Biological treatment of palm oil mill effluent by using a downflow hanging sponge reactor

N Rapi*, C Jane, M E Azni, S M S Hitam, R Mohamad, R Noorain*

Malaysian Institute of Chemical Engineering Technology, University Kuala Lumpur, Lot 1988 Kawasan Perindustrian Bandar Vendor, Taboh Naning, 78000, Alor Gajah, Melaka, Malaysia.

*Email: sitinoorain@unikl.edu.my

Abstract. The performance of biological treatment using a downflow hanging sponge reactor as post-treatment of agro-industrial wastewater has been recognized worldwide. However, the effectiveness of the system in treating POME is remain unknown. Therefore, with this background in mind, an anaerobic treatment operated at COD loading rate of 0.4-0.6 kg/m³/day, HRT of 2 hours and input pH of 7.6 was run using a DHS reactor packed with sponge media for the treatment of POME. During the study, the output concentration performances of COD (32-58%), Colour (41-76%), ammoniacal nitrogen (66-92%) and phosphate (54-87%) were recorded. These results indicate comparable performances with the existing biological treatment system. However, it also offered additional credits in-term of fouling and clogging issues, practically as well as lower cost and energy consumption. Thus, it will be great to have a system that is capable to provide a simple technology which is affordable to various industrial scales.

1. Introduction

Oil palm tree or its scientific name ‘Elaeis guineensis’ is one of the fastest-growing equatorial crops that mostly planted in tropical country such as Malaysia, Indonesia, and Thailand [1] [2]. A very special kind of oil is extracted from the fruit of oil palm tree for food manufacture, cosmetics, detergents and to a small extent for renewable energy productions [3]. According to [4] the global market demand of palm oil is expected to increase at annual growth rate of 3.1% by year of 2027. As the economy in palm oil mill industry grows broadly, large amount of wastes also will produced such as palm kernel shell (5%), mesocarp fibre (12%), empty fruit bunch (23%) and palm oil mill effluent (POME) (60%) [5] [6]. It is estimated that around 2.5 to 3.5 tonnes of POME are produced for every ton of crude palm oil production [7]. POME is identify as an environmental pollutant that is 100 times more polluted than the municipal sewage [8] which contains with high in biochemical oxygen demand (BOD) (30,000 mg L⁻¹), chemical oxygen demand (COD) (50,000 mg L⁻¹), oil and grease (O&G) (4000-8000 mg L⁻¹), total solid (40,500 – 63,000 mg L⁻¹) and suspended solid (SS) (18,000 – 30,000 mg L⁻¹) [9] [10]. Furthermore, due to high of organic contains of carotene (8 ppm), pectin (3400 ppm), tannin, phenolic (5800 ppm), and lignin (4700 ppm) had resulted in a brownish color of POME [11]. Thus, the discharge of untreated POME into natural water body not only creates adverse impact on the aquatic life but also impacted for surrounding environment pollution [12].
Due to the environmental implication associated with POME untreated disposal, the principle and criteria of roundtable for Sustainable Palm Oil (RSPO), Malaysian sustainable palm oil (MSPO) and Indonesian sustainable palm oil (ISPO) standard were followed as a reference for sustainable palm oil products through trustworthy global standard and engagement of stakeholders [13]. In addition, an integrated waste treatment approach on POME also had been introduced which involving the conventional treatment with several advanced treatment technologies for zero waste production [14]. [15] reported that an effective and sustainable treatment technology of POME are related with the concepts of low energy consumption with cost-effective processes and maximal recovery of valuable compounds present in the wastewater. In Malaysia, the development and utilization of effective POME treatment technology is still lacking especially at small scale industries areas which mostly dominated with an open or closed ponding treatment system [14] [16] [17]. Similarly, Indonesia also facing similar situation where more than 95 percent of POME treatment is operated using aerobic or anaerobic processes using ponding treatment system due to low operational and maintenance cost [18] [19] [20] [21]. However, although ponding treatment offered great economic value nevertheless due to several aspect such as large area requirement, longer retention time, emission of greenhouse gases and low effluent quality had restricted its installation and application [22] [23]. Therefore, if these disadvantages can be reduced, definitely the implementation of an efficient wastewater treatment can be installed for various of industrial activity and level.

