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Novel Bacterial Consortium for Mitigation of Odor and Enhance Compost Maturation Rate of Municipal Solid Waste: A Step Toward a Greener Economy

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ABSTRACT

Composting is an integral component of sustainable Municipal Solid Waste (MSW) management within the circular bio-economy platform. However, it faces challenges due to malodorous emissions that impact environmental and societal equilibrium. The present study aims to minimize odorous emissions and expedite compost maturation using a novel, efficient microbial consortium. Bacteria sourced from open dump sites in Sri Lanka were carefully screened based on concurrent enzyme production. Five developed consortia were tested for their performance in reducing malodors during the composting process of MSW. Consortium No. 5 (C5), comprised of Bacillus haynesii, Bacillus amyloliquefaciens, and Bacillus safensis, demonstrated outstanding performance with a significant ($p < 0.05$) reduction in odorous emissions. Additionally, consortium C5 exhibited impressive control over gas emissions, maintaining VOC, CH_4, NH_3, and H_2S concentrations within ranges of 0.5-6 ppm, 0.5-0.8 ppm, 0.3-0.5 ppm, and 0.5-0.6 ppm, respectively, compared to control concentrations of 4.5-10.2 ppm, 0.5-5.5 ppm, 0.3-5.5 ppm, and 0.5-6.4 ppm, respectively. Additionally, comprehensive Electronic nose (E-nose) analysis substantiated C5's efficiency in attenuating Methane-Aliphatic compounds, Sulfur and Aromatic compounds, along with low-polarity aromatic and alkane compounds, all with statistical significance ($p < 0.05$). Further, the developed consortium could reduce the composting time from 110 ± 10 days to 17 ± 3 days, offering a sustainable solution for global MSW management.

INTRODUCTION

The relentless advance of rapid industrialization and urbanization has resulted in a mounting tide of Municipal Solid Waste (MSW) globally, leading to severe environmental conditions of unprecedented scale. A detailed analysis of waste footprints reveals that annual MSW production exceeds an astounding 2 billion tons, with a projected increase of 70% by 2050 (Gautam & Agrawal 2021, Golroudbary 2019, Zhao et al. 2022). In this troubling scenario, the average individual contributes 0.74 kg of waste daily, with a staggering 70% destined for landfills and dumpsites (Golroudbary 2019, Pal & Tiwari 2023). Given this escalating crisis, composting has emerged as an eco-friendly solution, providing a sustainable approach to solid waste management within the circular bio-economy. Composting involves controlled, aerobic processes that transform organic materials into a nutrient-rich soil amendment or mulch through natural decomposition (Ayilara et al. 2020, Wijerathna et al. 2023).

The introduction of bacterial consortia adds a dynamic element to the rapid degradation of various organic materials within a greener framework. This approach has proven integral to novel composting technologies (Behera et al. 2021, Wijerathna et al. 2022). The heterogeneous nature of bacterial consortia can synergistically enhance both mesophilic and thermophilic composting phases, leading to an accelerated transformation driven by their combined metabolic and co-metabolic mechanisms (Ali et al. 2021).

Particularly, the emission of greenhouse gases (GHGs), including ammonia and methane, results in significant environmental consequences. Methane has a global warming potential 28 times greater than carbon dioxide (Silva et al. 2017) and is primarily emitted from the anaerobic fermentation of wastes (Costa et al. 2021). Additionally, odorous compounds such as hydrogen sulfide (H_2S), methyl mercaptan, dimethyl sulfide, ammonia, and Volatile Organic Compounds (VOCs) are generated during the composting process (Jiang et al. 2017). Ammonia emission is especially problematic in sewage sludge composting, as it can cause odor issues and lead to nitrogen loss in the mature compost (Shou et al. 2019). Therefore, mitigating odor during the composting process is a major concern.

Different techniques have manifested through a variety of physicochemical symphonies, where bio-filters, odor neutralizers, sorbent materials, compost covers, and bulking agents have all played their part in the grand stage of odor control (Wysocka et al. 2019). Yet, most of these physico-chemical methods are not economically feasible and have limited accessibility. Thus, the exploration of the serenading potential of microbial consortia targeting odor reduction and enhancing the composting process is noted as a greener approach to meeting sustainability in solid waste management.

The synergistic collaborations within microbial consortia may reduce the generation of odor-causing compounds while improving the composting efficiency, fostering an environment of mutual nourishment (Bajpai Bajpai 2018, Kumar et al. 2022, Zhang & Dong 2022, Pacheco et al. 2019). Thus, this study focuses on the analysis of the performances of microbial consortia for their capacity to transmute malodorous compounds and accelerate compost maturation.

