

# Potential of Sulphate Reducing Bacteria on Reducing Soluble Sulphate Level in Phosgate Medium with Various pH at Laboratory)

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## ABSTRAK

Acid sulphate land in Indonesia covers 6.71 million ha spread on the islands of Kalimantan, Papua and Sumatra. Acid sulphate land is found in areas that have topography including flat (<3%). Judging from the extent, topography and availability of water, the presence of acid sulphate land is being considered for the development of agricultural land, especially with the decreasing fertile land for agriculture. The land has the potential for food crops and annual development. Some of the land has been cleared for transmigration and planted with rice, pulses and fruits with results that are generally below crop production potential (Nugroho et al., 1992).

The agronomic constraints faced in acid sulphate soils are the direct effects of acidity mainly due to the increase in solubility / poisoning of Al and Fe (III), Mn and H ions, phosphate defects because they are fixed by Fe and Al to form Fe-P and Al-P, low base saturation and nutrient deficiency and salinity. If stagnant conditions are found, iron (II) poisoning, H<sub>2</sub>S poisoning, CO<sub>2</sub> poisoning and organic acids are encountered. Whereas soil physical constraints are root developmental barriers at the sulfate horizon because of the stress of water deficiency for plants (Dent, 1986). The agronomic constraints faced in acid sulphate soils are the direct effects of acidity mainly due to the increase in solubility / poisoning of Al and Fe (III), Mn and H ions, phosphate defects because they are fixed by Fe and Al to form Fe-P and Al-P, low base saturation and nutrient deficiency and salinity. If stagnant conditions are found, iron (II) poisoning, H<sub>2</sub>S poisoning, CO<sub>2</sub> poisoning and organic acids are encountered. Whereas soil physical constraints are root developmental barriers at the sulfate horizon because of the stress of water deficiency for plants (Dent, 1986).

Agriculture in acid sulphate land requires new technology input so that the production produced is good enough and the environment is well preserved and there is no land degradation. BPS application can reduce the concentration of sulfate in the soil, improve soil chemical properties, as indicated by changes in pH, C-organic (Siagian et al., 2015; Widyati, 2007). Giving organic material as an electron source followed by flooding to break oxygen supply as an electron acceptor will stimulate SRB activity (Widyati, 2007; Doshi, 2006). The results of research conducted by Siagian et al., (2015), showed that the administration of SRB reduced the sulfate content of former coal mines as well as increased the growth of rubber plants in the polybags after one month of SRB isolates.

From the results of research by Sitingjak et al. (2016), obtained 20 SRB isolates from three sources, namely from Kuala Simpang acid sulphate soils, Lau Sidebuk-debuk sulfur hot springs, Tanah Karo Regency and paper mill waste PT. Toba Pulp Lestari Porsea (TPL). The results of the study showed that SRB was tested at various pHs of growing media (liquid postgate) able to increase the pH of the growing medium. Further research is needed to determine the potential of SRB for dissolved sulfate levels in growing media carried out in the laboratory. Therefore, the authors are interested in conducting research for the test.

## INTRODUCTION

This study consisted of three stages of research, namely the preparation stage of the research, the isolation stage of sulfate reducing bacteria and the potential test stage in liquid postgate media. In this study liquid media and postgate solid media were used as growth media and test media.

Sampling used as a source of sulfate reducing bacteria (SRB) was taken from three locations, namely sulfur hot water of Lau Sidebuk-debuk in Brastagi, waste paper from Toba Pulp Lestari (TPL) in Porsea, acid sulphate soil that had been oxidized from PT. Mopoli Raya in Aceh Tamiang. SRB handling includes two tests, namely qualitative and quantitative tests. Both tests are useful for selecting isolates that will be used in potential tests. SRB isolates from each selected source will continue to the isolation stage.

## Isolation and Purification of Sulfate Reducing Bacteria

The Sulfate Reducing Bacteria that have been tested are grown back into the postgate solid medium. The Sulfate Reducing Bacteria which grows in a tube is scraped to the surface so that it is inserted with the needle into the test tube and scratched onto the surface of the solid Postgate media with a zigzag technique. Petridish was again closed and incubated in Anaero jar containing anaerobic kit for 5-6 days in the incubator.

The colonies that have been grown are then purified by transferring to a test tube containing solid Postgate media according to their respective isolates and labeled. Colonies that have been successfully purified are ready to proceed to the potential test stage

Potential Test of Sulfate Reducing Bacteria in Liquid Postgate Media Sulfate Reducing Bacteria which have been successfully purified carried out the potential test stage which aims to obtain superior the Sulfate Reducing Bacteria isolates that

are able to increase pH and tolerate low pH. The isolates were tested at various pHs of liquid media whose pH levels were adjusted, namely 2.5, 3, 3.5, 4, 4.5, 5 and 5.5. The design used was a Non Factorial Randomized Block Design (RBD) with 2 replications. The potential test stage is by regenerating pure isolates grown in Posgate solid media in posgate liquid media and then incubated

at 350C for 5-6 days. After a growing period, pure SRB isolates (1 mL) were grown on the medium which had been adjusted to the acidity level, then the media which had been inoculated by pure SRB was incubated at 35oC for 14 days. Observation of media sulfate levels was carried out at the end of the incubation period (14 days)

## A. RESULTS AND DISCUSSION

The results of observations of dissolved sulfate levels in liquid media are listed in Table 1.

