

**UTILIZATION OF PALM OIL MILL EFFLUENT (POME) AS
SUBSTRATE FOR MYCELIAL GROWTH BY THE FUNGUS
*Neurospora sitophila***

(Bachelor Thesis)

By

**Galuh Septa Nugraha
2114051039**



**FACULTY OF AGRICULTURE
LAMPUNG UNIVERSITY
BANDAR LAMPUNG
2025**

ABSTRACT

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The increasing production of palm oil in Indonesia has led to the generation of large volumes of Palm Oil Mill Effluent (POME), an organic-rich wastewater with significant environmental impacts. Despite its pollutant load, POME contains essential nutrients such as carbon, nitrogen, and minerals, offering potential for microbial biomass cultivation. This study investigated the use of POME as a substrate for the growth of *Neurospora sitophila*, a filamentous fungus, through submerged fermentation. Four parameters were optimized to achieve maximum biomass production: dilution ratio, supplementation, initial pH, and agitation speed. The optimal conditions identified were a 1:10 dilution ratio of POME, supplementation with 5 g/L peptone, initial pH adjusted to 3.5, and agitation at 125 rpm for 72 hours. These conditions yielded a maximum fungal biomass of 4,7408 g/L (dry basis). Nutritional analysis of the biomass showed a protein content of 31,63%, fat content of 18,66%, ash content of 4,10% (dry basis), crude fiber content of 2,94% (dry basis), and moisture content of 79,99%. Compared to conventional feed ingredients such as soybean meal and palm kernel meal, the fungal biomass demonstrated competitive nutritional values, indicating its potential as a sustainable protein source for feed applications. This study highlighted the dual benefit of utilizing POME for waste mitigation and the production of value-added fungal biomass, contributing to circular bioeconomy practices in the palm oil industry.

Keywords: Palm Oil Mill Effluent (POME), fungal biomass, *Neurospora sitophila*

ABSTRAK

PEMANFAATAN LIMBAH CAIR PABRIK KELAPA SAWIT (LCPKS) SEBAGAI SUBSTRAT UNTUK PERTUMBUHAN MISELIA OLEH JAMUR *Neurospora sitophila*

Oleh

Galuh Septa Nugraha

Peningkatan produksi kelapa sawit di Indonesia telah menyebabkan terbentuknya limbah cair pabrik kelapa sawit/*Palm Oil Mill Effluent* (POME) dalam jumlah besar, yaitu limbah cair yang kaya akan bahan organik dan berdampak signifikan terhadap lingkungan. Meskipun memiliki beban pencemar, POME mengandung nutrisi penting seperti karbon, nitrogen, dan mineral yang berpotensi dimanfaatkan untuk budidaya biomassa mikroba. Penelitian ini bertujuan untuk memanfaatkan POME sebagai substrat untuk pertumbuhan jamur *Neurospora sitophila*, menggunakan metode fermentasi terendam. Empat parameter dioptimalkan untuk memperoleh produksi biomassa maksimal, yaitu rasio pengenceran, suplementasi, pH awal, dan kecepatan agitasi. Kondisi optimal yang diperoleh adalah dengan rasio pengenceran POME 1:10, suplementasi 5 g/L pepton, pH awal 3,5, dan kecepatan agitasi 125 rpm selama 72 jam. Kondisi ini menghasilkan biomassa jamur maksimum sebesar 4,7408 g/L (basis kering). Analisis nutrisi terhadap biomassa menunjukkan kandungan protein sebesar 31,63%, lemak 18,66%, abu 4,10% (basis kering), serat kasar 2,94% (basis kering), dan kadar air 79,99%. Biomassa jamur menunjukkan nilai gizi yang kompetitif dibandingkan dengan bahan pakan konvensional seperti bungkil kedelai dan bungkil inti sawit, sehingga memiliki potensi sebagai sumber protein berkelanjutan untuk aplikasi pakan. Penelitian ini menunjukkan manfaat ganda dari pemanfaatan POME, yaitu mitigasi limbah sekaligus produksi biomassa jamur yang memiliki nilai tambah dan berkontribusi terhadap praktik ekonomi sirkular dalam industri kelapa sawit.

Kata kunci: limbah cair pabrik kelapa sawit, biomassa jamur, *Neurospora sitophila*

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Bachelor Thesis

**As one of the requirements for achieving a degree
BACHELOR OF AGRICULTURAL TECHNOLOGY**

In

**Agricultural Product Technology Department
Faculty of Agriculture Lampung University**



**FACULTY OF AGRICULTURE
LAMPUNG UNIVERSITY
BANDAR LAMPUNG
2025**

Bachelor Thesis Title

**: UTILIZATION OF PALM OIL MILL
EFFLUENT (POME) AS SUBSTRATE
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Name of Student

: Galuh Septa Nugraha

Student Identification Number

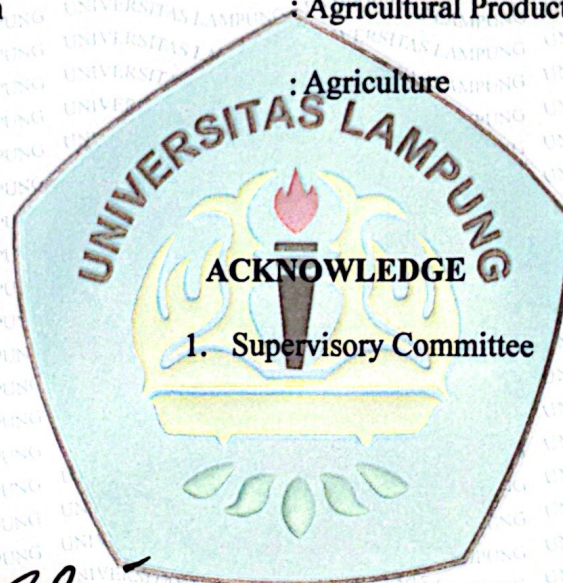
: 2114051039

Study Program

: Agricultural Product Technology

Faculty

: Agriculture



1. Supervisory Committee

Prof. Dr. Ir. Udin Hasanudin, M.T.
NIP. 19640106 198803 1 002

Rachma Wikandari, S.T.P., M. Biotech., Ph.D.
NIP. 19860126 201803 2 001

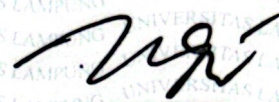
2. Head of Agricultural Product Technology Department

Dr. Erdi Suposo, S.T.P., M.T.A., C.EIA.
NIP. 19721006 199803 1 005

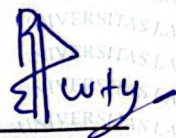
VALIDATE

1. Examination Committee

Chairman : Prof. Dr. Eng. Ir. Udin Hasanudin, M.T.



Secretary : Rachma Wikandari, S.T.P., M. Biotech., Ph.D.



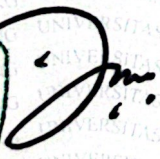
Examiners : Prof. Mohammad Taherzadeh
Not an advisor



2. Dean Faculty of Agriculture



Dr. Ir. Kuswanta Futas Hidayat, M.P.
NIP. 19641118 198902 1 002



Date of passing the thesis examination : July 1st 2025

STATEMENT OF ORIGINALITY

I am Galuh Septa Nugraha, student identification number 2114051039.

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 from other people's work, except for those that are explicitly cited from sources and properly listed in the bibliography.

