UTILIZATION OF PALM OIL MILL EFFLUENT (POME) AS SUBSTRATE FOR MYCELIUM PRODUCTION BY THE FUNGUS *Rhizopus oligosporus*

(Bachelor Thesis)

By

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AGRICULTURAL PRODUCT TECHNOLOGY DEPARTMENT
FACULTY OF AGRICULTURE
LAMPUNG UNIVERSITY
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ABSTRACT

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Global demand for palm oil has resulted in the large-scale production of Palm Oil Mill Effluent (POME), a nutrient-rich but environmentally harmful wastewater generated during the milling process. In Indonesia, the management of POME remains a significant environmental challenge due to its high organic content and volume. However, POME also contains essential nutrients such as carbon, nitrogen, and phosphorus, making it a potential substrate for microbial fermentation. This study explores the utilization of POME as a growth medium for *Rhizopus oligosporus*, a filamentous fungus known for its application in mycoprotein production. Four key parameters such as POME dilution, media supplementation, pH level, and agitation speed, were optimized to enhance biomass yield. The results showed that the highest fungal biomass yield, 18.5991 g/L dry basis, was obtained under the following conditions: undiluted POME, supplemented with 5 g/L tryptone, adjusted to pH 5.5, and agitated at 110 rpm for 72 hours. These conditions promoted optimal hyphal development and nutrient assimilation. The nutritional analysis of the resulting fungal biomass demonstrated promising characteristics for feed or food applications, including a protein content of 19.95%, fat content of 56.07%, ash content of 2.85% (dry basis), and moisture content of 74.01%. Compared to conventional feed ingredients such as palm kernel meal and fish meal. This finding highlights the feasibility of valorizing agro-industrial waste through microbial biotechnology, contributing to circular economy practices in the palm oil sector. Moreover, it provides a sustainable alternative to conventional protein sources while addressing environmental concerns associated with POME disposal.

Key words: POME, fungal biomass, *Rhizopus oligosporus*

ABSTRAK

PEMANFAATAN LIMBAH CAIR PABRIK KELAPA SAWIT (LCPKS) SEBAGAI SUBSTRAT UNTUK PRODUKSI MISELIUM OLEH JAMUR *Rhizopus oligosporus*

Oleh

Shabrina Maharani

Permintaan global terhadap minyak sawit telah menyebabkan produksi limbah cair pabrik kelapa sawit (LCPKS) dalam skala besar, yaitu limbah cair yang kaya nutrisi namun berpotensi mencemari lingkungan dan dihasilkan selama proses pengolahan minyak sawit. Di Indonesia, pengelolaan LCPKS masih menjadi tantangan lingkungan yang signifikan karena tingginya kandungan bahan organik dan volumenya yang besar. Namun, LCPKS juga mengandung nutrisi penting seperti karbon, nitrogen, dan fosfor, sehingga memiliki potensi sebagai substrat fermentasi mikroba. Penelitian ini mengeksplorasi pemanfaatan LCPKS sebagai media pertumbuhan untuk *Rhizopus oligosporus*, yaitu jamur berfilamen yang dikenal dalam produksi mikoprotein. Empat parameter utama seperti pengenceran LCPKS, suplementasi media, tingkat pH, dan kecepatan agitasi dioptimalkan untuk meningkatkan hasil biomassa. Hasil menunjukkan bahwa hasil biomassa jamur tertinggi sebesar 18,5991 g/L berat kering diperoleh pada kondisi berikut: POME tanpa pengenceran, disuplai dengan 5 g/L tripton, disesuaikan pada pH 5,5, dan diaduk dengan kecepatan 110 rpm selama 72 jam. Kondisi ini mendukung perkembangan hifa yang optimal dan penyerapan nutrisi secara efisien. Analisis nutrisi dari biomassa jamur yang dihasilkan menunjukkan karakteristik yang menjanjikan untuk aplikasi sebagai pakan atau pangan, dengan kandungan protein sebesar 19,95%; lemak 56,07%; abu 2,85% (berat kering); kadar air 74,01%. Hasil ini menunjukkan potensi pemanfaatan limbah agroindustri melalui bioteknologi mikroba yang mendukung praktik ekonomi sirkular di sektor kelapa sawit. Selain itu, hal ini memberikan alternatif berkelanjutan terhadap sumber protein konvensional sekaligus mengatasi permasalahan lingkungan akibat pembuangan POME.

Kata kunci: LCPKS, biomassa jamur, *Rhizopus oligosporus*

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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 "Utilization of Palm Oil Mill Effluent (POME) as Substrate for Mycelium Production by The Fungus *Rhizopus oligosporus*" as a requirement for obtaining a Bachelor's degree in Agricultural Technology from the University of Lampung. 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. INTRODUCTION

1.1 Background and Problem

The global production of palm oil was around 77.28 million tonnes in the year 2023/2024 (USDA, 2023). In that period, Indonesia was the leading exporters and producer of palm oil worldwide with 57% of global palm oil production can be fulfilled by Indonesia alone. Despite being the top of all countries in exporting and producing palm oil, Indonesia was having a decrease in 2023 as many as 2.38% in export of Crude Palm Oil (CPO) and Palm Kernel Oil (PKO), the number dropped from 33.15 million tonnes in 2022 to 32.21 million tonnes in 2023 (GAPKI, 2024). This phenomenon happened because the demand for vegetable oil has been decreased and this thing lead to global vegetable oil price decline (KEMENTAN, 2023). Though the export was decreasing, it is still a massive production for our country. The massive production need to be supported by massive plantation also, according to Badan Pusat Statistik (BPS) in 2023, there are nearly 15.4 million hectares of palm oil plantation across the country.

Massive production in palm oil industry generates amount of wastewater, also known as Palm Oil Mill Effluent (POME). POME is the mixed effluent generated from two major sources, sterilizer and separator during the extraction of palm oil (Prasertsan and Binmael, 2018). According to data from GAPKI (2024), Indonesia produced 50.07 million tonnes of CPO in 2023 and the average for Oil Extraction Rate (OER) in palm oil industry in Indonesia is 22%. We can calculate that almost 227.59 million tonnes of Fresh Fruit Bunch (FFB) used in the process, estimated POME that comes from production is about 136.554 million tonnes. POME that comes out from the production has to be treated first before the disposal of it, because it contain several chemicals which unbeneficial and will harm the environment if it disposed without any treatment and there are some water-discharge requirements that need to be fulfilled before the POME discharge to environment. Industrial wastewater generally treated by physical, chemical, and biological methods (Prasertsan and Binmael, 2018), for POME there are several ways to treat POME and it include ponding, aerobic digestion, anaerobic digestion, physicochemical treatments, and biomass production using filamentous fungi.

