University of Tehran Press

Pollution

Print ISSN: 2383-451X
Online ISSN: 2383-4501

https://jpoll.ut.ac.ir/

Optimization of Sulphate-Reducing Bacteria for Treatment of Heavy Metals-Containing Laboratory Wastewater on Anaerobic Reactor

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Article Info

Article type:
Research Article

Article history:
Received: 24 Aug 2022
Revised: 15 Nov 2022
Accepted: 13 Jan 2023

Keywords:
Anaerobic reactor
Heavy metals
Laboratory wastewater
Sulphate-reducing bacteria

ABSTRACT

Laboratory wastewater is categorized as hazardous waste that should not be released into the environment since it poses a serious threat to environmental safety. In the present study, the use of Sulphate-Reducing Bacteria (SRB) colonies in an anaerobic reactor to treat heavy metals-containing laboratory wastewater was examined. SRB was initially cultivated with the treatment of fermented compost and Postgate's medium before being attached to the laboratory-size anaerobic reactor to treat laboratory waste containing heavy metal. Within the 15 days of initial incubation under the room temperature of 28 °C, we discovered that SRB optimally grew on the medium with the composition of 5% Postgate B solution, 30% fermented compost liquid, and 5% active suspension liquid, with a total population of cell colonies was 1.2 x 10^5 CFU/ml. After SRB colonies from the most optimum medium were affixed to the reactor, the reactor attained 89% of lead (Pb) removal, 69.78% of iron (Fe) removal, and 48.93% of copper (Cu) removal for 15 days treatment periods. On the 21st days of treatment time, the removal efficiency increased significantly to 91.57%, 78.09%, and 83.56% of Pb, Fe, and Cu removed, respectively.

Cite this article: Suyasa, W. B., Sudiartha, G. A. W., & Pancadewi, G. A. S. A. (2023). Optimization of Sulphate-Reducing Bacteria for Treatment of Heavy Metals-Containing Laboratory Wastewater on Anaerobic Reactor. *Pollution*, 9 (2), 545-556. http://doi.org/10.22059/POLL.2022.347642.1588

 Publisher: University of Tehran Press.
DOI: http://doi.org/10.22059/POLL.2022.347642.1588

INTRODUCTION

The laboratory wastewater can be categorized as hazardous and toxic waste. Most of the hazardous elements contained in laboratory wastewater are in the form of heavy metals, residual organic and inorganic solvents and washing water. In addition, the characteristics of laboratory wastewater are also high with dissolved solids (TDS), Ammonia (NH_3) and Nitrile (NO_2) and acid or alkaline pH (Mahendra & Alvarez-Cohen, 2006). As a result, it will be extremely dangerous for both environmental pollution and human health once laboratory wastewater is disposed in the environment and require thorough treatment process before discharged to water bodies. The presence of heavy metal in laboratory waste is a major concern in many laboratories

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as heavy metal is one of the most hazardous components that are extremely soluble in aquatic environments, easily absorbed by living creatures, and hard-to-breakdown by conventional biological treatment (Kinuthia et al., 2020). Heavy metals are substances with atomic weight ranging between 63.5 – 200.6 and specific gravity higher than 5 (Soliman & Moustafa, 2020).

In several laboratory wastewater and chemical industries wastewater, copper (Cu) and lead (Pb) are some of the most common heavy metals contained in the wastewater (Mosivand, Kazeminezhad, & Fathabad, 2019; Amanze et al., 2022). The toxicity level of some heavy metals are defined in the following sequence of cobalt (Co) < aluminum (Al) < chromium (Cr) < lead (Pb) < nickel (Ni) < zinc (Zn) < copper (Cu) < cadmium (Cd) < mercury (Hg) (Mansourri & Madani, 2016). In order to limit the harmful and dangerous effects of heavy metals contained in laboratory wastewater, it is necessary to have proper wastewater treatment for laboratories that easy to operate and significantly remove heavy metals content. Chemical precipitation, chemical stabilization, ion-exchange, chemical coagulation, membrane filtration, adsorption, chemical oxidation, and electrochemical treatment are just a few of the numerous techniques that scientists have utilized to date to treat water, in which each of these techniques has some benefits and some drawbacks (P. Xu et al., 2013; Naushad et al., 2015; Obaid et al., 2018). At the end, the most effective method for treating wastewater or well water will be chosen based on several variables, including the initial heavy metal concentration, wastewater composition, capital investment and operational costs, plant flexibility, dependability, and environmental impact (Mosivand, Kazeminezhad, & Fathabad, 2019).

