Effectivity of Methanotrophic Bacteria and Ochrobactrum Anthropi as Biofertilizer and Emission Reducer of CH_4 and N_2O in Inorganic Paddy Fields

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***Abstract*—Global warming is the increasing of earth's surface temperature which can occurred due to agricultural activities. Agricultural activities contribute to the global warming as sources of CH_4 and N_2O emissions. Application of methanotrophic bacteria, *Ochrobactrum anthropi*, *Azotobacter* and *Azospirillum* combination could reduce the emission of CH_4 and N_2O. In addition, these bacteria can fix nitrogen (N_2) to enhance the plant growth. The aim of this study was to determine the effectivity of methanotrophic bacteria, *Ochrobactrum anthropi*, *Azotobacter* and *Azospirillum* combination as biofertilizer and emission reducer of CH_4 and N_2O in the inorganic paddy fields. This experiment was arranged by treating 100% dosage of inorganic fertilizer as positive control, 25% dosage of inorganic fertilizer and 25% dosage of inorganic fertilizer mixed by biofertilizer. The observations were made on the growth parameters and the rate of gas fluxes. The combination of bacterial isolates could increase rice growth, grain yield productivity by 67.53% and they also could reduce CH_4 and N_2O emission.**

***Index Terms*—biofertilizer, CH_4, methanotrophic, N_2O, paddy**

I. Introduction

Global warming is the increasing of earth's surface temperature which occured by the increasing of greenhouse gases emissions in atmosphere every year. Greenhouse gases those cause global warming are CH_4, CO_2, N_2O and CFC. Based on International Panel of Climate Change's data in 2007, the increasing of the greenhouse gases emissions was up to 70% between 1970 and 2004**.** Various human activities such as mining, industry, transportation, farming and agriculture are some sources of CH_4, CO_2 and N_2O emissions into the atmosphere. Indonesia as an agricultural country has paddy field more than 8 million hectares. Wetlands such as rice fields become one of the main source of CH_4 and N_2O. CH_4 in paddy fields derived from the activity of methanogenic bacteria in the soil through the process of methanogenesis. The use of inorganic fertilizer such as urea to support the agricultural activities increases the change rate of N_2O [1]. CH_4 in paddy fields can be oxidized by methanotrophic bacteria by produce methane monooxygenase (MMO) enzyme. N_2O emissions can be reduced by denitrifying bacteria which has the N_2O reductase enzyme activity like *Ochrobactrum anthropi*.

Biofertilizer is one of the solution to increase agricultural productivity but still environmentally friendly. *Azotobacter* and *Azospirillum* are a group of nitrogen-fixing bacteria (diazotroph bacteria) which have been widely used as a biological fertilizer component. Both bacteria are also capable of producing diazotroph growth regulating substances that can trigger the growth of plants [2]. These bacteria combination with methanotrophic bacteria and *Ochrobactrum anthropi* also have the capability as nitrogen fixer [3], [4]. It also stated that the combination of diazotroph bacteria such as *Azotobacter* and *Azospirillum* and methanotrophic bacteria could increase the levels of ammonium cultures and decrease methane concentration in the culture headspace [5], [6]. Moreover, the combination of methanotrophic bacteria and *Ochrobactrum anthropi* increase the levels of ammonium culture, CH_4 oxidation and N_2O reduction [7], [8]. The combination of these bacteria are very potential to be developed as a biofertilizer which at the same time can reduce the greenhouse gas emissions in paddy field. Therefore, it is necessary to study the combination effectivity of *Ochrobactrum anthropi*, *Azotobacter* and *Azospirillum* as biofertilizer, CH_4 and N_2O emmision reducer in the inorganic field.

II. Materials and Methods

This research used three treatments which were fertilized by inorganic fertilizer NPK (nitrogen, phosphate, potassium) 250 kg/ha (dosage 100% from recommendation) (K), fertilized by NPK 62.5 kg/ha (dosage 25% from recommendation) mixed biofertilizer (C) and fertilized with NPK 62.5 kg/ha (dosage 25% from recommendation) without biofertilizer (TC).

