Malaysian Journal of Microbiology, Vol 18(4) 2022, pp. 408-423
DOI: http://dx.doi.org/10.21161/mjm.221508

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Bio-valorization of palm oil mill effluent waste for the potential production of renewable biomass fuel pellets

Nurul Alia Syufina Abu Bakar and Siti Baidurah*

School of Industrial Technology, Universiti Sains Malaysia, 11800 Gelugor, Penang, Malaysia.
Email: sitibaidurah@usm.my

Received 12 April 2022; Received in revised form 1 June 2022; Accepted 16 June 2022

ABSTRACT

Aims: The primary aim of this study was to utilize abundant palm oil mill effluent (POME) waste and turn it into a value-added product of biomass fuel with high calorific energy value (CEV) via fermentation and drying process, then simultaneously reduce abundant liquid waste.
Methodology and results: POME is available abundantly in Malaysia and only a small portion of it is utilized to produce other value-added products. In this study, fermentation of POME in the presence of bacteria (*Lysinibacillus* sp.) and fungus (*Aspergillus flavus*) separately at 37 °C, 180 rpm for 5 days, followed by overnight oven-drying at 85 °C was conducted. Four fermentation medium conditions were performed, *viz*.: (1) autoclaved POME, (2) autoclaved POME with the addition of *Lysinibacillus* sp., (3) autoclaved POME with the addition of *A. flavus* and (4) POME as it is (non-sterile).
Conclusion, significance and impact of study: Among all conditions, fermentation utilizing autoclaved POME in the presence of *A. flavus* evinced the highest CEV of 25.18 MJ/kg. The fermentation in the presence of *Lysinibacillus* sp. strain revealed high COD and BOD removal efficiency of 59.20% and 320.44 mg/L as well as the highest reduction of oils and grease among other groups with the value of 15.84%. Future research directions are proposed for the elucidation of co-fermentation in the presence of both *Lysinibacillus* sp. and *A. flavus*.

Keywords: Aspergillus flavus, biomass fuel, *Lysinibacillus* sp., palm oil, renewable energy

INTRODUCTION

In lieu of Malaysia being one of the largest producers of palm oil, this industry generates tonnes of waste every year, which in turn creates significant waste disposal issues (Abdullah and Sulaiman, 2013). As the oil palm industry expands, the waste generated increases and causes serious environmental pollution to its surroundings. The wastes from the oil palm industry can be categorized into two: (1) solid wastes and (2) liquid wastes. Solid wastes of palm oil include empty fruit bunch (EFB), mesocarp fruit fibers (MF), oil palm frond (OPF) and palm kernel shells (PKS), while palm oil mill effluent (POME) is classified as liquid waste (Abdullah and Sulaiman, 2013). Large portion of liquid wastes are generated in the decanter during the wet process of palm oil extraction. The POME is brownish in colour, high viscosity sludge in which composed of water, oil and fine cellulosic materials generated from sterilization of fresh fruit bunch (FFB), clarification of crude palm oil (CPO) and hydro-cyclone separation of the kernel (Abdullah and Sulaiman, 2013; Hassan *et al.*, 2013).

Although POME is considered a non-toxic waste, upon discharge without extensive treatment, POME can cause environmental pollution due to its high biochemical oxygen demand (BOD), chemical oxygen demand (COD), as well as high oil and grease content (Kamyab *et al.*, 2018). POME is released at a larger output than the desired product, which is crude palm oil (CPO), with a ratio of 3.12 to 1 (POME:CPO) in volume (Cheng *et al.*, 2019). Consequently, voluminous POME waste is generated, and a large portion is left underutilized. Treatment of POME is necessary prior to discharge to the environment. In detail, the BOD should be in compliance with the regulatory limit of 100 mg/L imposed by the Malaysian Department of Environment (DOE, 1999; Chan and Chong, 2019).

In 2014, POME waste generated, amounting to *circa* 64 million tonnes during the whole milling process (Loh, 2017) and is expected to increase yearly in Malaysia (Kamyab *et al.*, 2018). In 2021, it was recorded that POME makes up to *circa* 60% of processed FFB in which 53 million cubic meters are generated annually. It should be noted that the current open pond system adopted in most mills are inadequate to efficiently treat this amount of POME (Dashti *et al.*, 2021; Shamsuddin *et al.*, 2021). Throughout the years, various efforts in POME treatment processes have been explored. Generally, in Malaysia,

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 408-423
DOI: http://dx.doi.org/10.21161/mjm.221508

obsolete open ponding systems are employed to treat POME, whereby it is treated continuously and deploys both anaerobic and aerobic conditions. This approach is widely applied due to its economical operating costs, low upfront capital and minimum technical requirements (Ng, 2021). Obsolete open ponding systems are composed of the preliminary physical screener, cooling pond, acidification pond, anaerobic ponds, facultative ponds, aerobic ponds and settling ponds (Liew *et al.*, 2015; Mohammad *et al.*, 2021b). The drawbacks of this system are the hydraulic retention time (HRT) of organic pollutants was long (6-7 months) and anaerobic ponds require periodic cleaning of biomass produced as well as production of greenhouse gases such as methane (Ng, 2021).

Due to limitations of the conventional obsolete open ponding systems treatment of POME, enhanced attention has been paid in alternative approaches to overcome these problems. The most straightforward approach is to use POME as compost for plant fertilizer (Dominic and Baidurah, 2022). This method can reduce the weight or volume of the sludge generated. This method is deemed time-consuming due to the composting process requiring *circa* 40 days to complete. Furthermore, there are also approaches to entrapping the greenhouse gases produced by the pyrolysis, combustion and gasification, for example, methane and hydrogen gases (Tabassum *et al.*, 2015; Onoja *et al.*, 2019; Ng, 2021). These entrapped gases can be utilized as heat and electricity generators (Loh, 2017). Although some of the studies produced significant energy yields, this approach necessitates the use of a specific instrument, which results in a large capital cost investment on an industrial scale (Hassan *et al.*, 2013; Onoja *et al.*, 2019, Mohammad *et al.*, 2021b).

The valorization of agricultural waste to other value-added products via fermentation is deemed practical and has been widely applied by many researchers (Sen *et al*., 2019; Kassim *et al*., 2020; Sen and Baidurah, 2020; Boey *et al*., 2021; Low *et al*., 2021). Various advanced treatment technologies were reported utilizing POME waste as raw material and turn into valuable products such as energy sources (Mohammad *et al*., 2021b; 2021b). Currently, researchers are focusing on treating the POME waste via biological treatment approaches such as fermentation in the presence of microorganisms to produce biomass fuel.

To date, commercially available biomass fuel has a considerably low calorific energy value (CEV) in comparison to coal, with the total CEV of coal approximately 32 MJ/kg (Demirbas, 2007) and 15-20 MJ/kg for biomass fuel (Malmgren and Riley, 2018). To achieve a higher CEV of biomass fuel, the high composition of carbon is required. This can be achieved by subjecting the POME via biological treatment, whereby complex organic compounds will be degraded and releasing simpler carbon elements by the reaction of microorganisms. Withal, a limited study was conducted utilizing POME as a fermentation medium in the presence of *Lysinibacillus* sp. and *Aspergillus flavus* as a biological treatment of POME sludge.

The ultimate analysis provides an indication of the presence of major elements such as carbon, oxygen and minor elements, mostly inorganic-based, such as potassium, chlorine, calcium and sulphur in the POME. In terms of product application for biomass fuel, high carbon content evinced high energy and portrayed in enhanced CEV (Loh, 2017).

In this research, the effects of POME fermentation with and without the presence of microbes on producing high CEV of biomass fuels were studied. Bacteria and fungus identified as *Lysinibacillus* sp. and *A. flavus*, isolated from POME are considered as a robust strain that can proliferate in extreme conditions. Valorization of POME into a useful energy source is explored, specifically as biomass fuel pellets production. The pellets can be applied as raw material for burning in a boiler to generate energy. Four fermentation medium conditions were performed, *viz*.: (1) autoclaved POME, (2) autoclaved POME followed by the addition of *Lysinibacillus* sp., (3) autoclaved POME followed by the addition of *A. flavus* and (4) non-autoclave POME (as it is non-sterile). The parameters fixed for the four conditions were 180 rpm of agitation speed and temperature at 35 ± 2 °C. The CEV and crack analysis of the obtained pellets was studied to determine the potential application as a renewable and sustainable energy source. Withal, POME fermentation effectiveness in the presence of *Lysinibacillus* sp. and *A. flavus* was elucidated by the determination of BOD, COD, moisture content (MC), oil residue as well as carbon, hydrogen and nitrogen analysis. The BOD and COD are analyzed to obtain information regarding the degradation effectiveness in the presence of bacteria or fungus in the POME during and after the fermentation process. The degraded matter will be in the simpler form of carbon matter which contributes to the high CEV.

MATERIALS AND METHODS

Palm oil mill effluent (POME) sampling

POME was retrieved from the clarifier tank prior to entering the pond treatment at United Oil Palm (UOP) Industries Sdn. Bhd. Located in Nibong Tebal, Pulau Pinang, geographical coordinates at 5°09'22.3" N and 100°30'32.3" E. POME was kept in a carboy container and stored at room temperature.

Bacteria inoculum preparation

Two microorganisms were isolated from POME and used for the fermentation in which the bacteria strain is previously identified as *Lysinibacillus* sp. NBRC 103108^T (NR_114207). The bacteria is the stock culture and identification was previously performed in a separate study via 16S rRNA gene sequence similarities using the BLAST programs in the National Centre for Biotechnology Information (NCBI) database (Mohammad *et al*., 2021a).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 408-423
DOI: http://dx.doi.org/10.21161/mjm.221508

Lysinibacillus sp. was activated from the 20% (v/v) glycerol stock stored at –80 °C (Model 952, Thermo Scientific). The strain was thawed for 10-15 min. After thawing, the cells were centrifuged at 5,000× *g* for 10 min to obtain the cell pellet. The supernatant was discarded upon centrifugation and the pellet was inoculated into a 100 mL sterile nutrient broth (NB) medium for cell activation purposes. This step is crucial to confirm that the cells is viable and healthy to be used in the next fermentation stage. The culture was then placed inside the incubation shaker with an agitation speed of 180 rpm at 35 ± 2 °C for 16 h. The cells were inoculated into a fermentation medium once the optical density (OD) reached 0.6 ± 0.05 at 600 nm wavelength (Mohammad *et al*., 2021a).

