ENHANCEMENT OF METHANE OXIDATION WITH EFFECTIVE METHANOTROPHIC MIXED CULTURES

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ABSTRACT The emission of CH_4 from landfill is ranked third among the anthropogenic CH_4 sources and ranged between 19-40Tg/yr. The Microbial oxidation of landfill methane plays a significant role in reducing the emissions to the atmosphere. This study was carried out to the investigate the impact of several parameters on methane(CH_4) oxidation, using compost as biocover. Addition of dedicated methanotrophic bacterial cultures was also included.. Experiments with different concentrations of methanotrophic mixed cultures ranging from 2.33X 10^7 CFU/g to 11.33X 10^7 CFU/g showed that the highest oxidation rate with addition of 5.33 X 10^7 CFU/g was 4.166 X $10^3 ugg^{-1}h^{-1}$. Experiments with different incubation temperature showed that highest oxidation rate of 4.166 X $10^3 ugg^{-1}h^{-1}$ was at 35°C. Similar oxidation rates were obtained with the addition of mixed culture at 60% moisture content. The highest bacterial count was obtained at 35°C at 12.33 X 10^7 CFU/g while lowest was at 45°C. The moisture at 60% showed the highest bacterial count at 10.66 X 10^7 CFU/g whereas 30% moisture showed the lowest count at 3 X 10^7 CFU/g. From this study we concluded that the addition of methanotrophic mixed culture gave a significant increase in methane oxidation compared to the control at the optimal temperature and moisture content.

(**Key words**: methane oxidation rate, methanotrophic mixed culture, compost, Biocover)

INTRODUCTION

The amount of Municipal Solid Waste (MSW) generated by human population is constantly growing, especially in developing countries. In Malaysia, the waste generation increased at 3% annually due to the rapid increase in population size and urbanization [1]. Most of the 30,000 tonnes/day of MSW are disposed into non engineered dumpsites. The emission of CH_4 from landfill are ranked third among the anthropogenic CH_4 sources and ranged between 19-40Tg/yr [2]. CH_4 and CO_2 are greenhouse gases (GHG) and CH_4 is recognized as a potent GHG with a global warming potential (GWP) approximately 25 times that of CO_2 [3]. The anaerobic decomposition of solid waste in landfill generates methane (CH_4) [4]. Malaysia shows an average of 1.3-7.5 L/kg/year of methane gas generation [5]. Malaysia's total green house gas (GHG) emission is equivalent to 1.5 $\times 10^{11}$ CDE in 2004 [5]. Global warming is caused by the passive release of GHG to the atmosphere leading to an increased radioactive forcing of the Earth's climate.

Previous studies have shown that microbial CH_4 oxidation in landfill cover soil can be enhanced using substrates that are rich in organic matter, such as compost, rather than pure clay covers [6, 7]. Studies by Streese and Stegmann [8] on compost as a biofilter material for microbial degradation and they reported that high degradation rates of up to 63 g CH_4 $m^{-3}h^{-1}$.The microbial oxidation of methane plays a significant role in reducing the emission of methane to the atmosphere [9]. The oxidation of methane is usually mediated by a group of bacteria called the methanotrophs. Methanotrophs are aerobic microorganisms that use oxygen to oxidize CH_4 to CO_2 and biomass [10].The methane produced in the landfill can be converted to carbon dioxide which is 25 times less harmful greenhouse gas by the oxidation process. Methane oxidation is controlled by several factors, including soil temperature, moisture, exture, as well as pH and nutrient content [11].

The aim of this study was to isolate methanotrophic bacteria that are capable of oxidizing methane gas and addition of these indigenous bacteria, as an effective microbial methanotrophic mixed cultures, to the biocover material under controlled conditions and analyze the methane oxidation rate. The studies also carried out included the impact of temperature and moisture content to determine the optimal condition for methane oxidation in tropical conditions.

MATERIALS AND METHOD

Composting

Compost was obtained by composting a mixture of 75% grass clippings and 25% cow dung .The materials were uniformly mixed to ensure even

distribution of microbes for optimum composting. Heap method was used and composting was carried out under a shade. Water was added to the compost mixture to maintain the moisture level at 60 %. Aerobic condition was maintained by manual turning of composting mixture with daily mixing for the first 8 days and then mixing on alternate days. Temperature of the composting mixture was measured daily using electronic thermometer (Model Oregon Scientific SA880SSX). The moisture content was determined gravimetrically by oven drying compost at 104°C for 24 hours and expressed as the mass ratio of water to drying compost, following the ASTM (2004) procedure. The pH of the compost was measured using the pH meter model HANNA HI 8424. The organic matters was obtained according to ASTM 830-97 standard method. The total Carbon was obtained according to ASTM 777-87 (96) method and Total Nitrogen obtained according to ASTM E778-87 respectively.