MATERIALS AND METHODS

Isolation and Screening of Potential Bacteria

The potential bacteria for effective solid waste degradation was isolated from soil/solid waste and leachate samples collected from MSW dump sites in Sri Lanka. The bacteria were isolated following the standard pour plate method on a nutrient agar medium, and morphologically different pure cultures were obtained following the streak plate method. The isolated microorganisms were subjected to the primary screening following plate assays for Cellulase, Amylase, lipase, and Proteinase enzymes on Congo Red Agar, Carboxyl Methyl Cellulose (CMC) agar starch-agar and skimmed milk agar medium (Sarkar & Chourasia 2017). The secondary screening for cellulase, amylase, lipase, and proteinase enzymes was performed using the method described by Sarkar & Chourasia (2017). The best potential isolates used for the consortia preparation are based on their concomitant enzyme production in an artificial broth media containing Cellulose, Starch, Lipids, and protein substrates.

Experimental Design for Comparative Study

Based on the enzyme production potential, five bacterial consortia (C1-C5) were prepared (Table 2) using the most potential bacterial species. They were inoculated to the compost bins to evaluate their efficiency on compost maturation and odor reduction potential. The composting experiment was conducted employing eighteen regular compost bins (each measuring 150 cm in height and 45 cm in diameter) as triplicates for both control groups and five distinct bacterial consortia setups. Ninety-five kg of a mixed organic portion of municipal solid waste was added into each bin. Nutrient broth 1% bacterial inoculum (v/w) was added into every bin except for the control group. Throughout the study, different composting indicators, pH, electrical conductivity, moisture content, total organic carbon, and total Kjeldahl nitrogen were monitored daily to observe and analyze the dynamic changes occurring within each compost bin.

Sampling and Odor Analysis

Gaseous odor emissions from various compost setups were assessed using both portable gas analyzers and Electronic Nose (E-Nose) devices. The odorous gases, including NH_3, CH_4, H_2S, and Total Volatile Compounds (TOC), were quantified in the cap space of the compost bins. The probes of gas analyzers (Aeroqual HH S500L analyzer with a range of 0–10,000 ppm and 5 IR multi-gas detector with a range of 0–50,000 ppm) were directly exposed to the cap space of the compost bins at intervals of every 3 days.

Odor Characterization Using E-Nose

Gas samples were collected from the cap space of the compost bins employing syringes and subsequently transferred to GCMS vials to facilitate Electronic Nose (E-Nose) analysis. Weekly characterization of the gaseous composition was carried out using an automated E-Nose comprising ten distinct sensors. For the instrumental sensory analysis, an E-Nose system (Model - PEN3 Airsense Analytics GmbH, Schwerin, Germany) equipped with an automated sampling device was employed. This E-Nose system featured ten thermo-regulated (ranging from 150°C to 500°C) metal oxide semiconductor sensors, each demonstrating slight sensitivity to different classes of chemical compounds, as noted by Jonca et al. (2022) and Eusebio et al. (2016).

These ten sensors, adept at discriminating compounds within samples, are outlined in Table 1. The measurement process was orchestrated through a computer program, and each measurement phase spanned 50 seconds, a duration sufficient for the sensors to attain stable readings. Data collection was carried out at 1-second intervals. A flush time of 3 min was interposed between successive samplings, and the flow rate was maintained at 300 $mL.min^{-1}$. To ensure signal stability and encompass sample variability, the procedure was iterated three times for each sample, as

Table 1: The list of sensors used to discriminate compounds in samples.

Sensor Number	Sensitive compounds
R1	Aromatic
R2	Broad range Polar and NOx
R3	Aromatic compounds, ketones, and aldehydes
R4	Hydrogen
R5	Low polarity aromatic and alkane
R6	Broad-methane
R7	Sulphur organic
R8	Broad alcohols, ketones, and partially aromatic
R9	Sulfur and aromatic
R10	Methane-aliphatic

corroborated by Cipriano & Capelli (2019). At operating conditions, relationships with volatiles from the headspace and the sensing device's surface caused alterations in the conductivity of the semiconductor. Thus, the ratio G/G0 (in which G and G0 represent the resistance of a sensor when detecting a gas and when inhaling clean air, respectively) was recorded by the e-nose dedicated software (Ottoboni et al. 2018).

RESULTS AND DISCUSSION

Isolation and Screening of Potential Bacterial Species

After 24-h incubation at 30°C, 226 morphologically different bacterial colonies were isolated from the soil, leachate, and solid waste sample. Out of all isolates, 15 bacterial isolates were positive for cellulase, 15 for amylase, 15 for proteinase, and four were positive for lipase. According to the secondary screening results, Cellulase-positive bacterial isolates *Bacillus haynesii* exhibited the highest cellulase activity at 6.2 ± 0.2 $U.mL^{-1}.min^{-1}$ and the highest amylase activity at *Bacillus haynes* and *Bacillus velezensis* with values of 6.5 ± 0.1 $U.mL.min^{-1}$ and 6.2 ± 0.3 $U.mL.^{-1}min^{-1}$, respectively. Similarly, the most significant proteinase activity was found in bacteria *Bacillus amyloliquefaciens* and *Bacillus velezensis*, with 7.2 ± 0.2 $U.mL.min^{-1}$ and 6.5 ± 0.1 $U.mL^{-1}.min^{-1}$ enzyme activities, respectively. Further, Bacteria *Bacillus velezensis* and *Bacillus safensis* exhibited the highest lipase activity, with enzyme activity values of 4.2 ± 0.2 $U.mL^{-1}.min^{-1}$ and 3.8 ± 0.2 $U.mL.min^{-1}$, respectively. The bacterial isolates with the highest extracellular enzyme activities were subjected to the consortia preparation. Table 2 demonstrates the prepared five consortia for evaluating odor reduction and compost maturity potential. The odor reduction potential of prepared five consortia was evaluated following the standard methods.

