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Laboratory Wastewater Treatment Using a Combination of Anaerobic Bioaccumulation Systems and Plant Biofiltration

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ABSRACT

Laboratory waste that is disposed of into the environment will have an impact on environmental pollution and threaten human health. Efforts to treat laboratory wastewater must be carried out. This research aimed to analyze the effectiveness of Anaerobic Bioaccumulation Systems with sulfate-reducing bacteria (SRB) in reducing heavy metals and sulfate ions as well as the effectiveness of plant biofiltration (PB) in reducing biological oxygen demand (BOD), chemical oxygen demand (COD) and heavy metals in laboratory wastewater compared to quality standards. In this research, a plant biosystem which uses the principle of phytoremediation was used to reduce the heavy metal content in wastewater. This anaerobic reactor is cylindrical with r of 0.23 m and t of 0.93 m and has a volume of 1.5 m^3. In this tank, there is an SRB initial growth column with a volume 6.7 L. SRB media in the form of 30% compost fermentation solution, Postgate B nutrients with 10% sulfate added to the column. It stimulated with SRB seeds that had been isolated previously, then laboratory waste is added until it fills the column. After being acclimatized for 15 days, the laboratory wastewater began to flow slowly into anaerobic bioaccumulation system. The next stage was a plant filtration system in a size of basin 3.0x1.0x1.0 m with of 4 vertical layers. The bottom layer consists of 20% limestone, 30% coral and 50% sand. The plant used was *Sansevieria trifasciata*. The research results showed that the SRB Anaerobic Bioaccumulation was effective in reducing heavy metals and sulfate ions by up to 80.6% with a residence time of 24 hours after growing SRB for 15 days PB is effective in reducing BOD, COD and heavy metal content to meet the specified quality standards with a residence time of 30 hours after plant acclimatization for 15 days. The combination of the SRB anaerobic accumulation and PB system worked effectively with a total residence time of 2.25 days, which was marked by a decrease in all test parameters to below the specified quality standards.

Keywords: laboratory wastewater, anaerobic bioaccumulation, plant system.

INTRODUCTION

Laboratory liquid waste has the potential to contain the materials that are categorized as hazardous and toxic, because they come from the disposal of chemical residues and washing equipment. The use of various chemical elements and compounds in analytical activities in the laboratory can produce liquid waste containing residual chemical solvents, heavy metals and organic compounds which can be used as dissolved materials (TDS) and suspended materials (TSS) (Saunders et al., 2016). Laboratory waste that is disposed of into the environment will have an impact on environmental pollution and threaten human health. The efforts to treat laboratory wastewater must be carried out. Effective and efficient processing is needed to be widely implemented. Considering this, it is necessary to have alternative waste processing for laboratories that can be applied more easily. One way to deploy appropriate bacteria in environmental remediation methods is through the bioaugmentation technique (Jałowiecki et al., 2024). Phytoremediation using aquatic plants can be used to treat domestic wastewater (Ryanita et al., 2020). Meanwhile, plant biosystems that use the principle of phytoremediation have been used to reduce the heavy metal content in wastewater (Ju & Zhang., 2015; Indrawan et al., 2017). The biosystems that rely on processes in the root rhizosphere and ion absorption by plants provide good heavy metal reduction efficiency and are able to remove organic compounds (BOD, COD), suspended, and color with fairly good results (Tangahu & Putri, 2017). The advantages of the wastewater treatment process with a biofilter are that it is easy to operate and maintain, the operating costs are relatively cheap, the sludge produced is relatively small, the air supply for aeration is relatively small. It can be used for wastewater with a fairly large BOD load and it also can remove ammonia and suspended solids (SS) well (Ju & Zhang., 2015). The acidity level (pH) and metal element treatment ponds are used as pretreatment for plant biofiltration. Processing the degree of acidity (pH) and metal elements in this Artificial Wetland system utilizes the interactions between organic materials, micro-organisms and plants (Saunders et al., 2016). The acidity degree (pH) and metal element treatment pond consist of several compartments which take into account the required and available Artificial Wetland area, waste water flow process settings, and maintenance in each compartment. Organic materials will produce organic acids which then react chemically to reduce the sulfate concentration, precipitate and trap metal elements, as well as increase the degree of acidity (pH). Sulfate-reducing bacteria will grow and actively reduce sulfate in acid mine water (bioremediation). Absorption of metal elements by plants (phytoremediation). Dead leaves and plant organs will provide input of organic material into the Artificial Wetland system so that its sustainability can be maintained (Kuroda et al., 2015; Ratnawati et al., 2014).

