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Lipid Production from Palm Acid Oil (PAO) as a Sole Carbon Source by *Meyerozyma guilliermondii*

Noor-Afiqah Ahmad Zain †, Kar Ling Tan †, Prihardi Kahar * and Chiaki Ogino

Department of Chemical Sciences and Engineering, Graduate School of Engineering, Kobe University, 1-1 Rokkodai, Nada, Kobe 657-8501, Japan; noorafiqah51@gmail.com (N.-A.A.Z.); ochiaki@port.kobe-u.ac.jp (C.O.)
* Correspondence: pri@port.kobe-u.ac.jp
† These authors contributed equally to this work.

Abstract: *Meyerozyma guilliermondii* is an interesting oleaginous yeast with considerable potential for biotechnological applications. This yeast demonstrates the ability to utilize palm acid oil (PAO), a low-cost and renewable feedstock, as a carbon source, making it a sustainable candidate for single-cell oil production. Under optimal conditions with 4% of PAO, *M. guilliermondii* can accumulate lipids to approximately 45% of its cell dry weight (CDW). Notably, the expression level of *PEX14*, a gene associated with peroxisomal biogenesis, increases with higher PAO concentrations, coinciding with the formation of multiple small lipid bodies. These findings highlight the metabolic adaptability of *M. guilliermondii* and its potential for industrial lipid production using waste-derived feedstocks.

Keywords: *Meyerozyma guilliermondii*; oleaginous yeast; palm acid oil (PAO); lipid

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1. Introduction

Palm oil serves as an essential commodity for the world, since it can be used as a raw material for various essential products, including cooking oil, food, and cosmetics [1]. Concurrently, with the increasing demand for clean and renewable energy sources to reduce reliance on fossil fuels, palm oil has also been found to be a promising source for production of biofuel, including biodiesel and biogas [2]. The diverse applications of palm oil have led to a substantial rise in global demand, with crude palm oil production in the world exceeding 80 million tons in 2021 compared to approximately 50 million tons in 2011 [3]. However, this growing demand presents a dual challenge, as it requires the expansion of palm oil plantations to meet global needs, often leading to more land clearing and associated environmental impacts. To avoid competition with food supplied and reduce reliance on edible resources, attention has shifted toward exploring an alternative biofuel source derived from non-edible biomass such as organic waste. As a result, efforts have been focused on the production of biogas and biodiesel using waste products such as waste tallow and food waste [4–7].

Palm oil mill effluents (POMEs) are viscous liquid wastewater by-product generated during palm oil extraction processes, including sterilization and fruit washing in palm oil refineries [8]. Processing one ton of fresh fruit bunches generates wastes comprising approximately 60% POME, which consists of 95–96% water and 4–5% total solid, including palm acid oil (PAO) [9]. Raw POME generally contains a high concentration of organic matter, total solids, oil, and grease, along with high levels of chemical oxygen demand (COD) and biological oxygen demand (BOD). Although POME is classified as a non-toxic waste product, its untreated discharge into water bodies can cause severe environmental

pollution [10]. For instance, methane gas emissions resulting from anaerobic processes in POME treatment ponds contribute substantially to greenhouse gas accumulation, with an estimated 12.36 kg of methane released per ton of POME treated [11].

To mitigate this problem, several treatments have been proposed, including coagulation-flocculation, anaerobic digestion, membrane-based treatment, and photocatalytic treatment [12,13]. The cost of treating POME varies significantly based on the treatment method employed, plant capacity, and local economic factors. For instance, coagulation-flocculation treatment can cost approximately 0.4 USD/m^3, whereas advanced membrane-based systems may range from 1.6 to 26 USD/m^3 due to the need for specialized equipment and membrane replacement [13,14]. While effective, these methods are often associated with high operational costs and extensive use of chemicals. Thus, an eco-friendly and cost-effective way of disposal aligned with sustainability goals is highly desirable [15]. One promising approach is the use of microbes to convert POME into value-added products, such as single-cell oils, biofuels, or bioplastics, which not only reduce waste but also enhance the economic value of palm oil processing. Various oleaginous microorganisms like yeast, algae, mold, and bacteria are reported to be able to utilize waste such as POME and accumulate lipid in the form of single-celled oil (SCO) [16]. Recycling waste to culture microorganisms for lipid production is definitely more advantageous, as it could recover value-added products at the end of the treatment process by adopting a zero-waste discharge concept. This microbial bioconversion could provide a sustainable alternative with lower overall treatment cost while simultaneously supporting the palm industry's transition to a circular economy.

