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Pressmud Compost for Improved Nitrogen and Phosphorus Content Employing Bacillus Strains

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Abstract: Pressmud, a by-product of sugarcane processing, is typically disposed of through incineration or landfilling, though it has considerable potential in organic agriculture. This study explored the composting of pressmud through bioaugmentation using specific bacterial strains. Two experimental setups were created: E2 with a cellulolytic and phosphorus-solubilizing strain, *Bacillus amyloliquefaciens*-ASK11, and E3 with a nitrogen-fixing strain, *Bacillus megaterium*-ASNF3. A control setup (E1) was also maintained without bacterial augmentation. Results indicated that the Bacillus-enhanced composts in E2 and E3 showed significant increases of 129% and 83% in nitrogen and of 49% and 91% in phosphorus contents, respectively, after 60 days. Additionally, organic matter decomposition improved by 49–50% in the bioaugmented setups after 60 days. FTIR analysis revealed organic phosphate peaks and P-O-C stretching bands at 1025 cm^{-1} in the E2 compost, while a nitrogen vibration band at 3849 cm^{-1} in E3 indicated significantly higher nitrogen content compared to the control. The Bacillus-enriched pressmud compost not only accelerated the composting process but also enhanced nutrient levels, positioning it as a promising biofertilizer for rehabilitating barren lands.

Keywords: sugar industry waste valorization; phosphorus bioavailability; pressmud compost; biofortified compost; nutrients; bioamended compost

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1. Introduction

Pakistan, a country renowned for its rich agricultural heritage, faces the challenge of rehabilitating around 80–90% of its soils deficient in nitrogen and phosphorous [1,2]. As a developing nation, Pakistan requires cost-effective strategies to enhance agricultural productivity, particularly in response to the demands of a growing population and the need to bolster its economy. Researchers have been actively exploring innovative approaches to improve fertilizers, with a notable focus on utilizing agricultural waste as a sustainable resource for soil enrichment [3–5].

Pressmud, a by-product of the sugarcane industry, represents a promising avenue for enhancing soil fertility [6] (Bokhtiar et al., 2015). Pakistan, home to 90 sugar mills with a sugarcane production of 89.5 million metric tons, generates around 2.7 million tons of sugarcane pressmud (SPM) annually [7] (Fiead et al., 2012). This by-product, rich in organic matter and essential nutrients, has gained attention as a valuable resource for biofortification in agriculture. Composting, a well-established method for stabilizing organic matter, plays a critical role in transforming pressmud into a nutrient-rich biofertilizer. The process involves the microbial decomposition of organic material, resulting in the formation of humic substances that improve soil health.

The use of pressmud as a soil amendment has been widely studied for its ability to enhance soil fertility, particularly in terms of increasing phosphorus (P) and nitrogen (N) as

well as potassium (K) contents. Several studies have demonstrated that when pressmud is applied in conjunction with conventional fertilizers, there is a significant improvement in the availability of P and N in the soil. For instance, research has shown that pressmud can act as a slow-release fertilizer, providing essential nutrients over time, thereby enhancing the soil's nutrient profile [8,9].

However, there is limited literature focusing on the efficacy of pressmud in enhancing P and N levels through bacterial augmentation without the addition of traditional fertilizers. Most studies on pressmud have primarily investigated its role as an organic amendment in combination with fertilizers rather than exploring its potential as a standalone biofertilizer when inoculated with specific bacterial strains. Bacterial augmentation, particularly with phosphorus-solubilizing and nitrogen-fixing Bacillus strains, is a promising area of research, as it could further enhance the bioavailability of these critical nutrients in the soil. Despite its potential, the research on this approach remains sparse, and more studies are needed to fully understand and optimize the use of pressmud with bacterial inoculants for soil fertility enhancement [10,11]. This literature provides a basis for understanding the role of pressmud in soil fertility enhancement, particularly in terms of P and N content, while also highlighting the need for further research on bacterial augmentation as a standalone approach.

2. Results

2.1. Moisture Content

Moisture content is a critical factor in maintaining the composting process. A 49% reduction in moisture content was observed in E2 after 60 days of composting, while E3 showed a 58% reduction over the same period, compared to a 73% decrease in the control (E1). Detailed moisture content measurements at 15, 30, 45, and 60 days are illustrated in Figure 1.

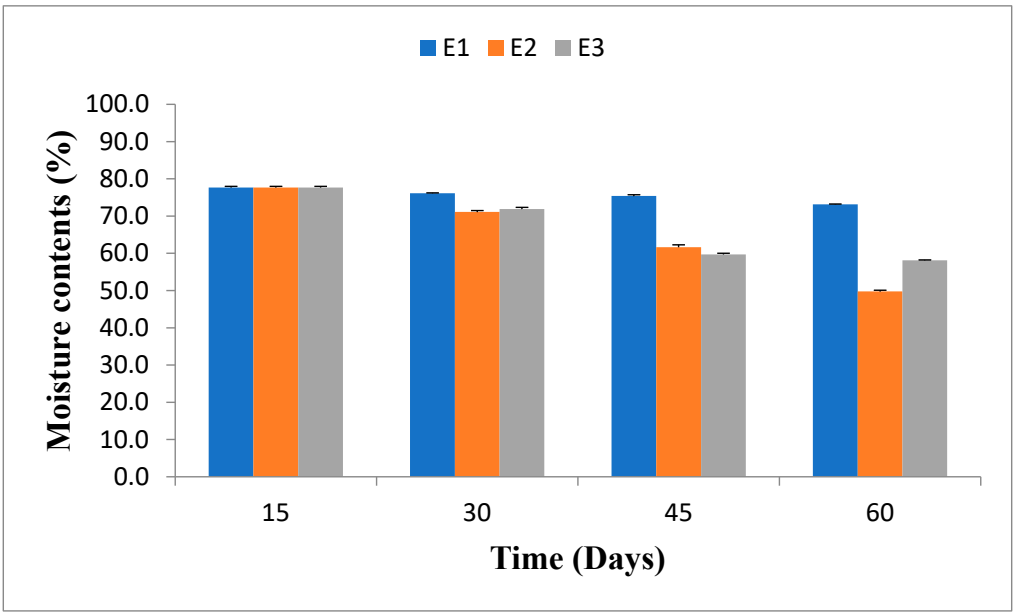

Figure 1. Moisture reduction of different pressmud compost groups enriched with or without phosphorus-solubilizing and nitrogen-fixing bacteria (E1: Indiginous bacteria; E2: Indiginous bacteria + *B. amyloliquefaciens*-ASK 11; E3: Indiginous bacteria + *B. megaterium*-ANF3) after varying time intervals.

