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PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan



Research Article

Enrichment of Empty Oil Palm Fruit Bunch Biochar with Actinomycetes Bacteria as a Microbial Inoculant Carrier

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Abstract

Background and Objective: Biochar is widely recognized for its porous structure, stability and large surface area, making it a promising carrier for beneficial soil microorganisms. However, limited information exists regarding its efficiency in supporting actinomycete enrichment at varying bacterial densities. This study addressed this gap by evaluating the colonization potential of biochar enriched with actinomycetes. The objective was to determine the optimal bacterial density and dilution level for maximizing colony formation under controlled laboratory conditions. **Materials and Methods:** The experiment was conducted from July to October 2024 at the Laboratory of Plant Biotechnology, Centre for Research Activities (CRA), Hasanuddin University, using a randomized group design with three replications. Actinomycetes were inoculated onto biochar at three bacterial densities (10^4 , 10^8 and 10^{12} CFU/mL) and three dilution levels (10^{-5} , 10^{-6} and 10^{-7}). Colony observations were recorded weekly for four weeks. Visual colony morphology was documented and environmental conditions (pH, temperature and humidity) were monitored. Data were tabulated and analyzed using ANOVA and significant treatment effects ($p < 0.05$ or $p < 0.01$) were further compared using Tukey's HSD test at $\alpha = 0.05$. **Results:** Actinomycete isolates consistently exhibited round, raised colonies with irregular edges and smooth to wrinkled surfaces. The highest average colony count occurred at pH 7.93, 24.8°C and 66% humidity. The 10^8 CFU/g enrichment showed the greatest colony formation during the first week, reaching 4.5×10^9 CFU/g, significantly higher than the 10^4 and 10^{12} CFU/g treatments. Among dilution levels, 10^{-7} produced the highest colony count (4.5×10^9 CFU/g), showing a significant difference compared with 10^{-6} and 10^{-5} . **Conclusion:** Biochar demonstrated strong potential as a suitable carrier medium for actinomycete enrichment, with the 10^8 CFU/g density and 10^{-7} dilution yielding optimal growth. These findings highlight biochar's promise as an effective microbial inoculant for enhancing soil health and supporting sustainable agriculture. Further research should explore field-scale validation and long-term microbial stability.

Key words: Biochar, pyrolysis organic waste, actinomycetes, microbial stability

Citation: Ady, M.R., B. Patandjengi, Nasaruddin and I. Ridwan, 2026. Enrichment of empty oil palm fruit bunch biochar with actinomycetes bacteria as a microbial inoculant carrier. Pak. J. Biol. Sci., 29: 121-130.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sustainable agriculture is important for meeting long-term agricultural needs while using natural resources without damaging the environment. However, land resources are currently degrading due to excessive use of inorganic fertilisers, over-exploitation of groundwater, poor drainage leading to increased soil salinity, rising chemical residue levels in groundwater and loss of biodiversity caused by long-term monoculture¹.

Organic fertilisers containing microorganisms are an effective way of supporting sustainable plant growth. These fertilisers can break down organic compounds in the soil into simpler compounds and form new ones. They also function as soil conditioners by changing the soil's physical condition into stable aggregates. There are many other beneficial functions of organic fertilisers for the soil itself². Microbial fertilisers can also enhance the decomposition of organic matter in the soil, increasing nutrient availability³.

Biochar is a natural soil conditioner formed by burning biomass or organic material in the absence of oxygen. This process enables the soil to utilise it as a source of organic matter. It is highly effective and can remain in the soil for hundreds to thousands of years once mixed in. It is derived from agricultural residues or waste, such as wood, empty oil palm fruit bunches, coconut shells and rice husks and can enhance soil productivity by improving its physical, chemical and biological properties. It accumulates in the soil, maintaining a balance of properties to minimise the risk of nutrient leaching and ultimately improves environmental quality⁴.

Using organic materials, such as biochar, is one strategy for managing the environment in agriculture. Various studies have shown that biochar can enhance the physical, chemical and biological properties of soil, thereby promoting plant growth⁵. Biochar can significantly improve soil quality, such as water-holding capacity, soil aggregate stability and nutrient retention^{6,7}. Biochar can also improve retention⁸ and increase organic carbon and phosphorus content⁹.

