Omni-Akuatika, 14 (3): 96–105, 2018
ISSN: 1858-3873 print / 2476-9347 online
Research Article

journal homepage: http://ojs.omniakuatika.net

The Ability of *Gracilaria Sp*. to Absorb Ammonia (NH_3-N) and its Effect on Chlorophyll Content and Growth

Yudi Nurul Ihsan[1,*], Rizky K. Bangsa[1], Kalysta Fellatami[1], Tri Dewi K. Pribadi[2]

[1]*Department of Marine Science, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran, Jatinangor 45363 West Java, Indonesia*
[2]*Department of Biology, Faculty of Mathematic and Natural Science, Universitas Padjadjaran, Jatinangor 45363 West Java, Indonesia*

[*]*Corresponding author: yudi.ihsan@unpad.ac.id*

Received 31 July 2018; Accepted 28 November 2018; Available online 30 November 2018

ABSTRACT

One of the most commonly found component of domestic waste in coastal areas is ammonia. Bioremediation techniques can be done to reduce ammonia concentration, including the use of macroalgae *Gracilaria* sp., as in this study. This study aimed to measure the optimum ability of *Gracilaria* sp. in absorbing ammonia and its effect towards the primary productivity of water exposed to ammonia. The experiment was done for 120 hours in room temperature with three replicates for each treatment. Parameter being measured was comparison of reduction of ammonia in media seawater, and the ammonia concentration in *Gracilaria* sp. The results of ammonia reduction by macroalgae in treatment A, B, C and D were 41.21%, 50.27%, 47.37% and 43.05% respectively. The initial concentration results of ammonia in *Gracilaria* sp. was 20.75 mg/kg and the final concentrations in treatment A, B, C and D were 21.08 mg/kg, 21.20 mg/kg, 22.87 mg/kg and 24.44 mg/kg respectively. The chlorophyll-a concentration in water coloumn ranged between 0.015 - 0.027 mg/l.

Keywords: *Ammonia, chlorophyll, Gracilaria sp., bioremediation*

1. Introduction

Indonesian citizens who live in coastal areas are about 60 %, and its population continue to increase, pressuring the coastal natural resources such as beach degradation and waste disposal to the sea. Waste pollution, especially domestic waste in coastal areas is a serious problem. Domestic activities such as household activities that could be very complex have the potential to pollute coastal and marine ecosystems. Increased household activities in coastal areas cause greater volume and more types of waste produced from time to time. As a result, the burden on water bodies that have been used to dispose waste becomes too much that would eventually harm humans and the environment.

One of the most commonly found component in household waste is ammonia. The source of ammonia in water is from the breakdown of organic and inorganic nitrogen. Organic nitrogen comes from protein, urea and the decomposition of organic materials by microbes and fungi in dead plants and marine biota. Under anaerobic conditions, high concentrations of ammonia in water is toxic and it contributes to the occurence of eutrophication. Eutrophication can cause blooming of certain algae that will block sunlight from entering water and disrupt photosynthesis. This condition can cause the concentration of dissolved oxygen in the water to decrease. If dissolved oxygen levels in water bodies decreases, may not directly kill an organism, but may significantly increase its susceptibility to other environmental stresses and diseases. (Wilson 2010).

One of the methods to reduce ammonia in water is by using bioremediation techniques. Several marine organisme can be used as an agent of bioremediation in absorb hydrocarbon and organic waste from coastal area (Syakti, 2013). One of the organism that is efficient and has the potential to be used as a bioremediation agent is macroalgae.

bioremediation that uses macroalgae has the advantage of utilizing its traits, which is bio-extraction and biodegradation (Black, 1995). Bioextraction is where the thallus walls of *Gracilaria* sp. absorb and store nitrogen in its cells which would then be degraded with the help of photosynthesis to form energy and cells as a reflection of the macroalgae clusters's growth (Boyajian & Carriera, 1997) (Burken & Schnoor, 1997). Examples of nitrogen absorbed by macroalgae in the form of inorganic nitrogen are nitrate and ammonia.

