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Fermentation of Palm Oil Mill Effluent in the Presence of Lysinibacillus sp. LC 556247 to Produce Alternative Biomass Fuel

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Abstract: A bacterial strain, identified as Lysinibacillus sp. LC 556247 POME, was isolated from palm oil mill effluent (POME). The present article highlights the potential utilization of POME as a sole fermentation medium by Lysinibacillus sp. LC 556247 to produce biomass fuel via aerobic fermentation. The fermentation was performed in a shake flask with a working volume of 300 mL, agitated at 180 rpm, incubated at 35 ± 2 °C for various fermentation hours, ranging from 1, 2, 3, 4, 24, 48, 72, 96, and 120 h, and was followed by a drying process. Elucidation of the POME characteristics, calorific energy values (CEV), moisture content (MC), oil and grease content, chemical oxygen demand (COD), biochemical oxygen demand (BOD), dissolved oxygen (DO), total suspended solids (TSS), pH, total nitrogen, and the colony-forming unit (CFU) were performed. The results demonstrate that the highest CEV, of 21.25 ± 0.19 MJ/kg, was obtained at 48 h fermentation. High amounts of extractable oil and nitrogen content were retrieved at the highest CEV reading of the fermented and dried POME samples, which were 17.95 ± 0.02% and 12.80 ± 0.08%, respectively. The maximum removal efficiencies for the COD (50.83%), the BOD (71.73%), and the TSS (42.99%) were achieved at 120 h of fermentation, with an operating pH ranging from 4.49–4.54. The XRF analysis reveals that the fermented and dried products consisted of elements that had a high amount of carbon and potassium, and a significantly low amount of silica, which is sufficient for the effective burning of biomass fuel in the boiler.

Keywords: Lysinibacillus sp.; palm oil mill effluent treatment; batch fermentation; biomass fuel; calorific energy value

1. Introduction

The oil palm industry is the largest contributor to biomass in Malaysia. Over the years, oil palm biomass has been constantly produced in massive amounts, with a small portion being converted into value-added products, while a large portion is left and underutilized. The oil palm biomass, originating from plantations and mills, can be categorized into two groups in solid and liquid forms. In the solid form are empty fruit bunches (EFB), palm fronds (PF), palm trunks (PT), mesocarp fibers (MF), palm kernel shells (PKS), and the liquid form of palm oil mill effluent (POME). The later liquid waste of the POME becomes...
the most significant pollutant from the oil palm mills since it has been generated in vast amounts, and it concomitantly creates serious environmental problems [1]. POME exists as a viscous brownish liquid containing more than 94–95% water, together with oils and total solids, mainly from the fruit debris, and is known to be a nontoxic substance. However, POME contains high organic content of COD, BOD, and a high quantity of oil and grease, with numerous amounts of plant nutrients. As a rich source of biomass, POME has been developed into potential products for varied applications to methane-rich biogas, fertilizers, biochar, bio-oil, and biomass fuel [2].

To date, Malaysia has begun to valorize POME for the biogas treatment plant as a source of renewable energy because it has been claimed that this is a promising alternative source of raw materials for the biogas and biomass industries [3]. It is known that Malaysia is one of the world’s primary palm oil producers and that it has been taking steps to promote the use of renewable energy. The valorization of renewable energy sources is considered to be viable for oil palm waste, and it can contribute to the country’s sustainability of the energy supply and minimize the current negative impacts on the environment. The valorization of industrial waste to other value-added products via fermentation is deemed practical and has been widely applied by many researchers [4–8].

Oil palm wastes, especially POME, is a major problem in palm oil factories because of their abundance and disposal problems. Currently, POME has been valorized using fermentation techniques, such as aerobic, anaerobic, or dark conditions to produce various value-added products [9–12]. The valorization of industrial wastewater as a feedstock to produce alternative renewable biofuels, in lieu of the over-reliance on fossil fuels, could be an ideal option and could simultaneously minimize the environmental burden posed by the oil palm factories. Several researchers have reported on the utilization of various feedstocks, viz., sawdust [13], POME sludge and PF mixture [14], pine trees [15], sewage sludge [16], banana waste [17], and EFB [18] as alternative fuels and have investigated their energy properties [19]. Purvanto et al. [20] reported on the utilization of EFB, rubberwood, and coconut fiber to produce biomass fuel with a CEV ranging from 16.59–17.41 MJ/kg. Briquette and pellet fuel, derived from EFB and MF, has an average CEV of 17.84–18.23 MJ/kg and is produced by the Malaysian Oil Palm Board [21]. Furthermore, a local Malaysian company supplied an EFB pellet with a considerably low CEV of 17.16 MJ/kg. Onoja et al., utilized oil palm fronds (OPF) as an alternative source of biofuel, yielding higher CEVs of 19.21 MJ/kg with 46.98% C, 22.10% K, and 19.20% Si as the major elements [22].

POME exists as an acidic substance with an unpleasant odor and high values of COD (15,000–100,000 mg/L), BOD (10,250–43,750 mg/L), and TSS (5000–54,000). Other elements have also been reported: C 51.0%, O 35.3%, Na 0.0632%, Mg 1.09%, Al 0.215%, Si 0.552%, P 0.429%, S 0.553%, Cl 2.75%, K 6.77%, Ca 1.09%, Mn 0.0243%, Fe 0.141%, and Rb 0.0286% [12].

Although the palm oil milling process generates typical wastewater with its polluting characteristics, POME contains a nutrient-rich organic substrate with varying essential minerals, such as potassium, calcium, magnesium, aluminum, and other nutrients. These major and minor nutrients are essential for microbial growth processes [23]. The amount of mineral ion POME varies accordingly because of the various processing methods among the facilities, which contribute to the variation in terms of the qualities and properties of the extracted oils. In this regard, POME has the strong potential to be developed as a feasible and versatile fermentation feedstock for multiple applications, including energy and nonenergy products [24].

The aforementioned researchers have exploited the varieties of microorganisms for fermentation processes to further increase the CEV. The current utilization of pretreated oat straw was enzymatically saccharified, fermented to produce ethanol by Saccharomyces cerevisiae J672 (industrial isolate obtained from Agroetanol Norkopping, Sweden, Germany), and subjected to biogas digestion, yielding approximately 9.50–9.80 MJ/kg for the applications for biofuel energy [25]. Additionally, biomass derived from the rice industry, such as rice husk and rice straw, displayed a slightly higher CEV, with approximately 15 MJ/kg, but the hindrance of
the high silica content in both feedstocks limits their applications at the industrial scale [26]. To some extent, a researcher conducted simultaneous saccharification and co-fermentation to produce bioethanol by *Candida tropicalis* [27]; however, the resultant CEV was considerably low. Considering that the POME is still underutilized at the industrial scale for alternative biomass fuel applications, the fundamental experiments are deemed crucial prior to proceeding with the comprehensive studies on the detailed physicochemical properties of POME-derived fuel products. On the basis of the ISO 17225 standard, the best quality of solid biomass fuel or pellets must possess MC ≤ 10%, a net CEV of ≥16 MJ/kg, and a reduced percentage of fines fraction (≤6%) [28].

Limited studies have reported on the aerobic fermentation process for the treatment of organic pollutants in POME, especially for biomass fuel utilization with a high CEV. Hence, the aerobic fermentation of POME using a locally isolated strain from a POME source, *Lysinibacillus* sp. LC 556247, is deemed attractive and the novelty has been ascertained in the studied parameters. In the present study, POME was utilized as fermentation media in the presence of a locally isolated bacteria strain from Malaysian soil, identified as *Lysinibacillus* sp. LC 556247. The main objective was to further increase the CEV via fermentation for the potential application as an alternative biomass fuel.

2. Materials and Methods

2.1. Isolation and Identification of Bacterial Strain from the POME

POME samples, prior to entering the treatment pond used in this study, were collected from a palm oil mill located at Pulau Pinang, Malaysia (geographical coordinates were ranged at 5°09′22.3″ N and 100°30′32.3″ E). Approximately 1 mL of POME samples (serially diluted up to 10^8 CFU/mL) were spread onto a sterile nutrient agar (NA) plate and incubated at 35 ± 2 °C for 24 h. Upon the formation of single colonies on the NA plates, a colony was streaked again onto a new NA plate and incubated overnight to obtain a pure single bacterial colony. Then, the bacterial colony was cultured in the nutrient broth (NB) medium at 35 ± 2 °C, with agitation at 180 rpm, for approximately 14–16 h. The cells were harvested by centrifugation at 12,000 rpm and 4 °C. Then, the supernatant part was removed, and the bacterial pellet was subjected to DNA extraction. The single colony formed on the NA plate was stained using methylene-blue and safranin, followed by observation with a compound microscope (Model CX41, Olympus Corp., Tokyo, Japan).

