

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Physico-chemical properties of Biocover Material

The physico-chemical properties of compost used in this study is shown in Table 4.1. The compost moisture content was 62.17%. One of the important functions of compost is the ability to retain the water content and maintain the bacteria population required for CH₄ oxidation. A study by Humer and Lechner (1999) also indicated that CH₄ emission was controlled by soil moisture content. According to Hilger and Humer (2003) and Wilshusen *et al* (2004) compost can offer good water holding capacity needed to optimize CH₄ oxidation. CH₄ oxidation becomes limited due to physiological stress to methanotrophs if moisture content is low (Pawloska, 2008). Similarly, the pH of the compost should be neutral to slightly acidic in order to optimize CH₄ oxidation (Moldes *et al.*, 2007). In this study, the pH of the compost was slightly acidic (pH of 6.33). Zehnder *et al* (1982) showed that compost works efficiently at a narrow pH range of 6-8. Suitable pH is necessary to favor a balanced microbial population particularly for the optimization of CH₄ oxidation activities. Also, the results showed 52% of organic matter content within the compost. Just as Chanton and Liptay (2000) stated that, CH₄ oxidation is higher in organic rich soils, therefore organic matter serves as the main carrier for the methanotrophic microorganisms and improves the soil properties and substrate.

Table 4.1: Physiochemical properties of compost used for methane oxidation

Test Parameter	Test Method	Result
Moisture content (%)	ASTM 2004	62.17%
pH	ASTM 2004	6.33
Organic matter (%)	ASTM 830-97	52%
Total Carbon (%)	ASTM 777-87 (96)	20.30%
Total Nitrogen	ASTM E778-87	1.20%
Carbon: Nitrogen Ratio	USEPA 3050B	17
Total Potassium	ASTM E 926-94	690.9 ppm
Total Boron	USEPA 3050B	183.6 ppm
Calcium	USEPA 3050B	372.7 ppm
Iron	USEPA 3050B	23.8 ppm
Manganese	USEPA 3050B	3.66 ppm
Magnesium	USEPA 3050B	55.0 ppm
Sodium	USEPA 3050B	0.75 ppm
Copper	USEPA 3050B	0.627 ppm
Total Phosphorus	ASTM D 5198-92	183.6 ppm
Zinc	USEPA 3050B	2.67 ppm
Lead	USEPA 3050B	ND (< 0.01) ppm
Cadmium	USEPA 3050B	ND (< 0.01) ppm
Mercury	APHA 3112B	ND (< 0.002) ppm
Chromium	USEPA 3050B	0.018 ppm
Nickel	USEPA 3050B	0.014 ppm
Aluminium	USEPA 3050B	8.51 ppm
Total Sulphur	EDAX	145 ppm

In fact, field and laboratory trials showed that organic rich soil material have high capacity for CH₄ oxidation (Humer and Lechner, 2001). The C:N ratio of the compost was 17 and it should be noted that maturity of the compost material is crucial for efficient CH₄ consumption (Boeckx *et al.*, 1996). Study by Mor *et al.*, (2006) with garden waste compost is agreeable with the present study as it showed that the C:N ratio ranged from 11.2 to 26.9. The compost used in this study has 1.2% N value which is also within 1.08 to 1.54% value for the garden waste used by Mor *et al.*, 2006. The compost has low copper (Cu) (0.627ppm). Such condition will be deemed favourable since Kjeldsen *et al* (2004) showed that CH₄ oxidation will be higher when the Cu contents are low considering that the methanotrophs only express the sMMO at low Cu concentration.

4.2 Batch experiment on CH₄ Oxidation activity with Matured compost

Batch experiment on the CH₄ oxidation activity was carried out using matured compost. Headspace gas which was analyzed for CH₄, O₂ and CO₂ is illustrated in Figure 4.1. Complete CH₄ oxidation was observed on the fourth day. Depletion of O₂ gas and the production of CO₂ were also observed during the oxidation process. The presence of water droplets in the surface of Wheaton bottle also depicted an oxidation process. According to Scheutz *et al* (2009) the process of CH₄ oxidation is the conversion of CH₄ in the presence of O₂ into water, CO₂ and biomass by microbial activity. Compost took a short acclimatization period (1 day) and it may be due to very high content of organic matter in the compost, hence enhancing the growth of methanotrophic microorganisms (Christensen *et al.*, 1996). Matured compost which

was used in this study oxidized 20% CH₄ within a day. A similar study carried out by Navarani (2009) using black soil and compost residue took 2 and 4 days, respectively, to oxidize similar amount of CH₄. This indicated that the matured compost used in this study has the potential to oxidize CH₄ at a higher rate. Organic components in the compost play an important role in the microbial activity and determination of CH₄ oxidation process (Fauziah, 2009; Insam and Wett, 2008; He *et al.*, 2008; Ritzkowski *et al.*, 2006; Prant *et al.*, 2006).

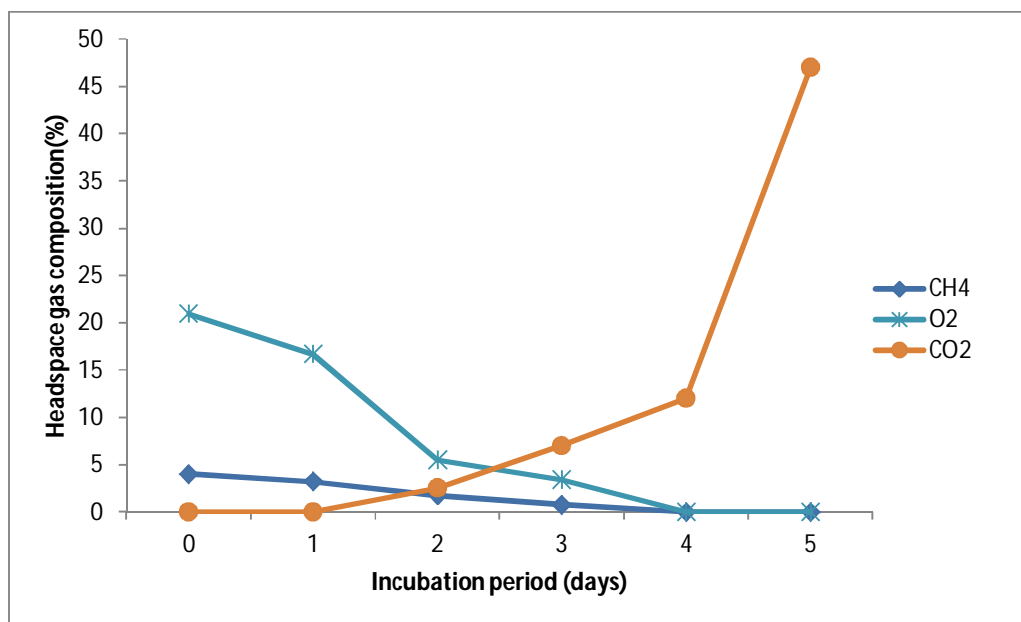


Figure 4.1: Headspace gas analysis for CH₄, O₂ and CO₂

Rapid decline in CH₄ concentration was observed after the acclimatization period. Similar decline in CH₄ concentration between day 3 and 5 was also observed by Perdikea *et al* (2007) while working with garden waste compost. According to

investigations by Humer and Lechner (1999) and Wilshusen *et al* (2004), optimal CH₄ oxidation activity was obtained from diverse, mature and well structured compost materials compared to other cover materials. CH₄ oxidation peak was observed between day 2 to 3. The physico-chemical properties of compost contributed directly to enhance CH₄ oxidation activity by the matured compost material. Methanotrophic bacteria favor slightly acidic environment in undergoing CH₄ oxidation activity and this is typical of compost used in the study. The CH₄ oxidation might have been influenced by the moisture content of compost and this can be supported by findings from Whalen *et al* (2001) who stated that increase in moisture to optimum level will significantly increase CH₄ oxidation activity. Batch experiments carried out by Perdikea *et al* (2007) using compost from garden waste took 6 days for complete CH₄ oxidation whereas similar compost used in this study took only 4 days. Methanotrophic bacterial activity in the compost material used in the study was also observed to be very active, hence a rapid decline in CH₄ concentration was obtained between day 1 and day 3. Comparison between sterilized compost materials and non sterilized compost materials showed that no CH₄ oxidation took place in sterilized compost of the bottle experiment, thereby indicating that bacterial activity is the main mechanism for CH₄ oxidation. Even though O₂ level is low or minimal on day 3 of the experiment, CH₄ oxidation still occurred because methanotrophic bacteria are capable of oxidizing CH₄ at low O₂ concentration (Pawloska, 2008). When O₂ and CH₄ levels decreased, CO₂ level increased. The increase in CO₂ is explained by the fact that when CH₄ is oxidized with the help of O₂, the byproduct of oxidation process will be CO₂. Similar increase in CO₂ level was obtained by Charlotte and Kjeldsen (2000) and Perdikea *et al* (2008) when CH₄ oxidation occurred. Compost used in this

study showed a higher good CH₄ oxidation capacity compared to other biocover materials used by previous researchers such as black soil and compost residue. According to previous researchers diverse, mature and well structured compost materials exhibit higher CH₄ oxidation capacity (Wilshusen *et al.*, 2004). Different types of compost such as MSW compost, sewage sludge compost, biowaste compost and leaf compost have been tested by previous researchers to evaluate the CH₄ oxidation efficiency (Huber and Humer, 2004; Scheutz *et al.*, 2009; Felske, 2003; Wilshusen *et al.*, 2004b). Therefore compost have 2 -3 fold CH₄ oxidation potential than soil (Wilshusen *et al.*, 2004).

Figure 4.2 shows the percentage of CH₄ reduction. At day 1, 20% of CH₄ was oxidized followed by 56% on day 2 and 81% in day 3 and 100% CH₄ oxidation occurred in day 4. Slope gradient of compost used in this study was 26.1. Navarani (2009), recorded a slightly higher rate of 27.55 while using similar biocover material. Value of slope gradient indicated a higher CH₄ oxidation potential of the compost. In another study by Perdikea *et al* (2008), garden waste was used and the value of slope gradient obtained for CH₄ oxidation activity was 15.63 which is low compared to the value obtained from this study. The lower value of slope gradient may be because of the longer time taken for CH₄ oxidation.

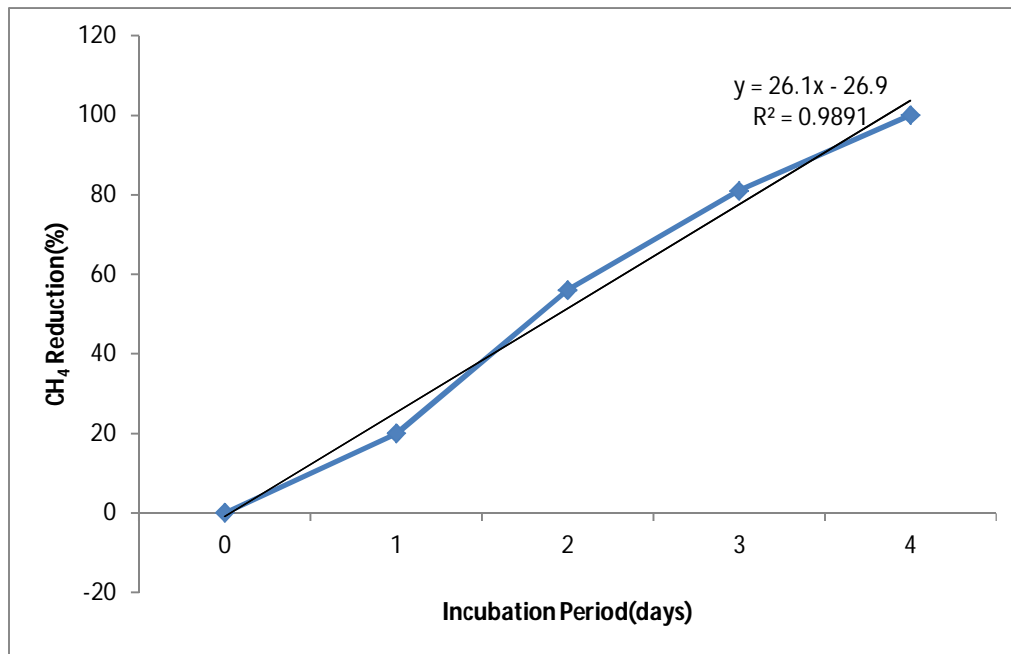


Figure 4.2: Percentage of CH₄ reduction

4.3. Isolation and identification of methanotrophic bacteria

Potential methanotrophic bacteria were isolated and identified as *Methylomonas* sp, *Methylococcus* sp 1 and *Methylococcus* sp 2 based on their characteristics and the ability to consume CH₄. The bacteria were aerobic and gram negative species that used CH₄ as sole energy source. Table 4.2 shows the basic characteristics of the three bacteria, based on Bergey's Manual and phenotypic characteristics of methanotrophic bacteria by Bowman *et al* (1993) and Hanson and Hanson (1996).

Table 4.2: Characteristics of methanotrophic bacteria isolated

Characteristics	<i>Methylobacter</i> sp	<i>Methylobacter</i> sp 1	<i>Methylobacter</i> sp 2
Cell morphology	Rods	Cocci	Cocci
Length(μm)	0.8	2.1	2.3
Width (μm)	0.7	0.8	0.7
Cyst formation	Yes	Yes	Yes
Cell capsule	Yes	Yes	No
Motility	Yes	No	No
Chain formation of the cell	Yes	No	No
Colony morphology	Opaque	Opaque	Opaque
Pigmentation	Pink	Buff	White
Growth in static liquid culture	Surface pellicles	Evenly dispersed	Evenly dispersed
Growth at different pH	pH 6-7	pH 6-8	pH 5-8
Optimum pH	pH 6	pH 7	pH 7
Growth at different incubation temperature	30 - 40°C	35-45 °C	30-40 °C
Optimum temperature	37 °C	40 °C	35 °C
Growth in NaCl (1.5%)	Yes	Yes	Yes
Serine pathway	No	No	No
Oxidase test	Yes	Yes	No
Nitrate to nitrite reduction	No	Yes	No
Urease test	Yes	No	Yes
Phosphatase test	No	No	No
Esculin test	No	No	No
Nitrogen source utilization			
L-Alanine	Yes	Yes	No
L-Arginine	Yes	Yes	Yes
L-Aspartate	Yes	Yes	Yes
L-Citrulline	Yes	Yes	Yes
L-Glutamine	Yes	Yes	Yes
Lysine	No	Yes	Yes
Ornithine	No	No	Yes
Tryptophan	No	Yes	Yes

4.4 CH₄ depletion efficiency by pure culture of methanotrophs (4% CH₄ introduced)

Figure 4.3 depicts the depletion of CH₄ upon the addition of three different types of methanotrophic bacteria isolated and identified as *Methylobacter* sp, *Methylobacter* sp 1 and *Methylobacter* sp 2. Pure culture of methanotrophs (single colony in 10ml of NMS broth) tested for CH₄ oxidation were used as individual cultures to study their CH₄ oxidation consequent upon addition of 4% CH₄. *Methylobacter* sp 1 showed highest CH₄ oxidation activity compared to *Methylobacter* sp and *Methylobacter* sp 2, as it took only 24 hours for complete CH₄ oxidation while *Methylobacter* sp and *Methylobacter* sp took 36 and 48 hours, respectively.

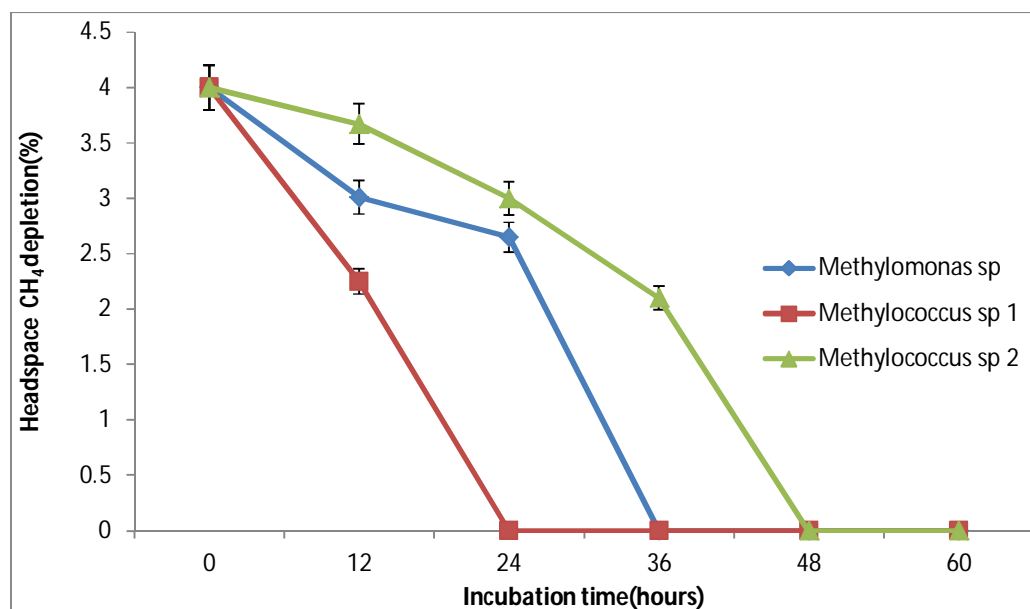


Figure 4.3: CH₄ depletion with addition of pure methanotrophic cultures. (Bar indicates standard error, n=3).

Statistical analysis ($P < 0.05$) showed the significant difference between the three bacteria for CH_4 oxidation potential. Previous researchers tested *Methylobacter* sp for oxidation of CH_4 which was produced from gas phase bioreactors, and had observed that a significant amount of CH_4 were removed or oxidized, hence concluded that this bacteria is also capable of oxidizing CH_4 in mine atmosphere (William *et al.*, 1990). According to Stern *et al* (2007) and Fauziah (2009) the rate of CH_4 oxidation activity depends on the microbial community. Liu *et al* (2011) also observed potential isolates of methanotrophic bacteria which help in reducing the CH_4 produced from coal bed generation process.

4.5 Batch experiment on CH_4 oxidation activity with addition of methanotrophic bacteria individual culture to the compost at different concentration

Batch experiment which was carried out with addition of *Methylobacter* sp to the compost at different bacterial concentration is shown in Figure 4.4. Bacterial test concentration tested ranged from 2×10^7 CFU/g to 1.43×10^8 CFU/g. At 4×10^7 CFU/g to 1.43×10^8 CFU/g, *Methylobacter* sp exhibited a higher CH_4 oxidation activity rate ($4.16 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) which was double the rate obtained from the Control experiment ($2.08 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$).

Further increase in the *Methylobacter* sp bacterial concentration after the optimal concentration of 7×10^7 CFU/g showed a slight decrease in the CH_4 oxidation rate activity. At 1.06×10^8 CFU/g the CH_4 activity was slightly lower at the rate of $3.77 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ whereas 1.43×10^8 CFU/g the CH_4 oxidation rate was similar to the

Control rate, $2.08 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$. This may be due to the changes in the moisture level of the biocover with the addition of the bacterial culture. The CH_4 oxidation rate is influenced by certain factors which include climate variables such as moisture content and the temperature (Bogner *et al.*, 1995; Borjesson and Svensson, 1997). A laboratory experiment by Buvid (1980) and Rees (1980) showed that the methane production rate increased with increased moisture content.

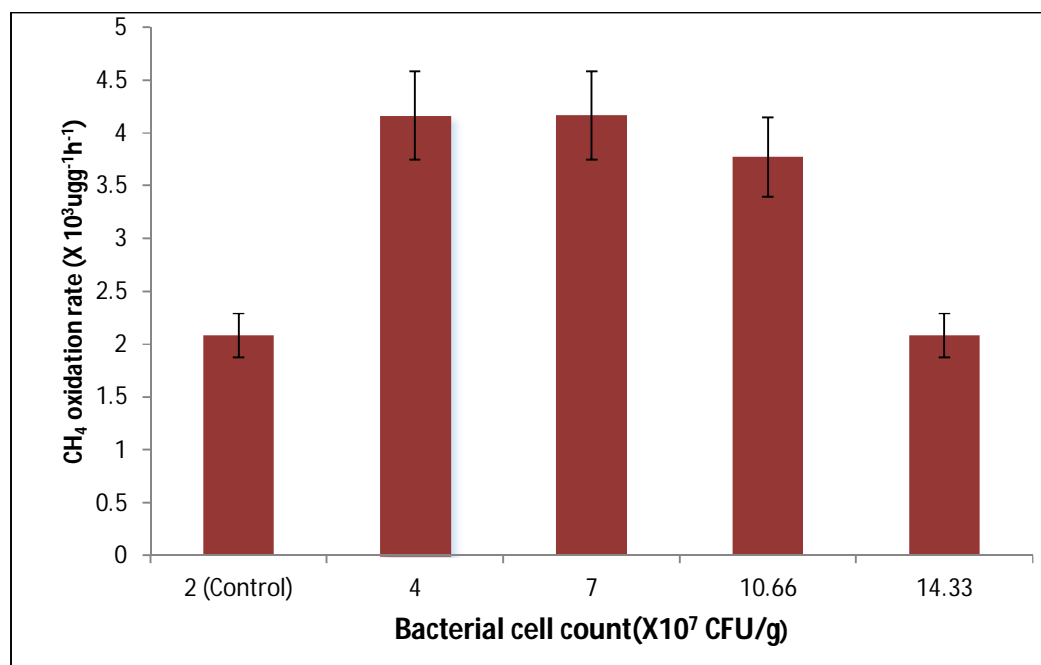


Figure 4.4: Influence of *Methylomonas* sp bacterial culture concentrations on the CH_4 oxidation rate. (Bar indicates standard error, $n=3$).

Figure 4.5 represents the batch experiment with addition of *Methylococcus* sp 1 to the compost at different cell concentrations. Concentrations of bacteria culture tested ranged from 6.33×10^7 CFU/g to 14×10^7 CFU/g. Highest CH_4 oxidation was observed with addition of *Methylococcus* sp 1 at 10.33×10^7 CFU/g with a

corresponding CH₄ oxidation rate of $8.33 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ which is 3 fold increase when compared with $2.08 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ recorded from the Control experiment. Optimum CH₄ oxidation was observed at this concentration. Increased bacteria population at 10.33×10^7 CFU/g could as well be one of the reasons for higher CH₄ oxidation activity compared to Control which had the least bacterial count (2×10^7 CFU/g). At 6.33×10^7 CFU/g CH₄ oxidation rate was similar to that of Control. No significant increase in CH₄ oxidation was observed at this concentration. Further increase in the bacterial concentration of *Methylococcus* sp 1 after the optimum concentration even showed decrease in CH₄ oxidation activity. It may be due to higher concentration of culture which affects the activity of CH₄ oxidation. The CH₄ oxidation activity was observed to be 50% lower, at any further increase in bacterial concentration beyond 10.33×10^7 CFU/g (optimal bacterial concentration).

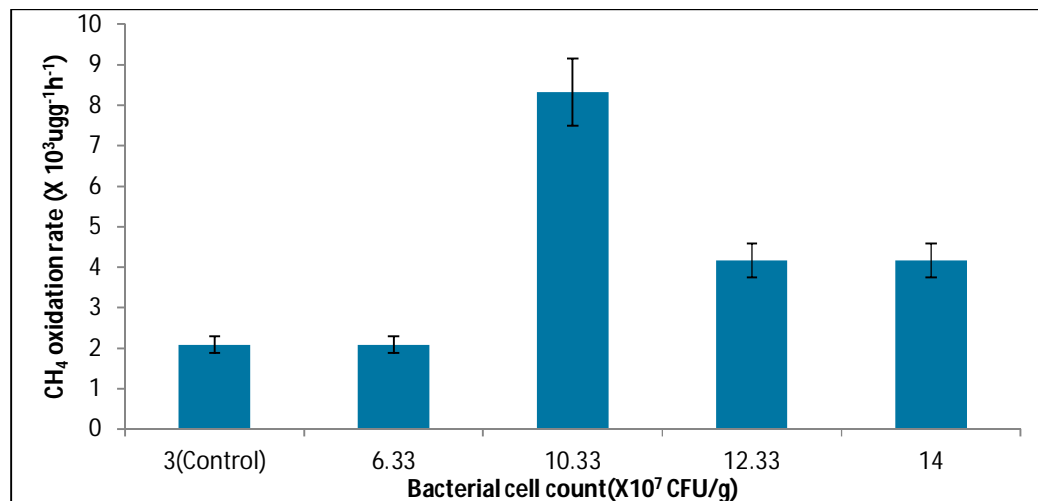


Figure 4.5: Influence of *Methylococcus* sp 1 bacterial culture concentration on the CH₄ oxidation rate. (Bar indicates standard error, n=3).

Similarly, Figure 4.6 shows the influence of *Methylococcus* sp 2 bacterial concentration on the compost CH₄ oxidation rate. The optimal CH₄ oxidation activity was observed in the control and with the addition of *Methylococcus* sp 2 at 7.33 X10⁷ CFU/g, the CH₄ oxidation rate was also 2.08 X 10³ µg g⁻¹h⁻¹. This is reduced compared to higher concentration of 1.19 X 10³ µg g⁻¹h⁻¹ when *Methylococcus* sp 2 concentrations was increased (9 X10⁷ CFU/g to 12.66 X10⁷ CFU/g). However no significant (P<0.05) increase and enhancement in the CH₄ oxidation activity was observed with addition of *Methylococcus* sp 2 bacterial culture to the compost. Unfavorable environment for bacterial growth could be one of the reasons for low CH₄ oxidation activity.

