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Deposition of Heavy Metals on Sulphate Reducing Bacteria Bioreactor Treatment

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ABSTRACT

Heavy metal sedimentation of acid mine waste (AMW) with sulphate reducing bacteria (SRB) isolated from estuary dam ecosystem sediment of Denpasar was studied in detail. Three isolates of SRB namely DPS 1703, DPS 1705 and DPS 1711 were selected. The bacterium was straight rods, motile, spore-forming and able to grow in simple or defined media. According to physiology and biochemical characterization, the isolates were identified as *Desulfotomaculum orientis*. The bacterium survived at pH 3 and showed abundant growth at pH 4.0 to 7.0. Reactor with SRB consortium was applied to precipitate heavy metal ions from acid mine waste. The experiment used compost as a simple organic matter source. Growth of SRB in the anaerobic reactor significantly reduced sulphate to sulfide (92.77 -94.52%) and increased the pH from 3 to 6.2-6.7. Soluble heavy metal ions reduced to the much less mobile or insoluble state. The efficiency achieved for Cd(II) was 85.45-90.00%, Pb(II) 95.10-97.95%, Mn(II) 99.79-99.89%, Zn(II) 99.87-99.90%, Cu(II) 99.23-99.43% and Fe(III) 99.93-99.96%.

INTRODUCTION

Estuary dam Suwung is a lagoon which is on the estuary of the Badung River of Denpasar city. Badung River has long experienced pollution due to waste disposal from various activities in Denpasar city. The high burden of pollutants continuously enters the estuary and leads to a decrease in the quality of the waters and the formation of oxygen deficit zones at the bottom of the waters. This condition affects the composition of biological species with biogeochemical cycles of a number of chemical elements. The acidic properties can trigger the formation of reactive metals in the form of their ions, thereby causing metal contamination in the aquatic environment. Meanwhile, sludge from wastewater treatment becomes its own problem with heavy metal content and acid. The high level of danger to life from heavy metal pollution requires the attention and development of an acid and heavy metal sludge management system. The technology of a concern that is being developed is a biosystem using bacteria. One of the advantages of this is to reduce the level of poisoning pollution element without putting the environment at risk of any side effect. The reduction is accomplished by precipitation of the contaminant in the reduced form of a liquid by the activity of the microorganism. Furthermore, on a certain scale, pollutants that have been isolated can be recovered.

Under anaerobic conditions, biological processes encourage sulfide formation by sulphate reducing bacteria (SRB) (Paulo et al. 2013). SRB is a heterotrophic group that uses simple organic compounds as carbon source. With metabolic respiration, the bacteria utilize sulphate, thiosulphate, sulphites and other reducible sulphur compounds as electron acceptor. The sulphur in the oxidized state will be reduced to sulfide in the anaerobic environment (Kato 2016). SRB is a true anaerobic microorganism with a primitive dissimilatory respiration pathway capable of living in extreme environments. The group of bacteria is generally isolated from aquatic sediments with extreme conditions such as temperature, pH, alkalinity, sulphate content, iron, manganese, ammonium and phosphate (Rückert 2016). This paper discusses the isolation and characterization of SRB from sediment waters contaminated with domestic waste and its use in treating the acidic mine waste.

MATERIALS AND METHODS

Growth of SRB with compost as a carbon source: As a carbon source, compost is fermented by mixing it with water and sewage sludge in 10v tube and aerated for more than 10 days. The fermented compost then insulated for more than 7 days. Rejuvenated compost is readily available as a substrate for SRB growth. Fifty grams of the compost is introduced into the reactor, then the nutrient solution and 10 mL of the SRB suspension were added. The reactor was then topped up with the media and tightly closed. The sus-