Filamentous fungi also known as mould is a type of fungi that can be identified because of its hyphae that look like long white-thread and it form a network called mycelium (Schulthauz and Shaw, 2015). Filamentous fungi have been known over the years to be a potential source for protein, namely mycoprotein. Filho *et al* (2018) stated that these are the fungi which can produce protein, such as *Aspergillus oryzae, Fusarium venenatum, Monascus purpureus, Neurospora intermedia, and Rhizopus sp.* Mycoprotein has used widely across the globe to create meat-like product to substitute animal meat, because it has been recognized as a high-quality alternative protein source, offering superior nutritional value compared to most plant-based proteins. It contains a high proportion of protein (approximately 45–54%) and a well-balanced profile of essential amino acids, with a Protein Digestibility Corrected Amino Acid Score value (0.996) comparable to that of milk (Saaed *et al*., 2023). Moreover, it can also be used for animal feed as it contains lots of nutrient. For example, there is PEKILO, a mycoprotein derived from fungus *Paecilomyces variotii* that already been used as feed for poultry and fish (Amara *et al.,* 2023).

Filamentous fungi contribute to degrading nearly all hydrocarbon wastes and POME is one of the wastes that can be treated by fungi as stated above (Amara *et al.,* 2023). The filamentous fungi with potential protein to grow on POME is *Rhizopus oligosporus*. *R. oligosporus* have been used for centuries in the food, especially fermentation field. *R. oligosporus* is edible fungi and commonly used in Indonesia for producing tempeh. *R. oligosporus* has demonstrated the ability to reduce the levels of harmful mycotoxins such as aflatoxins (Kusumaningtyas *et al*., 2006) and significantly decrease phytic acid content (Toor *et al*., 2021), which improves nutrient bioavailability. Furthermore, from an economic perspective, *R. oligosporus* is affordable and available in Indonesia.

The production of fungal biomass from *R. oligosporus* on POME must consider several factors such as dilution, media nutrition, pH, and agitation speed to achieve high biomass yields. Hereinafter, there is no single research has been conducted on the optimization of *R. oligosporus* growth on POME. Therefore, this study aims to assess the potential of POME as a medium for fungal biomass production and determine the optimum conditions for achieving the highest biomass of *R. oligosporus*, considering dilution, media nutrition, pH, and agitation. Additionally, to assess the chemical characteristics and nutritional value of the produced fungal biomass, characterization is conducted, including protein, carbohydrate, crude fiber, and proximate.

1.2 Objectives

The objectives from this research are as follows:

1. To investigate the characteristics of POME and its potential as a medium for fungal biomass production
2. To determine the optimum conditions for achieving the highest *R. oligosporus* biomass production
3. To characterize the nutritional properties of the fungal biomass.

1.3 Research Framework

Filamentous fungi grow by forming hyphae, the hyphae intertwine with one another and branch out, creating mycelium. The fungi consume nutrients that present in a substrate which makes it grow. This growth generates amount of mycelium and resulting in the production of fungal biomass. Fungal biomass is a versatile protein source, that can easily grow on various substrate. In the food industry, it is one of promising alternative protein source due to its nutritional richness and its capability to grow on various media (Baltork *et al*., 2020). Furthermore, the used of fungal biomass not only limited to food-related only,

many actions have been done using filamentous fungi to treat wastewater and it will lead to bioconversion into animal feed and compost. More *et al*. (2010) stated that the effect of using filamentous fungi to treat wastewater can increase the degradability, settleability, and dewaterability.
Agroindustrial waste still contain rich nutrients which make it capable for fungal cultivation. The nutrients include proteins, minerals, sugar, and also it is considered as a low-cost substrates for fungal cultivation (Aty, 2023). Palm Oil Mill Effluent (POME) is potentially enough to become substrate for fungal growth, considering the composition of nutrients in it, such as K, N, Mg, Ca, P, Fe, B, Zn, Mn, Cu which support fungal growth including growth of *R. oligosporus* (Sim *et al*., 2016). Therefore, POME shows suitable nutrients as a medium for filamentous fungi growth to produce biomass.

The optimal growth of *R. oligosporus* are influenced by physical and physicochemical parameters that need to be followed precisely in order to produce high biomass. It include of dilution, media supplementation, pH, and agitation. These environmental conditions can alter growth rates and they can act as stimuli that guide fungal development. These conditions also influence how fungi adapt and evolve in response to their environment (Mustafa *et al*., 2023). POME need to be diluted first because it is such viscous liquid, with dilution applied, it is easier to find the maximum treatment efficiency of the fungal strains.

Dilutions also reduce the organic and nutrient concentrations in POME to the suitable range for the growth of microorganisms, as it contains an excessive amount of organic and nutrients in it (Dominic and Baidurah, 2022). Haruna et al. (2017) in their research using microalgae to treat POME recorded that a high diluted POME allowed the microalgae to grow better because there are not much excess nutrients in high diluted POME, so that it would have not caused toxicity and obstructed the growth of microalgae. Furthermore, the dilution allowed light to penetrate so that, it can adopt a phototropic growth mode instead of mixotrophic growth mode. Prasertsan and Binmael (2018) in their research stated that the optimum POME concentration was obtained by diluting POME in the ratio 1:1. Karim *et al.* (2019) in their research using *B. cereus* to treat POME stated that, 50% of dilution was found to have potential to produce highest biomass as much as 8.09 g/L.

In order to determine highest production of biomass in supplemented media, it is important to add various supplementation into the media such as nutrients or yeast exract (Nazir *et al*., 2022). Either using complex or natural media for fungi cultivation, it still requires supplementation from inorganic nutrients to gratify the necessity of the fermenting organism (Kampen, 2014). Based on research conducted by Jin *et al.* (1999) using *R. oligosporus*, ammonium sulphate and phosporus supplementation resulting in high biomass yield, which is, 4.72 g/L and 5.21 g/L, respectively. However, this finding inversely proportional with the research conducted by Wikandari *et al*. (2023), they revealed that medium supplemented with yeast extract gave the highest biomass yield (5.72 g/L) compared to supplementation with minerals salts and urea. The yeast extract contains 10% of nitrogen and 2.5% phosporus. Therefore, in this study to enhance the growth of both fungi, supplementation of yeast extracts, mineral salts, and tryptone are added.

