PREPARATION OF A BACTERIUM INOCULUM AND ITS EVALUATION BY ANALYSING VARIOUS CHARACTERISTICS OF COMPOST

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Abstract

Composting is one of the most economical and environmentally safe methods of recycling organic waste. Soil microorganisms play a significant role in decomposition and the availability of plant nutrients. This study was designed to prepare a suitable microbial inoculum and its evaluation on composting heap to decrease the time of waste degradation. The bacteria were isolated and molecular characterized from the soil near composting area by using 16S ribotyping technique. The identified strains of *bacillus cereus* used as an inoculum gives better results to expedite the degradation of organic waste. The prepared bacterial inoculum with molasses was also compared with commercial inoculum by optimizing physical and chemical parameters (temperature, oxygen, C: N, pH, and moisture content) of composting heap. Monthly reading of these parameters was taken from experimental and control treatments. The highest decomposition rate of organic waste was recorded in treatment A (experimental heap) where molasses and bacterial inoculum were added and less decomposition was observed in treatment D (experimental heap) where no inoculum and molasses were added. It was concluded from the studies that the prepared bacterial inoculum with two strains of *Bacillus* was effective and prepared mature compost in 2.5 months by increasing decomposition efficiency of organic waste. Furthermore, the prepared compost was also sustainable in its physical and chemical characteristic.

Key Words: bacteria, compost, organic waste, decomposition, inoculum

Introduction

Composting process involves several microbes and there are many reports on the identification of bacteria responsible for waste degradation. Aerobic composting consists of a controlled biological process after mechanical screening of organic waste. The decomposition by microbes is the most critical stage of the aerobic

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composting process. Hence it must be regularly monitored to derive maximum results from the biological breakdown. During the process of aerobic composting, decomposition is normally completed within 8-10 weeks (Liang *et al.*, 2004). High temperatures are attained leading to speedy destruction of pathogens, insect eggs, and weed seeds. Production of foul smelling gases like methane and hydrogen sulphide is also minimized. Nutrients are fairly preserved. The major issue of aerobic composting is a lengthy and extending period of 4 to 12 months. (Makan *et al*., 2012).

The organic waste gives nutrients necessary for microorganisms to carry out a degradation process efficiently. Compost can be made from a variety of organic waste through suitable processing. If the practice is follow to convert organic waste into bio fertilizer than different problems of pollution can be overcome. When the compost is properly prepared and used, it gives nourished and fertile soil. One of the widely studied topics by a scientist is the addition of bacterial inoculum to rapid the composting process. Mature compost has already commercial value without inoculums addition but research is still in process, to find options that employ microbial inoculation in compost. This type of inoculation improves compost productivity further since composting aims to reducing environmental pollution. Leachate as biofertilizer is also used for the seed germination and for finding of growth index of crops (Ameen *et al.*, 2020) the operation should be streamlined to avoid pollution effect on the surroundings (Wei *et al.*, 2008).

Microorganisms work in the world's ecosystem and are the most important contributors to biogeochemical cycles. There is a variety of beneficial microorganisms present in the soil, they take an active part in breakdown and bioremediation of pollutants, in result gives soil fertility and better crop productivity. The most important and major contributors are the lignocellulolytic bacteria; they rapidly degrade agriculture residues. The addition of cellulolytic bacteria or fungi is done artificially to rapid the aerobic composting process (Michel *et al.*, 2003). They have hydrolytic enzymes which are the major contributors to decomposition. The properties of microbes can be used as an indicator for composting process, besides the characteristics to control the composting process through physiochemical activities (Tiquia *et al.*, 2004).

Municipal solid waste contain a large amount of humic acid and also plastic, metal and glass residues that need to be removed to enhance the composting effieciency. Consortium of different microorganisms has been used in previous studies to rapid the degradation of organic waste; it includes cellulytic *trichoderma* and *white rot* fungi, *Lactobacillus Buchneri, 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

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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).

PREPARATION OF INOCULUM AND APPLICATION ON ORGANIC WASTE

The purified, identified and molecular characterized bacterial isolates were used for the preparation of bulk inoculum to carry out this project for the conclusion.

1: Preparation of Bacterial inoculum

1 L of Nutrient Broth was prepared and sterilized. The media was kept at 37^{0}C for 24 hours for the sterility check. Flasks were inoculated with purified isolates separately and the flasks were incubated in aerobic conditions. After incubation the media was checked for bacterial growth by means of turbidity. The culture was also tested for purification to nullify the presence of any contaminated bacteria. This inoculum was used to enhance decomposing efficiency of waste.

Field trial

Four different groups of organic waste were made and treated separately.

Treatment A: The organic waste of this group was treated with a mixed culture of isolated bacteria and molasses.