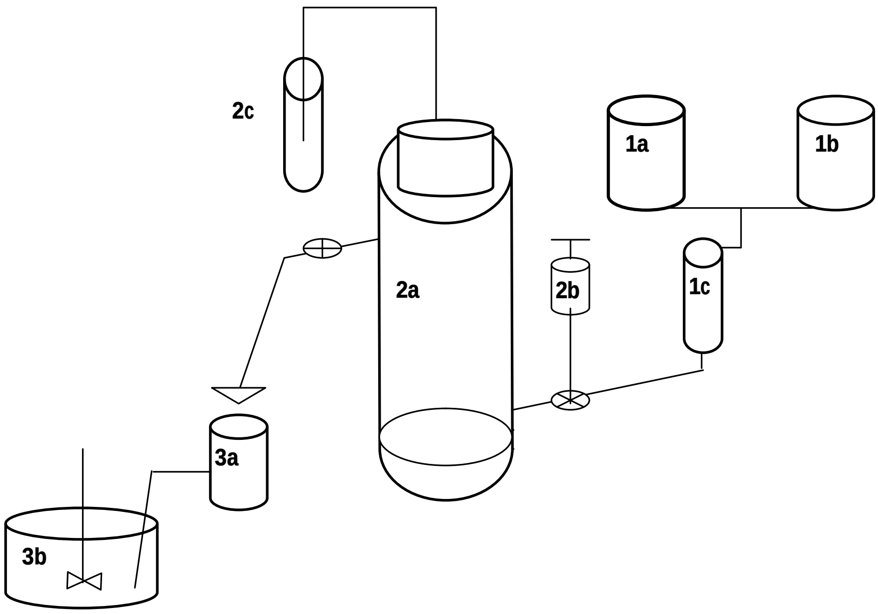

Fig. 1: SRB bioreactor.

pension was incubated at room temperature. The same treatment was performed on the other isolates and the growth of SRB was observed.

Sedimentation of heavy metals in an SRB reactor: Wastewater containing heavy metals is treated in an anaerobic reactor with a bulk system. The prototype of the acid waste treatment reactor is shown in Fig. 1. Acidic waste generally contains high sulphate, low pH and hazardous heavy metals. The acid waste treatment used a reactor with SRB DPS 1703 isolated from estuary dam sediment ecosystem at Denpasar. The reactor comprises of a waste reservoir to be processed, a nutrient pool (starters) and mixing tube (mixer).

The reactor is an airtight processing column with input regulator, wastewater feeder, isolates inoculation tank, and gas flowing. The reactor was also equipped with sampling port and effluent container. The reactor was filled with a suspension of organic compounds and the acid mine waste to be processed. 10% of the isolate DPS 1703 was inoculated into the reactor. Organic fermented to produce lactate and other simple organics substances.

The simple organic carbon such as lactate and pyruvate were main carbon source for the SRB growth in the reactor.

RESULTS AND DISCUSSION

SRB Growth in Liquid Media Containing Sodium Lactate and Compost

To be used as a carbon source for SRB, compost should be treated aerobically and anaerobically to produce simple organic compounds. Decomposition was done in two stages, firstly with sufficient aeration for more than 7 days until pH decreased. Secondly, anaerobic decomposition produces short-chain organic acids. The pH of the sludge and the pH decrease are observable parameters of the measurement, where the decrease in pH is an indication of the formation of simple organic compounds. In this treatment, the pH changed from 7.2 to 5.

The growth of DPS 1703, shown in Fig. 2, reveals that it has a similar growth profile under both the media (sodium lactate and compost). The relationship between dissolved sulphate (SO_4^{2-}) ions, hydrogen sulfide (H_2S), dissolved metal ions, and sulphides insoluble metal (Fig. 3), shows the role of SRB in the sulphate reduction and heavy metal precipitation. Oxidized sulphur minerals produce sulphate ions which result in the acidic properties of the surface waters. Fig. 3 describes how the SRB increases pH and precipitates heavy metals. The process catalysed by bacteria is a redox reaction. Lactate oxidation produces 8 electrons used to reduce sulphate to S^{2-}. The loose S^{2-} ions immediately react with hydrogen and heavy metal cations to form sulfide compounds. The SRB group uses sulphate ions as electron acceptor with simple organic compounds as the electron donor. Chemical reactions catalysed by these bacteria generate energy, producing sulphides (Kato 2016).

At low pH, the metals are ionized. The nucleophile S element will readily react with the cations to form metal sulphides. Metal cations can react with both S^{2-} and H_2S to form precipitated metal sulphides. The description of precipitation of metals through the formation of metal sulphides catalysed by SRB is shown in Fig. 3.

Physico-Chemical Parameters and Nutrient Requirement for SRB Growth

Composition of the materials in the detoxification process of mine acid waste: The essential media composition for the bacterial growth is given in Table 1. Minimal conditions and requirement for SRB growth were obtained from the experiment on each parameter to determine the lowest content of each material resulting in optimum growth of the isolate. Breeding of isolates with posterior B media composition is an attempt to efficiently apply SRB on acid waste process on a larger scale. The observed SRB isolates require a medium (environment) with minimal physico-chemical condition requirements to grow, as given in Table 1.