The high-income countries such as Japan were very concerned with improving water quality to maintain environmental qualities and provide an alternative water source. Thus, sewage treatment technology, referred to DHS reactor has been proposed due to cost-effectiveness, advanced and systematic technology [24]. A downflow hanging sponge (DHS) reactor is currently used to treat various kinds of wastewater, such as domestic, aquaculture, and industrial wastewater [25] [26]. In addition, recent studies by [26] also shown the application of DHS have come into wide use in fields other than sewage treatment such as toluene gas treatment, minor metal recovery from wastewater and dissolved methane, arsenic elimination from water, regeneration and oxidation, and microorganism enrichment. The application of DHS reactor contained polyurethane sponge as referring to [27] and [28] it acts as the supporting media which offered a huge amount of biomass production and high pollutant removal capabilities. Moreover, according to [29] the sponge is easy to access, the material is non-biodegradable, simple and cheap applications. [27] also stated that the DHS reactor effectively eliminates residual COD, nitrogen and pathogens. Thus, it has been widely applicable in most countries such as Thailand, India, Egypt and Vietnam for various kinds of wastewater treatment [26]. However, the effectiveness of DHS reactor in treating high strength industrial wastewater especially POME wastewater is still remains unknown. Therefore, this research aims to investigate the performance of the biological treatment process by DHS reactor technology in treating POME wastewater.

2. Materials and methods

2.1. Experimental setup

A column with a height of 100cm and an inner diameter 4cm was used to treat POME in this study. A string of 25 carriers connected to each other in series was hung in the column (Figure 1). The activated carbon sponges connected in series with string and were soaked with inoculum collected from a wastewater treatment plant's aeration tank that treated municipal sewage, taken from Indah Water Konsortium (IWK) Sdn.Bhd. Each carrier consisted of activated carbon sponge cube (volume: $2 \times 2 \times 2 \text{ cm}$ each; total volume: 200cm$^3$) with a plastic frame (framed sponge). The schematic of the POME biological treatment in an anaerobic (DHS) reactor (Figure 1) shown the sample in the input tank (influent) was fed from the top of the reactor and flowed down through the sponges and discharged at the bottom of the reactor (effluent). The output gas was collected using a gas bag connected to the
reactor. The pH of the sample was set at pH 7.6, hydraulic retention time (HRT) of 2 hours, and temperature is monitored at 25°C – 31°C.

In the sample tank, minerals, trace elements, and phosphate buffer were added. The composition of the mineral, trace elements and phosphate buffer added in 10 L of the diluted POME was follow: minerals \([\text{CaCl}_2\cdot2\text{H}_2\text{O}} \text{(0.05mg/L), MgSO}_4\cdot7\text{H}_2\text{O} \text{(0.2mg/L), Fe}_2\text{SO}_4\cdot5\text{H}_2\text{O} \text{(0.1mg/L)}]\), trace elements \([\text{CuSO}_4\text{(0.025 mg/L), NiCl}_2\cdot6\text{H}_2\text{O} \text{(0.019mg/L), CoCl}}_2\cdot6\text{H}_2\text{O} \text{(0.024mg/L)]}\) and phosphate buffer \([\text{KH}_2\text{PO}_4\text{(1 mg/L), K}_2\text{HPO}_4 \text{(6 mg/L)}]\). In this study, minerals, trace elements, and phosphate buffer were acted as a supplement for microbial growth and help to enhance its metabolism in treating the sample. Initially, the input sample tanks were purged with nitrogen gas \((\text{N}_2)\) for an anaerobic condition.

2.2. Operational conditions
The reactors were operated for 40 days. This experiment was conducted under constant hydraulic retention time (HRT) of 2 hours for 2 phases with volumetric flowrate, \(Q_L\) of the influent sample was 0.0048 m\(^3\)/day. Loading rate used in phase 1 was 0.6 kg/m\(^3\)/day, and phase 2 was 0.4 kg/m\(^3\)/day as shown in Table 1.

| Parameter / Phase | 1  | 2   |
|------------------|----|-----|
| \(Q_L\) (m\(^3\)/day) | 0.0048 | 0.0048 |
| Loading rate (kg/m\(^3\)/day) | 0.6 | 0.4 |

2.3. Sampling and analyses
The POME sample was collected from Bell Kilang Sawit Kuala Linggi and was transported using a closed 2 L tank container to avoid contamination from the environment. The sample was kept in the refrigerator at a temperature 4 °C to prevent any biological biodegradation by microbial in the sample. The presence of the coarse solids in the sample was filtered 2-3 times by using filter cloth to prevent clog. The analysis of COD, pH, colour, ammoniacal nitrogen (NH\(_3\)-N), phosphates (PO\(_4^{3-}\)), and the presence of microbial were carried out in this study. The chemical oxygen demand (COD) was measured using Standard method 5220, colour using PtCo, Standard method 15 – 500 colour units, NH\(_3\)-N using Method 8155 powder pillows, and PO\(_4^{3-}\) using Method 8048 powder pillows, by using Hach colorimeter (Hach Co.Dr900). The pH was measured using a pH meter (EcoSense pH100A).
Biomass was sampled from the sponge’s carriers of the reactor after completing the experiment. The sludge samples taken from the sponges by squeezed sponges for microbial community analysis. DNA extraction was performed using FastDNA®SPIN Kit Soil according to the method reported by [30]. Then, the DNA samples were identification and characterization under Gram staining.