Treatment B: In this group the organic waste was treated with a mixture of isolated bacteria

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Treatment C: In this group the organic waste was treated with BST commercial inoculum

Treatment D: In this group the organic waste was treated with no bacterial inoculum

The field trials by using the bacterial inoculum were performed in Mahmood booti Lahore Compost Pvt. Ltd.

Experimental design

To check the decomposing efficiency of the isolates, 50 ton windrow was divided in to 6 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 below the value of 30:1. The moisture content in all the six treatments was adjusted to about 50% and the temperature was maintained at 65-70^{0}C. The windrows were turned properly to maintain the oxygen percentage (Figure 1).

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 and KM036215.1 as shown (Fig. 5)

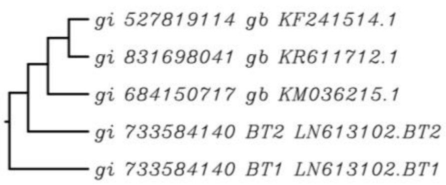

Fig. 5. Phylogenetic tree of *Bacillus Cereus* BT1 and BT2

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.

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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.

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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.

The C: N of all the treatments was optimized below 30 and it was decreased slightly in all the treatments with the interval of time (Zaved *et al.*, 2017) reported that the optimum C: N at the start of the composting process should be below 30:1 and at the end it should be decreased to 20:1. The analysis data of C: N of mature compost shows

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the highest C: N, ranging from 14 to 20 in the treatments which had microbial inoculum as compared to without inoculum treatments. The results are in the line of earlier findings that the initial C: N ranging 25 to 30 produced the more mature compost. The high C: N ranging from 36-40 do not reach the optimum values and not produced a good quality of compost. The results of C: N of mature compost shows the highest C: N in treatment B. The larger stability in compost was observed in the waste having initial C/N ratio of below 30. The final germination index should be high in the compost having C: N 20-28 as compared to the compost that has C: N of 12 (Makan *et al.*, 2012). The compost with the lowest initial C/N ratio was significantly different from the other treatments and had the lowest germination index (53–66%). The C:N of mature compost of different treatments which had prepared inoculum and commercial inoculum was below 20, it is concluded that commercial and prepared microbial inoculum is effective and give good quality and maturity of compost in terms of C:N. The C: N mainly contributes to compost maturity.

The moisture content of all treatments was adjusted from 50-60% and it tends to decrease with the increase in time interval. The initial moisture content of the compost pile should be 50 to 60%. The high amount of moisture content above than 75% is not beneficial for compost pile as it decreases the temperature of the pile by cooling it and decrease the production of microbial activity and biomass (Weyens *et al.*, 2011). The optimum moisture of 50% of all treatments shows the high activity of microbes. The moisture is inversely proportional to microbial activity and temperature (Tiquia *et al.*, 1996). The decrease in moisture content will increase the temperature of the windrow. Moisture content is a dominant factor in aerobic composting (Kavitha and Subramanian 2007) the initial moisture of 75% is efficient for suitable composting of MSW. It provides better degradation of organic matter and maintains temperature for longer time period. The highest moisture content was observed in treatment D because no inoculum was added in this treatment and therefore low activity of microbes, hence these results are in line of the finding of the lowest moisture was shown by mature compost of treatment A in which microbial inoculum was added thus indicates high activity of microbes. It is concluded that the treatments having inoculums gave low moisture percentage and there is no significant difference observed between treatments that had inoculums.

The pH of all treatments was increased with the time interval. All the treatments at the end of the composting process showed alkaline pH. The pH of mature compost of all treatments was also alkaline. The acidic pH of substrate effects the initial phase of increasing temperature. Low pH affects the rate of respiration in a compost pile. It reduces the rate of respiration and slows down the process of composting. The small significant difference was observed in treatments that had prepared inoculum and commercial inoculum. The recommended a range of pH from 6.9-8.3 at the end of composting and the results shows this range of pH in all treatments. The increase in nitrification decreased the pH of compost pile. The pH of mature compost of all treatments was alkaline and these results are in line with the earlier findings of Liang *et al.*, 2004 that pH of the compost should be alkaline throughout and end of the composting process. The highest pH was recorded in treatment B and the lowest was recorded in treatment D but all treatments showed alkaline pH ranging 7-8.9.