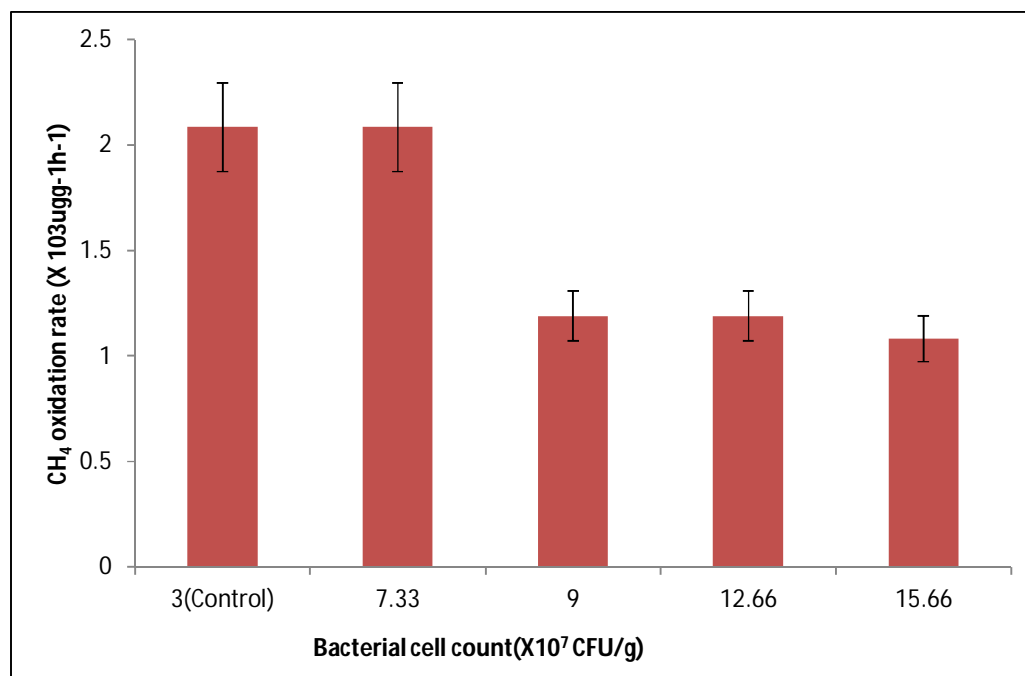


Figure 4.6: Influence of *Methylococcus* sp 2 bacterial culture concentration on the CH₄ oxidation rate. (Bar indicates standard error, n=3).

A study carried out by Wang *et al* (2011) using nitrate mineral salt media (NMS) as nutrient supply to enhance methanotrophic bacteria showed increase in the CH₄ oxidation activity with addition of 0.5ml to 1kg of compost material while further increase in the nutrient to 1.0ml showed a decrease in CH₄ oxidation activity. This shows that NMS without bacteria have impacts to the CH₄ oxidation. It can be explained that even though higher bacteria population is tested in this study, CH₄ oxidation activity are minimal after certain concentrations because NMS media volume plays critical role and affects the CH₄ oxidation activity in higher concentration. Comparison between the three types of bacteria at the optimum concentration showed highest CH₄ oxidation activity with the addition of *Methylococcus* sp 1 ($8.166 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) compared to *Methylomonas* sp ($4.16 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) and *Methylococcus* sp 2 ($2.08 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$). CH₄ oxidation activity with addition of *Methylomonas* sp showed 50% less CH₄ oxidation activity compared to *Methylococcus* sp 1. This may be due to slower bacteria activity of *Methylomonas* sp. Addition of *Methylococcus* sp 2 showed 75% lower CH₄ oxidation activity compared to *Methylococcus* sp 1 and 50% lower activity compared to *Methylomonas* sp.

4.5.1 Batch experiment: Influence of temperature on the CH₄ oxidation rate with the addition of individual cultures *Methylomonas* sp, *Methylococcus* sp 1 and *Methylococcus* sp 2 to compost.

Temperature is one of the important factors in determining the efficiency of methanotrophic activity. The influence of temperature on the CH₄ oxidation activity are tested with addition of individuals cultures of *Methylomonas* sp, *Methylococcus* sp

1 and *Methylococcus* sp 2 at a fixed concentration obtained from previous experiment as shown in Figures 4.4 to 4.6 to the compost. Incubation temperature tested was between 30°C and 60°C. The influence of temperature on the CH₄ oxidation rate was tested with the addition of *Methylobacter* sp to the compost as shown in Figure 4.7. Addition of *Methylobacter* sp shows highest CH₄ oxidation activity at the temperature of 35°C and 40°C and the CH₄ oxidation rate was $4.16 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ compared to lower incubation temperature of 30°C ($1.19 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$). Also at higher incubation temperature of 45°C to 60°C shows lowest CH₄ oxidation activity at rate of $0.83 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$. The addition of the *Methylobacter* sp culture to the compost showed a significant ($P < 0.05$) increase in the CH₄ oxidation rate at incubation temperature of 35 °C to 40 °C compared to the Control. The CH₄ oxidation rate with addition of *Methylobacter* sp shows 50% increase in the CH₄ oxidation activity compared to the Control. *Methylobacter* sp is a mesophilic bacteria and the optimum temperature for the growth is between 35°C to 40 °C. In a study carried out by Siti Aishah (2011), the optimal CH₄ oxidation activity was observed at 35°C where the rate of CH₄ oxidation activity was $2.08 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ using garden waste compost. Similar findings by William (2005) recorded that mesophilic bacteria grow and undertake maximum CH₄ oxidation at 35°C. For the core process for the microbial conversion of CH₄ to CO₂, temperature plays a very important role in the whole oxidation process. No significant CH₄ oxidation activity is obtained with the further increase in the incubation temperature.

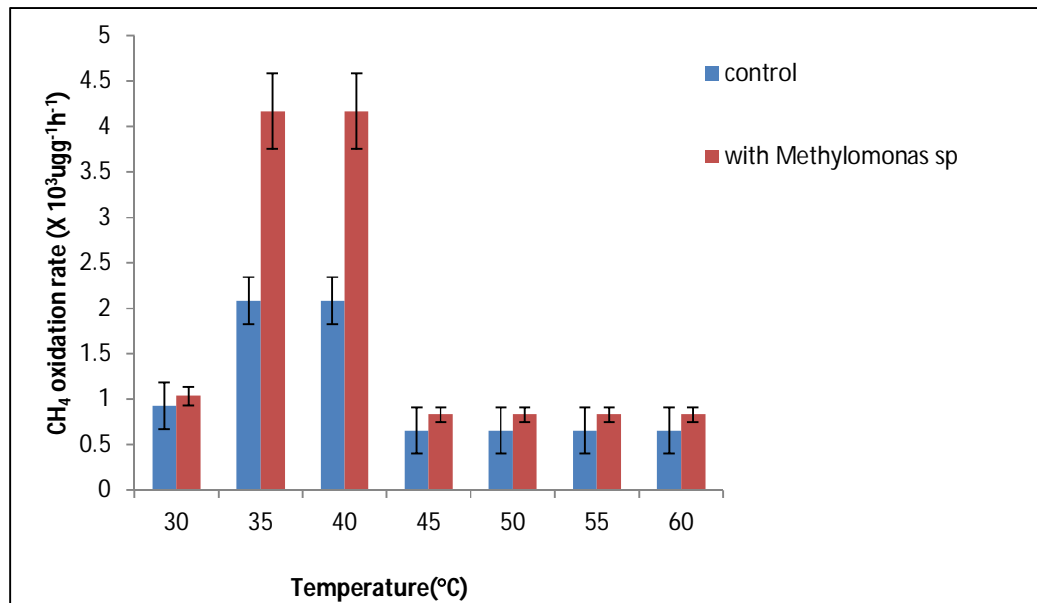


Figure 4.7: Influence of temperature on CH₄ oxidation rate with the addition of *Methylobacterium* sp. (Bar indicates standard error, n=3).

Similar CH₄ oxidation activity was observed between 35°C and 40°C, which may be attributed to the temperature range typical of tropics, hence the microbes have adapted to the environment. Study carried out by Whalen *et al* (1990) using sandy clay soil from landfill cover tested within 5°C to 46°C and the highest CH₄ oxidation activity rate of 1.75X 10³ µg g⁻¹h⁻¹ was recorded at 31°C. But in this study, the highest CH₄ oxidation occurred between 35°C and 40 °C. This may be due to difference in cover materials used and varying environmental conditions of the landfill. Whalen *et al* (1990) also reported that increase in temperature after 31°C showed a smooth decline in methanotrophic bacteria activity and caused a rapid drop to almost zero at 46°C. In this study, further increase after the optimum temperature showed decrease in CH₄ oxidation activities. Similar CH₄ oxidation rate was observed within 45°C to 60°C incubation temperature. This clearly indicates that methanotrophic activities are very

high in mesophilic conditions and temperature rise showed that the activities of bacteria become slower. It may be due to inactivation of enzymes activities due to temperature effect that reduce cellular activity (Fauziah, 2009). A similar study by Pawloska (2008) indicated that methanotrophs prefer mesophilic conditions with the optimum temperature. Changes in incubation temperature affect the growth of biomass and activity of methanotrophic bacteria (Naranjo *et al.*, 2004).

Similar study carried out with the addition *Methylococcus* sp 1 at different incubation temperature was shown in Figure 4.8. Addition of *Methylococcus* sp 1 also showed that the highest CH₄ oxidation activity ($8.3 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) occurred between 35°C and 40°C while the lowest was at 60°C ($0.69 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$). The addition of *Methylococcus* sp 1 at optimal incubation temperature of 35°C- 40 °C showed increase in the oxidation rate which was almost 75% more than obtained in the Control. This may be due to the bacteria adaptation to the environment which encourage rapid CH₄ oxidation. Temperature has been identified to have profound effect on CH₄ oxidation activities. *Methylococcus* species showed an optimal temperature at 37°C and is known to grow within 30°C -50°C (Bergey, 1984). Einola *et al* (2007) also observed higher or optimum CH₄ oxidation at 35°C though the CH₄ oxidation rate was slightly lower. At lower incubation (30°C), the CH₄ oxidation rate was also observed to be very low. Further increase in the incubation after the optimum temperature of 35°C-40°C also showed similar trend with the addition of *Methylomonas* sp.

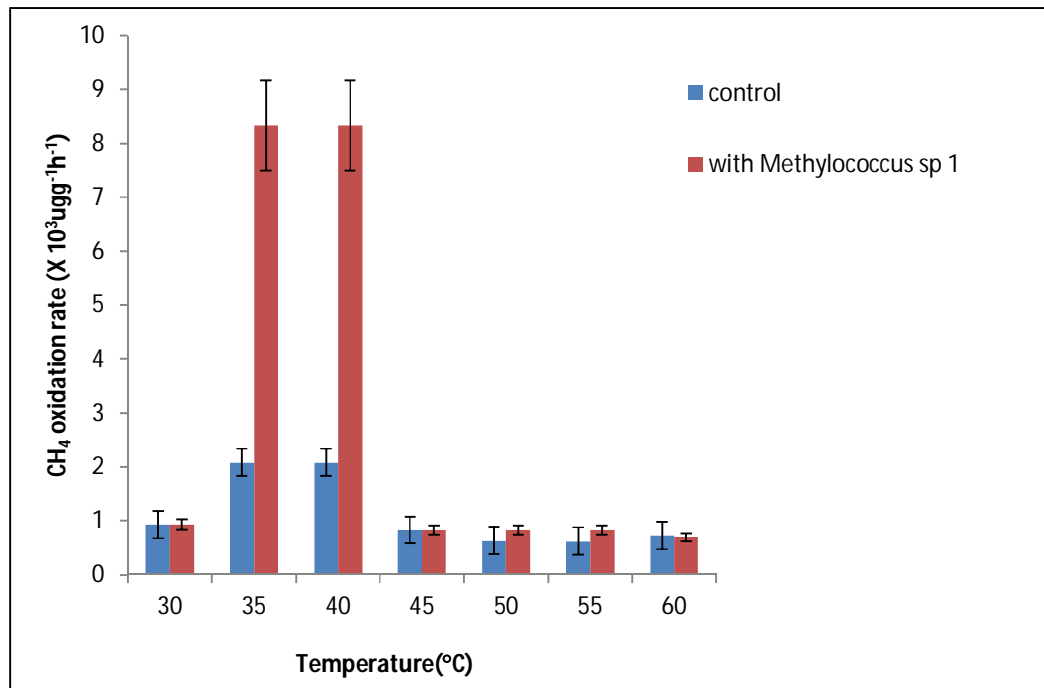


Figure 4.8: Influence of temperature on CH₄ oxidation rate with the addition of *Methylococcus* sp 1. (Bar indicates standard error, n=3).

The influence of temperature on the CH₄ oxidation rate with the addition of *Methylococcus* sp 2 to compost is shown in Figure 4.9. CH₄ oxidation activity when *Methylococcus* sp 2 was added to the compost and tested across different incubation temperature showed highest CH₄ oxidation activity at 35°C with a corresponding oxidation rate of $2.08 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$. At 30°C the CH₄ oxidation rate was $0.757 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ whereas at 40 °C it was $1.38 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ followed by 45 °C to 60 °C ($0.909 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$). However comparison between control and the addition of *Methylococcus* sp 2 showed no significant ($P < 0.05$) difference.

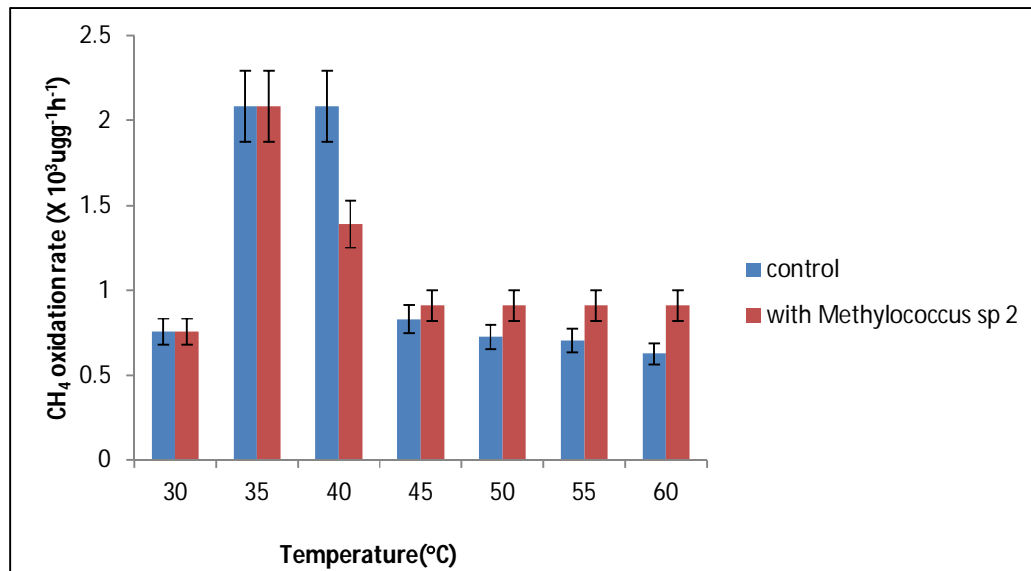


Figure 4.9: Influence of temperature on CH₄ oxidation rate with the addition of *Methylococcus* sp 2. (Bar indicates standard error, n=3).

The comparison at 35°C with the addition of *Methylobacter* sp, *Methylococcus* sp 1 and *Methylococcus* sp 2 showed that the highest CH₄ oxidation rate was obtained with the addition of *Methylococcus* sp 1 since the rate was 50% higher than that for *Methylobacter* sp 1 and 75% higher than *Methylococcus* sp 2. In tropical environment, temperature is the dominant factor controlling CH₄ oxidation and ranged between ~30 to 36°C (Visvanathan *et al.*, 1999). Figure 4.10 shows the bacterial count at the end of experiment at different incubation temperature for the three cultures. The highest count was with the addition of *Methylobacter* sp (1.53 X10⁸ CFU/g) before *Methylococcus* sp 1 and *Methylococcus* sp 2 that recorded 1.23 X10⁸ CFU/g and 1.2 X10⁸ CFU/g respectively.

It also indicates that further increase in the temperature showed lower bacterial count. There was drastic decline in the bacterial count for all the bacteria culture when the temperature was increased from 40°C to 45°C. An increase in temperature to any value exceeding the optimum temperature caused a rapid drop in the CH₄ activity, hence it was almost zero at 46°C (Navarani, 2009).

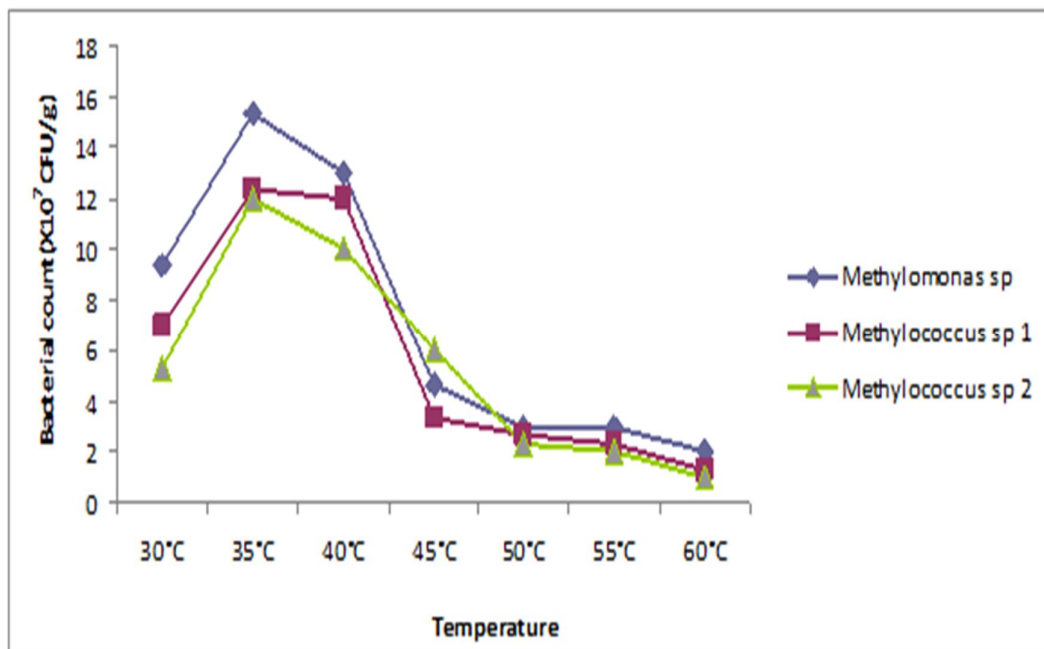


Figure 4.10: Bacterial counts at end of experiment for temperature with the addition of bacteria cultures.

4.5.2: Batch Experiment: The influence of moisture content on the CH₄ oxidation rate with the addition of individual cultures of *Methylobacter* sp, *Methylobacter* sp 1 and *Methylobacter* sp 2 to compost.

Moisture content of biocover material is one of the important factors that influence methanotrophic activity. The influence of moisture content on the CH₄ oxidation activity efficiency was tested with the addition of individual cultures of *Methylobacter* sp, *Methylobacter* sp 1 and *Methylobacter* sp 2 at fixed concentrations obtained from previous experiment as shown in Figure 4.4 to 4.6 to the compost. Biocover moisture content tested ranged from 30% (v/v) to 70% (v/v). The result as shown in Figure 4.11 indicates the CH₄ oxidation rate at different moisture content level upon the addition of *Methylobacter* sp. The highest CH₄ oxidation rate was obtained with the addition of *Methylobacter* sp at 60% (v/v) moisture and the rate was $4.16 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ while the lowest CH₄ oxidation activity was recorded at 30% (v/v) moisture level and the CH₄ oxidation rate was $0.69 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$. Control experiment at 60% (v/v) moisture level showed 50% lower CH₄ oxidation activity when compared to situation where *Methylobacter* sp was added.

Addition of *Methylobacter* sp bacteria has a significant implication on CH₄ oxidation activity and enhanced the microbial activity. At 50% (v/v) moisture the CH₄ oxidation activity was at $2.08 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ when *Methylobacter* sp was added. The lack of water decreased the rate of CH₄ oxidation. The CH₄ oxidation activity shows an increasing pattern when the moisture is increased towards the optimal moisture level and shows a sharp decrease when the moisture is at 70% (v/v). At moisture content of

70% (v/v) the CH₄ oxidation rate was $1.66 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ for both Control and with the addition of *Methylobacter* sp.

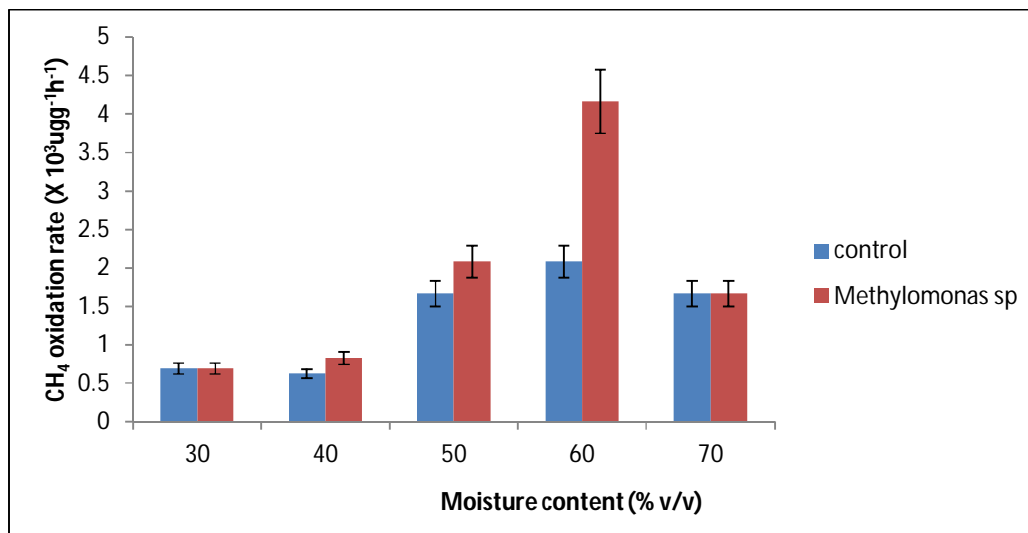


Figure 4.11: Influence of moisture on CH₄ oxidation rate with the addition of *Methylobacter* sp. (Bar indicates standard error, n=3).

Boeckz *et al* (1996) indicated that water content widely regulates the activity of methanotrophic organisms. The activity of *Methylobacter* sp shows inhibition in oxidizing CH₄ when the water content is too low or high. Optimum water content is essential for the activity of methanotrophs. The microorganisms tends to be inactive when the minimum and maximum humidity are not achieved (Bender, 1992). Similar pattern have been obtained with the addition of *Methylococcus* sp 1 and *Methylococcus* sp 2 as indicated in Figures 4.12 and 4.13. CH₄ oxidation with the addition of *Methylococcus* sp 1 to compost shows higher CH₄ oxidation activity when

moisture level is 60% (v/v) ($4.16 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) against the other moisture levels. At the optimal moisture level of 60% (v/v), the CH_4 oxidation was also observed to have doubled with addition of *Methylococcus* sp 1 when compared to the Control. Similarly, upon the addition of *Methylococcus* sp 2 the highest CH_4 oxidation activities were recorded at 60% (v/v) moisture level ($2.083 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) compared to lower moisture level 30% (v/v) ($0.694 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) and highest moisture level of 70% (v/v) ($1.10 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$). As the moisture level in the biocover increased CH_4 oxidation also increased until at 60% (v/v) moisture level and any moisture increase beyond that level shows a gradual decrease in the CH_4 oxidation rate.

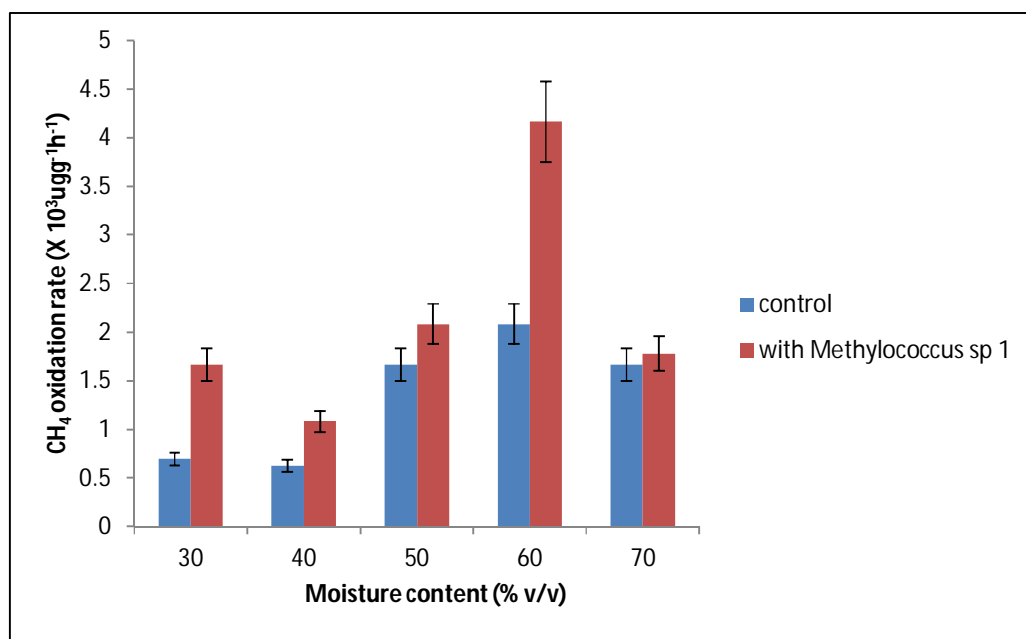


Figure 4.12: Influence of moisture on CH_4 oxidation rate with the addition of *Methylococcus* sp 1. (Bar indicates standard error, n=3).

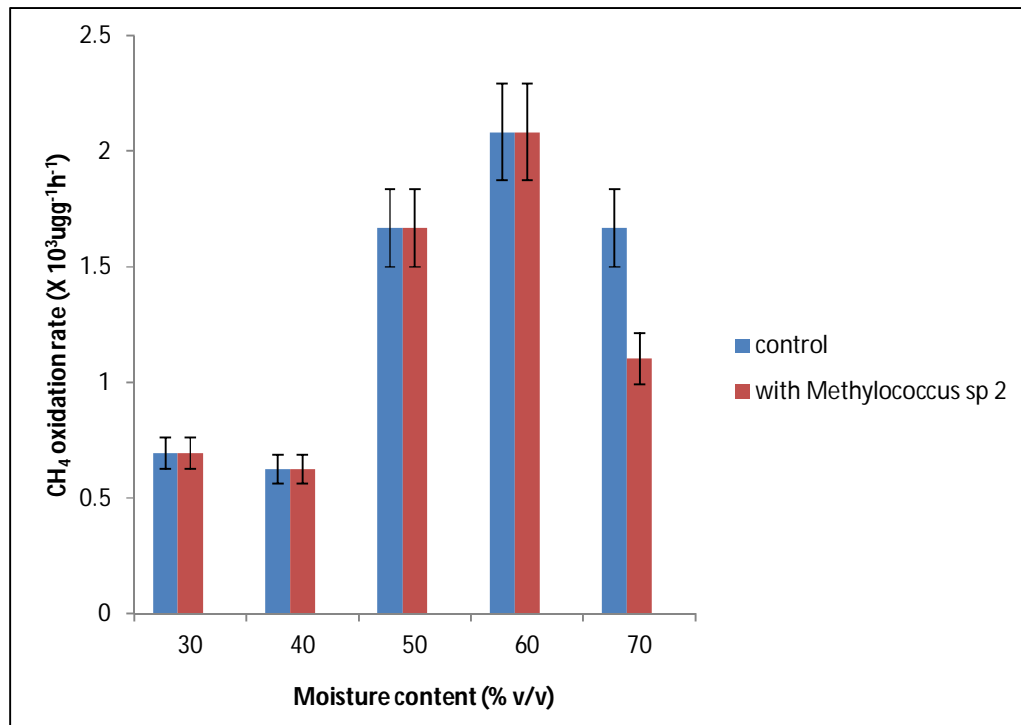


Figure 4.13: Influence of moisture on CH₄ oxidation rate with the addition of *Methylococcus* sp 2. (Bar indicates standard error, n=3).

