



## Characterization and morphological study of microbes in treated palm oil mill effluents

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### Abstract

Palm oil mill effluent (POME) is wastewater generated by palm oil milling. Due to its extremely polluting qualities, it must be treated before being discharged into the water course. This study was aimed to evaluate the bacterial growth of raw and treated POME as well as identifying indigenous microorganisms by determining the morphological characteristics of bacteria that were found in the POME. The bacterial growth was identified by bacterial enumeration of colony forming units (CFU). Besides, the morphological identification of bacteria was determined by using gram staining. The results show the best bacterial growth curve is from serial dilution factor of  $10^{-6}$  with a total of  $2.24 \times 10^{-6}$  CFU/mL in raw POME and optimum growth on day seven. While for the treated POME, the total is  $1.97 \times 10^{-6}$  CFU/mL and recorded the optimum growth on day ten of incubation. The growth curve indicates the number of colonies in raw POME is higher than treated POME. It concluded that treated POME still has the bacteria although it has been treated. Apart from that, from the morphological identification by gram staining, the bacteria were *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*. From a gram staining, this research obtained all gram positive in purple colour from the POME samples. Two of them in treated POME were in *Bacillus* shape while the other two from raw and treated POME were in coccus shape, respectively.

Keywords :

Bacteria, bacterial growth, morphological identification, palm oil mill effluent, gram staining

## 1 Introduction

Palm oil production has expanded rapidly in response to growing global demand for fats and oils, becoming a critical in several tropical countries, including Malaysia (Tan and Lim 2019; Wadchaisit et al., 2021). Malaysia produces more than 10 million tonnes of palm oil annually, making it one of the largest producers in the world (Mahlia et al., 2019; Salleh et al., 2020). However, when a large amount of industrial effluent is dumped into water sources, the booming palm oil industry unavoidably creates environmental problems (Adela et al., 2014; A Aziz et al., 2020; Ratnasari et al., 2022). Palm oil manufacturing consumes approximately 5-7.5 tonnes of water, with half of that water ending up as palm oil mill effluents (POME) (Kahar et al., 2022). POME also has high concentrations of suspended particles and total dissolved solids in the range of 18,000 mg/L and 40,500 mg/L, respectively (Ding et al., 2020; Jamali et al., 2021). These solids are commonly referred to palm oil mill sludges (POMS) (Cui et al., 2021; Rajani et al., 2019). In addition, there are solid waste generated during palm oil extraction that consists of the leaves, trunk, decanter cake, empty fruit bunches, seed shells, and mesocarp fibre (Bala et al., 2018; Choong et al., 2018; Mustamu et al., 2021).

An effluent from palm oil mills consists of brown sludge made up of organic solids (4-5%), residual oil (0.5-1.0%), and water (95%) that is produced during the numerous processing stages of crude oil production (Akhbari et al., 2021; Sodri and Septriana 2022; Ratnasari et al., 2021). Due to its high biological oxygen demand (BOD) and chemical oxygen demand (COD), POME is known to contribute to environmental problems such as eutrophication and water pollution (Blair et al., 2021; Liew et al., 2021).

Anaerobic digestion, aerobic processes, membrane separation, water evaporation, and solid removal by coagulant-flocculant are only a few of the methods currently being investigated to treat POME. In the absence of oxygen, anaerobic digestion is the breakdown of organic matter by bacteria (Rupani et al., 2022; Uning et al., 2020). Although this occurs naturally within a landfill, the word typically refers to a process that is purposefully accelerated in closed vessels to produce a somewhat stable solid residue (Dominic and Baidurah 2022). During anaerobic digestion, mostly methane and carbon dioxide are produced as biogas, which can be used as a chemical feedstock or as a fuel (Rhezqy Furwati 2020). Historically, the majority of palm oil mills have treated POME using an open ponding system due to its simplicity, ease of maintenance, and economic effectiveness. POME effluent is generated in large quantities during the crude palm oil extraction process, as previously mentioned (Hasanudin et al., 2021). The discharge of this massive amount of wastewater, without treatment, into freshwater, estuarine, and marine environments might alter aquatic habitats, injure aquatic animals and endanger human health. POME has a number of well-documented physicochemical characteristics (Akhbari et al., 2021; Sodri and Septriana 2022; Ratnasari et al., 2021). For its part, the microbiological component

