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Enhancing Methane Oxidation in Landfill Cover Soil Using Microbially Activated Biochar: A Sustainable Approach for Mitigating Greenhouse Gas Emissions

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Abstract:- Landfill gas, primarily composed of methane (CH_4) and carbon dioxide (CO_2), poses significant environmental and health risks due to its contribution to greenhouse gas emissions and potential explosive hazards. This study proposes an efficient way to lower methane emission amended cover soil with biochar and microbially activated biochar (MAB), Because microbial acclimation causes an initial lag phase, adding biochar to landfill cover soil lowers the rate of CH_4 oxidation. Activated biochar, which is produced by converting biochar induced with methane oxidizing bacteria, shortens the acclimation period and increases the methane oxidation activity. In this study series of Batch experiments were conducted to evaluate the methane oxidation capacity of soils amendment with various proportions of biochar. Results demonstrated that MAB-amended soils significantly enhanced methane oxidation rates (213 mg/g) compared to non-activated biochar–amended soils (195.11 mg/g) and biochar alone (32 mg/g). Microstructural, chemical, and mineralogical analyses using SEM, EDX, and XRD revealed that MAB provides an ideal environment for microbial colonization, thereby improving methane oxidation. This study highlights the potential of MAB as an effective strategy for mitigating methane emissions from landfills.

Keywords*: Methane Oxidation, Landfill Gas Emissions, Biochar Amendment, Microbially Activated Biochar (MAB), Methanotrophic Bacteria, Gas Chromatography (GC), Greenhouse Gas Mitigation, Sustainable Waste Management, Soil Amendment, Methane Reduction Strategies.*

1. Introduction

Urban waste disposal remains a major environmental challenge, with landfills being the most widely used method for managing municipal solid waste (MSW) [6]. The rapid increase in MSW production, driven by population growth and rising socio-economic levels, has exacerbated the demand for effective waste management solutions [14] Landfills, while offering a designated area for the safe disposal of non-recyclable refuse, play a critical role in reducing environmental waste loads. Without these controlled disposal sites, improper waste handling could severely threaten public health and surrounding ecosystems. In addition to their role in waste consolidation, transport, and processing [9] landfills generate a variety of gases during waste decomposition. The composition of landfill gases (LFG) typically includes 45% to 65% methane (CH_4) and 40% to 60% carbon dioxide (CO_2), alongside trace amounts of nitrogen, oxygen, ammonia, sulfides, hydrogen, carbon monoxide, and non-methane organic compounds (NMOCs) such as trichloroethylene, benzene, and vinyl chloride [10].

The release of landfill gas is a significant environmental concern due to its contribution to greenhouse gas emissions and the associated health and safety risks. Methane generation is more when organic wastes break down anaerobically in landfills [15]. Methane contributes to climate change and global warming by trapping heat in the atmosphere, along with other greenhouse gases such as CO_2 and nitrous oxide. The negative effects of these emissions extend beyond rising temperatures, influencing air quality, altering climate patterns, disrupting ecosystems, and impacting human health. As the global carbon cycle is closely linked to soil organic carbon (SOC) stocks, effective management of landfill cover soils is essential for controlling methane emissions and maintaining carbon balance in the environment.

Reducing greenhouse gas emissions from landfills is a crucial strategy for mitigating climate change. Biocovers, which serve as engineered landfill covers, have emerged as an important tool for managing low-level methane emissions that escape LFG extraction systems. In recent years, there has been a growing interest in utilizing organic amendments to promote microbial activity and improve methane reduction in landfill soils [16]. Studies have demonstrated the potential of organic materials, such as biochar, to improve methane removal through microbial processes [2] [4] and [18]. Methane-oxidizing bacteria, or methanotrophs, naturally enrich landfill cover soils when exposed to CH_4 emissions. These bacteria metabolize methane as their primary carbon and energy source, converting it into CO_2 and water in the presence of oxygen [3] [14].

The chemical reaction, shown in Eq. (1), illustrates this process (Hanson and Hanson, 1996; Bowman, 2006):

$$CH_4 + 2O_2 \rightarrow 2O_2 + H_2O$$

Biochar, is carbon-rich material produced derived from biomass pyrolyze method, has been identified as a promising amendment to increase methane oxidation in soils [8]. Although biochar does not chemically react with CH_4, its porous structure facilitates the adsorption of methane, thereby creating a favorable environment for methanotrophic bacterial colonization and activity. When mixed with soil, biochar supports microbial growth, which can enhance CH_4 oxidation rates. However, biochar-amended soils typically require an acclimation period for microbial colonization to reach optimal methane oxidation levels. Studies have further suggested that activating biochar with methylotrophic bacteria could accelerate this process [17].

