NUEJ
Naresuan University
Engineering Journal

Effects of Aeration and Quantity of Effective Microorganisms (EM) Balls for Water Quality Restoration

Yumatorn Mingmongkol[1,2], Khakhanang Ratananikom[3], Duangdao Channei[4], Auppatham Nakaruk[5], Wilawan Khanitchaidecha[1,2,*]

[1]Department of Civil Engineering, Faculty of Engineering, Naresuan University, Phitsanulok, Thailand
[2]Centre of Excellence for Innovation and Technology for Water Treatment, Faculty of Engineering, Naresuan University, Phitsanulok, Thailand
[3]Department of Science and Mathematics, Faculty of Science and Health Technology, Kalasin University, Kalasin, Thailand
[4]Department of Chemistry, Faculty of Science, Naresuan University, Phitsanulok, Thailand
[5]Department of Industrial Engineering, Faculty of Engineering, Naresuan University, Phitsanulok, Thailand

* Corresponding E-mail: wilawank1@gmail.com

(Received: 11 May 2021, Revised: 19 July 2021, Accepted: 7 September 2021)

Abstract

The simple and low cost water treatment of effective microorganisms (EM) technology was applied for water quality restoration; NH_4-N and carbon content removal. The EM balls were prepared from EM stock, molasses and dried bran. The 30-L opened reactors were operated under different air supplies (namely no aeration and aeration reactors) and quantity of EM balls (namely 1EM, 2EM and 3EM reactors). The results revealed that the aeration benefited to rapid pollutants reduction due to the increasing microorganisms activity under sufficient dissolved oxygen concentration. The concentration of NH_4-N and COD in the aeration reactor was notably lower than that in the no aeration reactor during the experimental period. The aeration reactor achieved 83% for total nitrogen removal and 80% for COD removal after 17 days. On the other hand, the lower total nitrogen and COD removals of 77% and 60% respectively was observed in the no aeration reactor. In the meanwhile, the quantity of EM balls had significant impacts on the pollutants removal, because the number of effective microorganisms were dependent on quantity of EM balls. The best performance was observed in 2EM reactor, and followed by 1EM reactor and 3EM reactor respectively. This was because the excessive EM balls led to very high NH_4-N and COD releasing from substrates decomposition. According to this study, the DO should be maintained around 6 mg/L and the optimal quantity of EM balls was two for achieving the efficient NH_4-N and COD removals with a high NH_4-N removal rate.

Keywords: Effective microorganisms technology, EM balls, Water quality, NH_4-N removal, Carbon content removal

1. INTRODUCTION

The effective microorganisms (EM) technology was developed during the 1970's by Professor Teruo Higa at the university of Ryukyus in Okinawa, Japan. The EM are multi-culture of aerobic and anaerobic beneficial microorganisms, which is capable to decompose organic matter by converting it to carbon dioxide (CO_2), methane (CH_4) or use it for growth and reproduction. The main species include lactic acid bacteria (i.e., *Lactobacillus casei*), photosynthetic bacteria (i.e., *Rhodopseudomonas palustrus,*), yeasts (i.e., *Saccharomyces cerevisiae, Candida utilis*), actinomycetes (i.e., *Streptomyces albus*) and fermenting fungi (i.e., *Aspergillus oryzae*, *Mucor hiemalis*) (Victoria & Mahaeswari, 2016). Recently, the EM technology has been widely used in developing countries for enhancing sewage and wastewater treatment (Boruszko, 2017; Lananan et al., 2014), increasing plants growth under salt stress (Abd El-Mageed et al., 2020), reducing residual sludge in septic tanks, livestock development and household uses (Higa & Kanal, 1996). Significant advantages of using EM were ease of use and relatively inexpensive technology. In addition, it can be applied in various fields such as agriculture, environment, etc.

Aeration (or dissolved oxygen concentration; DO) and amount of EM were important factors for water quality improvement by EM technology. The ratio of activated EM culture and domestic wastewater was 100:2000 mL in a literature (Namsivayam et al., 2011). After 20 days, the COD was decreased from 164 mg/L to 109 mg/L and the DO was low as 1.7 mg/L due to no air supply. According to Thepnarong et al. (2015), the effluent from aquaculture was treated by EM balls; the aeration was controlled at 2.5 L/min and the quantity of EM balls was two. The organic content in term of BOD and NH_4-N was decreased to < 20 mg/L (initial 25 mg/L) and < 0.5 mg/L (initial 1.5 mg/L) respectively, which were acceptable concentrations for effluent standard in Thailand (PCD, 2021). The DO of treated wastewater was in the range of 4-5 mg/L.

