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About TREPSEA 2021

In the last half century, Asia has experienced the fastest economic growth and rapid increase of human prosperity, compared to other continents. However, ecological risks due to significant land use changes, global environmental change and climate change-related disasters have also occurred rapidly in Asia. The global deterioration of soils and landscape poses a significant threat to poverty reduction, sustainable food production, and climate change adaptation and mitigation. A recent study by the International Food Policy Research Institute (IFPRI) has estimated that the annual cost of land degradation due to "land use and cover change" (LUCC) and the use of land-degrading management practices on static crop and grazing land is about $300 billion. This does not include the costs related to deterioration of ecosystem services, which may increase the estimates significantly. However, global hunger also increases, reaching 815 million people in 2017, where about 2 billion people suffer from micronutrient-deficiencies.

In Indonesia, agriculture grows at 3.87 percent per year, which is not very low, although it does not contribute much to poverty reduction and job creation in the country. The poverty level in September 2019 was 24.79 (9.22 percent of total population), where the majority (14.93 million or 12.60 percent) of them are living in rural area, and the rest 9.86 million (6.56 percent) are living in urban area. Income inequality in Indonesia is considered high, where the Gini coefficient generally has increased in the past decade or so, from 0.32 in 1998 to 0.38 in 2007, increasing to 0.41 in 2012 and started to decrease in recent years to 0.382 in September of 2019 (BPS, 15 January 2020). The hunger level in Indonesia has also decreased significantly from 44.1 million in 2004-2006 to 22.0 million in 2016-2018. Interestingly, the hunger level in urban area is 8.2 percent of total population, whereas that in rural area is 7.57 percent. The level of stunting among children has also decreased from 32.9 percent in 2013 to 29.9 percent in 2018, whereas the level of anemia among pregnant women has increased from 37.1 percent in 2013 to 48.9 percent in 2018.

Higher ecological risks and more severe climate change-related disasters have more serious consequences on food and nutritional security in Indonesia and other parts of Asia. Hunger and malnutrition have multiple causes, and in facts many of these challenges transcend national borders. Thus, sustainable, integrated and trans-disciplinary innovative solutions to such problems require a more holistic approach in formulating the development strategies to diversify agricultural and food production, increase productivity, hence generating income and employment. Tran-disciplinary approach in mitigating and adapting the climate change related disaster are also required for more sustainable development strategies, coupled with better strategies for disaster risk reduction and management, workable and effective programs to sustainable development and environmental conservation, and better access to food and improved nutritional security.

Every other year, the International Conference of Transdisciplinary Research on Environmental Problems in Southeast Asia (TREPSEA) is conducted in several cities in Indonesia. The first TREPSEA was conducted in Makassar in 2014, the second was in Bandung in 2016, and the third was in Gorontalo in 2018. The fourth TREPSEA Conference will be conducted as an online conference on September 16-18, 2021. The purpose of International Conference of the Transdisciplinary Research on Environmental Problems in Southeast Asia (TREPSEA) is to conduct integrative research of interactions between natural environment and human-social systems in Southeast Asia to solve the environmental problems in Southeast Asia. Its scope thus includes topics of sustainable development, agricultural and resource economics, economics, social science, environmental science, engineering, medicine, sociology, education, etc. Transdisciplinary Research (TDR) is defined as research efforts conducted by investigators from different disciplines and non-academic participants working jointly to create new conceptual, theoretical, methodological, and transnational innovations. Related stakeholders include academics, governments, development organizations, business and industries, sponsoring institutions, civil society organizations, the media, etc.

The theme of 4th International Conference of TREPSEA will address important themes of "Managing Ecological Risks and Natural Disasters in Southeast Asia: Challenges for Food Security, Public Health, and Economic Welfare". The conference will serve as an arena for stimulating academic exercise and policy dialogues on trans-disciplinary dimensions of environmental problems in Southeast Asia, primarily on the themes of managing ecological risk, disaster mitigation, environmental conservation, food and nutritional security, social and economic welfare, and other related issues in sustainable development in general. The 4th International Conference of TREPSEA will be held as an online conference on September 16-18 of 2021.

