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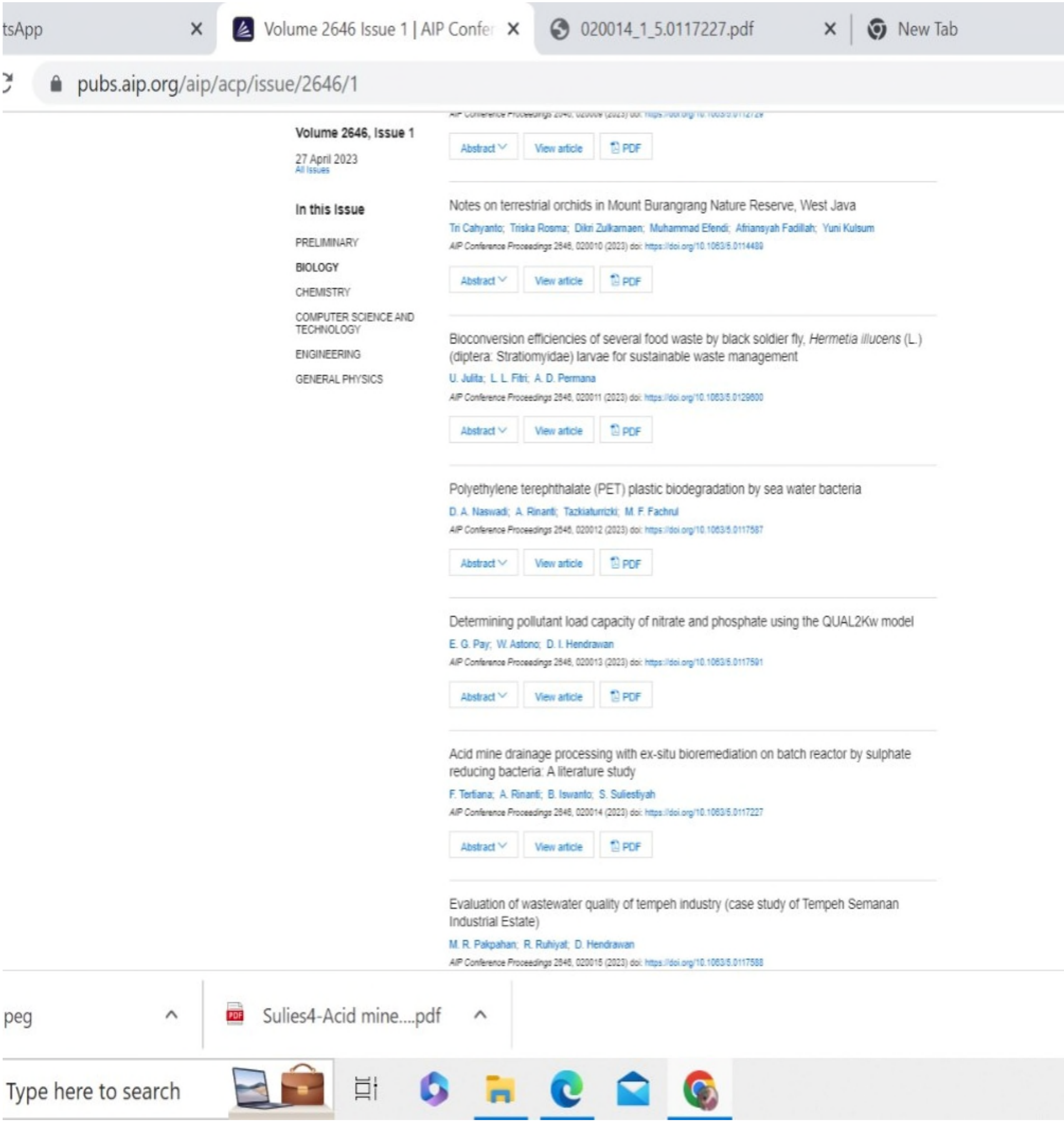

