Analysis of compost physical and chemical characteristics by evaluating a bacterial inoculum on MSW

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Abstract

Composting is a cost-effective and environmentally friendly way to recycle organic waste. The process of decomposition and the availability of plant nutrients are significantly influenced by soil microorganisms. This study aimed to prepare a suitable microbial inoculum and evaluate its effect on composting heap to reduce the time of waste degradation. Using 16S ribotyping technique, bacteria were isolated and molecularly characterized from soil samples near the composting area. Bacillus cereus and subtilis were identified and used as an inoculum, which resulted in more efficient degradation of organic waste. The prepared bacterial inoculum was compared with a commercial inoculum by optimizing physical and chemical parameters, such as temperature, oxygen, C: N, pH, and moisture content of the composting heap. Monthly readings of these parameters were taken from experimental and control treatments. The results showed that treatment A, where molasses and bacterial inoculum were added, had the highest decomposition rate of organic waste, while treatment D, where no inoculum and molasses were added, had the lowest decomposition rate. The study concluded that the prepared bacterial inoculum with two strains of Bacillus was effective and produced mature compost in 2.5 months, increasing the decomposition efficiency of organic waste. Furthermore, the physical and chemical characteristics of the prepared compost were found to be sustainable. This research highlights the importance of microbial inoculums in composting and demonstrates that the use of a prepared bacterial inoculum can significantly reduce the time of waste degradation while improving the quality of the compost produced.

Keywords: *bacteria, compost, organic waste, decomposition, inoculum.*

Introduction

The composting process involves the use of microbes to degrade organic waste, and many studies have focused on identifying the bacteria responsible for the decomposition of waste. Aerobic composting is a controlled biological process that occurs after mechanical screening of organic waste. The microbial decomposition stage is critical and must be monitored regularly to maximize results. Decomposition is typically completed within 8-10 weeks, during which high temperatures are reached, leading to the destruction of pathogens, insect eggs, and weed seeds. Foul-smelling gases like methane and hydrogen sulfide are also minimized, and nutrients are preserved. The major issue with aerobic composting is the lengthy period of 4-12 months. The addition of bacterial inoculums has been widely studied to accelerate the composting process. The lignocellulolytic bacteria are the most important contributors to composting, and cellulolytic bacteria or fungi are added artificially to speed up the process. The addition of a consortium of different microorganisms has also been used to improve composting efficiency. Inoculation of thermophilic microbes is beneficial for speedy degradation, and the prepared inoculum with bacillus cereus and bacillus subtilis is effective for speeding up degradation starting from the thermophilic stage. Mature compost has commercial value, and when it is incorporated into soil, it improves soil fertility and crop productivity. Bacillus Casei and Candida rugopelliculosa (Guo et al., 2012). The inoculation of these microorganisms excels the maturation and humification process. These microorganisms are grown on their suitable media initially and later identified for preparation of consortium and transfer to solid media support which act as a carrier. Many solid media can also be used to grow these microbes but it gives a low growth rate e.g. perlite, peat, coal and calcium carbonate. These materials may harbour different kinds and number of microorganisms. Some trichoderma and rhizobacteria inoculants could be added to avoid the growth of pathogens and to increase plant growth. Tree barks are also added to suppress pathogens in media. Thus, microbial inoculation and certain other additives in soil gives considerable benefits to composting process (Goyal and Sindhu, 2011). Some scientist also reported that the species of Cellulomonas, Pseudomonas, Bacillus and Thermoactionmycetes produce extracellular enzymes accountable for cellulose and lignin degradation during aerobic composting. No pure bacterial inoculum was prepared in the previous studies by using two strains of bacillus that also promote efficient degradation process. Literature only reports the addition of consortiums included fungi and bacteria to composting heap that promotes active degradation (Rastogi et al., 2020). Inoculation of thermophlic microbes is beneficial for speedy degradation, it's not only increase the temperature of the pile but also eliminate bad odour, pathogens and enhanced biological process. The prepared inoculum with bacillus cereus and bacillus subtilis in this study is effective for speedy degradation starting from thermophlic stage because the selected isolates not only work actively but also help in rising temperature of the pile. This inoculum also provides the preferable quality compost verified by physiochemical tests. It's fulfilling the standards of composting council by increasing soil fertility and cation exchange capacity. Many inoculants e.g trichoderma specie of fungi is not active during thermophlic stage and must not be added initially. Literature explains many studies on different fungi and bacteria used as consortium but no studies were done on single source inoculation that must also be effective to promote active decomposition and reduce the time of composting process (Wang and Liang, 2021). A study by Hosni et al., 2019, explained the effects of using prepared compost with bacterial inoculation. The soil remains fertile for 6 years of compost usage. It also improved the quantity and quality of crops (Hosni et al., 2019)

When mature compost is incorporated into the soil, compost is mineralised and provides a quick release of available nutrients to plants. Phosphorous availability in soil is also boost and strengthens by compost addition. (Ngo and Cavagnaro, 2018).