With current increasing population growth and socio-economic development, the quality and quantity of water resource are gaining the attention. The improper management of waste and wastewater causes negatively impacts on water resource and human health from pollutants exceeding the standards, unpleasant by-products and odor. The sustainable development incorporating both socio-economic and environmental perspectives is essential task in order to prevent the environmental deterioration. Various advanced technologies such as nanoparticle-activated carbon hybrid (Kamaraj et al., 2020) and innovative hybrid membrane-ultrasound (Naddeo et al., 2020) are in practice for improving water quality as well as pollutants removal, however most of them are costly and generate secondary waste. Therefore, the EM is one of promising ways for improving water quality, due to its ecofriendly nature, less cost and capital requirement. However, several aspects of its operating conditions are unclarified.

In the present study, the performance of EM technology was evaluated to remove ammonium-nitrogen (NH_4-N) and carbon content (as presented in chemical oxygen demand; COD) which are common pollutants in water resource and causing an unpleasant odor. The influence of aeration and amount of EM on both pollutants removal were clarified and discussed.

2. MATERIALS AND METHOD

2.1 Preparation of EM ball and synthetic water

The EM stock was collected from Land Development Office Section 8 (Phitsanulok, Thailand), and 12.5 g of EM stock was mixed with 25 mL of molasses and 1 L of deionized water. The EM solution was kept at room temperature (~25°C) for 2 days. Later, the EM solution was slowly mixed in 2 kg of dry bran, and the mixture was molded into spherical balls each of diameter 3 cm (namely EM balls). The EM balls were kept at room temperature (~25°C) for 7 days before using in the experiments.

2.2 Reactor setup and operation

The opened cylinder plastic reactors with 30-L working volume (28 ∅ cm x 65 cm) were used in this study. The synthetic water was prepared by following chemicals (g/L); 1.13 of $CH_3COONa{\cdot}3H_2O$, 0.11 g of NH_4Cl, 0.24 g of $NaHCO_3$ and 0.01 g of KH_2PO_4. The synthetic water contained approximately 30 mg/L of NH_4-N and 600 mg/L of COD. Various quantity of EM balls and air were supplied via aquatic aerators to the reactors, as summarized in Table 1. No fresh water was fed to the reactors during the experiments. The water was sampled, collected and analyzed every 1-2 days for water quality analysis.

Table 1. Operating conditions for the experiments.

Reactor	Amount of EM (balls)	Air supply (L/min)	Approximately DO concentration at the steady state (mg/L)
Influence of aeration			
1	2	0	0.7
2	2	2	6.0
Influence of amount of EM			
1	1	0	0.7
2	2	0	0.7
3	3	0	0.6

2.3 Analytical methods

Three forms of nitrogen including NH_4-N, NO_2-N and NO_3-N were determined by phenate, colorimetric and ultraviolet spectrophotometric screening methods, respectively in accordance with the standard methods for the examination of water and wastewater (APHA, 2017). The COD was determined by closed reflex method (APHA, 2017). The in-situ DO, pH and turbidity were measured every 1-2 days by DO meter (DKK-TOA DO-31P), pH meter (Proline B210) and turbidity meter (HACH 2100Q).

Equations used for calculations

At the end of experiment (day 17), the performance of EM on water quality improvement was indicated by total nitrogen removal and COD removal, as presented in Equations 1-2.