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Objectives

The objectives of the 4th International Conference of TREPSEA will address important themes of "Managing Ecological Risks and Natural Disasters in Southeast Asia: Challenges for Food Security, Public Health, and Economic Welfare" are as follows:

1. to stimulate academic exercise and policy dialogues on academic exercise and policy dialogues on trans-disciplinary dimensions of environmental problems in Southeast Asia;

2. to encourage trans-disciplinary research dimensions on of environmental problems, primarily on the themes of managing ecological risk, disaster mitigation, environmental conservation, food and nutritional security, and

3. to update some research progress and new knowledge on managing ecological risk, disaster mitigation, environmental conservation, food and nutritional security, and other related issues in sustainable development in general.

Topics of TREPSEA 2021

"***Managing Ecological Risks and Natural Disasters in Southeast Asia: Challenges for Food Security, Public Health, and Economic Welfare***" as the main theme of the conference and sub-themes are as follows:

1. Environmental Issues and Public Health
2. Ecological Risks and Food Security
3. Disaster Risks Reduction (DRR) and Disaster Management (DM)
4. Sustainable Agriculture and Equitable Development
5. Resources Management and Global Value Chain
6. Legal Issues and Business Ethics in Resource Management
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9. Sustainable Development Goals (SDGs)

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Effectiveness of using of brown algae alginate to immobilize the indigenous bioremediation bacteria for reducing waste water from shrimp culture

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Effectiveness of using of brown algae alginate to immobilize the indigenous bioremediation bacteria for reducing waste water from shrimp culture

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Abstract. *Bacillus coagulans* T1.2, an indigenous bacterium from shrimp ponds in East Lampung, has been found to reduce total ammonia nitrogen (TAN). The purpose of this study was to know the effectivity of immobilization of bacterial by sodium alginate from marine brown algae to reduce wastewater pollution from shrimp culture. The brown algae, *Sargassum* sp. and *Padina* sp., were collected from Pesisir Barat and Ketapang beach, Lampung. Alginate was extracted from both *Sargassum* and *Padina* by alkali method. The bacterial immobilization beads were made by mixed *Bacillus coagulans* suspension with alginate (1:3 v/v) and formed beads by 1,5 ml syringe without needle. In the in vitro study, the immobilized bacteria were submerged in artificial wastewater (2 beads ml^{-1}) and incubated for 20 days. The control group includes the same bacteria without immobilization. The TAN was measured and the viability of bacteria was evaluated after the incubation period. The results showed that using alginate from *Sargassum* sp. and *Padina* sp. as a matrix of immobilization indigenous bacterial *Bacillus coagulans* effectively and significantly reduce the content of Total Ammonia Nitrogen (TAN) in wastewater. The viability of bacteria immobilized with the alginate of *Sargassum* sp. and *Padina* sp. better than the bacterial treatment without immobilization.

1. Introduction

In the last five years, the national shrimp production volume has shown a positive growth trend with an average growth of 15.7% [1]. Shrimp production that occurs continuously will cause problems of decreasing environmental carrying capacity. This happens because biochemical processes involving suspended particles, inorganic nitrogen, and phosphorus nutrients in the cultivation environment will have a direct impact on the content of ammonia, nitrite, hydrogen sulfide (H_2S) compounds, and carbon compounds that are toxic for cultivation system [2].

Bioremediation is one way to overcome these problems. The utilization of bioremediation bacteria derived from external products is not effective to reduce organic waste in shrimp ponds due to unequal environmental conditions. Indigenous bacteria *Bacillus coagulans* are expected to accelerate the degradation process of pond waste because the habitat of these bacteria is same as current environmental conditions. Based on previous research, *Bacillus coagulans* bacteria has the potential as a

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bioremediation agent. This bacterium is not pathogenic and can reduce levels of Total Ammonia Nitrogen (TAN) in vitro [3].

Storing isolates for a certain time can cause a decrease in bacterial bioactivity so that their potential is not optimal. Technology improvement is needed to increase the activity of these bacteria, a potential alternative is to improve its performance through immobilization using sodium alginate. The use of sodium alginate was chosen because its presence is easily obtained from the extraction of brown seaweed *Sargassum* sp. and *Padina* sp. which are plentiful in the coastal area of Lampung. Utilization of this type of brown seaweed as a source of alginate is expected to support the potential of *Bacillus coagulans* to optimally degrading TAN.