Yeast is a preferable microorganism for microbial oil production compared to filamentous fungi, microalgae, or bacteria. Unlike microalgae, yeast does not require land for cultivation, has a higher growth rate, and can utilize a broader range of carbon sources. Yeast offers advantages in lipid production, including higher lipid content, and the process of scaling up is also easier with yeast [17]. Additionally, yeasts can tolerate living in extreme environmental conditions, such as low pH conditions, which can be used to control bacterial contamination, as most bacteria are inhibited under low pH conditions [18]. Yeasts capable of accumulating lipids more than 20% of their dry cell weight (DCW) are classified as oleaginous [19]. Presently, several oleaginous yeast genera, including *Yarrowia*, *Candida*, *Rhodotorula*, *Rhodosporidium*, *Cryptococcus*, *Trichosporon*, and *Lipomyces*, have been extensively studied for their lipid accumulation capabilities [16].

Meyerozyma guilliermondii is another oleaginous genera widely recognized for its diverse applications, including xylitol production, enzyme production, and bioremediation [20–25]. *M. guilliermondii* is capable of utilizing diverse carbon sources, including lignocellulosic hydrolysates, waste materials, and other renewable feedstocks [26]. This yeast is recognized for its relatively fast growth rate and metabolic versatility, which make it an attractive candidate for biotechnological applications. In addition, *M. guilliermondii* demonstrates adaptability to various environmental conditions and resistance to inhibitors typically found in industrial processes [20,27]. Therefore, the use of *M. guilliermondii* for industrial-scale lipid production holds promise. However, despite its versatility, research on its potential for lipid production remains limited. Current studies on lipid accumulation in *M. guilliermondii* have primarily focused on substrates such as glucose, glycerol, and inulin, suggesting its capacity for lipid biosynthesis [26,28]. Nevertheless, these studies have not extensively explored its performance when utilizing waste-derived feedstocks.

To the best of our knowledge, there are no comprehensive studies reporting the use of PAO derived from POME as a substrate for single-cell oil production by any strain of *M. guilliermondii*. PAO has limited applications, primarily being utilized for production of fatty acids, animal feed, and low-grade laundry soup [29,30]. In this study, we demonstrate

the ability of our *M. guilliermondii* strain to accumulate lipids in nutrient-rich media supplemented with PAO as the sole carbon source. Additionally, we investigate the effect of varying concentrations of PAO on cell growth and subsequent lipid production.

2. Materials and Methods

2.1. Yeast Strain and Growth Condition

Meyerozyma guilliermondii from a private collection (No. PK01) at Kobe University was used in the study. The yeast strain was preserved in 20% (*w*/*w*) glycerol and revived by streaking on a potato dextrose agar (PDA) plate. For short-term storage, the yeast strain was grown on a yeast extract peptone dextrose (YPD) agar plate and maintained at 4 °C. The composition of YPD agar included 10 g/L of yeast extract, 20 g/L of peptone, 20 g/L of glucose, and 15 g/L of agar. The culture was subcultured regularly to a new plate at least once a month to maintain its freshness. A single colony of the strain from YPD agar was inoculated into 12 mL of YPD broth in a 100 mL Erlenmeyer flask. The composition of YPD broth was 10 g/L of yeast extract, 20 g/L of peptone, and 20 g/L of glucose. The seed cultures were incubated in an orbital shaker incubator (BioShaker BR-43FH MR, Taitec Corp., Tokyo, Japan) at 29 °C and 190 rpm for 18–24 h before inoculation. Shake flask fermentation was conducted in a 100 mL Erlenmeyer flask with 12 mL of working volume. The seed volume of the inoculum was adjusted to an initial optical density (OD_{600nm}) of 16–18 to obtain an appropriate amount of active cells for higher lipid production. Afterward, the seed culture was transferred to each YP medium containing different concentrations of PAO. The cultivation mode was by batch, and the fermentation was terminated after 4 days (96 h).

2.2. Media Preparation

The fermentation medium used in this study consisted of yeast extract peptone (YP) broth supplemented with varying concentration of PAO 1% to 10% (*w*/*v*) [31]. The composition of the YP medium included 10 g/L of yeast extract, 20 g/L of peptone and the specified concentrations of PAO. The percentage values for PAO supplementation were based on a mass/volume (*w*/*v*) ratio. Additionally, YP media without PAO supplementation was prepared as a negative control, containing only 10 g/L of yeast extract and 20 g/L of peptone.

