The potentials of biochar from agricultural waste as a carrier material of biofertilizer for swamplands

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Abstract. Biochar has pores suitable for microbial habitat, and it contains carbon which can be used as an energy source by microbes. The research aims to determine the potential of some biochar as a carrier of biological fertilizer for swamplands. The treatment given was the type of biochar (rice husk, coconut shell, and palm empty fruit bunches) and the microbial type ie decomposer, Phosphate Solubilizing Bacteria (PSB), N Fixing Bacteria, and a consortium microbial. The design used was a Factorial Complete Randomized Design, 3 replications. Biochar analysis included organic C, total N, pH, CEC, SiO2, ash content and water content. Calculation of microbial populations was done at 2, 6 and 10 weeks after inoculation. Biochar rice husks, oil palm empty fruit bunches and coconut shells were very effective as decomposer carrier material. The highest of population of N-fixing bacteria and PSB at 6 weeks after inoculation was in the biochar rice husk reached 0.33 x 106 cfu/g for N-fixing bacteria and 54.8 x 106 cfu/g for P solvent bacterial. Rice husk biochar can be used as a carrier material both for a single biofertilizer, as well as a consortium biofertilizer that consists of decomposer fungi, P solubilizing bacteria and N-fixing bacteria.

1. Introduction
Swampland has great potentials to be used as a productive agricultural area. Swampland in Indonesia covers 34.12 million Ha [1]. However, swamps have several problems among others because of high soil acidity and relatively low availability of nutrients in the soil [2,3]. Optimizing the swamplands requires improvements in water, soil, land and plant management. One of the tipping points to increase swampland productivity is needed the support the availability of fertilizers both organic and inorganic.

The continuous use of inorganic fertilizers not only requires high cost, but also negatively impact soil quality. Increased awareness and understanding of the negative impacts of the use of inorganic fertilizers encourage the development of alternative technologies or fertilizer products that are more environmentally friendly [4,5]. Biofertilizer is an alternative type of fertilizer which, when used in accordance with soil conditions and its intended purpose, is able to increase soil fertility and plant productivity, efficient use of inorganic fertilizers, and reduce environmental impacts.

In general, microorganisms in biological fertilizers include N-fixing, Phosphate Solubilizing Bacteria, organic decomposers, and producing siderophores and growth hormones such as IAA, gibberellin, cytokinin and ethylene [6,7]. Shinha [8] suggested that biological fertilizers provide benefits for plant growth and increase yields through its role of fixing N, dissolving P and K, releasing plant growth regulators, and producing antibiotics, and decomposing organic matter.
One of the quality of biological fertilizer is determined by the carrier material. Biochar can be used as an effective carrier material for microbes that play an important role in the soil so that it can play a role in the manufacture of biological fertilizers [9]. Biochar has an ideal pore size for soil microbial growth [9,10]. The presence of biochar in the soil can be beneficial for bacteria, fungi, and other soil microbes. Chen [11] reported that biochar microbes are protected from external conditions, such as pH, toxic materials and competition with other microbes. Fungi can sporulate in biochar micro pores because in these pores the competition that occurs with other saprophytes is quite low [12]. Biochar can increase the diversity of microorganisms in the soil [13]. These advantages make biochar as usable as a soil enhancer and carrier agent [14,15,16]. This research aims to study the potentials of various types of biochar as a biological fertilizer carrier for swamps.

2. Materials and methods

2.1. Materials

The research was conducted in the laboratory of the Indonesian Swampland Research Institute from February to October 2015. Equipment for laboratory activities used namely: laminar flow (ESCO), autoclave (All American), incubator, analytical balance, petri dish, erlenmeyer, baker, measuring cup, glass spatula and stainless steel, etc. The materials used include decomposer isolates, Phosphate Solubilizing Bacteria (PSB) and N fixing bacteria, rice husk biochar, coconut shell biochar, oil palm empty fruit bunch biochar, and chemicals (merck), such as: Ca(NO₃)₂, KCl, MgSO₄·7H₂O, MgSO₄·7H₂O, (NH₄)₂SO₄, glucose, yeast, K₂HPO₄, NaCl, CaCl₂, Na₂MoO₄ and aquadest.

2.2. Methods

The treatment given was the type of biochar (rice husk, coconut shell, and palm empty fruit bunches) and the type of microbial decomposer, Phosphate Solubilizing Bacteria (PSB) and N fixing bacteria, and a consortium microbial i.e decomposer, PSB, N fixing bacteria. The design used was a Factorial Complete Randomized Design, three replications.