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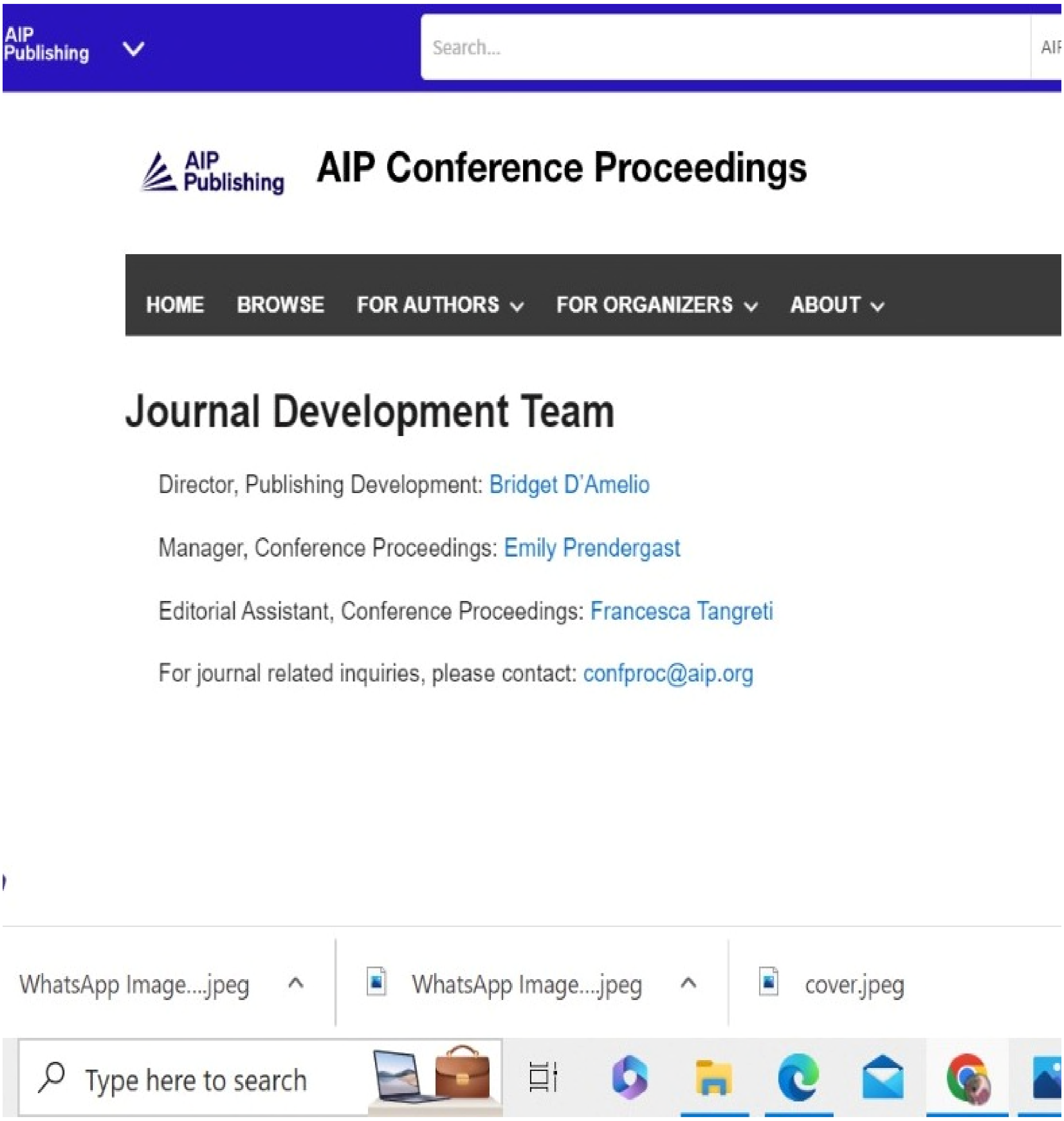

