FERMENTATION EFFECTIVENESS OF VARIOUS RATIOS OF PALM OIL MILL EFFLUENT (POME) AND EMPTY FRUIT BUNCH (EFB) MIXTURE BY *LYSINIBACILLUS SP.* FOR SOLID BIOMASS FUEL PRODUCTION

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by

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BOD	Biochemical oxygen demand
CEV	Calorific energy value
CHP	Combined heat and power
COD	Chemical oxygen demand
CPO	Crude palm oil
EFB	Empty fruit bunch
EQA	Environmental quality act
GDP	Gross domestic product
POME	Palm oil mill effluent
EFB	Empty fruit bunch
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
g	Gram
GCMS	Gas chromatography–mass spectrometry
GDP	Gross domestic product
GHG	Greenhouse gas
GM	Genetically modified
GMO	Genetically modified organism
HAc	Acetic acid
HBu	Butyric acid
HCl	Hydrochloric acid
HPr	Propionic acid
HRT	Hydraulic retention time
HVa	Valeric acid
IC	Inorganic carbon
IEA	International Energy Agency
LCA	Life cycle assessment
MF	Mesocarp fruit
mg	Milligram
m	Millilitre

MySTIE	Malaysian Science, Technology, Innovation and Economy
NB	Nutrient broth
NSTIP	National Science, Technology and Innovation Policy
OPF	Oil palm frond
OPS	Oil palm shell
OPT	Oil palm trunks
PA6	Polyamide 6
PKC	Palm kernel cake
PKS	Palm kernel shells
POME	Palm oil mill effluent
PPF	Palm press fiber
Pt-Co	Platinum-cobalt color
SDG	Sustainable development goals
sp.	Species
TC	Total carbon
TOC	Total organic carbon
TSS	Total Suspended solids
UNESCO	United Nation of Educational, Scientific and Cultural Organization
VFA	Volatile fatty acids

LIST OF SYMBOLS

°C	Degree celsius
°C/min	Degree celsius per minute
C_6H_{14}	n-hexane
C_7H_{16}	n-heptane
CH_4	Methane
CH_4BF_3O	Methanolic boron trifluoride
CO_2	Carbon dioxide
g biomass^{-1} m$^{2\ -1}$ day^{-1}	Gram per biomass per metre^2 per day
H_2S	Hydrogen sulphide
K_2SO_4	Potassium bisulfate
KCl	Potassium chloride
kg CO_2-eq/t	Kilogram of carbon dioxide equivalent per ton
KOH	Potassium hydroxide
Kg	Kilogram
Kn	Kilonewton
kPa	Kilopascal
kWh	Kilowatt hour
L	Litre
MPa	Megapascal
mg/L	Milligrams per liter
Million tons CO_2/year	Million tons carbon dioxide per year
MJ/kg	Megajoules per kilogram
mL	Millilitre
mL/min	Milliliter per minute
Mm	Millimetre
mm/sec	Millimeter per second
MW	Megawatt
Na_2SO_4	Sodium sulfate
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NH_3-N	Ammoniacal nitrogen

NO_x	Nitric oxide
tons ha^{-1} $year^{-1}$	Ton per hectare per year
wt %	Percentage by weight
µm	Micrometre

LIST OF APPENDICES

KEBERKESANAN PENAPAIAN PELBAGAI NISBAH CAMPURAN EFLUEN MINYAK SAWIT (POME) DAN BUAH TANDAN KOSONG (EFB) OLEH *LYSINIBACILLUS* SP. UNTUK PENGHASILAN BAHAN API BIOJISIM

ABSTRAK

Biovalorisasi sisa agroindustri untuk menghasilkan sumber tenaga alternatif seperti bahan api biojisim pepejal semakin mendapat perhatian di seluruh dunia kerana potensinya untuk menghasilkan produk berharga yang mampan. Dalam kajian ini, potensi sisa buangan efluen minyak sawit (POME) dan buah tandan kosong (EFB) sebagai bahan mentah biojisim diterokai. Penapaian POME dan EFB telah dijalankan dalam bioreaktor atas bangku pada 37 ± 2 °C, 180 rpm selama lima hari dengan kehadiran bakteria *Lysinibacillus* sp., diikuti dengan pengeringan ketuhar. Dua kategori utama media penapaian terdiri daripada campuran POME dan EFB yang diautoklaf dan tidak diautoklaf diterokai untuk menyiasat aktiviti metabolik *Lysinibacillus* sp. Selain itu, nisbah komposisi yang berbeza bagi campuran POME dan EFB bagi media penapaian dijelaskan untuk menilai keadaan penapaian yang ideal, terutamanya kesannya terhadap CEV untuk pengeluaran bahan api biojisim pepejal. Antara keadaan yang diuji, Kumpulan 4 mempamerkan nilai tenaga kalori (CEV) tertinggi iaitu 29.54 MJ/kg. Penapaian dengan kehadiran *Lysinibacillus* sp. menunjukkan penyingkiran BOD pada 27.64 ± 0.53 %, COD pada 70.42 ± 0.01 %, minyak dan gris pada 87.68 ± 0.14 %, dan jumlah pepejal terampai (TSS) 93.94 %. Kepekatan nitrogen ammonia (NH_3-N) kekal tidak berubah sebelum dan selepas penapaian dalam semua keadaan. Semua keadaan penapaian yang dijalankan menunjukkan pH antara 4.4 – 8.1 sepanjang lima hari penapaian dengan Kumpulan 1, 2, 3, 4 dan 7 kekal dalam keadaan berasid manakala Kumpulan 5 dan Kumpulan 6 masing-masing beralih kepada neutral dan alkali. Analisis kandungan lembapan (MC) menunjukkan bahawa Kumpulan 4 mencapai MC terendah (0.58 ± 0.01 %), selepas semalaman proses pengeringan dalam ketuhar. Sebatian asid lemak utama yang dikenal pasti dalam sampel eksperimen ialah asid miristik, asid palmitik, asid elaidik, dan asid linoleik, dan asid palmitic sebagai juzuk dominan. Analisis XRF mikro mengesahkan kehadiran beberapa unsur utama dan kecil dalam biojisim pepejal, termasuk aluminium, silika, fosforus, sulfur, klorin, kalium, kalsium, titanium, mangan, besi, nikel, kuprum, zink, arsenik, bromin, rubidium, strontium, zirkonium, ruthenium, rhodium, dan paladium. Tiada logam berat berbahaya dikesan. G1 D5 mengandungi kandungan karbon yang lebih tinggi (55.53 %) dan hidrogen (13.26 %) walaupun CEV yang direkodkan lebih rendah berbanding G4 D5. Kumpulan 4 mempamerkan daya mampatan beban tertinggi (0.337 ± 0.037 kN). Kajian ini berjaya menunjukkan biovalorisasi sisa POME yang banyak kepada bahan api biojisim pepejal dengan CEV yang tinggi serta pengurangan sisa cecair secara serentak. Bahan api biojisim pepejal yang dibangunkan mempunyai potensi tinggi untuk digunakan sebagai bahan pembakaran di dalam dandang untuk penjanaan tenaga dalam dandang. Tambahan pula, inisiatif ini sejajar dengan pelbagai matlamat kemampanan, termasuk Matlamat Pembangunan Lestari Pertubuhan Bangsa-Bangsa Bersatu 7: Tenaga Mampu dan Bersih, 9: Industri, Inovasi dan Infrastruktur, 12: Penggunaan dan Pengeluaran Bertanggungjawab, Sains, Teknologi, Inovasi dan 10-10 Malaysia. Rangka Kerja Ekonomi (MySTIE), dan Dasar Sains, Teknologi dan Inovasi Negara (NSTIP) 2021-2030.