This study explores the effectiveness of microbially activated biochar (MAB) in enhancing methane oxidation within landfill cover soils. Unlike conventional biochar amendments, the novelty of this research lies in the use of biochar pre-activated with a high-density mixed culture of methylotrophic bacteria to accelerate microbial activity. Through a series of batch experiments, this study aims to assess whether MAB can significantly improve methane oxidation rates compared to non-activated biochar, providing a more efficient and sustainable solution for reducing methane emissions from landfills.

2. Materials

2.1 Soil and Biochar Collection:

The primary materials for this study were soil and biochar, sourced from specific locations in Bengaluru, India. The soil was collected from the Mittigenhalli waste yard, a representative landfill site, and biochar was obtained from the Peenya industrial area. The biochar used in this study is a byproduct of pinewood gasification, produced at a maximum temperature of 530°C. Before being used in the experiments, the soil samples were oven-dried at 105°C to bring out moisture and then sieved through a 4.75 mm mesh to ensure uniform particle size. The biochar was similarly processed to ensure consistency in the experimental procedure.

2.2 Methylotrophic Bacteria Culture:

Methylotrophic bacteria, known for their ability to oxidize methane, were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh (MTCC-60036). These bacteria were stored at temperatures below -5°C. A subculture was prepared from the mother culture to ensure adequate bacterial growth for the experiments. Methylobacteria, as a group of microorganisms, utilize one-carbon compounds, including methane, methanol, and methylamines, as their sole source of carbon and energy.

3. Experimental Method

3.1 Material Properties:

The physical and chemical properties of the cover materials used in this study were tested according to ASTM standards. Particle size distribution and specific gravity were determined using ASTM D6913 and ASTM D792 protocols, respectively. The dry density was obtained from the compaction test, which measured the weight of the dry material. The water holding capacity (WHC) of the soil and biochar was evaluated by arranging a known mass of the sample into a funnel lined with filter paper and adding a require amount of distilled water. After allowing the sample to soak for 2-3 hours, the material was drained under gravity, and the retained moisture content was calculated to determine the WHC [25]. Atterberg limits were assessed following ASTM D4318 standards, while pH values were measured using a pH meter according to ASTM D1293 standards. All tests were conducted in triplicate, and the results findings were averaged to ensure consistency.

3.2 Biochar Sample Preparation:

Oven-dried biochar sample, each weighing 5 grams, were placed in 100 mL glass vials. The biochar was then thoroughly mixed with 1.5 mL of distilled water. The vials were filled with a gas mixture containing 50% carbon dioxide (CO_2) and 50% methane (CH_4) [14] and sealed with rubber septa to ensure an airtight environment, preventing gas leakage. Gas samples were collected at intervals of 0, 2, 4, 6, 8, and 10 days. The collected gas samples were analyzed using gas chromatography (GC) to determine the gas uptake by biochar over time.

3.3 Biochar Amendment in Soil (Varying Proportions):

Biochar-amended soil samples were prepared by mixing oven-dried biochar with soil in varying proportions (10%, 20%, 40%, 60%, 80%, and 100% by weight). The biochar and soil were thoroughly mixed in a container to ensure an even distribution of biochar throughout the soil [15]. The prepared samples were placed into labelled glass vials, and a mixture of 50% methane and 50% carbon dioxide was introduced into each vial. The gas concentration in each sample was then analysed using gas chromatography equipped with a Flame Ionization Detector (FID).

