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Research Article

The Effect of Agung Semeru Banana Peel Extract as Biostimulation of Indigenous Bacteria in Reducing Ammonia

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ABSTRACT

The purpose of this study was to determine the effectiveness of the agung semeru banana peel extract as additional nutrition for indigenous bacteria in reducing ammonia levels. This research was conducted in the Biology laboratory, PGRI Argopuro University, Jember. This study uses a simple bioreactor as a tool to determine the decrease in ammonia levels in various concentrations of banana peel extracts (0%, 5%, 10%). Based on the analysis of research data, it showed that Agung Semeru banana peel extract as an additional nutrient for indigenous bacteria was effective in reducing ammonia levels. At a concentration of 10% banana peel extract, the lowest ammonia level was in the range of 0.25-0.5 compared to the control concentration. The conclusion of this study is that a higher concentration of banana peel extract can reduce the ammonia content in water contaminated with rubber waste.

Key words: *Banana Peel Extract; Biostimulation; Indegenous Bacteria; Reducing Ammonia*

Introduction

Indonesia is one of the second largest rubber producing countries in the world. This country has the largest rubber plantation in the world, with a total area of 3.6 million hectares (ha) with rubber production reaching 3.6 million tons per year (Andriani et al., 2019).

Large rubber production has an impact on the generation of waste which is a by-product of industrial plants that cannot be recycled, and if this waste continues to accumulate in the environment, it will have an impact on nature and public health Jember is one of the industrial rubber factories which also produces harmful waste for the surrounding community (Hatta, 2020; Hamimed & Kthiri, 2021). Waste treatment that is still not effective is a major problem in rubber-producing countries in Indonesia itself, many complaints from residents because liquid rubber waste is directly dumped into the river so that, the ecosystem around the river flow which has an impact on water and air pollution (Yasin, 2018).

The method most recommended by BLH worldwide is bioremediation because bacteria can live even in extreme conditions and do not cause side effects to the environment (Wardani & Santoso, 2015). According to Suryani's research (Suryani, 2011), it is stated that indigenous bacteria found in the waste can reduce hazardous

content in waste, this is also supported which states that indigenous bacteria found in rubber waste are able to reduce ammonia. contained in rubber liquid waste (Nainggolan et al., 2015), because there is an organic substrate in rubber liquid waste (Yasid, 2014).

To provide good nutrition to bacteria in this study, researchers will use banana peel extract as a nutrient because banana peels are organic waste that is widely available in the environment and people still have not utilized it optimally (Febriyanti & Kusnadi, 2015). Banana peel itself is in the form of water, cellulose, fat, calcium, phosphorus, protein, iron, B vitamins, and vitamin C (Rosmania & Yanti, 2020).

Until now, there is still no research that shows the results of giving agung Semeru banana peel extract as a nutrient for indigenous bacteria to reduce ammonia levels, which are then used as a learning resource in microbiology courses. So, in this study, the researchers wanted to test whether the administration of nutrients from the extract of Semeru banana peel extract could help indigenous bacteria in reducing ammonia levels in rubber liquid waste.

Materials and Methods

1. Tools and Materials

In this study, the tools used were petri dishes, glass beaker, measuring pipette, Bunsen, pH meter, autoclave, TDS, ammonia tester and simple bioreactor. While the materials used in this study were sterile aquadest, *Nutrient Agar* (NA), industrial rubber liquid waste, spirit, cotton, aluminum foil, markers, label paper, wood paper.

2. Sterilization of Tools and Materials

The first step is wrapping the tool, the petri dish and measuring pipette are wrapped using newsprint, a beaker glass filled with NA media, and then wrapped by closing the hole using cotton then coating it with aluminum foil, then putting it into an autoclave, sterilization of tools and materials is carried out at a temperature of 121°C with time 15 minutes (Wiegand et al., 2008).

3. Preparation of *Nutrient Agar* (NA)

Nutrient Agar (NA) media as much as 2.8gr using a digital scale, dissolve 2.8gr of NA powder into 100ml of distilled water using a magnetic stirrer, put the NA solution into a beaker then cover with cotton and aluminum foil, then put it in an autoclave for sterilization with temperature of 120oC to avoid microbes that grow, then prepare a Bunsen fire and a sterilized petri dish, then put 5 ml of sterile NA solution into a sterilized petri dish near a bunsen fire to avoid contamination (Wiegand et al., 2008).

4. Isolation of Microbes Indigenous

Prepare a petri dish containing 5 ml of NA media near a bunsen fire, take 1 ml of a liquid waste sample that has gone through a 10-1 dilution using a measuring pipette then open the petri dish using your left hand near a bunsen fire then quickly enter 1 ml of liquid waste sample, close the petri dish then stir by turning the petri dish like number 8 after finishing the petri dish tape so it is not contaminated with other bacteria.

