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Alcaligenaceae and *Chromatiaceae* as reliable bioindicators present in palm oil mill effluent final discharge treated by different biotreatment processes

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ABSTRACT

This study was undertaken to confirm the reliability of the proposed potential bioindicators, *Alcaligenaceae* and *Chromatiaceae* to specifically indicate contamination in river water by palm oil mill effluent (POME) final discharge. The use of bioindicators could assist in determining the specific cause of contamination in water bodies. *Alcaligenaceae* and *Chromatiaceae* were shown by a previous study to be present in river water contaminated with the final discharge from the treatment of POME. In the present study, the compositions of the bacterial communities in the POME final discharge obtained from four different palm oil mills which used different biotreatments of POME were elucidated using high-throughput MiSeq. The four POME final discharges studied showed different species richness and evenness among them. However, the bacterial community compositions in the different final discharges exhibited almost similar patterns in that the phylum *Proteobacteria* was dominant in all the samples. The proposed bioindicators, the *Alcaligenaceae* and *Chromatiaceae* families, were found to be present in all the four final discharges despite the different characteristics of the mills and the different biotreatment processes used by them. These bioindicators were also strongly and positively correlated with the biochemical oxygen demand (BOD_5) concentration. This makes them reliable bacterial indicators to detect the presence of POME final discharge in river water.

1. Introduction

The generation of palm oil mill effluent (POME) from the palm oil industry has become a serious issue that not only affects the industry, but also people and the environment. POME is known as a high strength agro-industrial wastewater that contains a large amount of organic matter which eventually increases both the biochemical oxygen demand (BOD) and the chemical oxygen demand (COD) (Bala et al., 2015). The anaerobic digestion applied for the treatment of POME could increase the rate of biodegradation (Poh and Chong, 2009), in addition to the conventional POME treatment using the ponding system. However, if not managed efficiently, the discharge of treated or partially treated POME into a nearby river could lead to severe environmental pollution (Rupani et al., 2010).

The current determination of physicochemical properties in assessing the level of pollution is considered inaccurate as the affected river water may have other anthropogenic sources such as from residential areas and agricultural practices. Therefore, it is crucial to have a reliable indicator for assessing the specific cause of pollution in the effluent receiving river water. A study conducted by Shade et al. (2012) showed that the compositions of microorganisms and their functions were most sensitive to disturbances. Therefore, the use of bacteria as an indicator of the presence of pollutants is now widely regarded as a potential approach in determining the actual cause of contamination in water bodies. The use of a microbial source tracking method in identifying fecal contamination was suggested as a complement to the traditionally used bacterial indicators and environmental variables (Zhang et al., 2014). Furthermore, an assessment of the bacteriological water quality and the monitoring of microbial contamination could also serve as biomonitoring standards and hence would be beneficial in

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Table 1
Capacity of fresh fruit bunch (FFB), anaerobic retention time and total retention time of palm oil mill effluent (POME) for the treatments A, B, C, and D (presented in Fig. 1) and the compositions of nutrients in the different POME final discharges.

	A	B	C	D
Characteristics				
Capacity (tonne of FFB/hour)	40	30	54	54
Anaerobic retention time (days)	40	45	40	45
Total retention time of POME (days)	61	100	142	82
Composition of nutrients (%)				
Nitrogen	1.89 ± 0.02	0.84 ± 0.03	1.05 ± 0.02	0.27 ± 0.04
Nitrate	2.04 ± 0.05	1.01 ± 0.02	1.16 ± 0.01	0.27 ± 0.02
Ammonium	1.26 ± 0.02	0.42 ± 0.03	0.63 ± 0.04	0.25 ± 0.01
Phosphorus	0.15 ± 0.03	0.15 ± 0.02	0.16 ± 0.03	0.05 ± 0.02
Potassium	0.12 ± 0.04	0.14 ± 0.02	0.04 ± 0.01	0.22 ± 0.02

Table 2
Number of raw reads, high-quality reads, operational taxonomic units (OTU) and alpha-diversity indices measured as Shannon-Weaver index (H′) and Evenness (E′) in the palm oil mill effluent (POME) final discharge for the treatments A, B, C, and D (presented in Fig. 1).

	A	B	C	D
Number of raw reads[a]	132,259	66,563	64,275	87,752
Number of high-quality reads[b]	118,100	55,093	56,467	69,806
Number of OTU[c]	98,613	37,787	56,364	68,509
Shannon-Weaver index (H')[d]	2.252	2.097	1.331	1.788
Evenness (E')[e]	0.144	0.145	0.059	0.096

[a] Image data output from the sequencing machine was transformed by base calling into sequence data and stored in fastq format.
[b] Poor quality sequences were discarded (*i.e.*, sequences of < 200 bp with an average quality score of < 25 and ambiguous characters).
[c] Clustered according to the similarity to one another at 97% threshold.
[d] Calculated as $H = \Sigma(pi * \ln pi)$, where *pi* is the abundance of each species.
[e] Calculated as $E = H/\ln s$, where S is the total number of species.

environmental planning and management (Baghel et al., 2005).

