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Performance of biofilm enriched Eppawala rock phosphates over triple super phosphates in rice cultivation

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Abstract

Eppawala Rock Phosphates (ERP) has a greater potential to be used as an alternative for Triple Super Phosphate (TSP) if phosphorous (P) bio-solubility is increased. A certain biofilm (BF3) has been identified as the most efficient P bio-solubilizer for ERP. Thus, this study was designed to test the potential of biofilm-enriched ERP to replace the TSP in rice cultivation. Two experiments were conducted; soil leaching tube and pot experiments under controlled conditions. A modified chemical fertilizer (CF_M) mixture was developed by replacing TSP from ERP in the existing chemical fertilizer (CF_E) mixture for rice recommended by the Department of Agriculture (DOA). Nitrogen (N) and potassium (K), levels were maintained according to the DOA recommendation. Eleven treatments were used with all possible combinations of CF_E and CF_M at 50% or 100% rates alone or together with the BF3. Soil alone was used as the control. Treatment of 50% CF_M + BF3 was denoted as biofilm-enriched ERP. The experiments were conducted in a Completely Randomized Design (CRD) with three replicates. Biofilm enriched ERP showed no added advantage over the CF_E, with lower cumulative solubilized

P in leachates. At the end of the pot experiment, biofilm-enriched ERP showed significantly ($p<0.05$) higher P retention in soil and significantly ($p<0.05$) lower grain yield compared to the CF_E. The overall results conclude that the biofilm-enriched ERP performed poorly in comparison to the DOA recommended TSP dosage. Thus, further studies are required to enhance the performance of biofilm-enriched ERP to use as an alternative for TSP in rice cultivation.

Keywords: *Biofilm, Eppawala rock phosphate, Rice cultivation, Triple super phosphate*

1. Introduction

Triple Super Phosphate (TSP) is the major P fertilizer in rice cultivation due to its high solubility and easy application. TSP is manufactured from rock phosphate excavated from the earth. Hence, TSP consists of many trace elements such as cadmium (Cd), nickel (Ni), copper (Cu), lead (Pb) etc. Further, the TSP contains Cd levels about 24 µg g^{-1}, which is higher than the maximum permissible levels (10 µg g^{-1}) identified by the Sri Lanka Standards Institution (SLSI). In comparison, ERP contains about 1.92 µg g^{-1} of Cd, much smaller than that in TSP (Premarathne *et al.,* 2011). According to a study conducted by Chandrajith *et al*. (2010), the agricultural fields where TSP has been used as the leading P supplement have recorded notable trace elements such as aluminium, chromium, nickel, cadmium, lead, and uranium. The results suggest that the long-term application of TSP can lead to contamination of soils with trace elements, highlighting the importance of finding an alternative P fertilizer in place of TSP. The ERP is considered a cheap and environmentally-friendly alternative to TSP, despite few constraints. Due to the low water solubility of ERP, its direct application as a P fertilizer has been limited to few perennial crops such as tea, rubber, coconut, export cash crops etc. Due to the slow release of P, ERP is not recommended for short-term crops (Appleton, 1994).

The solubility of ERP can be enhanced with Phosphorous solubilizing microorganisms (PSMs) present in biofilm mode. The National Institute of Fundamental Studies (NIFS) has developed biofilm formulations (BF1, BF2, BF3 and BF4) with bio-solubilizing ability of P in rock phosphates (Weerasundara *et al.,* 2014; Kumarihami *et al.,* 2015). Among them, BF3 was identified as the most effective biofilm formulation for bio-solubilization of ERP following a series of experiments conducted under laboratory conditions (Jayaneththi *et al.,* 2017). Thus, the present study was conducted to test the potential of replacing TSP with biofilm (BF3) enriched ERP in rice cultivation.