Given the availability of agricultural waste as a raw material for biochar, the potential for its utilisation is enormous, especially for waste that is difficult to decompose or has a high C/N ratio. Empty oil palm fruit bunches (EOPFB) are a promising source of biochar. In Indonesia, agricultural waste such as EOPFB, oil palm shells and corn cobs has not yet been fully utilised as a source of organic material¹⁰. This biomass is produced continuously in significant quantities. Indonesia has the largest oil palm plantation area in Asia, spanning 16.38 million hectares and produces 50.07 million

tonnes of crude palm oil (CPO) per year. During production, approximately 70-90% of the total oil palm biomass remains as residual material¹¹. This equates to 45.06 million tonnes of waste with the potential to be used as a source of organic material, 23% of which is EOPFB and 5.5% palm kernel shells¹².

Due to its properties that are highly conducive to microbial life, biochar is a highly effective carrier for microbial inoculants, particularly in agricultural and environmental applications. Rich in organic matter, nitrogen, phosphorus and potassium, biochar possesses high porosity and water-holding capacity. Its high porosity and specific surface area provide space for microbial life. The abundance of functional groups, such as hydroxyl, carboxyl, sulfonic acid, amino and hydroxyl-carboxyl, is highly effective in promoting microbial cell adhesion and proliferation, thereby enhancing microbial persistence. Biochar's good physicochemical properties allow for the immobilisation of bacteria on its surface, thereby removing organic contamination¹³.

Soil microorganisms play a crucial role in maintaining soil health and productivity. They support sustainable agricultural production by enhancing plant growth and increasing resistance to biotic and abiotic stress. They also restore soil contamination, recycle nutrients, manage soil fertility, mineralise rocks and improve the efficiency of fertiliser and pesticide use in agriculture¹⁴. It is important to understand the role of soil microbes, which include various organisms such as bacteria, fungi and microscopic algae that play an important role in the soil nutrient cycle and the decomposition of organic matter, thereby increasing nutrient availability for plants. They also contribute to pest and disease control processes through complex interactions with plants¹⁵.

The presence of microorganisms, such as bacteria, fungi, protozoa and nematodes, on and in the soil surface will affect a plant's ability to obtain the macro- and micronutrients necessary for growth. Biochar stimulates the formation of a new ecosystem, creating a mutualistic symbiosis between microorganisms and plant roots. This expands the roots' range to obtain the necessary nutrients and water for growth¹⁶. Actinomycetes bacteria are among the most commonly found microorganisms that form a mutualistic symbiosis with plant root systems¹⁷. Several types of these bacteria can produce plant growth-promoting (PGP) substances in the root system, supporting plant growth and the solubilisation of phosphorus¹⁸, as well as the production of siderophores¹⁹. Actinomycetes play a crucial role in decomposing organic matter and producing bioactive compounds that benefit plants. These bacteria are widely distributed throughout soil ecosystems. They are highly adaptable to extreme conditions and play a significant role in supporting plant growth, particularly in the rhizosphere²⁰.

This study demonstrates that, due to its high organic carbon content and the presence of nutrients such as nitrogen, phosphorus and potassium that support microbial life, biochar is an effective carrier for actinomycete microbial inoculants in agricultural and environmental applications.

MATERIALS AND METHODS

Place and time: This study was conducted at the Plant Biotechnology Laboratory, Centre for Research Activities (CRA) of Hasanuddin University, from July, 2024 to October, 2024.

Tools and materials: The equipment used included: Pyrolyser, Microscope, Infrared Thermometer (MESTEK), pH meter, cutting tool (knife/machete), digital scale, petri dishes, spatula, laminar air flow (LAF), incubator (Memmert), autoclave (All American), electric stove (Masption), analytical scale (Radwag), Petri dishes (Pyrex), glass beakers (Pyrex), 1 mL volume pipettes (Pyrex), Erlenmeyer flasks (Pyrex), reaction tubes (Pyrex), spatulas, forceps, Bunsen burners, tweezers, knives, cameras and other writing tools. The materials used were NA medium (Nutrient Agar), empty oil palm fruit bunches (TKKS), Actinomycetes bacteria, NA medium (Nutrient Agar), SNB medium (Starch Nitrate Broth), distilled water and 70% ethanol.