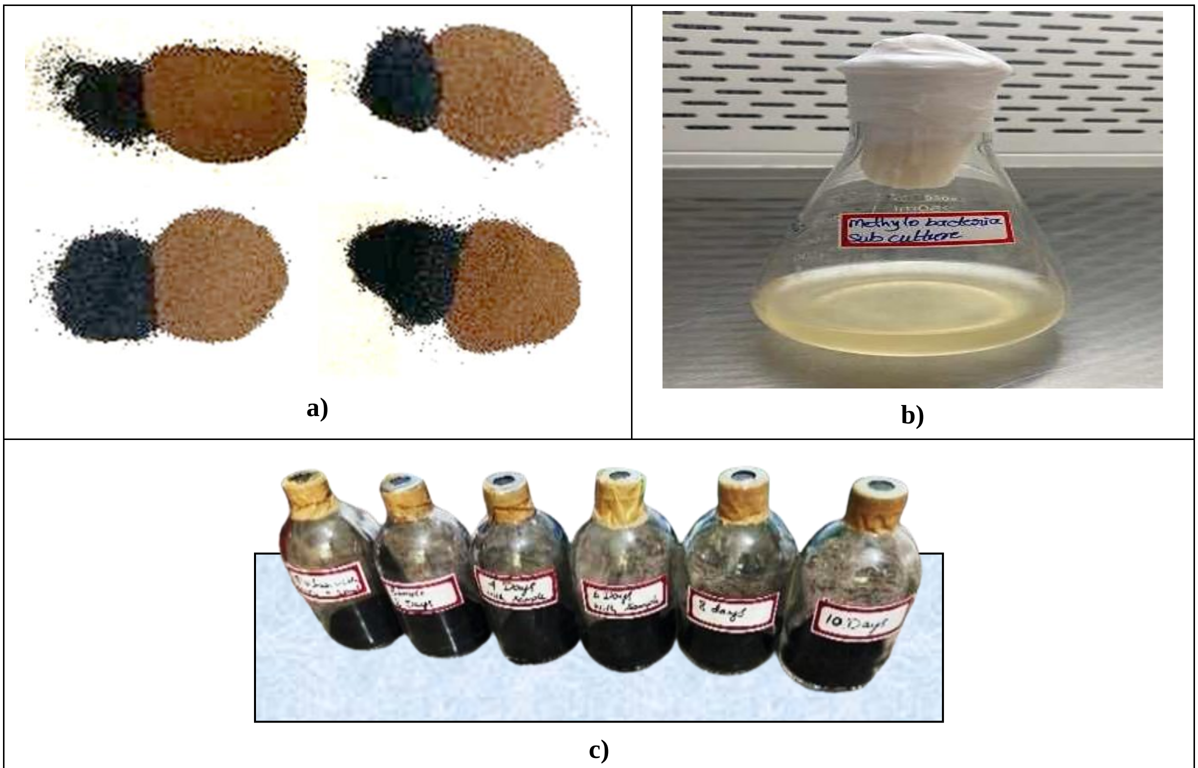

Figure1: (a) Biochar amendment soil with varying proportions; (b) Methylo bacteria subculture, (c) Biochar sample preparation;

3.4 Activated Biochar Preparation with Methylotrophic Bacteria Culture:

The methylotrophic bacterial culture was prepared by growing the bacteria in nutrient broth (NB) in a 50% CH_4 and 50% CO_2 gas mixture at room temperature (23°C). This culture was used to colonize biochar with the bacteria.

The biochar was activated by inoculating 10 grams of biochar with 5 mL of the methylotrophic bacterial culture. The inoculated biochar was incubated in a 50% CH_4 and 50% CO_2 gas mixture at 23°C under static conditions. Headspace gas concentrations were systematically monitored through the collection and analysis of gas samples via gas chromatography [11]. Figure 1 shows the activated biochar in glass vials during the incubation period.