FERMENTATION EFFECTIVENESS OF VARIOUS RATIOS OF PALM OIL MILL EFFLUENT (POME) AND EMPTY FRUIT BUNCH (EFB) MIXTURE BY *LYSINIBACILLUS* SP. FOR SOLID BIOMASS FUEL PRODUCTION

ABSTRACT

Biovalorization of agro-industrial wastes to produce an alternative energy source such as solid biomass fuel is gaining attention worldwide due to its potential to produce sustainable valuable products. In the current study, the potential use of palm oil mill effluent (POME) waste and empty fruit bunches (EFB) was explored for the production of solid biomass fuel. Fermentation of POME and EFB was carried out in a benchtop bioreactor at 37 ± 2 °C, 180 rpm for five-days in the presence of *Lysinibacillus* sp. bacteria, followed by oven drying. Two main categories of fermentation media consisted of autoclaved and non-autoclaved mixture of POME and EFB are explored to investigate the metabolic activities of *Lysinibacillus* sp. Additionally, the different composition ratio of POME and EFB mixture of the fermentation media is elucidated to assess the ideal fermentation conditions, particularly their impact on CEV for solid biomass fuel production. Among the tested conditions, Group 4 exhibited the highest calorific energy value (CEV) of 29.54 MJ/kg after fermentation at day 1. Fermentation in the presence of *Lysinibacillus* sp. showed efficient removal of biochemical oxygen demand (BOD) at 27.64 ± 0.53 %, chemical oxygen demand (COD) at 70.42 ± 0.01 %, oil and grease at 87.68 ± 0.14 %, and total suspended solids (TSS) 93.94 ± 2.03 %. The concentration of ammoniacal nitrogen (NH_3-N) remained unchanged before and after fermentation in all conditions. All fermentation conditions indicated pH ranging from 4.4 – 8.1 throughout five-days fermentation with Group 1, 2, 3, 4 and 7 remained in acidic conditions while Group 5 and Group 6 shifted to neutral and alkaline respectively. Moisture content (MC) analysis revealed that Group 4 achieved the lowest MC (0.58 ± 0.01 %), after overnight oven drying process. The major fatty acid compounds identified in the experiment samples were myristic acid, palmitic acid, elaidic acid, and linoleic acid, with palmitic acid being the dominant constituent. Micro XRF analysis confirmed the presence of several major and minor elements in the solid biomass, including aluminum, silica, phosphorus, sulfur, chlorine, potassium, calcium, titanium, manganese, iron, nickel, copper, zinc, arsenic, bromine, rubidium, strontium, zirconium, ruthenium, rhodium, and palladium. No hazardous heavy metals are detected. G1 D5 contained higher carbon (55.53 %) and hydrogen (13.26 %) content despite lower CEV recorded compared to G4 D5. Group 4 exhibited the highest compressive force of load (0.337 ± 0.037 kN). This study successfully demonstrated the biovalorization of abundant POME waste into solid biomass fuel with high CEV and simultaneous reduction of liquid waste. The current material utilized for solid biomass fuel has high potential as burning materials for energy generation in boilers. Furthermore, this initiative aligns with various sustainability goals, including United Nations' Sustainable Development Goals 7: Affordable and Clean Energy, 9: Industry, Innovation and Infrastructure, 12: Responsible Consumption and Production, the 10-10 Malaysian Science, Technology, Innovation and Economy (MySTIE) Framework, and the National Science, Technology, and Innovation Policy (NSTIP) 2021-2030.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Oil palm is an estate crops that contributes important roles in development of Malaysia economy. The introduction of oil palm commodity to Malaysia has improved the country's agricultural sector and economy standard. In 2021, Malaysia's contributions to the global production and export of oils accounted for 9.1 % and 19.7 % respectively, with a production of 18,116,354 tons of crude palm oil (CPO), an export of 24,279,569 tons of diverse processed palm oil products, and an oil palm plantation area of 5.90 million hectares (Parveez et al., 2021; MPOB, 2021; Dominic & Baidurah, 2023). Despite being one of the largest palm oil producers, this industry generated tonnes of waste every year which in turn created major waste disposal issues and contributed to environmental degradation.

The oil palm wastes originated from plantations and mills can be categorized into two groups: liquid and solid biomass wastes. Palm oil mill effluent (POME) is the only waste classified as liquid waste produced by palm oil mill, whilst empty fruit bunch (EFB), mesocarp fruit fibers (MF), oil palm frond (OPF), and palm kernel shells (PKS) are classified as solid (Abdullah & Sulaiman, 2013; Hambali & Rivai, 2017). POME is the most voluminous waste generated at the final stage of palm oil milling activities with approximately 2.5 to 3.5 tonnes production for every 1 tonne of CPO produced (Ng et al., 2023; Tan et al., 2023). The production of POME is not considered as toxic waste because no chemicals are involved during the processing of CPO. However, the release of POME into the environment without any extensive treatment will cause environmental pollutions due to its high biochemical oxygen demand (BOD), chemical oxygen demand (COD), oil and grease content as well as its acidic and viscous characteristics. Aesthetical value of surrounding environment will be compromised due to the colour and foul odour. EFB, the second most voluminous oil palm waste produced is a highly fibrous biomass and disposed into the environment either by burning or left at the landfill to decompose due to its bulky nature and low commercial value. Decomposition of EFB in open space contributes to greenhouse gas (GHG) emission such as carbon dioxide and methane. Additionally, burning and decomposition of EFB in an open environment causes air pollution and insect manifestation.

