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## RESEARCH ARTICLE

### ROLE OF BIOINOCULANTS IN THE BIODEGRADATION OF LINGO-CELLULOSIC WASTE (BAGASSE)

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#### ABSTRACT

Lignocellulose, the major component of biomass, makes up about half of the matter produced by photosynthesis. It consists of three types of polymers- cellulose, hemicellulose and lignin that are strongly intermeshed; therefore their recycling is indispensable for the carbon cycle. Each polymer degraded by a variety of microorganisms which produce a number of enzymes that work on the waste material. For the present research work agro-industrial waste such as bagasse were selected this was good source of lingo-cellulose material. Bagasse were inoculated with fungal strains such as *Pleurotus sajor-caju*, *Trichoderma harzianum* and *Aspergillus niger* and *Chaetomium globosum* and allowed composting process for 40 days with above different combinations of fungal strains. Temperature and moisture content 60-80% maintained throughout the experimental work. Analysis of initial, control and compost samples showed degradation of cellulose, hemicellulose and lignin content. Cellulose decreased from 27.10% to 11.6%, hemicellulose 18.10% to 5.71% and lignin 9.70% to 3.46%. In the future, processes that use lingo-cellulolytic enzymes or are based on microorganisms could lead to new, environment friendly technologies. This study suggests that agro-industrial wastes could be converted into some value added products such as compost.

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#### INTRODUCTION

Waste is an unavoidable by-product of most human activity. Currently, the management and disposal of agro-industrial waste production is one of the most critical environmental issues. Therefore, the study related on safe reuse and management of agro-industrial waste is important. According to Appelhof (1981), the appropriate disposal of waste should involve both maximum cost effective recovery of recyclable constituents and transformation of non-recoverable material into forms, which do not present environmental hazards. According to the Collivignarelli *et al.* (2004), in the developing countries solid waste management such as agricultural and agro-industrial wastes deals with various complex resources covering collection and transfer costs only, leaving no resources for safe final disposal. These situations pose a serious public health risks and have also resulting in environmental degradation in a number of cities of the developing world (Diaz *et al.*; 1999). Agro-industries account for production of large quantity of wastes such as coir industry waste, paper and dairy industry waste, biscuit industry waste, fruit pulp industry waste, oil refineries and breweries wastes, stems, leaves, flowers from aromatic oil extraction units etc.

Apart from sugar and alcohol as primary products, sugar industries and fermentation units also produce many by-products such as press mud, bagasse, distillery waste, and boiler ash and fermentation yeast sludge. All these wastes serve as an excellent source of nutrients. Bioinoculants defined as preparations containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilising or cellulolytic micro-organisms used for application to seed, soil or composting areas with the objective of increasing the extent of the availability of nutrients in a form which can be easily assimilated by plants. Bioinoculants are living organisms containing strains of specific bacteria, fungi or algae. Lignocelluloses, the composite of the predominant polymers of vascular plant biomass, are composed of polysaccharides like cellulose and hemicelluloses and the phenolic polymer lignin. Hence, the capacity of microorganisms to assimilate organic matter depends on their ability to produce the enzymes needed for degradation of the substrate components i.e., cellulose, hemicellulose and lignin. The percentage of cellulose, hemicellulose and lignin is different to each waste since it varies from one plant species to another. Usually, cellulose is the dominant fraction in the plant cell wall (35-50%), followed by hemicellulose (20-35%) and lignin (10-25%). The use of inoculants in composting processes has been clear from some of the research conducted which shows that inoculation in composting processes can be a useful tool to increase the humification degree in the final product and therefore, to improve the agricultural quality of compost by

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achieving a higher stabilization and maturity levels. The process by which the fungi are capable of bringing about the decomposition of organic matter is by the secretion of various types of enzymes, such as cellulases, hemicellulases, proteases, pectinases and lignolytic enzymes (phenoloxidases, laccases, peroxidases) into the environment i.e. extracellular secretion which degrade a specific molecule into its simpler components, making it to go into solution.

## MATERIALS AND METHODS

**A. Source of agro-industrial waste (Bagasse):** After utilizing sugarcane in the sugar factory the waste material remains behind that is good source of substrates used for experimental purpose. Bagasse was collected from Datta sugar factory, Kolhapur (Maharashtra).