Gracilaria verrucosa extract is similar to immunostimulant molecules which can improve survival and growth of shrimp (Jasmanindar et al., 2018). Macroalgae is a component of autotrophs that can produce their own oxygen, where oxygen is the result of photosynthesis. The results of photosynthesis carried out by plants which have chlorophylls are called primary productivity. Photosynthesis plays a very important role in regulating the metabolism of communities living in aquatic environment. In marine ecosystems, the dominant group which photosynthesis is algae. Macroalgae is among the most productive, for example kelp forests in the Indian Ocean have a primary productivity value of 2000 g c/ m^2/ year.

2. Materilas and Methods

2.1 Tools and materials

The materials which are used include *Gracilaria* sp. that is obtained from cultivation at Karangsong Beach, Indramayu; seawater as a medium for the experiment and ammonia solution as pollutant. The materials used to measure ammonia concentration are aquades, $ZnSO_4$, NaOH, tartate and nessler solution.

The tools used to test the absorption capabilities of ammonia is an aquarium with a capacity of 4 liters. The tools that are used to measure water quality: thermometer, refractometer, pH meter and DO meter; to measure ammonia concentration: a UV-Vis spectrophotometer; and to measure primary productivity: Winkler bottles.

2.2. Method

The research method used was an experimental method using a Completely Randomized Design (CRD) which consists of 4 treatments with 3 repetitions, which are presented in **Tabel 1**.

Table 1. Treatment Concentration of Ammonia and *Gracilaria* sp.

Treatment	NH_4Cl concentration (mg/ l)	*Gracilaria* sp. (gram)
A (control)	0	14
B	0 .5	14
C	1	14
D	1. 5	14

The study was conducted for 120 hours. The physical-chemical parameters observed in this study were used as supporting data to determine the absorption pattern of ammonia and to determine its effect on the growth of *Gracilaria* sp. Here are the physical-chemical parameters measured during the observation: temperature, salinity, DO and pH. The primary productivity was observed using oxygen method (dark bottle-bright bottle) with incubation for 6 hours. To figure out the gross primary productivity, it is calculated using the formula (Umaly and Culvin,1998) :

$$\mathrm{GPP} = \frac{(O_2\ \mathrm{BB}) - (O_2\ \mathrm{DB})(1000)\ \times 0.375}{(\mathrm{PQ})\mathrm{t}}$$

$$\mathrm{R} = (\mathrm{O_2\ IB}) - (\mathrm{O_2\ DB})$$

Information:

GPP = *Gross Primary Productivity* (gross productivity value) (mg c/ m^3 /hour)
O_2 = dissolved oxygen (mg/ l)
BB = bright bottle
DB = dark bottle
IB = initial bottle
PQ = results for photosynthesis (1,2)
t = incubation time (hours)

3. Results and Discussion

3.1. Physical and chemical parameters

The physical-chemical parameters observed in this research were used as supporting data to determine the absorption pattern of ammonia as well as determine its effect on the growth of *Gracilaria* sp. The following are physical-chemical parameters measured during the observation: temperature, salinity, DO and pH. The physical and chemical parameters measurement on the experiment's media was carried out after the addition of ammonia and observations were carried out for 120 hours. The measurement results of the physical-chemical parameters are presented in **Table 2**.

The temperature experienced fluctuation during the research, but the values did not vary much among all treatments, which ranged between 23.9 – 25 °C. The temperature measurement results in the study are still in a safe range for the growth of *Gracilaria* sp. According to Anggadiredja et al. (2006), the optimum water temperature for the growth of *Gracilaria* sp. is around 20 – 30 ℃ .

The salinity also experienced fluctuation during the research as it is affected by the enviromental's temperature concentration (Ihsan, et al., 2015). It ranges between 25 - 27 ppt and is still in a suitable range for the growth of *Gracilaria* sp.

Table 2. The Measurement Results of Physical and Chemical Parameters

Parameter	Range of the research results	Optimum range for the growth of *Gracilaria* sp.
Temperature	23.9 - 25 °C	20-30 °C (Anggadiredja et al. 2006)
Salinity	25 - 27 ppt	15 - 30 ppt (Amalia 2013)
pH	7.0 - 8.2	8.2 - 8.7 (Aslan 1998)
DO	6.2- 8.2 mg/ l	> 4 mg/ l (Aslan 1998)

The acidity level in the study showed that the average values are between 7.5 to 8.2. The results obtained tend to be base. This happens due to the release of oxygen into the water from the product of photosynthesis by macroalgae which utilized CO_2, hence reducing the availability of CO_2 (Rukmi, et al., 2012). The pH value obtained is still tolerated for the growth of *Gracilaria* sp. According to Aslan (1998), *Gracilaria* sp. grows well in a pH range of 6.0 - 9.0 and has an optimum growth at pH 8.2 - 8.7.