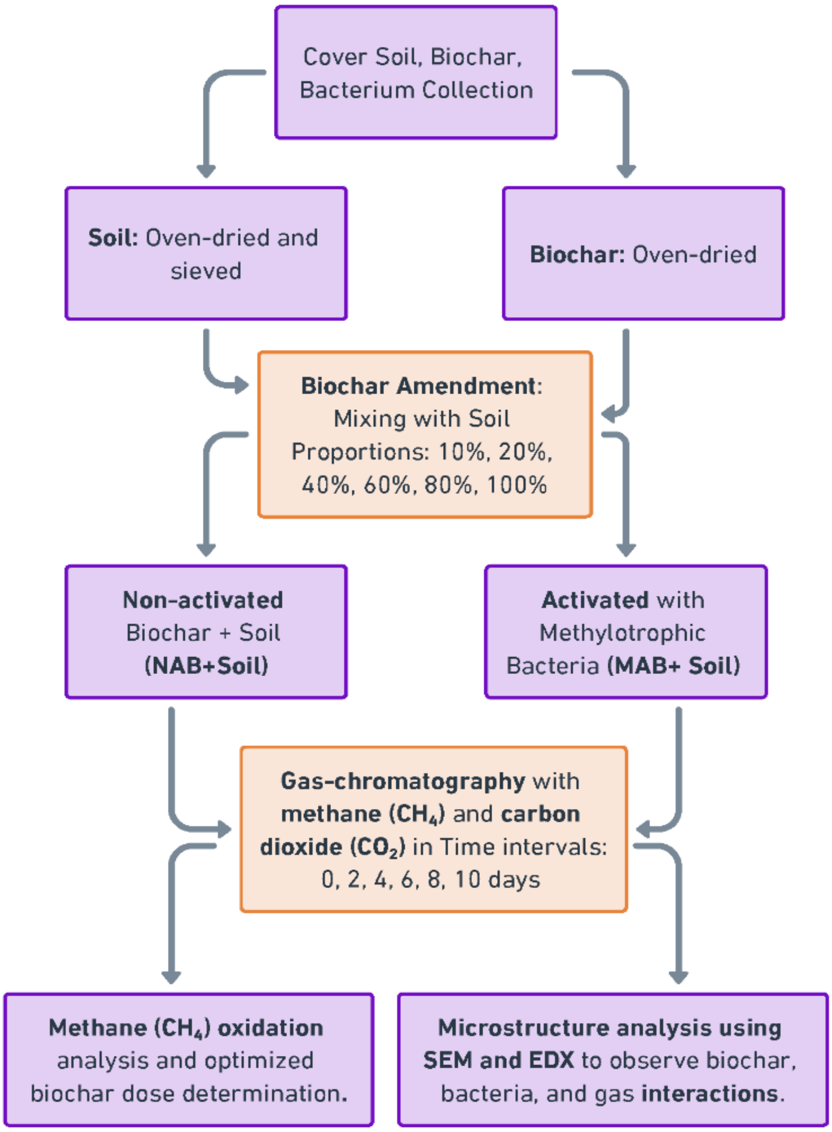

Figure 2. Flow chart of the methodology

3.5 Batch Testing:

Batch tests were conducted using 100 mL serum vials containing soil material and biochar. The moisture content of each sample was adjusted to 30% (w/w) using distilled water. Soils were amended with biochar at varying proportions (10% to 100% w/w). Crimp camps and rubber septa were used to ensure the vials were airtight. Landfill gas made up of methane and carbon dioxide was used to replace the air in the vial's headspace. Gas sample collection and analysis using gas chromatography allowed for the long-term monitoring of changes in headspace concentration [11].

3.6 Gas Analysis:

Gas sample were analysed at regular intervals using an AGILENT gas chromatograph equipped with a Flame Ionization Detector (FID) and a column designed for simultaneous analysis of CH_4 and CO_2. A 1 mL gas-tight syringe was used to collect samples from the vials and inject them into the gas chromatograph [12].

3.7 SEM and EDX Analysis:

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX) were employed to analyse the physical and chemical structure of the biochar. These methods were selected to investigate the microstructural characteristics of both the non-activated and microbially activated biochar. Understanding the surface morphology and pore structure of biochar is crucial, as these properties directly influence its capacity for methane adsorption and microbial colonization, both of which are key factors in enhancing methane oxidation. The SEM provided detailed images of the surface area and porosity, while the EDX analysis helped identify the elemental composition and chemical bonding in the biochar. This characterization allowed for a deeper understanding of how activated biochar's structural properties contribute to its effectiveness in reducing methane emissions.

4. Results and Discussion

4.1 Soil and Biochar physicochemical properties

pH and Water Holding Capacity:

The pH of the biochar is neutral, measured at 7, while the soil exhibits a slightly acidic pH of 5. This variation in pH between the soil and biochar can affect microbial activity, particularly bacteria growth that thrive in neutral to slightly acidic conditions. The water holding capacity (WHC) of biochar is significantly lower (34.2%) than that of the soil (71%), which indicates that the biochar, while porous, has a lower capacity to retain moisture. However, its structure still promotes gas diffusion and microbial colonization, which is essential for methane oxidation.

Atterberg Limits:

The Atterberg limits for the soil indicate that it has moderate plasticity, with a liquid limit of 34%, a plastic limit of 20.1%, and a plasticity index of 15.4%. Biochar, on the other hand, is non-plastic. This non-plasticity is expected, as biochar is a porous, rigid material. The addition of biochar to soil could reduce soil plasticity, improving aeration and gas flow within the landfill cover material, which is beneficial for enhancing microbial methane oxidation.

Specific Gravity and Dry Density:

The specific gravity of the soil is measured at 2.5, while biochar is non-plastic and does not have a specific gravity value. The dry density of the soil is 13.1 g/cm³, indicating its compactness. Biochar, due to its porous nature, lacks this level of compactness, making it more suitable for enhancing the aeration and porosity of soil, which is important for promoting methane oxidation in the landfill cover.

Particle Size Distribution:

The particle size distribution of the soil reveals that it contains 2.8% gravel, 13.1% sand, and 84.1% fines. The fines dominate the soil composition, which typically reduces aeration. The inclusion of biochar, with its highly porous structure, helps counterbalance this by increasing soil porosity, allowing better gas movement and microbial activity.

Colour:

The colour difference between the materials is also noteworthy. The soil is brown, while biochar is black, which reflects the carbon-rich nature of biochar. This high carbon content plays a role in supporting microbial colonization, contributing to the biochar’s role as an effective medium for enhancing methane oxidation.

Table 1: The physical and chemical properties of soil, biochar

SN	Properties	Method	Soil	Biochar
1	**Atterberg limits**	ASTMD4318		
	Liquid limit (%)		34	Non-plastic
	Plastic limit (%)		20.1	
	Plasticity index (%)		15.4	
2	Specific gravity	ASTMD792	2.5	0.5
3	Water holding capacity (%)	ASTMD2980	71%	34.20%
4	**Particle size distribution**	ASTMD6913		
	Gravel (%)		2.8	44
	Sand (%)		13.1	56
	Fines (%)		84.1	1
	Coefficient of curvature Cc		1.2	0.82
	Coefficient of uniformity Cu		1.1	2.42
5	Dry density (g/cm³)	ASTMD7263	1.7	1.12
6	pH	ASTMD1293	6	7
7	Color		Brown	Black