Total nitrogen removal (%)

$$= (1 - \frac{[NH_4 - N]_{day17} + [NO_2 - N]_{day17} + [NO_3 - N]_{day17}}{[NH_4 - N]_{day0}}) \times 100 \quad (1)$$

$$\text{COD removal (\%)} = (1 - \frac{[COD]_{day17}}{[COD]_{day0}}) \times 100 \quad (2)$$

3. RESULTS AND DISCUSSION

3.1 Influence of aeration and no aeration to water quality

Two fed-batch-mode reactors containing 2 EM balls were operated without air supply (namely no aeration reactor) and with air supply of 2 L/min (namely aeration reactor) to investigate the effect of aeration and DO concentration on the pollutants reduction. The no aeration reactor referred to the condition of natural water restoration which obtaining dissolved oxygen from the air diffusion, whereas the aeration reactor referred the condition of excessive dissolved oxygen from external aerator. In the no aeration reactor, the NH_4-N was continuously decreasing from initial concentration of 30 mg/L to 14 mg/L in day 5 (reduction rate of 2.8 mg/L-day), whereas the slight NO_3-N concentration was observed. Later, the NH_4-N was increasing to the maximal concentration of 26 mg/L and this concentration was again decreasing until the end of experiment (see in Figure 1). Similarly, three changing concentration profiles of COD were observed in the no aeration reactor; 1) the concentration was started to decrease, 2) it was raised in sequencing days, and 3) finally it was constantly decreased (Figure 2).

The biological mechanisms relating to the reduction of NH_4-N and COD were suggested in Equations 3-5 (Alikhani et al., 2017; Tenore et al., 2020). The nitrogen and organic carbon was oxidized by microorganisms existing in EM balls to synthesize the new cells, as described in Equation 3. The initial DO concentration was around 5.8 mg/L, which was in the range of surface water quality from 4 to 6 mg/L (PCD, 2021), and it was sharply decreased to approximately 0.7 mg/L at the steady state. Since the dissolved oxygen was still remained in the no aeration reactor (DO of ~0.7 mg/L), the nitrification was occurred to transform NH_4-N to NO_3-N, as described in Equation 4. The generated NO_3-N was continuous transformed to gaseous N_2 by denitrification, as shown in Equation 5. Although the conventional denitrifying microorganisms were active in anaerobic condition, the literatures reported that the denitrifying microorganisms were also effective in a low DO condition (Song et al., 2020). The EM balls consisted of multi-culture microorganisms, therefore various microbial species including nitrifying, denitrifying, autotroph and heterotroph were co-exited and caused synergistic effects on pollutants removal. However, the other organic contents such as molasses and bran in EM balls were decomposed, causing the increasing NH_4-N and COD concentrations during day 4-9. However, those active microorganisms can remove the decomposed matters via their microbial activity consequently. At the end of experiment, the no aeration reactor achieved 77% of total nitrogen removal and 60% of COD removal.

Oxidation and synthesis:

$$COHNS + O_2 + \text{nutrients} \rightarrow CO_2 + NH_3 + C_5H_7NO_2 + \text{other end products} \quad (3)$$

Nitrification:

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \rightarrow 0.021C_5H_7NO_2 + 0.98NO_3^- + 1.041H_2O + 1.88H_2CO_3 \quad (4)$$

Denitrification:

$$NO_3^- + 1.08CH_3OH + H^+ \rightarrow 0.065C_5H_7NO_2 + 0.47N_2 + 0.76CO_2 + 2.44H_2O \quad (5)$$

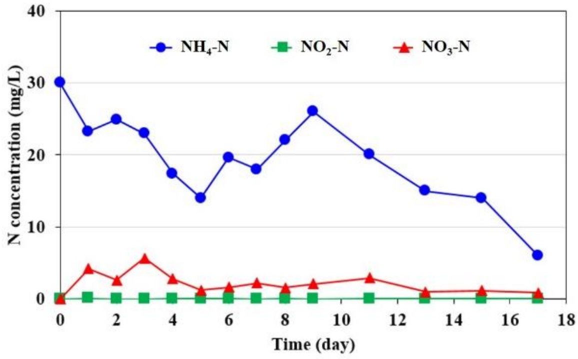

Figure 1 Change of nitrogen forms in the no aeration reactor

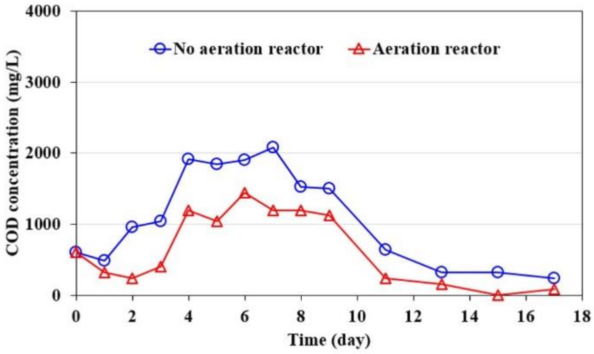

Figure 2 Comparison of COD trends in the no aeration reactor and aeration reactor