The reduction in CH₄ oxidation rate at 70% (v/v) moisture level is a reflection of the negative effects of the higher water content in the cover materials (Viswanathan *et al.*, 1999). One of the main effects of increased water content is limitation on oxygen transport from the atmosphere which is supposed to facilitate exchange of substrate, nutrients buffer and possible dilution of inhibitors and the spreading of the microorganisms between the micro environments (Navarani, 2009).

Bacterial count at end of experiment carried out across the different moisture levels with the added bacteria cultures is shown in Figure 4.14. Highest bacterial count was at 60% (v/v) moisture level compared to other moisture level. Bacterial count for

experiment with *Methylomonas* sp at 60% (v/v) moisture level was 11.33×10^7 CFU/g while at lower moisture level 30% (v/v) the bacterial count was 3×10^7 CFU/g which was 73.5 % lower. At higher moisture level (70% (v/v)) the bacterial count was 6.66×10^7 CFU/g.

The bacterial count with addition of *Methylococcus* sp 1 showed similar trend as *Methylomonas* sp where the highest bacterial count was observed at 60% (v/v) (14.33×10^7 CFU/g) while the lowest bacterial count was at 30% (v/v) (2.33×10^7 CFU/g) moisture level. The addition of *Methylococcus* sp 2 also exhibited highest bacterial count at 60% (v/v) moisture. In fact, the bacterial counts at this point accounted 11.33×10^7 CFU/g whereas lowest count was obtained at 30% (v/v) moisture level (2.33×10^7 CFU/g). This indicates that a suitable moisture level is essential for the bacteria to grow and enhance the CH₄ oxidation. Another study by Cabral *et al* (2004) showed that too much moisture may inhibit the process of movement of the gas in the compost or cover material, because the molecular diffusion in water was 10^4 times slower than in the air. Moisture in soil has a strong effect on methanotrophic activity (Pawloska, 2008).

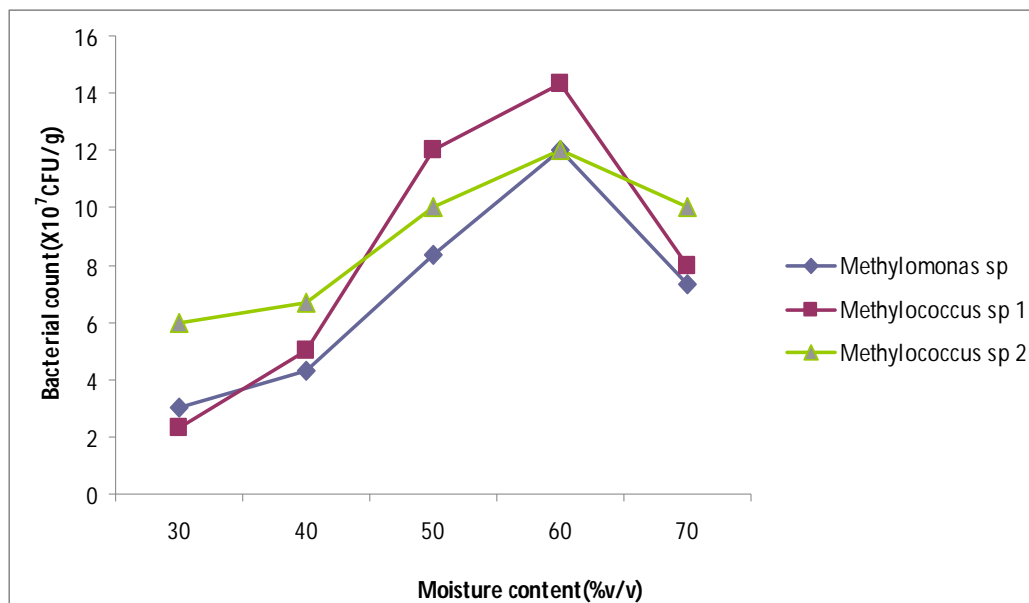


Figure 4.14: Bacterial count at end of experiment for moisture content experiments with the addition of bacteria cultures

4.5.3: Batch experiment: The influence of pH on the CH₄ oxidation rate with the addition of individual cultures of *Methylobacterium* sp, *Methylococcus* sp 1 and *Methylococcus* sp 2 to compost.

Biocover pH has a direct impact in methanotrophic activity. Influence of biocover (compost) pH on the CH₄ oxidation activity efficiency was tested with the addition of individual cultures of *Methylobacterium* sp, *Methylococcus* sp 1 and *Methylococcus* sp 2 to the compost at fixed concentration which were obtained from the previous experiment. The pH test range was pH 5 -8. The influence of pH on CH₄ oxidation rate, with the addition of *Methylobacterium* sp is shown in Figure 4.15. It clearly shows that the highest CH₄ oxidation rate ($4.16 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) was obtained at pH 6 with

the addition of *Methylobacter* sp bacteria at $1.66 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$, $2.08 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ and $0.72 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ recorded across pH 5, 7 and 8, respectively.

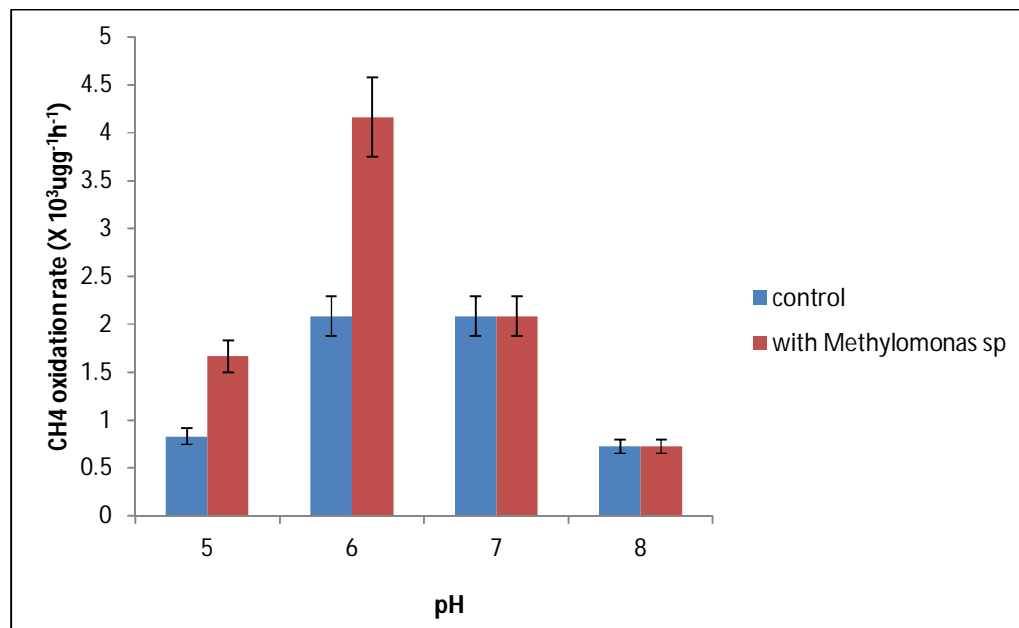


Figure 4.15: Influence of pH on CH₄ oxidation rate with the addition of *Methylobacter* sp. (Bar indicates standard error, n=3).

Control also showed highest CH₄ oxidation activity at pH 6 and 7 but the CH₄ oxidation rate was lower when compared to addition of *Methylobacter* sp. The CH₄ oxidation rate of control was $2.08 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$. According to Hutsch (1994) the highest CH₄ oxidation rate is usually observed at pH values of 6-7, while according to McBean *et al* (1995) the top cover of landfill pH should range from 5 to 8. This shows that the methanotrophic grows well at a narrow pH of 6. Previous researcher also reported that almost all type of methanotrophic bacteria grow at pH more than 5 (Pawloska, 2008).

Figure 4.16 shows the effect of pH on CH₄ oxidation rate with the addition of *Methylococcus* sp 1 to the compost. Similarly the CH₄ oxidation difference between pH 6 ($4.16 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) and pH 5 ($1.67 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) was almost 60%. In the same vein, at pH 7 and 8 the CH₄ oxidation rates ($2.08 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ and $0.72 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) were 50% and 83%, respectively, less than rate obtained at pH 6. It clearly indicates that the activity of methanotrophs are inhibited when the pH value are altered. Addition of *Methylococcus* sp 1 at pH 6 shows 50% increase in CH₄ oxidation activity compared to the control. An increase in pH after pH 6 shows very limited methanotrophic activity since CH₄ oxidation rate shows declining trend. Rozej and Stepniewski (2008) observed a strong increase in CH₄ oxidation activity when pH of cover material was less than pH 8.

The CH₄ oxidation activity with the addition of *Methylococcus* sp 2 (Figure 4.17) shows the highest CH₄ oxidation activity at pH 6, with a corresponding CH₄ oxidation rate at $2.77 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ unlike pH 7 and 5 that had CH₄ oxidation rate of $2.08 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ and $1.517 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$, respectively. Similar to experiment carried out with addition of *Methylobacter* sp and *Methylococcus* sp 1 the optimal pH for maximum CH₄ oxidation was observed at pH 6. The influence of the biocover pH shows a direct implication in the CH₄ oxidation, and optimal CH₄ oxidation occurs in slightly acidic conditions.

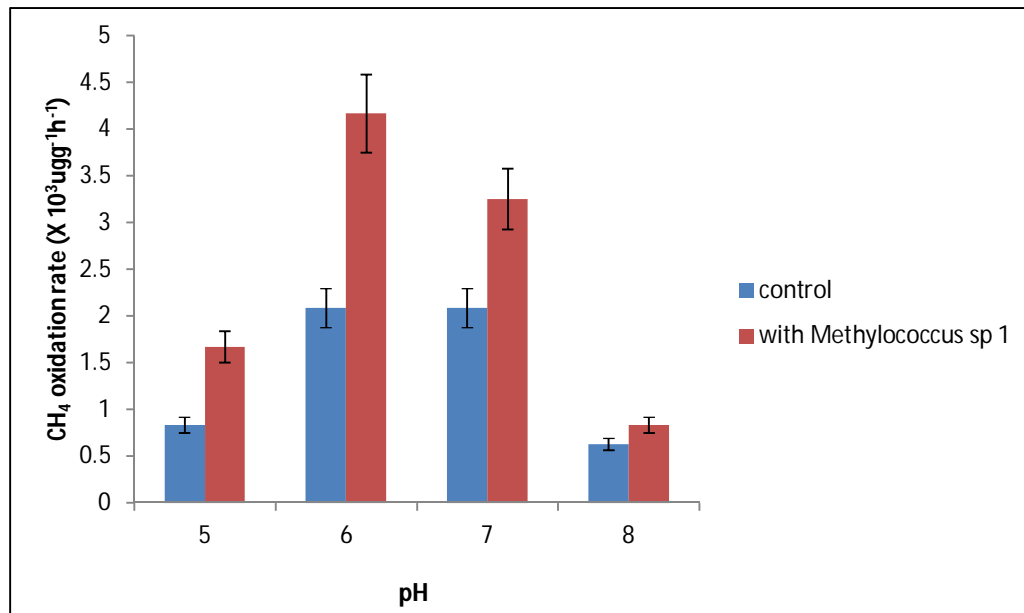


Figure 4.16: Influence of pH on CH₄ oxidation rate with the addition of *Methylococcus* sp 1. (Bar indicates standard error, n=3).

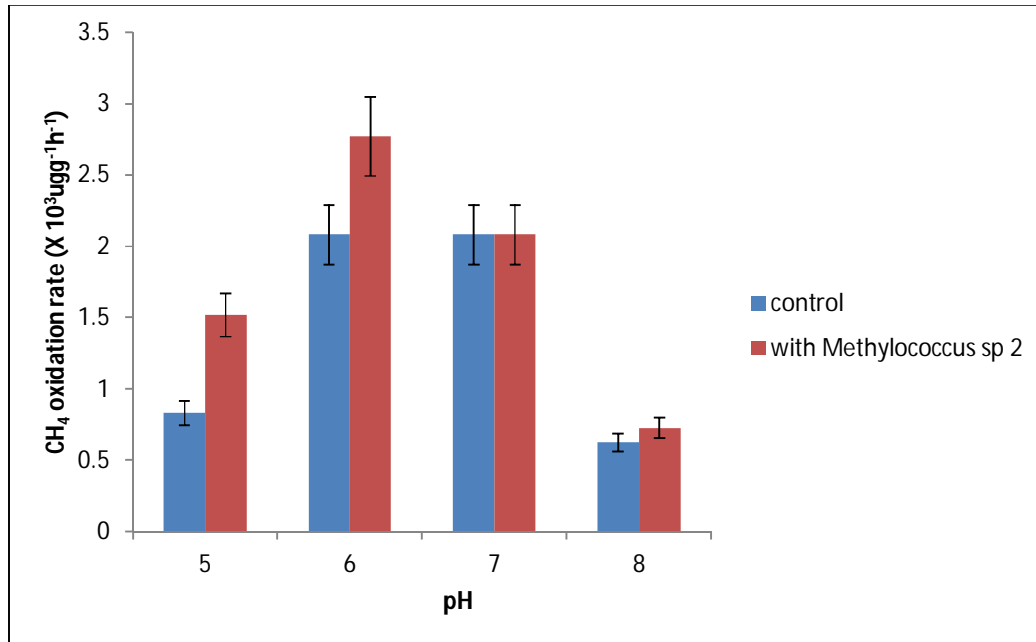


Figure 4.17: Influence of pH on CH₄ oxidation rate with the addition of *Methylococcus* sp 2. (Bar indicates standard error, n=3).

Comparison of CH₄ oxidation activity between *Methylococcus* sp 2 with *Methylomonas* sp and *Methylococcus* sp 1 at pH 6 showed 34% lower CH₄ oxidation activity. The lower CH₄ oxidation of *Methylococcus* sp 2 may be due to lower methanotrophic bacterial activity.

Among different biocover pH (5-8) tested with the addition of *Methylomonas* sp (Figure 4.18) the highest bacterial count was observed at pH 6 (11×10^7 CFU/g), followed by pH 7 (7.66×10^7 CFU/g) and pH 8 (5.33×10^7 CFU/g) whereas the lowest count was at pH 5 (4.66×10^7 CFU/g). Similar to addition of *Methylomonas* sp the highest bacterial count with addition of *Methylococcus* sp 1 was at pH 6 and lowest bacterial count was at pH 5. At pH 6 the bacterial count was 11.33×10^7 CFU/g while at pH 5 the bacterial count was 4.66×10^7 CFU/g. Variation in the bacterial count may be due to changes in biocover pH which limits or enhance bacterial growth. Methanotrophs grow well at slightly acidic pH but lower pH the environment became toxic to the bacteria. The bacterial count with addition of *Methylococcus* sp 2 (Figure 4.18) shows highest count at pH 6 and lower count at pH 8. At pH 6 the bacterial count was 7.66×10^7 CFU/g and pH 8 count was 3.66×10^7 CFU/g.

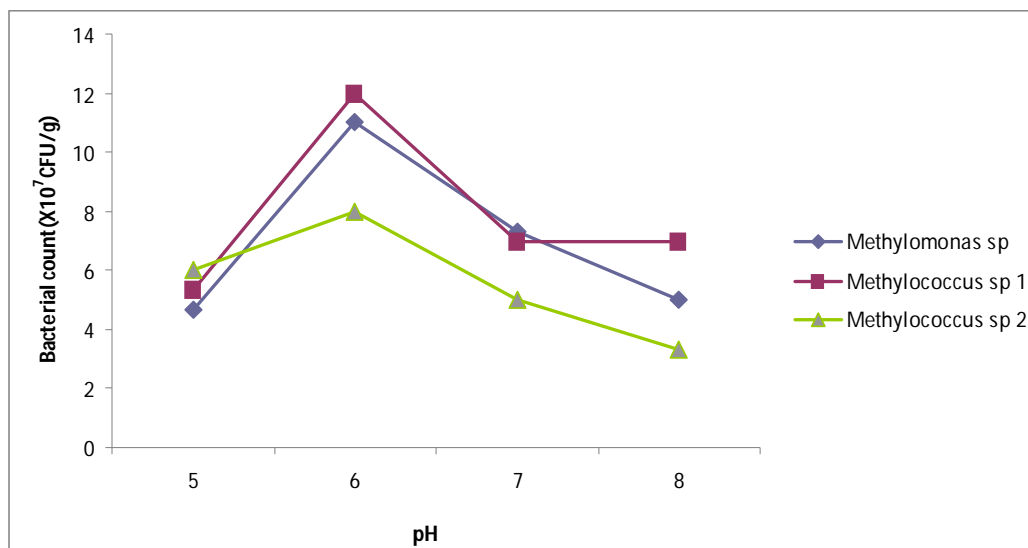


Figure 4.18: Bacterial count at end of experiment for pH experiments with the addition of bacteria culture

4.6: Batch experiment on CH₄ oxidation activity with addition of methanotrophic bacteria mixed culture (*Methylobacter* sp, *Methylobacter* sp 1 and *Methylobacter* sp 2) to the compost at different concentration.

Figure 4.19 shows the CH₄ oxidation rate of the compost with the addition of methanotrophic bacteria mixed culture. The highest oxidation rate was obtained at $4.16 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ with the addition of 5.33×10^7 CFU/g of methanotrophic mixed culture compared to lower concentrations of mixed culture 3.66×10^7 CFU/g ($2.08 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) and 4.66×10^7 CFU/g ($3.77 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) with difference of almost 10%. At higher concentration of 6×10^7 CFU/g onwards there was a gradual reduction in the CH₄ oxidation rate. Optimum CH₄ oxidation activity was at 5.33×10^7 CFU/g and further increase in the bacterial concentration showed lower CH₄

oxidation activity. This may be due to changes in the biocover moisture level with increase in bacterial concentrations which affects the CH₄ oxidation activity. High moisture level in biocover will result in lower CH₄ oxidation.

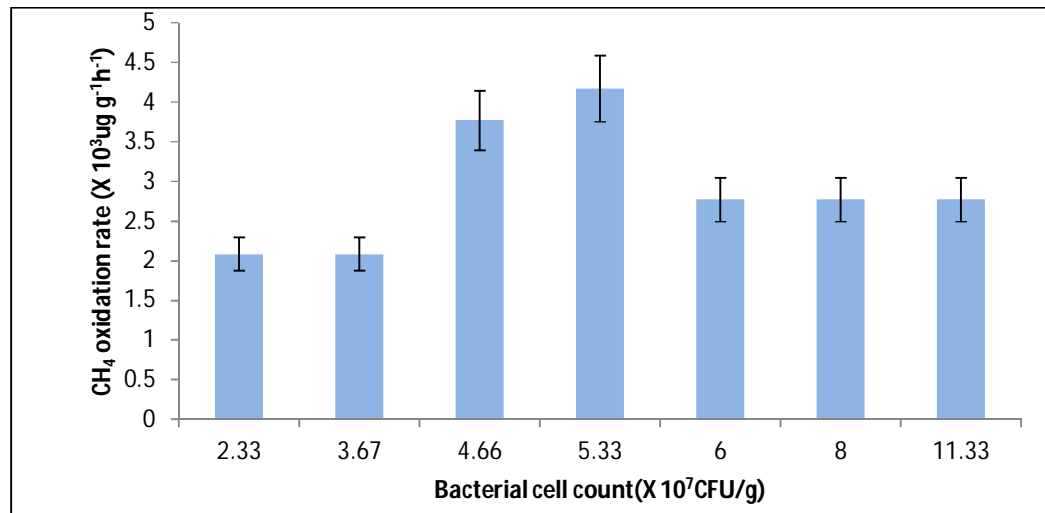


Figure 4.19: CH₄ oxidation rate with addition of different concentration of mixed culture. (Bar indicates standard error, n=3).

4.6.1: The influence of temperature on the CH₄ oxidation rate with the addition of mixed microbial culture (*Methylomonas* sp, *Methylococcus* sp 1 and *Methylococcus* sp 2).

The influence of temperature on the CH₄ oxidation rate with the addition of mixed microbial culture is shown in Figure 4.20. The highest CH₄ oxidation rate was obtained at 35°C with the addition of mixed culture, where the oxidation rate was 4.16X 10³ μg g⁻¹h⁻¹ compared 3.77X 10³ μg g⁻¹h⁻¹ and 1.19X 10³ μg g⁻¹h⁻¹ obtained at

40°C, 30°C and 45°C, respectively. The comparison with 35°C shows that the CH₄ oxidation at 30°C and 45°C was almost 71.43% slower.

Findings by Fauziah *et al* (2004) reported highest CH₄ oxidation at 35°C for the batch experiment using landfill cover soil. The incubation temperature is very important for the survival of bacteria and the ability of these bacteria to oxidize methane. An indirect effect of the higher incubation temperatures is that the biocover material will easily dry out especially in the upper layers just as soil column dry out and result in lower oxidation capacity (Visvanathan *et al.*, 1999). The findings by Humer and Lechner also indicates that at 35°C, the methanotrophic activity was high compared to the other incubation temperature studied (15°C to 35°C). Temperature can affect the CH₄ solubility in water, which eventually alters the CH₄ oxidation by the change in the CH₄ uptake rate (Barlaz *et al.*, 2004).

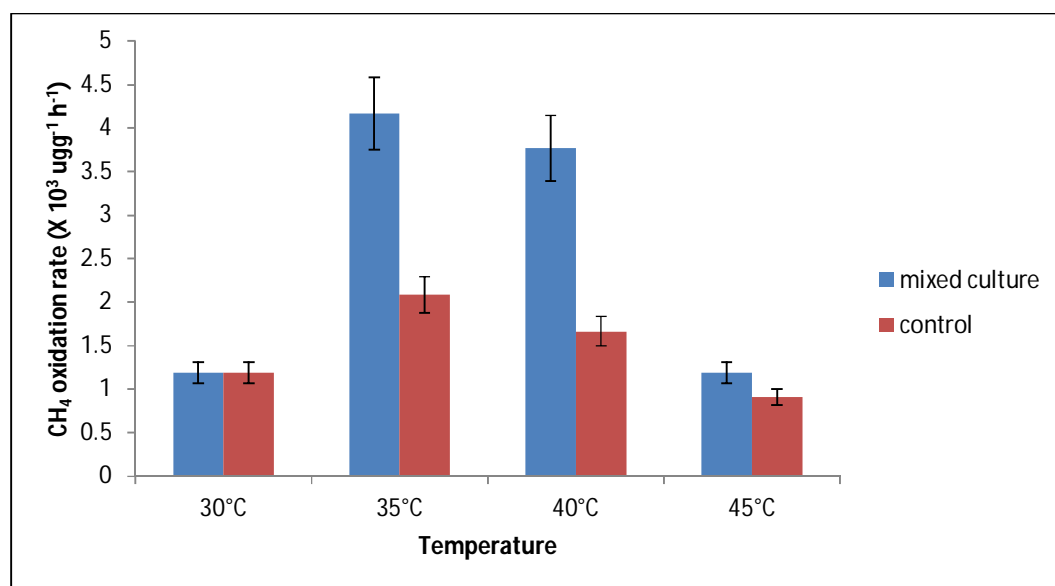


Figure 4.20: Influence of temperature on CH₄ oxidation rate with addition of mixed culture to the compost. (Bar indicates standard error, n=3).

The bacterial count from Figure 4.21 also indicated that at the end of the experiment the bacterial count was highest at 35°C (12.33×10^7 CFU/g) while the least was recorded count obtained at 45°C (3.33×10^7 CFU/g). The difference in the bacterial count at end of the experiment might be due to the influence temperature where the methanotrophic bacteria grows best at 35°C compared to other incubation temperature. According to Pawloska (2008) methanotrophic bacteria prefers mesophilic conditions as shown in this study.

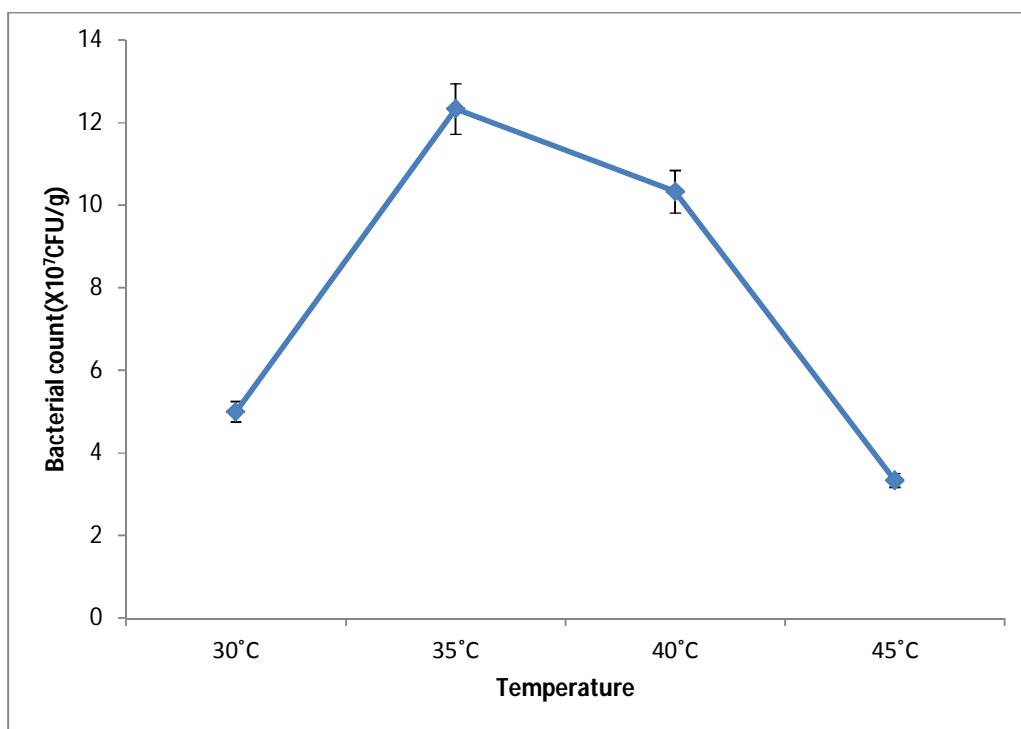


Figure 4.21: Bacterial count for influence of temperature on CH₄ oxidation with addition of bacterial culture. (Bar indicates standard error, n=3).

