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Research Article

Biostimulation of Indigenous Fungi with Agung Banana Semeru Lumajang Peel Extract in Reducing Ammonia Levels

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Abstract

Indonesia is the second rubber producer in the world, in its processing rubber produces liquid waste that contains quite high ammonia levels. This study aims to provide an alternative solution to the problem of water pollution due to ammonia in rubber industry liquid waste using a bioremediation process with a simple bioreactor. This study used indigenous fungi treatment of 4% and banana peel extract of 0%, 5%, and 10%. This study studies the potential of indigenous fungi with banana peel extract nutrients to reduce ammonia levels in water. The parameter measured is the level of ammonia in the water in the bioreactor. The results showed that indigenous fungi with banana peel extract nutrients had an effect on ammonia parameters. Treatment with a concentration of indigenous fungi 4% and banana peel extract 10% had a significant effect on reducing ammonia levels. The more indigenous fungi inoculum and banana extract used, the more effective it is in reducing ammonia in water.

Keywords*: Ammonia; Banana Agung Semere Peel Extract; Fungi; Indigenous.*

Introduction

Indonesia is one of the countries that has the largest plantations in the world and has the potential to be the largest rubber producer. Indonesia is also the second rubber producer in the world after Thailand according to data from FAO [1], [2]. In its processing, rubber not only produces the desired product, but rubber also produces waste that can damage the environment. The liquid waste of rubber factories is generated from the process of washing, shearing, grinding, sanding, drying, and pressing. Rubber factory liquid waste contains organic matter with high nitrogen levels such as protein, phosphate and ammonia [3], [4].

Waste that is not treated first and directly discharged into the environment is the cause of environmental pollution, one of which is water pollution by ammonia. Rubber waste treatment in Indonesia generally uses activated sludge, anaerobic and facultative ponds which are quite expensive and the treatment only reduces carbon levels but phosphorus and nitrogen compounds are still relatively high [5], so that other techniques are needed in its management. One of the methods used to reduce ammonia levels of rubber factory waste in the aquatic environment, one of which is by using a bioremediation method that has been proven to be more effective [6].

Bioremediation does not only use bacteria, fungi can also decompose some organic compounds in waste that are larger than bacteria. Fungi contain extracellular enzymes that are able to break down complex compounds through nonspecific oxidation reactions [7]. Other advantages of fungi compared to bacteria are the ability to survive in low pH, low temperatures, as well as improve the decomposition of orgnic materials [8]. In the environment of waters that have been polluted with ammonia, there are several indigenlus fungi, namely *Aspergillus sp.*, *Fusarium sp., Penicillium sp.*, and several yeast groups, which have the ability to act as bioremediation agents [5].

Fungi need nutrients to reproduce and in their survival. Although fungi can survive in a state of minimal nutrients, fungi cannot survive for a long time in a state of minimal nutrients and cannot decompose organic compounds such as ammonia to the maximum extent [9]. An alternative in overcoming the lack of nutrients as a factor for fungal growth is by utilizing organic waste from banana peels, one of which is the Agung Semeru Lumajanag banana peel which can effectively reduce ammonia levels in polluted water [9]. Banana peels can be used as an alternative to adding nutrients to indigenous microbes, namely bacteria [10] or culture media for fungi, because it contains 14.4% cellulose which can meet the nutritional needs of fungi [11]. Based on this background, the research on the use of Agung Semeru Lumajang banana peel extract as a nutrient biostimulation agent in indigenous fungi can reduce ammonia levels in polluted waters.

Materials and Methods

1. Tools and Materials

The tools used in this study are petri cups, glass beakers, measuring pipettes, tweezers, stirrers, Bunsen, magnetic stirrer, Erlenmeyer, digital scales, pH meters, ammonia level measuring instruments, autoclaves, and scissors. The materials used in this study are sterile aquatics, PDA, banana peels, and liquid waste from the rubber industry, physiological salts, spirtus, cotton, aluminum foil, markers, label paper, wood paper, and indigenous fungi [12].

2. Sterilization of tools and materials

All tools and materials to be used are washed and dried. The mouthpiece of the pipette, and the Erlenmeyer are covered with cotton then coated with aluminum foil while the petri cup is wrapped in paper. Then the whole appliance is wrapped in wooden paper/brown paper, then sterilized with an autoclave 1 atm, 121ºC for 120 minutes [13].