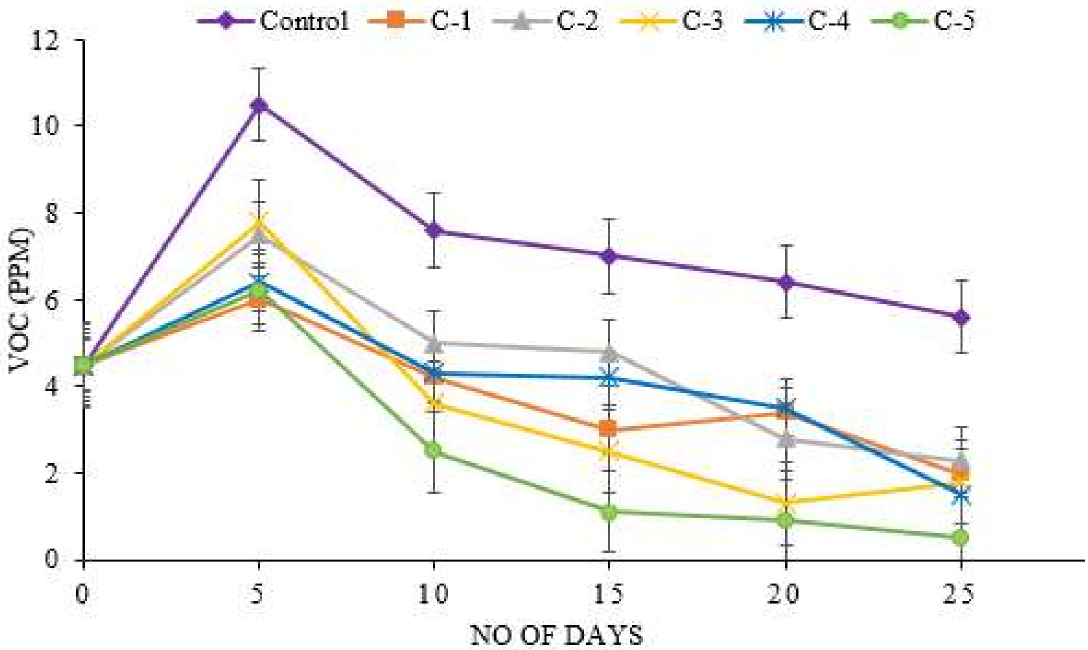
Table 2: Different bacterial consortia used for the experimental setup.

Set up name	Bacterial combination in the prepared consortium
C1	*Bacillus haynesii, Bacillus hisashii, Bacillus licheniformis*
C2	*Bacillus haynesii , Bacillus licheniformis , Bacillus subtilis,*
C3	*Bacillus haynesii, Bacillus safensis, Bacillus velezensis*
C4	*Bacillus haynesii, Bacillus amyloliquefaciens, Bacillus subtilis,*
C5	*Bacillus haynesii, Bacillus amyloliquefaciens, Bacillus safensis*
Control	Not Applicable

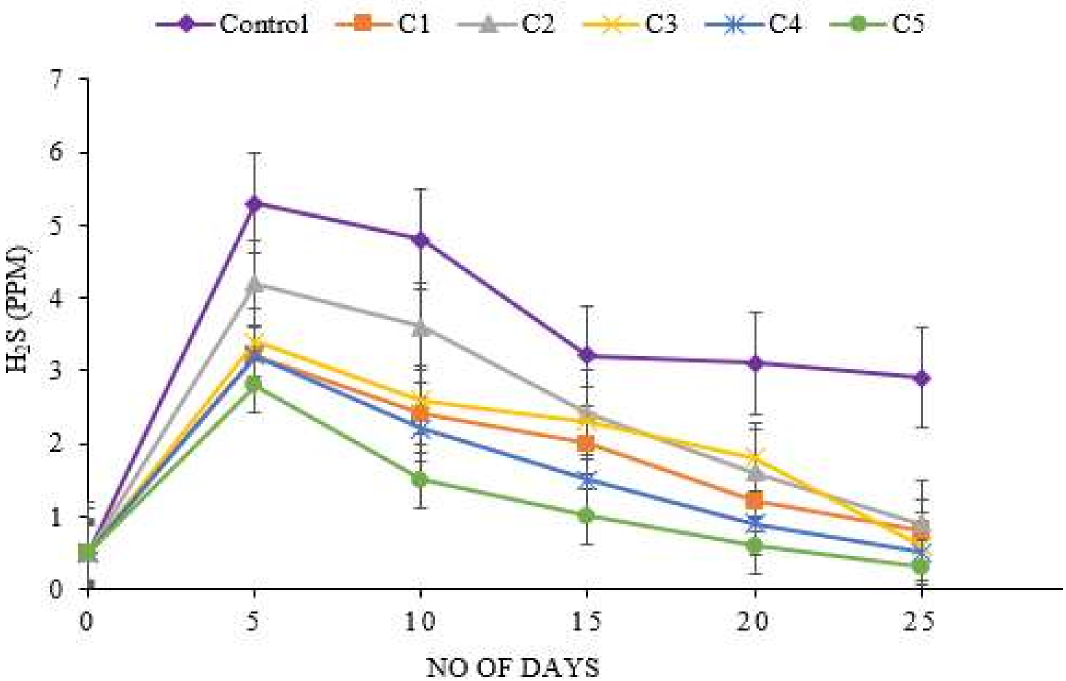
Odor Reduction of Prepared Bacterial Consortia

