

# ASSESSMENT OF GHGs EMISSION FROM DIFFERENT COW-PAT TREATMENT METHODS AND EFFECTIVENESS OF EM SUPPLEMENTATION

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## ABSTRACT

Greenhouse gas (GHGs) emission is a great concern in the agriculture industry. These gases mainly consist of methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>), and nitrous oxide (N<sub>2</sub>O). GHGs might originate from rice farming, livestock, or manure storage. In countryside areas, there are many approaches to archive manure, however, no evidence for GHGs emission evaluation has been conducted. In this study, we aim to testify the GHGs emission among the common cow-pat treatment approaches, including basking, and in-box inoculation with and without surface lid. Furthermore, effective microorganisms (EMs) named Balasa No.1 and EM Balasa No.5 were also deployed in this study to appraise their effects on cow-pat decomposition and GHGs emissions. Results suggest that the basking method releases the least GHGs as compared to in-box inoculation. In addition, the surface lid generates more CO<sub>2</sub> than to group without a lid for two weeks of observation. The amendment of EMs rises the temperature of the chamber, preferably increasing CH<sub>4</sub> emission in Balasa No.1 treatment while elevating CO<sub>2</sub> production in EM Balasa No.5 treatment. To compromise between decreasing GHGs emissions and cow-pat decomposition/ fertilizer transformation, EM Balasa No.5 seems to be the safe choice per this study.

*Keywords:* Greenhouse gases (GHGs), cow-pat, methane, carbon dioxide, effective microorganisms EM.

## 1. INTRODUCTION

Agriculture activities create significant greenhouse gas (GHGs) emissions, accounting for one-third GHGs released by human activities [1]. The GHGs are mainly methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>), and nitrous oxide (N<sub>2</sub>O). These GHGs partly come from ruminant fermentation or during animal housing and manure storage. Many studies have been funded to investigate and figure out the effective approaches in an attempt to reduce GHGs release [2]. It could be breeding to lower mortalities while gaining more cattle with higher resistance to heat or disease and therefore decreasing an individual number of cattle. Another approach is feeding efficiency. This approach mainly focuses on improving feed efficiency and cattle performance via monitoring the materials of nutrients (eg., corn and legume produce less CH<sub>4</sub> than grass) and keeping from overfeeding nutrients to reduce manure production. Moreover, some additives such as ionophores and some oils are believed to lower CH<sub>4</sub> emission and aerobic manure management is also a considerable choice for lower CH<sub>4</sub> production. Interestingly, composting is a low-cost method to limit CH<sub>4</sub> emission via encouraging aerobic

fermentation while repressing anaerobic fermentation and therefore partly reducing GHGs released. Therefore, attenuation of GHGs release in agricultural activity is possible and could help minimize the negative impacts of climate change.

Microbes play an important role in either serving as GHGs generators or being able to consume, recycle and transform GHGs into soluble nutrients for soil and organisms [3, 4]. Among them, effective microorganisms (EMs)-the mix of microbes, are believed to enhance the turnover of organic waste during composting. Typically, EMs are consisting of three basic types of microorganisms. First, lactic acid bacteria play an important role to maintain low pH conditions, which inhibits pathogenic microbe growth and facilitates the survival of methane-producing microorganisms. The second ingredient is yeast which allows the fermentation initiation. Moreover, photosynthetic bacteria are also important for EM activity. These bacteria metabolize both organic and inorganic substances and convert them into basic cellular materials for amino acids, sugar, or nucleic acid synthesis [5]. With those advantages, EMs might be a useful factor for the attenuation of GHGs in agriculture activity. In this study, we aim to evaluate various local practices of cow-pat treatment, including basking, and in-box inoculation with and without a surface lid. In addition, the amendment of EMs during in-box inoculation is also appraisal. These data would benefit the prevention of GHGs released during cow-pat treatment while suggesting the potential of EM Balasa No.5 in organic waste decomposition.