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2. Methodology

2.1 Initial soil analysis

Paddy soils (great soil group Reddish Brown Earth, Order - Alfisols, Sub order -Ustalfs, Great group -Rhodustalfs) (Panabokke, 1996) were collected from a farmer field, Puliyankulama, Anuradhapura, and analyzed for pH with 1:2.5 soil suspension using a Fisher Accumet AB 15 pH meter. The soil sub samples (5g) were analyzed for available P (Murphy and Riley, 2002) using 100 ml of 0.5 M sodium bicarbonate extracts (Olsen *et al.,* 1954)), available N (Bremner, 1982) using soil extracts from 100 ml of 1M KCl. Exchangeable K was determined using soil extracts from 100 ml of 1M ammonium acetate (Matula,1996) employing inductively coupled plasma-optical emission spectroscopy (ICP-OES; Thermo I CAP 6500 Duo). Microbial biomass C of soils were determined by the chloroform fumigation-incubation technique (Jenkinson and Powlson, 1976; Vance *et al.,* 1988) and organic carbon by digestion with an acid dichromate solution and titrating with ferrous ammonium sulphate (Nelson and Sommers, 1982) at the commencement of the experiment.

2.2 Leaching Column Experiment

The leaching column experiment was conducted in the Soil and Water Science Laboratory at the Faculty of Agriculture, Rajarata University of Sri Lanka. Paddy soil (RBE) was collected from a farmer field at Puliyankulama in Anuradhapura, which belongs to the DL_{1b} agro-ecological region with an average annual temperature of 27^0C and average annual rainfall of 1,368 mm.

2.2.1 Preparation of treatments

Biofilm biofertilizer (BFBF) for rice reduces the application of DOA recommended level of N, P_2O_5 and K_2O in chemical fertilizers (CF) by 50% (Seneviratne *et al.,* 2011), and hence, for the treatments 50% of CF_E and CF_M were used with the BFBF application, as explained in Table 1. In this study, BF3 was used as BFBF, since BF3 also acts as a PGP biofilm formulation (Weerasundara *et al.,* 2014; Kumarihami *et al.,* 2015). This prevented complicating the experiment, if two different biofilm formulations would have been applied separately to solubilize ERP-P and biofertilizer for the rice crop.

The biofilm-enriched ERP was developed by spraying BF3 on to ERP at a rate of 1.7 L per 100kg as recommended by the NIFS. The existing DOA chemical fertilizer (CF_E) for rice recommended in 2013 was modified (CF_M) by replacing TSP (22 kg ac^{-1} /55 kg ha^{-1}) from BF3-enriched ERP (37 kg ac^{-1}/ 92 kg ha^{-1}). This replacement was done by considering the

P_2O_5 contents of fertilizers. The average P_2O_5 contents of TSP and ERP were taken as 46% and 27% respectively (Dahanayake, 1988). Eleven treatments were used in the experiment and they were arranged in a completely randomized design with three replicates for each treatment (Table 1).

Table 1: The treatments used for the leaching tube experiment and pot experiment.

Treatments	
T1	100% CF_E
T2	50% CF_E
T3	50% CF_E+ BF3
T4	100% CF_M
T5	50% CF_M
T6	50% CF_M + BF3
T7	100% CF_E(only N,K)
T8	50% CF_E(only N,K)
T9	50% CF_E(only N,K)+ BF3
T10	BF3 (only)
T11	Control (soil alone)

CF_E -DOA recommendation for (N,P and K)-TSP as the sole P source

CF_M- DOA recommendation for (N, P and K)- BF3 enriched ERP as the sole P source

CF_E (only N, K) - DOA recommendation for (N and K)-only nitrogen and potassium

2.2.2 Leaching column study

Field soils and river sand were sieved (using a 2 mm sieve) separately before mixing them at a weight ratio of 1:1. The soil: sand mixture was sieved again using a 2 mm sieve before use them in the experiment. The leaching columns (50 ml) were filled with the 100g of soil: sand mixture and placed a glass wool pad (about ¼ - inch) on the soil surface to avoid dispersion. The initial weight of each column was measured and recorded. The measured amounts of fertilizers for the treatments were mixed thoroughly with 25 ml distilled water and added to each leaching tube. The rate of fertilizer applications (inorganic fertilizers and biofilm-enriched fertilizers) was aligned with the DOA and NIFS recommendations for rice (NIFS Annual Review Report, 2012). After adding of the treatments, columns were stoppered on top. Weights of all columns were measured once in 3 days and maintained the initial weight with the help of distilled water addition. The leachates were collected in two week intervals (Stanford and Smith, 1972) and analyzed for available P in leachates using molybdate-ascorbic acid method (Murphy and Riley, 2002). At the end of the study period (3 months), cumulative solubilized P was calculated. The soil remaining in the leaching columns were analyzed for biomass C using chloroform fumigation- incubation method (Jenkinson and Powlson, 1976; Vance *et al.,* 1998), soil organic carbon using acid dichromate digestion followed by titration with ferrous ammonium sulphate (Nelson and Sommers, 1982) and pH with 1.25 soil suspension using a Fisher Accumet AB 15 pH meter at the end of the 3 months.

