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Research Article
Ability of Indigenous Microbial Consortium in the Process of Ammonia Oxidation of Livestock Waste

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Abstract

Background: This study was performed to determine ammonia oxidation ability of the livestock farm waste by a microbial consortium and to reduce the amount of ammonia gas from the manure. **Materials and Methods:** The study consisted of five treatments: Without the addition of isolates as control, the addition of *Pseudomonas* sp., LS3K, the addition of *Candida* sp., LS3T, the addition of *Arthrobacter* sp., LM1KK and the addition of microbial consortium. The observed parameters consisted of microbial growth and the oxidation ability of the microbial consortium to minimize the concentration of ammonia. The obtained data were analyzed using one-way analysis of variance and the mean differences between treatments were tested by Duncan's Multiple Range Test (DMRT). **Results:** The results showed that determined by Optical Density (OD), the microbial consortium was capable of growing with the addition of more than 10% ammonia with a value of maximum growth (μ max) 4.02 h^{-1}. The number of colonies that grew on the addition of 0, 2.50, 5, 7.50 and 10% $(NH_4)_2SO_4$ were 1.03×10^5, 0.94×10^5, 1.60×10^5, 0.82×10^5 and 0.86×10^5 CFU mL^{-1}, respectively. The reduction of the ammonia concentration in the liquid medium with $(NH_4)_2SO_4$ was 15.37 ppm. Ammonia emission from the chicken excreta, dairy cows feces and beef cattle feces was lower after the treatment with the addition of microbial consortium which was 28.72, 71.47 and 56.50 ppm, respectively compared than that of the control. **Conclusion:** The result can be concluded that the ability of oxidation by the microbial consortium was the most optimal in the oxidizing ammonia in beef cattle feces. The capability oxidizing of microbial consortium can reduce as much as 15.34 ppm concentration of ammonia in beef cattle feces.

Key words: Ammonia emission, ammonia oxidation, *Arthrobacter* spp., *Candida* spp., livestock waste, microbial consortium, nitrification-denitrification, *Pseudomonas* spp.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Livestock farm produced animal by-product in the form of waste either solid, liquid or gas. However, feces, excreta and livestock waste at farms just were buried away on the ground and they were lived a long time there. Deposition of livestock waste has the potential for air pollutions and it also causes nitrate pollution in rainwater that would lead to acid rain[1].

Major cases of environmental pollutions caused by farm businesses were from the content of NH_3 that accumulated in animal manure. In the case of high humidity and low temperature conditions, synthesis of ammonia gas increased and large quantities of NH_3 gas was emitted from manure[2]. Moreover, it was known that ammonia has high toxicity to all living things, for example, LD_{50} for fish is in the range of 0.20-2 mg L^{-1} and the concentration of 0.20-9 mg L^{-1} for higher organisms. In the past decade, several countries in Europe and America have discussed for their solutions in order to overcome the ill effects of farm waste, in particular, ammonia gas (NH_3) emissions from agriculture-livestock activities[1] and it has been expected to develop treatment process of reducing ammonia from livestock wastes.

Some microbes could oxidize ammonia existing environment and several researchers have tried to screen ammonia-oxidizing microbes in order to develop treatment process of reducing ammonia from livestock wastes using the microbes[3]. Until now, it has been reported some strains belonged to *Nitrosospira tenuis*, *Nitrosomonas marina*, *Nitrosomonas eutropha* and *Nitrosococcus mobilis* as ammonia-oxidizing microbes in aquaria[4]. Moreover, environmentally-friendly handling of the NH_3 gas oxidation process has been carried out by using of commercial bacterial EM^5_4. However, there was a few report described indigenous microbes in Indonesia for the ammonia waste oxidizing and it has not been constructed NH_3 gas oxidation system using indigenous microbes in Indonesia in the farm wastes.

Until now, it already isolated several indigenous ammonia-reducing microbes, *Pseudomonas* sp., LS3K, *Candida* sp., LS3T and *Arthrobacter* sp., LM1KK. Those strains have been proven with specific capabilities to oxidize high concentration of ammonium (NH_4OH) in the wastes. For example, on the addition of high ammonium stressor, *Pseudomonas* sp., LS3K is still able to grow optimally, *Candida* sp., LS3T can grow by utilizing excess ammonium stressor and *Arthrobacter* sp. LM1KK was able to grow optimally utilizing high ammonium concentrations as nutrient growth. It assumed that one alternative effort to reduce air pollution by waste ammonia can be overcome by the oxidation of ammonia using not only each strain, *Pseudomonas* sp., LS3K, *Candida* sp., LS3T and *Arthrobacter* sp., LM1KK, but also the microbial consortium consisting of these strains. In this study, it indicated that the indigenous microbial consortium of ammonia-oxidizing microbes was expected to break down farm waste efficiently and in a friendly way for the environment.