## 2. MATERIALS AND METHODS

### 2.1. Materials preparation

Per the local practices of cow-pat treatment, the amount of 540 kg cow-pat was divided into three different measures of cow-pat treatment, including basking, and box-inoculation with and without lid. Each group was triplicate and each experiment was duplicated (Figure 1a). *Group 1*, basking, 60 kg cow-pat was basked in the square of canvas 2.8 x 2.8 x 0.1 m (L x W x H) within 7 days and raked twice per day. *Group 2*, 60 kg cow pat was incubated in a foam box and covered with a lid. The distance between cow-pat and lid is 50 cm and monitored within 30 days. *Group 3*, 60 kg cow pat was incubated in foam box without lid within 30 days. For the experiment to evaluate the contribution of effective microorganisms (EMs), 540 kg of cow-pat was divided into three groups, cow-pat was mixed with EM Balasa No.1 or EM Balasa No.5 or without EMs (Figure 1b). As the manufacturer's guide, EMs was pre-processed and activated with rice bran as a ratio of 1 kg EMs:10 kg rice bran: 10 L of distilled water, and anaerobically incubated within 3 days. During pre-processing, EM temperature was maintained below 50°C. Activated EMs were sprinkled into cow-pat evenly before proceeding study.

EM Balasa No. 1 contains 04 main strains of microorganisms: *Streptococcus lactis*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, and *Thiobacillus sp* (NN3b). Meanwhile, EM Balasa No.5 includes strains of microorganisms: *Bacillus subtilis*, *Nitrosomonas*, *Nitrobacter*, *Lactobacillus acidophilus*, *Saccharomyces sp*, *Thiobacillus sp*.

### 2.2. Experiment model

To evaluate the GHGs in each group, the experiment was illustrated in Figure 1a. Chamber is designed at dimension (3 x 3 x 2.5 m). On the top of the chamber, the Polyvinyl chloride (PVC) pipeline (2.8 m, Φ60) was put on and there are 3 rows of holes (dimension 10 mm) and the gap between the holes is 10 cm. The PVC pipeline is connected with the corrugated pipe (Φ34), which is jointed with another PVC pipeline (Φ60). This PVC pipeline

serves as the GHGs collecting tube via two holes covered by rubber stoppers. On the other end, the PVC pipeline is attached to the air vacuum (0.75 kW) and the gases are exhausted via the pipe  $\Phi 34$ . The chamber face is covered by plastic PE to prevent the gases from leaking.

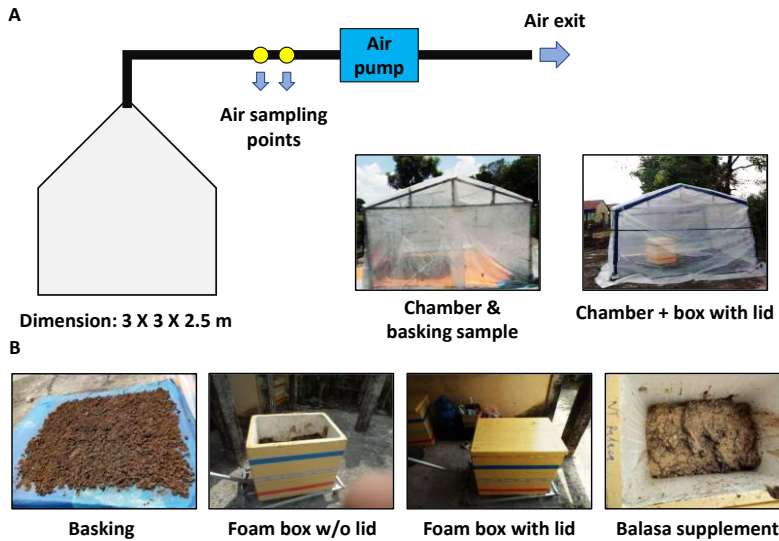


Figure 1. Model for green-house gas evaluating the experiment

## 2.3. Methods

### 2.3.1. Gases sampling and measurement

Criteria such as  $\text{CH}_4$  and  $\text{CO}_2$  were evaluated by the Institute of Animal Sciences for Southern Vietnam as illustrated by Thompson *et al.* 2001 [6]. GHGs ( $\text{CH}_4$ ,  $\text{CO}_2$ ) were measured as protocol below: once/a day in 7 first days, afterward once/3 days from day 8-30. Different groups were put in the chamber with a fan and ventilating air-load was measured by Extech SDL350 (24/24h auto-reader). Air samples were collected and measured by a Gaset FT-IR gas analyzer. Temperature in-door and out-door were recorded by a regular thermometer at  $100^\circ\text{C}$ , immovably hanged inside and outside of the chamber daily at 13h. The pH was determined directly using a pH meter.

