GREENHOUSE GAS (GHG) EMISSION MITIGATION FROM PALM OIL MILL EFFLUENT (POME) USING FILAMENTOUS FUNGI

(Bachelor Thesis)

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ABSTRACT

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Palm Oil Mill Effluent (POME) not only causes environmental pollution due to its hazardous characteristics but also contributes to significant greenhouse gas (GHG) emissions, particularly methane (CH4), which is 28 times more potent than CO_2. This study aimed to determine the effect of filamentous fungi on the pH, total solids (TS), volatile solids (VS), and chemical oxygen demand (COD) of POME and to assess their impact on GHG emission reduction. The research involved three treatments (POME without inoculation, inoculation with *Rhizopus oligosporus,* and inoculation with *Neurospora sitophila*), each with three replications. Effluent pH, TS, VS, and COD were analyzed after biomass separation, and GHG reduction potential was calculated from COD values. Results showed that filamentous fungi significantly neutralized POME's pH from an initial 4.58 to 6.47 with *R. oligosporus* and 7.26 with *N. sitophila*. Filamentous fungi also achieved 61.90% reduction in POME's TS with *R. oligosporus* and 28.57% reduction with *N. sitophila*. Furthermore, these fungi reduced POME's VS 70.27% with *R. oligosporus* and 40.54% with *N. sitophila*. Lastly, filamentous fungi were effective in reducing POME's COD 70.78% with *R. oligosporus* and 37.76% with *N. sitophila*. The potential GHG emission reduction POME treatment with *R. oligosporus* was 241.87 kg CO2e/Ton FFB , which is equivalent to 88.80%. Potential GHG emissions from POME treated with *N. sitophila* emission reduction was 207.38 kg CO2e/Ton FFB , equivalent to 76.13%.These findings highlight the promising potential of filamentous fungi in POME bioremediation, offering an environmentally friendly solution for waste management and GHG emission mitigation.

Key words: palm oil mill effluent, wastewater treatment, filamentous fungi

ABSTRAK

MITIGASI EMISI GAS RUMAH KACA (GRK) DARI LIMBAH CAIR PABRIK KELAPA SAWIT (LCPKS) MENGGUNAKAN JAMUR BERFILAMEN

Oleh

YOSNITA ANGGRIANI

Limbah Cair Pabrik Kelapa Sawit (LCPKS) merupakan limbah industri kelapa sawit yang tidak hanya mencemari lingkungan akibat sifatnya yang berbahaya, tetapi juga menyumbang emisi gas rumah kaca (GRK) yang signifikan, khususnya metana (CH_4) yang memiliki potensi pemanasan global 28 kali lebih besar dibandingkan karbon dioksida (CO_2). Penelitian ini bertujuan untuk mengetahui pengaruh jamur berfilamen terhadap pH, total padatan (TS), padatan volatil (VS), dan kebutuhan oksigen kimia (COD) POME serta mengkaji dampaknya terhadap pengurangan emisi GRK. Terdapat tiga perlakuan yang diuji, yaitu POME tanpa inokulasi, POME diinokulasi dengan *Rhizopus oligosporus*, dan POME diinokulasi dengan *Neurospora sitophila*, masing-masing dengan tiga ulangan. Analisis dilakukan terhadap pH, TS, VS, dan COD setelah pemisahan biomassa, sedangkan estimasi penurunan emisi GRK dihitung berdasarkan nilai COD. Hasil penelitian menunjukkan bahwa jamur benang mampu menetralkan pH POME dari 4,58 menjadi 6,47 dengan *R. oligosporus* dan 7,26 dengan *N. sitophila.* Selain itu, *R. oligosporus* mampu menurunkan kadar TS sebesar 61,90% dan VS sebesar 70,27%, sedangkan *N. sitophila* menurunkan TS sebesar 28,57% dan VS sebesar 40,54%. Penurunan COD juga signifikan, yakni 70,78% oleh *R. oligosporus* dan 37,76% oleh *N. sitophila.* Berdasarkan penurunan COD tersebut, potensi penurunan emisi GRK pada perlakuan *R. oligosporus* mencapai 241,87 kg CO_2e/ton tandan buah segar (TBS), setara dengan 88,80%. Sementara itu, perlakuan *N. sitophila* menghasilkan potensi penurunan sebesar 207,38 kg CO_2e/ton TBS atau setara dengan 76,13%.Dengan demikian, penggunaan jamur benang khususnya *Rhizopus oligosporus*, menunjukkan potensi besar dalam bioremediasi POME secara ramah lingkungan sekaligus menjadi strategi mitigasi emisi GRK yang efektif dalam industri kelapa sawit.

Kata kunci : limbah cair pabrik kelapa sawit, pengolahan air limbah, jamur berfilamen

Bachelor Thesis Title : **GREENHOUSE GAS (GHG) EMISSIONS MITIGATION FROM PALM OIL MILL EFFLUENT (POME) USING FILAMENTOUS FUNGI**

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STATEMENT OF ORIGINALITY

I am Yosnita Anggriani, student identification number 2114051052.

I hereby declare that what is written in this work is my own original work based on the knowledge and information I have obtained. This work does not contain material that has been previously published or, in other words, is not the result of plagiarism form other people's work.

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AUTOBIOGRAPHY

The author was born in Bumi Jaya, Central Lampung, on January 31, 2004, as the first child of Tommy Pitoyo and Ani Fitriani. The author completed elementary education at SDN 103 Bengkulu Utara in 2015, junior high school at SMPN 32 Bengkulu Utara in 2018, and senior high school at SMAN 6 Kabupaten Tangerang in 2021.