In the aeration reactor, the NH_4-N was sharply decreased and reached the minimal concentration of 3 mg/L which was lower than the no aeration reactor (Figure 3). The results exhibited the enhancing nitrification activity under continuous air supply (DO of ~6.0 mg/L) with a high reduction rate of 8.8 mg/L-day. In the meanwhile, the increase in NH_4-N from substrates decomposition of EM balls was also happened. The aeration can cause the improvement of microbial activity to utilize the organic carbon, as indicated by the lower COD trend in the aeration reactor rather than the no aeration reactor (Figure 2). Finally, the aeration reactor with EM balls reached the 83% of total nitrogen removal and 80% of COD removal, which was better performance than the no aeration reactor with EM balls.

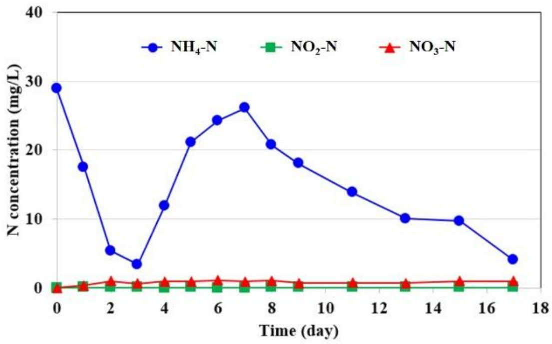

Figure 3 Change of nitrogen forms in the aeration reactor

Turbidity is one of important water quality factors for residential satisfaction. The impact of using EM balls on the water turbidity was examined. The EM balls were immediately separated after adding in the reactors (Figures 4a and 4b). This resulted the suddenly increasing turbidity value at day 1 (see in Figure 5). The continuous air supply had significant impact on the dispersion of microorganisms and microorganisms' growth, thus the high turbidity of 30 NTU was detected in the aeration reactor. The greater number of suspended microorganisms in the aeration reactor led to agglomerate and consequently the microorganisms were settled down at the reactors base (Figure 4c). This phenomenon led to the lower turbidity of aeration reactor (of 9 NTU) rather than the no aeration reactor (of 15 NTU) at the end of experiment. The increase in total heterotrophic bacteria and yeast population in the EM system was reported in a previous study (Namsivayam et al., 2011). The change of EM balls in the reactors was illustrated in Figure 4. Furthermore, the settling microorganisms provided anaerobic condition for microbial reactions (i.e., denitrification). Therefore, either no aeration reactor or aeration reactor was facultative system for pollutants removal. Although the pollutants removal efficiency was improved via continuous air supply, the increase in operating cost and on-site condition should be concerned for EM balls implementation.