2.2. Temperature Variation

Temperature fluctuations were recorded at 15, 30, 45, and 60 days. At the end of the experiment, E2 exhibited a temperature of 54 ± 0.02 °C, while E3 showed 49 ± 0.09 °C,

compared to 40 ± 0.09 °C in the control group. These temperature values at each interval are shown in Figure 2.

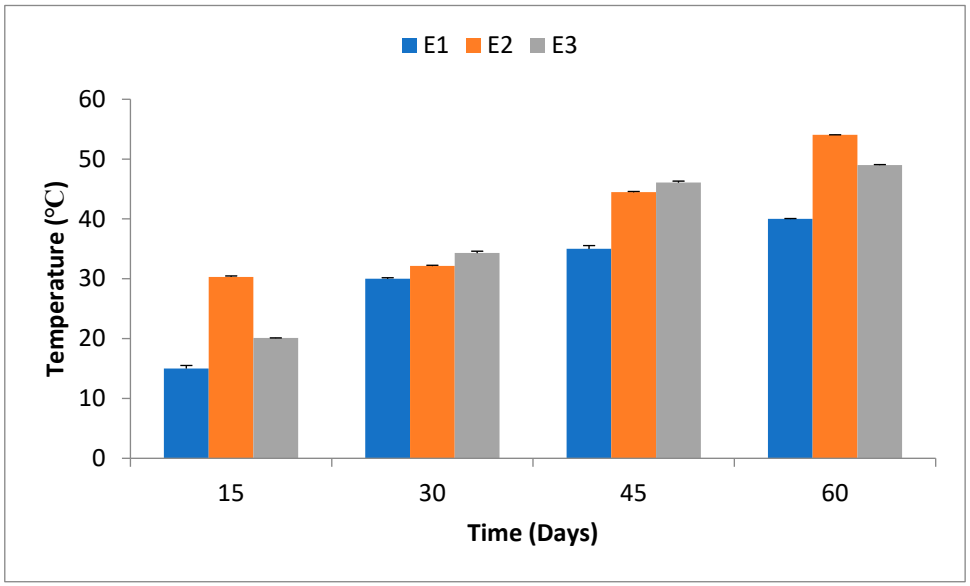

Figure 2. Temperature (°C) of different pressmud compost groups enriched with or without phosphorus-solubilizing and nitrogen-fixing bacteria bacteria (E1: Indiginous bacteria; E2: Indiginous bacteria + *B. amyloliquefaciens*-ASK 11; E3: Indiginous bacteria + *B. megaterium*-ANF3) after varying time intervals.

2.3. pH of the Compost

The pH of the compost was measured at 15, 30, 45, and 60 days. In E2, the pH values ranged from 7.1 ± 0.05 at day 15 to 8.2 ± 0.12 at day 60. For E3, pH increased from 7.1 ± 0.05 to 8.6 ± 0.03 over the same period, while the control group (E1) showed a pH of 7.3 ± 0.06 after 60 days. The pH data are illustrated in Figure 3.

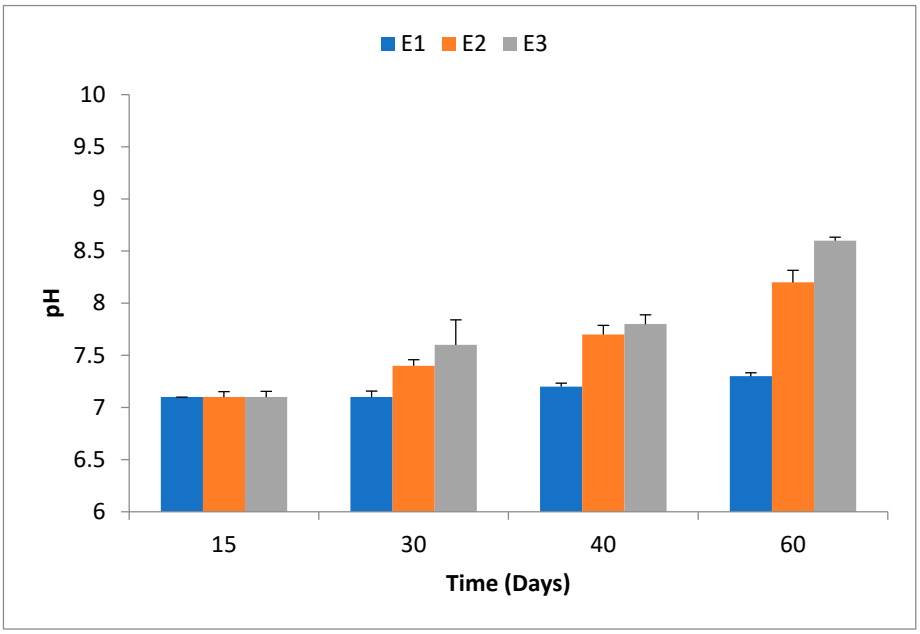

Figure 3. pH of different pressmud compost groups enriched with or without phosphorus-solubilizing and nitrogen-fixing bacteria bacteria (E1: Indiginous bacteria; E2: Indiginous bacteria+ *B. amyloliquefaciens*-ASK 11; E3: Indiginous bacteria + *B. megaterium*-ANF3) after varying time intervals.

2.4. Electrical Conductivity (EC)

At the beginning of the experiment, the EC increased due to the release of soluble salts. After 60 days, the EC in the control group was 0.68 ± 0.01 mS/cm, whereas in E2 and E3, the EC was recorded as 0.31 ± 0.05 mS/cm and 0.35 ± 0.04 mS/cm, respectively. The data are illustrated in Figure 4.

