STM JOURNALS

Journal of
Polymer & Composites

ISSN: 2321-2810 (Online)
ISSN: 2321-8525 (Print)
Volume 13, Special Issue 3, 2025
DOI (Journal): 10.37591/JoPC

https://journals.stmjournals.com/jopc

Research JoPC

Characterization Of Bagasse Ash for Polymer Composite Applications: Reinforcement and Biocompatibility

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Abstract

In present investigation, comparison of the survival rate of Bacillus megaterium inoculated in bagasse ash and charcoal is done which is widely used as a carrier material. An Isolate SB1 was isolated from the soil. This isolated SB1 was subjected for a study of cultural, morphological, and biochemical characteristics. By comparing all the studied characteristics and with reference to Bergey's manual of systematic bacteriology, Vol. II, an isolate SB1 was tentatively identified as Bacillus megaterium. Biomass of isolate SB1 i.e. Bacillus megaterium was obtained, the biomass was inoculated separately in charcoal and test material i.e. Bagasse Ash. This was stored as biofertilizer and the efficacy of test material for supporting the growth of inoculants was checked by carrying out a Total Viable Count (TVC) of inoculants after every seven days from the date of inoculation. This was compared with that of charcoal. This study proved the ability of Bagasse Ash to support the survival of inoculum i.e. Bacillus megaterium which is slightly inferior to charcoal.

Keywords: *Bacillus megaterium*, bagasse, ash, inoculants, TVC, biobased.

INTRODUCTION

Biofertilizers

Biofertilizers are formulations comprising live or dormant cells of beneficial microorganisms that enhance nutrient uptake in crop plants through rhizospheric interactions upon seed or soil application. They accelerate targeted microbial processes in the soil, thereby enhancing the availability of nutrients in a readily absorbable form for plants [1].

The integration of bio-based fertilizers is a pivotal component of comprehensive nutrient management, offering both economical and sustainable sources of plant nutrients [2] They complement chemical fertilizers, contributing to sustainable agriculture practices. Various microorganisms and their symbiotic relationships with crop plants are harnessed in the manufacturing of biofertilizers, highlighting the diverse strategies employed to enrich soil productivity and stimulate plant development [3].

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Received Date: April 02, 2024
Accepted Date: December 12, 2024
Published Date: April 28, 2025

Citation: Anagha A. Raut, Shilpa Ruikar, G.R. Pathade. Characterization of Bagasse Ash for Polymer Composite Applications: Reinforcement and Biocompatibility. Journal of Polymer & Composites. 2025; 13(Special Issue 3): S374–S378p.

CARRIERS FOR BIOFERTILIZERS

Conveyor Substrates

Introduction

Microbial fertilizers are commonly designed as carrier-based inoculants containing beneficial microorganisms. Carriers, which are inert materials, are utilized for mixing with broth to facilitate the handling, packaging, storage, transportation, and application of inoculants. [4] Various types of carriers are blended with broth for this purpose. Incorporating microorganisms into conveyor

substrates ensures convenient handling, prolonged storage, and improved effectiveness of biofertilizers, thus optimizing their usefulness in agricultural practices. [5]

The Characteristics of an Ideal Carrier Material for Seed Inoculation Include

Non-toxic to both the inoculant bacterial strain and the plant, possessing good moisture absorption capacity, Easy to process, free of lump-forming materials, and sterilizable by autoclaving or gamma-irradiation, readily available in sufficient quantities and cost-effective, exhibiting good adhesion to seeds, pH buffering capacity, and ensuring the survival of the inoculant bacteria on the seed.

Characteristics of Carriers Used for Inoculants in India

Various carriers are utilized in biofertilizer production. In India, powdered farmyard manure and charcoal powder are regarded as suitable alternatives to peat and lignite [6]. A quality carrier culture contains an adequate amount of Rhizobial cells, typically ranging from 1000×106 to 4000×106 rhizobia per gram of carriers. The carrier material is powdered and sun-dried to achieve a moisture level of around 5%, then screened through 100-200 mesh sieves. Neutralization is carried out by mixing with CaCO3, followed by sterilization through autoclaving at 15 PSI. [7]. The inoculant is left for 2-10 days, covering the trays with polythene, at temperatures of 22-24°C, while maintaining moisture levels at approximately 35-40% on dry weight. It is crucial to ensure that the rhizobial count remains at 3×10 cells per gram of carrier. Subsequently, the Rhizobium inoculant can be used directly or packed and stored for future application. This meticulous process ensures the quality and efficacy of the biofertilizer product. [8]

Bagasse Ash

Boiler ash is recognized as a valuable organic waste source, capable of providing essential plant nutrients to the soil. Research [9] has shown enhanced water use efficiency in soil and increased yields of wheat crops with the addition of fly ash.