Fig. 1 illustrates the spectrum of odor-inducing gases produced throughout the composting process, with Fig. 1(a) specifically highlighting the dynamic changes in Volatile Organic Compounds (VOCs). As shown in Fig. 1(a), the control sample exhibited a pronounced and statistically significant increase in VOC production compared to all treatment samples. Notably, the experimental setup with the C5 consortium inoculation resulted in the lowest VOC production during composting. This reduction is likely due to the efficient bioconversion of solid waste facilitated by the bacterial consortia, leading to lower VOC emissions (Rastogi et al. 2020). Additionally, setups C4, C3, and C2 each demonstrated a statistically significant reduction ($p < 0.05$) in VOC levels compared to the control, confirming the effectiveness of the inoculated microbial consortia.

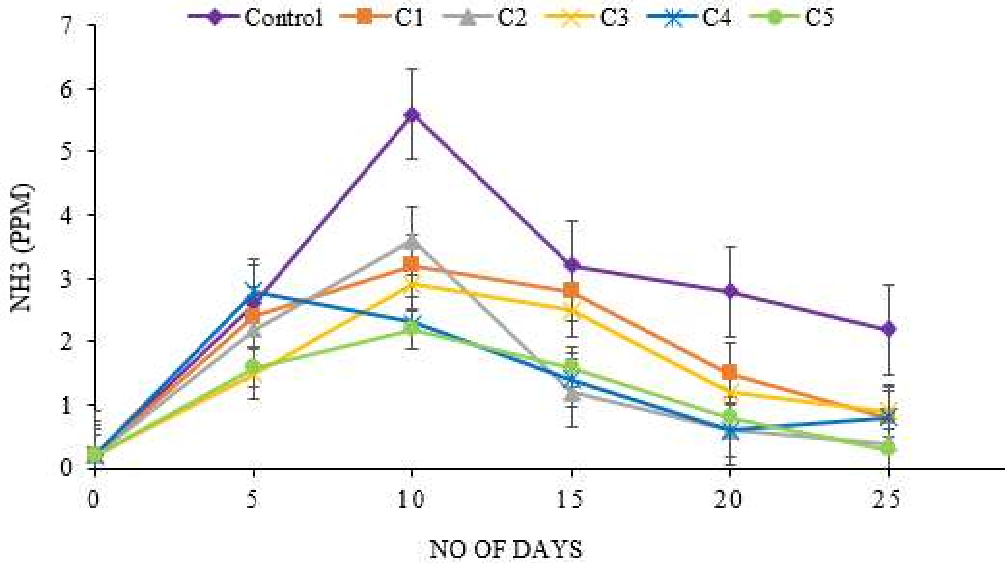
VOCs contain a diverse array of chemical constituents, including alkanes (such as Butane, Pentane, Cyclopentane, Heptane, Octane, and Decane), Terpenes (including Propylene and Limonene), aromatic compounds (encompassing Benzene, Toluene, Ethylbenzene, p-xylene, and o-xylene), halogenated compounds (like Chloromethane, Methylene chloride, Chloroform, Carbon tetrachloride, Ethyl chloride, Styrene, and Chlorobenzene), oxygenated compounds (Ethanol, Acetone, Ethyl acetate, and 2-Hexanone), and Sulfur-containing compounds (such as Carbon disulfide, Methanethiol, Dimethyl Sulfide, and Dimethyl disulfide) which can harm the environment and social well-being through air pollution, smog and its contribution to global warming (Halios et al. 2022, Saraga et al. 2023). According to Hu et al. (2016), a specific bacteria consortium consisting of *Zoogloea resiniphila* HJ1 *Methylobacterium rhodesianum* found similar results: lowered release of volatile organic compounds during composting. In addition, Awasthi et al. (2020) have studied the effect of biochar and bacterial inoculum additions on cow dung composting for the reduction of VOC emission and have achieved a successful reduction of VOC generation. Preferred species of organic-