### 2.3.2. Greenhouse gases release estimation

Quantification of total gases (including  $\text{CH}_4$  and  $\text{CO}_2$ ):

$$\text{Airflow} \left( \frac{\text{l}}{\text{min}} \right) = \left( \frac{D}{2 \times 1000} \right)^2 \times \pi \times V \times 1000 \times 60 \quad (1)$$

Whereas:

D: The dimension of PVC pipeline serving as gases collecting pipe (mm)

V: Velocity of releasing gases (m/s)

Released  $\text{CH}_4$  quantification

$$\text{CH}_4 \text{ cowpat (ppm)} = \text{CH}_4 \text{ measured (ppm)} - \text{CH}_4 \text{ from environment (ppm)} \quad (2)$$

$$\text{CH}_4 \text{ released} \left( \frac{\text{l}}{\text{min}} \right) = \left( \frac{\text{CH}_4 \text{ cowpat (ppm)} \times V \left( \frac{\text{l}}{\text{min}} \right)}{1000,000} \right) \quad (3)$$

$$\text{weight of CH}_4 \text{ released } \left(\frac{g}{\text{min}}\right) = \left(\frac{\text{CH}_4 \text{ released } \left(\frac{l}{\text{min}}\right)}{22.4}\right) \times \text{MW of CH}_4 \quad (4)$$

Released CO<sub>2</sub> quantification

$$\text{CO}_2 \text{ cowpat (ppm)} = \text{CO}_2 \text{ measured (ppm)} - \text{CO}_2 \text{ from the environment (ppm)} \quad (5)$$

$$\text{CO}_2 \text{ released } \left(\frac{l}{\text{min}}\right) = \left(\frac{\text{CH}_4 \text{ cowpat (ppm)} \times V \left(\frac{l}{\text{min}}\right)}{1000,000}\right) \quad (6)$$

$$\text{weight of CO}_2 \text{ released } \left(\frac{g}{\text{min}}\right) = \left(\frac{\text{CO}_2 \text{ released } \left(\frac{l}{\text{min}}\right)}{22.4}\right) \times \text{MW of CO}_2 \quad (7)$$

### 2.3.3. Statistical analysis

Data are expressed as mean ± SD (Standard Deviation). Experimental differences were examined using ANOVA and Student's *t*-tests, as appropriate by Graphpad prism 6.01. *P* values < 0.05 were considered to indicate statistical significance. Each experiment was duplicated.

## 3. RESULTS AND DISCUSSION

### 3.1. In-box inoculation accelerates GHGs emission

Livestock activities, especially manure composting, have been creating a huge amount of GHGs which mainly are CH<sub>4</sub> and CO<sub>2</sub> emitted from the organic waste. These GHGs contribute to global warming. Therefore, in an attempt to evaluate GHGs emissions from organic wastes in local areas, we have tested different organic waste treatments, popular in almost the countryside of Vietnam, including basking, in-box inoculation with and without living, and cow-pat is employed for this study. Results showed that the CH<sub>4</sub> amount emitted in the basking group exhibited the lowest level after 7 days (D1, D3, D5, D7) observation while in-box inoculation with and without lid, created a higher amount of CH<sub>4</sub> at day 7 (p-value < 0.01 on with a lid and < 0.05 without lid, respectively) (Figure 2a). Regarding CO<sub>2</sub> release, in-box inoculations also created higher CO<sub>2</sub> versus to basking group and increased time-dependently (Figure 2b). Furthermore, in-box inoculation with a lid released the highest amount of CO<sub>2</sub>, compared to without a lid (p-value < 0.001) and the basking group (p-value < 0.0001). Both in-box inoculations increased the temperature of the in-door and out-door chambers as compared to basking treatment (Figure 2c-d). These data suggest that organic waste treatment via in-box inoculation releases a higher amount of GHGs and rises the temperature of the chamber. These results are quite understandable. In basking treatment, organic wastes, for instance, cow-pat, have more chance to contact oxygen and consequently trigger aerobic fermentation. In contrast, CH<sub>4</sub> production is preferable in anaerobic conditions, leading to the higher CH<sub>4</sub> emission in the in-box inoculation as compared to the basking group. Furthermore, the increase of temperature in in-box inoculation is believed to facilitate GHG emission (CH<sub>4</sub>, N<sub>2</sub>O) [7]. Therefore, basking treatment proved itself as the least model in GHGs emissions.