**B. Source of fungal bioinoculants:** The 4 different fungal strains were used for the composting process. The fungal strain such as *Aspergillus niger*, *Trichoderma harzianum* and *Chaetomium globosum* were procured from Agharkar Research Institute (NFCCI), Pune and *Pleurotus sajor-caju* was obtained from Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli.

**C. Culturing of fungal strains:** All the four fungal strains were inoculated on PDA (Potato Dextrose Agar) and PDB (Potato Dextrose Broth) plates and slants, kept in incubator at 26°C after inoculation. After sufficient growth, all cultures were used for further composting of substrates (Bagasse).

**D. Experimental set up:** 15 plastic drums were prepared for experiment. 15 kg bagasse was collected; sun dried and was pasteurized by dipping it overnight in 0.1% formalin. (Each drum contains 1 kg substrates).

**E. Composting of paddy straw with fungal inoculants:** Bagasse substrate was inoculated with fungal strains in various combinations for composting process. Pure cultures of *Pleurotus sajor-caju*, *Trichoderma harzianum*, *Aspergillus niger*, *Chaetomium globosum* (1gm of mycelium of each fungal strain per 1 kg substrate) were inoculated in different combinations as given experimental set up. All 15 experimental drums were divided into 5 categories such as

**BAGASSE:** (Each category done in triplicates)

**Category 1:** Control (Only Bagasse) (B)

**Category 2:** Bagasse+ *Pleurotus sajor-caju* (B+P.s)

**Category 3:** Bagasse + *Pleurotus sajor-caju* + *Trichoderma harzianum* (B+P.s+T.h)

**Category 4:** Bagasse+ *Pleurotus sajor-caju* + *Trichoderma harzianum*+*Aspergillus niger* (B+P.s+T.h+A.n)

**Category 5:** Paddy straw + *Pleurotus sajor-caju* + *Trichoderma harzianum* + *Aspergillus niger*+ *Chaetomium globosum* (B+P.s+T.h+A.n+C.g)

**F. Factors controlling composting process:** The moisture was maintained around 60-80% throughout the experiment.

Temperature also maintained. Turning/Aeration was done manually after every 4 days.

**G. Chemical analysis:** Analysis of Cellulose done by Anthrone method, Hemicellulose and Lignin by Reflux method (Sadasivam, S. and Manickam A. 2005).



Figure 1. Fungal bioinoculants pure cultures from authentic Research Institutes



Figure 2. Temperature maintaining in control (Bagasse) experimental set up



(From left to right Control B+P.s, B+P.s+T.h, B+P.s+T.h+A.n, B+P.s+T.h+A.n+C.g)

Figure 3. Composted sample of treated Bagasse after 40 days period

## RESULTS AND DISCUSSION

The results of the experiments are summarized in Table 1 and 2, which showed changes in the cellulose, hemicelluloses and lignin content after composting process.

**Table 1. Composition of initial Bagasse**

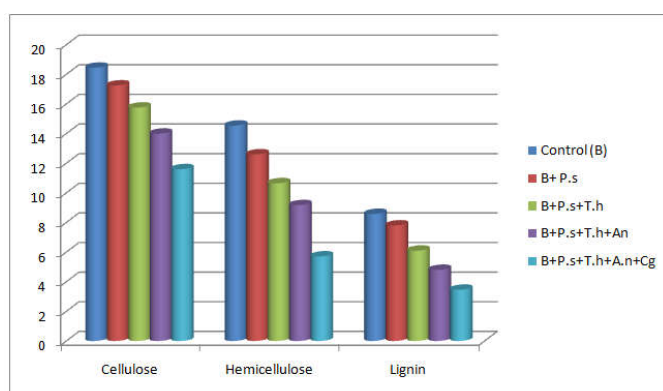
NO.	PARAMETERS	VALUES IN PERCENTAGE (%)
1	Cellulose	27.70
2	Hemicellulose	18.10
3	Lignin	9.70

**Table 3. Composition of Bagasse after composting with fungal bioinoculants for 40 days**

Parameters	Control (B)	B+P.s	B+P.s+T.h	B+P.s+T.h+An	B+P.s+T.h+A.n+Cg
Cellulose	18.45±0.75	17.25±0.15	15.76±0.36	14±1.1	11.6±0.8
Hemicellulose	14.54±0.51	12.60±0.40	10.65±0.35	9.17±0.87	5.71±0.67
Lignin	8.58±0.32	7.8±0.1	6.1±0.2	4.8±0.3	3.46±0.27

Note: All values are mean and standard deviation of three replicates (M± SD).

