Effectivity of Methanotrophic Bacteria and Ochrobactrum Anthrophi as Biofertilizer and Emission Reducer of CH$_4$ and N$_2$O in Inorganic Paddy Fields

Gloria. Maria Foster Pingak$^{1a}$, Hendri. Sutanto$^{1b}$, Alina. Akhdiya$^{2c}$, and Iman. Rusmana$^{1d}$

$^1$Department of Biology Bogor Agricultural University, Bogor, Indonesia
$^2$Indonesian Centre for Agricultural Biotechnology and Genetic Resources Research and Development, Bogor, Indonesia

Email: $^{1a}$gloriamfp, $^{1b}$greg.hendrisutanto@gmail.com, $^{2c}$a2khdiyar@yahoo.co.uk, $^{2d}$rusmana13@yahoo.com

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$_2$O emissions. Application of methanotrophic bacteria, Ochrobactrum anthrophi, Azotobacter and Azospirillum combination could reduce the emission of CH$_4$ and N$_2$O. In addition, these bacteria can fix nitrogen (N$_2$) to enhance the plant growth. The aim of this study was to determine the effectiveness of methanotrophic bacteria, Ochrobactrum anthrophi, Azotobacter and Azospirillum combination as biofertilizer and emission reducer of CH$_4$ and N$_2$O 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 combinations of bacterial isolates could increase rice growth, grain yield productivity by 67.53% and they also could reduce CH$_4$ and N$_2$O emission.

Index Terms—biofertilizer, CH$_4$, methanotrophic, N$_2$O, paddy

I. INTRODUCTION

Global warming is the increasing of earth’s surface temperature which occurred by the increasing of greenhouse gases emissions in atmosphere every year. Greenhouse gases those cause global warming are CH$_4$, CO$_2$, N$_2$O 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$_2$O 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$_2$O. 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$_2$O [1]. CH$_4$ in paddy fields can be oxidized by methanotrophic bacteria by produce methane monooxygenase (MMO) enzyme. N$_2$O emissions can be reduced by denitrifying bacteria which has the N$_2$O reductase enzyme activity like Ochrobactrum anthrophi.

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 diazotrophic growth regulating substances that can trigger the growth of plants [2]. These bacteria combination with methanotrophic bacteria and Ochrobactrum anthrophi 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 anthrophi increase the levels of ammonium culture, CH$_4$ oxidation and N$_2$O 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 anthrophi, Azotobacter and Azospirillum as biofertilizer, CH$_4$ and N$_2$O emission 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).
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°C for 3-7 days. The bacteria from purification process were cultured in 500 ml liquid media. The culture then incubated in shaker (± 37°C) for 5-7 days until the total of cell was 10⁸ cell ml⁻¹.

B. Planting

Each plot measured approximately 150 m² 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 plant 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 °C 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 (CH₄ and N₂O). 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 CH₄ and N₂O 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>
<thead>
<tr>
<th>Treatment</th>
<th>Days after planting</th>
<th>Plant height (cm)</th>
<th>Number of tillers</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>47</td>
<td>73.75 b</td>
<td>79.37 b</td>
</tr>
<tr>
<td>C</td>
<td>50.83 a</td>
<td>69.875 a</td>
<td>81.750 a</td>
</tr>
<tr>
<td>TC</td>
<td>49.667 a</td>
<td>68.750 a</td>
<td>82.063 a</td>
</tr>
</tbody>
</table>

*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 I. PLANT GROWTH PARAMETERS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Clump (g)</th>
<th>Root (g)</th>
<th>Percentage of empty grain</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>1.1999 a</td>
<td>55.29 a</td>
<td>45.96 b</td>
</tr>
<tr>
<td>C</td>
<td>1.4697 a</td>
<td>44.47 a</td>
<td>73.26 a</td>
</tr>
<tr>
<td>TC</td>
<td>1.201 a</td>
<td>40.88 a</td>
<td>59.11 b</td>
</tr>
</tbody>
</table>

Dry weight (g)*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Clump (g)</th>
<th>Root (g)</th>
<th>Percentage of empty grain</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>3.205 a</td>
<td>18.64 a</td>
<td>36.66 b</td>
</tr>
<tr>
<td>C</td>
<td>4.52 a</td>
<td>22.27 a</td>
<td>61.41 a</td>
</tr>
<tr>
<td>TC</td>
<td>4.404 ab</td>
<td>23.90 a</td>
<td>50.70 a</td>
</tr>
</tbody>
</table>

*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.