The genomic DNA (gDNA) of an isolated strain was extracted using Nucleospin® Tissue (Takara, Otsu, Japan). Upon completion of the gDNA extraction, the region containing the 16S rRNA gene was amplified using the universal bacterial primers, 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGCTACCTTGTTACGACTT-3′), and gDNA using Q5 high-fidelity DNA polymerase (New England BioLabs, MA, USA) by polymerase chain reaction (PCR). The nucleotide sequence of the 1.5-kb amplified fragment, separated on 0.8% agarose gel electrophoresis, was determined by Eurofins Genomics (Tokyo, Japan). The nucleotide sequences of the 16S rRNA gene of the *Lysinibacillus* sp. LC 556247 strain of POME was deposited in the DDBJ/EMBL/GenBank database, under the accession number, LC556247. The 16S rRNA gene sequence similarities were searched using the BLAST programs in the National Centre for Biotechnology Information (NCBI) database. The nucleotide sequences of the 16S rRNA gene of the Bacillaceae were obtained from NCBI. The multiple alignments with the Clustal W program, and the construction of a phylogenetic tree with a maximum likelihood algorithm (bootstrap repeat, 1000), were performed using the MEGAX program version 10.1.8 [29].

2.2. Microorganism, Inoculum Preparation, and Growth Profile

The isolated bacteria, *Lysinibacillus* sp. LC 556247, were grown and enriched by incubation in a rotary shaker at 35 ± 2 °C and 180 rpm, overnight in a sterile NB medium. Once the optical density (OD) of the bacteria cells reached 0.6–0.8 at 600 nm, the bacteria were inoculated (10% w/v) into the fermentation medium. POME was used as the sole feedstock in the fermentation. The growth profile of *Lysinibacillus* sp. LC 556247 was performed using...
a 250 mL shake flask with a working volume of 100 mL, in which approximately 10% (w/v) of the strain was inoculated into a sterile NB medium. The culture was then incubated at 35 ± 2 °C, with an agitation speed of 180 rpm, for 68 h of fermentation. A UV-Vis spectrophotometer (Shimadzu UVmini-1240, Shimadzu Corp., Kyoto, Japan) was used to determine the bacterial cell growth at a wavelength of 600. The OD reading of the POME during fermentation was performed every 2 and 4 h, and a growth curve was plotted.

2.3. Batch Fermentation of the Lysinibacillus sp. LC 556247

The fermentation was performed under three different conditions: (1) Group 1: autoclaved POME with the addition of Lysinibacillus sp. LC 556247; (2) Group 2: autoclaved POME without Lysinibacillus sp. LC 556247; and (3) Group 3: nonsterile POME (as it is). The nonsterilized POME (unprocessed, as it is) was included in this study in order to elucidate the enhancement of the CEV from the degradation mechanisms of the bacteria, and other consortiums of microbes, in its natural condition. A total volume of 300 mL of POME was subjected for batch fermentation utilizing Lysinibacillus sp. LC 556247. The fermentation experiment was performed in triplicate. The temperature was set at 35 ± 2 °C, and the agitation at 180 rpm, for various fermentation times, ranging from 1 to 120 h. The pH was recorded throughout the experiment. Upon the fermentation’s designated time, of 1, 2, 3, 4, 24, 48, 72, 96, and 120 h, all of the fermented POME samples were dried overnight at 105 °C using an oven (Binder World FD056UL, Binder GmbH, Tuttlingen, Germany) [30]. Then, the dried POME powder samples were subjected to further analyses.

2.4. Analytical Methods and Characterization of POME

The MC of the samples was determined using a standard oven-drying method, at 105 °C, until a constant weight was obtained [30]. The CEV was determined by employing an oxygen bomb calorimeter (ParrTM 6200, Fisher Scientific International Inc., Pittsburgh, USA) using approximately 0.5–0.6 g of dried sample, which was finely ground and sieved. The sample was weighed in a stainless-steel crucible (43AS), placed in the combustion vessel, and filled with oxygen at 99.95% purity until the pressure reached 450 psi (3.0 ± 0.2 MPa). The combustion vessel was inserted into the bucket and ignited at the following conditions: pre-fire: 3 m; post-fire: 5 m; fuse wire length: 10 cm; bucket and jacket temperatures: 13–33 °C. The colony-forming unit (CFU) was performed by diluting 100 µL of POME samples (serially diluted up to 1020 CFU/mL) and then spreading them on NA plates, for each of the designated sampling hours, during the 5 days of fermentation. Afterwards, the CFU was calculated after 24 h to evaluate the growth kinetics of Lysinibacillus sp. LC 556247. The total nitrogen content (%) was determined by using the Kjeldahl-digestion method [30]. The pH of the POME, as a fermentation medium, was measured using a pH meter (Eutech, Thermo Fischer Scientific Instruments, Massachusetts, USA). The oil and grease content were evaluated by using the Soxhlet-extraction method utilising, utilizing n-hexane as a solvent. The elemental analysis of the dried POME powder samples was performed by using X-ray fluorescence (ZSX Primus II, Rigaku Corp., Tokyo, Japan) and micro-X-ray fluorescence (Micro XRF, Bruker Corporation, Berlin, Germany).

The organic contents present in the POME (COD, BOD, and TSS) were determined using APHA methods [31]. The dissolved oxygens (DO) were measured using a Hanna portable DO meter (HANNA HI 198193, Hanna Instruments, Woonsocket, RI, USA). The removal efficiency of each parameter was calculated by using Equation (1) [32]: whereby $Ci$ is the initial concentration of pollutants in the POME prior to treatment, and $Cf$ is the final concentration of those contents after 5 days of treatment. All tests were performed in triplicate to confirm the reproducibility.

\[ \text{Removal Efficiency} \ (%) = 100 - \left[ \frac{Ci - Cf}{Cf} \times 100 \right] \]
2.5. Statistical Analyses

All results were expressed based on triplicate determinations. A one-way ANOVA ($\alpha = 0.05$) was used to analyze the data, and a value of less than 0.05 was considered significant. Significant data were analyzed with an F-distribution to compare the means between treatments. The F-statistics were more than the F-critical value considered significantly different. A significant difference between treatments was obtained if the $p$-value was less than 0.05. LEAD Technologies, MINITAB Version 17.1.0, was used to perform the statistical analyses.

3. Results and Discussion

3.1. Strain Identification and General Characteristics of the Isolated Bacteria

A bacterial strain was isolated from POME and identified as *Lysinibacillus* sp. LC 556247. In order to identify the taxonomy of the strain, the nucleotide sequence of the 16S rRNA gene of the strain was determined for the partial nucleotide sequence (616 bp, accession number in NCBI: LC556247) of the 16S rRNA gene. This strain showed a 100% identity as *Lysinibacillus boronitolerans* (NR_114207.1), *Lysinibacillus capsici* (PXXX01000046.1), *Lysinibacillus fusiformis* (NR_112569.1), and *Lysinibacillus macroides* (NR_114920.1). Moreover, the phylogenetic analysis indicated that the bacteria strain was genus *Lysinibacillus* (Figure 1). A related species of *Lysinibacillus* sp. LC 556247, *Lysinibacillus boronitolerans* is known to be abundant in the environment.