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Bandar Lampung, July 1st, 2025



Galuh Septa Nugraha
SID Number 2114051039

AUTOBIOGRAPHY

The author was born in Bandar Lampung, Lampung on September 30, 2003. The author is the first child of Aris Susanto and Tri Feriyani. Since childhood, the author has lived in Lampung. The author completed elementary education at SDN 1 Harapan Jaya in 2015, junior high school at MTsN 2 Bandar Lampung in 2018, and senior high school at SMAN 5 Bandar Lampung in 2021. In 2021, the author was admitted to the Agricultural Product Technology study program at the Agriculture Faculty, Lampung University.

In January–February 2024, the author participated in the community service program (KKN) in Rangai Tri Tunggal Village, Katibung District, South Lampung Regency. Later, in July–August 2024, the author carried out a internship at PT Madubaru, Madukismo Sugar Factory, Yogyakarta, with a project entitled “*A Study on Quality Control of the Final Product of White Crystal Sugar at PT Madubaru*”.

During the author’s academic journey, the author actively engaged in student organizations. In 2024, the author served as the Chairman of the Himpunan Mahasiswa Jurusan Teknologi Hasil Pertanian Fakultas Pertanian Universitas Lampung (HMJ THP FP Unila). Previously, in 2023, the author was entrusted with the role of Head of the Education and Reasoning Division of the same organization. In the 2023/2024 academic year, the author also contributed as a teaching assistant for the Basic Chemistry course..

DEDICATION

Alhamdulillah 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 “Utilization of Palm Oil Mill Effluent (POME) as Substrate for Mycelial Growth by The Fungus *Neurospora sitophila*” as a requirement for obtaining a Bachelor’s degree in Agricultural Technology from the 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:

1. Dr. Ir. Kuswanta Futas Hidayat, M.P., as the Dean of the faculty of Agriculture, Lampung University.
2. Dr. Ir. Erdi Suroso, S.T.P., M.T.A., C.EIA., as the Head of the Agricultural Product Technology Department, Faculty of Agriculture, Lampung University.
3. Prof. Dr. Ir. Samsul Rizal, M.Si., as the Coordinator of the Agricultural Product Technology Study Program, Department of Agricultural Product Technology, Faculty of Agriculture, University of Lampung.
4. Mrs. Dyah Koesoemawardhani, S.Pi., M.P., as Academic Advisor, who has continually provided guidance, advice, motivation, and suggestions from the beginning to the end of my studies.
5. Prof. Dr. Ir. Udin Hasanudin, M.T., as First Supervisor, who has provided the opportunity, permission for research, motivation, facilities, guidance, and suggestions throughout my studies, enabling me to complete this thesis.
6. Mrs. Rachma Wikandari, S.T.P., M. Biotech., Ph.D. from Gadjah Mada University, as Second Supervisor, who has given the opportunity, permission for research, motivation, facilities, guidance, and suggestions for completing this thesis.

7. Prof. Mohammad J. Taherzadeh from University of Borås, Sweden, as Examiner, who has given the opportunity, provided suggestions, feedback, and evaluation of my thesis work.
8. The lecturer of study program Agricultural Product Technology who have taught, guide, and help the author throughout study period.
9. The faculty members, staff, and employees of the Agriculture Technology Department who have taught, guided, and assisted with the administration of this bachelor thesis.
10. My parents, Aris Susanto and Tri Feriyani, who have never ceased to offer their support, motivation, prayers, and encouragement throughout the completion of this thesis.
11. My best friend, Puteri Syalaisya Farianto, who always provided support, motivation, assistance, and companionships through both joyful and challenging times, helping me complete this thesis.
12. My research team, Shabrina and Yosnita, who always help each other during research.
13. To all individuals at the Waste Management Laboratory, in particular Mr. Joko, as well as Vico and Jannah, for their invaluable guidance, support, and encouragement during my laboratory research. Their contributions significantly contributed to the successful completion of this thesis.
14. My friends, Aliefuddin, Zhafran, Gani, Alfian, Naufal, Duta, Primasetya, Nyoman, Randi, Haris, Mellisa, Yasmeen, Nurul, Frily, Nabila, and all my friends from the 2021 batch of the Agricultural Technology Department, who have provided support, motivation, assistance and companionships through both joyful and challenging times, helping me complete this thesis.

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 1st 2025

Author

Galuh Septa Nugraha

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I. INTRODUCTION

1.1 Background and Problem

Palm oil is currently the most widely consumed vegetable oil globally, with approximately 60% of packaged products in supermarkets worldwide containing palm oil (GAPKI, 2025). Indonesia is the world's largest producer, contributing over 47.5 million metric tons annually, followed by Malaysia, Thailand, Colombia, and Nigeria (USDA, 2025). The industry plays a critical role in the national economy, particularly in regions such as Sumatra and Borneo. However, the rapid expansion of palm oil production has raised environmental concerns, especially due to the large volume of waste generated; chief among them, Palm Oil Mill Effluent (POME) (Sar et al., 2024). POME is major by-product of palm oil processing that has raised serious concerns due to its high pollutant load and treatment challenges (Iwuagwu & Ugwuanyi, 2014).

POME is a major by-product of crude palm oil (CPO) extraction. For every ton of fresh fruit bunches (FFB) processed, approximately 0,5 to 0,75 m³ of effluent are produced (Sukson et al., 2020). This wastewater is typically acidic (pH < 5,5), has a high temperature (80–90°C), and contains large concentrations of pollutants such as total solids (40,500–75,000 mg/L), oil and grease (2,000–8,300 mg/L), and suspended solids (18,000–47,000 mg/L) (Madaki & Seng, 2013b). It also contains nitrogen (400–800 mg/L), ash (3,000–42,000 mg/L), and extremely high levels of biochemical oxygen demand (BOD: 25,000–54,000 mg/L) and chemical oxygen demand (COD: 50,000–100,000+ mg/L); often exceeding those of municipal sewage by more than a hundredfold (Iwuagwu & Ugwuanyi, 2014). Without proper treatment, POME poses a serious threat to aquatic ecosystems and surrounding environments (Okereke & Gikinawa, 2020).

Several treatment technologies have been developed for POME, including anaerobic and aerobic digestion, physicochemical methods, and ponding systems.. Among these, open ponding is still the most common method in Indonesia. While low in cost, it is inefficient, requires long retention times, and contributes to greenhouse gas emissions (Poh et al., 2020). In this context, biological treatment using filamentous fungi has gained attention as a promising alternative. These fungi can facilitate both bioremediation and value-added biomass production by utilizing POME as a substrate, given its high organic content (Sar et al., 2024).

Neurospora sitophila, a filamentous fungus traditionally used in the fermentation of oncom, holds potential as a biotechnological agent for the valorization of POME (Fitriyah et al., 2024). Unlike many other fungi, *N. sitophila* is known to grow rapidly on various organic waste media (Asad et al., 2006; Ramadhani et al., 2024). However, its application in POME-based cultivation has yet to be extensively studied. The submerged fermentation method, which allows for controlled conditions, could further enhance biomass productivity (Confortin et al., 2019). Studying the growth of *N. sitophila* in POME-based media could thus provide an alternative waste management solution while producing useful fungal biomass.

This research aims to evaluate the potential of POME as a substrate for the production of *N. sitophila* biomass using submerged fermentation. The study will optimize four key parameters: dilution ratio, nutrient supplementation, initial pH, and agitation speed, to determine the most effective conditions for maximizing fungal biomass yield. By integrating waste valorization with fungal biotechnology, this study contributes to sustainable palm oil processing and opens new pathways for bioresource development.

1.2 Objectives

The objectives of this study are:

1. To characterize of POME and evaluate its potential as a growth medium for fungal biomass production.
2. To determine the optimum conditions for maximizing *N. sitophila* biomass yield.
3. To assess the nutritional composition of the resulting fungal biomass.