Currently, there is an emerging technology that use biomass of particular microbial communities that was found effective and cost-efficient to be utilized to treat heavy metals containing wastewater. Several past studies have reviewed the potential of heavy metals removal by SRB communities and the mechanisms of SRB in treating heavy metals (Ayangbenro, Olanrewaju, & Babalola, 2018; Liu et al., 2018; Y. N. Xu & Chen, 2020; Neria-González & Aguilar-López, 2021). SRB is one of the reducing agents with a bioprecipitation mechanism that is capable of producing sulphides to precipitate metals (Ju & Zhang, 2015). The SRB communities can be easily obtained from muddy soil in anoxic environments such as sediments in the polluted aquatic environments that are characterized by the smell of hydrogen sulphide gas. Li et al. (Jing, Yang, & Wang, 2018) reported 91.7 – 98.2% of Cu removed by the SRB colonies with microalgae as carbon source during 45 days hydraulic retention time (HRT). Santini et al. (Santini, Degens, & Rate, 2010) discovered 96% Pb removal achieved by SRB communities after incubated for 50 days in the temperature of 18 – 30 °C with straw and ethanol as carbon source. Pinto et al. (Pinto, Al-Abed, & McKernan, 2018) even required longer HRT of 500 days to achieve 98% of Cu removal and 70.59% of Fe removal in treating mining-influenced water using SRB communities.

From the previous research, it can be observed that SRB required more than 45 days to remove the majority of heavy metals contained in the wastewater. Hence, present study was aimed to improve the degradation of heavy metals (especially Cu, Fe, and Pb) in less than 30 days of treatment by variating the proportions of several substrates, such as fermented compost and Postgate B, that were considered effective to enhance the biodegradability of heavy metals by SRB communities.

MATERIALS AND METHODS

As much as ± 50 grams of black sediment obtained from Tukad Badung river in Denpasar City, Bali Province, Indonesia. A total of 10 grams of black mud sediment was put into a 500 ml Erlenmeyer flask mixed with Postgate B media, then subsequently sealed, and incubated at room temperature for 10 days. Afterwards, 250 ml of the suspension formed in the Erlenmeyer flask was taken for the second phase cultivation by pouring it into a new 500 ml Erlenmeyer flask that filled with media and incubated at room temperature. The growing phase of SRB was characterized by the formation of a black precipitate (ferrous iron) which appeared several days after inoculation (Seong et al., 2007). Afterwards, the SRB inoculum cultures were cultivated on Postgate B media in 1 L clear glass vials and incubated at 30 °C for 24 hours in the no-light condition. Tubes that did not exhibit blackish colour change were considered in absence of the SRB communities. To perform microbial analysis, the population density of SRB was calculated using the 3 series tube MPN method.

SRB growth supporting media comprised Postgate B liquid media without sodium lactate, mixed with $MgSO_4.7H_2O$, NH_4Cl, $CaSO_4$, $FeSO_4.7H_2O$ and ascorbic acid. As co-digestion to support the growth of SRB, fermented compost liquid was made by mixing the compost solution with 3 g/L yeast and incubated for 3 weeks. Afterwards, the fermented compost was blended with Postgate B, SRB active suspension, laboratory wastewater, and SO_4^- ion with three different compositions as presented in Table 1.

In the present study, laboratory wastewater was taken from the Integrated Laboratory of Chemistry, Udayana University and divided into 3 variations of pH levels between 4.5 to 5.4 and TSS between 1500 - 2500 mg/L, which also contained several heavy metals, namely Cu (20 mg/L), Fe (50 mg/L), Pb (5 mg/L), as shown in Table 2.

The schematic diagram of laboratory wastewater treatment using pilot-scale anaerobic reactor can be seen in Figure 1. The anaerobic reactor was made from fiberglass with a total volume of 5 L and built in the form of an airtight column, to maintain anaerobic condition inside of the reactor to support the growth of SRB, equipped with control of the inflow and outflow, cleanout system, and the isolate inoculation section, which was also connected to the gas reservoir. The treatment reactor was equipped with an iron frame at the bottom of the column which was utilized as a barrier between the compost and the liquid portion of the column. Anaerobic conditions stimulated the growth of anaerobic SRB to breakdown complex organic materials into the simple organic materials. The use of the SRB group to reduce sulphate and precipitate heavy metal residues requires anaerobic culture conditions with low redox potential (Bitton, 2010).

