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EFFECT OF NUTRIENTS ON METHANOTROPHIC OXIDATION ACTIVITY IN BATCH EXPERIMENTAL STUDIES

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ABSTRACT

Methane biofiltration (MBF) has been proposed as an effective and cost efficient method for mitigating methane emissions from anthropogenic sources. Previous MBF studies have successfully demonstrated effective methane oxidation using compost, soil and biodegradable organic materials as the filter medium because they are a natural source of nutrients, organic matter content, and provide adequate space to support microbiological growth. However, these media types will inevitably decompose over time and lose their effectiveness. This study investigates the use of biologically stable media mixtures of lava rock and biochar in batch oxidation studies to determine their ability to support a methanotrophic bacterial population for the oxidation of methane. A screening experiment was performed using an unreplicated 2^3 factorial design to understand the influence of nutrient content, water holding capacity (WHC), and mixture ratios of lava rock and biochar, by volume, on the methane oxidation rate (MOR). Virtually no activity was observed when 3300 ppm of nitrogen were added to the batches. Methane oxidation activities were only observed when lower nutrient additions were made at 96 ppm of nitrogen with values reaching 2.2 - 2.5 μmol/mL·hr when the WHC was adjusted to 50% regardless of the media composition. Subsequent replicates performed at the lower nitrogen level additions and 30% WHC demonstrated similar MORs. Results demonstrate the supplementation of nutrients to a mixture of lava rock and biochar is possible in supporting a methanotrophic population and that lava rock can be used as a bulking agent.

Keywords: Methane oxidation, biochar, lava rock, biofilter, batch studies, methanotrophs, greenhouse gas

1. INTRODUCTION

Methane (CH_4) is a potent greenhouse gas (GHG) with a global warming potential of 34 over a 100 year time horizon relative to CO_2 (IPCC 2013). The oxidation of CH_4 by methanotrophic bacteria serves as an important sink for the removal of CH_4 that otherwise would escape from terrestrial and aquatic environments into the atmosphere. Microbiological oxidation of CH_4 in granular media is a biological air pollution technology that utilizes microorganisms that are attached to a solid surface for the biodegradation of chemicals in the gaseous state—otherwise known as methane biofiltration (MBF). MBFs comprising of soils and similar granular media mixtures has been proposed as an inexpensive method for the mitigation of CH_4 from heavy oil well sites, landfills, and other terrestrial systems. Previous MBF studies that have utilized organically labile filter bed materials, such as compost and landfill cover soil, commonly observed 100% CH_4 oxidation but eventually would taper off to a lower level of performance (Gebert, et al. 2003, Hilger, et al. 2000, Huber-Humer, et al. 2011, Wilshusen, et al. 2004).

Recently, there has been a growing interest in utilizing biologically stable materials in place of compost and landfill cover soil in MBFs to overcome declining performance issues due to biodegradation (Avalos Ramirez, et al. 2012, Kim, et al. 2013, Nikiema and Heitz 2009). Biochar is a biomass that has undergone thermochemical conversions through pyrolysis, gasification, or by hydrothermal carbonization in an O_2 limited environment (Qian, et al. 2015).

High temperature pyrolysis conditions produce biologically stable biochar (Keiluweit, et al. 2010, Zhang, et al. 2014) and thereby making them suitable materials to support methanotrophic growth in MBFs. The physical and chemical properties of biochar that are of interest include their adsorption capacity for CH_4; ability to buffer against sudden changes to moisture content; and providing increased aeration, porosity, and surface area for methanotrophic growth (Kinney, et al. 2012, Reddy, et al. 2014).

The purpose of this research is to evaluate the effect of two different media types or their mixtures, by volume, including lava rock and biochar, nutrient addition, and moisture content (based on the water holding capacity (WHC) of the mixture) on the methane oxidation capacity. Lava rock (also known as volcanic rock) is investigated here to determine its suitability as a bulking agent without compromising the methane oxidation activities and to, ultimately, reduce the cost of operating a field-scale biologically stable MBF.