![Figure 1. A phylogenetic tree of the bacterial isolate from the POME based on the 16S rRNA gene sequences. Bootstrap values (based on 1000 replicates) are given at branch points. Nucleotide sequences of the 16s rRNA gene from the POME-type strains of *Lysinibacillus* and Bacillaceae were used for phylogenetic analysis. The superscript ‘T’ indicates the type of strain. Bar: 0.01 substitutions per nucleotide position. The accession numbers of the gene sequences in the GenBank database are shown within brackets.](image-url)
Figure 2 shows a picture of the compound microscope view of the *Lysinibacillus* sp. LC 556247 after the incubation period of 24 h, stained using methylene blue and safranin. At 1000× magnification, the picture exhibited the healthy growth of rod-shaped bacterial cells. *Lysinibacillus* sp. LC 556247 is a Gram-positive motile rod-shaped round-spore-forming and boron-tolerant bacterium. It was apparent that the bacterial cells were about 3.0–5.0 mm in length, and 0.8–1.5 mm in diameter, with circular or flat colonies on the agar media [33,34]. At the time of writing, the genus had the following species with validly published names: *L. fusicornis*; *L. sphaericus* [35]; *L. parviboronicapiens* [36]; *L. xylanilyticus* [37]; *L. macroides* [38]; *L. mangifer-alumii* [39]; *L. sinduriensis; L. massilienis; L. odyssey* [40]; *L. tabacifolii* [41]; *L. chungkukjangi* [42]; *L. fluoroglycophenilyticus* [43]; *L. alkalosili* [44]; *L. louembei* [45]; and *L. manganicus* [46].

This type of bacteria, *Lysinibacillus* sp. LC 556247, is ubiquitous in soil [35] and is isolated from plant tissues [47], the plant-seed-fermented products of soybean foods, and even from pufferfish liver specimens [34]. It is well known that POME is rich in carbohydrates, proteins, nitrogenous compounds, fatty acids, lipids, minerals, celluloses, hemicelluloses, and lignin [48]. *Lysinibacillus* sp. LC 556247 is expected to have the potential to consume the oils and carbon sources that exist in POME [49].

### 3.2. Growth Profiles of *Lysinibacillus* sp. LC 556247 and Colony-Forming Units (CFU)

The bacterial growth profile of *Lysinibacillus* sp. LC 556247 is delineated in Figure 3. Similar to other microorganisms, bacteria go through different stages of growth (lag phase, logarithmic phase, stationary phase, and death phase) [50]. Upon inoculation into a fresh growth medium, the bacteria cells enter a brief lag phase, whereby they are biochemically active but not dividing. A lag phase occurs over the first two hours, known as the latency phase [51]. The lag phase refers to the initial growth phase, in which some cells remain relatively constant prior to rapid growth, also known as adaptation time. During this phase, the bacterial cells are metabolically active, synthesizing ribosomes and enzymes, and adapting to their new physical and chemical environment before the cells starts to divide [51]. The length of an organism’s lag phase is dependent on its ability to adjust to new population sizes and environmental conditions, such as temperature, pH, oxygen intake and uptake, salt concentrations, nutrients, and others. After two hours of incubation, the log phase was observed and remained until 20 h, whereby the growth occurred
exponentially. In the log phase, the bacterial cells begin to grow most rapidly, as they have been introduced to the new environmental conditions. At this stage, the population of the bacterial cells has increased up to $10^9$ to $10^{10}$ cells/mL [52].

![Growth profiles of Lysinibacillus sp. LC 556247](image)

**Figure 3.** Growth profiles of *Lysinibacillus* sp. LC 556247 in nutrient broth (NB) medium until 68 h of incubation. Error bars indicate the mean ± standard deviation of triplicate experiments.

After 20 h of incubation, the growth rate was in the stationary phase condition, and began to decline after 60 h. At this stage, the cell metabolism becomes slow, and there is no rapid cell division. In this study, the *Lysinibacillus* sp. LC 556247 was chosen to acclimatize overnight (10–12 h), which indicates the logarithmic phase of the *Lysinibacillus* sp. LC 556247 growth profile. During the log phase, the optical density at 600 nm of the bacterial cells reached 0.6 to 0.8, demonstrating that the bacterial cells were matured enough and ready to be inoculated into a new fermentation medium. The numbers of *Lysinibacillus* sp. LC 556247 colonies in the POME medium are presented (Figure 4) and expressed in the log10 CFU.

![The numbers of Lysinibacillus sp. LC 556247 colonies in the various conditions of the POME medium](image)

**Figure 4.** The numbers of *Lysinibacillus* sp. LC 556247 colonies in the various conditions of the POME medium, expressed in log10 CFU. Error bars indicate the mean ± standard deviation of triplicate experiments.
Figure 4 shows that the numbers of the bacterial colonies in Group 1 increased from 0 h to 48 h. The population ranged from 8.42–9.31 log10 CFU during the initial stage of fermentation, and achieved maximum growth at 48 h of fermentation, with a population of 20.84 log10 CFU. The colonies remained stagnant from 72 h to 120 h of fermentation. The *Lysinibacillus* sp. LC 556247 used in the fermentation process is an indigenous bacterial isolate from the POME source itself. With regard to Group 2, autoclaved POME, and without the addition of *Lysinibacillus* sp. LC 556247, indicated no viable colony throughout the 120 h of fermentation time. The population of consortium microbes in the nonsterile POME for Group 3 (as it is, utilized without any pretreatment or autoclave) ranged from 11.76–20.95 log10 CFU at the initial stage of the fermentation process, and started to yield maximum growth at 48 h, with 21.08 log10 CFU. From 72 h to 96 h, with the utilization of fermentation, the colonies reduced slightly, and become stagnant at 20.80 log10 CFU. The POME in the presence of *Lysinibacillus* sp. LC 556247 in Group 1 showed slightly lower cell growth as compared to the nonsterile POME medium in Group 3. This can be attributed to the single inoculation of a bacteria type in Group 1, in lieu of the mixed consortium of bacterial and fungus in the nonsterile POME medium. Additionally, the lower cell growth log10 CFU obtained was due to the adaptational stage of *Lysinibacillus* sp. LC 556247 growth. However, there were copious organic compounds and other nutrient sources present in the POME, which promote the acclimated bacteria to proliferate and rapidly consume substrates. The decline of the cell growth after obtaining the maximum growth was due to a higher load of byproducts, such as phenolic compounds and long-chain fatty acids (LCFA), which act as inhibiting agents and provoke bacterial death. Simultaneously, these factors could affect the metabolic process and the growth of the *Lysinibacillus* sp. LC 556247 in POME fermentation as well [9]. These components, such as the inhibiting agents that have been found to exist in POME, have been widely reported to have both phytotoxic and antibiotic properties [53].

### 3.3. Batch Fermentation Utilizing POME as the Sole Carbon Source for Biomass Fuel Application

The batch fermentation of POME in the presence of *Lysinibacillus* sp. LC 556247 was performed under aerobic conditions. The POME properties depend on the quality of the raw material and the palm oil production processes in the respective mills. The POME was initially collected at a high temperature of about 80–85 °C at the mill, and the sample appeared in high-viscosity liquid and was brownish in color, with colloidal suspension, in which water was contained at 95–96% (w/v), oils at 0.6–0.7% (v/v), with 4–5% (w/v) total solids, and 2–4% suspended solids. The POME medium, with the varied conditions, was fermented for 1, 2, 3, 4, 24, 48, 72, 96, and 120 h, and oven-dried at 105 °C until a constant weight was observed. The detailed results regarding the weight reduction, MC, and CEV are delineated in Table 1 for each designated fermentation time.

With regard to Table 1, the weight reduction of all of the POME samples was in the range of 94.00–95.70%, parallel with the MC values of the POME samples on a dry weight basis. Overall, the MC values were reduced to less than 3%. For the biomass fuel application, a sample with a high MC requires extra energy for heating and evaporating the existing water and will defer to the combustion process. The incomplete combustion process will lead to less energy per kg released and renders a low CEV. Thus, it is preferable to obtain a low MC for the biomass fuel product. The CEV were defined as the feedstock’s energy as estimated from the heat released during combustion. For Group 1, the POME fermentation in the presence of *Lysinibacillus* sp. LC 556247 at 48 h demonstrated the highest CEV of 21.25 ± 0.19 MJ/kg. The same fermentation conditions at 72 h of fermentation showed the second highest CEV of 20.27 ± 0.18 MJ/kg. The fermentation of POME without *Lysinibacillus* sp. LC 556247 at 120 h demonstrated the highest CEV of 20.04 ± 0.02 MJ/kg. In contrast, the fermentation of the nonsterile POME showed the highest CEV at 1 h of fermentation with 18.74 ± 0.05 MJ/kg. The overall performances were significantly tested at a significance level of α = 0.05. The fermentation without the presence of *Lysinibacillus* sp. LC 556247 and the nonsterile POME portray slightly lower CEVs, as compared to that
containing *Lysinibacillus* sp. LC 556247. The low CEVs are due to the low bacterial activity required to degrade the organic content and, consequently, the low production of the carbon element in the POME. A detailed elaboration regarding the carbon element produced, and the CEV, are discussed below.