The treatment efficiency of the anaerobic reactor was determined based on the number of concentrations of pollutant removed from the wastewater. The removal of total suspended solid (TSS), pH, and the levels of heavy metals Cu, Fe and Pb were used as indicator of the treatment efficiency. The treatment performance were considered remarkable and efficient if the pollutant

Table 1. Treatment variations with different compositions

Treatment	**Fermented compost** (%)	**Postgate B** (%)	**SRB active suspension** (%)	**Wastewater** (%)	**SO_4^- Ion (ppm)**
T1	10	25	5	60	100
T2	20	15	5	60	100
T3	30	5	5	60	100

Table 2. Physical and chemical content of Chemistry Laboratory, Udayana University, wastewater

Treatment	**Metals**			**SO_4^-**	**pH**	**TSS**
	Cu	Fe	Pb	**mg/L**		**mg/L**
A1	20	50	5	100	5.4	2000
A2	20	50	5	100	5.1	2100
A3	20	50	5	100	4.5	2500

removal percentage reached 50-80% and above. The percentage of reduction in the levels of pollutants were determined based on the following equation:

$$\%removal = \frac{C_a - C_t}{C_a} x100\%$$

where:
C_a = initial concentration of pollutant (mg/l)
C_t = concentration of pollutant at the time, t (mg/l)

RESULTS AND DISCUSSION

The growth of SRB in the initial treatment was indicated by the change of the color of suspension into black as shown in Figure 2. Active suspension with SRB was obtained by activation for 15 days on soil/water sediments with Postgate B solution. The composition of the growth medium consisted of Postgate B solution (5%), Fermented compost liquid (30%) and active suspension liquid (5%). Changes in color density to black (dark). SRB consumes hydrogen and sulphate

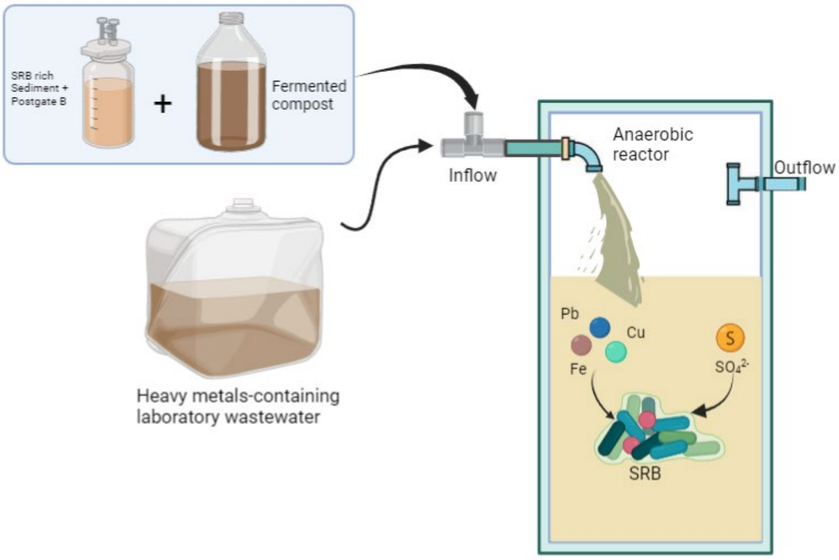

Fig. 1. Schematic diagram of anaerobic reactor treating heavy metals-containing laboratory wastewater

Fig. 2. SRB active suspension (a) before cultivation and (b) after cultivation

as an electron acceptor to degrade organic compounds and volatile fatty acids (VFA) which contribute to the production of sulphide gas (H_2S) and bicarbonate ions (HCO_3^-) (F. Wang et al., 2022; Sudiartha, Imai, & Hung, 2022). MPN 3 series tube analysis was carried out to determine the population density of SRB cells in the SRB solution. The results of MPN analysis showed that the total colony of SRB cells was around 1.1 x 10^5 CFU/ml. The number of colonies was considered adequate for the sewage treatment process (>10^4 CFU/ml) (Seong et al., 2007).

The performance of the SRB-containing anaerobic reactor in the treatment of wastewater from the Integrated Chemistry laboratory, FMIPA Udayana University, was influenced by the different nutrient-to-waste composition. In the present study, the treatment was carried out in three nutrient-to-waste variations as previously mentioned in Table 1. The metals removal performance of the SRB-containing anaerobic reactor can be seen in Figure 3, 4, and 5. As shown by Figure 3, T1 showed a slower removal rate of Cu concentration than that exhibited by the T2 and T3 composition. However, in the first 9 days, the Cu concentration was fluctuating around 15-20 mg/l without showing any significant removal. This finding indicated that the SRB communities in the present study required 9 days, and at least 15 days for T1, for acclimatization