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appears to have been overlooked; as a result, there appears to be a dearth of understanding about the microbiota, indicating that a more in-depth examination of this is required (Bala et al., 2018; Rupani et al., 2022). The difficulty is that because of the high concentrations of organic and inorganic substances in POME, it has significant COD and BOD levels, as well as a wide variety of bacteria. In light of these unique characteristics of complexity, it is critical to isolate and identify the indigenous species of bacteria that are present in POME in order to identify the key functional bacterias that were responsible for the reaction in POME. An abundant and diversified microbial community has been demonstrated to be beneficial to POME (Gray et al., 2019). The common genera that were usually involve in the anaerobic digestion for POME remediation were *Methanosarcina*, and *Methanosaeta* (Ma et al., 2021; Vítězová et al., 2020).

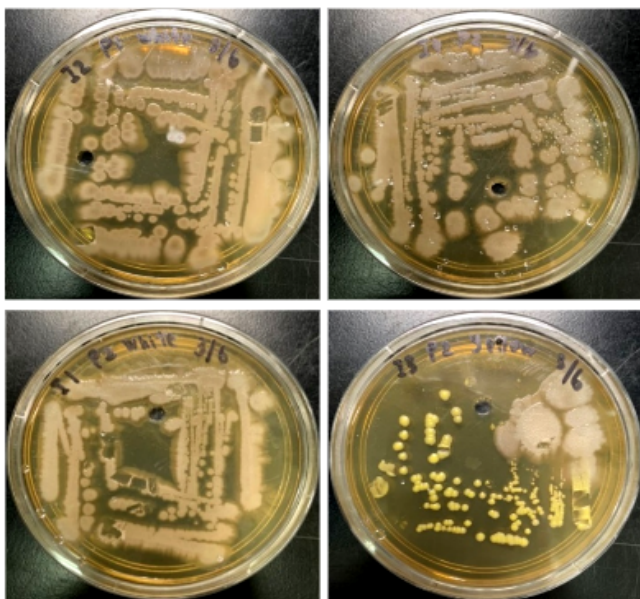
This study aims to identify the bacterial biodiversity through morphological identification and molecular technique. The biodiversity of bacteria detected in treated POME were determined using a molecular method. Microbial diversity is the diversity of unicellular organisms such as fungi, bacteria, protists, and archaea. Numerous microorganisms survive throughout the biosphere, defining the boundaries of life and fostering the survival and evolution of other living things. Significant contribution of the study is the evaluation of the bacterial growth profile in raw and treated POME which has been lacking in literature.

## 2 Materials and method

### 2.1 Collection of samples

Raw POME was collected aseptically from the MALPOM Industries Bhd, in Nibong Tebal, Penang. The POME sample was kept in the refrigerator at the Faculty of Civil Engineering postgraduate laboratory, Universiti Malaysia Perlis. Anaerobic digestion treatment was used to treat raw POME to produce the treated POME as the samples in this study. The POME sample was kept in the refrigerator at the Faculty of Civil Engineering postgraduate laboratory, Universiti Malaysia Perlis and preserved at 4°C until further experiment in order to stop the wastewater from undergoing biodegradation due to microbial action.

### 2.2 Colony forming units assessment



**Figure 1** Isolated bacterial pure culture

The spread plate method was conducted to determine the population density of the bacteria by estimating the present cell num-

ber based on their ability to give rise to colonies under specific conditions. The spread plate method was chosen as this method is easier for quantifying bacteria in solution form. A series of dilutions up to  $10^{-7}$  were made to reduce the cells in the samples. The spread plate method was used wherein 0.1  $\mu\text{L}$  each of diluted samples from all serial dilution ( $10^{-5}$ ,  $10^{-6}$ , and  $10^{-7}$ ) were spread across the surface of a nutrient agar (NA) plate and allowed to dry before incubation for counting. Samples were incubated in an incubator with a temperature 37°C and bacterial counts were recorded 24 hours after plating as shown in Fig. 1.

