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AZOTOBACTER AND TOTAL BACTERIAL POPULATION IN ENRICHED COMPOST: PRELIMINARY STUDY

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ABSTRACT

Compost enrichment with nitrogen fixing Azotobacter is a method to enhance the used of biofertilizer in sustainable agriculture. The objective of a laboratory experiment was to verify the change in Azotobacter and total bacteria population in two kind of compost after inoculation with nitrogen fixing *A. chroococcum*. The experiment was setup in completely randomized design with four combination treatments of sterilized and unsterilized composted manure as well as vermicompost. The control treatments are the sterilized one. The results verified that irrespective of treatments, Azotobacter enrichment increased Azotobacter but reduced total bacteria population after 18-day incubation compared to the composts before treatment. At the end of experiment, the population of Azotobacter in both sterilized and unsterilized vermicompost were slightly lower than in manure; but the count total heterotroph bacteria in both organic matter was similar. The acidity of both enriched compost was neutral while their humidity was declined to 18-19% at the end of experiment. This experiment showed that inoculating the compost with Azotobater maintain the Azotobacter count but decline the total bacteria. This suggested that negative interaction between Azotobacter and other heterotroph bacteria in compost might be occured.

Contribution/Originality: This study is one of very few studies about the viability of total bacteria and Azotobacter in Azotobacter-enriched compost. Compost enrichment with Azotobacter enhanced Azotobacter population but decreased total bacteria decreased in the compost. This enriched compost can serve as organic matter and Azotobacter inoculant once introducing to the soil.

1. INTRODUCTION

Organic fertilizer as well as biofertilizer is suggested as natural input to optimize plant growth and yield crops in sustainable agriculture. Intensive agriculture in general introduce high rates of inorganic fertilizer which cause a detrimental effect on soil. Organic matter amendment has been proven to maintain soil health since organic carbon (C) in soil support the dynamic living system that in turn support microbial diversity and nutrient cycle [1]. Maintaining organic C stock in arable soils is importance for nutrient supplying system which accommodate by microbial activity [2]. Nowadays, the application on biofertilizer is not as intensive as organic fertilizer. In Indonesia, the constraint of using biofertilizer is - among others - time consuming. The growers need to allocate significant time to inoculate the plants during crops cultivation. Usually, the growers mix organic matter with soil during the soil preparation and dress chemical (and bio-) fertilizer when the crops are already grown. In order to

overcome this constraints, enriching compost with biofetilizer is an efficient way to introduce the biofertilizer at the same time of organic matter amendment. Composting is an appropriate and accepted system elsewhere to stabilize organic matter [3] so that composts are used elsewhere in Indonesia.Non-symbiotic nitrogen (N) fixing Azotobacter now is broadly used as active ingredients in biofertilizer industry. This plant growth promoting rhizobacteria induce plant growth not only by N fixation but also by phytohormones and exopolysaccharides production [4-6]. Enriching compost before introducing to soil with Azotobacter inoculant become a very simple method to introduce N fixing bacteria. In tropics, the soil usually contains low amount of N because of high precipitation and daily temperature [7] and farmers anticipate the low avilability N during plant growth by introducing significant amount of N fertilizer, mainly urea. By using N fixing biofertilizer, the level of N fertilizer will be reduced and hence protect the soil health. Compost enrichment with microbial inoculant has a substantial effect to increase the consumption of biofertilizer. Enrichment of compost has been shown to enhance the microbial count and nutrient content of compost [8]. The count of beneficial microbes includes Azotobacter increased significantly in the compost during 30-day incubation although the microbial enrichment but didn't affect the level of major macronutrients N, P as well as K [9]. The phytohormones content was higher in the microbial-enriched compost [10]. Change in microbial population is reported following microbial enrichment on compost. The total count of N-fixing *Azospirillum brasilense* and *Rhizobium leguminosarum* in enriched vermicompost was high during the early phases of incubation but the population was decreased at 180-day storage [11]. Enrichment compost of mushroom logs with Azotobacter-Pseudomonas consortia results in the enhancement of Azotobacter population in compost at 21-day storage up to 10^6 CFU/g and then the population reduction is recorded at 7 days later [12].

The quality of compost include chemical and biological traits is depending largely on the organic material composition before composting. Moreover, the nutrient composition of similar plant-origin organic matter is determined by the soil where they grow as well as nutrient management during growing period. The microbial enrichment of composted organic matter will result in the different count of target microbes in the compost. The objective of this preliminary study was to verify the population of Azotobacter and total bacteria, as well as the acidity and humidity of two kind of compost after *Azotobacter chroococcum* enrichment.