MATERIALS AND METHODS

Cultivation and research design: Excreta was taken from the hen house layer of the Universitas Gadjah Mada (UGM). The feces of the dairy cow was taken from the stables of dairy cows in UPT Dairy Farm Faculty of Animal Science. Feces of beef cattle was taken from the shed of the fattening cow of each 100 g and then it was diluted with distilled water to a ratio of 1:1 and stirred to homogeny. Manure was divided into five erlenmeyer. Each erlenmeyer was added with microbial isolates of *Pseudomonas* sp., LS3K, *Candida* sp., LS3T, *Arthrobacter* sp., LM1KK and as much as 1% consortium as well as the control treatment without the addition of microbial isolates. Each treatment of each manure was cultivated for 5 days and research was repeated 3 times.

Measurement of ammonia concentration in the culture medium: Steps were performed by adding the isolates at 1 mL pre-culture in a liquid medium that has been added to the 5% $(NH_4)_2SO_4$ and then shaker was cultivated at 120 rpm. In every day, 1 mL samples were taken at every 4 h to measure the optical density by putting in an eppendorf tube and were continued by centrifugation at the speed of 8000 rpm at 4°C for 10 min. After the cells had been separated from the supernatant and the precipitate formed, the supernatant was taken and put into the sterile eppendorf tube and added with 0.20 mL nessler reagent and allowed to stand for 10 min. Ammonia concentration of samples was measured using the spectrophotometer UV-1601 PC Shimadzu and recorded to the results. When the nessler reagent reacts with ammonium in an alkaline solution, it will form colloidal dispersions yellow-brown. The intensity of the color that will happen is in line to the concentration of ammonium. Color formed spectrophotometer was measured at a wavelength of 425 nm.

Measurement of ammonia gas emission in manure (excreta, feces of dairy cow, beef cattle feces): The total amount of 100 g manure samples from each treatment was put into the 500 mL erlenmeyer. The ammonia gas emitted from manure was driven by air from the constant speed aerator through the plastic hose into the other erlenmeyer

which contained 200 mL of 0.02 N boric acids to capture the NH_3 gas. The ammonia gas was dissolved in water and formed some ammonium ions. Furthermore, boric acid has the capability to maintain the low pH for the maximum absorption of ammonia gas. Periodically the ammonium concentration was measured spectroscopically using nestler reagent. The nessler solution will produce yellow to reddish brown when reacting with ammonium. The color formed was measured by a spectrophotometer absorbance at the wavelength of 425 nm.

Data analysis: The significance of differences between means of the data was statistically calculated by one-way analysis of variance (ANOVA) followed by Duncan's new multiple range test. The difference between means was considered significant when $p<0.05$. The obtained data were expressed as means±standard errors.

RESULTS AND DISCUSSION

Bacterial consortium has an ability to grow under high $(NH_4)_2SO_4$ stressor: The growth of the bacterial consortium in the liquid medium was observed by optical density (Fig. 1).The bacterial consortium can grow well on the medium under the ammonium stress until a dosage of 10%, although they slightly showed growth defect under stress. This growth of microbes could be due to that microbial consortium was included in heterotrophic microbial species, that can utilize the source of nutrients from an inorganic material or organic compounds. The heterotrophic microbes have the ability for rapid growth in oxidized ammonium and can reach a long period of life[6]. The medium used in optical density observation contained 2.5-10% ammonium sulfate in each erlenmeyer flask and it was used as an inorganic nitrogen stress or which contained at 100% stock solution (the stock solution consists of 1 g meat extract, 1 g bacteriological peptone and 0.5 g NaCl diluted in 100 mL aquadest). The *Pseudomonas* played a major role in the process of nitrification and denitrification in aerobic conditions[6]. Currently aerobic conditions, *Pseudomonas* can oxidize ammonia to nitrite, nitrate and directly degrading nitrate into N_2O and/or N_2. *Candida* spp., was an organism that can oxidize ammonium to nitrate[7]. The *Arthrobacter* strains are the predominant bacteria found in the soil[8]. This microbial life in the soil that contained ammonium and it grew by degradation ammonium so that it has the capability of nitrification.