3. Results and discussion

3.1. System performance

The experiments were conducted in two different phases considering the influence of HRT and loading rates on the system performance, as illustrated in Figure 2. According to [31] HRT and loading rate are the important parameters for anaerobic bioreactor as it helps in deciding the quantity of substrate to be fed into the reactor. The reactor was operated at constant hydraulic retention time (HRT) of 2 hours with volumetric flowrate, \( Q_h \) of the influent sample was 0.0048 m\(^3\)/day, corresponding to organic loading rates (OLRs) was 0.6 kg/m\(^3\)/day (Phase 1). At the beginning of the experiment (Phase 1), pH influent was set at constant pH 7.6 and the output effluent pH was recorded within pH 8 (Figure 2a). The increase in pH indicate that methanogenic bacteria were activated to treat the wastewater. For proper anaerobic digestion, a suitable pH range is required for microbial development. Methanogens seek an anaerobic environment with a pH range of 6.5 to 7.5 [32]. Low pH able to cause accumulation of volatile fatty acid while high pH gives toxic environment for the methanogenic bacteria and inhibit the growth of bacteria [31].

The effects of HRT and loading rates on the color and COD removal efficiencies (Phase 1) were shown in Figure 2b and Figure 2c. The output of COD removal efficiencies in phase 1 were varying between 32% to 58%. The COD removal efficiencies in day 0 – 4 at maximum was 0.42 kg/m\(^3\)/day and constant removal in the range of 0.37 kg/m\(^3\)/day at day 4 – 11 with 41% removal efficiency. The active utilization of COD removal indicated that bacteria are involved in the COD degradation of organic and inorganic compounds in wastewater in an indirect manner [33]. The presence of high levels of COD in wastewater may led to the death of aquatic biota. Next, it was observed that fluctuation on the colour removal performance was occurred and a maximum removal efficiency of 76% was achieved within day 0 – 4. At day 4 – 11, the colour removal was recorded with ±70% of removal efficiency. This removal efficiency of COD and colour can be explained by the growth of the bacteria in reducing the organic contents.

During day 0 – 4, the anaerobic bacteria start to grow and adapt with the environment inside the reactor. COD and colour removal were higher at this period as the anaerobic bacteria need more food to grow and undergo cell division. At day 4 – 11, the growth of the bacteria cell was in the healthiest condition and cell division process were rapidly occurred which help in consuming more organic matter, thus reducing the COD and colour concentration of the effluent [34]. After day 11 – 19, the performance of the system starts to fluctuate in colour removal as the anaerobic bacteria was on the stationary phase where the number of the new cells was the same as the number of dying cell. However, the COD removal were still in the stable removal which illustrated that the anaerobic bacteria like to consume COD more than colour. Figure 2d shown the results of ammonia nitrogen production for Day 1-20, the level was at the constant removal rate of 12 mg/L/day with removal efficiency of 83%. The release of ammonia due to protein synthesis caused an increase in alkalinity, which led to a steady increase in pH values to 8 (Figure 2a). According to [32] the ammonia nitrogen released during protein degradation is often in the form of unionised free ammonia (NH\(_3\)), which is more harmful to the formation of methane than ionised ammonium (NH\(_4^+\)) at high pH due to NH\(_3\) diffuses more quickly across the cell membrane, obstructing cell function by disturbing the potassium-proton balance within the cell. From the observation shown in Figure 2e the biological phosphorus removal was happened based on the results decreases in phosphorus rate from output results. PO\(_4^{3-}\), removal was constant at 22 mg/L/day with maximum removal efficiency achieved was 58%. Increase in phosphorus release due to happening of breakdown of polyphosphate molecules. The graph shows
that the DHS reactor able to remove NH$_3$N (92%) compared to PO$_4^{3-}$ (58%). These inorganic matters were reduced by using adsorption process by the sponge cubes attached inside the DHS reactor as it cannot be break down by anaerobic bacteria. Besides, some of the nutrients have been consumed by the anaerobic bacteria as supplement which contribute to the reduction of excessive nutrient in the effluent sample. It is shows that the activity of bacteria in consuming the organic matters was really happening in the treatment system as the results of COD, colour, NH$_3$N and PO$_4^{3-}$ also reduced constantly along the Day 1 – 20.

