Role of phosphate solubilising bacteria on availability phosphorus in Oxisols and tracing of phosphate in corn by using ^{32}P

Tri Candra Setiawati[A] and Eko Handayanto[B]

[A]Department of Soil Science, Faculty of Agriculture, Jember University, Jember – Indonesia, Email candra_setiawati@yahoo.com
[B]Department of Soil Science, Faculty of Agriculture, Brawijaya University, Malang – Indonesia, Email ehn-fp@brawijaya.ac.id

Abstract
Phosphorus deficiency is one of the most growth-limiting factors in acid soils in the tropics. Phosphorus fixation results in low P use efficiency in acid soils. Using isotopes as tracers, the percentage of utilization by plants of the P derived from either organic or inorganic fertilizer can be determined. This investigation was conducted with soybean biomass and corn biomass treatments, phosphate-solubilising bacteria (PSB), and rock phosphate (RP). ^{32}P-free carrier was applied to thirty six pots and another set of thirty six pots which were identical with the first in all aspects but without ^{32}P labeling. Corn was grown on a Typic kandiudox from Depok, West Java, Indonesia for 8 weeks. The radioisotope technique with $KH_2{}^{32}PO_4$ carrier free was used to trace the distribution of P in shoot of corn, to calculate the P-fertilizer use efficiency and also to detect phosphate uptake by corn. The ^{32}P activity in corn was measured by Liquid Scintillation Counter. The result of this study showed that application of PSB in biomass significantly increased available-P in an Oxisol. Uptake of P by corn from biomass or RP was higher than from PSB activity. In addition, P-fertilizer use efficiency of corn was very low and less than 5%.

Key Words
Acid soil, *Bacillus*, phosphate-solubilising bacteria, radiotracer ^{32}P, rock phosphate.

Introduction
The term available-P is often used to describe the amount of soil P that can be extracted from solution or taken up by plant roots and utilized by the plant to growth and develop during its life cycle. The concentration of available-P is always low because of continuous plant uptake. Phosphorus fertilizer efficiency in acid soils is less than 20% due to P fixation through P precipitation by soluble Fe and Al, and adsorption by Fe oxides. Phosphorus sorption may decrease with pH increase in acid soils that is caused by precipitation of amorphous Fe and Al oxides, a greater competition of OH^- with phosphate ions for sorption and an increase of negative charges on soil particles. Organic anions with low molecular mass may coordinate with soluble Fe or Al to make some complexes more stable than Fe or Al phosphates that prevent formation of Fe or Al phosphates (Srivastava *et al.* 2007), and also with allophane minerals (Violante and Gianfreda 2000). Phosphate-solubilising bacteria (PSB) inoculants have been assayed but their effectiveness in the soil-plant system is still unclear. In addition, the role of the inoculated PSB that supplies P to the plant seems limited because the transient nature of the compounds released by PSB responsible for phosphate solubilization, and because the possible re-fixation of phosphate ions on their way to the root surface, if any solubilization does take place (Barea *et al*. 2007). Many researchers prove that PSB plays a key role in soil organic P (P*o*) transformations (Frossard *et al.* 1995) through excretion of phosphatase enzymes (Eichler *et al.* 2004), mineralization of P from organic sources (Gressel and McColl 1997), and also synthesis and release of P*o* (Oberson *et al.* 2001). In addition, microorganisms can solubilize sparingly soluble P*i* forms (Iyamuremye *et al*. 1996). Isotopic dilution method is one of the methods used to evaluate of the agronomic effectiveness of P fertilizers. In the isotopic dilution method, which is realized with or without carrier, the isotope must be applied in the same chemical and physical form as the element to be determined. The objectives of this study were to determine the effect of phosphate-solubilising bacteria on available-P in an Oxisol and to tracer of phosphate on corn.

Methods
A Typic kandiudox from Depok, West Java was used in this study. The soil used for this study has the average top soil depth of 0.10 - 0.25 m with the following characteristics: pH (H_2O) 4.3; 1.82% organic C; 0.19% total N; 35 mg/kg available P, cation exchange capacity 14.16 $cmol_{(+)}$/kg; 0.09 K^+, 2.57 Ca^{2+} and 0.99 Mg^{2+} $cmol_{(+)}$/kg. *Bacillus sp.* Phosphate-solubilising bacteria in corn and soybean biomass and rock phosphate were used as treatments. The chemical composition of soybean biomass and corn biomass used as bacteria carriers were 31.53% and 27.5% organic C; 1.76 % and 0.67% total N; 3.45 % and 10.27% sucrose; 11.90 % and 10.28 %

glucose; 6.32% and 18.55 % cellulose. Treatments applied for this experiment were as follows:

1. no application of solubilising bacteria and rock phosphate (as control treatment).
2. application of soybean biomass with no addition of rock phosphate,
3. application of phosphate-solubilising bacteria in soybean biomass with no addition of rock phosphate,
4. application of corn biomass with no addition of rock phosphate,
5. application of phosphate-solubilising bacteria in corn biomass with no addition of rock phosphate,
6. direct inoculation of phosphate-solubilising bacteria into soil with no addition of rock phosphate,
7. application of rock phosphate only,
8. application of soybean biomass with addition of rock phosphate
9. application of phosphate-solubilising bacteria in soybean biomass with addition of rock phosphate,
10. application of corn biomass with addition of rock phosphate
11. application of phosphate-solubilising bacteria in corn biomass with addition of rock phosphate,
12. direct inoculation of phosphate-solubilising bacteria into soil with addition of rock phosphate,

Each treatment was mixed with 7 kg of soil and placed in a plastic pot. Approximately 80g of each soybean and corn biomass containing $\pm 10^{10}$ CFU of *Bacillus sp* per gram and 4.5 g of rock phosphate were applied accordingly. The twelve treatments were arranged in a completely randomized design with three replicates than constituting thirty six pots. A second set of thirty six pots that were identical with the first set in all aspects but without ^{32}P labeling, were maintained to study the effect of the treatments on the corn growth. The radiotracer ^{32}P applied was from a stock solution of ^{32}P -free carrier of which 20 mL was added to each of the first set of thirty six pots to give an activity of 52.36 MBq/pot.

Three pre-germinated seeds of corn cultivar Arjuna from Indonesia were planted in each pot and grown in a glasshouse. After 8 weeks, corn shoots were harvested by cutting 5 cm above the soil surface. Dry matters, ^{32}P, P content and total P-uptake of plant materials were then analyzed. The ^{32}P activity was counted by the Carenkov method using a liquid scintillation counter carried out at the Centre for Research and Development of Isotopes and Radiation Technology, National Nuclear Energy Agency of Indonesia. From the radio assay data, the % P derived from fertilizer (Pdff), % P derived from soil (Pdfs), and P use efficiency (PUE) were computed. Soil samples from the second set of thirty six pots were analyzed for available P (Bray I) and pH (1:5).

Results and discussion

Effect of treatments on soil pH and available-P

The pH value for all treatments increased if compared with control (Figure 1a). The effect of phosphate-solubilising bacteria in biomass carrier on pH was likely associated with the production of OH^- ions by ligand exchange mechanisms that occurred between organic acids and hydroxyl Fe and Al in soils (Iyamuremye *et al*. 1996). Phosphate-solubilising bacteria in soybean biomass with addition of RP increased available-P higher than that in corn biomass and that without solid carriers (Figure 1b). This seems to be related to the different composition of the biomass. The soybean biomass has N content higher that the corn biomass but has lower C/N and lignin ratios than the corn biomass. Residue factors include chemical composition, C/N ratio, lignin content, and the size of residue particles (Johnson *et al.* 2007). Residue C/N ratio is a common indicator of residue quality but is not necessarily an accurate predictor of decomposition rate (Handayanto *et al.* 1994). Available-P of all treatment increased around 5.34% -

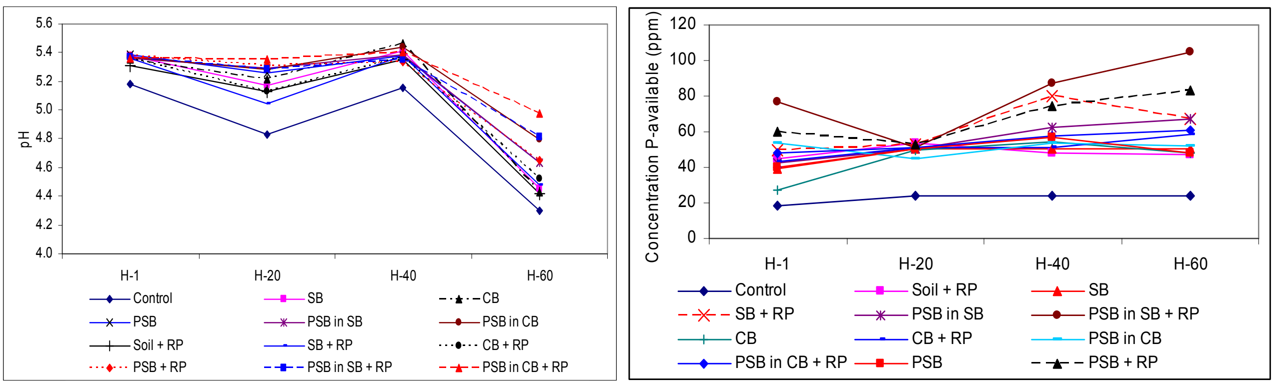

Figure 1. Concentration of soil pH (a) and soil available-P (b) affected by appllication of phosphate-solubilising bacteria (PSB) in biomass with and without RP. SB = soybean biomass; CB = corn biomass; RP= rock phosphate.