2. MATERIAL AND METHODS

2.1. Azotobacter Inoculant and Compost

The *Azotobacter chroococcum* liquid inoculant was provided by Soil Biology Laboratory, Faculty of Agriculture Universitas Padjadjaran, Indonesia, located in tropical area at the altitude of 735 m above the sea level. The liquid inoculant was produced by using molasses-based broth enriched with low concentration of N. During 30-day storage, the population of Azotobacter in the liquid inoculant was 10^7 CFU/mL. This experiment enriched two kind of compost. The cow manure-based compost from Universitas Padjadjaran and the vermicompost was produced by sugar factory in Jatitujuh which belong to PT Rajawali Nusantara. The acidity of compost was 6,8 with organic C 10,85%, N 0,57%, P_2O_5 0,65% and K_2O 0,88%. The vermicompost has the pH of 7.1 and contained 1.12% N, 2.31 % P_2O_5 and 1.04 %, K_2O. The humidity of compost and cast before experiment was 29%.

2.2. Experimental Setup

The design of laboratory trial was in completely randomized design with four replications and four treatments that consisted of sterilized and unsterilized composted manure and vermicompost. The control treatments are the sterilized one. Sterilization method was wet sterilization by using autoclave at 120°C for 20 minutes. A total of 100 g compost and vermicompost were placed in transparent polyethylene bag and autoclaving before Azotobacter enrichment in the next day. Before introducing liquid inoculant on the 100 g of compost, 10 mL liquid inoculant of Azotobacter (cell density of 10^7 CFU/mL) was diluted with 10 mL of sterilized 0,85% sodium chloride and mixed evenly in sterilized test tube. The compost was removed from polyethylene bag and placed in clean black

polyethylene bag. Diluted liquid inoculant was pouring evenly on the surface of compost and mixed evenly by using sterilized stick. After homogenization, the bags were covered with perforated thick white paper to avoid high evaporation but maintain air circulation. This enrichment method resulted in the population of Azotobacter up to about 10^6 CFU/mL in compost. The humidity of both compost after enrichment was 29%. Enriched compost then stored at room temperature (15-27°C) for 18 days without direct sun light.

2.3. Parameters and Statistical Analysis

Azotobacter and total bacteria population has been counted once in three days during 18-days storage. The first count has been carried out at inoculation day; precisely about one hours after inoculation. The methods of population counting was serial dilution plate method [13] in N-free Asbhy plate for Azotobacter and Nutrient Agar plate for total aerobic-heterotroph bacteria. The acidity and humidity of enriched compost and vermicompost were measured at the end of experiment by AOAC Methods [14]. All data were subjected to analysis of variance with $p<0.05$. If the sum square of the treatment was significant then the Least Significance Difference (LSD) tests with $p<0.05$ were performed. The Statistical analysis used Minitab 18 program.

3. RESULTS AND DISCUSSION

3.1. Bacterial Population

To verify the viability of Azotobacter and total bacteria in compost without interaction with indigenous microbes, sterilized compost and vermicompost were the control treatments. Two hours after enrichment, the population of Azotobacter in both sterilized and unsterilized compost was about 6 $\log_{10}$ CFU/g (Figure 1a).

The Azotobacter count increased during 6 days and declined from 6-18 days. However, after 18-days storage the highest population of Azotobacter demonstrated by sterilized compost either compost or vermicompost (Figure 1a). The growth peak of Azotobacter population in compost was recorded at 6-day incubation but highest Azotobacter count in sterilized compost was at day 9. At the end of experiment, population of Azotobacter in both enriched sterilized compost was 1 log higher than before inoculation compared to unsterilized one.