Research methodology: This study was conducted as an experiment using a randomised block design (RBD) with six treatments and three replications. Biochar enrichment using actinomycete bacteria consisted of three bacterial density levels: Bacterial density 10^4 (k1), bacterial density 10^8 (k2) and bacterial density 10^{12} (k3), with three dilution levels: 10^{-5} (p1), dilution 10^{-6} (p2) and dilution 10^{-7} (p3). There were nine treatment combinations for each replicate. Actinomycetes bacterial inoculation was performed on sterilised, empty oil palm bunch biochar samples at bacterial density concentrations of 10^4 , 10^8 and 10^{12} before being stored in an incubator. The bacterial inoculum source was isolated by placing one gram of biochar material that had been isolated for one week into a reaction tube containing 9 mL of water solution. This was then diluted sequentially to concentrations of 10^{-5} , 10^{-6} and 10^{-7} . From each dilution tube (dilutions 10^{-5} , 10^{-6} and 10^{-7}), 0.1 mL of the bacterial suspension was transferred to a Petri dish containing solid medium. The bacteria were spread evenly across the entire surface of the medium using a spatula. The bacteria were then incubated for 2-3 days. After this time, observations and calculations of the

parameters of the emerging bacterial colonies were conducted. The same process was performed in the second, third and fourth weeks after the biochar was inoculated with bacteria. Periodic observations and calculations were conducted for pH, biochar temperature, moisture/water content and the total number of actinomycete bacterial colonies during the four consecutive weeks. The number of colonies was expressed in Colony Forming Units (CFU) and calculated using the formula²¹:

$$\text{CFU/gr} = \frac{\text{Number of actinomycetes colonies}}{\text{Dilution factor}} \times 1$$

Data analysis: Data is collected and tabulated in a table. The data is then analysed using Analysis of Variance (ANOVA). Treatment effects showing significant ($p < 0.05$) or highly significant ($p < 0.01$) will be further analysed using Tukey's significant difference test (HSD) at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Microbial morphology: Table 1 shows the morphology of actinomycete bacteria isolates from the first week to the fourth week. From observing the isolates weekly, it can be seen that, visually, all isolates generally show the same morphology due to the same inoculum source. Isolates with a dilution of 10^{-5} (p1) in all density treatments-Bacterial Density 10^4 (k1), Bacterial Density 10^8 (k2) and Bacterial Density 10^{12} (k3)-clearly showed a greater number of colonies than dilutions of 10^{-6} (p2) and 10^{-7} (p3) in each observation period. However, the average of the largest number of colonies was 4.5×10^9 CFU/g in bacterial isolates with a bacterial density of 10^8 (k2), at a dilution level of 10^{-7} (p3), in week 1 after inoculation. The same was true for bacterial isolates with the same treatment in weeks 2, 3 and 4.

Across bacterial density and dilution levels, the actinomycete isolates exhibited clear differences in colony density and morphology (Table 1). During Week 1, colonies were small and sparse at higher dilutions but dense at 10^{-5} (p1). By Week 2, colony numbers and diameters increased across all treatments, with bacterial density of 10^8 CFU/g (k2) and 10^{12} CFU/g (k3) showing visibly higher growth intensity. In Week 3, colonies developed typical Actinomycetes characteristics, including chalky and powdery textures, with greater expansion at lower dilutions. By Week 4, colonies in k2 (10^8 CFU/g) and k3 (10^{12} CFU/g) at 10^{-5} (p1) began to merge due to extensive growth, while higher dilutions maintained

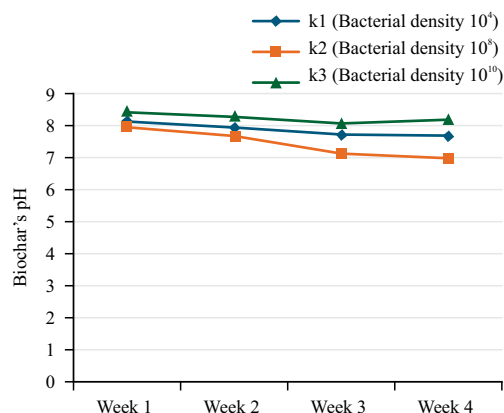


Fig. 1: pH values of the biochar after inoculation with actinomycetes bacteria at weeks 1, 2, 3 and 4

Table 1: Colonies of actinomycetes bacterial isolates in different dilution levels

Week	Bacterial density (k) and dilution level (p)								
	k1 (10 ⁴ CFU/g)			k2 (10 ⁸ CFU/g)			k3 (10 ¹² CFU/g)		
	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷
1									
2									
3									
4									

well-separated, countable colonies. Overall, higher bacterial densities consistently produced more abundant and faster-maturing colonies, while increasing dilution effectively reduced colony number and improved separation.

