

Innovation with The Use of Probiotics as an Eco-friendly Tool for Sewage and Palm Oil Mill Effluent Treatment

Teow Yeit Haan^{1,2*}, Nurul Ain Asyikin bt Mohamad Zaimi², Noorini Izzati Mohamad Mazuki², Ho Kah Chun^{1,2} and Maha Mohammad Hussein Al-Rajabi^{2,3}

¹Research Centre for Sustainable Process Technology (CESPRO), Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia.

²Department of Chemical Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia.

³Chemical Engineering Department, School of Engineering, The University of Jordan, Amman, Jordan.

ABSTRACT

This research study is aimed to explore the potential of using probiotic, active microorganisms for both sewage and palm oil mill effluent (POME) treatment. Probiotic strain contains 10 types of Bacillus strains including Bacillus catenulatus, Bacillus careus, Bacillus drementensis, Bacillus firmus, Bacillus flexus, Bacillus megaterium, Bacillus niaci, Bacillus subtilis, Bacillus teguilensis, and Bacillus thuringiensis was used in this study. Bench-scale batch system was used to study the performance of probiotic for the treatment of sewage and aerobic digested POME. The treatment process was conducted for 10 days where the water sample was collected every consecutive 2 days for the analysis on total suspended solids (TSS), chemical oxygen demand (COD), phosphorus, biological oxygen demand (BOD), ammonia, and nitrate. The results depicted that probiotic was able to break down concentrated organic matters into simple amino acid, thereby reduces the sludge, COD, BOD, and odour. 300 mL probiotic presented the greatest performance in which the percentage of COD, BOD, TSS, phosphorus, NO₃-N, and NH₃-N removal was recorded as 100.00%, 100.00%, 0.00%, 64.66%, 0.00%, and 97.36%; and 75.44%, 100.00%, 17.24%, 69.77%, 0.00%, and 81.45% for sewage treatment and aerobic digested POME treatment, respectively after 10 days of retention time.

Keywords: Palm Oil Mill Effluent, Probiotics, Sewage, Sustainability, Wastewater Treatment.

1. INTRODUCTION

Malaysia is one of the largest palm oil producers after Indonesia. As reported by Malaysian Palm Oil Board, Malaysia had produced 19.52 million tonnes of crude palm oil in 2018 [1]. Along with the huge amount of palm oil production attributed by high market demand, large quantity of palm oil mill effluent (POME) had been generated and it required a proper treatment before discharge to the environment [1]. POME is a brownish colloidal suspension containing high concentration of organic matters, oil and grease, and total solids. Due to its large discharge volume, high biological oxygen demand (BOD) and chemical oxygen demand (COD) value, POME is considered as the major pollutant to aquatic environment as it will deplete the dissolved oxygen in water body, thereby affecting the survival of aquatic organisms [2]. Ponding system is the conventional POME treatment method which has been practice for many decades. It consists of aerobic pond and anaerobic pond to break down the organic substances contained in POME. However, conventional ponding system has several drawbacks including the releasing of greenhouse gases during the treatment process, it requires a large land area for operations, a long hydraulic retention time is needed, and it produces a bad odour.

*Corresponding Author: yh_teow@ukm.edu.my

On the other hand, Malaysia has generated 2.97 billion cm³ sewage per year [3]. Discharge of untreated sewage will cause dissemination of water borne disease due to microorganisms contained in sewage, depletion of dissolved oxygen in water body, and nauseating odour to the environment. Conventional sewage treatment processes involve physical treatment and chemical treatment are complicated, costly, and at the same time generates chemical treatment waste.

Nowadays, the use of beneficial microorganisms to improve the water quality has become a trend [4]. *Bacillus* is a Gram-positive, rod shaped, spore forming, aerobic or facultative anaerobic bacterium. Attributed to its several advantages, such as stability for long periods due to spore formation, has immune-modulatory ability, and able to react antagonist towards the pathogens, *Bacillus* has the potential to be applied as a probiotic strain for water remediation [5, 6, 7, 8]. Hong *et al.* [9] reported that *Bacillus* is an important candidate for developing commercial biological agents for nitrogen removal and water quality enhancement. Chen *et al.* [10], Laloo *et al.* [11], and Rui *et al.* [12] also agreed that some *Bacillus strains* such as *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus cereus* are able to exhibit strong nitrite removal ability. Yusuf *et al.* [13] found that the *Bacillus subtilis* was successful in removing 87.6 % and 91.7% of COD and total suspended solids (TSS), respectively from tannery wastewater after 84 hours of treatment process.

