

The soil sulphate effect and maize plant (*Zea mays* L.) growth of sulphate reducing bacteria (SRB) inoculation in acid sulfate soils with the different soil water condition

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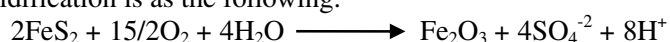
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Abstract. The objective of the study was to determine the potential application of sulphate reducing bacteria on acid sulfate soil with different water content in the green house. The research was carried out in the Laboratory and Green House, Faculty of Agriculture, Universitas Sumatera Utara. This research used Randomized Block Design with two treatments factors, ie sulphate reducing bacteria (SRB) isolate (control, LK4, LK6, TSM4, TSM3, AP4, AP3, LK4 + TSM3, LK4 + AP4, LK4 + AP3, LK6 + TSM3, LK6 + AP4, LK6 + AP3, TSM4 + TSM3, TSM4 + AP4, TSM4 + AP3) and water condition (100% field capacity and 110% field capacity). The results showed that application of isolate LK4 + AP4 with water condition 110% field capacity decreased the soil sulphate content (27.38 ppm) significantly after 6 weeks. Application of isolate LK4 + AP3 with water condition 110% field capacity increased soil pH (5.58) after-week efficacy 6. Application of isolate LK4 with water condition 110% field capacity increased plant growth (140 cm; 25.74 g) significantly after week 6. The best treatment was application isolate LK4 with water condition 110% field Capacity (SRB population 2.5×10^8 ; soil sulphate content 29.10ppm; soil acidity 4.78; plant height 140cm; plant weight 25.74g).

1. Introduction

Based on Bos [1], there are about 12 million ha of acid sulphate soil worldwide and 1.5 million ha of which are found in Indonesia. Adhi and Alihamsyah [2] also reported if combined this soil association with peat and saline soils of acid sulphate in Indonesia to 6.7 million ha . Sludge sulphate is widely used for rice crop (232 ha), annual crops (59,237 ha) and annual crops / plantations (4.0 million ha) but faces many obstacles, including high soil acidity, increased solubility of toxic elements such as Al and Fe and low nutrient availability.

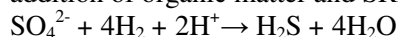
Konsten dan Sarwani [3] explained that the pyrite (FeS_2) which is widely contained in acid sulphate soils is stable if it is in a reductive condition, but if the sulfuric soil is drained the pyrite will oxidize, causing the formation of H_2SO_4 compounds which can increase soil acidity, in this condition soil pH can reach Less than 3.5 (pH <3.5). The reaction equation that describes the oxidation of pyrite and causes soil acidification is as the following:



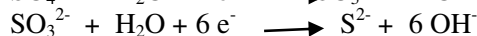
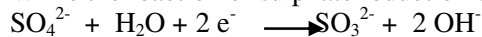
Widyati [4] stated that arrangement of land and water system in accordance with the characteristics of the land, selection of appropriate commodities and varieties, and the application of appropriate amelioration and fertilization technology is a comprehensive effort that can be done to ensure the successful management of sulphate sulphate into productive agricultural land. The results showed that if sulphate sludge is properly managed, almost all food commodities, horticulture, and plantations are well developed and can produce well. High acidity has a negative impact on chemical properties and soil microbial activity because not all soil microbes are able to survive in very acid conditions. Therefore, a technology package is required to improve and improve the productivity of the land. Sulphate Reducing Bacteria (SRB) is a bacteria that can survive in acidic conditions. The bacteria can be utilized to increase the productivity of acid sulphate soil and SRB likes acid conditions then the bacteria can also be applied to sulfur contaminated environments such as post-coal mining land, drainage water and sludge paper waste disposal sites.

Hanafiah et al [5] stated that sulphate reducing bacteria are anaerobic obligate bacteria that use H_2 as an electron donor (chemolithotroph). SRB can reduce sulphate under anaerobic conditions into sulfides, then the resulting H_2S can precipitate toxic metals (Cu, Zn, Cd) as sulphide metal. SRB requires organic substrates derived from short-chain organic acids such as pyruvic acid. Under natural conditions, the acid is generated by other anaerobic activities.