In phase 2, again the pH of influent also stables at pH 7.6 and was operated at constant hydraulic retention time (HRT) of 2 hours with volumetric flowrate, Q$_L$ of the influent sample was 0.0048 m$^3$/day, corresponding to organic loading rates (OLRs) was 0.4 kg/m$^3$/day (Phase 2). Then the results of effluent pH shown that the pH achieved was constant at pH 7.6 thus shows that the anaerobic bacteria work efficiently and grow inside the treatment system. The COD constant removal was 0.2 kg/m$^3$/day with 44% removal efficiency and the maximum results achieved was on day 0 – 11 with 0.17 kg/m$^3$/day COD removal efficiencies shown. However, the results of the COD removal seem to have obvious fluctuation might be due to the technical error occurred on the equipment used to read the results. In addition, the percent of color removal for this phase 2 were reduced to ±200 PtCo, with maximum removal efficiency 73%. This result shows that the anaerobic bacteria have a maximum limit to consume the organic matter which is up until in the range 70% despite the changes amount of loading rate was supplied. The results of ammonia nitrogen (NH$_3$N) production in phase 2, shown constant removal was at 12 mg/L/day and 76% removal efficiency despite there were fluctuation in the reading of the results. The PO$_4^{3-}$ removal achieved was up to 87% with constant reading was 12 mg/L/day. Low loading rate seem to have a good performance in treating PO$_4^{3-}$ in the sample.

![Figure 2](image-url)  
*Figure 2. Performance of reactor system at varies parameters; a) pH; b) COD; c) colour; d) Ammoniacal nitrogen; e) phosphate.*
3.2. Microbial community identification

The bacteria identification was identified by Gram Staining technique. Bacteria sample from the sponge of the anaerobic reactor after the full biological treatment reactor were collected to differentiate groups of bacteria based on their different cell wall constituents, either gram-negative or gram-positive, by colouring these cells red or violet. The bacteria sample was observed under compound microscope under different total magnification are 40x (4x times 10x), 100x (10x times 10x), 400x (40x times 10x) and 1000x (100x times 10x) after the Gram Staining technique were apply. The staining results under magnification 1000x were observed and identified as gram-negative bacteria on the microscope, as illustrated in Figure 3 and there was no present of any gram-positive of bacteria although there is a present of oxygen in the raw sample. Gram negative bacteria were identified because the cell stain in red. Red color regarding to the attributed to a thinner peptidoglycan wall, which does not retain the crystal violet during the decoloring process. According to [35] gram-negative bacteria have a thinner coating of peptidoglycan (10%) and lose the CV-iodine complex during decolorization with the alcohol rinse, but maintain the counter stain safranin, giving them a reddish or pink appearance. They also have a lipid-containing outer membrane that is divided from the cell wall by the periplasmic space. It is assumed that types of Protobacteria were present during before and after the treatment using anaerobic DHS reactor. The possible types of Proteobacteria present are Alphaproteobacteria, Betaproteobacteria and Deltaprotobacteria [25].

To support this finding, study conducted by [25] concluded that Phyla Firmicutes, Bacteroides and Proteobacteria are the group of microbes mostly found in DHS sludge that able to treat wastewater. Therefore, this study showed the possibility of Proteobacteria species contributed to the treatment of POME by using DHS reactor system.

![Gram staining image for reactor at different image resolution](image)

**Figure 3.** Gram staining image for reactor at different image resolution.

4. Conclusions

The HRT and OLR played a crucial role in performance of anaerobic Down Hanging Sponge (DHS) reactor on treating colour, COD, NH$_3$N and PO$_4^{3-}$ of POME. From the results obtained, the pH and efficiency removal achieved for phase 1 were [(pH 7.6; colour (70%); COD (41%); NH$_3$N (83%); PO$_4^{3-}$ (58%)] and phase 2 were [(pH 7.6; colour (73%); COD (44%); NH$_3$N (76%); PO$_4^{3-}$ (87%)]. Based on gram staining on sludge sample taken along the anaerobic DHS reactor, gram-negative bacteria were detected, and it is assumed that Proteobacteria was present in the treatment system. Overall, this research helps in developing an idea on how to operate the anerobic DHS reactor. The technology can improve the efficiency of wastewater treatment and it is useful to be applied for various industrial scale.
References