The result of the maximum growth of microbial consortium based on the absorbance values of optical density was equal to 4.02 h^{-1} with Ks value (saturation constant) of 0.29 mg L^{-1}. The rate of microbial growth would be equal to the concentration of substrate in the medium[9]. The growth of bacteria will reach maximum stages in the high concentration of substrate. However, in a high dose of nitrogen in the medium, many of bacteria strain will be eliminated due to the poison of high concentration of nitrogen. Fortunately, all strain in the consortium could encounter the nitrogen stressor at the level of 10%.

Growth of bacterial consortium in liquid medium: From three replications of research which has performed, the number of colonies after 24 h of cultivation period from each treatment with additional of 0, 2.50, 5, 7.50 and 10% $(NH_4)_2SO_4$ was observed 1.03×10^5, 0.94×10^5, 1.60×10^5, 0.82×10^5 and 0.86×10^5 CFU mL^{-1}, respectively (value of the number colonies is shown in Table 1).

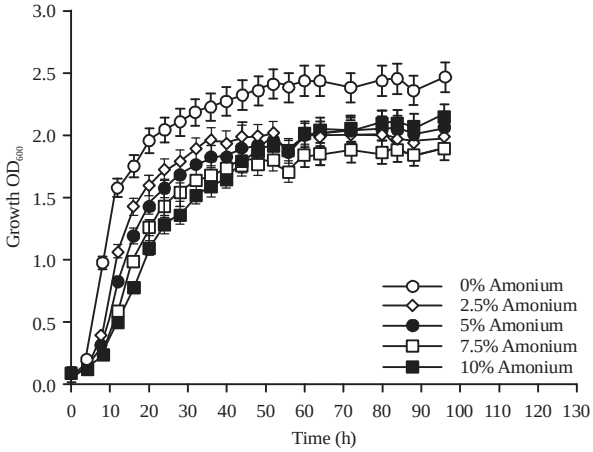

Fig. 1: Growth of the consortium bacteria in 1/100 meat extract liquid medium with the various addition of $(NH_4)_2SO_4$as inorganic N stressor. The growth was observed by Optical Density (OD_{600nm})

Table 1: Growth of the consortium bacteria in 1/100 meat extract solid medium with various stressor of $(NH_4)_2SO_4$. The growth is expressed in the number of colonies appeared in petri dish agar medium (10^5 CFU mL^{-1})

$(NH_4)_2SO_4$ (%)	No. of colonies
0	1.03±0.08[a]
2.50	0.94±0.43[a]
5	1.60±0.24[b]
7.50	0.82±0.20[a]
10	0.86±0.29[a]

[ab]Different superscripts in the same row shows a significantly different effect ($p<0.05$)

The addition of 5% $(NH_4)_2SO_4$ has given a significantly higher in the colony number ($p<0.05$) as an indication that the growth of the strains is better compared to the treatment with the addition 0 and 10% $(NH_4)_2SO_4$. The number of colonies that grew in the addition of 2.50 and 5% $(NH_4)_2SO_4$ was not shown a different effect to the treatment with the addition of 0 and 10%. The growth of consortium microbes with the addition of 5% $(NH_4)_2SO_4$ showed the best result and faster acceleration compared to the other treatments. It was suggested that the optimal condition of consortium microbes for growing with the additional of an organic nitrogen supplementation was at dosage 5% and the growth will decrease when the addition concentration of $(NH_4)_2SO_4$ was more than 5% (Table 1).

The number of colonies appeared in the solid agar medium after 24 and 48 h cultivation were 0.65×10^6 and 1.56×10^6CFU mL^{-1}, respectively. The growth of *Candida* spp., was clearly observed after 24-48 h[10]. On the other hand, the optimal growth of *Arthrobacter* spp., was after 2 days[11]. Furthermore, *Pseudomonas* spp., was growing in less than 48 h and will increase the growth after 48 h[12].

Ability of the consortium microbes for growing at acid condition: Based on the observation of counted colonies after 24 h cultivation period on 100% agar petri dish containing 1% stock solution of liquid medium for 72 h with the addition of 5% $(NH_4)_2SO_4$ and adjustment pH to 5.50, the number of colonies of each microbial additions LS3K, LM1KK and consortium were 1.56×10^5, 0.97×10^5 and 1.18×10^5 CFU mL^{-1}, respectively, while the colony of strain LS3T could not be confirmed.