©2014 Engineering and Technology Publishing
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).

D. Activity of Gas Emmisions

\(\text{CH}_4\) 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 \(\text{CH}_4\) gas were from box C treatment by -19.57 mmol m\(^{-2}\)hr\(^{-1}\) (Fig. 3). The highest fluxes of \(\text{N}_2\text{O}\) gas achieved by the box K when vegetative phase by 238 µmol.m\(^{-2}\)hr\(^{-1}\). The fluxes of \(\text{N}_2\text{O}\) gas at box C decreased with the lowest value by -127.19 µmol.m\(^{-2}\)hr\(^{-1}\) (Fig. 4).

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 \textit{Azotobacter}, \textit{Azospirillum}, methanotrophic bacteria and \textit{Ochrobactrum anthropi} as nitrogen-fixing bacteria in the biofertilizer. The ability of bacteria to bind nitrogen as it has \textit{nifD} and \textit{nifH} 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 \textit{Azotobacter} and \textit{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 insufficient. At this treatment, the inorganic fertilizer given cannot fulfill 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 fulfill the assimilates during panicle fulfilment.

A controlled usage of inorganic fertilizers can reduce N$_2$O emissions [13]. As in N$_2$O observation at K treatment that used a highest dosage of inorganic fertilizer than in other treatment, showed an increasing of N$_2$O emissions. The C treatment which used inorganic fertilizer less than K treatment, showed a decreasing of N$_2$O emissions. It showed that the usage of higher inorganic fertilizers lead increasing in N$_2$O gas. As reported before that the increasing usage of inorganic nitrogen fertilizers changing the global nitrogen cycle thereby increasing the rate of N$_2$O 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$_2$O 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$_2$O emissions. The growth parameters at C treatment which are indicated by consisting of methanotrophic bacteria, nitrogen fertilizers lead increasing in N$_2$O and temperature interaction,“ Soil Biol Biochem., vol. 47, pp. 36-45, November 2012.


Gloria. Maria Foster Pingak was born at Quelicai, October 31st 1991. She finished her Bachelor of Science (S.Si) at biology major, Department of Biology, Bogor Agricultural University, Indonesia in 2013.

She joined some internships such as Supervision of fresh plants and food at Soekarno-Hatta International Airport Association of Agricultural Quarantine, Indonesia in 2012. Breeding & Selection of Improved Corn Seed at PT BISI International Tbk, Indonesia in 2012 and Laboratory Assistant at Department of Biology, Bogor Agricultural University, Indonesia in 2013. She also got the Ministry of Education Scholarship Republic of Indonesia in 2010-2011 and Woman International Club Scholarship in 2012-2013.

She took some trainings such as IT Training : Web Hacking at Indonesia in 2010 and Hazard Analysis and Critical Control Point (HACCP) in 2013. She joined the Nature Exploration of Bodogol, Indonesia in 2011. Since 2006-2013 she joined some seminars such as IPBS’s Dedication for Education Sharing and Inspiring People for Better Education, Unclocking Potential College Conference, BIONIC Human Reproduction and Health and Indonesian Association for Microbiology (PERMI). Her research interests are in environmental microbiology especially about methanotrophic bacteria and denitrification bacteria as biofertilizer, CH$_4$ and N$_2$O emissions reducer in inorganic paddy fields.

Hendri. Sutanto was born at Jakarta, January 1st 1990. He finished his Bachelor of Science (S.Si) at biology major, Department of Biology, Bogor Agricultural University, Indonesia in 2013. He is now taking Master Degree in Microbiology Major, Bogor Agricultural University Graduate School, Indonesia.

