



## Production of Phosphate Biofertilizer Using Lignocellulosic Waste as Carrier Material

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Bagasse, a lignocellulosic waste of sugarcane industry is utilized for producing the phosphate biofertilizer. Phosphate biofertilizer is suited for all varieties of crops. It solubilizes the bound phosphate and contributes 20-30 % of phosphate required for crops. Selection of *Bacillus megaterium* was made due to its capability to survive and solubilize the rock phosphate as compared to other bacteria. By using biological and organic fertilizers a low input system can be carried out and it can be helpful for achieving the sustainability of farms. In order to reduce the cost and also to manage the waste, sugarcane bagasse, *i.e.*, lignocellulosic waste is used in this experiment to replace the commercially used carrier material (lignite). The chemical parameters (protein, carbohydrate, chlorophyll contents) in *vigna unguiculata* plant grown using bagasse biofertilizer and lignite biofertilizer are comparable. The soil analysis also indicated signs of improvement in nutrient content, hence bagasse could be replaced in part of lignite in commercial production of biofertilizer.

**Keywords:** Lignocellulosic waste, Phosphate biofertilizer, Cost effective, *Vigna unguiculata*, *Bacillus megaterium*.

### INTRODUCTION

One of the major concerns in today's world is soil infertility. The use of chemical fertilizers and pesticides has caused tremendous harm to the environment. An answer to this is the biofertilizer, an environmental friendly fertilizer now used in most countries. Therefore, maintaining soil quality can reduce the problems of land degradation, decreasing soil fertility and rapidly declining production level. Thus, biofertilizer can be important components of integrated nutrient management. Biofertilizer is mostly produced as a carrier based inoculants. Lignite is used as a carrier in the commercial biofertilizer production. In order to reduce the cost and also to manage the waste, sugarcane bagasse, a Lignocellulosic waste is used in this experiment to replace the commercially used carrier material (lignite)<sup>1</sup>. Utilization of lignocellulosic waste for producing biofertilizer serves two purposes; it supplies nutrients to the soil and acts as soil conditioner. It is also a solution for disposal of huge amounts of bagasse produced by sugarcane as industry waste<sup>2</sup>. *Bacillus megaterium* is a phosphate solubilizing bacteria have been advocated as effective and economical bioinoculant to use in the integrated nutrient and pest control system<sup>3</sup>. It has the high ability to improve plant growth and yield based on increasing the capacity of roots to mobilize and take up nutrients and substances for overall reproductive plant fitness<sup>4</sup>. The purpose of the research was to utilize the bagasse for producing phosphate biofertilizer.

The objective of our study was to utilize lignocellulosic waste (sugarcane bagasse) as carrier material to produce phosphate Biofertilizer and to estimate the soluble phosphorus, Organic acids and phosphatase activity of the inoculants produced and to compare the activity of inoculants with sugarcane Bagasse and lignite as carrier material by analysis with *Vigna unguiculata* plant and soil.

### EXPERIMENTAL

The mother culture of *Bacillus megaterium* is acquired from the TNAU, Coimbatore, Tamil Nadu. The stain is further confirmed under digital microscope.

Sugarcane bagasse is collected from juice shop near thiruvottiyur, Chennai, India.

**Analysis of carrier material:** The carrier material has to be analyzed before the preparation of biofertilizer. pH analysis is performed using pH meter. Moisture content analysis of bagasse and lignite is determined using azeotropic distillation method. Ash content analysis of bagasse and lignite is determined using dry ashing method<sup>5</sup>.

**Chemical analysis:** The culture broths were analyzed for the amount of soluble phosphorus using Mo-blue method<sup>6</sup> at 450 nm using Spectrophotometer (Shimadzu, UV 1800, Japan). Phosphatase was assayed using *p*-nitrophenyl phosphate (pNPP) by spectrophotometer method as described by Tabatabai and Bremner<sup>7</sup>. Analysis of organic acid secreted in the culture filtrate was performed by using GC-MS<sup>8</sup>.