The bagasse ash currently under investigation exhibits characteristics such as low organic matter content, a calcareous nature, high pH values, and deficiencies in nitrogen (N), phosphorus, and zinc. Conversely, the boiler ash analyzed is rich in calcium, magnesium, phosphorus, sulphur, potassium, and all micronutrients (Table 1).

In a study conducted by Seth Nishtha Gaurav Bhushan and Jaspal Singh[9], the effects of various treatments of bagasse ash on soil fertility parameters and nutrient availability were investigated. The findings revealed significant improvements in soil fertility with the application of bagasse ash.[10-11] Specifically, the levels of available phosphorus and potassium increased progressively with increasing rates of boiler ash application.[12-13] Furthermore, there was a notable enhancement in the availability of micronutrients in the soil. These results suggest that bagasse ash has the potential to serve as a viable carrier material for bioinoculants.

Table 1. Physico-chemical characteristics of bagasse ash:

Characteristics	Unit	Bagasse ash (value)
pH	Mg /kg	9.2
HCO_3^{-1}	Mg /kg	1525
CO_3^{2-}	Mg /kg	36
Cl^{-1}	Mg /kg	1808
SO_4^{2-}	Mg /kg	8108
Ca^{+2}+ Mg^{+2}	Mg /kg	773
Available P	Mg /kg	110
Available K	Mg /kg	210
Copper	Mg /kg	55.0
Ferrous	Mg /kg	267.0
Magnesium	Mg /kg	194.0
Zinc	Mg /kg	65

Aims and Objectives

A current investigation was therefore undertaken to study the impact of Bagasse ash as carrier material by comparing the survival rate of Bacillus megaterium inoculated in bagasse ash and charcoal i.e. widely used carrier material.

1. To isolate *Bacillus megaterium* from the soil.
2. Characterization of *Bacillus megaterium*
3. Biomass production of *Bacillus megaterium*
4. Formulation of solid biofertilizer by using charcoal and Bagasse ash as carriers.
5. To carry out comparative studies on Bagasse ash and charcoal as carriers.

MATERIAL AND METHODS

Collection of samples

The Bagasse ash was collected from Sahydri Sahakari Sakhar Karkhana, Karad.and the Charcoal was brought from Deepak coal supplier, Raviwar Peth, Satara.

Isolation of Bacillus Megaterium

Initially, 1gm of the soil sample is moved into 10 ml of purified distilled water and blended completely to create a soil suspension. Then the soil suspension is then subjected to heat treatment by placing it in a boiling water bath at 80°C for a duration of 10 minutes. This step aims to eliminate any vegetative microbial contaminants present in the soil sample. Following heat treatment, 1 ml of the heated soil suspension is moved into another test tube containing 9 ml of purified distilled water. And then A small volume of each diluted suspension is streaked onto sterile Nutrient agar plates using a sterile inoculating loop. The plates are then allowed to grow at 28°C for a period of 24 hours. This latent phase facilitates the growth and proliferation of viable microbial colonies present in the soil sample until visible colonies were observed. A typical, well-isolated colony was selected. Suspension was prepared and subjected to Gram staining, spore staining and sub-cultured to get pure culture. The pure culture was preserved at 4°C. It was designated as SB1. The pure culture was confirmed by different biochemical tests.

Characterization of Isolate

In the studies on the cultural characteristics and colony characteristics of the isolates were studied. Morphological characteristics were studied by Gram staining (Hucker's modification) and spore staining (Dornor's Method). The motility was observed by hanging drop preparation.

The biochemical characteristics of the bacterial isolate was studied by performing the test such as Mannitol Fermentation test, Arabinose Fermentation test, Catalase test, Caseinase test, Amylase test detailed by Cruckshank (1975).