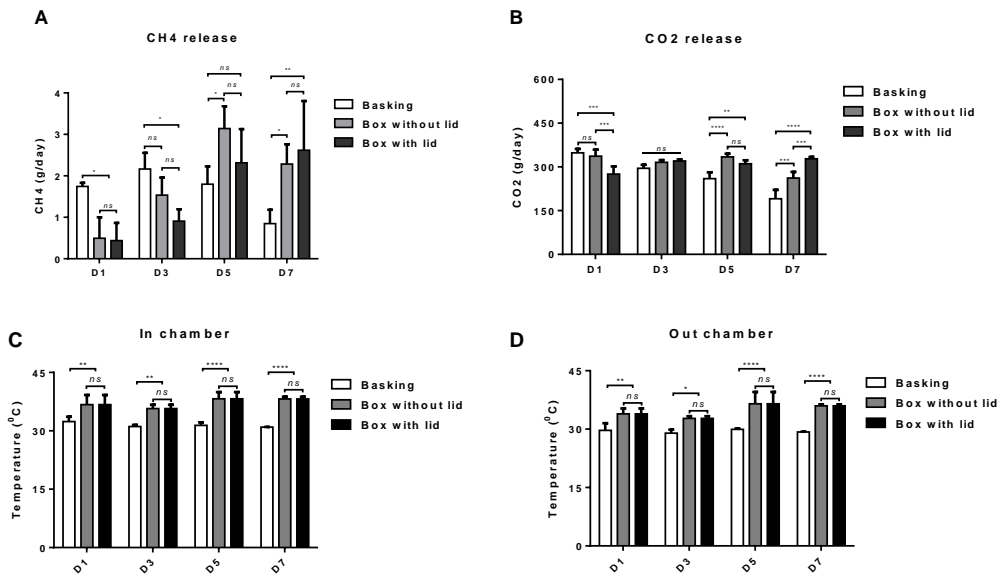


Figure 2. Comparison of various cow-pat treatments to GHGs release. Experiment was conducted within 7 days of observation (D1-D7), and repeated twice. Data are mean  $\pm$  SD,  $n = 3$ , \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ , *ns* means not significant (student's *t*-test).

### 3.2. Long-term lid in-box inoculation is prone to produce more CO<sub>2</sub> emissions than without a lid

Previous data showed that in-box inoculation creates more amount of CH<sub>4</sub> and CO<sub>2</sub> within 7 days. Therefore, we wonder if, in long-term treatment such as 4 weeks, there is any change in GHGs released between with and without lid groups.

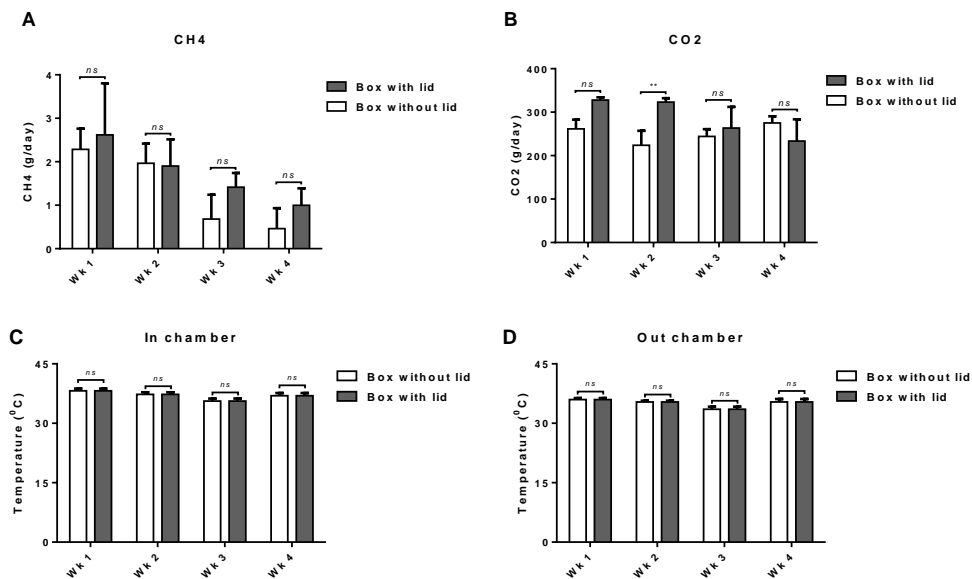


Figure 3. Long-term evaluation of in-box inoculation with and without lid. Data are mean  $\pm$  SD,  $n = 3$ , \* $p < 0.01$ , *ns* means not significant (student's *t*-test).