2.3. Carbon Sources

Palm acid oil (PAO) is a semi-solid waste derived from POME. The PAO was obtained from PT. Agricinal Palm Oil Mills (Bengkulu, Indonesia). PAO was heated at 70 °C overnight to obtain the liquid form prior to adding the medium.

2.4. Determination of Total Lipid Content by Gravimetric Analysis

Gravimetric analysis was used to quantify the total lipid produced in the harvested cells based on the modified Folch method [32]. A triplicate of freeze-dried samples was transferred to 2.0 mL of polypropylene microvial with an O-ring-sealed cap containing 0.5 mm of zirconia beads and 1.5 mL of Folch solvent (2:1 $CHCl_3$: MeOH, *v*/*v*). Cells were pulverized using a Shake Master Neo ver.1.0 (BMS-M10N21, BMS, Tokyo, Japan) at 1500 rpm for 15 min. Afterward, the cells were centrifuged at 14,000 rpm for 5 min, the supernatant was removed by pipetting, and the remaining pellets were washed with 1.5 mL of deionized water. The cells were then re-pulverized for a second time and centrifuged, and the water was removed from the pellet. The lipid content was calculated based on the weight difference and was expressed as the percentage of cell dry weight.

2.5. Determination and Quantification of Total Fatty Acid Methyl Esters (FAMEs)

The transesterification process was performed according to the protocol provided by the fatty acid methylation kit (Nacalai Tesque, Inc., Kyoto, Japan). The extracted FAME in the light phase were analyzed using a gas chromatography–mass spectrometer (GC-MS) (Shimadzu, Kyoto, Japan) equipped with a DB-23 capillary column (0.25 mm × 30 m) (J&W Scientific, Folsom, CA, USA). Helium gas was employed as the carrier at a flow rate of 0.8 mL/min with a split ration of 1:5. The initial column temperature was 250 °C, followed by an increase of 50 °C for 1 min, and then increased by 25 °C/min to 190 °C. Subsequently, the temperature was increased 5 °C/min to 235 °C, which was held for 4 min. Caprylic acid (C8:0) was used as an internal standard in each sample. The composition of FAME was determined as the percentage of each fatty acid relative to the total fatty acids produced during fermentation.

2.6. BODIPY Staining

BODIPY (493/505) is used as a dye to visualize the triacylglycerols (TAGs) in the cell. BODIPY has various advantages over other staining methods. It can be taken up efficiently with minimal dye needed, and the lipid droplets can be easily spotted in bright green fluorescence [33]. BODIPY stock solution was prepared by dissolving 10 mg of BODIPY powder with 1 mL of dimethyl sulfoxide (DMSO) to make the stock concentration 10 g/L. During sampling, 100 µL of fermentation culture was withdrawn, pelleted, and washed with 0.9% (*w*/*v*) phosphate saline buffer (PBS). After washing, the pellet was suspended in 45 µL of 0.9% (*w*/*v*) PBS. An amount of 5 µL of BODIPY stock solution was added and mixed well by pipetting. The cell suspension was kept in the dark for at least 1 h. Fluorescence images of the BODIPY-stained cells were viewed and subsequently captured on a digital inverted fluorescence microscope (Keyence Biorevo BZ-9000, Keyence, Osaka, Japan).

2.7. Lipase Activity Assay

Lipase activities were measured from the supernatant separated after centrifugation using p-nitrophenyl-butyrate (pNPB) in 10 mM of sodium phosphate buffer, pH 7.0, at 37 °C. Hexane was used as a solvent to wash and remove the residual POME from the supernatant. The supernatants were washed twice before being used as a sample for lipase activity evaluation. One unit of activity was defined as the amount of enzyme that releases 1 µmol of p-nitrophenol per minute.

2.8. Quantitative PCR

The RNA of each sample was isolated and purified by using the NucleoSpin RNA kit (Takara Bio, San Jose, CA, USA) according to the manufacturer's instruction. The purified RNA was quantified by measuring the absorbance at 260 nm and 280 nm using a Thermo Scientific Nanodrop One (Thermo Fisher Scientific, Waltham, MA, USA). After the concentration of RNA was determined and its purity confirmed, it was used as a template for cDNA synthesis. Reverse transcription, the process of generating cDNA from single-stranded RNA, was performed using the ReverTra Ace qPCR RT Master Mix (Toyobo, Osaka, Japan) following the manufacturer's protocol. The product was subsequently amplified, and its concentration was measured using Thermo Scientific Nanodrop One. Real-time quantitative PCR (qPCR) is used to quantify gene expression. The qPCR was performed using an Applied Biosystems StepOnePlus using SYBR Green RT-PCR Master Mix (Toyobo, Osaka, Japan). Amplification was performed in a 20 µL reaction mixture solution containing 10 µL of 2× KOD SYBR qPCR mix, 0.4 µL of 50×ROX reference dye (1:10 diluted), 5 µL of cDNA template (final concentration ≈ 12.5 ng/µL), 0.8 µL of each