Biochar chemical characteristics due to actinomycetes inoculation: Figure 1 shows the changes in the pH values of the biochar following the inoculation of the actinomycete bacteria from week 1 to week 4. The graph shows that the pH of the biochar ranges from 6.94 to 8.41. In general, the pH decreased in all bacterial density treatments from week 1 to week 4. The lowest pH (6.94) occurred in biochar with a bacterial density of 10⁸ (k2) in week 4, while the highest pH (8.41) occurred in biochar with a bacterial density of 10¹² (k3)

in week 1. The largest colony count, 4.5 × 10⁹ CFU/g, was found in biochar bacterial isolates with a bacterial density of 10⁸ (k2), a dilution of 10⁻⁷ (p3) and a pH of 7.93 in week 1 after inoculation.

Figure 2 shows the results of observations of biochar temperature after inoculation of actinomycete bacteria from weeks 1 to 4. The lowest temperature, 24.8°C, occurred in biochar with a bacterial density treatment of 10⁸ (k2), while the highest temperature, 27.3°C, occurred in biochar with a bacterial density treatment of 10¹² (k3). The largest colony count, 4.5 × 10⁹ CFU/gram, was found in biochar bacterial isolates with a bacterial density treatment of 10⁸ (k2), a dilution of 10⁻⁷ (p3) and a temperature of 24.8°C which occurred in week 1 after inoculation.

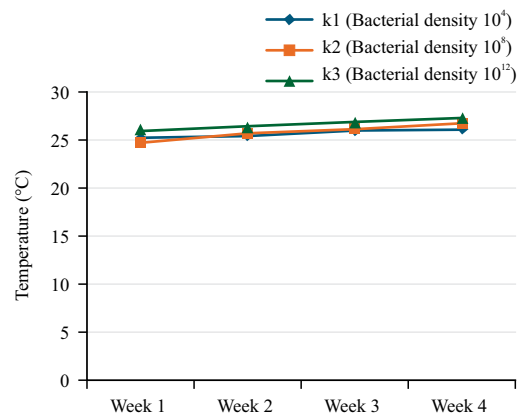


Fig. 2: Biochar temperature after inoculation with actinomycetes bacteria at weeks 1, 2, 3 and 4

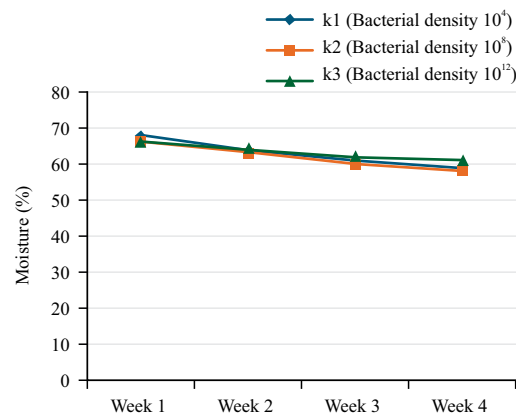


Fig. 3: Biochar moisture content after inoculation with actinomycetes bacteria at weeks 1, 2, 3 and 4

Figure 3 shows that the moisture in biochar continues to decline from week 1 to week 4 after inoculation with Actinomycetes bacteria. The decrease in biochar moisture values occurred in all bacterial density treatments. The 10⁴ bacterial density (k1) decreased biochar moisture from 68% in the first week to 59% in the fourth week. The 10⁸ bacterial density (k2) decreased biochar moisture from 66% in the first week to 58% in the fourth week, as did the 10¹² bacterial density (k3), which decreased from 66% in the first week to 61% in the fourth week. The decrease in biochar moisture was slow up to week four as the temperature value increased in each bacterial density treatment. The largest colony count, 4.5 × 10⁹ CFU/g, was found in the biochar bacterial isolate with a bacterial density of 10⁸ (k2), a dilution of 10⁻⁷ (p3) and 66% humidity. This occurred in week 1 after inoculation.

Microbial colonization of actinomycetes: Figure 4 presents the results of observations of the average number of Actinomycetes bacterial colonies in weeks 1, 2, 3 and 4 after inoculation. The Fig. 4 presents the temporal changes in

Actinomycete colony counts in oil palm empty fruit bunch (EFB) biochar inoculated with three bacterial densities of 10⁴ CFU/g, 10⁸ CFU/g and 10¹² CFU/g (Fig. 4c), shown by Fig. 4a-c, respectively, over four weeks. Across all treatments, colony counts generally increased between Weeks 1-2, peaked or stabilized around Week 3 and then shifted differently depending on density. Lower initial densities showed steady but moderate colonization, while medium densities produced sustained growth. In contrast, the highest bacterial density exhibited a dynamic boom-collapse-recovery pattern, reflecting density-dependent microbial interactions within the biochar environment.