### 2.3 Isolation of bacteria

The nutrient agar was used to isolate the bacterial strains from POME samples. An inoculum of the sample was spread onto the agar surface and then incubated at room temperature and allowed to grow for 48 hours. Single-developed colony was picked up from the nutrient agar plates and subcultured to obtain the pure culture using the streak plate method as in Fig. 1. All work was done with aseptic technique to prevent the contamination.

### 2.4 Bacterial staining

On a glass slide, a smear of bacteria was deposited and thoroughly air-dried. It was stained for 1 minute in crystal violet solution, 1 minute in iodine mordant, washed with distilled water prior to decolorization with 95% ethyl alcohol, and then counterstained for 45 seconds with safranin. The stain was washed gently with tap water and dried with filter paper. The procedure was repeated for additional bacterial smears. All of the stained slides were covered with glass slides, and a microscope was used to look at them under oil at 100x magnifications. It was decided whether the bacteria were gram positive or gram negative by looking at the colour that formed after they were stained.

## 3 Results and discussion

### 3.1 Bacterial numeration

Fig. 2 shows the bacterial growth curve of each dilution factor for raw POME and treated POME samples. After 24 hours of incubation, the both samples still remain in the lag phase where the turning on metabolic and replication or little cell division takes place. During this phase cells change very little because the cells do not immediately reproduce in a new medium. After that, it is increased to the log phase or exponential phase on day 3 where the bacteria in rapid exponential growth and optimal growth take place. Stationary phase results from a situation in which growth rate and death rate are equal. The phase is starting to slowly decrease as on day 10 with the lower number of colonies growing on the nutrient agar. Bacterial growth can be measured by simple observation of how many colonies are present. The results show the best bacterial growth curve is from serial dilution factor of  $10^{-6}$  with a total of  $2.24 \times 10^6$  CFU/mL in raw POME and optimum growth on day seven. While for the treated POME, the total is  $1.97 \times 10^6$  CFU/mL and recorded the optimum growth on day ten of incubation. It can be concluded there are a lot of factors that affect the microbial growth that lead to the pattern of bacterial growth curve. The raw POME stated the highest of CFU similar to the previous study (Bala et al., 2018).

In these results, the bacteria growth is in slow condition which is indicates by the gentle slope in the growth curve as it lacks nutrients. The nutrients are one of the factors that affect the growth of bacteria. Another study reveals that the concentration of nutrients in POME play a significant role during microbial growth. It is possible that the high nutrient content of POME will stimulate not only their growth but also the synthesis of other cellular components from waste (Gray et al., 2019; Dominic and Baidurah 2022).

Changes in pH occur quickly in a closed environment, such as with nutritional broth in a tube. In this research, the pH value in raw and treated POME is 6.8 from anaerobic digestion treatment. It can say that the bacteria grow best at a pH of roughly 7.0, however they can grow in a pH range of 5.0-8.0 such as *Streptacidiphilus pinicola* (Ratnasari et al., 2021). Bacteria are cultured at various pH levels to observe the influence of pH (Furwati and Jufri 2020). Bacteria are inactive at low temperatures (<4 °C), but cannot survive at very high temperature as (>121 °C) (Cheng et al., 2021). The temperature involved in raw effluent is around 80 to 90 °C. This leads one to the conclusion that the wastewater from palm oil mills contains a significant variety of microorganisms due to the high levels of chemical oxygen demand (COD) and biological oxygen demand (BOD) (Patel et al., 2021).