In 2021, the author was admitted and registered as a student in the Department of Agricultural Product Technology, Faculty of Agriculture, University of Lampung, through the SBMPTN pathway. The author completed an internship at PT Madubaru Yogyakarta with the title "The Production Process and Polarization Analysis of White Crystal Sugar Final Products at PT Madubaru PG PS Madukismo, Yogyakarta."

During her time as university students, the authors served as practicum assistants for Basic Chemistry, Agricultural Product Analysis, and Agro-industrial Waste Management. The author was also actively involved in various student activities, serving as a member of the Funding and Business Division of the Student Association of the Department of Agricultural Product Technology, Faculty of Agriculture, University of Lampung (HMJ THP FP Unila) during the 2023-2024 period. In addition to campus organizations, the author also participated in an off-campus organization, Ruang Pangan, as General Secretary. The author also received an incentive for PKM-AI in 2024.

DEDICATION

Alhamdulillahi rabbil ' alamin. I express my gratitude and thanks to Allah SWT, who has granted blessings and grace, enabling me to complete this bachelor thesis entitled "Greenhouse Gas (GHG) Emission Mitigation from Palm Oil Mill Effluent (POME) using Filamentous Fungi " as a requirement for obtaining a Bachelor's degree in Agricultural Product Technology from Lampung University. I acknowledge that the completion of this thesis has received extensive guidance, support, and advice both directly and indirectly, and I would like to extend my thanks to :

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I hope that Allah SWT rewards the kindness shown and that this thesis may be beneficial to me, specifically, and to readers, generally.

Bandar Lampung, July 21st 2025
Author

Yosnita Anggriani

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I. INTRODUCTION

1.1. Background and Problem

Greenhouse gases (GHGs) are one of the main causes of climate change facing the world today. GHG emissions originate from various sectors, including the agricultural industry, which significantly contributes to rising global temperatures and shifting weather patterns. One source of emissions from the agricultural sector is the palm oil industry, which produces waste in the form of palm oil mill effluent (POME). POME not only pollutes the environment but also emits methane gas (CH_4). Methane (CH_4) is estimated to be 28 times more potent than carbon dioxide (CO_2) in contributing to climate change (IPCC, 2021). According to Hosseini and Wahid (2015) every 1 ton of crude palm oil (CPO) produced can produce about 1102,325 CO_2eq of greenhouse gas (GHG) emissions, including from waste such as POME, energy use, and other production processes.

In addition to greenhouse gas emissions, POME generated from the production process has characteristics that are harmful to the environment because it contains high levels of Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), total nitrogen, oil, and fat, and has a low pH. Characteristics of palm oil liquid waste include BOD levels of 20,000-100,000 mg/L, COD 50,000-120,000 mg/L, TSS 10,000-30,000 mg/L, and pH ranging from 3.5 to 5.0 (Karno et al., 2024). This effluent can degrade the water quality in the environment around the discharge and can cause serious environmental pollution if not handled properly. Discharging POME into rivers can inhibit aquatic life by reducing sunlight penetration and disrupting photosynthesis (Wun et al., 2017). Furthermore,untreated

POME disposal can significantly alter soil chemical and biological properties, including decreased enzyme activity, changes in pH, and accumulation of excess nutrients. This leads to soil contamination, which results in reduced soil fertility and production potential (Nwachukwu et al., 2018). Therefore, mitigation measures are needed to reduce the environmental impact of this industry.

In the past five years, various POME treatment technologies have been developed and implemented, such as the Integrated Anaerobic-Aerobic Bioreactor (IAB), membrane filtration, coagulation-flocculation, photocatalysis, and Moving Bed Biofilm Reactor (MBBR). One of the latest innovations is the Zero Liquid Discharge (ZLD) technology, which is capable of treating POME to the point of producing no liquid waste at all. However, it requires a large initial investment cost. These methods have been widely developed and applied in the last five years as a more sustainable and efficient effluent treatment solution. However, conventional methods such as anaerobic ponding treatment (APT) systems are still the main choice in the field due to their low cost (Pauzi et al., 2022). Although this system is relatively simple and has low costs and energy, this treatment method has several disadvantages, such as requiring long retention times and large treatment areas. In addition, the anaerobic digestion process in the pond system produces large amounts of methane gas, which can cause greenhouse gas (GHG) emissions and contribute to global warming (Jumadi and Wong, 2020). Given these drawbacks, more effective treatment alternatives are needed that can reduce greenhouse gas emissions and improve overall effluent treatment efficiency.

Biological treatment of POME to reduce effluent water content is one of the potential methods that can be adopted to address the pollution problems faced by the palm oil industry, as it is more environmentally friendly and affordable than physical and chemical methods (Sar et al., 2024). Researchers have widely reported the use of various types of microorganisms, such as bacteria, fungi, and microalgae, in POME treatment due to their ability to significantly reduce pollutant loads. Among these microorganisms, bacteria are the most commonly used biological agents in the biochemical decomposition process of wastewater (Dominic and Baidurah, 2022). In addition to bacteria, filamentous fungi have significant potential in organic waste treatment, although their application is still rare on an industrial scale (Ferreira et al., 2020).

Several species of filamentous fungi, such as *Aspergillus oryzae* (Barker and Worgan, 1981), *Trichoderma viride* (Karim and Kamil, 1989), and *Rhizopus oryzae* (Prasertsan and Binmaeil, 2018) have been proven effective in biologically treating POME. *Rhizopus oligosporus* and *Neurospora sitophila* are filamentous fungal species that have the potential to bioconvert organic waste into biomass (Wikandari et al., 2023; Beuchat et al., 1978). However, to date, there has been no study examining the potential and effectiveness of these two species in POME treatment. Based on this description, it is necessary to conduct research on mitigating POME greenhouse gas emissions using filamentous fungal production.