3. Test Microbial Suspension Manufacturing

The manufacture of the test microbial suspension is carried out with a pour plate. Inoculation of 1 ml of suspension from river water polluted by waste is then put into a petri dish aseptically. The special nitrified media is poured and flattened by rotating on a flat plane to form the number 8 so that the media and suspension are homogeneous, then the media is incubated at a temperature 25^0C for 48 hours [8].

4. Making Agung Semeru Banana Peel Extract Lumajang Variety

The making of the Great Semeru Banana Peel Extract is by way the Lumajang variety Agung Semeru banana peel is cleaned first and then dried using an oven with a temperature of 45ºC for ±3 days, the banana peel that has been curned is blended until smooth, after that it is sifted and then soaked with aquaades. Then weigh as many as 100 grams of Agung Semeru banana peel of the Lumajang variety that has been crushed and then soaked with 300 ml of aquaades, then the beaker glass is closed using aluminum foil and stored in a pending cabinet for 24 hours. The extract obtained after soaking is a thick extract that is ready to use [14].

5. Treatment in bioreactors

500 mL of liquid waste from the rubber factory is placed into a bioreactor that has been prepared in advance [15]. The administration of microbial inoculum given in this study was 4% (20 mL) of the total volume of waste. The determination of the magnitude of the concentration given refers to research that has been conducted that microbes given as much as 4% of the volume of liquid waste are able to degrade ammonia consistently [16]. According to Parapouli *et al* [16], the greater the use of fungal inoculums, the more effective ammonia degradation is. Based on the results of the study, it is stated that inoculum microorganisms can degrade organic waste with a ratio of inoculum and domestic waste, which is 1:4 (v/v) [15]. Agung Semeru Lumajang banana peel extract used as an additional nutrient medium during bioremediation takes place at 5% of the waste volume [17].

Bioreactor Treatment, namely Positive Control (Ammonia); Negative Control (Ammonia waste); P0F4 (0% Banana Peel + 4% Fungi); P5F4 (Banana Peel 5% + Fungi 4%); and P10F4 (Banana Peel 10% + Fungi 4%) with a waste volume in the bioreactor of 500 mL. Measurement of Ammonia content parameters was carried out during mass incubation by following the standard protocol of each method. Ammonia measurements are carried out in situ with an Ammonia meter in the laboratory every 3 days for 15 days. In addition to ammonia parameters, other parameters that were calculated to strengthen the results of this study were pH and Temperature. pH is measured using a pH meter while temperature is measured using a TDS meter.

Results and Discussion

The results of observation of ammonia, pH and temperature levels in river water polluted by rubber factory liquid waste were carried out by a simple bioreactor method in which each bioreactor used treatment with indigenous fungi and Agung Semeru banana peel extract with different concentrations for 15 days.

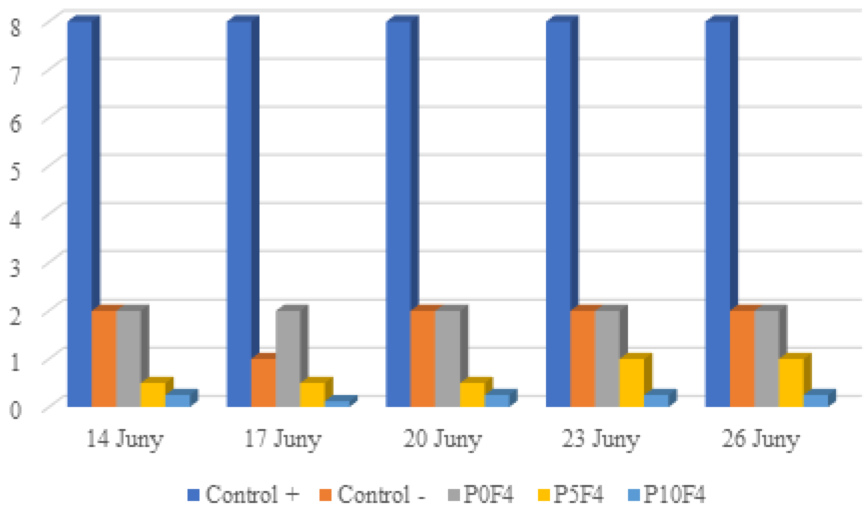

Figure 1. Graph of observation of ammonia levels (ppm) after treatment at various concentrations every 3 days for 15 days.