1.3 Research Framework

Filamentous fungi are increasingly recognized for their metabolic versatility, ability to survive under harsh environmental conditions, and capacity to produce high-value biomass. Unlike bacteria, fungi can tolerate low pH, high organic loads, and nutrient-limited conditions, making them suitable for treating complex industrial effluents such as POME (Dhanavade & Patil, 2023). These fungi secrete extracellular enzymes to break down organic matter, contributing to efficient bioremediation (Singh & Vyas, 2022). Additionally, fungal biomass can be utilized for various applications, such as animal feed, enzyme production, and bioactive compounds (Karimi et al., 2018). While species such as *Aspergillus*, *Rhizopus*, and *Pleurotus* have been commonly used in bioconversion research (Sar et al., 2024), *Neurospora sitophila* remains relatively under explored despite its historical use in food fermentation and its rapid growth on agricultural waste (Fitriyah et al., 2024; Ramadhani et al., 2024). Investigating its ability to utilize POME as a substrate represents a novel and potentially valuable approach to fungal biotechnology.

POME is particularly well-suited for fungal cultivation due to its high concentrations of carbohydrates, proteins, lipids, and micronutrients such as nitrogen, phosphorus, and potassium (Sar et al., 2024). Despite being a pollutant, its nutrient-rich composition makes it ideal for fungal biomass production when used in a controlled biotechnological process. Moreover, POME's liquid nature makes it suitable for submerged fermentation, a method known to enhance fungal

growth and enzyme production (Hermansyah et al., 2018). This research, therefore, explores the dual benefit of using POME for waste treatment and fungal biomass production.

The selection of *N. sitophila* is based on its ability to form fast-growing mycelia, its established safety in food contexts, and its adaptability to protein-rich, acidic environments. It has been shown to thrive on substrates such as rice bran, bagasse, and coconut oil cake (Kanti et al., 2020; Oguntimein et al., 1992). *N. sitophila* exhibits strong sporulation and hyphal development, attributes crucial for rapid biomass accumulation. Its safety profile and acceptance in food contexts also open opportunities for future valorization of the biomass. Nevertheless, its growth in oily wastewater systems like POME has not been adequately studied, presenting a unique research opportunity.

Several factors influence fungal growth in POME, starting with the dilution ratio. Raw POME may contain toxic levels of solids and oils that inhibit microbial activity (Dominic & Baidurah, 2022). Dilution reduces these effects while retaining adequate nutrients (Gonzalez & Aranda, 2023). Previous studies have shown that fungi like *Rhizopus oryzae* grow better in diluted POME (Prasertsan & Binmaeil, 2018). This study will test multiple dilution levels (undiluted, 1:10, 1:15, 1:20) to identify the most favorable condition.

Media supplementation is also crucial in supporting fungal metabolism and enhancing biomass production, especially when certain limiting nutrients are absent in POME (Pratama et al., 2025). While POME is rich in carbon, its nitrogen content may be insufficient for optimal fungal protein synthesis and growth (Iwuagwu & Ugwuanyi, 2014). Supplementation with organic nitrogen sources such as peptone, tryptone, or yeast extract has shown to significantly improve fungal biomass yield (Hamza et al., 2024). This study will assess these three supplements to identify the most effective and economical option.

Initial pH is another key variable. It affects enzyme activity, nutrient solubility, and cell membrane permeability. Therefore, maintaining optimum pH of the substrate is important for maximum fungal growth. In (Prasertsan & Binmaeil, 2018) study of *Rhizopus oryzae* strain growth on POME, the experiment was conducted at eight different initial pH values as 3,0; 3,5; 4,0; 4,5; 5,0; 5,5; 6,0; 6,5 whichin the optimum pH was determined to be 4,5, which gave the highest biomass (18,3 g/L). In another study conducted by Toghiani et al. (2025), it has been reported that optimum initial pH of *Neurospora intermedia* is 5,5 (6,7 g/L). This study will explore initial pH levels of 2.5, 3.5, 4.5, and 5.5 to determine the best condition for *N. sitophila* biomass production.

Lastly, agitation speed plays a role in oxygen transfer and nutrient mixing in submerged fermentation. Optimal agitation speeds vary between fungal species and are influenced by the viscosity of the medium (Bhattacharya et al., 2012; Kirsch et al., 2016; Mohamad et al., 2015). The summary of previous research on optimal agitation sped for fungal biomass production can be seen in Table 1.

Table 1. Summary of previous research on optimal agitation speed for fungal Biomass production

Fungi	Substrate	Variation of Agitation Speed	Optimal Agitation Speed	Source
<i>Neurospora intermedia</i>	Mushroom Complete Media	50 rpm, 70 rpm, 90 rpm, 110 rpm, and 130 rpm	130 rpm (highest speed)	(Mohamad et al., 2015)
<i>Pleurotus ostreatus</i>	Mineral salt medium	50 rpm, 100 rpm, 120 rpm, 150 rpm, 180 rpm, 200 rpm	180 rpm	(Bhattacharya et al., 2012)
<i>Pleurotus albidus</i>	Standart Medium	120 rpm, 150 rpm, 180 rpm	180 rpm (highest speed)	(Kirsch et al., 2016)

As seen in Table 1, optimal agitation speed varies significantly depending on fungal species and medium viscosity. For example, *Neurospora intermedia* achieved highest biomass at 130 rpm, while *Pleurotus* species responded better at higher speeds (up to 180 rpm). These findings suggest that optimal agitation is substrate- and species-specific, requiring experimental validation. In this study,

agitation speeds of 110, 125, 140, and 155 rpm will be evaluated to determine the most favorable condition for *N. sitophila* biomass production in POME

1.4 Hypotheses

The study is guided by the following hypotheses:

1. POME contains sufficient nutrients to support the growth of *N. sitophila* and can serve as an effective medium for fungal biomass production.
2. Optimization of key parameters; dilution ratio, supplementation, initial pH, and agitation speed; will significantly influence fungal biomass yield..
3. The fungal biomass produced under optimized conditions will exhibit favorable nutritional characteristics suitable for value-added applications.

II. LITERATURE REVIEW

2.1 Filamentous Fungi

Filamentous fungi are mostly saprophytic, eukaryotic organisms. As saprophytes, they obtain carbon by decomposing non-living organic matter. To break down complex macromolecules and insoluble polymers, these fungi secrete extracellular enzymes, allowing digestion to occur outside the cell. The digestion products then diffuse through the cell wall for absorption. Structurally, the basic unit of filamentous fungi is the hypha, and a mass of hyphae forms a mycelium. Several filamentous fungi have industrial importance, including *Aspergillus*, *Rhizopus*, *Trichoderma*, *Penicillium*, *Fusarium*, and *Neurospora* (Wikandari et al., 2022). These organisms play a key role in the decomposition of organic matter and have been widely utilized in biotechnology, particularly in enzyme production, fermentation, and wastewater treatment. Furthermore, filamentous fungi are capable of biosorption, bioaccumulation, and biodegradation of various pollutants, making them suitable for mycoremediation applications (Sar et al., 2024).