4.6.2: Batch Experiment: The influence of moisture content on the CH₄ oxidation rate with the addition of mixed microbial culture (*Methylobacter* sp, *Methylococcus* sp 1 and *Methylococcus* sp 2).

Figure 4.22 shows the CH₄ oxidation rate at different moisture content with addition of mixed culture to the compost. The moisture content tested ranged from 30 to 80%. Highest CH₄ oxidation activity was at 60% (v/v) moisture level with addition of mixed culture compared with the other moisture levels. This indicates that suitable moisture content is important for the CH₄ to be oxidized. CH₄ oxidation rate at 60% (v/v) moisture level was at $4.16 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ compared to $0.92 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ obtained at 80% (v/v). Statistically, the result showed significant difference ($P < 0.05$) in the CH₄ oxidation between 60% v/v and 80% v/v moisture levels. This indicates that suitable water content is very important for methane oxidation to occur. However according to Dang Wang *et al* (2011) optimum CH₄ oxidation was observed at 40% moisture. The water content of the cover soil is an important factor controlling the methane emissions from landfill (De Visscher, 2001). This indicated that the biocover materials ability to retain water is important in sustaining the microbial population required for CH₄ oxidation. Inhibition effects on the methane oxidation at low moisture content have been reported (Humer and Lechner, 2001). The CH₄ oxidation rate showed a gradual decrease when the moisture increased to 70% ($1.19 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) and 80% ($0.92 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$). There is no significant difference between the control and with addition of microbial culture. Barlaz *et al* (2004) reported that the compost covers oxidized more CH₄ in field trials, but warned that compost covers can also produce CH₄ if the moisture content is too high. Meanwhile according to Boeckx

et al (1996) a multiple regression analysis shows that moisture content has more influence on CH₄ oxidation activity compared to temperature.

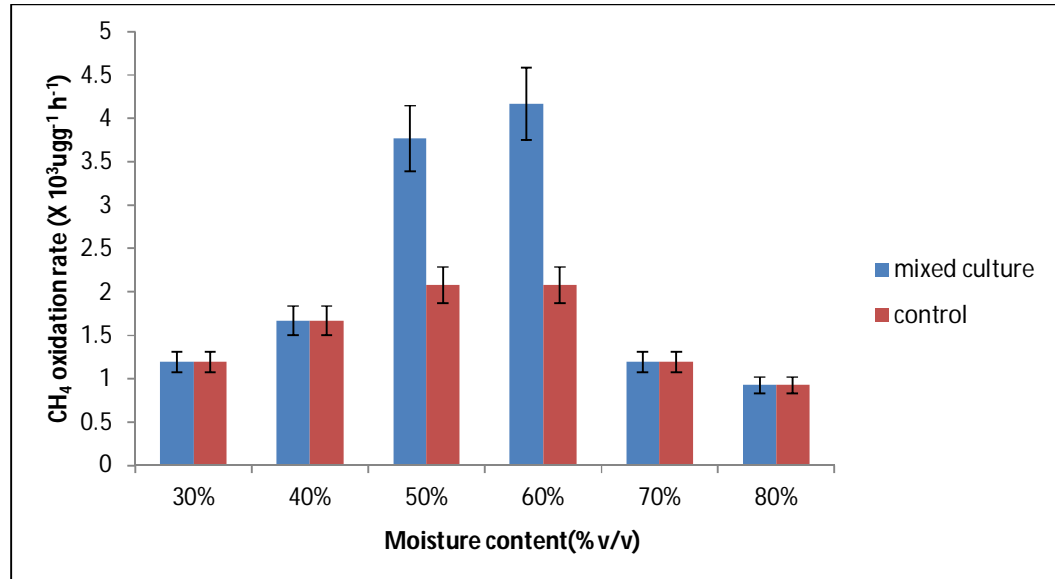


Figure 4.22: Influence of moisture content on CH₄ oxidation rate with addition of mixed culture to the compost. (Bar indicates standard error, n=3).

Highest bacterial count was obtained when the moisture content is 60% (v/v) as shown in Figure 4.21. The count was 10.6X 10⁷ CFU/g while lowest bacterial count was obtained when the moisture content was 30% (3X10⁷ CFU/g). Whalen *et al.*, (1990) also indicated that a decrease in methanotrophic activity when the moisture content ranged from 30% to 50% (v/v). Boeckx *et al* (1996) indicated that the water content widely regulates the activity of methanotrophic bacteria.

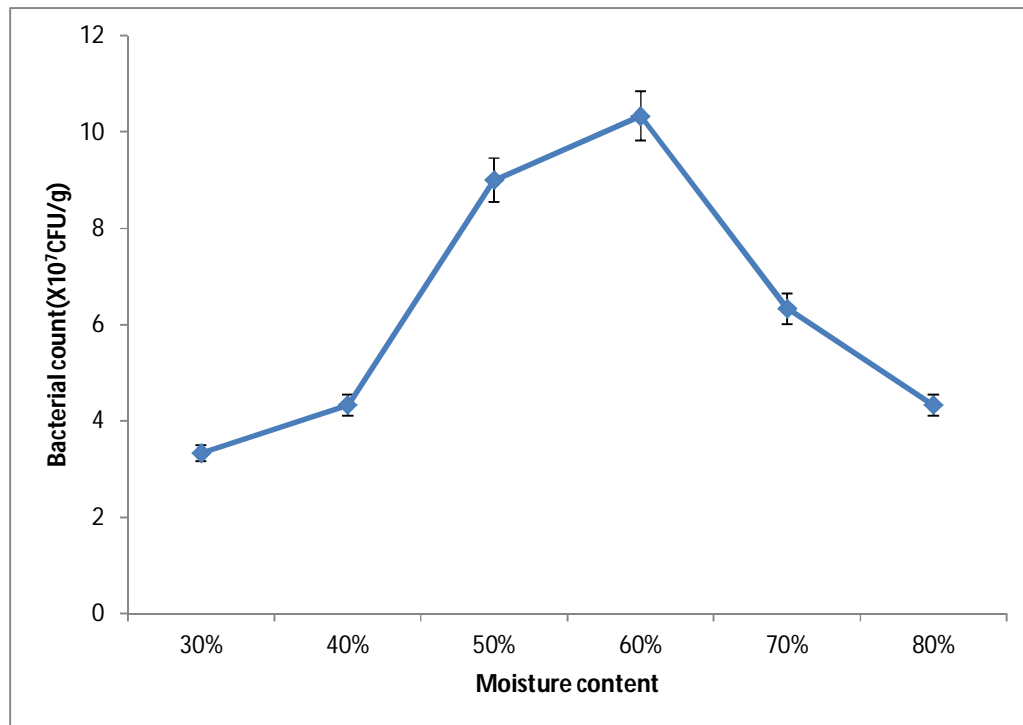


Figure 4.23: Bacterial count of the influence of moisture content on CH₄ oxidation with addition of bacterial culture. (Bar indicates standard error, n=3).

4.6.3: Batch Experiment: The influence of pH on the CH₄ oxidation rate with the addition of mixed microbial culture (*Methylobacter* sp, *Methylococcus* sp 1 and *Methylococcus* sp 2).

Influence of Biocover pH on CH₄ oxidation activity is shown in Figure 4.24. Highest CH₄ oxidation rate was observed at pH 6 with addition of methanotrophic mixed culture. The CH₄ oxidation rate was $4.16 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ $1.38 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ at pH 5. At lower pH values, reduction in CH₄ concentration is possible, and may be due to activity of yeast which easily adapt to the acidic environment that favor methanotrophic activity (Pawloska, 2008). Further increase in the biocover pH

showed a decreasing trend in CH₄ oxidation activity. At pH 7, the addition of mixed culture showed CH₄ oxidation rate rate $2.77 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ while at pH 8 $1.38 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ was obtained under the same condition. According to Figueroa (1993) methanotrophic activity is observed between pH 5.5 and pH 8.5. Moldes *et al* (2007) stated that pH is one important factor that influenced CH₄ oxidation and can range from neutral to slightly acidic situation in order to favor balanced microbial population and optimize methane oxidation activity. Changes in pH have direct implications on methanotrophic activity.

As shown in Figure 4.25, the highest bacterial count at the end of the experiment under varying pH levels showed highest rate at pH 6. The bacterial count for pH 6 was $12.33 \times 10^7 \text{CFU/g}$. At pH 5, the bacterial count was $5.66 \times 10^7 \text{CFU/g}$ while at pH 7 and pH 8, the bacterial counts were $9.66 \times 10^7 \text{CFU/g}$ and $7 \times 10^7 \text{CFU/g}$. Lowest count was at pH 5 and according to Pawloska (2008), almost all type of methanotrophic bacteria grow at pH higher than pH 5. This may be one of the reasons for lowest bacterial count at pH 5. The growth of methanotrophs was limited in acidic environment. According to McBean *et al* (1995) at a lower pH the environment for bacterial growth became toxic and inhibits the growths.

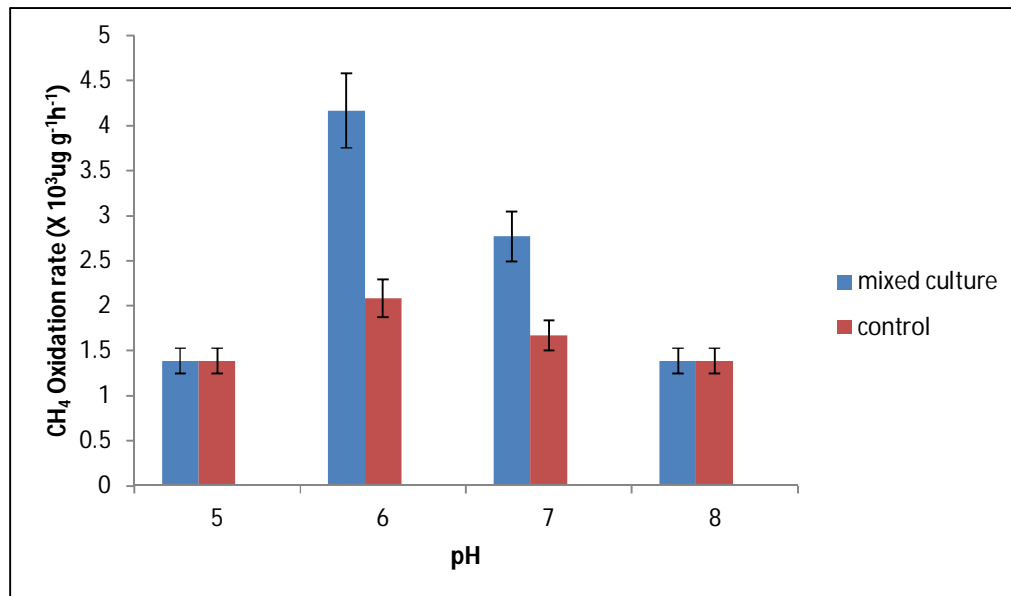


Figure 4.24: Influence of pH on CH₄ oxidation rate with and without addition of bacteria culture to the compost. (Bar indicates standard error, n=3).

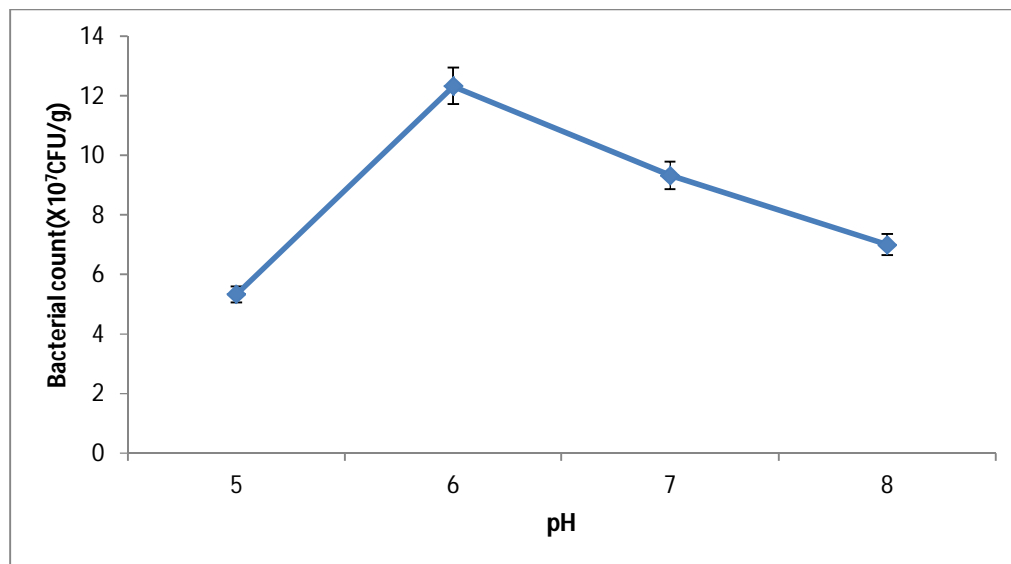


Figure 4.25: Bacterial count for the influence of pH on CH₄ oxidation with addition of bacterial culture. (Bar indicates standard error, n=3).

4.7 Batch experiment with different concentration of CH₄ tested with individual methanotrophic cultures and mixed culture

Influence of CH₄ concentration (4% to 16%) on methanotrophic activity in compost was tested as in Figure 4.26. At 4% CH₄ concentration, the highest CH₄ oxidation activity was observed with the addition *Methylococcus* sp 1 to the compost (CH₄ oxidation rate was $8.33 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) while *Methylomonas* sp and mixed culture exhibit same oxidation rate ($4.16 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$). Control and with addition of *Methylococcus* sp 2 added compost recorded lower CH₄ oxidation rate ($2.08 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$).

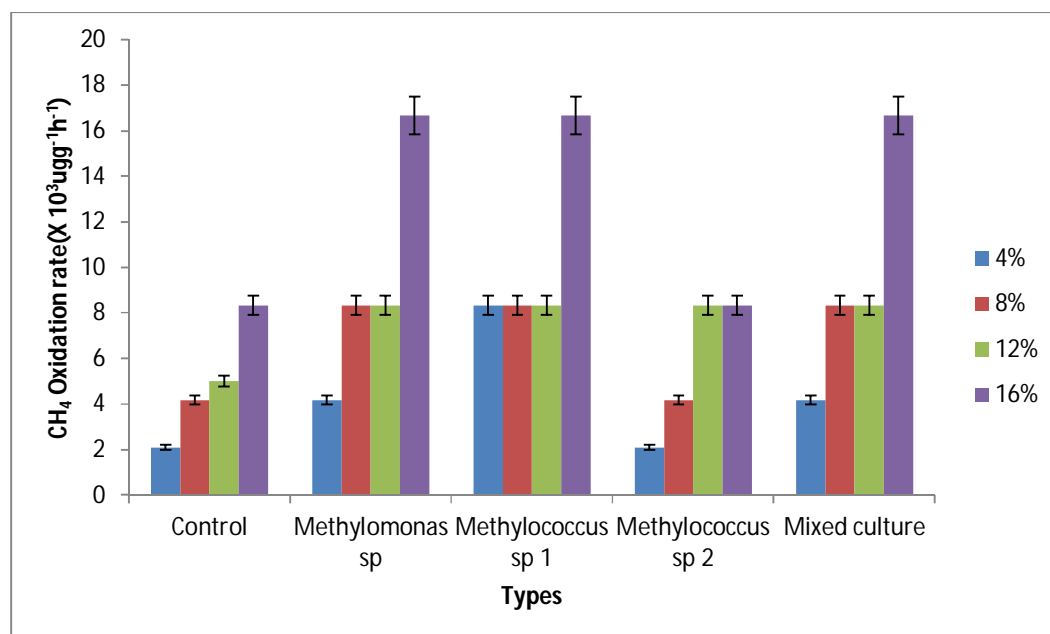


Figure 4.26: Influence of CH₄ concentration on methanotrophic activity. (Bar indicates standard error, n=3).

Lower CH₄ oxidation activity may be due to lack of minimum bacterial activities. With 8% CH₄ concentration, higher and similar CH₄ oxidation was observed with addition of *Methylomonas* sp, *Methylococcus* sp 1 and mixed microbial culture (CH₄ oxidation rate was $8.33 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) compared to the addition of *Methylococcus* sp 2 and control ($2.08 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$). Comparison between 8% CH₄ load and 4% CH₄ load showed 50% increase in CH₄ oxidation activity for *Methylomonas* sp and mixed culture. According to Balasingam (1999), as CH₄ concentration increased, the amount of CH₄ oxidized also increased. At 12% of CH₄ addition of *Methylomonas* sp, *Methylococcus* sp 1, *Methylococcus* sp 2 and mixed culture showed no difference or similar CH₄ oxidation activity compared to 8% CH₄ load. However Control showed an increase in CH₄ oxidation activity compared to the 8% load where the CH₄ oxidation rate was $5 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$. CH₄ oxidation rate for control was low compared to addition of methanotrophic bacteria cultures.

According to Huber Humer (2004) the population of methanotrophic bacteria increases with increasing methane supply in a substrate and this is supported by several other researchers (Whalen *et al.*, 1990; Jones and Nedwell, 1993; Bender, 1992; Mancinelli, 1995). CH₄ oxidation rate is similar possibly due to no significant increase in CH₄ concentration to methanotrophs. While according to Visvanathan *et al.* (1999) sometimes the CH₄ oxidation activity is not proportional to the supply rates, when a column test was carried out with low CH₄ and high CH₄ concentrations the activity was higher in low CH₄ concentration. At higher CH₄ concentration of 16% *Methylomonas* sp, *Methylococcus* sp 1 and mixed microbial culture recorded highest oxidation rate $16.66 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ whereas the CH₄ oxidation rate of the Control and

addition of *Methylococcus* sp 2 was $8.33 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$. A general trend found in this study is that as CH_4 concentration increases the CH_4 oxidation also increases. Also the addition of *Methylobacter* sp, *Methylococcus* sp 1 and mixed microbial culture showed higher CH_4 oxidation activity when compared to the control and *Methylococcus* sp 2. According to Huber Humer (2004) the amount of supplied methane determines the competition and growth between Type I and Type II methanotrophs in oxidizing CH_4 .

4.8: Batch experiment: The comparative study on compost and sterilized compost with the addition of cultures at fixed amounts and fixed parameters.

Activity of methanotrophic bacteria used in this study was tested and compared by adding bacterial culture to sterilized and non sterilized compost in order to identify whether the activity of methanotrophic bacteria is from biocover material or from the culture added. Figure 4.27 shows the CH_4 oxidation activity of sterilized and non sterilized compost. Sterilized compost without addition of methanotrophic bacteria showed zero CH_4 oxidation activity while addition of bacteria to the sterilized compost shows that CH_4 oxidation activity took place.

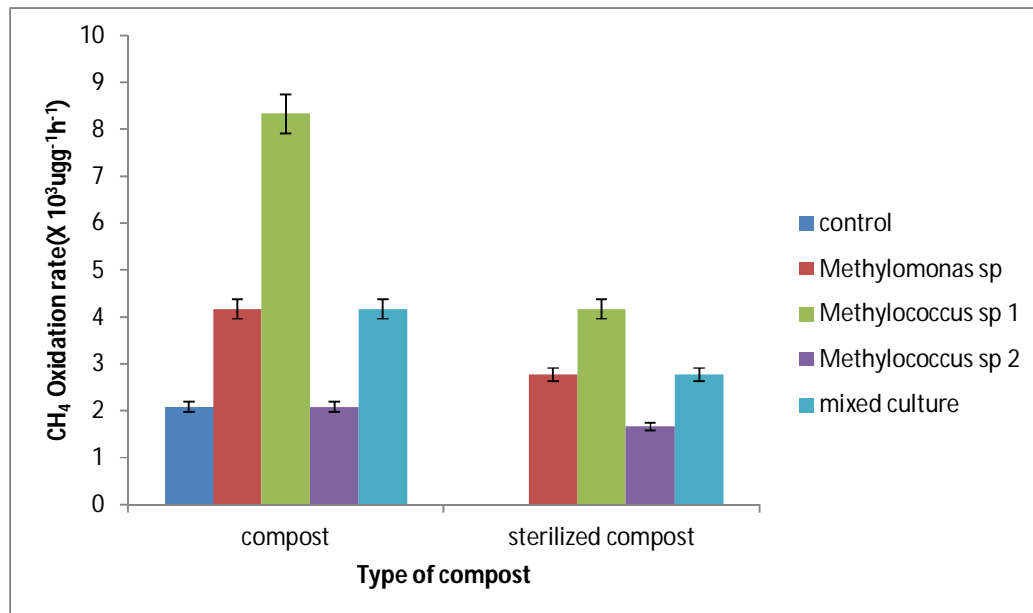


Figure 4.27: Comparison study with compost and sterilized compost with addition of cultures. (Bar indicates standard error, n=3).

Addition of *Methylococcus* sp 1 to compost showed higher CH₄ oxidation activity of $8.33 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ while addition of same bacteria to sterilized compost showed lower CH₄ oxidation activity at the rate of $4.166 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$. Similar trend was also observed with addition of *Methylomonas* sp, *Methylococcus* sp 2 and mixed culture. The addition of *Methylomonas* sp to non sterilized compost shows 33% higher activity compared to sterilized compost while the addition of *Methylococcus* sp 2 shows 20% higher activity. Non sterilized compost has a significant effect on CH₄ oxidation because it is rich in organic matter and promote growth of methanotrophs and contribute to higher CH₄ oxidation activity. Previous researcher reported that well matured compost offers higher CH₄ oxidation capacity because it is rich in organic matter and does not require additional nutrients for growth of methanotrophs (Nikiema *et al.*, 2005). Bacterial count at the end of experiments (Figure 4.28) also recorded higher count in non sterilized compost for control and also with addition of

methanotrophic bacteria compared to sterilized compost. This implies that non sterilized compost promotes more bacterial growth when compared to sterilized compost, even though equal amounts of bacterial culture were added. Addition of *Methylomonas* sp in non sterilized compost recorded highest count (1.5×10^8 CFU/g) followed by mixed culture and *Methylococcus* sp 1 (1.33×10^8 CFU/g and 1.2×10^8 CFU/g , respectively) whereas the least count was found with the addition *Methylomonas* sp (10×10^7 CFU/g), mixed culture (8.66×10^7 CFU/g) and *Methylococcus* sp 1 (9.33×10^7 CFU/g) when added to sterilized compost. Significant difference ($P < 0.05$) in the bacterial count between sterilized compost and non sterilized compost was observed with the addition of *Methylomonas* sp, mixed culture and *Methylococcus* sp 1.

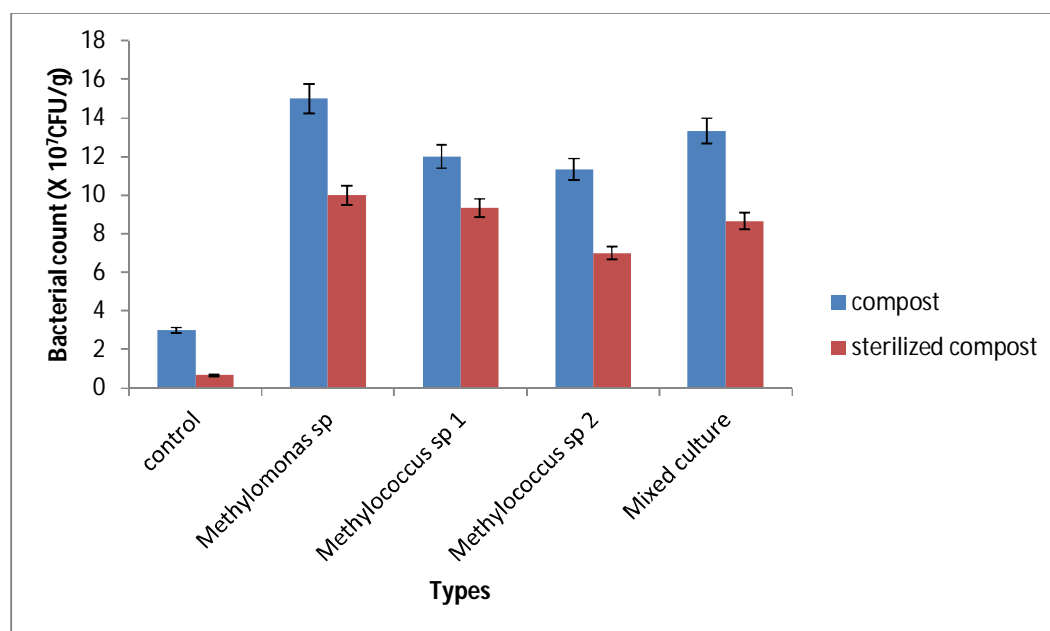


Figure 4.28: Bacterial count at end of experiment set up for comparison study with compost and sterilized compost with addition of cultures. (Bar indicates standard error, n=3).

4.9 Batch experiment with different bacteria type combination

Batch experiment with different combinations of bacteria culture to the biocover material was carried out to determine the best bacterial combination. The experiment was carried out at fixed parameters (Temperature 35°C, Moisture content 60% (v/v), pH 6). Figure 4.29 shows the combination of *Methylomonas* sp and *Methylococcus* sp 1 which was tested at different cell concentrations. Higher CH₄ oxidation activity was observed when the cell concentrations were 7.33X 10⁷CFU/g and 8X 10⁷CFU/g (CH₄ oxidation rate 4.16X 10³ µg g⁻¹h⁻¹) while at 2 X10⁷CFU/g and 4 X10⁷CFU/g the CH₄ oxidation rate was 50% lower (2.08 X 10³ µg g⁻¹h⁻¹). Even at higher concentrations, 9 X10⁷CFU/g to 12 X10⁷CFU/g the CH₄ oxidation rate was 2.77 X 10³ µg g⁻¹h⁻¹.