The cow-pat in-box inoculation with and without lid for 4 weeks has been compared and results showed that there was no significant difference in CH<sub>4</sub> released in both groups (Figure 3a). On the other hand, CO<sub>2</sub> emission in the in-box inoculation group with a lid was higher as compared to the without lid group at week 2 (Figure 3b). Moreover, there was no difference in temperature of in and out-door chambers (Figure 3c-d). These data suggest that 1-2 weeks is the time point that GHGs release reached the peak and starts the plateau phase, and therefore 1-2 weeks of inoculation could be the endpoint for further experiment.

### 3.3. The supplement of effective microorganisms accelerates GHGs released in in-box inoculation

The supplement of EMs has been verified to promote the decomposition of organic wastes [8]. However, we wonder whether the supplement of EMs to cow-pat treatment could increase GHGs emissions. Therefore, the effect of EMs on GHG release within 16 days of observation has been evaluated. Results showed that the amount of CH<sub>4</sub> significantly increased on day 1 and day 4 in the group supplemented with Balasa No. 1 while there was not much difference between EM Balasa No.5 and non-EM groups in all the rest of the observing days (Figure 4a). Regarding CO<sub>2</sub> release, the supplement of EMs increased CO<sub>2</sub> emission at all the time points of observation as compared to the non-EM group, especially in the group added with EM Balasa No.5 while Balasa No.1 creates lesser CO<sub>2</sub> (Figure 4b). In addition, the EM supplement accelerated temperature both in and out chamber from day 4 to day 10 (Figure 4c-d). This evidence suggests that the GHGs release would be minimized in case of in-box inoculation with a lid without EM supplement. However, supplementing with EM Balasa No. 1 leads to higher CH<sub>4</sub> release and EM Balasa No.5 tends to emit more CO<sub>2</sub>.

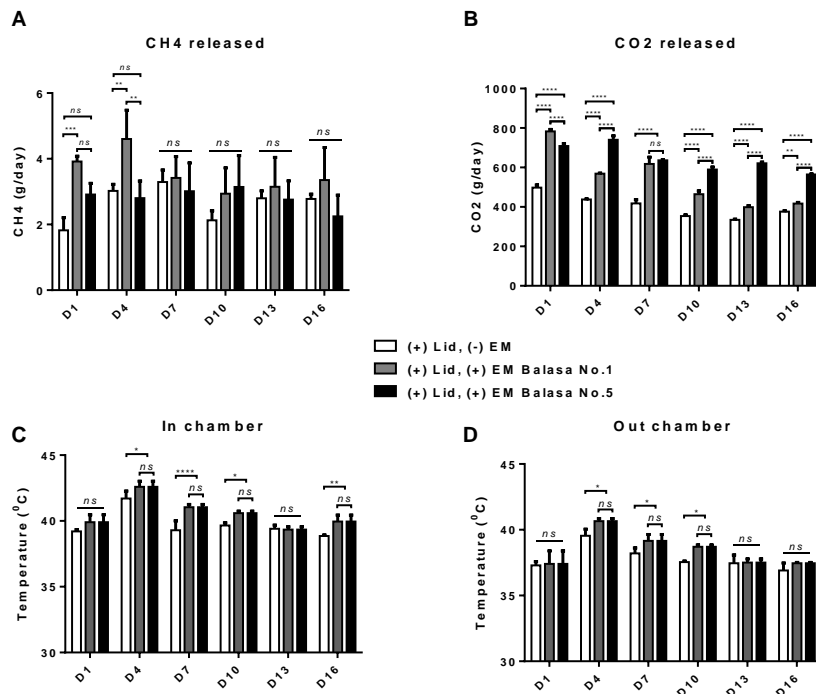


Figure 4. The impacts of EMs supplement to In-box cow pat treatment

The experiment was conducted during 16 days of observation (D1-D16) and repeated twice. Data are mean ± SD, n = 3, \*\*\*\**p* < 0.0001, \*\**p* < 0.01, \**p* < 0.05, ns means not significant (student's *t*-test).