The dissolved oxygen concentration during the research ranged from 6.2 - 8.2 mg/l. It is still within the safe limit for macroalgae growth. According to Aslan (1998), the optimum limit of dissolved oxygen for macroalgae growth is more than 4 mg/l. The results for DO concentrations in the 120 hours observation has a decreasing pattern. This is caused by an increase in oxygen consumption by macroalgae for respiration. Dissolved Oxygen requirements for maintenance of health and reproduction differ for different fish and invertebrate species. Also, dissolved oxygen is the important element for respiration process and decomposition by macroalgae. (Wilson, 2010).

The highest average concentration of dissolved oxygen is at T0 with a value of 8.2 mg/l. This is because the sea water that was used as the experiment's medium has been aerated using aerators for 3 days.

3.2. The effectivity of *Gracilaria* sp. in reducing ammonia (NH_3-N)

The largest ammonia measurement in the experiment's media with an effectivity percentage for *Gracilaria* sp. is owned by treatment B of 50.27 % and the smallest is 41.21 % from treatment A. The results of the initial and final concentration of ammonia in the experiment's media are presented in **Table 3** .

Table 3. The effectivity of ammonia decrease by *Gracilaria* sp.

Treatment	Average Ammonia Concentration (mg/l)		Effectiveness (%)
	initial	Last	
A	1.76	1.03	41.21
B	1.82	0.91	50.27
C	1.90	1.00	47.37
D	1.94	1.11	43.05

Based on **Table 3**, it is known that *Gracilaria* sp. reduces ammonia most effectively in treatment B. It is assumed that treatment B has the right ammonia concentration for the growth of *Gracilaria* sp., which allowed ammonia in the experiment's media to be used optimally as nutrients that supports their growth. The right ammonia concentration can maintain the osmotic pressure inside the cell so that the exchange of water and nutrients can run smoothly. These results are still lower when compared to the researcg conducted in natural conditions. Izzati's reseach (2010) obtained results which says that the ecosystem model for shrimp windu-*Gracilaria* can inhibit the accumulation of ammonia in aquatic ecosystems up to 69.09 %. In his study, the ammonia concentration obtained in the experiment's medium for 4 weeks without *Gracilaria* was 0.022 mg/l, while the medium which used *Gracilaria* was 0.0068 mg/l.

Treatment A has the lowest effectivity compared to others. This happened as there was no addition of ammonia in its medium, resulting in few absorption as well. In **Table 3**, treatments C and D have low effectivity values. It is suspected that the concentration of ammonia contained in the experiment's media is too high.

Based on **Figure 1**, it is shown that at the beginning of the study, *Gracilaria* was able to play a good role in absorbing ammonia; this can be seen from 0-h to 48-h where the declination rate of ammonia happened quickly. This occurred due to the high dissolved oxygen content from the aeration process in the beginning of the study. Dissolved oxygen requirements for maintenance of health and reproduction differ for different fish and invertebrate species. Also, dissolved oxygen is the important element for respiration process and decomposition by macroalgae. (Wilson 2010).

Gracilaria sp. has a high absorption capacity for ammonia. A section of *Gracilaria* sp. which only consists of thallus, shortens the transport and regulation system of foreign substances that enter the body. Based on **Figure 1**, ammonia fluctuation occurred at 72-h till 120-h in the experiment's media which occurred due to the decreasing concentration of dissolved oxygen. The availability of dissolved oxygen in the media is used by macroalgae for respiration. Respiration in plants is the process of taking O_2 to break down organic compounds into CO_2, H_2O, and energy.

