Methane Oxidation: Methanotrophic Bacteria's Response to Methane Addition in a Northern Temperate Lake

BIOS 35502: Practicum in Environmental Field Biology

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Abstract

Methane is among the most potent greenhouse gases and is 23 times more effective at trapping heat per mole than carbon dioxide; accordingly added methane may increase atmospheric warming. With human population increasing at an unprecedented rate the need for food is becoming essential causing nutrient additions to crops a standard practice to promote maximum yields of produce. It is now known that fertilizer (nutrient) run-off from agriculture into lake systems inhibits oxidation of methane in lake systems. When this occurs methane may be released directly into the atmosphere rather than getting oxidized by methanotrophs within the water column. A methane oxidation rate in the oxic water column is still a poorly known process. To better understand the oxidation process we amended various amounts of water from the oxic water column with methane to determine if methanotrophs would respond. Adding methane to oxic water showed observations of significant oxidation of methane within our samples compared to our control as well as observed atmospheric levels of lakes on the UNDERC property.

Introduction

Methane (CH_4) is an odorless, colorless, greenhouse gas and is 23x (Duc, Nguyen Thanh et. al., 2010) more effective at trapping heat in the Earth's atmosphere than Carbon Dioxide (CO_2) making it a strong contributor to the warming of the Earth's atmosphere. Methane is a major by-product of decomposition of organic matter in the sediment of aquatic ecosystems. It can either be oxidized or emitted into the Earth's atmosphere. Methane concentrations have increased in the atmosphere by more than 157% since 1750 (Lehours, et al., 2008). A causal factor to this situation is the well-studied excess nutrient run-off from non-point sources into lakes as well as other aquatic systems (Edmondson & Lehman, 1981; Smith,2003; Bosch et al., 2009). Lakes that received these nutrients are characterized by accelerated eutrophication. (Sharpley et al., 1994; Schindler et al., 2008). Once eutrophication occurs there is a nutrient rich anoxic environment suitable for fermentation thus leading to CH_4 production by methanogens (Conrad, 1999). Subsequently within the substrate of lakes, CH4 production is increased due to

agricultural sources. Lake cover accounts for approximately .9% of total land area on Earth (Downing et. al., 2006) but emits an estimated 6-16% of CH_4 (Bastviken et. al., 2004) compared to less than 1% of all methane produced from oceans combined (Rhee et. al., 2009).

Methane is produced by microorganisms known as Methanogens under anoxic conditions in a process known as Methanogenesis. Optimum conditions to produce CH_4 exist under bodies of both marine and freshwater, wetlands, tundra, stomachs of ruminants and termite guts. Anthropogenic sources can include rice paddies, stomachs of ruminants (domestic) and landfills. (Hanson et. al., 2008) Within lake systems availability of organic material is key to the structure and activity of the microbial community (Graf et al. 1982; Sander and Kalff 1993). Input associated with an algal bloom results in a in a response from that community (i.e. Methanogens) within the sediment of a lake (Goedkoop et al. 1997; Tornblom and Rydin 1998).

Typically offsetting much of the production of CH_4 in lakes and the focal point of our study is the oxidation of CH_4 by methanotrophs. This bacterium oxidizes CH_4 to CO_2 in the oxic water column (R S Hanson et al. 1996). Oxidation of methane could presumably reduce emissions of methane in aquatic ecosystems. Reducing methane concentrations by enhancing methane oxidation in the water column of lakes may have significant impacts on methane emissions from on a global scale. By understanding how methanotrophs respond to increases in CH_4 production; mitigation efforts can be developed in order to reduce methane emissions from aquatic ecosystems.

Our study sought to determine how methanotrophs respond to increases of methane concentrations within the oxic water column of lakes. To do this we amended epilimnion water samples obtained from Bay Lake on the Upper Peninsula of Michigan at depth of 7.5m with various concentrations of CH_4.

Methods

Sample site

Bay Lake is an oligotrophic lake that is approximately 170 acres (U.S. Forest Service) in surface area. It is found on the property of the University of Notre Dame Environmental Research Center (UNDERC) at 46° 14' 37" North by 89° 29' 52" West. On 26 June 2012 we took 18 samples of water at 200 ml each using 500ml Viaflex plastic containers (saline solution IV bags) at a depth of 7.5 meters using a Van Dorn water sampler. Water profile at this depth (7.5m) was; 10.8 °C, DO=3.99mg/L, pH=10.35 (Figure 1).

