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Formulation of Novel Microbial Consortia for Rapid Composting of Biodegradable Municipal Solid Waste: An Approach in the Circular Economy

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ABSTRACT

Urbanization and rapid industrialization have led to the escalation of municipal solid waste generation and accumulation. Composting is widely recognized as a sustainable solution for solid waste management. However, its long-term investment is considered a disadvantage. The present research study discusses the rapid biotransformation of solid waste into valorized compost. Bacteria were isolated from soil, solid waste, and leachate samples from open dump sites. From the 18 different bacterial consortia created using potential isolates, the five most promising consortia were selected based on concurrent different enzyme production. These selected consortia were incorporated into typical compost bins with Municipal Solid Waste (MSW). Daily monitoring of enzymatic activity, pH, conductivity, bulk density, moisture, and temperature, along with other composting parameters, was conducted. The study's results demonstrated that consortium No. 5, comprising *Bacillus haynesii*, *Bacillus amyloliquefaciens*, and *Bacillus safensis*, exhibited significant ($p<0.05$) enzyme activity of cellulase, amylase, lipase and proteinase enzymes during composting compared to the control and other treatment setups. Consortium No. 5 also facilitated rapid and successful composting, as evidenced by significant alterations of composting parameters by exhibiting a shorter average composting time, reducing it from 110±10 days to 20±3 days, showcasing the potential applicability of formulated bacterial consortium as a sustainable and greener approach to the global solid waste problem. The novelty of this study lies in the isolation of local bacterial strains from open dump sites soil, MSW, and MSW leachate samples, which were then utilized in the composting organic fraction of MSW, enhancing the potential for effective waste management.

1. INTRODUCTION

Rapid urbanization, industrialization and agricultural modernization-related anthropogenic activities cause a massive generation and accumulation of Municipal Solid Waste (MSW) globally. Simultaneously, the worldwide MSW generation in 2016 was about 2,010 MT, projected to grow to 3,400 MT by 2050 (Pal and Tiwari, 2023). Consequently, the improper MSW management practices severely impacted natural ecosystems and public health circumstances in numerous ways (Wang et al., 2023; Wijerathna et al., 2023).

Recovering useful materials or energy from garbage has become standard in the circular economy. The need for environment-friendly solutions is increasing rapidly, yet recent technological

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advancements have elevated solid waste management to a stable foundation. Most waste is composed of easily recyclable organic materials, with estimates ranging from 40-50% (Goushterova et al., 2020). Composting is considered one of the popular eco-friendly and cost-effective approaches in which the organic waste coverts into valorized stable humic soil amendments (Chen et al., 2021; Wang et al., 2023).

The application of high-quality compost for agricultural lands is beneficial to minimize the cost of chemical fertilizers, improve crop yield and enhance the soil structure (Goushterova et al., 2020). Conversely, still, traditional composting consists of the areas where the entire process could be improved to overcome the issues of low treatment efficiency and poor final product quality (Pal and Tiwari, 2023). The significant time requirement is one of the major limitations in traditional composting which both delays production and expands the land area required. Inoculation of the effective microbial consortium which has a greater organic waste degradation potential is a promising greener approach for efficient organic waste composting (Wang et al., 2023).

The full biotransformation of waste into valorized composts necessitates the synergistic action of various hydrolytic enzymes in microbes, allowing the development of such a thing an even better value (Shah et al., 2022; Koyama et al., 2022). Nevertheless, the lignocellulolytic oxidative enzymes in numerous fungal and bacterial species combine to depolymerize complex substrates which include cellulose, hemicellulose, and lignin in waste (Andlar et al., 2018; Kumar et al., 2022). Besides, the efficiency with which microorganisms break down organic waste correlates to their structural and functional stability (Andlar et al., 2018). For example, microorganisms in the soil around waste are constantly exposed to new substrates and chemicals, strengthening their natural defenses and making them more resilient. Keeping the waste breakdown rate comparable to garbage disposal may be possible by inoculating waste with potential microorganisms that secrete extracellular hydrolytic enzymes at higher levels, such as cellulase, amylase, protease, pectinase, and lipase (Sarkar and Chourasia, 2017).

The performance of a developed microbial consortium relies on factors which include the nature of the substrate, different climatic or environmental factors and the synergistic interactions with the existing microbial community (Kumar et al., 2022). Besides, numerous studies have been conducted on the development of an efficient microbial consortium for enhancing composting and waste management (Koyama et al., 2022; Wang et al., 2023). However, still, the current available microbial solutions have been unable to answer all the issues in composting MSW efficient and sustainable manner.

The focus of the present study was to develop a novel bacterial consortium with locally isolated potential bacterial strains to enhance the composting process of organic MSW as a cost-effective greener approach (Kumar et al., 2022). Furthermore, this research study may lead to a promising waste management technology in meeting agenda 2030 by highlighting the importance of achieving SDGs 11 and 12, sustainable cities and communities and responsible consumption. Furthermore, the findings of this research would be beneficial in the real-world composting industry and society to meet critical factors of SGDs 9, 1, 3, 6, and 14; Industry, innovation and infrastructure, no poverty, good health and well-being, clean water and sanitization (Chen et al., 2021).

2. METHODOLOGY

2.1 Collection of samples for bacteria isolation

Sample sites were selected based on the accessibility to natural MSW whereby the onsite microbial community could predominantly be adapted to break down MSW by nature which could be isolated easily in large numbers. Three major dumps including Karadiyana (6°48'58.23"N, 79°54'9.27"E), Meethotamulla (6°56'18.65"N, 79°53'25.00"E) and Kerawalapitiya (7°30'33.63"N, 80°21'12.24"E) in Sri Lanka were selected for the isolation of bacteria from soil, solid waste and leachate. Leachate samples were collected into sterilized amber-coloured glass bottles, whereas soil and solid waste samples were collected into sterilized press-seal bags. The samples were transported to the lab in an ice box and immediately subjected to microbiological analysis (Gunaratne et al., 2024). Figure 1 illustrates the sampling locations of this study.

2.2 Isolation of bacteria from the soil, leachate, and solid waste

The isolation of bacteria was performed following the standard serial dilution method. One gram of the composite soil/solid waste sample or 1 mL of leachate sample were individually transferred to test tubes filled with 10 mL of sterilized saline water and mixed well, followed by subsequent tenfold dilution by transferring 1 mL solution to sterile 9 mL deionized

water. Finally, 100 µL of the solution from each dilution was transferred onto a sterile solid nutrient agar medium (1.5%, pH 7) to prepare spread plates and then incubated at 37ºC for 24 h. Bacterial colonies with different morphological features were isolated, purified and stored at 4ºC for future studies (Sarkar and Chourasia, 2017).

