Biofilm biofertilizer increase mustard growth and nutrient status of dry land Lithosols

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Abstract. Almost all sectors including agriculture have the risk of being affected by global climate change. It need strategies and prepare adaptation action programs with the support of innovative and adaptive technologies to anticipate. This research aimed to find out an appropriate dosage of organic fertilizer decomposted by biofilmed biofertilizer (OF-Bio2) that optimally increases mustard growth as well as N, P and K status at Lithosols. The experiment was done in a single factor randomized completely block design (RCBD) with eight dose levels, i.e. 0, 3, 6, 9, 12, 15, 18, and 21 ton ha⁻¹ of OF-Bio2 on 2 x 3 m² plot and 15 x 20 cm planting spaces. OF-Bio2 was incorporated thoroughly within 20 cm depth of the soil and one mustard plantlet was planted in each planting hole. Plant height, fresh and dry weights were observed and soil organic carbon (SOC), total-N, av-P and exch-K content were analysed at harvest time. Data were analysed by F-test followed by Duncan multiple range test (DMRT) at 95% of confidence level. The result showed the use of OF-Bio2 increased mustard growth linearly as well as N, P and K status concomitantly with the increase of OF-Bio2 doses. An optimal dose of OF-Bio2 for mustard yield was not achieved yet, because of low organic matter content of the soil. The use of biofilm biofertilizer to decompose organic fertilizer will support sustainable soil nutrient management with more effective soil microbes enable to increase nutrient available.

1. Introduction
The farmer all over the world may has been used biofertilizers to enhance nutrient availability. Biofertilizers are commercially produced and widely used. The use of biofertilizers has increased due to the demand for healthy products, enhancement in nutrient uptake efficiency (NUE), environmentally friendly technology and low cost [1]. However, the adoption of biofertilizers by farmers is still limited. The reasons for the slow rate of adoption includes doubts concerning biofertilizer’s effectiveness and consistency to enhance plant growth and yield at any condition. Inoculum formulation with good quality and stable effect under field condition is needed [1]. Innovative technology of biofertilizer has to be revitalized to use it as nutrient management strategy, which can give beneficial effects consistently in diverse agro-ecological condition [2]. According to O’Toole et al. [3], Microbial biofilm can be defined as one or several species of microorganism communities attached to an abiotic or biotic surface. These may be structured communities of algae, bacteria or other types of cells enclosed in a self-produced polymer matrix attached to an inert or living surface. Microbial biofilms are heterogeneous communities that are highly organized and attached to artificial substrates. According to Seneviratne et al. [4], biofilms are complex communities of many microbial species attached to or located near plant roots. These biofilms can be developed in vitro and in high cell density can be used as biofertilizer or biocontrol...
agents in plants. Formerly, the biofilms developed were phosphate solubilizers and dinitrogen-fixing microbes. In the in vitro culture, microbes usually attach to and colonize the fungal mycelia to form biofilms called fungal-bacterial biofilms (FBB). Biofilms formed from rhizobium species are called fungal-rhizobial biofilm (FRB). Biofilm biofertilizer is more resistant to environmental pressures, predators and antagonists and has a higher ability to increase crop yields than conventional biological fertilizers. For example in rice plants, based on weight of dry stover, conventional biofertilizer increased growth by only 7%, biofilm biofertilizer by 25% compared to the controls, and biofilm biofertilizer + ½ dose of fertilizer recommendation by 55% compared to the treatment with a full dose of NPK fertilizer. Biofilm-based biofertilizers have better abilities than the ones that are not, both in improving nutrients availability, production of plant growth substances (e.g. IAA) and inhibition of plant pathogens [4]-[10]. Various countries have developed and utilized biofertilizers and biopesticides to increase fertilizer efficiency, crop yields, preservation of natural resources and food safety [11]-[15].