Table 1. Weight reduction, moisture content, and calorific value of dried POME powder samples throughout 120 h fermentation.

| Fermentation Time (h) | Group | Weight Reduction (%) | MC (%) | CEV (MJ/kg) |
|-----------------------|-------|----------------------|--------|-------------|
| 0                     | G1    | 95.52                | 1.41 ± 0.01 | 18.04 ± 0.05 d |
|                       | G2    | 95.70                | 2.75 ± 0.04 | 17.78 ± 0.02   |
|                       | G3    | 95.72                | 2.18 ± 0.04 | 18.10 ± 0.02   |
| 1                     | G1    | 95.60                | 1.54 ± 0.01 | 17.89 ± 0.22 c |
|                       | G2    | 95.69                | 2.42 ± 0.00 | 17.48 ± 0.07   |
|                       | G3    | 95.60                | 2.10 ± 0.01 | 18.74 ± 0.05   |
| 2                     | G1    | 95.32                | 1.67 ± 0.01 | 19.16 ± 0.10 d |
|                       | G2    | 95.37                | 2.52 ± 0.01 | 18.59 ± 0.33   |
|                       | G3    | 95.32                | 1.75 ± 0.05 | 18.58 ± 0.04   |
| 3                     | G1    | 95.29                | 1.27 ± 0.01 | 19.36 ± 0.04 c |
|                       | G2    | 95.15                | 2.27 ± 0.01 | 16.85 ± 0.56   |
|                       | G3    | 95.10                | 1.49 ± 0.03 | 18.13 ± 0.07   |
| 4                     | G1    | 95.55                | 1.42 ± 0.01 | 18.06 ± 0.13 d |
|                       | G2    | 95.57                | 2.03 ± 0.01 | 17.71 ± 0.19   |
|                       | G3    | 95.02                | 1.76 ± 0.09 | 18.05 ± 0.04   |
| 24                    | G1    | 95.48                | 1.32 ± 0.02 | 18.23 ± 0.29 d |
|                       | G2    | 95.72                | 1.42 ± 0.02 | 18.01 ± 0.46   |
|                       | G3    | 94.90                | 1.25 ± 0.05 | 17.73 ± 0.06   |
| 48                    | G1    | 94.39                | 1.21 ± 0.01 | 21.25 ± 0.19 a |
|                       | G2    | 94.17                | 1.21 ± 0.01 | 18.86 ± 0.96   |
|                       | G3    | 94.62                | 1.37 ± 0.05 | 17.64 ± 0.01   |
| 72                    | G1    | 94.42                | 1.08 ± 0.02 | 20.27 ± 0.18 b |
|                       | G2    | 94.60                | 1.05 ± 0.01 | 18.39 ± 0.70   |
|                       | G3    | 94.42                | 1.39 ± 0.03 | 17.50 ± 0.01   |
| 96                    | G1    | 94.12                | 0.76 ± 0.04 | 19.29 ± 0.02 c |
|                       | G2    | 94.26                | 0.34 ± 0.04 | 18.25 ± 0.78   |
|                       | G3    | 94.00                | 1.10 ± 0.02 | 17.11 ± 0.04   |
| 120                   | G1    | 94.02                | 0.67 ± 0.01 | 18.69 ± 0.00 c,d |
|                       | G2    | 94.00                | 0.43 ± 0.03 | 20.04 ± 0.02   |
|                       | G3    | 94.01                | 1.26 ± 0.06 | 17.56 ± 0.42   |

Note: G1: Autoclaved POME with *Lysinibacillus* sp; G2: Autoclaved POME without *Lysinibacillus* sp; G3: Nonsterile POME (as it is). Superscript: Means with the same letter are not significant at *p* < 0.05. Means that do not share a letter are significantly different at *α* = 0.05.

In the present study, at the designated time, the fermented and dried POME samples with the highest CEVs of 21.25 ± 0.19 MJ/kg were analyzed by micro XRF for the elemental analysis, as shown in Table 2. Here, high amounts of C 49.70%, O 36.30%, and K 21.44% were detected, while other elements were in minute amounts. Researchers have validated that the high C content simultaneously leads to a high CEV [54]. The high C content, coupled with a low O content, were preferable for the effective combustion of biomass fuel. Here, the fermented and dried POME samples displayed a much lower O content (36.30%), in contrast with the reported value (46.90%) by Loh [54]. This observation is highlighted as one of the novelties of this POME fermentation in the presence of *Lysinibacillus* sp. LC 556247. On the basis of the tabulated data, the fermented and dried POME samples consisted of potassium as one of the major elements. This is also a huge advantage, since the POME is a byproduct disposed from the oil palm mills prior to treatment. Minerals,
especially key metal ions, are often essential and crucial determinants of bacterial growth and fermentation performance.

Table 2. Elemental analysis and CEV of fermented and dried POME samples during batch fermentation in the presence of Lysinibacillus sp. LC 556247.

| Element (wt, %) | POME 0 h | POME 48 h | POME |
|----------------|----------|----------|------|
| Carbon, C *    | 48.60    | 49.70    | 50.01 |
| Oxygen, O *    | 37.80    | 36.30    | 46.90 |
| Potassium, K   | 22.67 ± 0.65 | 21.44 ± 0.81 | 1.85 |
| Chlorine, Cl   | 2.68 ± 0.08 | 2.43 ± 0.07 | 0.10 |
| Silica, Si     | ND       | ND       | 39.60 |
| Calcium, Ca    | 2.99 ± 0.07 | 2.92 ± 0.09 | - |
| Manganese, Mn  | 0.10 ± 0.01 | 0.10 ± 0.01 | - |
| Iron, Fe       | 2.40 ± 0.06 | 2.38 ± 0.06 | 0.05 |
| Zinc, Zn       | 0.04 ± 0.00 | 0.04 ± 0.00 | - |
| Bromine, Br    | 0.03 ± 0.00 | 0.04 ± 0.00 | - |
| Strontium, Sr  | 0.02 ± 0.00 | 0.02 ± 0.00 | - |
| Ruthenium, Ru  | 3.08 ± 0.20 | 3.30 ± 0.14 | - |
| Rhodium, Rh    | 4.34 ± 0.18 | 4.64 ± 0.08 | - |
| Palladium, Pd  | 3.86 ± 0.16 | 4.05 ± 0.07 | - |
| CEV (MJ/kg)    | 18.04 ± 0.05 | 21.25 ± 0.19 | 16.99 ± 0.58 |

Note: POME fermentation with Lysinibacillus sp. LC 556247. Means ± standard deviations, n = 2. ND = Not detected (Si lowest detection limit: < 10 ppm). * Analyzed by XRF ZSX Primus II, Rigaku Corp., Tokyo, Japan.

However, potassium’s nature and concentration is a key example of the ‘bulk’ minerals needed in millimolar concentrations, while Ca, Na, Fe, Zn, Cu, Mn, and Sr are required in micromolar concentrations for bacterial growth. It is interesting to note that POME fermentation, in the presence of Lysinibacillus sp. LC 556247, has an extremely low silica content (lowest detection limit: less than 10 ppm) as compared to the data obtained by Loh [54]. In this finding, the Cl of 2.68% was obtained prior to fermentation and was reduced to 2.43% at 48 h of fermentation. Overall, the POME samples show a positive reduction in some of the major elemental compositions at 48 h of fermentation, except for Ru, Rh, and Pd. This observation suggests that POME can be utilized as the sole carbon source, which is feasible for alternative biomass fuel applications because of its high carbon and potassium content, high calorific energy value, and extremely low silica content.

