Application of ameliorant and microbials fertilizer as bioagent for enhancing the health of rhizomicrobiome and yield of soybean on marginal soils ecosystem

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Abstract. The sustainability of agricultural production depends on the health of soils ecosystem and soil properties. Rhizomicrobiome act as external digestive organs of the plant plays a magnificent role for enhancing the nutrient availability, soil health and plant growth on marginal soils. The intensive use of agrochemical such as inorganic fertilizers has accelerated the soil health degradation and inefficiency of fertilizers use. Bioameliorant or bioagent containing activated carbon, organic extract, humic substances and beneficial rhizobacteria was developed and formulated as soil conditioner and microbial fertilizer for improving the soil properties and increasing the microbial population in rhizosphere microbiome (rhizomicrobiome) and as well as to increase the yield of soybean. The experiment was set up as randomized block design, consisted of 7 treatments (0, 4, 8, 12, 16 kg ha⁻¹ of bioagent, 1 ton ha⁻¹ dolomite and 1 ton ha⁻¹ manure) and provided with 4 replications. The chemical properties (organic carbon, pH and CEC), beneficially bacterial population in rhizomicrobiome (Azospirillum sp., Pseudomonas sp. and Bacillus sp.) and soybean grain yield were significantly increased by the low dosage application of organic bioagent. Application of 8-12 kg ha⁻¹ of bioagent had increased the grain yield of soybean significantly (about 47.5-51.1% higher than control). The obtained yield was not different significantly either with application 1 ton ha⁻¹ dolomite or 1 ton ha⁻¹ organic manure. Consequently, application of 8-12 kg ha⁻¹ of bioagent can be recommended to substitute the use of conventional ameliorant and to enhance the bacterial biodiversity in rhizomicrobiome for increasing the soil health and soybean yield.

1. Introduction
Soybean belongs to the strategic and the most important food crops in Indonesia. The need of soybean now is about 2 million tons per year and it demand increase along with the growth of population and...
soya based of food industry or product (tofu, tempe, soya milk, etc.). The consumption of soybean is about 14-16 kg capita\(^{-1}\) year\(^{-1}\) [1]. The Indonesia demand of soybean grain is highly depend on import, while the domestic production only meet about 20-30\% [2]. In addition, the average productivity of soybean is about 1.2 ton ha\(^{-1}\) and its still relative low compared to its potential yield (3-4 ton ha\(^{-1}\)) [3, 4, 5]. An effort to increase the soybean production is by expanding and intensifying on marginal soil or suboptimal, particularly on acid soils of dry land ecosystem. This soils covered about 70 million hectares (69\%) of total area of Indonesia. The main constraint of this agricultural soils are low pH, low organic matter, low availability of macro nutrient (N, P, K, Ca, Mg) and micro elements (B, Mo) and associated with relative high content of toxic element, such as Al, Fe and Mn [6].

The growth and development of crops are highly influenced by the low pH and low organic matter. The low pH usually correlated with solubility of Al and the excess H\(^{+}\) ions and caused the roots growth significantly [7]. Currently, about 90\% of dry in Indonesia are belong to sick soils (low organic carbon and high acidity) [8, 9]. In addition, the low content of organic matter is usually correlated with: (1) water and essentially nutrient retention, (2) activity of beneficially rhizobacteria in rhizosphere and (3) the efficiency of fertilizers. A low content of organic matter in soils will lead to decline of crops response on fertilizers application [10, 11].

Many efforts had been done to increase the soybean productivity on acid agricultural soils since the last 30 years, such as the application of agricultural liming (dolomite and calcite), organic fertilizers, application of biofertilizers (Bradyrhizobium japonicum) and the development of acid tolerant soybean cultivars. The application of 1-2 tons dolomite ha\(^{-1}\) or 1-2 ton organic fertilizers (compost, cow manure) combine with N biofertilizers has improve the soil chemical properties (reduce the Al toxicity), the plant growth and increased the productivity of soybean significantly [12, 7]. The lime or organic ameliorant are usually deployed and incorporated into the soil homogeneously about 2 weeks before planting. The availability of dolomite or organic manure as conventional ameliorant in some areaal become limited due to the relative high dose of usage and transportation cost. The advance of soil biotechnology has opened another alternative or possibility to overcome the constraint of acid soils for cultivation the food crops by improving the soil health and quality locally or in situ treatment (microsite). It had been formulated bioagent or bioameliorant (activated carbon or biochar, organic extract, humic substance and beneficially rhizobacteria as biofertilizers and soils conditioner [10, 13]. Its application is expected to produce more organic substance (exudate), increase the chelating of toxic element, such as Al in rhizosphere and stimulate/promote the roots and plant growth. Biofertilizers application can increase the yield by about 25-50\% and reduce the application inorganic fertilizers until 25-50\% for nitrogen and about 25\% for phosphor nutrient [8, 14, 15, 16, 17]. In addition, biofertilizers can improve soil health and provide protection against drought and some soil borne diseases [18, 16, 13]. This research is focused to investigate the formulated of bioagent for substituting the conventional ameliorant (dolomite and cow manure) and improving the soil quality, rhizomicrobiome health and increasing the growth of soybean on acid soils ecosystem.