The performance of probiotic is much dependent on the waste excretion property. Therefore, the combination of several *Bacillus strains* could be a great solution in achieving high efficiency degradation and removal of organic substances from wastewater. With the existence of sunlight as the energy source and carbon dioxide as the carbon source, these *Bacillus strains* could degrade the organic substances and convert it into simple organic substances, such as amino acids. This will eventually result in a reduction of several water quality parameters including COD, BOD, TSS, and nutrients. Up-to-date, there are limited studies on the potential application of probiotic for sewage and POME treatment. As such, this study intends to explore the potential of probiotic, a multi active microorganisms in treating both sewage and POME.

2. MATERIAL AND METHODS

2.1 Probiotic Strain

Probiotic strain, BANIK 303 manufactured by Banik Yuli Sdn. Bhd. (Malaysia) was used in this study. BANIK 303 contains 10 types of *Bacillus strains* including *Bacillus catenulatus*, *Bacillus careus*, *Bacillus drementensis*, *Bacillus firmus*, *Bacillus flexus*, *Bacillus megaterium*, *Bacillus niaci*, *Bacillus subtilis*, *Bacillus teguilensis*, and *Bacillus thuringiensis*. It is formulated with nitrogen, phosphorus, potassium, magnesium, calcium, boron, silicon, sulphur, iron, zinc, copper, and manganese to promote the growth of *Bacillus strains* and to stimulate the food chain for in-situ treatment.

2.2 Wastewater

POME used in this study was collected from aerobic digester pond at Sime Darby East Mill located at Carey Island, Selangor, Malaysia. Meanwhile, sewage was collected from a primary treatment tank at Indah Water Konsortium (IWK) Unit Langat STP-A, Cyberjaya, Malaysia. Both aerobic digested POME and sewage were characterized immediately after sampling. Table 1 summarizes the characteristics of both aerobic digested POME and sewage.

Table 1 Characteristics of aerobic digested POME and sewage

Parameter	Aerobic digested POME	Sewage
Temperature (°C)	21.30	28.20
pH	6.66	8.25
TSS (mg/L)	31.33	290.00
NH ₃ -N (mg/L)	19.80	469.00
NO ₃ -N (mg/L)	0.10	91.00
Phosphorus (mg/L)	11.80	118.00
COD (mg/L)	90.00	7900.00
BOD (mg/L)	11.43	335.00

2.3 Experiment Set-up

Bench-scale batch system was used to study the performance of probiotic for the treatment of sewage and aerobic digested POME. 30 L of aerobic digested POME was filled into the cylindrical treatment tank. 150 mL, 300 mL, and 450 mL of probiotic were added into 3 different cylindrical treatment tanks containing the aerobic digested POME. Air flow of 4 L/min was purged into the cylindrical treatment tank. The treatment process was conducted for 10 days where the water sample was collected every consecutive 2 days for the analysis on total suspended solids (TSS), COD, phosphorus, BOD, ammonia, and nitrate. The experiment was repeated using sewage as the feed. Figure 1 presents the schematic diagram of bench-scale probiotic batch treatment system. Whereas, Table 2 summarizes the experiment set-up for bench-scale probiotic batch treatment system.

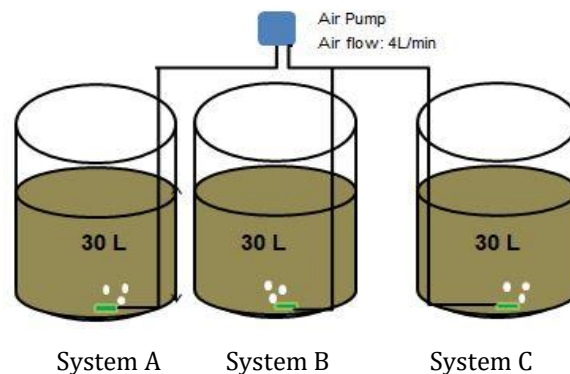


Figure 1. Schematic diagram of bench-scale probiotic batch treatment system.