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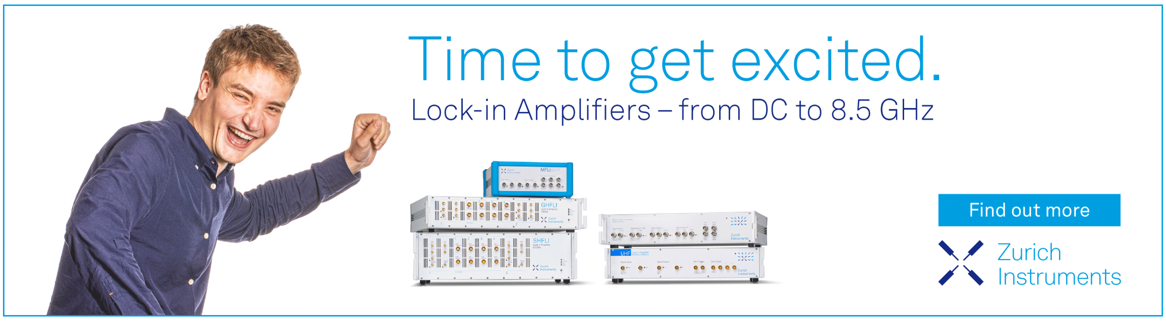

Acid Mine Drainage Processing with Ex-Situ Bioremediation on Batch Reactor by Sulphate Reducing Bacteria: A Literature Study

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Abstract. Environmental damages around mining area caused by acid mine drainage (AMD) require effective yet environmental friendly methods. The objective of this research is to develop AMD processing with ex-situ bioremediation on a batch system reactor to increase pH level and remove heavy metal compounds. This literature study is conducted to obtain secondary data on the performance of Sulfate Reducing Bacteria (SRB), the influencing environmental factor, and carbon source as growth media for SRB. Cow dung and chicken manure were added as carbon sources. The abundant availability of SRB can trigger sulphate removal and accelerate AMD decrease on a natural way. Mobilized SRB and FeRB were able to lower more than 60% of sulphate and 90% of iron (Fe) rates. Chicken manure is ideal to reduce sulphate but it's even better to neutralize pH when cow dung is added as carbon source. Batch bioreactor design was produced with an Anaerobic Sequential Batch Reactor (ASBR), the height of the reactor is at 3.8 m with radius of 3.73 m. If the AMD concentration composition is at 10% (v/v), the mix culture is at 30% (v/v) and the nutrient concentration is at 60% (v/v), to lower AMD concentration we will require 49044 L SBR and 98088 L nutrient. The residence time required for SRB to remove sulphate is 42 hours. AMD bioremediation by SRB on a reactor can become a solution to process AMD with environmental friendly method because the process does not produce any secondary pollution.

INTRODUCTION

Acid Mine Drainage (AMD) is the main waste produced from mining activities with highly acidic nature and contains heavy metal compound. AMD is formed through natural oxidation of sulphide minerals such as pyrite, chalcopyrite, pyrrhotite, or arsenopyrite, which will become highly acidic wastes when they are exposed to open air. Heavy metal compound in AMD could be passively mobilized into the environment through rain water flowing outside the mining area [1,2]. AMD can be chemically processed with neutralization process method, which is conducted by adding chemical compounds such as hydrated lime ($Ca(OH)_2$) or calcium carbonate ($CaCO_3$), caustic soda (NaOH) or natrium carbonate (Na_2CO_3). AMD processing can also be conducted physically through coagulation, flocculation, and sedimentation processes. Chemical and physical processing can run effectively, but they require relatively high maintenance and operational costs, and also has the potential to produce secondary pollution [3-5]. These facts have driven researchers to conduct numerous researches to develop and improve bioremediation as a sustainable solution to recover the environment impacted by AMD [6].

Sulphate Reducing Bacteria (SRB) has the potential to absorb heavy metal and reduce sulphate contained by AMD [7-9]. A number of researches have proven that more than 99.9% of heavy metal compound in AMD can be removed

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by SRB [10-13]. Sulphate Reducing Bacteria (SRB) is able to lower around 90% of sulphate concentration in 20 days [14] and produce hydrogen sulphide that deposit Fe^{2+}, Zn^{2+}, and Cu^{2+} metals underground. Bacteria that play important role in AMD bioremediation are Fe Reducer Bacteria (FeRB) with acidophilic nature and SRB due to their high level of tolerance on AMD acidity [15].

By utilizing natural ability of SRB, bioremediation process can be highly profitable. The cost of bioremediation implementation is relatively lower than any other chemical-physical processing methods and does not produce secondary pollution, which is environmentally friendly [3,4,5,16,17].