2. Materials and Methods
The experiments to investigate the effectiveness of formulated bioagent as a biofertilizer and soils conditioner for improving the soils quality, rhizomicrobiome health and increasing the soybean productivity has been conducted since March 2016 at the experimental farm of Agriculture Faculty of Padjadjaran University in Jatinangor, located about 700 m above sea levels.

The soil texture belongs to clay soils (62\% clay, 32\% silt and 7\% sand) and it has relative low fertility (pH 5.9, 1.5\% organic-C, 0.15\% total-N, 0.74 ppm of available-P, and 22.3 cmol kg\(^{-1}\) of CEC). The formulated organic based bioagent containing: (1) activated carbon enrich with organic extract and humic substances, (2) symbiotic N–fixer (Bradyrhizobium japonicum) about 2.0 \(\times 10^8\) CFU g\(^{-1}\), (3) non symbiotic N–fixer (2.2 \(\times 10^7\) CFU g\(^{-1}\) of Azotobacter sp., and 2.1 \(\times 10^8\) CFU g\(^{-1}\) of Azospirillum sp.), and (4) phosphate solubilizing bacteria about 2.1 \(\times 10^8\) CFU g\(^{-1}\) (Pseudomonas sp. and Bacillus sp.). The experiment was arranged as randomized block design, consisted of 7 treatments (0, 4, 8, 12, 16 kg ha\(^{-1}\) of bioagent, conventional ameliorant (1 ton ha\(^{-1}\) of dolomite or 1 ton ha\(^{-1}\) of cow
manure) and provided with 4 replications. Dolomite (30% CaO and 20% MgO) and cow manure (23.3% organic-C and 2.14% total-N) were used as conventional ameliorant. The bacterial isolates for bioagent formulation were obtained from the culture collection of Soil Microbiology Laboratory of Agriculture Faculty of Padjadjaran University. The bioagent was applied either as seed treatment and land application. Soybean seed was moisted with a bit water and subsequently 20 g of bioagent was mixed homogeneously with 1 kg of soybean seed.

For field application, the bioagent according to the treatment was mixed with 100 kg of phosphate fertilizer (SP-36 contain 36% of P2O5) and applied into plant hill. The soybean was planted 2 seed per hill at planting space 40 x 15 cm in plots (4 x 5 m). The inorganic fertilizers (25 kg N and 60 kg K2O) were applied 7 days after planting into drilled hole about 10-15 cm distance from the plant. The composite soil sample (0-20 cm depth) for microbial and chemical analyses were taken from plant rhizosphere at maximum vegetative stage (6 weeks after planting) using specially modified auger bor.

The population beneficial bacteria were enumerated with plate count methods [19] using selective N-free media [20, 21]; (1) yeast extract mannitol agar (YEMA) for Bradyrhizobium japonicum, (2) mannitol Ashby agar medium for Azorhizobium sp. [22], (3) the Okon media for Azospirillum sp. [23] and (4) the Pikovskaya media for phosphate solubilizing bacteria [24, 19]. The observed responds were analyses statistically [25].

3. Result and Discussion

3.1. Soil Chemical Properties

The chemical properties (pH, organic-C and CEC) were influenced significantly by bioagent or conventional ameliorant (dolomite or organic manure) (Table 1).