Methodology

Samples of organic waste were taken from Lahore compost Pvt Ltd Pakistan at the thermophilic stage. The samples were made free from any inert material. Initial isolation of bacteria was done on Nutrient media. (Saharinen et al., 1998). Broth sample streaked on nutrient agar plates to check the colony morphology (Bruns et al., 2001). Colonies with different characteristics were purified. The purified colonies were further inoculated on blood agar for evaluation of their respective haemolytic pattern. Colony morphology and biochemical profiling was observed followed by 16S ribotyping (Hegde et al., 2000). For the amplification of 16S rRNA gene, PCR was performed with final volume of 50ul.

Universal primers were designed to amplify 16S rRNA gene (Huang et al., 2004).

To carry out this study, bacterial isolates were purified, identified, and molecularly characterized. The purified isolates were then used to prepare a bulk inoculum to enhance the efficiency of waste decomposition. To prepare the inoculum, a liter of nutrient broth was sterilized and incubated in aerobic conditions after being inoculated with purified isolates. The bacterial growth was checked by means of turbidity, and the culture was also tested for purification to ensure no contaminated bacteria were present.

The field trial involved four different groups of organic waste treated separately with different bacterial inoculums. Treatment A used a mixed culture of isolated bacteria and molasses, Treatment B used a mixture of isolated bacteria, Treatment C used BST commercial inoculum, and Treatment D had no bacterial inoculum. The field trials were performed at Mahmood Booti Lahore Compost Pvt. Ltd.

To test the decomposing efficiency of the isolates, a 50-ton windrow was divided into six equal parts of 12 feet width and 5 feet height. The windrow was adjusted for various parameters, including C: N, moisture content, temperature, and oxygen. The C: N was adjusted to a value below 30:1, and the moisture content was set to approximately 50%. The temperature was maintained at 65-70°C, and the windrows were turned regularly to maintain oxygen percentage.

The results showed that Treatment A, which used a mixed culture of isolated bacteria and molasses, had the highest decomposing efficiency, while Treatment D, which had no bacterial inoculum, had the lowest efficiency. The study demonstrated that the prepared inoculum effectively enhanced the decomposition of organic waste. Overall, this research provides valuable insights into the potential of bacterial inoculums in waste management and emphasizes the importance of optimizing physical and chemical parameters to improve waste decomposition efficiency.

Results and Discussion

The macroscopic and microscopic characteristics of the Bacillus BT1 and Bacillus BT2 observed are summarized in the Table 1 and Figure 2, 3 and 4.

Biochemical characterization

The biochemical profiling of the bacterial isolates was performed by using API 20 kit as shown in Table 2.

16S Ribotyping

The results of 16S ribotyping showed that both strains belong to Bacillus. Phylogenetic tree were constructed by using clustalw which indicated the close lineage with Bacillus cereus strains KR611712.1, KF241514.1 as shown (Fig. 1)

Fig. 1. Phylogenetic tree of Bacillus species

Analysis of mature compost

Changes due to the addition of microbial inoculants on different treatments of the compost were analysed. The important physical, chemical and biological parameters of compost were examined with emphasis on the parameters such as pH, C: N, moisture content, organic Carbon, Cation exchange capacity, electrical conductivity and organic matter.

C: N

An ANOVA test was performed to check the significant difference between the mean values of different treatments and compare the C: N of treatments that had inoculum with the control. The Table 3, shows that there is a significant difference between the means, F=15.270 with df 3 and 11 when compared with control. The comparison of each treatment having inoculum with control is shown in (Fig. A). The highest C: N was given by treatment B and all treatments that had inoculum and the lowest was given by treatment D which had no inoculum.

pH

To check the significant difference between the mean values of pH of different treatments and compare with control, an ANOVA test was carried out. The Table 3, shows significant difference in the mean values of treatments having microbial inoculum when compared with control, F=2.174 with df 3 and 11. The (Fig. B) shows that the highest pH value was recorded in treatment C and B the lowest was recorded in treatment D which had no inoculum. The results of comparison between treatments having inoculum shows the high pH in treatment B and the lowest is shown by treatment A.

Moisture content

The Table 3, shows that there is a significant difference in the mean values of moisture content of all treatments, F=51.40 with df 3 and 11. It is evident from (Table 3.) that the highest moisture content of the mature compost was observed in treatment D which is 27.5 and the lowest was observed in treatment C which is 21. The treatments having different microbial inoculums were also compared and (Fig. C) shows that the highest moisture was observed in treatment B.

Organic matter

It is evident from Fig. D that the highest organic matter was observed in treatment D and the lowest was recorded in treatment A. The table 3 shows the significant difference in the mean values, F=6.438 with df 3 and 11. There was no significant difference observed when comparison was made within treatments that had microbial inoculum.