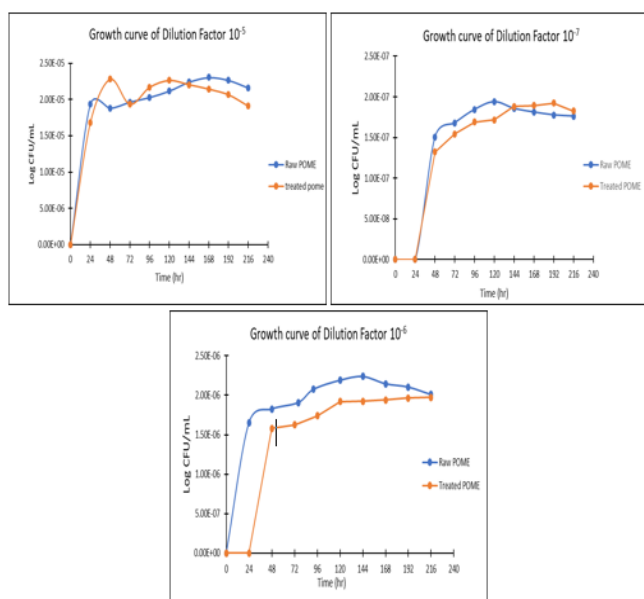


Figure 2 Bacterial growth curve of each dilution factor

### 3.2 Morphological identification of isolate bacteria

Table 1 describes the morphological characteristics of the bacterial isolates with the presumed organisms. Gram staining and hanging drop methods were applied to identify morphology of isolated bacteria. There are four bacteria that were used in morphological identification by observation through macroscopic and microscopic characteristics.

Table 1 Morphological characteristics of the bacteria isolates

Isolates Name	Morphological characteristics	Gram reaction	Shape	Expected bacteria
Isolate-1	White, circular	Gram-positive	Bacillus	<i>Bacillus cereus</i>
Isolate-2	White, circular	Gram-positive	Bacillus	<i>Bacillus subtilis</i>
Isolate-3	White, circular	Gram-positive	Coccus	<i>Staphylococcus aureus</i>
Isolate-4	Yellow, circular	Gram-positive	Coccus	<i>Micrococcus luteus</i>

The macroscopic morphology of *Bacillus cereus* is large in size and opaque. Besides that, the colour is grey to yellow to white and it has flat colonies with a diameter around 5-10 mm. The size of *Bacillus cereus* in diameter reached 3.9 cm after 3 days of incubation with an optimum temperature. The *Bacillus cereus* in microscopic view as in Fig. 3 (A). Moreover, when *Bacillus subtilis* isolate is grown on regular nutrient agar, it forms a round colony that

is rough, opaque, fuzzy, white or slightly yellow. Like other bacteria in the genus *Bacillus*, it can make an endospore to protect itself from high temperatures and dry conditions. The colony growth reached 2.6 cm in diameter after 3 days of incubation. When cells divide into spores, they make brown and white pigments Fig. 3 (B). Next, the bacterial morphology of *Staphylococcus aureus* on nutrient agar circular shape, convex, glistening with an entire edge large, shiny surface and pigmented in golden yellow as in Fig. 3 (C). The colony grew rapidly and fast and the size reached 2.16 cm after 3 days of incubation with the optimum temperature. The last bacterium is a colony of *Micrococcus luteus* that was growing fast after 3 days of incubation. The colonies appear in circular shape, smooth, entire, convex and usually pigmented in shades of yellow or red. Some strains may produce matted colonies. *Micrococcus luteus*'s cell wall teichuronic acid (TUA) is a long-chain polysaccharide made of disaccharide repeating units that is covalently attached to the peptidoglycan on the inner cell wall and extended to the outside surface of the cell envelope as shown in Fig. 3 (D) through the microscopic view under oil immersion at 100x magnifications. The morphological characteristics of the isolated bacteria are similar with description reported in the previous studies (Adela et al., 2014; Bala et al., 2018).