Table 1. The chemical properties (pH, organic-C and CEC) of treated plots with bioagent (BA) compared with conventional ameliorant (DL = dolomite or CM = cow manure).

| Treatments     | pH_H2O | Organic-C (%) | CEC (cmol kg⁻¹) |
|---------------|--------|---------------|-----------------|
| T0 = control  | 5.9 ab | 1.34 a        | 22.43 a         |
| T1 = 4 kg ha⁻¹ of BA | 5.9 ab | 1.44 ab      | 29.03 b         |
| T2 = 8 kg ha⁻¹ of BA | 6.1 b  | 1.70 bc      | 29.01 b         |
| T3 = 12 kg ha⁻¹ of BA  | 5.9 ab | 1.71 bc     | 30.78 b         |
| T4 = 16 kg ha⁻¹ of BA  | 5.8 a  | 1.92 c       | 29.46 b         |
| T5 = 1 ton ha⁻¹ of DL  | 6.3 c  | 1.64 bc     | 24.01 a         |
| T6 = 1 ton ha⁻¹ of CM  | 6.0 ab | 1.54 bc    | 33.14 b         |

Mean values followed by the different letters within each column are significantly different (P≤0.05).

The highest soil pH was obtained by the application of 1 ton dolomite ha⁻¹, while the other treatments except the application of 8 kg ha⁻¹ of bioagent were not different significantly with control. The relative high content of Ca and Mg in dolomite contribute directly for enhancing the soil pH [12, 7]. The improving of fertility were seen clearly on the increasing of organic-C and cation exchange capacity (CEC). The content of organic-C of treated plots with 8-16 kg ha⁻¹ of bioagent or one ton dolomite or organic manure were much higher than control. In addition, the application of bioagent with relative low dosage has resulted a no significant different with conventional ameliorant. This result indicated that the activity and biodiversity of beneficial microbes and the roots growth was increased in the rhizosphere by the application organic based ameliorant. The higher of CEC of treated plots with 4-16 kg ha⁻¹ bioagent or organic mature than control or plot receipt 1 ton dolomite is a clear indication that CEC is highly correlated with organic content and microbial activity. The relative high content of humic substance of bioagent and the biodiversity of rhizobacteria contribute the increasing of CEC in soils [7, 10, 11].
3.2. Population of Beneficially Bacteria in Rhizomicrobiome

The population of beneficial rhizobacteria except Azotobacter sp. was increased significantly by the application bioagent or conventional ameliorant (Table 2).

Table 2. Population of beneficial rhizobacteria treated with bioagent (BA) compared with conventional ameliorant (DL = dolomite or CM = cow manure) at 6 weeks after planting.

| Treatments | N-Fixer Bacteria (CFU g⁻¹) | P-Solubilizers Bacteria (CFU g⁻¹) |
|------------|---------------------------|----------------------------------|
|            | Azotobacter sp. (x 10⁷)   | Azospirillum sp. (x 10¹⁰)         | Pseudomonas sp. (x 10⁸) | Bacillus sp. (x 10⁶) |
| T₀ = control | 3.62 a                    | 3.41 a                            | 1.86 a                  | 5.96 a            |
| T₁ = 4 kg ha⁻¹ of BA | 5.92 a                    | 3.63 ab                           | 1.42 a                  | 9.59 ab          |
| T₂ = 8 kg ha⁻¹ of BA | 4.08 a                    | 4.14 ab                           | 2.60 a                  | 11.26 bc         |
| T₃ = 12 kg ha⁻¹ of BA | 4.87 a                    | 4.95 bc                           | 2.76 a                  | 11.74 bc         |
| T₄ = 16 kg ha⁻¹ of BA | 3.65 a                    | 5.95 cd                           | 5.12 b                  | 17.68 c          |
| T₅ = 1 ton ha⁻¹ of DL | 3.51 a                    | 6.64 d                            | 5.62 b                  | 8.02 ab          |
| T₆ = 1 ton ha⁻¹ of CM | 3.84 a                    | 5.89 cd                           | 4.80 b                  | 6.15 ab          |

Mean values followed by the different letters within each column are significantly different (P≤0.05).

Compared to control plot, the higher population of Azospirillum sp. as endophytic N-fixers was obtained by the application of 12-18 kg ha⁻¹ of bioagent or one conventional ameliorant, while the high population of Pseudomonas sp. was obtained in the treated plots with 16 kg ha⁻¹ of bioagent or one ton ha⁻¹ dolomite or organic manure. In contrast, population of Bacillus sp. was relative high in the treated plots with 8-16 kg ha⁻¹ of bioagent compare to the control. This results indicated that the activity and the biodiversity of rhizobacteria in rhizosphere correlated with the roots growth and supply of organic carbon or roots exudate [8, 10, 18]. Moreover, the domination and high activity or biodiversity of N-fixers or PSB as biofertilizers or as plant growth promoting rhizobacteria (PGPR) in soils will improve the nutrients status and phytohormone production for supporting the plant growth [26, 18, 8].