Control
C-1
C-2
C-3
C-4
C-5
VOC (PPM)
12
10
8
6
4
2
0
0
5
10
15
20
25
NO OF DAYS

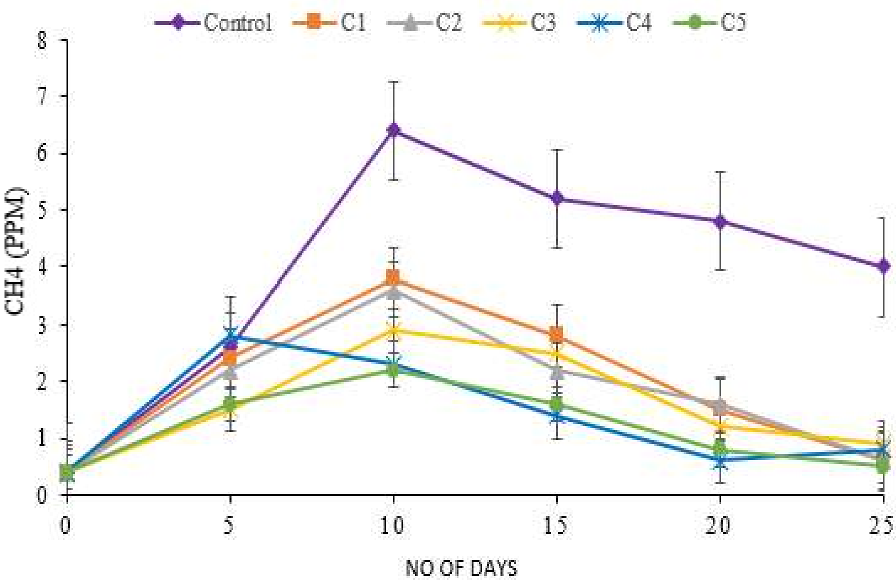
(a)


Control
C1
C2
C3
C4
C5
H_2S (PPM)
7
6
5
4
3
2
1
0
0
5
10
15
20
25
NO OF DAYS

(b)



(c)



(d)

Fig. 1: VOC (a), H_2S (b), NH_3 (c), and CH_4 (d) concentrations of different treatments.

digesting microorganisms include bacteria of *Bacillus*, *Clostridium*, and *Deinococcus*, particularly *Bacillus subtilus*, *Bacillus licheniformis*, and VFAs. Additionally, these microorganisms produce lytic enzymes such as urease, phytase, lipases, proteases, cellulases, xylanase, hemicellulases, and amylases that degrade organic matter and reduce odor. Further, some bacteria can eliminate odors and reduce gaseous pollutants via their biochemical digestive reaction processes (Barbusinski et al. 2021).

Hydrogen sulphide (H_2S), another odorous gas released during composting, arises from the microbial transformation of protein substrates rich in Sulphur (Yao et al. 2022).

The dynamics of H_2S are shown in Fig. 1(b) throughout composting. Parallel to the VOCs, a distinct pattern took place, with the control sample exhibiting a significant surge in H_2S production, while the C5 sample exhibited the most lowered H_2S emissions. Among the compost bins, the peak of H_2S concentration was noticed as 5.5 ± 0.4 ppm within the cap space of the control setup on the fifth day of composting, followed by a gradual decline. This behavior could be attributed to the gradual reduction of Sulphur-containing protein compounds during the mesophilic phase of composting. Fig. 1(c) presents the dynamic evolution of Ammonia (NH_3) levels throughout the composting process. Ammonia emerges as a primary odorant compound, predominantly generated through the ammonification process during the transformative conversion of nitrogen-rich organic materials (Ahmad et al. 2021). As the figure depicts, the control sample exhibited the highest NH_3 production, notably peaking on the 10th day of composting.

Furthermore, the C5 treatment displayed comparatively lower NH_3 emissions during the entirety of the composting period. This alignment highlights the potential of these setups in mitigating NH_3 production. The genesis of ammonia during composting is influenced by factors including moisture content, aeration, temperature, pH of composting materials, and the carbon-to-nitrogen (C/N) ratio of the waste substrate. Lowered ammonia synthesis may result in higher nitrogen content in the compost, leading to delayed breakdown and a buildup of nitrogenous molecules (Anas et al. 2020). Kim et al. (2014) showed that the bacterium release of bacteriocins, which prevent the establishment of many bacteria that are responsible for odor generation, may be connected to the lowering of NH_3. As an alternative, a variety of extracellular enzymes may be responsible for the reduction of ammonia (Leite et al. 2022). Further, the bacterial genus *Bacillus* can help keep the slurry's pH levels stable by producing acidic substances, which might reduce the rate of hydrolysis of urea and other nitrogen molecules being delaminated, resulting in the emission of NH_3 (Nowocień & Sokołowska 2022).