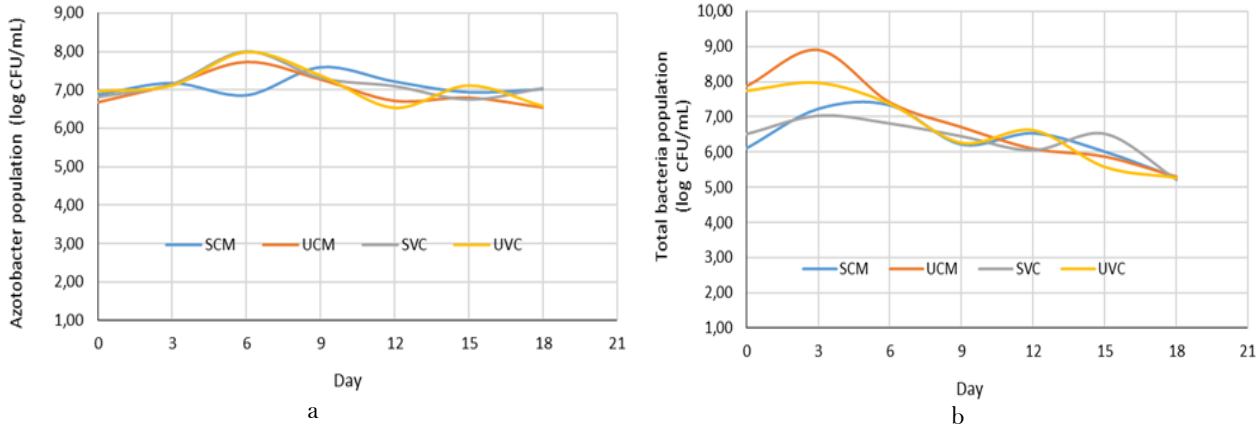

Figure-1. The population of Azotobacter (a) and total bacteria (b) in sterilized and unsterilized organic matter during 18 days Azotobacter inoculation. SCM: sterilized composted manure, USM: unsterilized composted manure; SWC: sterilized vermicompost; UWC: unsterilized vermicompost.
Source: Primary data of the research.

Total bacteria counted by nutrient verified that bacterial population was ecreased sharply after 18-day incubation (Figure 1b). The bacteria grown in plate agar were aerobic and heterotrophic bacteria since the isolated media was nutrient agar that contain organic C. At the day of inoculation, the population of total bacteria reached 6 $\log_{10}$ of CFU/mL and 7 $\log_{10}$ CFU/mL in sterilized and unsterilized compost which is almost equal to the population before inoculation The population increased during 6-day incubation and declined from day 9 to day 18.

Based on Duncan test, at day 6, Azotobacter population in sterilized vermicompost was significantly higher than in sterilized compost Table 1. However, at day 18 the its density in unsterilized compost were 1 log higher than sterilized one.

Table-1. The difference between Azotobacter and total bacteria population in sterilized and unsterilized compost after at 6, 12 and 18-day enrichment with Azotobacter.

Treatments	Log_{10} CFU/mL Azotobacter			
	Day 0	Day 6	Day 12	Day 18
Sterilized composted manure	6.88 b	6.86 b	7.22 a	7.02 a
Unsterilized composted manure	6.68 c	7.72 a	6.72 b	6.54 b
Sterilized vermicompost	6.81 b	8.01 a	7.10 a	7.04 a
Unsterilized vermicompost	6.97 a	7.98 a	6.54 c	6.59 b
Treatment	Log_{10} total bacterial population			
	Day 0	Day 6	Day 12	Day 18
Sterilized composted manure	6.11 d	7.34 a	6.54 a	5.27 a
Unsterilized composted manure	7.87 a	7.41 a	6.09 b	5.29 a
Sterilized vermicompost	6.52 c	6.82 b	6.06 b	5.22 a
Unsterilized vermicompost	7.73 b	7.36 a	6.61 a	5.26 a

Numbers followed by the same letters are not significantly differ based on LSD test $p<0.05$.

Lower bacterial population showed by sterilized vermicompost at 6-day incubation but 9 days later, all bacterial population in unsterilized compost were lower than sterilized one. At the end of experiment, total bacterial count decreased up to 5 log_{10} CFU/mL and there was no cell count difference between both sterilized and unsterilized compost.

3.2. Acidity and Humidity of Compost

Analysis of variance showed that the Azotobacter enrichment to different compost didn't affect humidity but decreased the acidity compare to untreated compost. Nonetheless, based on LSD test ($p<0.05$), the acidity and humidity of sterilized/unsterilized compost and vermicompost was not differ at 18 day-incubation (Figures 2a and 2b). Before inoculation, the acidity of compost and vermicompost were 6.8 and 7.1 respectively. Slightly increased in compost acidity was demonstrated at the end of incubation (Figure 2a). The humidity of both compost after Azotobacter liquid inoculant application was up to 29%; and at the end of incubation they reduced to less than 20% (Figure 2b).

