absorption process was inhibited. Furthermore, wastewater on the 0<sup>th</sup> day of treatment underwent a continuous nitrification process. The biofilter treatment of *G. verrucosa* was apparent on the 30<sup>th</sup> day, while in the control treatment (without biofilter) showed increasing nitrate concentration. *G. verrucosa* started to absorb nitrate after the 20<sup>th</sup> day.

### 4.3. Phosphate ( $\text{PO}_4$ )

Phosphate is the phosphorous form that can be used by various plants such as water plants [9]. The increasing of phosphate concentration is caused by the evaporation of wastewater so that the phosphate concentration is higher than the previous. The pH condition during the research was ranged 7.42–8.83. The pH condition which higher than 7 will cause phosphate element in the form of  $\text{H}_2\text{PO}_4^-$  is reduced to  $\text{H}_2\text{PO}_4^{2-}$  or in the form of  $\text{PO}_4^{3-}$  which furthermore is harder to absorb [6]. The phosphate concentration absorption done by *G. verrucosa* occurs on the 30<sup>th</sup> day, which means that *G. verrucosa* as biofilter treatment is more prominent, while the control treatment without biofilter showed increasing. *G. verrucosa* biofilter becomes actively absorb the phosphate content after the 30<sup>th</sup> day.

The decreasing phosphate content on the 30<sup>th</sup> day occurred almost on all treatments except the control which experiences increase, indicating that *G. verrucosa* can absorb the phosphate content and make it as the nutrient for its growth. Phosphate compound has an interrupted cycle because it is reactive or easily bound to sediment but is hardly dissolve to the water [13]. *G. verrucosa* is considered effective to inhibit the increase of phosphate content found in the shrimp pond wastewater. The using of *G. verrucosa* resulted in a lower value of phosphate than control. The plant weight treatment did not affect the phosphate content on the wastewater.

#### 4.4. *G. verrucosa* Biomass

Seaweed growth can be explained using a comparison between Carbon and Nitrogen (C/N ratio). The growth variously influenced by some environmental factors [4]. During the research, the biomass of *G. verrucosa* experienced increasing on the 10<sup>th</sup> day. It was revealed that *G. verrucosa* can absorb the nutrient for its growth. However, the biomass tends to reduce on the 20<sup>th</sup> and 30<sup>th</sup> day since waste nutrient content decreased. It is also confirmed by Páez-Osuna *et al.* [12], that the less nutrient content in the water resulted in decreasing microalga biomass. Furthermore, the limited light factor in the laboratory has interrupted the photosynthesis process; hence, the biomass of *G. verrucosa* was decreased at the end of the treatment. Significant differences in the biomass of *G. verrucosa* showed on the 10<sup>th</sup>, 20<sup>th</sup>, 30<sup>th</sup> days during the study period.

#### 4.5. Absorption

Seaweed requires nitrogen and phosphorus elements for its growth. In general, the phosphorus element is absorbed in the form of orthophosphate, while nitrogen is in the form of nitrate, ammonium, and urea [1]. According to Nelson *et al.* [11], 500 grams of fresh seaweed *Gracilaria* sp. can reduce the total nutrient N concentration of 1.2 mg.L<sup>-1</sup> to 0.4 mg.L<sup>-1</sup>.

The result indicates that nitrogen (N) and phosphorus (P) content absorbed in the tip of *G. verrucosa* experienced increasing on all treatments to the end of the research, which caused by the ability of *G. verrucosa* in absorbing the nitrogen (N) and phosphorus (P) content to heal the tissue and grow the new one. The absorbed nitrogen (N) and phosphorus (P) content by *G. verrucosa* will cause its biomass addition, and increase the water quality.

The nitrogen (N) content in *G. verrucosa* tissue increased at the end of the research, from 0.14% to 0.19%. It is lower than the N and P absorption on *G. lemaneiformis* [17]. The absorption of nutrient waste by phytoremediation influenced by contact duration and phytoremediation density [10]. It becomes evident that *G. verrucosa* can absorb the content found in the waste for its growth. According to Abreu *et al.* [1], *G. vermiculophylla* absorption is decreased as the research duration and N accumulation on the tissue. Furthermore, N accumulation will be at the maximum value with the increase of N and NH<sub>4</sub><sup>+</sup> concentration. The phosphorus content (P) in *G. verrucosa* tissue increased at the end of the research, from 0.72% to 0.92%. It was observed that *G. verrucosa* can absorb N and P in wastewater for its growth that can be proved from the increasing of *G. verrucosa* fresh weight, and water quality.

The research concluded that *G. verrucosa* as biofilter can improve the water quality of wastewater by reducing the ammonia effectively on the 10<sup>th</sup> day, nitrate on the 20<sup>th</sup> day, and phosphate on the 30<sup>th</sup> day. *G. verrucosa* absorbed inorganic nitrogen (N) content by 0.08%, and phosphorus (P) content by 0.35%.

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