Sansevieria is a genus of ornamental plants that are quite popular as decoration inside the house because they can grow with a little water and sunlight (Alwi et al., 2018). Sansevieria has tough, succulent, erect leaves, with pointed tips. Sansevieria is known as the mother-in-law's tongue plant because of its sharp shape (Yumna et al., 2018). Sansevieria is not only an ornamental plant, but also has benefits for fertilizing hair, treating diabetes, hemorrhoids and malignant cancer. Meanwhile, the fiber is used as clothing material. In Japan, Sansevieria is used to deodorize indoor furniture. Compared to other plants, Sansevieria has the privilege of absorbing toxic, such as carbon dioxide, benzene, formaldehyde and trichloroethylene. Sansevieria is divided into two types, namely the type that grows lengthwise upwards with a size of 50–75 cm and the type with short, circular leaves in a rosette shape with a length of 8 cm and a width of 3–6 cm. This plant has thick leaves and contains succulent water, so it is drought resistant. However, under damp or wet conditions, Sansevieria can grow well. Sansevieria leaf colors vary, from dark green, light green, gray green, silver, and a combination of white and yellow or green and yellow. The specialty of Sansevieria has high adaptability to the environment and is naturally able to reduce pollution. The groove motifs or lines found on the leaves also vary, some follow the direction of the leaf grain, are irregular, and some are zig-zag (Mosivand et al., 2019; Hendrasari, 2016).

Regarding the problem of laboratory waste, this research aimed to analyze the effectiveness of anaerobic bioaccumulation systems with sulfate reducing bacteria (SRB) in reducing heavy metals and sulfate ions as well as the effectiveness of plant biofiltration (PB) in reducing biological oxygen demand (BOD), chemical oxygen demand (COD) and heavy metals in laboratory wastewater compared to quality standards.

MATERIAL AND METHODS

Laboratory wastewater contains dangerous elements originating from expired substances, consumables, laboratory process products, products of waste handling efforts, residual chemicals that have been used, water used to wash equipment, and remaining samples tested. In this research, the waste treated was laboratory wastewater from the integrated chemistry laboratory of the Faculty of Mathematics and Science, Udayana University. The quantity of waste water produced was 1.46 m^3/day and quality of laboratory wastewater contains BOD and COD which exceed the quality standards, namely 123.39 mg/L and 1182.89 mg/L respectively. The laboratory wastewater also contains heavy metals that exceed the standard, namely Pb (0.23 mg /L), Cd (0.19 mg/L) and total Cr (0.82 mg/L), and waste is acidic with a sulfate content of 186 mg/L.

This research used 3 compartments, namely an anaerobic tank with sulfate reducing bacteria, a plant system biofiltration tank and a processed product storage tank.

Anaerobic bioaccumulation with SRB

The anaerobic tank is a cylinder with r of 0.23 m and t of 0.93 m and has a volume of 1.5 m^3. In this tank there is an initial SRB growth column with a volume of 6.7 L. The SRB media is a 30% compost fermentation solution, Posgate B nutrients with 10% sulfate are added to the column. Next, the SRB seeds that have been grown are added, then add laboratory waste is slowly added until they fill the SRB growth column. The length of waste treatment time or residence time is in accordance with the tank capacity and waste water discharge.