The main difference between EM Balasa No.1 and EM Balasa No.5 is *Streptococcus lactis* (also known as *Lactococcus lactis*), instead of *Nitrosomonas*, *Nitrobacter*, and *Lactobacillus acidophilus* (*L. acidophilus*). Both types of EMs elevate the temperature of the chamber, representing the intensive activity of decomposition. However, the CO<sub>2</sub> level supplemented with EM Balasa No.5 seems to be maintained at a high level, therefore the amendment of EM Balasa No.5 may promote the aerobic condition while Balasa No.1 amendment exhibits the anaerobic fermentation. Indeed, *Nitrosomonas* and *Nitrobacter* are aerobic bacteria while *L. acidophilus* can act in both aerobic and anaerobic conditions [3, 9]. This explains why CO<sub>2</sub> emits higher with EM Balasa No.5 treatment. *Lactococcus lactis* is a facultative anaerobic lactic acid bacterium, therefore resulting in high CH<sub>4</sub> production in the group with Balasa No.1 amendment [3,10]. Nevertheless, CH<sub>4</sub> is believed to negatively impact climate change, 25 time-fold than CO<sub>2</sub> [11], therefore with a certain study purpose, mitigation of GHGs emissions is the priority. Taken together, this evidence firmly clues for EM Balasa No.5 application in an attempt to reduce GHGs emissions while enhancing the decomposition of cow-pat.

### 3.4. In-box inoculating with lid, accelerates cow-pat decomposition

The decomposition of cow-pat reflects CO<sub>2</sub> emission due to the digestion of aerobic microorganisms [3]. Therefore, measurement of CO<sub>2</sub> release would be a powerful indicator for organic waste decomposition progress. In this study, we have collected the air samples for 16 days and measured them every 3 days/time. Results showed that in-box inoculation with a lid, triggered higher CO<sub>2</sub> emissions as compared to the group without a lid (p-value < 0.05, Figure 5).

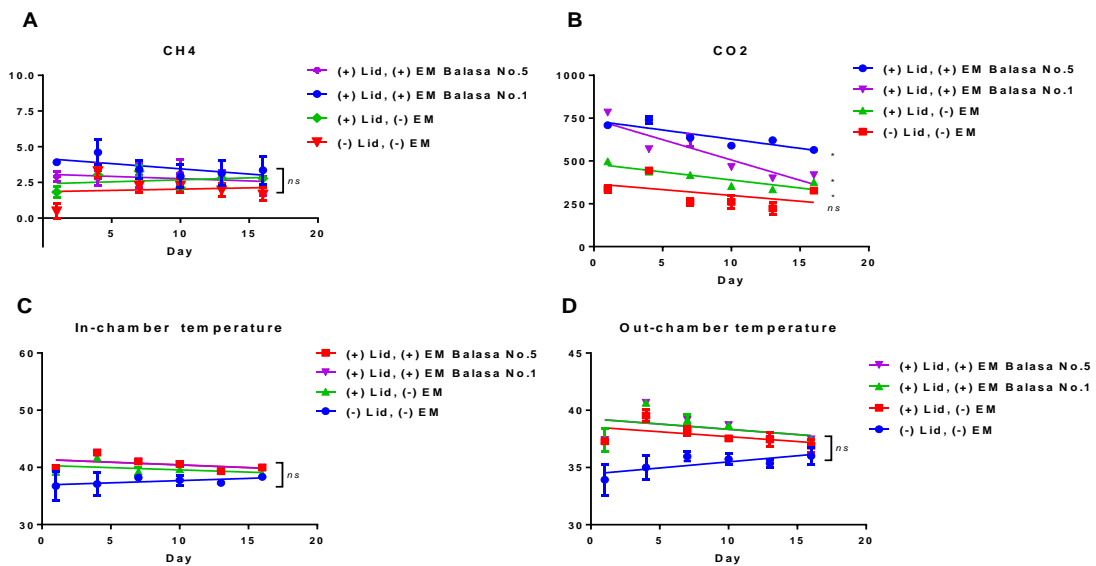


Figure 5. Linear regression between inoculating time and parameters  
The experiment was repeated twice. Data are mean  $\pm$  SD, n = 3, \*p < 0.05, ns means not significant (student's t-test).

Furthermore, the supplementation of EMs resulted in the tremendous CO<sub>2</sub> emission versus non-EM supplement (regressive slope in non-EM, EM Balasa No.1 and EM Balasa No.5 are  $-9.349 \pm 2.679$ ;  $-23.72 \pm 5.337$ ;  $-10.71 \pm 2.887$ , respectively). These data suggest that Balasa No.1 seems to promote impressively the activities of anaerobic-methanogen

microorganisms, leading to the reduction of CO<sub>2</sub> release while EM Balasa No.5 and non-EM present the promotion of aerobic microorganism activity.