2. METHODS

2.1 Media Material

A biologically stable biofilter media mixture was tested in an unreplicated 2^3 factorial design comprising of 3-20 mm diameter lava rock (Burnco, Calgary, Alberta) and wood-based biochar (Diacarbon, Burnaby, British Columbia). The biochar was produced using fast pyrolysis at 550°C with 20-30 minutes residence time. As the feedstock for the biochar is primarily in the form of sawdust, there is no pretreatment of the material and, therefore, the overall size and morphology of the individual particles following treatment is similar to the parent material and ranges from 0.5 to 1 cm long slivers. Compost from East Calgary Landfill in Calgary, Alberta was used as a source for methanotroph starter material. The compost was acquired in July 2015 and sieved through a no. 8 sieve (2.38 mm) prior to use.

2.2 Physical and Chemical Characterization Testing

Duplicate samples of compost were sent to ALS environmental laboratories for analysis of total and available phosphorus and nitrogen. In addition, the three media types were analyzed for moisture content, bulk density and WHC prior to the batch incubation experiments. Following the completion of the experiments, the moisture content was redetermined.

2.3 Batch oxidation experiments

In order to test the oxidation capacity of using biologically stable media mixtures of lava rock and biochar, batch oxidation experiments were conducted in the laboratory. Three factors were chosen to study their potential interactive effects on the methane oxidation rate (MOR) including nutrient level, WHC, and mixture ratio by volume using an unreplicated 2^3 factorial design (see Table 1). Three center-points were included to test for curvature. Each treatment combination was monitored for a total of 10-11 days. The center-points were monitored for a total of 18 days.

Table 1: 2^3 Methane oxidation rate experimental factors and levels. Each treatment combination consisted of 10% (v/v) of compost as the source of microbes. Here AN and TN denote the bioavailable and total nitrogen level of the ECL compost used in this study.

Factor	Treatment Level	
	Low	High
A* (lava rock: biochar)	100% biochar	90% lava rock, 10% biochar
B (WHC)	10%	50%
C (nutrient level)	96 ppm Nitrogen (¼ AN)	3300 ppm Nitrogen (¼ TN)

* The mixture ratios are on a volume basis.

An overall depth of 2.5 cm of media mixture was used for each test in order to ensure a consistent volume of media mixture was used in each batch and to allow for direct comparison between treatment combinations on a volume basis. Previous batch studies with a single media type, such as compost, typically have used a ratio of 10 g of media per 250 mL—equating to approximately 1 – 1.5 cm of overall depth (Perdikea, et al. 2008, Stein and Hettiaratchi 2001). In this study, the large variation in mass between lava rock and biochar requires that the comparisons between the different treatment combinations are to be done a volume basis; as a result, the diameter to depth ratio of 10 g of compost that would occupy a 250 mL amber bottle was calculated to determine an appropriate depth of media for the 1 L amber bottles used in this study. A larger depth was not used in this study in consideration of the gas diffusion potential through the media and to account for gas withdrawal from the headspace for periodic gas analysis. The source of starter microbes was provided in the form of compost at 10% (v/v) added to each bottle. The remainder 90% of the volume was then prepared using the assigned media mixture ratio as identified in Table 2. Based on the moisture content and WHC of each media material, a predetermined amount of distilled water was added into each amber jar, as necessary, in order to attain the target WHC.

Nutrients were added to match the target bioavailable nitrogen content in the same volume of compost for both the low and high level as outlined in Table 1. An all-purpose garden fertilizer (Miracle Gro®) at a N:P:K ratio of 24:8:16 was used for nutrient addition. As is common in commercial garden fertilizers, the phosphorus is actually in the form of P_2O_5 (phosphorus oxide) and therefore, the actual elemental nitrogen to phosphorus ratio in the fertilizer is 24:3.5 since the weight percent of elemental phosphorus in P_2O_5 is 43.6%. The pelletized garden fertilizer was dissolved into the distilled water and added together into each treatment combination. To ensure comparability between treatment combinations, the amount of nutrients added to the batches for the low level and high level conditions was based on approximately ¼ of the amount of bioavailable and total nitrogen, respectively, that would be present in ~166 mL of compost (i.e. 2.5 cm depth). Additional nutrients were added in the form of the fertilizer after compensating for the nutrients already present in the 10% (v/v) of compost. As a result, nutrients were added to obtain a nitrogen concentration of 96 ppm or 3300 ppm (Table 1) for the low and high level, respectively.