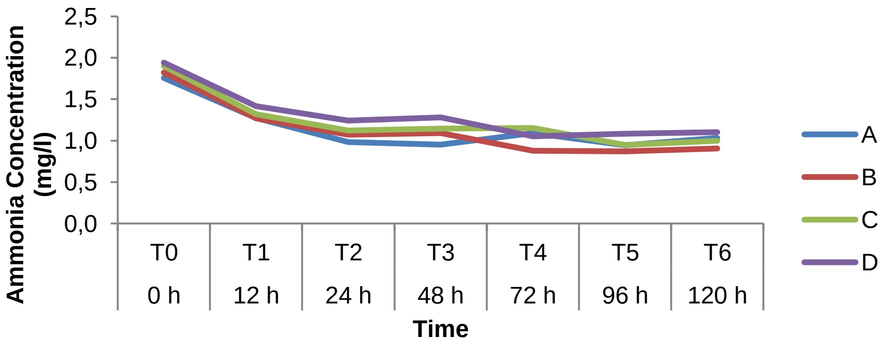

Figure 1. Ammonia concentration graph of the experiment's media

3.3. Ammonia consentration in the thallus of *Gracilaria* sp

Ammonia measurements in *Gracilaria's* sp thallus found out that the initial concentration of ammonia in the thallus amounts to 20.75 mg/kg. At the end of the study, the highest average ammonia concentration is find in treatment D at 22.44 mg/kg and the lowest was from treatment A with a value of 21.08 mg/kg.

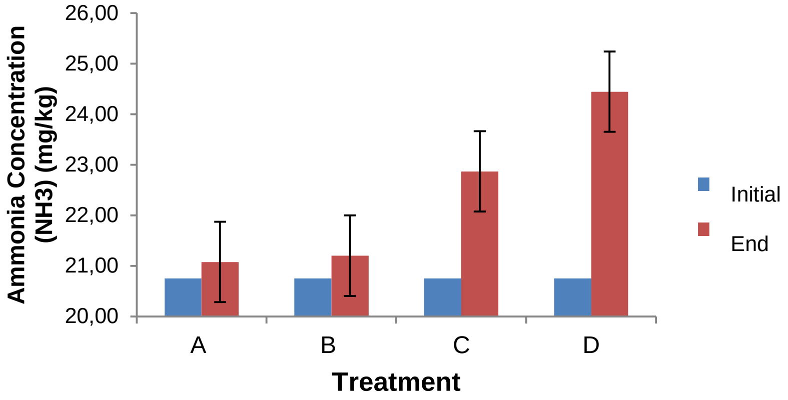

Figure 2. Average value graph of ammonia concentration in *Gracilaria* sp.

Figure 2 shows that the higher the ammonia concentration is in the media, the more the ammonia concentration found in the thallus of the macroalgae. This happens because with an increase concentration added to the media, the possibility of interaction between the dissolved ammonia with the thallus tissues becomes greater; hence, so is the absorption level. Lobban and Harrison (1997) stated that the amount of nutrients that move into the cells by osmosis depends on the nutrient concentration inside and outside the cell. The number of ammonia in the media was greater than inside *Gracilaria* sp. Therefore, ammonia diffused freely into the cell as needed. At the end of the research, the lowest average ammonia concentration in *Gracilaria* sp. was from treatment A with a value of 21.08 mg/kg. This happened as there was no addition of ammonia in the treatment, hence little absorption occurs.

3.4 Protein concentratin in *Gracilaria* sp.

Protein measurements were carried out at the beginning of the research before the treatments, so each treatment had the same initial protein content of 1.47 % of its gross weight. At the end of the study, the largest protein concentration value was 2.90 % in treatment D and the smallest was 2.15 % in treatment A. The effect of ammonia towards protein concentration in *Gracilaria* sp. i s presented in **Figure 3**.

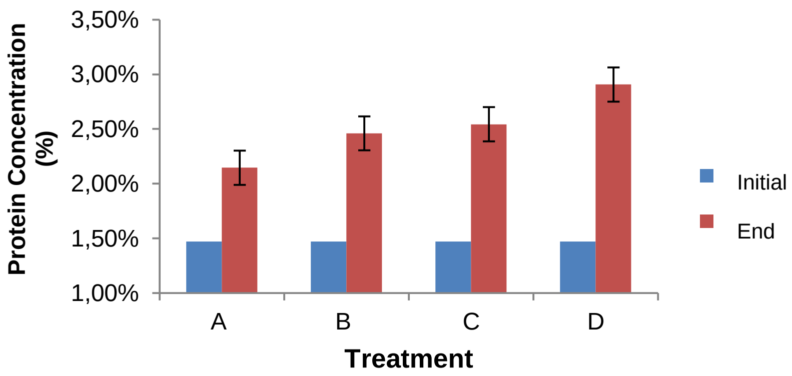

Figure 3. Protein Average (%) in *Glacillaria* sp.