The growth characters of *Pseudomonas* spp., were optimum at the range of pH from 5.50-11[13]. The colonies of *Candida* sp., LS3T did not appear at pH 5.5 indicated that *Candida* sp., LS3T can not grow at pH 5.50. This result describes that the *Candida* sp., LS3T was not able to grow under acidic conditions. The growth of *Arthrobacter* sp., LM1KK was very low. However, *Arthrobacter* sp., LM1KK still able to grow at acidic pH conditions. Consortium microbial colony growth was better compared to the single strain. This condition could be due to the accumulation of growth between *Pseudomonas* sp., LS3K and *Arthrobacter* sp., LM1KK from the consortium.

Ability of the consortium bacteria for growing at alkali condition: Based on the observation of counted colonies after 24 h cultivation period on 100% agar petri dish containing 1% stock solution of liquid medium for 72 h with the addition of 5% $(NH_4)_2SO_4$ and adjustment pH to 8, the number of colonies of each microbial additions LS3K, LM1KK and consortium bacteria were 1.14×10^5, 1.30×10^5 and 1.53×10^5 CFU mL^{-1}, respectively. While the colony of strain LS3T could not be confirmed. The colonies of *Candida* sp., LS3T was also unable to grow at pH 8.5. *Candida* sp., LS3T was also not able to grow under alkaline pH conditions. The growth of *Arthrobacter* sp., LM1KK was the significant amount to 1.30×10^5 CFU mL^{-1}, which means that *Arthrobacter* sp., LM1KK was accomplished by growing under alkaline pH conditions. This was in line with previous study which reported that *Arthrobacter* sp., could grow optimally at alkaline pH[14]. At pH 8, the colony of consortium microbes growth was the largest amount of 1.53×10^5 CFU mL^{-1} compared to the single strain. It was due to the accumulation of growth between *Pseudomonas* sp., LS3K and *Arthrobacter* sp., LM1KK at the same level.

Decreasing of ammonia concentrations from liquid medium: Research to investigate a reduction of the ammonia concentration was carried out by the addition of 5% $(NH_4)_2SO_4$ as nitrogen resources and data were shown in Fig. 2. It have decided to add 5% $(NH_4)_2SO_4$ due to the best condition at previous research while the consortium microbes have a good ability to grow with the different kinds of ammonia concentration that has been observed by optical density measurement (Fig. 1). The consortium microbes growth with the addition of 5% $(NH_4)_2SO_4$ in a liquid medium in100% meat extract medium were able to reduce the concentration of ammonia from the $(NH_4)_2SO_4$. The growth of consortium bacteria that continued for 4 days can decrease the concentration of ammonia from 49.08-33.71 ppm. It was the evidence that the consortium microbes can metabolize ammonia which can be applied to the waste manure livestock. The consortium microbes that consisted of *Pseudomonas* sp., LS3K, *Candida* sp., LS3T and *Arthrobacter* sp., LM1KK were among the heterotrophic microbial species. Heterotrophic microbes can utilize organic and inorganic materials[6]. The benefits of ammonia oxidation carried by heterotrophic microbes were that nitrification rate was faster than the other types[15].

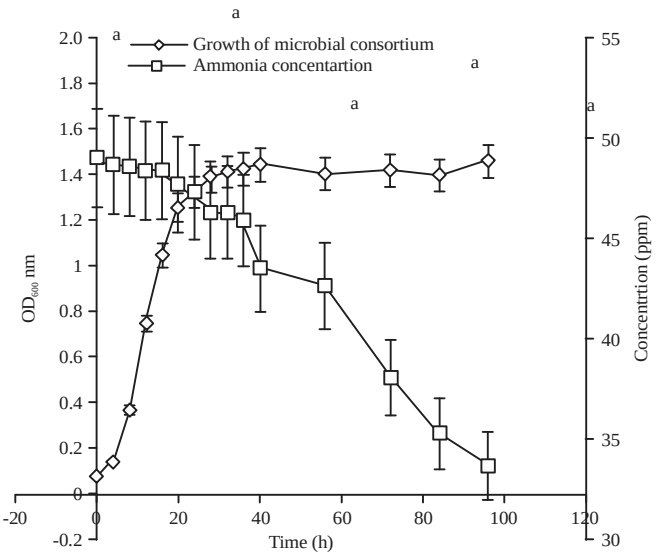

Fig. 2: Growth of the consortium bacteria and the decreasing of the ammonia concentration by a microbial consortium. [a]Same superscript in the figure shows the effects were not significantly different (p>0.05)