2.3 Pot experiment

A greenhouse experiment was also conducted at the Faculty of Agriculture, Rajarata University of Sri Lanka. Thirty-three plastic pots (0.016 m^3) were used for 11 treatments in triplicate, and the pots were arranged in a completely randomized design. Each pot was filled with 1kg of paddy soil (RBE) collected from a farmer field, Puliyankulama, Anuradhapura, from a depth of 0-15 cm.

Following the DOA and NIFS recommendations (NIFS Annual Research Review, 2012), the inorganic and biofilm-enriched fertilizer treatments were broadcasted into pots since all fertilizers are in the form of powder (biofilm enriched ERP) and granular (TSP, Urea and MOP) forms. In each pot, three seedlings of *Oryza sativa* (BG 352 variety) were transplanted at equal distance. Initially, an equal amount of water (1 L) was added to each pot and then 500 ml of water was added daily until the panicle initiation. Other management practices such as weeding, pest and disease management were carried out according to the recommendations given by the DOA, Sri Lanka. During the study, the maximum temperature inside the greenhouse was about 40°C and the minimum was 28°C. After 14 weeks, the grain yield (per pot) was recorded. The soils in the pots were also analyzed for available P (Murphy and Riley, 2002) using 100 ml of 0.5 M sodium bicarbonate extracts (Olsen *et al.,* 1954).

2.4 Statistical analysis

The statistical analysis was performed using one-way ANOVA to determine the significant difference of P solubilization among treatments. The normality of data was tested using Shapiro-Wilk statistic under the UNIVARIATE procedure. SAS version 9.3 (SAS Institute, 2011) was used and the means were separated using Tukey's HSD test.

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3. Results and Discussion

3.1 Initial Soil Analysis

Table 2 shows the properties of the soil samples collected from the farmer field, Puliyankulama, Anuradhapura. According to the results, the soil was low in organic matter content (1.65%), neutral in soil reaction (pH= 7.3), and it had a moderate level of available N. Available P content was close to critical or deficient level while K was at the sufficient level, but not int he optimum range for rice plant growth (Portch and Hunter, 2002). Soil microbial biomass of the studied samples was very low. This might be due to the intensive application of chemical inputs to the rice crop prior to this experiment.

Table 2: Properties of soils collected from the farmer field, Puliyankulama, Anuradhapura.

Soil Property	Mean ± SD
pH	7.3±0.09
Available P (mg kg-1)	14.31 ± 1.75
Available N (mg kg-1)	77.32 ± 0.34
Exchangeable K (mg kg-1)	102.6 ± 1.5
Organic matter (%)	1.65± 0.02
Microbial biomass C (mg g-1)	0.48± 0.06

3.2 Solubilized P in Leachate

The highest solubilized P was recorded in 100% CFE (T1) treatment at all sampling events, and it was significantly greater than the other treatments (Table 3). Further,100% CFE (T1) treatment solubilized P rapidly with an increasing trend (Fig. 1) compared to other treatments. The 50% CF_M + BF3 (T6) showed lower P solubilization performance compared to 100% CF_E (T1) throughout the study period.

Table 3: Average solubilized P (mg kg^{-1}) of leachates.