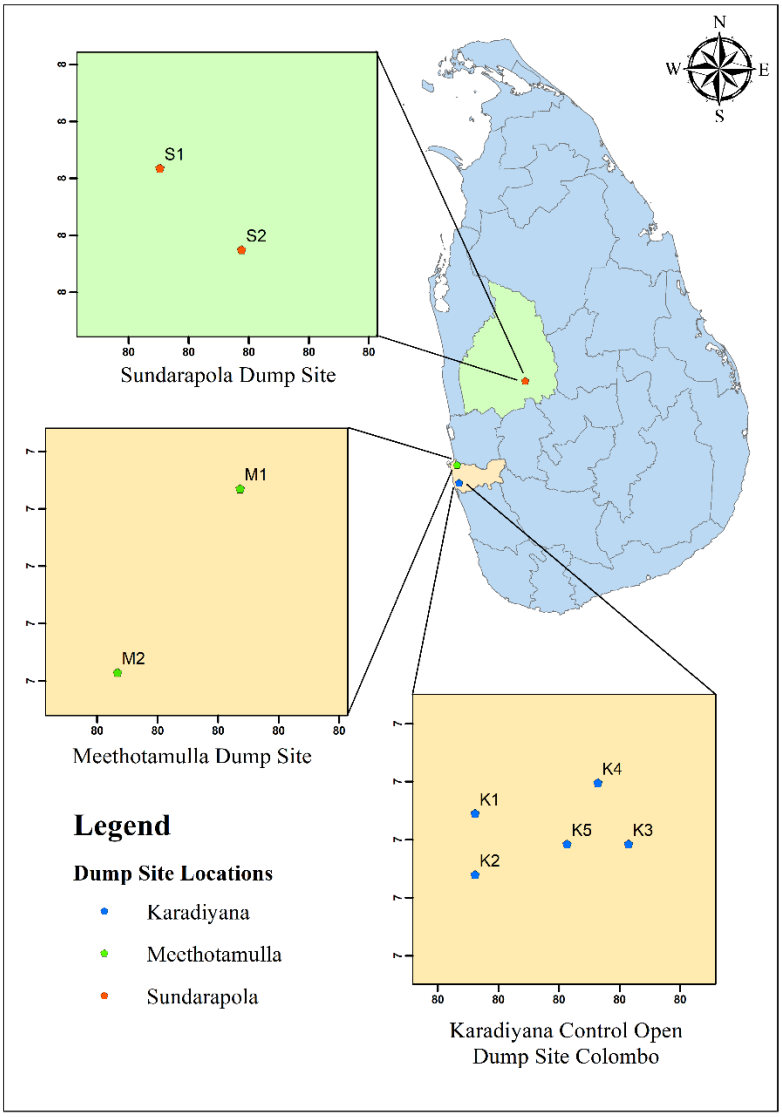

Figure 1. Sampling locations

2.3 Primary Screening of bacteria for extracellular enzymes production potential (enzyme assays)

The isolated microorganisms were subjected to the primary screening following the clear zone formation on different substrates. The cellulase enzyme producers were screened following the Congo Red method using Carboxyl Methyl Cellulose (CMC) as the substrate (Zhang et al., 2021; Harindintwali et al., 2020). The amylase-positive bacteria were screened following the starch-agar medium, and proteinase-positive bacteria were screened using skimmed milk agar medium. Finally, the lipase-positive bacteria were screened using the Phenyl Red agar medium and olive oil as the substrate (Sarkar and Chourasia, 2017).

2.4 Secondary screening of different enzyme-producing bacteria

Secondary screening was conducted for all the positive (enzyme-producing) isolates obtained from primary screening to select the best enzyme-producing strains to be used for final consortia preparation. Four different enzyme assays were performed for selected bacteria from the primary screening to quantify the extracellular cellulase, amylase, lipase, and proteinase enzyme production.

The test tubes were heated at 100ºC for 5 min and cooled to room temperature. Finally, the solutions were diluted by adding 5 mL of distilled water and absorbance values were recorded at 540 nm. The glucose standard curve was used to determine the amylase activity (Dhiman et al., 2021).

2.4.1 Amylase assay

The Di-Nitro Salicylic acid (DNS) method was used to determine amylase activity. A 1% starch broth was made and 50 mM phosphate buffer (pH 7) was prepared as a substrate. Tubes were filled with substrate, crude enzyme, and buffer following incubation for 30 min under 37ºC. Then, the enzyme blank and 1 mL of DNS reagent were added into each test tube. The test tubes were heated at 100ºC for 5 min and cooled to room temperature. Finally, the solutions were diluted by adding 5 mL of distilled water and absorbance values were recorded at 540 nm. The glucose standard curve was used to determine the amylase activity (Dhiman et al., 2021).

2.4.2 Cellulase assay

The standard DNS method was used to determine cellulase enzyme activity and the assay was carried out in the same manner as the amylase assay. Substrates for the assay were prepared by adding 1% of Carboxy Methyl Cellulose (CMC) broth in 50 mM phosphate buffer (pH 7) solution. Similar to the amylase assay, the substrate, crude enzyme extract and buffer solutions were mixed and heated in the same procedure of amylase assay. The absorbance values were recorded at 540 nm and the cellulase activity was measured using the glucose standard curve. One unit of enzyme activity is referred to as the amount of enzyme that releases one mole of reducing sugar per minute (U/mL/min) under assay conditions (Sarkar and Chourasia, 2017).

2.4.3 Protease assay

Protease enzyme activity was measured spectrophotometrically by Sigma's universal protease assay using Casein as the substrate. One per cent casein broth was prepared in 50 mM potassium phosphate buffer (pH 7.5) as the substrate. The crude enzyme, substrate and buffer were added to tubes and incubated at 37ºC for 10 min. Finally, the absorbance was measured under 660 nm, and one unit of enzyme activity is defined as the amount of enzyme that releases 1 mol of tyrosine per minute under assay conditions (U/mL/min) (Patel et al., 2019).

2.4.4 Lipase assay

Lipase enzyme activity was measured titrimetrically using tributyrin oil as the substrate. First, 1 mL of the substrate was into a test tube containing 3 mL of 50 mM Tris-HCl buffer (pH 8). Next, 1 mL of the crude enzyme broth was mixed with the solution and incubated for 30 min at 37ºC. After incubating, 1% phenolphthalein was mixed with the broth and titrated with 50 mM NaOH a pinkish colour developed. One unit of enzyme activity is defined as the amount of enzyme that releases 1 mol of fatty acid per minute under assay conditions (Sarkar and Chourasia, 2017).

2.5 Antagonistic effects

Eighteen consortia were formulated using the best potential isolates with the most significant single enzyme-producing ability for cellulase, proteinase, lipase and amylase enzymes. The antagonistic effects were checked for each consortium, and the cross-streaking method was used to test antagonisms. The incubated plates were examined for inhibition zones, which indicate the antagonistic effects of bacteria in the consortium. The consortia which did not show any antagonistic activity, were subjected to concomitant enzyme production (Zhimo et al., 2020).

2.5.1 Concomitant enzyme production by the consortium

The selected consortia were subjected to concomitant enzyme production by culturing in a modified medium containing specific cellulose, starch, protein and lipids substrates. After that, an equalized 1% broth consortium was inoculated in 50 mL of synthetic medium which contains 0.5% of each of mineral salts, CMC, starch, and skimmed milk. After that, the broth cultures were incubated for 7 days under 37ºC at 150 rpm (Sarkar and Chourasia, 2017). The enzyme activity for each specific enzyme was carried out for the samples withdrawn every 24 h, and the concomitant enzyme production of each consortium was measured (Ma and Liu, 2019).

2.6 Preparation of consortia broth cultures and the field experimental setup for the compost study

Municipal solid waste was obtained from Maharagama municipal council, western province, Sri Lanka, for the field experiment. Standard-size (height 150 cm, diameter 45 cm) eighteen concrete compost bins were used for the field experiment, and the bins were placed on a flat open field area, labelled and each

one filled with 100 kg of composited municipal solid waste. The automated sensor set-up was established inside the compost bins to read the temperature and relative humidity of the compost bins' cap space every 30 min and all treatments were triplicated. The best five consortia (a, d, f, h, j) from the concomitant enzyme assay were selected for the composting field application set-up, and they were named C1, C2, C3, C4, and C5, respectively.

A loop full of bacteria in each consortia was separately inoculated to the 100 mL of sterilized nutrient broth medium and incubated at 30ºC for 48 h under shaking conditions of 50 rpm. After the broth cultures were separately centrifuged under 6,000 rpm for 10 min to remove the supernatant. The microbial pellet was washed twice with a sterilized saline solution to remove medium residues. The pallets were dissolved in sterilized saline water and equalized for 590 nm wavelength to 0.35 absorbance value using a UV visible spectrophotometer. The same volume of equalized (5 mL) of bacterial cell suspension of each bacterial type in the consortia was mixed to prepare the consortia broth and a 1% v/w of consortium broth was inoculated to the respective compost bins. Figure 2 shows the field experimental set-up of the composting study.

Figure 2. The experimental field set-up of the composting study

2.7 Characterization of solid waste and the final compost

2.7.1 Solid waste characterization

A composite sample was taken from fresh MSW and the experiments were carried out immediately. The solid waste was characterized by measuring the initial pH and conductivity using an aqueous mixture of 1:10 diluted (w/v) using a multipara meter (Model Star 2001-Thermo Scientific); Total Kjeldahl Nitrogen (TKN) concentrations were analyzed for a standard composite mixture of a solid waste sample using the standard methods (Awasthi et al., 2018).

The concentration of lipids, proteins, carbohydrates, starch and free sugars was analyzed according to standard methods (Awasthi et al., 2018). Further, the composite solid waste's bulk density was determined using a standard metalcore 100 cc cylinder. The Initial microbial count was recorded following the standard serial dilution method and the standard pour plate method on the Nutrient Agar Universal medium.

2.7.2 Characterization of the final compost

To determine the quality of the final compost sample, the TKN, total organic carbon (TOC), C:N ratio, total phosphate and total ash content were measured in the final compost following the standard methods (Mahapatra et al., 2022; Parameswaran et al., 2024). The Germination Index (GI) was calculated in all samples following the equation given using green gram seeds.

$$GI = \frac{\text{Number of germinated seeds in sample} \times \text{Root length in the sample} \times 100}{\text{Number of germinated seeds in control} \times \text{Root length in control}}$$

2.7.3 Mesophilic and thermophilic viable cell count

The mesophilic and thermophilic bacteria were cultured on a nutrient agar medium by preparing a compost suspension by mixing 1 g of compost in 9 mL sterilized distilled water and a tenfold dilution series was prepared from the initial sample. The standard pour plate method was used to grow bacteria. The plates were incubated for 72 h at either 28ºC or 55ºC to determine the number of colony forming units (CFU) of mesophiles and thermophiles, respectively (Lin et al., 2022).

