

## 学位論文の内容の要約

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## 【論文の内容の要約】

Biogas slurry (BS), a by-product of biogas production, is attracting attention as an organic fertilizer use in crop production since it contains valuable nutrients for plant. However, its application to agricultural soils may have potential risks to soil and environment due to its contaminants and thus, the effect of its application on the soil ecosystem need to be investigated before their wider use. Since rice field is the major source for CH<sub>4</sub> emission and C is the major substrate for CH<sub>4</sub> producing bacteria, the utilization BS in rice cultivation may have a potential risk of CH<sub>4</sub> emission due to its C contents. Moreover, since BS contains also some heavy metals, its uptake by plant and contamination in soil should also be studied for sustainable utilization of BS. On the other side, plant biomass production and soil C sequestration was investigated as positive effects of its utilization. Moreover, since CH<sub>4</sub> emission may vary depend on rice cultivars, this study also investigated the effect of different whole crop rice cultivars on CH<sub>4</sub> emission combining with BS application. Besides, the changes in population and activities of methane consuming bacteria, methanotrophs, also called methane oxidizing bacteria (MOB) were also evaluated to relate with CH<sub>4</sub> emissions in this study. Thus, this study emphasized the effect of BS application on environmental impacts and benefits as mentioned above in relation to a conventional system using chemical fertilizer in field (for five years) and pot (one year) experiments.

Firstly, field experiment was done with four-year (2009-2012) consecutive application of biogas slurry (BS) at rates of 0 (NF), 100 (BS100) and 300 (BS300) kg N ha<sup>-1</sup> in comparison with a conventional rate of chemical fertilizer CF100 (100 kg N ha<sup>-1</sup>). High biomass productions were obtained from BS applications (1.9±0.1 and 2.1±0.1 dry matter m<sup>-2</sup> in

BS100 and BS300, respectively) compared with CF100 ( $1.8 \pm 0.1 \text{ kg m}^{-2}$ ), indicating BS has a potentiality to substitute chemical fertilizer. Four year's average  $\text{CH}_4$  emissions were significantly ( $P < 0.05$ ) highest in BS300 ( $43.7 \pm 18.2 \text{ g m}^{-2}$ ), followed by BS100 ( $32.1 \pm 3.1 \text{ g m}^{-2}$ ) and then NF ( $23.6 \pm 8.2 \text{ g m}^{-2}$ ) and CF100 ( $20.3 \pm 3.3 \text{ g m}^{-2}$ ), confirming a potential risk of  $\text{CH}_4$  emission from BS application. In this study, no significant differences in Cu and Zn uptakes by the rice plant were observed between BS100 and CF100, but significantly higher Zn content was observed in the grain of BS300 in 2011, indicating a potential risk of Zn uptake in higher application rate, BS300. Compared with CF100, no significant higher Cu and Zn accumulations in soil were observed with four years of consecutive BS application. Higher dosage of BS (BS300) showed higher MOA while there was no different MOA between BS100 and CF100 in each measurement time (at about reproductive stage) of each year, indicating the stimulation effect of N on MOA. Although MOA was higher in BS300, it could not mitigate all the produced  $\text{CH}_4$  in that treatment due to the higher C availability for methane producer. The application of BS to rice fields at the conventional rate ( $100 \text{ kg N ha}^{-1}$ ) may be considered to substitute chemical fertilizer utilization without additional environmental impacts in the greenhouse gas emission and heavy metal uptake from this study.