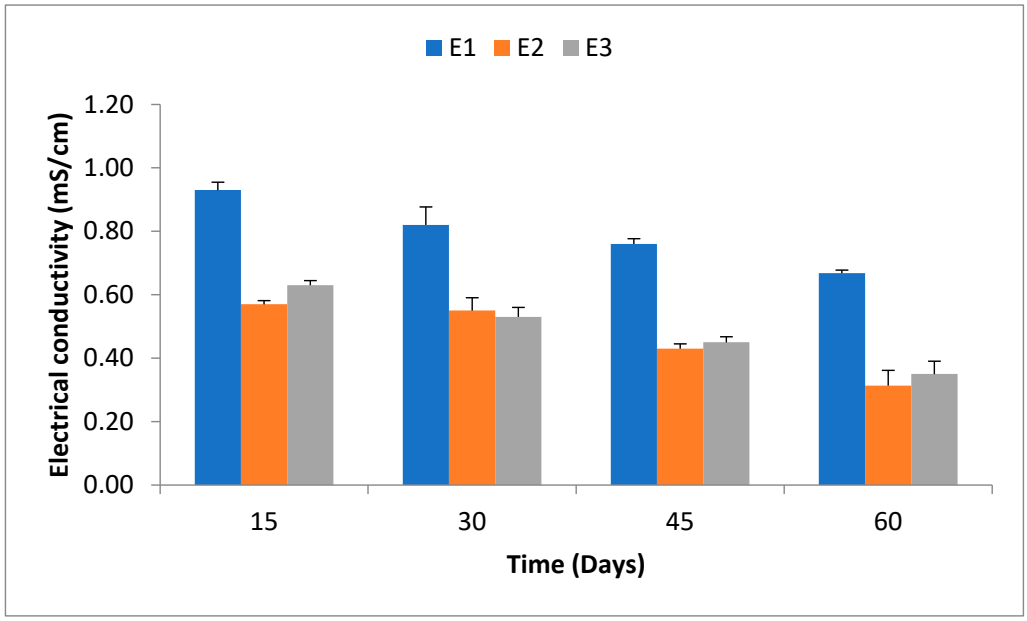

Figure 4. Electrical conductivity (mS/cm) of different pressmud compost groups enriched with or without phosphorus-solubilizing and nitrogen-fixing bacteria (E1: Indiginous bacteria; E2: Indiginous bacteria + *B. amyloliquefaciens*-ASK 11; E3: Indiginous bacteria + *B. megaterium*-ANF3) after varying time intervals.

2.5. Organic Matter (%)

The organic matter content varied during the experiment, increasing gradually. After 60 days, E2 had 49.41 ± 0.35% organic matter, and E3 had 50.55 ± 0.28%, compared to 12.63 ± 0.19% in the control group (E1). Detailed organic matter content at different stages is provided in Figure 5.

2.6. Waste Mass Degradation

A rapid decrease in waste mass was observed across all experimental groups at the onset of the experiment. In particular, the E2 group exhibited the highest degradation, with approximately 60% of the mass degraded by day 45. This represents a 58% increase in degradation in the E2 group compared to the control group. Although the difference between the E2 and E3 groups was minimal, the control group showed only 0.3% mass degradation, likely due to the slower bacterial activity. Both E2 and E3 groups produced a blackish-brown material with an earthy, woody odor, which was markedly different from the control group (Figure 6).

2.7. Total Nitrogen (N) Content

Nitrogen content increased as composting progressed. E2 and E3 showed an increase of 129% and 83% in total nitrogen content, respectively, and 1% after 60 days, significantly higher than the 65% increase in the E1 group's nitrogen content. The detailed nitrogen content at different stages is provided in Figure 7.

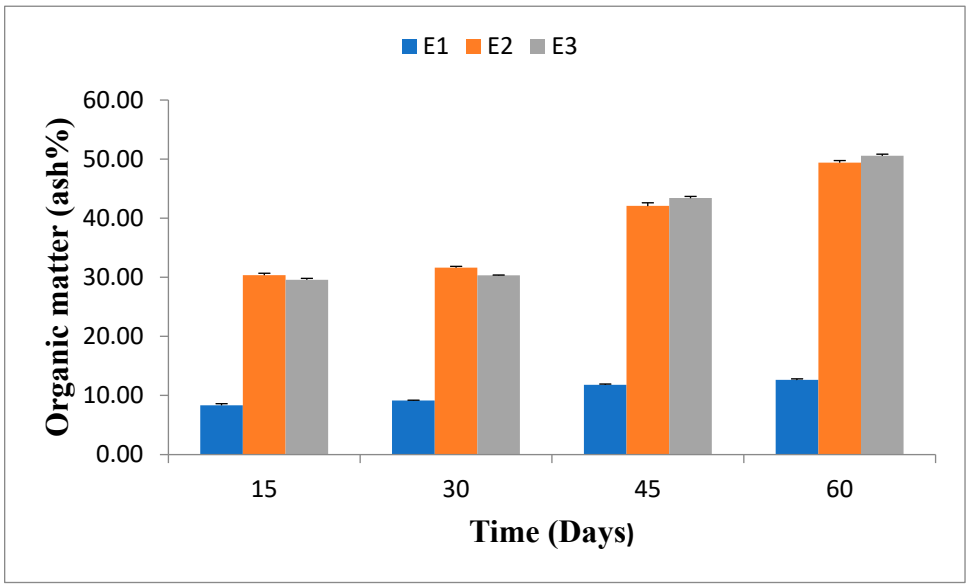

Figure 5. Organic matter of different pressmud compost groups enriched with or without phosphorus-solubilizing and nitrogen-fixing bacteria (E1: Indiginous bacteria; E2: Indiginous bacteria + *B. amyloliquefaciens*-ASK 11; E3: Indiginous bacteria + *B. megaterium*-ANF3) after varying time intervals.

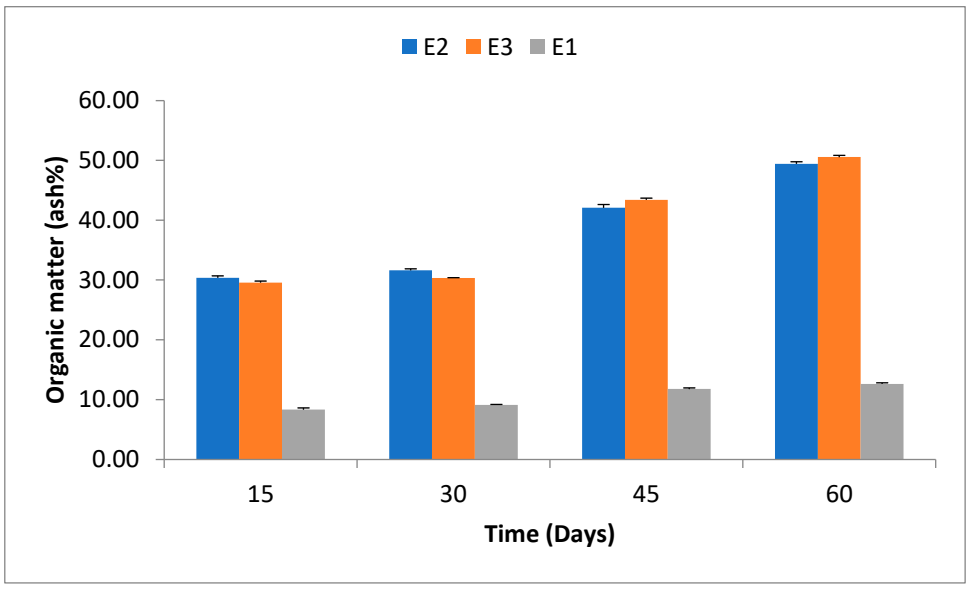

Figure 6. Waste mass degradation (%) in different pressmud compost groups enriched with/without phosphorus-solubilizing and nitrogen-fixing bacteria (E1: Indiginous bacteria; E2: Indiginous bacteria + *B. amyloliquefaciens*-ASK 11; E3: Indiginous bacteria + *B. megaterium*-ANF3) over various time intervals (days).