primer (forward + reverse), and 3.8 µL of RNA-free water. The qPCR conditions were 98 °C for 5 min, followed by 60 cycles of 98 °C for 10 s, 60 °C for 11 s, and 68 °C for 30 s. The specificity of each pair of primers was checked by melting curve analysis (98 °C for 1 min, 60 °C for 30 s, and 98 °C for 30 s). The primer sequences and target genes are listed in Table S1 and Table S2, respectively. The gene expression data were analyzed using the $2^{-\Delta\Delta Ct}$ method [34].

3. Results

3.1. Growth of Meyerozyma guilliermondii in PAO

The strain was cultivated in a nutrient-rich medium (YP broth) supplemented with PAO as the sole source of carbon (C). Yeast extracts and peptone serve as the main source of nitrogen (N) supporting both cell growth and lipid accumulation in the cells. The alteration of the carbon/nitrogen (C/N) ratio of the media can significantly affect the overall lipid production. Excess carbon combined with nutrient limitations, such as a lower N source in the media, is generally optimal for lipid accumulation in oleaginous yeast [19]. However, optimizing these parameters was not explored in the current study. In a previous study, the cell biomass and lipid yield were reported to remain high under a low C/N ratio [35], consistent with findings from earlier studies. In this study, a similar approach was applied to another oleaginous yeast, *Meyerozyma guilliermondii*, using PAO as the source of carbon. The growth of this strain was assessed by generating a cell dry weight (CDW) profile over a 4-day cultivation period at various PAO concentrations (Figure 1). We selected a 4-day period because the preliminary experiments indicated that the strain exhibited its highest growth and lipid accumulation within this timeframe.

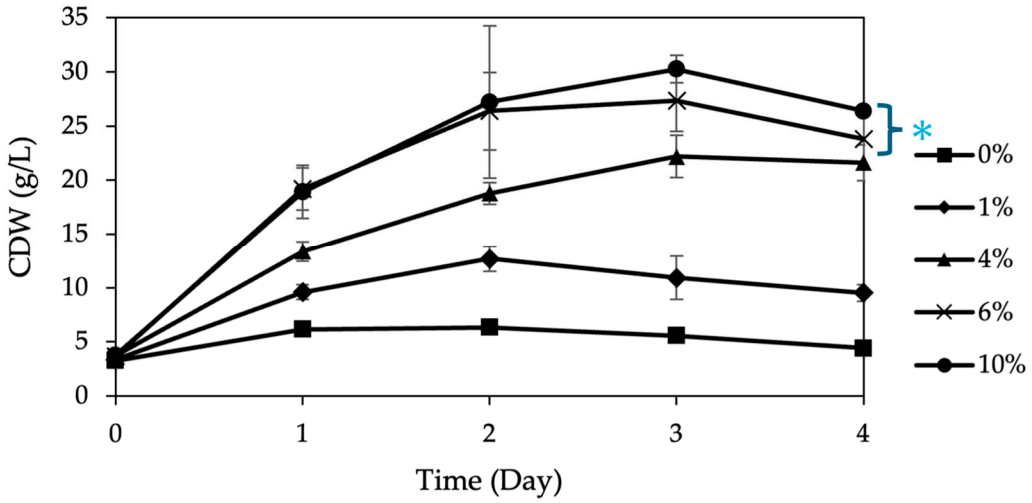


Figure 1. Biomass yield (CDW) of *M. guilliermondii* PK01 cultivated in different concentrations of PAO (%, *w*/*w*) at 0, 1, 4, 6, and 10. All experiments were performed in triplicate. Error bars represent the standard deviation (SD) of the mean. Statistical significance was determined using Student's *t*-test (* $p < 0.05$). All values are significantly different from each other across concentrations.