Bioenergy is regarded as a viable sustainable energy alternative to conventional fossil fuels for promoting energy security, mitigating global warming, and accelerating global population growth (Prasad et al., 2021). Biomass fuel derived from feedstock such as lignocellulosic biomass, agricultural biomass, agrochemical biomass and bio-waste can easily be obtained due to their abundance, low commercial value, no competition with food security and contain high calorific energy value (CEV). Less carbon dioxide emitted through the burning of biomass fuel is captured and sequestered by natural reservoirs, including trees and plants during the process of photosynthesis. Therefore, carbon cycle and neutrality are maintained in equilibrium. Additionally, this initiative will stimulate sustainable development among rural communities in Malaysia.

To the present day, open ponding system is the most common treatment approach employed by palm oil millers to treat POME prior discharge into the environment at industrial scale due to convenience, low capital cost and simple technological requirements. Various treatment technologies were invented to valorize POME and EFB as an alternative energy source such as biomass fuel. However, there are few challenges that need to be addressed; long hydraulic retention time (HRT), large land space, and final discharge parameter. Therefore, an alternative approach with shorter HRT and small space requirement which produces sustainable by-product is essentially needed.

POME bioremediation using *Lipomyces starkeyi* and indigenous *Bacillus cereus* enhanced lipid accumulation and pollutant removal, demonstrating the potential of this microbial lipid for biodiesel production with a maximum biomass of 8.89 g/L and lipid production of 2.27 g/L (Karim et al., 2021). The indigenous *Bacillus anthracis* strain PUNAJAN 1 demonstrated a significant fermentative hydrogen production potential when using POME wastewater as a substrate, with the highest hydrogen yield reaching 2.42 mol H^2/mol substrates consumed (Mishra et al., 2017). Indigenous *Bacillus toyonensis* strain BCT-71120 and *Stenotrophomonas rhizophila* strain e-p10 were employed in an 18-day anaerobic fermentation process, resulting in the highest methane content of 41.05 % in the biogas (Said et al., 2021). The valorisation of POME and EFB into sustainable biomass fuel in the presence of indigenous microorganism, *Lysinibacillus* sp. significantly enhanced the effectiveness of utilization of palm oil waste due to its high carbon composition.

This study is designed to be parallel with Sustainable Development Goals (SDGs) Goals 7: Affordable and Clean Energy, 9: Industry, Innovation and Infrastructure, 12: Responsible Consumption and Production adopted by United Nation of Educational, Scientific and Cultural Organization (UNESCO), 10-10 Malaysian Science, Technology, Innovation and Economy (MySTIE) Framework, and National Science, Technology and Innovation Policy (NSTIP) 2021-2030.

1.2 Problem Statement

The increasing CPO production from 14 % from 27,756.00 MT in 2018 to 32,054.00 MT in 2020 indicating an upward trend of POME and EFB rises by 14 %

101,978.00 MT in 2018 to 119,586.00 MT in 2020 and rises by 23 % from 25,041.19 MT in 2018 to 32,579.48 MT in 2020 respectively (Jafri et al., 2021). These residues are abundance in nature making them as highly promising biomass feedstocks for the biobased economy due to their ready availability as a localized resource at the processing mill, and their limited value at the mill. Even though the residues are non-toxic, they are highly pollutant because they contains high concentration of organic substances with BOD values ranging from 18,000 - 48,000 mg/L and COD values ranging from 45,000 - 65,000 mg/L, total suspended solids ranging from 18,000 – 50,000 mg/L and which are difficult to decompose, thereby affecting the content of dissolved oxygen while the total nitrogen content in the palm oil industry wastewater ranges from 500-800 mg/L (Ahmad et al., 2016; Munir & Wahyuningsih, 2021). The organic component of this effluent makes it particularly hazardous to the environment if it is not treated efficiently before being discharged into waterways. Oil palm biomass is consistently produced in significant quantities every year, but only a small portion is transformed into value-added products, leaving a substantial percentage underutilized with current utilization by mills, a large quantity of oil palm biomass is left underutilized (Onoja et al., 2019; Norrrahim et al., 2022). Being remarkably rich in organic content, POME has the potential to be valorized into a wide range of bioproducts, namely, biomass fuel, methane-rich biogas, fertilizers, charcoal, and bio-oil.

Till date, a local commercially available biomass fuel has considerably low calorific value ranging from 16.00 to 17.16 MJ/kg. Even though the CEV of the above said biofuel achieved the New European standard for energy pellet ENplus A1, A2 and A3 ($\geq$ 16.5 MJ/kg), biomass fuel with higher CEV is preferred because it can produce more energy output for the same fuel consumed, making them more energy-efficient, cost saving and reduces environmental impact.

CEV is the main priority of the current study. However, the biodegradation of POME and EFB by *Lysinibacillus* sp. through the observation of COD and BOD is also conducted to understand the metabolic characteristics of the indigenous bacteria. Thus far, there are limited studies available on the degradation of organic matter by *Lysinibacillus* sp. and focuses on the fermentation effectiveness of various ratios of POME and EFB mixture for solid biomass fuel production by locally isolated *Lysinibacillus* sp. bacteria. Moreover, indigenous bacteria are frequently employed in bioaugmentation to mitigate environmental degradation owing to their proficient adaptive capabilities (Widawati & Suliasih, 2019). Therefore, an autochthonous bacterial in a POME ecosystem identified as *Lysinibacillus* sp. was subjected for fermentation in this study to intensify the degradation of organic matter in POME and simultaneously offers tailorable biomass fuel formation with enhanced CEV. Moreover, indigenous bacteria are frequently employed in bioaugmentation to mitigate environmental degradation owing to their proficient adaptive capabilities (Widawati & Suliasih, 2019).

The utilization of palm oil mill residues as biomass fuel has the potential to significantly decrease greenhouse gas emissions. This is because the palm oil plants that are the source of biomass fuel for energy absorbs nearly the same quantity of CO_2 during their growth through photosynthesis as is released and may offset the CO_2 emission when biomass is burned, making the biomass fuel a potentially carbon-neutral energy source. The utilization of a benchtop bioreactor with controllable parameters when conducting this study in a controlled environment for this novel fermentation technology will significantly enhance the CEV.

1.3 Objectives

The objectives of the present research study are:

1. To elucidate the effect of various ratios of POME and EFB mixture on the fermentation effectiveness, in terms of CEV as well as obtaining ideal ratio of the solid biomass fuel.
2. To determine the dynamic degradation of organic matter by *Lysinibacillus* sp. through fermentation by observing the changes of oil content, elemental analysis, total organic carbon (TOC), total suspended solids (TSS), ammoniacal nitrogen (NH_3-N), pH, BOD and COD.
3. To investigate the properties of biomass pellet by compression strength analysis.