[1] Singh R P, Ibrahim M H, Esa N, Iliyana M S 2010 Composting of waste from palm oil mill: A sustainable waste management practice *Reviews in Environmental Science and Biotechnology*, 9(4) 331–344

[2] Al-Amshawee, S K, Yunus, M Y, & Azoddein, A A 2020 A Review Study of Biofilm Bacteria and Microalgae Bioremediation for Palm Oil Mill Effluent: Possible Approach. *IOP Conference Series: Materials Science and Engineering*, 736(2)

[3] Zainal, N H, Jalani, N F, Mamat, R., & Astimar, A A 2017 A review on the development of palm oil mill effluent (POME) final discharge polishing treatments. *Journal of Oil Palm Research*, 29(4) 528–540

[4] Oil, P, Size, M, Oil, P, Size, M, Oil, K, Use, B E, Region, B, & Forecasts, S 2021 *End-use Insights* 2020–2027

[5] Abidin, C Z A, Fahmi, Ibrahim, A H, Rahmat, N R, Ahmad, R, Hussein, N F M, Choong, P S, & Singa, P K 2021 Effect of electrode materials on the degradation of palm oil mill effluent by electro-oxidation process *IOP Conference Series: Earth and Environmental Science*, 646(1)

[6] Ahmad, A L, Ismail, S, & Bhatia, S 2003 Water recycling from palm oil mill effluent (POME) using membrane technology. *Desalination*, 157(1–3) 87–95

[7] Osman, N A, Ujang, F A, Roslan, A M, Ibrahim, M F, & Hassan, M A 2020 The effect of Palm Oil Mill Effluent Final Discharge on the Characteristics of Pennisetum purpureum *Scientific Reports* 10(1) 1–10

[8] Kamyab, H, Chelliapan, S, Din, M F M, Rezania, S, Khademi, T, & Kumar, A 2018 Palm Oil Mill Effluent as an Environmental Pollutant *Palm Oil* [9] Rana, S, Singh, L, Wahid, Z, & Liu, H 2017 A Recent Overview of Palm Oil Mill Effluent Management via Bioreactor Configurations *Current Pollution Reports* 3(4) 254–267

[10] Loh, S. K, Lai, M E, Ngatiman, M, Lim, W S, Choo, Y M., Zhang, Z, & Salimon, J 2013 Zero discharge treatment technology of palm oil mill effluent *Journal of Oil Palm Research* 273–281

[11] Abdulsalam, M, Man, H C, Idris, A I, Yunos, K F, & Abidin, Z Z 2018 Treatment of palm oil mill effluent using membrane bioreactor: Novel processes and their major drawbacks *Water (Switzerland)* 10(9)

[12] Nasrullah, M, Singh, L, Mohamad, Z, Norsita, S, Krishnan, S, Wahida, N, & Zularisam, A W 2017 Treatment of palm oil mill effluent by electrocoagulation with presence of hydrogen peroxide as oxidizing agent and polialuminum chloride as coagulant-aid *Water Resources and Industry* 17 7–10

[13] Nasution, M A, Wulandari, A, Ahamed, T, & Noguchi, R 2020 Alternative POME treatment technology in the implementation of roundtable on sustainable palm oil, Indonesian sustainable palm oil (ISPO) and Malaysian sustainable palm oil (MSPO) standards using LCA and AHP methods *Sustainability (Switzerland)* 12(10)

[14] Sayuti, S C, & Azoddein, A A M 2015a Treatment of palm oil mill effluent (pome) by using electrocoagulation as an alternative method (Rawatan Efluen Kilang Sawit Menggunakan Elektrokoagulasi Sebagai Kaedah Alternatif) *Malaysian Journal of Analytical Sciences* 19(4) 663–668

[15] Hasanudin, U, Sugiharto, R, Haryanto, A, Setiadi, T, & Fujie, K 2015 Palm oil mill effluent treatment and utilization to ensure the sustainability of palm oil industries *Water Science and Technology* 72(7) 1089–1095

[16] Rupani, P, & Singh, R 2010 Review of current palm oil mill effluent (POME) treatment methods: Vermicomposting as a sustainable practice *World Applied Sciences* 11(1) 70–81