Fungus isolation, identification and inoculum preparation

The fungus was isolated in this study from POME and identified by its morphological characteristics at the School of Biological Sciences, Universiti Sains Malaysia. Colony appearance such as texture, colour, pigmentation and elevation was examined. A mycelial disc was prepared by punching 7-day-old potato dextrose agar (PDA) culture with a sterilised cork-borer (1.0 cm diameter) and it was inoculated at the centre of a new malt extract agar (MEA) and Czapek yeast extract agar (CYA) plates.

PDA is prepared by weighing a total of 250 g of peeled potatoes, cut into small cubes and boiled in 500 mL of distilled water to make them soft. The boiled potatoes were sieved to collect its broth. Twenty grams of dextrose powder and 25 g of agar straws were added with potato infusion and the mixture was transferred into a Schott bottle. Distilled water was added to make the volume up to 1000 mL. The medium was sterilised at 121 °C, 15 psi for 30 min. The sterilised medium was cooled at room temperature before being poured into sterile Petri dishes (Booth, 1971).

The inoculated plate was then incubated at 30 ± 2 °C for 7 days. The colony appearance and pigmentation of the fungal isolate were assessed after 7 days of incubation using cultures from MEA CYA plates. One litre of MEA consists of 50 g malt extract agar powder and 15 g agar straw (Reddish, 1919). One litre CYA consists of 51.4 g Czapek yeast extract agar powder and 15 g of agar straws (Atlas, 2004). Both media were sterilized at 121 °C, 15 psi for 30 min. The sterilized medium was left to cool at room temperature before being poured into sterile Petri dishes. The plates were left to dry for at least one day prior to usage.

The structures of conidia and conidiophores were examined under a light microscope (OLYMPUS CX41) using the same cultures. Morphological identification of the isolated fungal was performed by referring to the description in Food and Indoor Fungi by Samson *et al*. (2010).

For inoculum preparation, the fungus was cultured on PDA agar for approximately 5-7 consecutive days at 35 ± 2 °C incubation temperature. The spores were then harvested with sterile distilled water containing 0.1% (w/v) Tween 80. Then, the suspension was sieved through 0.5 mm sieve to eliminate mycelia. The spore concentration was counted by direct microscopic counting using a hemacytometer. The inoculation volume is 15 mL with 1×10^6 spore concentration. Serial dilution method was employed to standardize the concentration of spores inoculated into the fermentation media.

Batch fermentation of POME as a feedstock

Figure 1 shows the overall experiment procedure conducted in this study. POME from the clarifier tank was utilized as the sole carbon source in the batch fermentation using a shake flask. The POME from the clarifier tank were selected due to its high viscosity and high oil content as compared to waste-derived at the treatment pond. The fermentation was performed under four different conditions with a total working volume of 250 mL; (1) autoclaved POME and without *Lysinibacillus* sp., (2) autoclaved POME followed by the addition of 10% (v/v) *Lysinibacillus* sp., (3) autoclaved POME followed by addition of 10% (v/v) *A. flavus* and (4) POME without autoclave (as it is). The first group is the control experiment of this study while the second and third groups is to study the fermentation effectiveness of bacteria (*Lysinibacillus* sp.) and fungus (*A. flavus*) in POME as a sole fermentation medium. The fourth group is to study the fermentation effects of existing microbes in POME.

The fermentation was performed with an agitation speed of 180 rpm at 35 ± 2 °C for 5 consecutive days (120 h). A total volume of 30 mL from the fermented products was collected at every 24 h interval and subjected for determination of the following analyses: colony forming unit (CFU), chemical oxygen demand (COD), biochemical oxygen demand (BOD), moisture content (MC), calorific energy value (CEV), crack analysis, oil residue content, elemental analysis of carbon, hydrogen and nitrogen content.

Determination of colony forming unit (CFU) using serial dilution method

Colony forming unit (CFU) was conducted to observe the growth of microorganisms throughout the fermentation in POME medium. In this study, CFU analysis was performed every 24 h intervals for five consecutive days of fermentation. Initially, for each group, one mL of POME sample was retrieved and transferred into an Eppendorf tube. A total volume of 100 µL was transferred into tubes containing 900 µL of sterile distilled water. The serial dilution was prepared from 10^{-1} until 10^{-9} and the spread plate was performed from 10^{-5} until 10^{-9}. A total volume of 50 µL sample was spread onto sterile nutrient agar (NA) plates and PDA plates (only for Group 3; autoclaved POME with the addition of *A. flavus*). Then, the plates were incubated inside an incubator at 35 ± 2 °C. Upon 24-48 h, the colony was observed and counted. The

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 408-423
DOI: http://dx.doi.org/10.21161/mjm.221508

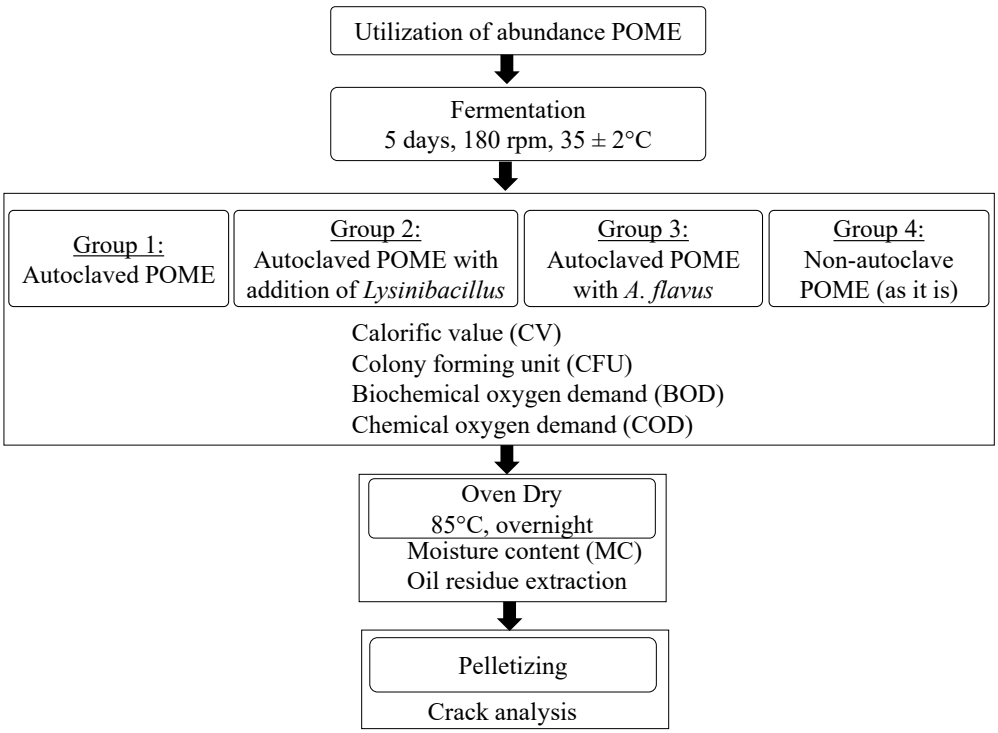

Figure 1: Overall experiment procedure in this study.

colony growth during fermentation were observed and calculated using Equation 1.

Cell concentration (CFU/mL)
= (Number of colony observed × dilution factor)/Sample volume (Equation 1)

Determination of calorific energy value

Calorific energy value (CEV) is a measurement of energy or heat released as a result of complete combustion in the presence of oxygen that can be expressed in unit of MJ/kg or kJ/m^3 (Rena and Kumar, 2019). CEV is directly proportional to the efficiency of fuel; hence, a high CEV represents a high efficiency of fuel. In this study, the CEV was determined throughout the 5 days of fermentation duration.

Approximately 24 mL of fermented products was collected from each group and transferred into a glass petri dish. The sample was then dried inside a drying oven (Binder, Germany) at 100 ± 2 °C overnight until constant weight is observed. Then, the sample was removed from the oven and placed inside a desiccator to cool at room temperature. The samples were homogenized using mortar and pastel, sieved, then packed inside a zip-lock plastic prior CEV analysis. The CEV measurement was performed using an oxygen bomb calorimeter (Model: Parr 6200, USA). Approximately 0.5-0.6 g of homogenized and dried sample was placed in the combustion vessel and filled with oxygen 99.95 % purity until the pressure reached 450 psig (3.0 ± 0.2 Mpa). The combustion vessel was inserted into the combustion vessel bucket and ignited at the following condition: pre-fire 3 min, post-fire 5 min, fuse wire length 10 cm, bucket and jacket temperature 13-33 °C.

Determination of chemical oxygen demand (COD)

Chemical oxygen demand (COD) is conducted to determine the amount of organic matter in a liquid sample. The method applied in this study is 5220 D COD closed reflux with a colorimetric method approved by EPA. COD reagent was prepared by mixing two different solutions, A and B. Solution A was prepared by dissolving approximately 18 g of silver sulphate in 800 mL of concentrated sulphuric acid and Solution B was prepared by dissolving 14.8 g potassium dichromate in 100 mL of distilled water. The mixture was left stable for two days prior to usage.