Multiple species such as *Pleurotus*, *Phanerochaete*, *Aspergillus*, and *Penicillium* have demonstrated the ability to degrade dyes and pollutants in industrial effluents via enzymatic mechanisms. Key enzymes include ligninolytic enzymes such as laccases, peroxidases, and azoreductases, which are responsible for breaking down harmful compounds into simpler, less toxic forms (Sar et al., 2024). For example, laccases can oxidize a wide variety of phenolic and non-phenolic compounds without the need for cofactors, making them highly efficient for degrading xenobiotic substances found in waste streams like coffee pulp, textile effluent, and olive mill waste (Dhanavade & Patil, 2023). In addition to live fungal cultures, cell-free enzyme extracts and fungal consortia have also been

used effectively for pollutant removal. One notable example is *Aspergillus ochraceus*, which has been shown to remove 56–90% of several synthetic dyes through thermostable laccase production (Telke et al., 2010). These findings highlight the flexibility of filamentous fungi in adapting to different pollutant types and wastewater compositions.

A further promising application in bioconversion is the formation of mycelial pellets; compact, spherical aggregates of fungal mycelium with high porosity, surface area, and mechanical stability. The formation of pellets is influenced by inoculum size, media composition, aeration, and agitation. These pellets offer several advantages in bioprocessing, including: they settle more easily in bioreactors, facilitate biomass recovery, and can be used as biosorbents for heavy metals. For instance, *Rhizopus oryzae* pellets have shown promising results in removing toxic metal ions such as copper and cadmium from aqueous solutions (Fu et al., 2012). In addition to structural benefits, the pellets support high enzymatic activity and efficient pollutant degradation. Overall, the morphological and biochemical properties of filamentous fungi make them highly attractive for the mycoremediation of industrial effluents such as POME, transforming waste into value-added fungal biomass (Sar et al., 2024).

Mycoremediation of industrial effluent wastes can be affected by several operational conditions such as pH value, temperature, pollutant concentration, carbon and nitrogen type and amounts, oxygen, mass transfer, and agitation rate. Therefore, it is crucial to carefully adjust these parameters to ensure the acquisition of reliable data and results. Among these parameters, pH plays a critical role during mycoremediation and essentially affects the use of filamentous fungi for industrial purposes. In addition to pH, under highly acidic (pH 2.0–4.0) or alkaline conditions (pH 10), fungal-mediated systems tend to fail rapidly (Sar et al., 2024).

2.2 The Potential of Palm Oil Mill Effluent

Palm oil mill effluent or POME is produced from the processing of fresh fruit bunches (FFB) of oil palm for palm oil production. POME comes from the condensate of boiling, hydrocyclone water, and separator sludge (Wu et al., 2010). Palm oil mills produce 0.7-1 m³ of POME for every ton of fresh fruit bunches processed. Freshly produced POME is generally hot (temperature 60°-80°C), acidic (pH 3.3-4.6), viscous, brownish in color with high content of solids, oil and fat, chemical oxygen demand (COD), and biological oxygen demand (BOD). POME contains a large amount of nitrogen, phosphate, potassium, magnesium, and calcium, making it a good fertilizer for oil palm plantations. Economically, both biogas and the final waste produced from the degradation process can be processed and utilized. Characteristics of POME are shown in Table 2.

The composition and characteristics of POME depend on the raw material quality, season, time of operation, and treatment (Madaki & Seng, 2013a). POME effluent has a foul odor, can reduce water quality, will affect the active organisms in the vicinity, and can cause serious environmental pollution if not handled properly. POME discharged into water bodies causes the waters to turn brown, smelly, and slimy and causes deoxygenation. Ahmad et al., (2003) noted that raw or partially treated POME has an organic matter which is due in part to the presence of unrecovered palm oil. This highly polluting wastewater according (Ahmad et al., 2003), can cause pollution of water –ways due to oxygen level in rivers leads to anaerobic conditions and the release of noxious gases, particularly hydrogen sulphide. Discharging untreated POME into the soil will alter its physical and chemical properties and nutritional status, causing an undesirable decrease in pH and an increase in salinity.

Table 2. Characteristics of palm oil mill effluent (POME)

Parameter	Value
pH	3.4 to 5.2
Oil and grase	130 to 180.000
Biochemical oxygen demand (mg L ⁻¹)	10.250 to 43.750
Chemical oxygen demand (COD) (mg L ⁻¹)	15 000 to 100 000
Total solid (SS) (mg L ⁻¹)	11 500 to 79 000
Total suspended solid (TSS) (mg L ⁻¹)	5 000 to 54 000
Total volatile solid (TVS) (mg L ⁻¹)	9 000 to 72 000
Total nitrogen (TN) (mg L ⁻¹)	180 to 1 400
Ammoniacal nitrogen (mg L ⁻¹)	4 to 80
Colour (ADMI)	> 500
Pottasium (mg L ⁻¹)	1 281 to 1 928
Calcium (mg L ⁻¹)	276 to 405
Magnesium (mg L ⁻¹)	254 to 344
Phosphorus(mg L ⁻¹)	94 to 131
Manganese (mg L ⁻¹)	2.1 to 4.4
Iron (mg L ⁻¹)	75 to 164

Source : (Rajani et al., 2019)

2.3 *Neurospora sitophila*

Neurospora sitophila, a filamentous fungus widely recognized for its use in producing the traditional fermented food oncom. It is also known as red bread fungus or orange bread fungus because of its distinctive color (Fitriyah et al., 2024). *N. sitophila* has a high genetic diversity and can adapt to different environmental conditions. It can be found in various habitats, such as soil, plant debris, and animal dung. It can also colonize burned areas after forest fires. It suggests that *N. sitophila* has a good ecological niche and can thrive in diverse environments. The following is the taxonomy of *N. sitophila* (Medtigo, 2024).

Kingdom : Fungi
 Phylum : Ascomycota
 Class : Sordaiomycetes
 Order : Sordariales
 Family : Sordariaceae
 Genus : *Neurospora*
 Species : *N. sitophila*

N. sitophila is a microscopic filamentous fungus within the Ascomycota division. It forms characteristic filamentous hyphae and produces conidia-bearing structures adapted for solid-state as well as submerged culture systems. The fungus operates optimally around 35-37°C and slightly acidic to neutral pH (5,0-5,5), conditions commonly used in food-grade fermentations. Under these conditions, *N. sitophila* exhibits robust growth and enzyme secretion, highlighting its versatile metabolism. Its ability to thrive on lignocellulosic substrates makes it well-suited for converting agricultural residues into protein-rich biomass (Moo-Young et al., 1992).

Enzymatically, *N. sitophila* is notable for secreting cellulolytic enzymes such as endoglucanases and β -glucosidases, which facilitate the breakdown of cellulose into fermentable sugars. It also produces phytases that have demonstrated potential in enhancing feed digestibility, achieving enzyme activities comparable to *N. crassa* under solid-state fermentation. These enzymes typically display optimal activity at pH 5,0-5,5 and temperatures between 50-55°C, with moderate thermal stability up to 40°C (Kanti & Sudiana, 2016). In addition, *N. sitophila* has been successfully applied in submerged and solid-state fermentation using diverse substrates: corn bagasse and corn stover. In these systems, it converts lignocellulosic material into valuable biomass, producing enzymes that have applications in animal feed, bioremediation, and nutrient recycling (Oguntimein et al., 1992).

III. METHODOLOGY

3.1 Place and Time of Research

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

3.2 Materials and Equipment

3.2.1 Materials

The materials used include Palm Oil Mill Effluent (POME) from the first pond obtained from PTPN VII Bekri, Lampung, and microorganism *N. sitophila*. The microorganism *N. sitophila* is procured from the Faculty of Agricultural Technology at UGM. The medium used to grow the strain was Potato Dextrose Agar (PDA). The supplementation media used are tryptone, peptone, and yeast extract. Other materials used include H₂SO₄, NaOH, 70% alcohol, tween 80, 20% lactic acid, and distilled water.