The mouth of the amber bottles were lined with Teflon® tape and sealed with a polypropylene cap and Teflon®/silicone liner and septum—where gas withdrawal was made for analysis. Electrical tape was used as an additional sealant around the outside of the cap. A blank bottle served as a control and was prepared in the same manner as the other bottles but without any media, water, or nutrient addition in order to measure CH_4 leakage. All bottles and bottle caps were autoclaved prior to use in order to avoid contamination from previous experiments. In between measurements, the bottles were placed in an incubator-shaker (G24, New Brunswick Scientific Co, Inc.) at 30°C and shaken at the lowest speed setting in order to ensure that the CH_4 gas is evenly distributed throughout the bottle and to minimize the opportunity of it separating from the air phase due to differential density.

Approximately 50 mL of air was withdrawn from the headspace of each bottle and replaced with CH_4 gas (99.9% purity, Praxair, Calgary, Alberta) to achieve a headspace concentration of 5.5 – 6.0 % (v/v). Gas samples were obtained using a 5 mL luer lock syringe and non-coring needle fitted with a two-way stainless steel stop-cock (Model 1005 SL SYR, Hamilton®, Reno, Nevada). Following each headspace sampling, a layer of fresh silicone was then applied to the septum to prevent gas leakage in between measurements. The samples were then immediately injected into a Varian CP4900 micro gas chromatograph (Varian Canada Inc.) equipped with a thermal conductivity detector (TCD). Results were analyzed on "Galaxie Chromatography Data System" (Version 1.9.3.2, Varian Inc.). The micro GC is outfitted with two columns including a 10 m in length MolSieve (MMS) and a 10 m PPU. The MMS and the PPU have the following specifications: injector temperature of 110ºC and 100ºC respectively; an oven temperature of 80ºC for both columns; and a carrier gas pressure of 29 psi and 40 psi, respectively. Helium was used as the carrier gas. Gas samples were obtained once per day until the CH_4 concentration began to deplete which provided an indication that the methanotrophic bacterial population was establishing itself. At this point, the bottles were flushed with air and reinjected with CH_4 gas. As soon as the O_2 content dropped below ~14% (v/v) or if ~10% of the headspace volume had been withdrawn the samples were flushed with air and reinjected with CH_4 in order to reduce potential stress on the methanotophs due to the reduction of O_2 or the build-up of negative pressure in the bottles, respectively. A minimum of 3 gas measurements were then taken over the course of 6 hours per day in order to calculate the CH_4 oxidation rate each day. Prior to making measurements, the GC was calibrated using two ultra-high purity CH_4 standards at 1 and 80% CH_4 (v/v), respectively.

3. RESULTS AND DISCUSSION

3.1 Physical and Chemical Characterization

Table 2 provides the results of the media characterization tests for biochar, lava rock, and compost. The bulk density (based on wet weight, ww) of biochar was lowest of the three material as expected since it is also the lightest. Although the pyrolysis process of manufacturing biochar would remove any trace of water from the material, in preparing the biochar for shipment the manufacturer treats it with a small amount of water in order to reduce the amount of dust production; therefore, both the bulk density and the moisture content of the biochar varies from batch to batch; as a result, only one batch of biochar was used for these set of experiments. Compost had the highest moisture content as it was freshly obtained from the landfill. The compost displayed a WHC of 153% (dw) and is roughly half the amount of the biochar.

Table 2: Physical properties of biochar, lava rock, and compost.

Media	Bulk density (g/mL, ww)	Moisture Content (% ww)	Water holding capacity (% dw)
Compost	0.63 ± 0.02	36.6 ± 0.19	153 ± 7
Lava rock	0.80 ± 0.01	0.1 ± 0.03	12 ± 3
Biochar	0.23 ± 0.01	7.6 ± 0.04	330 ± 12

As neither the biochar nor the lava rock is a significant source of nutrients for the methanotrophs (Zhang, et al. 2015), the compost was analyzed for both bioavailable and total nitrogen and phosphorus at ALS environmental laboratories (Calgary, Alberta). Table 3 are the results of the nutrient analysis. The ratio between total nitrogen and total phosphorus is approximately 6:1 in the ECL compost. As a result, the garden fertilizer with an actual elemental N:P ratio of 24:3.5 was chosen for nutrient matching. An additional benefit of using the all-purpose garden fertilizer is that it contains copper at 0.07% (w/w), which is a critical micronutrient for the methanotrophs (Dedysh and Dunfield 2014).