2.8. Phosphorus Concentration

During the composting process, total phosphorus was measured at various intervals, specifically on days 15, 30, 45, and 60, and reported in parts per million (ppm) as presented in Figure 8. Among the experimental groups, E2 demonstrated the highest phosphorus concentration, reaching 5.41 ppm by day 60. In contrast, the E3 group had a phosphorus concentration of 4.1 ppm at the end of the experiment. The control E1 exhibited the lowest phosphorus concentration, with a value of 1.98 ppm.

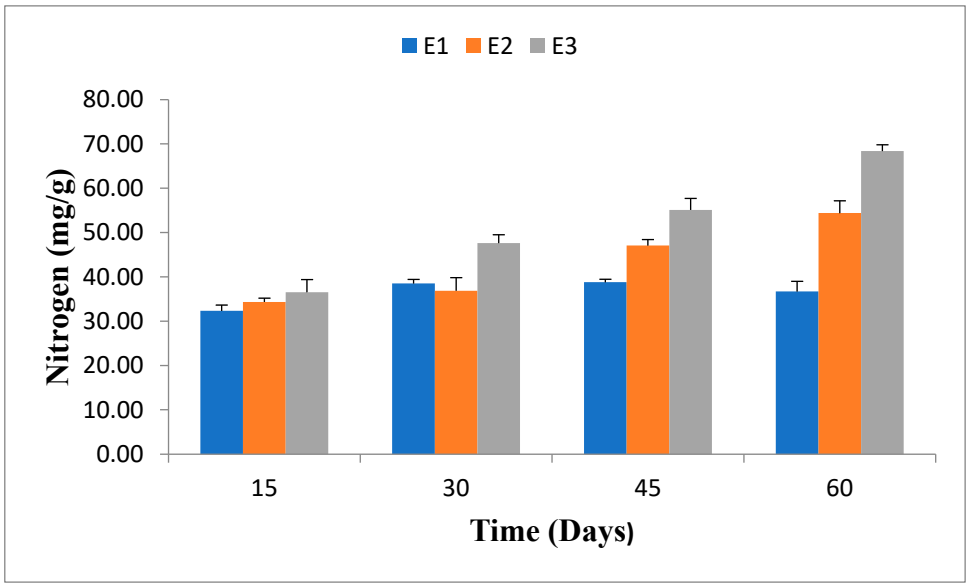

Figure 7. Nitrogen content (%) of different pressmud compost groups enriched with or without phosphorus-solubilizing and nitrogen-fixing bacteria (E1: Indiginous bacteria; E2: Indiginous bacteria+*B. amyloliquefaciens*-ASK 11; E3: Indiginous bacteria + *B. megaterium*-ANF3) after varying time intervals.

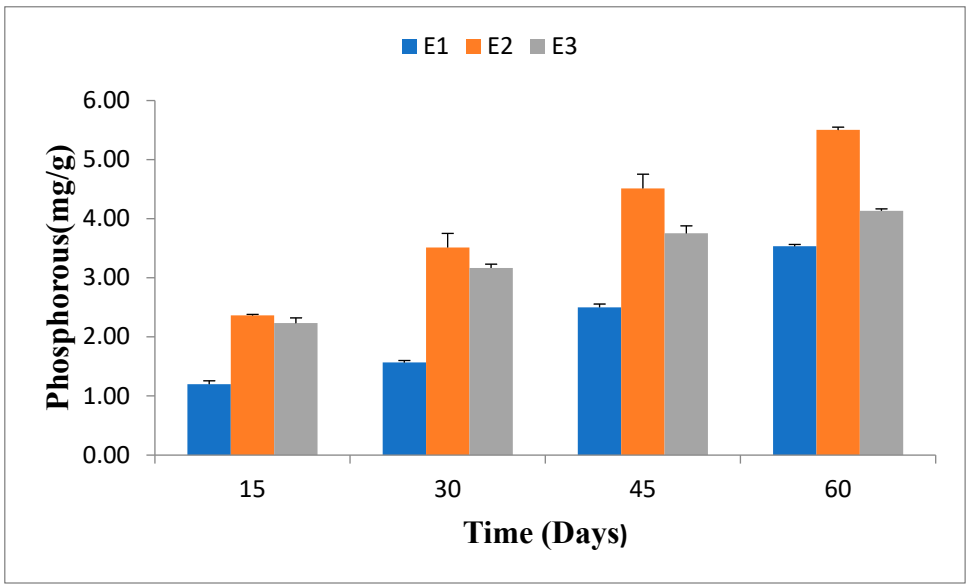

Figure 8. Total phosphorus concentration (mg/g) in different pressmud compost groups enriched with/without phosphorus-solubilizing and nitrogen-fixing bacteria (E1: Indiginous bacteria; E2: Indiginous bacteria + *B. amyloliquefaciens*-ASK 11; E3: Indiginous bacteria + *B. megaterium*-ANF3) over various time intervals (days).

2.9. FT-IR Analysis of Compost

2.9.1. FT-IR Spectrum of E2 Compost

The FT-IR spectrum of the biofortified compost sample from experimental setup E2 showed distinct absorbance peaks (Figure 9). The broad band at 3334 cm^{-1} and the smaller peak at 3567 cm^{-1} represent O-H and N-H stretching vibrations. These peaks indicate hydrogen-bonded hydroxyl groups from water or alcohols and amine groups from proteins or nitrogenous organic matter in the compost. The sharp peaks at 1220 cm^{-1} and 1026 cm^{-1} correspond to phosphate group (P=O) stretching vibrations, indicating the

presence of phosphorus compounds from mineral or organophosphates in the compost. The peaks at 1634 cm^{-1}, 1558 cm^{-1}, and 1520 cm^{-1} represent amide I and II bands, indicating proteins or peptides, characterized by C=O stretching and N-H bending of nitrogen-containing organic matter.