1.2 Objectives

The objectives of this research are as follows:

1. To determine the effect of using filamentous fungi on the pH, total solid (TS), volatile solid (VS), and chemical oxygen demand (COD) from POME.
2. To determine the effect of using filamentous fungi on greenhouse gas emission reduction from POME.

1.3 Reseacrh Framework

Palm oil liquid waste (POME) is known to contain carbon-rich organic compounds as well as important nutrients such as nitrogen, which can be utilized as a source of nutrients for microorganisms. POME contains approximately 613 mg/L of ammonia nitrogen, a key nutrient for microbial cell growth (Ibrahim et al., 2012). In addition, in the research of Sudirman et al. (2011), POME was shown to play a role in improving the carbon-to-nitrogen (C/N) ratio in the media, where a decrease in this ratio contributes to the acceleration of the decomposition process of organic compounds.

Therefore, POME has potential as a growth medium for microorganisms, especially filamentous fungi.

Filamentous fungi can produce various extracellular enzymes, such as cellulases, lipases, proteases, pectinases, xylanases, and lignin-degrading enzymes, which are important in degrading complex organic compounds. These enzymes provide filamentous fungi with metabolic versatility, enabling them to degrade complex substrate such as lignocellulose, starch, protein, and fat (Ferreira et al., 2020). Through this enzymatic activity, filamentous fungi are effective in POME treatment, potentially having a significant impact on effluent characteristic parameters, such as pH, total solid (TS), volatile solid (VS), and chemical oxygen demand (COD). According to Zanellati et al. (2020) the decrease in TS and VS indicates the success of the decomposition process of organic solid matter, where most of these compounds are converted into fungal biomass or converted into simple compounds. This change in fungal metabolism can also affect the pH of the media. Filamentous fungi have the ability to adapt to a wide range of pH ranges, and during the biodegradation process, fungal metabolic activities can cause pH changes. In addition, the decrease in COD reflects the ability of fungi to reduce the load of dissolved organic matter, which is generally difficult to decompose naturally. According to Ziliwu and Lase (2025) this is because the process of organic matter degradation involves various biochemical mechanisms triggered by extracellular enzymes produced by the microbes. By converting waste components into their cell biomass rather than into gas, filamentous fungi effectively contribute to mitigating greenhouse gas emissions.

Biological treatment of POME using filamentous fungi has been carried out by various researchers to utilize the potential of this waste more sustainably. Barker and Worgan (1981) reported that *Aspergillus oryzae* was able to grow well on POME-based media which initially had a pH of 4.6 and Total Solid (TS) of 59.1 g/L. This treatment produced 50 g of biomass for every 100 g of available organic matter along with a 75% to 80% reduction in COD and a 76.6% reduction in TS. In addition, the pH of the media increased from 4.5 to 7.5 to 8.0 during fungal growth. The study by Karim and Kamil

(1989) utilized *Trichoderma viride* and achieved a reduction in COD levels of more than 95% within 10 to 14 days of fermentation, accompanied by mycelial biomass production of 1.37-1.42 g/L. A study by Prasertsan and Binmaeil (2018) showed that *Rhizopus oryzae ST29* was able to grow optimally in POME that had an initial pH of 4.5 and total solid of 71.5 g/L. This treatment yielded a biomass of 16.9 g/L, accompanied by a COD reduction of up to 80% and a total solid reduction of 66.4% within just 4 days. During cultivation, the pH also increased slightly from 4.5 to 5.2-5.5.

In this study, the fungal species to be used for POME treatment are *Rhizopus oligosporus* and *Neurospora sitophila*. These fungal species were chosen based on their proven ability to bioconvert organic waste into biomass. Research by Wikandari et al. (2023) proved that the filamentous fungus *R. oligosporus* is effective in the bioconversion of residual water waste from tempeh factories into biomass, with production reaching 7.76 g/L. *R. oligosporus* utilizes organic compounds contained in the residual water of the tempeh factory, such as reducing sugars and proteins, as a source of nutrients for its growth. Through enzymatic activity, especially proteolytic enzymes, this fungus degrades proteins into amino acids and assimilates dissolved organic acids to convert these waste components into biomass. Meanwhile, research by Beuchat et al. (1978) proved that the filamentous fungus *N. sitophila* is effective in fermenting alkaline waste from vegetable (rutabaga and potato) and fruit (peach) peeling operations, as well as rinse waste. Submerged fermentation for 4 days was able to reduce the COD value of the stripping effluent between 42% to 68% of the initial value and the rinse effluent between 17% to 25%. The process also increased the total amino acid content of potato effluent biomass by almost four times, while the total amino acid content of peach effluent doubled after 1 day of fermentation.

The selection of these fungal species and their optimal treatment conditions, including initial pH, supplementation, media dilution, and agitation speed, have been established through a series of preliminary studies to ensure the highest biomass yield. This treatment approach uses aerobic fermentation, which naturally does not produce methane gas (CH4). Research by Beuchat et al. (1978) showed that fermentation using

N. sitophila was carried out under aerobic conditions with incubation on a gyratory shaker. Similarly, Wikandari et al. (2023) also applied aerobic fermentation for the cultivation of *R.oligosporus* in the residual wastewater of a tempeh factory, which was evident from the use of agitation at 125 rpm and the importance of aeration for higher biomass production. Thus, the utilization of *R.oligosporus* and *N. sitophila* under aerobic conditions is expected to provide a more environmentally friendly and efficient solution for reducing GHG emissions while reducing the load of other POME pollutants. Based on this description, this study was conducted to determine the effect of using filamentous fungi on pepH, total solid (TS), volatile solid (VS), and chemical oxygen demand (COD) from POME, as well as to determine its effect on reducing greenhouse gas emissions generated from the waste.