Studies on dairy manure management practices found that manure processing contributes to reducing GHG emissions such as CH<sub>4</sub> and CO<sub>2</sub>. Therefore, GHG emissions can vary between 2.2 to 12 tCO<sub>2</sub>e per ton of manure from collection [12]. GHG emitted from the current manure management system (manure is treated in various forms and the rest will be directly disposed into the environment) into the atmosphere is around 400.08tCO<sub>2</sub>/month, respectively a pig emitted about 0.0076t CO<sub>2</sub>/head/month [13].

#### 4. CONCLUSION

The study primarily provides evidence of comparison between various local cow-pat treatments and results displayed that in-box inoculation with and without a lid generates more CH<sub>4</sub> and CO<sub>2</sub> as compared to the basking method during 7 days of observation. For a long-term of observation, the amount of CO<sub>2</sub> in in-box inoculation with a lid creates more CO<sub>2</sub> emission versus without a lid at week 2 (p-value < 0.01), while there CH<sub>4</sub> remains unchanged. The amendment of EMs Balasa No.1 triggered higher CH<sub>4</sub> release (p-value < 0.01), whereas EM Balasa No.5 amendment seems not. Regarding CO<sub>2</sub> emission, EM Balasa No.5 generated higher CO<sub>2</sub> release after 16 days of observation while CH<sub>4</sub> remain invariable after 4 days in comparison to (-) EM treatment. This evidence suggests that supplementing with EM Balasa No.5 minimizes the emission of CH<sub>4</sub> to the environment while accelerating the cow-pat decomposition via intensifying CO<sub>2</sub> release. In summary, the amendment of EM Balasa No.5 would enhance the decomposition of cow-pat, facilitating the fertilization transformation, and could be a friendly option for anti-global warm strategies.

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## TÓM TẮT

### ĐÁNH GIÁ PHÁT THẢI KHÍ NHÀ KÍNH TỪ CÁC PHƯƠNG PHÁP XỬ LÝ PHÂN BÒ KHÁC NHAU VÀ TÍNH HIỆU QUẢ CỦA VIỆC BỔ SUNG CHẾ PHẨM SINH HỌC EM

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Sự phát thải của khí nhà kính (GHGs) là mối lo ngại lớn trong sản xuất nông nghiệp. Những khí chủ yếu bao gồm khí CH<sub>4</sub>, CO<sub>2</sub> và N<sub>2</sub>O. Khí nhà kính có thể bắt nguồn từ các hoạt động trồng lúa, sản xuất chăn nuôi hoặc quá trình ủ phân. Ở một số vùng nông thôn, có nhiều quy trình ủ phân theo cách thức truyền thống, tuy nhiên chưa có đánh giá về hiệu quả của các quy trình trên. Trong nghiên cứu này, nhóm tác giả đánh giá hiệu quả phát thải khí nhà kính ở các biện pháp khác nhau, bao gồm phơi khô, ủ trong thùng có đậy nắp hoặc không đậy nắp. Bên cạnh đó, các vi sinh vật hữu hiệu (EM) bao gồm Balasa No.1 và EM Balasa No.5 cũng được sử dụng trong nghiên cứu, nhằm đánh giá tác động của EM đến quá trình ủ phân và khả năng phát thải khí nhà kính. Kết quả cho thấy rằng, quá trình phơi khô, giải phóng ít khí nhà kính hơn so với phương pháp ủ thùng. Thêm vào đó, việc đậy nắp tạo ra nhiều khí cacbonic hơn so với không đậy nắp. Quá trình bổ sung EM làm tăng nhiệt độ của thùng ủ và Balasa No.1 có khuynh hướng tăng khí CH<sub>4</sub>, trong khi đó EM Balasa No.5 giúp tăng khí CO<sub>2</sub>. Dựa vào kết quả nghiên cứu cho thấy, để cân bằng việc giảm khí thải nhà kính, đồng thời thúc đẩy quá trình ủ phân, phương án bổ sung EM Balasa No.5 là sự lựa chọn được đề xuất.

*Từ khóa:* Khí nhà kính (GHGs), phân bò, CH<sub>4</sub>, CO<sub>2</sub>, chế phẩm sinh học EM.