One mL of samples was transferred into a 15 mL Falcon tube and tightly capped. POME sample was diluted with 9 mL of sterile deionized water. Two mL of diluted sample was filtered using 0.22 µm (nylon membrane) filter into 3 mL of COD reagent inside a digestion tube. The sample was placed inside a heater block at 150 °C for 2 h and then let cool at room temperature. The COD value (mg/L) for each sample was measured using a spectrophotometer (HACH DR 2800). The blank consisted of 3 mL COD reagent and 2 mL of sterile deionized water. The COD was measured for its removal efficiency (%) using the following Equation 2 (Makhtar and Tajarudin, 2020):

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 408-423
DOI: http://dx.doi.org/10.21161/mjm.221508

COD removal (%)
= [(COD_i – COD_f)/COD_i] × 100 (Equation 2)

Where, COD_i is the initial COD value and COD_f is the final COD value.

Determination of biochemical oxygen demand (BOD)

Biochemical oxygen demand (BOD) is a measure of dissolved oxygen required by biological organisms to break down the organic material contained in the water sample. The dissolved oxygen level was measured every 24 h throughout the fermentation.

Five mL of sample and 10 mL of seed solution were diluted with 285 mL of sterile deionized water and transferred into 300 mL of BOD bottles. A volume of 300 mL of deionized water was transferred into a BOD bottle and used as a control (blank). A dissolved oxygen (DO) meter (HANNA Instruments HI 98193) was used to measure the initial DO concentration (mg/L) in each bottle. Each bottle was then placed inside a dark incubator at 20 ± 2 °C for five days. Upon designated period, the final DO concentration was measured. The final DO reading was then subtracted from the initial DO reading to obtain the final BOD concentration. The following equation is applied to determine BOD level of the sample.

BOD (mg/mL)
= [(Final DO reading – Initial DO reading)/Total volume of flask] × dilution factor (Equation 3)

Determination of moisture content

Moisture content (MC) analysis was conducted to characterize the quality of the fuel in terms of moisture presence. Low MC produces a high quality of fuel briquette or pellets (Safana, 2018). This experiment was conducted using only the initial fermentation sample (Day 0) and final fermentation sample (Day 5). Dried samples weighing 0.5-0.8 g were placed onto the plate of the moisture analyzer (Model: Sartorius, Germany) and MC was recorded as a percentage (%).

Crack analysis

The purpose of conducting crack analysis is to observe the dimensional stability of the dried POME pellets. This experiment is conducted to samples with the highest CEV of each group.

One g of homogenized dried sample from each group was weighed using an electronic weighing scale (Mettler Toledo AL 204) and recorded as initial weight. The weighed sample was then transferred into a pellet press (Model: Parr, U.S.A.). The sample was pressed and compacted into a pellet with a dimension of 12 mm in diameter and a height of approximately 8 mm. The pellet was subjected to crack analysis whereby each pellet was dropped from 1 m high onto the floor. The cracks and final weight of the sample were observed and recorded.

Determination of oil residue by soxhlet extraction

Soxhlet-extraction was conducted to determine the amount of oil contained in the POME clarifier tank before and after fermentation. Approximately 1 g of dried samples was weighed and transferred into a cellulose thimble with a dimension of 22 × 80 mm (Smith, USA). Then, the oils were extracted *circa* 2-3 h using *n*-hexane as the extraction solvent at 60 °C. After the extraction process, the solvent was evaporated using a drying oven (Binder, Germany) at 115 °C until constant weight is observed. Upon an hour of drying, the oil extracted inside the round-bottom flask was put inside a desiccator for 30 min. The final weight of each sample was weighed. The oil extracts were then stored at 20 °C for further analysis.

The percentage of oil recovered from the pellet samples was calculated using Equation 4 (Bala *et al.*, 2014):

Oil and grease (%)
= [Final flask weight after evaporation (g) – Flask initial weight (g)]/Sample weight (g) × 100% (Equation 4)

Carbon, hydrogen and nitrogen analysis

Carbon, hydrogen and nitrogen (C, H and N) analysis was conducted to quantify the elements in the dried POME samples. This analysis was conducted using CHN Elemental Analyzer (Perkin Elmer 2400 Series II). *Circa* 1.5 mg of homogenized dried sample was placed inside the instrument and the set-up of the instrument was as follow: gas pressure settings (helium 20 psi, oxygen 20 psi and compressed air 60 psi). Acetanilide was used as the standard sample in this experiment.

Statistical analysis

The statistical analysis was performed using SPSS version 27. One-way ANOVA was employed to determine the statistically significant difference of the means for each group. The significance of the results was determined at $p<0.05$.

RESULTS AND DISCUSSION

Fungus identification

Figure 2 illustrates the morphological characteristics of the isolated fungus derived from POME. Based on the colony appearance and pigmentation grown on MEA Figure 2(a, b) and CYA Figure 2(c, d), the fungus is identified as *A. flavus*. Figure 2(e, f) indicates a clear observation of the conidia and conidiophores. Withal, the morphology exhibited by the culture can be described as green conidia, dominated colony appearance with plain, flat at edges and raised at the centre. The colony formed also has a wrinkled cerebriform pattern as well as encircled by a white border and pale inner side.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 408-423
DOI: http://dx.doi.org/10.21161/mjm.221508

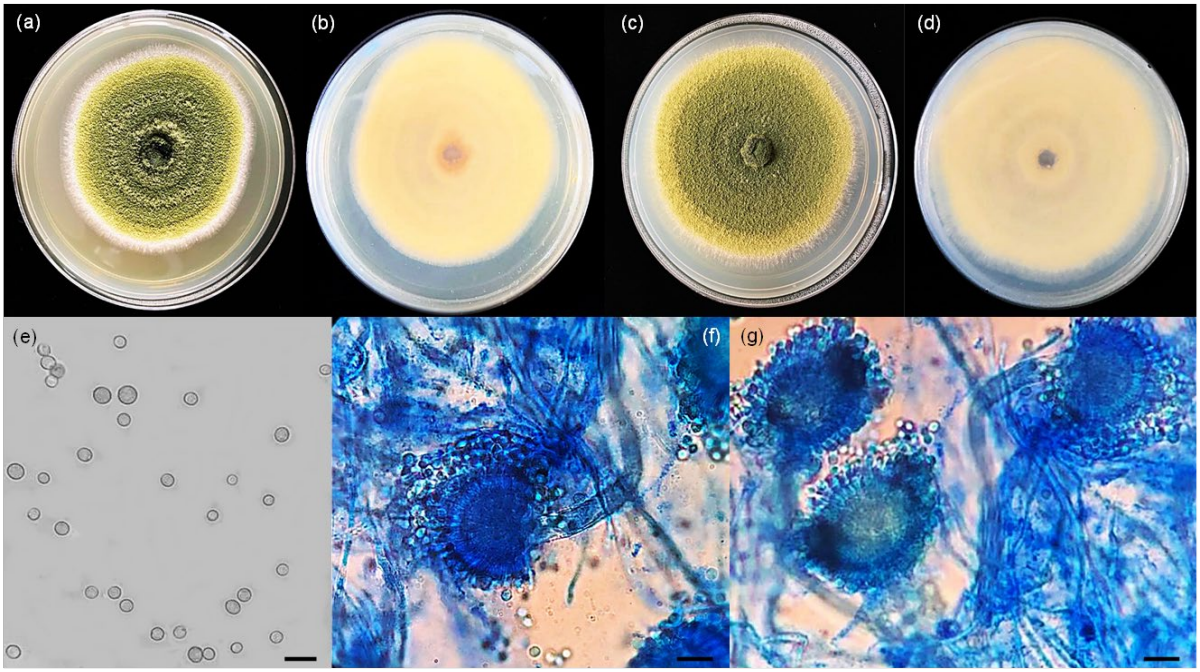

Figure 2: Morphological characteristics of *A. flavus*. (a, b) Colony appearance and pigmentation on MEA, (c, d) Colony appearance and pigmentation on CYA, (e) Conidia and (f, g) Conidia and conidiophores. Scale bar = 10 µm.

Table 1: The population of microorganisms in POME medium for 5 days of fermentation.

Group	Cell concentration (CFU/mL)					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
1	-	-	-	-	-	-
2	117850	40300	260800	591	1091	2199
3	1266.74	349.928	0	0	0	0
4	2356667	2256667	221500	253000	109414	952500

Colony forming unit (CFU)

The colony growth during fermentation were calculated using Equation 1 and the data are recorded in Table 1. Referring to Table 1, for Group 1, as predicted, there was no population of microorganisms were observed during the fermentation. *In lieu* of Group 1, the remaining Group 2, 3 and 4 shows the microorganisms' growth pattern throughout the 5 days of fermentation. The population of *Lysinibacillus* sp. in Group 2 throughout the fermentation shows a decreasing trend from 0 h to the first day of fermentation, indicating the cells were acclimatizing in the medium, followed by an increase in population on the second day of fermentation. On the third until the fifth day, a slight fluctuation of the cells counts was observed. This observation pinpointed that *Lysinibacillus* sp. is a robust strain that can survive in POME medium without any dilution prior to inoculation.

Group 3 consists of autoclaved POME followed by the addition of *A. flavus*. Here, the fungus concentration is in decreasing trend from 0 h to the first day of fermentation, followed by no population detected beyond the second day. This observation is plausibly owing to the unsuitable living conditions in the media, which renders the declination of fungus concentration. Even though no fungus was observed on day 2, the fungus reaction of degrading complex carbon material from day 0 to day 1 still exists in the POME medium, which can be monitored by means of high CEV.

The growth of microorganisms in Group 4 in NA portrayed the same morphology as Group 2, which are circular in shape, opaque white colour and elevated margin. Since this group is not a pure single culture fermentation, observation for Group 4 can only be interpreted as a consortium of microorganisms. Throughout the fermentation utilizing non-sterile POME, the microbe's population shows almost constant numbers with negligible changes throughout 5 days of fermentation. POME accommodates various microorganisms *viz*. *Bacillus* sp., *Pseudomonas* sp. and *Staphylococcus* sp. as well as fungal species such as *Aspergillus* sp. (Soleimaninanadegani and Manshad, 2014).