Isolation of methanotrophs.

A modified Nitrate Mineral Salt(NMS) medium was used. The medium contained the following, per 1,000 ml of distilled water: $NaNO_3$, 850 mg; K_2SO_4, 170 mg; $MgSO_4 \cdot 7H_2O$, 37 mg; and $CaCl_2 \cdot 6H_2O$, 7 mg. After autoclaving, 10 ml of phosphate buffer solution, 0.5 ml of trace element solution, and 1 ml of iron solution were added. The trace element solution (pH 4.0) contained the following, per liter of distilled water: $ZnSO_4 \cdot 7H_2O$, 10 mg; $MnCl_2 \cdot 4H_2 \cdot 4H_2O$, 3 mg; H_3BO_4, 30 mg; $Na_2MoO_4 \cdot 2H_2O$, 3 mg; $CaCl_2 \cdot 6H_2O$, 20 mg; $NiCl_2 \cdot 6H_2O$, 2 mg; and $CuCl_2 \cdot 2H_2O$, 1 mg. The phosphate buffer contained 1.4 g of KH_2PO_4 and 3.6 g of Na_2HPO_4 in 100 ml of distilled water, pH 6.8. The iron solution contained 1.12 g of $FeSO_4 \cdot 7H_2O$ in 5 ml of 0.25 M H_2SO_4 in 100 ml of distilled water. Methanotrophs were isolated in liquid culture as well as on agar plates. Enrichment cultures (3% [vol/vol] CH_4 atmosphere, NMS medium) from landfill soil samples where previously exposed to CH_4 atmosphere were made and transferred several times to the NMS media.

Cells from these cultures were diluted in NMS medium (10-fold dilution) with a 3% (vol/vol) methane atmosphere to obtain pure cultures of methanotrophs. Cells from enrichment cultures medium were also spread on agar plates. The plates contained NMS medium and 1% (wt/wt) Noble agar. After 2 weeks of incubation in 3% (vol/vol) CH_4, milky colonies from the agar plates were transferred into liquid medium. The absence of heterotrophic contaminants was tested by using complex agar (pH 7.4) containing the following, per liter of distilled water: meat extract, 0.5 g; Bacto Peptone, 0.5 g; yeast extract, 0.1 g; KH_2PO_4, 0.1 g; NaCl, 50 mg; and agar, 15 g. The uniform cell shape of pure cultures was examined by phase-contrast microscope. [12].

Preparation of the methanotrophic bacterial mixed culture.

Three bacteria isolated from the above method were directly inoculated into 10ml NMS media in Wheaton bottle sealed with rubber septa and aluminium foil and 3%(v/v) methane was introduced and incubated for 5 days until the culture turned turbid.

Batch experiments

Batch experiments were carried out using Wheaton bottles. All experiments were performed in triplicates. 20g of compost were transferred into 125ml bottle and the methanotrophic bacteria mixed culture was introduced to the compost ranging from 0.5ml to 3ml concentration and sealed with rubber septa and aluminium seal to ensure gas tight. Then 15 ml of air from the headspace of the Wheaton bottle was withdrawn using an airtight syringe and replaced with 10 ml of O_2 gas (99.98% purity) and 5 ml of CH_4 (99.9% purity) .This amount provided a mixing ratio of approximately 4% of CH_4 (v/v) and 8% O_2(v/v) in headspace. O_2 gas was added into the bottles to ensure that aerobic conditions prevailed during the experiment. The concentration of CH_4, O_2 and CO_2 in the headspace was measured daily using Gas chromatography (Model Shimadzu 8A). The procedures was repeated with temperature and Moisture content variations.

The Influence of incubation temperature

To determine the influence of incubation temperature on methane oxidation, temperature ranging from 30°C to 45°C were tested. Each parameter was done in triplicates with bacterial culture where the cell count are fixed to $5.33\pm0.33X10^7$ CFU/g.