This study aimed to analyze the effectiveness of immobilization of indigenous *Bacillus coagulans* bacteria to reduce TAN using sodium alginate from *Sargassum* sp. and *Padina* sp.

2. Methods

2.1. Algae Collection

This study used the brown algae *Sargassum* sp. and *Padina* sp. obtained from coastal areas in Lampung Province. The *Sargassum* sp. was obtained from Pesisir Barat beach, and *Padina* sp. was obtained from Ketapang Beach.

2.2. Design Experimental. This study was conducted using four treatments of immobilized bacteria to degrade TAN in Sewage medium, both for sodium alginate from *Padina* and *Sargassum*. The four treatments were control group that was without adding sodium alginate and bacteria (A), adding sodium alginate without bacteria (B), adding immobilized bacteria using sodium alginate (C), and adding bacteria without immobilization (D).

2.3. Alginate Extraction and Characterization.

2.3.1. Alginate Extraction. Alginate extraction was carried out on *Sargassum* sp. and *Padina* sp. which has a moisture content of <15%. The alginate extraction process was preparing 100 g of *Sargassum* sp. and *Padina* sp. powder then soaked in distilled water (1:10). After that, added 5% Na_2CO_3/50μM EDTA for 24 hours. The immersion results were filtered and added with 0.13 M KCL. Then, precipitation was carried out with 96% cold ethanol (1:1) while vigorously stirring until homogeneous and allowed to stand for 24 hours. The precipitation results were centrifuged at 3,500 rpm for 5 minutes. The results of the alginate extraction were then collected and dried in an oven at 60 ˚C overnight [4].

2.3.2. FTIR Analysis. Extraction results of *Sargassum* sp. and *Padina* sp. the functional groups were analyzed using the FTIR (Fourier Transform Infra-Red) Spectroscopy method. The test compared extracted alginate from *Sargassum* sp. and *Padina* sp. with commercial alginate. This test was weighing of 1 mg samples, then mixed with 100 mg of KBr. Then pressed for ± 10 minutes in 8-10 psi, until a thin pellet is obtained. The pellet was inserted into the cell holder and the spectra were made for analysis [5].

2.4. Bioremediation Bacteria Culture and Immobilization

The immobilization followed an entrapment method using *Bacillus coagulans* bacteria. *Bacillus coagulans* is an indigenous bacterium from traditional tiger shrimp ponds in Mulyosari Village, Pasir Sakti District, East Lampung Regency, Lampung Province. These bacteria have the potential as biodegradation agents to reduce TAN [3].

Bacillus coagulans bacteria were cultured in 1000 mL liquid SWC (Sea Water Compete) medium. The SWC medium consists of 5 g bacto peptone, 1 g yeast extract, 3 ml glycerol, 75% distilled water, and 25% sterile seawater. And then the bacteria were incubated for 24 hours at 30 °C. These bacterial culture was mixed with Na-Alginate solution extracted from *Sargassum* sp. (5%) for *Padina* sp. (10 %) in a ratio (1:3) (bacteria:Na-alginate(v/v)).

The mixture was printed using a syringe 0.1 mL and dripped it drop by drop in a container that already contains a 0.2 M CaCl2 solution for the gelatinization process to form alginate beads (balls). The alginate beads were stored in the refrigerator for 6 hours. After that, the alginate beads were washed with physiological NaCl and put in 150 mL of sewage medium [6].

2.5. Sewage medium assay

Sewage medium is a TAN simulation medium consisting of 13.5 g K_2HPO_4; KH_2PO_4 0.7 g; $MgCl_2.6H_2O$ 0.1 g; $NaHCO_3$ 0.5 g; $FeCl_3.6H_2O$ 0.014 g; $CaCl_2.2H_2O$ 0.18 g; NH_4Cl 0.1 g; EDTA 0.2 g [7]. The using of immobilized *Bacillus coagulans* bacteria 1 – 2 balls/mL into sewage medium. Incubation was carried out at 24˚C using a shaker (100 rpm) for 20 days [8].