At low density of 10⁴ CFU/g (Fig. 4a), the number remained relatively stable across the four weeks, with only minor fluctuations among dilution levels. A modest increase occurred in Week 3, particularly at 10⁻⁵, suggesting slow but steady colonization of the biochar matrix. In medium density (10⁸ CFU/g), the treatment exhibited the strongest early response, with colony counts increasing sharply in Week 2 across all dilutions (Fig. 4b). This was followed by a gradual

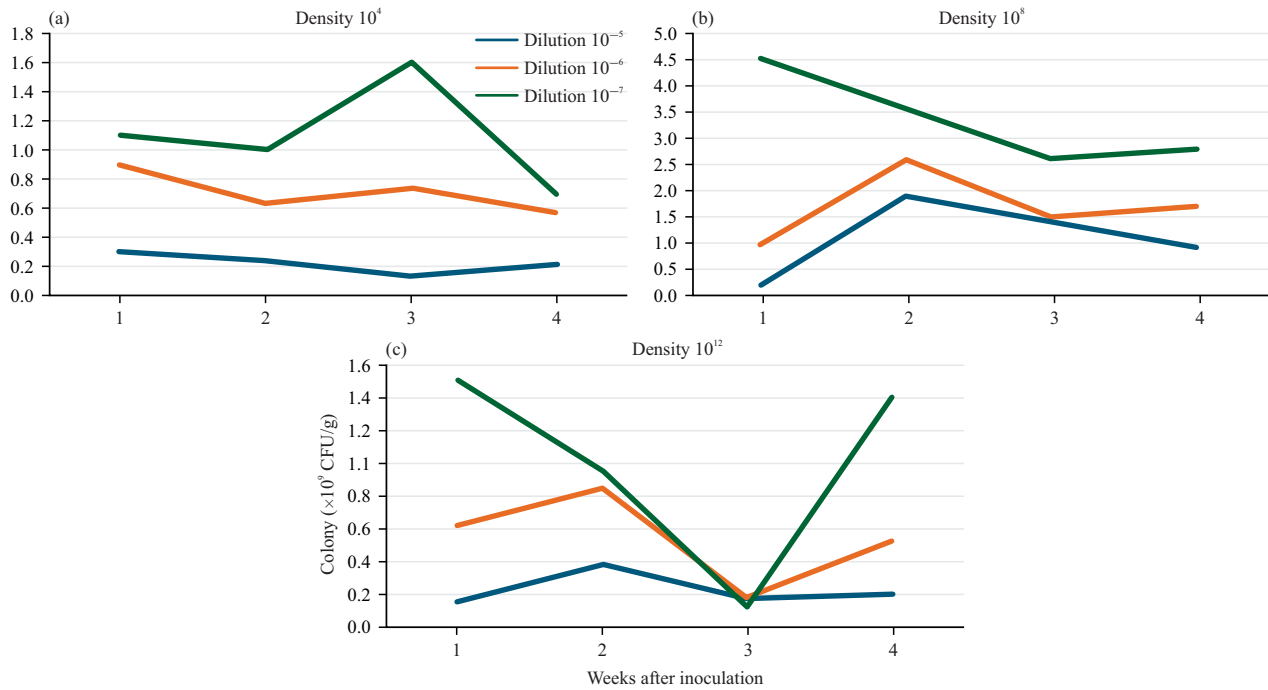


Fig.4(a-c): Changes in actinomycete colony counts in oil palm empty fruit bunch (EFB) biochar at different bacterial densities: (a) 10^4 CFU/g, (b) 10^8 CFU/g and (c) 10^{12} CFU/g, across dilution levels during weeks 1-4 after inoculation

Table 2: Average number of actinomycetes colonies in the oil palm empty fruit bunch biochar of week 1 after inoculation

Bacterial density (K)	Dilution level (P)		
	10^{-5} (p1)	10^{-6} (p2)	10^{-7} (p3)
10^4 (k1)	3.0×10^{9a} _q	8.9×10^{9a} _{pq}	1.1×10^{10b} _p
10^8 (k2)	2.2×10^{9a} _r	1.0×10^{9a} _q	4.5×10^{9a} _p
10^{12} (k3)	2.2×10^{9a} _q	6.3×10^{9a} _q	1.5×10^{10b} _p

Numbers followed by the same letter in column (a,b) and row (p,q,r) are not significantly different in the BNT test α at 0.05

decline toward Week 4. The pattern indicates that this inoculation level supported rapid establishment followed by stabilization as microbial competition increased. On the other hand, at 10^{12} CFU/g (Fig. 4c), colony counts dropped substantially in Week 3 at all dilution levels. This decline was followed by a clear recovery in Week 4, most prominently at the lowest dilution (10^{-5}). The mid-study drop suggests temporary inhibition likely due to overcrowding or nutrient limitation before microbial regrowth resumed.