1.4 Scope of Study

The present study highlighted the effectiveness of various ratios of POME and EFB fermentation using benchtop bioreactor in producing biomass fuel with high CEV. This proposed research pinpointed the fundamental aspect of changes and breakdown of organic matter in POME during fermentation by evaluating the elemental content, prior to and after the fermentation process in the presence of indigenous bacteria identified as *Lysinibacillus* sp. LC 556247 isolated from POME. In this study, seven fermentation medium conditions are performed, namely:

Group 1 = 7:3 autoclaved POME and EFB mixture + *Lysinibacillus* sp.

Group 2 = 8:2 autoclaved POME and EFB mixture + *Lysinibacillus* sp.

Group 3 = 9:1 autoclaved POME and EFB mixture + *Lysinibacillus* sp.

Group 4 = 10:0 autoclaved POME + *Lysinibacillus* sp.

Group 5 = Non-autoclaved POME + *Lysinibacillus* sp.

Group 6 = Non-autoclaved POME only (POME as it is).

Group 7 = Autoclaved POME only (Control group).

The parameters for all seven fermentations conditions are fixed at agitation speed of 180 rpm, temperature of 37 ± 2 °C. The ideal ratio of POME and EFB was deduced based on the potential production of the pellet as biomass fuel with the highest CEV. The CEV of obtained pellet was determined using bomb calorimeter. Furthermore, dynamic degradation of various ratio of POME and EFB mixture fermentation with and without the presence of *Lysinibacillus* sp. are determined by observing the changes of oil content, elemental analysis, TOC, TSS, NH_3-N, pH, BOD and COD. The biomass pellet properties of the ideal POME and EFB mixture is tested based on its ability to withstand loads or compressive strength based on compression strength analysis. The data are analysed statistically using SPSS software version 27.

CHAPTER 2

LITERATURE REVIEW

2.1 Palm Oil Industry

Oil palm is one of the estate crops that play important roles in Malaysia's economy. In Malaysia, oil palm has grown to occupy over 5.67 million hectares of land space by 2022 (MPOB, 2021). Generally, the oil palm plants are planted in the South-East Asia region, such as Thailand, Indonesia, and Malaysia (Chong et al., 2017). Significantly, Indonesia and Malaysia account for over 85 % of global production of palm oil (Alam & Begum, 2015; Arsyad et al., 2020). This is because the favourable soil condition, suitable rainfall and adequate sunlight found in South-East Asia are ideal for the oil palm plant to grow well (Kushairi, 2017).

The introduction of oil palm tree to Malaysia has helped to improves the standards of the country's economy and agricultural sector. The palm oil has proven to be the most efficient oil corps and become one of the 17 major oils and fats in the world's market with the largest yield quantity in the global market (Abdullah & Sulaiman, 2013; Al-Sabaeei et al., 2022). The oil palm tree becomes a significantly valuable source of vegetable oil because its production for palm oil exceeded rapeseed, soybean and sunflower (Chong et al., 2017). This is because it offers a far greater yield at a lower cost of production than other vegetable oils. Furthermore, 62 % of the export market was dominated by palm oil while soybeans at 13%, sunflower oil at 12%, and rapeseed oil at 6 % (USDA, 2018; Sarmidi et al., 2022). Moreover, the country has spurred the development of oil palm due to the increased demand for palm oil in the form of vegetables oils (Dungani et al., 2018). In 2020, the production of crude palm oil in Malaysia was recorded at 19.14 million tonnes (Nordin et al., 2021). Consequently, there are 457 palm oil mills operating in Malaysia (Parveez et al., 2021).

It is also worth noting that the aforementioned pandemic had unexpectedly positive ramifications for the country's oil palm industry, which portrayed a massive gain of 29.2 percent from the previous year (RM2079.00 per tonne), as well as an 8.4 percent increase in overall export earnings (Parveez et al., 2021).

Nevertheless, the thriving of the oil palm industry has contributed to environmental degradation. Significantly, the input and output of oil palm mill activities have contributed to environmental degradation (Abdullah & Sulaiman, 2013). Due to the global production of palm oil, the waste released from the palm oil mill has caused serious pollution (Orji et al., 2006; Kamyab et al., 2018). Similarly, the increasing agricultural waste can be captured through its annual production of palm oil. Therefore, Malaysia has enacted the Environmental Quality Act (EQA) 1974 to prevent, abate and control pollution. Environmental laws and regulations developed to restrict and control activities that posed a risk to the environment while expanding oil palm plantations have shown to be effective in reducing pollution and improving environmental friendliness (Alam & Begum, 2015; Kamaruddin et al., 2018).

2.1.1 Oil Palm

Oil palm tree or scientifically known as *Elaeis guineesis* is a very common species of palm oil plant planted in Malaysia for the use of palm oil production in the form of vegetable oil. A mature oil palm plant can be described as a single stemmed trunk with pinnate type of leaves known as frond that can reach approximately 3 – 4 meters long. The oil palm tree can reach a height of 7 to 13 m and a diameter of 45–65 cm, measuring 1.5 m above ground level (Khalil et al., 2010; Pulingam et al., 2022). The palm oil tree has an economic life span of about 25 years and contributes significantly to agricultural waste in Malaysia (Khalil et al., 2012; Apresian et al., 2020). Furthermore, it has the highest oil-yielding capability when compared to other vegetable oil producing plants such as the sunflower, soybean and rapeseed. According to Sumathi et al. (2008) and Omar et al. (2018), palm fruits weigh between 10 and 20 kg in huge bunches and are around the size of a small plum. A bunch can contain up to 2000 fruits, each of which consists of a hard kernel (seed) enclosed by a shell (endocarp) and surrounded by a fleshy mesocarp (Nair & Nair, 2021). Each of the oil palm trees produces approximately 90 % of biomass residue and 10 % of palm oil (Kurnia et al., 2016). The major products produced from the endosperm and mesocarp of fresh fruit bunches (FFB) are crude palm kernel oil and crude palm oil respectively. Figure 2.1 illustrates the anatomy of an oil palm tree and its residues.