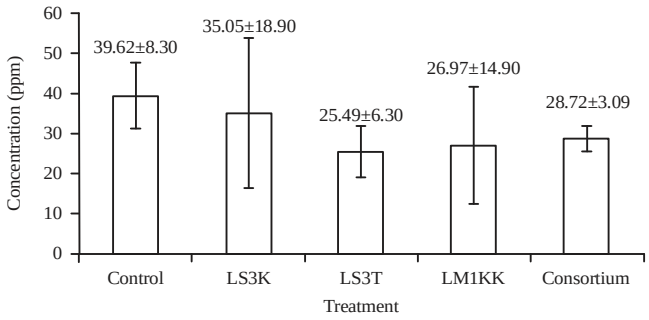

Fig. 3: Comparison of the ability in reducing the ammonia emission from various treatment using chicken excreta as ammonia resources. The concentration of ammonia was measured after 5 days of microbes cultivation

Ammonia emissions from excreta treatment: Figure 3 shows the ammonia emission level from the chicken excreta after 5 days added by the microbes as a treatment. The overall ammonia emission was increased from the first day to the 5th day. Furthermore, from 2nd-5th day, the increasing of higher ammonia concentrations can be seen in the control treatment. The low C/N ratio of the excreta and the high N content also allow the releasing of ammonia from the excreta. The average emission of ammonia of three replication on five treatments oxidation of the control, LS3K, LS3T, LM1KK and consortium has confirmed 39.62, 35.05, 25.49, 26.97 and 28.72 ppm, respectively. The chemical composition of N in excreta was equal to 13,500 ppm with a C/N ratio of 17.71. The addition of microbial treatment strain LS3K, LS3T, LM1KK and the consortium gave effect for not significantly different compared to the control treatment on the emission of ammonia concentration in excreta. *Candida* sp., LS3T was the most efficient in reducing the emission of ammonia in the excreta but the overall capability of the microbial consortium was also quite effective to reduce ammonia in excreta.

Candida spp., was the type of microorganisms high levels of yeast species[10]. The nitrification conducted by *Candida* spp., for 7 days can reduce ammonium by producing nitrate as much as 20.10 ppm[7]. It was consistent with the results of the measurement of the ammonia concentration that was oxidized by *Candida* sp., LS3T. The results of ammonia oxidation by *Candida* sp., LS3T has a difference of 14.14 ppm lower than the control treatment.

Ammonia emission from dairy cow feces treatment: Figure 4 show the ammonia emission level from the dairy cow feces after 5 days added by the microbes as a treatment. The

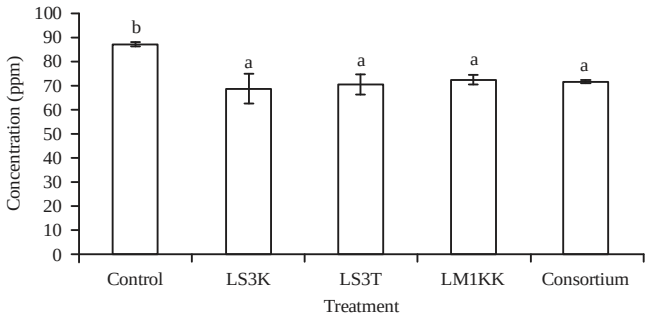

Fig. 4: Comparison of the ability in reducing the ammonia emission from various treatment using dairy cow feces as ammonia resources. The concentration of ammonia was measured after 5 days of microbes cultivation. [ab]Different superscripts in the figure shows a significantly different effect ($p<0.05$)

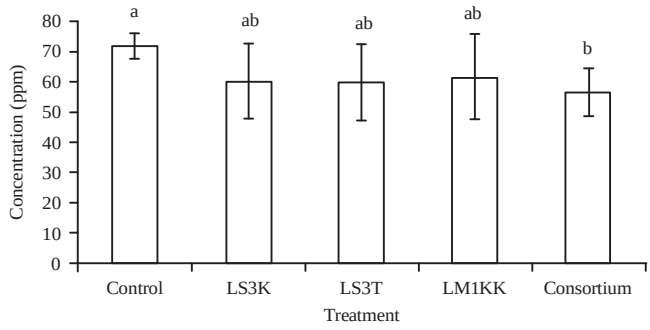

Fig. 5: Comparison of the ability in reducing the ammonia emission from various treatment using beef cattle feces as ammonia resources. The concentration of ammonia was measured after 5 days of microbes cultivation. [ab]Different superscripts in the figure shows a significantly different effect ($p<0.05$)