Table 1. Sulfate levels in solutions at various pH levels after 14 days of incubation

Isolat Code	Levels of Sulfat (ppm)						
	pH 5.5	pH 5	pH 4.5	pH 4	pH 3.5	pH 3	pH 2.5
AP 1	41.44 abcd	32.81 ab	101.28	130.16 fg	130.78	140.97 cde	151.00
AP 3	47.78 abcd	39.59 ab	113.44	113.16 bcdef	133.56	144.03 cde	156.00
AP 4	113.50 e	43.94 ab	53.59	107.5 bcdef	<b>114.94</b>	136.44 bcd	154.03
AP 6	49.97 abcd	29.28 ab	120.28	128.91 efg	123.94	146.75 cde	151.25
AP 7	43.03 abcd	23.19 ab	84.22	120.72 cdefg	135.66	157.28 de	145.31
AP 8	43.78 abcd	44.25 ab	54.78	131.81 fg	145.00	159.63 de	152.94
AP 9	77.69 d	32.97 ab	89.38	147.28 g	134.16	143.09cde	157.88
AP 10	122.06 e	90.34 cd	115.53	126.25 efg	127.22	158.75 de	157.94
LK 1	66.56 cd	21.28 ab	<b>36.25</b>	<b>77.65 a</b>	132.44	156.31 de	155.88
LK 2	22.38 a	24.34 ab	82.72	93.63 abc	134.97	156.44 de	137.03
LK 3	25.56 ab	13.34 a	<b>28.91</b>	96.94 abcd	<b>120.00</b>	149.28 de	148.56
<b>LK 4</b>	30.03 abc	50.44 ab	<b>41.19</b>	<b>86.13 ab</b>	<b>114.25</b>	138.25 cde	<b>118.16</b>
<b>LK 6</b>	144.22 abcd	95.31 cd	51.56	<b>88.62 ab</b>	134.84	<b>107.81 a</b>	143.66
LK 7	63.41 bcd	30.06 ab	56.59	110.06 bcdef	150.72	123.72 abc	<b>103.09</b>
TSM 1	23.56 ab	24.19 ab	140.97	127.47 efg	124.47	160.44 e	153.91
TSM 2	72.00 d	94.59 cd	140.16	125.44 efg	135.31	155.31 de	152.13
TSM 3	68.59 cd	46.41 ab	89.38	102.03 abcde	132.16	158.34 de	152.41
TSM 4	127.63 e	29.16 ab	68.81	122.91 defg	148.13	<b>116.03 ab</b>	134.22
TSM 5	43.09 abcd	58.72 bc	99.72	123.63 defg	132.38	139.65 cde	<b>111.84</b>
TSM 6	119.84 e	108.97 d	113.56	125.31 efg	149.09	144.16 cde	137.47

Description: Figures followed by the same letter show no significant difference at  $\alpha$  5% according to the Duncan Multiple Range Test

The results of observations in Table 1 show that in growing media with a pH of 2.5 overgrown by LK7, TSM5, LK4 containing the lowest soluble sulfate, whereas in growing media with pH 3.0 isolates LK6 and TSM 4 which have the ability to reduce sulfate at the most high. For growing media with a pH of 3.5, isolates of LK4, AP4, LK3 isolates which have the highest ability to reduce sulfate are indicated by lower dissolved sulfate levels. At pH 4.0 isolates of LK1, LK4, LK6 isolates which have more role in reducing sulfate. This is in accordance with Yusron et al., (2009) states that the group of sulfate reducing bacteria is strongly influenced by environmental conditions, environmental acidity, sediment depth,

availability of energy from organic matter and sulfate content

The Sulfate Reducing Bacteria is a bacterium that lives in moderate or mesophilic temperatures. thatthe Sulfate Reducing Bacteria comes from and factory paper waste is taken from warm temperature sludge waste so that the isolate is able to live well during the incubation time in the incubator for two weeks. Yani (2005) states that one of the parameters that must be maintained in processing sludge waste biologically is temperature. The optimal temperature for bacterial growth is 360C -380C. Widyati (2007) also stated that the Sulfate Reducing Bacteria from the origin of paper waste is very close to its characteristics

with the *Desulfovibrio* genus in which the mesophilic life environment is at room temperature (25 °C - 30 °C).

The ability of the Sulfate Reducing Bacteria when viewed from reducing sulfate with a difference in pH of the medium solution, in general the ability of isolates from paper waste factories has good ability with a mean value of lower sulfate levels compared to the others. This result is in accordance with Yusron et al., (2009) which states that differences in the condition of micro-ecosystems of waste storage ponds cause differences in bacterial isolates that grow and adapt to the condition of the ecosystem.

## B. CONCLUSION

From all sources of isolates taken from sulfur hot water, paper mill waste and acid sulphate soil with successive codes AP, LK and TSM can reduce sulfate. Among all the isolates tested, it was found that LK4 and LK 6 isolates derived from paper mill waste had the ability to reduce the highest media sulfat content compared to others and good life ability in all pH conditions of the solution.

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