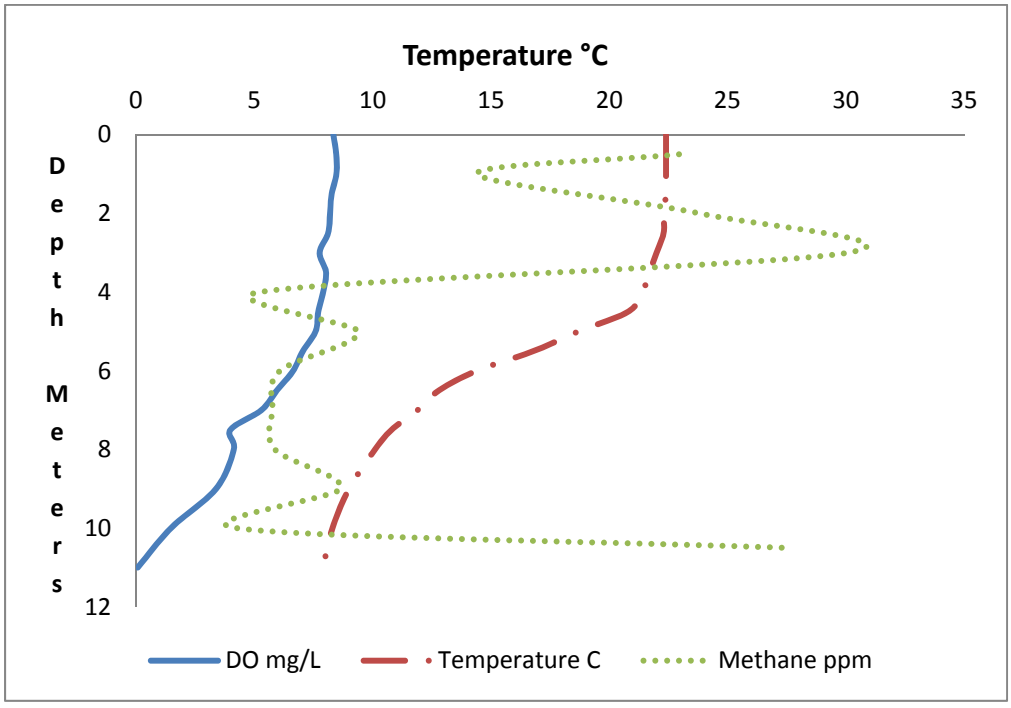

Fig. 1 Bay Lake depth profile of dissolved oxygen (solid line), CH_4 concentration (dash-dot line) and water temperature (solid line). Methane in ppm has been divided by 10 to keep the continuity of the graph.

Methane amendments, incubation and CH_4 measurements

Upon returning to the lab, water samples contained in Viaflex bags were injected with quantities of 99% CH4 for a total bag concentration of 116.8ppm, 2430.6 ppm, 4970 ppm, 24,365 ppm, 47,571 ppm and 90,818 ppm. Each treatment was performed in triplicate. These samples were then shaken vigorously and refrigerated at a temperature of 4°C for approximately an hour. After this time period, the following day and five days later, 45ml of the sample was then extracted with a syringe and 15 ml of Nitrogen (N_2) was added to create headspace within the syringe. Each gas sample was than measured on an Agilent 6890 gas chromatograph (GC) (Agilent Technologies, Santa Clara, CA, U.S.A.) equipped with a Flame Ionizing Detector (FID) and Thermal Conductivity Detector (TCD) using a GS carbon plot column with a length, diameter, and filter size of 30 mm, 0.32 mm, and 3.0µm respectively. Initial concentrations of the water samples were determined and were then re-quantified ~119 hours (5 days) later. During this time period the samples were again refrigerated at a temperature of 4°C.

Statistical Analysis

One way Anova was used to determine the significance of the methane additions to methane oxidation rates. Data was log transformed to normalize results. Tukey's HSD (Honestly Significance Difference) post-hoc test was also run with a 95% family-wise confidence level to compare all samples to each other. A Shaprio-Wilk normality test confirmed that the data was normally distributed.