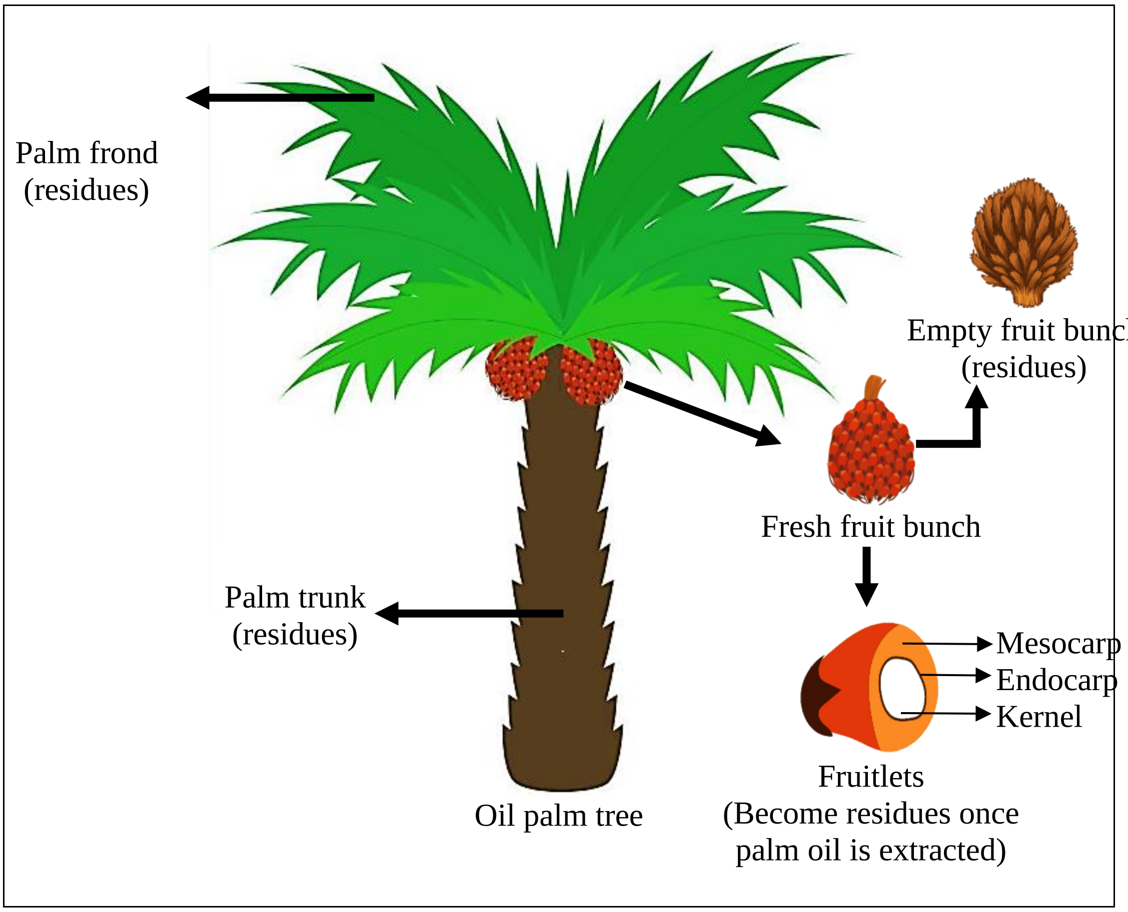

Figure 2.1 Anatomy of an oil palm tree and its residues (Drawn using BioRender).

2.1.2 Oil Palm Waste

The oil palm industries produce a large amount of biomass, measured in millions of tonnes per year. Palm oil mill effluent (POME), empty fruit bunch (EFB), oil palm fronds, oil palm trunks (OPT), palm press fiber (PPF), and palm kernel cake (PKC) are all produced during the processing of fresh fruit bunches (FFB) of oil palm. These wastes account for approximately 70-75 percent of FFB and are primarily in the form of palm fiber, steriliser condensate, oil palm shell (OPS), EFB, and POME (Otti et al., 2014; Olawepo et al., 2021). The palm oil mill waste can be categorised into two states of condition, solid waste and liquid waste. The solid waste is comprised of palm kernel shells (PKS), fruit fibers (MF) and EFB, while the liquid waste is comprised of palm oil mill effluent (POME). Table 2.1 tabulates the amount of palm oil mill waste produced per year in Malaysia.

Table 2.1 Production of palm oil mill waste per year (Umor et al., 2021; Deraman et al., 2022; Soo et al., 2022; Palm Kernel Shell Indonesia, 2023; MPOB, 2023; Hariz et al., 2023).

Palm Oil Mill Waste	Production per year (Metric Tonnes/Year)
Palm Oil Mill Effluent (POME)	43.41 million
Empty Fruit Bunch (EFB)	20.00 million
Mesocarp Fiber (MF)	6.95 million
Palm Kernel Shells (PKS)	4.00 million
Palm Kernel Cake (PKC)	1.54 million
Oil Palm Trunk (OPT)	405.40
Oil Palm Shell (OPS)	5.12

The input and output of palm oil milling operations have significantly contributed to environmental degradation. On the input side, crude palm oil mills require a lot of energy and water in their production operations, while on the output side, manufacturing activities produce a lot of solid waste, air pollution and wastewater known as POME (Abdullah & Sulaiman, 2013; Foong et al., 2021). The discharge of the palm oil mill waste has changed the environmental appearance in terms of its biodiversity, ecosystem, and properties. These include vegetation death, reduction of biodiversity, abnormal soil pH, eutrophication, and acidification of water bodies (Okereke & Ginikanwa, 2020). Nitrous oxide and methane are produced when the organic waste is disposed at shallow depth for a few days (Ermolaev et al., 2015). Significantly, it also leads to release of greenhouse gas (GHG) into the atmosphere. Nevertheless, disposal problems can be solved easily, and value-added products can be produced when the biomass residue is utilized appropriately as tabulated in Table 2.2. For example, fibers from EFB can be manipulated to produce pulp for paper making. EFB and MF can be compressed to create an engineered wood product boards known as medium density fiberboard. Biomass from palm oil mill waste can be combusted or pyrolyzed to generate biomass fuel and biofuel, such as biogas, bioethanol, or biodiesel and used as an alternative to traditional petroleum-based diesel fuel. EFB and MF can be used in agricultural practices as organic materials for producing fertilizers.

Table 2.2 Potential sustainable bioproducts of palm oil mill waste (Extracted from Mahlia et al., 2019; Awoh et al., 2023).

Palm Oil Mill Waste	Industries	Application
EFB	Paper and pulp	Pulp for paper making
EFB, MF	Construction	Medium density fiberboard, reinforced roofing slates, particleboards, concrete
EFB, MF, POME, PKS	Energy	Biomass fuel, biofuel, biochar
EFB, MF, POME, PKS	Agriculture	Fertilizers, biochar

In this research, palm oil waste such as POME and EFB are utilized as potential biomass fuel via fermentation in the presence of *Lysinibacillus* sp. This is because the plants that change into biomass feedstock use carbon dioxide during the growing stage and repel the same amount of carbon to the atmosphere when they are incinerated. Therefore, the utilization of POME as biomass fuel in the research can be regarded as the potential initiative to solve the pollution issues and the promotion of renewable energy in Malaysia parallel with the 10-10 Malaysian Science, Technology, Innovation and Economy (MySTIE) Framework, and the National Science, Technology, and Innovation Policy (NSTIP) 2021-2030.