The incorporation of microbes into fertilizer formula will make the microbes closer to the nutrients needed, so they can grow and move in the soil better. According to Sudadi et al. [16][17] incorporation of P-solubilizing fungi, Aspergillus niger and sulfur-oxidizing fungi, Penicillium nalgiovensis together with phosphate rock, sulfur and organic matter to form biosulfo fertilizer improves available P and S simultaneously as well as shallot yield under acidic, neutral and alkaline conditions. In addition, Sudadi et al. [18] and Hadiwyono et al. [19] stated that the use of biological control agents enrich-biofilm biofertilizer (bca-Bio2) increased available P in the soil and its uptake as well as shallot yield and decreases basal rot disease in Alfisols, Entisols and Vertisols. Biological control agents enrich-biofilm biofertilizer (bca-Bio2) is the integration of biofertilizer consortia microbes as nutrient provider and biological control agents when applied to soil or on plant body will form microbial biofilms. Integration of biological agents microbes and nutrient solubilizer in a formula of biofertilizer is proposed to further ensure their growth and activity in plant roots, forming a microbial biofilm and better performance in improving nutrients availability and absorption by plant and prevent the development of pathogenic microbes. It was a further step of improving biofilm biofertilizers to increase their effectiveness in soil nutrient availability, plant growth, and yield enhancement.

Vegetables are absolutely necessary for their many benefits in human nutrition. Some of which includes mustard, spinach, peppers, tomatoes, eggplant, beans, and cucumbers. Vegetable crops are usually cultivated in light texture, fertile soils. According to Pinthukas [20] cultivate vegetable organically has some constraints, 1) soil available water and fertility, and plant disease and pest, 2) economic limitations such as high cost material, high cost labor, high transportation cost, and market access, 3) are lack of understanding and management skills about pest and disease management, crop-environment, added value from farm processing and postharvest management, and 4) social limitations.

Litosol was relatively young soil order that it solum was generally thin (<10 cm) and located above the parent material [21][22]. According to Sarief [23], Low productivity of lithosol due to poor physical, chemical and biological properties of soil. The coarse lithosol soil texture tends to make the soil porous and has a low water capacity. The soil organic matter content of lithosol is very low and sometimes zero. Soil organic matter is a supplier of nutrients. The low organic matter content causes the soil to become nutrient poor. Low levels of organic matter also cause low soil cation exchange capacity (CEC) [24], Lithosols is generally distributed in highland areas and is a dry soil element. The land in this arrangement actually has the potential to be fertile if it is developed as a cultivated land using intensive organic fertilizers. For this reason, it is necessary to develop lithosol soil fertility for vegetable production using biofilm bio-fertilizers enriched with biological control agents which are expected to overcome problems with lithosols, especially those with N, P and K. status.

2. Material and methods
The isolates of functional microbes as nutrient providers and biological control agents used as microbial consortia on biofilm biofertilizer were obtained from [18]. For phosphate solubilizing microbes, the isolates used were A. niger YD17, bacterial isolates of PBH 17 and TBH 18. Sulfur-oxidizing microbes used were isolates of P. nalgiovense and bacteria isolates of NBH12 and TBK3. Potassium solubilizing
microbes were fungal isolates of JH 7, JK 7 and JK 17. Isolate of Azotobacter AG 17 used as non-symbiotic nitrogen-fixing bacteria. Isolates of Beauveria and Trichoderma were taken from BPP Palur, Mojolaban, Karanganyar District. These microbial consortia then used to make biofilm biofertilizer inoculum by cultured them together in liquid medium composed of molasses (3% v/v), coconut water (30% v/v), rice wash-waste water (30% v/v) and clean water. One reaction tube of agar slant culture of each isolate was inoculated into 15 liters liquid medium and incubated for 48 hours in room temperature, stirred fermentor.

The biofilm inoculum is then used to decompose organic fertilizers which composition is rock phosphate, feldspar, sulfur, quail droppings, rice husks, molasses, lime and clean water. Phosphate, feldspar and sulfur rock are ground through a 100 mesh sieve. All ingredients, except water, are then stirred evenly with the following composition (per 100 kg of organic fertilizer to be made) 10 kg of rock phosphate + 5 kg of feldspar + 1 kg of sulfur + 5 kg of lime and 79 kg of manure. Five liters of liquid biofilm biofertilizer inoculum were then added. The water content of the mixture is then adjusted to about 60% by adding clean water. The organic fertilizer material is then made in piles of 0.5 m high and covered with tarpaulin sheets and composted for two weeks.