The ability of *Gracilaria* sp. to absorb ammonia in water is related to the protein concentration in its thallus. Based on **Figure 3**, the more ammonia is given, the higher the protein value would be. This shows that there was an occurrence of ammonia absorption in the water media by *Gracilaria* sp. which was then converted to protein. The protein value obtained is relatively small compared to Handayani's *et al*. (2014) research. In his study, the protein values in red macroalgae ranged from 6 – 20 % of its gross weight.

3.5. Chlorophyll-a concentration in the experiment's media

Chlorophyll-a measurements were carried out at the beginning of the research before the treatments, so each treatment had the same initial concentration of chlorophyll-a which is 0.015 mg/l. At the end of the study, the highest average chlorophyll-a was 0.027 mg/l in treatment B and the lowest was 0.018 mg/l in treatment A. The results are shown in **Figure 4.**

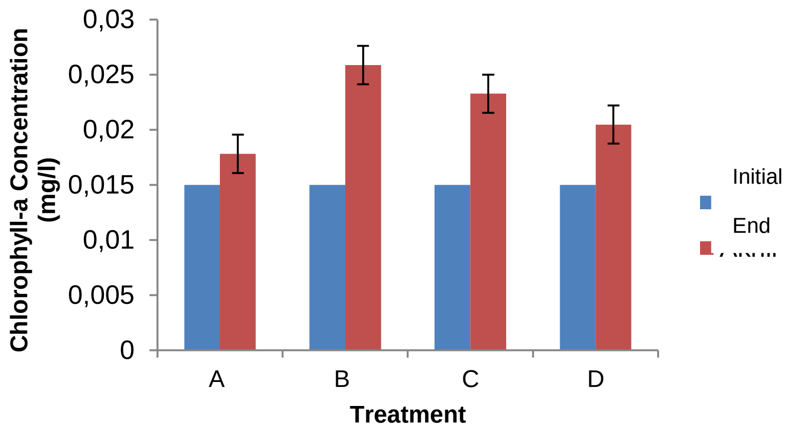

Figure 4. Average Concentration Graph of Chlorophyll-a in Media

Based on **Figure 4**, the lowest chlorophyll-a value is 0.018 mg/l in treatment A, where no ammonia was added so as to deprive nutrients from the macroalgae, resulting in inhibition of the metabolic process. High and low chlorophyll concentrations are affected by several physical-chemical parameters of water such as temperature, light, intensity and nutrients.

The measurement results of chlorophyll-a concentration ranged between 0.015 - 0.027 mg/l. The range of chlorophyll-a concentrations fit into the category of moderate condition. This is based on the quality standards of the Decree of the State Minister of Environment (2004); Chlorophyll-a category: less than 0.015 mg/l is categorized as in good condition, 0.015 - 0.03 mg/l as moderate and more than 0.03 mg/l as bad condition. The result is thought to occur due to ammonia concentrations that was too high in the media, causing the chlorophyll synthesis and photosynthetic process to be inhibited. Medium with high nutrient content can disrupt photosynthesis (Lobban & Harrison, 1997). **Figure 4** shows that ammonia treatments with more than 0.5 mg/l leads to a lower chlorophyll-a concentration. It can be assumed that high ammonia concentration can disrupt the photosynthesis process which makes the chlorophyll-a concentration in the water becomes low.

3.6. Total chlorophyll in the thallus of *Gracilaria* sp.

The initial concentration of chlorophyll measurement was carried out at the beginning of the research before the treatments, so each treatment had the same initial chlorophyll concentration which is 0.7 mg/100g. At the end of the study, the highest average value of total chlorophyll was 1.6 mg/100g from treatment B and the lowest was 0.8 mg/100 g from treatment A. The average value of total chlorophyll in *Gracilaria* sp. is presented in **Figure 5** .