3.3. Soybean Grain Yield

Application of bioagent or conventional ameliorant (dolomite or organic manure) gave a significant effect on enhancing the soybean grain yield (Table 3). The grain yield was increased about 18.7-51.1% of treated plots with 4-16 kg ha⁻¹ of bioagent and about 52.7-57.4% in the plots receipt a ton ha⁻¹ of dolomite or organic manure. Overall, the application of 8-16 kg ha⁻¹ had produced of grain yield as high as obtained in plots receipt one ton ha⁻¹ ameliorant.
Table 3. Soybean grain yield in responds to bioagent (BA) and conventional ameliorant (DL = dolomite and CM = cow manure).

| Treatments | Grain Yield | Increment (%) |
|------------|-------------|---------------|
|            | (g plot⁻¹)  | (ton ha⁻¹)    |               |
| **T₀ = control** | 352.50 a    | 0.70          | -             |
| T₁ = 4 kg ha⁻¹ of BA | 418.25 ab   | 0.84          | 18.7          |
| T₂ = 8 kg ha⁻¹ of BA | 520.00 bc   | 1.04          | 47.5          |
| T₃ = 12 kg ha⁻¹ of BA | 530.00 bc   | 1.06          | 50.3          |
| T₄ = 16 kg ha⁻¹ of BA | 532.75 bc   | 1.07          | 51.1          |
| T₅ = 1 ton ha⁻¹ of DL | 555.00 c    | 1.11          | 57.4          |
| T₆ = 1 ton ha⁻¹ of CM | 538.25 bc   | 1.08          | 52.7          |

Mean values followed by the different letters within each column are significantly different (P≤0.05).

This result was also seen in field plant growth performance during the experiment and supported either by the improved of soil chemical properties (Table 1) or increased biological activity (Table 2). Some studied has recommended the application of a relative high dosage conventional ameliorant to improve the soil properties and to increase the productivity of soybean in acid soils ecosystem [7, 12]. This finding revealed the application of relative low dosage (8-16 kg ha⁻¹) of formulated bioagent could be applied to substitute the usage conventional ameliorant. Consequently, the effectiveness of bioagent are depend on the biological active ingredients and the content of humic substances for enhancing the activity and biodiversity of beneficial rhizobacteria to stimulate the roots growth and to chelate the toxic substance in rhizosphere. Chelating agent (exudate, slime, organic acids) produced by biological activity will contribute in inactivating or reducing the toxical element (Al, Fe and Mn) in rhizosphere and promote the roots and plant growth [27, 18, 8, 28].

4. Conclusion
Enriched ameliorant with beneficial bacteria, humic substance, organic extract and others additive named as bioagent has improved: (1) the soil health and fertility, (2) increased the population of beneficially rhizobacteria (N-fixer and phosphate solubilizers bacteria) and (3) increased the soybean grain. Application of 8-12 kg ha⁻¹ of bioagent increased the grain yield of soybean significantly (about 47.5-51.1% higher than control), and the obtained grain yield was not different significantly either with application 1 ton ha⁻¹ dolomite or 1 ton ha⁻¹ organic manure. Consequently, application of 8-12 kg ha⁻¹ of formulated bioagent could be applied to substitute the use of conventional ameliorant and increase the soil health and productivity of soybean in acid soils. The furtherer researchs are need to enrich and develop the bioagent and its application on acid soils ecosystem to support the soybean growth or others food crops.

