Effect of sulphate reducing bacteria and ameliorants on nutrient status of N and P in acid sulphate soil

A S Hanafiah, Sarifuddin*, D S Hanafiah and A Araffat
Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia.

E-mail: *sarifuddin@usu.ac.id

Abstract. The application of sulphate-reducing bacteria is expected to reduce the use of lime in increasing the fertility of acid sulphate soils and the growth of corn plants. This study aimed to determine the effect of sulphate-reducing bacteria and ameliorant on shoot dry weight and N and P nutrient status in corn. This research was conducted in the Greenhouse and Soil Laboratory, Faculty of Agriculture, Universitas Sumatera Utara using factorial randomized block design with 2 factors. The first factor was sulphate-reducing bacteria, namely B0 = without bacteria application (Control) and B1 = with sulphate-reducing bacteria and the second factor was soil ameliorant, namely P0 = without ameliorant (control); P1 = OPEFB Organic Material; P2 = OPEFB Organic material + NPK fertilizer; P3 = CaCO3 + NPK fertilizer with 4 replications. The results showed that sulphate-reducing bacteria could increase the shoot dry weight but the application of ameliorants, especially organic matter + NPK and CaCO3 + NPK, besides increasing the shoot dry weight also increasing the plant N uptake and P uptake. The interaction of Sulphate Reducing Bacteria and Ameliorants did not significantly affect all parameters observed.

1. Introduction
Acid sulphate soil is a soil that has several sulfidic materials in the soil which when oxidized will produce sulphuric acid, causing the soil to become acidic to very acidic [1]. However, acid sulphate soils can be an alternative to the extensification of agricultural land to increase food production. The problem with acid sulphate soils is the pyrite layer (FeS2), which when oxidized will cause the soil pH to drop dramatically. Pyrite, in the aerobic atmosphere, becomes unstable because it reacts with oxygen from the air. The oxidation reaction of pyrite with oxygen is slow but can be accelerated by the presence of the *Thiobacillus ferrooxidans* bacteria. The result of this reaction is the release of Fe$^{3+}$ and SO$_4^{2-}$ ions which when dissolved will produce H$^+$ ions and cause Al$^{3+}$ solubility which is toxic to plant roots [2].

The amelioration of the problem in acid sulphate soils can be done by applying sulphate-reducing bacteria, ameliorants, and fertilizers. Ameliorant material can be in the form of lime (calcite or dolomite), organic matter such as husk ash, manure, and others. The results of research in several areas of acid sulphate soil showed that the diversity of microbial populations was influenced by the differences in the organic matter [3]. Currently, cost-effective and environmentally friendly sulphate treatment techniques are employed through the use of plants and microorganisms, although in some cases they have not been successful due to location or technological limitations. Isolation of acid-tolerant sulphate-reducing bacteria can convert sulphuric acid into hydrogen sulphide and produce alkalis in the soil [4]. One research results showed that the application of LK4 bacteria, isolated from...
paper sludge waste from PT. Toba Pulp Lestari at 110% field capacity was able to increase acid sulphate soil pH and decrease soil sulphate levels [5]. This treatment also significantly increased the dry weight of the corn plant. Because, corn has wide adaptations and is relatively easy to cultivate in very diverse environments such as dry land, paddy fields, lowlands, and tidal fields with various types of soil [6], hence, the corn also be planted on acid sulphate soils. This study aimed to determine the effect of sulphate-reducing bacteria and ameliorant and their interaction on the shoot dry weight and N and P nutrient status of corn on acid sulphate soils.

2. Materials and methods
This research was conducted in greenhouses and soil laboratories, Faculty of Agriculture, University of Sumatera Utara - Medan. The soil used is included in the acid sulphate type originating from PT. Mopoli Raya Kebun Paya Rambe II, Seruway Sub-district, Aceh Tamiang District. The soil was taken at a depth of 20 - 40 cm and there is a layer of pyrite. The ameliorant used is compost of oil palm empty fruit bunches (OPEFB), calcite lime (CaCO₃), urea, SP36, and KCl fertilizer. This research used a factorial randomized block design with 2 factors. The first factor was sulphate-reducing bacteria, namely B₀ = without bacteria application (Control) and B₁ = with sulphate-reducing bacteria application with a population of ≥ 10⁸ CFU/ml and the second factor was soil ameliorant, namely P₀ = no ameliorant (control); P₁ = 120 g of OPEFB/8 kg of soil; P₂ = 120 g of OPEFB + (1.2 g urea + 1.1 g SP36 + 0.65 g KCl)/8 kg of soil; P₃ = 30 g of CaCO₃ + (1.2 g urea + 1.1 g SP36 + 0.65 g KCl)/8 kg of soil with 4 replications. The observed parameters were shoot dry weight, plant N and P uptake (% N and P plants x plant dry weight). During the research, soil moisture was maintained at 110% field capacity. The sulphate-reducing bacteria before application were inoculated into a sterile compost weighing 120g. The sulphate-reducing bacteria isolate was put into compost as much as 20% of the weight of the compost used. Then the compost inoculum was incubated in an incubator with a temperature of 35-40°C for ± 4 days.