Results:

CH_4 Oxidation Rates

We observed significant effects of CH_4 oxidation in our amended samples (one way ANOVA, F=36.47, P=9.19e-08, d.f.=6). (Fig. 2) Amendments above 24,365ppm also showed significant results (24,365ppm-P value 0.0026797, 47,571ppm-P value 0.0003460, 90,818ppm-P value 0.0000094). All statistical analysis was conducted on R. **(**version 2.15.1 Copyright 2012© The R Foundation for Statistical Computing)

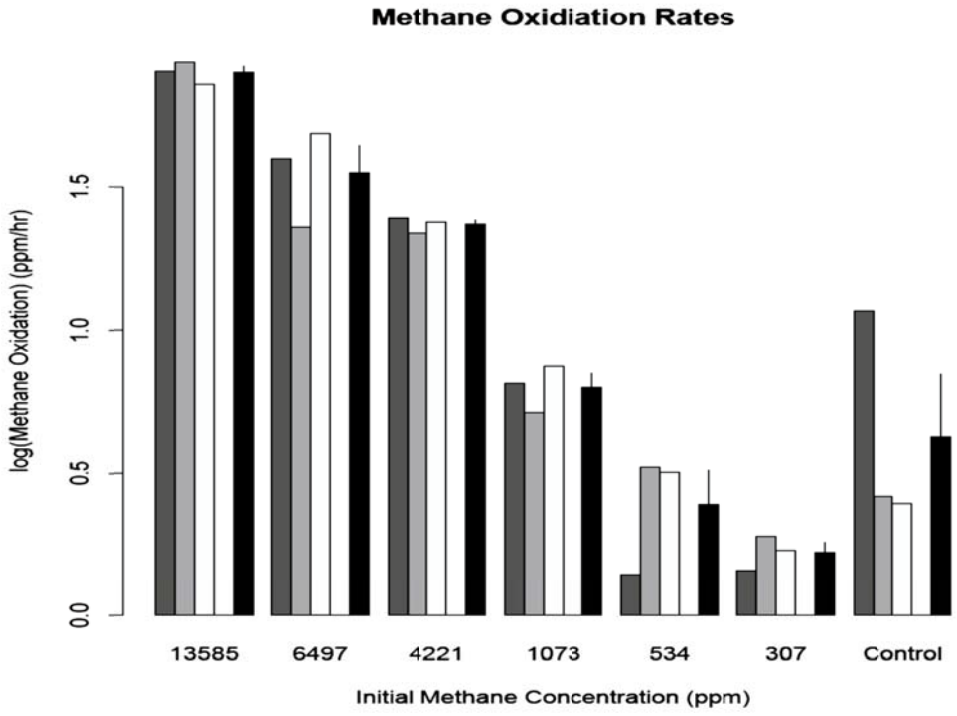

Fig. 2 Three treatments and average bar with standard deviation added. ANOVA: (F=36.47, P=9.19e-08, Df=6,)

Discussion

Our goal was to determine the how oxidation rates by methanotrophs were affected by various concentrations of CH_4. With the addition of CH_4 in our water samples we saw, compared to our controls, (F=36.47, P=9.19e-08, Df=6,) methane oxidation. In future studies it may be useful to determine oxidation rates at higher concentrations than we used. We intended to find the point where amendment concentration is no longer effective which may be useful for future research on CH_4 oxidation. At this time we do not know this concentration but suspect the amount is greater than our highest concentration (Fig 3.). With our experiment we observed CH_4 oxidation within our test samples with concentrations greater than 1073 ppm.

Compared to our amended samples, methane concentrations were observed as low as atmospheric concentrations and as high as 16970ppm in the water column of UNDERC lakes. Treatments in our study were within this range and therefore our study suggests that methanotrophs respond to concentrations observed within the water column of temperate lakes.

A biological feedback loop is observed within this system. With increased production of CH_4, oxidation of CH_4 also increases as a check to balance out the system. The exact amount of oxidation to production is not known at this time but would be well worth investigating.

The importance of this research has far reaching implications; rate of CH_4 oxidation responds to increased CH_4 production, further, there is little study of this process. This research could provide data that could enhance further research into the larger picture of climate change due to increased CH_4 production.

