

Role of degradative enzyme-producing microbial consortia in agricultural waste composting

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India is an agriculture-based country and everyday huge amount of agro-wastes are generated. Almost half of the wastes find their fate in burning which creates considerable air pollution and soil degradation. Decomposition of these heterogeneous organic matters into compost will help in a great way to solve the problems. Several fungi and bacteria like Cellulomonas sp., Trichoderma viride, Aspergillus niger, Pleurotus, Trichoderma, Talaromyces, Bacillus, and Chryseobacterium spp. can produce different types and amounts of degradative enzymes which enhances the process of composting. They can be utilized as microbial consortia after isolation from the environment and passing through the proper screening process. The isolated microbes are screened for degradative enzyme production, and then enzyme profile needs to be prepared. The potential microbes should be further characterized and ideal growth parameters should be defined. The microbes then have to be checked for compatibility with each other for making consortia. Composts should be prepared in pits along with microbial consortia and nitrogen-rich organic sources to improve the process. Proper moisture and aeration should be maintained for good quality compost, which is of great importance in organic farming. Composting gives an alternative to environment-friendly waste treatment and thus gives us an opportunity to convert waste to wealth..

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Sustainable agriculture involves producing food and raising livestock over an extended period while minimizing adverse impacts on the environment. One key focus of agricultural sustainability is the preservation and restoration of soil fertility. The diverse range of agricultural activities contributes to an increased generation of agro-wastes, resulting in elevated levels of environmental pollution and waste production. Among the total crop residue burned, 60% are paddy straw, while wheat and sugarcane accounted for 22% and 2% respectively. In India, massive amounts of post-harvest rice, wheat, and sugarcane residues are eliminated through open-air field burning, which is considered as a cheap and easy means of disposal of excess residues. This practice

appends to air pollution, increases soil erosion, affects soil fertility, and decreases the efficacy of soil-applied herbicides like isoproturon. Moreover, it also causes respiratory problems and increases fog incidences even in distant cities. Direct incorporation of agro-residues like rice straw in the field solves the problem of air pollution but it is not feasible due to the short time gap between harvesting and sowing for next season. The rate and extent of residue transformation in the soil depends on the type, quality, and quantity of residues produced and how and when residues are treated. Besides, it involves an additional cost of labour, irrigation, and extra tillage.

Most of the agricultural wastes are lignocellulosic materials which majorly consist of cellulose,

hemicellulose, and lignin. This can be converted to rich organic matter by utilizing specific microorganisms for enhancement of the natural biodegradation process. The use of crop residues (paddy, wheat, and sugarcane) for composting is the best alternative to manage this resource, along with its use for the restoration of soil health. Composting, a managed process that utilizes microorganisms to transform degradable organic materials into stable products, emerges as a solution for agro-waste management. Compost, rich in organic matter, possesses a distinctive capacity to enhance the chemical, physical, and biological attributes of soils. It addresses issues such as water retention in sandy soils and enhances soil structure in clayey soils by reinforcing the stability of

soil aggregates. The incorporation of compost into soil yields benefits such as improved soil fertility, enhanced cation exchange capacity, and a potential reduction in fertilizer needs by as much as 50%. This addition activates microbial life in the soil, rendering it more resistant to soil-borne and foliar pathogens. Various microorganisms, including fungi and bacteria, contribute to the composting process by producing degradative enzymes like cellulase and hemicellulose. Efficient microbial consortia play a crucial role in hastening the decomposition of agricultural waste during composting. This article aims to present a comprehensive overview of the necessity, preparation strategies, and application methods associated with microbial consortia for agricultural waste composting.