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References
[1] Sutyorini S and Waryanto B 2013 Agriculture Statistics 2013 (Indonesia: Center for Agriculture Data and Information System, Ministry of Agriculture Republic of Indonesia) p 316
[2] Marwoto, Simatupang P and Swastika D K S 2008 Soybean development and policy research in Indonesia (in Indonesian) ed A K Makarim et al. Proc. of the Workshop on Soybean Development at Suboptimal Land (Indonesia: Indonesian Agency for Agricultural Research and Development) pp 1–18
[3] Nainggolan K and Rachmat M 2014 Self sufficiency prospect of soybean in Indonesia Jurnal Pangan 23 (1) 83–92
[4] Kuswantoro H 2014 Potential yield of soybean promising lines in acid soil of central Lampung, Indonesia International Journal of Plant Biology 5 55–66
[5] USDA 2017 World Agricultural Production Circular Series WAP 05-17 May 2017
[6] Mulyani, Sukarman A and Hidayat A 2009 Prospect of soybean extensification in Indonesia Jur. Sumberdaya Lahan 3 (1)
[7] Wijanarko A and Tauqif A 2016 Effect of lime application on soil properties and soybean yield on tidal land AGRIVITA 38 (1) 14–23
[8] Simarmata T 2013 Tropical bioresources to support biofertilizer industry and sustainable agriculture (in Indonesian) Proc. Int. Seminar on Tropical Bioresources for Sustainable Bioindustry 2013: from Basic Research to Industry (Bandung: ITB)
[9] Simarmata T, Setiawati M R, Herdiyantoro D and Fitriatin B N 2018 Managing of organic-biofertilizers nutrient based and water saving technology for restoring the soil health and enhancing the sustainability of rice production in Indonesia IOP Conf. Series: Earth and Environmental Science DOI: 10.1088/1755-1315/205/1/012051
[10] Simarmata T, Hersanti, Turmukti T, Fitriatin B N, Setiawati M R and Purwanto 2017 Application of bioameliorant and biofertilizers to increase the soil health and rice productivity HAYATI Journal of Biosciences 23 181–184
[11] Sudjana B, Jingga A and Simarmata T 2017. Enriched rice husk biochar ameliorant to increase crop productivity on typic hapludults Global Advanced Research Journal of Agricultural Science 6 (5)
[12] Suryantini 2014 Effect of lime, organic and inorganic fertilizer on nodulation and yield of soybean (Glycine max) varieties in Ultisol soils Journal of Experimental Biology and Agricultural Sciences 2 (1)
[13] Simarmata T, Fitriatin B N, Setiawati M R, Herdiyantoro D, Suryatmana P and Hindersah R 2019 Development and formulation of beneficial rhizobacteria consortia to improve soil health and agricultural practice sustainability in Indonesia ed RZ Sayyed et al. Plant Growth Promoting Rhizobacteria (PGPR): Prospects for Sustainable Agriculture (Singapore: Springer Nature Singapore Pte Ltd) pp 63–74
[14] Board N 2012 Biofertilizers and Organic Farming (Delhi: NPCS)
[15] Ghany T A M, Alawlaqi M M and Al Abboud M A 2013 Role of biofertilizers in agriculture: a brief review Mycopath 11 (2) 95–101
[16] Purwanto, Yuwariah Y, Sumadi and Simarmata T 2017 Nitrogenase activity and IAA production of indigenous diazotroph and its effect on rice seedling growth AGRIVITA Journal of Agricultural Science 39 (1) 31–37
[17] Setiawati M R, Suryatmana P, Hindersah R, Fitriatin B N, Nurbaity A, Herdiyantoro D, Kamaluddin N N and Simarmata T 2018 Characteristic and capability of plant growth promoting endophytic bacteria of rice plant tissue in saline ecosystem Asian Jr. of Microbiol. Biotech. Env. Sc. 20 (3) 791–797
[18] Singh T and Purohit S S 2011 Biofertilizers Technology (India: Agrobiios)
[19] FNCA Biofertilizer Project Group 2006 Biofertilizer Manual (Japan: Japan Atomic Industrial Forum)
[20] Somasegaran P and Hohen H J 1994 Handbook for Rhizobia: Methods in Legume-Rhizobium Technology (New York: Springer-Verlag) pp 332–341
[21] Rosemary O, Gloria OT and Cecilia I 2013 Isolation and characterization of nitrogen-fixing bacteria in the soil Int. J. Life Sc. Bt & Pharm. Res. 2 (3)
[22] Thompson J P 1989 Counting viable Azotobacter chroococcum in vertisols Plant and Soil 117 9–16
[23] Okon Y 1985 Azospirillum as a potential inoculant for agriculture Trends in Biotechnology 3 223–228
[24] Gaur A C 1981 Phospho Microorganism and Various Transformation in Compost Technology *Project Field Document No. 13* (Rome: FAO)

[25] Gomez K A and Gomez A 1984 Statistical Procedures for Agricultural Research (New York: John Wiley & Sons)

[26] Hayat R, Ali S, Amara U, Khalid R and Ahmed I 2010 Soil beneficial bacteria and their role in plant growth promotion: a review *Ann. Microbiol.* DOI 10.1007/s13210-010-0117-1

[27] Vessey J K 2003 Plant growth promoting rhizobacteria as biofertilizer *Plant and Soil* 255 571–586

[28] Fitriatin B N, Khumairah F H, Setiawati M R, Suryatmana P, Hindersah R, Nurbainty A, Herdiyantoro D and Simarmata T 2018 Evaluation of biofertilizer consortium on rice at different salinity levels *Asian Jr. of Microbiol. Biotech. Env. Sc.* 20 (4) 1108–12