5. Preparation of Bacterial Suspension

Bacteria that have been grown in NA media for 1x24 hours at room temperature are then dissolved in 0.9 % NaCl solution (Saline) then 100 l of dissolved bacteria are pipetted, 100 l of bacteria that have been pipetted are added to 10 ml of nutrient both so that a bacterial suspension of 1-2 X 106 CFU/ml was obtained. The turbidity level of the test bacterial suspension was compared with the standard 0.5 Mcfarland (Doraja et al., 2012).

6. Procedure for Semeru Lumajang Banana Peel Extract

The peel of the Semeru banana is first cleaned using running water so that there is no dirt attached, then dried by airing and then in the oven at 45°C for 5 consecutive days. Samples that have been in the oven for 5 days are then finely blended until they become powder, the resulting powder is sieved using a 60 mesh sieve to obtain a smooth and uniform simplicia powder.

7. Treatment in The Bioreactor

Rubber liquid waste is placed into a bioreactor that has repared previously as much as 500 mL of waste (Suriani et al., 2013). The microbial inoculum given in this study was 5% (25 mL) of the total volume of waste.

For each bioreactor consists of a mixture of rubber factory waste, liquid extract of Agung Semeru Lumajang banana peel, microbial inoculum which is filled into each reactor, with details: A negative control containing 500 mL rubber factory liquid waste without giving Agung Semeru Lumajang banana peel extract or microbial inoculum; Treatment 1: bioreactor containing microbial inoculum at a concentration of 5 % of the waste volume + 500 mL rubber factory liquid waste; Treatment 2: bioreactor containing microbial inoculum at a concentration of 5 % of the waste volume + Agung Semeru Lumajang banana peel extract 5 % of the waste volume + 500 mL rubber factory liquid waste; and Treatment 3: bioreactor containing microbial inoculum at a concentration of 5 % of the waste volume + Agung Semeru Lumajang banana peel extract 10 % of the waste volume + 500 mL rubber factory liquid waste.

8. Data Analysis

Ammonia and pH parameters were measured during the incubation mass of 5 days. Ammonia and pH levels were measured in the laboratory every 3 days for 18 days. Ammonia levels were measured at the beginning, middle, and end of treatment. Data previously will be tested using homogeneity, normality, then continued to Kruskall Wallis test at 95 % (α = 0,05) using SPSS version 23.

Results and Discussion

Kruskal Wallis test is an alternative test used by researchers if the data they get is not normally distributed or not homogeneous, the Kruskal Wallis test is used to see if there is a difference in each treatment given. Based on the Kruskal Wallis test that has been carried out, the significance value is 0.01 < 0.05. This shows that the use of Agung Semeru banana peel extract as an additional nutrient for indigenous bacteria has an effect on reducing ammonia levels in wastewater.

Based on the observations that have been made for 15 days after the treatment of ammonia levels, pH, and temperature of the rubber factory wastewater, the results of these observations can be seen in table 1, table 2, and table 3.

In the observation of ammonia (table 1) the P10B5 treatment showed that the ammonia level was less than the control treatment and the ammonia level increased on June 20, 2021 and then fell again on June 23, 2021, while in the P0B5 treatment, the ammonia level in each observation did not change the same once since the beginning of the observation.

Table 1. Average Ammonia levels at various concentrations

Treatment	Average Ammonia Levels (Observation Day to-)				
	3	6	9	12	15
Control (+)	8	8	8	8	8
Control (-)	1,33	1	1,83	1,83	1,83
P0B5	2	2	2	2	2
P5B5	0,83	0,5	0,29	1	1
P10B5	0,5	0,25	0,37	0,2	0,25

Description: Control- (Rubber factory liquid waste), Control+ (pure ammonia), P0B5 (Rubber factory liquid waste with the addition of 5% Indigenous Bacteria), P5B5 (Rubber factory liquid waste with the addition of 5% banana peel extract and 5% Indigenous Bacteria), P10B5 (Waste of rubber factory with the addition of 10% banana peel extract and 5% Indigenous Bacteria).

In the observation of temperature (table 3) the values displayed are different for each treatment. The P0B5 treatment showed an increase at the beginning of the observation until the 9 th day, but on the 12th day the temperature value decreased and increased again on the 15th day. In the P5B5 treatment, the temperature increase can be seen on days 3 - 6, but it decreases on days 9. At the last observation (day 15) there was an increase in temperature. The P10B5 treatment for the 3-6 th day observation was the same as the P5B5 treatment, but there was a decrease on the 12th day. This treatment increased in the last observation.

In observing the pH, for the P0B5 and P5B5 treatments it shows that there was an increase in pH on each day of observation. On treatment P1B5 showed an increase until the 9 th days of observation, and it was on treatment P10B5 showed an increase until the 9th day of observation, and it was seen starting to decrease on the 12th and 15th days (table 2).

Table 2. Average pH at various concentrations

Treatment	Average Ammonia Levels (Observation Day to-)				
	3	6	9	12	15
Control (+)	10,9	10,9	10,9	10,9	10,9
Control (-)	7,57	7,92	8,34	8,47	8,58
P0B5	7,53	7,79	8,34	8,51	8,54
P5B5	6,41	6,42	6,58	7,1	7,48
P10B5	5,42	5,69	6,8	6,31	6,1

Description: Control- (Rubber factory liquid waste), Control+ (pure ammonia), P0B5 (Rubber factory liquid waste with the addition of 5% Indigenous Bacteria), P5B5 (Rubber factory liquid waste with the addition of 5% banana peel extract and 5% Indigenous Bacteria), P10B5 (Waste of rubber factory with the addition of 10% banana peel extract and 5% Indigenous Bacteria).