2.8 DNA extraction and identification of bacteria using 16S r RNA

The 16S rRNA identification was done only for the bacteria in the best-performed (C5) consortia. A

loop full of bacteria from each isolate was transferred to prepared sterilized nutrient broth media and incubated at 30ºC for 48 h. After that, a 200 µL of each broth culture was transferred to sterilized Eppendorf tubes and centrifuged under 10,000 rpm for 2 min. The supernatant was discarded and the procedure was repeated until obtaining a clear bacterial pellet. The obtained bacterial pellet was filled with 200 µL of cell lysis solution (Promega®, Cat No: A7933) and mixed thoroughly. The DNA extraction was carried out using a two-times repeated freeze-thaw cycle followed by heating under 120ºC for 10 min and freezing on ice for 10 min. The DNA solution was again centrifuged under 10,000 rpm for 2 min and the extracted DNA was transferred to a sterilized Eppendorf tube (Goushterova et al., 2020). The extracted DNA was subjected to the 16S rRNA gene sequencing, sending the bacterial DNA to Macrogen, Korea. The gene sequence was used to carry out BLAST with the NCBI Gene Bank database and aligned using multiple alignment software programs.

2.9 Figures and statistical analysis

Statistical analysis was carried out using the Minitab 17 version and a one-way ANOVA test was used. Figures were created using MS Excel 2010 version.

3. RESULTS AND DISCUSSION

3.1 Isolation, primary, and secondary screening of bacteria

Following a 24-hour incubation at 37ºC, a total of 226 morphologically different bacterial colonies were isolated from the soil, leachate, and solid waste sample. The isolated bacteria were screened for potential extracellular hydrolytic enzyme dynamic as a strong indicator which can determine the rate of biomass transformation during composting. Microbes secrete several hydrolytic enzymes, such as cellulase, protease, lipase, and amylase, which can degrade the predominant substrates of MSW (Zhang et al., 2021).

Figure 3 represents the positive results obtained for each enzyme plate assay. Based on the primary screening results, the CMC agar plate assay revealed the presence of 15 cellulase-positive bacterial strains. Similarly, the starch agar assay identified 15 amylase-positive bacterial strains and the skimmed milk agar assay revealed 15 proteinase-positive bacterial strains. Lastly, the lipase plate assay revealed 7 lipase-positive isolates.

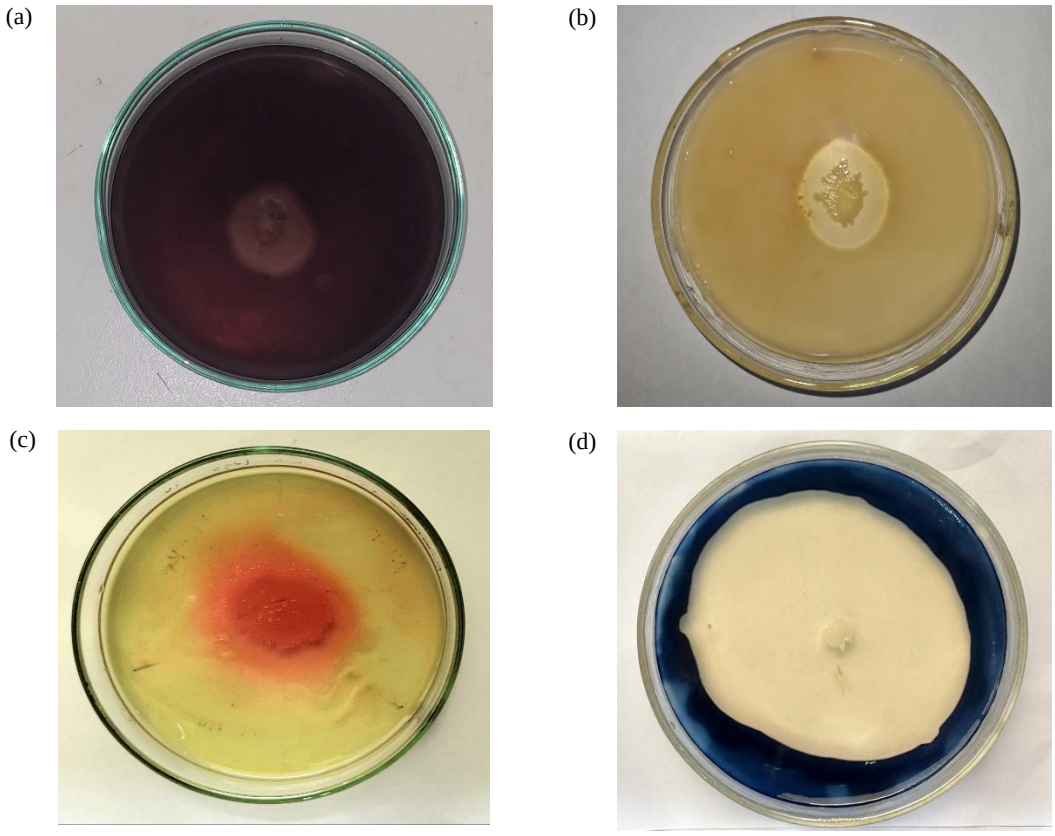

Figure 3. Positive isolates for the cellulase (a) *Bacillus amyloliquefaciens strain* BAC1-PP391056, proteinase; (b) *Bacillus haynesii* strain BHC1-PP391133; lipase (c) *Bacillus safensis* PP391033); and amylase (d) *Bacillus amyloliquefaciens* strain AMWC-PP391615 tests.

The number of lipase-positive isolates has recorded as a lower number compared to the cellulase, amylase and protinase-positive isolates. This might be attributed to the predominant bacterial community in the dump site environment may have adapted to degrade carbohydrates and protein substrates (Harindintwali et al., 2020). Further, Chukwuma et al. (2023) have depicted the potentiality of lingo-cellulolytic bacteria *Bacillus* spp. from MSW landfill, which can break down biomass by producing hydrolytic enzymes.

According to the secondary screening results (Table 1) of the study, cellulase-positive bacterial isolates K11 and K50 exhibited the highest cellulase activity at 6.2±0.2 U/mL/min and were selected for subsequent consortia formation. The highest amylase activity was observed in bacterial isolates K21 and K29, with values of 6.5±0.1 U/mL/min and 6.2±0.3 U/mL/min, respectively. Similarly, the most significant proteinase activity was found in bacterial strains K21 and K29, with measurements of 7.2±0.2 U/mL/min and 6.5±0.1 U/mL/min, respectively. Bacteria K29 and K17 exhibited the highest lipase activity, with values of 4.2±0.2 U/mL/min and 3.8±0.2 U/mL/min, respectively indicating the efficient degradation potential lipid substrates.

Similarly, Sarkar and Chourasia (2017) have researched bio-fortified compost using developed microbial consortia which were able to produce amylase, cellulase, and lipase activities of 4.18 U/mL/min, 2.35 U/mL/min, 15.62, and U/mL/min, respectively. Besides, the studies carried out by Zhang et al. (2021) have recorded the 0.8 U/mL/min of cellulase activity for the hydrolysis of lignocellulosic sugarcane bagasse.

Table 1. Enzyme activities of isolated bacterial strains

Positive isolates	Cellulase enzyme activity (U/mL/min)	Amylase enzyme activity (U/mL/min)	Proteinase enzyme activity (U/mL/min)	Lipase enzyme activity (U/mL/min)
KX2	3.5±0.1	ND	5.8±0.2	ND
K01	3.8±0.2	3.5±0.1	ND	ND
K05	4.2±0.1	ND	ND	2.6±0.2
K07	3.5±0.1	ND	ND	ND
K04	3.2±0.1	4.2±0.1	6.8±0.2	ND
K19	3.8±0.1	ND	ND	ND
K20	2.5±0.2	ND	3.2±0.2	ND
K17	2.1±0.2	ND	1.8±0.2	3.8±0.2
K18	1.5±0.1	5.2±0.1	4.5±0.1	ND
K13	1.1±0.1	ND	ND	ND
K14	5.2±0.1	3.8±0.1	ND	ND
K11	6.2±0.3	ND	6.2±0.3	ND
K50	6.2±0.2	ND	5.2±0.1	ND
K32	4.2±0.1	4.2±0.1	ND	ND
K21	ND	6.5±0.1	7.2±0.2	ND
K29	ND	6.2±0.3	ND	4.2±0.2
K30	ND	4.8±0.1	ND	1.5±0.1
K39	ND	1.6±0.1	ND	ND
K37	ND	5.7±0.1	ND	ND
KX2	ND	0	5.8±0.2	ND

*Note - All the data were the average of the three replicates, ND - Not Detected

3.2 Antagonistic effects

The performance of a microbial consortium relies on several conditions including the microbial composition, metabolic mechanisms of microbes and the synergistic interactions of the individual strains in the microbial community (Blair et al., 2021). Particularly, the antimicrobial substances that are secreted by some microorganisms can selectively kill or inhibit the growth of other bacteria in their vicinity (Lin et al., 2022).