The influence of moisture content

To determine the influence of moisture content on the methane oxidation, moisture content ranging from 30% to 80% were tested, each parameter was done in triplicates with bacteria culture where the cell count are fixed to $5.33\pm0.33X10^7$ CFU/g. The moisture content of the compost was adjusted by adding sterile distilled water to increase the moisture content and by evaporating the water content to reduced the

moisture content to the desired level. Samples were incubated at 35°C.

Table 1: The physiochemical properties of the compost used for methane oxidation.

Moisture content	62.17±0.14 % v/v
pH	6.33±0.12
Organic matter	63.6%
Total Carbon	20.30%
Total Nitrogen	1.20%
Carbon: Nitrogen Ratio	17

CH_4 Oxidation rate Calculation

$$\text{The } CH_4 \text{ Oxidation rate} = \frac{(CH_4)_0 - (CH_4)_n}{W \times N}$$

$(CH_4)_0$= Initial concentration of CH_4 (ml)
$(CH_4)_n$ = Concentration of CH_4 at a time n(ml)
W= the amount of compost(g)
N= time taken for complete methane oxidation (hours)

Statistical Analysis

Statistical analysis of data was carried out using SPSS Statistics version 17.00 for Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

Table 1 shows the physiochemical properties of the compost material. The compost moisture content was 62.17and the pH 6.33.The ability of compost to retain the water is important to sustain the microbial population for the methane oxidation. According to Hilger and Humer[13] compost can offer a good water holding capacity to optimize CH_4 oxidation. The pH of the compost should be neutral to slightly acidic to optimize the CH_4 oxidation[14]. The organic matter of the compost was 63.6%. According to the Chanton and Liptay [15] the methane oxidation is higher in organic rich soils. The C:N ratio of the compost was 17. A high maturity of the compost material is crucial for efficient CH_4 consumption[16].

Figure 1 shows the CH_4 oxidation rate of the compost with addition methanotrophic bacterial mixed culture. The highest oxidation rate was obtained at 4.166X 10^3ug $g^{-1}h^{-1}$ with the addition of 5.33 X 10^7 CFU/g of methanotrophic mixed culture. The comparison with the control shows that the oxidation rate with 5.33 X 10^7 CFU/g of culture was almost double where the oxidation rate of control are 2.083 X 10^3ug $g^{-1}h^{-1}$. The oxidation rate shows a significant reduction after 5.33 X 10^7 CFU/g onwards.

The CH_4 oxidation rate with the addition of 4.66 X 10^7 CFU/g culture shows a difference of almost 10% with the rate a 3.77 X 10^3ug $g^{-1}h^{-1}$.The addition of 6 X 10^7 CFU/g onwards shows a gradual reduction in the CH_4 Oxidation rate where the rate was 2.77 X 10^3ug $g^{-1}h^{-1}$ respectively. The highest CH_4 oxidation rate by compost was obtained at the temperature of 35°C with the addition of bacterial culture, where the oxidation rate was 4.166 X 10^3ug $g^{-1}h^{-1}$ in Figure 2. The comparison without addition of bacteria shows a decrease of almost 50% in the oxidation rate. According to Fauziah *et al.*, [17] the highest oxidation rate was obtained at 35°C for the batch experiment with the landfill cover soil. At 40°C the CH_4 oxidation rate with addition of culture was 9.5% low compared to 35°C where the oxidation rate was at 3.77 X 10^3ug $g^{-1}h^{-1}$.

The oxidation rate at 30°C and 45°C were very low where the oxidation rate are 1.19 X 10^3ug $g^{-1}h^{-1}$. The comparison with 35°C shows that the oxidation rate was almost 71.43% slower. The incubation temperature is very important for the survival of bacteria and the activity of these bacteria to oxidize methane. The findings by Humer and Lechner [18] also indicated that at 35°C, the methanotrophic activity was highest compared to the other incubation temperature studied ranging from 15°C -35°C. Temperature can affect the CH_4 solubility in water, which eventually alters the CH_4 oxidation by the change the CH_4 uptake rate [19].

