Evaluating the effect of different biochar application sizes on methane emission reduction from rice cultivation

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Abstract. Application of biochar to the soil has been reported as one of the mitigation technologies of CH₄ emission from rice cultivation due to its unique characteristics of high porosity and surface area. The application of small particle size of biochar is rich in surface area that may enhance the mitigation potential. Rice cultivation and soil incubation experiments were conducted to evaluate the effect of two groups of biochar particle size on CH₄ emission and production in order to show the mitigation potential. This experiment consists of three treatments including no biochar (CT), small particle size (0.5-2 mm) biochar (SB), and large particle size (2-4 mm) biochar (LB). Both biochar sizes were amended at 10 t ha⁻¹ equivalent rate and all treatments were applied chemical fertilizer at 100 kg N ha⁻¹ equivalent rate. The results demonstrated that SB and LB reduced cumulative CH₄ emission by 24.0% and 17.1% and cumulative CH₄ production by 24.6% and 15.0% as compared to CT, respectively. Our results showed that SB achieved higher mitigation potential than LB by an average of 8.47%, although it was not significant. The mitigation of both biochar sizes was supported by the significant change of soil methanogens and methanotrophs abundances. The suppression of methanogens abundance and the stimulation of methanotrophs abundance indicated in the ratio of mcrA to pmoA was significantly reduced in SB (68.0%) which higher than in LB (56.3%) as compared to CT. Both application sizes also increased soil oxidation capacity through soil Eh increase which no difference between SB and LB. In term of grain yield, SB and LB were not different and both did not show the significant change as relative to CT. The application of small size biochar in this study affected more mitigation potential of CH₄ emission as compared to larger size, therefore there is a need of further study on typical size of biochar in order to recommend the most mitigation potential of biochar application.

Keywords: Biochar size, Methane emission, Methane production, Rice cultivation, Soil incubation
1. Introduction
Paddy rice field is recognized as one important source of anthropogenic methane (CH$_4$) emissions that affect global climate change. CH$_4$ is produced by organic matter (OM) decomposition of methane-producing bacteria (methanogens) in the soil under anaerobic conditions [1] as result of continuous flooded of water management practice. The acceptable technology for CH$_4$ mitigation from rice growing with yield increase is biochar [2, 3]. Biochar is a charcoal-like material that produced from pyrolysis with low oxygen [4]. Many studies reported the reduction of CH$_4$ emission by biochar amendment around 34 to 91% [5, 6] but its mechanism has not been clearly explained. The most discussion related to the increase of electron acceptors (such as nitrate and sulphate) that were absorbed on biochar surface as result to inhibit CH$_4$ production under anaerobic environment [7]. The promotion of CH$_4$ oxidation outpace CH$_4$ production because of stimulating CH$_4$ oxidizing bacteria by providing bacteria habitat [8] and improving soil aeration [2] as result of biochar’s porosity and surface area. It showed that porosity and surface area of biochar play a major role on CH$_4$ emission reduction from rice paddy soil. In addition, these unique properties of biochar also reduce nutrients leaching, resulting in enhancing nutrients availability for plant growth development and yield production [9]. Therefore, the amendment of different porosities and surface areas of biochar (as result of different particle sizes) into the soil may reveal the different mitigation potential and yield production that lead to the higher benefit of biochar utilization in the rice paddy soil. This study aimed to evaluate the effect of different biochar sizes (0.5-2 mm and 2-4 mm) that affected different porosities and surface areas on soil oxidation capacity and CH$_4$ producing-oxidizing bacteria (methanogens and methanotrophs) to describe the change of CH$_4$ production and emission from rice cultivation soil as well as investigate the rice yield.

2. Materials and methods

2.1. Paddy soil and biochar properties
Paddy topsoil that classified as Vertisols with clay loam texture was collected from Ratchaburi Province, Thailand (13°30’28”N & 99°57’12”E). This soil contains pH (H$_2$O) of 7, CEC of 23 cmol kg$^{-1}$, OM of 1.12 %, total C of 0.65%, total N of 0.06%, available P of 9.47 mg kg$^{-1}$, available K of 88.0 mg kg$^{-1}$, and bulk density of 1.54 g cm$^{-3}$. Mangrove (Rhizophora apiculata) biochar was produced by the pyrolysis at Yisarn community in Samut Songkarm Province of Thailand. Biochar was ground to size of 0.5-2 mm and 2-4 mm before amended into the soil. These two sizes were chosen from the recommended size that suitable for amendment into the paddy field. Biochar properties contain pH (H$_2$O) of 7.80, CEC of 54.4 cmol kg$^{-1}$, total C of 59.5%, total N of 0.28%, total P of 0.23%, and total K of 0.14%. The average of pore volume of 0.5-2 and 2-4 mm were 0.61 and 1.01 cm$^3$ g$^{-1}$, while surface area were 78.45 and 39.76 m$^2$ g$^{-1}$, respectively.