Therefore, the effectiveness of bacterial consortia relies on the compatibility of the other bacteria in the same consortium. The results indicated that there was no antagonism exhibited between any

bacterial species in consortia A to J, respectively, based on the results of the antagonistic assay (Table 2) for all bacteria consortia. However, it revealed an adverse, antagonistic interaction in consortium K which caused the K50 species of bacteria to obstruct the growth of K04 and K21. Producing hazardous substances may hamper growing conditions and degrade consortium performance. The consortia that showed no signs of antagonism were subjected to further study.

Table 2. Prepared different consortia and their antagonistic effects

Consortium No.	Bacterial combination	Antagonistic effects
A	K01, K04, K21, K29	ND
B	K01, K04, K21, K17	ND
C	K01, K04, K29, K17	ND
D	K01, KX2, K21, K29	ND
E	K01, KX2,K21, K17	ND
F	K01, KX2,K29, K17	ND
G	K50, K04, K21, K29	ND
H	K50, K04, K29, K17	ND
I	K50,KX2, K21,K 29	ND
J	K50, KX2, K21,K 17	ND
K	K50, K04, K21, K17	Detected
L	K50, KX2, K29, K17	ND

*Note - ND (Not Detected)

3.3 Screening of best potential bacterial consortia for field application

The concomitant production of each enzyme was used to select the best 5 consortia for the field application. According to Figure 4, the consortia a, d, f, h, j (denoted by C1-C5 respectively in the Table 3) were shown the highest concomitant enzyme production, whereas demonstrating the enzyme activity of 21.2±0.2, 20.4±0.2, 15.3±0.1, 19.4±0.1, and 20.5±0.2 U/mL/min, respectively. The most potential five consortia (Table 3) were separately inoculated to each composting setup and their respective composting rates were determined. Similarly, Al-Dhabi et al. (2019) have studied the concomitant enzyme production of developed consortia to enhance solid waste composting.

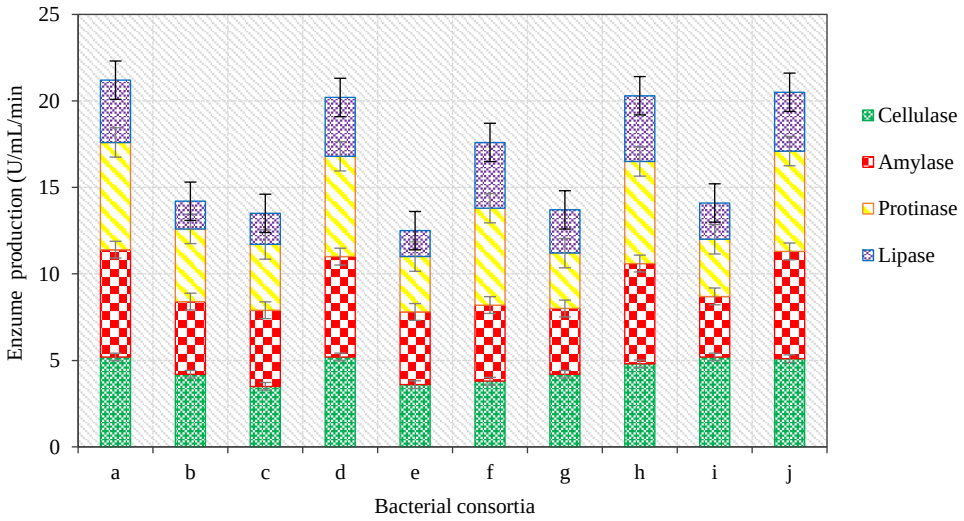

Figure 4. Concomitant enzyme production by formulated consortia (Note: All the data were the average of the three replicates)

Table 3. Selected potential bacterial consortia for the field study

Bacterial consortium	Bacterial species of the consortium
C-1	K01, K04, K21, K29
C-2	K01, KX2, K21, K29
C-3	K01, KX2,K29, K17
C-4	K50, K04, K29, K17
C-5	K50, KX2, K21, K17

3.4 Solid waste characterization

In Table 4, the basic characteristics of the municipal solid waste used in the composting study are presented. The data obtained from the waste samples indicated that they exhibited typical waste characteristics, and no extreme deviations were observed. This suggests that the waste samples used in the study were representative of municipal solid waste and can be considered suitable material for the composting process (Kumar et al., 2022).

Table 4. Preliminary characterization of the solid waste

Parameter	Mean value
pH	7.5±0.3
Electrical conductivity (µS/cm)	750.21±1.50 µS/cm
Moisture %	65±2 %
Ash content	62.5±3 %
Bulk density	285±10 kg/m^3
Total nitrogen	7.2±0.5 g/kg
Total phosphate	0.8±0.5 g/kg
Total lipid content	15.10±0.50 g/kg

Note - Data were the average of the three replicates

3.5 Changes in composting parameters

Compost maturity and stability are vital aspects of compost quality that are directly related to the degree of decomposition (Mahapatra et al., 2022). Figure 5 illustrates the fluctuations in temperature (a), pH (b), electrical conductivity (c), and moisture (d) during the composting process.

Temperature (Figure 5(a)) is one of the crucial factors that reflects microbial metabolism during composting. It affects the rate of reactions and contributes to pathogen and seed eradication, ensuring the sanitation of the composting process. The optimal temperature range for composting is typically considered to be 40-65ºC (Sun et al., 2019). The composting process consists of four phases: the mesophilic phase (25-40ºC), thermophilic phase (45-70ºC), cooling phase (second mesophilic phase), and maturation phase (Awasthi et al., 2018).

In the study, a significant difference ($p<0.05$) was observed between the five inoculated samples and the control sample in terms of compost temperature. The highest temperature of 65.5±0.2ºC was recorded on the fourth day for the C5-added sample. Interestingly, the C5 consortium inoculated sample rapidly entered the thermophilic phase within two days, while the other samples indicated a slower transition pattern. Importantly, the control sample exhibited a meagre temperature increment rate, indicating a prolonged entry into the thermophilic phase with slower composting (Rashwan et al., 2021). A rapid enhancement in temperature throughout the early stage of composting leads to the speedy breakdown of degradable organic substrates relies on accelerated microbial metabolism. Additionally, several researchers have recorded the maximum temperature in the thermophilic phase as 60-70.2ºC that were similar to the present study (Sun et al., 2021; Chen et al., 2022). However, most of the composting studies have reported that the theomorphic phase has started after 5-7 days of composting (Finore et al., 2023; Wan et al., 2020). Importantly, the results of the present study were better compared to most of the previous studies indicating a rapid solid waste degradation due to the favorable synergistic interactions of bacteria in the C5 consortia with the environmental microbial community.

The pH levels depicted in Figure 5(b) followed the same pattern across all treatments, following a sudden pH drop which indicates the formation of organic acids and favourable conditions for the breakdown of lignin and cellulose by the mesophilic microorganisms. Notably, treatment C5 exhibited less time exceptional performance by reaching this phase within the first 2-3 days, whereas the control took 9-11 days. Furthermore, an increase in pH (6-9) indicates compost maturity. Treatment C5 reached the mature pH after five days, while the control took 17-19 days. The control sample showed a significantly slower composting dynamic compared to the treatments with added consortia. A significant correlation of pH was observed between C2 and C5 treatments compared to the control treatment ($p<0.05$). Microbial activities are greatly influenced by the pH, and a neutral pH is optimum for composting. After day 3, when the thermophilic phase prevailed, the pH increased due to possible ammonia evolution (Sun et al., 2021; Awasthi et al., 2018; Wijerathna et al., 2024). Previous research revealed that the presence of H^+ induced a reduction in pH values after the maximum pH as ammonia and nitrification volatilized which relies on the results of the present study (Wan et al., 2020).