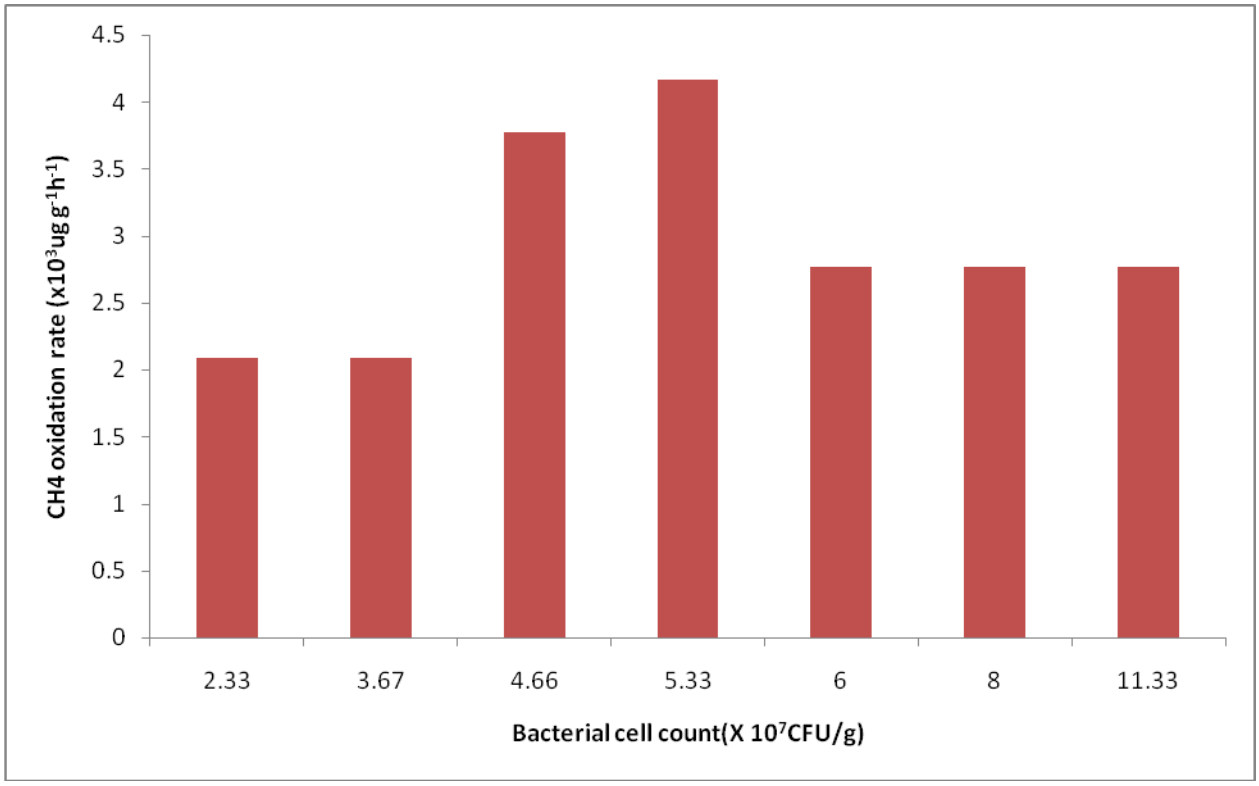

Figure 1: The CH4 oxidation rate with addition of different concentration of bacteria culture.