Plant biofiltration

PB system with dimensions of 3.0×1.0×1.0 m consists of 4 vertical arrangements of materials. The bottom layer is 20% limestone, 30% coral and 50% sand. The plant used is a type of *Sansevieria trifasciata* which is sold on the market. In the initial planting, saplings with 3 leaves are planted with a height of between 30–40 cm and a spacing of 20 cm. Before use, the plants are cared for by watering them regularly and ensuring the plants grow well in the sand media. The interval between planting and PB operation is 15 days. The length of time for waste treatment after the PB is ready for use or residence time is in accordance with the PB capacity and wastewater discharge.

Storage compartment

The wastewater processing tank is used to control the quality of processed water and the performance of the anaerobic bioaccumulation with SRB and PB tanks. Figure 1 shows a diagram of a processing installation with SRB and PB. The pollutant removal efficiency of each compartment and system is calculated using the Equation 1(Neria-González and Aguilar-López, 2021):

$$\textit{Removal efficiency}\ (\%) = \frac{(C1-C2)}{c1} \times 100 \qquad (1)$$

where: $C1$ – initial concentration(input), $C2$ – final concentration (output). Removal efficiency is measured in SRB, PB and its combination.

The residence time in each compartment and its combination is determined by the Equation 2:

$$\textit{Residence time} = \frac{V\ basin}{Q} \qquad (2)$$

where: *V basin* – volume anaerobic basin (m^3), Q – treated wastewater discharge (input) in m^3/day, *V basin* – volume biofilter plant basin (m^3), Q – treated wastewater discharge (input) in m^3/day).

RESULTS AND DISCUSSION

The characteristics of processed laboratory waste have relatively low TSS and TDS values, namely 32 mg/L and 759.11 mg/L, respectively. When compared with the Regulation of the Minister of Environment and Forestry of The Republic of Indonesia Number 5, 2021, Regarding quality standards for waste water disposal and utility activities, the laboratory wastewater has the BOD and COD contents that exceed the quality standards, namely 123.39 mg/L and 1182.89 mg/L respectively. The laboratory wastewater also contains heavy metals that exceed the standard, namely Pb (0.23 mg /L), Cd (0.19 mg/L) and total Cr (0.82 mg/L). However, the reduction effectiveness achieved from wastewater treatment plant treatment reached 79.06% and 48.76%, respectively. This treatment increases the clarity of the wastewater produced by leaving TSS of 6.7 mg/L and TDS of 389 mg/L. In the 54 hours residence time at the combined waste water treatment plant, the quality of the processed water produced was classified as good, because all

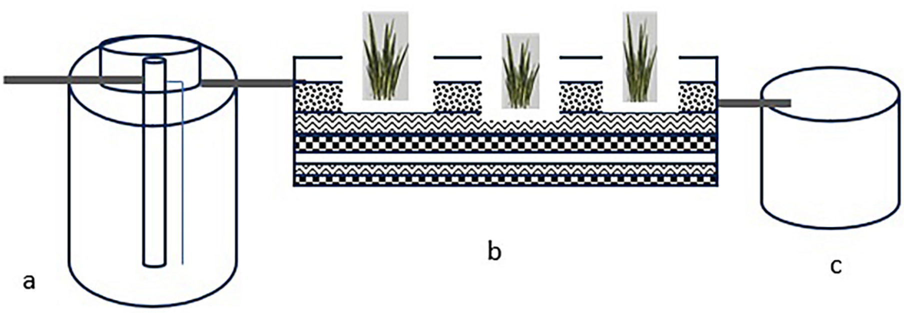

Figure 1. Wastewater treatment plant (a) anaerobic bioaccumulation with SRB, (b) plant biofilter system and (c) storage compartment

the parameters tested were in accordance with the specified standards.

In processing liquid waste containing organic materials, processing is generally carried out using biological processes or a combination of biological processes and chemical-physical processes. Biological processing processes can be aerobic and anaerobic processes (Ratnawati et al., 2014). In this research, anaerobic methods were used related to the growth of groups of sulfate-reducing bacteria (SRB) to remove heavy metals. SRB are grown by providing nutrients (postgate b), in an anaerobic tank with the addition of sulfate if necessary. Advanced biofiltration uses a plant system with sand, coral and limestone media.