Figure 5 delineates the total nitrogen content profile during the fermentation process. Nitrogen is originally present in POME in the form of organic (protein) nitrogen and is gradually transformed into ammoniacal nitrogen [23]. The aforementioned researchers have reported on the existing ammoniacal nitrogen and total nitrogen content in POME, circa 35 mg/L and 750 mg/L, respectively [19]. The excessive amount of nitrogen in POME wastewater should be minimized prior to discharge to avoid severe environmental problems, especially eutrophication and toxicity. The highest concentration of total nitrogen in the fermented POME in the presence of the Lysinibacillus sp. LC 556247 strain was achieved at 48 h of fermentation (12.80%), and gradually decreased to 11.61% at 72 h of fermentation. Then, the nitrogen was depleted until 0.82% at 120 h of fermentation. In comparison with the nonsterile POME medium, the total nitrogen content was 8.89% at the earlier stage of fermentation, and continuously reduced until the final amount of 0.14% nitrogen, which is lower than the autoclaved POME without Lysinibacillus sp. LC 556247 (0.42%). These results reveal that Lysinibacillus sp. LC 556247 was able to grow in a nitrogen-limiting medium and was able to metabolize the organic materials present in the effluents. Several researchers have confirmed and reported that the higher dilution of POME samples (50–85%) may also reduce the amount of nitrogen, and other nutrient compositions, in the POME [55]. In this study, POME with a high moisture content (94–95%) was utilized instead of concentrated POME. Thus, the existing nitrogen in the POME medium would be
completely consumed by the *Lysinibacillus* sp. LC 556247 and the consortium of microbes present in the nonsterile POME.

![Graph](image)

**Figure 5.** Total nitrogen content in the fermented and dried POME samples during the fermentation process.

With regard to Figure 5, the nitrogenous compound was in the range of 0.14–12.80%, indicating a high value as compared to the other studies (1.987 ± 0.18%) [56]. In another report, *Bacillus cereus* 103PB was reported to facilitate the denitrification process, which involved the removing of nitrogenous compounds, either in aerobic or anoxic conditions [56]. The nitrogen source in the POME medium was seen to affect the microbial growth of *Lysinibacillus* sp., along with the CEV increment. The overall findings reveal that the complete fermentation process was obtained during 48 h of fermentation, which corresponds to the higher CEV in POME samples in the presence of *Lysinibacillus* sp. LC 556247. In the biomass fuel applications, it is preferable to have a high oil content, as it may contribute to a high CEV and, simultaneously, a better performance of the combustion properties. In this study, it is crucial to determine the oil and grease content in the fermented and dried POME samples. The dried POME samples with the highest CEV from each group were subjected to the oil and grease analysis and the obtained data are summarized in Table 3. In Table 3, POME at 48 h of fermentation with *Lysinibacillus* sp. LC 556247 displays the highest oil and grease content at 17.95%, followed by the nonsterile POME medium with 15.03%, and the POME samples without *Lysinibacillus* sp. LC 556247 with a value of 12.03%. The highest oil and grease content at 48 h fermentation in Group 1 shows the highest CEV at 21.25 ± 0.19 MJ/kg. A high oil content produces a high CEV of biomass fuel. As mentioned by Loh [54], the highly extractable oil content, via the n-hexane solvent, led to high and desirable heating values or calorific values. POME has been revealed to display the highest oil and grease content (4000 mg/L), as compared to the MF, PKS, and other byproducts from the palm oil industry [19]. This data pinpoints that more energy can be retrieved as heat upon the biomass being combusted with the oxygen [54].

In this study, *Lysinibacillus* sp. LC 556247 was isolated from a POME source and was mooted to have the ability to hydrolyze lipid (oil). The POME samples used in this study only consisted of 0.6–0.7% lipid, which may be comprised of major fatty acid components, such as palmitic acid 22.45%, oleic acid 14.54%, myristic acid 12.66%, stearic acid 10.41%, linoleic acid 9.53%, pentadecanoic acid 2.21%, and others [23]. During the aerobic or facultative anaerobic condition, *Lysinibacillus* sp. LC 556247 may consume all the lipids in the POME and convert them into fatty acid and alcohol. This condition is in parallel
with the nutrient consumption, especially the carbon- and nitrogen-source consumption during the fermentation process. Furthermore, the oil and grease secretion in the POME samples at 48 h of fermentation exhibited the complete fermentation of *Lysinibacillus* sp. LC 556247, thus demonstrating complete substrate degradation as well. These properties highlight the novelty of this strain as a highly potential source in biomass fuel applications. *Lysinibacillus* sp. LC 556247 has the potential to degrade the carbon source present in the POME. Various substrates can be completely oxidized by *Lysinibacillus* sp. LC 556247, such as D-ribose, D-tagatose, D-, L-alanine, glycogen, inosine, L-alanyl glycine, β-cyclodextrin, 2-aminoethanol, L-histidine, L-leucine, L-proline, L-threonine, acetic acid, D-, L-lactic acid, L-malic acid, pyruvic acid, propionic acid, adenosine, α-hydroxybutyric acid, α-ketovaleric acid, bromosuccinic acid, and others [35]. Most of the aforementioned substrates are present in the POME, indicating the ability of *Lysinibacillus* sp. LC 556247 to degrade sugar, amino acids, organic acids, and fatty acids.

**Table 3.** Oil and grease content of fermented dried POME samples during batch fermentation in the presence of *Lysinibacillus* sp. LC 556247 for samples with the highest CEV from each group.

| Parameters       | G1       | G2       | G3       |
|------------------|----------|----------|----------|
| Fermentation time (hours) | 0 H | 48 H | 0 H | 120 H | 0 H | 1 H |
| Oil and grease content (%)  | 13.93 ± 0.03 | 17.95 ± 0.02 | 13.95 ± 0.14 | 12.03 ± 0.02 | 13.90 ± 0.02 | 15.03 ± 0.08 |
| CEV (MJ/kg)      | 18.04 ± 0.05 | 21.25 ± 0.19 | 17.78 ± 0.02 | 20.04 ± 0.02 | 18.10 ± 0.02 | 18.74 ± 0.05 |

Note: G1: POME fermentation with *Lysinibacillus* sp.; G2: POME fermentation without *Lysinibacillus* sp.; G3: nonsterile POME. Means ± standard deviations, n = 2.

### 3.4. Biodegradation Study of POME Fermentation

POME contains high concentrations of degradable organic matter that can be quantitatively monitored by COD (50,000 mg/L), BOD (25,000 mg/L), total solids (40,500 mg/L), and total suspended solids (18,000 mg/L) [19]. Figure 6 delineates the batch fermentation performance of *Lysinibacillus* sp. LC 556247. In detail, Figure 6a shows that the COD concentration profile reduced significantly until 120 h of fermentation. POME fermentation in the presence of *Lysinibacillus* sp. LC 556247 indicated the highest COD reduction of 12.24 g/L, followed by nonsterile POME at a 5.16 g/L reduction, and then POME fermentation without *Lysinibacillus* sp. LC 556247 at 0.64 g/L. Figure 6b represents the COD removal efficiency of the POME fermentation. The highest reduction, of 50.83% COD in POME fermentation with *Lysinibacillus* sp. LC 556247, was attributed to organics degradation because the bacteria transformed the organics into their corresponding simplest forms for the assimilation purpose. Only a small reduction in COD removal efficiencies was observed in the nonsterile POME medium (19.88%), followed by the POME without *Lysinibacillus* sp. LC 556247 (11.53%). A similar trend was reported in a recent study, with a significant reduction in the COD concentration of POME and an increase in bacterial growth [57].

The DO concentration, shown in Figure 6c, has a significant role in POME fermentation. Low DO concentrations concomitantly limit the oxygen penetration into the POME medium. Thus, the maintenance of a minimum DO concentration of 2 mg/L, on the final day of the fermentation process, is a reasonable benchmark [58,59]. In this study, the operating DO was found to be 2.23–7.50 mg/L in POME with *Lysinibacillus* sp., followed by 6.77–7.13 mg/L in POME without *Lysinibacillus* sp. LC 556247, and 2.47–7.35 mg/L of nonsterile POME (2.47–7.35 mg/L). The DO concentration indicates the oxygen uptake by the bacteria during the POME fermentation. Interestingly, *Lysinibacillus* sp. LC 556247 was predicted to be categorized as a facultative anaerobe that can survive with or without oxygen. This condition is in a good agreement with the study by Cheng et al. [60] which indicated that the majority of the native microbes in POME, especially *B. cereus, P. vermicola, K. pneumonia*, and *B. subtilis*, were categorized as facultative anaerobes. Under hostile environments, most native POME microbes revert to the dormant stage via sporula-
tion to resist the extremely acidic conditions, high temperatures, anoxic conditions, and oily ambience [57].