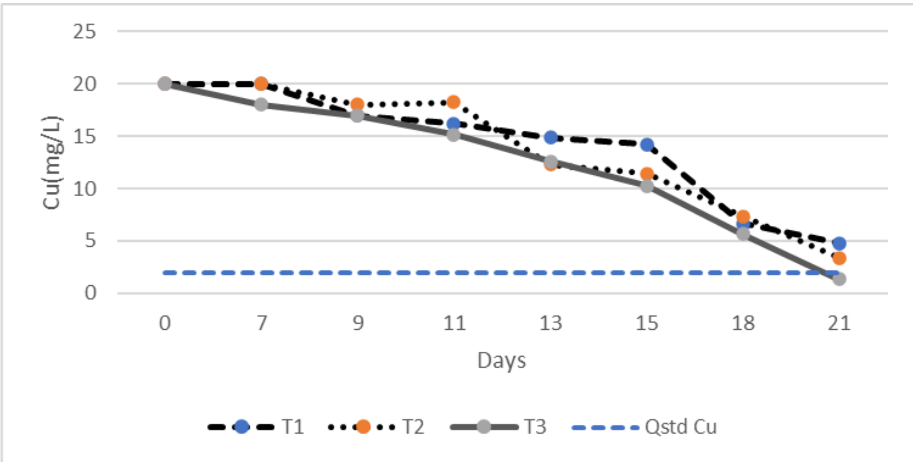

Fig. 3. SRB-containing anaerobic reactor treatment performance of removing Cu metal

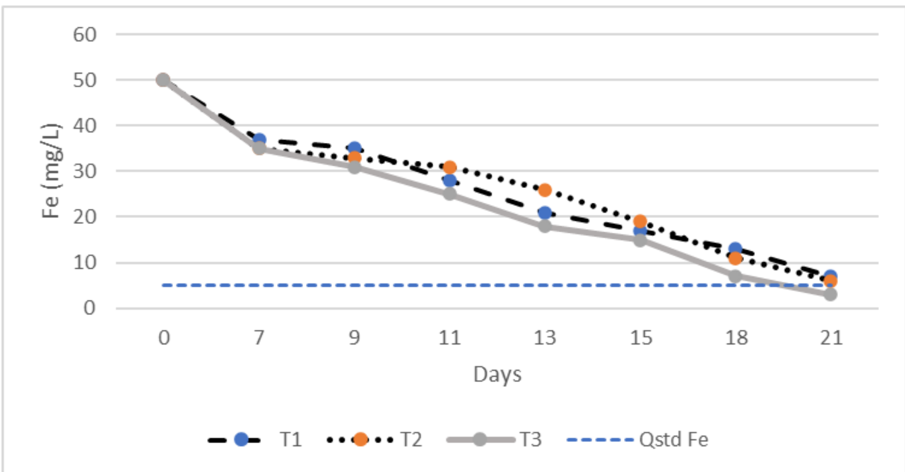

Fig. 4. SRB-containing anaerobic reactor treatment performance of removing Fe metals

after exposed to heavy metals-containing wastewater. This result signified that the lower the composition of fermented compost, the slower the degradation occurred. Qiu et al. (Qiu et al., 2009) achieved a faster SRB growth in which only required 4 days to reach maximum growth, and demonstrated 100% Cu removal on the day 7th. However, the researchers used a low Cu concentration of 0.2 mM, in which below 10 mg/l, exposing less inhibition risk to the SRB growth compared to the present study.

Meanwhile, Jong et al. (Jong & Parry, 2003) demonstrated total removal of Cu by SRB communities in roughly 8 days. The researchers obtained SRB population by isolating water samples collected from a wetland filter at the mine site. Several previous research have presented that the waste streams from acid-mine drainage and wastewater treatment plant contained high quality SRB communities as SRB grow exponentially in mildly acid wastewater that has pH around 6 – 7 (Jong & Parry, 2003; Ayangbenro, Olanrewaju, & Babalola, 2018; Liu et al., 2018). However, in some cases, the pH acid-mine drainage varied around 2 – 3 which seriously inhibited the growth of SRB and destroyed its metabolism (Haixia Wang et al., 2022). Hence, in the present study, the pH was controlled around pH 4 – 7 as can be seen in Figure 6. In harmony