	Average Solubilized P concentrations (mg kg^{-1}) ± SD					
	2 Weeks	**4 Weeks**	**6 Weeks**	**8 Weeks**	**10 Weeks**	**12 Weeks**
T1	0.070± 0.118 ^{a}	0.074 ±0.116 ^{a}	0.075±0.088 ^{a}	0.079±0.061 ^{a}	0.081±0.055 ^{a}	0.092±0.185 ^{a}
T2	0.029±0.251 ^{e}	0.045±0.025 ^{c}	0.057±0.035^{c}	0.063±0.053^{b}	0.074±0.026 ^{b}	0.076±0.190 ^{b}
T3	0.047±0.168 ^{d}	0.044±0.104 ^{d}	0.040±0.158 ^{e}	0.034±0.081 ^{e}	0.035±0.063 ^{i}	0.025±0.190 ^{j}
T4	0.022±0.003 ^{j}	0.012±0.003 ^{k}	0.018±0.016 ^{j}	0.031±0.002 ^{g}	0.028±0.001 ^{j}	0.028±0.003 ^{i}
T5	0.024±0.004 ^{i}	0.028±0.002 ^{g}	0.029±0.010 ^{g}	0.031±0.001 ^{g}	0.037±0.001^{h}	0.038±0.002 ^{h}
T6	0.026±0.002 ^{g}	0.033±0.002 ^{f}	0.034±0.007 ^{f}	0.033±0.002 ^{f}	0.043±0.003^{f}	0.044±0.003 ^{g}
T7	0.059±0.002 ^{b}	0.049±0.002 ^{b}	0.067±0.012 ^{b}	0.059±0.001 ^{c}	0.060±0.004 ^{c}	0.068±0.002 ^{c}
T8	0.048±0.004 ^{c}	0.044±0.001 ^{e}	0.052±0.012 ^{d}	0.054±0.003^{d}	0.058±0.001^{d}	0.062±0.006 ^{d}
T9	0.025±0.004 ^{h}	0.025±0.001 ^{h}	0.026±0.009 ^{h}	0.027±0.001 ^{h}	0.027±0.002 ^{k}	0.030±0.006 ^{k}
T10	0.028±0.003 ^{f}	0.015±0.002 ^{i}	0.021±0.014 ^{i}	0.030±0.002 ^{h}	0.050±0.002 ^{e}	0.060±0.008 ^{e}
T11	0.028±0.003^{f}	0.021±0.004 ^{i}	0.034±0.003 ^{f}	0.033±0.002 ^{f}	0.041±0.003 ^{g}	0.055±0.001 ^{f}

According to Tukey's mean comparison test, different letters above each column are statistically significant at 5% probability level.

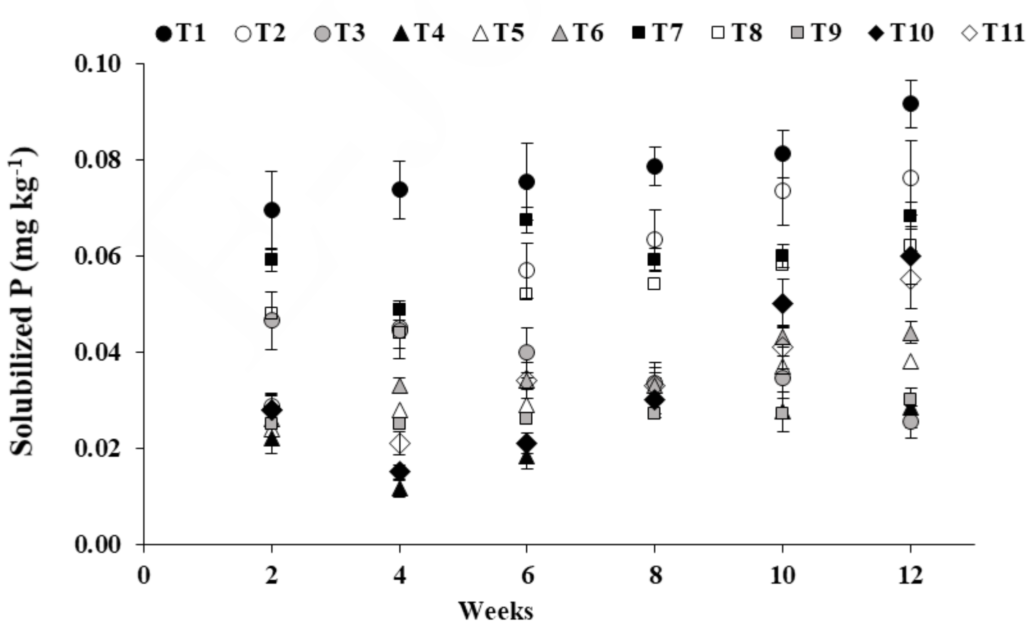

Fig. 1: Solubilized P (mg kg^{-1}) of leachates in 2 weekly intervals

3.2. Cumulative Solubilized P

Biofilms have been observed in many environments, but little is identified as P solubilizers. BF3 has proven its efficient solubilization of mineral P in ERP (Jayaneththi *et al.,* 2017). However, BF3 enriched modified CF mixture recorded significantly *($p<0.05$)* lower cumulative solubilized P than T1 (Fig 2). In every two weeks, significantly *($p<0.05$)* highest solubilized P content was recovered in TSP containing the existing CF mixture (T1) since over 90% of the total P in TSP is water-soluble and rapidly available for plant uptake.