Table 2: Chemical and physical properties of soil and Biochar amended soil

Physical properties	**10% BC**	**20% BC**	**40% BC**	**60% BC**	**80% BC**	**100% BC**
Specific gravity	2.4	2.2	2.1	1.8	1.5	1.3
Atterberg's limit						
Liquid limit	35	38	39	40	41	42
Plastic limit	21	23	27	28	28	29
Plasticity index	14	14	12	13	14	16
Initial water content	2.6	2.8	2.92	3.2	3.5	3.8
Optimum moisture content (%)	18	18	18	18	19	19
Maximum dry density (KN/m3)	12	12.5	12.62	12.8	12.9	13.1
Chemical properties	**10% BC**	**20% BC**	**40% BC**	**60% BC**	**80% BC**	**100% BC**
pH	7.1	7.4	7.8	7.7	7.9	8.1
color	Brown	Black	Black	Black	Black	black

4.2 Chemical and Mineralogical Composition of Soil and Biochar:

Table 3 presents the chemical and mineralogical composition of the soil and biochar used in the study. It shows that biochar contains higher amounts of CaO (0.41%) and MgO (0.34%) compared to the soil, which has lower concentrations of these oxides. The soil, however, has significantly higher levels of SiO_2 (55.06%) and Al_2O_3 (32.96%), indicating a mineral-rich composition, whereas biochar is less mineralized. These variations in chemical composition are important as they influence the material's reactivity and potential to enhance methane oxidation.

Table 3: Chemical and mineralogical composition of soil and biochar

Oxides	Soil	Biochar
CaO	0.14	0.41
SiO_2	55.06	34.34
Al_2O_3	32.96	0.81
Fe_2O_3	3.46	-
MgO	1.02	0.34

4.3 Variation of pH of Soil-Biochar mix:

Table 4 presents the pH values of soil amended with different proportions of biochar. The unamended soil (100% soil) has an acidic pH of 6, which is typical for landfill soils like those from the Mittigenhalli waste yard due to breakdown of organic matter and the production of acidic byproducts. As the proportion of biochar increases, the pH becomes more neutral, reaching values between 6.1 and 7.2. Biochar, with its neutral to slightly alkaline nature, helps to buffer the soil, reducing its acidity. This pH adjustment creates a more favorable environment for microbial activity, particularly methane-oxidizing bacteria, which thrive in neutral conditions.

Table 4: pH test for Biochar amendment soil

BC amendment Soil proportion	pH value
Cover soil	6
10% BC	7.1
20% BC	7.4
40% BC	7.8
60% BC	7.7
80% BC	7.9
100% BC	8.1

Gas consumption profile

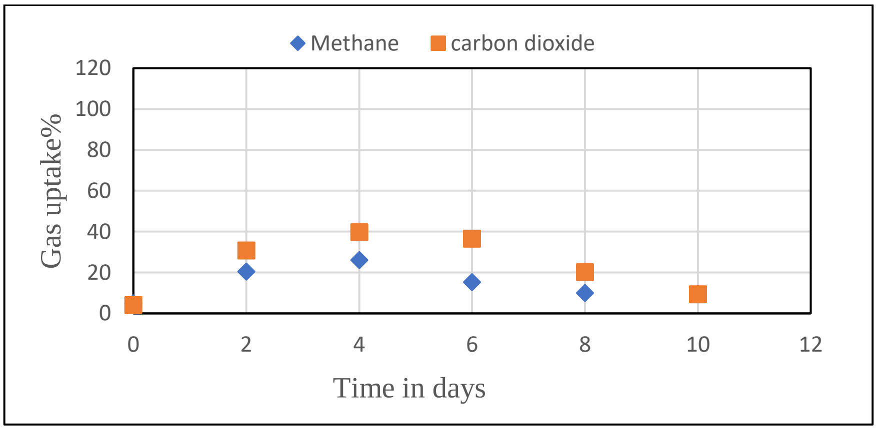

Figure 3: Variation of CH_4 and CO_2 with time

Figure 3 illustrate that gas uptake with biochar as shown in this graph with respect to time or days. There are no organisms present in the biochar material, so oxidation process is less in non-activated biochar. The results from the non-activated biochar indicate that it was capable of oxidizing CH_4 when mixed with soil, without imposing substrate limitations on the microbes. Therefore, carbon dioxide production is little more in non-activated biochar material