The Tukey's test ($\alpha = 0.05$) in Table 2 showed that the average number of colonies in week 1 was highest on biochar with a bacterial density of 10^8 (k2), which is 4.5×10^9 CFU/g, which is significantly different from the bacterial densities of 10^{12} (K3) and 10^4 (K1). At the level of biochar dilution, the highest number of colonies occurred at a dilution of 10^{-7} (P3), which is 4.5×10^9 CFU/g, which is significantly different from the dilution of 10^{-6} (p2) and extremely different from the dilution of 10^{-5} (p1).

The Tukey's test ($\alpha = 0.05$) shows that the average number of colonies in week 2 (Table 3) is highest on biochar with a bacterial density of 10^8 (k2), which is 2.7×10^9 CFU/g. This is significantly different from bacterial densities of 10^{12} (k3) and 10^4 (k1). The highest number of colonies occurred at a biochar dilution level of 10^{-7} (p3), with 1.8×10^9 CFU/g. This is significantly different from the 10^{-5} (p1) dilution but not significantly different from the 10^{-6} (p2) dilution.

The Tukey's test ($\alpha = 0.05$) shows that the average number of colonies at week 3 in Table 4 is highest on biochar with a bacterial density of 10^8 (k2), which is 1.9×10^9 CFU/g, which is significantly different from the bacterial densities of 10^4 (k1) and 10^4 (k3). At the biochar dilution level, the highest number of colonies occurred at a dilution of 10^{-7} (p3), which was 1.8×10^9 CFU/g, which is significantly different from the dilutions of 10^{-6} (p2) and 10^{-5} (p1).

Table 3: Average number of actinomycetes colonies in the oil palm empty fruit bunch biochar in week 2 after inoculation

Bacterial density (K)	Dilution level (P)			Average
	10 ⁻⁵ (p1)	10 ⁻⁶ (p2)	10 ⁻⁷ (p3)	
10 ⁴ (k1)	2.4 × 10 ⁸	6.3 × 10 ⁸	1.0 × 10 ⁹	6.2 × 10 ^{8b}
10 ⁸ (k2)	1.9 × 10 ⁹	2.6 × 10 ⁹	3.6 × 10 ⁹	2.7 × 10 ^{9a}
10 ¹² (k3)	3.8 × 10 ⁸	8.5 × 10 ⁸	9.6 × 10 ⁸	7.3 × 10 ^{8b}
Average	8.7 × 10 ^{8q}	1.3 × 10 ^{9pq}	1.8 × 10 ^{9p}	

Numbers followed by the same letter in column (a,b) and row (p,q) are not significantly different in the BNT test $\alpha = 0.05$

Table 4: Average Number of Actinomycetes colonies in the oil palm empty fruit bunch biochar in Week 3 after inoculation

Bacterial density (K)	Dilution level (P)			Average
	10 ⁻⁵ (p1)	10 ⁻⁶ (p2)	10 ⁻⁷ (p3)	
10 ⁴ (k1)	1.3 × 10 ⁸	7.4 × 10 ⁸	1.6 × 10 ⁹	8.2 × 10 ^{8b}
10 ⁸ (k2)	1.4 × 10 ⁹	1.5 × 10 ⁹	2.6 × 10 ⁹	1.9 × 10 ^{9a}
10 ¹² (k3)	1.7 × 10 ⁸	1.8 × 10 ⁸	1.2 × 10 ⁹	5.4 × 10 ^{8b}
Average	5.8 × 10 ^{8q}	8.3 × 10 ^{8q}	1.8 × 10 ^{9p}	

Numbers followed by the same letter in column (a,b) and row (p,q) are not significantly different in the BNT test $\alpha = 0.05$

Table 5: Average number of actinomycetes colonies in the oil palm empty fruit bunch biochar of week 4 after inoculation

Bacterial density (K)	Dilution level (P)			Average
	10 ⁻⁵ (p1)	10 ⁻⁶ (p2)	10 ⁻⁷ (p3)	
10 ⁴ (k1)	2.1 × 10 ^{8b} _p	5.7 × 10 ^{8b} _p	7 × 10 ^{8b} _p	
10 ⁸ (k2)	9.3 × 10 ^{8a} _r	1.7 × 10 ^{9a} _q	2.8 × 10 ^{9a} _p	
10 ¹² (k3)	2 × 10 ^{8b} _q	65.2 × 10 ^{8b} _q	1.4 × 10 ^{9b} _p	