Content comparison of media, compost, and acid waste for optimal bacterial growth is given in Table 2. It is important to determine the composition of the material in a reactor column. Determination of the optimal material composition is done by measuring the parameters of growth rate, optical density and increasing the pH of the media.

From Table 2, it can be seen that the composition of A2, A3 and B1 has intense bacterial growth, with high optical density, relatively fast growth (12 to 3 days) with pH of 4.9, 5.3 and 5.1, respectively; while the composition of A1, B2, and B3 has slower growth with low optical density at relatively high acidity. For further applications, A3 composition is chosen because it gives good growth with highest increase of pH.

DPS 1703, DPS 1705 and DPS 1711 isolate growths in acid waste treatment reactor: The growth of SRB isolates from DPS 1703, DPS 1705 and DPS 1711 in mine acid effluent reactor was observed on the basis of changes in optical density in the specified time, i.e. 7, 15, 20, 25, 30 and 35 days. The optical density measurements calibrated with blanks (solution without inoculum) were performed to determine the growth rate of each isolate from the adjustment phase to the SRB mortality phase.

Table 3 describes the increasing trend of the optical density of the three isolates after 15 days, peaked between 20 to 25 days and decreased on the 30th day. DPS 1703 has the highest optical density at the peak of its growth but grows slightly slower compared to DPS 1711. DPS 1703 also shows faster growth and higher optical density when compared to DPS 1705. The growth comparison curve of all the three isolates is shown in Fig. 4.

All three isolates show similar growth profiles. Each of those isolates shows sluggish growth (adjustment time up to 13 days), but when it reaches growth period (exponential phase) bacterial growth progresses quickly to stationary phase. Isolate DPS 1703 has the highest optical density in the exponential phase up to the stationary phase compared to the other two isolates.

Sulphate reduction of solution using DPS 1703, DPS 1705 and DPS 1711 isolates: Sulphate ions (SO_4^{2-}) are naturally dispersed in natural waters in a large range of sulphate contents, from a few to thousands of milligrams per litre. The effluent from mining waste can release an enormous amount of sulphate to the environment, derived from pyrite oxidation. Sulphate compounds such as sodium sulphate and magnesium sulphate which are widely used in pharmaceuticals, can also cause sulphate contamination in the environment.

Chemical release of heavy metal uses a solution of sulphuric acid to control the pH of the media (2.4 to 1.7), while biological release uses iron oxidizing bacteria such as *T. ferrooxidans*, by converting iron (II) to iron (III) and sulphuric acid. Likewise, the release of metals naturally involves sulphate as a result of pyrite oxidation, which proceeds through the interactions of chemical and biological processes (Xin et al. 2017). Table 4 gives the observed reduction of liquid media sulphate in bioreactors grown with DPS 1703, DPS 1705 and DPS 1711 isolates. In general, the decrease of sulphate ions started to be seen on the 15th day, and a drastic decrease was seen on the day 20, and then slowly decreased to the 30th day, in accordance with the growth curve (Hwa et al. 2016). In general, the three isolates were in a period of rapid growth on day 20 and reached their

Table 1: Physico-chemical media parameters for SRB growth.

Parameter	Range for Immense SRB Growth
pH	2.5-8.0
Temperature	20°C-40°C
Redox Potential	<120 mV
Na-Lactate	> 500 mg/L
Sulphate	> 100 mg/L
Growth on Compost	> 9 days

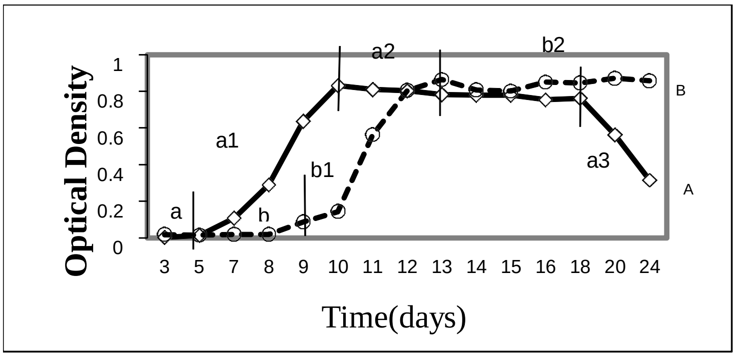

Fig. 2: Growth curve of DPS 1703on sodium lactate and compost.
Note: A=DPS 1703 growth on Na-lactate (correction factor=0.023) B=DPS 1703 growth on compost (correction factor=0.667) a=lag phase A, a1=exponential phase A, a2=stationary phase A, a3=death phase A, b=lag phase B, b1=exponential phase B, b2=stationary phase B.