The pH level also supporting the production of biomass, it is happened due to the difference in optimal pH for each fungus. Looking back to the study conducted by Wikandari *et al*. (2023) using tempeh residual boiling water as substrate, revealed that the optimal pH is 4.5 to obtain highest biomass up to 7.76 g/L dry basis. The same pH (4.5) also reported to be the best pH to produce biomass from *R. stolonifer*, which is 15.73 g/L (Cobos *et al*., 2020). Mostly, the pH for *R. oligosporu*s to germinate are ranging between acidic environment, which is 4.0-6.0 (Turgeman *et al.,* 2016). Hence, to produce biomass for both fungi in this study, we will use pH ranging from 3,5; 4,5; 5,5; 6,5 with controlled pH conditions.

R. oligosporus is an aerobic fungi that need an oxygen for their growth, that is why agitation is an important thing in this fermentation, because it supplies an oxygen in closed vessels. Several studies revealed different optimal agitation speed, because it depends on the resistance of the hyphae and their physiological state. However, almost every study that have been conducted agreed that the excessive agitation rate will increase shear forces and resulting in lesser product obtain. The range of agitation speed of *R. oligosporus* in tempeh residual boiling water are 100 and 125 rpm, in this case higher agitation resulting in higher fungal biomass due to better mixing (Wikandari *et al*., 2023). Therefore, this study employs agitation speeds within range 110 rpm, 125 rpm, and 140 rpm, and 155 rpm to determine the optimal agitation speed for the production of *R. oligosporus* in POME.

1.4 Hypothesis

The hyphotesis of this research are as follow:

1. POME contains essential nutrient for *R. oligosporus* growth, thus potentially serving as medium for fungal biomass production
2. Optimization of conditions (dilution, media supplementation, adjusting pH, and aeration) influence the biomass yield of *R. oligosporus*
3. The produced fungal biomass exhibits favorable nutritional characteristics

II. LITERATURE REVIEW

2.1 Palm Oil Mill Effluent

Palm oil mill effluent is defined as the voluminous liquid waste produced from the production of palm oil. This voluminous liquid originates from two major sources which is sterilization and separator (Mosunmola and Olatude, 2020). POME is thick, brownish liquid that primarily composed of over 95-95% water. This substantial water content is accompanied by approximately 0.6-0.7% oils, which contribute to its overall composition, as well as 2-4% suspended solids that mainly comes from the remnants of fruit debris processed during palm oil extraction. Moreover, POME also known to contains high composition and concentration of carbohydrate, protein, nitrogen compound, lipid, and minerals. The brownish colour presents in POME is due to some organic compound, which are, carotene, pectin, tannin, phenolic, and lignin. Thus, making it a nutrient-rich substance and recognized as non-toxic substance, though it has rich organic content (Mohammad *et al*., 2021).

As it is stated above that POME is non-toxic substance, it is usually still contain heavy metals such as as zinc (Zn) and iron (Fe) (Jumadi *et al*., 2020). Based on research conducted by Poh *et al.* (2010) the characteristics of POME depends on days of harvesting, factories batches, processing techniques, type of fruit used, and the chopping season. Due to some chemical presents in POME, it must be properly managed to prevent it from polluting aquatic life. Table 1. show the characteristics of POME that was collected from Lampung, precisely from PT. Perkebunan Nusantara VII.

Table 1. Characteristics of Sumatera's Palm Oil Mill Effluent (POME)

Parameter	**Mellyanawaty *et al*., 2018**	**Sagala *et al*., 2024**	**Unit**
pH	4.09	4 - 5.5	-
COD	10,000 – 16,000	80,000 – 120,000	mg/L
Protein	0.15	-	%
Temperature	-	70-90	°C
Nitrogen	0.0295	-	%
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

2.2 Mycoprotein

Mycoprotein is an alternative source to produce protein and it made from filamentous fungi. Mycoprotein commonly used to substitute animal meat because it form a texture that similar to meat and it hold the amount of nutrients needed for human's body. It is rich in essential amino acids (EAAs) with 41% as percentage of total protein. Although the percentage of total protein still lower compare to animal protein, mycoprotein's composition compares favorably with human muscle (Vliet *et al*., 2015). Additionally, mycoprotein contains various fatty acids, minerals, and vitamins (Finnigan *et al*., 2011). However, utilization of mycoprotein is not limited for human only, it can also be used as feed's poultry (Amara and Baky, 2023) and ruminants (Conceição *et al*., 2022). Conceição *et al*. (2022) stated that by giving animal feed that has been enrich by filamentous fungi can favor the efficiency of biomass use by the animal, and reduce the need for antibiotics. Status quo right now, show that the feed cultivation is the biggest contributor in rising of greenhouse gass (GHG) emissions, water and arable land use, and other environmental problems (Hal *et al*., 2019).

The characteristics of mycoprotein such as flavors, textures, healthy lipid contents or probiotic properties may vary to one another, and also the biomass and protein yield could be different because it depends on species and cultivation parameters (Barzee *et al*., 2021). Mycoprotein also contain high fibers that consist of approximately one-third of chitin and two-third of beta-glucan that can lower blood cholesterol concentration (Denny *et al*., 2008). High protein content, high growth rate, and the ability to grow in filamentous form are the major criteria that applied when selecting fungal strain. In order to produce excellent filamentous fungi, it must be supported by selecting agricultural by-products that will match the characteristics of fungi used. It matters because each by-products have their own uniqueness of chemical and physical characteristics and it will lead to capability of fungi used to utilize the nutrients that presents as a substrate to grow hyphae (Barzee *et al*., 2021). If criteria above are followed, it can lead to nifty mycoprotein result. Table 2. shows mycoprotein nutritional composition.

Table 2. Mycoprotein nutritional composition

Composition/100g	Wet basis	Dry basis
Moisture (g)	75	0
Protein (g)	11.25	45
Fat (g)	3.25	13
Fiber (g)	6.25	25
Carbohydrate (g)	3	10
Ash (g)	0.85	3.4
Energy (Kcal)	85	340

Source: Finnigan *et al*., 2017

The most used fungus to develop mycoprotein is *Fusarium venetatum*, it is well-known because it already been commercialized by company named Quorn® to create food that resemble animal meat. Moreover, mycoprotein can be cultivated by any filamentous fungi, such as *Aspergillus oryzae*, and *Rhizopus oligosporus*. There are two types of cultivation that commonly used for producing mycoprotein, first is submerged cultivation, and second is solid-state cultivation. The production using submerged cultivation (aerobic fermentation) is easier to conduct than the solid-state cultivation. To conduct aerobic fermentation successfully, it requires maintaining conditions, including temperature, pH, nutrient availability, dissolved oxygen, and growth rate, specific to the type of fungus cultivated (Barzee *et al*., 2021). Cultivation media also need to consider the availability of carbon and nitrogen in order to create suitable environment for optimal fungal growth (Hägerdal *et al*., 2005).