The negative control (YP without PAO) exhibited the lowest CDW, with a decline observed after 1 day of cultivation. This observation aligns with the fundamental role of carbon as an essential element for building blocks of living organisms, enabling cells supplemented with carbon sources to proliferate more effectively. In general, the biomass yield, represented by the CDW obtained, increased with a higher concentration of PAO. The highest CDW was obtained in the medium containing 10% (*w*/*v*) of PAO. The growth rates of the strain grown in media with 6% (*w*/*v*) and 10% (*w*/*v*) of PAO were similarly rapid during the beginning of the fermentation. However, growth in the medium with 6%

(*w*/*v*) of PAO started to plateau after Day 2, while growth in 10% (*w*/*v*) of PAO medium continued to increase, though at a slower rate than at the beginning, until Day 3. These findings suggest that higher concentrations of PAO can effectively promote cell growth, demonstrating its potential as a promising carbon substrate.

3.2. Effect of PAO on Biomass Yield and Lipid Production

The harvested cell biomass and lipid production of *M. guilliermondii* were analyzed to evaluate the effects of PAO concentration (Figure 2). The results demonstrate a clear correlation between increased PAO concentration and simultaneous increases in biomass yield and total lipid production. As the carbon source concentration in the fermentation medium increases, the overall C/N ratio also increases. The availability of excess carbon is metabolized and stored as intracellular lipids, which are essential for cell growth, stress response, and species survival [36]. Notably, the increase in biomass yield is more pronounced between 0% (*w*/*v*) and 4% (*w*/*v*) of PAO compared to the increment observed between 4% (*w*/*v*) and 10% (*w*/*v*) of PAO.

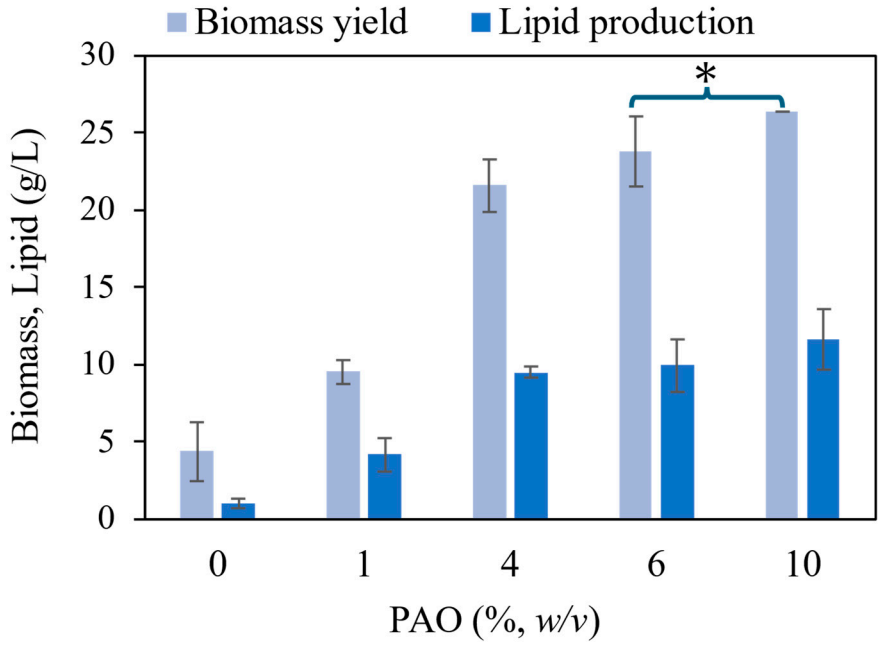


Figure 2. Biomass yield and total lipid production of harvested cells grown in different concentrations of PAO after 4 days of cultivation. Statistical significance was determined using Student's *t*-test (* $p < 0.05$) in Microsoft Excel. All experiments were performed in triplicate. Error bars represent the standard deviation (SD) of the mean.

The highest lipid content (45.33% *w*/*w*) was obtained when the strain was cultivated in 4% of PAO (Table 1). However, the lipid yield obtained in 4% (*w*/*v*) of PAO was approximately 2.1 g/L lower than that observed in 10% (*w*/*v*) of PAO. Several factors may influence lipid accumulation and the subsequent lipid content calculated under each condition. PAO tends to solidify and clump together after being added to the medium, potentially resulting in a final PAO concentration that does not accurately reflect the actual concentration intended to be prepared. Additionally, uneven distribution of PAO within the medium could limit the cell's access to the carbon substrate, which will affect the ability of the cell to accumulate lipids [31]. Nevertheless, constant agitation during fermentation may help reduce these inconsistencies. A summary of the biomass yield and lipid production is presented in Table 1.