The researchers found that *Bacillus cereus* anaerobic digestion of POME produced the highest COD and BOD removal efficiency at 79.35%, 72.65% and 0.2 L of working volume at 35 °C, and HRTs of six days. It can be clearly said that the *Bacillus cereus* helps in treatment of wastewater (Karim et al., 2019; Mustamu et al., 2021). Besides that, *Staphylococcus aureus* is one of the bacteria that was isolated from lipolytic, proteolytic and cellulolytic bacteria from palm oil mill effluent found by another researcher. In that research, there are two distinct groups of consortium bacteria, each of which is capable of lipolysis or proteolysis (Hasanudin and Faizal 2021). On the other hand, *Micrococcus luteus* can be found on human skin, as well as in the flora of soil, dust, and water. On nutrient agar, these bacteria typically form colonies that are bright yellow in colour. This bacterium is able to withstand extremely high doses of ultraviolet radiation and also possesses the ability to break down pollutants such as gasoline (Adela et al., 2014; Bala et al., 2018).

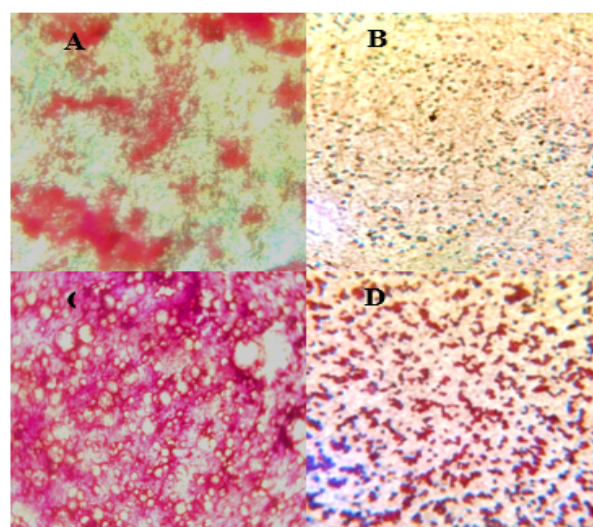


Figure 3 The microscopic view of bacteria after being stained: (A) Gram-positive (*Bacillus cereus*), (B) Gram-positive (*Bacillus subtilis*), (C) Gram-positive (*Staphylococcus aureus*), (D) Gram-positive (*Micrococcus luteus*) as examined under oil immersion at 100 x magnifications

## 4 Conclusion

The aim of the present study was to identify the bacterial biodiversity through morphological identification and molecular tech-

nique. *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus Luteus* were among expected bacteria found in raw and treated POME. The best bacteria growth curve can be applied in serial dilution of  $10^{-6}$  by using a spread plate method to count CFU. It can be concluded there are a lot of factors that affect the microbial growth that lead to the pattern of bacterial growth curve. Findings of the current investigation demonstrated the presence of a wide variety of microorganisms that have made their homes in the wastewater from palm oil mills. The use of molecular techniques such as DNA extraction, PCR and 16s rRNA sequencing can further help in enhancing the genus, species and name of bacteria that are found in these raw and treated POME.

## Declaration of competing interest

The authors declare no known competing interests that could have influenced the work reported in this paper.