The pH changes in the fermentation system have been known to influence the microbial growth; however, the quantitative effects remain unclear. Throughout the fermentation time, the POME was in acidic conditions, with a pH ranging from 3.95–4.54 due to the accumulation of organic acids. In detail, the operating pH in the POME with *Lysinibacillus* sp. LC 556247 was 4.49–4.54 (Figure 6d), followed by 4.41–4.47 in the POME without *Lysinibacillus* sp. LC 556247 (4.41–4.47), and 3.94–4.52 in the nonsterile POME. These values indicate that, throughout the fermentation process, the pH remained acidic (below 5), in all designated groups. These findings reveal that pH was not the critical factor that determined the performance of fermentation, especially towards *Lysinibacillus* sp. LC 556247. However, this implies that the active growth of the microorganisms can be observed and sustained in the acidic condition.

The BOD, COD, and TSS removal efficiencies were elucidated for each designated group of POME fermentation, as presented in Table 4. The substantial reduction in the organic load, particularly in BOD (71.73%) and COD (50.83%), was obtained in POME fermentation in the presence of *Lysinibacillus* sp. LC 556247. This suggests that the isolated strain, *Lysinibacillus* sp. LC 556247, was effective for the BOD reduction. The nonsterile POME medium indicated slightly lower BOD (66.39%) and COD removal (19.88%). The removal efficiency of TSS indicates the ability of the bacteria to degrade the organic matter content and suspended solids present in the POME samples. The observed data indicates that the TSS removal in the POME samples with *Lysinibacillus* sp. LC 556247 (42.99%) was slightly higher than in the nonsterile POME (40.93%). These results reveal that the single pure cultures performed better than the consortium bacteria in the nonsterile POME.
medium. POME fermentation without the presence of *Lysinibacillus* sp. LC 556247 portrays the lowest reduction of all the parameters because of the absence of any bacteria to degrade the organic pollutants. The overall performances of the final COD, BOD, and TSS concentrations upon treatment were unable to meet the current discharge effluent standard [61]. Generally, after the biological treatment of POME, a second-stage chemical or physical treatment will be conducted. In this study, after the biological treatment, the product can be directly dried to obtain the powdered output. In lieu of the direct discharge of these wastes, pelletization has been suggested, upon drying, for the application of biomass fuel.

### Table 4. Biodegradation study of POME fermentation.

| Parameters (mg/L) | DOE [61] | This Study G1 | G3 |
|-------------------|----------|----------------|----|
|                   | Discharge Effluent Limit | Before Treatment | After Treatment | Removal Efficiency (%) | Before Treatment | After Treatment | Removal Efficiency (%) |
| BOD               | 100      | 99.33          | 351.30          | 71.73                  | 109.33          | 325.30          | 66.39                  |
| COD               | -        | 24,080         | 11,840          | 50.83                  | 25,950          | 20,790          | 19.88                  |
| TSS               | 400      | 10,620         | 6054            | 42.99                  | 10,330          | 6101            | 40.93                  |

Notes: G1: Autoclaved POME in the presence of *Lysinibacillus* sp.; G3: Nonsterile POME.

The efficiency of the *Lysinibacillus* sp. LC 556247 on degrading substrates, or in POME samples, is summarized in Table 5. The *Lysinibacillus* sp. LC 556247 efficiency was comparable with the *Bacillus* spp., as reported by other researchers in previous work. The high COD removal obtained in this study reveals its broader potential utilization for the treatment of other substrates, aside from POME. This could be because of the greater tolerance of *Lysinibacillus* sp. LC 556247 towards extremely acidic conditions and protective endospore-forming natural properties [35]. A recent published study on *Lysinibacillus* sp. was performed for the 3HHx bioplastic degradation [62], whereas the *L. loumbei* sp. was employed for the alkaline fermentation of cassava leaves [45]. However, no data on the biodegradation ability of *Lysinibacillus* sp. for POME treatment has been compiled. Bala et al. [63] successfully employed mixed cultures of *B. cereus* 103 PB and *B. subtilis* 106 PB, yielding high BOD and COD removal efficiencies of 93.11% and 90.64%, respectively. The high percentage of removal was due to the superior performance of the *Bacillus* sp. isolated from the POME source, which has been well-documented previously [9,62,63]. The microbe was touted to have the metabolic ability to biodegrade and metabolize organic compounds, such as oil, into simpler byproducts of organic acids, volatile fatty acids, carbon dioxide, methane, and water (63). On the basis of the current findings, it can be concluded that the *Lysinibacillus* sp. LC 556247, cultivated in POME waste medium as the sole carbon source, can enhance the CEV of fermented POME, and significantly reduce the BOD, COD, and TSS efficiencies.

### Table 5. Comparison of *Lysinibacillus* sp. and the efficiency of other *Bacillus* strains in fermentation.

| Strain          | Substrate      | Fermentation Condition | Treatment Duration (days) | COD Removal (%) | BOD Removal (%) | TSS Removal (%) | Ref.     |
|-----------------|----------------|------------------------|---------------------------|----------------|----------------|----------------|----------|
| *Lysinibacillus* sp. | POME           | Batch/Aerobic          | 5                         | 50.83          | 71.73          | 42.99          | This study |
| *L. loumbei* sp. | Cassava leaves | Alkaline               | 6                         | 79.35          | 72.65          | 65.91          | [45]     |
| *Lysinibacillus* sp. | 3HHx           | Submerged              | 30                        | NA             | NA             | NA             | [62]     |
| *B. cereus* MF661883 | POME (50%, v/v) | Batch/Aerobic         | 6                         | 90.64          | 93.11          | NA             | [9]      |

NA: Not available.
4. Conclusions

The potential use of *Lysinibacillus* sp. LC 556247 was evaluated in terms of POME treatment for biomass fuel production. The highest CEV (21.25 ± 0.19 MJ/kg) was obtained at 48 h of POME fermentation in the presence of *Lysinibacillus* sp. LC 556247. High oil (17.95 ± 0.02%) and nitrogen (12.80 ± 0.08%) contents were achieved at the highest CEV reading. The bacterium had removed a significant amount of BOD, COD, and TSS, demonstrating removal efficiencies of 71.73%, 50.83%, and 42.99%, respectively. The elemental analysis indicates that the obtained fermentation products consisted of high amounts of carbon and potassium, and a significantly low amount of silica, which is preferable for the effective burning of biomass fuel in the boiler. The overall findings of this research work tout the utilization of *Lysinibacillus* sp. LC 556247 as a potential bacterium for biomass fuel application through POME fermentation. However, further research is required to evaluate the applicability of *Lysinibacillus* sp. LC 556247 at the industrial scale, along with the POME wastewater remediation concept. Thus, POME fermentation utilizing a locally isolated strain from a native POME source, *Lysinibacillus* sp. LC 556247, could be beneficial and feasible because of the ease of cultivation and the remarkable properties discovered in this biomass fuel study.