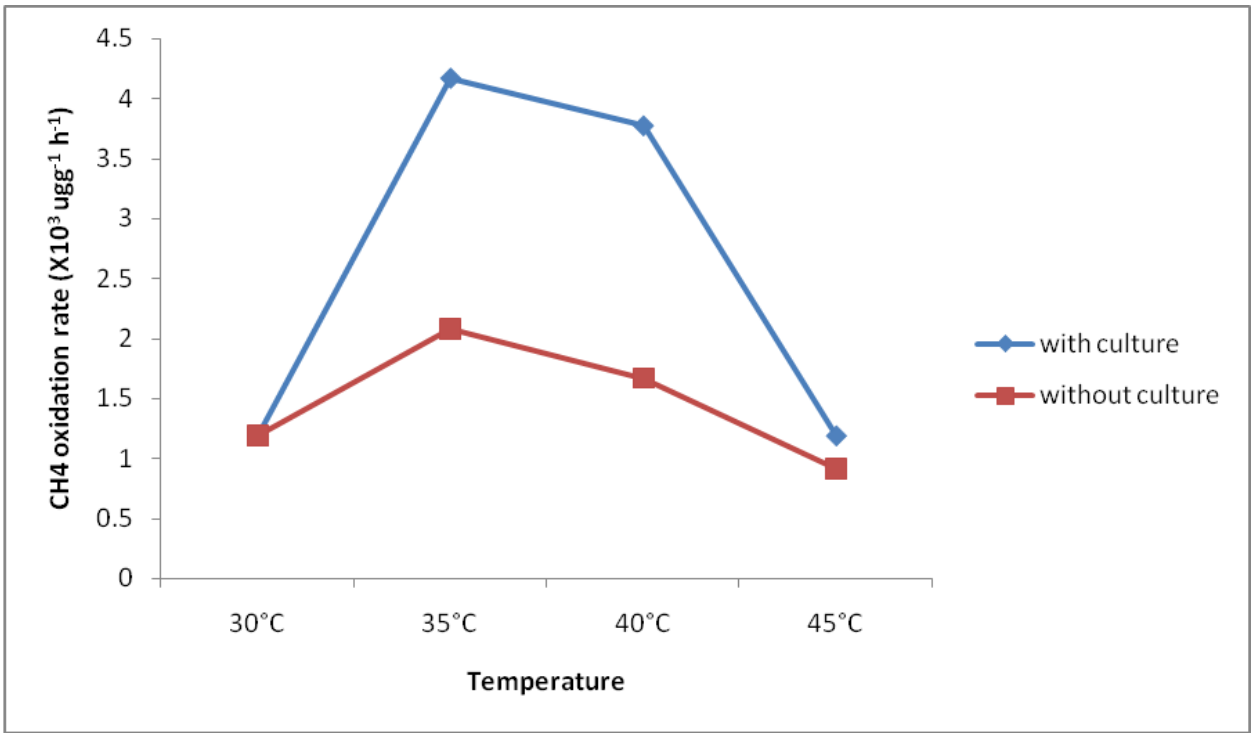

Figure 2: The Effect of temperature on methane oxidation rate with and without addition of bacteria culture to the compost

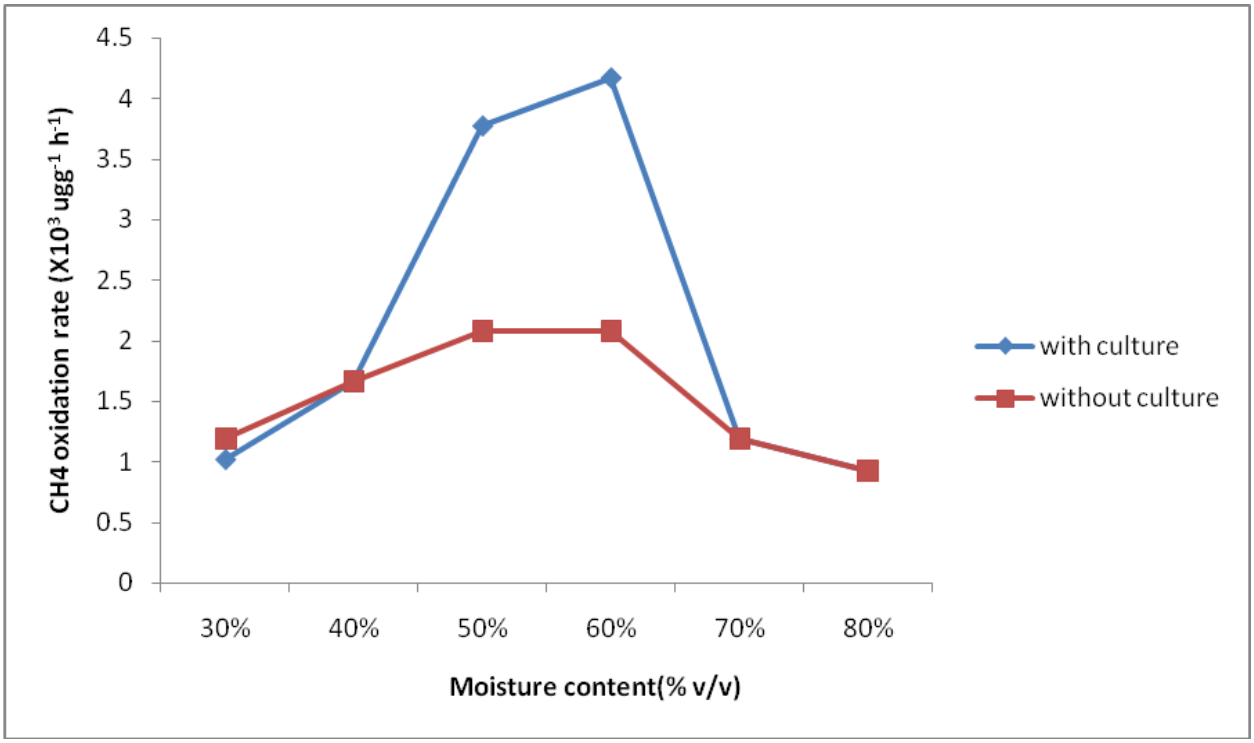

Figure 3: The Effect of moisture content on methane oxidation rate with and without addition of bacteria culture to the compost