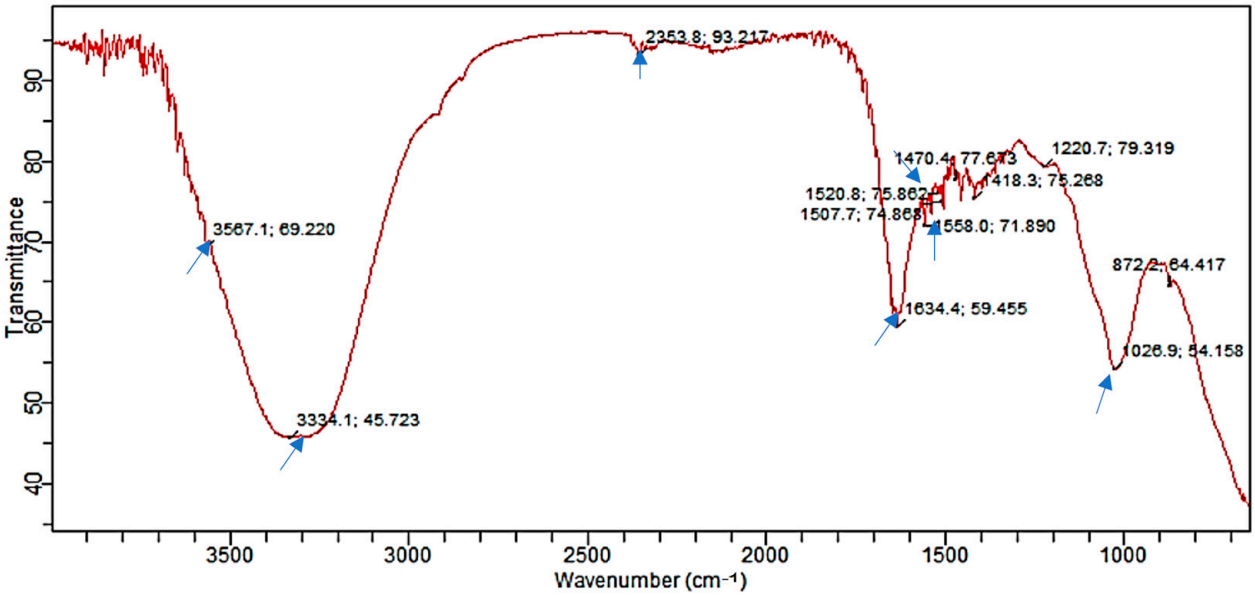

Figure 9. FT-IR spectra of mature pressmud compost from experimental setup E2 bioaugumented with *Bacillus amyloliquefaciens*-ASK 11 after 60 days.

2.9.2. FT-IR Spectrum of E3 Compost

The FT-IR spectrum of the compost sample from experimental setup E3 provided insights into the composting process and maturity (Figure 10). The broad absorption band at 3326 cm^{-1} (with smaller peaks at 3567 cm^{-1} and 3849 cm^{-1}) represents O-H and N-H stretching vibrations, suggesting the presence of hydrogen-bonded hydroxyl groups (O-H) and amine groups (N-H). These functional groups are common in proteins, amino acids, or nitrogenous organic matter in the compost. A broad band at 2905 cm^{-1} corresponded to C-H stretching. Several peaks at 1507 cm^{-1}, 1520 cm^{-1}, and 1558 cm^{-1} were attributed to aromatic carbon rings (C-H). A sharp peak at 1634 cm^{-1} was associated with unsaturated double and triple bonds, as well as the amide group. The fingerprint region contained peaks related to ethanol and C-C stretching bonds, with lignin present at 1472 cm^{-1} and organic sulfate at 1418 cm^{-1}. An aliphatic compound was identified at 1026 cm^{-1}. Additionally, stretching bands at 1090–1110 cm^{-1} suggested the presence of polysaccharides. The bands at 1680–1638 cm^{-1} were associated with protein origins, corresponding to amide I and II. The absorption bands at 2820 cm^{-1} and 2950 cm^{-1} were attributed to aliphatic methylene groups, indicating the presence of lipids and fats. The bands at 3750 cm^{-1} and 3900 cm^{-1} were representative of O-H stretching vibrations.

2.9.3. FT-IR Spectrum of E1 Compost

The FT-IR spectra of the control sample (E1) revealed a peak at 3649 cm^{-1} corresponding to C-H stretching bands, with no evidence of hydrogen-linked groups. A peak at 2353 cm^{-1} was attributed to cyclic carbons (C-C), graphite, and alcoholic groups. The sharp peak at 1770 cm^{-1} indicated the presence of aldehyde, ketone, and ester bonds. A sharp peak at 1558 cm^{-1} was characteristic of aromatic ring compounds. Methyl and symmetric bands were observed at 1455 cm^{-1} and 1418 cm^{-1}, while a narrow band at 872 cm^{-1} was associated with aromatic C-H stretching bonds (Figure 11).

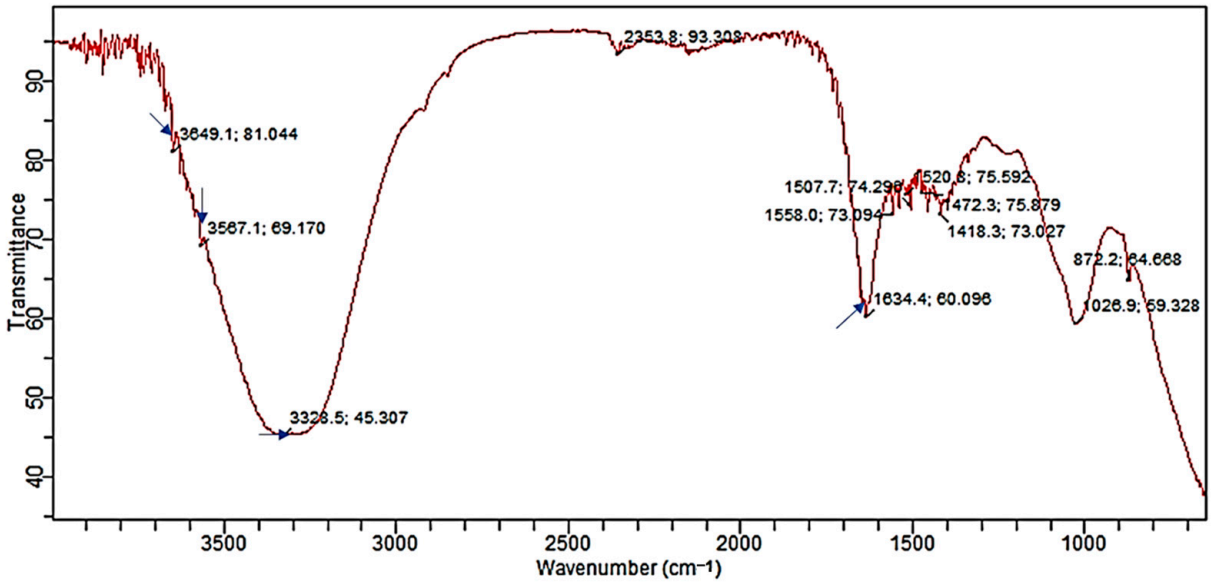

Figure 10. FT-IR spectra of mature pressmud compost from experimental setup E3 bioaugumented with *B. megaterium*-ANF3 after 60 days.