2.2 Palm Oil Mill Effluent (POME)

Palm oil mill effluent (POME) is the only liquid waste produced through the oil palm process, and it is described as a high viscosity, brownish sludge made up of fine cellulosic materials, water, and oil. The brownish colour for the sludge is attributed by the fulvic acid-like components, and humic acid (Lee et al., 2019). The colloidal suspension-like liquid waste contains 95-96 % water, 0.6-0.7 % oil and 4-5 % total solids including 2-4 % suspended solids (Wu et al., 2007; Mohammad et al., 2021). Liquid waste can be obtained at the final stages of palm oil mill production in the mill (Abdullah & Sulaiman, 2013; Kamyab et al., 2018). The liquid waste can be acquired from hydro-cyclone, sterilizer condensate, and clarifier tank with 4 %, 36 % and 60 % contribution to POME respectively (Liew et al., 2015; Tan & Lim, 2019; Mohammad et al., 2021). Table 2.3 gives details about characteristics of raw POME.

Table 2.3 Characteristics of raw POME.

Parameters	Value	Standard discharge		References
		DOE discharged limit (1986 onwards)	Environmental Quality Act	
pH	4.16 – 4.35	5.0	5.0-9.0	Zainuri et al., 2018; Dashti et al., 2022
Colour	58,000 – 60,0000	NA	NA	Zainuri et al., 2018; Dashti et al., 2022
COD	55,100 – 56,000	1,000	1,000	Zainuri et al., 2018; Dashti et al., 2022
Total suspended solid (TSS)	4,000 – 9,000	400	400	Zainuri et al., 2018; Wulandari & Senda, 2022
BOD	64, 440	50	100	Zainuri et al., 2018; Mishra et al., 2021
Oil and grease	14,110	50	50	Zainuri et al., 2018; Sani et al., 2021
Total nitrogen	800	200	200	Zainuri et al., 2018; Nasrullah et al., 2017
Total phosphorus	90	NA	NA	Zainuri et al., 2018; Nasrullah et al., 2017
Total solids	20,000 ± 30	1,500	1,500	Zainuri et al., 2018; Mohamad et al., 2022
Total volatile solid	18,000 ± 200	NA	NA	Zainuri et al., 2018; Mohamad et al., 2022

Note: All values are stated in mg/L except for color (PtCo) and pH. NA = Not available.

It is estimated that for every tonne of crude palm oil produced, about 2.5 to 3.5 tonne of POME is generated (Madaki & Seng, 2013; Tan et al., 2023). Significantly, POME is 100 times more toxic than municipal sewage (Kamyab et al., 2018). The presence of high solid, oil, grease, biological oxygen demand (BOD), and chemical oxygen demand (COD) in palm oil mill effluent (POME) is a major concern due to their adverse effects on many forms of life (Lee et al., 2019). If POME is released without sufficient treatment, its acidic, viscous, and contaminating characteristics can cause major water pollution and disrupt aquatic ecosystems (Tan & Lim, 2019). Hence, it requires effective treatments prior to discharge into the environment because it possesses high polluting properties.

In Malaysia, POME is considered as the main source of water pollution due to the high BOD and COD (Osman et al., 2020). When POME is released into the watercourse, the biodiversity and aquatic ecosystem would reduce. This is because as the BOD and COD increase, the dissolved oxygen level decreases. Consequently, water quality reduces. Furthermore, POME could contribute to global warming up to 25 times more than carbon dioxide as it can produce an enormous amount of methane gas by almost 600 million m^3 per year (Shakib & Rashid, 2019). Moreover, a large amount of POME generated per day will make it difficult to manage, and the treatment of wastewater is expensive (Osman et al., 2020).

Nevertheless, POME is known as a good source of nutrients (Kamyab et al., 2014; Kamyab et al., 2018). This is because it contains a significant amount of nutrients which are essential for microbial growth process. For example, calcium, copper, magnesium, and iron (Osman et al., 2020). For every 100 ton of POME contains the average amount of; 55 kg of nitrogen, 9 kg phosphate, 85 kg of potassium and 18 kg of magnesium (Lelyana et al., 2013; Sakiah & Wahyuni, 2018). These major and minor nutrients are essential for microbial fermentation processes (Iwuagwu & Ugwuanyi, 2014; Mohammad et al., 2021). In this regard, POME is considered to have a high potential to be developed as a feasible and versatile feedstock for multiple applications including energy products (Kamaruddin et al., 2018).

2.3 Empty Fruit Bunch (EFB)

Oil palm empty fruit bunch (EFB), which is basically the stem of harvested oil palm bunches, is an abundant biomass waste, with 32,000 metric tons generated annually by palm oil mills in Malaysia alone (Jafri et al., 2021). EFB is one of the most abundant palm oil biomass residues that have been identified as the most abundant bioenergy resources in Malaysia (Foo et al., 2011; Derman et al., 2018).

EFB appears to have significant potential for contribution to renewable energy ever since the government shifted from conventional energy sources to increase energy security (Zahraee et al., 2020). This is because EFB has a great potency as basic raw materials used for the fermentative production which contain 37.3 – 46.5 % cellulose, and 25.3 – 33.8 % hemicelluloses (Sudiyani et al., 2013; Mardawati et al., 2022). Studies have shown that EFB is suitable for fast pyrolysis and for microwave-assisted pyrolysis (Brunerová et al., 2018). Significantly, EFB has been demonstrated successfully for energy generation. EFB that was previously disposed of as waste has been repurposed as fuel (Han & Kim, 2018). It is also proven that EFB produces approximately one third of the power from the direct combustion system as compared to a similar amount of methane used to produce electricity (Kaniapan et al., 2021).

EFB are composed of lignin and volatile matter which are responsible as natural binder and easier ignition respectively (Faizal et al., 2016; Kaniapan et al., 2021). Lignin in EFB allows the biomass fuel pellets to be bound during densification process. The fiber tends to hold the briquette together more securely due to its fibrous composition (Faizal et al., 2016). Furthermore, the densification process may decrease dust production and enhance combustion ability of biomass materials such as burning rate, moisture content and calorific energy value (Faizal et al., 2016). Consequently, the shape of the biomass fuel can be retained during transportation and storage. The higher volatile matter in EFB contributes to efficient ignition of the solid biomass fuel pellet.

