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Prospect of phosphate solubilizing microorganisms for acid sulphate soil bioremediation

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ABSTRACT

Acid sulphate soil (ASS) have a large distribution in the world (107-108 ha) and in Indonesia (6.71x 106 ha). ASS characterized by high concentration of Al^{3+} and Fe^{2+} ions and also high acidity resulted pyrite oxidation. The presence of these ions and lowering of pH causes some problem such as loss of agricultural productivity and environmental damage. To improve agriculture productivity, ASS requires remediation to minimize of Fe and Al activity and to increase soil pH. One of remediation techniques is microorganisms use (bioremediation). Bioremediation is a process that using living organisms to decrease toxic elements. Phosphate solubilizing microorganisms (PSM) remediate metal-contaminant in soil through decreasing metal toxicity by transforming metal species into immobile form. Some compounds which are excreted PSM and play a role in remediation are organic acid, exopoly saccharides and fosfatase enzyme. Organic acids and fosfatase enyme inactivate Al^{3+} and Fe^{2+} through the process of chelate and precipitate respectively. Absorption of H^+ from solution by exopolysaccharides increase soil pH. Another mechanisms remediation by PSM is its accumulation of metal contaminated in cell surface. Decreasing of Al_{3+} and Fe_{2+} activity and acidity can increase agricultural productivity. This review is an effort to emphasize how PSM can be used to remediate acid sulphate soil dominated by Al, Fe and SO_4^{2-}.

Keywords: *acid sulphate soil, bioremediation, phosphate solubilizing microorganisms*

INTRODUCTION

Acid sulphate soils are estimated to cover an area of 6.71 million hectares in Indonesia. They are environmentally unfriendly soils if they are disturbed or drained. The soil is characterized by the presence of pyrite (FeS2). Under flooded environment, the pyrite is known to be stable. When water table drops due to drought or drainage canal, the pyrite is exposed and oxidized. The drainage generates acidification and also may be adverse effect on groundwater.

The oxidation of pyrite produces high concentration of ferrous ions, and sulfuric acid, which in turn attacks clay mineral and produces high concentration of monomeric aluminum and other acid-soluble metals. Subsequent leaching of these toxic products into adjacent water bodies rapidly increases the stress on ecosystem (Vuai et al., 2003).

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In the soil, metal ion such as Fe2+ and Al3+ cannot be degraded by microorganisms. These ion can only be transformed into other form that do not toxic for the plant and environment. Usually, the technique used for acid sulphate soil remediation is amelioration by lime or organic materials that known as chemoremediation. However, amelioration sometimes influences the soil become very more acidic.

It is known, that some microorganisms are able to remediate degraded land, increase land productivity and crop production. PSM is one of the microorganisms that is able to inactivate heavy metal such as Al3+ and Fe2+ and increase soil pH. Therefore PSM has good prospects for bioremediation of acid sulphate soil.

PROBLEM ACID SULPHATE SOIL

Acid sulphate soils are difficult to be managed for crop production. It is caused the pyrite oxidation in acid sulphate soils (ASS), produce high concentration of strong acid (H2SO4) and Fe2+ in the soil. The acid reacts with soil mineral and dissolves aluminum and other acid-soluble metals. The element and compound resulted pyrite oxidation become suspended in the water when the acid sulphate soil is flooded (Mustafa and Sammut, 2007). During rainfall, they are flushed and discharged into terrestrial and aquatic environments (Vuai et al., 2003). It depends on a number of factors, including exposure to oxidizing condition, the nature and extents of the sulfidic characteristics of material, the capacity to self neutralization and the buffering capacity of the receiving environment (ENVIRONMENT, 2016).

The research in Okinawa Island shows that the runoffs were very acidic with pH range of 2.87 to 4.29, and minimum values were close to the pH values of the soil. The dominant species were SO42-, Al3+ and Fe2+ and also they were well correlated with pH. The acidity of the runoffs caused dissolution of metals in the following order Mn>Zn, Cu=Cd. The quality of runoffs was found to be poor and mainly governed by the interaction of rain and soil. A comparison between the stream waters collected during rain event and on fine day showed a marked changes in stream water chemistry during rainfall as follows: alkalinity was reduced by 3.7 times, sulphate concentration was increased by 3 times whereas pH was decreased by 0.18 units (Vuai et al., 2003).

According to Mustafa and Sammut (2007), soil pH correlated with available P in soil and concentration of Fe and Al. The decrease of soil pH caused increase of soil Fe and Al but decrease of available P. It is caused the available P precipitated by Fe and Al. The pKa of Al is 5 (Rosilawati et al., 2014). When water pH was below, Al3+ will be activated.