Table 3: Bioavailable and total phosphorus and nitrogen nutrient content in East Calgary Landfill compost (dry mass basis).

	Bioavailable phosphorus (mg/kg)	Total phosphorus (mg/kg)	Bioavailable nitrogen (mg/kg)	Total nitrogen (mg/kg)
Compost sample 1	611	2300	404	13,300
Compost sample 2	608	2252	399	14,200
Average	609.5	2275	401.5	13,750

3.2 Batch oxidation experiments

The results from the single unreplicated 2^3 factorial design are provided in Table 4. Of the 8 treatment combinations, only 4 demonstrated CH_4 oxidation activity and they all coincided with low level nutrient additions (i.e. ¼ of AN or 96 ppm nitrogen). The high level nutrient (i.e. ¼ of TN or 3300 ppm nitrogen) treatment combinations showed peak activity on Day 1 but dropped thereafter to virtually no activity (data not shown); however, all of the ¼ TN demonstrated very minimal activity. In fact, some of the high level nutrient experiments displayed slightly negative oxidation rates over the course of the experiments. Although it is unlikely that these treatment combinations were producing CH_4 since the O_2 content was always maintained at > 14% (v/v) and therefore the development of an anaerobic environment for methanogenesis to take place (Feng, et al. 2012) is unlikely. The O_2 drop in the high level nutrient treatment combinations coincided with CO_2 increases and is indicative of the presence of biological activities; therefore, in the presence of nutrient rich media the methanotrophs may have been outcompeted by other bacteria that are better able to metabolize the higher level of nutrients and thereby outgrow and utilize all of the available space. In a recent study on the effects of NH_4-N addition to a biofilter column Hernandez, et al. (2015) similarly observed a drop in CH_4 removal efficiencies with increasing N concentrations; however, in their study, the impact on the response

was attributed to inhibitory effects related to NH_4-N. Another possible reason may be the hydrolysis of urea from the fertilizer, which makes up 20.5% of the total nitrogen content with the remaining 3.5% as ammoniacal nitrogen, into NH_4-N which has been demonstrated to be inhibitory to methanotrophs (Bedard and Knowles 1989). Similarly, Jugnia, et al. (2012) observed minimal improvement on methanotrophic activity to a landfill cover soil following the amendment of urea at three different incubation temperatures: 5°C, 15°C and 25°C.

Table 4: Peak methane oxidation rate on the unreplicated 2^3 factorial experiment on mixture ratio, by volume, of lava rock: biochar; % water-holding capacity; and nutrient content. All treatment combinations were inoculated with 10% (v/v) compost. Three center-points were included to test for curvature. Low level nutrient additions (¼ AN) corresponded to 96 ppm and high level nutrient additions (¼ TN) 3300 ppm.

Run Label	Factor A Mixture	Factor B % WHC	Factor C Nutrient	Methane oxidation rate ($\mu mol \cdot mL^{-1} \cdot h^{-1}$)
(1)	100% biochar	10	¼ AN	0.92
a	90% lava rock: 10% biochar	10	¼ AN	0.68
b	100% biochar	50	¼ AN	2.52
ab	90% lava rock: 10% biochar	50	¼ AN	2.24
c	100% biochar	10	¼ TN	0.005
ac	90% lava rock: 10% biochar	10	¼ TN	0.009
bc	100% biochar	50	¼ TN	0.029
abc	90% lava rock, 10% biochar	50	¼ TN	0.009
Center-point 1	45% lava rock: 55% biochar	30	⅛ TN	0.01
Center-point 2	45% lava rock: 55% biochar	30	⅛ TN	0.22
Center-point 3	45% lava rock: 55% biochar	30	⅛ TN	0.02

* Methanotrophic oxidation rates for all of the ¼ TN and the three center-point treatment combinations subsequently dropped following the peak MORs reported here.

The time profile of the MORs for all four ¼ AN treatment combinations are shown in Figure 1. Treatment combination *b* (100% biochar, 50% WHC, and ¼ AN addition) and *ab* (90% lava rock: 10% biochar, 50% WHC, and ¼ AN addition) demonstrated the highest MOR at 2.5 $\mu mol \cdot mL^{-1} \cdot h^{-1}$ (Day 4) and 2.2 $\mu mol \cdot mL^{-1} \cdot h^{-1}$ (Day 8), respectively.