Table 2 summarizes the microorganisms capable of utilizing POME as feedstocks to produce biomass fuel such as biogas, microbial fuel cells as well as biodiesel.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 408-423
DOI: http://dx.doi.org/10.21161/mjm.221508

Table 2: Microorganisms capable utilizing POME as feedstocks to produce biomass and biogas fuel.

Microorganisms	Feedstocks	Parameters and duration	Final product	References
Mix culture of POME	Raw POME	Continuous mode	15.43 MJ/g_{COD} of biogas	Garritano *et al.* (2018)
Mix culture of POME	Raw POME	Continuous mode, feed flow rate at 42,000 kg/h	1,358.34 kg/h biogas	Lok *et al.* (2020)
Mix culture of POME and *Klebsiella oxytoca* culture	Raw POME	10 consecutive days batch fermentation, 35 °C at 150 rpm	MFCs with power density 207.28 mW/m^3	Islam *et al.* (2016)
Lysinibacillus sp. LC 556247	Raw POME	Batch shake flask fermentation, 120 h, pH 4.49-4.54, 180 rpm, 35 ± 2 °C	Biomass fuel with a calorific energy value of 21.25 ± 0.19 MJ/kg	Mohammad *et al.* (2021a)
Bacillus cereus *Bacillus cereus* activated overnight in LB broth	Sterilized 50% POME	Inoculation at 10% v/v, 13 consecutive days batch fermentation, incubation temperature of 35 °C at 150 rpm	MFCs with power density 3880 mW/m^3	Islam (2017)
P. aeruginosa, *A. oryzae*, *A. peroxydans*, *S. variicoloris* and existing culture in POME	1000 mg/L POME	20 consecutive days batch fermentation, 29 ± 1 °C with 1 kΩ external resistance	MFCs with power per unit area 107.4 mW/m^2	Baranitharan *et al.* (2015)
K. variicola and *P. aeruginosa*	Sterilized 50% POME	11 consecutive days batch fermentation, 37 °C at 150 rpm with 1 kΩ external resistance	MFCs with power 14,780 mW/m^3	Islam *et al.* (2018)
A. oryzae	Sterilized POME	Four consecutive days of batch fermentation, 4 g POME, 446 U of immobilized lipase, 40 °C reaction temperature at 35 rpm	Biodiesel	Rachmadona *et al.* (2021)

The mix cultures are generally bacteria such as *Micrococcus luteus*, *Stenotrophomonas maltophilia*, *Bacillus cereus*, *Bacillus subtilis*, *Klebsiella pneumoniae* as well as *Providencia vermicola* (Garritano *et al.*, 2018) and fungi such as *A. oryzae*, *A. peroxydans*, *A. fumigatus*, *A. nomius*, *A. niger* and *Meyerozyma guilliermondii* (Baranitharan *et al*., 2015; Barapatre *et al.*, 2016). *A. flavus* strain is a remarkable fungus that can metabolize lignin as its primary carbon source. This fungus has a ligninolytic enzymatic system in which able to efficiently degrade complex carbon materials in the POME medium (Barapatre *et al.*, 2016). Generally, higher inoculum size (10-20 vol%) will contribute to enhancing fermentation effectiveness. The presence of microbes in POME will promote the bioconversion and biodegradation of organic matter in POME. Howbeit, environmental-sensitive microbes are prone to microbial inhibitions, which can deter bioconversion. Thus, the selection of microbes that can stand the extreme environmental condition such as extreme acidity or alkalinity, high-strength wastewater, and limited oxygen supply is of utmost required to ensure efficient bioconversion and rapid microbial proliferation.

Calorific energy value (CEV)

Figure 3 delineates fermentation performance; (a) calorific energy value, (b) COD concentration profile, (c) COD removal efficiency profile, (d) DO concentration profile, throughout 5 days of fermentation for each group. The CEV is a measure of the energy released upon combusted in the presence of oxygen and here represented by the unit of MJ/kg. This analysis was

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 408-423
DOI: http://dx.doi.org/10.21161/mjm.221508

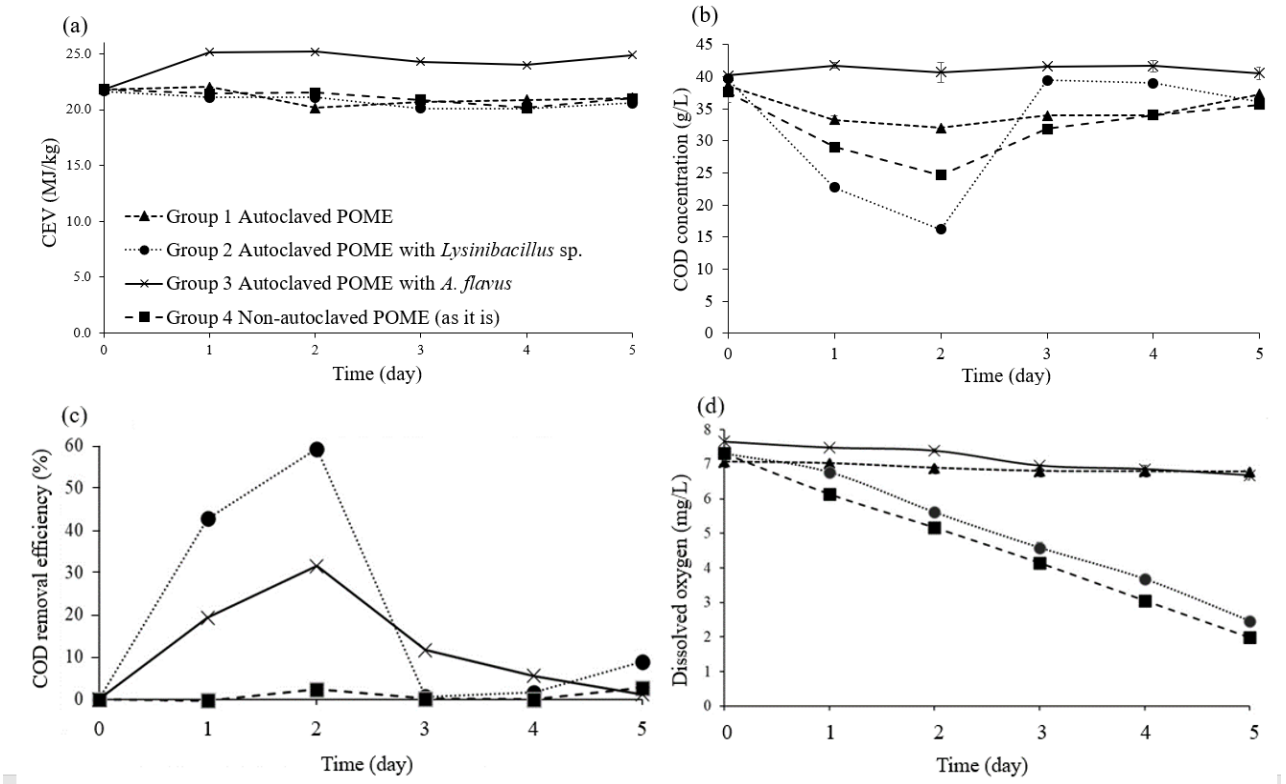

Figure 3: Fermentation performance throughout 5 days for each group: (a) Calorific energy value, (b) COD concentration profile, (c) COD removal efficiency profile and (d) DO profile.

conducted to elucidate the changes of CEV upon treatment in different fermentation conditions throughout five consecutive days. In this experiment, the fermented, dried and homogenized POME samples were subjected to an oxygen bomb calorimeter. The combustible properties of POME biomass fuel can be characterized by determining the CEV of the samples.

Referring to Figure 3(a), the highest CEV is 25.18 MJ/kg (Group 3), followed by 22.01 MJ/kg (Group 1), 21.80 (Group 4) and 21.65 MJ/kg (Group 2), respectively. High CEV observed on the second day of fermentation from Group 3 was due to the fermentation efficiency of *A. flavus* presence in the autoclaved POME medium. *A. flavus* is a soft rot fungus that are able to degrade complex carbon molecule into their corresponding simpler monomer units, as the strain produced ligninolytic enzymes which are proficient in metabolizing lignocellulose materials (Soleimaninanadegani and Manshad, 2014; Asemoloye *et al.*, 2021). The degradation products will produce carbon elements and directly increase the CEV (Abdel-Aal, 2021). The CEV or heating value of a fuel can be affected by the presence of a long chain of carbon as well as alkyl groups in the complex molecules, whereby a long carbon chain without or less alkyl groups attached together in the molecule are highly favoured in attaining high heating value (Abdel-Aal, 2021).

Generally, the CEV of oil palm biomass materials is relatively lower than 20 MJ/kg (ÖzyuğUran and Yaman, 2017; Aminu, 2018; Onoja *et al.*, 2019) as compared to coals, with CEV of 27.07 MJ/kg (Wood, 2019). This observation is due to several factors affecting the combustion characteristics of the fuel, such as fixed carbon content, moisture content, oil content, volatile matter and ash contents presence in the biomassic fuel materials (Hassan *et al.*, 2013; Atnaw *et al.*, 2014; Loh, 2017; ÖzyuğUran and Yaman, 2017). A high CEV fuel results from a high fixed carbon content of the biomass. Therefore, high volatile matter content is not preferable as volatile matter can also be formed from non-combustible gases such as carbon dioxide and water (ÖzyuğUran and Yaman, 2017). In contrast, biomass with high content of combustible gases will, in exchange easily ignited, then gasified or oxidized (Loh, 2017).

Chemical oxygen demand (COD)

The method employed in the experiment is approved by EPA, which is 5220 D COD closed reflux with the colorimetric method. This experiment was conducted to observe the efficiency of *Lysinibacillus* sp., *A. flavus* as well as mix cultures of microbes in treating POME via fermentation. In this experiment, the sampling was conducted every 24 h intervals and the result is delineated in Figure 3(b) and (c).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 408-423
DOI: http://dx.doi.org/10.21161/mjm.221508

Table 3: BOD concentrations of every fermentation group.