Figure 3 shows the CH_4 oxidation rate at different moisture content .The figure clearly shows that the highest oxidation was obtained when the moisture content was 60% , with the addition of culture. This

indicates that suitable moisture content is important for the methane to be oxidized. The oxidation rate was at 4.166 X 10^3ug $g^{-1}h^{-1}$ with the addition of bacteria culture. The Statistical analysis revealed that there were significant differences between with and without addition of microbial culture, at 60% moisture content.

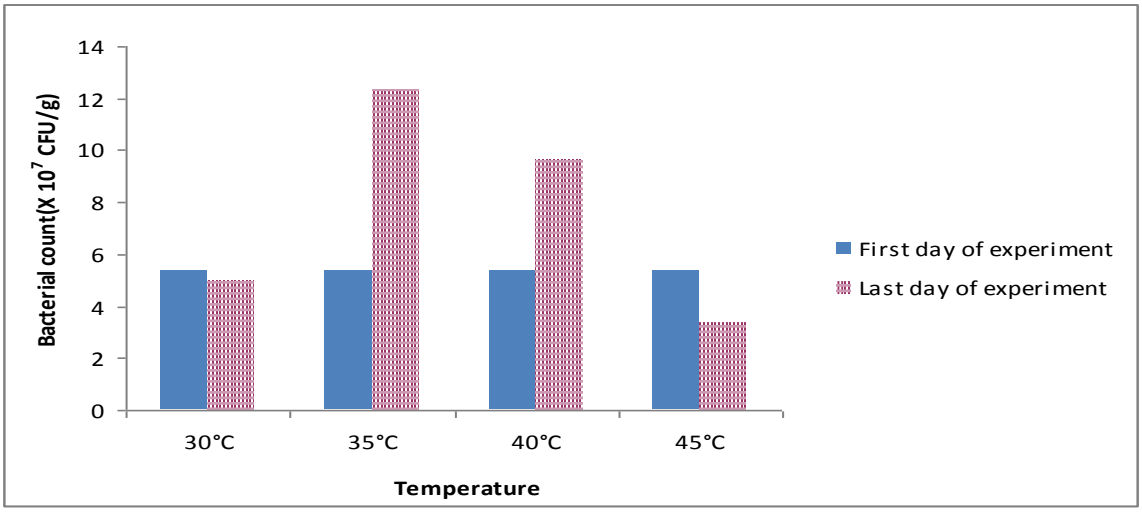

Figure 4: The bacterial count for the effect of temperature on CH_4 oxidation with addition of bacterial culture.

At 40% moisture content the oxidation rate are 1.667 X 10^3ug $g^{-1}h^{-1}$ with addition of culture which is very low compared to the 60% moisture content. The difference in the oxidation rate is almost 60% lower. This indicate that suitable water content is very important for the methane oxidation to occur .The water content of the cover soil is an important factor controlling the methane emissions from landfill[20, 21].

This indicated that the biocovers materials ability to retain water is important to sustain the microbial population for the CH_4 oxidation. Inhibition effects on the methane oxidation at low moisture content have been reported [18]. The CH_4 oxidation rate shows a gradual decrease when the moisture increased to 70% and 80% where the CH_4 oxidation rate was 1.19 X 10^3ug $g^{-1}h^{-1}$ and 0.925 X 10^3ug $g^{-1}h^{-1}$ respectively. There is no significant difference between the control and with addition of microbial culture. Barlaz et al., [19] reported that the compost covers oxidized more CH_4 in field trials, but warned that compost covers can also produce CH_4 if the moisture content is too high.