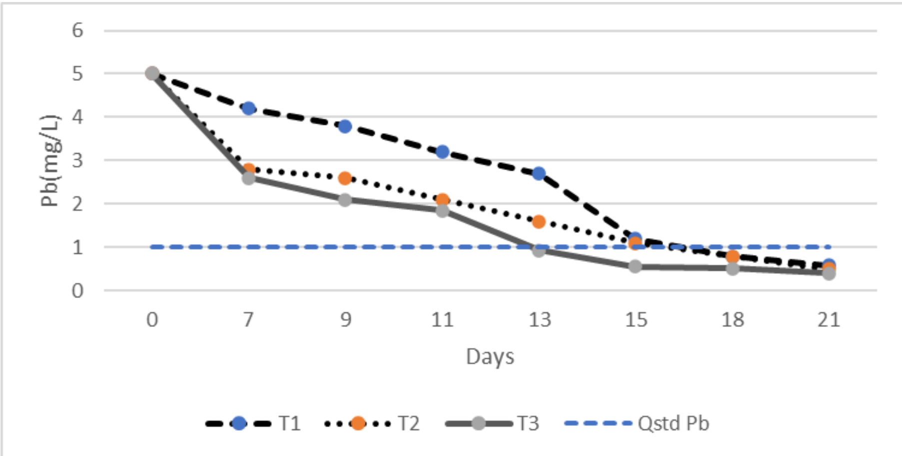

Fig. 5. SRB-containing anaerobic reactor treatment performance of removing Pb metals

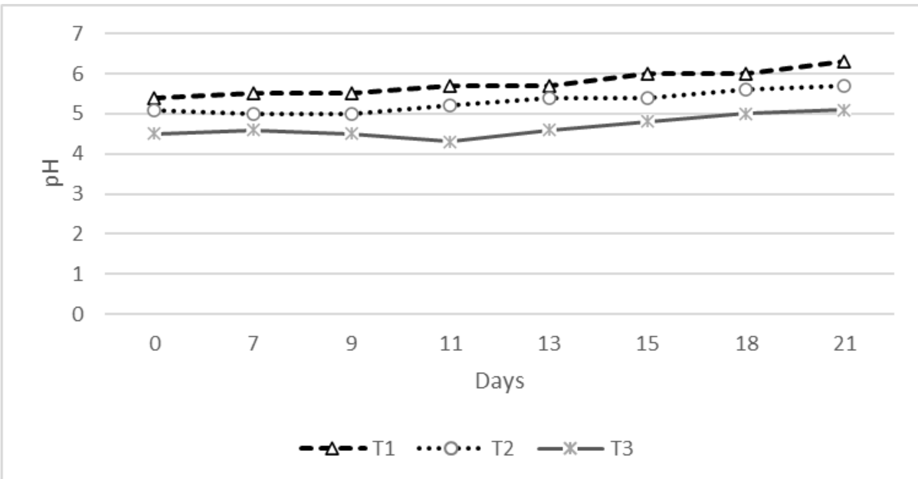

Fig. 6. Variation of pH during the treatment of laboratory wastewater in the anaerobic reactor

to the increase of the pH, the metals removal rate increasing significantly, substantiated by firm degradation after the pH leaving the acid zone of pH 4 – 5 on day 11, for T2 and T3 composition. Meanwhile, T1 composition showed slow degradation when the pH ranging around pH 4 – 5 then managed to optimally precipitate metals after day 15 when the pH was above 5.

In Figure 4, T1 managed to outperform T2 in Fe removal especially in the day 13 where T1 degraded Fe concentration from 50 mg/l to 20 mg/l, compared to T2 that showed a slightly higher Fe concentration in the same treatment period. On the final treatment day, T3 managed to remove Fe concentration below the water quality standard (Qstd) of 5 mg Fe/l, while T1 and T2 are still slightly above the water quality standard. In the present study, the anaerobic reactor managed to significantly degrade the Fe concentration faster than the Cu removal despite that the metal sulphides formed by Cu precipitation is more insoluble than the product of metal sulphides by Fe precipitation (Jong & Parry, 2003). However, on the final day of the treatment, anaerobic reactor only achieved total Fe removal of maximum 78% while Cu deposition reached 84% in total. Despite that Cu is more toxic for the microbial metabolism which perturbs the growth of SRB in the first 15 days, however Cu tends to precipitate faster than Fe due to the insolubility of the sulphides form of Cu. Since that CuS is more insoluble than the FeS, Cu would be easily deposited than Fe as similarly mentioned by previous research (Gopi Kiran, Pakshirajan, & Das, 2018; Yan et al., 2018).

Compared to any other metals observed in the present study, Pb was the fastest metal removed from the wastewater by the SRB-containing anaerobic reactor. As shown by Figure 7, T3 managed to remove Pb concentration into the water quality standard on day 13, while T1 and T2 achieved the quality standard on day 15. On the day 18, both T1 and T2 achieved maximum removal for Pb of 88%, higher than Fe and Cu removal that below 80%, as seen in Figure 7. While in T3, up to 91% of Pb was removed from the day 13 until the end of treatment period, and 81% of Cu was precipitated on the day 18.

Several previous research supports the findings in the present study which also denoted a higher removal efficiency of Pb concentrations, compared to any other metals observed, which was achieved during the treatment using either free or immobilized SRB microbial communities (Zhang, Wang, & Han, 2016; Gopi Kiran, Pakshirajan, & Das, 2018; Yan et al., 2018). There have been several previous research that explored the potential of SRB microbial communities in degrading heavy metals pollutant in wastewater, using inexpensive substrates, as can be seen in Table 3.