Table 2 Experiment set-up for bench-scale probiotic batch treatment system

Feed	Treatment system	Volume of water sample (L)	Volume of probiotic (L)
Sewage	A1	30	0.150
	B1	30	0.300
	C1	30	0.450
Aerobic digested POME	A2	30	0.150
	B2	30	0.300
	C2	30	0.450

2.4 Water Sample Analysis

The efficiency of probiotic for treating both sewage and aerobic digested POME was evaluated based on several water quality parameters, including TSS, ammonia-nitrogen (NH₃-N), phosphorus, nitrate-nitrogen (NO₃-N), BOD, and COD followed the HACH standard method. The percentage of removal for each parameter was calculated using equation (1).

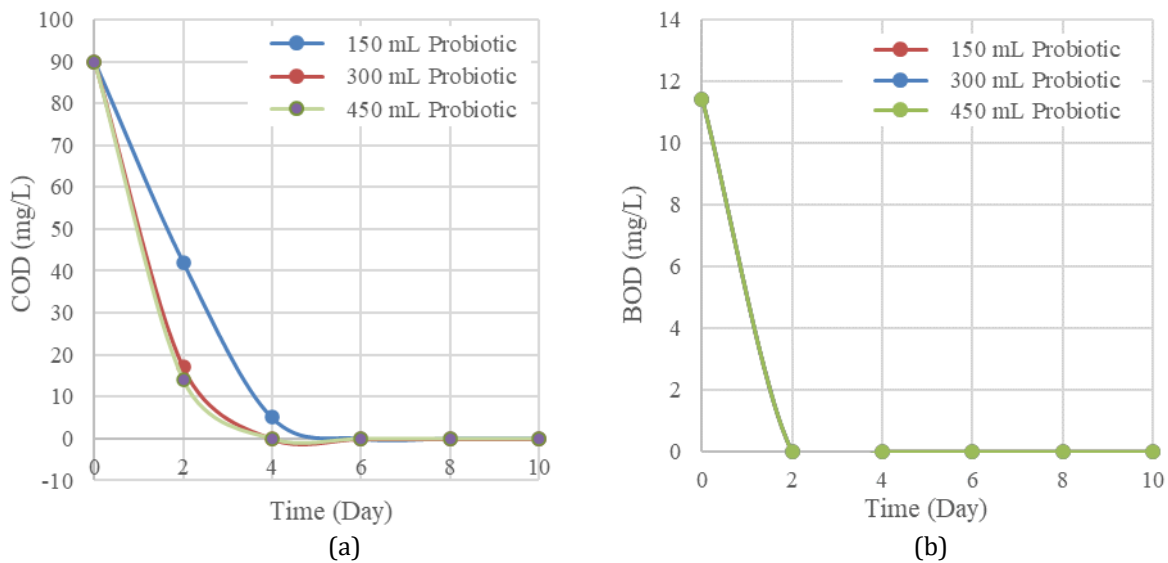
$$R = \frac{C_i - C_f}{C_i} \times 100\% \quad (1)$$

C_i and C_f denote the initial concentration of the water sample and the final concentration of the water sample, respectively.

3. RESULTS AND DISCUSSION

3.1 Sewage Treatment

Figure 2 shows the performance of bench-scale probiotic batch treatment system for sewage treatment whereas Table 3 summarizes the percentage of removal of each water analysis parameter after 10 days of retention time. As presented in Figure 2, COD, BOD, phosphorus, and $\text{NO}_3\text{-N}$ decreases with the increase of retention time except for TSS and $\text{NO}_3\text{-N}$. The existence of probiotic does not have any significant effect in removing TSS from sewage. Aerated bubbles created in bench-scale probiotic batch treatment system prohibits the suspended solids in sewage from settling down at the bottom of the treatment tank. Rapid circular motion therefore maintains the suspended solids concentration in sewage sample. Additionally, probiotic treatment is a biological treatment process, the *Bacillus strains* will not able to degrade or ingest the suspended solids in water sample. Attribute to this, the suspended solids content in sewage does not decrease.