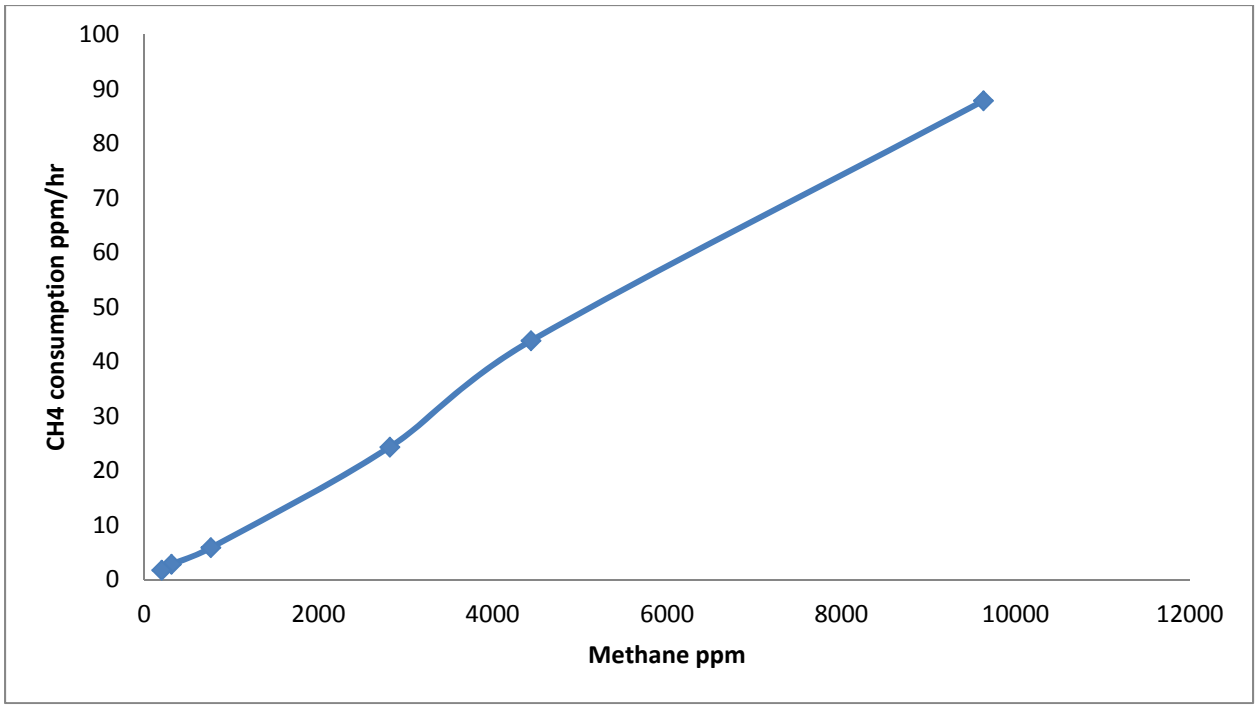

Fig. 3. Initial methane concentrations in viaflex bags plotted vs methane oxidation rates.