Current scenario

According to data from the Directorate of Economics and Statistics for the year 2020-21, India's production includes 122.27 million tonnes of rice, 109.52 million tonnes of wheat, and 399.25 million tonnes of sugarcane. Among various crops, the primary sources of crop residue are rice, wheat, and sugarcane. The Ministry of New and Renewable Energy (MNRE) reports an annual generation of approximately 500 million tonnes (MT) of crop residue in India. Out of this annual crop residue, 50% is utilized for purposes like roofing material, animal feed, fuel, and packing material, while the remaining 50% exists a surplus of 140 MT, with 116 MT being burned each year. The overall crop residue (dry matter) generated has shown a notable increase from 80 MT in 1950-51 to 517 MT in 2017-18. Among the states, three states viz. Uttar Pradesh, Punjab, and Maharashtra, along with Gujarat - collectively contribute around 29% of the total crop residue. Uttar Pradesh leads with 60 MT (11%), followed by Punjab with 51 MT and Maharashtra with 46 MT, while Gujarat accounts for 29.32 MT. The data reveals a significant increase in crop residue burning, with the total biomass burned escalating from

18 MT in 1950-51 to 116 MT in 2017-18, representing a sixfold increase over the years.

Composting

Composting is a transformative process that involves the decomposition and humification of diverse organic matter. Serving as a robust source of organic material, compost plays a crucial role in sustaining soil fertility, contributing to the promotion of sustainable agricultural productivity. The incorporation of compost into the soil not only enhances its physiochemical and biological properties but also complements the use of agricultural inputs such as fertilizers and pesticides. The positive impacts of compost-amended soil include heightened potential for increased yields and resistance to external stressors like drought, diseases, and toxicity. This technique fosters higher nutrient uptake and facilitates active nutrient cycling through an augmentation of microbial activity in the soil. In recent times, there has been a growing interest in composting driven by societal demands for environment friendly waste treatment practices and the rise of organic farming. Composting stands out as a widely accepted method for recycling organic matter and agricultural residues.

Agricultural residue composting, particularly managing lignocellulosic waste, proves to be an easily controllable process with notable economic efficiency attributed to the actions of lignocellulolytic bacteria. Various organic wastes, including paddy straw, sugarcane waste, and other agricultural residues, find a sustainable disposal solution through composting. The mediation

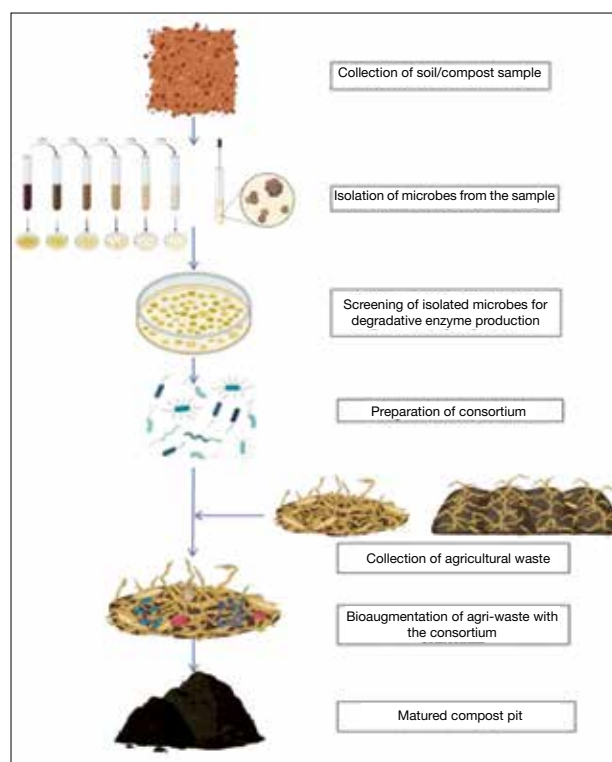


Fig. 1. Steps involved in conversion of agricultural waste to compost

of composting is facilitated by bacteria, fungi, and actinomycetes naturally present in organic materials or deliberately introduced to expedite the breakdown process. Biological maturity occurs when organic matter from plant or animal sources breaks down into substances with shorter molecular chains under aerobic conditions. The result of this process is a reliable, sanitary, and humus-rich compost that brings advantages to crops and facilitates the recycling of soil organic matter. While cellulose-degrading microorganisms have traditionally played a role in breaking down crop residues, such as straw and leaves, the time taken for composting has often been a limiting factor. To overcome this constraint, incorporating lignin-degrading microorganisms in conjunction with cellulose-degrading counterparts can expedite the composting process. The steps of preparation of compost is given in Fig. 1 which represents the whole process as a flow-chart.