**Preparation of phosphate biofertilizer:** Bagasse was collected from the local sugarcane juice sellers. It was initially sundried and then pulverized with the help of flour mill. Prepared bagasse was stored at room temperature. Prepared Pikovskaia's media was inoculated with *Bacillus megaterium*. The prepared bagasse and broth was mixed in the ratio of 1:2. The resulting biomass was termed as biofertilizer<sup>9</sup>.

**Evaluation and comparison of bagasse biofertilizer and lignite biofertilizer:** Soil samples (100 g each) were incubated separately with 20 mL biofertilizer. Each sample was analyzed for the amount of phosphate solubilized and enhanced at an interval of 2 days up to 12 days<sup>10</sup>. Plantation experiment was carried out using the plant *Vigna unguiculata*<sup>11</sup>. The plant sample was analyzed for protein content using Lowry's method<sup>12</sup>, carbohydrate and Chlorophyll contents using arthrone method<sup>13</sup>.

**RESULTS AND DISCUSSION**

**Analysis of carrier material:** The result was obtained in an expected manner. For a material to be used as a carrier material, it should accomplished with the characteristics of high moisture content, low ash content and pH to be of 6.5-7. With the results obtained bagasse possess high moisture content, low ash content than lignite (commercially used). So bagasse could be used in the place of lignite (Fig. 1).

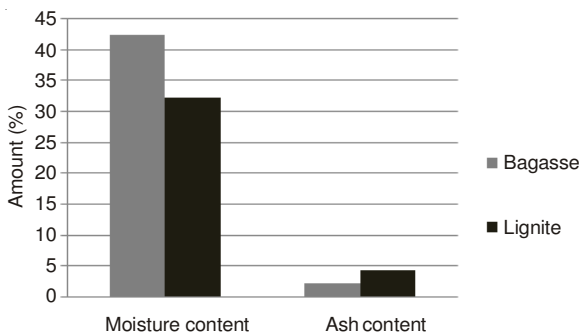


Fig. 1. Analysis of moisture content and ash content result

**Analysis of innoculant (*Bacillus megaterium*):** The culture filtrate of the bacterial sample was analyzed for release of inorganic phosphate, which showed 65 µg/mL of culture filtrate (Fig. 2). Acid phosphatase was recorded as maximum of 2.57 U/mL. Phosphorous solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids mainly of gluconic and keto gluconic acids.

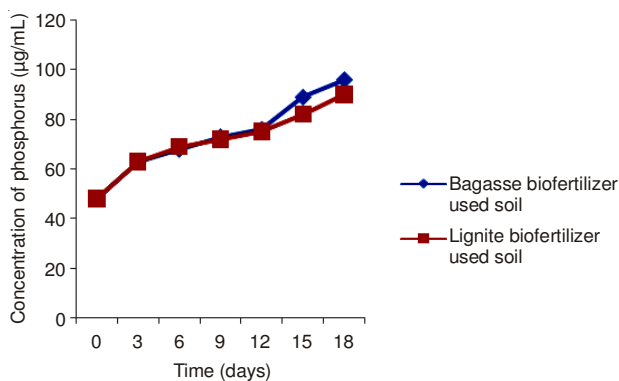


Fig. 2. Increase in phosphorus concentration of the soil incubated with bagasse biofertilizer and lignite biofertilizer

**Evaluation and comparison of bagasse biofertilizer and lignite biofertilizer:** These are the final analysis in which bagasse biofertilizer to be used as an alternative to lignite biofertilizer. The results consists of viable cell count method, soil evaluation by estimating the amount of soluble phosphorous with incubation of both the biofertilizer (Fig. 3). In addition, the results of protein, carbohydrate and chlorophyll content in *Vigna unguiculata* plant grown using lignite and bagasse biofertilizer are shown in Figs. 4 and 5.