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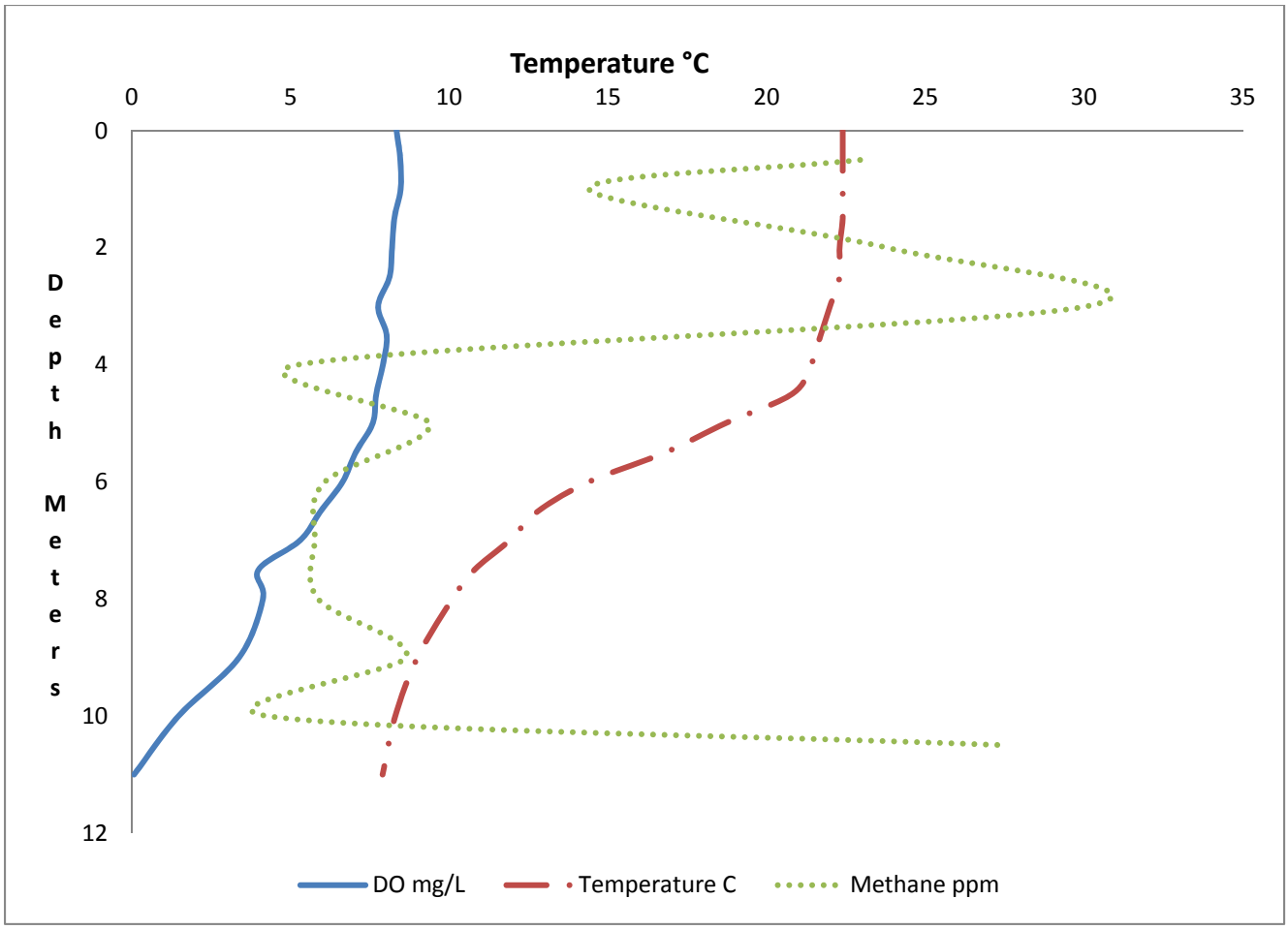

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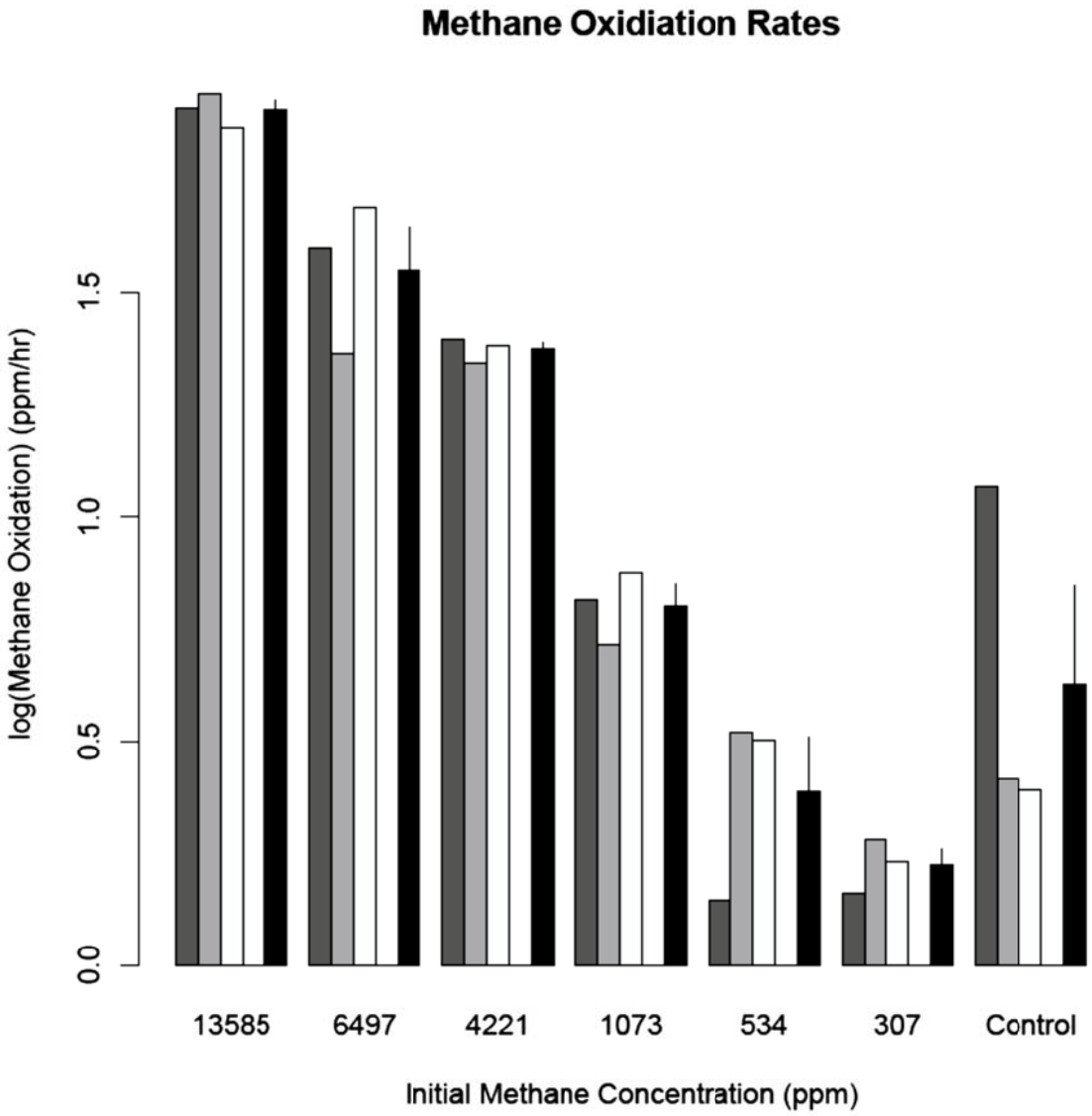