Widyati [4] explained that in sulphate reduction process, SRB uses sulphate as an energy source that is an electron acceptor and uses organic matter as a carbon source (C). Decreasing sulphate concentration will increase soil pH. This occurs because of several interrelated processes, namely due to inundation, addition of organic matter and SRB activities. The sulphate reduction reaction by SRB is as follows:



While the reaction of sulphate reduction by water is as follows:



Based on Sitinjak's [7] research also explained that sulphate reducing bacteria can be found in virtually every environment on earth including sour soil sulphate, paper sludge and sulfur hot water. Isolation, potential test, and benchmarking of sulphate reducing bacteria from paper waste, sulfur hot water and acid sulfuric soil have been found in the same growth medium in laboratory scale. Based on that, it is necessary to test the potential of sulphate reducing bacteria with different isolate sources on acid sulphate soil media with indicator of maize plant.

2. Materials and Methods

This research was conducted in Laboratory of Soil Biology and in the Green house at Faculty of Agriculture, University of Sumatera Utara. The study was conducted from April 2016 to April 2017. The materials used in this research were SRB collection of Soil Biology Laboratory (LK4, LK6, TSM4, TSM3, AP4, AP6), Postgate E (Postgate, 1984) consisting of (g / L): KH_2PO_4 (0.5); NH_4Cl (1.0); Na_2SO_4 (1.0); $CaCl_2 \cdot 6H_2O$ (1.0); $MgCl_2 \cdot 7H_2O$ (2.0); Yeast extract (1.0); Ascorbic acid (1.0); $FeSO_4 \cdot 7H_2O$ (0.5); In order (18.0); Sodium lactate (8 ml); Thioglycollic acid (0.76 ml) for growth of SRB, anaerobic kit as source of SRB CO_2 , label as marker of each sample, sample plastic for sample place, acid sulfuric soil that has been oxidized source PT. Mopoli Raya, Kebun Paya Rambe Aceh Tamiang, polybag 5 kg, plastic hose as a watering hose, BISI hybrid crown seeds as planting material, straw compost as bacteria carrier media, NPK fertilizer as basic fertilizer, and other supporting research materials.

Equipments used included anaerobic jar, incubator, test tube, concrete nail, soil drill, ice box, petridish, inoculation needle, analytical scale, pipette scale, pH meter, microscope, laminar air flow, oven, autoclave, bottle, hoe, ground sieves, 10 kg-scale, gauges, cameras, and other glass tools used for laboratory analysis.

This research used Factorial Random Block Design with two treatments and 2 replication factors, which the treatment, factor I was soil water content with 2 treatment levels; T1 = 100% Field Capacity; T2 = 110% Field Capacity and treatment factor II was SRB isolates with 16 different Isolate; B0 = Without giving inoculum (control); B1 = Isolate LK4; B2 = Isolate LK6; B3 = Isolate TSM4; B4 = Isolate TSM3; B5 = Isolate AP4; B6 = Isolate AP6; B7 = Isolate LK4 + Isolate TSM3; B8 = Isolate LK4 + Isolate AP4; B9 = Isolate LK4 + Isolate AP3; B10 = Isolate LK6 + Isolate TSM3; B11 = Isolate LK6 + Isolate AP4; B12 = Isolate LK6 + Isolate AP6; B13 = Isolate TSM4 + Isolate TSM3; B14 = Isolate TSM4 + Isolate AP4; B15 = Isolate TSM4 + Isolate AP3.

- LK4 (SRB isolate from waste paper sludge of PT. Toba Pulp Lestari code 4)
- LK6 (SRB isolate from waste paper sludge of PT. Toba Pulp Lestari code 6)
- TSM4 (SRB isolate from acid sulfate soil of PT. Mopoli Raya code 4)
- TSM3 (SRB isolate from acid sulfate soil of PT. Mopoli Raya code 3)
- AP4 (SRB isolate from hot water sulfur in Sidebukdebuk code 4)
- AP6 (SRB isolate from hot water sulfur in Sidebukdebuk code 6)

Data was analyzed by variance based on linear model as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \epsilon_{ijk}$$

The data was statistically tested using variance analysis at 5% level,

3. Results and Discussion

3.1. Total Population of Sulphate Reducing Bacteria

Based on Table 1, it appeared that the population of SRB was not much different in each treatment, but there is an increase in population with the added moisture content given. In the average population of sulphate predominant bacteria with soil moisture treatment, the treated water content of 110% Field Capacity ($2,07 \times 10^8$) was higher than the treatment of 100% Field Capacity ($2,01 \times 10^8$). This is because all the macro and micro pores on the planting medium of the treatment of 110% Field Capacity was filled with full of water, therefore it created anaerob condition which is preferred by SRB. The population of bacteria with treatments of groundwater level 100% of field capacity and 110% of field capacity was not statistically significant.