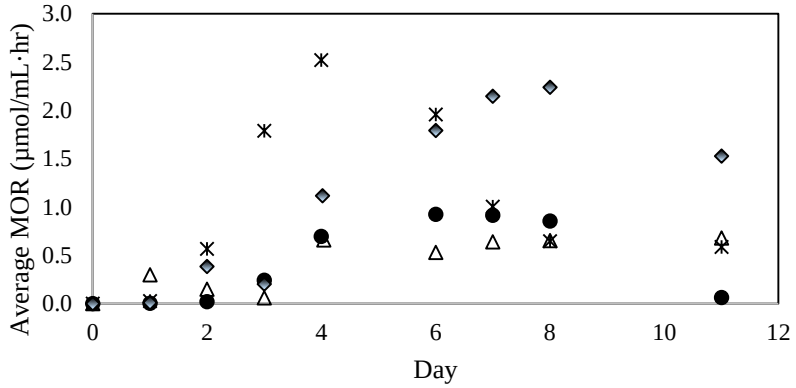

Figure 1: Time profile of the MOR over 12 days for the ¼ AN treatment combinations: (✳) denotes 100% biochar, 50% WHC, ¼ AN (treatment combination b); (●) denotes 100% biochar, 10% WHC, ¼ AN (treatment combination (1)); (△) denotes 90% lava rock: 10% biochar, 10% WHC, ¼ AN (treatment combination a); and (◆) denotes 90% lava rock: 10% biochar, 50% WHC, ¼ AN (treatment combination ab).

This peak oxidation state remained for only 1 day for both treatment combinations and declined thereafter. By Day 8, treatment combination *b* had dropped to nearly one-quarter of the peak activity level. Treatment combinations (1) (100% biochar, 10% WHC, and ¼ AN addition) and *a* (90% lava rock: 10% biochar, 10% WHC, and ¼ AN addition) maintained at their peak levels for more than one day but the highest MORs were not even half of the peak MORs for treatment *b* and *ab*. In fact, treatment *a* was able to sustain steady oxidation rates at around 0.6 $\mu mol \cdot mL^{-1} \cdot h^{-1}$ when

the experiments were ceased on Day 11. From the results on the ¼ AN experiments, higher WHC had a positive influence on MOR activities in both the 100% biochar and the 90% lava rock, 10% biochar mixtures—indicating that the use of lava rock produced comparable results to the 100% biochar mixture. This may suggest that only a small amount of biochar is required to support methanotrophic bacterial growth, in place of compost, and that the use of lava rock as a bulking agent is promising. Alternatively, it is also possible that the 100% biochar batch experiment, treatment *b*, was limited by CH_4 diffusion into the media since the addition of water to reach 50% WHC was observed to swell the material and increase the overall depth beyond 2.5 cm.

The center-point replicates were observed for a total of 19 days, 8-9 additional days from the other experiments, to observe if the methanotrophs could eventually acclimatize to ⅛ of the TN addition. The MORs peaked on Day 3 for CP1 and CP3 and Day 2 for CP2 before declining to nearly zero or slightly negative values. Day-to-day measurement variability on the micro GC may be a possible reason for the calculated negative MOR values since the changes in CH_4 concentration were minor from day to day. Although CP2 displayed a peak MOR that is approximately 10-20x of CP1 and CP2 it had dropped to 0.003 $\mu mol \cdot mL^{-1} \cdot h^{-1}$ by the end of the experiment. Similar to the high level nutrient experiments, all of the center-point replicates exhibited very minimal activity.

3.2.1 ANOVA Analysis

As a full ANOVA statistical analysis cannot be performed on an unreplicated factorial experimental design, due to the lack of an error term, the effects are plotted on a normality curve to observe for any main effects or interactions between the effects. A normality curve (not shown) of the effects revealed three outliers corresponding to the significance of % WHC (factor B), nutrient level (factor C), and their corresponding interaction (BC). These results are re-confirmed upon including the third-order interaction as the error term in an ANOVA: % WHC ($F(1,1) = 1082.88$, $p<0.05$), nutrient level ($F(1,1) = 3936.77$, $p<0.05$), and the interaction term between %WHC and nutrient level ($F(1,1) = 941.46$, $p<0.05$). Due to the significance of the interaction term, it is not possible to look at the % WHC and the nutrient level independently. When the nutrient is under ¼ AN conditions, an increase in % WHC produces a higher MOR, whereas at the ¼ TN level, virtually no activity occurs with an increase in % WHC.