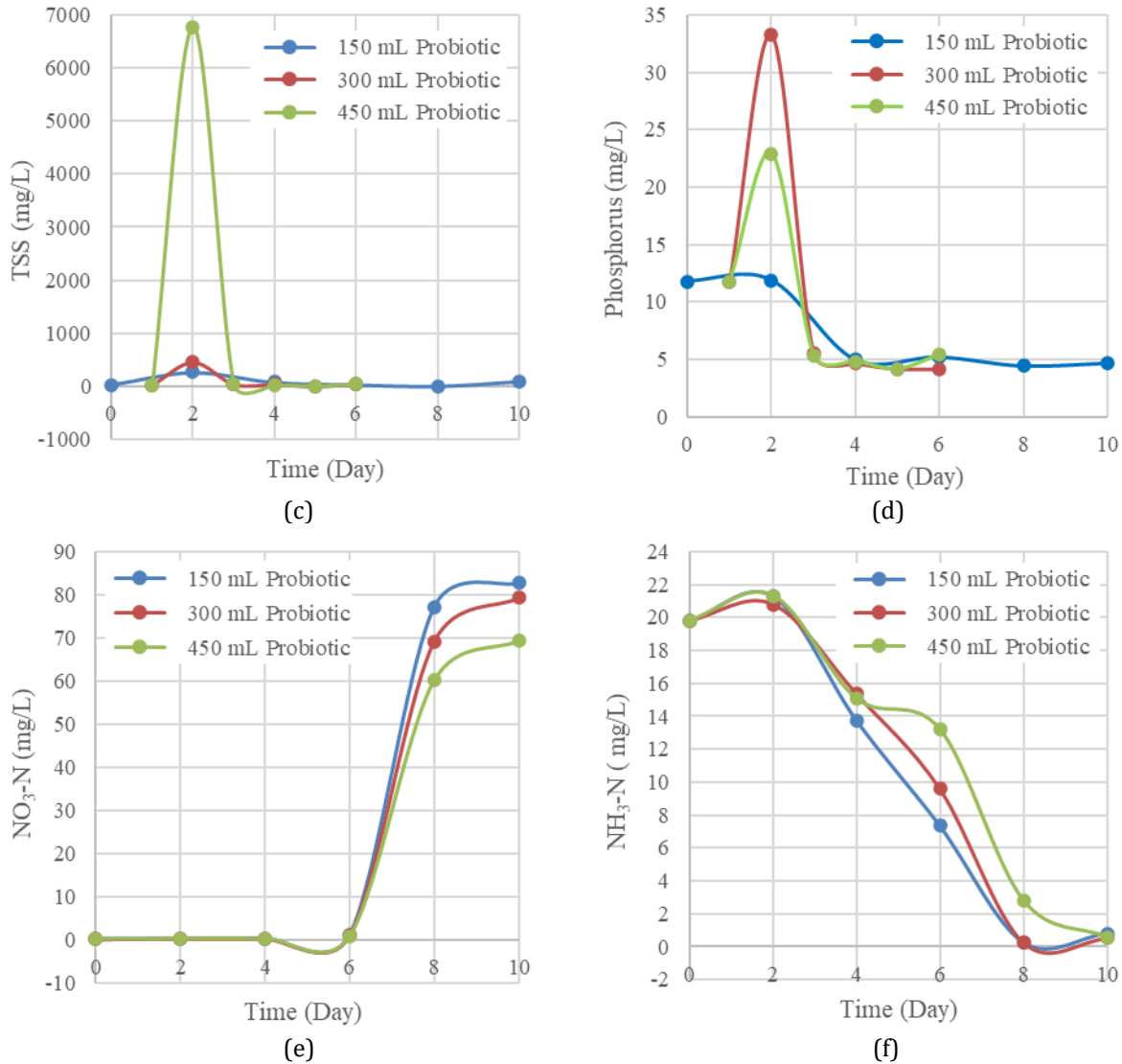


Figure 2. Performance of bench-scale probiotic batch treatment system for sewage treatment (a) COD (b) BOD (c) TSS (d) phosphorus (e) NO₃-N, and (f) NH₃-N.

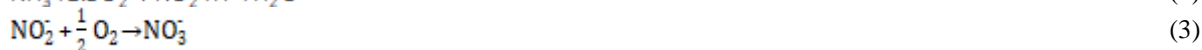
Table 3 Percentage of removal after 10 days of retention time for each water analysis parameter in sewage treatment

Parameter	Percentage of removal (%)		
	A1	B1	2
COD	100.00	100.00	100.00
BOD	100.00	100.00	100.00
TSS	0.00	0.00	0.00
Phosphorus	60.56	64.66	54.04
NO ₃ -N	0.00	0.00	0.00
NH ₃ -N	96.01	97.36	97.14