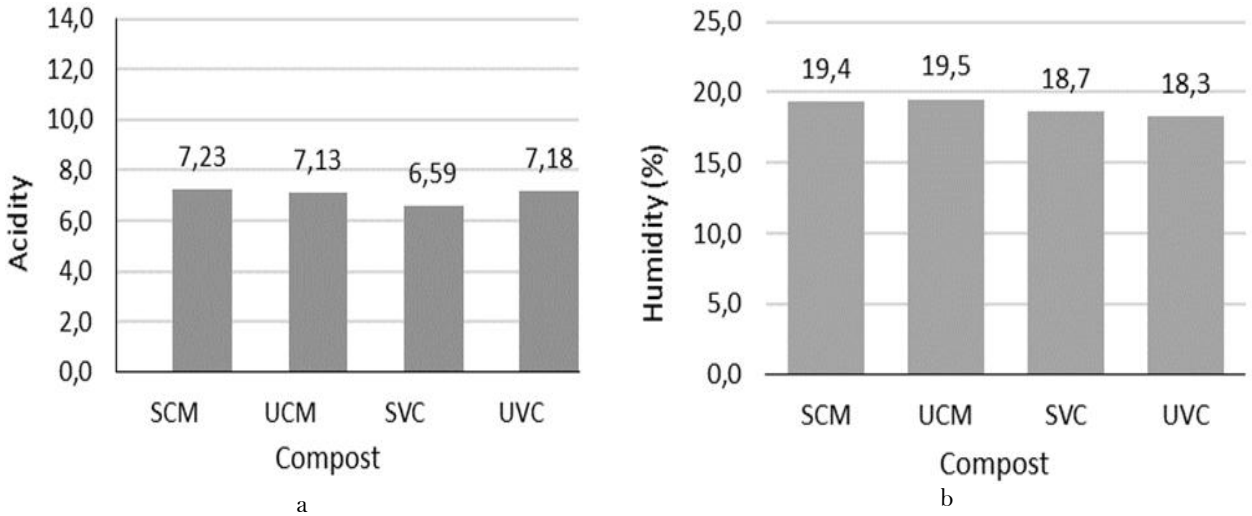

Figure-2. The acidity (a) and humidity (b) of manure compost and vermicompost 18 days after enrichment with Azotobacter liquid inoculant. SCM: sterilized composted manure, USM: unsterilized composted manure; SWC: sterilized vermicompost; UWC: unsterilized vermicompost

Composted manure as well as vermicompost serve as a source of nutrients and physical protection to proliferation of heterotrophic bacterial. In heterotroph metabolisms, microbes utilized organic matter as carbon source and to generate energy [15]. Both compost support the growth of Azotobacter but less supportive to maintain bacterial population during 18-day incubation.

Before the enrichment, the Azotobacter count in sterilized and unsterilized composted manure and vermicompost were less than 10^3 CFU/g (3 $\log_{10}$). The population of total bacteria in sterilized compost was 2 x10^5 lower than unsterilized one; almost 3 x 10^6 CFU/mL. This research showed that the population of Azotobacter was increased from day 3 to day 18; but the enrichment of organic matte compost with Azotobacter cannot maintain total bacterial population The increased of Azotobacter agreed with the similar trial by using waste mushrooms log compost vermicompost and farmyard manure [9, 12]. Both researcher verified that compost enable to maintain Azotobacter population up to 21 days.

Azotobacter is heterotroph rhizobacteria that forms cysts under dry condition [14]. At the end of experiment, the humidity of compost was declined from 29 % to up to 18%. The decrease of humidity induces the cyste formation [16]. Moreover, the acidity (pH) of either sterilized and unsterilized organic matters were about neutral. The Azotobacter proliferate better in neutral pH and they are sensitive to acid environment [17]. The decrease of total bacteria which is enumerated on nutrient agar plate, might be the results of the negative interaction with exogenous Azotobacter as well as abiotic stress condition. This antagonistic interaction due to abiotic stress hence influence the impact on plant performance [18]. The native bacteria in compost compete the nutrients and water with exogenous Azotobacter. Moreover, the low humidity distresses the metabolisms of native microbes. Both of condition resulted in significant reduced of total bacteria in compost between 3 – 18 days after inoculation.

Introducing beneficial microbes to both compost increased Azotobacter count. However microbial enrichment also will increase its quality but this research didn't analyze the chemical properties of compost. For further research. longer shelf life should be evaluated to ensure the viability of Azotobacter and the change of physico-chemical properties of enriched compost.

4. CONCLUSION

Compost enrichment with Azotobacter liquid inoculant resulted in the increase of Azotobacter count and decreased in total bacteria population after 18-day incubation at room temperature. The acidity of enriched compost was about neutral but the humidity was decreased from 29% before enrichment to 18-19% at the end of experiment. In order to increase the used of biofertilizer, compost enrichment by using beneficial soil microbes is suggested.

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