4.4 Gas profile and methane oxidation during number of days

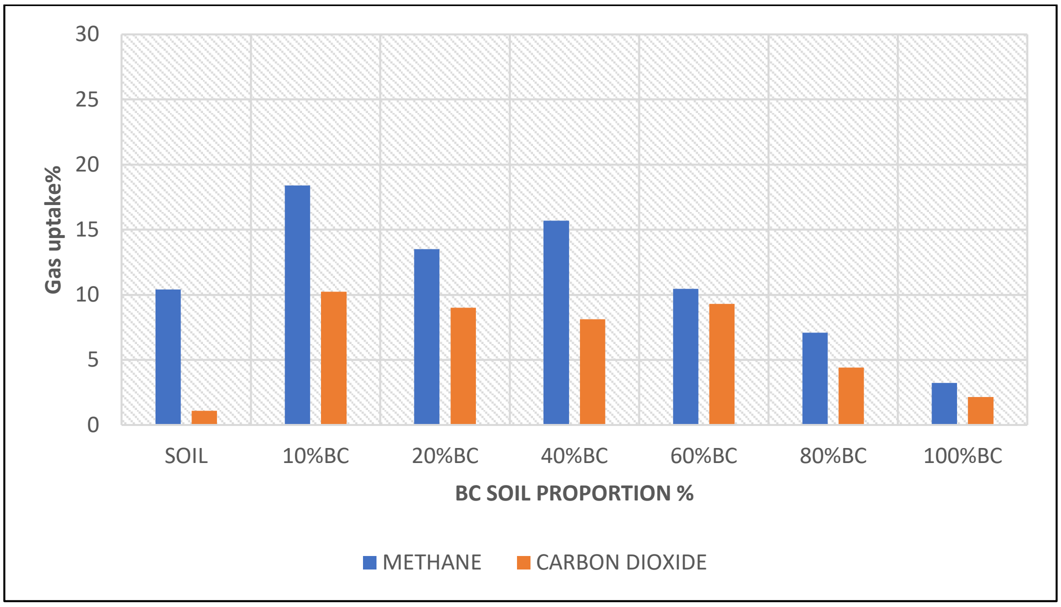

Figure 4: Biochar amendment soil with varying proportion

The figure 4 shows methane depletion, carbon dioxide production, and oxygen utilization at intervals for biochar-amended soil, respectively. To evaluate the effectiveness of different biochar proportion in methane removal such as 0%, 10%, 20%, 40%, 60%, 80%, 100% by weight can be analysis in gas chromatography which is required for measuring methane concentration. The study results represent minimal oxidation process in nonactivated biochar. However, the soil CH_4 oxidizing bacteria were not acclimated to the biochar in the biochar-amended soil, which may have contributed to the decreased CH_4 absorption. The zero-order kinetics were used to calculate the CH_4 oxidation rates for each experimental set. For the ten days in soil, the average CH_4 oxidation rate is 195.11 mg/g.

4.5 Gas reduction in activated biochar

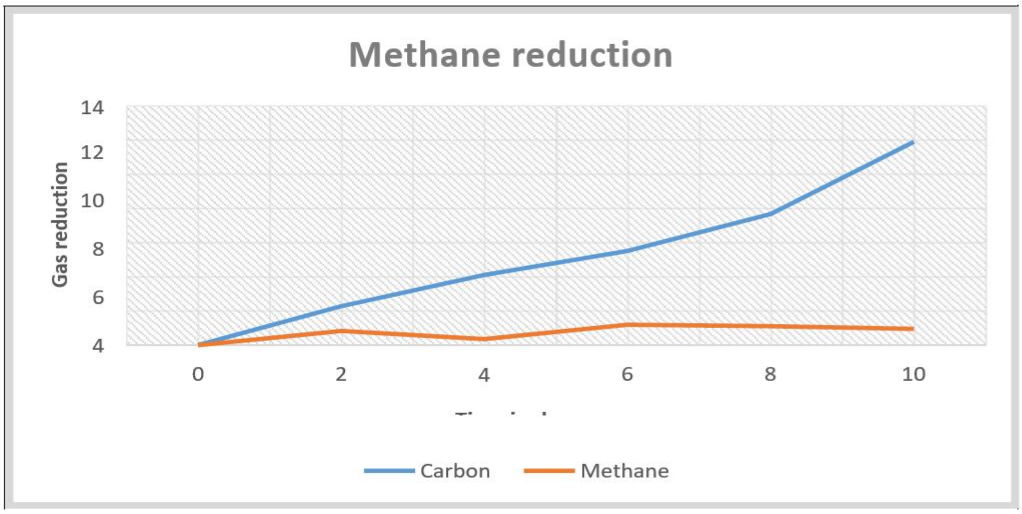

Figure 5: Methane and carbon dioxide uptake in Activated Biochar in days.

This study suggests that when incorporated into soil, methylo bacteria-activated biochar may enhance CH_4 mitigation more effectively than non-activated biochar-amended soils. While no remarkable CO_2 uptake was observed in the methylo bacteria-activated biochar, there was a notable increased in CO_2 levels, likely due to CH_4 oxidation by CH_4 oxidation bacteria. Overall, the methylobacteria successfully colonized the highly porous activated biochar, resulting in increased methane uptake over time, as shown in the figure. The results indicate that while no significant CO_2 uptake was detected by the methylobacteria-activated biochar, a rise in CO_2 levels occurred due to CH_4 oxidation by the bacteria. When incorporated into soil, methylo bacteria-activated biochar is likely to mitigate or control methane at a faster rate compared to non-activated biochar-amended soils [14]. The biochar–amended soil had higher CH_4 oxidation rate is (213mg/g) for 10 days.