2.2. Experimental design
This study has two experiments were rice cultivation and soil incubation. Both experiments comprised three treatments were no amendment soil (CT), small size (0.5-2 mm) of biochar (SB) and large size (2-4 mm) of biochar (LB). The experiment was carried out with three replications.

2.2.1. Rice cultivation. Biochar was mixed in the soil thoroughly at equivalent rate of 10 t ha$^{-1}$ at 16 days before sowing. Pathumthani 1 rice (Oryza sativa L.) cultivar was planted by the sowing method in the bucket with size of 60 (w) x 90 (l) x 60 (h) cm at King Mongkut’s University of Technology Thonburi, Bangkhuntien campus in Bangkok. The soil was flooded at level of 10 cm continuously since 20 day after sowing (DAS) till 90 DAS. After that, soil was naturally dried with no water adding for harvesting preparation. During cultivation season, fertilizer was used at 100 kg N ha$^{-1}$ equivalent rate that applied compound fertilizer (N-P$_2$O$_5$-K$_2$O) at 20 DAS (35 kg N ha$^{-1}$) and urea (CH$_4$N$_2$O) at 60 DAS (65 kg N ha$^{-1}$). The crop duration of all treatments were 110 days.

2.2.2. Soil incubation. 50 g of soil and 50 mL of deionized water were added in the 100 mL glass vial. Vial’s headspace was flushed with nitrogen gas (99.999%) to create anaerobic environment of soil. The
vial was covered with butyl rubber and aluminum caps. At 20 and 60 days after incubation (DAI), fertilizer solution (ratio 1:1 of fertilizer: water) was injected through septum and covered with laboratory film. All samples were incubated in the closed system at room temperature for 110 days.

2.3. \( \text{CH}_4 \) emission and production measurements

\( \text{CH}_4 \) emission from rice planting was conducted by using a closed-chamber method. Air samples in chamber's headspace were collected at 0, 5, 10, 15 and 20 mins after chamber closure in the mid-morning time during 20 to 110 DAS [10]. \( \text{CH}_4 \) production from soil incubation was measured by the collecting of air sample in vial's headspace during incubation time. Air samples from both experiments were immediately analyzed \( \text{CH}_4 \) concentration by a gas chromatograph (Shimadzu GC-2014, Japan) that equipped with a flame ionization detector (FID) and Unibead C Packed column that using helium as a carrier gas. \( \text{CH}_4 \) flux and cumulative emission were calculated by use the equations that reported in Minamikawa et al. (2015) [10]. \( \text{CH}_4 \) production rate and cumulative production were computed by using the equations given by Treesubsuntorn and Thiravetyan (2012) [11] and Case et al. (2012) [12], respectively.

2.4. Soil microbial analysis

2.4.1. DNA extraction. Soil samples of rice cultivation at 29, 45 and 70 DAS were extracted DNA [13] using a Magnetic bead (Agencourt AMPure XP; Beckman, USA). Optical density (OD) technique with 260 nm wavelength was used to measure the quality and concentration of extracted DNA by using a NanoDrop® ND-1000 spectrophotometer (Wilmington, USA).

2.4.2. Real-time quantitative polymerase chain reaction (qPCR). The extracted DNA was determined the methyl coenzyme M reductase (\textit{mcrA}) gene and particulate methane monoxygenase (\textit{pmoA}) gene in order to quantify the copy number of methanogens and methanotrophs, respectively. MLF/MLR and A189F/Mb661R primers were used for analysis of \textit{mcrA} and \textit{pmoA} genes by using amplification processes that were described in Wang et al. (2019) [14]. Each reaction was performed in a Quant ноя SYBR Green PCR Master Mix (Qiagen, Germany) using real-time qPCR technique with CFX96 Touch System (Bio-Rad, USA) and interpreted the results using CFX ManagerTM Software v3.1. Standard curves were created using 10-fold dilution series of plasmid DNA with the target genes. The reaction efficiencies were 104.5–106% and coefficient R\(^2\) was 0.997–0.999.

2.5. Soil Eh and rice yield measurement

The Eh of rice cultivation soil and incubated soil were analyzed by using a combination sensor: pH/ORP (YSI professional plus, USA) at the same time with gas sampling. Rice yield was sampled in the area of 50 x 25 cm with three points per treatment and weighed on the harvesting date.

2.6. Statistical analysis

Data were presented as mean ± standard deviation (SD). The significant difference was indicated by using one-way analysis of variance (ANOVA) with Tukey’s honesty significant difference (HSD) of the SPSS version 22.0 at a confidence level of 95% (\( p < 0.05 \)).