The measurement of the anaerobic tube was with radius (r) = 23 cm and height (t) = 93 cm, volume of the tank (V) = $\pi \times r^2 \times t$, namely $3.14 \times (23)^2 \times 93$ is 1.55 m^3) with an effective volume of 1.47 m^3. The residence time of wastewater in an anaerobic tank with SRB is an effective volume of 1.48 m^3 divided by the inlet discharge to BP of 1.46 m^3/day which is 1 day. The Biofilter Plant System compartment with dimensions (l × w × h) 3.0 × 1.0 × 1.0 m has an effective volume of 2.8 × 0.8 × 0.8, namely 1.792 m^3. The residence time of wastewater in the BT compartment is an effective volume of 1.792 m^3 divided by the inlet discharge to the BP of 1.46 m^3/day which is 1.23 days (30 hours).

The performance of anaerobic tanks with SRB for several parameters is presented in Table 1. With a retention time of 24 hours, the effectiveness of solids reduction ranges from 25 to 46.9%. The highest reduction occurred in sulfate ions up to 80%. This is related to SRB activity in anaerobic reactors which require sulfate as an electron acceptor and is reduced to sulfide.

The performance of anaerobic SRB shows good effectiveness in reducing sulfate levels and increasing pH, but is not effective in reducing TSS and TDS. Likewise, it is less effective in reducing the BOD and COD content with effectiveness values of 32.7 and 36.1, respectively. Anaerobic treatment carried out previously can reduce the BOD_5, COD and TSS levels by 60%, 60% and 80%, respectively (Regulation of the Minister of Environment and Forestry of The Republic of Indonesia Number 5, 2021; Said & Utomo, 2018). Anaerobic treatment is also able to remove pollutants significantly with BOD, COD and TSS parameters. However, another research using an anaerobic biofilter for industrial liquid waste (14 days residence time) showed a reduction in the COD, BOD and TSS concentrations with a COD efficiency of 20.67–30.88%; BOD 7.70–45.71%, and TSS 12.32–62.82% (Quan et al., 2018).

The sulfides formed from sulfate reduction by sulfate-reducing bacteria react with heavy metal ions in solution to form metal sulfides in the form of black deposits on the bottom and walls of the tank. In Table 2, the performance of the anaerobic reactor with SRB with HRT of 24 hours is presented to precipitate heavy metals in the form of metal sulfides. The effectiveness of reducing heavy metal ions ranged from 86.96 to 99.51%. The measured effluent heavy metal ion content completely meets the specified quality standards (Regulation of the Minister of Environment and Forestry of The Republic of Indonesia Number 5, 2021, Regarding Quality Standards for wastewater disposal and utility activities).

The performance of the Plant Biofilter System in reducing the pollutant content of laboratory wastewater as measured by several parameters is presented in Table 3. The performance of PB is measured in the residence time of wastewater in the PB for 30 hours. The influent that enters PB is the effluent from the SRB reactor. The effectiveness of reducing several pollutant levels ranged from 48.76 to 97.07%.

The performance of the plant system biofilter in reducing the content of several heavy metals in laboratory wastewater is presented in Table 4.

Table 1. The performance of anaerobic tanks with SRB for several parameters

No	Parameter	Unit	Input	Output*	% effective
1	pH		3.57	6.23	
2	TSS	Mg/L	32	17	46.9
3	TDS	Mg/L	759.11	569	25.0
4	BOD	Mg/L	123.39	83	32.7
5	COD	Mg/L	1182.89	756	36.1
6	Sulfate	Mg/L	186	36	80.6

Note: *hydraulic retention time (HRT)24 hours.