The results of ammonia observation in figure 1 show that the highest ammonia level is at (+) control of 8 ppm, while the lowest ammonia level is at 0.22 ppm in the P10F4 treatment. The pH observation results in figure 2 show the lowest pH level with a value of 5 - 6 in the P10F4 treatment. The results of temperature observation in figure 8 show the lowest temperature, namely in the P10F4 treatment with a temperature of 26.6 ºC. Ammonia (NH3) is one of the indicators of water pollution (figure 3). If the presence of ammonia in water exceeds the threshold or quality standard of ammonia levels in waters according to Government Regulation No. 82 of 2001, it will be toxic to living things in these waters and will damage the ecosystem in these waters [18].

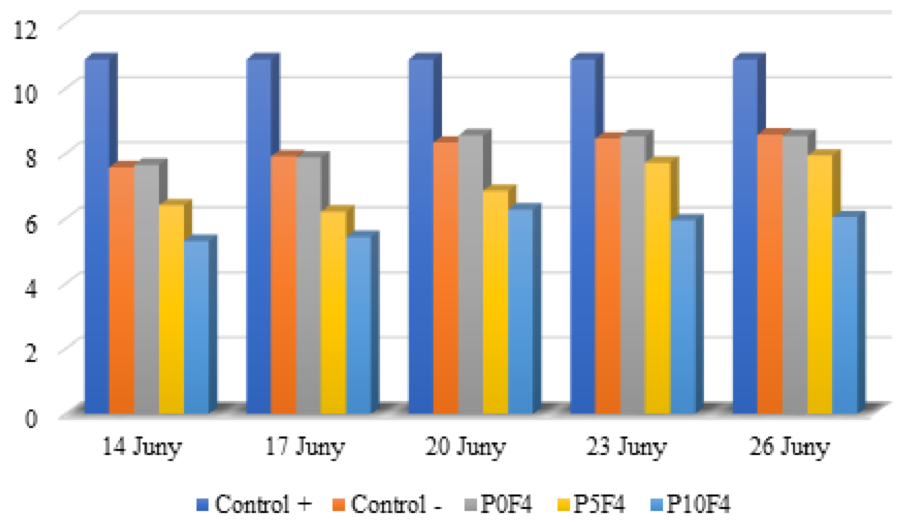

Figure 2. Graph of pH level observation after treatment at various concentrations every 3 days for 15 days.

Data analysis using *Kruskall-Wallis* showed that there was a significant difference between the treatment of indigenous fungi and the addition of Agung Semeru banana peel extract to reduce ammonia levels in river water polluted with liquid waste. The quality standard of ammonia in water is 0.5 ppm, based on the observation results of ammonia levels in river water polluted by liquid waste from rubber factories shows that the ammonia level exceeds the quality standard. If the level of ammonia in the water increases, it will be corrosive and toxic which is likely to have a bad impact on the aquatic environment [18]. The high level of ammonia in the river water is due to the liquid waste of rubber factories that are flowed into the river [19]. The Kruskl-Wallis statistical test shows the results of the Asymp Value. Sig. = 0.000 < 0.05 which means that there is a significant difference between treatments (Control (+) (-), P0F4, P5F4, P10F4) on the decrease in ammonia levels in river water contaminated with rubber factory liquid waste in a simple bioreactor. The results of Duncan's test showed which treatment groups had significant differences

Based on the results of Duncan's test (table 1), the decrease in ammonia levels in Control (+), Control (-), P0F4, P5F4, and P10F4 was significantly different except that Control (-) and P0F4 did not have a significant difference or no significant difference. This is due to the lack of nutrients in the fungi, in the P0F4 treatment the bioreactor only contains indigenous fungi with river water that is polluted with waste without Agung Semeru banana peel extract as an additional nutrient and cannot reduce ammonia levels optimally so that the data does not differ significantly.

Fungi that lack nutrients will cause stunted growth and are not optimal in decomposing organic compounds [20]. pH is one of the indicators of water that is interrelated with ammonia levels. The pH level is inversely proportional to the ammonia level, the higher the pH level (the more alkaline) in the waters, the lower the ammonia level in the waters [21]. This is one of the causes of different ammonia and pH levels in each treatment in the bioreactor. The higher the pH (the more alkaline) in the bioreactor proves that there is a decrease in ammonia levels in the bioreactor.