1.4 Hypotheses

The hypotheses of this research are as follows:

1. The use of filamentous fungi affects the pH, total solids (TS), volatile solids (VS), and chemical oxygen demand (COD) of Palm Oil Mill Effluent (POME).
2. The use of filamentous fungi contributes to the reduction of greenhouse gas emission from Palm Oil Mill Effluent.

II. LITERATURE REVIEW

2.1 Green House Gasses Emission

Greenhouse gas (GHG) are atmospheric components that significantly contribute to global warming through the greenhouse effect. These gases trap heat in the Earth’s atmosphere, functioning like a greenhouse and resulting in rising global temperatures (Sugiyono, 2006). Sunlight reaches the Earth as shortwave solar radiation, which is then absorbed and re-emitted as longwave infrared radiation. Greenhouse gases allow approximately 90% of visible solar radiation to pass through the atmosphere. All solar radiation that strikes the Earth is converted into longwave radiation in the form of infrared radiation as longwave infrared radiation.

While solar radiation penetrates the atmosphere, infrared radiation is trapped by greenhouse gases. As a result, the accumulation of radiant energy on Earth causes the Earth’s temperature to increase. The Earth absorbs a portion of solar energy and reflects the remainder as infrared radiation. Greenhouse gases in the Earth’s troposphere can emit most of the solar radiation, but also retain the infrared radiation contained in the reflection. However, when greenhouse gases cover the Earth in large quantities, the reflected infrared radiation gets trapped in the atmosphere, making the Earth’s temperature hotter than normal for a long period of time (Cengel, 1997).

The IPCC (Intergovernmental Panel on Climate Change) reports that the Earth's temperature has increased by 0.6°C during the 20^{th} century compared . At the beginning of industrialization in 1750. The Earth’s temperature is predicted to continue rising, with an average increase of 0.1°C to 0.2°C per decade over the next five decades (IPCC, 1997).

At the beginning of industrialization in 1750. The Earth's temperature is predicted to continue rising, with an average increase of 0.1°C to 0.2°C per decade over the next five decades (IPCC, 1997).Gases classified as GHGs include carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), perfluorocarbons (PFC), hydrofluorocarbons (HFC), and sulfur hexafluoride (SF6). These six GHGs are recognized in the Kyoto Protocol as being responsible for increasing global warming. These gases have a global warming potential that is calculated in terms of CO_2 potential, known as Global Warming Potential (GWP). GWP measures the magnitude of the GHG radiative effect compared to CO_2 (Suarsana and Wahyuni, 2011). The types of GHGs based on the Kyoto Protocol are presented in Table 1.

Table 1. Six Types of Greenhouse Gases Based on the Kyoto Protocol

Greenhouse Gasses (GHG)	Global Warming Potential (GWP)
Carbon dioxide (CO_2)	1
Methane (CH_4)	21
Nitrogenoxide (N_2O)	310
Hydrofluorocarbons (HFC)	140-11.700
Perfluorocarbons (PFC)	6.500-9.200
Sulfurhexachloride (SFC)	23.900

Source: Handayani (2008)

2.2 Palm Oil Mill Effluent (POME)

Palm Oil Mill Effluent (POME) is a colloidal suspension comprising 95–96% water, 0.6–0.7% oil, and 4–5% total solids. POME is released from the industry as a brown liquid with a discharge temperature ranging from 80°C to 90°C, a pH value of 4.0 to 5.0, and a strong sour odor (Directorate General of PPHP, Department of Agriculture, 2006). The palm oil industry generates waste in solid, gaseous, and liquid forms. Solid waste includes empty fruit bunches (approximately 23%), fiber (approximately 13.5%), and shells (approximately 5.5%). This solid waste can be utilized as fuel, fertilizer, or animal feed. Gas waste consists of emissions from boilers and incinerators used in the crude palm oil (CPO) production process. Liquid waste, known as Palm Oil Mill Effluent (POME), accounts for 55-67% of the total waste produced. POME consists of wastewater originating from boiled condensate, hydrocyclone water, and separator sludge. The characteristics of POME liquid waste presented in Table 2.

Table 2. Characteristics of Lampung Palm Oil Mill Effluent (POME)

Parameter	Value	Unit
pH	4.09	-
Protein	0.15	%
Nitrogen	0.0295	%
Fe	0.258	mg/L
Phosphate	37.175	mg/L
Phenol	≤ 0.0001	mg/L
Oil and grease	115	mg/L
Potassium	1,459.86	mg/L
Sulphate	1,032.93	mg/L
Ammonia	125	mg/L

Source: Mellyanawaty et al., 2018

The discharge of untreated Palm Oil Mill Effluent (POME) has been recognized as a major environmental pollutant due to its high organic load and potential toxicity to aquatic ecosystems. When released directly into water bodies, POME reduces water transparency, disrupts the photosynthesis process, decreases dissolved oxygen levels, and causes severe effects such as tumors and mortality in aquatic organisms, as well as health risks in humans including irritation, poisoning, gene mutations, and cancer (Valerie et al., 2018). Despite these harmful impacts, POME contains valuable organic materials such as carbohydrates, nitrogenous compounds, lipids, organic acids, and polyphenolic substances that support microbial growth and metabolism, making it a potential substrate for bioconversion (Crognale et al., 2006). The nutritional content of POME is relatively balanced, containing essential macronutrients like carbohydrates, proteins, and fats, which enables its use in microbial processes such as fermentation, composting, or biomass production.

However, if left untreated, the degradation of organic matter in POME leads to the accumulation of ammonia, which is toxic to aquatic life and contributes to offensive odors (Azwir, 2006). To address these issues, it is necessary to implement strict quality standards for liquid waste discharge from palm oil industries to minimize environmental damage and protect public health. Several technologies, including anaerobic and aerobic biological treatments, have been developed and applied to reduce the pollutant content in POME effectively. The quality standards for liquid waste from the palm oil industry presented in Table 3.