2.3 *Rhizopus oligosporus*

Rhizopus oligosporus is one of edible fungi that has been utilized for centuries especially in traditional Indonesia fermentation food, tempeh. *R. oligosporus* that is commonly used by human is not associated with production of potentially harmful metabolites (Jennessen *et al*., 2008). The following is taxonomy for *R. oligosporus*:

Kingdom	: Fungi
Phylum	: Zygomycota
Subphylum	: Mucoromycota
Ordo	: Murocales
Family	: Muroceae
Genus	: *Rhizopus*
Species	: *Rhizopus oligosporus*

R. oligosporus has the characteristics of white to gray-black thread-like hyphae that are not separated. Sporangiospore of *R. oligosporus* mostly irregular, approximately between 10% - 31% and larger than regular spores. This strain also differed from the other strains in the taxa by having the largest irregular spores up to 43 µm. This fungus also has colonies' colour of brownish gray with walls that appears to be smooth or slightly rough with a diameter of 10-18 µm (Jennessen *et al*., 2008).

R. oligosporus excellently forming hyphae at the temperature of 35ºC and the optimum growth time is 36 hours (Massa *et al*., 2024). *R. oligosporus* showed its ability to an acid environment because it can formed biomass, yielding 5.0 g/L at pH 3 (Massa *et al*., 2024). However, the research conducted by Sparringa *et al*. (2002) showed that this fungus can grow in acidic to neutral pH ranging from 4-7 with the optimum pH is 5.5. The water activity (Aw) of medium also plays an important role as it is associated with the optimum pH for *R. oligosporus* growth.

A nutrient-dense medium is essential for the cultivation of *R. oligosporus,* it requires high moisture content, carbohydrate, minerals, and protein in order to grow well (Surbakti, 2022). This genus plays a crucial role in the production of easily digestible protein, it prevents harmful substances such as aflatoxins and mycotoxin to form. Furthermore, this fungus has the ability to produce various enzymes which beneficial for producing high quality food and feed (Egbune *et al*., 2022). Enriched agricultural waste can be suitable match for medium to cultivate this fungus.

2.4 Factors Influencing Fungal Biomass Production

The optimal growth of *R. oligosporus* is influenced by physical and physicochemical parameters such as dilutions, media supplementation, pH level, and agitation speed. Below are the impact of these factors towards the growth of both fungi.

2.4.1 Dilution

POME is known as nutrient wastewater, because it has high concentration of carbohydrate, protein, nitrogen compound, lipid, minerals, and some organic compound. Wastewater that contain lots of nutrient in it usually have high COD and BOD, also unbalanced C/N ratio (Chen *et al.,* 2020). POME which diluted first usually resulting in lower COD, BOD, and nitrogen content. The lower COD content in a medium will lead to better penetration of light which cause microorganism such as fungi, bacterial, and microalgae growing well (Dominic and Baidurah., 2022).

Dilution also affecting the concentrations of organic and nutrient compounds. In some cases, the excess nutrients could have caused toxicity and become inhibitor for fungal growth. When dilution applied in a medium, it will reduce the organic and nutrient compounds to acceptable concentration. The microorganism will adapt better to this type of environment and it will enhance the growth of microorganisms (Dominic and Baidurah., 2022).

2.4.2 Substrate nutrition

Medium used in submerged fermentation for fungal growth requires various nutrients from macro to micro. It is important for one medium to contains such nutrients, because it favors the growth and product formation at high yield. Oxygen, carbon, hydrogen, nitrogen, phosporus, potassium, sulfur, and magnesium are needed large amounts. On the other hand, the micronutrients which include manganese, iron, zinc, copper, and molybdenum are needed in small amounts (Papagianni *et al*., 2004). Furthermore, the C/N ratio play an important role towards the biomass yield and protein content. Chen *et al*. (2020) stated that it could increase the protein content of photosynthetic bacteria up to 90%.

Fermentation media can be either formulated chemically (synthetic) or complex. The fungal cell structure is mostly made up from carbon along with hydrogen, oxygen, and nitrogen. That is why carbon is needed for fungi to convert it into cellular biomass and forming products. Carbon can be found commonly in the form of glucose, fructose, and sucrose, namely simple sugars. Moreover, in many agricultural wastewater, it contain complex substrate such as lignocellulose that can be utilized for fungal growth also. To utilize lignocellulose, the fungi need to break it down first into simple sugars using extracellular enzymes (Barzee *et al*., 2021).

The presence of nitrogen in a medium is crucial because it’s substantial for fungal growth and metabolism as the fungi able to metabolize a wide variety of nitrogen sources. Nitrogen also become a building block for amino acids, ribonucleic acids, vitamins, and minerals that used by the fungi (Tudzynski, 2014). There is no different effects between two types of nitrogen sources, which is, organic and inorganic. The example of inorganic nitrogen such as ammonium and nitrate and the organic nitrogen such as urea, soybean meal, yeast extract, and peptone (Itoo and Reshi, 2014).

2.4.3 Acidity level (pH)

Most of the time, filamentous fungi including *R. oligosporus* can grow over a wide range of pH, and most tolerable pH ranging from 4 to 9. The pH may be differ depends on fungi used and its application (Barzee *et al*., 2021). The pH level of a medium is one of the parameters to achieve suitable environment for fungal growth. It is a measurement of H+ ions concentration that presents in solution, meanwhile each fungus has their own optimum pH in order to grow optimally. Medium pH affects the transport of nutrients, nutrient solubility, and enzyme reactions. The composition of the medium also need to be adjusted, it can't be too acidic or too alkaline, otherwise it will inhibit fungal growth. While it has an important role, pH drifts during fungal growth will still be occurred as a result of fungal nutrient degradation and to tackle the drifts of the pH from its prevailing pH still difficult to achieve (Papagianni, 2004).

2.5.4 Agitation speed

Submerged fermentation (SmF) is one of the methods for cultivating fungi, this cultivation take a place in a liquid medium contain rich-nutrient and closed vessels (Ouedraogo and Tsang, 2021). Large-scale production usually used bioreactors to conduct SmF, meanwhile, for a small-scale production such as in laboratorium, it can be conducted in Erlenmeyer flask. *R. oligosporus* is aerobic fungi that need oxygen for their growth, and the agitation speed plays important role for mixing the broth to ensure sufficient oxygen troughout the flask (Papagianni, 2004). The mechanical forces from the shaking influence shear forces, mass, and heat transfer in the flask. More forces reported to have positive impact in mycelial growth due to increased oxygen transfer, while the enzyme production dropped at excessive agitation speed.