Fermentation condition	BOD (mg/L)
Group 1: Autoclaved POME	19.33
Group 2: Autoclaved POME with *Lysinibacillus* sp.	320.44
Group 3: Autoclaved POME with *A. flavus*	65.33
Group 4: POME as it is	355.56

By using Equation 2, the COD removal efficiency were calculated and plotted in Figure 3(c). This equation determines where the removal was at the highest throughout the five consecutive days of fermentation. Figure 3(c) does not include the COD removal of Group 1 due to zero removal throughout the experiment condition. Referring to Figure 3(c), the highest efficiency of eliminating COD in POME was observed in Group 2; autoclaved POME with the addition of *Lysinibacillus* sp. at the second day of fermentation with a COD removal percentage of 59.20% while the highest COD removal from Group 3 and 4 was at 31.51% (Day 2) and 2.69% (Day 5), respectively. The COD removal efficiency of POME is one of the utmost important factors as POME is regarded as toxic wastewater because of its high content of COD as well as BOD (Muzzammil and Loh, 2020). Moreover, the Department of Environment (DOE), Malaysia enforces a standard in which stated the COD concentration level allowed to be discharged, which are 100 mg/L should be complied by all stakeholders (Bala *et al.*, 2014; Salihu and Bakar, 2018; Muzzammil and Loh, 2020).

In correlation with colony forming unit (CFU) analysis, the highest population of *Lysinibacillus* sp. was observed at the second day of fermentation as well. Hence, it can be deduced that *Lysinibacillus* sp. pure culture was able to remove COD more efficiently than the mixed cultures of microorganisms in Group 4 and an isolated *A. flavus* culture in POME. The presence of mixed populations of microorganisms in the POME medium might have inhibited the COD from removing microorganisms during the fermentation, similar to a study reported by Bala *et al*. (2014).

Biochemical oxygen demand (BOD)

Biochemical oxygen demand (BOD) is a measure of dissolved oxygen required by biological organisms to break down the organic material contained in the water sample and it is commonly presented by the unit milligrams of oxygen per liter of the sample (mg/L) (Shmeis, 2018). Figure 3(d) delineates the dissolved oxygen level observed every 24 h interval for each group throughout five consecutive days.

Referring to Figure 3(d), only a slight reduction of the DO concentration throughout the fermentation for Group 1 was observed. The DO level of Group 1 at the beginning and end of the experiment was 7.07 mg/L and 6.78 mg/L, respectively. This observation is due to the lack of microorganisms present in the POME medium. DO concentrations for Group 2 showed apparent changes as shown in Figure 3(d), whereby, initially, the DO concentration was recorded at 7.30 mg/L and at the end of the fermentation was significantly reduced to 2.50 mg/L. The distinguish DO reduction indicates the *Lysinibacillus* sp. activity in utilizing the oxygen in the shake flasks. The DO levels in Group 4 showed more noticeable changes in which the initial and final concentration was at 7.32 mg/L and 1.98 mg/L, respectively. This reduction indicates the vigorous activity of the *Lysinibacillus* sp. population as well as the cumulative activity of microorganisms consortium in their respective POME medium (Chan and Chong, 2019). Group 3 recorded initial DO concentrations of 7.66 mg/L and 6.68 mg/L at the final day of fermentation. Group 3, in relation with colony forming unit (CFU) results, shows that there were hardly any changed in DO concentration in the fermentation medium due to the fact that there was only a population of *A. flavus*. When focusing on the reduction of DO throughout the whole fermentation time, Group 4 showed the highest reduction, followed by Group 2. Group 3 indicates a slight DO decrease on Day 2 and 3. Whilst Group 1 showed the least DO reduction. The fungus population presented in the medium of Group 3 expressed the most negligible reduction of DO.

With the obtained DO concentration levels of each group, BOD level was calculated using Equation 3 and tabulated in Table 3. It can be summarized that the groups in which lacked microorganisms portray a low BOD level. In detail, Group 1 at 19.33 mg/L and Group 3 at 65.33 mg/L while fermentation groups in which had active microorganism populations in the medium expresses higher BOD concentration which are Group 2 and Group 4, with BOD concentration at 320.44 mg/L and 355.56 mg/L, respectively.

BOD levels are affected by the population of microorganisms that are actively consumed oxygen and therefore DO levels in the sample tested were reduced (Bajpai, 2018; Bendicho and Lavilla, 2019). Referring to Table 3, a consortium of bacteria exists in non-sterile POME consumed oxygen the most, followed by Group 2, with the presence of *Lysinibacillus* sp., Group 3, sterile POME with the addition of *A. flavus* and lastly, Group 1 in which did not have any microorganism in the medium.

Moisture content (MC)

Moisture content (MC) is one of the main factors affecting the quality of a fuel (Hassan *et al*., 2013; Atnaw *et al.*, 2014; Loh, 2017; ÖzyuğUran and Yaman, 2017). This is because low moisture content fuel produces high-quality fuel briquettes or pellets (Safana, 2018). This experiment was conducted on homogenized oven-dried samples at initial fermentation (Day 0) and final fermentation (Day 5)

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 408-423
DOI: http://dx.doi.org/10.21161/mjm.221508

Table 4: The weight difference of POME pellet before and after the crack analysis.

Sample	Initial weight (g)	Final weight (g)	Difference (g)
Group 1	1.0092	0.9773	0.0319
Group 2	1.0093	0.9588	0.0505
Group 3	1.0092	0.9796	0.0296
Group 4	1.0093	0.9986	0.0107

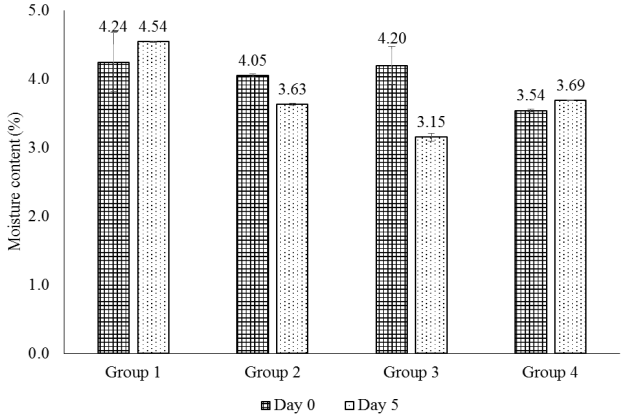

Figure 4: Moisture content of dried samples from each group whereby; Group 1: Autoclaved POME, Group 2: Autoclaved POME with *Lysinibacillus* sp., Group 3: Autoclaved POME with *A. flavus* and Group 4: POME as it is (non-sterile).

for each group using a moisture analyzer (Model: Sartorius, Germany). Figure 4 shows the MC upon overnight drying of each group with different fermentation conditions.

Referring to Figure 4, all oven-dried samples of POME resulted in low moisture content; below 5% in which the highest moisture percentage can be seen from Group 1 with the initial and final moisture, 4.24% and 4.54%, respectively. The change in moisture from Group 2 and Group 3 both shows a decreasing trend with MC from 4.05% to 3.63% and 4.20% to 3.15%, which the difference in Group 3 is the biggest among the other three groups while Group 4 exhibits an increasing trend similar with Group 1 from 3.54% to 3.69%. Overall, all MC of samples are below 5%, which is desirable to be applied as biomass fuel.

MC of solid biomass fuel is an important aspect that affects the burning characteristics of the fuel. A high MC resulted in slowed ignition of fuel because wet fuel requires extra energy for heating and evaporating the water prior to combustion take place. Hence, the energy per kg produced was less during combustion, which resulted in lower CEV (Loh, 2017). In contrast, lower MC will increase the efficiency of pyrolytic conversion of the fuel (Nyakuma *et al.*, 2014).

Since high MC can lead to ignition difficulties, the drying method employed is essential in decreasing the moisture of fuel (Atnaw *et al.*, 2014; Safana, 2018). To reduce moisture of the biomassic material, a few methods can be employed, such as mechanical, thermal, or natural methods under atmospheric conditions (Safana, 2018). In this study, the drying method employed was the oven dry method at 85 ± 5 °C overnight.

By reducing moisture of the biomassic material, the oxygen content of the biomass will decrease in turn increases the carbon content in the biomass; thus, an increment of CEV can be expected (Awad *et al.*, 2017). Water content in the biomass material prolonged the ignition time resulting in low efficiency of biomass fuel combustion. Apart from the loss of energy due to moisture in biomass, the stability of the flare of the fuel can also be affected during combustion (Awalludin *et al.*, 2015). Furthermore, low moisture fuel produces a preferable stable flare. High moisture fuel produces low concentration of carbon dioxide, which indicates less combustion occurred (Atnaw *et al.*, 2014).

To summarize, the characteristics of biomass fuel is significantly affected by MC of the biomass material and therefore initial drying method employed is essential to reduce the moisture and water content of the fuel in order to increase the combustion efficiency, obtain stable flare combustion, and achieve greater CEV.

Crack analysis

In this study, crack analysis was performed to evaluate the compactness and dimensional stability of the dried POME pellet in each group by observing the cracks exhibited from the pellets after being dropped from one meter high. To further clarify the difference between each sample, the initial and final weight of the sample was recorded and changes in weight difference were tabulated in Table 4. This experiment was only conducted for the highest CV samples in each group.

The purpose of observing cracks on the sample pellets was to determine the porosity of the biomass pellets by observing the cracks and fragments detached from the pellet after dropping. Pictures of each sample before and after the experiment are depicted in Figure 5.