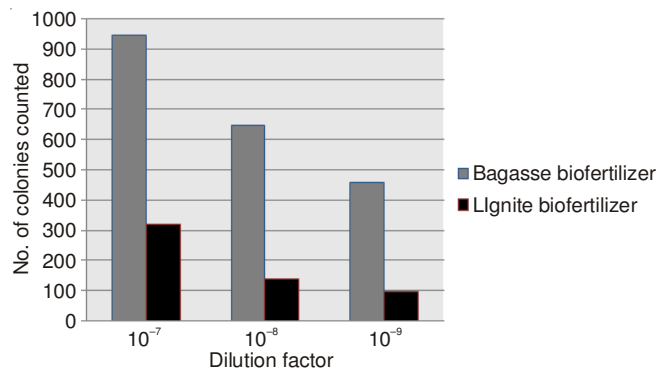


Fig. 3. Evaluation of colony forming unit of two samples of biofertilizers

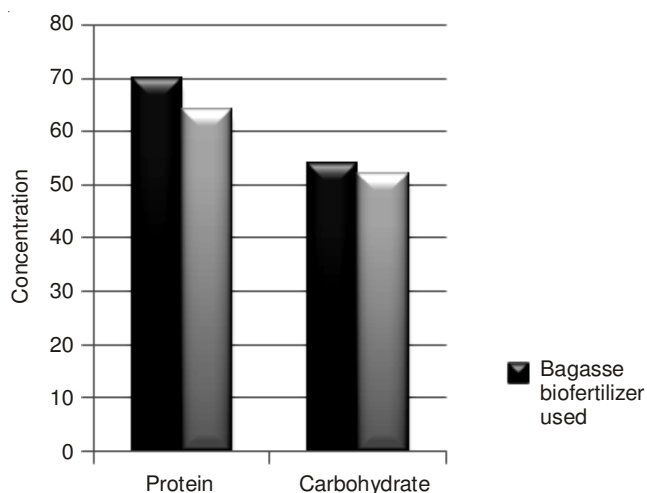


Fig. 4. Analysis of protein content and carbohydrate content in *Vigna unguiculata* plant treated with bagasse biofertilizer and lignite biofertilizer

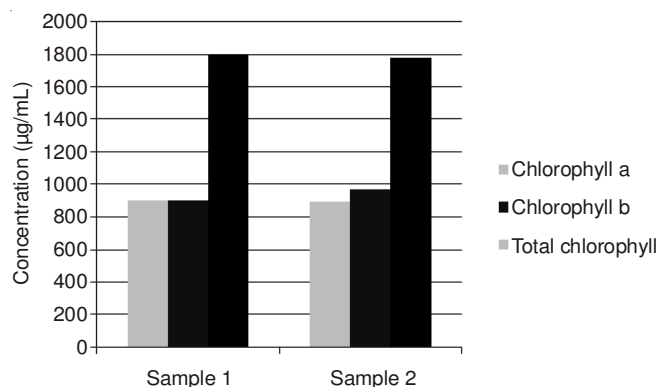


Fig. 5. Analysis of chlorophyll content in *Vigna unguiculata* plant treated with bagasse biofertilizer and lignite biofertilizer. (Sample 1: Leaf extract of the plant grown using Bagasse Biofertilizer). (Sample 2: Leaf extract of the plant grown using Lignite Biofertilizer)

## Conclusion

The metabolism of *Bacillus megaterium* could produce a large number of dissolving phosphorus and plants can obtain surplus available phosphorus produced by *Bacillus megaterium*. Furthermore, Bagasse as to replace part of the use of lignite reduces the cost and dumping of waste. The soil analysis also indicated signs of improvement in nutrient content. It can be concluded that chemical parameters (protein, carbohydrate, chlorophyll contents) in *Vigna unguiculata* plant grown using bagasse biofertilizer and lignite biofertilizer are comparable. Small differences were found in all the parameters in *Vigna unguiculata* plant in relation of bagasse biofertilizer and lignite biofertilizer. Hence bagasse could be replaced in part of lignite in commercial production of biofertilizer.

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