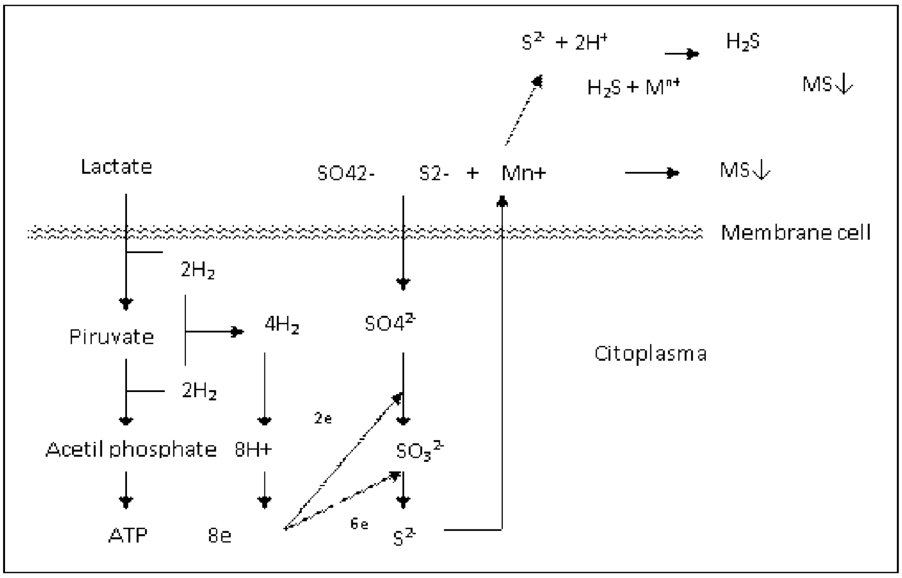

Fig. 3: Reduction schematic of sulfate and heavy metals by SRB.

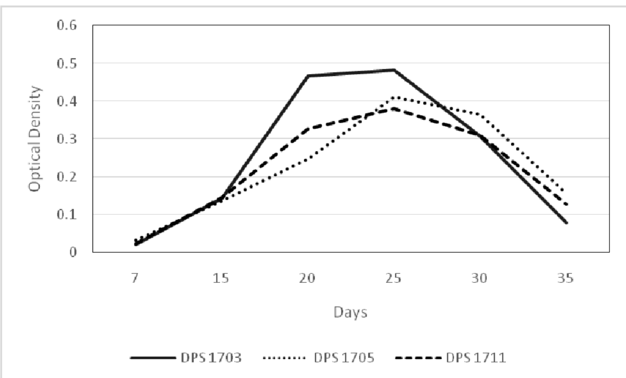

Fig. 4: DPS 1703, DPS 1705 and DPS 1711 growth curve in varied incubation time.

peak on day 25. The three isolates entering the mortal phase after 30 days.

pH increase in acid waste treatment reactor: Natural waters are mostly acidic, which are caused mainly by peat. Acidity is caused by the high content of mineral acids. In the acidic atmosphere the binding of cations is very low, so metals present as free cations. Acidity (low pH) can introduce toxic elements that affect the type and structure of substances in the environment.

The acid mine wastes from the mining areas in Irian Jaya are acidic with pH ranging from 3 to 4. The acidity of the waste is caused by the high sulphate content, which turns to sulphuric acid. The toxicity of the waste is very high, in addition to the acidity and heavy metal ion contents. The

Table 2: Material composition and condition in the detoxification of acid waste.