However, probiotic had successfully reduced the COD, BOD, and NH₃-N concentration in sewage to 96-100% after 10 days of retention time. COD is the measurement of the capacity of water to consume oxygen during the decomposition of organic substances and the oxidation of inorganic chemicals. On the other hand, BOD is the oxygen uptake for the decomposition of biodegradable organic carbon [14]. Along with the probiotic treatment process, *Bacillus strains* from probiotic ingests the high molecular weight of organic and inorganic substances in sewage due to the

presence of oxygen provided by aeration system and breaks it down into simpler substances. Therefore, it is not surprising that the COD and BOD concentration in sewage decreased with the increase in retention time. $\text{NH}_3\text{-N}$ on the other hand is a nutrient for the growth of probiotic [15]. By prolonging the retention time, probiotic gradually grows increasing its population. Therefore, $\text{NH}_3\text{-N}$ is being consumed. On top of that, there is also a reduction of phosphorus content in the sewage with the probiotic treatment process. The $\text{NH}_3\text{-N}$, phosphorus was also consumed by the probiotic as a source of nutrient for continuous growth of the population.

On the other hand, the performance of a bench-scale probiotic batch treatment system on $\text{NO}_3\text{-N}$ removal has shown an opposite trend compared to the other water analysis parameter in which the concentration of $\text{NO}_3\text{-N}$ increased with the retention time. It is presumed that other than the consumption of $\text{NH}_3\text{-N}$ by probiotic, probiotic is also involved in the nitrification process. A study conducted by Koops and Moller [16] states that *Bacillus strains*, especially *Bacillus subtilis* contribute to nitrification in aquatic systems by utilizing ammonium ions as the nitrogen source for its growth under aerobic condition. During the nitrification process, $\text{NH}_3\text{-N}$ is converted into nitrate. Eq. (2) and Eq. (3) are used to explain the nitrification process [17]. As a result, the concentration of $\text{NH}_3\text{-N}$ decreases while the concentration of $\text{NO}_3\text{-N}$ increases with the increasing retention time.



By comparing the percentage of removal for each water analysis parameter after 10 days of retention time in sewage treatment, it shows that the addition of 300 mL of probiotic into the treatment system has a better performance in reducing most of the water analysis parameter. The percentage of removal for COD, BOD, TSS, phosphorus, $\text{NO}_3\text{-N}$, and $\text{NH}_3\text{-N}$ are 100.00%, 100.00%, 0.00%, 64.66%, 0.00%, and 97.36%, respectively.

3.2 POME Treatment

Figure 3 shows the performance of a bench-scale probiotic batch treatment system for aerobic digested POME treatment whereas Table 4 summarizes the percentage removal of each water analysis parameter after 10 days of retention time. Basically, the bench-scale probiotic batch treatment system shows a similar performance as both sewage and aerobic digested POME. However, due to the high TSS content in aerobic digested POME, the circulation motion in the treatment tank causes a great fluctuation on the TSS concentration. Meanwhile, bacterial biomass was also largely created by the microorganisms in the aerobic digested POME along with the treatment process.