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References

1. Singh, R.P.; Ibrahim, M.H.; Esa, N.; Iliyana, M.S. Composting of waste from palm oil mill: A sustainable waste management practice. *Rev. Environ. Sci. Biotechnol.* 2010, 9, 331–344. [CrossRef]
2. Abdullah, N.; Sulaiman, F. The Oil Palm Wastes in Malaysia. In *Biomass Now-Sustainable Growth Use*; IntechOpen: Rijeka, Croatia, 2013.
3. Onoja, E.; Chandren, S.; Razak, F.I.A.; Mahat, N.A.; Wahab, R.A. Oil palm (*Elaeis guineensis*) biomass in Malaysia: The present and future prospects. *Waste Biomass Valoriz.* 2019, 10, 2099–2117. [CrossRef]
4. Low, T.J.; Mohammad, S.; Sudesh, K.; Baidurah, S. Utilization of banana (*Musa* sp.) fronds extract as an alternative carbon source for poly (3-hydroxybutyrate) production by *Cupriavidus necator* H16. *Biocatal. Agric. Biotechnol.* 2021, 34, 102048. [CrossRef]
5. Sen, K.Y.; Hussin, M.H.; Baidurah, S. Biosynthesis of poly (3-hydroxybutyrate) (PHB) by *Cupriavidus necator* from various pretreated molasses as carbon source. *Biocatal. Agric. Biotechnol.* 2019, 17, 51–59. [CrossRef]
6. Sen, K.Y.; Baidurah, S. Renewable biomass feedstocks for production of sustainable biodegradable polymer. *Curr. Opin. Green Sustain. Chem.* 2020, 27, 1–6. [CrossRef]
7. Boey, J.Y.; Mohamad, L.; Khok, Y.S.; Tay, G.S.; Baidurah, S. A Review of the Applications and Biodegradation of Polyhydroxyalkanoates and Poly (lactic acid) and Its Composites. *Polymers* 2021, 13, 1544. [CrossRef] [PubMed]
8. Kassim, M.A.; Meng, T.K.; Serri, N.A.; Yusoff, S.B.; Shahrin, N.A.M.; Seng, K.Y.; Bakar, M.H.A.; Keong, L.C. Sustainable Biorefinery Concept for Industrial Bioprocessing. In *Biorefinery Production Technologies for Chemicals and Energy*, 1st ed.; Kuila, A., Mukhopadhyay, M., Eds.; Wiley & Sons: Hoboken, NJ, USA; Scrivener Publishing LLC: Beverly, MA, USA, 2020; pp. 15–53.
9. Karim, A.; Islam, M.A.; Yousuf, A.; Khan, M.M.R.; Faizal, C.K.M. Microbial lipid accumulation through bioremediation of palm oil mill wastewater by Bacillus cereus. *ACS Sustain. Chem. Eng.* 2019, 7, 14500–14508. [CrossRef]

10. Wakil, S.M.; Fasiku, S.A.; Adelabu, A.B.; Onilude, A.A. Production of bioethanol from spontaneous fermentation of palm oil mill effluent (POME). *Researcher* 2013, 5, 28–35.

11. Zainal, B.S.; Zinatizadeh, A.A.; Chyuan, O.H.; Mohd, N.S.; Ibrahim, S. Effects of process, operational and environmental variables on biohydrogen production using palm oil mill effluent (POME). *Int. J. Hydrogen Energy* 2018, 43, 10637–10644. [CrossRef]

12. Mohammad, S.; Baidurah, S.; Kobayashi, T.; Ismail, N.; Leb, C.P. Palm Oil Mill Effluent Treatment Processes—A Review. *Processes* 2021, 9, 739. [CrossRef]

13. Bergström, D.; Finell, M.; Gref, R. Effects of extractives on the physical characteristics of Scots pine sawdust fuel pellets. *For. Prod. J.* 2010, 60, 640–644. [CrossRef]

14. Hassan, S.; Kee, L.S.; Al-Kayiem, H.H. Experimental study of palm oil mill effluent and oil palm frond waste mixture as an alternative biomass fuel. *J. Eng. Sci.* 2013, 8, 703–712.

15. Zeng, W.S.; Tang, S.Z.; Xiao, Q.H. Calorific values and ash contents of different parts of Masson pine trees in southern China. *J. For. Res.* 2014, 25, 779–786. [CrossRef]

16. Jiang, L.; Yuan, X.; Xiao, Z.; Liang, J.; Li, H.; Cao, L.; Wang, H.; Chen, X.; Zeng, G. A comparative study of biomass pellet and biomass-sludge mixed pellet: Energy input and pellet properties. *Energy Convers. Manag.* 2016, 126, 509–515. [CrossRef]

17. Gumisiriza, R.; Hawumba, J.F.; Okure, M.; Hensel, O. Biomass waste-to-energy valorisation technologies: A review case for bioenergy from palm tree waste in Uganda. *Biotecnol. Biofuels* 2017, 10, 1–29. [CrossRef]

18. Abdulrazik, A.; Elsholkami, M.; Elkamel, A.; Simon, L. Multi-products productions from Malaysian oil palm empty fruit bunches (EFB): Analyzing economic potentials from the optimal biomass supply chain. *Evol. Microbiol.* 2014, 25, 1547–1549. [CrossRef] [PubMed]

19. Sirrajudin, M.S.; Rasat, M.S.M.; Wahab, R.; Amini, M.H.M.; Mohamed, M.; Ahmad, M.I.; Moktar, J.; Ibrahim, M.A. Enhancing the energy properties of fuel pellets from oil palm fronds of agricultural residues by mixing with glycerin. *ARPN J. Eng. Appl. Sci.* 2016, 11, 6122–6127.

20. Purwanto, W.W.; Supramono, D.; Fisasafarni, H. Biomass waste and biomass pellets characteristics and their potential in Indonesia. In Proceedings of The 1st International Seminar on Fundamental and Application ISFACHe, Bali, Indonesia, 3–4 November 2010; p. C004-1.

21. May, C.Y. The untapped potential of oil palm biomass and its potential applications: Challenges and Opportunities. In Proceedings of the Ranch and Teach Friends and Friends, Putrajaya, Malaysia, 25 January 2011.

22. Onoha, E.; Attan, N.; Chandren, S.; Razak, F.I.A.; Keyon, A.S.A.; Mahat, N.A.; Wahab, R.A. Insights into the physicochemical properties of the Malaysian oil palm leaves as an alternative source of industrial materials and bioenergy. *Mal. J. Fund. Appl. Sci.* 2017, 13, 623–631. [CrossRef]

23. Wu, T.Y.; Mohammad, A.W.; Jahim, J.M.; Anuar, N. A holistic approach to managing palm oil mill effluent (POME): Biotechnological advances in the sustainable reuse of POME. *Biotecnol. Adv.* 2009, 27, 40–52. [CrossRef]

24. Chiew, Y.L.; Shimada, S. Current state and environmental impact assessment for utilizing oil palm empty fruit bunches for fuel, fiber and fertilizer—A case study of Malaysian oil palm biomass. *Biomass Bioenergy* 2013, 51, 109–124. [CrossRef]

25. Dererie, D.Y.; Trobro, S.; Momeni, M.H.; Hansson, H.; Blomqvist, J.; Passoth, V.; Schnürer, A.; Sandgren, M.; Ståhlberg, J. Improved bio-energy yields via sequential ethanol fermentation and biogas digestion of steam exploded oat straw. *Bioresour. Technol.* 2011, 102, 4449–4455. [CrossRef] [PubMed]

26. Lim, J.S.; Manan, Z.A.; Alwi, S.R.W.; Hashim, H. A review on utilisation of biomass from rice industry as a source of renewable energy. *Renew. Sustain. Energy Rev.* 2012, 16, 3084–3094. [CrossRef]

27. Oberoi, H.S.; Vadliani, P.V.; Brijwani, K.; Bhargav, V.K.; Patil, R.T. Enhanced ethanol production via fermentation of rice straw with hydrolysate-adapted Candida tropicalis ATCC 13803. *Process Biochem.* 2010, 45, 1299–1306. [CrossRef]

28. Zanetti, M.; Costa, C.; Greco, R.; Grigolato, S.; Ottaviani Aalmo, G.; Cavalli, R. How wood fuels’ quality relates to the standards: A class-modelling approach. *Energies* 2017, 10, 1455. [CrossRef]

29. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 2018, 35, 1547–1549. [CrossRef] [PubMed]

30. Association of Official Analytical Chemists (AOAC). *Official Methods of Analysis of AOAC International*, 18th ed.; AOAC International: Gaithersburg, MD, USA, 2010.

31. American Public Health Association (APHA). *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association: Washington, DC, USA, 2005.