The success of biotechnology to remediate AMD is influenced by environmental factors such as pH level, temperature, and dissolved oxygen concentration. The contaminant and nutrition must be available to SRB to make this happen. Besides that, the activity and content of SRB inside the processed waste must be adequate [18]. The number of dissolved carbon on AMD is usually very low (<10 mg/L), which makes that electron donor addition such as hydrogen molecule or organic compound as carbon sources are essential to improve SRB activation and bioreactor performance [19-21]. The objective of this research is to analyze AMD processing by SRB which has been effectively proven on various levels of pH, AMD concentration, and carbon sources as electron donor, so that we are able to produce AMD processing planning on a pilot scale in a batch system reactor.

AMD BIOREMEDIATION

This research was conducted by gathering literature data. Analysis was conducted with a systematic review, specifically with a meta-analysis method, which is an effort to analyze quantitative data obtained from primary sources, starting from preparation, categorization based on keywords which are acid mine drainage; bioremediation; sulphur reduction bacteria; passive system; carbon source; bioreactor, until the composition of this paper.

Mine acid drainage characterization is vital to conduct to acknowledge sulphate, pH, and heavy metal levels. SRB can be isolated from the soil around mining area, which are commonly dark coloured and reek as the signify SRB [22]. After the isolation, the bacteria are inoculated on Postgate media [23]. Organic substrate as carbon source that will be evaluated are animal waste (cow dung, chicken manure, and goat dung), sawdust, mushroom compost completed with silica sand, and pebbles [24-27]. These organic substrates are commonly used as effective carbon source to cultivate SRB on AMD removal efforts. The pH level was measured by pH meter, sulphate analysis was measured with turbidimetry method or UV-VIS spectrometer, and the liquid metal concentration was analyzed by utilizing ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometry).

The initial and final concentrations of heavy metal were determined with ICP-EOS. The efficiency of heavy metal removal was calculated by utilizing the following formula (1).

$$\%\ \text{Adsorption} = \frac{(C_0 - C_{eq})}{C_0} \times 100 \qquad (1)$$

C_0 represents initial heavy metal concentration and C_{eq} represents heavy metal concentration equilibrium in a solution (mg/L) [28].

The bacteria growth rate kinetic was determined to acknowledge substrate utilization on bacteria growth activity. The biomass specific growth rate (μ) represents the growth rate of each biomass unit [29]. The specific growth rate can be calculated with the following formula:

$$q = \frac{1}{Yt}\mu + \text{b} \qquad (2)$$

Y_g = lost substrate; Y_A = total biomass of each lost substrate unit; q = specific substrate utilization (time^{-1}); b = constant that represents substrate utilization per biomass unit on each period of time (time^{-1})

Lawrence and McCarty (1970) explained that to determine reaction order, calculations by using formula (3) and formula (4) is required. Order 0 happens when Ks < S and Order 1 happens when Ks>S.

$$-\left(\frac{dS}{dt}\right)_{\text{u}} = \frac{kxS}{Ks+S} \qquad (3)$$

If we predetermined that Ks is 0, therefore,

$$So - S = kxt \text{ and } -\ln\frac{So}{S} = Kxt \quad (4)$$

According to Grau *et al*., (1975), formula (5) is more accurate to calculate substrate utilization rate for order 2 reaction.

$$Kxt = \frac{1}{S} \quad (5)$$

S_o = Initial substrate concentration; S = substrate concentration; K1 = constant of substrate utilization rate; Ks = saturation concentration rate.

RESULTS AND DISCUSSION

Mine Acid Drainage Characteristic

The characteristic of AMD obtained from PT. Kaltim Prima Coal possess high sulphate rate, low pH and high heavy metal compound (Table 1). AMD characteristic and composition are highly varied. They tend to have different nature based on local conditions and environmental factors.