Table 3. Waste water quality standards for palm oil businesses or industrial activities

Parameter	Maximum Concentration (mg/L)	Highest Pollution Load (kg/ton)
BOD	100	0,25
COD	350	0,88
TSS	250	0,63
Oil and Fat	25	0,0063
Nitrogen Total	50	0,125
pH	6,0 – 9,0	
Highest effluent discharge	2,5 m^3/ton CPO	

Source : Regulation of the Minister of Environment of the Republic of Indonesia, No.5/2014. Concerning Waste Water Quality Standards.

2.3 Filamentous Fungi

Filamentous fungi are ubiquitous in nature and play a crucial role in maintaining ecological balance through the decomposition of organic matter, nutrient recycling, and symbiotic interactions (Kues, 2015). These fungi are phylogenetically diverse, with members of the Ascomycota, Basidiomycota, and Zygomycota phyla most commonly associated with pollution mitigation research and commercial applications (Troiano et al., 2010). Their widespread ecological role and extensive use in research stem from their macroscopic filamentous growth, phylogenetic diversity, ability to produce a wide array of extracellular and intracellular enzymes, potential for generating value-added products, production of biosurfactants, cell wall sorption capabilities, and synergistic potential when used in co-culture systems.

While bioconversion processes can also utilize bacteria, yeasts, and algae. However, in view of the macroscopic filamentous structure which is easily recoverable from the medium, processes using filamentous fungi are economically less sensitive to the choice of cell mass recovery strategies. Their biomass can be easily separated from the medium through simple sieving, whereas other microbial groups typically require energy-intensive recovery processes such as centrifugation. For example, microalgal biomass recovery is one of the major bottlenecks in its large-scale feasibility, and the use of filamentous fungi to induce flocculation and facilitate easier biomass recovery has been investigated (Muradov et al., 2015) . As a result, processes utilizing filamentous fungi not only simplify biomass extraction but also contribute to the reduction of chemical oxygen demand (COD) and lower the viscosity of the medium (Ferreira et al., 2018).

2.4 Acidicity Level (pH)

The pH value indicates the degree of acidity or alkalinity, reflecting the concentration of hydrogen ions (H^+) in the waste. Changes in pH significantly affect the physical, chemical, and biological processes that influence aquatic organisms. The pH level strongly influences pollutant toxicity, gas solubility, and the chemical forms of substances in water. The pH value is used to express the hydrogen ion concentration in wastewater, indicating its level of acidity or alkalinity. The pH scale ranges from 0 to 14, where 0–6 indicate acidity, 7 is neutral, and 8–14 indicate alkalinity. Wastewater that is either too acidic or too alkaline can disrupt biological treatment processes (Sutrisno, 2010).

2.5. Total Solid (TS)

Total Solid (TS) are solid particles contained in wastewater, both from organic and inorganic materials or all solids that remain as residue after evaporation and drying at a temperature of 103 to 105°C. Total solids consist of both dissolved solids and insoluble solids. They significantly affect wastewater quality, ranging from large to very small dispersions. Measurements of total solids are based on water samples that are dried at a temperature above the water vapor point for a specific duration until all the water has evaporated. The weight of the remaining sample is recorded as the total solid weight per unit liter (mg/L) (Sutrisno, 2010).

2.5. Volatile Solid (VS)

Volatile solid (VS) is the fraction of total solids that can vaporize or burn into gas when heated further, usually at temperatures around 550°C. This fraction represents the organic portion of the solids, and is therefore often used to estimate the content of biodegradable organic matter. The organic fraction plays a crucial role in determining the effectiveness of biological waste treatment, its concentration influences microbial activity, substrate availability, and overall treatment efficiency. Measurement of this component is essential, as it is directly related to the stability of the process and the rate of degradation achieved. Therefore, it serves as a key parameter in evaluating the performance and sustainability of waste treatment systems. (Saragih, 2010).

2.6 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) is the amount of oxygen, expressed in ppm or milligrams per liter, required to chemically oxidize organic matter in wastewater using potassium bichromate ($K_2Cr_2O_7$) under acidic conditions. This process will convert organic waste into carbon dioxide (CO_2), water (H_2O) and chromium ions. COD values are generally higher than ultimate BOD due to the presence of organic compounds that are difficult to break down biologically such as lignin, as well as the possibility of inorganic substances being oxidized by dichromate. Therefore, COD is used as one of the important parameters to determine the level of organic matter pollution in wastewater (Nurhasanah, 2009).

III. METHODOLOGY

3.1. Time and Place

This research will conduct from January to June 2025 at the Agroindustrial Waste Management Laboratory and Microbiology Laboratory, Faculty of Agriculture, Major of Agricultural Product Technology, Lampung University, Lampung.

3.2. Materials and Equipment

3.2.1 Materials

The materials used in this study included Palm Oil Mill Effluent (POME) from the first pond obtained from PTPN VII Bekri, Lampung. Pure *Rhizopus oligosporus* and *Neurospora sitophila* fungal cultures were obtained from the Center for Food and Nutrition Studies, Gadjah Mada University. The medium used to grow the pure cultures was PDA (Potato Dextrose Agar). The supplementation media used are peptone and tryptone. Other materials used include H2SO4, NaOH, 70% alcohol, tween 80, COD reagent and distilled water.