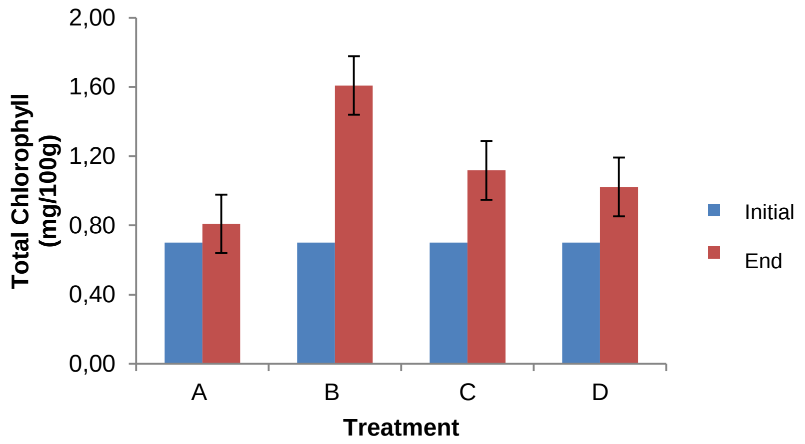

Figure 5 . The average value graph of total chlorophyll in *Gracilaria* sp.

Based on **Figure 5**, the lowest chlorophylls average was 0.8 mg/100 g in treatment A, where no ammonia added to the medium, resulting a nutrient deficiency for the macro algae and disrupt its metabolic process. Ammonia is nitrogen source for the growth of macro algae as nitrogen is important for the synthesis of chlorophyll, proteins and enzymes. Whereas in treatment D, the amount of ammonia given was too high resulting a distruption in the synthesis of chlorophyll and photosynthesis process.

3.7. The weight growth of *Gracilaria* sp.

Weight measurement was carried out before the treatment began, so each treatment has the same initial weight of 14 grams. At the end of the research, the largest average weight was from treatment B with a value of 14.15 grams and the lowest was 14.06 grams from treatment D. The weight growth results of *Gracilaria* sp. are presented in **Figure 6**.

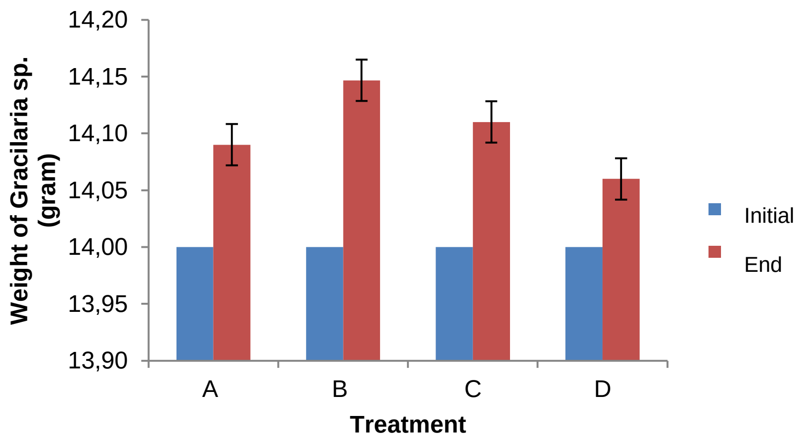

Figure 6. The average weight value graph of *Gracilaria* sp.

Based on **Figure 6**, it is known that treatment B has the highest average growth weight, where the ammonia concentration is the right concentration for the growth of *Gracilaria* sp. In treatment B, *Gracilaria* sp. was able to grow and develop well as well as able to utilize ammonic in the medium as a nutrient source, i.e. as a source of nitrogen. Lobban & Harrison (1997) stated that at the right nutrient concentration, the osmotic pressure in the cell does not change rapidly which allows the exchange of water and nutrients to run smoothly, and a good metabolic process leads to optimum growth. These nutrients will increase the activity of cell metabolisms by entering cells little by little and then developing vacuoles that are inside the cell. Vacuoles play a very important role in life because the plant's survival mechanism depends on the ability of vacuoles to maintain the concentration of dissolved substances in them. Plants grown in nutrient rich conditions will deposit large quantities of such nutrients in vacuoles of vegetative tissues, this allows the plant to survive subsequent periods of nutrient deficiency by mobilizing the vacuolar store. Thus, the solute composition of vacuoles is highly dynamic and reflects changes in the environment and developmental stage (Isayenkov et al., 2010).