Methane (CH_4) is a key contributor to the odor released during composting, as shown in Fig. 1(d), which displays the changing CH_4 concentrations in the composting bins' cap space. The control setup had notably higher CH_4 production compared to the bins with consortia, indicating that the consortia might help with composting while reducing gaseous CH_4 emissions. After the 10th day of composting, CH_4 levels gradually decreased in all samples. The production of methane in composting primarily occurs through a microbial process called methanogenesis. Archaea, which are primarily methanogenic microbes, can decompose organic matter in anaerobic environments and flourish in low-oxygen circumstances. The sequence of events in this process includes hydrolysis, acidogenesis, acetogenesis, and, finally, methanogenesis (Koniuszewska et al. 2020, Rath et al. 2022). Some bacterial groups utilize methane as their sole carbon and energy source via the enzyme methane monooxygenase, which is capable of the co-metabolism of some persistent compounds concurrently (Wang et al. 2022). These bacteria serve as biofilters for the oxidation of methane produced in anaerobic environments, and when oxygen is present in soils, atmospheric methane is oxidized (Ray et al. 2023, Qiu et al. 2022). Therefore, the bacterial species (*Bacillus haynesii, Bacillus amyloliquefaciens,* and *Bacillus safensis*) in the C5 treatment may contain methanotroph enzymes or synergistic interaction to oxidize methane by mitigating CH_4 emissions.

Electronic Nose (E-nose) Odor Profile for the Compost Samples during Composting Period

Fig. 2 shows the fluctuation patterns of major gas types detected by ten distinct sensors of the E-Nose throughout the composting duration. Analysis of gas levels during the first week of composting revealed a noticeable increase in odorous emissions from the control sample. The G/G_0 ratio for a wide range of polar compounds and NOx, as measured by the R2 sensor, was 4 for the control sample, while the ratios for the C1, C2, C3, C4, and C5 samples were distinctly lower at 0.6, 0.6, 0.7, 0.6, and 0.5, respectively. Additionally, the ratio of low-polarity aromatic and alkane compounds showed a significant increase ($p < 0.05$) in the control sample, with a value of 5. In contrast, the ratios for the C1, C2, C3, C4, and C5 samples were 0.7, 0.6, 0.7, 0.7, and 0.5, respectively.

Sulfur-containing organic compounds, as detected by the R7 sensor, showed a higher G/G_0 ratio in the control samples (4), compared to lower values of 2.3, 2.2, 2.3, 1.3, and 1.8 for the C1, C2, C3, C4, and C5 samples, respectively. Similarly, odor-forming sulfur and aromatic compounds, as detected by the R9 sensor, were more prevalent in the control sample, with a G/G_0 ratio of 6. In contrast, the ratios for the C1, C2, C3, C4, and C5 samples were 2, 2, 2, 1.5, and 1.3, respectively. Sulfide derivatives can result from both biological (decomposition of proteins) and non-biological reactions. Certain microbial groups decompose compost material to produce SO_2 as incomplete oxidation products. H_2S is produced under anaerobic conditions and is often detectable during the initial stages of composting (Kacprzak et al. 2023). The latest study suggests that *Bacillus* species can lower substances, including NH_3, H_2S, and SO_2. Kim et al. (2014) looked at how well *Bacillus amyloliquefaciens* reduced odor emissions from swine manure in anaerobic environments and observed a noticeable odor reduction during the process of anaerobic fermentation.