Unfortunately, the diagnostic checks on the residuals indicate that these results may not reveal all of the significant effects. Linearity of the factor effects is the underlying assumption of the 2-level factorial design. Upon inclusion of the three center-points, the ANOVA confirms that curvature is significant ($F(1,2) = 5646.25$, $p<0.05$) as is evident in the corresponding MORs (**Error! Reference source not found.**). Therefore, it is not possible to use the results of the above factorial analysis to prepare a linear regression model.

The moisture content of the different media mixtures were compared before and after the batch oxidation experiments. In most cases, the moisture content had dropped following the experiments, with the exception of treatment combination *ac* (90% lava rock: 10% biochar, 10% WHC, and ¼ TN addition) and *abc* (90% lava rock: 10% biochar, 50% WHC, and ¼ TN addition) indicating that the loss of water from the system is greater than the production by the methanotrophs. The moisture content reduction may be due to the consumption by other microorganisms present in the media or due to evaporation during air flushing before CH_4 reinjection.

3.3 Center-point batch oxidation experiments at available nutrient levels

Due to the inhibitory nature of the ¼ TN levels, 3 center-point replicates were prepared for the existing results on the treatment combinations of ¼ AN (Figure 2). All three replicates demonstrated a similar pattern of MOR of up to ~2.4 $\mu mol \cdot mL^{-1} \cdot h^{-1}$ between Day 5 and Day 7 before moving on a downward decline. This MOR activity is similar in response to treatment *b* and *ab* which indicates that the % WHC of the mixture may be the determining factor on MOR when nutrient is held constant. On Day 12, each replicate was supplied with additional nutrients at three different levels: ¼ AN, 1¼ AN, and 2½ AN in approximately 3-4 mL of distilled water. Center-point no 1 (CP1) responded immediately on the following day with an MOR of 4.3 $\mu mol \cdot mL^{-1} \cdot h^{-1}$, the highest MOR observed in all the experiments, which suggests that the bacteria respond best to repeated low-level nutrient additions rather than higher dosages at any given time.

Additional nutrient was supplied to CP1 on Day 14 at a dosage of ½ AN following the decline in MOR observed on Day 13. The response to ½ AN increased more gradually and did not peak to the same levels as the previous ¼ AN addition but the MOR rose quicker than the 1¼ AN additions made to center-point 2 (CP2) on Day 12—although also declining sooner. Conversely, CP2 and center-point 3 (CP3) required more time to adapt to the higher nutrient level additions including up to 7 days for CP3. Not only did CP2 respond sooner than CP3 but the MOR fluctuated less and were generally higher. These observations provide further evidence that the methanotrophs prefer low levels of nitrogen and phosphorus. For this reason, compost is a great media to support methanotrophic growth as the nutrients are slowly released over time; however, compost also biodegrades over time and, inevitably, loses their overall efficacy (Wilshusen, et al. 2004). On Day 21, all three center-point replicates were removed from the incubator-shaker and left on the laboratory bench at room temperature. MOR activities subsequently dropped for all three treatments but this may have also been due to the exhaustion of nutrients; however, CP2 at 1¼ AN supplementation appeared to be relatively steady and maintained MOR activities at ~1.3 $\mu mol \cdot mL^{-1} \cdot h^{-1}$. Therefore, it is unlikely that the nutrient supply in CP3 was depleted but rather the higher nutrient content was causing instability.