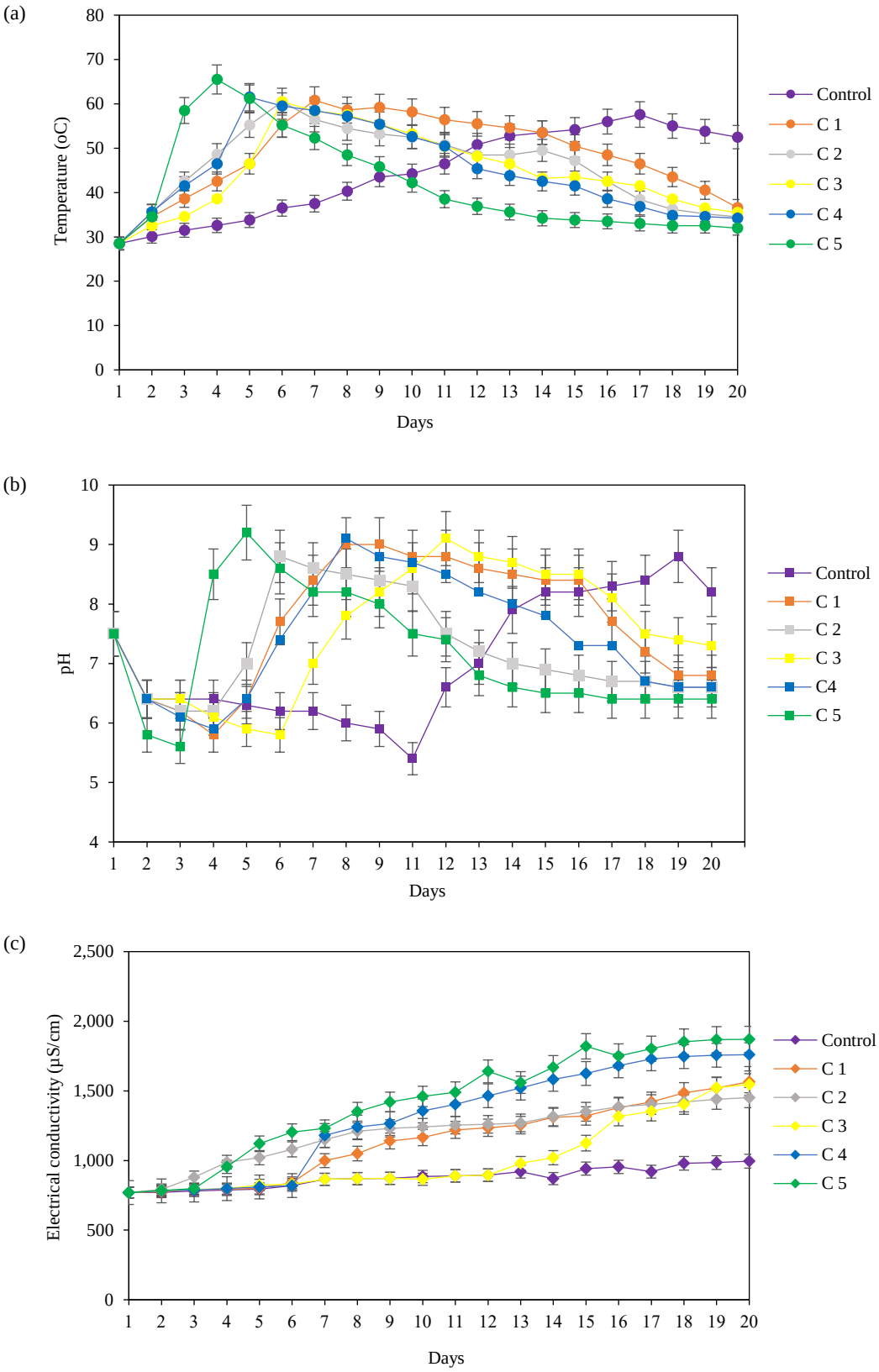

Figure 5. Changes of the temperature (a), pH (b), electrical conductivity (c), and moisture (d) during the composting period (*Note - All the data were the average of the three replicates)

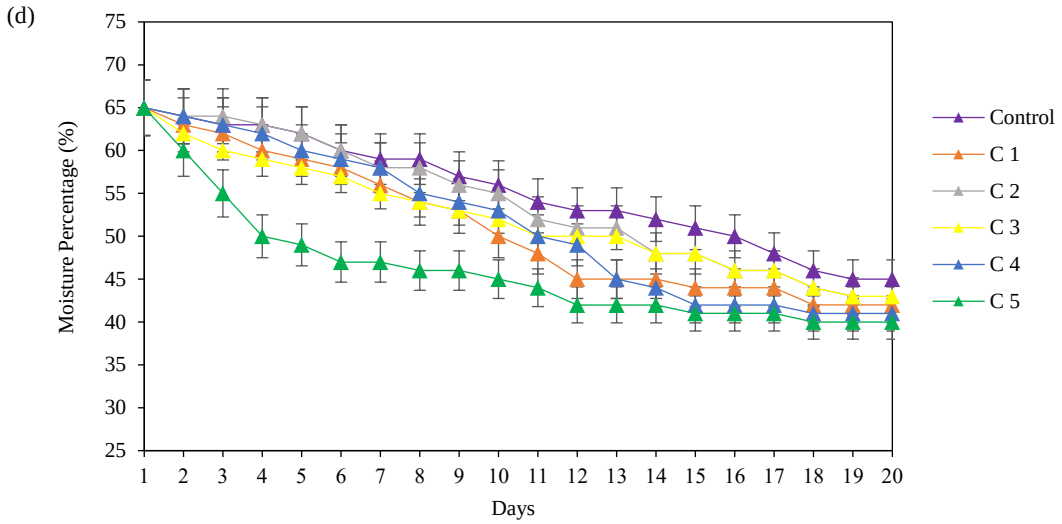

Figure 5. Changes of the temperature (a), pH (b), electrical conductivity (c), and moisture (d) during the composting period (*Note - All the data were the average of the three replicates) (cont.)

Electric conductivity (EC) reflects the level of salinity in the final compost product, which impacts nutrient availability for plant growth (Rashwan et al., 2021). Figure 5(c) illustrates that changes in EC which all the consortium-inoculated samples were significant ($p<0.05$) compared to the control sample. The C5 inoculated treatments exhibited the highest EC, indicating rapid degradation of solid waste. During composting, the EC values increase due to the production of inorganic compounds and ion release (Rashwan et al., 2021). It is generally desirable for mature compost to have an EC value below 4 mS/cm, as excessive EC can hinder plant growth. In this study, all composts had EC values below 4 mS/cm, reflecting good compost quality (Rashwan et al., 2021).

Regarding moisture changes, all consortium-added samples displayed a significant moisture loss ($p<0.05$) compared to the control throughout the composting period. The C5 samples showed the most rapid moisture loss due to their accelerated temperature increase during the thermophilic phase. Moisture is a crucial parameter closely associated with microbial activities (Awasthi et al., 2018). Initial moisture values below 30% can lead to accelerated dehydration and biologically unstable compost. On the other hand, moisture values above 80% can create anaerobic respiration conditions, reduce compost porosity, and cause leachate and unpleasant odour conditions (Awasthi et al., 2018).

Figure 6 depicts the changing pattern of bulk density over time for all the consortium-added samples compared to the control. The results reveal that the bulk density increased in all samples. However, there were variations in the rate of bulk density increment among the different treatments. The control sample exhibited a relatively smaller increase in bulk density, while consortiums 4 and 5 showed a rapid increase during the first and second weeks of composting.

The increase in bulk density can be attributed to the mineralization of organic matter, resulting in a rapid reduction in the mass and volume of MSW. However, certain inert materials such as soil minerals, metals, and other inorganic constituents do not decompose and instead remain as part of the finished compost. These inert materials contribute to the increment in bulk density observed during composting (Kumar et al., 2022). Overall, the increasing bulk density observed in the consortium-added samples indicates the progress of composting and the transformation of organic waste into a denser, more stable compost product (Awasthi et al., 2018; Chen et al., 2022).

3.6 Hydrolytic enzyme dynamics during composting

The dynamics of hydrolytic enzymes during the composting period were analyzed in Figure 7. The study observed changes in α-amylase, cellulase, proteinase, and lipase concentrations over time and among different treatments.