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A. Organic Fertilizer Making Process

Isolate of methanotrophic bacteria BGM 1, BGM 5, BGM 9, and SKM 14 were purified in *Nitrate Mineral Salt* (NMS)+1% methanol. Isolate of *Azotobacter* and *Azospirillum* purified in NMS+1% sucrose and NMS+1% malate acid. Isolate of *O. anthropi* BL1 and BL2 purified in solid denitrification media. Each isolate of bacteria is incubated at 27^oC for 3-7 days. The bacteria from purification process were cultured in 500 ml liquid media. The culture then incubated in shaker (± 37^oC) for 5-7 days until the total of cell was 10^8 cell ml^{-1}.

B. Planting

Each plot measured approximately 150 m^2 with 25×25 cm of planting distance. K treatment used a patch while C and TC treatments respectively used 4 patch. The paddy clump then planted in paddy fields. In the C treatment, the clumps were soaked with biofertilizers liquid for 15 minutes before being transferred. The untreated-biofertilizers clumps then separated into TC and K treatments. NPK fertilizer application has been done at 14 days after planting (D+14).

C. Soil Characteristic Analysis

100 grams of soil samples were taken from each plot then sent to the Soil Laboratory at Center for Agricultural Land Resources, Soil Research Institute, Bogor to analyze the physical-chemistry characteristic.

D. Plant Growth Parameters

Paddy growth was observed during the phase of vegetative (D+47), early reproductive (D+57), reproductive (D+71), and panicle ripening (D+98). The growth parameters observed were plant height and number of tillers with three plants samples in each plot. After the harvesting, wet weight and dry weight of plants were measured and also calculated to the grain productivity estimation. Plant dry weight measured by put the plants parts to the oven in 60 ℃ until reached the stable weight. Grain productivity was estimated by calculated the number of clumps per hectare through the planting distance. The weighing of dry grain weight per clumps result then converted to tones per hectare.

E. Gas Emissions Measurement Activities

Gas sampling has been done by closed the sample plots in the fields with the lid box. A box was placed in each treatment. Gas samples then taken from the inside the box using a 100 ml syringe then stored in vacuum glass bottle. Gas sampling was conducted on vegetative and generative phase. At each phase of growth, the gas was taken in three different times, they are shortly after the laying of the lid (t0), 4 hours (t4) and 16 hours (t16) after covering. Gas samples then sent to Greenhouse Gases Laboratory, Environmental Research Institute of Agriculture Jakenan Pati, Central Java to be analyzed the gas concentration (CH_4 and N_2O). The gas concentration results then converted to obtain the rate of gas change on day and night.

F. Data Analysis

Analysis of multiple data parameters (crop growth and activity of CH_4 and N_2O emissions) performed using the SAS program. SAS program used to view differences between treatments in the ANOVA and Duncan's Multiple Range Test (DMRT) with a significance level (α) of 5%.

III. RESULTS

A. Soil Characteristic Analysis

Soil analysis showed the composition of the soil with 15% of sand, 35% of silt and 50% of clay. Soil contained low category organic matter (1.75% of C and 0.15% of N), middle category of cation for Ca and Na (8.69 $Cmol.kg^{-1}$ and 0.62 $Cmol.kg^{-1}$) but high category for Mg and K (2.30 $Cmol/kg^{-1}$ and 0.88 $Cmol/kg^{-1}$). The soil pH value of 4.9 was categorized as acid soil category.

B. Plant Growth Parameters

The height of paddy plants in the K, C, and TC treatments were increased in every observation. Every treatments has a similar high accretion rate pattern, except for the K treatment. In the end of the observation, C treatment was able to produce the highest plant height and the highest number of clumps but instead for K treatment (Table I). Wet and dry weight of clumps also grain with C treatment has the largest weight (Table II). K treatment produced the highest value of wet root weight while the highest of dry root weight was resulted from the TC treatment.

TABLE I. PLANT GROWTH PARAMETERS

Treatment*)	Days after planting			
	47	57	71	98
	Plant height (cm)[a]			
K	-	60.625 b	73.125 b	79.375 b
C	50.833 a	69.875 a	81.750 a	90.125 a
TC	49.667 a	68.750 a	82.063 a	89.875 a
	Number of tillers[a]			
K	-	15.25 b	15.50 b	14.75 b
C	-	22.00 a	24.50 a	19.88 a
TC	-	19.75 a	19.25 ab	15.88 b

[a]Numbers at the same column which followed by the same word are not significantly different at significance level (α) of 5% (DMRT). *)Different fertilizer treatment; K: 100% inorganic fertilizer; C: biofertilizer+25% inorganic fertilizer and TC: 25% inorganic fertilizer. -)No observation has been done.