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## References

- A Aziz, M.M., Kassim, K.A., ElSergany, M., Anuar, S., Jorat, M.E., Yaacob, H., Ahsan, A., Imteaz, M.A., and Arifuzzaman. 2020. Recent advances on palm oil mill effluent (POME) pretreatment and anaerobic reactor for sustainable biogas production. *Renew. Sustain. Energy Rev.* 119, 109603
- Adela, B.N., Muzzammil, N., Loh, S., Choo, Y.J.A.J.o.M., Biotechnology, and Paper, E.S., 2014. Characteristics of palm oil mill effluent (POME) in an anaerobic biogas digester. *Asian J. Microbiol. Biotechnol. Environ. Sci.* 16, 225-231
- Akhbari, A., Onn, C.C., and Ibrahim, S., 2021. Analysis of biohydrogen production from palm oil mill effluent using a pilot-scale up-flow anaerobic sludge blanket fixed-film reactor in life cycle perspective. *Int. J. Hydrog. Energy.* 46, 34059-34072
- Bala, J.D., Lalung, J., Al-Gheeti, A.A.S., Hossain, K., and Ismail, N. J.T.L.s.r., 2018. Microbiota of palm oil mill wastewater in Malaysia. *Trop. Life Sci. Res.* 29, 131
- Blair, E.M., Dickson, K.L., and O'Malley, M.A., 2021. Microbial communities and their enzymes facilitate degradation of recalcitrant polymers in anaerobic digestion. *Curr. Opin. Microbiol.* 64, 100-108
- Cheng, Y.W., Chong, C.C., Lam, M.K., Leong, W.H., Chuah, L.F., Yusup, S., Setiabudi, H.D., Tang, Y., and Lim, J.W., 2021. Identification of microbial inhibitions and mitigation strategies towards cleaner bioconversions of palm oil mill effluent (POME): A review. *J. Clean. Prod.* 280, 124346
- Choong, Y.Y., Chou, K.W., and Norli, I., 2018. Strategies for improving biogas production of palm oil mill effluent (POME) anaerobic digestion: A critical review. *Renew. Sustain. Energy Rev.* 82, 2993-3006
- Cui, K., Xu, Q., Sheng, X., Meng, Q., Shang, G., Ma, Y., Zhang, Z., and Guo, K., 2021. The impact of bioaugmentation on the performance and microbial community dynamics of an industrial-scale activated sludge sequencing batch reactor under various loading shocks of heavy oil refinery wastewater. *Water.* 13, 2822
- Ding, G.T., Mohd Yasin, N.H., Takriff, M.S., Kamarudin, K.F., Saliho, J., Yaakob, Z., and Mohd Hakimi, N.I.N., 2020. Phycoremediation of palm oil mill effluent (POME) and CO<sub>2</sub> fixation by locally isolated microalgae: *Chlorella sorokiniana* UKM2, *Coelastrrella sp.* UKM4 and *Chlorella pyrenoidosa* UKM7. *J. Water Process. Eng.* 35, 101202
- Dominic, D., and Baidurah, S., 2022. Recent developments in biological processing technology for palm oil mill effluent treatment—A review. *Biology.* 11, 525
- Furwati, R., and Jufri. 2020. The effect of environmental factors on microbial growth. *Journal La Lifesci.* 1
- Gray, D.A., Dugar, G., Gamba, P., Strahl, H., Jonker, M.J., and Hamoen, L.W., 2019. Extreme slow growth as alternative strategy to survive deep starvation in bacteria. *Nat. Commun.* 10, 890
- Hasanudin, H., and Faizal, M., 2021. Isolates of lipolytic, proteolytic and cellulolytic bacteria from palm oil mill effluent and their potency as consortium. *Int. J. Adv. Sci. Eng. Inf. Technol.* 6, 15-30
- Jamali, N.S., Md Jahim, J., Mumtaz, T., and Abdul, P.M., 2021. Dark fermentation of palm oil mill effluent by *Caldicellulosiruptor saccharolyticus* immobilized on activated carbon for thermophilic biohydrogen production. *Environ. Technol. Innov.* 22, 101477
- Kahar, P., Rachmadona, N., Pangestu, R., Palar, R., Triyono Nugroho Adi, D., Betha Juanssilfero, A., Yopi, Manurung, I., Hama, S., and Ogino, C., 2022. An integrated biorefinery strategy for the utilization of palm-oil wastes. *Bioresour. Technol.* 344, 126266
- Karim, A., Islam, M.A., Yousuf, A., Khan, M.M.R., and Faizal, C.K.M., 2019. Microbial lipid accumulation through bioremediation of palm oil mill wastewater by *Bacillus cereus*. *ACS Sustain. Chem. Eng.* 7, 14500-08
- Liew, Z.K., Chan, Y.J., Ho, Z.T., Yip, Y.H., Teng, M.C., Ameer Abbas bin, A.I.T., Chong, S., Show, P.L., and Chew, C.L., 2021. Biogas production enhancement by co-digestion of empty fruit bunch (EFB) with palm oil mill effluent (POME): Performance and kinetic evaluation. *Renew. Energy.* 179, 766-77
- Ma, G., Chen, Y., and Ndegwa, P., 2021. Association between methane yield and microbiota abundance in the anaerobic digestion process: A meta-regression. *Renew. Sustain. Energy Rev.* 135, 110212
- Mahlia, T.M.I., Ismail, N., Hossain, N., Silitonga, A.S., and Shamsuddin, A.H., 2019. Palm oil and its wastes as bioenergy sources: a comprehensive review. *Environ. Sci. Pollut. Res.* 26, 14849-66
- Mustamu, N., Nasution, Z., and Sembiring, M. 2021. "Potential and phylogenetic of superior bacterial isolates in biogas sludge from anaerobic digestion of palm oil mill effluent." *IOP Conf. Ser.: Earth Environ. Sci.*, 012065
- Patel, G.B., Shah, K.R., Shindhal, T., Rakholiya, P., and Varjani, S., 2021. Process parameter studies by central composite design of response surface methodology for lipase activity of newly obtained Actinomycete. *Environ. Technol. Innov.* 23, 101724
- Rajani, A., Kusnadi, Santosa, A., Saepudin, A., Gobikrishnan, S., and Andriani, D., 2019. Review on biogas from palm oil mill effluent (POME): Challenges and opportunities in Indonesia. *IOP Conf. Ser.: Earth Environ. Sci.* 293, 012004
- Ratnasari, A., Syafiuddin, A., Boopathy, R., Malik, S., Aamer Mehmood, M., Amalia, R., Dwi Prastyo, D., and Syamimi Zaidi, N., 2022. Advances in pretreatment technology for handling the palm oil mill effluent: Challenges and prospects. *Bioresour. Technol.* 344, 126239
- Ratnasari, A., Zaidi, N.S., Syafiuddin, A., Boopathy, R., Kueh, A.B.H., Amalia, R., and Prasetyo, D.D., 2021. Prospective biodegradation of organic and nitrogenous pollutants from palm oil mill effluent by acidophilic bacteria and archaea. *Bioresour. Technol. Rep.* 15,
- Rupani, P.F., Embrandiri, A., Rezania, S., Shao, W., Domínguez, J.,