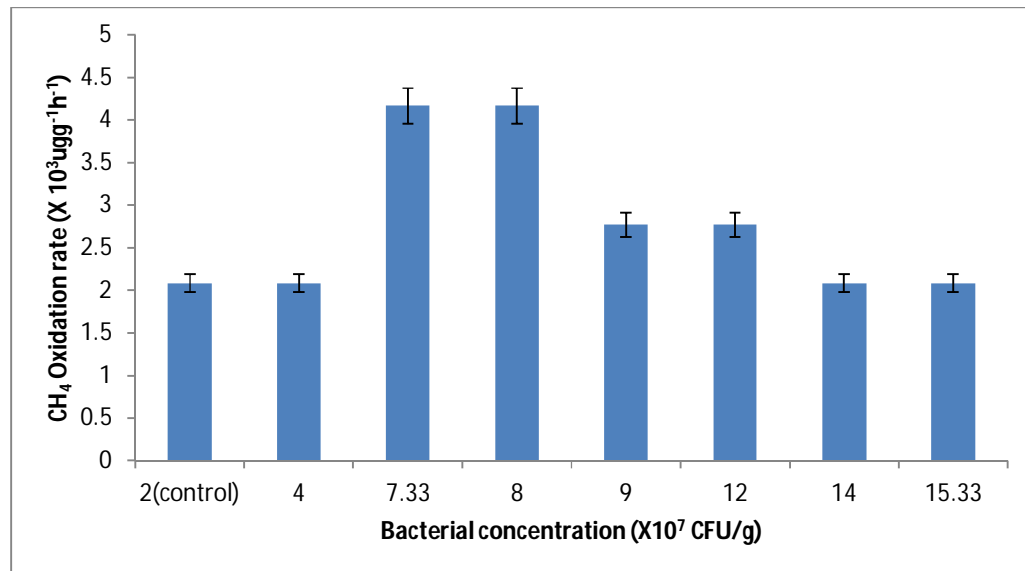


Figure 4.29: CH₄ oxidation activity at different concentration with bacterial combination of *Methylomonas* sp and *Methylococcus* sp 1(ratio of 1:1). (Bar indicates standard error, n=3).

Figure 4.30 shows the combination of *Methylobacter* sp and *Methylobacter* sp 2 which were tested at different concentration and showed highest CH₄ oxidation activity at rate of $4.16 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ with addition of bacterial concentration range of $7 \times 10^7\text{CFU/g}$ to $8.33 \times 10^7\text{CFU/g}$ compared to lower concentration of $3 \times 10^7\text{CFU/g}$ and $5.33 \times 10^7\text{CFU/g}$ ($2.77 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) and at higher concentration of $10 \times 10^7\text{CFU/g}$ ($3.77 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) and $11.33 \times 10^7\text{CFU/g}$ to $15.33 \times 10^7\text{CFU/g}$ ($2.77 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$). Any bacterial concentration below or higher than optimum concentration showed less CH₄ oxidation activity.

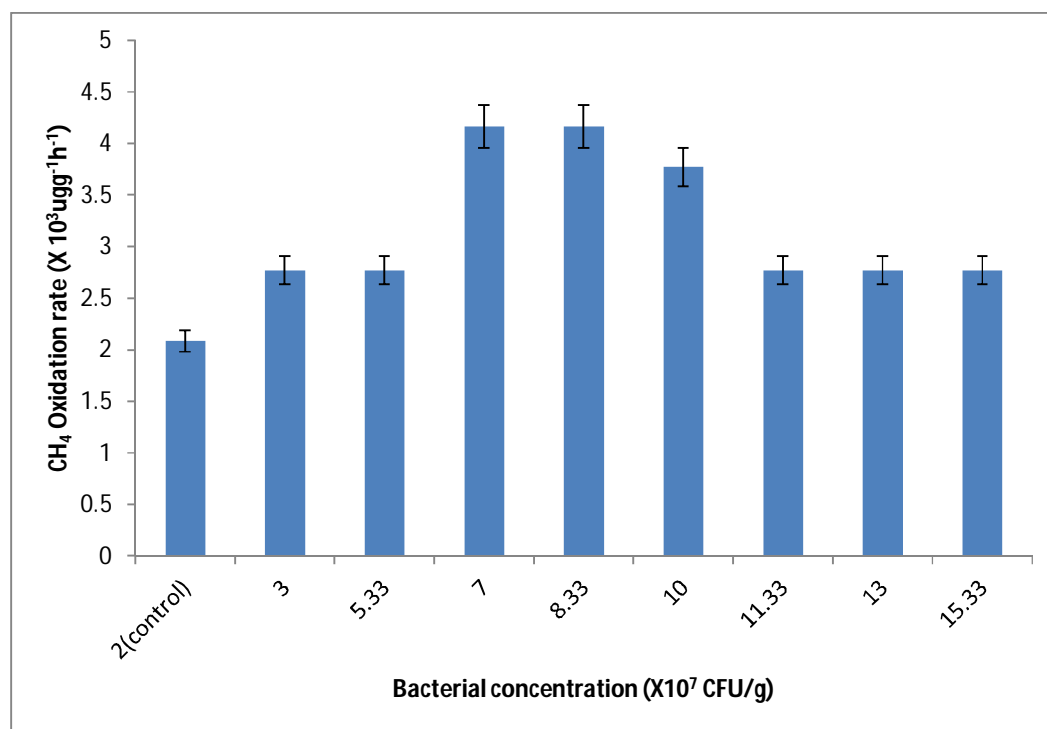


Figure 4.30: CH₄ oxidation activity at different concentration with bacterial combination of *Methylobacter* sp and *Methylobacter* sp 2 (ratio of 1:1). (Bar indicates standard error, n=3).

The third combination was between the species of *Methylococcus* sp 1 and *Methylococcus* sp 2, and the CH₄ oxidation activity is shown in Figure 4.31. The highest CH₄ oxidation activity was observed with addition of bacteria when the cell concentration was between 4.33×10^7 CFU/g and 1.2×10^8 CFU/g ($2.77 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$) compared to Control which recorded $2.083 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$. Even at higher concentration of bacteria, 13×10^7 CFU/g to 18×10^7 CFU/g CH₄ oxidation rate was same like the Control Experiment.

Combination namely, *Methylomonas* sp and *Methylococcus* sp 1, with *Methylomonas* sp and *Methylococcus* sp 2 recorded CH₄ oxidation of $4.16 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ as against $2.77 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ shown by the combination of *Methylococcus* sp 1 and *Methylococcus* sp 2. Previous experiment with individual cultures showed that both *Methylomonas* sp and *Methylococcus* sp 1 showed higher oxidation capacity compared to *Methylococcus* sp 2. However in this experiment when *Methylococcus* sp 2 was combined with *Methylomonas* sp, the CH₄ oxidation rate was higher compared to the combination with *Methylococcus* sp 1. It may be because of *Methylomonas* sp which dominantly carried out CH₄ oxidation activity and the reason for slower oxidation with combination of *Methylococcus* sp 1 and *Methylococcus* sp 2 may be as a result both bacteria being from same genera and as such influence each other in the CH₄ oxidation activity. Previous researchers also reported higher CH₄ oxidation capacity with addition of methanotrophic mixed culture but the genera of bacteria were not identified. According to Stralis *et al* (2006), *Methylomonas* sp is most abundant and most active methanotrophs. It is more dominant in carrying out CH₄ oxidation activity and could be one of the reasons for higher CH₄ oxidation rate.

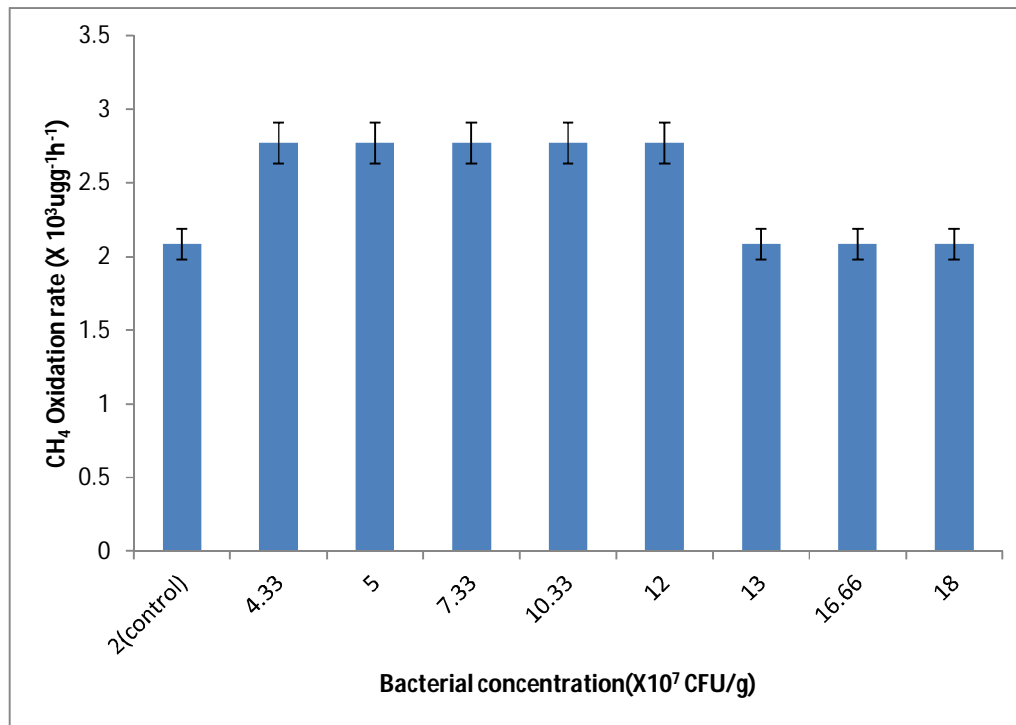


Figure 4.31: CH₄ oxidation activity at different concentration with bacterial combination of *Methylococcus* sp 1 and *Methylococcus* sp 2 (ratio of 1:1). (Bar indicates standard error, n=3).

4.10: Bioreactor Column Experiments with addition of methanotrophic bacteria

Column experiment is the advanced stage for oxidizing CH₄ and is carried out to determine suitable height of Biocover to be applied in landfill condition. Column experiment was carried out with the addition of methanotrophic bacteria at fixed concentrations to the compost. The amount of bacterial concentration was fixed in the column experiment based on the batch experiment trial discussed earlier. The column experiment was carried out in triplicates and was conducted at 35°C and 60% (v/v) moisture content and at different column heights (0cm to 100cm).

Column experiment with addition of methanotrophic bacteria cultures to the compost exhibits faster CH_4 oxidation activity compared to the Control (Figure 4.32) at all heights except at 40 cm and 50cm. Optimum CH_4 oxidation was observed at 50cm, 60cm and 70cm with addition of *Methylomonas* sp culture as shown in Figure 4.33. At this column height it took only 3 days to oxidize CH_4 compared to other column height tested with lowest CH_4 oxidation activity at 10cm and 20cm which took 9 days. Addition of *Methylococcus* sp 1 (Figure 4.34) showed similar oxidation activity at 60cm and 70cm (3 days for complete CH_4 oxidation) while it took 9 days for complete CH_4 oxidation at column height 10cm to 30cm. A similar experiment carried out by Pawloska *et al* (2006), recorded the highest CH_4 oxidation at around 66cm tested with landfill cover soils and garden waste compost.

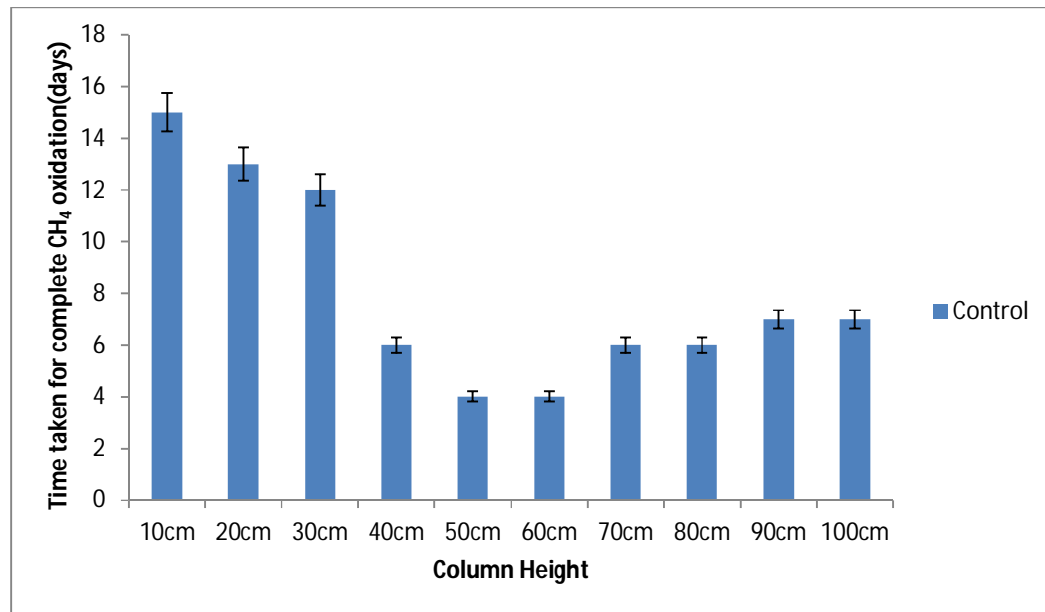


Figure 4.32: Column Experiment of Control without addition of bacteria. (Bar indicates standard error, n=3).

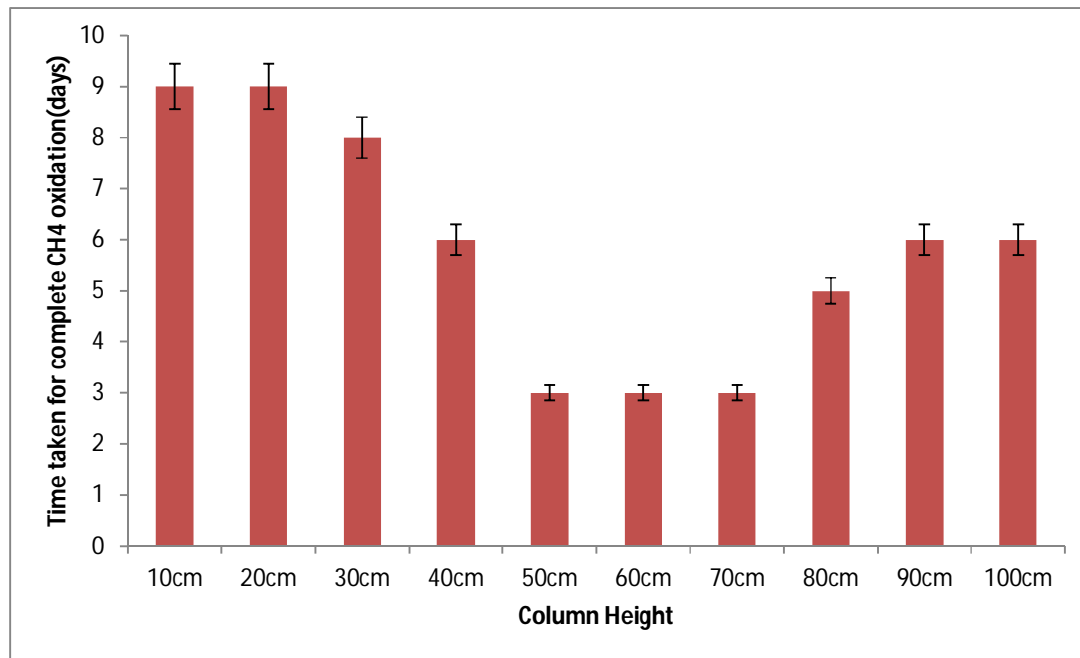


Figure 4.33: Column Experiment with addition of *Methylomonas* sp to compost. (Bar indicates standard error, n=3).

Another research carried out by Navarani (2009) also recorded highest CH₄ oxidation activity at 60cm using matured compost which took 4 days for complete CH₄ oxidation. Highest CH₄ oxidation activity at 60cm may due to suitable condition and amount of biocover at this height which provides more aerobic conditions and enhance the methanotrophs activity. Addition of *Methylococcus* sp 2 (Figure 4.35) shows similar CH₄ oxidation rate when compared to control at 60cm. CH₄ oxidation activity was observed to be low from 10cm to 40cm with the addition of bacteria and in the Control. Muna and Leta (2008) also obtained minimum oxidation activities at heights 10cm to 40cm, when compared to 50cm to 100cm. According to Navarani (2009) lowest CH₄ oxidation activity was observed at 10cm which took 18 days for complete CH₄ oxidation meanwhile in this study at 10cm the Control took 15 days,

addition of *Methylobacter* sp and *Methylococcus* sp 1 took 9 days, and *Methylococcus* sp 2 took 11 days.

Further increase in the column height beyond 70cm took a longer period to oxidize the CH₄ despite the addition of bacteria cultures even though the CH₄ oxidation rate was higher compared to the Control. Perdikea *et al* (2007) also reported that decrease in the CH₄ oxidation activity when the column height was increased above 66cm and according to the study it may be due to limited O₂ availability.

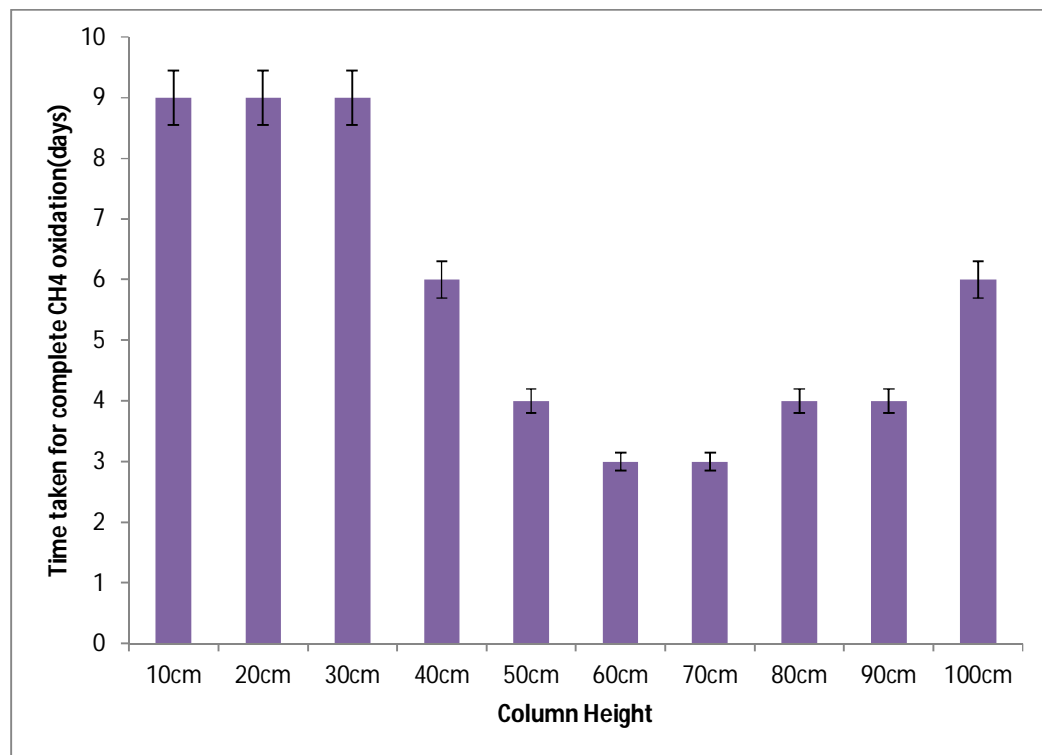


Figure 4.34: Column Experiment with addition of *Methylococcus* sp 1 to compost. (Bar indicates standard error, n=3).

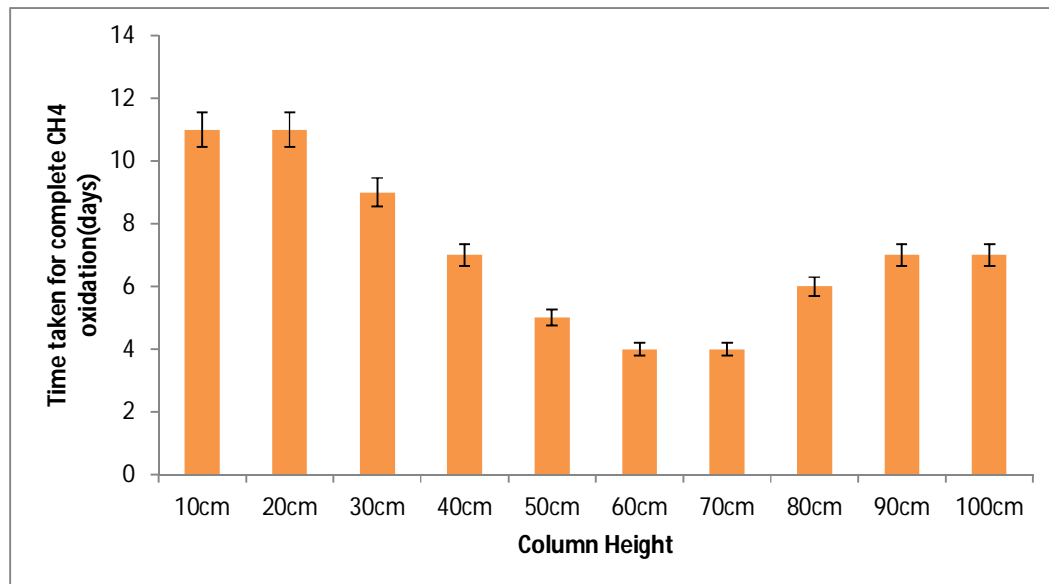


Figure 4.35: Column Experiment with addition of *Methylococcus* sp 2 to compost. (Bar indicates standard error, n=3).

Bacterial count at end of the experiment is represented in Figure 4.36. The highest bacterial count was obtained at 60cm with the addition of *Methylococcus* sp 1 which accounts for 1.7×10^8 CFU/g followed by *Methylomonas* sp, 1.6×10^8 CFU/g. No significant differences ($P < 0.05$) in amount of bacterial count at 60cm was observed between *Methylococcus* sp 1 and *Methylomonas* sp. Bacterial count of the Control was 8×10^7 CFU/g. In the Control, the low bacterial count at 60cm might be due to reduced CH₄ oxidation activity and none addition bacteria cultures. The lowest bacterial count was observed at 10cm for the Control and experiment with added bacteria culture. Perdikea *et al* (2007) reported very minimal CH₄ oxidation activity at column height of 10cm.

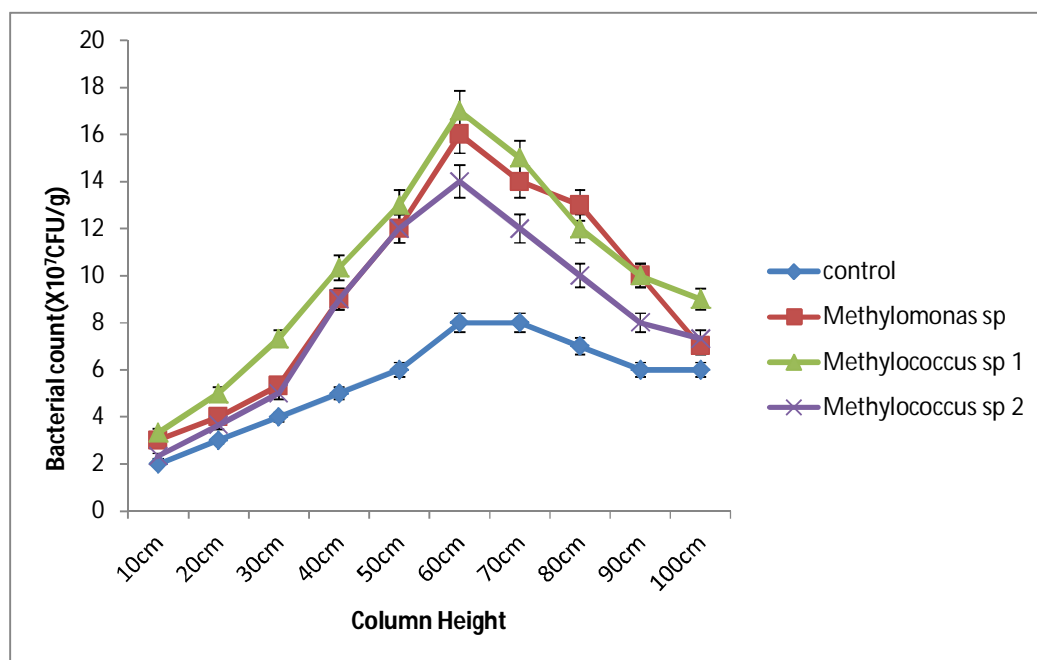


Figure 4.36: Bacterial count at the end of experiment for column experiment with compost. (Bar indicates standard error, n=3).

4.10.1 Column experiment tested at different incubation temperature

Column experiment was carried out at different incubation temperature to determine the best temperature for CH₄ oxidation with the addition of methanotrophic mixed culture to the compost at different column heights. Incubation temperature utilized were 30°C - 40°C. Reasons for narrowing down the incubation temperature was based on Wheaton bottle experiments which showed higher CH₄ oxidation activity at 35 °C - 40°C and at any temperature higher 45°C shows minimal CH₄ oxidation activity. Column experiment carried out at 30°C (Figure 4.37) with the addition of methanotrophic mixed culture exhibited higher CH₄ oxidation activity compared to the Control at all the column height. The highest CH₄ oxidation activity was observed

between 60cm to 70cm with addition of mixed culture which took 3 days for complete CH_4 oxidation whereas the Control took 5 days. At any point above or below 60cm to 70cm, H_4 oxidation activity was low. Longest time taken for complete CH_4 oxidation was 15 days at 10cm with the addition of mixed culture while the Control took 17 days.

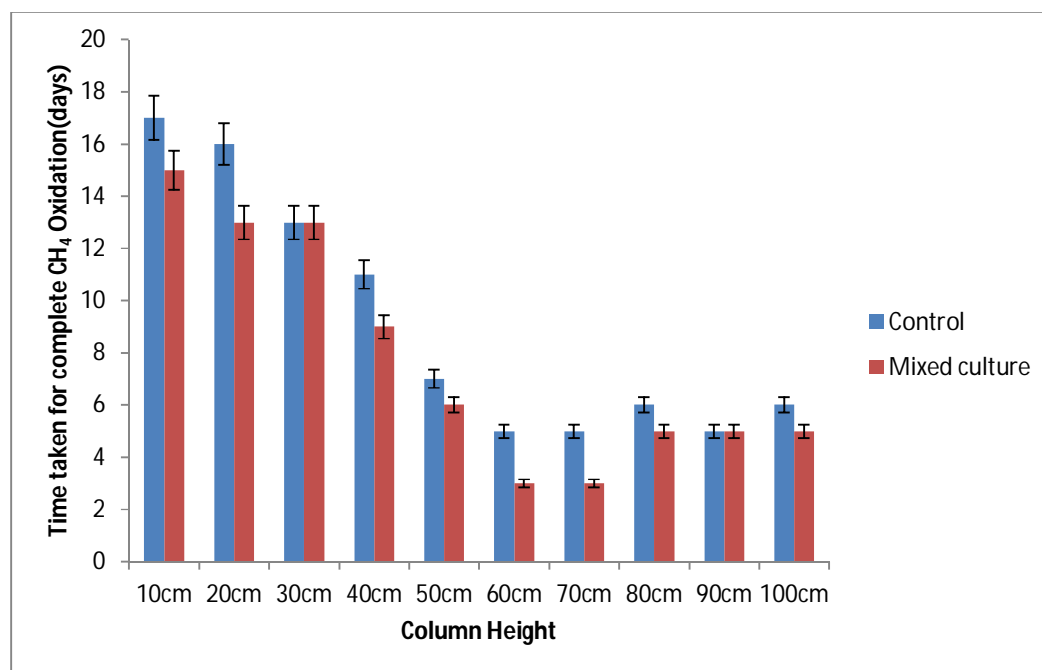


Figure 4.37: Column experiment with addition of mixed culture at 30°C (Bar indicates standard error, n=3).