Composition	Materials			Parameters				
	Media (%)	Compost (%)	Waste (%)	pH_1	COD (mg/L)	Incubation (days)	Optic density	pH_2
A_1	10	10	80	3.2	122	15	0.284	4.3
A_2	15	10	75	3.5	130	13	0.328	4.9
A_3*	20	10	70	3.5	132	12	0.381	5.3
B_1	20	5	75	3.4	130	13	0.315	5.1
B_2	5	5	90	3.2	154	15	0.263	4.6
B_3	15	5	80	3.2	203	16	0.205	4.3

Notes : * = composition with best result; pH_1 = pH before bacterial growth; pH_2 = pH after bacterial growth

Table 3: Optical density of DPS 1703, DPS 1705 and DPS 1711 isolates in varied incubation days.

Isolates	7 days	15 days	20 days	25 days	30 days	35 days
DPS 1703	0.022_a	0.142_a	0.469_a	0.486_a	0.352_a	0.069_a
DPS 1705	0.033_b	0.134_b	0.243_b	0.414_b	0.356_a	0.155_b
DPS 1711	0.025_a	0.147_c	0.325_c	0.345_c	0.349_b	0.124_c
Control	na	na	na	na	na	na

Notes: Distinguish alphabet notation in same column means real different (α = 0.05); na = no growth

Tabel 4: Sulphate reduction (ppm) by DPS 1703, DPS 1705 and DPS 1711 isolates in varied incubation days.

Isolat e	Sulphate concentration (ppm) in varied incubation days					
	Initial	7	15	20	25	30
DPS 1703	1776.53_a	1771.33_a	705.66_a	188.15_a	108.31_a	97.43_a
DPS 1705	1789.22_{ab}	1793.21_b	716.11_b	211.25_b	188.93_b	127.41_b
DPS 1711	1810.34_b	1795.61_c	719.56_c	269.72_c	197.41_c	131.65_c

Notes : Distinguish alphabet notation in same column means real different (α = 0.05)

Table 5: pH increasing by DPS 1703, DPS 1705 and DPS 1711 isolates in varied incubation days.

Isolate	pH on the incubation day					
	Initial	7	15	20	25	30
DPS 1703	3.4_{ab}	3.4_a	3.5_a	4.1_b	6.4_b	6.7_b
DPS 1705	3.5_b	3.5_a	3.6_a	3.8_a	5.5_a	6.2_a
DPS 1711	3.3_a	3.4_a	3.5_a	3.8_a	5.7_a	6.3_a

Notes : Distinguish alphabet notation in the same column means real difference (α = 0.05).

neutralization effort given so far is addition of limestone ($CaSO_4$). This effort is not effective and causes side effects, namely the formation of gypsum deposits ($CuSO_4$) which is also a hazardous waste.

The neutralization of acid mine waste by biotechnology using SRB is an alternative treatment investigated in this study. Table 5 gives the ability of DPS 1703, DPS 1705 and DPS 1711 to increase the pH of the treated medium, from (3.3-3.5) to (6.2-6.7). DPS 1703 isolate shows the highest ability to increase pH (3.3) compared to DPS 1711 (3.0) and DPS 1705 (2.7). The pH increased on day 20 and then increased sharply to day 25.

Reduction of Dissolved Fe (III), Mn, Zn, Cu, Cd and Pb in Acid Waste

The three isolates have similar ability in precipitating heavy metals through the formation of metal sulphides. As an example, DPS 1703 (Figs. 5 and 6) has the capability in reducing sulphate (94.52%), iron (96.96%), Mn (99.89%), Cd (90.00%), Zn (99.90%) and Pb (97.95%). However, unlike plants which can live on a soil with high heavy metal contents, bacteria can only live when the environment is supportive and provides sufficient certain elements.