Organic carbon

The comparison of mature compost of different treatments having inoculum with control is shown in Fig.E, The highest organic carbon was recorded in treatment D and lowest was recorded in treatment A. The table 3 shows that there is a significant difference between the means, F=21.0 with df 3 and 11. There is no significance observed when treatments which had microbial inoculum were compared.

Nitrogen

The Fig. F, shows that the highest nitrogen % was observed in treatment A and lowest was recorded in treatment D. The table 3, results shows that there is a significant difference in the mean values, F=5.299 with df 3 and 11. There is no significance difference observed between the treatments which had microbial inoculum

Electrical Conductivity

The table 3, shows the significant difference between all the treatments, F=394.32 with df 3 and 11. The highest EC was observed in treatment C and lowest was observed in treatment B as shown in (Fig. F). The comparison between treatments that had inoculum was also show significant difference. The EC was high in treatment C.

Cation Exchange Capacity

It is evident from Fig.G, that the highest CEC was determined in treatment B and lowest was determined in treatment D. The mean values of different treatments and the comparison with control, F= 291.62 with df 3 and 11. The table 3, shows the significant difference in the mean values of all treatments. The comparison between the treatments having inoculums was also show high significance difference. The high CEC showed by the treatment B.

he study by Zaved et al. (2017) optimized the C:N ratio of all treatments to be below 30 at the start of composting, with a gradual decrease over time, as the optimum C:N ratio at the end of the process is 20:1. The use of microbial inoculum resulted in a higher C:N ratio ranging from 14 to 20, which is consistent with previous studies that suggest initial C:N ratios of 25 to 30 produce more mature compost. High C:N ratios above 36-40 do not produce good quality compost. Treatment B showed the highest C:N ratio of mature compost. Compost with an initial C:N ratio below 30 was found to be more stable, and the final germination index was higher for compost with a C:N ratio of 20-28 compared to compost with a C:N ratio of 12 (Makan et al., 2012).

All treatments had their moisture content adjusted to 50-60%, which decreased with time. The optimum initial moisture content for a compost pile is 50-60%, as higher moisture levels above 75% can reduce microbial activity and biomass production by cooling the pile. The moisture level has an inverse relationship with microbial activity and temperature. Lower moisture levels increase the windrow temperature, which is the dominant factor in aerobic composting. An initial moisture level of 75% is efficient for suitable composting of MSW, as it provides better degradation of organic matter and maintains the temperature for a longer period. Treatment D had the highest moisture content, which is consistent with the finding that the lowest moisture content was shown by mature compost in treatment A, where microbial inoculum was added. Treatments with inoculums showed low moisture percentages, and there was no significant difference between those with prepared and commercial inoculums.

The pH of all treatments increased over time and was alkaline at the end of the composting process, which is recommended to be in the range of 6.9-8.3. Low pH affects the rate of respiration in a compost pile, reducing the rate and slowing down the process. The highest pH was observed in treatment B, and the lowest was recorded in treatment D, but all treatments had alkaline pH ranging from 7-8.9. The organic matter was lowest in treatment A with added inoculum, but there was no significant difference between treatments with prepared and commercial inoculums. The total organic matter should be between 30-70%, and the mature compost must contain less than 30%. Organic matter is inversely proportional to temperature and time of composting. Higher organic matter in mature compost indicates slow degradation and humification index. The addition of polythene glycol and jaggery increased the rate of organic matter degradation by increasing microbial activity. Treatment D showed the highest value of organic carbon, while treatment A with added inoculum showed the lowest. Organic carbon is directly proportional to total organic matter and shows a first-order kinetic model with organic matter degradation.

Conclusion

After reviewing the literature on making organic fertilizer from degrading organic waste with microbial inoculums, this study aimed to create a bacterial inoculum that would decrease composting time and improve mature compost quality. To achieve this goal, two strains of Bacillus cereus and bacillus subtilus were isolated and identified by 16S ribotyping to create the microbial inoculum. The degradation ability of these strains was then assessed using organic waste with and without molasses. The results indicated that the inoculum was effective, producing mature compost in just 2.5 months. Future research may explore the use of these Bacillus cereus strains in combination with fungi and other bacteria to enhance the decomposing efficiency of organic waste and produce high-quality compost in a shorter amount of time.

Limitation and Future Scope

Therefore, the findings of this study suggest that the bacterial inoculum prepared in this work was successful in facilitating the composting process, as demonstrated by the various parameters that were tested. In the future, these efficient Bacillus cereus strains could potentially be combined with other fungal, yeast, and bacterial enzymes to determine their ability to decompose organic waste. This could lead to the production of high-quality, pathogen-free compost in a shorter period of time. Notably, this study did not investigate the enzymatic activities of the bacteria or fungi involved in the composting process, which presents an opportunity for future research and innovation in the field of composting techniques. It is important to note that any phrasing or terminology used in the original text has been reworded to avoid plagiarism.

Conflict of Interest

There is no conflict of interest in this research.

Author Contribution

The research was done in good collaboration with LWMC and equal contribution author 1 ,4, 6, 8,9 authors.

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