TABLE 1. The Result of Mine Acid Drainage found in PT. Kaltim Prima Coal area

No	Parameter	Unit	Quality Standard 1)	Quality Standard 2)	Results
1.	pH (in-situ)	-	6-9	6-9	2.47
2.	Iron (Fe)	mg/L	0.3	7	30
3.	Mangan (Mn)	mg/L	0.1	4	8
4.	Sulphate (SO_4)	mg/L	400	-	1.039

Manganese (Mn) and iron (Fe) are important nutrients for our life. However, manganese is also toxic for human when the compound is excessively concentrated in water. Manganese can damage human central neural system [30]. Excessive iron input can also cause corrosion effect on human intestines and biological fluid. Iron can penetrate heart, liver, and brain cells. The toxicity of iron on plant cells have proven to cause tissue damage [31].

SRB Performance

SRB is able to decently grow on pH level of from 3.5 to 9, with a wide temperature tolerance which is -5ºC to 50ºC. The higher the AMD concentration, the higher the required SRB concentration will be. The abundant availability and variability of SRB can trigger sulphate removal and naturally enhance AMD reduction. Mobilized SRB and FeRB can lower up to 60% sulphate and up to 90% of iron rates. Manganese (Mn) removal was slightly disrupted, but 40% of it were removed although it is quite difficult to reach 90%. Bacteria that can be categorized as SRB are *Thermodesulfobium*, *Syntrophobacter*, *Desulfurella*, *Desulfomonile*, *Desulfovibrio* and *Desulfosporosinus* genus [32]. *Geobacter metallireducens* and *Shewanella putrefaciens* are examples of FeRB [33-35]. In relation with positive gram SRB (*Desulfosporosinus orientis*) and negative gram SRB (*Desulfovibrio desulfuricans*), Stanley [36] explained that positive gram SRB plays a more active role in the deposition of sulphate mineral. The occurrence of bacteria cell autolysis during endospore release will increase sulphate mineral aggression (gathering).

Carbon Source for SRB Growth

Chicken manure and cow dung were showing decent performances as carbon sources for SRB. The utilization of chicken manure and cow dung respectively produce sulphate reduction of 79% and 63% [24]. Cow dung additional was proven to be able to increase pH level up to 7. This result shows that chicken manure is also suitable to reduce sulphate, but to neutralize pH an additional of cow dung is required as carbon source.

The characterization result of organic substrate on animal wastes such as composted cow dung and chicken manure shows that animal wastes contain higher SRB level than grass silage, sawdust, and paper wastes. Compost produced from poultry wastes contains alkali pH meanwhile cow dung contains neutral pH. This will act as decent environment for SRB to grow inside a reactor that processing AMD [26].

AMD Processing on Pilot Scale

AMD processing on a pilot scale is recommended to be conducted in a reactor. Batch bioreactor design is utilizing Anaerobic Sequential Batch Reactor (ASBR) [10]. The excellence of Anaerobic Sequential Batch Reactor (ASBR) is that it can eliminate secondary pollutant sedimentation, high operation control efficiency, and simple operational procedure [37]. There are several researches regarding heavy metal removal from waste water which have proven that ASBR can be a low cost and high performance bioremediation alternative [10,37,38].

The volume of AMD from East Borneo Indonesia reaches 16,348.28 litres per day [39]. Reactor volume dimension on a pilot scale refers to the reactor and AMD volumes on laboratory scale, which are respectively at 200 ml and 20 ml [40], the calculation is as follows:

$$\frac{\text{Volume Reaktor (skala lab)}}{\text{Volume AMD (skala lab)}} = \frac{\text{Volume Reaktor (skala pilot)}}{\text{Volume AMD (skala pilot)}}$$

$$\frac{200\text{ mL}}{20\text{ ml}} = \frac{\text{Volume Reaktor (skala pilot)}}{16.348.000\text{ mL}}$$

Reactor volume (pilot scale) = 163.480.000 mL = 163.480 L = 163.48 m^3

Tube blanket = 2πrt; Base area = πr^2; Volume = $\pi r^2 t$ = 163.48 m^3; t= $\frac{163.48\, m^3}{\pi r^2}$; Area (A) = Blanket area + Base area; A= 2πrt + πr^2 = $2\pi r\frac{163,48\, m^3}{\pi r^2} + \pi r^2$; A = 326.96 $r^{-1} + \pi r^2$;

$$\frac{dA}{dr} = -326.96\, r^{-2} + 2\pi r$$

$$r = \sqrt[3]{\frac{326.96}{2\pi}} = 3.73\text{ m} \approx 3.7\text{ m};\ t = \frac{Volume}{\pi r^2} = 3.8\text{ m}$$

Based on the calculation, the reactor height should be at 3.8 m with radius of 3.73m (Fig. 1).