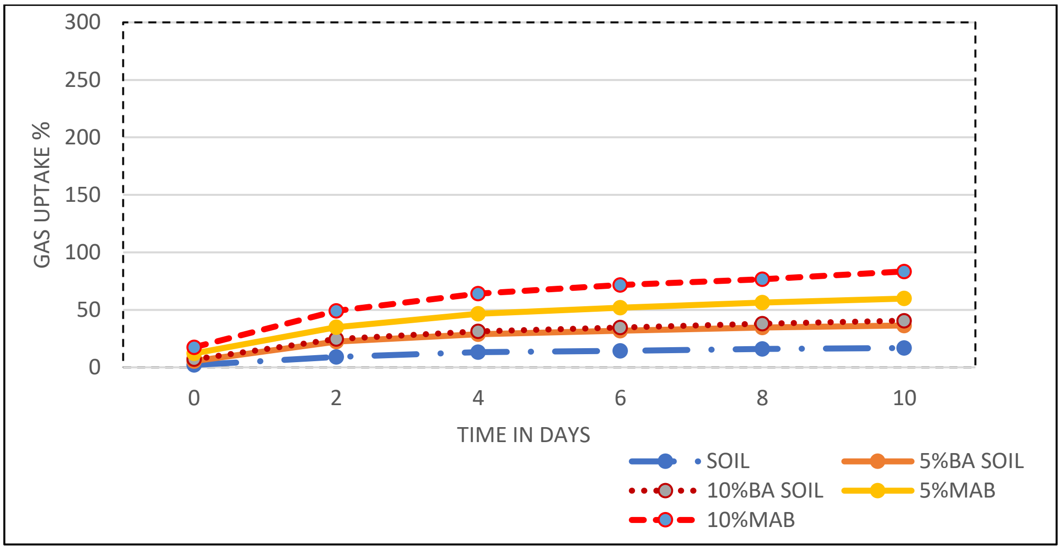

Figure 6: comparison graph for Activated Biochar, non-activated biochar and soil with number of days.

The activated biochar demonstrated the highest CH_4 removal efficiency, with a solid increase in uptake over the days. This performance can be attributed to the presence of CH_4-oxidizing bacteria inoculated into its porous structure, which provided an ideal environment for microbial activity. CO_2 levels initially increased due to CH_4 oxidation by the methylotrophic bacteria. However, no significant direct CO_2 uptake was observed. By the end of the observation period, activated biochar achieved the highest CH_4 oxidation. Non-activated biochar showed moderate CH_4 removal. Due to lack of microbial enhancement of activated biochar, its inherent properties, such as internal porosity and surface area, facilitated some CH_4 adsorption and oxidation. However, non-activated biochar exhibited minimal CO_2 removal, with no notable CO_2 production observed.

The cover soil exhibited the lowest CH_4 removal rate among the three materials. Its natural microbial population supported CH_4 oxidation but at a slower rate due to the lack of enhancement through biochar amendment or microbial inoculation, and also showed slight CO_2 uptake due to natural microbial activity. However, CO_2 production was more gradual compared to activated biochar, consistent with its lower CH_4 oxidation rate.

4.6 Scanning Electron Microscopy analysis (SEM)

The smallest porosities of the soil, biochar and biochar amended cover soil were computed by image processing software Pores (Elements). SEM images extract from the software [20]. By dividing the binary images into black and white areas, which stand for solid surfaces and voids, the micro porous areas were identified. A SEM was functioned in the high vacuum mode with an accelerating voltage ranging from 2 to 10 kV, depending on the sample charging, use a secondary electron detector. For the present study SEM of all three samples were taken at magnifications of 1kx to 2kx respectively [1]. The figure 5 displays SEM images of biochar and biochar amended soil that were analyzed at various magnification. The biochar macro and micro porous structure, which come from its beginning as wood pellets are visible in every images. At varying magnification, the presence of certain mineral particles is visible in both the biochar and biochar amended soil.

Biochar's macro and micropores offer methanotrophic bacteria an ideal environment in which to develop and proliferate. Additionally, the biochar can hold onto more moisture due to its high porosity.