Initial bagasse contains (Table 1) cellulose 27.70%, hemicellulose 18.10% and lignin 9.70%. Bagasse was rich source of lingo-cellulosic content which was beneficial for the fungal growth.

**Figure 4. Comparison of cellulose, hemicellulose and lignin in different treated set up after 40 days of composting**

The result clearly showed that (Figure 4) there was significant difference in cellulose, hemicellulose and lignin percentage after composting with fungal bioinoculants for 40 days in different combinations. Cellulose percentage in control (Bagasse) was 18.45%, and it was further decreased like 17.25% (B+P.s), 15.76% (B+P.s+T.h), 14% (B+P.s+T.h+A.n), 11.6% (B+P.s+T.h+A.n+C.g). Hemicellulose percentage in control was 14.54%, and it was further decreased like 12.60% (B+P.s), 10.65% (B+P.s+T.h), 9.17% (B+P.s+T.h+A.n) and 5.71% (B+P.s+T.h+A.n+C.g). Lignin showed 8.58% in control followed by 7.8%, 6.1%, 4.8% and 3.46% respectively as above. The result clearly indicates degradation of cellulose, hemicellulose and lignin from the agro-industrial waste. Dubey R.C. and Maheshwan D.K. (2005) have stated that the cellulolytic fungi, such as *Aspergillus*, *Trichoderma*, *penicillium* and *Trichurus* accelerate composting for efficient recycling of dry crop wastes with high C:N ratio and reduce the composting period for about one month. *P. sajor-caju* has varying enzyme activities (Buswell J. A and chang S. T, 1994). Capability of *Trichoderma* fungi in digesting cellulytic materials have been proved by researchers (Hataka, 1994, Meunchang *et al.*2005; A. Singh and S.Sharma, 2002). *Trichoderma* and *Aspergillus* degrade hemicelluloses and cellulose respectively, if these microfloras were added during pre-decomposition of the waste, the time of composting might be reduced (A. Singh and S. Sharma, 2002). Several fungi like *Trichoderma harzianum*, *Pleurotus ostreatus*,

*polyporus ostriformis* and *Phanerochaete chrysosporium* are known to play important role in composting of lignocellulosic materials (Singh S. et al, 2012). *Chaetomium globosum*, which have ability to produce both ligninolytic and cellulolytic activity (P. K. Nagdesi and Arun Arya, 2013). Pressmud showed chemical composition and high content of organic carbon, the usefulness of it as a valuable organic manure has been reported by several workers (Ramaswamy, P.P., 1999). Zeng et al (2010) showed the above higher activities of enzymes with *Phanerochaete chrysosporium* inoculation during agricultural waste composting.

The microbial community already existed in the waste helping the composting process effectively, the inoculation of residues with lingo-cellulytic microorganisms is a another strategy that could potentially enhance the way the process takes place or the properties of the final product. Inoculation with bacteria and fungi which can breakdown lingo-cellulytic material has been reported to be effective in composting (Nair J, Okamitsu K, 2010). Bagasse has been found to be the best substrate for the growth of cellulolytic and ligninolytic microorganisms (Poonam, N. and K.A. Prabhu.1986). Dan Lian Huang et al (2010) tested the microbial populations and their relationship to bioconversion of lingo-cellulosic wastes during composting.

## Conclusion

From the lingo-cellulosic waste degradation point of view, the results presented above suggest that this method would be the best for agro-industrial and other wastes which are rich source of lingo-cellulose content. Composting of agricultural residues through the action of lignocellulytic microorganisms is easier to manage and it recycles the lignocellulosic waste with high economic efficiency.

These bioinoculants (fungal) gave best result comparatively other bioinoculants. It degrades agro-industrial waste without polluting the environment. This study suggests that bio-stabilization of agro-industrial wastes with the help of fungal inoculants could be potential technology to convert noxious wastes into nutrient rich bio-fertilizer, which will be helpful to create sustainable land practices.

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