Bacterial count at end of experiment is shown in Figure 4.38 and it pointed out that the highest count at the optimum heights of 60cm and 70cm. However at 60cm the counts were slightly higher when compared to 70cm for both the Control and with the added mixed culture. Bacterial count at 60cm with the addition of mixed culture was

14.33 X 10⁷ CFU/g while the Control gave 10.33 X 10⁷ CFU/g. At 70cm the bacterial count was 13X 10⁷ CFU/g with the addition of mixed culture and 9 X 10⁷ CFU/g for Control which was 30 % lower.

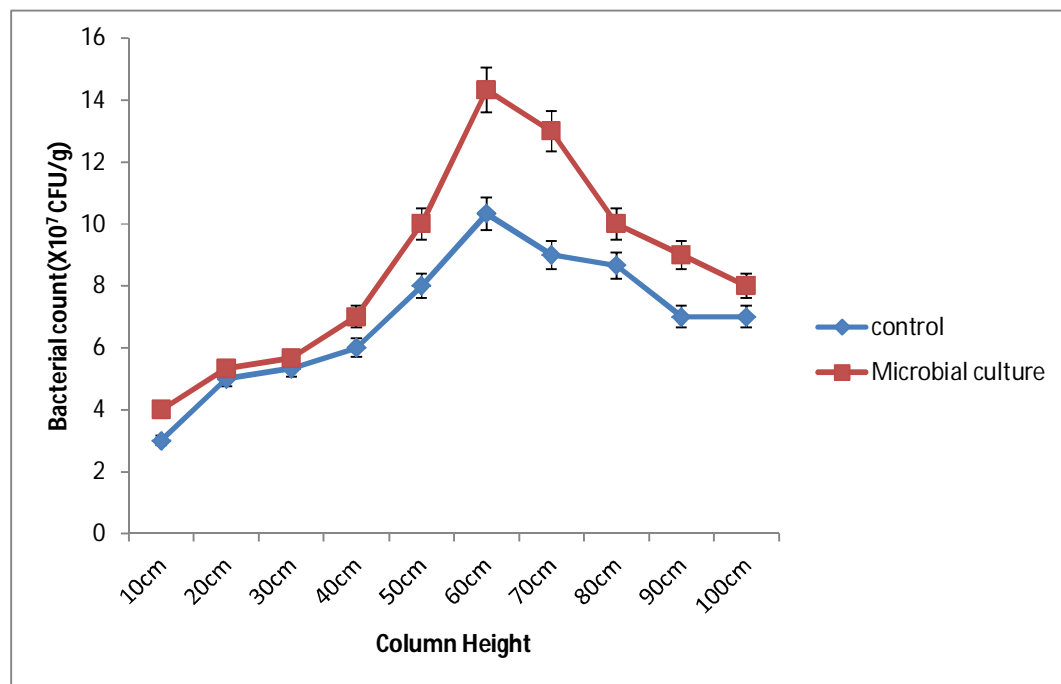


Figure 4.38: Bacterial population at end of the experiment at 30°C with the addition of methanotrophic mixed culture and control. (Bar indicates standard error, n=3).

Column experiment carried out at incubation temperature of 35°C is shown in Figure 4.39. The highest CH₄ oxidation activity was observed at 60cm to 70cm with the added mixed methanotrophic culture. It took only 2 days for complete CH₄ oxidation for both height (60cm and 70cm) while lower CH₄ oxidation activity was noted at 10cm (13 days). At 60cm the CH₄ oxidation activity was 50% higher when compared to the Control while at 70cm the CH₄ oxidation was 75% higher. It may be due to

higher bacterial activity that contributes to higher CH₄ oxidation activity at 60cm and 70cm column heights. Methanotrophic activity is higher in mesophilic conditions and may be the reason for higher CH₄ oxidation activity at 35°C. Significant test (P<0.05) carried out for 60cm column height with the addition of mixed culture and the Control in which there were significant differences in CH₄ oxidation activity. At 10cm to 70cm column height, the CH₄ oxidation activity was also observed to increase towards the optimum. Previous researchers also reported very high CH₄ oxidation activity at 60cm to 70cm and low CH₄ oxidation at lower column heights (Perdikea *et al.*, 2007; Pawloska *et al.*, 2006). At 35°C the CH₄ oxidation activity is observed to be higher and according to Park *et al* (2009) while using landfill cover soil collected from a tropical landfill, the highest CH₄ oxidation activity was at 35°C. Significant reduction in the CH₄ oxidation rate was also observed on CH₄ oxidation activity using landfill cover soil and concluded that at temperature higher than 35°C to 45°C, the CH₄ oxidation activity was very minimal and further increase to 50°C showed zero CH₄ oxidation activity (Stein and Hettiaratchi, 2001; Scheutz and Kjeldsen, 2004). The CH₄ oxidation activity above the optimum temperature was 90% less (Scheutz and Kjeldsen, 2004).

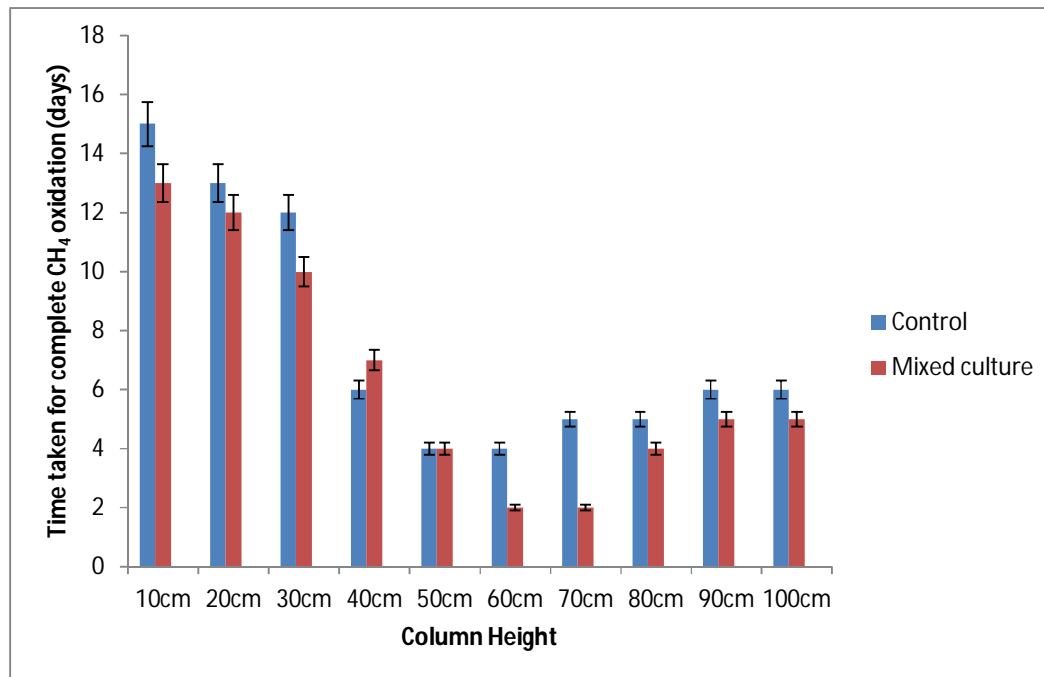


Figure 4.39: Column experiment with addition of mixed culture at 35°C (Bar indicates standard error, n=3).

The bacterial count at end of the experiment at 35°C (Figure 4.40) recorded the highest bacterial count at 60cm followed by 70cm with the added mixed culture. At 60cm the bacterial count with the addition of mixed culture was 1.4×10^8 CFU/g and at 70cm the bacterial count was slightly lower compared to 60cm (1.17×10^8 CFU/g) and lowest count was at 10cm (2.33×10^7 CFU/g). Similar to the CH₄ oxidation rate at the lower column height of 10cm to 50cm the bacterial counts was observed to be lower and as the column height was increased bacterial count also increased until the optimum column height was reached. After the optimum column height is reached the bacterial count decreased. However the bacterial counts obtained from 80cm to 100cm was higher compared to column height between 10cm to 50cm. Higher methanotrophic activity in the higher column height is one of the reasons for higher

bacterial count at 80cm-100cm when compared to 10cm to 50cm. At higher column height the CH_4 oxidation activity is higher simply due to higher microbial activity in the surface and aerobic conditions (Humer and Lechner, 1999).

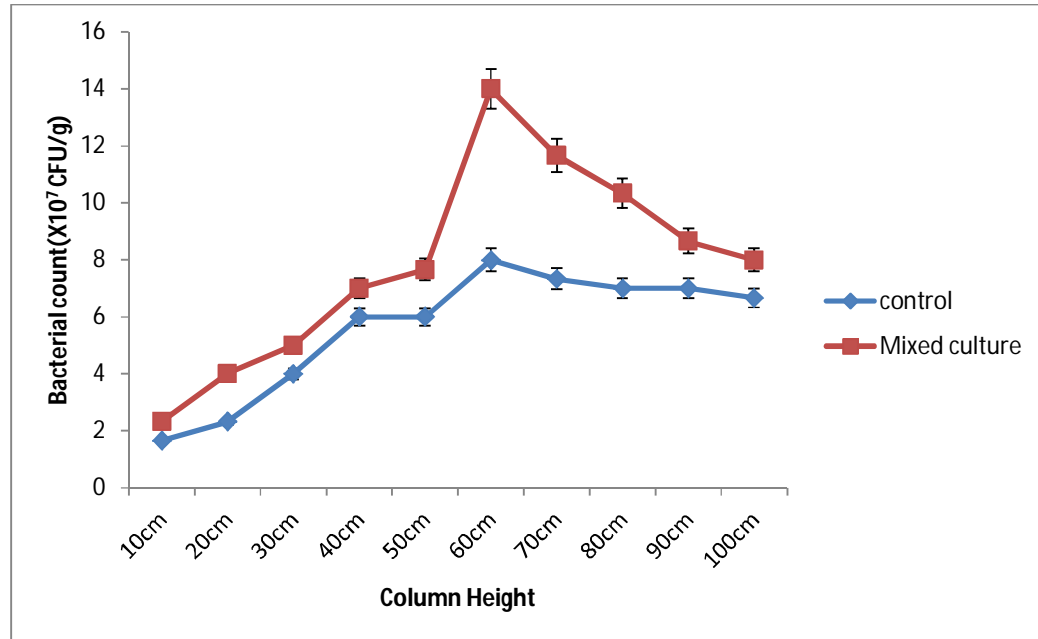


Figure 4.40: Bacterial counting at end of the experiment at 35°C with the addition of methanotrophic mixed culture and control. (Bar indicates standard error, n=3).

Column experiment with addition of mixed culture was carried out at 40°C and CH_4 oxidation activity is shown in Figure 4.41. The CH_4 oxidation activity at 60cm and 70cm with addition of methanotrophic mixed culture was similar to experiment carried out at 35 °C and it took 2 days for complete CH_4 oxidation. Rahedah (2012) also recorded high CH_4 oxidation activity in column experiment carried out in landfill condition between 60-70cm using mixture of sewage sludge and compost at 40°C. At lower column depth the CH_4 oxidation activity was low compared to column height above 60cm. At 10cm complete CH_4 oxidation took 12 days with the addition of

mixed methanotrophic cultures and as the biocover height increases the CH₄ oxidation also increased but at lower rate compared to the optimum heights.

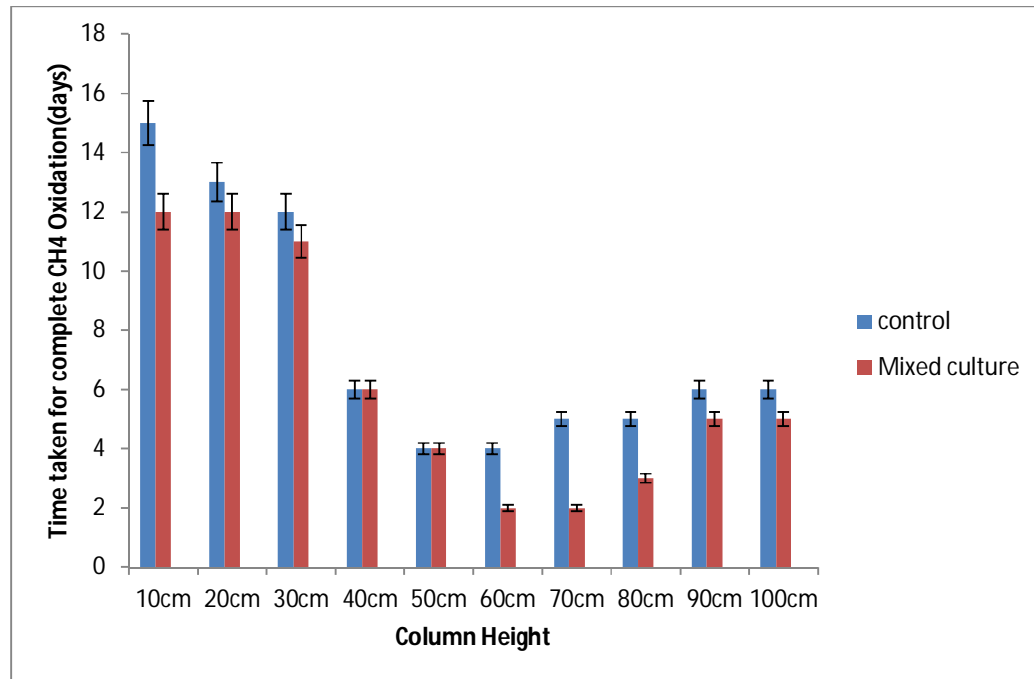


Figure 4.41: Column experiment with addition of mixed culture at 40°C (Bar indicates standard error, n=3).

Methanotrophic activity was found to be very limited at depths below 60 cm which may be due to low oxygen concentrations. According to Scheutz *et al* (2003) oxidative zone may occur at a greater depth at sites with low methane emissions with the installation of a gas extraction system. CH₄ oxidation activity above 70cm range from 80cm to 100cm showed slightly lower CH₄ oxidation activity. Significant test ($P < 0.05$) between column height 60-70cm and 80-100cm showed significant difference in the CH₄ oxidation activities for the experiment carried out with the added mixed methanotrophic culture.

Figure 4.42 shows the bacterial count at end of experiment for column experiment at 40°C. At 60cm the bacterial count was 1.23×10^8 CFU/g while the least count, (2.33×10^7 CFU/g) were obtained at column height was 10cm. Lack of nutrients for bacteria could be one of the reason for lowest count at this column height. Bacterial count also showed a steady increment trend when the column heights were increased towards the optimum column heights and further increase after the optimum showed significant ($P < 0.05$) decrease in bacterial count. Navarani (2009) also observed similar trend in column experiment carried out with matured compost and black soil at 25°C. Highest bacterial count at 60cm was due to bacterial distribution in column which was sufficient and resulted in highest count. According to Pawloska and Stepniewski (2006) lower bacterial count at column height above 60cm also may also be due to lack of oxygen permeability in the deeper zones.

From the study conducted with column experiment at different incubation temperature, we can conclude that higher CH₄ oxidation was observed between 60cm to 70cm for all the incubation temperature (both with and without addition of methanotrophic mixed culture). Comparison at 60cm between 30°C to 40°C showed higher oxidation activity at temperature of 35°C - 40°C with the addition of mixed culture. According to Park *et al* (2009) investigation carried out with compost samples and incubated at different temperatures showed maximum CH₄ oxidation activity between 35 °C to 40°C and very minimal or inhibition in CH₄ oxidation at higher or lower temperature.

It also concluded the suitability of composts as biocover material in enhancing the CH_4 oxidation.

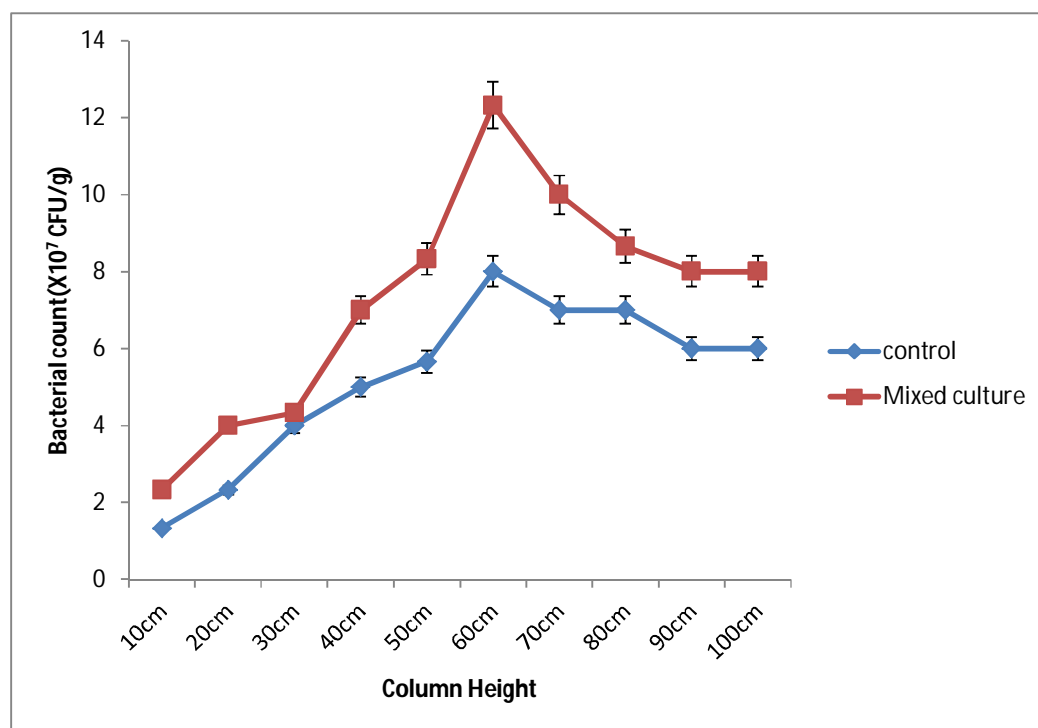


Figure 4.42: Bacterial counting at end of the experiment at 40°C with the addition of mixed culture and control. (Bar indicates standard error, n=3).

4.10.2: Column experiment tested at different moisture content with addition of methanotrophic mixed culture (50%-70% (v/v)).

Column experiment with different moisture content was carried out with the added mixed culture to the compost and 50% to 70% (v/v) was the moisture content utilized. Figure 4.43 depicts the column experiment at 50% moisture with addition of mixed culture and control at different column heights. Addition of mixed culture shows the optimal CH_4 oxidation activity at 60cm compared to other column heights. At 60cm, the complete CH_4 oxidation took 2 days when compared to lower column height of

10cm-50cm which took about of 4 to 14 days, while it took 3 to 5 days at higher column heights, 70cm to 100cm. Even though the experiments were conducted at fixed moisture content 50% (v/v), the CH₄ oxidation activity was observed to differ between different column heights. Active zone for optimum CH₄ oxidation activity was observed around 60cm and it may be due to distribution pattern of the microbes in the column. Navarani (2009) also observed highest CH₄ oxidation at 60cm column height using compost and black soil, and also found that the highest bacterial count was at this column height. Moisture has a very vital role in oxidizing CH₄. According to Chandrakanthi *et al* (2005) biofilter experiment with right amount of water or optimum moisture conditions will result into higher CH₄ oxidation activity.

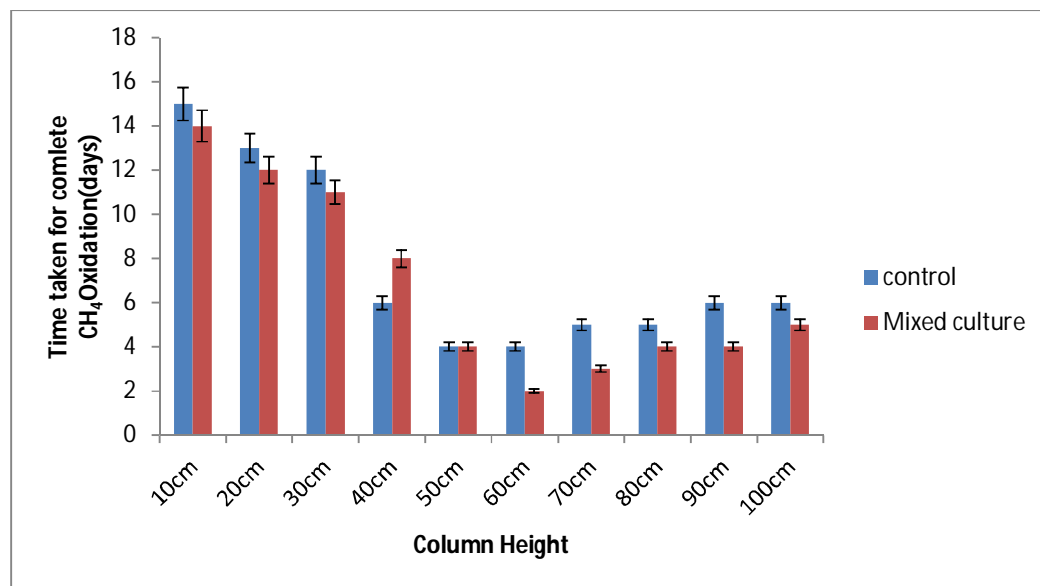


Figure 4.43: Column experiment with addition of mixed culture at 50% (v/v) moisture content. (Bar indicates standard error, n=3).

As in previous experiment, the bacterial count (Figure 4.44) was also higher at the optimum column height with addition of methanotrophic mixed culture. The higher bacterial count was at 60cm and lowest bacterial count was at 10cm. At 60cm column the bacterial count was 1.2×10^8 CFU/g while at 10cm the bacterial count was 3.66×10^7 CFU/g. At 70cm column the bacterial count was 16% less when compared to bacterial count at 60cm column heights.

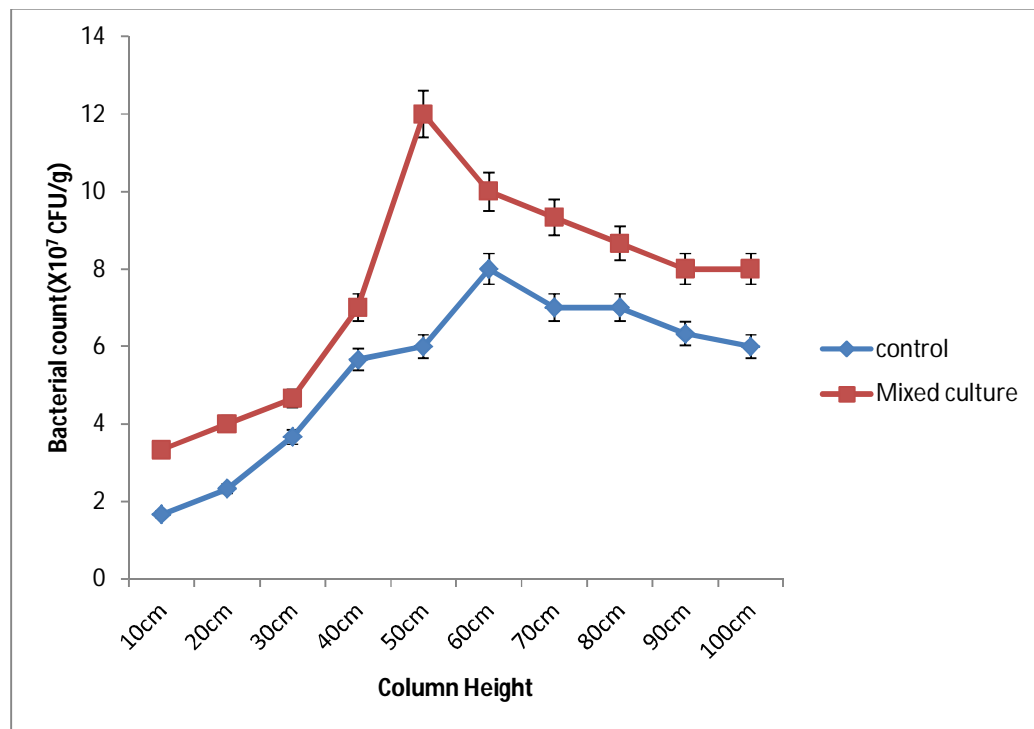


Figure 4.44: Bacterial counting at end of the experiment at 50% (v/v) moisture content with the addition of methanotrophic mixed culture and control (Bar indicates standard error, n=3).

Column experiment carried out below 60% (v/v) moisture content with the added mixed culture is shown in Figure 4.45. The experiment revealed that higher CH₄ oxidation activity was observed at 60cm to 70cm (2 days for complete CH₄ oxidation) with the added mixed culture compared to other column heights with lowest CH₄

oxidation activity at 10cm (13 days to oxidize similar amount of CH₄). At higher column height 80cm, the complete CH₄ oxidation took place within 4 days, while at 90cm - 100cm height 5 days was taken for such complete CH₄ oxidation. The comparison of CH₄ oxidation under two moisture levels, 50% (v/v) and 60% (v/v) at 60cm showed similarity but at 70cm the CH₄ oxidation activity was higher at 60% (v/v) moisture level. Higher moisture content in the biocover material could be one of the reasons for higher microbial activity which increase the CH₄ oxidation activity at 70cm. According to Gebert *et al* (2006) low moisture content of biocover will result in low CH₄ oxidation activity. Similar to 50% (v/v) moisture the CH₄ oxidation at lower column height for 60% (v/v) also observed to be lower and showed increasing trend as the column height increased. However the addition of methanotrophic bacteria culture showed significant (P<0.05) increase in the CH₄ oxidation at all column height compared to the Control.