The addition of Agung Semeru Lumajang banana peel extract, as much as 0 ml (P0B5), 25 ml (P5B5), and 50 ml (P10B5) (Table 4) had an effect on indigenous bacteria to reduce ammonia levels contained in liquid waste. This is because indigenous bacteria are able to act as bioremediation agents to reduce the ammonia content in these compounds. This research is supported by research conducted by Suryani (2011), which states that indigenous bacteria found in the waste can reduce the hazardous content of the waste (Lizayana et al., 2016).

In addition, the results of this study are supported by research by Nainggolan et al (2015), that indigenous bacteria found in rubber waste are able to reduce ammonia contained in rubber liquid waste, due to the presence of organic substrates in rubber liquid waste (Nainggolan et al., 2015). In the control treatment (-) and P0B5 there was no significant difference, but the mean results showed that there was a difference with the P5B5 and P10B15 treatments. Meanwhile, the P0B5 treatment showed a significant difference when compared to the control (+), P5B5 and P10B5.

Based on observations on the bioreactor, the P10B5 treatment seemed to contain more banana peel extract than the other treatments, causing the P10B5 bioreactor to be cloudier than the other treatments. The turbidity can be used as a parameter that the nutrient content contained in the P10B5 treatment is higher than the other treatments. This result is supported by research that in liquid waste that has high turbidity, more bacteria will grow (Retnaningdyah et al., 2009). High levels of turbidity have many deposits that are useful as nutrients for bacteria. The content contained in banana peel extract which is the main nutrient that bacteria can grow well is cellulose.

Table 3. Average temperatures at various concentrations

Treatment	Average Ammonia Levels (Observation Day to-)				
	3	6	9	12	15
Control (+)	28,2	28,3	28,3	28,3	28,3
Control (-)	28,9	29,1	29,1	27,8	28,2
P0B5	27,7	28,6	27,3	27,7	28,2
P5B5	27,6	28,5	27,3	27,4	28,4
P10B5	28,5	28,8	29,2	27,5	29,2

Description: Control- (Rubber factory liquid waste), Control+ (pure ammonia), P0B5 (Rubber factory liquid waste with the addition of 5% Indigenous Bacteria), P5B5 (Rubber factory liquid waste with the addition of 5% banana peel extract and 5% Indigenous Bacteria), P10B5 (Waste of rubber factory with the addition of 10% banana peel extract and 5% Indigenous Bacteria).

Table 4. Means of ammonia levels, in various treatments

Amonia Levels	K+	K -	P0B5	P5B5	P10B5
	$8,00 \pm 0,00^{d}$	$1,83 \pm 0,40^{c}$	$2,00 \pm 0,00^{c}$	$1,00 \pm 0,00^{b}$	$0,25 \pm 0,00^{a}$

Description: K = Control; P0B5 (Rubber factory liquid waste with the addition of 5% Indigenous Bacteria), P5B5 (Rubber factory liquid waste with the addition of 5% banana peel extract and 5% Indigenous Bacteria), P10B5 (Waste of rubber factory with the addition of 10% banana peel extract and 5% Indigenous Bacteria).

Ammonia levels during observations with Control (-) treatment of 1.83, P0B5 of 2, P5B5 of 1, P10B5 of 0.25, and Control (+) of 8. The decrease in high ammonia levels can be seen in the P10B5 treatment. because the indigenous bacteria that can reduce ammonia get good nutrition due to the provision of 50 ml of nutrients to the microbial inoculant, at the end of the observation there was an increase in ammonia levels in each treatment, except in the P0B5 treatment. The increase in ammonia levels at the end of the treatment can occur because the indigenous bacteria contained in the bioreactor can reduce nitrite to ammonia. The increase in ammonia levels in water is caused by the presence of nitrate-reducing bacteria that can reduce nitrate to ammonia (Kiding et al., 2015).

This temperature is the optimum temperature for bacterial growth which is generally found in bioremediation bacteria. This is reinforced by the results of research conducted by Kiding, that the growth of ammonia-reducing bacteria will be optimum at a temperature of 30°C (Kiding et al., 2015). Another factor that affects bacteria besides nutrition and temperature is the pH in which the bacteria live. This is supported by the research of Suriani *et al.*, which said that the minimum and maximum pH values for the average bacterial growth ranged from 6-9 (Lizayana *et al.*, 2016).

Conclusion

Based on the results of this study, Agung Semeru Lumajang banana peel extract, as an additional nutrient for indigenous bacteria, has an effect on reducing ammonia levels in rubber factory wastewater. Treatment P10B5 (0.25 ± 0.00^{a}) showed better results than the other treatments.

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