However, Chung *et al*. (2023) reported that excessive agitation speed generates shear stress. When the agitation speed above 150 rpm, it increase oxygen mass transfer and slightly increase the biomass, but it also comes with detrimental effect which affecting the fungal growth and morphology. It happened due to direct exposure to the broth and it cause cell collided one another. The effect of agitation rate on genus *Rhizopus* has been studied by Mustafa *et al.* (2023) and to obtain highest biomass it need the agitation rate ranging from 100-125 rpm. The difference on agitation speed also resulting in different kind of product. For instance, to form pellet it need agitation rate at least 100 rpm, the higher the rate will led to smaller size of the pellet (Purwanto *et al*., 2009).

III. METHODOLOGY

3.1 Place and Time of Research

This research will conduct from January 2025 to May 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 include Palm Oil Mill Effluent (POME) from the first pond obtained from PTPN VII Bekri, Lampung, and pure culture of *Rhizopus oligosporus* with strains code FNCC 6010. The pure fungal culture was obtained from the Faculty of Agricultural Technology at UGM. The medium used to grow the pure culture was PDA (Potato Dextrose Agar). The supplementation media used are yeast extract, tryptone and mineral solutions for *R. oligosporus* such as N_aNO_3, K_2HPO_4, $MgSO_4.7H_2O$. Other materials used include H_2SO_4, NaOH, 70% alcohol, tween 80, and distilled water.

3.2.2 Equipment

The research equipment used in this study included 500 mL Erlenmeyer flasks, rotary shaker, petri dish, filter cloth, a pH meter, analytical balance, 10 mL and 100 mL volumetric flasks, micropipettes, 1 mL blue-tips, laminar flow, autoclave, spatula, beaker glass, cotton plugs, plastic wrap, zip plastic, heating plates, and filter paper.

3.3 Research Methods

The study begin with the characterization of Palm Oil Mill Effluent, including analysis for pH, total solids, volatile solids, moisture content, ash content, CHN content, mineral content, and Chemical Oxygen Demand (COD). This research utilized filamentous fungi, specifically the pure culture of *R. oligosporus* with strains code FNCC 6010. The research method will employ 4 treatment including dilution, media supplementation, adjusting pH, and agitation speed with each of them will be having 3-4 treatment variations and 3 replications. This is conducted to obtain data on biomass yield and determine the best treatment for *R. oligosporus* to produce high biomass. Treatments that produced the highest fungal biomass, will further analyze for proximate composition, total fiber, carbohydrate, protein, 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) for further analysis.

3.4 Research Procedures

3.4.1 Preparation inoculum

The fungal culture used was an isolate of *R. oligosporus* 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 10^7 spores/mL.

3.4.2 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. (2023).

3.4.2.1 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, bacterial, and microalgae growth (Dominic and Baidurah, 2022). The variety of dilutions are 1:1 ; 1:5 ; 1:10; no dilutions (n = 3). The process for dilutions begin with preparation of media, 5-10 mL of POME added into 500 mL Erlenmeyer flasks, add distilled water untill the volume reach 100 mL and shaken to make it homogen, for the undiluted one, 100 mL of POME added into 500 mL Erlenmeyer flasks. Next step is pH checking, if it's within the range of pH 5-5.5 no adjustment is made. The Erlenmeyer 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 72 hours. After the shaking process done, harvesting was conducted by pouring it into filter cloths, and the remaining biomass was rinsed with water then squeezed. The flowchart of fungal biomass production stage 1 can be seen in Figure 1.

3.4.2.2 Stage 2 (Influence of supplementation)

Fungal biomass produced from POME using *R. oligosporus* fungus with variety of additional supplementation. To investigate the effect of medium supplementation in fungus *R. oligosporus*, 5 g/L yeast extract and 5 g/L tryptone were added to the medium. The mineral solutions concentration in the culture medium was 2 g/L N_aNO_3, 1.8 g/L K_2HPO_4, and 1.2 g/L $MgSO_4.7H_2O$. Medium without any supplementation was used as a control. Dilutions used is the best dilution from stage 1. The process begin with preparation of media, then pH checking; if it's within the range of pH 5-5.5 no adjustment is made. The Erlenmeyer 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 72 hours. After the shaking process done, harvesting was conducted by pouring it into filter cloths, and the remaining biomass was rinsed with water then squeezed. The flowchart of fungal biomass production stage 2 can be seen in figure 2.