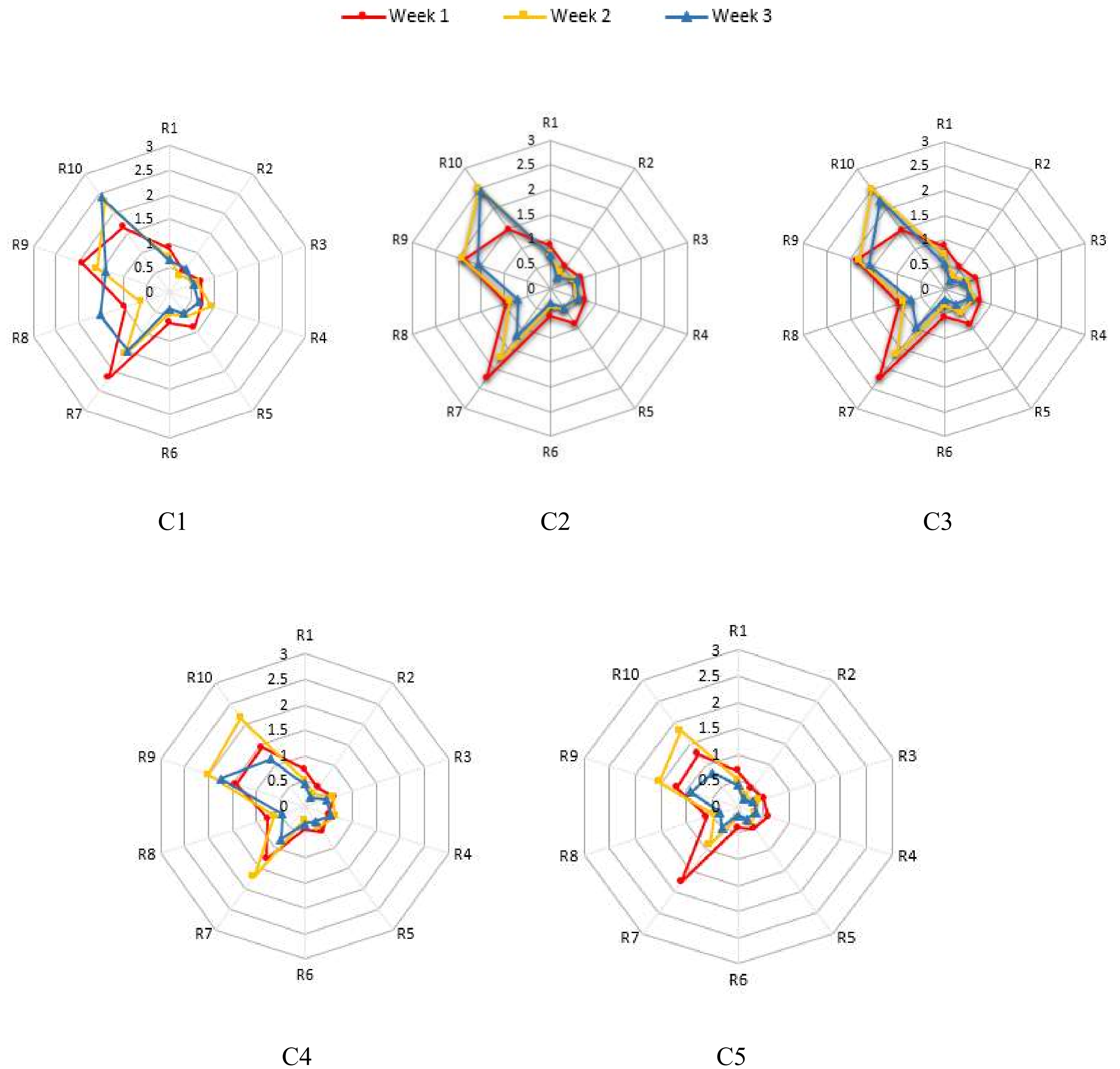


Fig. 2: E-nose odor profile in the compost treatments for three weeks.

In the present study, the second week of composting brought some changes in the gases released; the main gases detected were sulfur and aromatic compounds, along with methane-aliphatic compounds. This change might be because there was less oxygen in the composting bins and the progressed performance of the anaerobic microbes that break down the waste in the low-oxygen microenvironment (Nowocień & Sokołowska 2022). The G/Go ratio of methane-aliphatic compounds, which is detected by sensor 10, the control sample showed the highest ratio at 10.2, while for the C1, C2, C3, C4, and C5 samples, the ratios were lower, indicating less odor. Notably, among these, the C5 sample had the least odor (Mahapatra et al. 2022).

The results of the 3rd week of composting indicate that the overall odor profile of all the samples has been reduced compared to the 2nd week. This may be governed by the continuous odor emission and the conversion of the protein-based complex substrates into simple monomers, which reduce the microbial metabolism in the compost. However, in the control sample odor profile, it was recorded a significant ($P < 0.05$) greater methane-aliphatic (R10) concentration and broad methane (R6) concentration which was which were 6.6 and 6, G/Go ratio, respectively. Importantly, all the consortia inoculated samples recorded much lower odor values compared to the control, ranging from 0.4 -0.5 broad methane and 0.8 2.5 methane-aliphatic concentration, respectively. Out of all the consortia inoculated samples C5 sample showed a clear overall odor reduction compared to all other treatments.

The bacterial strains in the C5 consortium-*Bacillus haynesii*, *Bacillus amyloliquefaciens*, and *Bacillus safensis*, each bring unique biological attributes that collectively contribute to the promising results observed in the composting process. *Bacillus haynesii* is known for its ability to produce extracellular enzymes that degrade complex organic compounds into simpler forms, facilitating the breakdown of odor-causing substances. *Bacillus amyloliquefaciens* enhances this process through its robust metabolic activity and production of antimicrobial peptides, which suppresses unwanted microbial growth and reduces the overall microbial load responsible for odor production. *Bacillus safensis* adds value with its resilience in varying environmental conditions and its ability to metabolize a wide range of organic compounds, including those that are typically resistant to decomposition. The combined metabolic pathways and enzymatic activities of these strains effectively target and reduce volatile organic compounds (VOCs) and other odorous substances, leading to improved compost quality and reduced emissions.

Statistical Analysis of the Odor Parameters

The loading plot for the odor values detected by different sensors reveals that the odor components measured by R1, R7, R8, and R9 are positively correlated. Similarly, the components detected by R2, R3, R4, and R5 also show positive correlation. R6, however, appears to have minimal correlation with the other sensors, and no parameters were found to be negatively correlated. Additionally, the score plot indicates that the control sample released the majority of the odorant compounds, in contrast to the samples inoculated with bacterial consortia.