Based on **Figure 6**, treatment C and D produced a generally low growth of biomass for the macroalgae. This occurred because the concentration of ammonia in the media was not appropriate for the growth of *Gracilaria* sp. According to the Decree of the Minister of Environment (2004), the standard quality of ammonia (NH_3-N) for marine biota is 0.3 mg/l.

Besides nutrients, macro algae growth is affected by other several factors, such as light (Hasan, Rejeki, & Wisnu, 2015). At the time of the research, the lighting in the room was not evenly distributed which caused a reduction in the amount of light received and caused a disrupt in photosynthesis. If photosynthesis is disrupted, macroalgae cannot grow optimally. Other than the lack of light, it may also happen due to poor water movement in the media. The water parameters that support the growth of *Graciliaria* sp. seaweed include adequate nutrients and relatively stable water movement where mixing process occurs, thus helping macroalgae to absorb nutrients well and increase its growth (Putra, Aryawati, & Isnaini, 2013).

3.8. Ammonia bio-concentration factor in Gracilaria sp.

The bio concentration factor of ammonia is a useful parameter to determine the ability level of organisms or living creatures to absorb ammonia. The results of the ammonia bio-concentration factor are presented in Figure 7.

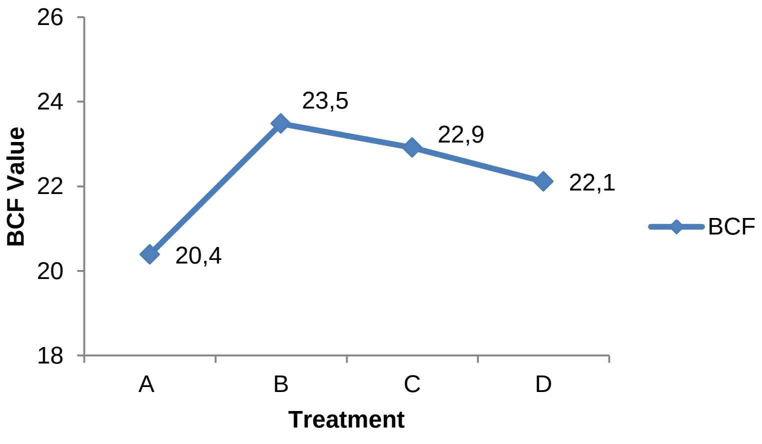

Figure 7. The value of ammonia bio-concentration factor in *Gracilaria* sp.

Based on Figure 7, the highest BCF value is 23.5 from treatment B and the lowest is 20.4 from treatment A. The values show that *Gracilaria* sp. has a high accumulation level for ammonia. This is based on 3 BCF categories of plants which are divided into accumulators, excluders and indicators. The accumulator has a BCF value of > 1, excluder has a BCF value of < 1 and an indicator plant has a BCF value close to 1 (Susana & Suswanti, 2013). Boyajian & Carriera (1997) stated that *Gracilaria* sp., with its phyto-extraction properties, can accumulate and store organic matter such as nitrogen in the form of ammonia in thallus cells .

4. Conclusion

Based on the research results and discussion, it can be concluded that:

1. *Gracilaria* sp has the ability as a bioemediation agent to reduce ammonia concentration in water with a consequence of interference towards the growth of *Gracilaria* sp. The higher the concentration of ammonia, the lower the growth of *Gracilaria* sp.
2. The chlorophyll concentration decreases with increasing ammonia concentration

References

[Kep. Men. LH] Keputusan Menteri Negara Lingkungan Hidup Nomor. 51 Tahun 2004. *Tentang Baku Mutu Air Laut. Jakarta.*

Amalia, D. R. 2013. *Efek Temperatur Terhadap Pertumbuhan Gracilaria verucosa.* Skripsi, Jurusan Fisika. Fakultas Matematika dan Ilmu Pengetahuan Alam. Universitas Jember.

Anggadiredja, J. T., Zatnika, A., Heri P, & Sri I. 2006. *Rumput Laut, Pembudidayaan, Pengelolaan, dan Pemasaran Komoditas Perikanan Potensial.* Jakarta: Penebar Swadaya.

Asela, E. 2016. *Keefektifan Metode Fitoremidiasi Dengan Pemanfaatan Tanaman Eceng Gondok Untuk Menurunkan Kadar Amoniak Limbah Rumah Sakit PKU Muhammadiyah.* Surakarta: Universitas Muhammadiyah Surakarta.