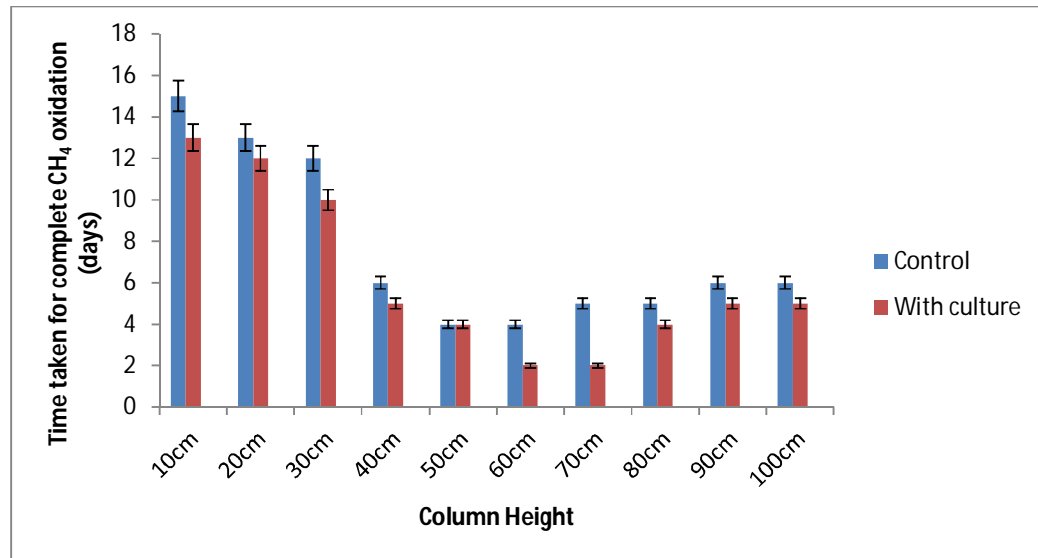


Figure 4.45: Column experiment with addition of mixed culture at 60% (v/v) moisture content. (Bar indicates standard error, n=3).

Figure 4.46 shows the bacterial count at end of experiment for the column experiments carried out at 60% (v/v) moisture content. Similar to previous experiment at 50% (v/v), the highest bacterial count was at 60cm (1.4×10^8 CFU/g) followed by 70cm (1.2×10^8 CFU/g) and lowest count at 10cm (4×10^7 CFU/g) all with the addition of methanotrophic mixed culture.

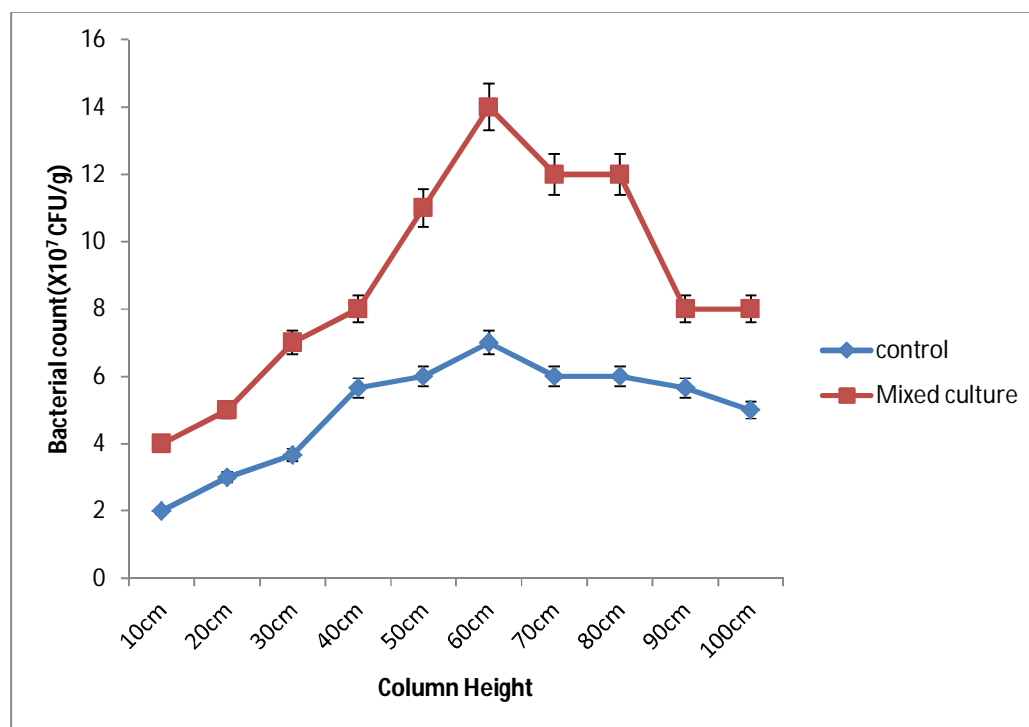


Figure 4.46: Bacterial counting at end of the experiment at 60% (v/v) moisture content with the addition of methanotrophic mixed culture and control. (Bar indicates standard error, n=3).

CH₄ oxidation activity carried out in column experiment with 70% (v/v) moisture content is shown in Figure 4.47. The highest CH₄ oxidation was observed at column height, 50cm to 80cm with the addition of mixed culture. The oxidation activity was

similar at column height experiment, 50cm to 80cm where it took 4 days for complete CH₄ oxidation to take place as against 13 days obtained at 10cm column height. The CH₄ oxidation activity at this range with the addition of mixed culture also showed higher CH₄ oxidation compared to the Control. However the CH₄ oxidation with the addition of mixed culture was not significant compared to Control. Very high moisture content of biocover could be one of the reasons for minimal methanotrophic activity. High moisture content in the biocover cause blockage or inhibition of the gas movement and often results into lower CH₄ oxidation activity (Scheutz *et al.*, 2009).

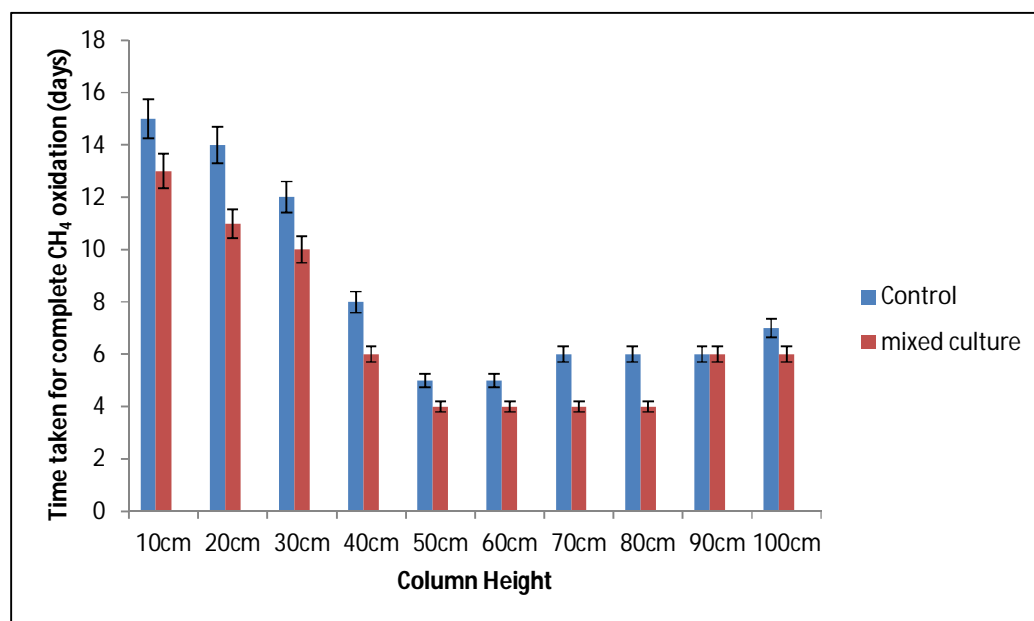


Figure 4.47: Column experiment with addition of mixed culture at 70% (v/v) moisture content. (Bar indicates standard error, n=3).

Bacterial count at end of column experiment with 70% (v/v) moisture level (Figure 4.48) shows highest bacterial count at 70cm. The population count was 1.13×10^8 CFU/g. However no significant differences at 95% confidence level ($P < 0.05$) was

observed between 70cm and 50cm to 80cm. This may be due to similar CH₄ oxidation activity at column height of 50-80cm. Population of bacteria was not significant and differ in less than 3%. The lowest bacterial count was observed at 10cm for both Control and with the addition of mixed culture. The bacterial count with addition of mixed culture was slightly higher compared to the control.

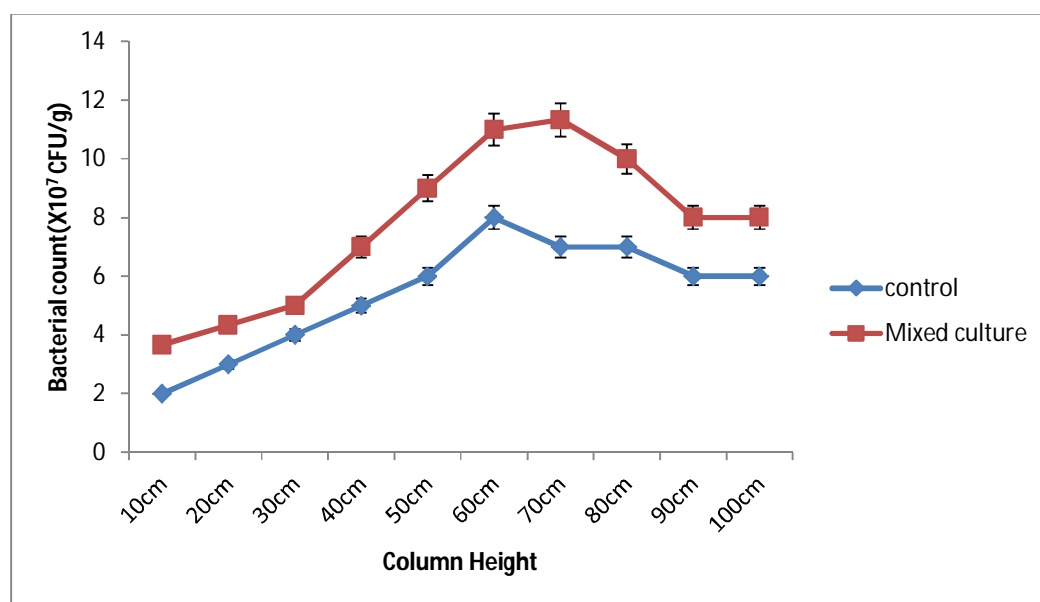


Figure 4.48: Bacterial counting at end of the experiment at 70% (v/v) moisture content with the addition of methanotrophic mixed culture and control (Bar indicates standard error, n=3).

Comparison of CH₄ oxidation at different biocover moisture content showed higher CH₄ oxidation under 50% (v/v) and 60% (v/v) at 60cm while the experiment with 70% (v/v) showed lower CH₄ oxidation capacity. The difference observed was almost 50%. Very high moisture in biocover above the optimum could be one of the reason for lower CH₄ oxidation rate. According to Christensen *et al* (2007) biocover material

supports very high porosity for microbial activity. However excess water results in lower or inhibition of CH₄ oxidation activity.

4.10.3: Column experiment with and without daily O₂ input (Mixed culture, fixed parameters)

Column experiment with addition of mixed culture was carried out with and without daily supply of O₂ as shown in Figure 4.49 and Figure 4.50. From Figure 4.49 highest CH₄ oxidation activity was at 50cm -70cm with addition of mixed culture (2 days for complete oxidation) meanwhile in Figure 4.50 highest CH₄ oxidation activity, took place at 60cm and 70cm within 2 days as well.

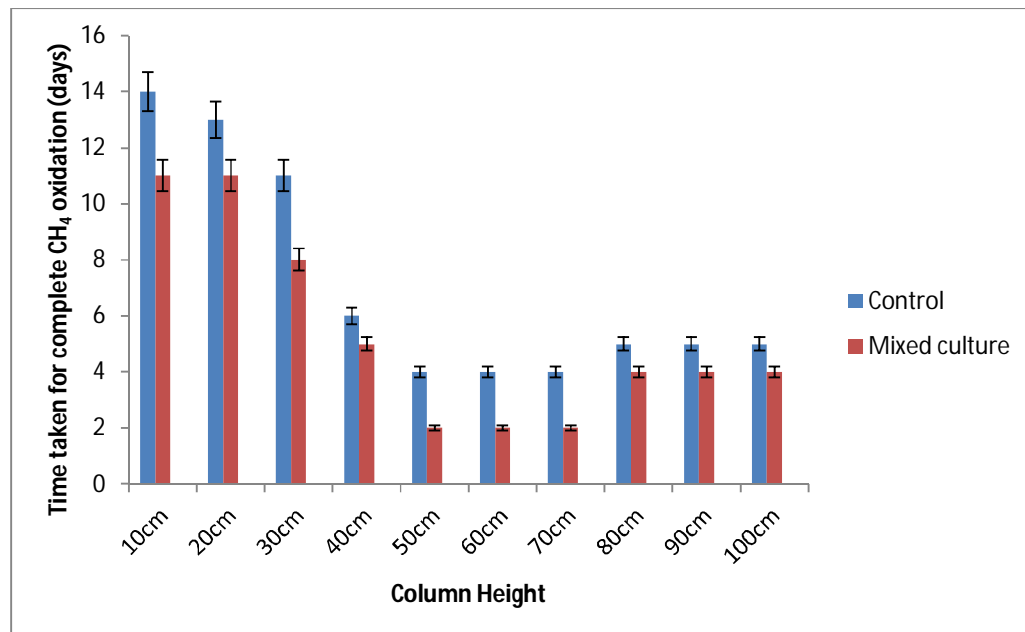


Figure 4.49: The column experiment with 8% O₂ supply daily. (Bar indicates standard error, n=3).

Comparison at 60cm and 70cm, with and without daily input of O₂, have no significant difference as observed in CH₄ oxidation activity. However at certain heights significant increase in CH₄ oxidation activity was observed. At 50cm with daily O₂ supply the oxidation was 50% higher compared to one time O₂ supply. Similar trend was also observed between 10cm to 40cm where shorter period was taken compared to one time O₂ supply. According to Pawloska and Stepniewski (2006) methanotrophic bacteria activity is higher or very active if there are higher oxygen supply. Besides that Humer and Lechner (1999) also reported that surface of biocover with high amount of O₂ under aerobic condition increase the CH₄ oxidation activity. Activity of CH₄ oxidation was also observed to increase at 90cm and 100cm heights with daily O₂ supply. At 90cm and 100cm with addition of mixed culture it took 4 days for complete CH₄ oxidation. According to Navarani (2009) the CH₄ oxidation will be limited if the amount of O₂ is very minimal.

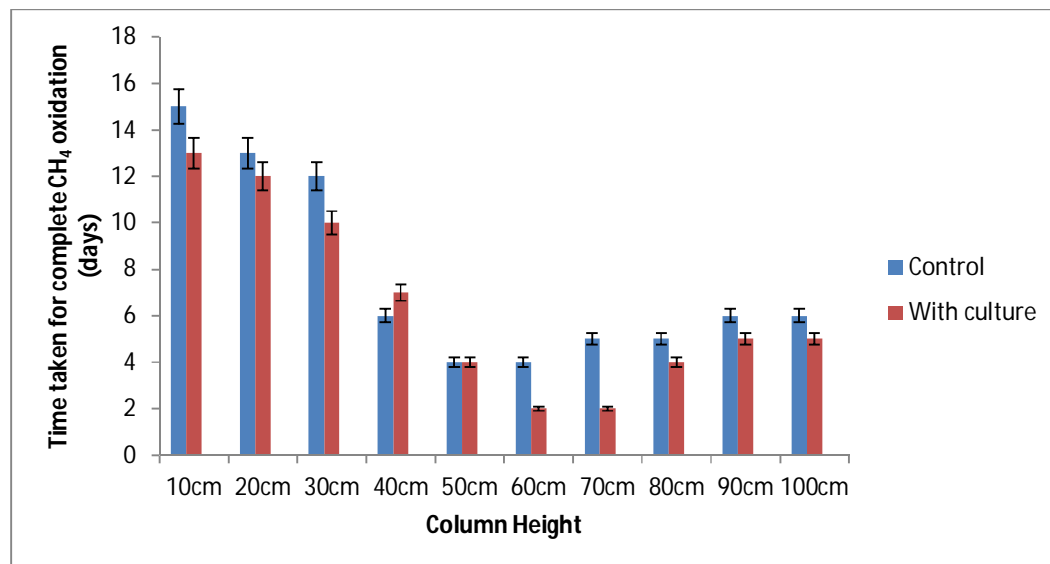


Figure 4.50: The column experiment with one time O₂ supply. (Bar indicates standard error, n=3).

Bacterial at end of the experiment as shown in Figure 4.51 and Figure 4.52 also indicated higher count with daily O₂ supply at all the heights with the addition of mixed culture. Highest count was at 60cm with addition of mixed culture and with daily O₂ supply where bacterial count was 14 X10⁷ CFU/g compared to the bacterial count with one time O₂ which counts for 12.66 X10⁷ CFU/g.

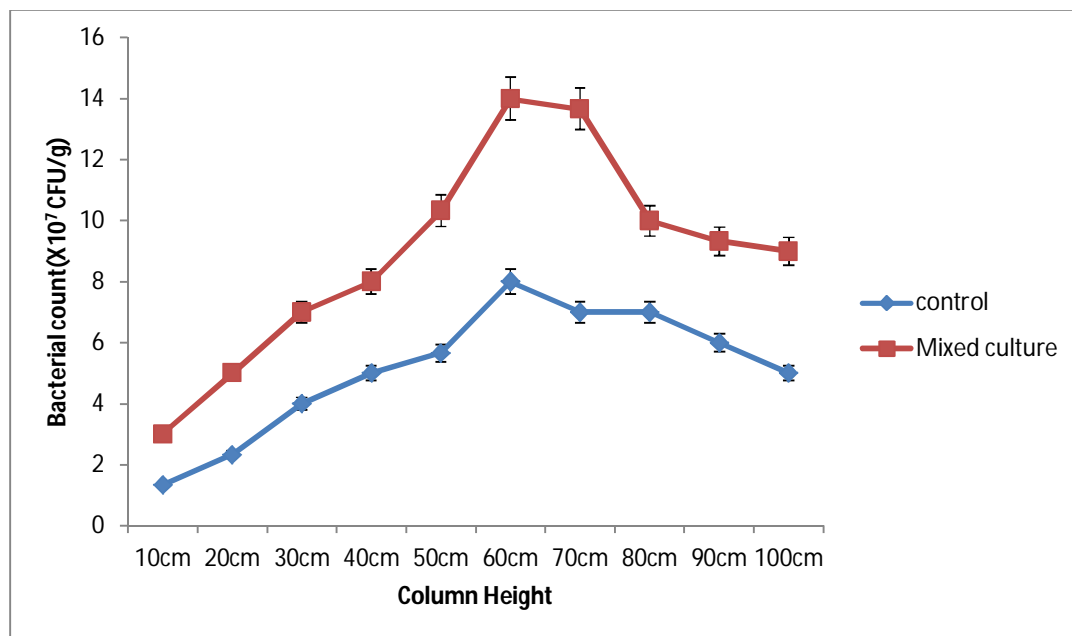


Figure 4.51: Bacterial counting at the end of experiment set up for column experiment for daily O₂ supply. (Bar indicates standard error, n=3).

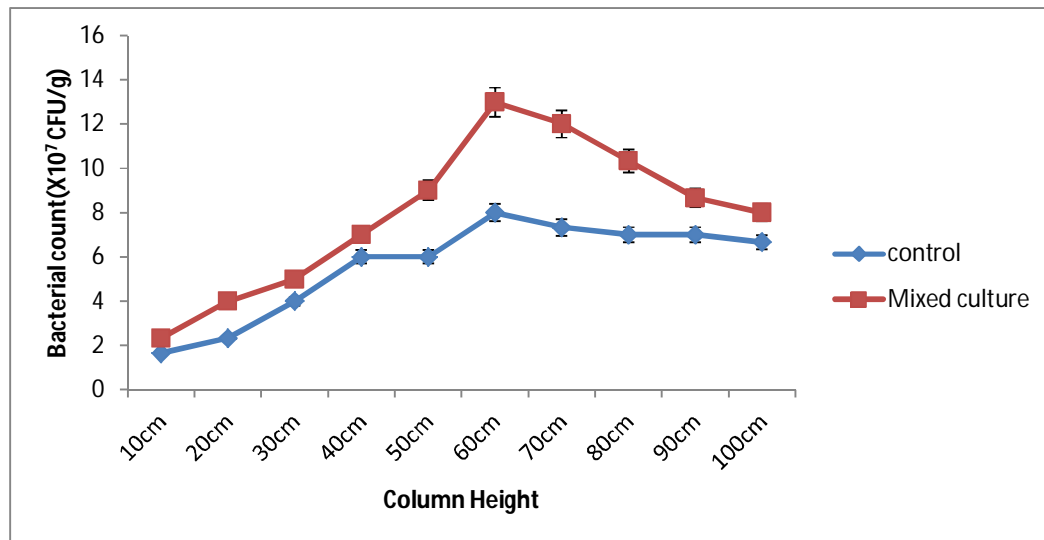


Figure 4.52: Bacterial counting at the end of experiment set up for column experiment for with one time O₂ supply. (Bar indicates standard error, n=3).

Daily O₂ supply favoured growth of methanotrophs and resulted into higher bacterial count as observed in this experiment. According to Schnell and King (1995) reductions of O₂ concentration affect the methanotrophic activity which may be one of the reasons for slower CH₄ oxidation as observed in the experiment carried out with one time O₂ supply.

4.11: Kinetic modeling to evaluate the efficiency of CH₄ oxidation

Kinetic modeling for the CH₄ oxidation of both the Control and with the addition of methanotrophic bacteria cultures experiments was evaluated by using Michaelis Menten equation. Volume of CH₄ (% v/v) was plotted against time (hours). Reaction rate was determined by measuring tangent of resulting curve.

Michaelis Menten equation:

$$R_p = R_{\max} \frac{1}{1 + (K_m / C)} \quad (\text{Equation 4.1})$$

Where

R_p = potential methane oxidation rate (ml/d)

R_{\max} = Maximum methane oxidation rate (ml/d)

K_m = Half saturation reaction rate (ml/d)

C = Initial CH_4 concentration (%)

Since C , is constant (4% v/v) for all the experiments conducted, C was eliminated from Equation 2 to modify the kinetics, where R_p is:

$$R_p = R_{\max} \frac{1}{1 + K_m} \quad (\text{Equation 4.2})$$

4.11.1 Kinetic modeling to evaluate the efficiency of batch experiments on CH_4 oxidation activity with the addition of methanotrophic bacteria at optimum parameters (Temperature 35°C , Moisture content 60%, pH 6).

Table 4.2 shows the calculation of the kinetic model for batch experiments with the addition of individual cultures and mixed cultures at the optimum parameters. The volume of CH_4 was calculated and plotted against time. Hence R_{\max} and K_m were obtained from the graph as shown in Figure 4.53 to Figure 4.57.

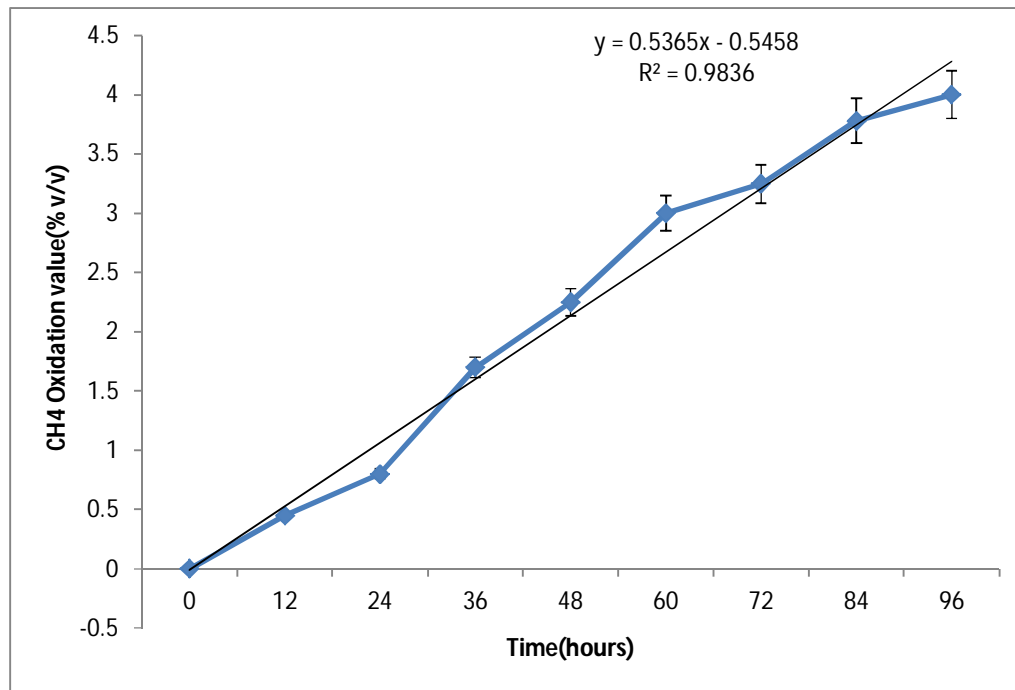


Figure 4.53: CH₄ oxidation value of control (Bar indicates standard error, n=3).

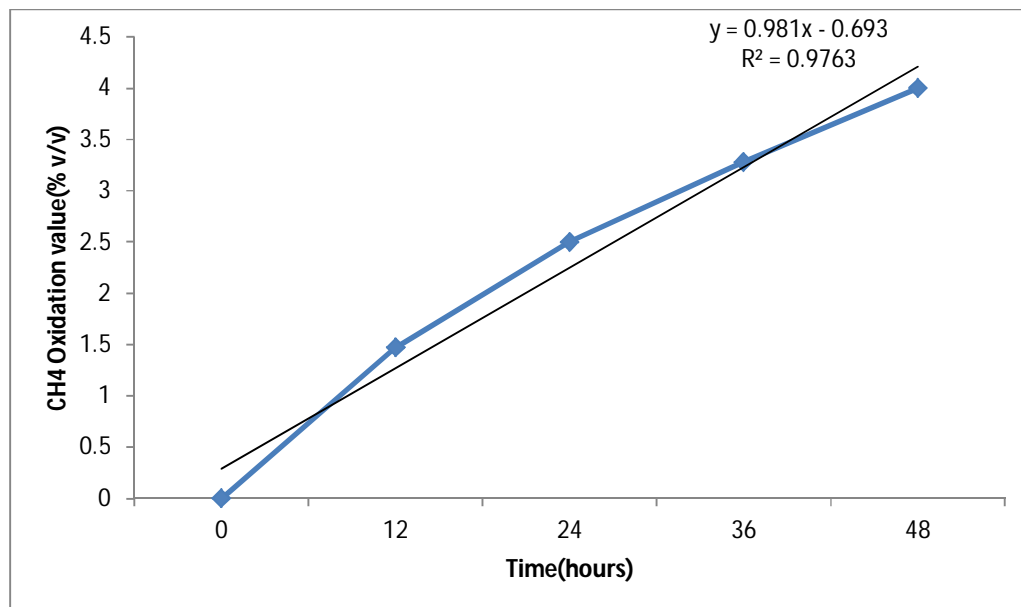


Figure 4.54: CH₄ oxidation value with addition of *Methylomonas* sp. (Bar indicates standard error, n=3).