overall ammonia emission was increased from the 1st-5th day. Furthermore, the 3rd-5th day, the increasing of higher ammonia concentrations can be seen in the control treatment. The content of the undigested N protein may affect the discharge of ammonia gas from feces. The chemical composition of N total feces was 0.70 g kg^{-1} or equal to 700 ppm[16]. The average ammonia emission of three replications from five treatments oxidation by control, LS3K, LS3T, LM1KK and consortium was 87.27, 68.57, 70.31, 72.287 and 71.47 ppm, respectively. The addition of microbial treatment LS3K, LS3T, LM1KK, the consortium gave a significantly different effect ($p<0.05$) compared to the control treatment on the emission of ammonia concentration of feces of a dairy cow. *Pseudomonas* sp., LS3K was the most efficient to reduce the ammonia emission in the feces of dairy cattle, but the overall concentration of ammonia in the feces of the dairy cattle on the outcome of oxidation treatment by *Candida* sp., LS3T, *Arthrobacter* sp., LM1KK and the consortium were also quite effective to reduce the ammonia emission from each treatment. The optimal conditions for microbes to oxidize ammonia were in range at neutral to the slightly alkaline pH condition. *Pseudomonas* spp., grew optimum for ammonia denitrification in the condition of the ratio between carbon (C) and nitrogen (N) at the limit of 4-8[12]. The result of the ammonia oxidation by *Pseudomonas* sp., LS3K has a slight difference of 18.69 ppm lower than the control.

Decreasing emission of the ammonia from beef cattle feces treatment: Figure 5 shows the level of ammonia emission from the beef cattle feces after 5 days cultivation by the microbes as a treatment. The result showed that the overall production of ammonia was increased from the first day till the 5th day. Start on the 1st day to the 5th, the increasing of higher ammonia concentrations can be seen in the control treatment. The ammonia gas was produced by the combination of nitrogen and hydrogen gas[17]. Millions of ammonia were produced annually. Undigested feed nitrogen and nitrogen soluble in the water came out with feces were 7.80 (7800 ppm) and 20.30 g kg^{-1} (20300 ppm), respectively[16]. The average production of ammonia of three replication on five treatments oxidation by control, LS3K, LS3T, LM1KK and

consortium was 71.84, 60.15, 59.87, 61.56 and 56.50 ppm, respectively. Control treatment has the highest concentration of ammonia that was 71.84 ppm.

The addition of microbial treatment of the consortium provided a significantly different effect ($p<0.05$) compared to the control. However, it was not significantly different with treatment LS3K, LS3T and LM1KK to the emission of ammonia concentration in beef cattle feces. The addition of microbial treatment LS3K, LS3T and LM1KK was not significantly different compared to the control treatment on the production of ammonia concentration in beef cattle feces. Microbial consortium oxidized ammonia of beef cattle feces at the most effective way and it was most widely in reducing the emission of ammonia from the waste of beef cattle feces compared to other treatments. The result of ammonia oxidation by the consortium microbes has a difference of 15.34 ppm lower than the control treatment. Production of ammonia from feces was quite high around the level from 70-80 ppm. The largest contributor to ammonia emissions from the agricultural sector was 50% from dairy farms, 20-22% from pork and 7-21% from the poultry. It was consistent with the production of ammonia generated from feces in the study[18].

CONCLUSION

Based on the study, it can be concluded that the ability of oxidation by the microbial consortium was the most optimal in the oxidizing ammonia in beef cattle feces. The capability oxidizing of consortium microbes can reduce as much as 15.34 ppm concentration of ammonia in beef cattle feces. Ammonia was most reduced by *Pseudomonas* spp., that was added to the feces of dairy cow waste which can reduce to 18.69 ppm.

SIGNIFICANCE STATEMENT

Mitigation for ammonia emission from animals manures using consortium nitrifying bacteria that consisted of 3 bacteria from *Pseudomonas* spp., *Arthrobacter* spp., and *Candida* spp., has not been performed yet by another researcher. This study shows how the consortium of bacteria, which isolated from the odorous region of a poultry farm at Yogyakarta city have the ability to reduce the ammonia emission from the chicken excreta, dairy cows manure and cattle beef manure. We hope that these three bacteria will have some advantages for ammonia reduction agent which can be implemented for odor reduction agent.

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