2.6. Total ammonia nitrogen (TAN) reduction

Analysis of TAN content was carried out at the beginning and end of the study. The steps in the analysis of TAN content include: standard ammonia solution prepared with a concentration of 0.1; 0.2; 0.4; 0.6; 0.8; 1 mg/L. This standard solution is used to determine the standard curve for ammonia. From each filtered sample, 10 mL was taken, and then 0.5 mL of phenol solution (C_6H_5OH) was added and homogenized. After that, 0.5 ml of 0.5% sodium nitroprusside ($C_5FeN_6Na_2O$) solution and 1 ml of oxidizing solution were added. The oxidizing solution consisted of an alkaline citrate solution ($C_6H_5Na_3O_7$) and 5% sodium hypochlorite (NaClO). The samples were allowed to stand for 1 hour (28–31˚C). The absorbance was measured using a spectrophotometer with 640 nm wavelength. The absorbance value was entered in standard curve formula to determine TAN content in sewage medium [9].

2.7. Statistical Analysis

Statistical analysis was tested with One Way ANOVA using the SPSS 22.0 program to determine the level of difference between treatments. If there was a difference between treatments, then further test of LSD (Least Significant Difference) with 95% confidence interval.

3. Results and Discussion

3.1. Yields

The yield is a percentage between the weight of the final product and the weight of dry samples used during extraction. Determination of yield is useful for knowing the results of Sargassum sp. and Padina sp extraction. The results showed that the yield of Na-Alginate from 100 g Sargassum sp. powder extraction was 19.80%. This value was lower than previous study that it reached 40.34% [10], but it higher than several previous studies 17.39% [11], and 12.88% [12].

Meanwhile, the yield of 100 g of *Padina* sp. extraction reached 14.44%. This value was lower than the results of previous studies, which reached 25% of the dry weight of Padina sp. extracted [13]. The factors that affect the yield of alginate include: type of seaweed used, conditions of seaweed grow place or habitat (light intensity, wave size or current, water-nutrition, etc.), climate, and the extraction method used, as well as how to handle it [14].

In the extraction process, using Na_2CO_3 solvent was considered to separate cellulose and alginate in brown algae cells. The concentration of Na_2CO_3 used during the extraction process also affects the yield produced. A high concentration of Na_2CO_3 can produce more yield [15].

3.2. FTIR (Fourier Transform Infra-Red) Spectra Analysis

The FTIR test was carried out at 4000 – 650 cm-1 wave length on 32 scanned samples and 4 cm-1 resolution. The test was conducted to determine the presence of chemical bonds in organic compounds in Sargassum sp. and Padina sp. extract. The results of functional group analysis were compared with data from various references to confirm the presence of functional groups representing a sodium alginate bond at a certain wavelength (Table 1).

Table 1. Functional group of alginate based on FTIR spectra.

Wavelength (cm^{-1})				
Na-Alginate *Sargassum* sp. Extract	Na-Alginate *Padina* sp. Extract	Na-Alginate Commercial	Functional Group Interpretation	Wavelength Reference (cm^{-1})
3332.2	3272.6	3227.9	Hydroxyl Group (O-H)	3200 – 3500[16]
2937.1	2929.7	2877.5	Aliphatic Group (C-H)	2850 – 3000[16]
1595.3	1595.3	1580.4	COO-asymmetric	1620[17]
1394.0	1401.5	1416.4	COO-symmetric	1410[17]
1028.7	1028.7	1319.5	Manuronic Acid and Carboxyl Group (C-O)	817,82 – 1315[18] and 1000 – 1300[16]
	857.3	1021.3	Guluronic Acid	817,82 – 1029[18]