3.2.2 Equipment

The research equipment used in this study included a 500 mL Erlenmeyer flask, shaker, filter cloth, pH meter, analytical balance, 100 mL volumetric flask, volumetric flask, beaker, micropipette, blue tip, laminar air flow, autoclave, spatula, cotton buds, ziplock plastic, and aluminum foil

The equipment used for analysis are test tubes, test tube racks, vortex, porcelain cups, measuring cups, digital scales, oven, furnace, desiccator, cup tongs, pH meter, rubber bulb, volumetric pipette, COD reactor DRB 200, and HACH spectrophotometer DR4000.

3.3 Research Methods

The study was conducted with three treatments, each with three replications: control POME (without fungal inoculation), POME with *Rhizopus oligosporus* inoculation, and POME with *Neurospora sitophila* inoculation. The determination of optimal conditions for the treatments (including initial pH, type, and concentration of supplementation, dilution level, and agitation speed) was based on the results of preliminary research that had been conducted to obtain the highest biomass production. After biomass separation, the effluent was analyzed for pH, TS, VS, and COD. The data were then analyzed descriptively. Greenhouse gas (GHG) emission reduction potential was calculated based on the COD value of the treated POME.

3.4. Research Procedures

3.4.1. Preparation Inoculum

The fungal culture used was an isolate of *Rhizopus oligosporus* and *Neurospora sitophila* grown on PDA medium. Spores were harvested from the surface of the slanted agar by adding 100 mL of 0.05% Tween 80 solution. This suspension contained 10^5 spores/mL.

3.4.2 Fungal biomass Production from *Rhizopus oligosporus*

Biomass production began with media preparation; 100 mL of POME effluent was added to each 500 mL Erlenmeyer flask. Then, 0.5 grams of tryptone supplementation was added to each Erlenmeyer flask and shaken to distribute the supplementation evenly. The medium with the added supplementation was then checked for pH; if it was within the pH range of 5.5, no adjustments were made. Erlenmeyer flasks were closed with cotton stoppers and wrapped in plastic for sterilization. Sterilization was performed using an autoclave at 121°C for 15 minutes. After sterilization, inoculation was carried out in the laminar room. In the laminar chamber, the cotton plug of the Erlenmeyer flask was opened, and 10 mL of spore solution of *R. oligosporus* containing 10^7 spores/mL was added. Next, the Erlenmeyer flask was closed with a cotton plug and paper and placed on a shaker with an agitation speed of 110 rpm at room temperature (28°C) for 72 hours. After 72 hours, harvesting was done by pouring the contents of the Erlenmeyer flask onto a filter cloth. The flow chart of mushroom biomass production is presented in Figure 1

3.4.2 Fungal biomass Production from *Neurospora sitophila*

Production began with the preparation of media that had been diluted 1:10 with distilled water. After that, 100 mL of diluted POME effluent was added to each 500 mL Erlenmeyer flask. Then, 0.5 grams of peptone supplementation was added to each Erlenmeyer flask and shaken to distribute the supplementation evenly. The medium with the added supplementation was then checked for pH; if it was within the pH range of 3.5, no adjustments were made. Erlenmeyer flasks were closed with cotton stoppers and wrapped in plastic for sterilization. Sterilization was performed using an autoclave at 121°C for 15 minutes. After sterilization, inoculation was carried out in the laminar room. In the laminar chamber, the cotton plug of the Erlenmeyer flask was opened, and 10 mL of spore solution of *N. sitophila* containing 10^7 spores/mL was added. Next, the Erlenmeyer flask was closed with a cotton plug and paper and placed on a shaker with an agitation speed of 125 rpm at room temperature (28°C) for 72 hours. After 72 hours, harvesting was done by pouring the contents of the Erlenmeyer flask onto a filter cloth. The flow chart of mushroom biomass production is presented in Figure 2.