According to Figure 7(a), all compost samples exhibited a rapid increase in α-amylase at the beginning of the composting process. This initial increment could be attributed to the presence of abundant starch content in the selected solid waste sample. The availability of starch created an optimal

environment for starch-degrading bacteria, leading to increased synthesis of the amylase enzyme. However, as the composting period progressed, the concentration of α-amylase showed a declining rate in all treatments. This decline was likely due to the depletion of starch in the piles over time.

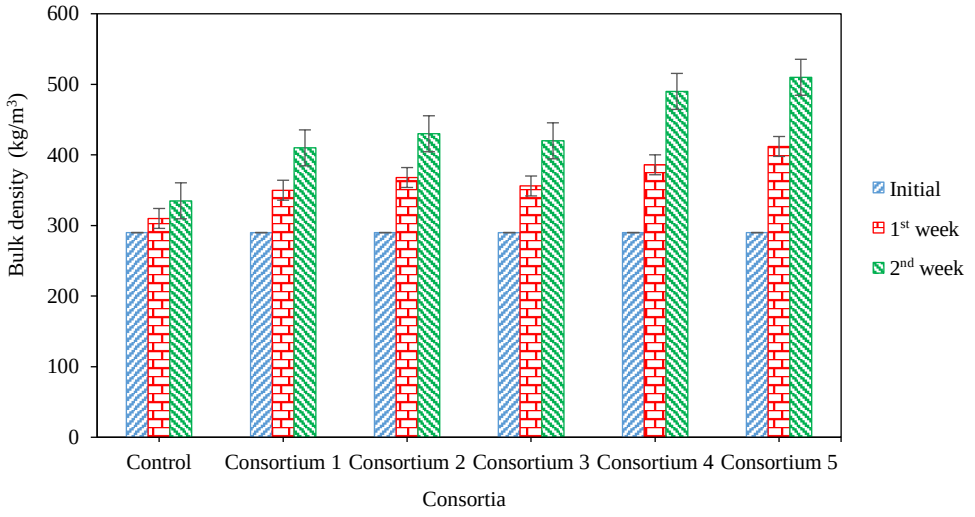

Figure 6. Changes in the bulk density (Note - All the data were the average of the three replicates)

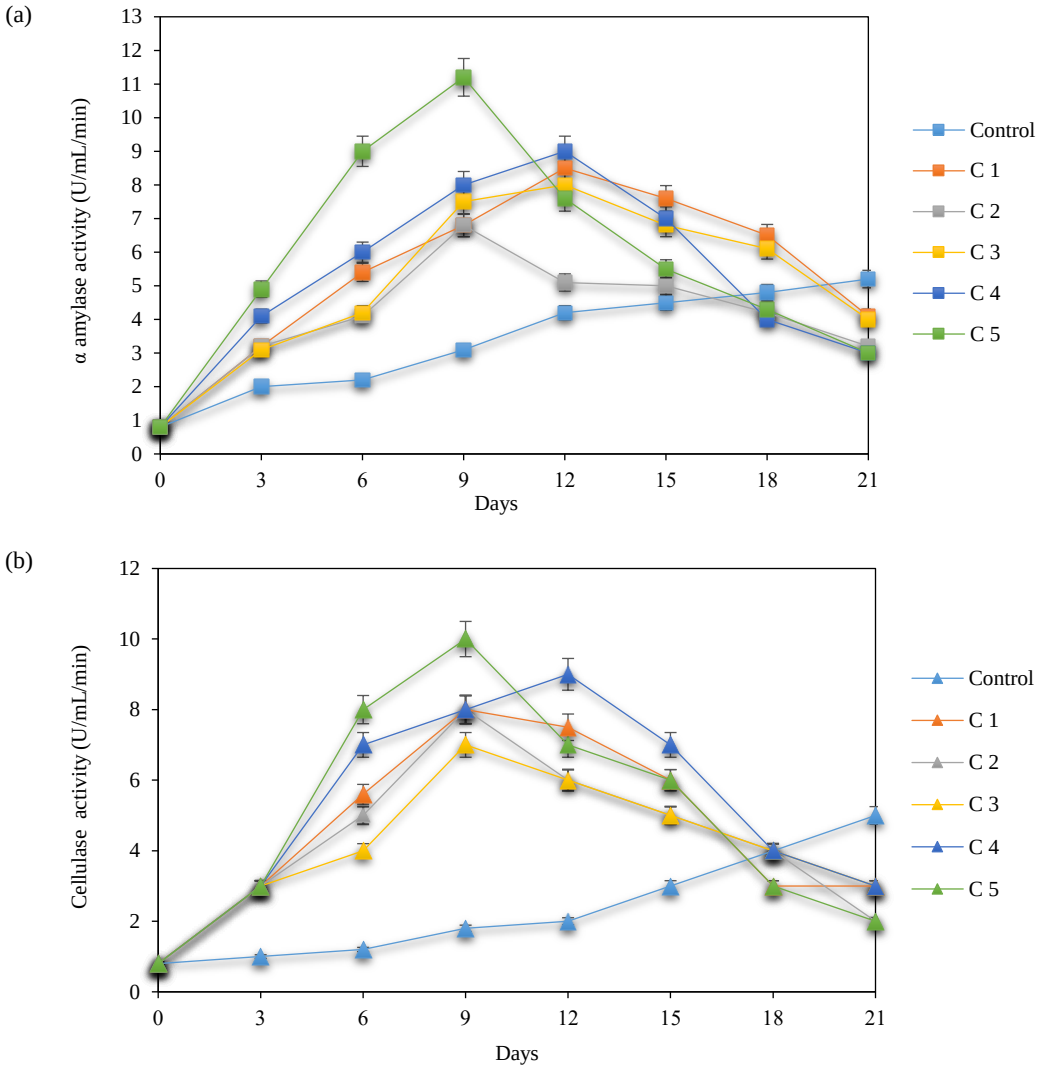

Figure 7. Hydrolytic enzyme dynamics during the composting period (Note - All the data were the average of the three replicates)

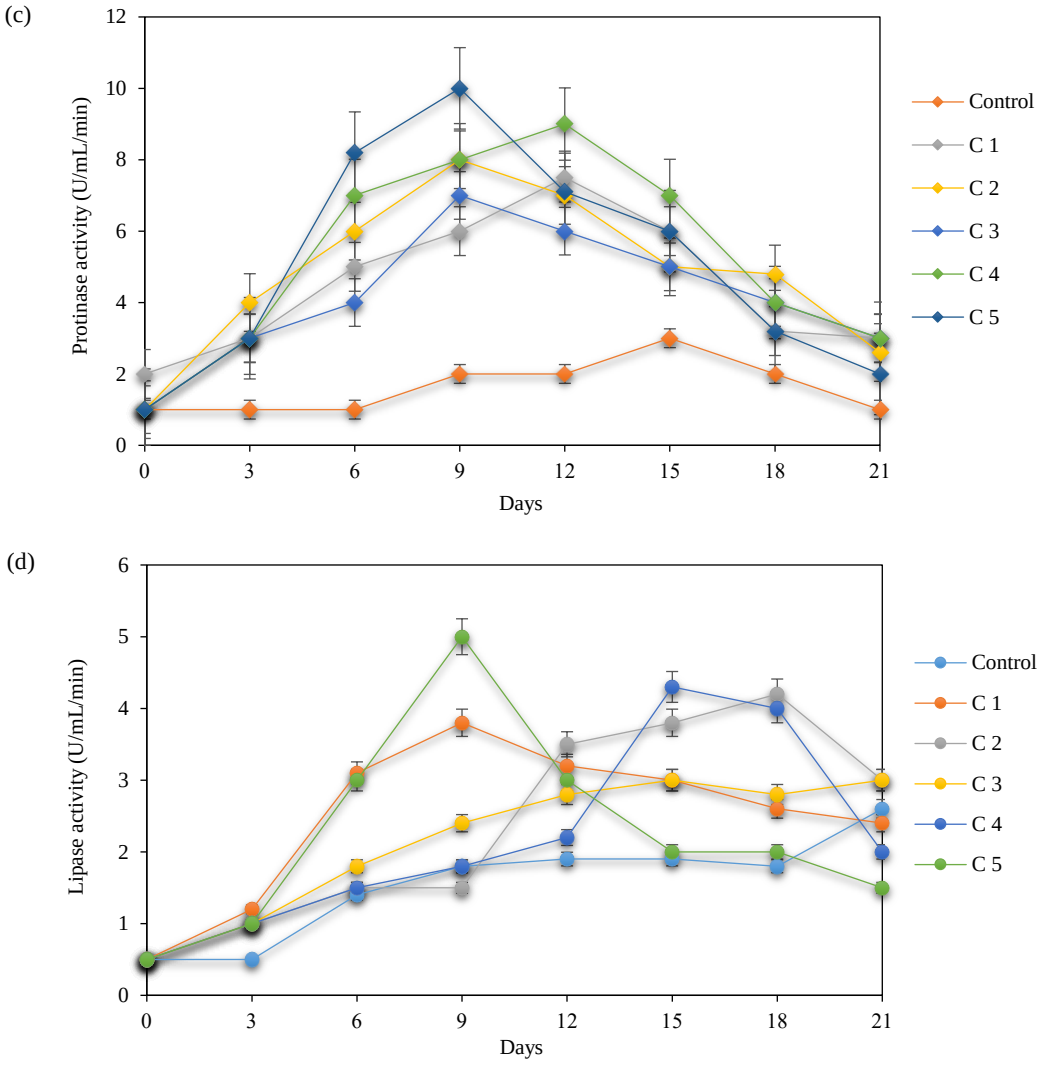

Figure 7. Hydrolytic enzyme dynamics during the composting period (Note - All the data were the average of the three replicates) (cont.)