Plants and bacteria are often used in the accumulation

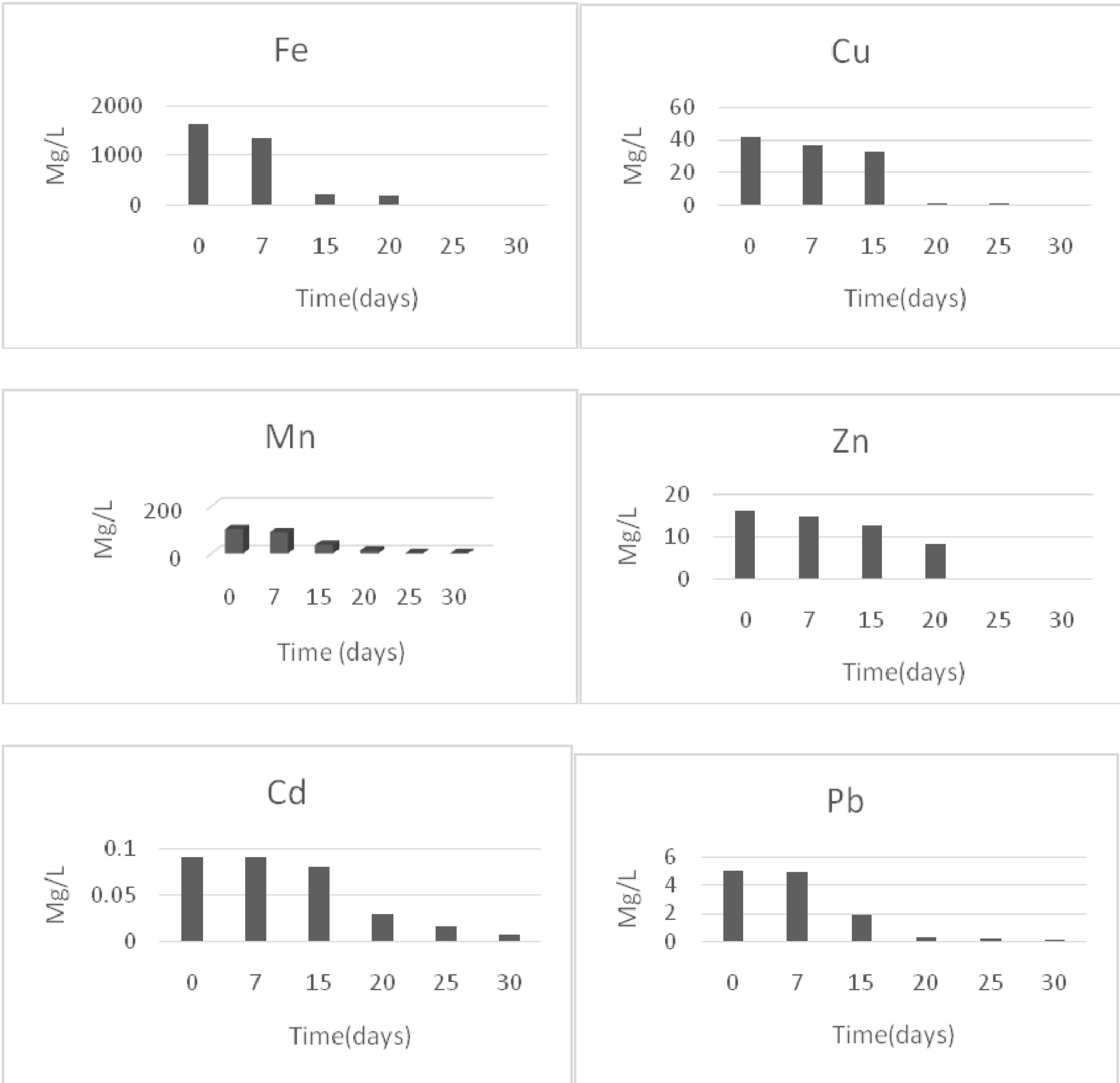

Fig. 5: Decrease of dissolved Fe(III), Cu, Mn, Zn, Cd and Pb concentrations in acid mine wastewater during reactor SRB periods.

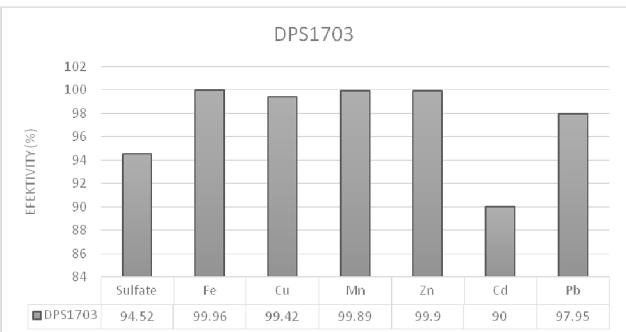

Fig. 6: Dissolved heavy metals reduction effectivity diagram by DPS 1703 after 30 days in ACMW reactor.

and transformation of certain biological elements, known as bioremediation. Biological treatment of chemical pollutants in the soil is conducted through the transformation of elements and compounds into their harmless forms. The degree of transformation depends on the reactions that occur, such as oxidation, reduction, functional group release and mineralization (Rukert 2016).