Aslan, L. 1998. *Budidaya Rumput Laut, Edisi Revisi.* Yogyakarta: Kanisius.

Black, H. 1995. *Absorbing Possibilities: Phytoremediation* (Vol. 103).

Boyajian, J., & Carriera, L. H. (1997, February). Pythoremediation: a clean transition from labotary to market place. *Nature Biotechnology, 15*, 127-128.

Burken, J. G., & Schnoor, J. L. 1997. Uptake and Metabolism of Atrazine by Poplar Trees. *Environmental Science and Technology*, 1399-1405.

Dawes, C. J. 1981. *Marine Botany.* Jhon Wiley & Sons, Inc.GESAMP. 1976. *Report and studies No.2: Review of harmful substances.* United Nations, New York.

Handriyani, R. 2013. *Pengaruh Medium yang Tercemar Amoniak (NH3) terhadap Kandungan Agar dan Morfologi Thallus Gracilaria verrucosa.* Skripsi, Universitas Airlangga, Surabaya.

Hasan, M. R., Rejeki, S., & Wisnu, R. 2015. Pengaruh Bobot Awal yang Berbeda terhadap Pertumbuhan Gracilaria sp. yang Dibudidayakan dengan Metode Longline di Perairan Tambak Terabrasi Desa Kaliwlingli Kabupaten Brebes. *Journal of Aquaculture Management and Technology*, 92-99.

Ihsan, Y. N., Aprodita, A., Rustikawati, I., & Pribadi, T. K. 2015. Kemampuan Gracilaria sp. Sebagai Agen Bioremediasi Menyerap Logam Berat Pb. *Jurnal Kelautan, 8.*

Izzati, M. 2010. Efektivitas Sargassum Plagyophullum dan Gracilaria Verrucosa dalam Menurunkan Kandungan Amonia, Nitrit dan Nitrat dalam Air Tambak. 64--71.

Jasmanindar, Y., Sukenda, Alimuddin, Junior, M. Z., & Utomo, N. P. 2018. The Chemical Composition of Gracilaria verrucosa Extract and its Utilization on Survival and Growth Litopenaeus vannamei. Omni Akuatika. 14(3). 1-9.

Komarawidjaja, W. 2005. Rumput Laut Gracilaria sp. Sebagai Fitoremedian Bahan Organik Perairan Tambak Budidaya. *J. Tek. Ling.*, 413.

Lobban, C. S., & Harrison, P. J. 1997. *Seaweed Ecology and Physiology.* United States of America: Cambridge University Press.

Putra, B. D., Aryawati, R., & Isnaini. 2013. Laju Pertumbuhan Rumput Laut Gracilaria sp. dengan Metode Penanaman yang Berbeda di Perairan Kalianda, Lampung Selatan. *Maspari Journal*, 36-41.

Rukmi, A. S., Sunaryo, & Djunaedi, A. 2012. Sistem Budidaya Rumput Laut Gracilaria verrucosa di Pertambakan dengan Perbedaan Waktu Perendaman di Dalam Larutan NPK. *Journal of Marine Research*, 90-94.

Susana, R., & Suswanti, D. 2013. Bioakumulasi dan Distribusi Cd pada Akar dan Pucuk 3 Jenis Tanaman Famili Brassicaceae: Imlementasinya untuk Bioremediasi. *Jurnal Manusia dan Lingkungan*, 221-228.

Syakti, A. D., Yani, M., Hidayati, N. V., Siregar, A. S., Doumenq, P., Sudiana, & I. M. M. 2013. The Bioremediation Potential of Hydrocarbonoclastic Bacteria Isolated From a Mangrove Contaminated by Petroleum Hydrocarbons on the Cilacap Coast, Indonesia. Bioremediation Journal. 17(1). 11-20, DOI: 10.1080/10889868.2012.731446.

Umaly, R. C. & Cuvin, L. A. 1988. Limnology: Laboratory and Field Guide Physico-Chemical Factors, Biology Factors. National Book Store Publ., Manila.

Wilson, P. Chris. 2010. Water Quality Notes: Dissolved Oxygen1. IFAS Extension, University of Florida.