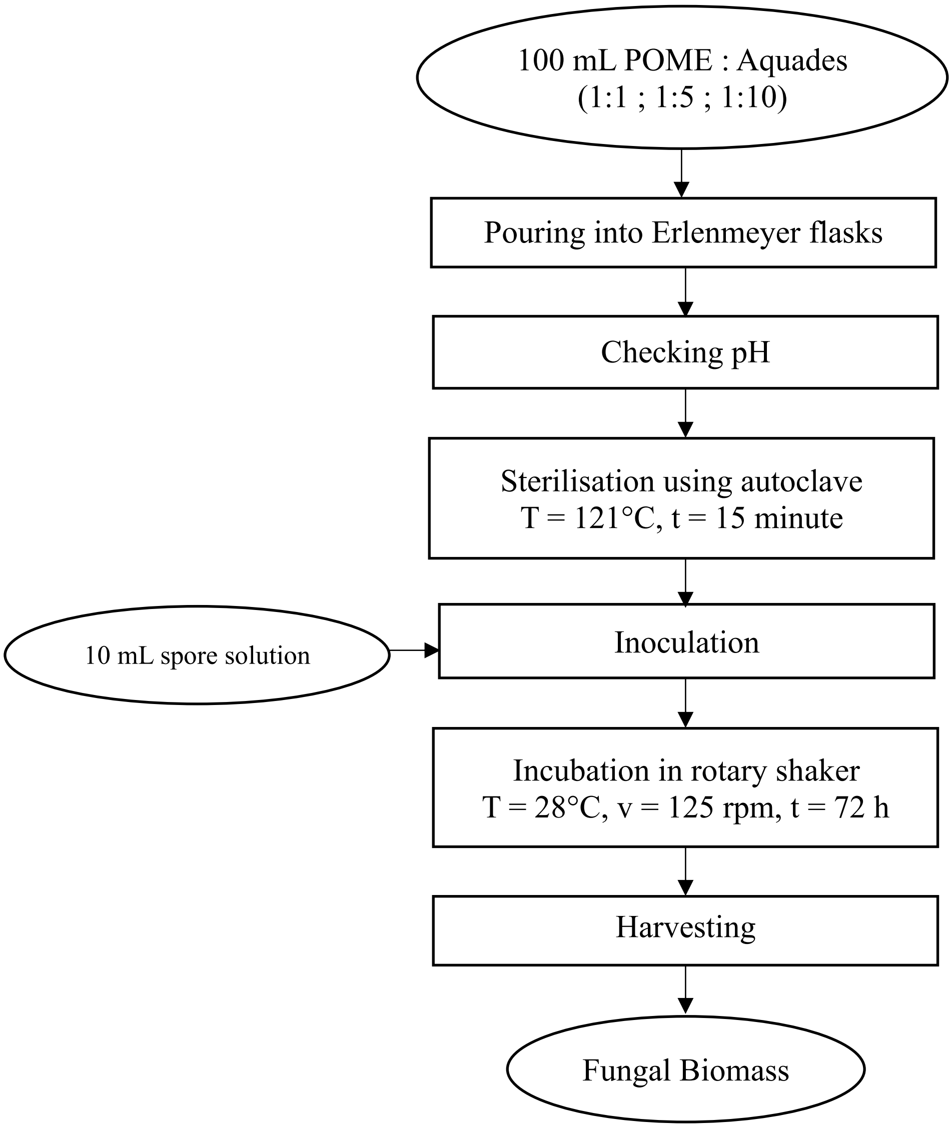

Figure 1. Stage 1 workflow diagram of *R. oligosporus* (Influence of Dilutions)

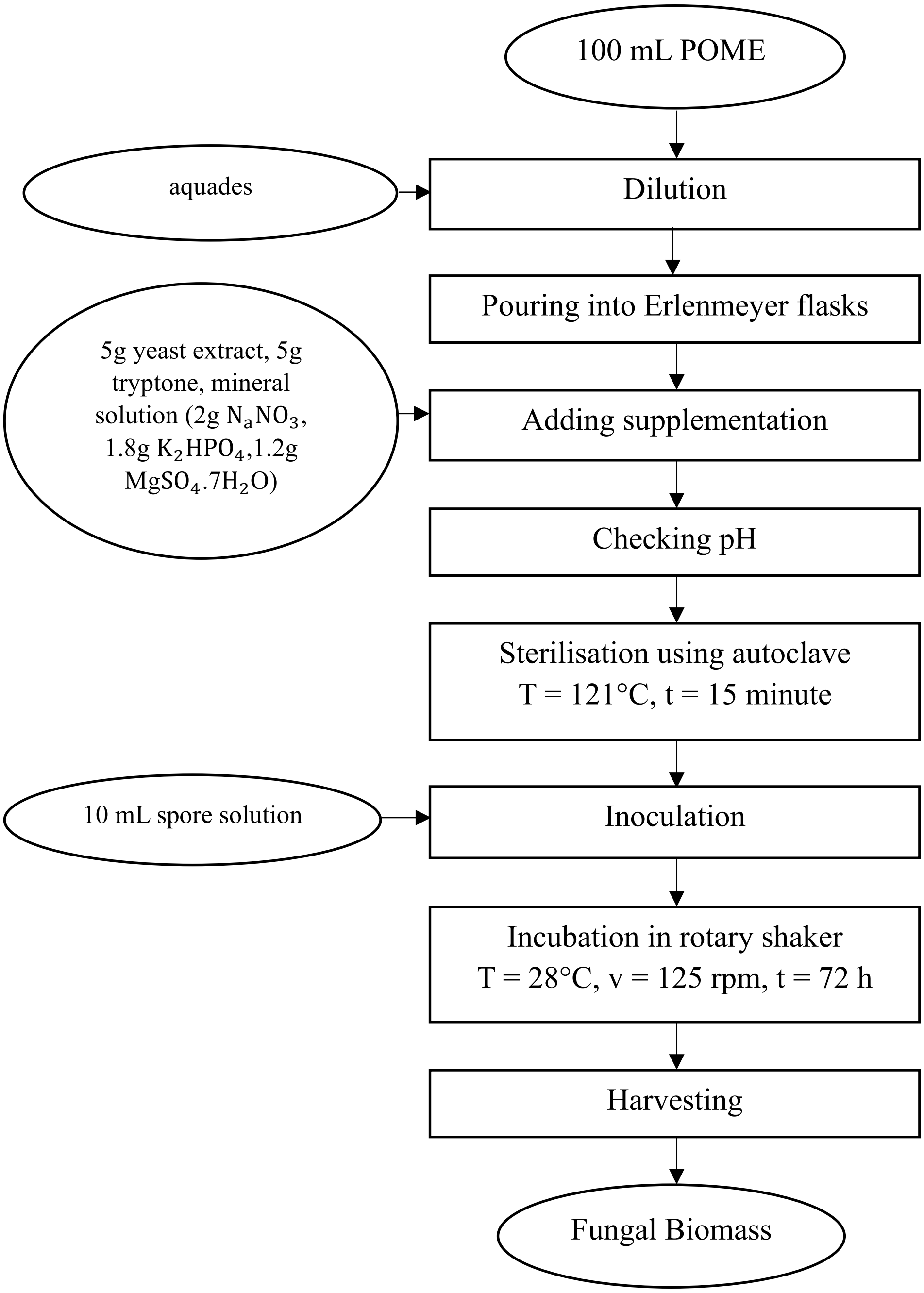

Figure 2. Stage 2 workflow diagram of *R. oligosporus* (Influence of Supplementations)

3.4.2.3 Stage 3 (Influence of pH)

Fungal biomass produced from POME using *R. oligosporus* fungus with variety of pH of 3.5; 4.5; 5.5; 6.5 (n=3). Dilutions used is the best dilutions from stage 1 and supplementations used is the best supplementation from stage 2. The process begin with preparation of media, 100 mL of 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 0.5N H_2SO_4 and 0.5N NaOH, using a pH meter. The Erlenmeyer 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 72 hours. After the shaking process done, harvesting was conducted by pouring it into filter cloths, and the remaining biomass was rinsed with water then squeezed. The flowchart of fungal biomass production stage 3 can be seen in figure 3.

3.4.2.4 Stage 4 (Influence of agitation speed)

Fungal biomass produced from POME using *R. oligosporus* fungus with variety of agitation speed of 110 rpm, 125 rpm, 140 rpm, and 155 rpm (n=3). Dilutions used is the best dilutions from stage 1 and supplementations used is the best supplementation from stage 2. The process begin with preparation of media, 100 mL of 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 best treatment from stage 3. The Erlenmeyer 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 110 rpm, 125 rpm, 140 rpm, and 155 rpm speed at room temperature (28°C) for 72 hours. After the agitation process done, harvesting was conducted by pouring it into filter cloths, and the remaining biomass was rinsed with water then squeezed. The flowchart of fungal biomass production stage 4 can be seen in figure 4.