Isolation and screening of microorganisms for composting

We can isolate microbes of desired characteristics from soil

or already degraded organic material. *Cellulomonas* sp. strain ASN2 isolated from soil samples is confirmed to have high cellulase activity. Supplementation of glucose, peptone, and cysteine to the fermentation medium favour enzyme secretion. The optimum pH and temperature for the activity of crude enzyme are 7.5 and 60°C, respectively. Among the cellulose-degrading fungus, *Trichoderma viride* and *Aspergillus niger* are significant. *T. viride* exhibits a maximum zone of hydrolysis for carboxymethylcellulose and produces higher titers of cellulases. Maximum enzyme production (1.76 U/ml) can be achieved at 30°C, pH 5.0 by *T. viride*, and on the 5th day of incubation.

Regarding selective delignification ability, microbes like *Pleurotus*, *Trichoderma*, *Talaromyces*, *Bacillus*, and *Chryseobacterium* spp. are found effective. Different phases of screening are discussed below.

Degradative enzymatic profiling of isolated microorganisms

The activity of microbial cellulase, hemicellulase, and lignolytic enzymes can be tested based on hollow zones around colonies. The appearance of the black colour zone of diffusion in esculin agar is a positive indication for the β -glucosidases activity test. *Aspergillus fumigatus* JCM 10253 is a potential producer of extracellular enzymes for lignocellulose degradation showing higher activity for cellulase as well as β -glucosidase. Simultaneously, their activity for xylanase can be assessed by plate assay methods. Maximum CMCase, FPase, β -glucosidase, and xylanase activities can be obtained after an incubation time of 144 h at 50°C under solid-state fermentation.

The ability to produce degradative enzymes can also be characterized by using different assays like starch agar for amylolytic activity, Winogradsky medium with asparagine for ammonification activity, Janshekar basal medium added to microcrystalline cellulose 0.5%, and aniline blue-black 0.005% for cellulolytic activity, Janshekar

basal medium added to xylan 0.5% for hemicellulolytic activity, Janshekar basal medium added to sodium caseinate 1.0% for proteolytic activity and NBRIP medium for phosphate-solubilizing activity. Ammonification, amylolysis, hemicellulolysis, and proteolysis are the more frequent activities among degradative enzyme-producing microbes, among which mesophilic bacteria and fungi are prevalent. Bacteria are mainly responsible for starch hydrolysis, while a higher percentage of hemicellulolytic and proteolytic isolates are ascribable to fungi.

Fungi and bacteria important for plant litter decomposition can be analysed for the enzymes β -glucosidase, β -xylosidase, cellobiohydrolase, β -N-acetylglucosaminidase, and phosphatase using fluorescent-linked artificial substrates, MUF- β -D-xylopyranoside, MUF-cellobioside, and MUF-phosphate. Leucine-aminopeptidase can be analysed by the fluorescent-linked artificial substrate L-leucine-4-methyl-7-coumarinylamide hydrochloride. Phenol oxidase activity can be measured by using L-3,4-dihydroxyphenylalanine.

Enzyme purification can be performed by ammonium sulfate precipitation, DEAE-cellulose, and CM-cellulose column chromatography, respectively.

Effect of physico-chemical parameters on growth and enzyme production

Cellulose-producing fungi typically exhibit optimal cellulase yields at 37°C within a pH range of 4 to 4.8. Notably, in the case of *Aspergillus*, significant enzyme production is often observed after a 7-day incubation period. The presence of cellulose boosts cellulase synthesis by approximately 10-fold, while the existence of glucose tends to repress it. The highest cellulase production is typically achieved after a fermentation period of 72 hours at 40°C and pH 5. For nitrogen sources, ammonium sulfate is found to be the most effective, and glucose stands out among carbon sources for

optimal cellulase production. When it comes to *A. niger* and *Penicillium* sp., these fungi can be advantageous for cellulase production due to their generally higher enzyme production rates compared to other fungi.