Table 2. The performance of anaerobic tanks with SRB for heavy metals

No	Parameter	Unit	Input	Output*	% effective
1	Pb	Mg/L	0.23	0.03	86.96
2	Cd	Mg/L	0.19	0.002	98.95
3	Zn	Mg/L	0.45	0.005	98.89
4	Cr	Mg/L	0.82	0.004	99.51
5	Cu	Mg/L	0.21	0.008	96.19

Note: *HRT 24 hours.

Table 3. The performance of the plant biofilter system for several parameters

No	Parameter	Unit	Input	Output*	% Effective
1	pH		3.57	7.82	
2	TSS	Mg/L	32	6.7	79.06
3	TDS	Mg/L	759.11	389	48.76
4	BOD	Mg/L	123.39	8.2	93.35
5	COD	Mg/L	1182.89	34.62	97.07
6	Sulfate	Mg/L	186	26.31	85.85

Note: *HRT 30 hours.

The performance of PB is measured in the residence time of wastewater in PB for 30 hours. The influent that enters PB is the effluent from the SRB reactor. The effectiveness of reducing several heavy metals is above 98% (Fig. 4).

The reaction of SRB under anaerobic conditions can be divided into two stages, namely the first stage is the process of reducing sulfate to metal sulfide. The next stage is the formation of insoluble metal sulfide from the reaction of hydrogen sulfide with dissolved metal ions such as Cu^{2+}, Zn^{2+}, Pb^{2+}, and Cd^{2+} to form acidic water (Francis, 2016). An important mechanism for this group of bacteria in reducing sulfate accompanied by the deposition of heavy metals requires anaerobic conditions and optimal environmental factors for maximum sulfide formation. Metabolic reactions of PB with lactate as the main carbon source is as follows:

$$2C_3H_5O_3^- + SO_4^{2-}\ 2CH_3COO^- + 2CO_2 + 2H_2O + S^{2-} \quad (3)$$

Sulfate reduction can occur over a wide range of pH, pressure, temperature and salinity (Fig. 5). However, in general carbon compounds as electron donors such as lactate, pyruvate and hydrogen molecules can be a barrier. Sulfate reduction is inhibited by the presence of oxygen, nitrate, and Fe(III) ions. Methanogenic bacteria are competitors for PB in utilizing electron donors (Fitri et al., 2016). The presence of sulfate is an advantage for sulfate reducers, but the rate of sulfate reduction is often limited by the presence of carbon compounds, resulting in habitat zonation. The hydrogen sulfide produced by PB will affect its habitat and population. Hydrogen sulfide is toxic to aerobic organisms, because the S element of the compound is very reactive to metal elements from the cytochrome system of organism cells.

SRB also plays a major role in the formation of sulfur deposits in nature. The PB group oxidizes organic materials and H_2 by using sulfate as an electron acceptor to produce hydrogen sulfide and bicarbonate, as in the following reaction:

Table 4. The performance of the plant biofilter system for heavy metals

No	Parameter	Unit	Input	Output*	% Effective
1	Pb	Mg/L	0.03	0.012	60
2	Cd	Mg/L	0.002	0.001	50
3	Zn	Mg/L	0.005	0.002	60
4	Cr	Mg/L	0.004	0.004	0
5	Cu	Mg/L	0.008	0.006	25

Note: *HRT 30 hours.