In 2013, the same fertilization experiment was continuously done at the same field using two different cultivars (TULT and Takanari) to know the effect of cultivars on  $\text{CH}_4$  emission. Methane emission was significantly highest in BS300 ( $80 \pm 19 \text{ g m}^{-2}$ ), followed by BS100 ( $52 \pm 27 \text{ g m}^{-2}$ ), CF100 ( $42 \pm 18 \text{ g m}^{-2}$ ) and NF ( $28 \pm 10 \text{ g m}^{-2}$ ) in TULT variety while it was not significantly different among treatments,  $26 \pm 2$ ,  $26 \pm 2$ ,  $32 \pm 4$  and  $29 \pm 8 \text{ g m}^{-2}$  in NF, CF100, BS100 and BS300 respectively in Takanari variety. Thus, risk of  $\text{CH}_4$  emission by BS application could be mitigated by selecting cultivar, Takanari. It might be due to the higher root biomass in Takanari ( $10.3 \text{ g/hill}$ ) than in TULT ( $8.9 \text{ g/hill}$ ), providing oxygen support to methane oxidizing bacteria (MOB) to oxidize the produced  $\text{CH}_4$  by methanogens, since significantly higher ( $P < 0.01$ ) MOB was found in Takanari than TULT. Although Takanari was effective in mitigating  $\text{CH}_4$  emission by BS application, Cu uptake in grain of Takanari showed a potential risk since its content in BS100 was significantly higher than NF. Although plant Zn contents showed no difference among treatments, Zn content in grain of TULT

showed significantly higher than Takanari, indicating different uptakes by plant depending on different elements. Thus, when selecting an appropriate cultivar to mitigate CH<sub>4</sub> emission, care should be taken for heavy metals uptake with the selected cultivar when BS was applied in rice field in a large amount.

In addition, the effect of different types and rates of biogas digestates on environmental impacts was also examined in a pot experiment in 2013. Treatments were CF300, DBD300, BS300, CF600, DBD600 and BS600 respectively using chemical fertilizer (CF), dry biogas digestate (DBD) and biogas slurry (BS) with two dosages (300 and 600 kg N ha<sup>-1</sup>). Mostly, high biomass production was obtained from both DBD and BS treatments compared with CF. Methane emission was significantly higher ( $p < 0.01$ ) in the BS treatments, followed by DBD treatments than CF under both of the application levels, indicating risk of CH<sub>4</sub> emission from application of biogas digestates. Although DBD treatments showed significantly higher CH<sub>4</sub> emission than CF, it was significantly lower than BS treatments; it might be due to the different types and concentration of C in BS and DBD and a high positive correlation ( $R^2 = 0.9268$ ,  $P < 0.001$ ) was also found between CH<sub>4</sub> emission and the applied amount of dissolved organic C (DOC) in this study, since BS contains high concentration of DOC than DBD. Thus, when applying organic matter in rice field, type of C and its concentration is also important to consider in mitigating CH<sub>4</sub> emission. Although higher CH<sub>4</sub> emission, DBD treatments showed no different Greenhouse gas intensity (GHGI, g CH<sub>4</sub> g<sup>-1</sup> biomass yield) compared with CF while BS showed significantly higher values, suggesting that DBD is suitable to use as an organic fertilizer for safe production and environment.

Thus, from all the above experiments, higher dosage of N showed stimulating effect on methane oxidizing bacteria and their activity, but risk of CH<sub>4</sub> emission was still occurring at the higher dosage of BS when using cultivars Leaf Star and TULT whereas no risk was found in cultivar Takanari in which more O<sub>2</sub> can be available due to the higher root biomass, indicating the importance of O<sub>2</sub> availability to stimulate the activity of MOB. Moreover, since CH<sub>4</sub> is the major substrate for MOB, the higher availability of CH<sub>4</sub> in higher dosage (BS300) may also stimulate the activity of MOB. Thus, this study pointed out the importance of availability of N, O<sub>2</sub> and CH<sub>4</sub> and any of them should not be neglected when evaluating CH<sub>4</sub> emission in rice soil to meet a clear mechanism.

For the conclusion of the whole study, biogas slurry can be used as an effective organic fertilizer to produce high biomass production. However, its application in rice soil caused risk of CH<sub>4</sub> emission although the level of CH<sub>4</sub> risk may vary depend on the type and amount of C contents in the biogas slurry used. Thus, to reduce CH<sub>4</sub> emission, biogas slurry with low C concentration and lower dosage (100 kg N ha<sup>-1</sup>) might be appropriate to use in rice field with selecting appropriate cultivars.