3.2.2 Equipment

Equipment used includes 500 mL Erlenmeyer flasks, rotary shaker, petri dishes, filter cloths, pH meter, analytical balance, volumetric flasks (100 mL and 1000 mL), micro- and macropipettes with tips (1 mL and 5 mL), laminar airflow cabinet, autoclave, spatula, volumetric pipettes, cotton plugs, plastic wrap, resealable plastic bags, heating plates, and filter paper.

3.3 Research Methods

The study begin with the characterization of Palm Oil Mill Effluent (POME), including analysis for pH, total solids, volatile solids, moisture content, ash content, Carbon, Hydrogen, and Nitrogen (CHN) content, mineral content, and Chemical Oxygen Demand (COD) to ascertain its potential as a medium for fungal biomass production. This research utilized filamentous fungi, specifically the microorganism *N. sitophila*. The research method will employ four treatment including dilution, media supplementation, initial pH, and agitation with each of them will be having four treatment variations and three replications. This is conducted to obtain data on biomass yield and determine the best treatment for the fungi. Treatments that produced the highest fungal biomass from the fungus will further analyze for proximate composition, total fiber, ammino acids and fatty acids. The data were processed using analysis of variance (ANOVA) at a 5% significance level, followed by Duncan's Multiple Range Test (DMRT) at significance level of 5% for further analysis.

3.4 Research Procedures

3.4.1 Carbon, Hydrogen, and Nitrogen (CHN) Analysis

The carbon (C), hydrogen (H), and nitrogen (N) content of the POME were analyzed using an Elementar vario EL cube elemental analyzer (Elementar Analysensystemen GmbH, Germany). Prior to analysis, the POME sample was dried in an oven at 105°C until a constant weight was achieved. Approximately 1-2 mg of the dried sample was weighed and sealed in a tin capsule. The sample was then subjected to high-temperature combustion at around 950-1.000°C in an oxygen-rich environment. Each analysis was conducted in duplicate, and the mean values were reported as weight percentages (% w/w) of carbon, hydrogen, and nitrogen.

3.4.2 Preparaton Inoculum

The filamentous fungus *Neurospora sitophila* (obtained from Universitas Gadjah Mada, Yogyakarta, Indonesia), was used in the current study. The fungal strain was first maintained on Potato Dextrose Agar (PDA) slants supplemented with 0,85 mL of 20% (v/v) lactic acid per 5 mL medium. The cultures were incubated aerobically for 4-5 days at 28°C, followed by storage at 4°C. For further propagation, the slant cultures were transferred to PDA plates and incubated at room temperature for 7 days. The plates were then stored at 4°C until spore suspension preparation. Spore solution for inoculation were prepared by flooding each plate with 100 mL of sterile 0,05% (v/v) Tween 80 solution and releasing the spores by agitation with a sterile spatula. All liquid cultures were inoculated with 10 mL of spore suspension containing 1×10^5 spores/mL.

3.4.3 Optimization of Fungal Biomass Production

Growth optimization research was conducted in four stages: the first stage is variations of dilutions, the second stage is variations of supplementation, the third stage is variations of pH, and the last stage is variations of agitation speed. The production of fungal biomass in this research employed a fermentation method in Erlenmeyer flasks as conducted by Wikandari et al. (2023).

3.4.3.1 Stage 1 (Influence of Dilutions)

Fungal biomass produced from Palm Oil Mill Effluent (POME) need to be diluted first because it allowed better light penetration for fungus, microalgae, and bacterial growth (Dominic & Baidurah, 2022). The variety of dilution are undiluted; 1:10; 1:15; 1:20 (n=3). The proses begin with preparation of media, the POME was diluted with distilled water in the ratio undiluted; 1:10; 1:15; 1:20 as much as 100 mL and added into 500 mL Erlenmeyer flasks, then pH checking; if its within the range of pH 3,5 no adjustment is made. The Erlenmeyer flasks were covered with cotton plugs and alumunium foil for sterilization. Sterilization performed using autoclave at the temperature 121°C for 15 minutes. After that,

the strain cells was inoculated inside a laminar. Afterward, place Erlenmyer flasks on the rotary shaker with cotton plugs and aluminium foil covering the top of it and begin to shake with 125 rpm speed at room temperature (28°C) for 3 days. After the shaking process done, harvesting was conducted by pouring it into filter cloths, and the remaining biomass was rinsed with distilled water then squeezed. The flowchart of fungal biomass production stage 1 can be seen in Figure 1.

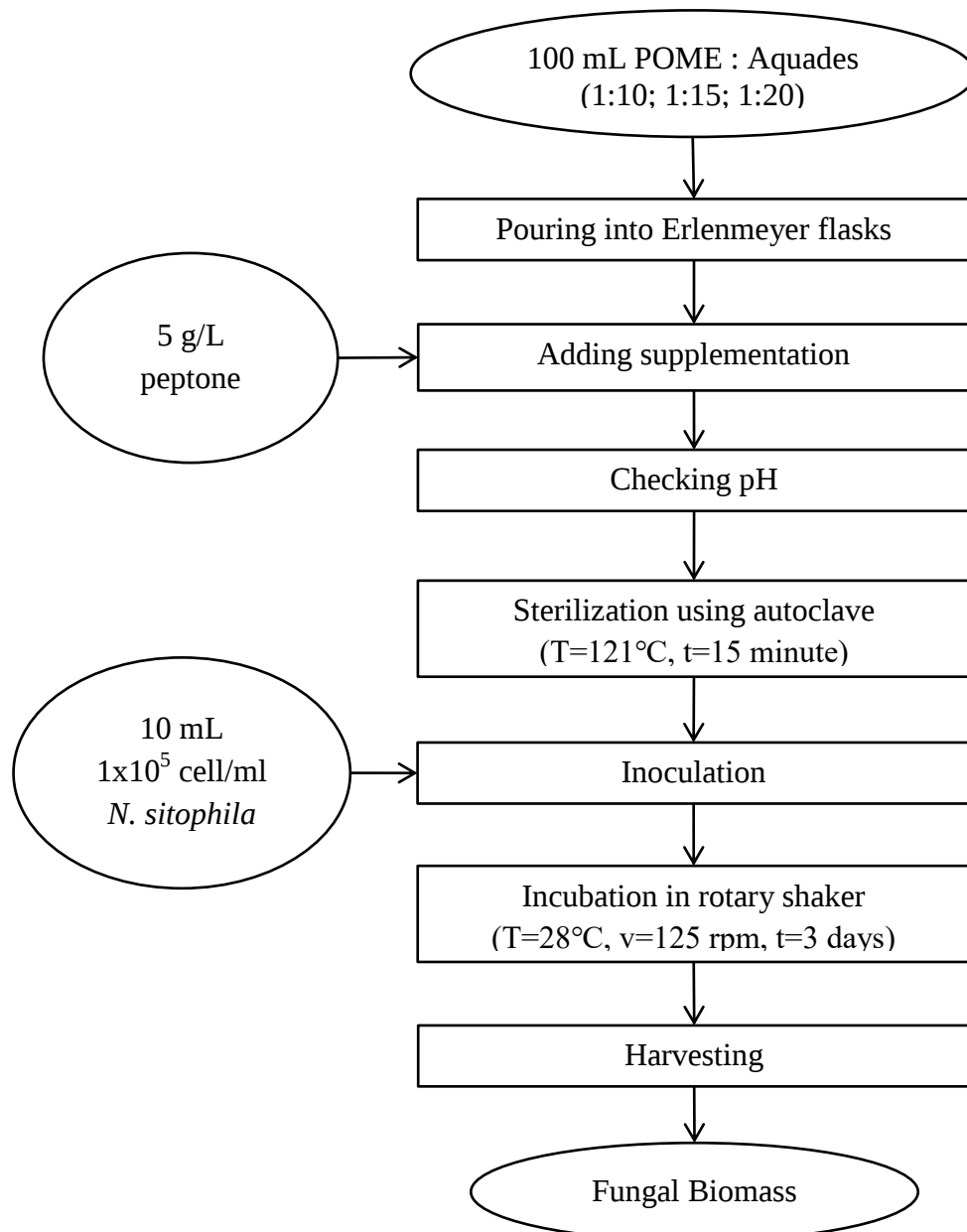


Figure 1. Stage 1 workflow diagram (influence of dilution)