Figure 8 shows the degradation of TSS during the treatment in anaerobic reactor. A sluggish removal was observed in the first 15 days of retention time. The significant decrease in TSS concentration occurred after 13 days of treatment along with the increasing activity of SRB and substantial deposition of metals content. Starting from 2000 mg/l TSS, T1 treatment process managed to decline the TSS concentration into 162 mg/l (92%). While T2 achieved total of 88% of TSS removal, T3 treatment only capable of reducing TSS up to 83%. Present study found that the decreasing removal efficiency of TSS was associated with the amount of fermented compost added as a carbon source. T1 consisted of 10% of fermented compost meanwhile T2 and T3 comprised 20% and 30% of fermented compost. The higher composition of fermented compost, the higher the TSS concentration as fermented compost was characterized by dense suspended solid and trigger biomass growth in the reactor system.

Metals degradation by SRB can be seen in Figure 9. Under anaerobic circumstances, SRB with various respiration types (litho-autotrophic, autotrophic, and heterotrophic) have been discovered (Hussain et al., 2016). While heterotrophic SRB may use a diverse range of substrates, including alcohols, hydrogen, organic acids, and fatty acids (FAs), autotrophic SRB use the CO_2 as a carbon source and H_2 as an electron donor to acquire energy for growth (Y. N. Xu & Chen, 2020). However, complex macromolecules substances need to be be breakdown into

Table 3. Recent studies regarding the removal of heavy metals using SRB

Carbon source	Wastewater type	Reactor type	Temperature	pH	Time	Heavy metals removal	References
Fermented compost	Laboratory wastewater	Culture medium + Sulphate-reducing bioreactor	Room temperature	4 – 6	21 days	Cu (76 – 84%), Fe (75 – 78%), Pb (88 – 91%)	Present study
Crushed crab shells	Mining-influenced water	Anaerobic experimental columns	27 °C	5 – 6.6	500 days	Cu (98.99%), Fe (70.59%)	(Pinto, Al-Abed, & McKernan, 2018)
Chicken manure	Acidic coal refuse	Culture medium	30 °C	5.5 – 6.6	20 days	Fe (99.7%), Cu (93.2%)	(Zhang & Wang, 2014)
Straw with ethanol	Acidic saline drainage	Culture medium	18 – 30 °C	5.1 – 5.9	50 days	Pb (96%)	(Santini, Degens, & Rate, 2010)
Sodium lactate	Artificial wastewater	Culture medium with graphene oxide (GO) and reduced GO	10 – 45 °C	2 - 12	80 hours	Pb (97.1%), Cu (89.2%), Fe (77%)	(Yan et al., 2018)

more digestible organic matter by other fermentative bacteria first, which then becomes carbon source for SRB growth. Equations (1) - (3) describe the process that occurs when lactic acid is employed as the carbon source for SRB (Tasaki et al., 1993).

$$2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2HCO_3^- + HS^- + H^+ \quad (1)$$

$$2CH_3CH_2OH + SO_4^{2-} \rightarrow 2CH_3COO^- + HS^- + H^+ + 2H_2O \quad (2)$$

$$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^- \quad (3)$$

SRB cannot directly convert sulphate to sulphide since sulphate is a thermodynamically stable oxidation form of sulfur, hence the process of sulphate reduction is carried out in stepwise manner as follows (Keller & Wall, 2011):

$$AMP^{4-} + SO_4^{2-} + H^+ \rightarrow APS^{2-} + HP_2O_7^{3-} \quad (4)$$

$$APS^{2-} + 2e^{2-} + H^+ \rightarrow HSO_3^- + AMP^{2-} \quad (5)$$

$$HSO_3^- + 6e^{2-} + 6H^+ \rightarrow HS^- + H_2O \quad (6)$$

$$H_2S + M^{2+} \rightarrow 2H^+ + MS(S) \quad (7)$$

Note: APS: adenosine 5-phosphosulphate; AMP: adenosine monophosphate; M: metal ion; MS: metal sulphide precipitation.