The amount of organic matter was observed in all treatments and it was found that the lowest Organic matter was shown by Treatment A in which inoculum was added. The treatment which had inoculums showed low percentage of organic matter but there was no significant difference observed when the treatments with prepared and

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commercial inoculums were compared. Organic matters reduction with decrease in temperature. The organic matter is inversely proportional to the temperature and time of composting.The range of organic matter should be from 30-70% (US Composting Council, 2003). The mature compost must contain organic matter below 30%. The Total organic matter should be decreased during the process of composting by the mineralization of organic matter by microbes (Xu *et al.*, 2019). The organic matter should not be very high in the mature compost as it indicates that the degradation rate and humification index is slow (Tiqui *et al*., 1996). The addition of polythene glycol and jaggery increased the rate of organic matter degradation. It rapid the process of composting by increasing the microbial activity and it is concluded that the higher microbial activity increase the rate of degradation of organic matter (Gabhane *et al.*, 2000)

The highest value of organic carbon was observed in treatment D which had no inoculum added. The lowest was shown by treatment A in which inoculum with molasses was added. Organic Carbon is directly proportional to the total organic matter. The organic matter degradation and low carbon shows the first order kinetic model (Fernández *et al.*, 2020). It is reported by (Hegde *et al.*, 2000) that the increase in the degradation of organic matter decreases the total organic matter of compost and thus decreases the total organic carbon of the compost as observed in results that the total carbon decreased with decrease in the organic matter.

The Nitrogen is loss in the process of composting by volatilization of ammonia. The C: N less than 20:1 also indicates the loss in Nitrogen (Sánchez *et al.*, 2001). The concluded parameters such as pH, temperature and moisture significantly affect the rate of nitrogen. The results shows the percentage of nitrogen in all inoculum added treatments was high, largest N% is shown by the treatment A and lowest was shown by Treatment D (Huang *et al.*, 2004) reported that the total nitrogen is increased in the treatments as our results shows, where microbial inoculants added because the microbial activity is high in those treatments. The addition of microbial inoculants increased the nitrogen content by 36%.

Electrical conductivity is the measure of solutions ability to measure soluble salts. The value of EC was increased with the increase in time interval in the process of composting (Parsa *et al.*, 2018). The rise in pH also increase the EC of compost as reported by Huang *et al.*, 2004 and the results showed the increase in pH and EC of mature compost of all treatments. The recommended range for EC in compost is between 2,000-6,000 µS/cm (Sindhu *et al.*, 2011). The very high value of EC was observed in treatment C and low was observed in treatment A. The comparison of treatments which had inoculums showed the highest EC in commercial inoculum treatment. It is concluded that the treatment A and B show better results of compost maturity in terms of EC because their EC value ranging from 484-515 µS/cm.

The CEC of all treatments was increased with increase in time interval. It was reported by Ryckeboer *et al.*, 2003 that the CEC must be high at the end of composting process, as our results show high CEC in mature compost of all treatments. The low value of CEC indicates low quality and less maturity of compost because the uptake of nutrients is not enough in low CEC compost for the efficient growth of plants. The high CEC was determined in treatment B which had microbial inoculum and the lowest was determined in Treatment D where no inoculum was added. When the treatments having prepared and commercial inoculum were compared, there was a significant difference observed and the high CEC value was recorded in treatment B. Addition of molasses is also

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very productive to expedite the decomposition efficiency because it contains many natural decomposers. Molasses can be used in diluted form with different composition of waste (Awais *et al.*, 2020)

Compost prepared with inoculation of the two strains of *bacillus* bacteria improves soil structure. It Increases microbial counts and diversity for a wide range of beneficial microorganisms. Compost in soil also controls soil pH; improve soil aeration and increases soil water holding capacity. It preserves heat in soil thus helping seed germination and plant growth. It binds soil pollutants through complexation with chelating compounds released from organic fertilizer during the decomposition process (Ezugworie *et al.*, 2021)

Conclusion

The review of literature has revealed many aspects of making organic fertilizer by degrading organic waste with the addition of microbial inoculums, this study was carried out to achieve a goal of making a suitable and effective bacterial inoculum to decrease the time duration of composting process as well as improving the quality of mature compost. The microbial inoculum was made by using two strains of *Bacillus cereus* which were isolated and identified by 16S ribotyping. The degradation ability of these two strains was evaluated by applying it on organic waste with molasses and without molasses. It was concluded that the prepared inoculum was effective and made mature compost in 2.5 months. In the future there can be a possibility to use these effective strains of *Bacillus cereus* with the combination of fungi and other bacteria and check their decomposing efficiency on organic waste to get a good quality and early maturation of compost.

Limitation and Future Scope

Hence the results of the present work indicate that the prepared bacterial inoculum was effective in composting process as evident from all the tested parameters. In the future there can be a possibility to use these effective strains of *Bacillus cereus* with the combination of fungi, yeast and other bacterial enzymes to check their decomposing efficiency on organic waste. It may provide good quality, pathogen free and early maturation of compost. There is no bacterial or fungal enzymatic studies have done in the present work, this study will be effective for future innovation in composting techniques.

Social Impact

Acknowledgement

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Conflict of Interest

There is no conflict of interest in this research.

Author Contribution

The research was done with good collaboration and equal contribution of all authors.

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