Biochar was prepared by pyrolysis at a temperature about 400°C using a retort kiln. The decomposer microbial used was *Trichoderma* sp, Phosphate Solubilizing Bacteria microbial was *Bacillus* sp and N-fastening microbial was *Azotobacter*. The microbes used had been tested by inter-isolate suitability. This test was carried out by inoculating 3-4 isolates namely microbial decomposers, Phosphate Solubilizing Bacteria, N fastening in one Petridish at room temperature (± 30°C). At five days after inoculation, the growth of each isolate was observed, whether inhibition occurred or not. Synergic growing microbes were selected for the test formulations of these carriers.

The carrier material (biochar) was dried (± 14% moisture content) and then mashed to a size of 2 mm. About 100 grams of each carrier was put in a plastic bag, plus water to get about 30% water content and then sterilized. In each of these carriers the inoculated decomposer (*Trichoderma* sp), Phosphate Solubilizing Bacteria (*Bacillus* sp) and N fixing bacteria (*Azotobacter*), and the combination of the three microbes. The combination of 12 types of treatment, and each treatment was repeated 3 times.

The analysis of the biochar raw materials carried out included lignin, cellulose, hemicellulose, organic C and total N. Biochar analysis included organic C content, total N, pH, CEC, SiO₂, ash content and water content. The media used for fungi using PDA media, P solvent bacteria using Pikhovskaya media and N-fixing bacteria using Nitrogen Free Bacteria (NFB) media. Calculation of microbial populations (Phosphate Solubilizing Bacteria, N-fixing bacteria and decomposer fungi) was done every week at 2, 6 and 10 weeks after inoculation.

3. Results and discussions

3.1. Biochar characteristics

The characteristics of the biochar raw materials used i.e rice husks, empty palm fruit bunches and coconut shells are presented in table 1. Organic materials used as biochar sources are of various quality. Coconut shell had the highest C/N ratio, and the lowest in rice husk. The content of lignin in
rice husk and coconut shell was almost the same, namely rice husk 32.69% while coconut shell 31.87% and lowest oil palm empty fruit bunch was 25.60%. Cellulose ranged from 43.46 to 49.63%, while hemicellulose was 19.65-23.16% (table 1).

Table 1. Cellulose content, hemicellulose and C/N ratio of several organic materials from biochar sources.

| Type of material          | Lignin | Cellulose | Hemicellulose | C    | N    | C/N |
|---------------------------|--------|-----------|---------------|------|------|-----|
| Rice husk                 | 32.69  | 43.46     | 23.16         | 49.07| 0.84 | 58.42|
| Empty palm fruit bunch    | 25.60  | 49.63     | 20.69         | 55.49| 0.70 | 79.27|
| Coconut shells            | 31.87  | 46.36     | 19.65         | 55.52| 0.42 | 132.19|

The characteristics of biochar produced from the pyrolysis process are presented in table 2. The content of organic C varied, the highest in oil palm empty fruit bunches and the lowest in rice husks. The highest SiO2 content of rice husk reached 34.83% and the lowest was at coconut shell 4.04%. The results of the biochar chemical analysis showed that rice husk biochar had a lower pH so it tended to be more suitable for microbes. Microbes can be affected by biochar both directly and indirectly through physicochemical changes. The pH values play a key role in microbial abundance [17]. Slightly alkaline or neutral conditions are preferred bacterial and fungal growth compared to weak acid conditions [18,19]. In general, biochar has a pH value that tends to be alkaline. There was a negative correlation between microbial enzyme activity and biochar pH value [20]. The characteristics of biochar produced from the pyrolysis process are presented in table 2.

Table 2. Characteristics of biochar used in research.

| Type of biochar   | C (%)  | pH   | CEC Cmol/1 kg⁻¹ | SiO2  | Ash content | N  | P  | K  | Ca  | Mg  | Fe  |
|-------------------|--------|------|-----------------|-------|-------------|----|----|----|-----|-----|-----|
| Rice husk         | 23.40  | 8.99 | 37.38           | 34.83 | 44.35       | 0.73| 0.48| 0.54| 0.21 | 0.18| 0.2 |
| Empty palm fruit  | 42.33  | 9.39 | 9.93            | 4.90  | 27.09       | 0.99| 0.49| 8.65| 0.43 | 0.67| 0.5 |
| Coconut shell     | 29.69  | 9.61 | 9.61            | 4.04  | 48.96       | 1.28| 0.52| 2.96| 0.29 | 4.43| 0.2 |

Fungal and bacterial populations are also related to pore spaces in biochar. Fungi and bacteria live and multiply in macropores. Microbial cells have a size of 0.5 – 5 μm, consisting mainly of bacteria, fungi, actinomycetes and algae in pores of 2 μm – 20 μm [21]. The existence and size, as well as the distribution of pores in biochar, provide suitable habitats for many microorganisms by protecting them from predators and drought, providing carbon for energy and minerals for nutrition [12,22]. Biochar pore structure, high surface area, ability to absorb dissolved organic matter, gases and inorganic nutrients tend to provide excellent habitat for microbes to move, grow and multiply, especially for bacteria, actinomycetes and arbuscular mycorrhizal fungi [23].