PREPARATION OF BIOMASS

Inoculum Preparation and Biomass Production

Suspension of the isolated colony from the master plate was prepared. A loopful of suspension was inoculated in 10 ml broth. The tube was incubated at 28°c for 48 hours. The incubated 10 ml broth was then transferred to 400 ml sterile nutrient broth. The flask was kept on a mixing plateform at 28°C for 2-3 days. The broth was harvested for inoculation on a sterile carrier when the cell count was about 10 cells/ ml, which was measured by SPC.

Preparation of Carrier Material

Bagasse ash was obtained from Sahyadri Sakhar Karkhana, Karad and charcoal was brought from Deepak coal supplier, Raviwar Peth, Satara. The pH of Bagasse ash and charcoal was adjusted to 7 by mixing with $CaCO_2$ (Calcium Carbonate) powder. Then about 200 g of Bagasse ash and charcoal were filled in autoclavable bottles and autoclaved for four hours at 120°C under 15 Psi continuously. The sterilized packets were allowed to cool to room temperature.

Mixing of The Carrier and the Broth Culture

About 25 ml of enriched culture broth was poured aseptically into each bottle of Bagasse ash and charcoal. Inoculated packets were blended to get uniform bacterial inoculants. The inoculated Bottle was stored at room temperature for the entire steady period.

Enumeration of Bacterial Culture in the Carrier

The population test was carried out for both Bagasse ash and charcoal-based inoculants one's in every 7 days after inoculation for 1 month by serial dilution techniques. 1gm of sample was added to 9 ml of purified distilled water and shaken well. The progressive dilutions were prepared. 0.1 ml from 10^8, 10^9, and 10^{10} of bagasse ash and charcoal were spread on a sterile nutrient agar plate. The plates were allowed to grow at 28°c for 24 hrs and the colony count was taken by colony counter.

TVC Was Obtained by the Following Formula

TVC = No. of Colonies x Dilution factor x aliquot factor

RESULTS AND DISCUSSION

The investigation of the cultural characteristics of the isolate are presented in Table 2.

The isolate SB1 was found to be Gram-positive, motile, and was spore former when stained by Donor's method. (Table 2)

The results of the Biochemical characters of the isolate are presented in Table No.3. The isolated SB1 formed acid as well as gas in both the sugars.i.e. mannitol and arabinose. When the growth of isolate SB1 was immersed in H_2O_2, the evolution of gas bubbles was observed. On milk agar plate, it showed a zone of clearance. i.e. hydrolysis of casein. SB1 was found to be positive for indole and VP. It also hydrolyzed starch as well as gelatin. By comparing the morphological, cultural, and biochemical characteristics of isolate SB1 with that of characters given in Bergey's manual of systematic bacteriology, Vol-II, it was tentatively identified as a *Bacillus megaterium.* The results of Total Viable Cell (TVC)/gm during storage of *Bacillus megaterium* are presented in Table No.4 Survival of Bacillus megaterium in sterilized Bagasse ash and charcoal carrier material (population X 10 cell/ g dry weight basis)

Table 2. Results of the Cultural characteristics and Gram's staining.

Isolate	**Colony characteristics**						
	Size	***Shape***	***Colour***	***Margin***	***Elevation***	***Consistency***	***Opacity***
SB1	1mm	Circular	White	Irregular	Convex	Dry	Opaque

Table 3. Results of Biochemical characteristics of the isolate.

S N	**Isolate**	**Biochemical test**	**Result**
1	SB1	Mannitol Fermentation	A+G
2		Arabinose Fermentation	A+G
3		Catalase	+
4		Caseinase	+
5		Amylase	+
6		Indol	-
7		Voges-Proskauer	-
8		Gelatinase	+

Table 4. Total Viable Cell (TVC)/gm during storage of Bacillus megaterium.

TVC Taken on the day	TVC/gm (Bagasse ash as carrier)	TVC/gm (charcoal as carrier)
1st	94	109
7th	92	95
14th	84	91
21st	78	79
28th	65	75

From table 4, it becomes clear that the number of *Bacillus megaterium* after incubation in bagasse ash goes on increases up to the 28th day of storage. The survival of Bacillus megaterium is better in charcoal than in bagasse ash.

CONCLUSION

A Bagasse ash supports the Survival of inoculated Bacillus megaterium biofertilizer. Its growth-supporting ability is slightly inferior to charcoal is a conventionally used carrier material. The amendment of bagasse ash with some organic material like agronomic wastes may in the future increase its potential as a carrier. Its efficacy with other organisms may prove it as a better bioinoculant carrier material.

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