Referring to Table 4, the highest weight difference was exhibited in Group 2, which is the autoclaved POME with the addition of *Lysinibacillus* sp. strain with the weight difference *circa* 0.0505 g followed by Group 1 *circa* 0.0319 g as well as Group 3 whereby *A. flavus* was inoculated into autoclaved POME medium recorded approximately 0.0296 g difference in weight. The least weight difference can be seen in Group 4, whereby the difference of weight was 0.0107 g. The huge weight difference implies that particles were detached from the pellet due to a lack of compactness.

The most cracks observed after dropping the pellet from 1 meter high was from Group 2 followed by Group 1

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 408-423
DOI: http://dx.doi.org/10.21161/mjm.221508

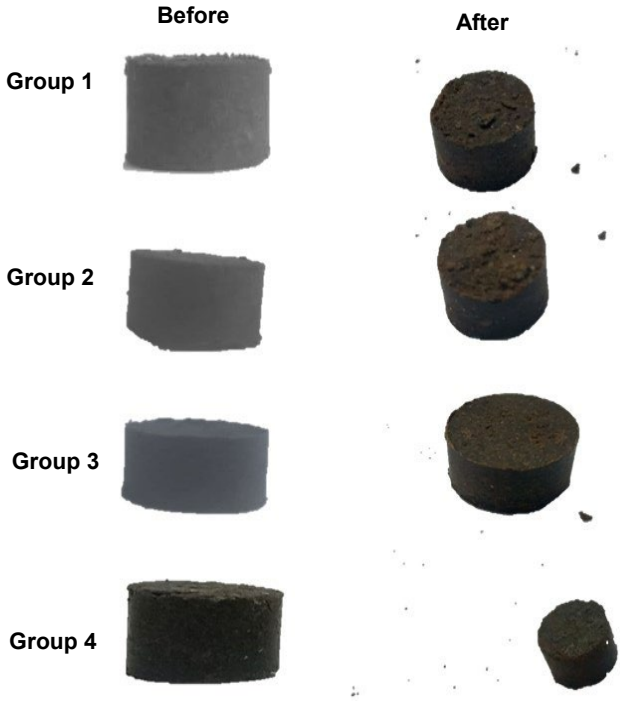

Figure 5: Observation of crack analysis for the POME biomass pellet.

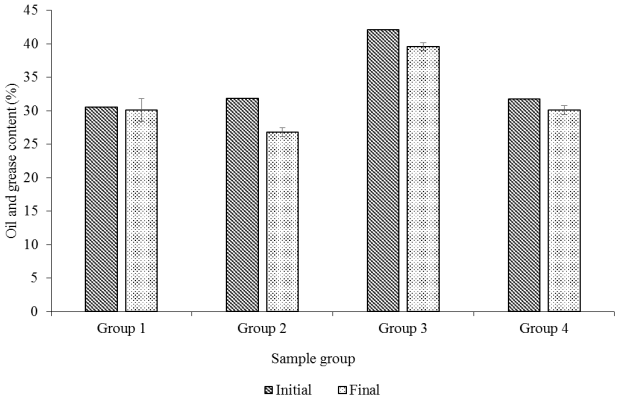

Figure 6: Oil and grease content (%) before and after fermentation of every fermentation group.

and Group 3, while Group 4 exhibited the least cracks of the pellet. This trend is similar when compared to the trend of weight difference of the pellet discussed earlier. The more cracks exhibited, the higher the porosity of the biomass pellet. The porosity of a pellet depends on the presence of binder materials in the biomass (Safana, 2018). In addition, the oily contents in the biomass help to hold the pellet together, thus strengthening the pellet and less cracks exhibited (Hassan *et al.*, 2013).

Apart from the presence of binder in the fuel pellets, the MC of the fuel materials also has significant effects on the porosity level of the pellets. The heating profile as well as handling and storage of solid fuel is more efficient when it is lower in moisture (Nyakuma *et al.*, 2014). In the case of handling and storage in Malaysia, which is considered to have warm weather all year with high humidity, high moisture of solid fuel will lead to undesired inconsistent clumping with holes or voids (Tabakaev *et al.*, 2017).

Overall, the high-quality pellet in which exhibits the least cracks and least weight difference was from Group 3. This was due to high oil content and suspended solids in addition to a low moisture content of the biomass that in turn, aids in binding the pellet firmly.

Oil residue extraction

Soxhlet extraction experiment was performed to determine the amount of oil and grease contained in the POME medium before and after fermentation when treated in different fermentation conditions. Figure 6 delineates oil and grease residue for samples in each group before fermentation (initial) as well as after fermentation (final). Referring to Figure 6, all groups revealed a reduction in oil and grease content after the fermentation. The least oil and grease reduction resulted from Group 1, which is the control of the experiment in this study. This observation is due to the fact that in which Group 1 did not have any microorganisms responsible for the degradation of oil and grease in the medium, thus, showed the least changes (1.57%) as compared to other groups. In contrast, Group 2 with the presence of *Lysinibacillus* sp. in the medium, showed the largest reduction (15.84%) followed by Group 3 (5.99%) and Group 4 (5.17%), respectively. The characteristics of oil extracted via soxhlet extraction conducted on each sample are similar and exhibited a yellowish to orange colour with a smell similar to cooking oil. In terms of wastewater treatment, the reduction of oil and grease is one of the important factors in compliance of the Department of Environment (DOE) Malaysia discharge requirement (Bala *et al.*, 2014). In contrast, a high-quality fuel deems to possess characteristics of ease to ignition as well as high heating value (CEV). High oils and grease content in the biomass fuel material is considered an added value (Loh, 2017; Suwanno *et al.*, 2017).

To conclude, oil and grease content in the samples showed a reduction across the fermentation, whereby the largest reduction was from Group 2. It can be deduced that *Lysinibacillus* sp. helps degrade oils and grease in the fermentation medium much more efficiently than the mix cultures of POME (Group 4) as well as Group 3, which has a population of *A. flavus* in the medium. Although a high content of oil and grease is favorable in obtaining a high CEV of biomass fuel, the reduction of oils and grease shows the potential of *Lysinibacillus* sp. role in wastewater treatment.

Carbon, hydrogen and nitrogen (CHN) analysis

CHN analysis was conducted for two samples from Group 3 (Autoclaved POME with the addition of *A. flavus*) on

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 408-423
DOI: http://dx.doi.org/10.21161/mjm.221508

Table 5: The current utilization of POME treatment processes to produce renewable energy.

Method	Experiment parameters and conditions	Yield	References
Anaerobic digestion	Mix cultures in POME	1.79 billion m^3 × 20 MJ/m^3 = 35.78 billion MJ	Loh (2017)
Aerobic and anaerobic biological treatment	Mix cultures of raw POME. Anaerobic expanded granular bed bioreactor (AnaEG), Nano air flotation (NAF), BioAX and microfiltration membrane	1170 m^3 h^{-1} × 20,000 kJ m^{-3} = 23400 MJ	Tabassum *et al.* (2015)
Aerobic fermentation	Batch shake flask fermentation, 120 h, pH 4.49-4.54, 180 rpm, 35 ± 2 °C	Biomass fuel with CEV of 21.25 ± 0.19 MJ/kg	Mohammad *et al.* (2021a)
Composting	Ratio of POME:EFB is 1:1.Require 40 days of composting process	Optimized C:N ratio of 12:4	Liew *et al.* (2015)
Direct combustion	Adiabatic bomb calorimeter	16.99 MJ/kg	Onoja *et al.* (2019)
Direct combustion	POME and OPF was dried, grind and pressed	Ratio of POME:OPF is 90%:10%, produced 30.85 MJ/kg	Hassan *et al.* (2013)
Direct combustion	Mix cultures of raw POME collected at separator and decanter	16.99 MJ/kg (Separator) and 16.13 MJ/kg (Decanter)	Loh (2017)
Microbial fuel cells (MFCs)	50% of sterilized POME with the addition of *K. variicola* and *P. aeruginosa*	Power per unit volume generated 14,780 mW/m^3	Islam *et al.* (2018)

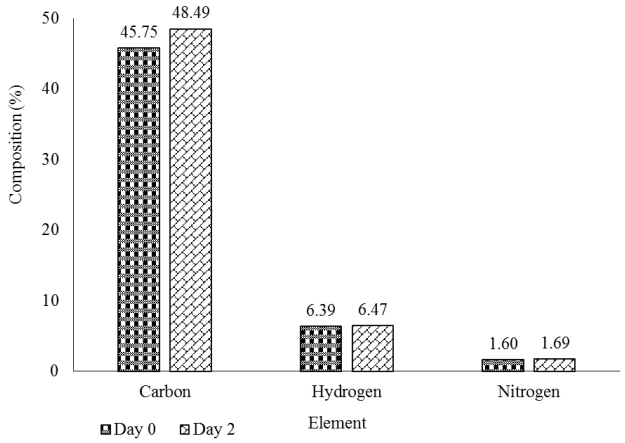

Figure 7: The carbon, hydrogen and nitrogen composition of Group 3 on the initial and second day of fermentation.

Day 0 and Day 2 to elucidate the changes in organic compounds (carbon, hydrogen and nitrogen). These two samples were selected because Day 2 of Group 3 yielded the highest CEV of 25.18 MJ/kg as compared to others and showed an increment from Day 0 (25.02 MJ/kg) of fermentation.

Figure 7 delineates the composition of carbon, hydrogen and nitrogen in the samples. Referring to Figure 7, the initial value of each element for carbon, hydrogen and nitrogen were 45.75%, 6.39% and 1.60%. With the elapsed of two days fermentation, all elements increased to 48.49%, 6.47% and 1.69%, respectively.

Both carbon and hydrogen are important elements in yielding a high CEV of biomass fuels. The result evinced positive validation of the former studies conducted on energy produced from biomass fuels, whereby high CEV was obtained with the increase of carbon and hydrogen values (Hassan *et al.*, 2013; Safana, 2018). A study reported that one mole of carbon gave approximately 33.5 kJ/kg energy while one mole of hydrogen gave 125.6 kJ/kg energy (Thangarasu and Anand, 2019). The low nitrogen content plays an advantageous role in POME biomass fuel CEV. This is because low nitrogen content will produce less nitrogen oxide (NO_X) from the biomass fuel combustion, thus, lower the risks of affecting the environment (Hassan *et al.*, 2013).