Biochar can be a microbial carrier because biochar is a relatively moist substance, the surface properties of biochar and slowly releasing minerals, the rate of decomposition is much slower than organic matter [23]. Soil microbial populations can be affected by the quality and quantity of biochar added. Biochar can function as a substrate of microorganisms depending on the composition of pyrolysis residual compounds [24, 25], however biochar can also be toxic to plants [26]. Biochar can act as a place provider, as well as a food source for microorganisms. Microorganisms that live on the surface of biochar are able to decompose enzymes needed for metabolism. Substrate C and inorganic nutrients absorbed on the surface of biochar and/or ash content become a food source for microorganisms.
3.2. **Fungi population**

The fungus used in this research was *Trichoderma* which acted as a decomposer. The results of the isolation study using PDA media at 2, 6 and 10 weeks as presented in table 3.

| Treatments                                      | Population mean $\times 10^4$(CFU/g) |
|-------------------------------------------------|--------------------------------------|
|                                                 | 2 WAI   | 6 WAI   | 10 WAI  |
| Rice husk biochar + *Trichoderma*               | Very abundant | Very abundant | Very abundant |
| Rice husk biochar + *Bacillus* sp               | 0       | 0       | 0       |
| Rice husk biochar + *Azotobacter*               | 0       | 0       | 0       |
| Rice husk biochar + microbial consortium        | 1,33    | Very abundant | Very abundant |
| Coconut shell biochar + *Trichoderma*           | Very abundant | Very abundant | Very abundant |
| Coconut shell biochar + *Bacillus* sp           | 0       | 0       | 0       |
| Coconut shell biochar + *Azotobacter*           | 0       | 0       | 0       |
| Coconut shell biochar + microbial consortium    | Very abundant | Very abundant | Very abundant |
| Empty palm fruit bunches biochar + *Trichoderma*| Very abundant | Very abundant | Very abundant |
| Empty palm fruit bunches biochar + *Bacillus* sp| 0       | 0       | 0       |
| Empty palm fruit bunches biochar + *Azotobacter*| 0       | 0       | 0       |
| Empty palm fruit bunches biochar + microbial    | Very abundant | Very abundant | Very abundant |
| consortium                                       |                                                   |

WAI= week after inoculation

All three types of biochar could be used as a fungi carrier media. It can be seen that the population of fungi in all the biochar types with the inoculation of *Trichoderma* until the 10th week was very high. Ogawa [27] suggested that biochar can be a habitat that is not suitable for saprophytic fungi, but not for mycorrhizal fungi. Biochar can be used as a carrier agent for arbuscular mycorrhizae because mycorrhiza can use biochar as a habitat for growth and a source of nutrition [28]. However, this will greatly depend on the nature of the biochar, as well as on the number of labile organic molecules present in biochar, because biochar can function as a source of C and energy for soil microbes. Biochar can function as a source of C and energy for soil microbes. Laborda [29] showed that fungi (*Trichoderma* and *Penicillium* spp) could contribute to coal depolymerization (hard coal, sub-bituminous coal and lignite) through the production of the phenoloxidase enzyme. Research by Ascough [30] which carried out research in the laboratory showed that by using SEM tools can clearly show the growth of fungi on the surface of the biochar and inside (interior) biochar for three months.

3.3. **Bacteria population**

The bacterial population in the pikovskaya media was used to identify P solubilizing bacteria as presented in table 4. The highest P-solubilizing bacterial population was in rice husk biochar media. Several studies have shown that the application of biochar can increase population and bacterial activity. Biochar increased the abundance of *Geobacter*, *Anaeromyxobacter* and *Clostridium* [31].

Provision of biochar can increase the population and activity of *Bacillus* in the soil, as well as increase P uptake, root and canopy growth and the combination of biochar and *Bacillus* is a prospect for sustainable agriculture [32]. Biochar rice husks can increase the population of the bacterium *Bacillus mucilaginosus* [33]. There was a positive correlation between the P content in biochar and the population of P solubilizing bacteria. The correlation of the effect of P content on biochar was in accordance with the regression equation $Y = -14844x^2 + 1888x - 3454$ $R^2 = 0.625$ [34].