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In agricultural practices, both of acidity and increased mobilization of soluble metal commonly Al and Fe may result directly plant toxicity and decreased of uptake and availability nutrient. According to Noor et al. (2012), iron (Fe) toxicity is a major constraint in rice production that decrease yield due to high level of soluble Fe. The level of Fe in a solution of ≥200 ppm Fe inhibited plant growth. The higher Fe concentration in the soil solution, the plant height, number of tillers, shoot and root dry weight decreased. The study from Fahmi et al. (2009) showed that Fe concentration of acid sulphate soil to be not given straw was 444 me/kg, while the one always given straw was 445 me/kg.

In the acid sulphate soil, Al3+ occur in high concentration which can be toxic to plants. The critical concentration of Al3+ in water for rice growth is 15μM (Elisa et al., 2011). At high Al concentration, the root surface was ruptured, leading to cell collapse.

MANAGEMENT OF ACID SULPHATE SOIL

The existence of pyrite in the topsoil is problem in managing the acid sulphate soil. Soil and water management is important in the agricultural development. The common acid sulphate soil management approach are prevention and minimizing pyrite oxidation, neutralising acidity and managing the movement/discharge of toxic oxidation products. Pyrite oxidation can be prevented by placing sulphidic material under anaerobic condition. Some technique for minimizing pyrite oxidation are shallow excavations and drainage measures so the sulphidic material at deeper levels, avoiding lowering of groundwater and/or surface water levels, and close the surface of acid sulphate soils materials.

Neutralizing acidity and managing the movement/discharge of toxic oxidation product are remediation technique for acid sulphate soil had been oxidized. Dolomite, magnesite and hydrate lime are required to increase soil pH and acid sulphate soil productivity. Acid sulphate soil productivity can also be increased by managing water at the micro and macro level, fertilizer and suitable variety (Suriadikarta, 2005).

Inundation of oxidized acid sulphate soil used freshwater or seawater can improve chemical characteristic soil. Sea water plays role in leaching of the pyrite oxidation product and as ameliorant. Seawater has the ability to extract exchangeable acidity (Al3+ and H+) so that it come out from cation exchange complex. The leaching by seawater with concentration 12.5% every 6 days for 8 times caused decrease Al3+ and H+ as much as 22.11 c mol(+)/kg and 5.18 c mol(+)/kg respectively. In the contrary, exchangeable-Mg increase as much as 5.61 c mol(+)/kg (Lestari et al., 2015). Thus the effect of mineral enrichment,

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increase in soil pH and leaching and neutralization of toxic ions by seawater can improve the chemical properties of acid sulphate soils that have oxidized.

PHOSPHATE SOLUBILIZING NICROORGANISME

Microorganisms are an important component of soil and directly or indirectly influence the soil health through their beneficial or detrimental activities. Rhizopheric microorganisms mediate soil processes such as decomposition, nutrient mobilization and mineralization, storage release of nutrients and water, nitrogen fixation and denitrification (Khan *et al.*, 2007).

Phosphate solubilizing microorganisms are the microorganisms possessing a phosphate-solubilizing ability which can convert the insoluble phosphatic compound into soluble form in soil and provide them available to the crop. They include bacteria, fungi and acinomycetes. Vazquez et al., (2000) had been isolated phosphate solubilizing bacteria and fungi. Some bacteria such as *Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus atropheous, Paenibacillus macerans, Vibrio proteolyticus, Xanthobacter agilis, Enterobacter aerogenes, Enterobacter taylorae, Enterobacter asburiae, Kluyvera cryocrescens, Pseudomonas stutzeri* and *Chryseomonas luteola. Burkholderia thailandensis, Spingomonas pituitosa* and *Burkholderia seminalis* are phosphate solubilizing bacteria from acid sulphate soil (Panhwar et al., 2014). Fungi which have ability to phosphate dissolve are *Penicillium sp.* and *Aspergillus sp.* (Sagala et al., 2015).

Low soil pH, especially acid sulphate soil contain low microorganisms. Phosphate solubilizing bacterial population were found in rhizosphere and endorhizosphere the higher compared with non rhizosphere. Population of phosphate solubilization bacteria in acid sulphate soil was 0-1.2x10^7 CFU/g soil, however fungi was 0.3-1.2x10^7 CFU/g soil (Dewi et al., 2017). Population of fungi was higher than bacteria because the fungi more tolerant to acidity.

Phosphate dissolution by PSM can be occurred through chemically and enzymatic processes. PSM solubilize of inorganic P by organic acid secreted, while organic P is mineralized by phosphatase and phytase enzymes (Kumar, 2016). In addition to dissolve phosphate, organic acid can also inactivate Al^{3+} and Fe^{2+} through a chelating process. While the phosphatase enzymes inactivate of Al^{3+} and Fe^{2+} through precipitation process.