- and Appels, L., 2022. Changes in the microbiota during biological treatment of palm oil mill waste: A critical review. *J. Environ. Manage.* 320, 115772
- Salleh, S.F., Mohd Roslan, M.E., Abd Rahman, A., Shamsuddin, A.H., Tuan Abdullah, T.A.R., and Sovacool, B.K., 2020. Transitioning to a sustainable development framework for bioenergy in Malaysia: policy suggestions to catalyse the utilisation of palm oil mill residues. *Energy Sustain. Soc.* 10, 38
- Sodri, A., and Septriana, F.E., 2022. Biogas Power Generation from Palm oil mill effluent (POME): Techno-economic and environmental impact evaluation. *Energies.* 15, 7265
- Tan, Y.D., and Lim, J.S., 2019. Feasibility of palm oil mill effluent elimination towards sustainable Malaysian palm oil industry. *Renew. Sustain. Energy Rev.* 111, 507-522
- Uning, R., Latif, M.T., Othman, M., Juneng, L., Mohd Hanif, N., Nadzir, M.S.M., Abdul Maulud, K.N., Jaafar, W.S.W.M., Said, N.F.S., Ahamad, F., and Takriff, M.S., 2020. A review of South-east Asian oil palm and its CO<sub>2</sub> fluxes. *Sustainability.* 12, 5077
- Vítězová, M., Kohoutová, A., Vítěz, T., Hanišáková, N., and Kushkevych, I., 2020. Methanogenic microorganisms in industrial wastewater anaerobic treatment. *processes.* 8, 1546
- Wadchaisit, P., Suksong, W., O-Thong, S., and Nuithitikul, K., 2021. Development of a novel reactor for simultaneous production of biogas from oil-palm empty fruit bunches (EFB) and palm oil mill effluents (POME). *J. Environ. Chem. Eng.* 9, 105209