TABLE II. DRY AND WET WEIGHT OF PADDY PLANT

Treatment*)	Clump	Root	Grain/clump	Percentage of empty grain
	Wet weight (g)[a]			
K	119.09 a	56.24 a	45.96 b	-
C	146.97 a	44.47 a	73.26 a	-
TC	120.31 a	48.08 a	59.11 b	-
	Dry weight (g)[a]			(%)[a]
K	32.50 b	18.64 a	36.66 b	4.198
C	45.20 a	22.27 a	61.41 a	3.130
TC	40.44 ab	23.90 a	50.70 a	4.209

[a]Numbers at the same column which followed by the same word are not significantly different at significance level (α) of 5% (DMRT).
*)Different fertilizer treatment; K: 100% inorganic fertilizer; C: biofertilizer+25% inorganic fertilizer and TC: 25% inorganic fertilizer. -)No observation has been done.

C. Grain Productivity Estimation

K treatment showed slower growth compared with other treatments. Estimation of grain yield productivity indicated that C treatment was able to produce grain with the highest number of 9.77 ton ha^{-1} while the K treatments produced grain with the lowest number of 5.83 ton ha^{-1} (Fig. 1).

Based on these results it can be seen that land with C treatment gave the highest grain yield productivity grains of 67.53% (Fig. 2).

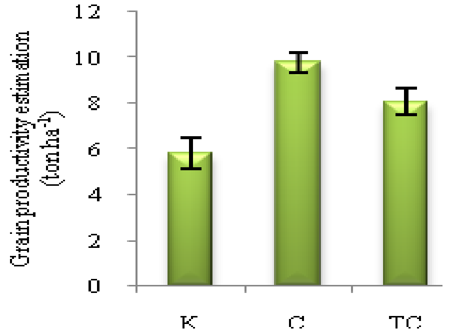

Figure 1. Estimation of grain productivity.

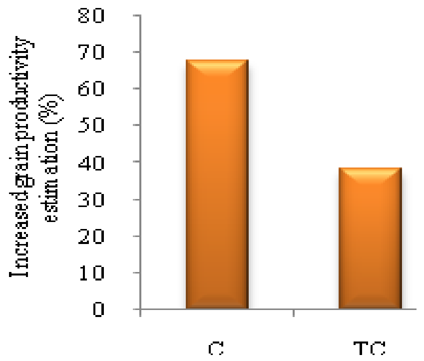

Figure 2. Estimation of increased grain productivity.

D. Activity of Gas Emmisions

CH_4 observations from the box K treatment were increasing the highest fluxes of gas when the vegetative phase by 18.31 mmol $m^{-2}hr^{-1}$. The lowest fluxes of CH_4 gas were from box C treatment by -19.57 mmol $m^{-2}hr^{-1}$ (Fig. 3). The highest fluxes of N_2O gas achieved by the box K when vegetative phase by 238 µmol.$m^{-2}hr^{-1}$. The fluxe of N_2O gas at box C decreased with the lowest value by -127.19 µmol.$m^{-2}hr^{-1}$ (Fig. 4).

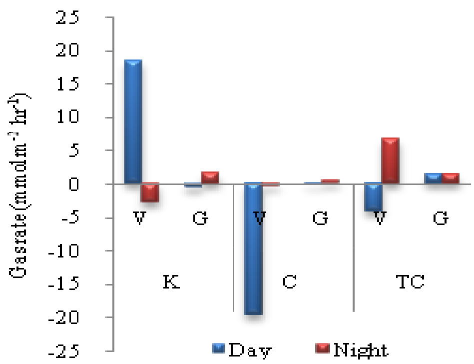

Figure 3. Gas rate of CH_4 at vegetative (V) and generative (G) phase.