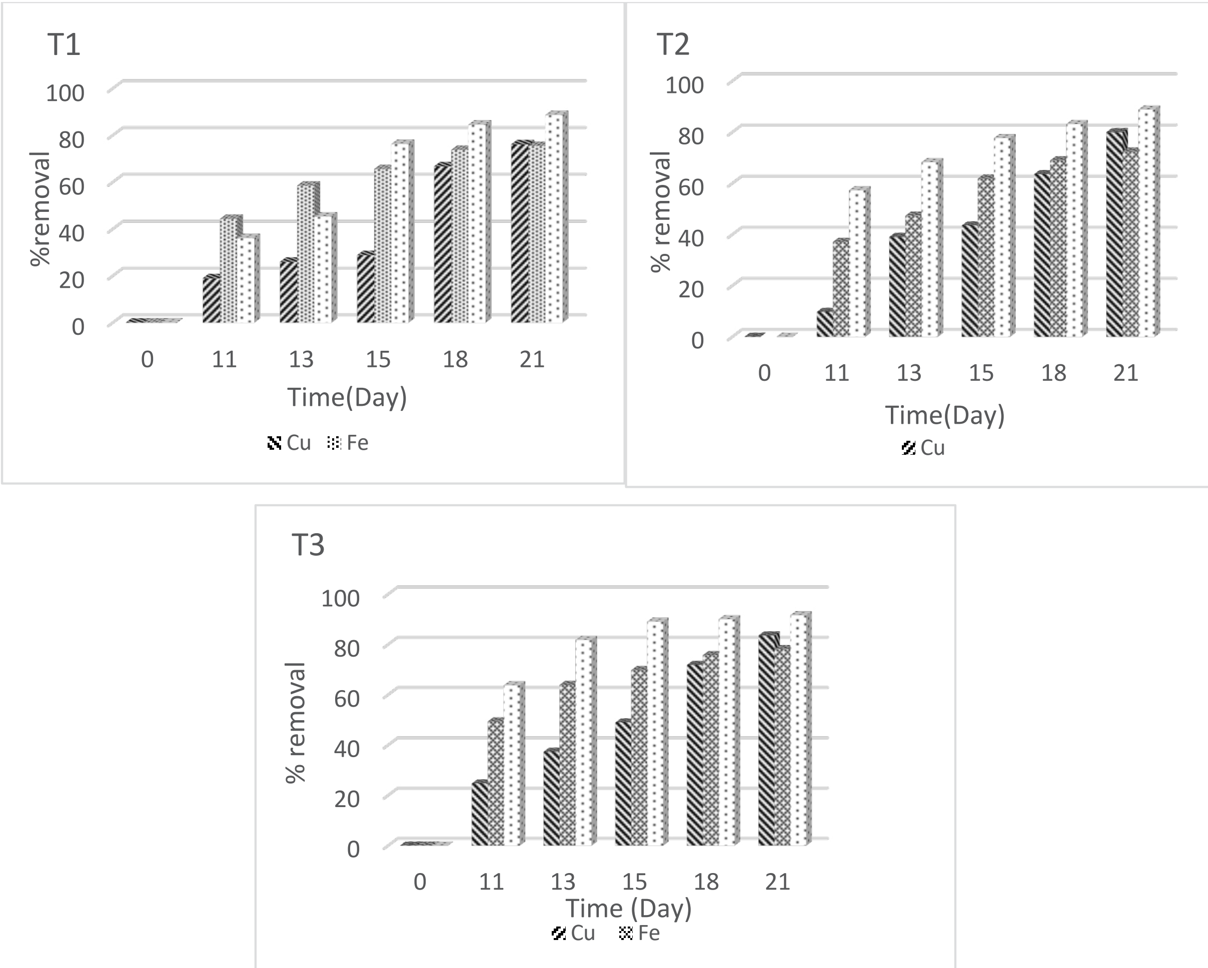

Fig. 7. Removal efficiency (in percentages) of Cu, Fe, Pb from three different substrates composition: T1, T2, and T3