32. Piro, P.; Carbone, M.; Tomei, G. Assessing settleability of dry and wet weather flows in an urban area serviced by combined sewers. *Water Air Soil Pollut.* 2011, 214, 107–117. [CrossRef]

33. Zhu, C.; Sun, G.; Chen, X.; Guo, J.; Xu, M. *Lysinibacillus varians* sp. nov., an endospore-forming bacterium with a filament-to-rod cell cycle. *Int. J. Syst. Evol. Microbiol.* 2014, 64, 3644–3649. [CrossRef]

34. Nam, Y.-D.; Seo, M.-J.; Lim, S.-I.; Lee, S.-Y. Genome sequence of *Lysinibacillus boronitolerans* F1182, isolated from a traditional Korean fermented soybean product. *J. Bacteriol.* 2012, 194, 5988. [CrossRef] [PubMed]

35. Ahmed, I.; Yokota, A.; Yamazoe, A.; Fujiwara, T. Proposal of *Lysinibacillus boronitolerans* Gen. *Nov*. Sp. *Nov.*, and Transfer of *Bacillus fusiformis* to *Lysinibacillus fusiformis* Comb. *Nov.* and *Bacillus spharicus* to *Lysinibacillus spharicus* Comb. *Nov.* *Int. J. Syst. Evol. Microbiol.* 2007, 57, 1117–1125. [CrossRef]
36. Miwa, H.; Ahmed, I.; Yokota, A.; Fujiwara, T. *Lysinibacillus parviboronicapiens* sp. nov., a low-boron-containing bacterium isolated from soil. *Int. J. Syst. Evol. Microbiol.* 2009, 59, 1427–1432. [CrossRef]

37. Lee, C.-S.; Jung, Y.-T.; Park, S.; Oh, T.; Yoon, J.-H. *Lysinibacillus xylanilyticus* sp. nov., a xylan-degrading bacterium isolated from forest humus. *Int. J. Syst. Evol. Microbiol.* 2009, 60, 281–286. [CrossRef] [PubMed]

38. Coorevits, A.; Dinsdale, A.E.; Heyrman, J.; Schumann, P.; Van Lantschoot, A.; Logan, N.A.; De Vos, P. *Lysinibacillus macroides* sp. nov., nom. rev. *Int. J. Syst. Evol. Microbiol.* 2012, 62, 1121–1127. [CrossRef] [PubMed]

39. Yang, L.-L.; Huang, Y.; Liu, J.; Ma, L.; Mo, M.-H.; Li, W.-J.; Yang, F.X. *Lysinibacillus mangiferahumi* sp. nov., a new bacterium producing nematicidal volatiles. *Antonie van Leeuwenhoek* 2012, 102, 53–59. [CrossRef]

40. Kim, J.-S.; Paek, W.; Stryrak, I.; Park, I.-S.; Sin, Y.; Paek, J.; Park, K.-A.; Kim, H.; Kim, H.-L.; Chang, Y.-H. Description of *Lysinibacillus sinduiriensis* sp. nov., and transfer of *Bacillus massiliensis* and *Bacillus odysseyi* to the genus *Lysinibacillus* as *Lysinibacillus massiliensis* comb. nov. and *Lysinibacillus odysseyi* comb. nov. with emended description of the genus *Lysinibacillus*. *Int. J. Syst. Evol. Microbiol.* 2011, 62, 2347–2355.

41. Duan, Y.-Q.; He, S.-T.; Li, Q.-Q.; Wang, M.-F.; Wang, W.-Y.; Zhe, W.; Cao, Y.-H.; Mo, M.-H.; Zhai, Y.-L.; Li, W.-J. *Lysinibacillus tabaciuli* sp. nov., a novel endophytic bacterium isolated from *Nicotiana tabacum* leaves. *J. Microbiol.* 2013, 51, 289–294. [CrossRef] [PubMed]

42. Kim, S.-J.; Jang, Y.-H.; Hamada, M.; Ahn, J.-H.; Weon, H.-Y.; Suzuki, K.; Wang, K.-S.; Kwon, S.-W. Lag phase is a dynamic, organized, adaptive, and evolvable period that prepares bacteria for cell division. *Bacteriol.* 2019, 201, e00697-18. [CrossRef] [PubMed]

43. Rolfe, M.D.; Rice, C.J.; Lucchini, S.; Pin, C.; Thompson, A.; Backman, P.A. Isolation of endophytic endospore-forming bacteria from *Nicotiana tabacum* leaves. *J. Microbiol.* 2019, 65, 4256–4262. [CrossRef]

44. Hii, K.L.; Yeap, S.P.; Mashitah, M.D. Cellulase production from palm oil mill effluent in Malaysia: Economical and technical perspectives. *Eng. Life Sci.* 2012, 12, 7–28. [CrossRef]

45. Melnick, R.L.; Suárez, C.; Bailey, B.A.; Backman, P.A. Isolation of endophytic endospore-forming bacteria from *Theobroma cacao* as potential biological control agents of cacao diseases. *Biol. Control.* 2011, 57, 236–245. [CrossRef]

46. Hui, K.L.; Yeap, S.P.; Mashitah, M.D. Cellulase production from palm oil mill effluent in Malaysia: Economical and technical perspectives. *Eng. Life Sci.* 2012, 12, 7–28. [CrossRef]

47. Gamaralalage, D.; Sawai, O.; Nunoura, T. Degradation behavior of palm oil mill effluent in Fenton oxidation. *J. Hazard. Mater.* 2019, 364, 791–799. [CrossRef] [PubMed]

48. Rolfe, M.D.; Rice, C.J.; Lucchini, S.; Pin, C.; Thompson, A.; Cameron, A.D.; Alston, M.; Stringer, M.F.; Betts, R.P.; Baranyi, J.; et al. Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. *J. Bacteriol.* 2012, 194, 686–701. [CrossRef] [PubMed]

49. Bertrand, R.L. Lag phase is a dynamic, organized, adaptive, and evolvable period that prepares bacteria for cell division. *J. Bacteriol.* 2019, 201, e00697-18. [CrossRef] [PubMed]

50. Maier, R.M. Bacterial Growth. In *Environmental Microbiology*, 3rd ed.; Pepper, I.L., Gerba, C.P., Gentry, T.J., Eds.; Academic Press Incorporation: San Diego, CA, USA, 2000; pp. 37–54.

51. Iswuagwu, J.O.; Ugwuanyi, J.O. Treatment and valorization of palm oil mill effluent through production of food grade yeast biomass. *J. Waste Manag.* 2014, 34, 439071. [CrossRef]

52. Loh, S.K. The potential of the Malaysian oil palm biomass as a renewable energy source. *Energy Convers. Manag.* 2017, 141, 285–298. [CrossRef]

53. Nwuche, C.O.; Aoyagi, H.; Ogbonna, J.C. Treatment of palm oil mill effluent by a microbial consortium developed from compost soils. *Int. Sch. Res. Not.* 2014, 2014, 762070. [CrossRef]

54. Bala, J.D.; Lalung, J.; Al-Gheethi, A.A.S.; Hossain, K.; Ismail, N. Microbiota of palm oil mill wastewater in Malaysia. *Trop. Life Sci. Res.* 2018, 29, 131–163. [CrossRef]

55. Soleimannanadegani, M.; Manshad, S. Enhancement of biodegradation of palm oil mill effluents by local isolated microorganisms. *Int. Sch. Res. Not.* 2014, 2014, 727049. [CrossRef]

56. Chan, Y.J.; Chong, M.F.; Law, C.L. Biological treatment of anaerobically digested palm oil mill effluent (POME) using a Lab-Scale Sequencing Batch Reactor (SBR). *J. Environ. Manag.* 2010, 91, 1738–1746. [CrossRef]

57. Al-Mutairi, N.Z. Aerobic selectors in slaughterhouse activated sludge systems: A preliminary investigation. *Bioresour. Technol.* 2009, 100, 50–58. [CrossRef]

58. Cheng, Y.W.; Chong, C.C.; Lam, M.K.; Leong, W.H.; Chuah, L.F.; Yusup, S.; Setiabudi, H.D.; Tang, Y.; Lim, J.W. Identification of microbial inhibitions and mitigation strategies towards cleaner bioconversions of palm oil mill effluent (POME): A review. *J. Clean. Prod.* 2020, 280, 124346. [CrossRef]
61. The Commissioner of Law Revision, M.; 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).

62. Mohapatra, S.; Samantaray, D.P.; Samantaray, S.M.; Mishra, B.B.; Das, S.; Majumdar, S.; Pradhan, S.K.; Rath, S.N.; Rath, C.C.; Akthar, J.; et al. Structural and thermal characterization of PHAs produced by *Lysinibacillus* sp. through submerged fermentation process. *Int. J. Biol. Macromol.* **2016**, *93*, 1161–1167. [CrossRef]

63. Bala, J.D.; Lalung, J.; Ismail, N. Studies on the reduction of organic load from palm oil mill effluent (POME) by bacterial strains. *Int. J. Recycl. Org. Waste Agric.* **2015**, *4*, 1–10. [CrossRef]