[17] Azmi, N S, & Yunos, K F M 2014 Wastewater Treatment of Palm Oil Mill Effluent (POME) by Ultrafiltration Membrane Separation Technique Coupled with Adsorption Treatment as Pre-treatment *Agriculture and Agricultural Science Procedia* 2 257–264
[18] Hazmi, A, Desmiarti, R, Waldi, E. P, & Emeraldi, P 2016 Preliminary study on treatment of palm oil mill effluent by sand filtration-dielectric barrier discharge system Journal of Engineering and Technological Sciences 48(1) 21–30

[19] Leela, D, & Nur, S M 2019 Processing technology POME-pond in Indonesia: A mini review IOP Conference Series: Earth and Environmental Science 365(1)

[20] Mohammad, S, Baidurah, S, Kobayashi, T, Ismail, N, & Leh, C P 2021 Palm Oil Mill Effluent Treatment Processes A Review 1–22.

[21] Sari, S P 2019 Optimising the utilisation of Palm Oil Mill Effluent (POME) for biogas power plants to achieve Indonesian target of bioenergy power plants in 2025 August

[22] Hosseini, S E, & Wahid, M A 2015 Pollutant in palm oil production process Journal of the Air and Waste Management Association 65(7) 773–781

[23] Chung, A Y K, Qamaruz Zaman, N, Yaacof, N, Yusoff, S, Abd. Manaf, F Y, Mohamed Halim, R, & Abd Majid, R 2019 The Effectiveness of Gas Recovery Systems for Managing Odour from Conventional Effluent Treatment Ponds in Palm Oil Mills in Malaysia Civil and Environmental Engineering Reports 29(3) 70–85

[24] Machdar, I, Muhammad, S, Onodera, T, & Syutsubo, K 2018 A pilot-scale study on a down-flow hanging sponge reactor for septic tank sludge treatment Environmental Engineering Research 23(2)195–204

[25] Hatamoto, M, Okubo, T, Kubota, K, & Yamaguchi, T 2018 Characterization of downflow hanging sponge reactors with regard to structure, process function, and microbial community compositions Applied Microbiology and Biotechnology 102(24) 10345–10352

[26] Maharjan, N, Hewawasam, C, Hatamoto, M, Yamaguchi, T, Harada, H, & Araki, N 2020 Downflow Hanging Sponge System: A Self-Sustaining Option for Wastewater Treatment. Wastewater Treatment

[27] Uemura, S, Okubo, T, Maeno, K, Takahashi, M, Kubota, K, & Harada, H 2016 Evaluation of water distribution and oxygen mass transfer in sponge support media for a down-flow hanging sponge reactor International Journal of Environmental Research 10(2) 265–272

[28] Nurmiyanto, A, & Ohashi, A 2019) Downflow Hanging Sponge (DHS) Reactor for Wastewater Treatment - A Short Review. MATEC Web of Conferences, 280(May) 05004

[29] El-Kamah, H, Mahmoud, M, & Tawfik, A 2011) Performance of down-flow hanging sponge (DHS) reactor coupled with up-flow anaerobic sludge blanket (UASB) reactor for treatment of onion dehydration wastewater Bioresource Technology 102(14) 7029–7035.

[30] Noorain, R, Kindaichi, T, Ozaki, N, Aoi, Y, & Ohashi, A 2019) Integrated biological–physical process for biogas purification effluent treatment Journal of Environmental Sciences (China) 83 110–122

[31] Emmanuel Alepu, O, & Li, Z 2016 Effect of Hydraulic Retention Time on Anaerobic Digestion of Xiao Jiahe Municipal Sludge International Journal of Waste Resources 6(3)

[32] Musa, M A, & Idrus, S 2020 Effect of hydraulic retention time on the treatment of real cattle slaughterhouse wastewater and biogas production from HUASB reactor Water (Switzerland) 12(2)

[33] Qadir, M, Drechsel, P, Jiménez Cisneros, B, Kim, Y, Pramanik, A, Mehta, P, & Olaniyan, O 2020 Global and regional potential of wastewater as a water, nutrient and energy source Natural Resources Forum 44(1) 40–51

[34] Bruslind, L 2021) Introduction to Microbiology Oregon State University

[35] Upadhyay, N, Vishawkarma, K, Singh, J., Mishra, M, Kumar, V, Rani, R, Mishra, R K, Chauhan, D K, Tripathi, D K, & Sharma, S 2017) Tolerance and reduction of chromium(VI) by Bacillus sp MNU16 isolated from contaminated coal mining soil Frontiers in Plant Science 8(May) 1–13
Acknowledgement
This research was supported by University Kuala Lumpur short term research grant (STRG), Grant Number STR19039.