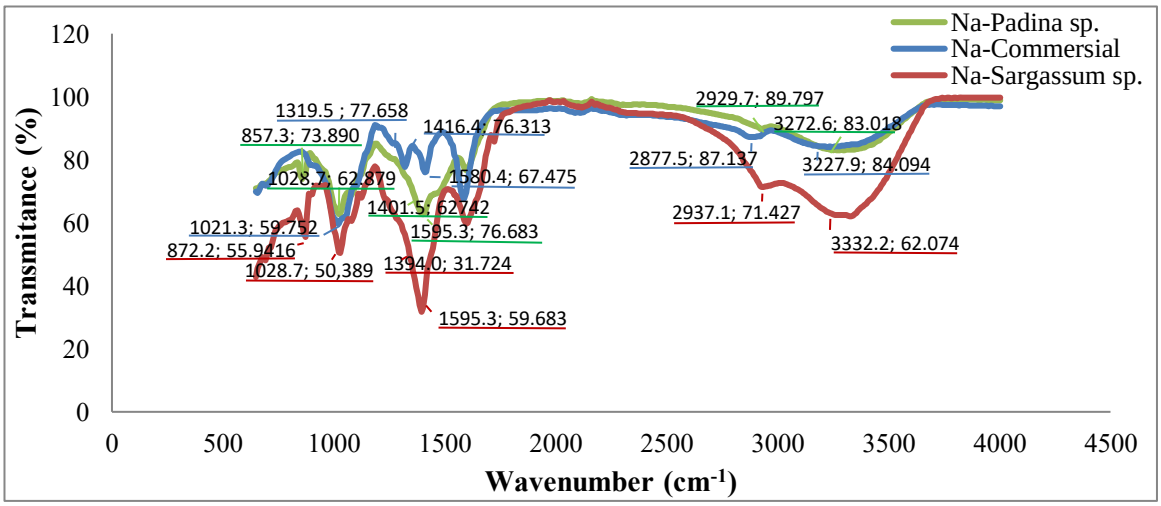

Figure 1. *FTIR spectra of sodium alginate.*

Vibration spectra at 3200 – 3500 cm-1 indicate the presence of a hydroxyl group (O–H) bonded to hydrogen, vibrations at 2850 – 3000 cm-1 indicate the presence of aliphatic compounds, and at 1000 – 1300 cm-1 indicate the presence of a carboxyl group (C– O) [16]. Vibration spectra at 1620 cm-1 indicated the presence of COO-asymmetric and at 1410 cm-1 indicated the presence of COO-symmetric [10]. Vibration spectra at 817.82–1315 cm-1 indicated the presence of mannuronic acid, and at 817.82–1029 cm-1 indicated the presence of guluronic acid [18]. The presence of mannuronic acid and guluronic acid means that the sample contains alginate compounds [19].

*The FTIR test results on samples of Sargassum sp., Padina sp., and commercial alginates (**Figure 1**.) showed slightly different spectral patterns. The sodium alginate extracted from Padina sp. has a lower spectrum pattern than sodium alginate extracted from Sargassum sp. and commercial alginates (**Figure 1**). This happens because other functional groups are also filtered during the sample purification process. However, the extraction results from Sargassum sp. and Padina sp. are still identified as sodium alginate because it has met the standard by having 3 spectral peaks consisting of hydroxyl groups, COO-asymmetric, and COO-symmetric [17].*

3.3. Analysis TAN (Total Ammonia Nitrogen)

Analysis of TAN content was carried out to determine the effectiveness of immobilization of Bacillus coagulans bacteria using sodium alginate from Sargassum sp. and Padina sp. The TAN content at the beginning treatment was 0.45 – 0.48 mg/L, then decreased until the end of the study. The decrease TAN content occurs because Bacillus coagulans bacteria can utilize TAN compounds as metabolism for their growth [3].

*The results showed the utilization of immobilized bacteria Bacillus coagulans with sodium alginate from Sargassum sp. and Padina sp. can increase the activity of bacteria better than free cells. This is known based on the decrease in TAN content in both treatments. Both treatments had a higher reduction in TAN levels than Bacillus coagulans without immobilization (**Figure 2**.). The utilization of immobilized bacteria can absorb nitrogen better than free cells [20].*