Fig. 7 illustrates the process of sulphate reduction and the precipitation of heavy metals by metabolic activity. The elements available as dissolved micro-nutrients may act as donors or electron acceptors in the oxidation-reduction (redox) reaction, which is directly involved in metabolic activity (Müller et al. 2014). With regards to SRB, the role of sulfide minerals is as electron acceptor with organic

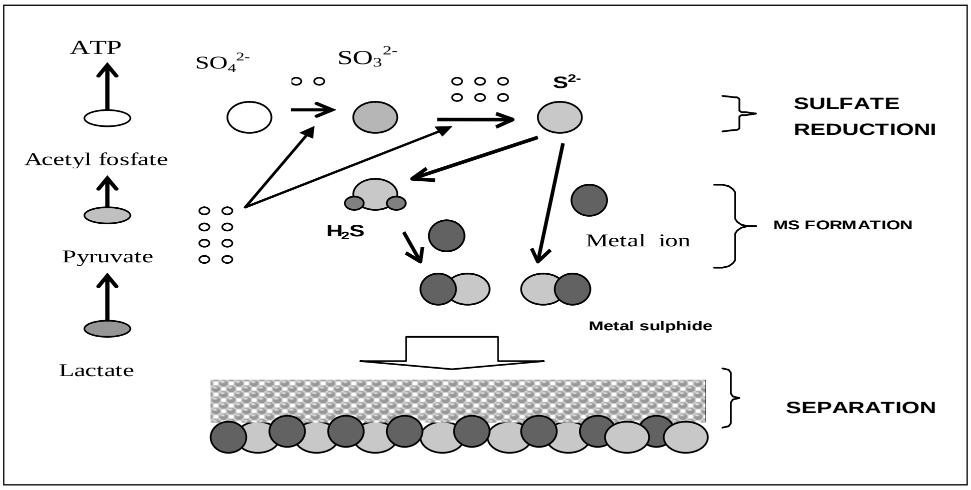

Fig. 7: Illustration of heavy metal precipitation by SRB.

material (heterotroph) as the electron donor.

The resulting chemical energy is used to produce new compounds of an organic material. SRB uses sulphates (SO_4^{2-}) thiosulphates ($S_2O_3^{2-}$), sulphites (SO_3^{2-}) and other reducible sulphur ions as terminal electron acceptor in metabolic respiration. In the process, the sulphur ion is reduced to sulfide. SRB requires organic substrates, generally short-chain organic acids such as lactic acid, pyruvate and so on. In nature, the substrate is provided through the fermentation activity by other anaerobic bacteria (Zhu et al. 2018).

Anaerobic respiration process of SRB can be explained into two stages, namely the sulphate reduction into metal sulfide and the formation of insoluble metal sulphides from the reaction of hydrogen sulfide with dissolved metal ions such as Cu^{2+}, Zn^{2+}, Pb^{2+} and Cd^{2+} as acid water formers (Dennis & Julia 2014). The metal contamination in water can be removed by precipitating the metal cations with the appropriate anions. Most metals are insoluble in water; therefore metal ions can be deposited in the form of sulphides. The reaction is shown by the following equation:

$$H_2S + M^{2+} \rightarrow MS\downarrow + 2H^+ \quad ...(1)$$

M^{2+} = cation

MS = sulfide of heavy metal

The precipitation of heavy metals in the form of sulphides varies greatly, depending on the affinity of the metal cations to react with the element S. The precipitation of certain metal cations is also affected by environmental conditions such as pH and other metal cations.

CONCLUSIONS

All SRB isolates investigated (DPS 1703, DPS 1705 and DPS 1711) have the ability to grow at pH 2.5, room temperature and on compost as organic substrate. This is an advantage, which is an important factor for the application of isolates in acid waste processing. The prototype bioreactor using SRB constructed of a bulk, non-aseptic, column-shaped system equipped with filter and output aeration as well as hydrogen sulfide gas capture, works effectively in treating acid waste. The acid waste successfully increased the pH from 2.5 to 6.2-6.7 with metal (iron, copper, manganese, zinc, cadmium and lead) reduction efficiency up to 85.45 - 99.96%.

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