3.4.3.2 Stage 2 (Influence of Supplementations)

Fungal biomass produced from POME as substrate using *N. sitophila* having 5g/L of different supplementations (triptone, yeast extract, and peptone). Medium without any supplementation was used as a control. The dilution ratio giving the best result in Stage 1 was selected to use. The process begin with preparation of media, 100 mL of diluted POME added into 500 mL Erlenmeyer flasks, shaken evenly to prevent clumping, then adjust pH to 3,5. The Erlenmyer flasks were covered with cotton plugs and plastic for sterilization. Sterilization performed using autoclave at the temperature 121°C for 15 minutes. After that, inoculation performed inside a laminar. Afterward, place Erlenmeyer flasks on the rotary shaker with cotton plugs and paper covering the top of it and begin to shake with 125 rpm speed at room temperature (28°C) for 3 days. After the shaking process done, harvesting was conducted by pouring it into filter cloths, and the remaining biomass was rinsed with distilled water then squeezed. The flowchart of fungal biomass production stage 2 can be seen in Figure 2.

3.4.3.3 Stage 3 (Influence of Initial pH)

Fungal biomass produced from POME as substrate using *N. sitophila* was analyzed by varying the initial pH (2,5; 3,5; 4,5; 5,5). Dilution used is the best dilutions from Stage 1 and supplementation used is the beset supplementation from Stage 2. The process begin with preparation of media, 100 mL of diluted POME added into 500 mL Erlenmeyer flasks, shaken evenly to prevent clumping. The media with added supplementation were then adjusted to the pH according to the treatment variation using 0,5 N H₂SO₄ and 0,5 N NaOH. The Erlenmeyer flasks were covered with cotton plugs and alumunium foil for sterilization. Sterilization performed using autoclave at the temperature 121°C for 15 minutes. After that, inoculation performed inside a laminar. Afterward, place Erlenmeyer flasks on the rotary shaker and begin to shake with 125 rpm speed at room temperature (28°C) for 3 days. After the shaking process done, harvesting was conducted by pouring it into filter cloths, and the remaining biomass was rinsed

with distilled water then squeezed. The flowchart of fungal biomass production stage 3 can be seen in Figure 3.

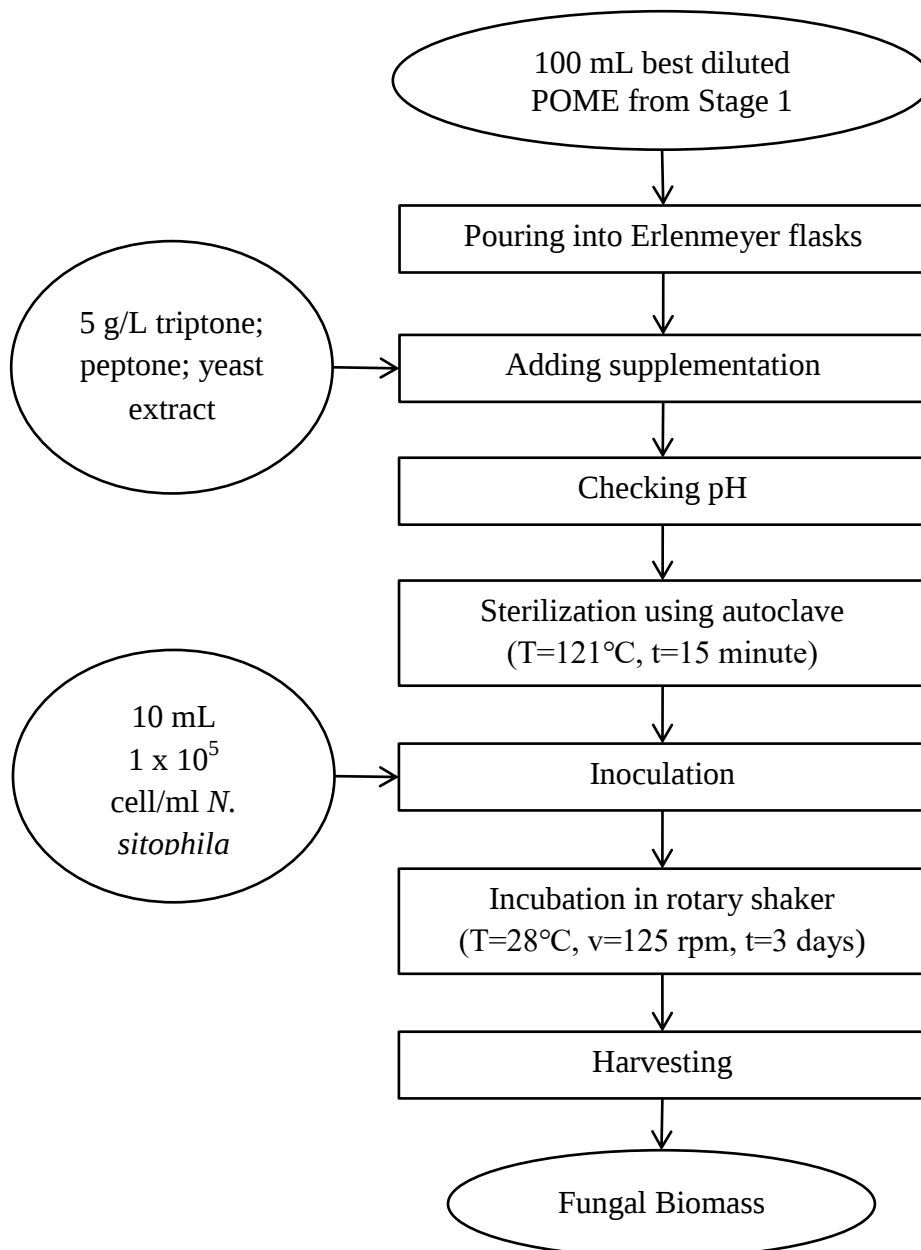


Figure 2. Stage 2 workflow diagram (influence of supplementation)