Numbers followed by the same letter in column (a,b) and row (p,q,r) are not significantly different in the BNT test $\alpha = 0.05$

The Tukey's test ($\alpha = 0.05$) shows that the average number of colonies at week 4 in Table 5 is highest on biochar with a bacterial density of 10⁸ (k2), which is 2.8 × 10⁹ CFU/g, which is significantly different from the bacterial density of 10¹² (k3) and very significantly different from the bacterial density of 10⁴ (k1). At the dilution level, the highest number of colonies occurred at a dilution of 10⁷ (p3), which was 2.8 × 10 CFU/g, which is significantly different from the dilution level of 10⁶ (p2) and significantly different from the dilution level of 10⁵ (p1).

DISCUSSION

The isolates generally appear round, with raised and convex elevations and flat, irregular edges. They have a smooth or rough/wrinkled surface and grow high above the medium. Like bacteria, actinomycetes are unicellular and lack a distinct cell wall. However, they can produce a non-septate, slender mycelium. Actinomycetes colonies are typically hard and rough and grow high above the surface of the medium. They form a flour-like consistency and adhere firmly to the surface of agar, producing fungus-like hyphae and spores²².

Actinomycetes bacteria colonies appear slowly, showing a powdery consistency and being firmly attached to the agar surface²³. Young actinomycete colonies resemble bacteria in

general, with round, convex, slippery surfaces that are firmly attached to the agar medium. These bacteria are Gram-positive and have a mushroom-like cell shape because their cells are mycelia that branch to form monopodial branching, ranging in length from 0.3 to 4 μ m. The mycelium is a vegetative hypha that grows on the surface, penetrates and fragments when isolated in an agar medium.

Actinomycetes constitute a diverse group of prokaryotic microorganisms displaying substantial variation in cellular organization. While some members occur as simple unicellular forms, others develop extensive filamentous networks that resemble fungal hyphae in overall appearance. These filamentous systems may either grow along the surface of the growth medium or extend above it, forming aerial structures²⁴. Reproduction in certain actinomycete taxa occurs through the formation of spores, a feature that historically contributed to their classification as organisms bridging characteristics of bacteria and fungi. Nevertheless, molecular and ultrastructural evidence firmly places actinomycetes within the bacterial domain, as they possess prokaryotic chromosomes and cell walls dominated by peptidoglycan²⁵.

Soil ecosystems represent the main ecological niche for actinomycetes, where these microorganisms form a substantial fraction of the resident microbial community. Their population density in soil can reach millions to billions of cells

per gram, highlighting their ecological dominance. Adaptation to this complex and competitive environment is supported by their remarkable metabolic versatility, particularly their ability to synthesize a wide array of extracellular enzymes. These enzymes enable actinomycetes to mineralize diverse organic compounds, facilitating nutrient acquisition from complex soil organic matter²⁶. Through enzymatic hydrolysis, actinomycetes are capable of degrading structurally recalcitrant polymers such as cellulose and lignin derived from plant residues, as well as chitin originating from arthropod remains, thereby playing a crucial role in soil carbon and nutrient cycling²⁷.

Microorganisms are sensitive to variations in environmental pH. Under field conditions, the optimal pH range for reproduction and viability is 6-. Acidity (pH) is one of the environmental factors that influence bacterial activity and growth, affecting enzyme activity and cell permeability and consequently metabolism and growth. Actinomycetes are acid-tolerant, rod-shaped, Gram-positive, anaerobic or facultatively anaerobic bacteria. However, Actinomycetes are not tolerant of acidic conditions and their numbers decrease in environments with a pH below 5. The interaction between biochar pH and bacterial density treatment affects actinomycete density. A recent 2025 review on the effects of liming and soil microbial distribution reported that actinomycetes are predominantly abundant in soils with neutral to slightly alkaline pH (approximately 6.5-0). Nevertheless, the review also highlighted the presence of acidophilic and acid-tolerant actinomycete taxa in acidic soils, indicating that although lower pH conditions generally suppress overall actinomycete abundance, certain taxa are able to persist across a wider pH range²⁸. Biochar pH is neutral to slightly alkaline and can provide some pH buffering capacity. Biochar commonly exhibits a neutral to moderately alkaline reaction, enabling it to influence soil chemical conditions through its buffering capacity²⁹. When biochar contains substantial amounts of basic cations, including calcium, magnesium and potassium, its application can contribute to the moderation of soil acidity. This adjustment in soil pH not only improves the chemical environment for plant growth but also limits the mobility and bioavailability of potentially harmful metal ions, particularly aluminium, within the soil matrix.