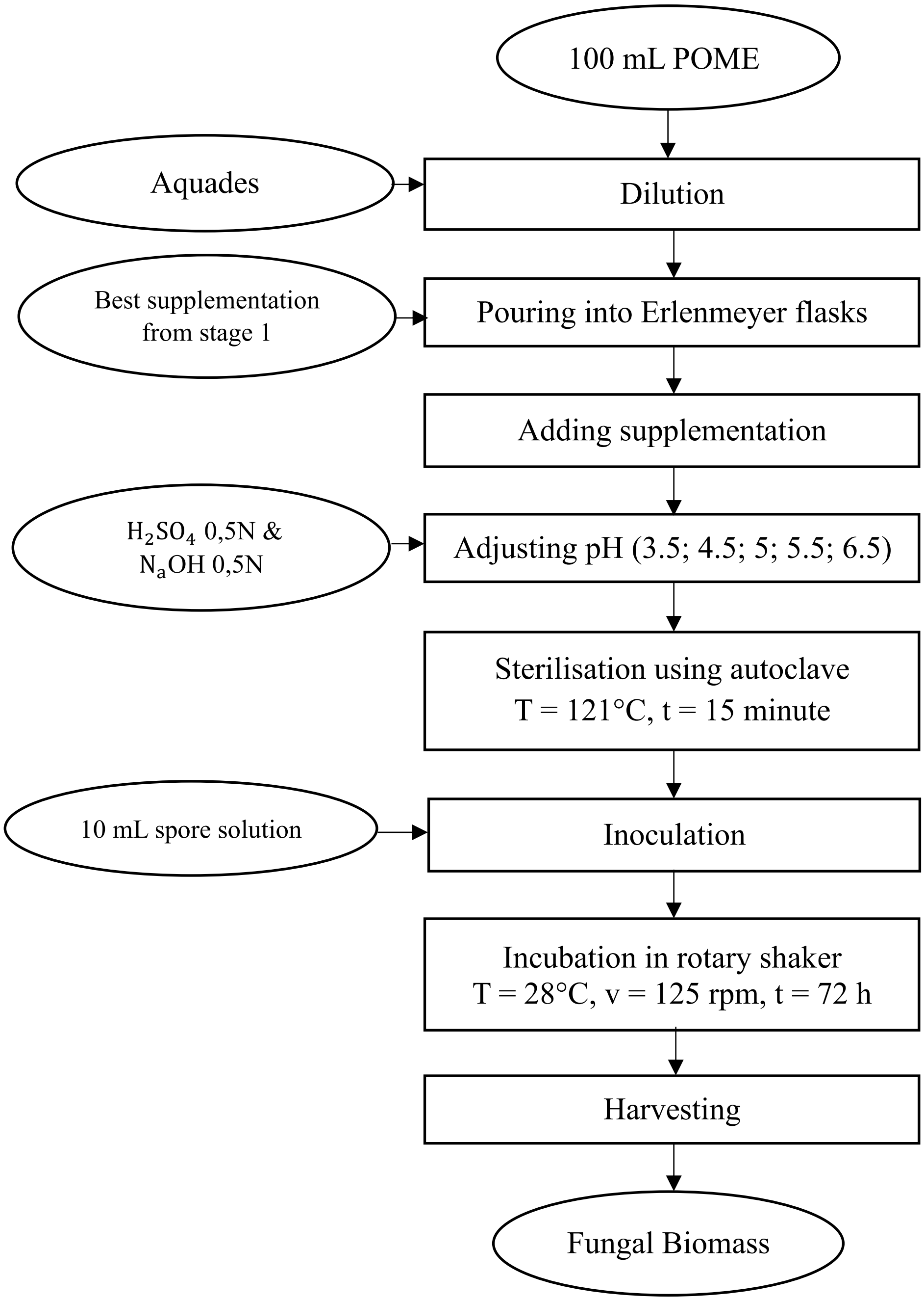

Figure 3. Stage 3 workflow diagram of *R. oligosporus* (Influence of pH)