There are also several groups of sulphate reducing bacteria that can live in aerobic conditions. This is supported by Yusron *et al.*[8], which states that sulphate reducing bacteria is an obligate anaerobic bacteria but there are several groups of sulphate reducing bacteria capable of growing under aerobic conditions.

In the treatment of addition of sulphate reducing bacteria into soil, there was no significantly different population increase compared to control. This is caused by sulphate reducing bacteria requiring time to adapt to very low acid soil pH conditions where the initial soil pH conditions are 2,17 and soil media conditions that are inconsistent with environmental conditions of isolate sources. Yusron *et al.*[8], explains that the growth and activity of sulphate reducing bacteria is influenced by pH. At low pH it will inhibit the bacterial enzymatic system resulting in inhibition of energy formation, and at a neutral pH of faster energy formation.

Table 1. Total Population of Sulphate Reducing Bacteria

Population of SRB (CFU)			
Treatments	Water Condition (Field Capacity)		Average
	T1 (100%)	T2 (110%)	
B0 (Control)	2.5 x 10 ⁸	2.5 x 10 ⁸	2.5 x 10 ⁸
B1 (LK4)	1.475 x 10 ⁸	2.5 x 10 ⁸	1.98 x 10 ⁸
B2 (LK6)	2.5 x 10 ⁸	1.47 x 10 ⁸	1.98 x 10 ⁸
B3 (TSM4)	1.29 x 10 ⁸	9.5 x 10 ⁷	1.12 x 10 ⁸
B4 (TSM3)	1.47 x 10 ⁸	2.5 x 10 ⁸	1.98 x 10 ⁸
B5 (AP4)	2.5 x 10 ⁸	2.5 x 10 ⁸	2.5 x 10 ⁸
B6 (AP3)	2.5 x 10 ⁸	2.5 x 10 ⁸	2.5 x 10 ⁸
B7 (LK4+TSM3)	1.72 x 10 ⁸	1.72 x 10 ⁸	1.72 x 10 ⁸
B8 (LK4+AP4)	2.5 x 10 ⁸	2.5 x 10 ⁸	2.5 x 10 ⁸
B9 (LK4+AP3)	2.5 x 10 ⁸	1.72 x 10 ⁸	2.11 x 10 ⁸
B10 (LK6+TSM3)	1.72 x 10 ⁸	2.5 x 10 ⁸	2.11 x 10 ⁸
B11 (LK6+AP4)	2.5 x 10 ⁸	2.5 x 10 ⁸	2.5 x 10 ⁸
B12 (LK6+AP3)	1.29 x 10 ⁸	5.2 x 10 ⁷	9.1 x 10 ⁷
B13 (TSM4+TSM3)	2.5 x 10 ⁸	2.5 x 10 ⁸	2.5 x 10 ⁸
B14 (TSM4+AP4)	7 x 10 ⁷	2.5 x 10 ⁸	1.6 x 10 ⁸
B15 (TSM4+AP3)	2.5 x 10 ⁸	1.72 x 10 ⁸	2.11 x 10 ⁸
Average	2.01 x 10 ⁸	2.07 x 10 ⁸	

Description: The numbers followed by the same letter are not significantly different at the 5% level according to the DMRT test

3.2. Soil Sulphate

The highest soil sulphate content was shown by treatment without SRB isolate and 100% soil water condition (B0T1) condition was 988,76 ppm and the lowest was on treatment of SRB LK4 + AP4 isolate and 110% field capacity on soil water condition (B8T2) that is 27.38 ppm (Table 2). The B8T2 treatment was able to significantly decrease the soil sulphate content statistically to 97.2% lower than B0T1. Treatment of B8T2 showed decrease of sulphate concentration to low category (40-99 ppm), which this category also showed by treatment of B1T2 (29.10 ppm), B9T2 (46 ppm), B10T2 (58,41 ppm), B7T2 (56 ppm), B6T2 (67,03 ppm), B14T2 (79,10 ppm), B5T2 (83,24 ppm), B15T2 (83,24 ppm), and B12T2 (84,97 ppm).

The provision of SRB was able to decrease sulphate concentration in acid sulphate soil to achieve the characteristic of medium level by giving isolates LK4 (B1), TSM4 (B4), AP4 (B5), LK4 + AP4 (B8) and isolate LK6 + AP3 (B12) The value of isolates were respectively 92.55 ppm, 98.07 ppm, 72.72 ppm, 67.21 ppm and 86 ppm, and capable of decreasing by 83%, 82%, 87%, 88% and 84% better than without provision of CPM (552.38 ppm). The decrease in sulphate content is influenced by good capability of SRB in reducing sulphate.