Similarly, T1 or TSP containing CF has recovered significantly *($p<0.05$)* highest cumulative solubilized P at the end of the experiment. T6 or 50% CF_M+ BF3 also recorded significantly lowest cumulative solubilized P. Therefore, 50% cut down of chemical fertilizer with the biofilm application is not sufficient for soil collected from RBE, from a farmer field, Puliyankulama.

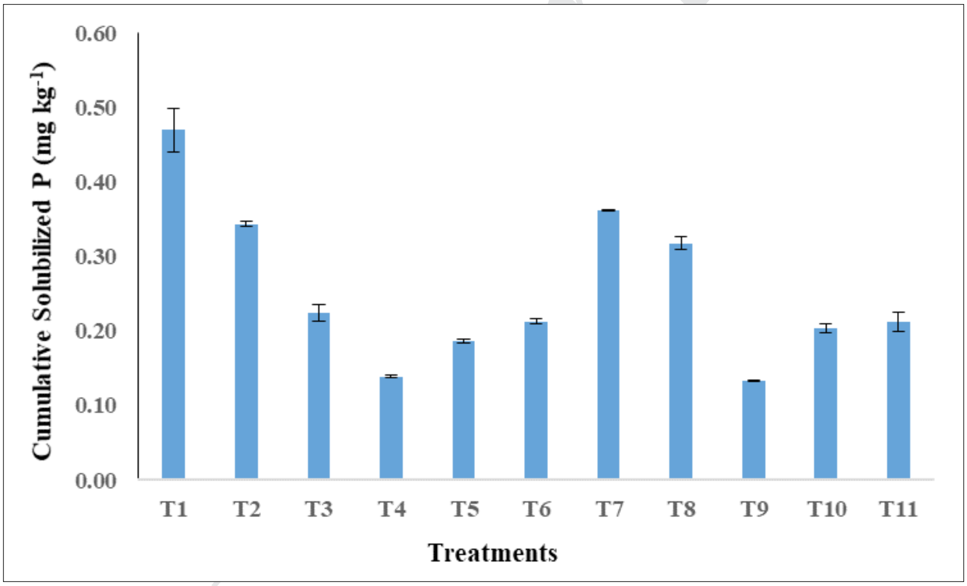

Fig. 2: Cumulative solubilized P (mg kg^{-1}) of the leachates at the end of the three months of leaching tube experiment. According to Tukey's mean comparison test, different letters above each bar are statistically significant at a 5% probability level.

3.3 P solubilizing Patterns

Tipple super Phosphate has several agronomic advantages that made it such a popular P source for many years. It rapidly releases the P for plants. Fig.3 clearly illustrates the P releasing rhythm of T1 or 100% TSP containing CF_E.

biofilm-enriched ERP to use as an alternative for TSP in rice cultivation.

Keywords: Biofilm, Eppawala rock phosphate, Rice cultivation, Triple super phosphate

Fig. 3: Phosphorous solubilization rhythms of treatments: (a). T1 (100% TSP containing CF_E) and (b). T6 (50% CF_M+ BF3)

The shelf life of microbes is the most critical point to mineral phosphate solubilization (Krishnaraj and Dahale, 2013). Phosphorous solubilization of biofilms varies with time. With the growth of microbes, solubilization was enhanced, and with the breakdown of fungal mass, solubilization was decreased. This process has happened up to the shelf life of microbes in biofilms. This nutrient release pattern is perfect for the plants and soils rather than the rapid release of nutrients as in T1.