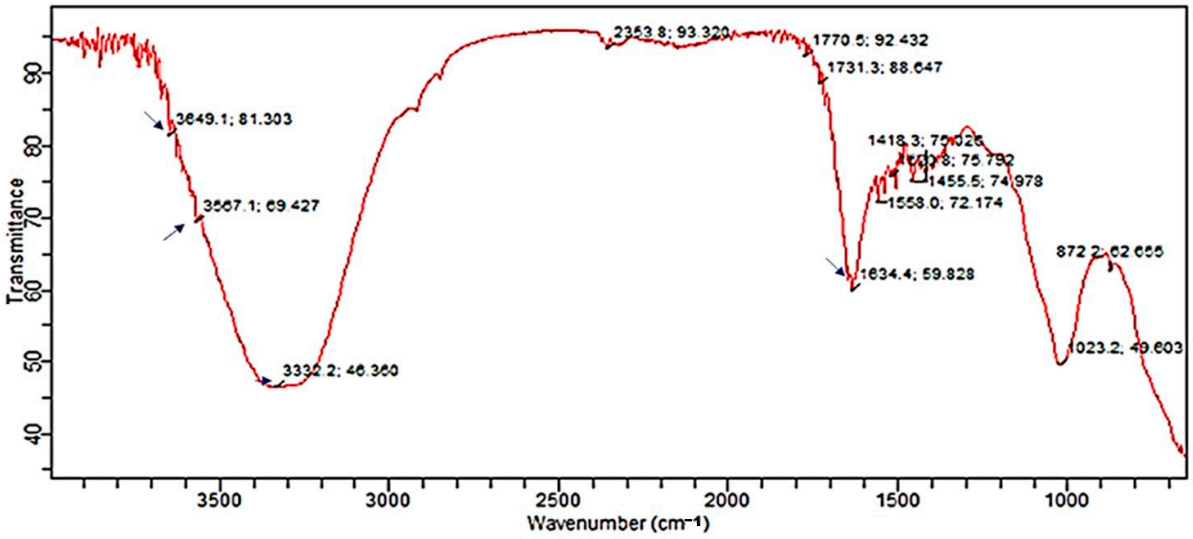

Figure 11. FT-IR spectra of mature pressmud compost of E1 group without bioaugumentation after 60 days.

3. Discussion

This study aimed to biofortify sugarcane waste compost through the application of nitrogen-fixing and phosphorus-solubilizing bacterial strains, with the objective of developing a potent biofertilizer. The biofortified compost primarily consists of essential nutrients, including calcium (Ca), nitrogen (N), phosphorus (P), and potassium (K), which are crucial for soil fertility and optimal plant growth [12,13].

Regular amendments of compost to soil have been shown to enhance nutrient concentrations significantly; however, these additions to soil may increase levels of heavy metals, raising environmental concerns [14,15]. It is therefore essential to develop compost formulations that not only provide nutrients in a controlled manner but also minimize the risk of metal accumulation. The introduction of metal-reducing bacteria, such as *Bacillus megaterium*-ASNF3 and *Bacillus amyloliquefaciens*-ASK11, into pressmud compost can produce a unique compost with dual benefits. These strains can enhance the bioavail-

ability of nitrogen and phosphorus while simultaneously reducing the toxicity of heavy metals. By facilitating the reduction of metal ions, these microbial strains help mitigate the potential environmental risks associated with metal accumulation, ensuring a safer and more effective biofertilizer for agricultural use [16,17].

In the current study, the nitrogen content in the compost was increased to 6.98% when bioaugumented with nitrogen-fixing bacteria, demonstrating a notable increase compared to the E1. This also aligns with findings from Wang et al. [18], where thermophilic nitrogen-fixing strains, including *Bacillus subtilis* (NF1) and *Azotobacter chroococcum* (NF2), were employed in cow dung compost. Their research indicated that these strains effectively raised total Kjeldahl nitrogen concentrations by 38.43% to 55.35% and extended thermophilic conditions, thereby improving overall compost quality.

Furthermore, phosphorus solubilizing bacteria, specifically *Bacillus amyloliquefaciens*-ASK11, demonstrated an increase in the phosphorus concentration of E2 by 5.41 mg/g compared to the compost without bioaugmentation. This release of bioavailable phosphorus from pressmud is crucial for plant utilization. The traditional composting methods involving composting of waste materials to enhance soil organic matter for higher crop yields may not sufficiently enhance phosphorus availability for crops [19]. Phosphate deficiency in soil is typically addressed with phosphorus fertilizers, but due to reactions with Ca^{2+}, Fe^{3+}, and Al^{3+}, most phosphorus becomes unavailable to plants, with only 5–25% efficiency. Its long-term use also leads to soil acidification, water pollution, and eutrophication [20]. Enhancing the bioavailability of insoluble phosphate is thus a key agricultural goal. The ability of microbes like *Bacillus megaterium* and other phosphorus-solubilizing bacteria to release phosphate from insoluble forms make them attractive candidates for microbial interventions in composting depending on factors like the availability of organic acids, temperature, and pH [21]. In some cases, the phosphorus release might be modest due to suboptimal composting conditions

A proper carbon-to-nitrogen (C/N) ratio is vital for effective composting, with an optimal ratio of approximately 30 [22]. Deviations from this ratio can lead to anaerobic conditions and nutrient loss, impacting microbial activity and compost quality [23]. In our study, *Bacillus megaterium*-ASNF3-bioaugumented pressmud compost exhibited a maximum temperature of 49 °C, while the compost with *B. amyloliquefaciens*-ASK11 reached up to 54 °C. The pH of the experimental compost cans changed from neutral to alkaline while a very slight shift of pH from neutral to slightly alkaline was observed. Consistent with findings from Rich and Bharti [24], our results indicated a maximum pH value of 8.6 for the E3 treatment, suggesting the stability of microbial communities under these conditions.