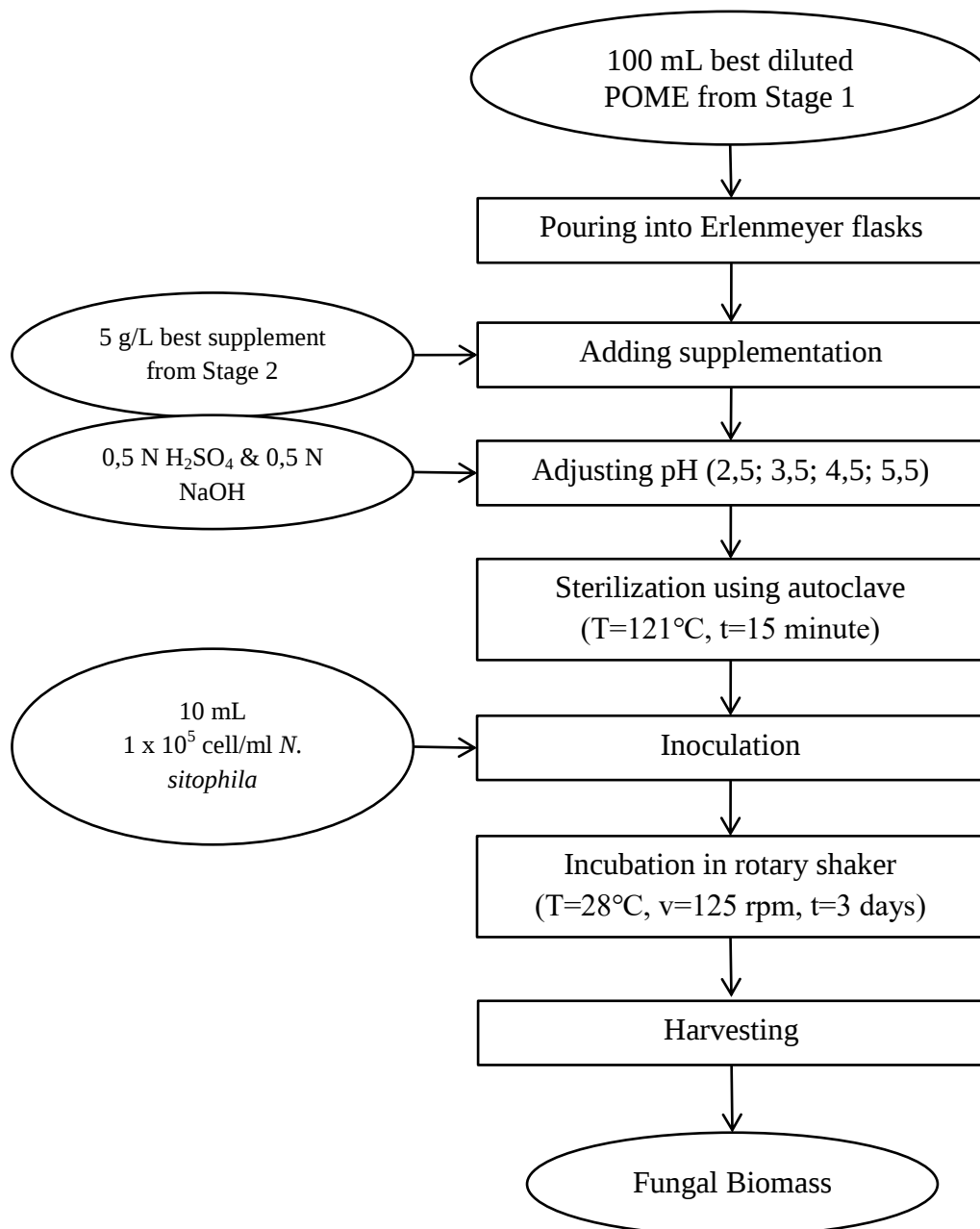


Figure 3. Stage 3 workflow diagram (influence of pH)

3.4.3.4 Stage 4 (Influence of Agitation Speed)

Fungal biomass produced from palm oil mill effluent using microorganism *N. sitophila* with agitation speed variations of 110 rpm, 125 rpm, 140 rpm and 155 rpm (n=3). The dilution used is the best dilutions from Stage 1 and supplementation used is the best supplementation from Stage 2. The media with added supplementation, then adjusted to pH according to the best treatment result

from Stage 3 using 0,5 N H₂SO₄ and 0,5 N NaOH, using a pH meter. The Erlenmeyer flasks were covered with cotton plugs and aluminium foil for sterilization at 121°C for 15 minutes. After sterilization, inoculation was carried out in the laminar. *N. sitophila* with concentration 1 x 10⁵ cells/ml were added as 10 mL. Then, the Erlenmeyer flasks were shaken to prevent clumping in the media. The Erlenmeyer flasks were placed on a shaker operated at speeds of 110 rpm, 125 rpm, 140 rpm and 155 rpm at room temperature (28°C) for 3 days. After 3 days, harvesting was conducted by pouring the contents of the Erlenmeyer flasks onto a filter cloth, and the remaining biomass was rinsed with distilled water and then squeezed. The flowchart of fungal biomass production optimization Stage 4 can be seen in Figure 4.

3.4.4 Determination of Biomass Quantity

The fungal biomass mycelium was harvested after 3 days of cultivation, followed by washing with water three times. Subsequently, the biomass was squeezed using a filter cloth, and weighed to determine the weight of fungal biomass.

3.4.5 Moisture Analysis

The moisture contents was analyzed by the gravimetric method AOAC (2016). The analysis commenced by oven-drying an empty porcelain crucible 105°C for 30 minutes, followed by cooling and placement in a desiccator for 15 minutes. Subsequently, the crucibles were weighed. This process was repeated until a constant weight of the crucible was obtained.. Next, a fresh sample (2-3 g) was placed into the porcelain crucible and weighed. The The crucible containing the sample was then dried in a hot air oven at 105°C for 3 hours or until a constant weight was reached. After drying, the porcelain crucible with the sample was cooled in a desiccator for 15 minutes and weighed. The moisture content was calculated using the following formula:

$$\text{Moisture (\%)} = \frac{a - (b - c)}{a} \times 100\%$$

a = Initial weight of fresh sample (g)

b = Porcelain crucible weight + dried weight (g)

c = Empty porcelain crucible weight (g)