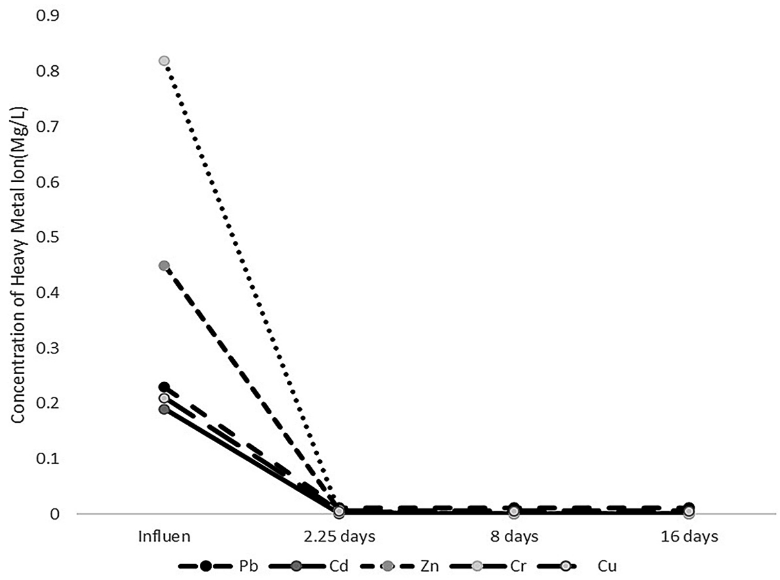

Figure 2. Decreased heavy metal concentration (mg/L) in total HRT of 2.25 days, 8 days and 16 days in SRB anaerobic tank and PB system treatment

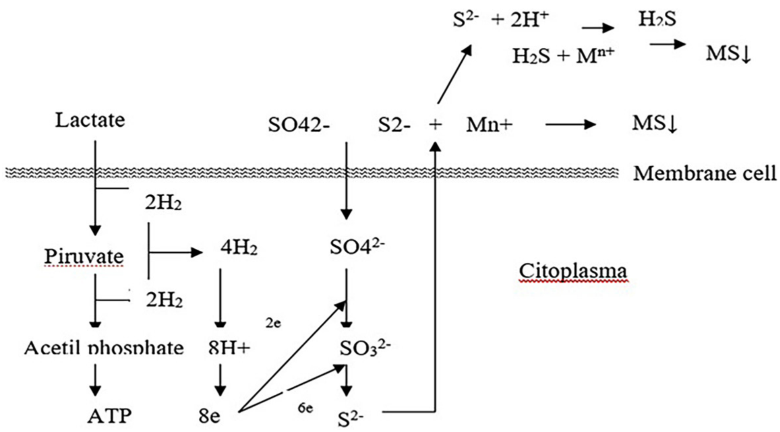

Figure 3. Sulfate reduction reaction pathway and heavy metal deposition

$$2CH_2O + SO_4^{2-}\ H_2S + 2HCO_3^- \quad (4)$$

$$5H_2 + SO_4^{2-}\ H_2S + 4H_2O + 2e \quad (5)$$

The producing sulfide will react with dissolved metal ions to form insoluble metal sulfide following the reaction below:

$$M^{2+} + S^{2-}\ MS\downarrow \quad (6)$$

The deposition of heavy metals in the form of sulfides varies greatly, depending on the affinity of the metal cation to react with the element sulfur (S). The deposition of certain metal cations is also influenced by environmental conditions, such as pH and the content of other metal cations (Goni et al., 2021). Related to the results of each processing stage, the combined performance between SRB and plant biofiltration can be seen in Table 5.

The performance of the combined treatment with anaerobic SRB and PB tanks is demonstrated by the suitability of achieving all parameters tested against the specified standards. In the residence time tests which is adjusted to the scale of each compartment, all the parameters tested have been proven to be effective with results in accordance with the specified quality standards (Regulation of the Minister of Environment and Forestry of The Republic of Indonesia Number 5, 2021, Regarding Quality Standards for waste water disposal and utility activities).

The advantage of the anaerobic system with SRB is compact management of heavy metal deposition with a regulated sulfate requirement, no need for large areas of land, relatively little sludge produced compared to the activated sludge process. Another advantage is that it can remove nitrogen and phosphorus which cause eutrophication, reduce BOD loads and suspended solids

Table 5. Performance of a combined anaerobic system with SRB and plant biofiltration on wastewater from the integrated chemistry laboratory of Faculty of Match and Science, Udayana University