Table 2. Soil Sulphate (ppm) by treatment with SRB isolates and soil water condition

Bacteria	Soil Sulphate (ppm)		Average
	Water Condition (Field Capacity)		
	T1 (100%)	T2 (110%)	
B0 (Control)	988.76a	116.00c	552.38
B1 (LK4)	156.00c	29.10c	92.55
B2 (LK6)	115.66c	117.03c	116.34
B3 (TSM4)	320.83c	130.14 c	225.48
B4 (TSM3)	108.41 c	87.72c	98.07
B5 (AP4)	62.21 c	83.24 c	72.72
B6 (AP3)	163.24 c	67.03 c	115.14
B7 (LK4+TSM3)	779.79ab	56.00 c	417.90
B8 (LK4+AP4)	107.03 c	27.38 c	67.21
B9 (LK4+AP3)	189.79 c	46.00 c	117.90
B10 (LK6+TSM3)	197.38 c	58.41 c	127.90
B11 (LK6+AP4)	226.34 c	65.31 c	145.83
B12 (LK6+AP3)	87.03 c	84.97 c	86.00
B13 (TSM4+TSM3)	169.10c	129.79 c	149.45
B14 (TSM4+AP4)	616.34b	79.10 c	347.72
B15 (TSM4+AP3)	123.93c	83.24 c	103.59
Average	275.74	78.78	

Description: The numbers followed by the same letter are not significantly different at the 5% level according to the DMRT test

In soil moisture content 110% of field capacity (T2) was able to decrease (Table 2). This is supported by Nenny [9], which states that in sulphate reduction reactions, not only H_2S is released but also hydroxyl ions (OH^-). Nenny [9], also states that the more reduced sulphate ions, the more OH^- -ions are produced so the pH will increase.

Treatment of groundwater content of 110% field capacity (T2) or slightly stagnant showed a 15% increase in pH value with an average pH of 4.39 compared to groundwater level 100% field capacity with a pH of 3.82. This is caused by the condition of 110% of the capacity of the field created conditions of reduction or anaerob while in 100% of the field capacity there is still some macro pore space that is not filled by water, thus it creates aerobic conditions. In the aerobic conditions of acid sulfate soils become more stable. Groudev *et al.* [10] states that the saturation of water causes the soil to become anaerobic because the oxygen that fills the soil pores is pushed and replaced by water. When sulphate accepts electrons from organic matter it will undergo reduction to form sulphide compounds as described by Foth [11].

In the study there were several treatments given by SRB with 100% groundwater content showed a lower pH value than without being given SRB with the same groundwater conditions. This might be caused by the death or decline in the ability of SRB due to the presence of oxygen or the presence of bacteria that are antagonistic to SRB isolate. Posgate [12] explained that there is a common occurrence that inhibits microbial growth especially anaerobic types: 1) the presence of oxygen 2) the presence of other bacteria that inhibit the growth of SRB.

3.3. Plant height

The highest plant height due to the application of sulphate reducing bacteria isolate and different soil conditions was indicated by treatment with LK4 bacteria and 110% field capacity water level (B1T2) of 103.25 cm and the lowest in treatment with LK4 + TSM3 bacteria with 100% field capacity (B7T1) is 41 cm. The treatment of B1T2 was not significantly different from the B9T2 treatment and was significantly different from B0T1, B0T2, B1T1, B2T1, B2T2, B3T1, B3T2, B4T1, B4T2, B5T1, B5T2, B6T1, B6T2, B7T1, B7T2, B8T1, B8T2, B9T1, B10T1, B10T2, B11T1, B11T2, B12T1, B12T2, B13T1, B13T2, B14T1, B14T2, B15T1, B15T2, B0T1, and B0T2.