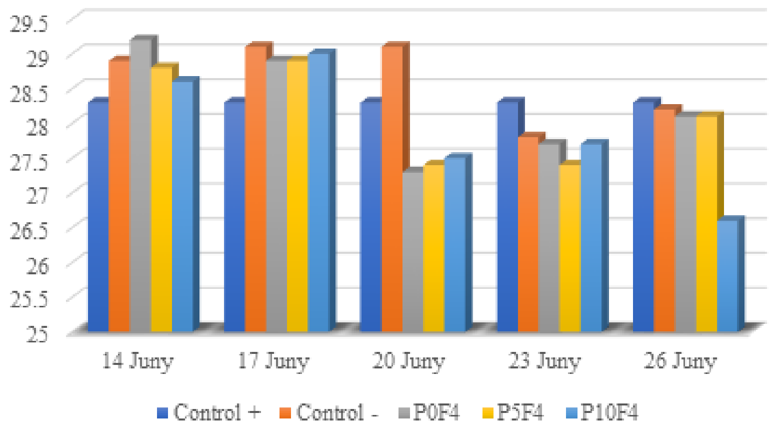

Figure 3. Observation graph of Temperature (°C) levels after treatment at various concentrations every 3 days for 15 days.

The results of the ammonia level reduction test using indigenous fungi with the addition of Agung Semeru banana peel extract as a nutrient can reduce the ammonia level of river water polluted by the liquid waste of rubber factories in the bioreactor. This can be seen from the lowest ammonia level, which is at the P10F4 concentration with an average ammonia level of 0.22 ppm, which means that the ammonia level of river water in the bioreactor is below the water ammonia quality standard. This is in accordance with the research of Parapouli *et al* [16], which researched on the reduction of ammonia levels in the rubber industry using *the culture of Saccharomyces Cerevisiae ATCC9763* which is known to reduce ammonia levels in the rubber industry. This happens because fungi have the ability to make organic compounds as nutrients for metabolism and survival. Indigenous fungi have the ability to degrade organic compounds in their environment [22].

Table 1. Average ammonia levels in various treatments

Bioreactor Treatment	Ammonia content (ppm)
Control +	8.00±0.00A
Control-	1.83 ± 0.40b
P0F4	2.00 ± 0.00b
P5F4	0.75 ± 0.27c
P10F4	0.22 ± 0.05d

Description: P= Banana peel extract, F= Fungi indigenous. a,b,c,d

In the process of decomposing organic compounds such as ammonia, fungi need sufficient nutrients for their survival [23]. Agung Semeru banana peel extract is one of the alternatives to provide the nutrients needed by fungi so that they are able to decompose ammonia in the environment. Agung Semeru banana peel contains calories, carbohydrates, protein fats, phosphorus, potassium fiber, vitamin A, vitamin B, glucose, cellulose and water which can help meet the nutritional needs of fungi [24].

Based on the results of the study, it was shown that the concentration of Agung Semeru banana peel extract of 10% had the lowest ammonia content with an average of 0.22 ppm which means that fungi at the concentration of P10F4 work optimally in degrading ammonia in the bioreactor, this is because the nutritional needs of fungi are met by the presence of banana peel extract [25], this is confirmed by the research of Dewi *et al* [19] that fungi can reduce ammonia levels in water. This is also strengthened by the research of Sari and Anitasari [5], that fungi can degrade ammonia if their nutritional needs are met. This proves that the hypothesis prepared is proven to be correct.

Several supporting parameters to strengthen the results of this study that were measured during the study were pH. In addition to ammonia, pH also has an effect on waters, one of which is on biochemical processes in waters, if the pH changes, it will inhibit the nitrification process. pH also determines the development of fungal growth, the optimal pH for fungal growth is 5, 6 and 7, if the pH is below 5, the growth of fungi will be inhibited and cause a decrease in pigment production in fungi [26]. The results of the measurement of supporting parameters showed the pH value obtained in the range of 5 - 8. This value shows that the pH of the water in the bioreactor is below the quality standard value in Government Regulation No. 82 of 2001, which is 6 - 9. From these results, fungi can grow optimally at a concentration of P10F4 with a pH of 5.80 which has a Total Plate Count (TPC) or the highest number of colonies, which is ± 402 colonies and water in the bioreactor can be used because the pH still meets the quality standards.

The results of measuring other supporting parameters, namely temperature, during the study obtained temperature values ranging from 27.8 – 28.24ºC. This value shows that the water temperature is in optimal conditions for fungal growth. Counter *et al* [26], stated that the optimum temperature for fungal growth is 28ºC. Temperature is one of the factors that affect cell metabolism, if the temperature increases, it can cause cell damage and inhibit the work of enzymes so that it affects the growth of fungi [26].

Conclusion

Treatment with a concentration of indigenous fungi 4% and banana peel extract 10% had a significant effect on reducing ammonia levels. The more indigenous fungi inoculum and banana extract used, the more effective it is in reducing ammonia in water.

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