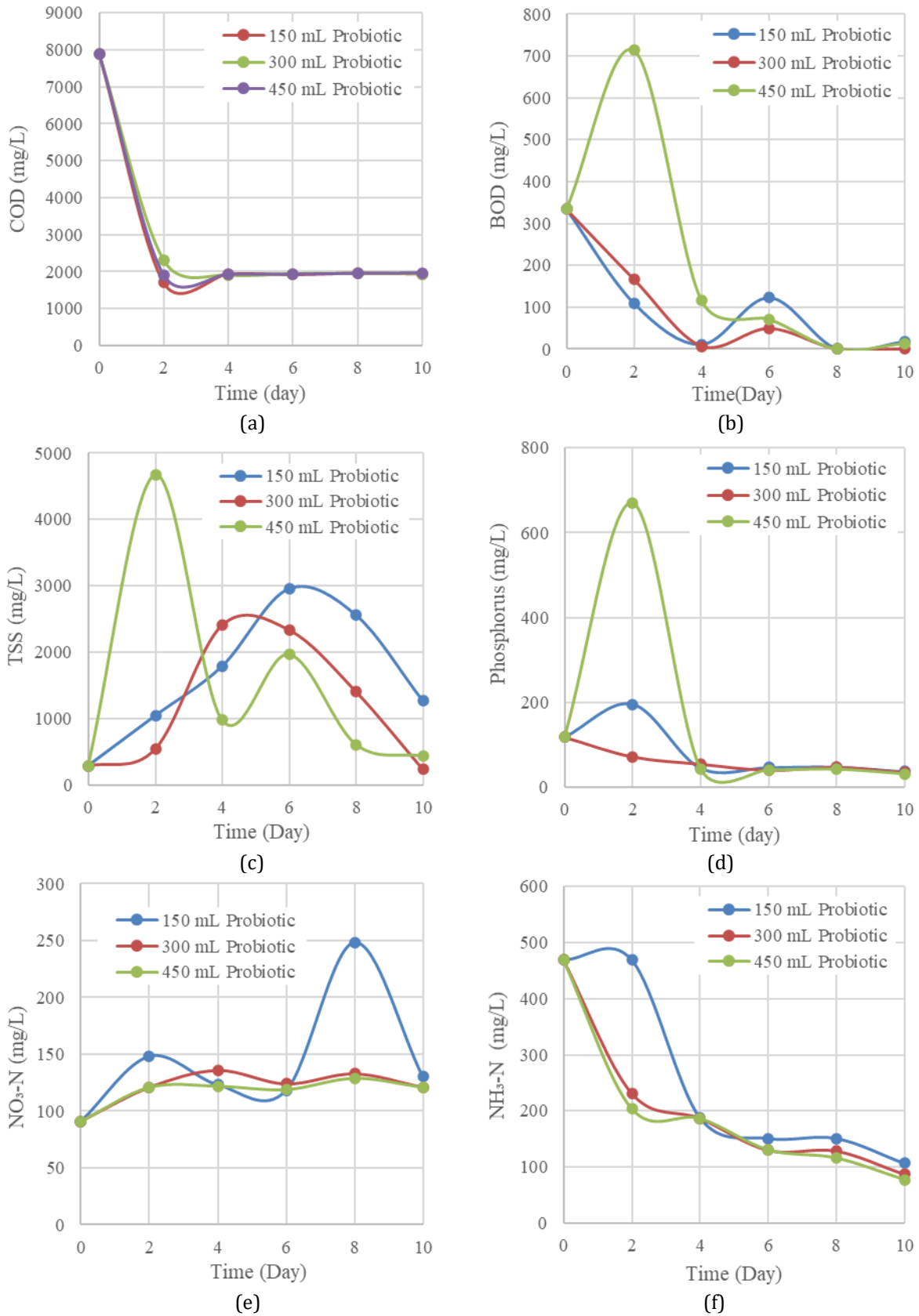


Figure 3. Performance of bench-scale probiotic batch treatment system for aerobic digested POME treatment (a) COD (b) BOD (c) TSS (d) phosphorus (e) NO₃-N, and (f) NH₃-N.

Table 4 Percentage of removal after 10 days of retention time for each water analysis parameter in aerobic digested POME treatment

Parameter	Percentage of removal (%)		
	A2	B2	C2
COD	75.44	75.44	75.19
BOD	94.93	100.00	95.97
TSS	0.00	17.24	0.00
Phosphorus	68.36	69.77	73.73
NO ₃ -N	0.00	0.00	0.00
NH ₃ -N	77.26	81.45	83.58

Analogous to probiotic treatment on sewage, by comparing the percentage removal of each water analysis parameter after 10 days of retention time in aerobic digested POME treatment, it shows that the addition of 300 mL of probiotic into the treatment system has a better performance in reducing most of the water analysis parameter. The percentage of removal for COD, BOD, TSS, phosphorus, NO₃-N, and NH₃-N are 75.44%, 100.00%, 17.24%, 69.77%, 0.00%, and 81.45%, respectively.

4. CONCLUSION

The potential of probiotic, a multi active microorganism in treating both sewage and aerobic digested POME has been explored in this study. A bench-scale probiotic batch treatment system equipped with aeration system has received partial success in treating both sewage and aerobic digested POME and has improved its water quality. The results show that the addition of 300 mL of probiotic into the treatment system has presented the most significant effect in the treatment process in which COD, BOD, TSS, phosphorus, NO₃-N, and NH₃-N were reduced to 100.00%, 100.00%, 0.00%, 64.66%, 0.00%, and 97.36%; and 75.44%, 100.00%, 17.24%, 69.77%, 0.00%, and 81.45%, for sewage treatment and aerobic digested POME treatment, respectively after 10 days of retention time. This great achievement by a bench-scale probiotic batch treatment system, confirms the potential application of probiotic as an eco-friendly tool for both sewage and POME treatment.