Changes in Composting Parameters

Solid waste composting is a process associated with both physico-chemical and microbial process mechanisms. Therefore, the maturity and stability of compost are vital to using compost as an agricultural supplement. The compost maturity and stability can be measured by monitoring the parameters including the compost pH, Temperature, Electrical conductivity, Bulk density, and mesophilic and thermophilic viable microbial cell counts. Based on the pH changing pattern, the pH ranged between 6.6 ± 0.1 – and 9.5 ± 0.1. All the consortia inoculated samples were shown a significant difference ($P < 0.05$) compared to the control samples. Importantly, C5 samples were shown an exceptionally faster rate of composting while rapidly moving to the acidification and stabilizing phases.

Temperature is another crucial factor that reflects microbial metabolism during composting, which affects the rate of microbial enzymatic reactions (Ge et al. 2020). In the study, a significant difference ($P < 0.05$) was observed

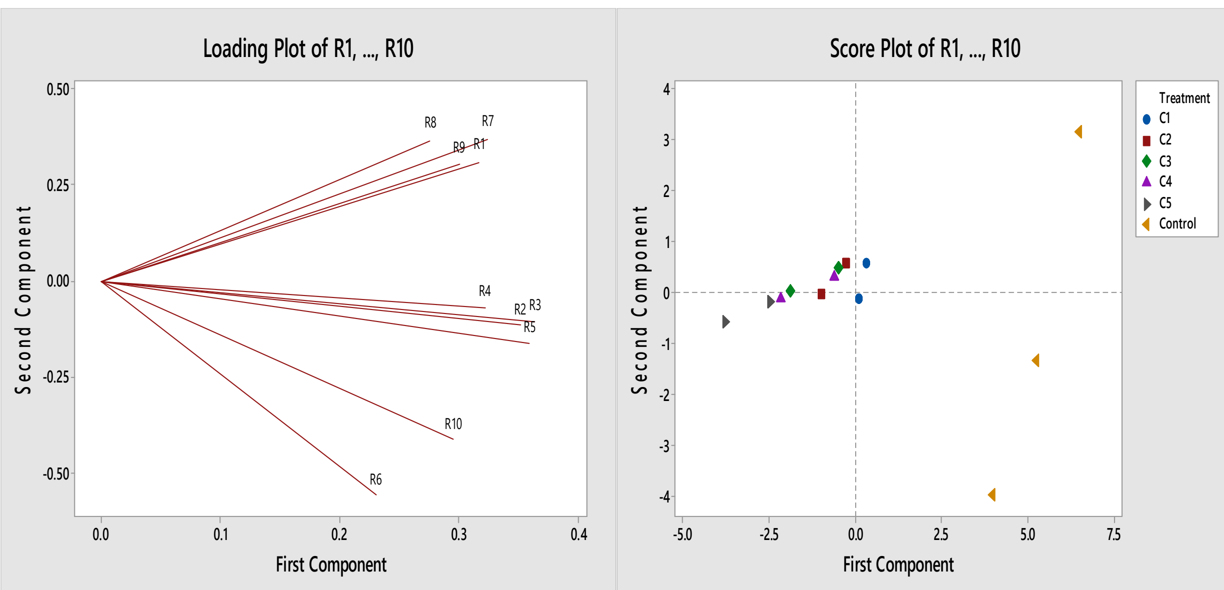


Fig. 3: Loading plot and Score plot for the PCA analysis of the odor parameters.

between the five inoculated samples and the control sample in terms of compost temperature, and it ranged between 30.1 ± 0.3°C and 65.5 ± 0.2°C. Interestingly, the added sample rapidly entered the thermophilic phase within two days, while the other consortia-inoculated samples showed a slower transition. Further, the Electric conductivity (EC) reflects the level of salinity in the final compost product, which impacts nutrient availability for plant growth (Asquer et al. 2019). The results showed that all the consortium-inoculated samples had a significantly higher EC ($P < 0.05$) compared to the control sample.

Further, the microbial dynamics of viable cell counts during the composting period also provide valuable information about the phase alteration of the composting period. In terms of the mesophilic bacterial count, a significant difference ($P < 0.05$) was observed between all the consortium-added samples and the control sample. Further, the thermophilic bacterial count of all the consortium-added samples demonstrated a significant increase ($P < 0.05$) compared to the control sample. In contrast, all analyzed compost parameters indicated that the inoculated bacterial consortia accelerated the overall composting process, and out of all inoculated consortia, the C5 consortia showed an exceptionally faster composting rate by producing mature compost within 17 ± 3 days.

CONCLUSIONS

The bacterial consortium (C5), comprising *Bacillus haynesii*, *Bacillus amyloliquefaciens*, and *Bacillus safensis*, exhibited significant odor reduction ($p < 0.05$) and an accelerated compost maturation rate in MSW composting. Specifically, the emission of malodorous VOCs, H_2S, NH_3, and CH_4 was significantly reduced ($p < 0.05$) by the inoculated bacterial consortium. Additionally, changes in pH, temperature, and bulk density were significant in the C5 consortium compared to the control and other bacterial consortia. These changes demonstrate the rapid degradation of MSW by the inoculated bacterial consortium through the acceleration of both mesophilic and thermophilic phases.

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