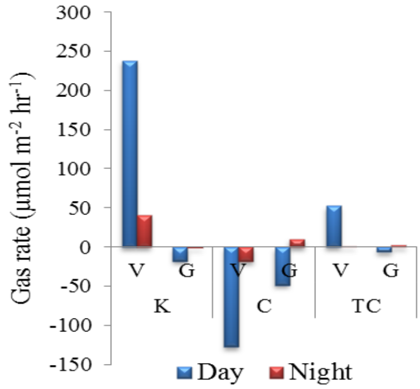

Figure 4. Gas rate of N_2O at vegetative (V) and generative (G) phase.

IV. Discussion

Soil analysis test result was classified as clay soil based on texture triangle grading diagram by USDA system (United State Department of Agriculture). Clay soil is characterised a bit slippery, slightly attached, can be formed as firm ball, roll formed, and rather easily destroyed [9]. It also showed that based on the soil chemical properties, the content of organic C and N were low. Other parameters were observed in rice growing is the number of tillers. The results showed an increase in the number of tillers on the vegetative phase. When entering the generative phase, the number of tillers were decreased. This situation showed when the growth of tillers is maximum, some of the seedling will die and not produce tassel. The dead tillers are also called unproductive tillers. The C treatment produced the highest number of tillers. It was because the plant growth is supported by *Azotobacter*, *Azospirillum*, methanotrophic bacteria and *Ochrobactrum anthropi* as nitrogen-fixing bacteria in the biofertilizer. The ability of bacteria to bind nitrogen as it has *nif*D and *nif*H genes that encode the nitrogenase enzyme [10]. This enzyme plays a role in catalyzing the process of nitrogen fixation. Nitrogen compound is one of the main constituent of plant cell components such as amino acids and nucleic acids. Therefore, nitrogen deficiency will inhibit tillers growth.

The results showed C treatment has the lowest roots weight (wet and dry) and the lowest number of root fibers. The state allegedly due to the influence of IAA compounds produced by *Azotobacter* and *Azospirillum* in biofertilizer. It also stated that excessive concentration of IAA will inhibit roots growth [11]. High IAA synthesis will be oxidized to ethylene which can inhibit the roots growth. The activity of auxin in inhibiting of root growth is mediated by ethylene [12]. Grain production is determined by the total assimilates during the panicles charging process. The number of assimilate will increase as the number of nitrogen needs. Highest grain yield achieved in C treatment which have no shortage of nitrogen. Unlike the other treatments, the plants need especially the nitrogen is insuficient. At this treatment, the inorganic fertilizer given cannot fulfil nitrogen needs because it was denitrificated, volatilizated, leached by the surface runoff. Nitrogen needs can be met at C treatment because of the active role of nitrogen-fixing bacteria in biofertilizer combination. Whereas K and TC treatment showed low productivity (grain yield) with a higher number of empty grain than C treatment. The emptiness is caused by the low capacity of the plant to fulfil the assimilates during panicle fulfilment.

A controlled usage of inorganic fertilizers can reduce N_2O emissions [13]. As in N_2O observation at K treatment that used a highest dosage of inorganic fertilizer than in other treatment, showed an increasing of N_2O emissions. The C treatment which used inorganic fertilizer less than K treatment, showed a decreasing of N_2O emissions. It showed that the usage of higher inorganic fertilizers lead increasing in N_2O gas. As reported before that the increasing usage of inorganic nitrogen fertilizers changing the global nitrogen cycle thereby increasing the rate of N_2O gas production [14]. Besides the usage of inorganic fertilizers is less, the reduction of gas emission presumably because of *Ochrobactrum anthropi* participation in the biofertilizer. This bacteria has nitrous oxide reductase enzyme that can convert N_2O into N_2 [15]. The application of biofertilizer consisting of methanotrophic bacteria, *Ochrobactrum anthropi*, *Azotobacter* and *Azospirillum* were effective in improving paddy growth, increasing grain yield productivity, and reducing CH_4 and N_2O emmisions. The growth parameters at C treatment which are indicated by the plant height, dry weight, and wet weight were found higher than other treatments. The treatment which has combination of biofertilizer and reduced inorganic fertilizer dosage could increase Ciherang paddy productivity by 67.53% and reduce CH_4 and N_2O emmisions.

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