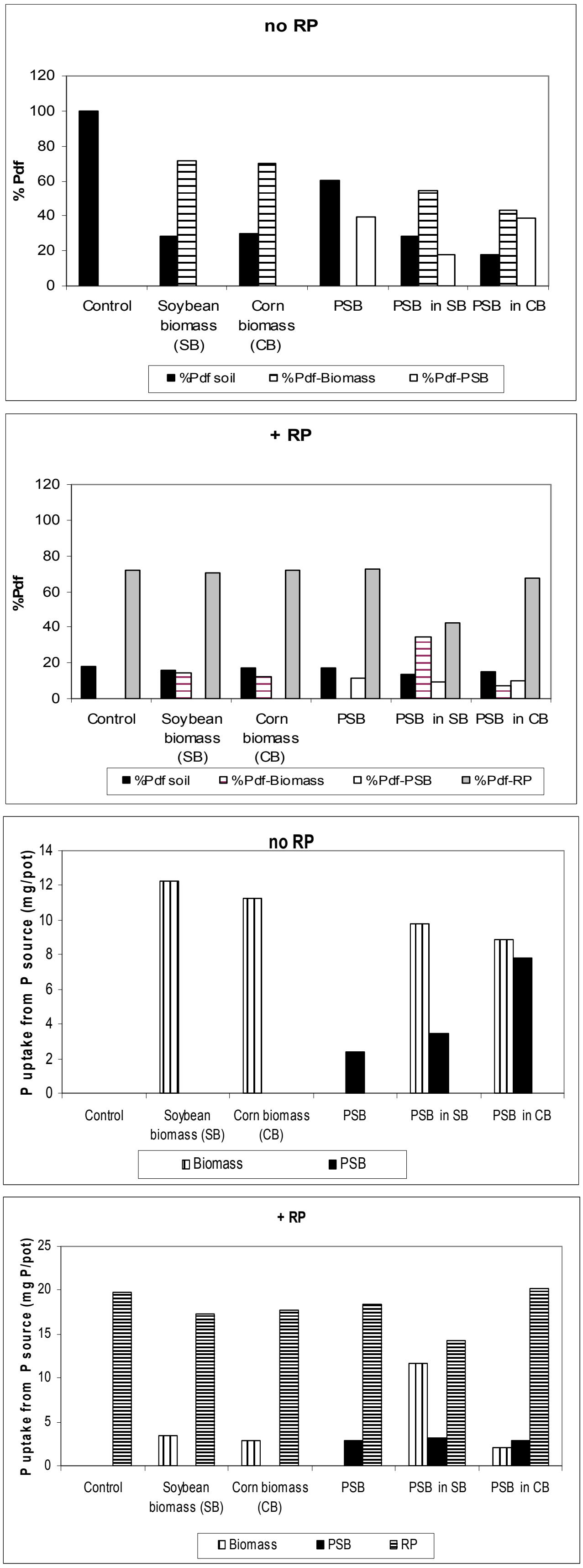

Figure 2. The %Pdff, uptake of P by corn and P use efficiency (PUE) affected by appllication of phosphate-solubilising bacteria (PSB) in biomass with and without RP.

76.71% at 1 to 60 days after planting. The mechanisms were seemed to be the followings: (i) decrease of soil pH because of solubility of Ca-P form; (ii) competition of organic anion with orthophosphate anion due to sorption sites; (iii) ligand exchange; (iv) organic P mineralization. There were no dominant mechanisms since correlation coefficients between available P with other variables (P-organic, P-inorganic and pH) are less than 0.5.

The %Pdff, uptake of P by corn and P use efficiency (PUE)
The PSB activity in both of biomass had a contribution in shoot phosphorus content of around 18.11% - 39.39%. Addition RP gave different model on P uptake by plant which was more uptake from RP than other source (Figure 2a and 2b). Apllication of PSB in CB without RP provided highest phosphate concentration on corn shoot about 16.64 mgP, there were 7.80 mg P from PSB activity, 8.80 mg P from RP and 3.69 mg P from soil. On the contrary, activity PSB in SB offer only 3.42 mg P. Effect of RP addition on concentration of phosphate uptake was significant, there was about 14.26 – 20.14 mg P/pot or 48.87 – 80.14% total concentration of P uptake by plant. The P-use efficiency (PUE) in corn ranged from 2.12 – 2.78% (biomass), while addition of RP about 1.39 – 3.53%. The PUE of corn was similar to that reported before (Hakim 2002), but was lower than the value of Mohanty *et al.* (2006).

Conclusion
This study showed that application of phosphate-solubilising bacteria in biomass significantly increased available-P in an Oxisol. Uptake of P by corn from biomass or RP was higher than from PSB activity. In addition, P-fertilizer use efficiency of corn was very low and less than 5%. This means that around 95%-97% of the P-fertilizer applied was still in the soil, and was expected to give a residual effect to next crops.

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