3. Results and discussion

3.1. Effect of different biochar sizes on soil Eh

Both sizes of biochar that mixed into the soil induced slightly higher soil Eh under anaerobic soil of both experiments (Figure 1 (a-b)). Small size increased higher soil Eh than large size in some period particularly in incubation experiment. The amendment of biochar might improve soil aeration with oxygen increase as result of high porosity and surface area, resulting in soil Eh has been risen [15].
3.2. Effect of different biochar sizes on methanogens and methanotrophs abundances

Both applications of biochar affected the change of soil microbial by suppressed methanogens abundance (mcrA) and stimulated methanotrophs abundance (pmoA) that consistent with the study of Dong et al. (2013) [16]. It resulted to the reduction of mcrA to pmoA ratio by 38%, 49% and 39% in SB and 47%, 57% and 41% in LB on 29, 45, and 70 DAS, respectively. These results showed the statistical difference except on 45 DAS of pmoA gene as compared to CT. Comparison among biochar sizes found that it did not show the significant change of microorganisms (Figure 2). The methanotrophs stimulation and methanogens suppression because of enhancing provision of oxygen in the soil by biochar that promote abundance of aerobic bacteria [17].

3.3. Effect of different biochar sizes on CH₄ production and emission

CH₄ production in incubated soil and CH₄ emission from rice cultivation were reduced in the most periods for both biochar application sizes. The result showed that large reduction of CH₄ emission was found in tillering and heading stages. In term of size comparison, SB produced and emitted lower CH₄ than LB particularly during 30-110 DAI and 40-77 DAS (Figure 1 (c-d)). The cumulative CH₄ production and emission was reduced by 25% and 24% in SB and by 15% and 17% in LB as compared to CT, respectively (Figure 3 (a-b)). SB reduced CH₄ production and emission by 11% and 8% as compared to LB. This indicated that small particle of biochar has more mitigation potential than large particle. However, this effect did not show the significant difference.

The reduction of CH₄ production in both applications of biochar amendment was supported by the enhancement of soil oxidation capacity and suppression CH₄ producing bacteria that indicated the low potential for CH₄ formation under anaerobic soil. It agreed with the study of Liu et al. (2011) [5] and Han et al. (2016) [18], showed that biochar significantly affected the reduction of soil methanogenic activity particularly in tillering and heading stages. CH₄ emission reduction due to the increase of CH₄

Figure 1. Eh of incubated soil (a) and cultivation soil (b), CH₄ production (c) and CH₄ emission (d).

Figure 2. Abundance of methanogens (a), methanotrophs (b) and ratio of methanogens to methanotrophs (c).
oxidation as result of the stimulation of methanotrophs abundance and oxygen availability increase [2]. Han et al. (2016) [18] demonstrated that biochar can increase CH\textsubscript{4} oxidation activity in the rhizosphere area particularly in heading stage. Feng et al. (2012) [8] reported that biochar reduced CH\textsubscript{4} emission by increasing methanotrophic bacteria and decreasing the ratio of methanogens per methanotrophs. These changes did not show the significant difference among SB and LB, so it was no effect of particle size in this study.

![Figure 3](image.png)

**Figure 3.** Cumulative CH\textsubscript{4} production (a), cumulative CH\textsubscript{4} emission (b) and grain yield (c).

### 3.4. Effect of different biochar sizes on rice yield
Figure 3 (c) showed no significant increase of grain yield in SB (2.2%) and LB (1.5%) as compared to CT. The increase of yield production in biochar amendment as result of soil fertility improving particularly carbon status [4], absorption increases of soil nutrients availability, and the increase of nutrients use efficiency [9]. It was according to the report of Zhang et al. (2010) [19] who showed the increase of rice yield in China as resulted of biochar addition. However, the yield increase in this study may be low as compared with other studies, it was might because of the low application rate of biochar. Zhang et al. (2012) [20] demonstrated that the difference of biochar application rates (10 to 40 t ha\textsuperscript{-1}) affected different enhancement of rice yield ranging from 9.21% to 27.6%.

### 4. Conclusions
Both biochar application sizes have potential to decrease CH\textsubscript{4} production and emission from anaerobic soil. The reduction as result of soil oxygen increase, anaerobic bacteria inhibition and refuge providing for aerobe bacteria, resulting in enhancing oxidation capacity of soil through soil Eh increase, stimulating methanotrophs and suppression methanogens as well as reducing the ratio of methanogens to methanotrophs bacteria. The higher mitigation potential was found in small size than large size by an average of 10%, although it did not show the statistical difference. In order to reveal the significance mitigation potential between particle sizes, detail of size differences should be studied. In addition to this, to avoid negative impact on water surface floating of biochar, limited small size of biochar should be concerned.

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