BOREMEDIATION BY PHOSHATE SOLUBILIZING MICROORGANISMS

According EPA Guideline (2007), basic principle of acid sulfate soil remediation are to avoid disturbing sulfidic material, neutralize acidity, dilute acidic leachate before discharging, re-flooding, leaching and bioremediation. Bioremediation is a remediation technique using microorganism to decrease or inactivate toxic element.

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Microorganisms are intimately involved in metal biogeochemistry with a variety of processes determining mobility and, therefore bioavailability. The balance between mobilization and immobilization varies depending on the organisms their environment and physicochemical condition. Metal mobilization can arise from a variety of leaching mechanisms, complexity by metabolites and siderophores and methylation, where the results in volatilization. Immobilization can be resulted from sorption to biomass or exopolymers, transport and intracellular sequestration or precipitation as organic an anorganic compounds (oxalates fungi and sulfides). In addition, reduction of higher valency species may affect mobilization eg. Mn(IV) to Mn(II) or immobilization, or Cr(VI) to Cr(III). In the term of bioremediation, solubilization of metal contaminants provides a means of removal from soil matrices, such as soil, sediments, dumps and other solid industrial waste. Alternatively, immobilization processes may enable metals to be transformed in situ and are particularly applicable to removing metal from aqueous solution (Gadd, 2004).

PGPB of the genus *Bacillus*, *Stenotrophomonas* and *Burkholderia* were capable of reducing the effects of Al toxicity. The main mechanism involved was the release of organic acids by PGPB that chelated Al. The common organic acid released by bacteria were oxalic, citric, and malic acid. Organic acids are known as Al-chelating molecules (Panhwar *et al*., 2015). According to Panhwar et al. (2012), the phosphate solubilizing bacteria are able to produce organic acid such as oxalic acid, malic acid, salicylic acid and propionic acid. Organic acids can supply protons and anions that form complexes with metal. Citric and oxalic anion can form stable complexes with a number of metals. Oxalic acid can form stable complexes with Al and Fe (Strasser et al., 1994 in Gadd, 2004).

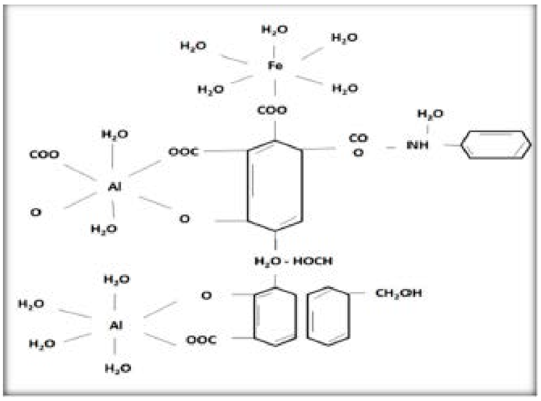

Figure 1. Mechanism of chelate formation by organic acids in the soil (Panhwar et al., 2016)

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The PSB is not only excreted organic acids that inactivated Al an Fe via chelation, but also increase soil pH in the level that precipitates Al as inert Al-hydroxides (Shamshuddin et al., 2017). According to Panhwar et al. (2014), PSB innoculation increased nutrient solution pH at different Al concentration in both plant or non plant system (Figure 2). The isolates were able to produce polysaccharides that might absorb H+ from the solution and increased pH (Panhwar et al., 2015).

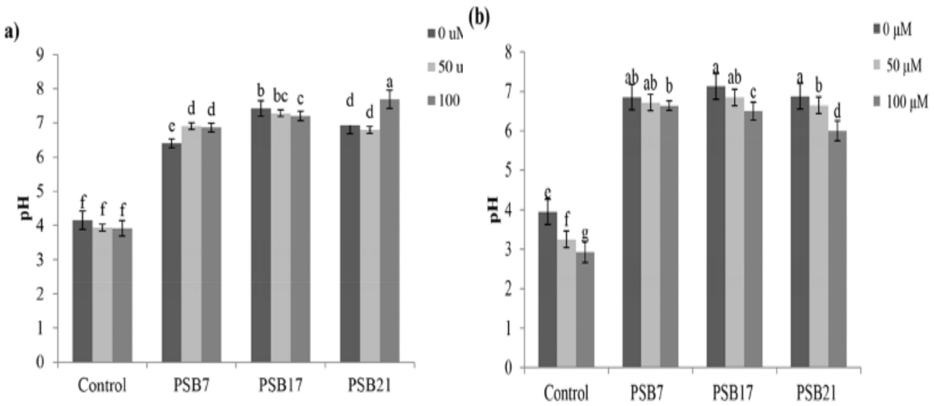

Figure 2. The effect of PSB inoculation on solution pH at different Al concentration (Panhwar et al., 2014)

Application of amendments (GML and basalt), biofertilizer and their combination reduced exchangeable Al and weakly-bound Al, on the other hand increased strongly-bound Al (Figure 3). Lower values exchangeable Al and weakly-bound Al were found in the bio-fertilizer in combination with GML treatment. However, the highest strongly-bound Al was found in the bio-fertilizer in combination with GML treatment too.