Experiments were carried out in the field with a Lithosol soil layout, in Geneng Duwur Village, Gemolong District, Sragen Regency, Central Java, Indonesia at an altitude of about 112 m above sea level. Soil processing is done manually with a hoe, to loosen the soil, remove grass and make beds measuring 2 x 3 m² for each treatment. The experiment was arranged in a single factor randomized complete block design (RCBD). Compost Organic Fertilizer with Biofilm Biofertilizer (OF-Bio2) with doses (0; 3; 6; 9; 12; 15; 18 and 21 ton ha⁻¹) with three replications. Spacing 15 x 20 cm with 3 seeds per planting hole. OF-Bio2 is evenly mixed with the soil until the soil preparation layer is according to treatment, then the soil is watered and incubated for three days before planting. The variables observed were plant height, fresh weight and shoot dry weight, soil organic matter content, soil pH, cation exchange capacity (CEC), total N, available P and K-swapped. Data analysis was performed with the F test at the 95% confidence level, followed by Duncan's multiple range test (DMRT) if there was a significant effect.

3. Results and discussion

On the status of some nutrients, cation exchange capacity (CEC), soil pH and organic matter content (Table 1), it can be said that the land field used for the study had low to moderate fertility. The main obstacle for the cultivation of horticulture, especially vegetables such as mustard is very low soil organic matter content. This will impact on the low availability of N and P. However, with high CEC, the problem of low nutrient status can be solved in this soil by the application of fertilizer and organic matter adequately. The use of organic matter will increase CEC thereby increasing the soil ’s ability to store and provide nutrients for plant.

| Soil chemical properties | Value | Status       |
|--------------------------|-------|--------------|
| pH                       | 7.47  | slightly alkaline |
| Organic-C (%)            | 1.23  | very low*    |
| total-N (%)              | 0.30  | Low          |
| Av-P (ppm)               | 3.65  | very low *   |
| Exch-K (me %)            | 0.19  | high*        |
| CEC (me %)               | 11.44 | medium*      |

* According to Cottenie et al. [25]

The use of OF-Bio2 increases soil organic matter content, total-N, available-P and exchangeable-K of Lithosol, but does not increase the soil pH because OF-Bio2 is an organic fertilizer which pH is lower than the initial soil pH. Total-N reaches optimum level at the dose of 15 ton ha⁻¹, while soil organic matter content, available-P and exchangeable-K did not reach optimum levels. The use of biofilm
biofertilizer increases available-P of Alfisol, Entisol and Vertisol soils [18][19]. The OF-Bio2 can increase soil nutrient status because it contains phosphate rock, feldspar, sulfur and quail manure and contain consortium of microbes which their function were nutrients providers. Buddhika et al. [26] reported that the use of biofilm biofertilizer increases soil microbials population.

**Table 2.** The effect of OF-Bio2 doses on soil organic carbon, soil pH and soil nutrient status of Lithosols.

| Doses of OF-Bio2 (ton ha⁻¹) | Org-C (%) | Total -N (%) | Av-P (ppm) | Exch-K (me %) | CEC (me %) | pH |
|----------------------------|-----------|--------------|------------|---------------|-----------|----|
| 0 (D₀)                    | 1.07 e    | 0.297 b      | 3.72 e     | 0.13 d        | 12.31 e   | 7.47 ns |
| 3 (D₁)                    | 1.08 e    | 0.267 b      | 4.27 e     | 0.16 d        | 15.03 d   | 7.53 ns |
| 6 (D₂)                    | 1.46 cd   | 0.287 b      | 5.28 d     | 0.18 cd       | 16.04 cd  | 7.57 ns |
| 9 (D₃)                    | 1.25 de   | 0.300 b      | 6.47 c     | 0.22 bcd      | 15.66 d   | 7.57 ns |
| 12 (D₄)                   | 1.88 ab   | 0.307 b      | 6.91 c     | 0.29 abc      | 16.24 cd  | 7.50 ns |
| 15 (D₅)                   | 1.63 bc   | 0.387 a      | 7.21 c     | 0.29 abc      | 17.49 bc  | 7.63 ns |
| 18 (D₆)                   | 1.82 ab   | 0.257 b      | 8.20 b     | 0.31 ab       | 18.88 ab  | 7.60 ns |
| 21 (D₇)                   | 2.00 a    | 0.287 b      | 9.81 a     | 0.38 a        | 19.81 a   | 7.67 ns |

Note: number followed by the same letter in one column is not significantly different by DMRT at 95% level of confidence.