In terms of ligninolytic enzyme production, *Trametes trogii* (MYA 28-11) demonstrates maximum efficiency at pH 4.5, with a peptone concentration of 12.5 g/L and the presence of CuSO₄ at 11 mM. This specific combination of conditions promotes the optimal production of ligninolytic enzymes from *Trametes trogii*.

Cultural, morphological, biochemical, and molecular characterization of microbes

Every microbe has a peculiar growth pattern which leads to its unique identity. There are several aspects for judging the characteristics of microbes under consideration. Firstly, we have to analyse the colony morphology as cultural characterization. One should look for typical characters like colony shape, colony colour, colony elevation, colony margin, colony surface, etc. For morphological studies of microbes, several characters are to be studied under a microscope, viz. shape and size of microbe, spore formation, spore shape and size, presence or absence of flagella, etc. Several biochemical tests like Gram staining, oxidase test, catalase test, protease test, amylase test, gelatinase test, etc. can be performed for characterization. Molecular characterization can be done by sequencing 16S rRNA gene for bacteria and 18S rRNA gene or ITS (Internal Transcribed Spacer) region for fungi. The Genetic diversity among the isolates can be assessed by RAPD (Random Amplified Polymorphic DNA), PCR (Polymerase Chain Reaction) fingerprinting, etc.

Consortia formulation and its application for composting of agricultural waste under controlled conditions

A microbial consortium is a combination of two or more interacting microbial populations performing certain activities

without interfering with each other. Potential microbes are tested for their compatibility with each other for consortia formulation. Consortia can be of two types, i.e., liquid consortia and solid consortia. Solid consortiums of fungi are generally viable for up to 90 days. Liquid consortium of bacteria and actinomycetes can be viable for up to 180 days based on colony count.

Composting, being a microbial degradation process, emphasizes the importance of the carbon-to-nitrogen (C:N) ratio in initiating the composting process. To accelerate composting, the C:N ratio should be approximately 50:1 during the initiation stage. Since major agricultural wastes, like paddy straw, have a high C:N ratio (e.g., 90:1), it becomes essential to lower down this ratio by introducing an exogenous nitrogen source like poultry droppings or fish meal.

The composting process involves collection of agricultural wastes and preparation of pits of 1 m³ size (Fig. 2). Agro-waste is then amended with poultry droppings (5 kg/pit) in an 8:1 ratio to achieve the desired C:N ratio for composting. Additionally, bioaugmentation with an efficient microbial consortium capable of degrading agro-waste is recommended. Regular mixing of the composting mixture and periodic sprinkling of water to maintain a 60% moisture level throughout composting are essential. Turning



Fig. 2. Compost pits

the composting piles biweekly is necessary to ensure proper aeration.

Inoculation with a microbial consortium results in faster carbohydrate metabolism, elevated temperature (73.2 vs. 71.2°C), an extended thermophilic phase (5 vs. 3 days), and significant shifts in microbial community composition and diversity. The use of the consortium in agro-waste composting enhances nutrient release and lowers the C:N ratio. The mature compost (Fig. 3) can then undergo assessments of physical and chemical properties, antifungal activities, and plant growth-promoting activities.

CONCLUSION

Keeping the burgeoning figure of agricultural waste on one side and the scenario of degraded land or poor quality of nutrition in soil on the other, we can realize very well how fast we need solutions to these problems to save humanity from upcoming miseries. The utilization of microbial consortia to convert agro-waste into composts will nourish the soil and will solve both problems in one go. Degradative enzyme-producing microbes are already there in the environment performing their job but getting suppressed via several chemicals applied in the agricultural fields. The isolation of such microbes and their proper utilization to prepare microbial consortia is highly required. These consortia are environmentally safe and can be used to improve organic waste management at both the community and industrial levels.



Fig. 3. Mature compost

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**Plant trees, Conserve water,
Protect environment.**