On this planning process, with the assumption of AMD concentrated composition is at 10% (v/v) [41], mix culture concentration is at 30% (v/v) [42], and nutrient concentration is at 60% (v/v), AMD concentration reduction will require 49044 L of SBR and 98088 L of nutrient.

Based on research data generated by Stanley [36], the calculation result of sulphate removal rate on AMD, it is acknowledged that the equation of order 1 reaction rate is Y = 0.0034x + 1.5156. R^2 value is at 0.4566 and bacteria growth rate constant is at 0.0034 hour^{-1}. If the curve equation of mix culture bacteria growth on exponential phase is at Y = 0.0635x + 7.677 [36], we can determine the residence time (t) required by SRB to remove sulphate on AMD. The calculation to measure residence time (t) with order 1 equation is as follows:

$$Y = 0.0034x + 1.5156;\ \text{Ln } S0/S = 0.0034t + 1.5156;\ t = \frac{\ln(\frac{S0}{S}) - \text{intersep}}{\text{slope}} = \frac{6.32 - \ln(s)}{0.0034}$$

The growth curve equation on exponential phase is Y = 0.0635x + 7.677; Y = 0.0635t + 7.677 [36]. The growth curve equation of exponential phase can be rewritten as follows:

$$t = \frac{\left(\frac{6.32 - \ln(s)}{0.0034}\right)}{0.0635t + 7.677}$$

If the final sulphate rate is at 127.3 mg/L [36], the required residence time calculation is as follows:

$$0.0635\, t^2 + 7.677\, t = \frac{6.32 - \ln(s)}{0.0034} = \frac{6.32 - \ln(127.3)}{0.0034} = 432.35$$

$0.0635\ t^2 + 7.677\ t - 432.35 = 0$

$$t = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} = \frac{-7.677 \pm \sqrt{7.677^2 - (4 \times 0.0635\ (-432.35))}}{2 \times 0.0635} = -162 \text{ hours} = 41.8 \text{ hours} \approx 42 \text{ hours}$$

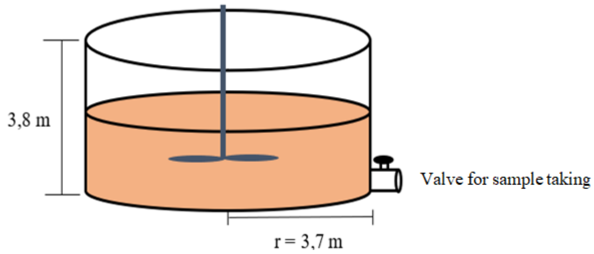

FIGURE 1. Pilot Scale Anaerobic Sequential Batch Reactor (ASBR) design

Based on the previous calculation, we discover that the required residence time for the bacteria to remove sulphate is at 42 hours. The implementation of AMD removal by SRB on pilot scale in the field is difficult to reach similar level of removal as the ones in the laboratory because of uncontrollable environmental condition that influence SRB performance [43]. Based on that, maximum safety factor of 30% is required to anticipate inefficiency in field removal.

CONCLUSION

Sulphate reducer bacteria including mobilized SRB are able to lower up to 60% of sulphate rate and 90% of FeRB rate. Manganese (Mn) removal faced a little difficulty but finally able to reach 40% of removal rate but it is quite difficult to reach 90% of removal rate. Cow dung, goat dung, and poultry wastes are proven effective as carbon sources with sulphate removal rate of 60% to 90%, Fe heavy metal removal of 90%, and more than 12% of Mn. Pilot scale implementation planning to process AMD from East Borneo at 16.348,28 litres per day is recommended to be conducted on a cylindrical batch reactor with 3.8m of height and 3.7m of radius. AMD removal requires 42 hours of contact time with order 1 reaction rate equation of Y = 0.0034x + 1.5156..

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