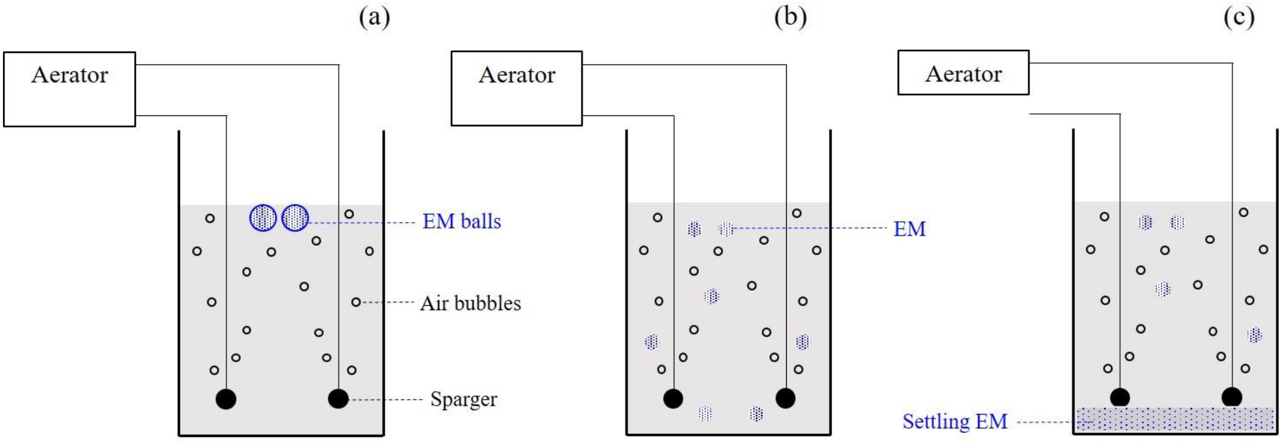

Figure 4 Change of EM balls in the reactors; (a) after EM balls addition, (b) EM balls was dispersed as effective microorganisms, and (c) effective microorganisms were settled down

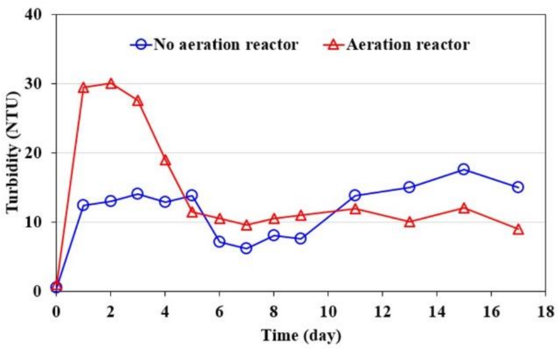

Figure 5 Comparison of turbidity trends in the no aeration reactor and aeration reactor

3.2 Influence of EM ball quantity to water quality

In this experiment, three no aeration reactors were operated under different number of EM balls to clarify the influence of EM balls on the pollutants removal; one EM balls (namely 1EM reactor), two EM balls (namely 2EM reactor) and three EM balls (namely 3EM reactor). From Figure 6, the NH_4-N reduction and its change were similar in the three reactors. In day 1-5, the NH_4-N reduction rate was ranged 2.8-3.0 mg/L-day in the 1EM and 2EM reactors, and it increased to 4.0 mg/L-day in the 3EM reactor. Although the greatest number of EM balls obtained the highest NH_4-N reduction rate in the beginning, the large amount of molasses and bran contents caused the excessive NH_4-N releasing from EM balls decomposition in day 6-10. This significantly affected on the deficient total nitrogen removal of 27% at the end, whereas the total nitrogen removal reached 63% for 1EM reactor and 77% for 2EM reactor.

The decomposition of substrates contents in EM balls causing pollutants releasing was proved by the trends of COD concentrations in Figure 7. The increasing COD reached the maximal concentration of 3,600 mg/L in the 3EM reactor, followed by 2,080 mg/L in the 2EM reactor and 1,280 mg/L in the 1EM reactor. In comparison, the highest COD removal of 60% was observed in the 2EM reactor, whereas only 3% of COD was removed in the 1EM reactor. On the other hand, none of COD was removed in the 3EM reactor after the experiment, and the higher concentration of 900 mg/L than the initial of 600 mg/L was found. From the above results, the number of EM balls was important factor for improving the water quality. The increasing number of EM balls led to the increasing number of active microorganisms for efficient pollutants removal, however the high decomposition of EM balls substrate can cause the poor quality of water resource. Due to the present study, the optimal number of EM balls was two.

The similar pH trends were presented in Figure 8. After adding the EM balls, pH of three reactors was immediately dropped, because the EM balls contained acidity property (pH < 3.5) with sweet-sour taste and smell (Victoria & Mahaeswari, 2016). Later, the pH was continuously increasing and stable in day 14; the pH value was around 7.8, 7.7 and 7.4 in the 1EM, 2EM and 3EM reactors respectively. The lowest pH in the 3EM reactor was possibly because the different dominant microorganisms due to the excessive organic substrates (i.e., molasses and bran), fermentation and acidogenesis process. However, the microbial mechanisms as well as the microbial community should be further clarified. Priya et al. (2015) suggested that the pH level of 7.4 was indication of healthy anaerobic environment and satisfactory methanogenic activity for EM technology. Due to previous studies, the continuously increasing pH was detected along with EM technology operation (Priya et al., 2015), and it was applicable to remove other pollutants such as phosphorus (> 90%) from improved fermentation in anaerobic zone (Rashed & Massoud, 2015).