*The highest decrease TAN content occurred in immobilization treatment of Bacillus coagulans bacteria using sodium alginate with Sargassum sp. The decrease TAN content in this treatment reached 60% **(Figure 2)**. At the end of study, the results of One Way ANOVA test showed that there were differences in concentration of TAN in each treatment ($\alpha<0.05$). The results of LSD test showed that each treatment had significantly different results. Thus, assumed that immobilization of Bacillus coagulans bacteria using sodium alginate matrix from Sargassum sp. and Padina sp. can to increase activity of these bacteria to reduce TAN significantly.*

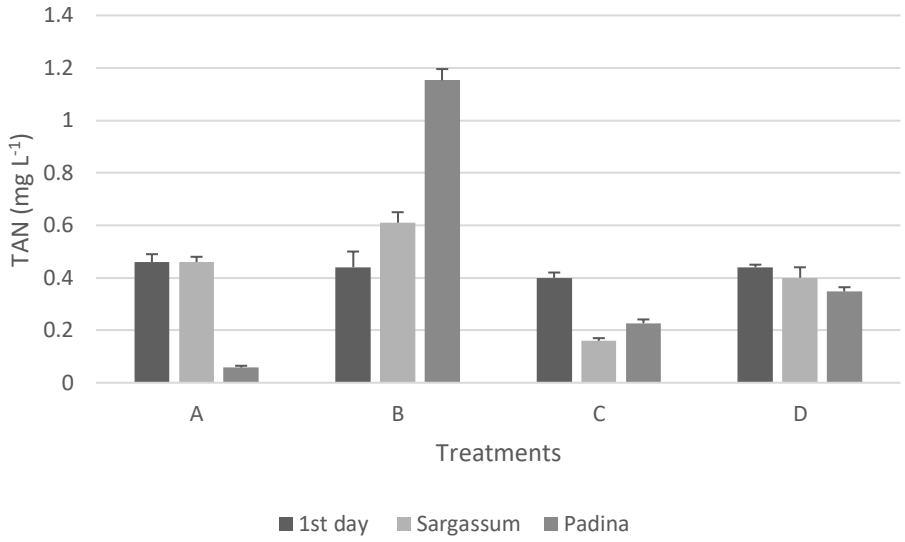

Figure 2. TAN Concentration in Sewage Medium: (A) Control (without adding sodium alginate and bacteria), (B) Adding sodium alginate (C) Adding immobilized bacterial using sodium aginate (D) Adding bacteria without immobilization. The values is average of TAN concentration ± standard deviation (SD).