3. Results and discussion
3.1 Shoot dry weight
The application of sulphate-reducing bacteria on acid sulphate soil was able to increase significantly the shoot dry weight as presented in Table 1. The increase in shoot dry weight was thought to be due to the application of sulphate-reducing bacteria being able to improve soil conditions such as reducing acidity and the level of Al and Fe poisoning in the soil, thereby increasing the availability of plant nutrients.

| Treatment | B₀ (Control) | B₁ (SRB) | Mean |
|-----------|-------------|----------|------|
| P₀ (Control) | 2.84 | 7.51 | 5.17 b |
| P₁ (Organic Matter) | 5.51 | 7.75 | 6.63 b |
| P₂ (Organic Matter + NPK) | 10.5 | 13.3 | 11.9 a |
| P₃ (CaCO₃ + NPK) | 13.7 | 17.1 | 15.4 a |
| Mean | 8.16 b | 11.43 a | 9.80 |

Information: The numbers followed by the same letter were not significantly different based on the DMRT at the 5% level.

The research results of [7], showed that the pH of ex-coal mining soil showed an increase in acid sulphate soil pH after 15 days of SRB incubation. The increasing of soil pH causes metal elements such as Al (Al (OH)₃) sediment and does not harm plant growth. Al poisoning is a major obstacle in...
planting on acid soils. Dissolved Al in soil solution is very active to be absorbed by plant roots yet is toxic. The main response to Al stress occurs in roots, besides that also appears in shoots and leaves [8].

The application of ameliorant was able to increase the plant shoot dry weight at 8 weeks after planting (WAP) with the highest value obtained in the CaCO$_3$ lime + NPK (P3) treatment. The application of CaCO$_3$ lime and NPK fertilizer can increase the growth of corn plants by increasing soil pH and soil nutrients because these two factors determine the plant growth. The research results on oil palm plants stated that the application of dolomite lime could significantly increase the pH of acid sulphate soils and produced the same pH value at doses of dolomite equivalent to 1 times exchangeable-Al and 2 times exchangeable-Al [9]. The appropriate liming will have a positive effect on soil properties in the form of increased availability of P, Ca, Mg, and biological activity as well as deactivating Al and Mn hence their potential toxicity decreases [10]. Plants that grow with the availability of essential nutrients (N, P, K) and energy from sunlight, can synthesize all the compounds needed for normal growth [11].

3.2 Plant N and P uptake
Table 2 showed that the highest plant N uptake was obtained in the treatment of sulphate-reducing bacteria (B1), namely 12.80 mg/plant, but it was not significantly different from the control treatment (B0). The ameliorant treatment significantly affected N uptake, and the highest N uptake was in the application of CaCO$_3$ lime + NPK (P3) which was 20.1 mg/plant and the lowest was in the control treatment (P0) which was 4.68 mg/plant.

| Treatment               | B0 (Control) | B1 (SRB) | Mean |
|-------------------------|--------------|----------|------|
| P0 (Control)            | 2.41         | 6.95     | 4.68 c |
| P1 (Organic Matter)     | 5.11         | 7.43     | 6.27 c |
| P2 (Organic Matter + NPK)| 13.0        | 15.5     | 14.3 b |
| P3 (CaCO$_3$ + NPK)     | 18.6         | 21.5     | 20.1 a |
| Mean                    | 9.82         | 12.8     | 11.3  |

Information: The numbers followed by the same letter were not significantly different based on the DMRT at the 5% level.

Table 3. Plants P uptake with the application of sulphate-reducing bacteria and ameliorants.

| Treatment               | B0 (Control) | B1 (SRB) | Mean |
|-------------------------|--------------|----------|------|
| P0 (Control)            | 0.41         | 0.54     | 0.48 b |
| P1 (Organic Matter)     | 0.52         | 0.65     | 0.59 b |
| P2 (Organic Matter + NPK)| 1.00       | 1.35     | 1.17 a |
| P3 (CaCO$_3$ + NPK)     | 1.38         | 1.40     | 1.39 a |
| Mean                    | 0.83         | 0.99     | 0.91  |

Information: The numbers followed by the same letter were not significantly different based on the DMRT at the 5% level.

Based on table 3, it was identified that the highest plant P uptake was obtained from the sulphate-reducing bacteria (B1) application with 0.99 mg/plant, but it was not significantly different from the
control (B0). The highest P uptake was 1.39 mg/plant from the CaCO₃ lime + NPK (P3) treatment and was not significantly different from the Organic matter + NPK (P2) treatment, while the lowest P uptake was obtained in the control (P0), namely 0.48 mg/plant and significantly different from the treatment of organic matter (P2) and (P1).

The application of ameliorant had a significant effect on increasing plant N and P uptakes. It’s happened because the amount of nutrient uptake is determined by plant nutrient content and plant shoot dry weight. Plants with better growth will absorb more nutrients and vice versa. Better growth occurs due to the availability of nutrients needed by plants. The application of ameliorants such as CaCO₃ lime and NPK fertilizers can increase the availability of plant nutrients. The application of lime can increase soil pH and assist the nutrients such as P which is bound by Al to be release [10]. Besides, N, P, and K fertilization also contribute to macronutrients that can support plant growth on acid sulphate soils, in which nutrient availability, both macro, and micronutrients, is generally low [12].