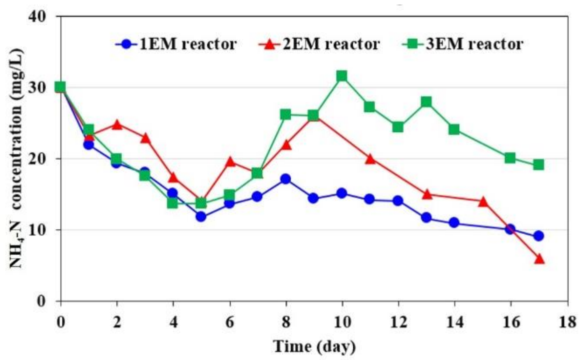

Figure 6 Comparison of NH_4-N trends in the 1EM, 2EM and 3EM reactors

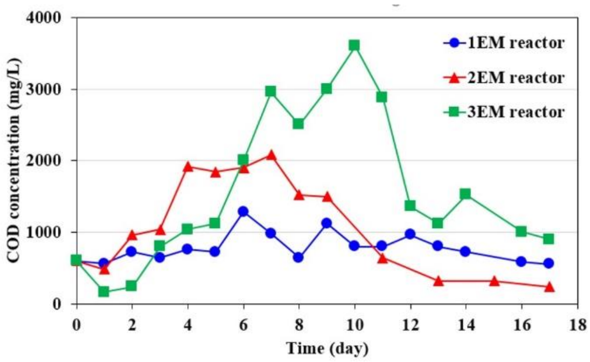

Figure 7 Comparison of COD trends in the 1EM, 2EM and 3EM reactors

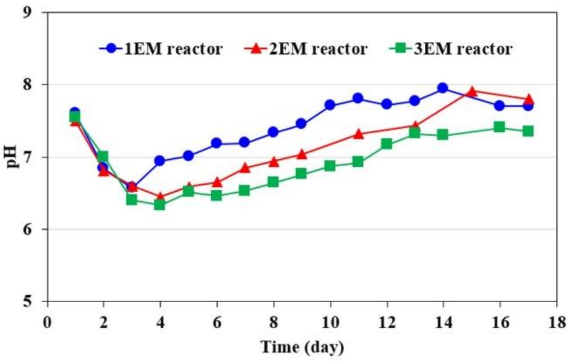

Figure 8 Comparison of pH trends in the 1EM, 2EM and 3EM reactors

4. CONCLUSION

The EM technology was able to remove NH_4-N and carbon content from the water resource. The aeration enhanced the DO concentration, microorganisms activity and their growth, resulting in the increasing NH_4-N reduction rate and pollutants removal (83% for total nitrogen and 80% for COD). In the meanwhile, the rapid growth and absolute dispersion of microorganisms in the aeration reactor caused high turbidity, however the microorganisms were agglomerated and settled at the end of experiment. The largest quantity of three EM balls also enhanced NH_4-N reduction rate in the beginning, however the NH_4-N and COD releasing from substrates decomposition caused that only 27% of NH_4-N was removed and relatively high COD was remained. Due to the present study, two EM balls were optimum and the DO value should be maintained around 6 mg/L for satisfied pollutants removal efficiency (83% for total nitrogen and 80% for COD). However, the pollutants types, volume of contaminated water and affordable treatment cost from aeration should be concerned and optimized for suitable and low-cost technology for water quality restoration.

5. ACKNOWLEDGMENTS

The authors gratefully acknowledge the post-graduate scholarship provided by Faculty of Engineering, Naresuan University. The authors also wish to acknowledge Kairop and Milint Nakaruk for the donation of their living allowance.

6. REFERENCES

Abd El-Mageed, T. A., Rady, M. M., Taha, R. S., Abd El Azeam, S., Simpson, C. R., & Semida, W. M. (2020). Effects of integrated use of residual sulfur-enhanced biochar with effective microorganisms on soil properties, plant growth and short-term productivity of Capsicum annuum under salt stress. *Scientia Horticulturae*, *261*, 108930 https://doi.org/https://doi.org/10.1016/j.scienta.2019.108930

Alikhani, J., Takács, I., Al-Omari, A., Murthy, S., & Massoudieh, A. (2017). Evaluation of the information content of long-term wastewater characteristics data in relation to activated sludge model parameters. *Water Science and Technology*, *75*, 1370-1389 . https://doi.org/10.2166/wst.2017.004

APHA. (2017). Standard Methods for the Examination of Water and Wastewater. 23rd Edn., American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.