The potential CH₄ oxidation rate, R_p was calculated and tabulated in Table 4.3. The highest potential CH₄ oxidation rate was observed with addition of *Methylococcus* sp 1 compared to the others with the R_p value of 44.64 followed by addition of *Methylomonas* sp which had R_p value of 41.39. Even though the CH₄ oxidation rate was similar between the *Methylomonas* sp and mixed culture, the potential CH₄ oxidation rate was observed to be lower with value of 37.35. This may be because of the different value of the CH₄ oxidation at such point of time. Control experiment potential CH₄ oxidation rate was observed to be 32.71 while lowest potential CH₄ oxidation rate with addition of *Methylococcus* sp 2 was 6.30. From this results, it is clearly indicated that *Methylococcus* sp 1, *Methylomonas* sp and methanotrophic mixed culture have higher CH₄ oxidation capacity.

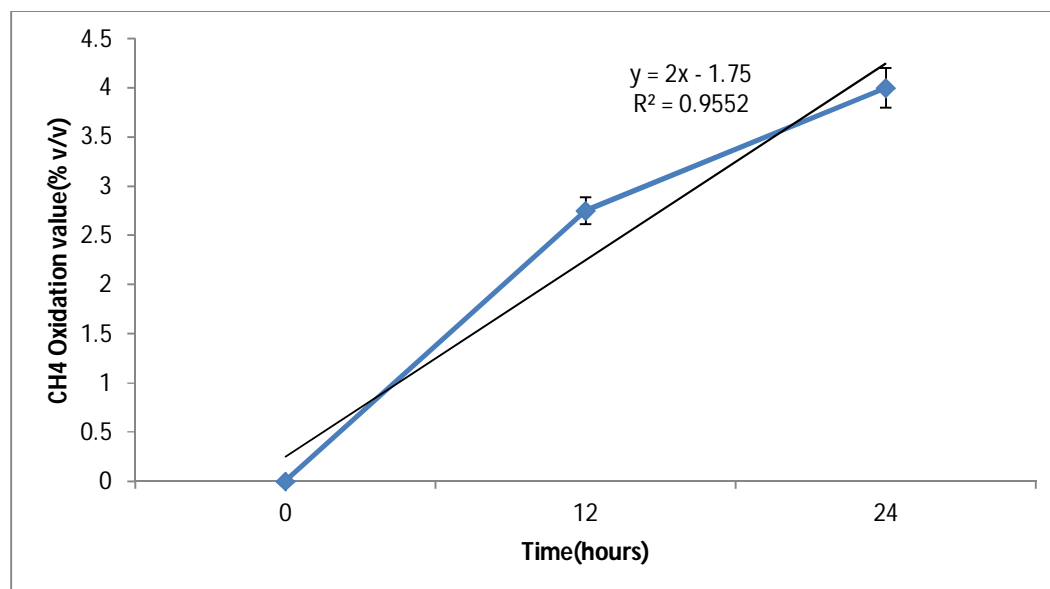


Figure 4.55: CH₄ oxidation value with addition of *Methylococcus* sp 1. (Bar indicates standard error, n=3).

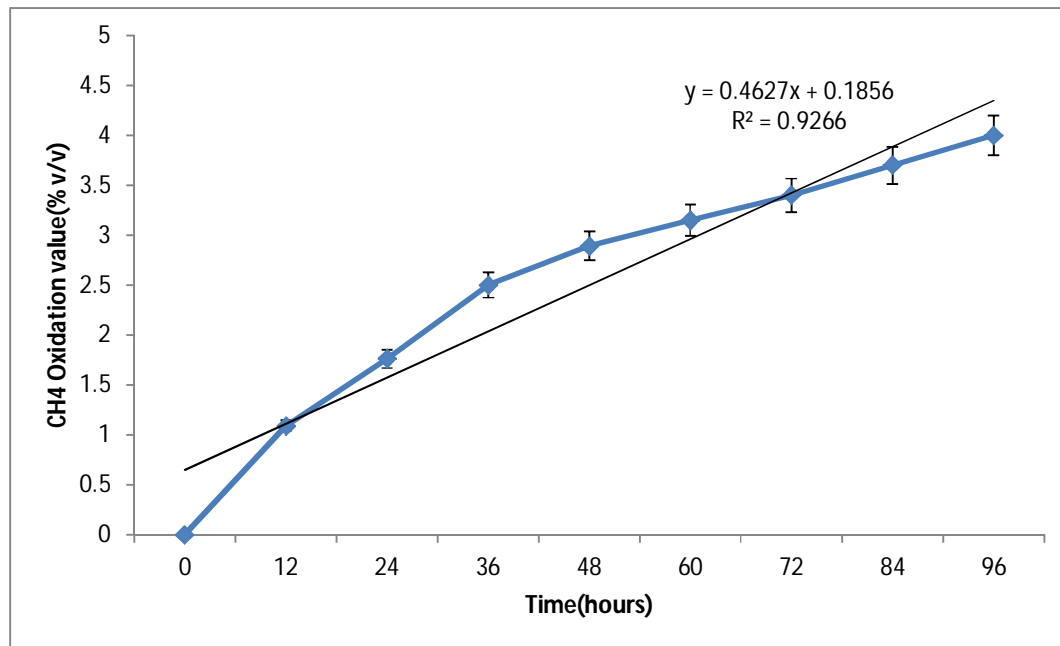


Figure 4.56: CH₄ oxidation value with addition of *Methylococcus* sp 2 (Bar indicates standard error, n=3).

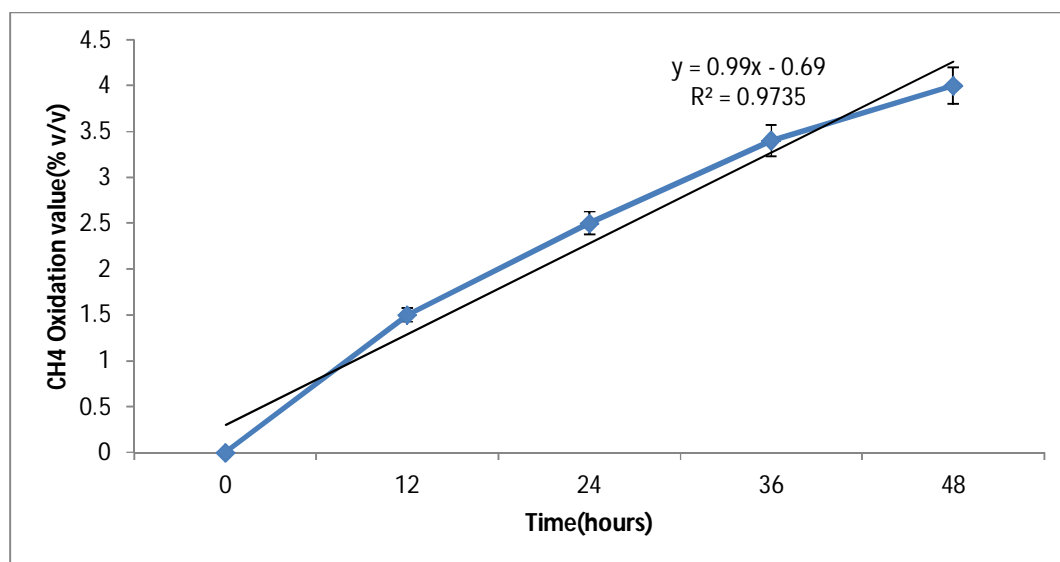


Figure 4.57: CH₄ oxidation value with addition of methanotrophic mixed culture. (Bar indicates standard error, n=3).

Other researchers also obtained similar trend on the potential CH₄ oxidation rate. Siti Aishah (2011) reported R_p value of 17.04 using compost and R_p value of 10.81. These values are slightly lower than the values obtained from this study. This may be due to difference in CH₄ oxidation rate. Another study by Pawloska and Stepniewski (2006) using different cover material tested with continuous CH₄ flow also recorded much lower value than value obtained in this study. Difference in experimental design is one of the reasons for different R_p value. Higher methanotrophic bacterial activity at optimum conditions could be one of reason for higher potential CH₄ oxidation rate.

Table 4.3: Potential CH₄ oxidation rate, R_p is calculated for batch experiment at optimum parameters

Types	K _m (mL/d)	R _{max} (mL/d)	R _p (mL/d)
control	0.9836	0.5365	$R_p = 0.5365 \frac{1}{1-0.9836} = 32.71$
<i>Methylomonas</i> sp	0.9763	0.981	$R_p = 0.981 \frac{1}{1-0.9763} = 41.39$
<i>Methylococcus</i> sp 1	0.9552	2.00	$R_p = 2.00 \frac{1}{1-0.9552} = 44.64$
<i>Methylococcus</i> sp 2	0.9266	0.4627	$R_p = 0.4627 \frac{1}{1-0.9266} = 6.30$
Mixed culture	0.9735	0.99	$R_p = 0.99 \frac{1}{1-0.9735} = 37.35$

4.11.2 Kinetic modeling for column experiment calculated for optimum biocover heights of 60cm with addition of methanotrophic bacteria.

Kinetic study on the column experiment was evaluated at the optimum heights of 60cm because at 60cm all the tested showed higher CH₄ oxidation activity compared to other column heights. Kinetic modeling at optimum biocover height of 60cm for CH₄ oxidation activity was calculated and plotted against time and R_{\max} and K_m are obtained from the graph. The potential CH₄ oxidation rate, R_p was calculated using the R_{\max} and K_m value obtained from Figure 4.58 to Figure 4.62 for both the Control and with the addition of methanotrophic bacteria cultures.

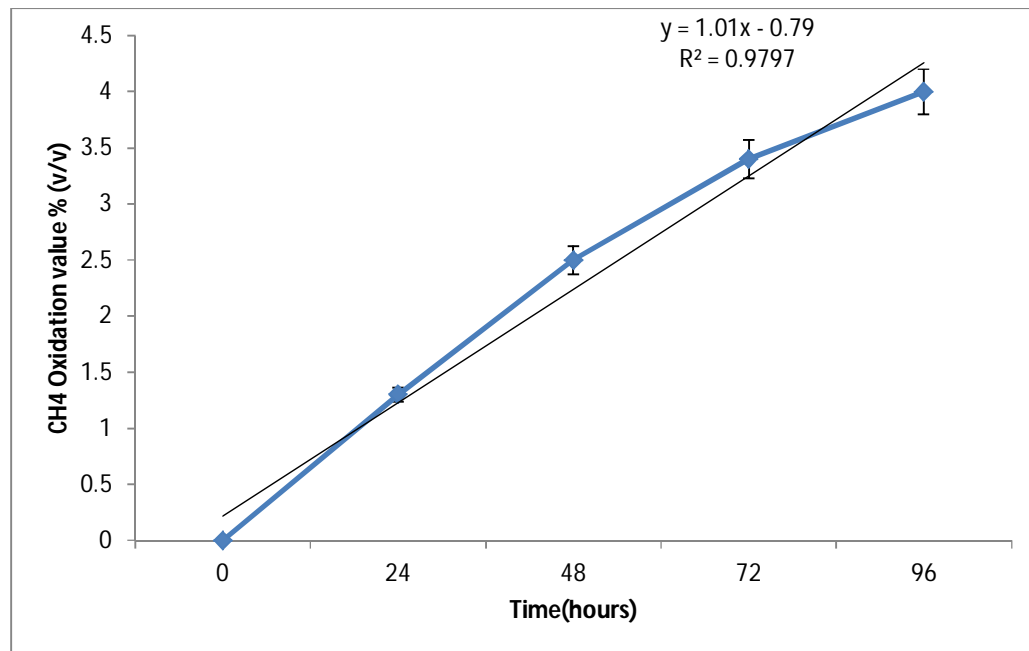


Figure 4.58: CH₄ oxidation value of column experiment at 60cm height for control (Bar indicates standard error, n=3).

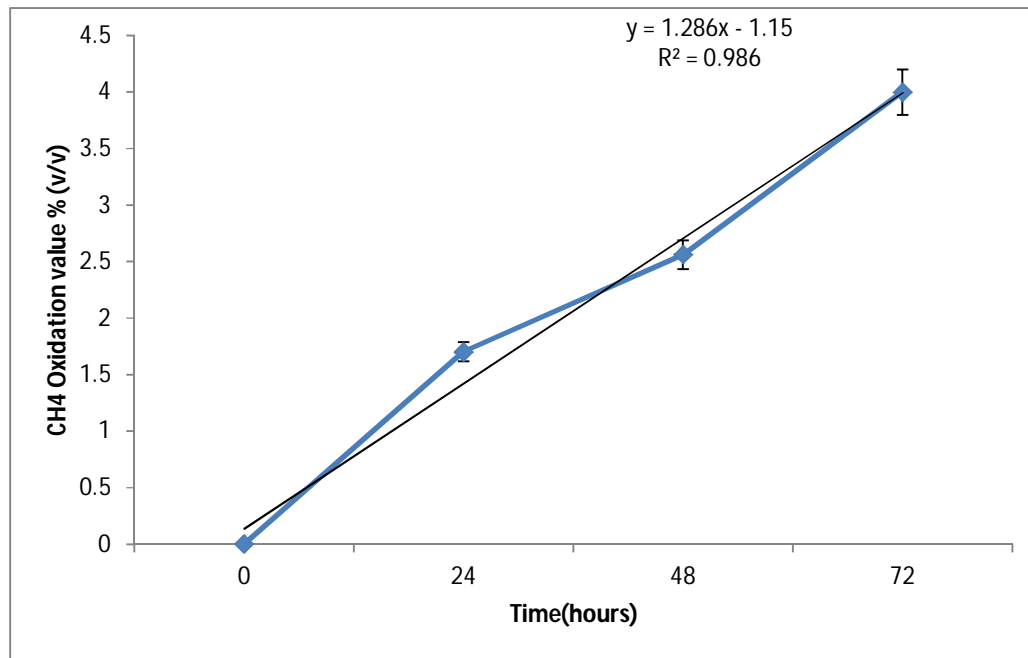


Figure 4.59: CH₄ oxidation value of column experiment at 60cm height with addition of *Methylomonas* sp. (Bar indicates standard error, n=3).

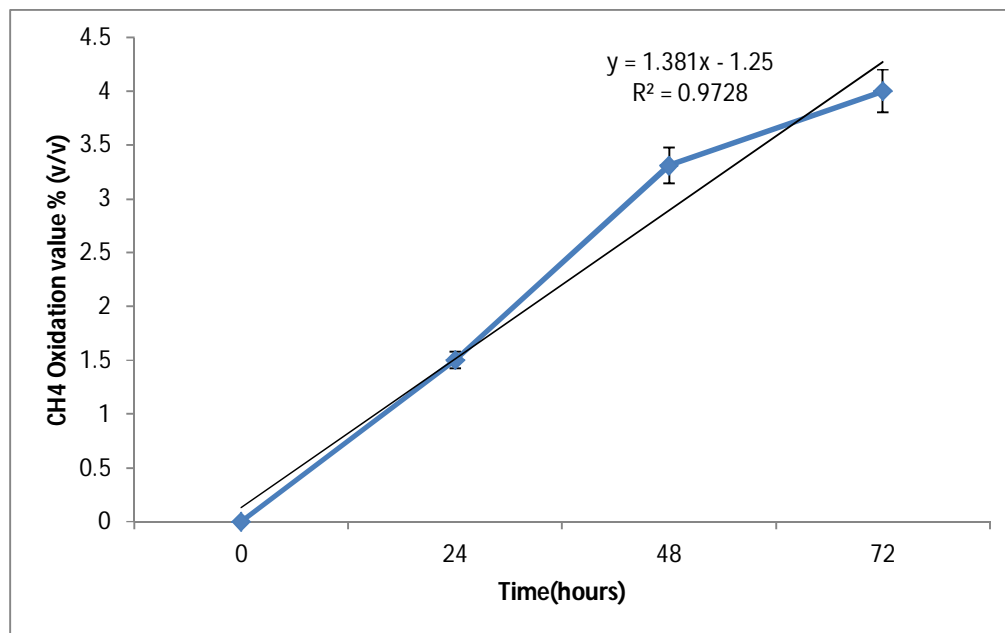


Figure 4.60: CH₄ oxidation value of column experiment at 60cm height with addition of *Methylococcus* sp 1. (Bar indicates standard error, n=3).

The potential CH₄ oxidation rate for this experiment as shown in Table 4.4 varied with addition of different types of bacteria. Higher R_p value was observed with the addition of mixed culture at 60cm height and the rate was 151.50 compared to the potential CH₄ oxidation rate with addition of *Methylococcus* sp 1 which was 98.64 followed by *Methylomonas* sp which scores 91.85. Addition of control showed potential CH₄ oxidation rate of 49.75 and *Methylococcus* sp 2 scored the lowest potential CH₄ oxidation rate (32.56).

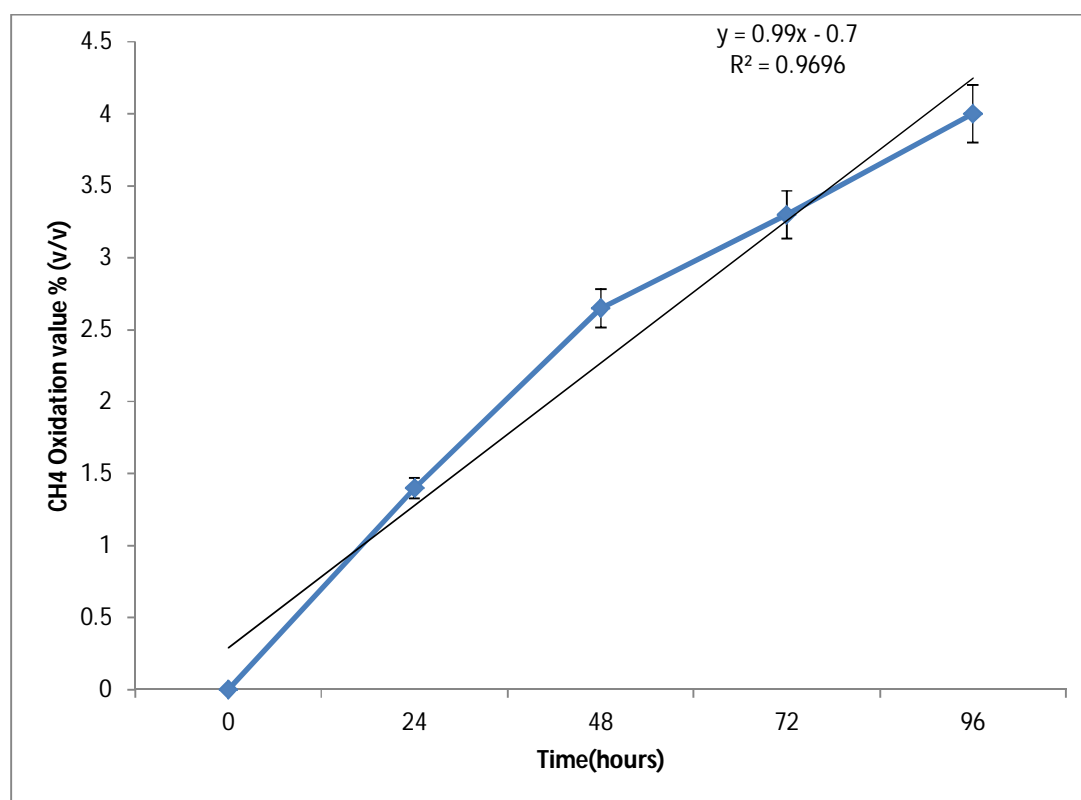


Figure 4.61: CH₄ oxidation value of column experiment at 60cm height with addition of *Methylococcus* sp 2 (Bar indicates standard error, n=3).

Potential CH₄ oxidation rate with addition of mixed culture exhibits higher rate compared to the Control and individual cultures. Addition of mixed culture shows high bacterial activity which contributed to higher CH₄ oxidation activity and higher potential for CH₄ oxidation rate. Mixed culture consists of three types of methanotrophic bacteria which adapted well to the environment and showed higher CH₄ oxidation. Previous researchers also calculated the potential CH₄ oxidation rate using the same kinetic model and obtained different values depending on type of biocover material, amount of CH₄ introduced and CH₄ oxidation rate (Pawloska and Stepniewski, 2006; Kightley *et al.*, 1995; Navarani, 2009). The K_m value obtained in column was also within the range of 0.08 to 2.54 (Pawloska and Stepniewski, 2006; Scheutz and Kjeldsen, 2004; Gebert *et al.*, 2003).

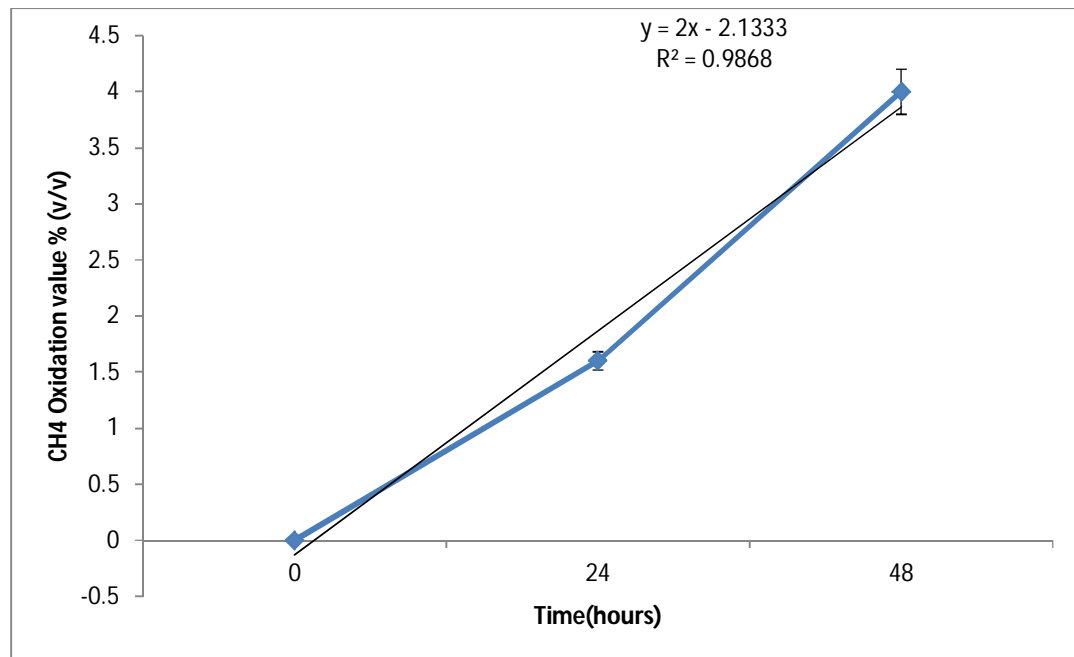


Figure 4.62: CH₄ oxidation value of column experiment at 60cm height with addition of mixed culture. (Bar indicates standard error, n=3).

Table 4.4: Potential CH₄ oxidation rate, R_p is calculated for column experiment at optimum heights of 60cm and optimum parameters.

Types	K _m (mL/ d)	R _{max} (mL/ d)	R _p (mL/ d)
control	0.9797	1.01	$R_p = 1.01 \frac{1}{1-0.9797} = 49.75$
<i>Methylomonas</i> sp	0.986	1.286	$R_p = 1.286 \frac{1}{1-0.986} = 91.85$
<i>Methylococcus</i> sp 1	0.9728	1.381	$R_p = 1.381 \frac{1}{1-0.9728} = 98.64$
<i>Methylococcus</i> sp 2	0.9696	0.99	$R_p = 0.99 \frac{1}{1-0.9696} = 32.56$
Mixed culture	0.9868	2.00	$R_p = 2.00 \frac{1}{1-0.9868} = 151.51$

CHAPTER 5

CONCLUSION

Landfill CH₄ emissions are major problem in Malaysian landfills. The microbial CH₄ oxidation is important to mitigate CH₄. Compost was identified as the best biocover material based on the physiochemical properties which able to oxidize CH₄ within 4 days. The potential methanotrophic bacteria isolated from landfill cover soil was identified as *Methylomonas* sp, *Methylococcus* sp 1 and *Methylococcus* sp 2 . All the three bacteria isolated were able to oxidize CH₄ in a very short period of time. Pure culture of *Methylococcus* sp 1 was able to oxidize CH₄ within 24 hours followed by *Methylomonas* sp which took 36 hours while *Methylococcus* sp 2 took 48 hours for complete CH₄ oxidation. Batch experiment carried out at different parameters (Temperature, Moisture content, pH) with addition of methanotrophic bacteria as individual culture or mixed culture to the compost exhibits higher CH₄ oxidation at temperature of 35°C- 40°C, moisture content of 60% (v/v) and pH 6. From this study it shows that *Methylomonas* sp, *Methylococcus* sp 1 and mixed culture showed a potential to oxidize CH₄ at higher rate when added to compost used as biocover material. The significant test at 95% confidence also showed significant difference between control and addition of *Methylomonas* sp, *Methylococcus* sp 1 and mixed culture to the compost. The addition of *Methylococcus* sp 1 recorded very high CH₄ oxidation activity rate of $8.33 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$ while *Methylomonas* sp and mixed methanotrophic culture (1:1:1) shows similar oxidation activity at rate of $4.16 \times 10^3 \mu\text{g g}^{-1}\text{h}^{-1}$.

The optimum conditions are needed in order for maximum CH₄ oxidation by the indigenous methanotrophic bacteria. The cultures are capable of oxidizing CH₄ at higher rate compared to control as individual culture and also as mixed culture in batch experiments. The bacterial count at end of the experiment also revealed that the highest count was obtained at the optimum parameters. The column experiment carried out with addition of methanotrophic bacteria shows complete CH₄ oxidation at height between 60cm to 70cm which only took 2 days compared to control at the optimum parameters. The suitable height is important for the CH₄ oxidation under landfill conditions.