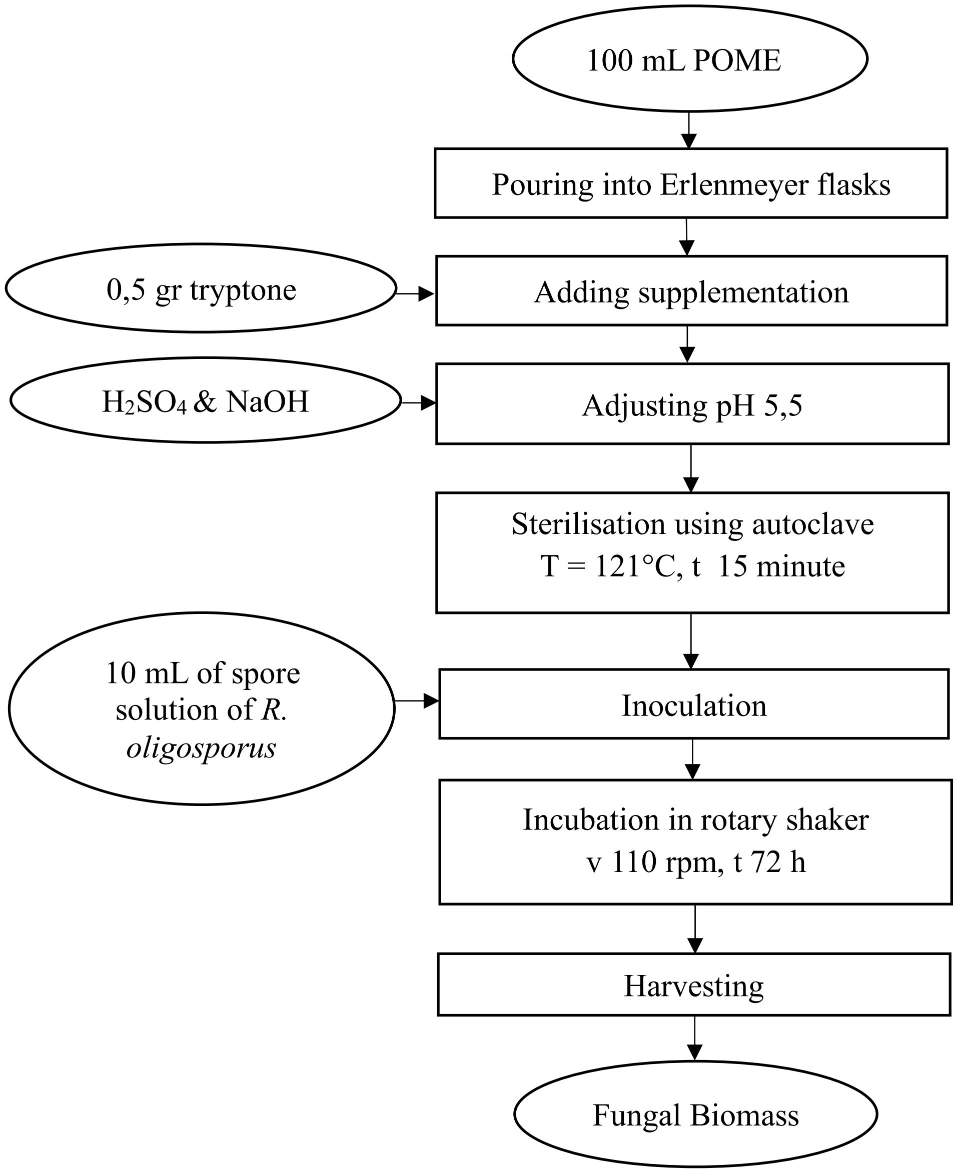

Figure 1. Workflow diagram of fungal biomasss production *R.oligosporus*.

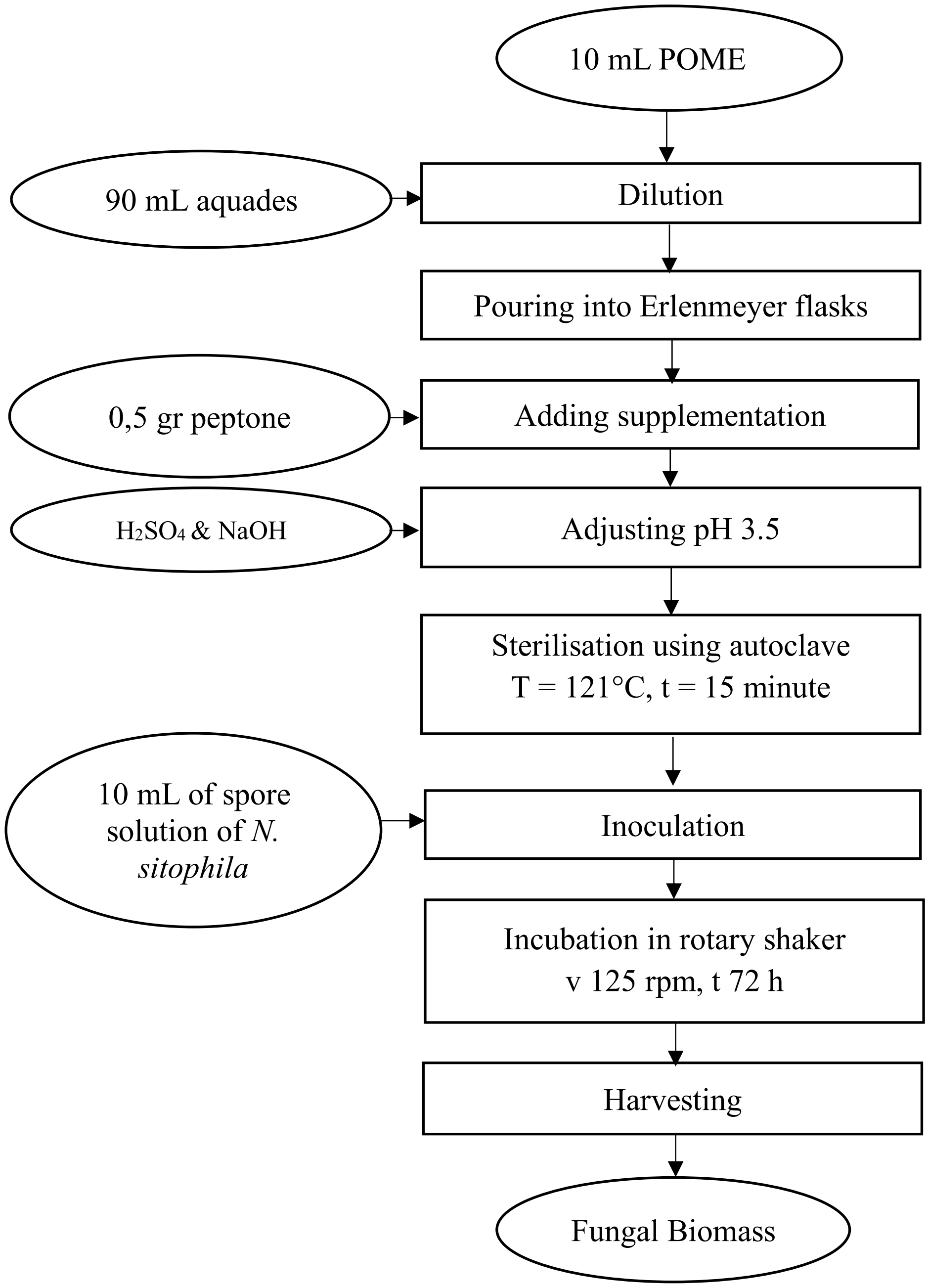

Figure 2. Workflow diagram of fungal biomasss production from *N. sitophila.*

3.4.3 Separation of biomasss from media

Mycelium biomass was harvested after cultivation. Furthermore, the biomass was squeezed using a filter cloth, then weighed to determine its weight. The effluent as media will be analyzed based on the observed parameters.