Boruszko, D. (2017). Research on the influence of anaerobic stabilization of various dairy sewage sludge on biodegradation of polycyclic aromatic hydrocarbons PAHs with the use of effective microorganisms. *Environmental Research*, *155*, 344-352. https://doi.org/https://doi.org/10.1016/j.envres.2017.02.019

Higa, T., & Kanal, A. (1996). *An Earth Saving Revolution: A Means to Resolve Our World's Problems Through Effective Microorganisms (EM)*. Sunmark Pub https://books.google.co.th/books?id=drOMQQAACAAJ

Kamaraj, M., Srinivasan, N. R., Assefa, G., Adugna, A. T., & Kebede, M. (2020). Facile development of sunlit ZnO nanoparticles-activated carbon hybrid from pernicious weed as an operative nano-adsorbent for removal of methylene blue and chromium from aqueous solution: Extended application in tannery industrial wastewater. *Environmental Technology & Innovation*, *17*, 100540 https://doi.org/https://doi.org/10.1016/j.eti.2019.100540

Lananan, F., Abdul Hamid, S. H., Din, W. N. S., Ali, N. a., Khatoon, H., Jusoh, A., & Endut, A. (2014). Symbiotic bioremediation of aquaculture wastewater in reducing ammonia and phosphorus utilizing Effective Microorganism (EM-1) and microalgae (Chlorella sp.). *International Biodeterioration & Biodegradation*, *95*, 127-134 https://doi.org/https://doi.org/10.1016/j.ibiod.2014.06.013

Naddeo, V., Secondes, M. F. N., Borea, L., Hasan, S. W., Ballesteros, F., & Belgiorno, V. (2020). Removal of contaminants of emerging concern from real wastewater by an innovative hybrid membrane process – UltraSound, Adsorption, and Membrane ultrafiltration (USAMe®). *Ultrasonics Sonochemistry*, *68*, 105237. https://doi.org/https://doi.org/10.1016/j.ultsonch.2020.105237

Namsivayam, S. K. R., Narendrakumar, G., & Kumar, J. (2011). Evaluation of Effective Microorganism (EM) for treatment of domestic sewage. *Journal of Experimental Sciences*, *2*, 30-32.

Pollution Control Department, Ministry of Natural Resources and Environment (2021). https://www.pcd.go.th/.

Priya, M., Meenambal, T., Balasubramanian, N., & Perumal, B. (2015). Comparative Study of Treatment of Sago Wastewater using HUASB Reactor in the Presence and Absence of Effective Microorganisms. *Procedia Earth and Planetary Science*, *11*, 483-490. https://doi.org/https://doi.org/10.1016/j.proeps.2015.06.048

Rashed, E. M., & Massoud, M. (2015). The effect of effective microorganisms (EM) on EBPR in modified contact stabilization system. *HBRC Journal*, *11*(3), 384-392 https://doi.org/https://doi.org/10.1016/j.hbrcj.2014.06.011

Song, J., Zhang, W., Gao, J., Hu, X., Zhang, C., He, Q., Yang, F., Wang, H., Wang, X., & Zhan, X. (2020). A pilot-scale study on the treatment of landfill leachate by a composite biological system under low dissolved oxygen conditions: Performance and microbial community. *Bioresource Technology*, *296*, 122344. https://doi.org/https://doi.org/10.1016/j.biortech.2019.122344

Tenore, A., Vieira, J., Frunzo, L., Luongo, V., & Fabbricino, M. (2020). Calibration and validation of an activated sludge model for membrane bioreactor wastewater treatment plants. *Environmental Technology*, *41*(15), 1923-1936. https://doi.org/10.1080/09593330.2018.1551940

Thepnarong K., Chiayvareesajja S., Kantachote D. (2015). Efficiency of fermented organic matter and effective microorganism (EM) ball for treating effluent from freshwater aquaculture. *Thaksin Journal*, *18*(1), 15-22.

Victoria, J., & Mahaeswari, U. N. (2016). Effective microorganisms – An incredible alternative for wastewater treatment. *International Journal of Development Research*, *6*, 9399-9403.