Statistical analysis

One-way ANOVA was conducted for CEV analysis and MC analysis, whereby all four different fermentation conditions were statistically compared to determine the significant difference between the means of each fermentation condition. The significant level was tested at α=0.05. The null hypothesis of both analyses is there are no significant differences between the means of the groups. Based on the obtained result, the *p*-value of CEV, as well as MC between groups, are 0.00 and 0.04, respectively. Since both obtained values are smaller than 0.05, hence, the null hypothesis can be rejected as there are statistical differences between the means of the groups proven. To summarize, the CEV and MC of each fermentation group expressed a significant difference between the groups. Hence, it can be concluded that different POME fermentation treatment conditions affect the parameters differently.

Applicability of POME to produce biomass fuel

Table 5 summarizes the current utilization of POME treatment processes to produce renewable energy.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 408-423
DOI: http://dx.doi.org/10.21161/mjm.221508

Table 6: Ideal characteristics of solid biomass fuel.

Properties	Safana (2018)	This study
Proximate analysis		
Moisture content (wt., %)	6-14	3.15-4.54
Ash content (wt., %)	<4%	-
Fixed carbon (wt., %)	9-25	-
Volatile matter (wt., %)	50-90	-
CEV (MJ/kg)	10-35	21.65-25.18
Ultimate analysis, wt., % (Elemental analysis)		
Carbon, C	40-55	48.49
Hydrogen, H	5-8	6.47
Oxygen, O	35-48	-
Nitrogen, N	0-1	1.69
Sulphur, S	0-2	-
Chloride, Cl	0-1	-

Biomass fuel is an alternative fuel source and usually exhibits a lower CEV than coal, as biomass fuel generally has less carbon content than coal. The average CEV for POME was at 16.99 MJ/kg (Loh, 2017; Onoja *et al.*, 2019), while when 90% of POME is used with the addition of 10% OPF the CEV obtained increased to 30.85 MJ/kg (Hassan *et al.*, 2013). Moreover, the nitrogen and sulphur content are very low in biomass fuel compared to coal and therefore less, greenhouse gases will be released into the atmosphere. The biomass fuel briquettes also exhibit less cracks owing to the oily POME properties, which act as a binder and in turn lower the porosity of the briquette (Hassan *et al.*, 2013).

Solid fuels are fuels that are densified into briquettes or pellets, which can be formed by utilizing highly abundant biomass. There are several factors that directly influence the briquettes or pellets formation as well as the burning qualities of the formed briquettes. Briquettes is a process of compressing materials in which mainly used to compact biomass such as fiber, wood and sawdust. Briquettes and pellets differ in terms of a size whereby the size of pellets is about 5 to 30 mm while briquettes are about 50 to 400 mm. The ideal properties of a high-quality solid biomass fuel were described in terms of two different aspects, which are proximate analysis and ultimate analysis, as shown in Table 6 (Safana, 2018).

Referring to Table 6, the drying process of the biomass fuel is an important step in overcoming the high moisture content, low fixed carbon and low heating value of the biomassic material. Drying is a process of eliminating water or moisture from solid material through thermal, mechanical or natural methods under atmospheric conditions. Withal, the storage of the briquettes is as equally important because storage at high temperature will make the briquettes too dry and hard to ignite, while low temperature can make the briquettes soft and less durable (Safana, 2018). Moreover, compaction pressure applied while making the pellet or briquette, helps in the densification of biomass, this process may also affect the porosity of the sample whereby higher pressures lead to diminishing the porosity of the biomass (Safana, 2018). In general, the obtained data in this study are within the requirement, low MC and high CEV without any addition of binder are successfully obtained. The water or moisture content of biomass fuel is considered an important characteristic owing to its effects on the biomass density and stability of the biomass briquettes or pellets. High moisture content will decrease the biomass briquette or pellet density and stability, and a coherent briquette will be challenging to obtain (Safana, 2018).

CONCLUSION

The abundant POME biomass generated in the palm oil mill industry was successfully utilized in this study to produce an alternative sustainable and renewable biomass fuel. POME was utilized as the fermentation medium in 4 different conditions to compare the CEV of the resultant products. The highest CEV obtained was fermentation in the presence of *A. flavus* (25.18 MJ/kg) followed by the control of the experiment, Group 1 in which yielded 22.01 MJ/kg, Group 4 (non-sterile POME), which yielded 21.80 MJ/kg and Group 2 (autoclaved POME with the addition of *Lysinibacillus* sp.) which produced 21.65 MJ/kg. In addition, the composition of carbon, hydrogen and nitrogen of the highest CEV (Day 2 of Group 3 – autoclaved POME with the addition of *A. flavus*) obtained overall are 48.49%, 6.47% and 1.69%, respectively. *A. flavus* is a fungus remarkable for its ability to degrade organic compounds such as lignin and metabolize the organic compound as it is carbon source. *Lysinibacillus* sp. treatment resulted poorly in increasing CEV of POME biomass fuel as well as exhibited the most cracks of the biomass pellet, howbeit, the treatment by *Lysinibacillus* sp. strain revealed high COD and BOD removal efficiency of 59.20% and 320.44 mg/L as well as the highest reduction of oils and grease among other groups with the value of 15.84%. In order to further improve the effectiveness of POME treatment, future research directions are recommended for the elucidation of co-fermentation in the presence of both *Lysinibacillus* sp. and *A. flavus*. This method is expected to reduce the COD, BOD and simultaneously obtain a biomass fuel with high CEV. In terms of pellet compactness, the addition of natural binders such as starch is suggested to further increase the density. This study evinced the usage

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

conceptualization of the strains for the fermentation process and another drying method such as an industrial scale drum dryer is suggested to be applied.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support from the Ministry of Higher Education, Malaysia, to the Universiti Sains Malaysia (USM), through the Fundamental Research Grant Scheme (FRGS), with a project code of FRGS/1/2021/STG01/USM/02/12.

REFERENCES

Abdel-Aal, H. K. (2021). Oil and gas industry overview. *In*: Economic Analysis of Oil and Gas Engineering Operations. CRC Press, Boca Raton. **pp. 3-8.**

Abdullah, N. and Sulaiman, F. (2013). The oil palm wastes in Malaysia. *In*: Biomass Now - Sustainable Growth and Use. Matovic, M. D. (ed.). IntechOpen, London.

Asemoloye, M. D., Marchisio, M. A., Gupta, V. K. and Pecoraro, L. (2021). Genome-based engineering of ligninolytic enzymes in fungi. *Microbial Cell Factories* **20, 20.**

Atlas, R. M. (2004). Handbook of Microbiological Media. CRC Press, Boca Raton.

Atnaw, S. M., Sulaiman, S. A. and Yusup, S. (2014). Influence of fuel moisture content and reactor temperature on the calorific value of Syngas resulted from gasification of oil palm fronds. *The Scientific World Journal* **2014, Article ID 121908.**

Awad, O. I., Mamat, R., Ibrahim, T. K., Hagos, F. Y., Noor, M. M., Yusri, I. M. and Leman, A. M. (2017). Calorific value enhancement of fuel oil by moisture removal and its effect on the performance and combustion of a spark ignition engine. *Energy Conversion and Management* **137, 86-96.**

Awalludin, M. F., Sulaiman, O., Hashim, R. and Nadhari, W. N. A. W. (2015). An overview of the oil palm industry in Malaysia and its waste utilization through thermochemical conversion, specifically via liquefaction. *Renewable and Sustainable Energy Reviews* **50, 1469-1484.**

Bajpai, P. (2018). Environmental impact: *In*: Biermann's Handbook of Pulp and Paper. Elsevier, Netherlands. **pp. 325-348.**

Bala, J. D., Lalung, J. and Ismail, N. (2014). Biodegradation of palm oil mill effluent (POME) by bacterial. *International Journal of Scientific and Research Publications* **4(3), 502-511.**

Baranitharan, E., Khan, M. R., Prasad, D. M. R., Teo, W. F. A., Tan, G. Y. A. and Jose, R. (2015). Effect of biofilm formation on the performance of microbial fuel cell for the treatment of palm oil mill effluent. *Bioprocess and Biosystems Engineering* **38(1), 15-24.**

Barapatre, A. and Jha, H. (2016). Decolourization and biological treatment of pulp and paper mill effluent by lignin-degrading fungus *Aspergillus flavus* strain F10. *International Journal of Current Microbiology and Applied Sciences* **5(5), 19-32.**

Bendicho, C. and Lavilla, I. (2019). Water analysis: Sewage. *In*: Encyclopedia of Analytical Science. Worsfold, P., Townshend, A., Poole, C. and Miró, M. (eds.). Elsevier, Amsterdam. **pp. 371-381.**

Boey, J. Y., Mohamad, L., Khok, Y. S., Tay, G. S. and Baidurah, S. (2021). A review of the applications and biodegradation of polyhydroxyalkanoates and poly(lactic acid) and its composites. *Polymers* **13(10), 1544.**

Booth, C. (1971). The Genus Fusarium. Commonwealth Agricultural Bureaux, Commonwealth Mycological Institute, Kew, United Kingdom.