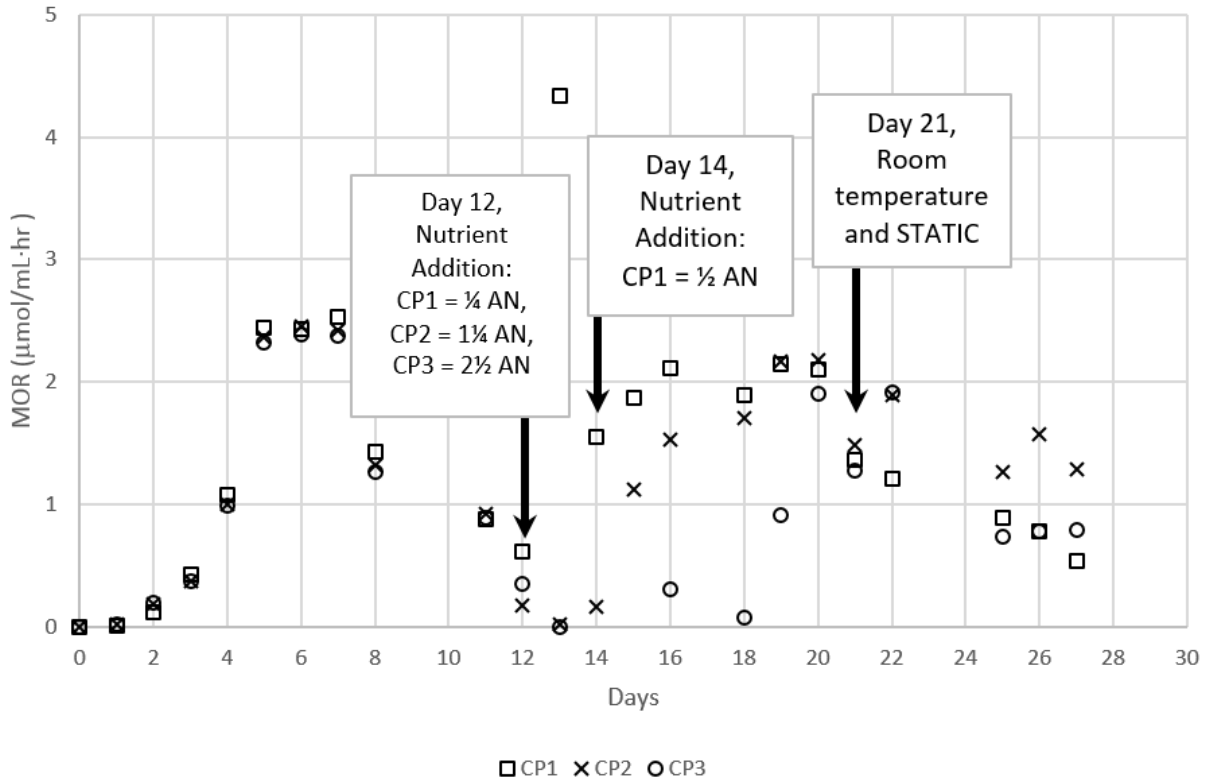

Figure 2: Center-point triplicates prepared using available nutrient addition followed by three different nutrient concentration additions on Day 12. Additionally nutrient addition was made to CP1 on Day 14. All three replicates were placed in an incubator shaker set at 30°C until Day 21 where they were removed to a stationary bench at room temperature.

4. CONCLUSION

This study investigated the influence of nutrient content, WHC, and the use of lava rock and biochar on the MOR. The unreplicated 2^3 factorial design revealed an interaction effect between WHC and nutrient content. However, diagnostics checks on the underlying model revealed that further investigations are required as the ANOVA uncovered significant curvature and therefore, the assumption of linearity in the 2-level factorial design did not hold. There were two conclusions drawn from these experiments. First, high nutrient content appeared to inhibit the growth of the methanotrophs as evidenced by the lack of CH_4 oxidation. Although CH_4 was not consumed, CO_2 continued to be produced while O_2 was depleted, this lends support to the conclusion that other microorganisms dominate the media mixture at higher nutrient content and likely displaces the methanotrophs by outcompeting them for nutrients and

space. At higher concentrations, the NH_4-N production through the hydrolysis of urea from the fertilizer may have also contributed to inhibitory effects. At compost ¼ AN levels of nutrient, MOR was found to be higher when the WHC was at least 30% regardless of the media type or mixture used and responded more favorably to further nutrient additions at low levels; however, in order to maintain peak MORs will require frequent nutrient additions. Second, the preliminary results indicating an indifference to media type suggests that only a minimum amount of biochar is required to support an effective methanotrophic population for methane oxidation activities. The use of lava rock as a bulking medium appears to be promising for future MBF applications. Further studies are required to examine the MOR at lower ranges of nutrient addition and with urea-free fertilizer in order to observe for potential interaction effects with media types and WHC and the duration of one full cycle of MOR consumption activities per nutrient level addition relative to the total lag time for the methanotrophs to adapt to the conditions.

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