Biochar could also be used as a carrier for N-fixing bacteria. In this study, *Azotobacter* bacteria were used to determine the effectiveness of biochar as a fungal-carrying agent. The results showed that only rice husk biochar could be used as this Azotobacter carrier agent (table 5).
Table 4. Average bacterial population numbers at 2, 6 and 10 weeks after inoculation on Pikovskaya media.

| Treatments                                          | Population mean x 10^6 (CFU/g) |
|-----------------------------------------------------|---------------------------------|
|                                                     | 2 WAI | 6 WAI | 10 WAI |
| Rice husk biochar + *Trichoderma*                   | 9.67  | 6.00  | 5.33   |
| Rice husk biochar + *Bacillus sp*                   | 37.33 | 54.67 | 28.30  |
| Rice husk biochar + *Azotobacter*                   | 39.00 | 18.67 | 14.00  |
| Rice husk biochar + microbial consortium            | 41.00 | 18.00 | 10.00  |
| Coconut shell biochar + *Trichoderma*               | 4.50  | 6.00  | 7.67   |
| Coconut shell biochar + *Bacillus sp*               | 24.67 | 6.67  | 4.67   |
| Coconut shell biochar + *Azotobacter*               | 5.00  | 19.00 | 11.00  |
| Coconut shell biochar + microbial consortium        | 5.00  | 8.67  | 0.33   |
| Empty palm fruit bunches biochar + *Trichoderma*    | 0.67  | 15.00 | 20.33  |
| Empty palm fruit bunches biochar + *Bacillus sp*    | 16.00 | 5.00  | 5.33   |
| Empty palm fruit bunches biochar + *Azotobacter*    | 0.67  | 6.33  | 4.67   |
| Empty palm fruit bunches biochar + microbial consortium | 6.33 | 0     | 0      |

WAI= week after inoculation

Table 5. The average bacterial population at 2, 6 and 10 weeks after inoculation on NFB media.

| Treatments                                          | Population mean x 10^6 (CFU/g) |
|-----------------------------------------------------|---------------------------------|
|                                                     | 2 WAI | 6 WAI | 10 WAI |
| Rice husk biochar + *Trichoderma*                   | 0     | 0     | 0      |
| Rice husk biochar + *Bacillus sp*                   | 0     | 0     | 0      |
| Rice husk biochar + *Azotobacter*                   | 7.67  | 0.33  | 0.33   |
| Rice husk biochar + microbial consortium            | 5.50  | 0.33  | 0      |
| Coconut shell biochar + *Trichoderma*               | 0     | 0     | 0      |
| Coconut shell biochar + *Bacillus sp*               | 0     | 0     | 0      |
| Coconut shell biochar + *Azotobacter*               | 0     | 0     | 0      |
| Coconut shell biochar + microbial consortium        | 5.30  | 0     | 0      |
| Empty palm fruit bunches biochar + *Trichoderma*    | 0     | 0     | 0      |
| Empty palm fruit bunches biochar + *Bacillus sp*    | 0     | 0     | 0      |
| Empty palm fruit bunches biochar + *Azotobacter*    | 0     | 0     | 0      |
| Empty palm fruit bunches biochar + microbial consortium | 0     | 0     | 0      |

WAI= week after inoculation

Azotobacter in addition to fixing N that is in the soil can also produce phytohormones such as cytokinins [35] and gibberellins, IAA [36] and exopolysaccharide production [35] which can increase root proliferation and plant growth. Azotobacter sp. able to increase growth through nitrogen supply, supply of growth regulators, and make soil conditions more favorable for plant growth [37].

Biochar could increase the activity of N fixation by the N fixing bacterial. Güereña [38] reported that fix N in the common bean crop (*Phaseolus vulgaris*) was enhanced by biochar rice husk biochar, biochar bagase, corn stover biochar, corncob biochar, *Eucalyptus* biochar, *Delonix* biochar, and biochar tea. Application of tea biochar showed high nodule formation in ordinary pea plants.

Biofertilizers consisting of microbial consortia ie decomposers, P solubilizing bacteria and N-fixing bacteria were more effective using rice husk carriers than coconut shells and palm empty fruit bunches...
The microbial population in the rice husk biochar media for *Trichoderma, Bacillus* and *Azotobacter* was the highest compared to the other biochar, and although at week 10 *Azotobacter* was not seen in all carriers. *Azotobacter* population in rice husk biochar was higher than in biochar cancorb and bamboo [39].

4. Conclusion
One of the quality of biochar is determined by biochar raw material. Biochar rice husks, oil palm empty fruit bunches and coconut shells are very effective as *Trichoderma* carrier material. However, rice husk biochar is better than coconut shell biochar and palm empty fruit bunches for *Bacillus* and *Azotobacter* carrier material. Rice husk biochar can be used as a carrier material both for a single biofertilizer, as well as a consortium biofertilizer that consists of decomposer fungi, P solubilizing bacteria, and N-fixing bacteria.

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