Table 2.4 summarizes the characteristics of EFB. Referring to Table 2.4, EFB contains high carbon and oxygen element at 47.10 wt % and 47.00 wt % respectively. Hydrogen and sulphur are present at a smaller quantity (6.05 wt % and 1.10 wt %) compared to carbon and oxygen. Nitrogen and chlorine present in trace amounts (0.30 wt % and 0.38 wt %). The CEV of EFB when it is combusted ranges between 14.80 - 18.60 MJ/kg. 17.49 wt % of fixed carbon which contributes to heat generation during burning remained in in EFB after volatile matter is driven off during combustion. EFB also contains high moisture content and ash at 34.40 wt % and 3.39 wt % respectively which are not desirable for energy value and negatively affect combustion efficiency. Nevertheless, EFB contains a high amount of 40.72 wt % volatile carbon and is desirable for efficient combustion. In terms of fiber composition, EFB contains high amount of cellulose (47.70 wt %) and considerable amount of hemicellulose (20.20 wt %) that contributes to the flammability of the fibers. Low composition of lignin at 10.50 wt % in EFB releases low methane. Low lignin content combined with high cellulose content influences the degradation pathway of the cellulose fibers, resulting in charring and incomplete combustion thus restricts their ability to contribute effectively to the heat generated during burning (Dorez et al., 2014; Rangabhashiyam & Balasubramanian, 2019).

Table 2.4 Characteristics of EFB.

Parameters	Values	References
Ultimate analysis:		
Carbon, C	47.10	Saritpongteeraka et al., 2022
Hydrogen, H	6.05	Saritpongteeraka et al., 2022
Oxygen, O	47.00	Srasri et al., 2022
Nitrogen, N	0.30	Srasri et al., 2022
Sulphur, S	1.10	Srasri et al., 2022
Chlorine, Ca	0.38	Srasri et al., 2022
Proximate analysis:		
CEV	14.80 -18.60	Srasri et al., 2022; Saritpongteeraka et al., 2022
Fixed carbon	17.49	Srasri et al., 2022
Moisture content	34.40	Srasri et al., 2022
Ash content	3.39	Srasri et al., 2022
Volatile carbon	40.72	Srasri et al., 2022
Fiber composition:		
Cellulose	47.70	Saritpongteeraka et al., 2022
Hemicellulose	20.20	Saritpongteeraka et al., 2022
Lignin	10.50	Saritpongteeraka et al., 2022

All values are stated in wt % except for CEV (MJ/kg).

2.4 Biological treatment of palm oil mill waste by *Lysinibacillus* sp. bacteria

Lysinibacillus sp. is a ubiquitous Gram-positive rod-shaped and boron-tolerant bacterium belonging to the family *Bacillaceae* (Mohammad et al., 2021). The bacterium can be isolated from soil, puffer fish liver specimen and even soybean based fermented food product (Ahmed et al., 2007; Nam et al., 2012; Mohammad et al., 2021). *Lysinibacillus* sp. LC 556247 was successfully isolated from POME and addressed as *Lysinibacillus* sp. in the thesis (Mohammad et al., 2021).

The bacteria can be characterised as circular or flat colonies on the agar media and able to produce ellipsoidal or spherical endospores which lie terminally in a swollen sporangium (Ahmed et al., 2007; Mohammad et al., 2021). *Lysinibacillus* sp. can grow within 3.0 to 5.0 μm in length and 0.8 to 1.5 μm in diameter (Nam et al., 2012; Mohammad et al., 2021). The bacterium can grow at a wide temperature range from 16 °C to 45 °C and pH from pH 5.5 to pH 9.5 where it grows optimally at 37 °C and pH 7.0 to pH 9.0 respectively (Ahmed et al., 2007; Hoa Bach et al., 2020).

Recently, the bacteria have been reported to have the potential to control pests, remediate heavy metal-contaminated environments, and increase crop yields (Ahsan & Shimizu, 2021). It is also contemplated as a proper microbial product agent due to its ability to produce endospores (Ahsan & Shimizu, 2021). Furthermore, it also can resist and survive in a variety of environmental stresses and adverse conditions and considered as very important microbiota due to its diverse ecophysiology, direct and indirect functions (Hayat et al., 2013; Jha & Mohamed et al., 2023).

Lysinibacillus sp. is also able to degrade synthetic polyamide 6 (PA6) a widely used engineered thermoplastics on the market and in industrial applications more quantitatively. The ability of these bacterial isolates to excrete specific enzymes capable of attacking the PA6 pellets and thus inducing the breakdown of the PA6 macromolecule reflected a good bacterial degradation capacity at 21 % within 48 days (Oulidi et al., 2022). The bacterial strain can adhere to and colonize the PA6 polymer surface, resulting surface damage, implying that they can acclimatize in an environment lacking nitrogen and carbon (Oulidi et al., 2022). Under oxygen-deficient mode, the bacteria can switch to anaerobic digestion mode which results in the production of acetate and hydrogen from fermentation products containing more than two carbon atoms, such as alcohols and aromatic fatty acids (Soleimaninanadegani & Manshad, 2014; Bakar et al., 2022). In another study, the bacteria could hydrolyse the products from hydrolysis stage into butyric acid, propionic acid, ethanol, acetic acid, carbon dioxide and hydrogen (Mohammad et al., 2021).

Lysinibacillus sp. is a boron-tolerant bacterium. It can grow in an environment containing high boron such as POME. Boron is hazardous to living cells above a specific threshold level (Ahmed & Fujiwara, 2010; Sezer et al., 2018). Due to the toxicity, the bacterium is applied to remediate heavy metal-contaminated environments (Ahsan & Shimizu, 2021).

Bioremediation offers efficient, cost effective, and eco-friendly techniques over traditional physicochemical methods (Archana & Jaitly, 2014; Salilh & Tarekegn, 2020). Microbial population will utilize toxic heavy metals as a source of nutrition (Verma & Kuila, 2019). Accordingly, it has become a crucial tool for environmental remediation of non-biodegradable heavy metals (Ahsan & Shimizu, 2021). In general, the bioremediation process by *Lysinibacillus* sp. involves two mechanisms. First, an enzymatic reduction by which toxic heavy metals are converted into non-toxic forms (Ahsan & Shimizu, 2021). It is reported that *Lysinibacillus* sp. can fix nitrogen as ammonia (Abbas et al., 2014; Gong et al., 2019). Nitrogen fixation by bacteria is accomplished through the catalytic action of complex enzyme system known as nitrogenase encoded by *Nif* genes (Ahsan & Shimizu, 2021). Studies showed that *Lysinibacillus* spp. harbour the *Nif* genes and produce nitrogenases (Shabanamol et al., 2018). Second, is biosorption, which is the binding of metal ions with metal-binding proteins present on the bacterial cell wall (Ahsan & Shimizu, 2021). *Lysinibacillus* sp. accumulates or remove toxic metals through biosorption process (Rahman et al., 2014; Ahsan & Shimizu, 2021). *Lysinibacillus* sp. that were reported to have bioremediation potential includes *Lysinibacillus sphaericus*, *Lysinibacillus fusiformis*, *Lysinibacillus xylanilyticus*, *Lysinibacillus massiliensis* and *Lysinibacillus macrolides* (Velásquez & Dussan, 2009; Lozano & Dussán, 2013; Kučić Grgić et al., 2021).