He joined the student exchange at Japan held by Ministry of Manpower and Transmigration, Republic of Indonesia and IMM Japan, High School Regional National Biology Olympiad Workshop in 2008, participated at International Genetically Engineered Machine (iGEM) Competition, HongKong University of Science and Technology in 2012 and achieved some awards in international choir competition since 2006. He joined internships as Laboratory Assistant at Department of Biology, Bogor Agricultural University, Indonesia in 2011-2012. He also got the Ministry of Education Scholarship of Indonesia in 2000-2013. In 2011, he did the field study research about The Diversity of Fresh Water Fish at Watat Mountain, Sukabumi West Java Indonesia and noted as Cytology Staining Laboratory Assistant at Cengkareng State Hospital, Jakarta Indonesia in 2012. His previous research was about the effectivity of methanotrophic bacteria and denitrification bacteria as biofertilizer on paddy growth in organic

ACKNOWLEDGMENT

This research was supported by Department of Biology, Bogor Agricultural University.

REFERENCES


paddy fields. His research interests are in environmental microbiology especially about the community of methanotrophic bacteria based on pMMO gene in inorganic paddy fields.

**Alina. Akhdiya** was born at Jepara, December 8th 1968. She was graduated from undergraduate school of biology study program at Bogor Agricultural University in 1994 and finished master degree in Microbiology Major at Bogor Agricultural University Graduate School, Indonesia in 2000.

She is working at Microbiology Laboratory, Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICBIOGRRD), Indonesia since 2001. She worked as Junior Researcher at pilot plan laboratory, Research Center for Crop Biotechnology, Bogor Indonesia in 1997-2001. Since 1997, she has been publishing her researchs on some journals and papers such as Isolation and Characterization of Methanotrophic Bacteria from Rice Fields published by Biotropia.16 (2):71-78, Evaluation of Soybean Germplasm for Its Resistance to several foliar pathogens in Indonesia published by J. Agric. Technol. 8:751-763, and Efficacy of Consortia Bacteria for Control Rice Disease under System of Rice Intensificatio n (SRI) in West Java-Indonesia published by AJAS. 12: 1-147. Her research interests are about development technology and application of methanotrophic bacteria as biofertilizers and methane emission reducers in rice fields, exploration of indigenous AHL-degrading bacteria.

Alina Akhdiya, MSi is now listed as the member of Indonesian Society for Microbiology (PERMI).

**Iman. Rusmana** was born at Tasikmalaya, July 20th 1965. He was graduated from undergraduate school of biology study program at Bogor Agricultural University, Indonesia in 1990, Master degree in Microbiology Major at Bogor Agricultural University Graduate School, Indonesia in 1997, and PhD in Environmental Microbiology at Essex University, Colchester, United Kingdom in 2003.

He worked as The Head of Microbiology Laboratory, Division of Fermentation at PT Miwon Indonesia in 1990-1991 and Microbiology Consultant at PT. Central Pertiwi Bahari (Integrated Shrimp Farming Company), Indonesia in 2004-2007. He is working as The Head of Department of Biology, Chief Editor Hayati Journal of Biosciences and Microbiology Lecturer at Bogor Agricultural University,Indonesia. He has been publishing his researchs since 2002 on some journals such as Nitrous oxide formation in the Colne estuary, England: the central role of nitrite published by Appl. Environ. Microbiol. 68:1240-1249, Use of chlorate as a selective inhibitor to distinguish membrane bound nitrate reductase (Nar) and periplasmic nitrate reductase (Nap) on dissimilative nitrate reducing bacteria in sediment published by FEMS Microbiology Ecology.48:379-386, and Characterization of lactic acid bacteria isolated from an Indonesian fermented fish (bekasam) and their antimicrobial activity against pathogenic bacteria published by Emirates Journal of Food and Agriculture 25(6). His research interest now are about the effectivity of methanotrophic bacteria and denitrification bacteria consortium as biofertilizer, CH$_4$ and N$_2$O emissions reducer and the bacteria community in paddy fields.

Dr. Ir. Iman Rusmana, MSi is now listed as the member of some professional societies such as Indonesian Association for Microbiology (PERMI) and Society for General Microbiology (SGM), United Kingdom.