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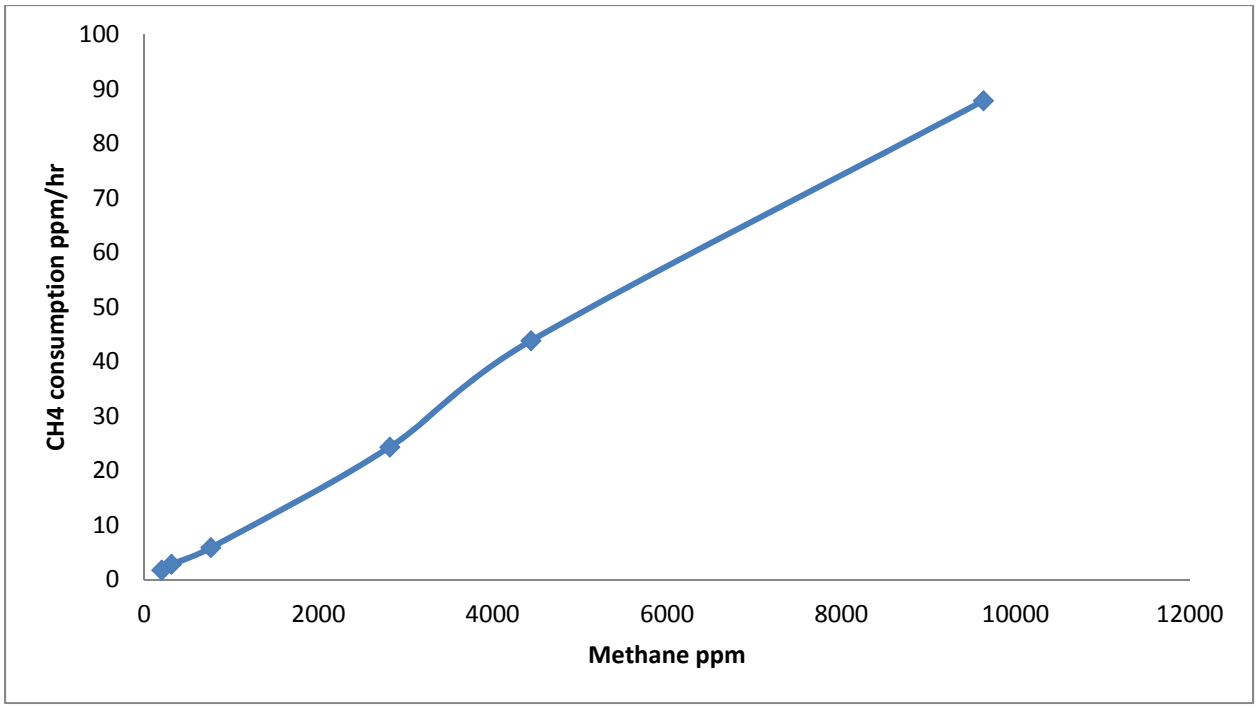

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