ACKNOWLEDGEMENTS

The authors wish to gratefully acknowledge the financial support of this work by Dana Penyelidikan Strategik (KRA-2017-06).

REFERENCES

- [1] MPOB, "Overview of the Malaysian Palm Oil Industry 2018", Malaysian Palm Oil Board, (2019).
- [2] Shian, Y., M. Omar, A.B. Kadir, & T. Tow, "Diversity in the antibacterial pnetics evaluation of anaerobic stabilization pond treatment of palm oil mill effluent", *Bioresource Technology*. **100** (2009) 4969–4975.
- [3] Engku Azman, T. M., S. Jamil, & V. K. How, "Wastewater Production, Treatment, and Use in Malaysia", (2012).
- [4] Xie, F., T. Zhu, F. Zhang, K. Zhou, Y. Zhao, & Z. Li, "Using *Bacillus amyloliquefaciens* for remediation of aquaculture water", *SpringerPlus*. **2** (2013) 119.

- [5] Elshaghabee, F. M. F., N. Rokana, R. D. Gulhane, C. Sharma, & H. Panwar, "Bacillus As Potential Probiotics: Status, Concerns, and Future Perspectives", *Frontiers in microbiology*. **8** (2017) 1490.
- [6] Lefevre, M., S. M. Racedo, G. Ripert, *et al.*, "Probiotic strain *Bacillus subtilis* CU1 stimulates immune system of elderly during common infectious disease period: a randomized, double-blind placebo-controlled study", *Immun. Ageing*. **12** (2015).
- [7] Ripert, G., S. M. Racedo, A. M. Elie, C. Jacquot, P. Bressollier, & M.C. Urdaci, "Secreted compounds of the probiotic *Bacillus clausii* strain O/C inhibit the cytotoxic effects induced by *Clostridium difficile* and *Bacillus cereus* toxins", *Antimicrob. Agents Chemother.* **60** (2016) 3445–3454.
- [8] Shobharani, P., R. J. Padmaja, & P. M. Halami, "Diversity in the antibacterial potential of probiotic cultures *Bacillus licheniformis* MCC2514 and *Bacillus licheniformis*", *Res. Microbiol.* **166** (2015) 546–554.
- [9] Hong, H. A., L. H. Duc, & S. M. Cutting, "The use of bacterial spore formers as probiotics", *FEMS Microbiology Reviews*. **29** (2005) 813–835.
- [10] Chen, S., & Y. Hu, "Use of *Bacillus subtilis* in purification of slightly-polluted water", *Acta Scientiae Circumstantiae*. **31** (2011) 1594–1601.
- [11] Laloo, R., S. Ramchuran, D. Ramduth, J. Gorgens, & N. Gardiner, "Isolation and selection of *Bacillus* spp. as potential biological agents for enhancement of water quality in culture of ornamental fish", *Journal of Applied Microbiology*. **103** (2007) 1471–1479.
- [12] Rui, M., H. LianSheng, X. BeiDou, H. Xiang, & L. YueYan, "Experimental study on purifying aquaculture wastewater between *Bacillus* and nitrifying bacteria", *Environmental Science & Technology (China)*. **32** (2009) 28–31.
- [13] Yusuf, R. O., Z. Z. Noor, M. A. A. Hassan, & S. E. Agarry, "A comparison of the efficacy of two strains of *Bacillus subtilis* and *Pseudomonas fragii* in the treatment of tannery wastewater", *Desalination and Water Treatment*. **51** (2013) 3189–3195.
- [14] Nazari, L., S. Sarathy, D. Santoro, D. Ho, M. B. Ray, & C. C. Xu, "3 - Recent advances in energy recovery from wastewater sludge", *Direct Thermochemical Liquefaction for Energy Applications*. (2018) 67–100.
- [15] Liu, J., E. Lkhagva, H. J. Chung, H. J. Kim, & S. T. Hong, "The pharmabiotic approach to treat hyperammonemia", *Nutrients*. **10** (2018).
- [16] Koops, H. P., & U. C. Moller, "The lithotrophic ammonia-oxidizing bacteria", In A. Balows, H.G. Truper, M. Dworkin, W. Harder and K.H. Schleifer, eds., *The prokaryotes*. Springer, Berlin, Germany, (1992), 2625–2637.
- [17] P, P., S. K, & V. K, "Efficacy of probiotics in improving water quality and bacterial flora in fish ponds", *African Journal of Microbiology Research*. **6** (2012) 7471–7478.