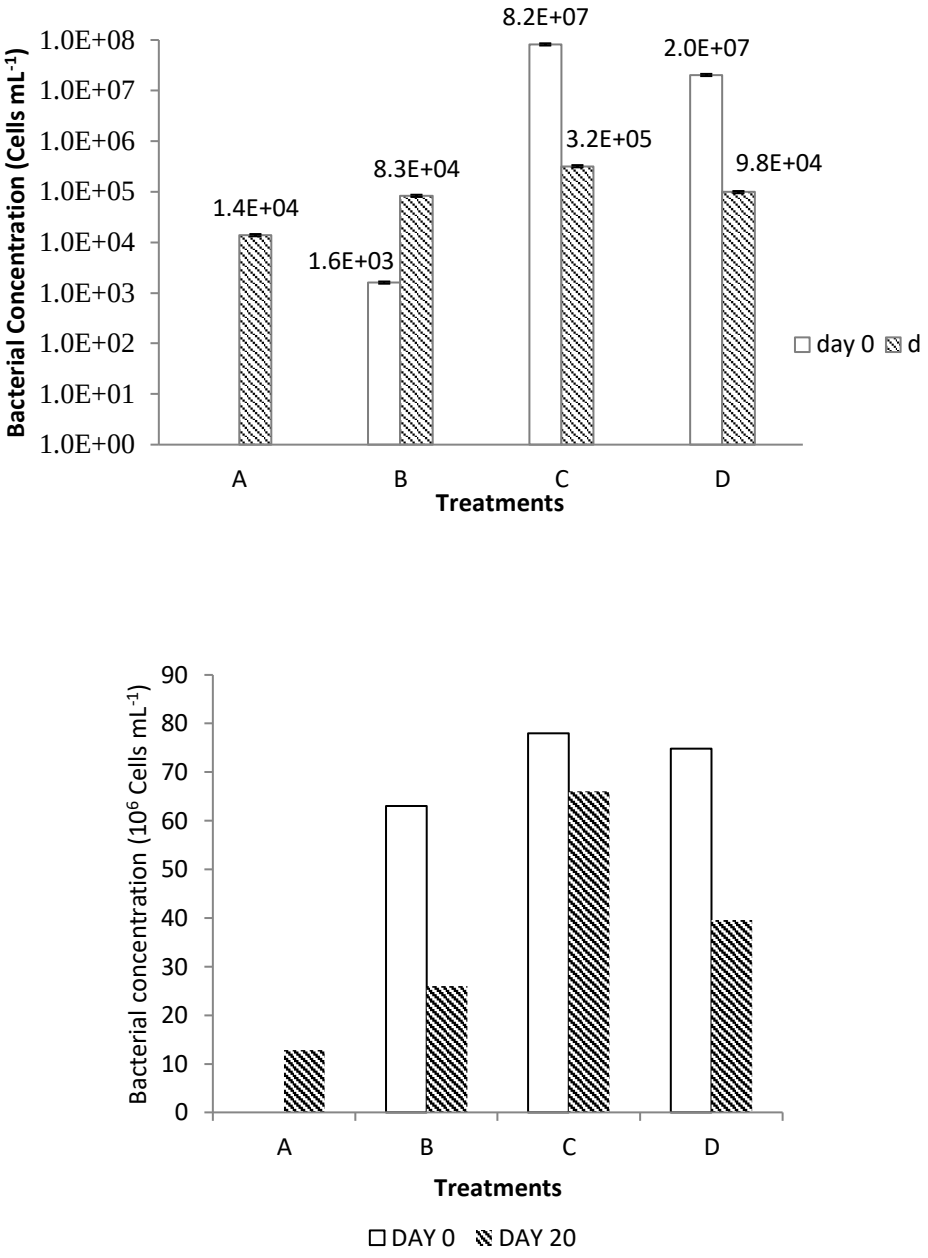

Figure 3. Viability of bacteria in Sewage medium which treated by sodium alginate immobilization matrix from *Sargassum* (A) and *Padina* (B); A: Control, B: Immobilization of *Bacillus coagulans* with Na-*Sargassum* sp., C: Immobilization of *Bacillus coagulans* with Na-*Padina* sp., D: *Bacillus coagulans* without immobilization.

3.4. Bacterial Viability

The bacterial viability test was performed to determine the density of bacteria that grew in sewage medium. This method has counted the colonies that grow on agar medium. The bacterial counts were performed on free cells detached from alginate beads. During the research process, bacteria were released from the alginate beads and counted as free cells. The cells released from the alginate beads

occurred due to the diffusion of nutrients in alginate beads, causing a decrease in density and rigidity of the gel matrix. The use of sodium alginate concentration also affects the strength of matrix protection in holding cells out of the alginate bead [21].

Figure 3 showed that immobilization of *Bacillus coagulans* bacteria using sodium alginate from *Sargassum* sp. had the highest viability and was more stable than other treatments. Meanwhile, in the treatment of immobilized *Bacillus coagulans* using sodium alginate from an extract of *Padina* sp. and *Bacillus coagulans* without immobilization occurred decrease in bacterial viability until the end of the study. The decreased viability of bacteria in a medium can be caused by a reduction source of nutrients in the media, and saturation of the growth of bacteria is achieved [22].

Some factors cause decreased bacterial viability, including availability and amount of bacteria concentration in the samples. Bacterial growth biomass can also be affected by nutrients in the media. The lack of nutrients in the media for the needs of microorganisms causes the metabolism of *Bacillus coagulans* bacteria is not optimal so that the bacterial density decreases [23].

4. Conclusion

Based on the results, it was concluded that the immobilization of endogenous bacterium *Bacillus coagulans* using sodium alginate from *Sargassum* sp. and *Padina* sp. extract effective to reduce the content of Total Ammonia Nitrogen (TAN). The treatment showed that the viability of bacteria was better than Indigenous *Bacillus coagulans* without immobilization.

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