Chan, Y. J. and Chong, M. F. (2019). Palm oil mill effluent (POME) treatment – Current technologies, biogas capture and challenges. *In*: Green Technologies for the Oil Palm Industry. Foo, D. C. Y. and Tun Abdul Aziz, M. K. (eds). Springer, Singapore. **pp. 71-92.**

Cheng, Y. W., Lee, Z. S., Chong, C. C., Khan, M. R., Cheng, C. K., Ng, K. H. and Hossain, S. S. (2019). Hydrogen-rich syngas production via steam reforming of palm oil mill effluent (POME) – A thermodynamics analysis. *International Journal of Hydrogen Energy* **44(37), 20711-20724.**

Dashti, A. F., Fatehah, M. O. and Zahed, M. A. (2021). Waste management of the palm oil industry: Present status and future perspective. *Journal of Environmental Engineering and Science* **17(2), 75-88.**

Demirbas, A. (2007). Effects of moisture and hydrogen content on the heating value of fuels. *Energy Sources, Part A: Recovery, Utilization and Environmental Effects* **29(7), 649-655.**

Dominic, D. and Baidurah, S. (2022). Recent developments in biological processing technology for palm oil mill effluent treatment – A review. *Biology* **11(4), 525.**

Garritano, A. N., de Oliveira Faber, M., De Sá, L. R. V. and Ferreira-Leitão, V. S. (2018). Palm oil mill effluent (POME) as raw material for biohydrogen and methane production via dark fermentation. *Renewable and Sustainable Energy Reviews* **92, 676-684.**

Hassan, S., Kee, L. S. and Al-Kayiem, H. H. (2013). Experimental study of palm oil mill effluent and oil palm frond waste mixture as an alternative biomass fuel. *Journal of Engineering Science and Technology* **8(6), 703-712.**

Islam, M. A., Rahman, M., Yousuf, A., Cheng, C. K. and Wai, W. C. (2016). Performance of *Klebsiella oxytoca* to generate electricity from POME in microbial fuel cell. *MATEC Web of Conferences*, ***38*.**

Islam, M. A. (2017). Bioelectrochemical behavior of wild type *Bacillus cereus* in dual chamber microbial fuel cell. *IIUM Engineering Journal* ***18*(2), 79-86.**

Islam, M. A., Ethiraj, B., Cheng, C. K., Yousuf, A. and Khan, M. M. R. (2018). An Insight of Synergy between *Pseudomonas aeruginosa* and *Klebsiella variicola* in a Microbial Fuel Cell. *ACS Sustainable Chemistry and Engineering* ***6*(3), 4130-4137.**

Kamyab, H., Chelliapan, S., Din, M. F. M., Rezania, S., Khademi, T. and Kumar, A. (2018). Palm Oil Mill Effluent as an Environmental Pollutant. *In*: Palm Oil. IntechOpen. **pp. 13-28.**

Kassim, M. A., Meng, T. K., Serri, N. A., Yusoff, S. B., Shahrin, N. A. M., Seng, K. Y., Bakar, M. H. A. and Keong, L. C. (2020). Sustainable biorefinery concept for industrial bioprocessing. *In*: Biorefinery Production Technologies for Chemicals and Energy. Kuila, A. and Mukhopadhyay, M. (eds.). John Wiley and Sons, USA. **pp. 15-53.**

Liew, W. L., Kassim, M. A., Muda, K., Loh, S. K. and Affam, A. C. (2015). Conventional methods and emerging wastewater polishing technologies for palm oil mill effluent treatment: A review. *Journal of Environmental Management* **149, 222-235.**

Loh, S. K. (2017). The potential of the Malaysian oil palm biomass as a renewable energy source. *Energy Conversion and Management* **141, 285-298.**

Lok, X., Chan, Y. J. and Foo, D. C. Y. (2020). Simulation and optimisation of full-scale palm oil mill effluent (POME) treatment plant with biogas production. *Journal of Water Process Engineering* **38(1), 101558**

Low, T. J., Mohammad, S., Sudesh, K. and Baidurah, S. (2021). Utilization of banana (*Musa* sp.) fronds extract as an alternative carbon source for poly (3-hydroxybutyrate) production by *Cupriavidus necator* H16. *Biocatalysis and Agricultural Biotechnology* **34, 102048.**

Makhtar, M. M. Z. and Tajarudin, H. A. (2020). Electricity generation using membrane-less microbial fuel cell powered by sludge supplemented with lignocellulosic waste. *International Journal of Energy Research* **44(4), 3260-3265.**

Malmgren, A. and Riley, G. (2018). Biomass power generation. *In*: Reference Module in Earth Systems and Environmental Sciences. Elsevier, Amsterdam, Netherlands.

Mohammad, S., Baidurah, S., Kamimura, N., Matsuda, S., Bakar, N. A S. A., Muhamad, N. N. I., Ahmad, A. H., Dominic, D. and Kobayashi, T. (2021a). Fermentation of palm oil mill effluent in the presence of *Lysinibacillus* sp. LC 556247 to produce alternative biomass fuel. *Sustainability* **13(21), 11915.**

Mohammad, S., Baidurah, S., Kobayashi, T., Ismail, N. and Leh, C. P. (2021b). Palm oil mill effluent treatment processes – A review. *Processes* **9(5), 739.**

Muzzammil, N. and Loh, S. K. (2020). Pilot scale integrated anaerobic-aerobic treatment of palm oil mill effluent. *Journal of Oil Palm Research* **32(2), 286-293.**

Ng, K. H. (2021). Adoption of TiO2-photocatalysis for palm oil mill effluent (POME) treatment: strengths, weaknesses, opportunities, threats (SWOT) and its practicality against traditional treatment in Malaysia. *Chemosphere* ***270*****, 129378.**

Nyakuma, B. B., Johari, A., Ahmad, A. and Abdullah, T. A. T. (2014). Comparative analysis of the calorific fuel properties of empty fruit bunch fiber and briquette. *Energy Procedia* **52, 466-473.**

Onoja, E., Chandren, S., Abdul Razak, F. I., Mahat, N. A. and Wahab, R. A. (2019). Oil palm (*Elaeis guineensis*) biomass in Malaysia: The present and future prospects. *Waste and Biomass Valorization* **10(8), 2099-2117.**

ÖzyuğUran, A. and Yaman, S. (2017). Prediction of calorific value of biomass from proximate analysis. *Energy Procedia* **107, 130-136.**

Reddish, G. F. (1919). A substitute for beer wort as a bacteriological culture medium. *Journal of Bacteriology* **3, 6-7.**

Rena, G. P. and Kumar, S. (2019). Landfill gas as an energy source. *In*: Current Developments in Biotechnology and Bioengineering: Waste Treatment Processes for Energy Generation. Kumar, S., Kumar, R. and Pandey, A. (eds.). Elsevier, Amsterdam. **pp. 93-117.**

Safana, A. A. (2018). The mixture of bio-oil and biochar produced from slow pyrolysis of oil palm wastes for briquettes production and combustion as solid fuels. M.Sc. Thesis. Universiti Sains Malaysia, Malaysia.

Salihu, S. O. and Bakar, N. K. A. (2018). Modified APHA closed-tube reflux colorimetric method for TOC determination in water and wastewater. *Environmental Monitoring and Assessment* **190(6), 369.**

Samson, R. A., Houbraken, J., Thrane, U., Frisvad, J. C. and Andersen, B. (2010). Food and Indoor Fungi. CBS Laboratory Manual Series. CBS-KNAW Fungal Biodiversity Centre, Netherlands.

Sen, K. Y., Hussin, M. H. and Baidurah, S. (2019). Biosynthesis of poly(3-hydroxybutyrate) (PHB) by *Cupriavidus necator* from various pretreated molasses as carbon source. *Biocatalysis and Agricultural Biotechnology* **17, 51-59.**

Sen, K. Y. and Baidurah, S. (2020). Renewable biomass feedstocks for production of sustainable biodegradable polymer. *Current Opinion in Green and Sustainable Chemistry* **27, 100412.**

Shamsuddin, R., Singh, G., Kok, H. Y., Hakimi Rosli, M., Dawi Cahyono, N. A., Lam, M. K., Lim, J. W. and Low, A. (2021). Palm oil industry – Processes, by-product treatment and value addition. *In*: Sustainable Bioconversion of Waste to Value Added Products. Inamuddin and Khan, A. (eds.). Springer, Cham. **pp. 121-143.**

Shmeis, R. M. A. (2018). Water chemistry and microbiology. *Comprehensive Analytical Chemistry* **81, 1-56.**

Soleimaninanadegani, M. and Manshad, S. (2014). Enhancement of biodegradation of palm oil mill effluents by local isolated microorganisms. *International Scholarly Research Notices* **2014, Article ID 727049**

Suwanno, S., Rakkan, T., Yunu, T., Paichid, N., Kimtun, P., Prasertsan, P. and Sangkharak, K. (2017). The production of biodiesel using residual oil from palm oil mill effluent and crude lipase from oil palm fruit as an alternative substrate and catalyst. *Fuel* **195, 82-87.**

Tabakaev, R., Shanenkov, I., Kazakov, A. and Zavorin, A. (2017). Thermal processing of biomass into high-calorific solid composite fuel. *Journal of Analytical and Applied Pyrolysis* **124, 94-102.**

Tabassum, S., Zhang, Y. and Zhang, Z. (2015). An integrated method for palm oil mill effluent (POME) treatment for achieving zero liquid discharge – A pilot study. *Journal of Cleaner Production* **95, 148-155.**

Thangarasu, V. and Anand, R. (2019). Physicochemical fuel properties and tribological behavior of aegle marmelos correa biodiesel. *In:* Advances in Eco-Fuels for a Sustainable Environment. Elsevier. **pp. 309-336.**

The Commissioner of Law Revision, Malaysia; Federal Subsidiary Legislation, Division of Environment. Environmental Quality (Prescribed Premises) (Crude Palm Oil) (Amendment) Regulations 1982. Available online: https://www.doe.gov.my/portalv1 /wp-content/uploads/2015/01/Environmental_Quality_Prescribed_Premises_Crude_Palm_Oil_Amendment_Regulations_ 1982_-P.U.A_183-82.pdf (accessed on 13 October 2021).

Wood, D. A. (2019). Transparent open-box learning network provides auditable predictions for coal gross calorific value. *Modeling Earth Systems and Environment* **5(2), 395-419.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538