Rapid mineralization rates of chemical fertilizers are not favorable for soil. It adversely affects the soil because it reduces soil P pool rapidly and the application of chemical fertilizer badly influences soil health and its quality, especially effects on soil pH, CEC etc. (Barak et al., 1997). Low mineralization rates help to maintain soil fertility level long term and increase soil health.

3.4 Biomass C, pH and organic matter

At the end of the 3 months, all the biofilms incorporated treatments, T3 (50% CF_E+ BFBF, T6 (50% CF_M + BF3), T9 (50% CF_E(only N,K)+ BF3) and T10 (BF3 only) showed significantly higher microbial biomass C contents compared to other inorganic fertilizer only applications in T1, T4, T5, T7 andT8(Fig. 4 a). Further, applying inorganic fertilizer only treatments and the control decreased the soil microbial biomass C considerably from its initial microbial biomass C levels prior to commencing the experiments (0.48 mg g^{-1}) (Table 2).

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At the end of the three-month study period, soil reactions of all treatments changed from the initial neutral pH (pH=7.3) level to slightly acidic (Table 2). Relatively higher acidic pH (5.43) was recorded with the biofilm alone applications (T10) (Fig. 4 b). Phosphorous solubilizing biofilms solubilize calcium bound P by excreting organic acids, accompanied by a drop in pH that results in the acidification of microbial cells and their surroundings (Tallapragada and Seshachala, 2012). Acidity directly influences the activity of microbes that are involved in the solubilization of insoluble inorganic phosphates.

The initial soil contained 1.65% of organic matter (Table 2), and the application of biofilms enhanced the organic matter contents in the soil over time. According to Fig. 4 c, treatments with biofilms (T3, T6, T9) showed significantly *(p<0.05)* higher organic matter contents than the treatments of chemical fertilizer only applications.

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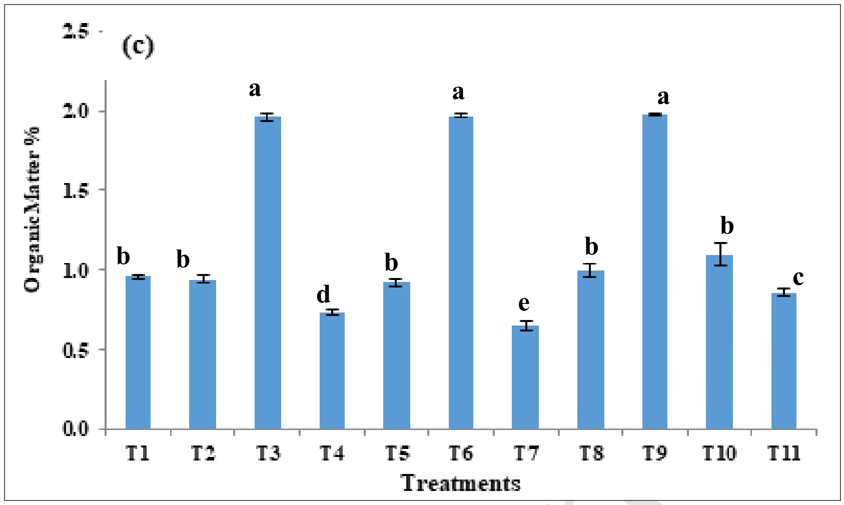

Fig. 4: (a) Biomass, C (b) pH, and (c) Organic matter content of soils remaining in leaching tubes at the end of the three months. Different letters above each bar are statistically significant at 5% probability level according to the Tukey's mean comparison test.

3.5 Available P in potting soils and grain yield

The soil used in this study initially contained 14.48 mg kg-1 of available P. Fig. 5 shows the available P levels in potting mixtures of different treatments after the 14 weeks (harvesting stage).

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4. Conclusions

This study was conducted to test the potential of biofilm-enriched ERP to replace the TSP in rice cultivation. However, the results of this research suggest that biofilm enriched ERP (50% CFM + BF3) shows no added advantage over the full dosage of TSP (100% CFE) on P supply to the rice plant. Moreover, it can be concluded that biofilm enriched ERP performs poorly compared to the DOA recommended TSP dosage in rice cultivation under controlled conditions. However, all the biofilms applied treatments significantly contributed to enhanced soil quality compared to other treatments. Therefore, further studies should be conducted by changing different ERP rates in the modified CF mixture and BF3 in greenhouse and field conditions.

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