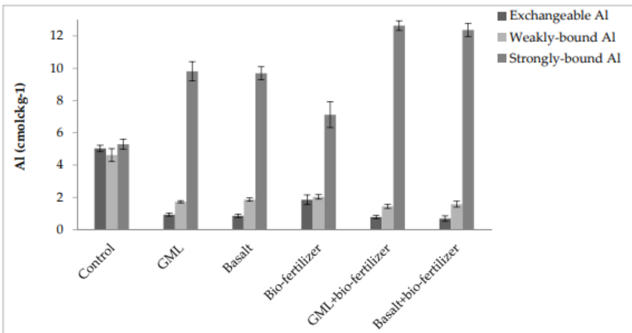

Figure 3. Effect of applying GML (Ground Magnesium Limestone) and basalt, or without bio-fertilizer on the form of Al in acid sulphate soil (Panhwar et al., 2014)

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Soil pH and P-available in acid sulphate soils increase due to amendments (GML and basalt), biofertilizers and a combination of both. On the contrary,it reduced exchangeable-Fe in acid sulphate soil (Table 1). Increasing soil pH followed feri-fosfat solubility so that increasing available-P. Decreasing exchangeable-Fe due to change in the form Fe2+ to Fe3+. According Basir Ciyo (2000), the equition reaction is:

$$FePO_4 + 2H^+ \rightarrow Fe^{3+} + H_2PO_4^{1-}$$

Table 1. Effect of applying GML and basalt, or withouth bio-fertilizer on soil pH, available-P and exchangeable-Al in the acid sulphate soil

Treatment	pH soil	Available-P (mg/kg)	Exchangeable-Fe (cmol/kg)
Control	3.85 e	18.64 d	189 a
GML	4.62 d	19.83 c	86 c
Basalt	4.88 b	20.16 c	79 c
Bio-fertilizer	4.11 d	29.23 b	141 b
GML + Bio-fertilizer	4.85 c	33.74 a	67 d
Basalt + Bio-fertilizer	5.15 a	30.26 ab	76 d

(Sumber: Panwar et al., 2016),
The numbers within the same column followed by the differerent letter are significantly different at $p<0.05$.

Panhwar et al. (2016) showed that application of bio-fertilizer, GML and basalt, either alone or in combination increased rice grain and straw yield (Figure 4). It showed that growth and productivity of rice in acid sulphate soil increased. The reason for this is that soil fertility of acid sulphate soil improve due to reduce Al3+ and Fe2+ activity.

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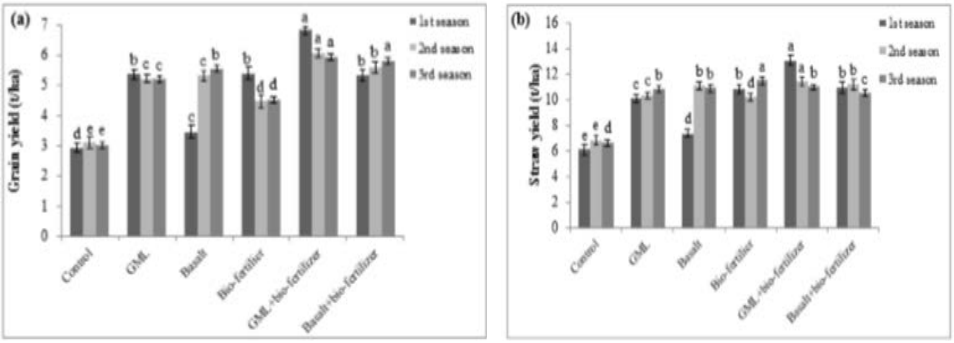

Figure 4. Effect of applying bio-fertilizer, GML and basalt, either alone or in combination to grain and straw yield (Panhwar et al., 2015)

CONCLUSSIONS

The low of acid sulphate soil productivity is caused by high acidification, low availability of macronutrient and the presence of toxic element such as Al3+ and Fe2+. Microorganisms such as PSM can be used to remediate acid sulphate soil. PSM have multifunction such as increase of P-availability and soil pH, decrease Al3+ and Fe2+ activity. Increasing soil pH due to H+ absorbtion by polysaccharide caused precipitated Al3+ and Fe2+. Meanwhile, organic acid excreated by PSM can form stable complex with Al and Fe. Decreasing of Al and Fe activity can increase acid sulphate soil productivity.

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