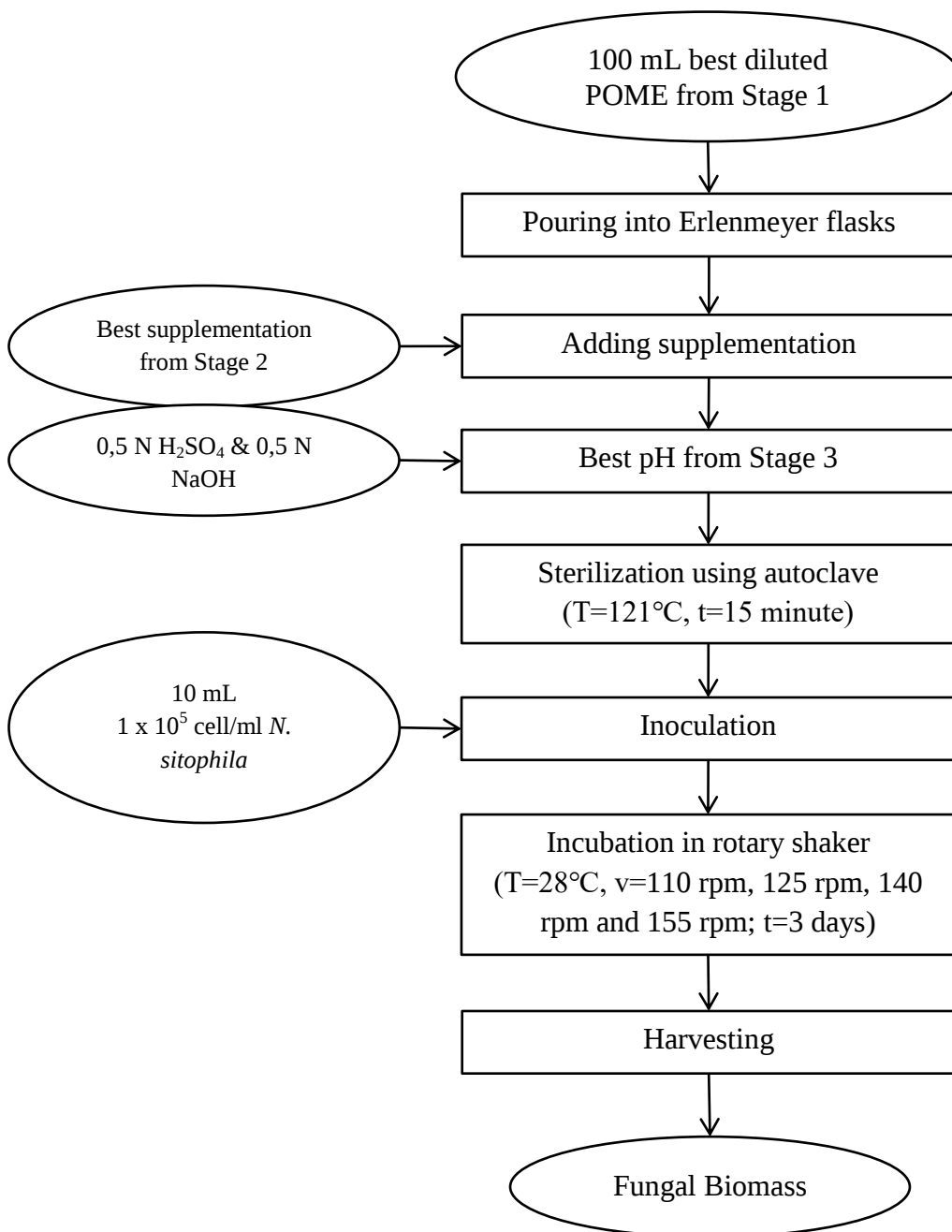


Figure 4. Stage 4 workflow diagram (influence of agitation speed)

3.4.6 Ash Content Analysis

The ash content was analyzed by the gravimetric method AOAC (2016). The analysis commenced by oven-drying empty porcelain crucibles at 105°C for 30 minutes, followed by cooling and placement in a desiccator for 15 minutes. Subsequently, the crucibles were weighed. This process was repeated until a constant weight of the crucible was obtained. Next, dried sample (1g) was placed into a porcelain crucible and weighed. The crucible containing the sample was ignited over a flame of a stove until no smoke was produced. Then, ashing was carried out using a furnace at 550°C for 3 hours until white ash was formed. The sample was then placed in a desiccator for 15 minutes and subsequently weighed until a constant weight was obtained. The ash content was calculated using the following formula:

$$\text{Ash content (\%)} = \frac{(c - a)}{(b - a)} \times 100\%$$

Note:

a = An empty porcelain crucible weight (g)

b = Porcelain crucible weight + sample weight (g)

c = Porcelain crucible weight + ash weight (g)

3.4.7 Protein Analysis

The protein content analyzed by CHNS (O) elemental, the analysis methodology is based on the principle of high-temperature combustion within an oxygen-rich environment, utilizing the Pregl-Dumas method under various static and dynamic conditions. The analysis commences with the combustion of the sample, generating gaseous compounds from the elements carbon, hydrogen, nitrogen, and sulfur. The first step that should be done is oven-drying the sample as much as 5 g. Dried sample then ground until become fine particles, then weigh the sample as much as 10–14 mg, and place it into closed tin. Next step is the sample undergoing high-temperature combustion at 1150°C within oxygen-enriched atmosphere. The result of combustion, including CO₂, H₂O, and NO₂ are

quantified through gas chromatography. Carbon, hydrogen, nitrogen, and sulfur are assessed simultaneously, while oxygen is evaluated in a subsequent pyrolysis step. The protein content was calculated by seeing the nitrogen content present in sample multiple by 6,25.

3.4.8 Fat Content Analysis

The fat content was analyzed by the Soxhlet method AOAC (2016). The analysis commenced by sample preparation, the sample was dried at 100°C for 6 hours. Subsequently, the Soxhlet flask was oven-dried at 100°C for 1 hour. Then, the flask was placed in a desiccator and weighed. A dry sample of 1-3 g was wrapped in filter paper with cotton placed on the top and bottom, forming a thimble. The Soxhlet setup included a heating mantle, fat flask, lead weight, Soxhlet extractor, and condenser. The sample was placed into the chamber of the Soxhlet apparatus, and petroleum ether solvent was added according to the size of the Soxhlet flask. The sample was then extracted for approximately 6 hours until the solvent returned through the siphon into the Soxhlet flask. After extraction, the Soxhlet flask was oven-dried at 105°C for 24 hours. Subsequently, the Soxhlet flask was placed in a desiccator and weighed until a constant weight was obtained. The determination of fat content was calculated using the following formula:

$$\text{Fat content (\%)} = \frac{(c - a)}{b} \times 100\%$$

Note:

a = An empty Soxhlet flask weight (g)

b = sample weight (g)

c = Weight of extracted Soxhlet flask after oven-dried (g)

3.4.9 Crude Fiber Analysis

The crude fiber was analyzed using the acid-base hydrolysis method referring to SNI 01-2891-1992 with modifications. The analysis began with the oven drying of empty fiber sleeves at 100°C for 30 minutes. Subsequently, the sleeves were

placed in a desiccator and weighed. Then, samples (1-2 g) placed into the sleeves. Place the sleeve containing the sample into the sleeve rack. Then, pour 500mL of 1.25% H₂SO₄ into a 1000mL beaker. Place the sleeve rack into the beaker. Position the beaker on a heating plate and place a cooling glass on top of the beaker. Heat the heating plate to 100°C for 30 minutes, rotating the sleeve rack to induce agitation in the sleeves containing the samples. After 30 minutes, turn off the heating plate, discard the 1.25% H₂SO₄, and rinse the sleeve rack and beaker containing the sleeves with flowing water. Next, repeat the process using 1.25% NaOH. Then, remove the sleeves from the sleeve rack and oven dry them at 100°C for 1-3 hours until a constant weight. The determination of crude fiber content was calculated using the formula:

$$\text{Crude Fiber (\%)} = \frac{a - b}{W} \times 100\%$$

Note:

a = Residue weight in dried sleeve (g)

b = Weight of empty sleeve(g)

W = Sample weight (g)

V. CONCLUSION & SUGGESTION

5.1 Conclusion

The conclusion of this study are as follows:

1. POME has the potential to serve as a substrate for fungal biomass cultivation, due to its high carbon content (60,05%), acidic pH (4.58), with C/N ratio (35,51), and phosphorus 3,10%. However, its low nitrogen content limits microbial growth, requiring nitrogen supplementation to achieve optimal results.
2. Among the tested variables, dilution ratio, media supplementation, initial pH, and agitation speed significantly influenced fungal biomass yield. The optimal conditions for *N. sitophila* biomass production were identified as POME diluted at a 1:10 (v/v) ratio, supplemented with peptone, adjusted to an initial pH of 3.5, and agitated at 125 rpm, resulting in the highest biomass yield of 4,7408 g/L (dry basis).
3. The fungal biomass produced under these optimal conditions showed favorable nutritional properties, including a protein content of 31,63% (db), fat content of 18,66% (db), ash content of 4,10% (db), and moisture content of 79,99%. These values indicate the potential of *N. sitophila* biomass as a protein-rich and energy-dense alternative feed ingredient, with its composition comparable to or exceeding that of several conventional protein sources.

5.2 Suggestion

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

1. Further research is recommended to evaluate the amino acid profile and protein digestibility of *N. sitophila* biomass to assess its suitability for different types of livestock feed.
2. Alternative nitrogen sources such as malt extract, urea, or inorganic nitrogen compounds may be explored to enhance cost-efficiency and biomass productivity.
3. Studies should be conducted to assess the safety, microbial stability, and storage characteristics of fungal biomass harvested from POME-based media, particularly after drying and processing.

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