4. Conclusions
The application of sulphate-reducing bacteria was able to increase the shoot dry weight of plants and the application of ameliorants, especially organic matter + NPK and CaCO₃ + NPK, could increase shoot dry weight, N and P uptakes of plants. The interaction of sulphate-reducing bacteria and ameliorant did not have a significant effect on the observed parameters.

References
[1] Noor M 2004 Lahan Rawa: Sifat dan Pengelolaan Tanah Bermasalah Sulfat Masam [Swamplands: Nature and Management of Acidic Sulfate Problematic Soils] (Jakarta: PT Raja Grafindo Persada)
[2] Balai Besar Penelitian dan Pengembangan Sumberdaya Lahan Pertanian [Center for Research and Development of Agricultural Land Resources] 2006 Karakteristik dan Pengelolaan Lahan Rawa [Swamp Land Characteristics and Management] (Bogor: Badan Penelitian dan Pengembangan Pertanian [Agricultural Research and Development Agency])
[3] Ling Y, Bush R, Grice K, Tulipani S, Berwick L and Moreau J W 2015 Distribution of iron- and sulphate-reducing bacteria across a coastal acid sulphate soil (CASS) environment: implications for passive bioremediation by tidal inundation (Australia) Front. Microbiol. 3 6 624
[4] Ayangbenro A S, Olenrewaju O S and Balalola O O 2018 Sulfat Reducing Bacteria as an Effective Tool for Sustainable Acid Mine Bioremediation (Mbabatho: North-West University)
[5] Sihombing Y S, Hanafiah A S and Sembiring M 2018 Uji Potensi Isolat Bakteri Pengurang Sulfat (SRB) Terhadap Perubahan Keasaman Tanah Sulfat Asam dan Pertumbuhan Tanaman Jagung dengan Kondisi Kelembaban Tanah Berbeda di Rumah Kaca [Potential Test of Sulphate Reducing Bacteria Isolates (SRB) Against Changes in Acidic Sulphate Soil Acidity and Growth of Corn Plants with Different Soil Moisture Conditions in Greenhouses] Jurnal Agroekoteknologi 6 3 pp 515-25
[6] Faesal and Syuryawati 2009 Kendala Dan Prospek Pengembangan Jagung Pada Lahan Sawah Tadah Hujan Di Sulawesi Selatan [Constraints and Prospects for Corn Development in Rainfed Rice Fields in South Sulawesi] (Sulawesi Selatan: Lembaga Penelitian Tanaman Serelia [Cereal Crops Research Institute])
[7] Widyati E 2006 Bioremediasi Tanah Bekas Tambang Batubara Dengan Sludge Industri Kertas Untuk Memacu Revegetasi Lahan [Bioremediation of Ex-Coal Mining Soil with Paper Industry Sludge to Spur Land Revegetation] (Bogor: Institut Pertanian Bogor)
[8] Mukhlis, Sarifuddin and H Hanum 2017 Kimia Tanah: Teori dan Aplikasi [Soil Chemistry: Theory and Applications] (Medan: USU Press)
[9] Ramadhan M, A S Hanafiah and H Guchi 2018 Respon Pertumbuhan Bibit Kelapa Sawit (Elaeis guineensis Jacq.) Terhadap Pemberian Dolomit, Pupuk dan Bakteri Pereduksi Sulfat Pada Tanah Sulfat Masam di Rumah Kaca [Response to the Growth of Oil Palm Seeds (Elaeis guineensis Jacq.)] Medan: Lembaga Penelitian Tanaman Serelia [Cereal Crops Research Institute]
Jacq.) To Giving Dolomite, Fertilizer and Sulphate Reducing Bacteria on Sulphate Soil in Greenhouses] Jurnal Agroekoteknologi 6 3 pp 432-41.
[10] Hanafiah K A 2014 Dasar-Dasar Ilmu Tanah [Basics of Soil Science] (Jakarta: Raja Grafindo Persada)
[11] Utomo M, T Sabrina, Sudarsono, J Lumbanraja, B Rusman and Wawan 2016 Ilmu Tanah: Dasar-Dasar dan Pengelolaan [Soil Science: Basics and Management] (Jakarta: Prenamadia Grup)
[12] Sagala D 2010 Peningkatan pH Tanah Masam di Lahan Rawa Pasang Surut Pada Berbagai Dosis Kapur Untuk Budidaya Kedelai [Increase in pH of acid soils in tidal swamplands at various doses of lime for soybean cultivation] J Agroqua 8 2

Acknowledgements
The authors would like to thank the Directorate General of Higher Education, Ministry of Research, Technology, and Higher Education of Indonesia. Through the Research Institute of the University of Sumatera Utara, which has provided funding for the 2020 Best Basic Higher Education Research. The authors also express their gratitude to the Rector of the University of Sumatera Utara and the Dean of the Faculty of Agriculture for providing permission and facilities to conduct the research.