Increased electrical conductivity at the experiment's outset correlated with microbial activity [25]. This underscores the potential of microbial interventions as environmentally friendly techniques for effective waste degradation and nutrient recycling. The E2 treatment exhibited a waste mass degradation rate of 2.9%, while E3 achieved 3.5%, corroborating the biodegradation capabilities of *B. amyloliquefaciens*-ASK11 noted by Ayilara et al. [26]. Various physical and chemical parameters significantly influence biodegradation efficacy, highlighting the interdependence of these factors on microbial activity [27].

Phosphorus in compost typically exists in stable, insoluble forms that are not easily accessible to plants unless solubilized by microbes, as supported by studies such as [28]. The FTIR spectra provide evidence of the presence of both nitrogen- and phosphorus-containing compounds in compost, primarily in the form of amides (proteins/peptides) and phosphates. Tiquia [29] explained that nitrogenous compounds are gradually mineralized into ammonium and nitrate throughout the composting process. However, the bioavailability of these nutrients may depend on further microbial activity or specific treatments to make them more accessible to plants.

Overall, this study demonstrates that the application of Bacillus strains can significantly enhance nitrogen and phosphorus contents in pressmud compost, making these nutrients more biologically available for plant uptake. The measured phosphorus con-

centration of 5.41 ppm in the E2 treatment at 60 days, along with the 6.98% nitrogen content attributed to the activity of *B. megaterium*-ASNF3, emphasizes the potential of these microbial agents in sustainable agriculture.

4. Material and Methods

4.1. Preparation of Consortia

Pure cultures of two bacterial strains named *Bacillus amyloliquefaciens*-ASK11 and *Bacillus amyloliquefaciens*-ASNF3 with accession No. KC527054 and KC527057, respectively, were collected from the Microbial Biotechnology Laboratory of the Punjab University, Lahore, and used in this study. These strains are well known for their nitrogen-fixing and cellulose-degrading potential together with their heavy metal reduction ability [30,31]. The phosphorous solubilizing ability of *Bacillus amyloliquefaciens*-ASK-11 was determined following the methods of Amri et al. [32].

4.2. Inoculum Preparation

Small amounts of the bacteria from the slants were revived in nutrient broth. For pure culturing, a single colony was picked up and transferred to an agar plate, and then equal quantities of bacterial consortia were added to the compost. For maturation of the compost, compost was left for 60 days; during maturation, physiochemical parameters were checked regularly. Bacterial refresh was conducted in nutrient broth for 36 h at 38 °C temperature. Later, to prepare the inoculum for the compost, 0.1 mL of the bacterial culture was inoculated into a 500 mL flask containing 300 mL of nutrient broth. The culture was incubated for 48 h under optimum growth conditions [30,31]. The growth was then harvested by centrifugation at 6000 rmp. The obtained microbial pellets were then suspended in sterilized distilled water after repeated washing with water. This culture was used for the corresponding compost treatment as the bacterial inoculum.

4.3. Compost Formation

The experiment for the compost formation was established in the microbial Biotechnology and aquatoxicology laboratory of the Zoology Department of GC Women University, Faisalabad, in plastic cans. Aeration was supplied with the help of sterilized piping connected to the aerator. The sterilization of the air was ensured by plugging piping with cotton. Compost made from sugarcane pressmud was prepared by adding three basic ingredients, i.e., (a) green material containing vegetable waste and fruit scraps from the kitchen and green grass clipping, (b) brown material, and (c) pressmud (Figure 12). Addition of (a) and (b) in the pressmud was carried out appropriately to maintain a C:N ratio between 26 and 36 [15]. Two experimental setups designated as E2 and E3 were established on the basis of the microbes processing the compost, as well as a control group E1 containing the indigenous microbiota without bioaugmentation. Experimental group E2 contained *B. amyloliquificens*-ASK11 while experimental group E3 contained *B. megaterium*-ASNF3. All three setups were processed together with their indigenous microbes.

4.4. Physiochemical Characteristics of Compost

The physical and chemical characteristics of biofortified pressmud compost are crucial indicators of its quality and suitability for agricultural use. There were some physical and chemical properties associated with the biofortified compost. The following parameters were determined in the compost: EC, pH, organic matter content, moisture content, waste mass degradation, and temperature.

4.4.1. Moisture Content

To determine the moisture content, a2 g sample of compost was weighed on a weight machine as W1. After weighing, the sample was spread thinly and evenly on a tray and placed in an oven at −70 °C (140–160 °F) for 5 h. After that, the sample was cooled and

weighed again as W2. The moisture content (%) was determined using the following formula [33]:

$$\text{Moisture Content } (\%) = \frac{(\text{W1} - \text{W2}) \times 100}{\text{Weigh of compost}}$$

(**a**) (**b**) (**c**)

Figure 12. The material used for composting: (**a**) green material, (**b**) brown material, and (**c**) pressmud for compost formation.

4.4.2. Variation in Temperature

Maintaining optimal compost temperature is essential for efficient decomposition, pathogen reduction, and the production of high-quality compost. The temperature of the compost was maintained by carefully selecting the seasons as well as the laboratory location. The temperature of the compost was monitored by using a thermometer inserted into the compost. The compost was turned or aerated if the temperature was raised while moisture levels were adjusted. Moisture level and proper insulation were ensured to regulate compost temperatures and promote efficient decomposition throughout the composting process [34].

4.4.3. Determination of pH Value

The value of the pH was determined by using a pH meter (inno TECH- pH 18 model). To determine the pH, a compost suspension (water mixture) was prepared with 1 g compost suspended into 10 mL of distilled water in a glass jar. The suspension was mixed, and the pH of the aqueous solution was measured afterwards. The electrode of the pH meter was dipped into the suspension, and the reading of the pH meter of each experimental group was noted [35].

4.4.4. Electrical Conductivity

To determine the EC, 50 mL fresh compost and 250 mL water were placed in a 500 mL Erlenmeyer flask. The suspension was shaken for 1 h with an agitator and filtered afterwards. The EC was measured using the water suspension with an EC conductivity meter [36].