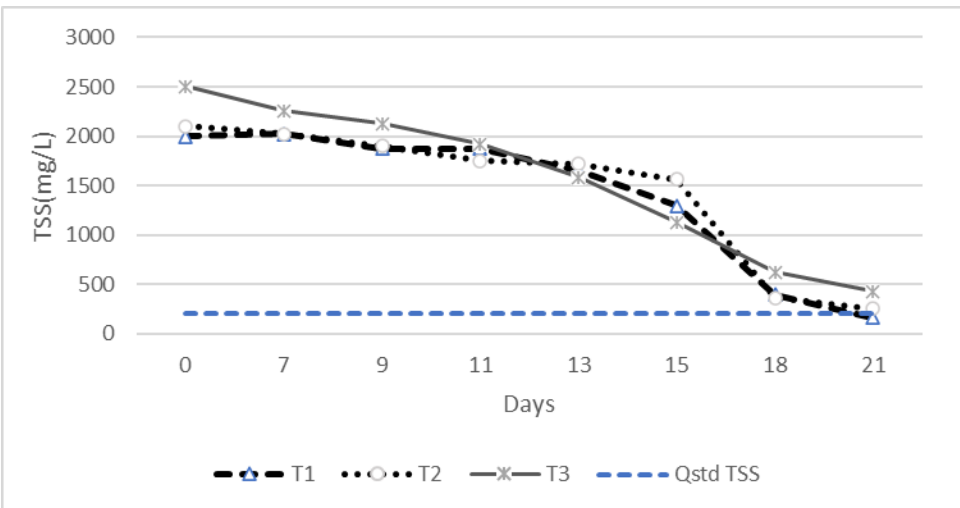

Fig. 8. TSS reduction during treatment of SRB-containing anaerobic reactor

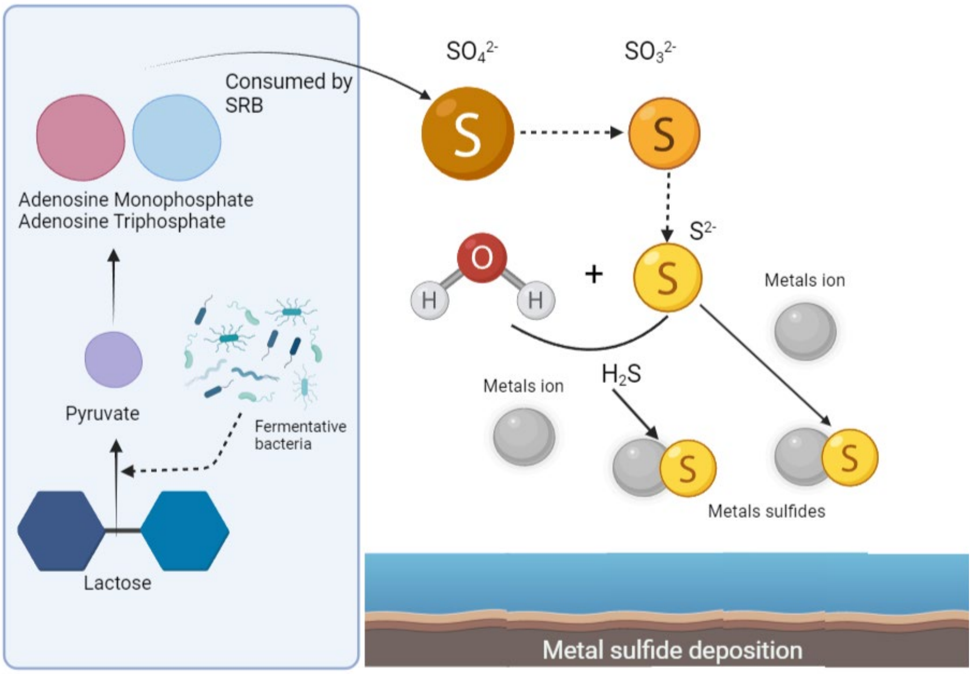

Fig. 9. Metals removal mechanism by SRB

The pH of the solution reduced during the process in equation (4) – (6) by the creation of sulphide, such as HS^-. Nevertheless, bicarbonates produced by electron donor oxidation (equations (1) and (3)) can balance the acidity (Huawei Wang et al., 2013). Equation (7) demonstrates that H2S may react with dissolved metals (such Cu^{2+}, Pb^{2+}, Ni^{2+}, and Fe^{3+}) to produce insoluble and settleable metal sulphide precipitates. Due to their advantageous densities, low solubilities, and effective dewatering and settling capabilities, metals in form of sulphide precipitates may be effectively removed from wastewater (Kieu et al., 2015). The level of metals removal depends on the solubility product constants (K_{sp}), the lower the K_{sp} of the metal sulphides, the higher the metals removal rate (Zhang & Wang, 2016).

CONCLUSION

This study investigated the potential of low-cost substrate of fermented compost and Postgate B in improving the degradation of heavy metals (especially Cu, Fe, and Pb) by SRB microbial communities treating heavy metals-containing laboratory wastewater. Among several substrate compositions, cultivating the SRB in the growth media consisting of 5% Postgate B, 30% fermented compost and 5% SRB active suspension (T3), showed the best growth with a total population of cell colonies reaching 1.1 x 10^5 CFU/ml. Furthermore, the T3 substrate composition also exhibited the best heavy metals removal compared to the other substrate compositions, by removing 91% of Pb, 84% of Cu, and 78% of Fe. Nevertheless, the TSS removal of T3 was found to be the worst due to high suspended solids contained in the fermented compost solution.

ACKNOWLEDGEMENT

Authors would like to appreciate the continuous support from the Research and Community Service Institute of Udayana University for funding research assistance through the Research Group scheme.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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