3.5 Observation

3.5.1 Acidicity Level (pH)

The pH analysis was carried out using the HI 2550 pH/ORP & EC/TDS/NaCl Meter Hanna Instruments. pH testing was carried out using the APHA AWWA WEF *23rd Edition* 2017 Part 4500 - H + B method. The steps in pH analysis begin with preparing the pH instrument equipment. First, the pH meter electrode is spelled using distilled water three times and dried using a tissue. Then, the pH meter electrode is immersed into the sample for ± 1 minute and then dried with a tissue. If the sample to be analyzed is more than one, then the stages of analyzing the next sample follow the stages of the previous sample. A new sample is prepared for analysis and the rinsed electrode is then immersed into the new sample until the pH meter shows a fixed reading. The number shown on the pH meter screen is the pH value of the measured wastewater. The measurement results are then recorded on the observation sheet

3.5.2. Total Solid (TS) Analysis Gravimetric Method

Total solid (TS) is all solids contained in wastewater, both dissolved and undissolved solids. Total solid is calculated based on the weight difference of solids left behind per liter of solution. Calculation of total solid is done by heating and weighing the cup as weight A Wastewater sample is put in a cup with a certain volume. The cup containing the sample was heated at 103-105 ℃ for 24 hours. The cup was cooled in a desiccator for 15 minutes. After cooling the cup is weighed as weight B. The result is calculated the amount of TS according to the following formula.

$$\text{TS (g/mL)} = \frac{\text{weight of cup oven (g)} - \text{weightof empty cup (g)}}{\text{sampel weight}}$$

(SNI 06-6989.26-2005)

3.5.3 Volatile Solid (VS) Analysis Gravimetric Method

Volatile Solid (VS) analysis is a measurement that serves to determine the weight of material lost or broken from the total solid during the heating process. VS analysis is a continuation of the TS analysis process by calculating the amount of material that evaporates. VS analysis where the cup containing the sample that has been oven and weighed, then put into the furnace for 1 hour at 550˚C. After that, wait until the temperature in the furnace drops to 30-33˚C to remove the cup and put it in a desiccator for 15 minutes. The cup was then weighed until the consant weight was obtained and the VS calculation was carried out using the formula as follows.

$$\text{VS (g/g)} = \frac{\text{weight of cup after furnance (g)} - \text{weightof empty cup (g)}}{\text{sampel weight}}$$

(SNI 06-6989.26-2005)

3.5.4 . Chemical Oxygen Demand *(*COD)

COD analysis was carried out by taking a sample solution of 0.2 mL using a 1 mL volumetric pipe. The sample was put into a vial or tube containing COD reagent and then heated with a DBR200 reactor unit at 150 °C for 2 hours. After heating, the vial is removed and allowed to cool (until it reaches room temperature) then the COD value is measured using HACH Spectrophotometry DR4000 at a wavelength of 620 nm (APHA, 1998)

3.6 Calculation of Greenhouse Gas Emissions

To determine the effect of this treatment on GHG, an analysis of the COD values will be conducted because this parameter is directly related to the significant potential for methane (CH4) formation. Below are several calculation formulas that will be used in the research.

$$BE = Qww \times CODinflow \times CODr \times MCF \times BOww \times UF \times GWPCH4$$
$$PE = Qww \times CODeffluent \times MCF \times BOww \times UF \times GWPCH4$$
$$ER = BE - PE$$

Notes :
BE = Baseline Emission
PE = Project Emission
ER = Emission Reduction

Qww = Volume wastewater (m^3/ Ton FFB)
$CODinflow$ = Chemical oxygen demand of the wastewater (kg COD/m^3)
$CODeffluent$ = Chemical oxygen demand of the wastewater (kg COD/m^3)
$CODr$ = COD removal
MCF = Methane correction factor
$BOww$ = Methane producing capacity of the wastewater (IPCC value of 0.25 kg CH_4/kg)
UF = Model correction factor to account for model uncertainties (0.89)
$GWPCH4$ = Global warming potential for methane
Source : (UNFCCC, 2019)

V. CONCLUSION & SUGGESTION

5.1 Conclusion

The conclusions of this study are as follows :

1. Filamentous fungi influence pH, total solids (TS), volatile solids (VS), and chemical oxygen demand (COD) in POME. Specifically, filamentous fungi helped neutralize pH, increasing it from an initial 4.58 to 6.47 with *R. oligosporus* and to 7.26 with *N. sitophila*. Filamentous fungi also achieved 61.90% reduction in TS with *R. oligosporus* and 28.57% reduction with *N. sitophila*. Furthermore, these fungi reduced VS 70.27% with *R. oligosporus* and 40.54% with *N. sitophila*. Lastly, filamentous fungi were effective in reducing COD 70.78% with *R. oligosporus* and 37.76% with *N. sitophila.*
2. Filamentous fungi influence greenhouse gas (GHG) emission reduction from POME. The potential GHG emission reduction POME treatment with *R. oligosporus* was 241.87 kg CO_2e/Ton FFB , which is equivalent to 88.80%. Potential GHG emissions from POME treated with *N. sitophila* emission reduction was 207.38 kg CO_2e/Ton FFB , equivalent to 76.13%.

5.2 Suggestion

Based on this study, the following recommendations should be considered for future research :

1. Combination with post-treatment methods to achieve the specified waste water quality standards for palm oil.

2. Potential study and economic analysis of the utilization of fungal biomass produced after POME treatment. This biomass can be used as animal feed or as a source for other bioproducts.
3. It is necessary to conduct a Life Cycle Assessment (LCA) for POME processing using filamentous fungi.

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