2.4.1 Utilization of indigenous microorganism for biological treatment.

Biological treatment also known as secondary treatment implies the use of microorganism namely bacteria, fungi, and consortium of microorganism to degrade complex pollutant such as organic carbon, nutrients, heavy metals, suspended solids, and inorganic salts present in wastewater (Dominic & Baidurah, 2022). The complex organic matter is oxidized into the cells of the microorganism under biological processing such as anaerobic or aerobic conditions and subsequently eliminated by the removal process or sedimentation which will be valorised into value-added products such as biomass fuel (Samer, 2015; Mohammad et al., 2021).

Said et al. (2019) successfully treated POME biologically via aerobic treatment using indigenous bacteria, *Bacillus cereus* ATCC 14579 (KP 1.1), isolated from POME with a working volume of 10 liters and HRTs of 24 hours at room temperature. BOD, COD, and TSS values decrease along the fermentation time when POME is degraded in the presence of the bacteria. A similar observation is recorded when other indigenous bacteria, namely, *Pseudomonas azotoformans* strain NBRC 12693 (KP 1.3) and *Burkholderia cepacia* ATCC 25416 (KP 2.2), isolated from POME, are applied during POME treatment. Given that POME contains a substantial amount of organic compounds primarily consisting of cellulolytic material derived from cellulose fruit debris, the facilitation of cellulolytic activity in a liquid medium is attributed to *B. cereus* owing to its capacity to secrete enzymes (Bala et al., 2015; Dominic & Baidurah, 2022).

Fungal isolates that showed highest lipase producing activity, namely *Emericella nidulans* NFCCI 3643, Trich*oderma reesei, Trichoderma harzianum*, *Aspergillus niger*, and *Aspergillus fumigatus*, were screened and isolated from POME dump sites for POME treatment under specific conditions: a working volume of 0.25 L, incubation at 30°C, and HRTs of 5 days (Lanka et al., 2014; Lanka & Pydipalli, 2018). BOD, COD and oil and grease values reduce along the fermentation time when POME is degraded in the presence of the fungi with *E. nidulans* NFCCI 3643 emerged as a remarkable biological agent, demonstrating significant reduction in the organic load of POME. Under optimal environmental and nutritional conditions, *E. nidulans* NFCCI 3643 achieved an 80.28 % reduction in COD, 88.23 % in BOD, and 87.34 % in oil and grease content (Lanka & Pydipalli, 2018; Dominic & Baidurah, 2022). Other isolates, including *A. niger, T. harzianum, A. fumigatus*, and *T. reesei*, also exhibited significant efficiency compared to the control experiment, which recorded lower reduction efficiencies of COD, BOD, and oil and grease at 13.88 %, 16.54 %, and 16.85 %, respectively (Lanka & Pydipalli, 2018). The overall results underscore the effectiveness of these indigenous fungal isolates, particularly *E. nidulans*, in reducing COD, BOD, and oil and grease content in POME, showcasing their potential in bioremediation efforts at POME dump sites.

Bala et al. (2018) successfully utilized indigenous bacterial stains (*Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus*

103PB, and *Bacillus subtilis* 106PB) and fungi strains (*Aspergillus fumigatus* 107PF and *Aspergillus niger* 109PF) isolated from POME. The aerobic mixed microbial consortium demonstrated high efficiency (90.23 % BOD, 91.06 % COD, 92.23 % TSS reduction) with working volume of 1000ml, a temperature of 30°C, and HRTs of 5 days evident that the indigenous bacterial and fungal have the capability to decrease organic load from POME and the current treatment approach demonstrated additional benefits as it necessitated no further physical or chemical treatments (Bala et al., 2018; Dominic & Baidurah, 2022).

2.5 Biomass Fuel Evolution

Biomass fuel is a renewable and sustainable organic material that comes from plants and animals used to produce heat or power by burning. It is one of the earliest sources of energy with very specific properties (Saidur et al., 2011; Toklu et al., 2017). Biomass fuels are made from vegetation that capture solar energy and convert it to chemical energy by lowering atmospheric carbon dioxide in photosynthetic form (Montoya et al., 2021). Plant leaves serve as biological solar collectors, whereas stems, branches, and roots function as batteries, accumulating complex carbon molecules that are loaded with energy (Montoya et al., 2021). As the plant died, the energy became trapped in the remains of the plants (Funabashi, 2016). Waste materials, biogenic elements in municipal solid wastes, animal manure, and human sewage are all biomass energy sources. The application of biomass for biomass fuel can be environmentally friendly because the biomass is reduced, recycled, and reused. The biomass removes carbon from the atmosphere via photosynthesis and returns it back when burn as energy source while achieving zero net carbon emission or as being carbon neutral. Biomass fuels are almost carbon-neutral because the carbon dioxide emitted when the biomass is burned equals the carbon dioxide sequestered during the plant's growth (Eloka-Eboka, 2019). Plants are a net carbon sink with approximately 2000 and 3000 billion metric tonnes of carbon stored on Earth due to the uptake of carbon dioxide (Craggs & Gilbert, 2018). It is also a renewable resource because plants capable of producing biomass may be cultivated repeatedly. These biomass energy sources can be converted to energy through various processes, including direct combustion, thermochemical, chemical, and biological.

The primary energy sources in the world are represented by fossil fuels, natural gas, and coal. These energy sources have benefited the community in many aspects. For example, the major advantage of fossil fuel is the generation of huge amounts of electricity in just a single location. Moreover, coal is abundant in supply and natural gas can be burned in power plants to generate electricity. Nevertheless, the reliance on the primary energy sources does not always guarantee an advantage. This is because these energies give off carbon dioxide when burned. Consequently, causing a greenhouse effect. In this regard, it has become a contributory factor to the global warming experienced by the earth. Furthermore, it is also predicted that these sources of energy will deplete within the next 50 years (Holechek et al., 2022).

Biofuels are categorized by first, second, third and fourth generations. The similarity of these biofuels is that each generation of biofuel strives to address global energy requirements while reducing environmental impacts (Mat Aron et al., 2020). The utilization of biomass fuel as a renewable energy resource in a sustainable way as compared to coal and fossil fuel is a much favourable way to mitigate climate change. The utilization of biomass fuel also provides economic, social, and environmental benefits such as financial net saving, conservation of fossil fuel resources, job