Our previous case study was carried out to determine the potential bioindicators that could be used to indicate the contamination of river water by POME final discharge. *Alcaligenaceae* and *Chromatiaceae* which were present in the downstream part of the river that receive the POME final discharge directly from the palm oil mill were reported to have originated from the POME treatment. Interestingly, these bioindicators were not detected in the upstream part of the river that was unpolluted due to POME final discharge (Sharuddin et al., 2017). Even though the goal of POME treatment is the same; to reduce the polluting power of

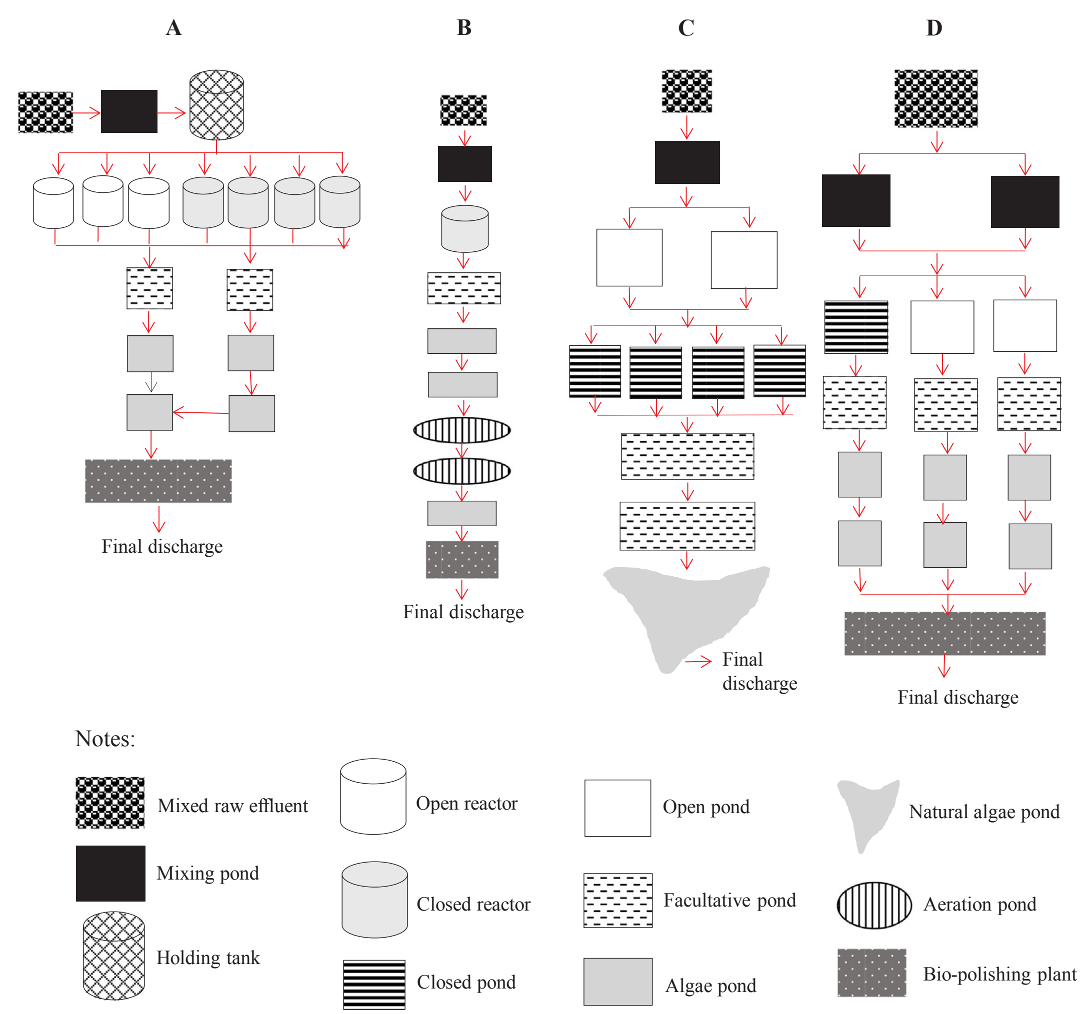

Fig. 1. Process flow schematics of the different stages of full-scale treatment of palm oil mill effluent (POME) in four typical palm oil mills, named as A, B, C and D. Different anaerobic digestion processes were applied either using open and closed reactors (A), closed reactor (B), closed ponds (C) or open and closed ponds (D). The facultative anaerobic (facultative pond) and the aerobic (algae pond) processes were also different with B and C adopting one series of ponding system, while A and D adopted two and three series of ponding system, respectively. All POME treatment systems except C adopted biopolishing as their tertiary treatment.