No.	Parameter	Unit	Anaerobic with SRB[1]		Plant biofilter (PB)[2]		Standard[3]	Effectivity (%)[4]	Suitability[3]
			Input	Output	Input	Output			
1	pH		3.57	7.49	6.23	7.82	6--9		accordance
2	TSS	Mg/L	32	4.5	17	6.7	200	79.06	accordance
3	TDS	Mg/L	759.11	3.72	569	389	2000	48.76	accordance
4	BOD	Mg/L	123.39	28.2	83	8.2	50	93.35	accordance
5	COD	Mg/L	1182.89	8.78	756	34.62	100	97.07	accordance
6	Sulfate	Mg/L	186	22.76	36	26.31		85.85	accordance
7	Pb	Mg/L	0.23	0.016	0.03	0.012	0.1	94.78	accordance
8	Cd	Mg/L	0.19	0.0013	0.002	0.001	0.05	99.47	accordance
9	Zn	Mg/L	0.45	0	0.005	0.002	2	98.44	accordance
10	Cr	Mg/L	0.82	0.0037	0.004	0.004	0.5	99.51	accordance
11	Cu	Mg/L	0.21	0.007	0.008	0.006	2	97.14	accordance

Note: 1 – HRT 24 hours (1 day); 2 – HRT 30 hours (1,25 days) 3 – regulation of the Minister of Environment and Forestry of The Republic of Indonesia Number 5, 2021, regarding quality standards for waste water disposal and utility activities, 4 – effectivity of process refer to formula 1.

well. Anaerobic biofilters can remove the BOD_5 levels with an efficiency of 70–95%, remove the COD levels with an efficiency of 70–95%, and remove the TSS pollutant levels with an efficiency of 80–95% (Nasoetion et al., 2017). The design criteria used for calculations are the residence time required for the anaerobic biofilter, baffle/pipe length, microbial culture bed volume, organic loading rate, and hydraulic loading rate (Yusup & Panca, 2019). On the basis of calculations, the residence time for the anaerobic biofilter is 34.94 m^3, the permissible baffle/pipe length is 50–60 cm, the volume of microbial culture media required is 0.92 m^3, the organic loading rate is 7.85 kg COD/m^3. days, and the hydraulic loading rate is 0.0036 m^3/m^2.hour. Anaerobic treatment with SRB also plays a neutralization role. This neutralization occurs due to the reduction of sulfate by SRB bacteria to sulfide. This anaerobic tub treatment with SRB was able to increase the pH from 3.57 (input) to 6.23 (output) and at the end of processing to 7.28 in a total residence time of 2.25 days.

The advantage of the PB system is easy operation and maintenance. Various processes in biological systems can support performance, namely degradation, transformation, distribution and accumulation. This process allows the removal of various pollutant contents, both organic and inorganic. With the porosity of the media and surface, PB also supports the aeration process. Aeration is an effort to supply oxygen to the media which increases the dissolved oxygen in the water (Hendrasari, 2016; Yuniarti et al., 2019). Some of the main functions of aeration are dissolving oxygen into the water to increase the dissolved oxygen levels, releasing dissolved gases in the water and helping to stir the water. The pH in water is directly related to dissolved oxygen, where when dissolved oxygen is low, the pH of the water also becomes low and vice versa so that the aeration process can increase the acidity of liquid waste to become close to neutral (Fitri et al., 2016; Sarwono et al., 2017).

CONCLUSIONS

On the basis of on the discussion of result, it can be concluded that the anaerobic bioaccumulation with SRB is effective by reducing the sulfate levels up to 80.6%, which has implications for pH neutralization. This performance also has the impact of reducing dissolved heavy metals to below quality standards by forming metal sulfides which precipitate. The performance is achieved on residence time of 24 hours after growing the SRB for 15 days in an anaerobic column. The PB basin can effectively reduce the BOD, COD and heavy metal content to meet the quality standards. Its performance on residence time of 30 hours after a 15 days plant acclimatization period on the plant system. The combination of the Combination of Anaerobic Bioaccumulation with SRB and PB system worked effectively in a total residence time of 2.25 days, as indicated by the reduction of all test parameters to below the specified quality standards.

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