Interestingly, the highest α-amylase enzyme activity was recorded in consortium C5 until the peak on the 9th day of composting. This finding suggests that consortium C5 exhibited hyperactivity in the presence of sufficient starch. In contrast, the control samples showed the lowest α-amylase activity until the other treatments peaked, but they demonstrated a steady and smooth increasing pattern throughout the study. Similarly, Pan (2021) recorded α-amylase activity of 3.7 U/mL/min from a thermostable bacillus sp. isolated from compost and observed a reduction of α-amylase activity with the increase of starch concentration, indicating substrate inhibition.

Figure 7(b) exhibits the cellulase activity of all consortia inoculated samples which indicates a rapid increase compared to the control. The control sample, however, displayed a prolonged rate of enzyme activity increase. Consortium C5 exhibited the highest cellulase activity peak at 10 U/mL/min on the 9th day of composting, while the other consortia-added treatments showed lower peaks compared to C5. Cellulase is crucial for cellulose degradation and requires the involvement of both fungi and bacteria, such as cracking bacteria and *Aspergillus* sp. The reason for observing the highest cellulase activity on the 10th day may be attributed to the comparatively slower degradation rate of cellulase which is mostly completed in the middle and late phases of composting (Finore et al., 2023). Similarly, several researchers have measured the cellulase activity during organic waste composting (Dantroliya et al., 2022; Malik and Javed, 2021). Further, Lin et al. (2022) have observed a similar finding to the present study indicating that the cellulase activity was significantly higher during the thermophilic phase (>55°C) than the other phases. Further, the obtained results demonstrate that the C5 consortium has successfully accelerated the cullose degradation of MSW by enhancing the cellulase activity.

Proteinase, an enzyme responsible for protein and peptide degradation in solid waste, also exhibited varying dynamics among the consortia treatments. The highest proteinase activity, 10 U/mL/min, was recorded from consortium C5 on the 9th day of composting. As the composting stages progressed, the availability of protein content gradually decreased, resulting in lower protease activity and eventually entering the stabilization phase. The control sample displayed the lowest proteinase production, with a maximum enzyme activity of 3 U/mL/min. Significant differences in proteinase activity were observed between the treatments, with consortia C1, C2, C3, and C4 showing increased activities of 8, 8, 7, and 8 U/mL/min, respectively. Similarly, Sarkar and Chourasia (2017) recorded the highest protinase activity of 16 U/mL/min on the 4th day of MSW composting which showed a bit higher enzymatic activity values than those recorded in the present study. This may be due to the lower protein content in the collected MSW. However, accelerated Proteinase activity was observed during the thermophilic phase of composting, which indicated that protease plays an important role in the thermophilic phase, similar to the findings of Awasthi et al. (2018).

Moreover, lipase, responsible for breaking down lipid substances in solid waste, exhibited relatively low activity compared to other enzymes. The highest lipase activities were recorded from consortia C5, C4, C3, C2, and C1, with values of 5, 4, 3, 3, and 4.2 U/mL/min, respectively. Lipids are more complex to degrade compared to other substrate types, and their abundance in the compost piles could impair oxygen transfer efficiency and microbial dynamics. The lower lipase activity suggests a relatively lower amount of lipid substrates in the MSW. Similarly, Ng et al. (2019) isolated the *Enterococcus* sp., *Staphylococcus* sp., *Bacillus* sp., *Providencia* sp., and *Morganella* sp. activity up to 15.40 U/mL/min and 15.62 U/mL/min. The majority of collected MSW contained the lignocellosis substrates. This may have led to the relatively low lipase production in the present study.

When considering the total hydrolytic enzyme activity of all consortia, consortium C5 exhibited slightly higher activity compared to the control and other treatments (C1, C2, C3, and C4). These findings provide insights into the enzymatic dynamics during composting, highlighting the influence of different consortia on the degradation of organic components in MSW.

3.7 Changes in mesophilic and thermophilic bacterial population

Figure 8(a) and (b) provides insights into the dynamics of viable cell counts during 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. As the composting process progressed, the mesophilic bacterial count gradually decreased due to the rise in temperature during the thermophilic phase. Similar to the observation of Rich et al. (2018) the control sample exhibited a slower reduction rate in mesophilic viable cell count, which can be attributed to its slower composting rate and a more prolonged mesophilic phase during the composting process. The mesophilic bacterial population was roughly steady during the thermophilic phase. This is in line with the findings of Ince et al. (2020) and Shah et al. (2022) who found a decrease in bacteria during the thermophilic phase, followed by an increase as temperatures dropped.

The thermophilic bacterial count of all the consortium-added samples demonstrated a significant increase ($p<0.05$) compared to the control sample. The thermophilic phase is characterized by elevated temperatures, which promote the degradation of complex waste substances into simpler monomers. Similar to the observations of Finore et al. (2023) the favourable environment in the consortium-added samples led to a higher population of thermophilic bacteria compared to the control, indicating more efficient decomposition and organic matter breakdown during composting.

Overall, the observations from Figure 8 highlight the effectiveness of the consortium-added samples in promoting bacterial activity and accelerating the composting process, particularly during the thermophilic phase. The higher thermophilic bacterial counts in the consortium-added samples suggest enhanced microbial activity and the potential for more efficient decomposition of organic waste materials (Finore et al., 2023).

3.8 Final compost characterization

Seed Germination Index (GI) (Table 5) is one of the important and sensitive indicators of compost quality that determine the phytotoxicity effect of the compost (Awasthi et al., 2018). The results indicate that the GI of the final compost ranged from 76±3 to 110±2. If the GI value is 26-65, the substrate is

classified as phytotoxic; if the GI value is 66-100, the product is classified as non-phytotoxic, stable, and can be used in agricultural production; and if the GI value is greater than 101, the product is classified as phytonutrient-phyto stimulant and can be used as fertilizer (Meena et al., 2021). According to the data, the GI value of C2-C5 compost samples was in the acceptable range. However, C5 consortia inoculated compost demonstrated an exceptionally favourable GI value (110.25±2.21) indicating the potential applicability of the C5 compost as a fertilizer (Meena et al., 2021; Awasthi et al., 2018). Further, Total Kjeldahl Nitrogen (TKN) and total organic carbon (TOC) and C:N values of all the compost samples ranged from 1.82±0.51 to 2.50±0.25, 14.40±0.16 to 26.66±0.50, respectively. Moreover, the C5 final compost sample demonstrated the maximum TKN of 2.50±0.25 indicating a good quality compost compared to the other samples (Awasthi et al., 2018).