Beneficial effect of organic fertlizer application on soil properties and plant yield were well known, especially on soil with marginal fertility. Nurmegawati et al. [27] stated that the use of cow manure compost on new-established rice field of marginal land with Oxisol soil type increased soil pH, exchangeable cation (K, Ca, Na), base saturation, and decrease exchangeable-H. Mukhtar et al. [28] stated the use of organic amendment (vermicompost, chicken and cattle manure) on Ultisols increase soil pH, total-N, available-P and decrease exchangeable aluminium. Minardi et al. [29] stated that the use of manure combined with zeolite increase soil pH, CEC and soil organic matter content on Alfisols.

**Table 3.** The effect of OF-Bio2 doses on mustard growth on Lithosols.

| Doses of OF-Bio2 (ton ha⁻¹) | Plant height (cm) | Plant fresh weight (kg plot⁻¹) | Plant dry weight (g plant⁻¹) |
|-----------------------------|-------------------|--------------------------------|-----------------------------|
| 0 (D₀)                      | 18.84 c           | 2.790 c                        | 19.55 c                     |
| 3 (D₁)                      | 19.81 c           | 6.110 b                        | 31.97 ab                    |
| 6 (D₂)                      | 20.92 bc          | 5.640 b                        | 25.04 bc                    |
| 9 (D₃)                      | 21.07 bc          | 7.810 ab                       | 28.90 abc                   |
| 12 (D₄)                     | 20.48 bc          | 6.307 b                        | 31.26 ab                    |
| 15 (D₅)                     | 22.21 ab          | 7.143 ab                       | 38.86 a                     |
| 18 (D₆)                     | 22.72 ab          | 9.077 a                        | 38.14 a                     |
| 21 (D₇)                     | 23.60 a           | 9.383 a                        | 30.40 abc                   |
| CV (%)                      | 5.34              | 20.51                          | 19.31                       |

Note: number followed by the same letter in one column is not significantly different by DMRT at 95% level of confidence.

Mustard is one of the many types of vegetables for consumption. Mustard also includes plants that are easily cultivated and has a relatively short harvesting time. The use of organic matter will usually improve it’s growth. The results showed that the use of OF-Bio2 improves mustard growth as indicated by increased height, mustard fresh and dry weights with increasing doses of fertilizer used (Table 3). This is because OF-Bio2 improves soil organic matter content as well as soil nutrient status (Table 2). On the Galelli et al. [30] stated that the use of *Bacillus subtilis* subsp. Spizenii as biofilm biofertilizer showed a higher benefit on *Lactusa sativa* than the planktonic form. Hettiarachchi et al. [31] said that the response of rubber seedling was more pronounced than that of full recommended inorganic fertilizer...
application. The highest fresh weight of mustard was obtained at a dose of 21 ton ha\(^{-1}\), while the highest dry weight of mustard was obtained at dose of 15 ton ha\(^{-1}\). Mustard is harvested in the form of fresh stover so increasing fresh weight is more important than dry weight. This means that the optimum dose of OF-Bio2 for mustard yield on Lithosols has not yet been reached. Presumably, this is as a result of the native soil organic matter levels being very low. Soil organic matter plays an important role in diving most of soil bio-physico-chemical processes. According to Nurmeegawati et al. (27) applied cow manure up to 10 ton ha\(^{-1}\) increase rice panicle and straw dry weight at Oxisols, while Sudadi et al. (29) stated that application of manure combined with zeolit can increase soybean yield at Alfisols. Sudadi et al. (18) state that the use of biofilm biofertilizer is able to increase growth and yield of onion on Vertisol. Similar results from (19) suggests the use of biofilm biofertilizer increases the yield of onion and suppresses incidence of basal rot disease of onion on Alfisol, Entisol and Vertisol.

4. Conclusion

Organic Fertilizer composted with Biofilm Biofertilizer (OF-Bio2) is suitable for the cultivation of mustard on Lithosol soil where organic matter content and nutrient status are low. It can increase soil organic matter content, total-N, available-P and exchangeable-K as well as mustard growth. The OF-Bio2 used did not reach the optimum dose yet for providing the highest soil nutrient availability of N, P and K, and mustard yield. Mustard yield increase linearly with the increase of OF-Bio2 dose of 21 ton ha\(^{-1}\). This maybe caused by the very low of native soil organic matter content of the soil.

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