4.4.5. Organic Matter (OM)

The organic matter was determined by following the method of Masood et al. [37]. For determination of OM, a crucible was cleaned and placed into a muffle furnace at 600 °C for 1 h. Then the empty crucible was cooled and weighed. Next, a 2 g compost sample was placed in the crucible and the weight was noted. The compost-filled crucible was placed into the muffle furnace at 550 °C for 4 h. This process indicates the complete oxidation of organic matter, with a white-grey substance called ash appearing; the ash which was

present in the crucible was weighed. The organic matter was calculated by using the following equation:

$$\text{Ash\%} = \frac{\text{difference of weight in ash} \times 100}{\text{Weight of sample}}$$

where:

(W1) = weight of the empty crucible;
(W2) = weight of the sample-filled crucible;
(W3) = weight of the ash;
Difference of weight in ash = (W1 − W2).

4.4.6. Waste Mass Degradation

Applying the density formula, waste mass degradation was calculated at different time intervals of 15, 30, 45 and 60 days [37].

$$\text{P} = \text{mass/volume}$$

4.4.7. Determination of Nitrogen in Sample

The Kjeldahl method [38] was used to determine the nitrogen content in the compost. The method consists of three basic steps: digestion, distillation, and quantification.

For digestion, a 1 g sample of compost was added into a clean crucible. Five grams of digestion mixture (potassium sulphate 100 g + copper sulphate 10 g + ferric sulphate 5 g) and 25–30 mL sulfuric acid were then added to the compost sample; the crucible was then placed it in digester for one and half hours or until a light green color appeared. Later, distilled water was added to bring the volume in the volumetric flask to 250 mL. Next, 10 mL of the sample solution was placed in a Kjeldahl distillation apparatus, and 10 mL of 40% NaOH solution was added. Another flask containing 10 mL of 4% boric acid was prepared taken. The water was allowed to boil so that steam entered into the Kjeldahl flask containing the sample. Fumes of ammonia gas were produced and passed through the condenser into the 4% solution of boric acid until the boric acid became colorless.

The colorless solution of the boric acid was titrated with N/10 H_2SO_4 until the color of the boric acid reappeared. The volume of acid used was recorded. The % N in the sample was then determined using the following formula:

$$\text{\% of Nitrogen} = \text{Reading of N/10 vol. used} \times \text{factor} \times \text{vol. used for dilution} \times 100$$

$$\text{Sample in g} \times \text{prepared sample in mL}$$

4.4.8. Analysis of Phosphorus in Compost

The sample (BFC) was ground, and a suitable acid, such as concentrated nitric acid (HNO3) or a mixture of nitric acid and perchloric acid (HNO_3 + $HClO_4$), was added to the sample for digestion. The mixture was heated in the incubator until the sample was completely digested and a clear solution was obtained. After digestion, the solution was cooled at room temperature. The solution was then filtered by using filter paper to remove any undigested residue. The solution was diluted 10 times. The phosphorus concentration in the sample was determined using a spectrophotometer at UV- 1800 nm via the molybdenum blue method [39,40].

4.4.9. FTIR Analysis of Compost

FTIR analysis was performed following the method reported by Lin et al. [41]. A 1 mg sample was ground and mixed with 200 mg of pre-dried KBr, pressed in a die at 10,000–15,000 lb/m^2, and held under air pressure for 3 min to form a KBr pellet. Light transmissivity was measured using a Shimazu FTIR spectrophotometer (Kyoto, Japan) over a range of 4000 to 400 cm^{-1}.

5. Conclusions and Future Suggestions

The biofortification of pressmud generated from the sugarcane industry through the application of microbial strains is an environmentally friendly technique. In this study, *Bacillus amyloliquefaciens*-ASK11 and *Bacillus megaterium*-ASNF3 enhanced nitrogen content up to 129% and phosphorus up to 91% after 60 days of composting of bioaugumented composts. This approach effectively degrades waste mass and recycles nutrients, thereby improving compost efficacy. The use of these bacterial strains facilitates the efficient utilization of by-products from sugarcane factories. Consequently, this waste material can be transformed into biofertilizers to enhance both the quality and quantity of crops, ultimately supporting increased agricultural yields to meet the demands of a growing population. Future studies regarding optimizing environmental factors (such as temperature, moisture, and aeration) during composting to enhance microbial activity could be explored to deepen our understanding and enhance the application of this environmentally friendly approach. Future large-scale field trials to assess the long-term impact of biofortified pressmud on different crops would help to determine the specific application rates and the benefits to particular plant types and overall crop yields.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/recycling9060104/s1, Table S1: Moisture content of different pressmud compost groups enriched with/without phosphorus solubilizing and N-fixing bacteria (E1: indigenous bacteria; E2: indigenous bacteria + *B. amyloliquefaciens*-ASK 11; E3: indigenous bacteria + *B. megaterium*-ANF3) after varying time intervals (Days); Table S2: Temperature (°C) of different pressmud compost groups enriched with/without phosphorus solubilizing and N-fixing bacteria (E1: indigenous bacteria; E2: indigenous bacteria + *B. amyloliquefaciens*-ASK 11; E3: indigenous bacteria + *B. megaterium*-ANF3) after varying time intervals (Days).; Table S3: pH of different pressmud compost groups enriched with/without phosphorus solubilizing and N-fixing bacteria (E1: indigenous bacteria; E2: indigenous bacteria + *B. amyloliquefaciens*-ASK 11; E3: indigenous bacteria + *B. megaterium*-ANF3) after varying time intervals (Days); Table S4: Electrical conductivity (mS/cm) of different compost groups enriched with/without phosphorus solubilizing and N-fixing (E1: indigenous bacteria; E2: indigenous bacteria + *B. amyloliquefaciens*-ASK 11; E3: indigenous bacteria + *B. megaterium*-ANF3) after varying time intervals (Days); Table S5: Organic matter (ash %) of different pressmud compost groups enriched with/without phosphorus solubilizing and N-fixing bacteria (E1: indigenous bacteria; E2: indigenous bacteria + *B. amyloliquefaciens*-ASK 11; E3: indigenous bacteria + *B. megaterium*-ANF3) after varying time intervals (Days); Table S6: Total nitrogen concentration (mg/g) of different press mud compost groups enriched with/without phosphorus solubilizing and N-fixing bacteria (E1: indigenous bacteria; E2: indigenous bacteria + *B. amyloliquefaciens*-ASK 11; E3: indigenous bacteria + *B. megaterium*-ANF3) after varying time intervals (Days); Table S7: Total phosphorus concentration (mg/g) of different pressmud compost groups enriched with/without phosphorus solubilizing and N-fixing bacteria (E1: indigenous bacteria; E2: indigenous bacteria + *B. amyloliquefaciens*-ASK 11; E3: indigenous bacteria + *B. megaterium*-ANF3) after varying time intervals (Days).

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