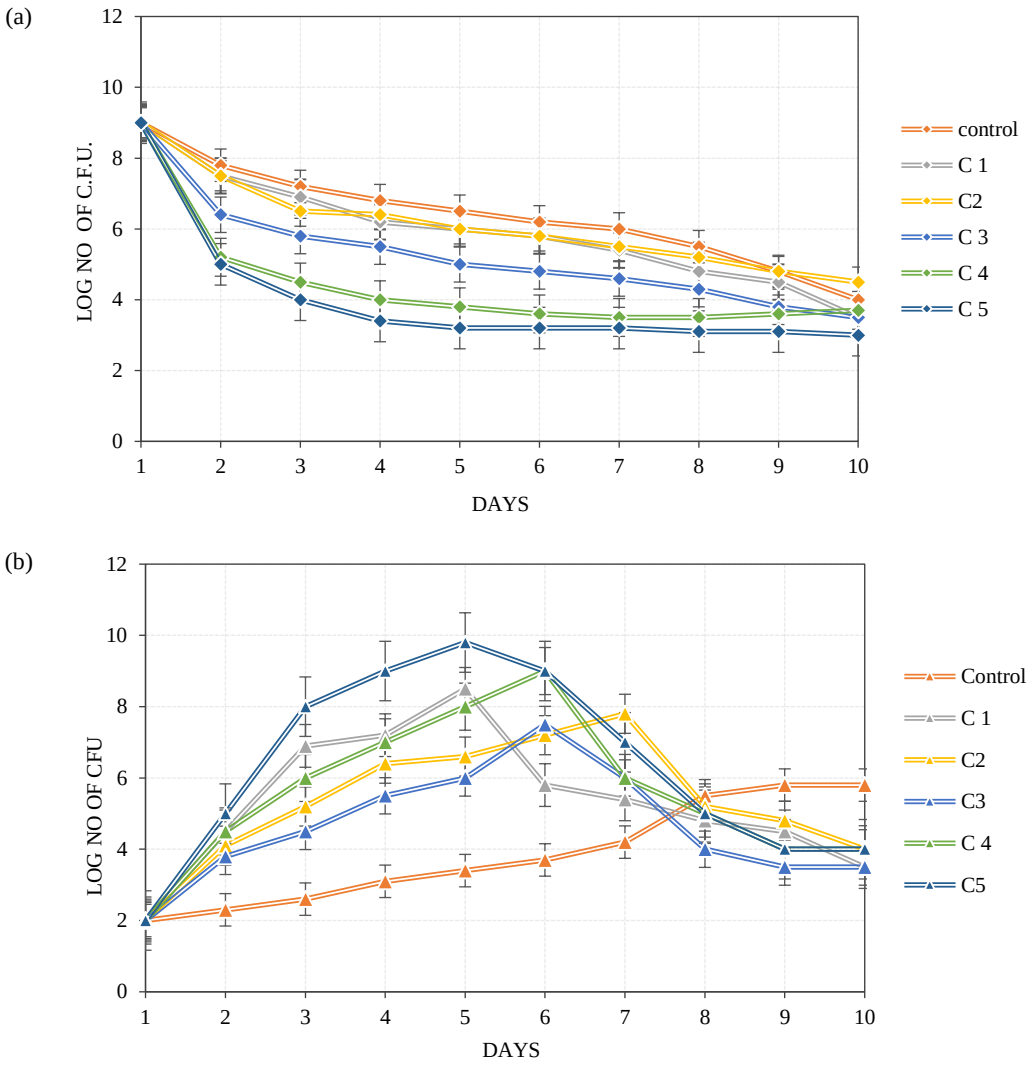

Figure 8. Mesophilic (a) and thermophilic (b) viable cell count during composting (Note - All the data were the average of the three replicates)

Table 5. Quality parameters of the final compost

Parameter	Compost sample					
	Control	C1	C2	C3	C4	C5
GI	76.53±3.21	72.43±4.25	80.15±3.51	84.51±2.22	84.50±2.31	110.25±2.21
TKN (g/kg)	1.82±0.51	2.22±0.42	2.10±0.25	2.20±0.52	2.00±0.25	2.50±0.25
TOC (g/kg)	48.25±0.52	45.23±0.45	44.15±0.52	46.12±0.22	40.25±0.50	36.34±0.50
C:N	26.66±0.50	20.42±0.52	20.90±0.34	20.90±0.32	20.00±0.15	14.40±0.16

3.8 Molecular identification

According to the 16S rRNA analysis results, the bacteria in the C 5 consortium belong to the *Bacillus haynesii* strain BHC1 (PP391133), *Bacillus amyloliquefaciens* strain BAC1 (PP391056), and *Bacillus safensis* (PP391033) and *Bacillus amyloliquefaciens* strain AMWC (PP391615). Recently, a few studies have been carried out for *Bacillus* inoculation for the lignocellocis biomass degradation (Wang et al., 2023; Zainudin et al., 2022). Mei et al. (2020) studied biomass lignin degradation using *B. amyloliquefaciens* and have achieved successful results. Further, Zaccardelli et al. (2020) have stated that *B. amyloliquefaciens* can be successfully applied to control plant soil-borne diseases. There are no records on the usage of *B. safensis* for lignocellocis waste degradation. Importantly, though the individual effect of *B. haynesii* and *B. amyloliquefaciens* on composting have been researched, the synergistic interaction of *B. haynesii* and *B. amyloliquefaciens* with *B. safensis* in the same consortia has not been studied. Additionally, Sahu et al. (2020) have recorded *B. haynesii* and *B. amyloliquefaciens* as plant growth-promoting bacterium while producing diverse growth-promoting substances. Figure 9 represents the phylogenic relationship of the bacterial strains in the prepared C5 consortium.

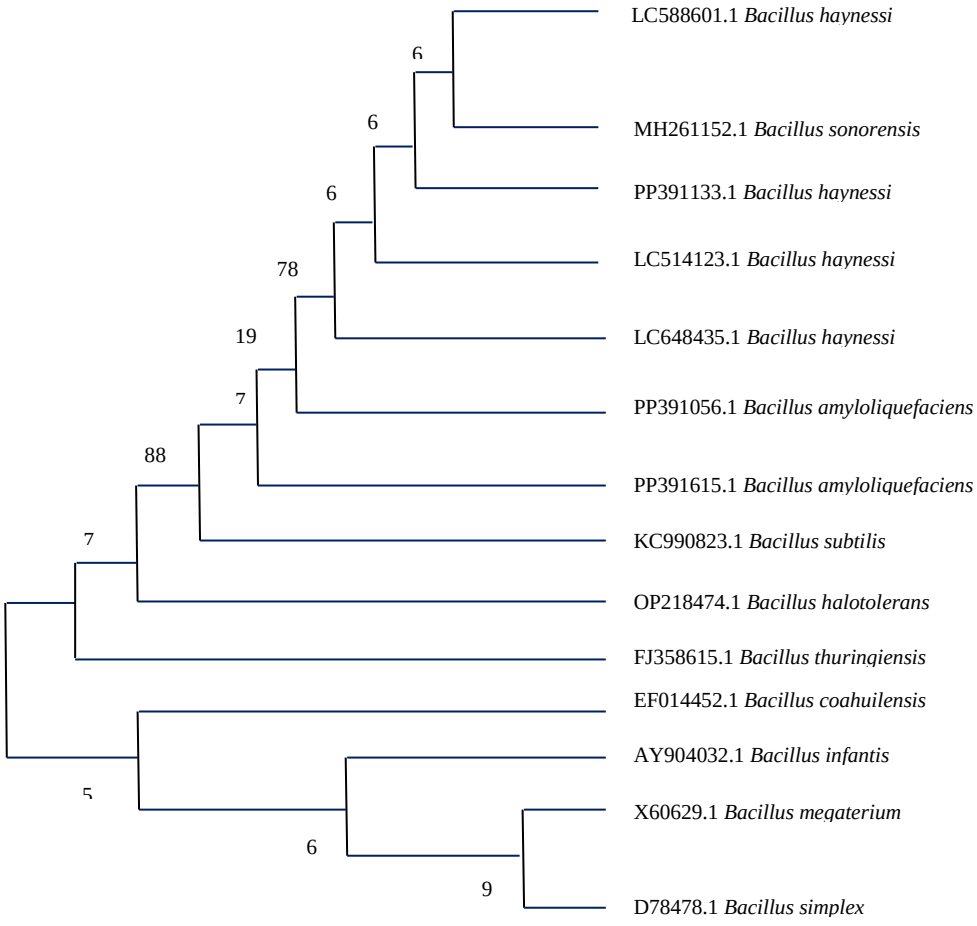

Figure 9. Phylogenic analysis of the bacterial strains in the C5 consortium

The evolutionary history was inferred by using the Maximum Likelihood method and General Time Reversible model using MEGA11.

4. CONCLUSION

Four *Bacillus* sp. were contained in the prepared novel bacterial consortium (C5) which includes *B. haynesii* strain BHC1 (PP391133), *B. amyloliquefaciens* strain BAC1 (PP391056), and *B. safensis* (PP391033), and *B. amyloliquefaciens* strain AMWC (PP391615). The consortium could significantly ($p<0.05$) alter the composting parameters which include pH, temperature, EC, and bulk density, enzyme dynamics by rapidly converting MSW into compost within 20±3 days. Further, the findings reveal that the prepared consortium has successfully reduced

the phyto toxicity of compost while enhancing the rate of compost maturity and stability. Therefore, the proposed novel consortium can be used as a potential green approach for sustainable MSW management.

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