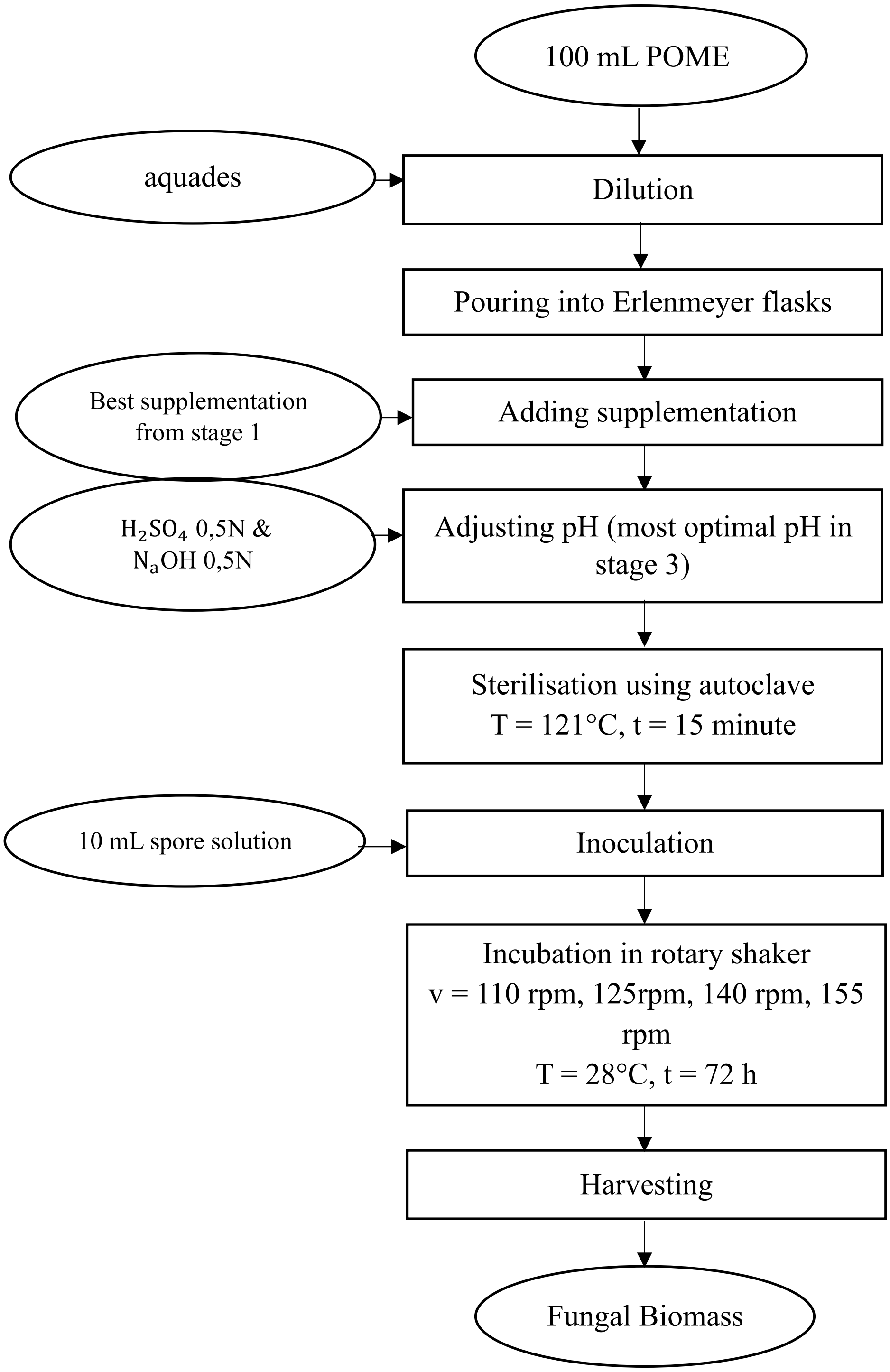

Figure 4. Stage 4 workflow diagram of *R. oligosporus* (Influence of Agitation Speed)

3.4.3 Determination of biomass quantity

The fungal biomass mycelium was harvested after 72 hours of cultivation, followed by washing with water three times. Afterwards, the biomass was squeezed using a filter cloth, then dried using an oven at 100°C for 24 hours and weighed to determine the weight of the fungal biomass.

3.4.4 Moisture content analysis

The moisture contents analyzed by the thermogravimetric method AOAC (2016). The analysis begin with oven-drying empty porcelain crucibles at 105°C for 30 minutes, followed by cooling and placed in a desiccator for 15 minutes. Afterward, the crucibles were weighed. This process of weighing was repeated until a constant weight of the crucible was obtained. Next, 1-2 g of sample was placed into a porcelain crucible and weighed. The crucible containing the sample was burned and then ashing was carried out using a furnace at 550°C for 3 hours. After that, the sample placed in a desiccator for 15 minutes and weighed until a constant weight was obtained. The ash content was calculated using the following formula:

$$\text{Moisture content (\%)} = (W\text{-}W1) \times \frac{100}{W}$$

Notes:

W = Initial sample (g)

W1 = Sample weight after drying process (g)

3.4.5 Ash content analysis

The ash contents analyzed by the gravimetric method AOAC (2016). The analysis begin with oven-drying empty porcelain crucibles at 105°C for 30 minutes, followed by cooling and placement in a desiccator for 15 minutes. Afterward, the crucibles were weighed. This process of weighing was repeated until a constant weight of the crucible was obtained. Next, 1-2 g of sample was placed into a porcelain crucible and weighed. The crucible containing the sample was burned and then ashing was carried out using a furnace at 550°C for 3 hours. After that, the sample placed in a desiccator for 15 minutes and weighed until a constant weight was obtained. The ash content was calculated using the following formula:

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

Notes:

a = An empty porcelain crucible weight (g)

b = Porcelain crucible weight + sample weight (g)

c = Porcelain crucible weight + ash weight (g)

3.4.6 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.

3.4.7 Fat content analysis

First step in conducting fat analysis using Soxhlet method is to prepare the sample, as much as 4 g sample placed into thimble that made from filter cloth and the top was closed using cotton and then placed into the Soxhlet. On the other hand, the Soxhlet flask was oven-dried at 100°C for 1 hour, then it was placed in a desiccator and weighed. The sample was placed into the chamber of the Soxhlet apparatus, and petroleum ether solvent was added approximately two times the volume of the tube. The extraction was carried out for about 6 hours until the solvent returned through the siphon into the Soxhlet flask. The flask containing result of extraction was then take out to be dried in an oven at 105°C for 24 hours. Later on, it was placed into the desiccator to be cooled and weighed until a constant weight was obtained. The fat content was calculated using the following formula:

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

Notes:

a = An empty Soxhlet flask weight (g)

b = Sample weight (g)

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

3.4.8 Fiber content 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. Afterward, the sleeves were placed in a desiccator and weighed. 1-2 g of samples placed into the sleeves and place it into the sleeve rack. 500mL of 1.25% H_2SO_4 was later on poured into a 1000mL beaker. A cooling glass was put on to the beaker which hold a sleeve rack inside of it and place it on to a heating plate for about 30 minutes with the heat set up until 100°C. After 30 minutes, turn off the heating plate, discard the 1.25% H_2SO_4, and rinse the sleeve rack and beaker. Repeat the exact same process using 1.25% NaOH. Lastly, after these sleeves have gone through those stages, they need to be dried in an oven at a temperature 100°C for 1-3 hours until a constant weight. The fiber content was calculated using the formula:

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

Notes:

a = Residue weight in dried sleeve (g)

b = Weight of empty sleeve (g)

W = Sample weight

V. CONLUSIONS & SUGGESTIONS

5.1 Conclusions

The conclusions of this study are as follows:

1. Palm oil mill effluent has the potential as a medium for fungal biomass growth, it contains lots of organic matter which can support the growth of the fungus, such as nitrogen 1.85%, organic carbon 60.05%, and phosphorus 3.097%. Although the supplementation is still needed to produce higher biomass.
2. The optimum conditions for achieving high biomass need suitable dilutions, media supplementation, pH level, and agitation speed. The optimal conditions produced 7.1535 g/100mL (wb) biomass, with the following conditions are without dilutions, supplementation with tryptone, pH 5.5, and agitation speed at 110 rpm.
3. The nutritional content of the fungal biomass produce under optimal conditions includes protein content of 19.95% (db), fat content 56.07% (db), ash content 3.85% (db), moisture content 74.01% (wb).

5.2 Suggestions

Based on the findings of this study, several recommendations are proposed for future research to further enhance the production and application of fungal biomass derived from Rhizopus oligosporus cultivated on palm oil mill effluent (POME):

1. Future research need to investigate the heavy metals in the final biomass to ensure biomass safety for its application in animal feed.

2. The relation between fermentation duration and agitation speed also need to be reviewed more to find the the best balance between biomass yield, protein content, and lipid accumulation.
3. The C/N ratio in POME is quite high, it is best if the future research can conduct various C/N ratio to see the optimal condition in POME.

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