The bacterial count from Figure 4 also indicated that at the end of the experiment the bacterial count was highest at 35°C where the counts was 12.33X 10^7 CFU/g. At 40°C the counts was 10.663X10^7 CFU/g. The difference in the bacterial count at end of the experiment was due to the incubation temperature where the methanotrophic bacteria grows best at 35°C compared to other incubation temperature.

The lowest count was obtained at 45°C where the counts was 3.33 X10^7 CFU/g. According to Pawloska [23] the methanotrophic bacteria prefers mesophilic conditions as shown in this study.The highest bacterial count was obtained when the moisture content is 60% shown in Figure 5. The counts was 10.66X 10^7 CFU/g. Boeckx *et al* [16] indicated that the water content widely regulates the activity of methanotrophic bacteria. The lowest bacterial count was obtained when the moisture content was 30% where the counting was 3X10^7 CFU/g.Whalen *et al* [23]also indicated that a decrease in the methanotrophic activity when the moisture content are ranged from 30% to 50% v/v.

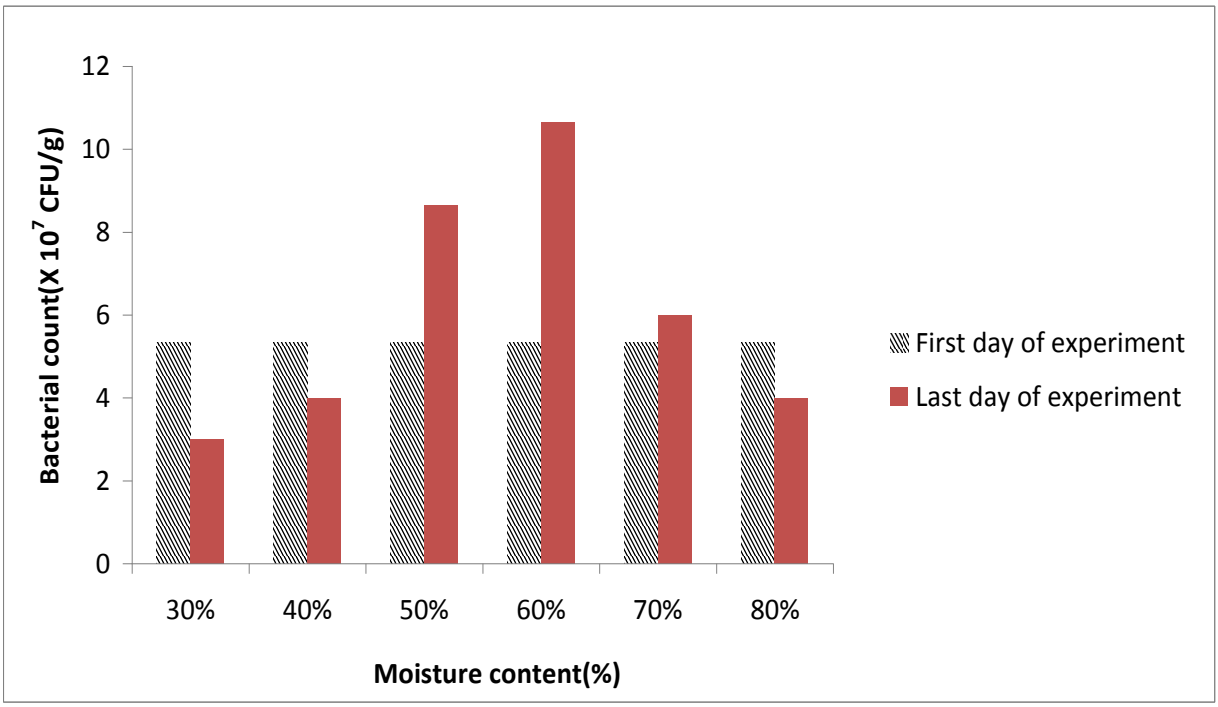

Figure 5: The Bacterial count for the effect of moisture content on CH_4 oxidation with addition of bacterial culture.

CONCLUSION

The isolation and addition of methanotrophic mixed culture to the compost shows an increased in the methane oxidation rate by almost 50% compared to the control. Temperature also play an important role in methane oxidation rate with addition of the culture at fixed amount where from this study we can conclude that the temperature at 35°C shows highest oxidation rate compared to the other temperature 30°C to 45°C. The moisture content also shows highest oxidation rate are at 60%. Therefore the optimal conditions for methane oxidation in this study were temperature of 35°C and moisture content of 60% .

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