Table 3. Plant Height (cm) by treatment with SRB isolates and soil water condition

	Plant height (cm)		Average
	Water Condition (Field Capacity)		
	T1 (100%)	T2 (110%)	
B0 (Control)	70defgh	50fgh	60
B1 (LK4)	66.5defgh	140a	103.25
B2 (LK6)	73.5cdefgh	92.5bcde	83
B3 (TSM4)	83cdefg	99bcd	91
B4 (TSM3)	76.5cdefg	106.5bc	91.50
B5 (AP4)	70defgh	92.5bcde	81.25
B6 (AP3)	50fh	72cdefgh	61
B7 (LK4+TSM3)	41h	89bcde	65
B8 (LK4+AP4)	68defgh	90.5bcde	79.25
B9 (LK4+AP3)	85cdef	123ab	104
B10 (LK6+TSM3)	83cdefg	88.5bcde	85.75
B11 (LK6+AP4)	52.50fgh	77.5cdefg	65
B12 (LK6+AP3)	49gh	82.5cdefg	65.75
B13 (TSM4+TSM3)	89bcde	83cdefg	86
B14 (TSM4+AP4)	59efgh	82.5cdefg	70.75
B15 (TSM4+AP3)	98bcd	83cdefg	90.50
Average	69.63	90.75	

Description: The numbers followed by the same letter are not significantly different at the 5% level by the DMRT test

3.4. Dry Shoot Weight

The increase of dry shoot weight by giving of sulphate reducing bacteria and difference of soil water condition compared with control showed a varied increase. The highest dry shoot weight of plant where treatment with LK4 isolate with the condition of soil water 110% field capacity (B1T2) is 25.74 g and the lowest is on treatment of LK6 isolate plus AP4 with 100% field capacity (B11T1) that is 0.69 g (Table 4). In the research obtained the highest plant weight on treatment with LK4 bacteria isolate with water content 110% field capacity (B1T2) is 25.774 g where the pH of the treatment is 4.78. The sulfate content of 29.10 ppm is better than the treatment without sulfate reducing bacteria isolate with 100% soil water level. Low soil pH and high sulfate content in the treatment of B0T1 inhibit the growth of corn crops.

The provision of sulphate reducing bacteria will decrease the sulfate content and soil pH increases. An increase in pH leads to a reduction in heavy metals that can interfere plant growth. This is in accordance with Widyati [13], which states that with the provision of SRB into acid sulphate soil will result in reduced sulfate so that the soil pH increases and the solubility of heavy metals will decrease so as not to disrupt the growth of plants or other soil microbes.

Table 4. Dry Shoot Weight of Corn Plant

Dry Shoot Weight			
Treatments	Water Condition (Field Capacity)		Average
	T1 (100%)	T2 (110%)	
B0 (Control)	4.14def	4.56def	4.35
B1 (LK4)	3.20def	25.74a	14.47
B2 (LK6)	3.38def	7.37bcdef	5.37
B3 (TSM4)	4.96def	7.79bcdef	6.38
B4 (TSM3)	5.25def	12.95bc	9.10
B5 (AP4)	1.63ef	1.69ef	1.66
B6 (AP3)	2.84def	4.00def	3.42
B7 (LK4+TSM3)	2.12ef	9.95bcd	6.04
B8 (LK4+AP4)	2.06ef	6.60bcdef	4.33
B9 (LK4+AP3)	6.52cdef	14.08b	10.30
B10 (LK6+TSM3)	6.50cdef	6.29cdef	6.39
B11 (LK6+AP4)	0.69f	2.25def	1.47
B12 (LK6+AP3)	2.48def	5.63cdef	4.05
B13 (TSM4+TSM3)	6.29cdef	5.16def	5.72
B14 (TSM4+AP4)	2.95def	7.32bcdef	5.13
B15 (TSM4+AP3)	8.99bcde	6.62bcdef	7.80
Average	4.00	8.00	

Description: The numbers followed by the same letter are not significantly different at the 5% level by the DMRT test

Plant height growth is the result of a long process in plant metabolism from the absorption of nutrients, carbon dioxide and sunlight. In some plants will form a certain metabolic system in a less favorable situation. When viewed on the terms of growth of maize crop included into the indicator plants that have broad growing requirements. Havlin *et al.* [14] states that plant growth is the result of a complex process through plants synthesizing solar energy, carbon dioxide, water and nutrients from the soil.

4. Conclusions

Sulphate reducing bacteria isolates was able to increase soil sulfuric acid pH and increase the growth of plant with the best SRB isolate types to increase soil acid saturation pH was LK4 isolate. Increased soil moisture content could also increase soil sulphate acid pH and increased the growth of crown crops. The best groundwater conditions to increase acid soil pH was 110% field capacity. The best interaction in reducing acid acid sulphate acidity and increasing the growth of crown plants was shown by LK4 isolates

with groundwater content of 110% KL (population of SRB 2.5×10^8 , soil sulphate 29.10 ppm, soil pH 4.78, plant height 140 cm, canopy dry weight 25.74 g).

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