Actinomycetes are aerobic, grow slowly and require a suitable growth temperature of 25-37°C. They are large in size and tend to form filaments³⁰. The optimum growth temperature is between 28 and 30°C, but some actinomycetes, especially the genera *Thermoactinomycetes* and *Streptomyces*, can grow in large numbers at

temperatures between 55 and 65°C²³. Stagnant water is not suitable for the growth of actinomycetes, while dry or semi-dry conditions can support large populations.

The appropriate humidity for the growth of Actinomycetes is 85%. Dry soil conditions form conidium. Biochar's ability to retain water (between biochar colloids) maintains moisture well. Adhikari *et al.*³¹ found that there is an increase in water holding capacity (WHC) in loamy sand soil after it is given biochar; the higher the proportion of biochar, the higher the soil's ability to hold water. Biochar can significantly improve soil quality by enhancing water holding capacity, soil aggregate stability and nutrient retention^{6,7}. Biochar can also improve water retention and increase organic carbon and phosphate content⁹.

Observations of actinomycete colony development across different inoculum density treatments indicate that biochar functions as an effective carrier material for microbial enrichment. Its suitability as a microbial support matrix arises from a combination of physicochemical characteristics that promote microbial attachment, persistence and activity in both agricultural and environmental contexts. Properties such as surface charge, pore architecture, functional groups and pH collectively influence the interaction between biochar and microbial cells. The highly porous structure and extensive specific surface area of biochar create protected microhabitats that facilitate microbial colonization and survival. These internal pore spaces not only shield microorganisms from biotic stressors, including predation by protozoa and soil fauna, but also provide reactive sites capable of binding organic and inorganic contaminants. As a result, biochar supports concurrent contaminant adsorption and microbially mediated degradation processes³².

Physicochemical characterization of biochar derived from oil palm empty fruit bunches indicated a near-neutral reaction and moderate nutrient content, with pH 6.85 organic carbon 19.25%, total nitrogen 0.95%, a C/N ratio of 20, phosphorus 0.21%, magnesium 0.58%, potassium 1.74% and a cation exchange capacity of 32.65. These properties suggest that the material provides both chemical and structural advantages when applied to soil systems. The well-developed pore structure of enriched biochar enables it to function as a reservoir for nutrients while simultaneously acting as an effective carrier for microbial inoculants, thereby enhancing the retention and delivery of nutrients and beneficial microorganisms in soil environments³³. Moreover, the favorable surface chemistry of biochar supports the attachment and stabilization of bacterial cells, facilitating the immobilization of microorganisms involved in the transformation or removal of organic pollutants¹³. The presence of diverse surface functional groups, including

hydroxyl, carboxyl, sulfonic, amino, imino and acylamino moieties, further promotes microbial adhesion and growth, reinforcing the role of biochar as a multifunctional amendment for soil improvement.

Biochar possesses a suite of physicochemical properties that collectively contribute to its capacity to stimulate microbial activity in soil systems. Characteristics such as a well-developed pore network, high water-holding potential, elevated pH and enrichment in organic carbon, essential macronutrients and cation exchange capacity create favorable conditions for microbial metabolism and persistence. The abundance of organic carbon and nutrients, including nitrogen, phosphorus and potassium, combined with enhanced porosity and moisture retention, enables biochar to function as a supportive microenvironment for soil microorganisms³⁴. When applied as a carrier for microbial inoculants, biochar has been shown to improve microbial establishment and longevity, promoting greater colonization and proliferation within bulk soil and the rhizosphere³⁵. Consequently, biochar-based microbial formulations have demonstrated effectiveness in supporting plant growth and facilitating the rehabilitation of polluted soils.

CONCLUSION

The study found that the highest average number of actinomycete bacterial colonies on biochar occurred in week 1 after inoculation at a bacterial density of 10^8 (k2), showing a significant difference compared with densities of 10^4 (k1) and 10^{12} (k3). Similarly, the greatest colony formation was recorded at a dilution level of 10^{-7} (p3), reaching 4.5×10^9 CFU/g, which was significantly higher than the colony count at 10^{-6} (p2) and very significantly higher than at 10^{-5} (p1). Optimal colony growth was observed under environmental conditions of pH 7.93, temperature 24.8°C and 66% humidity. Overall, the findings confirm that biochar serves as an effective carrier medium for actinomycete microbial inoculants, supported by its high organic carbon content and essential nutrients such as nitrogen, phosphorus and potassium that enhance microbial activity in agricultural and environmental applications.

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