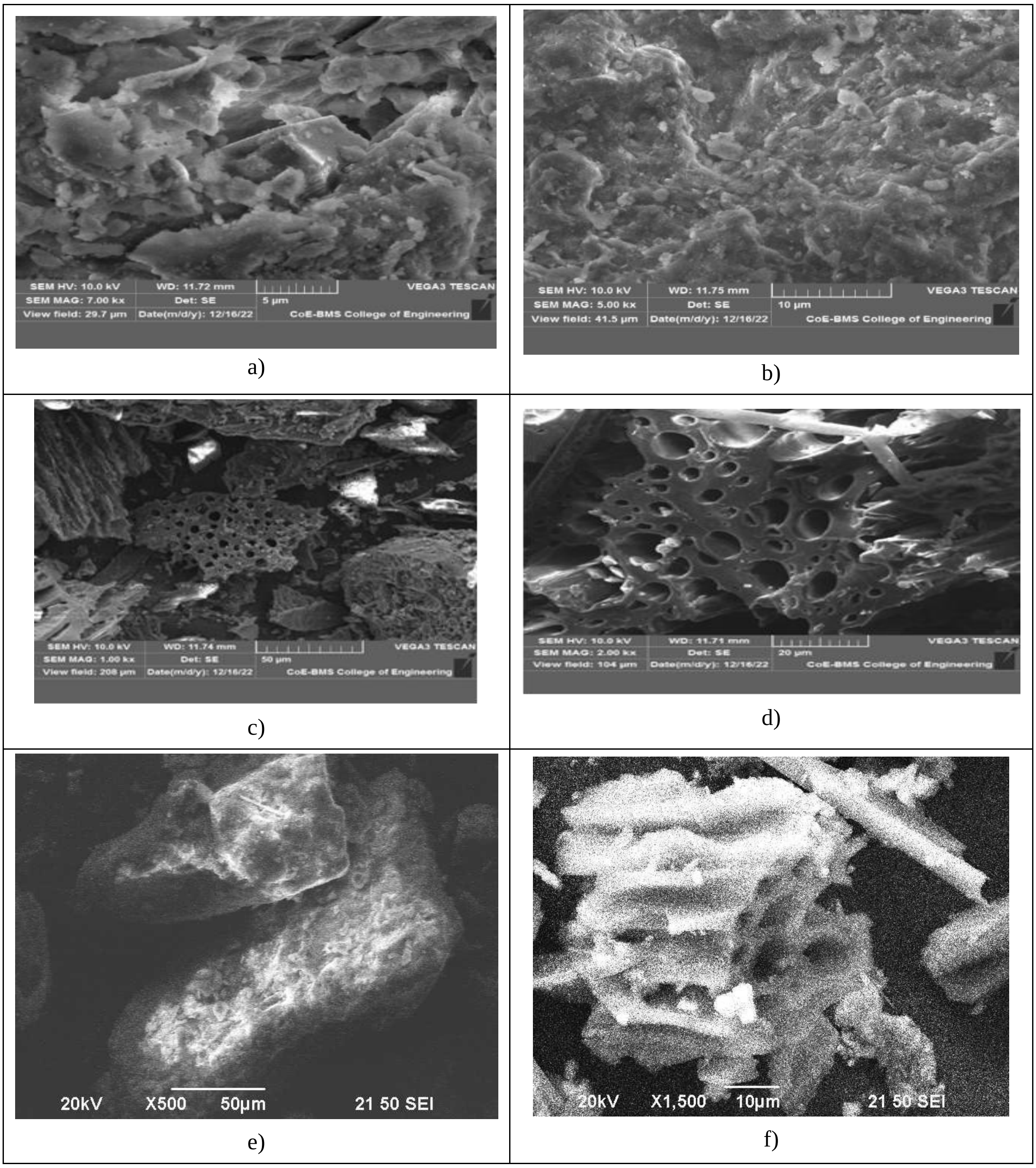

Figure 7: SEM analysis materials of Soil a), b) and Biochar c), d) and Biochar amended soil e), f)

This can also make biochar more liveable because water is a universal solvent biochar has high level of porosity also improves its ability to absorb gases. Difference between SEM images of biochar in its natural condition, after biochar amendment to the soil in figure (e) and (f) demonstrates that while biochar as is has wider pores than biochar interact more strongly when biochar is added to soil at different proportion and finer clay particles coat the smaller biochar particles more than the larger ones.

5. Conclusion

The CH_4 removal capability of the biochar-amended soil and the methanotrophic-activated biochar-amended soil at two distinct sets was evaluated. The findings show that, in comparison to soil that was not activated by biochar, the methylotrophic-activated biochar-amended soil exhibited a notable oxidation of CH_4. Among these, soil modified with activated biochar exhibited better CH_4 absorption than soil not altered with active biochar.

The physical and chemical properties of the landfill cover soil were enhanced by adding biochar. The addition of biochar raised the pH of the soil, which enhanced the conditions for the growth and colonization of microorganisms (methanotrophs). Consequently, the landfill cover soil will help mitigate methane emissions. The permeability of the landfill cover will rise when biochar is added. By raising the number of microorganisms in the landfill cover soil, that activity will increase the oxidation layer of landfills and enhance oxygen diffusion within the cover. Compared to other biochar and soil mixes, the most noticeable soil improvement was when 10% of biochar was added to the landfill cover soil.

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