Table 1. Lipid production of *M. guilliermondii* grown in YP supplemented with different concentrations of PAO.

Media	Biomass Yield (g/L)	Lipid Production (g/L)	Lipid Content (% *w*/*w*)
YP	4.40 ± 1.90	1.03 ± 0.31	21.87 ± 0.76
YP + 1% (*w*/*v*) PAO	9.52 ± 0.78	4.19 ± 1.07	43.59 ± 5.41
YP + 4% (*w*/*v*) PAO	21.61 ± 1.68	9.50 ± 0.34	45.33 ± 5.49
YP + 6% (*w*/*v*) PAO	23.81 ± 2.29	9.96 ± 1.69	41.70 ± 3.47
YP + 10% (*w*/*v*) PAO	26.37 ± 0.00	11.6 ± 1.95	38.49 ± 2.38

The lipase activity towards pNPB as the substrate was also determined (Figure 3). The results indicated that the lipase activity of cells grown in the presence of PAO was lower than that of the negative control. PAO contains high amounts of free fatty acids (FFAs), which constitute approximately 76% of its composition [37], with oleic acid constituting a significant fraction of the FFA (40.3%) [38]. Lipase catalyzes the hydrolysis of TAGs into glycerol and FFAs. However, PAO contains only about 13% TAGs [37]. This limited TAG availability in PAO likely contributes to the reduced lipase activity observed, as FFAs are already abundant in the medium, diminishing the need for TAG hydrolysis.

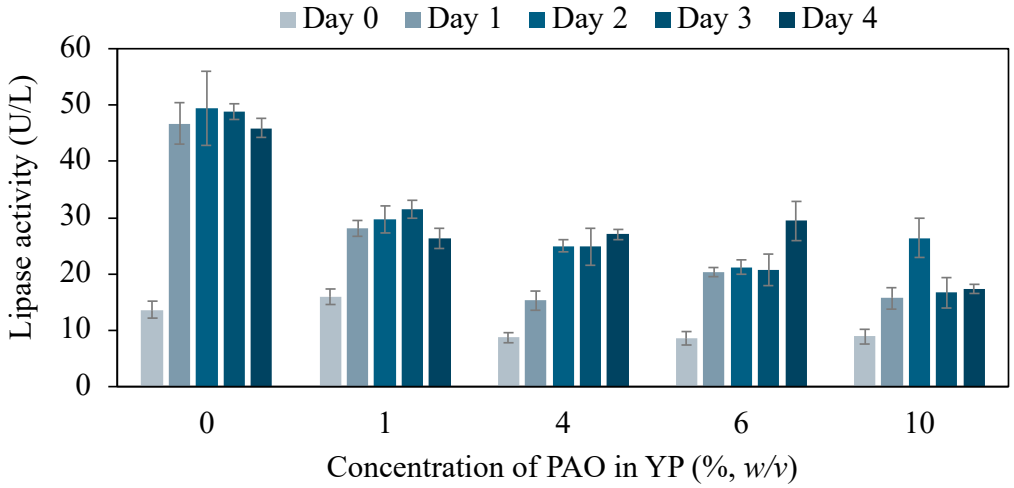


Figure 3. Lipase activities of *M. guilliermondii* PK01 cultivated in different concentrations of PAO in a YP medium. All experiments were performed in triplicate. Error bars represent the standard deviation (SD) of the mean.

3.3. Visualization of Lipid Bodies

The accumulated single-cell lipids were visualized, analyzed, and qualitatively compared using brightfield microscopy (which generates images with shadier objects on darker backgrounds) and fluorescence microscopy (which generates images with fluorescence) at Day 0 (Figure 4), Day 2, and Day 4 (Figure 5). BODIPY is a lipophilic dye commonly used to visualize neutral lipids, including TAGs and FFAs [33]. However, BODIPY staining does not specifically distinguish between TAGs and FFAs. In this study, the green fluorescence observed in images (b) and (c) is attributed to accumulated neutral lipids, predominantly TAGs. The intensity of green fluorescence likely reflects the relative amount of TAGs accumulated in the cells. In the absence of a carbon source, the fluorescence intensity noticeably reduced after 2 days of cultivation (Figure 5) compared to Day 0 (Figure 4), suggesting that the cells have metabolized their intracellular lipids, likely due to nutrient limitation, as there was no carbon source available in the medium. Oleaginous yeast tends to consume its food reserve under conditions of carbon starvation, indicating that lipid metabolism is a highly controlled cellular process in yeast [39].

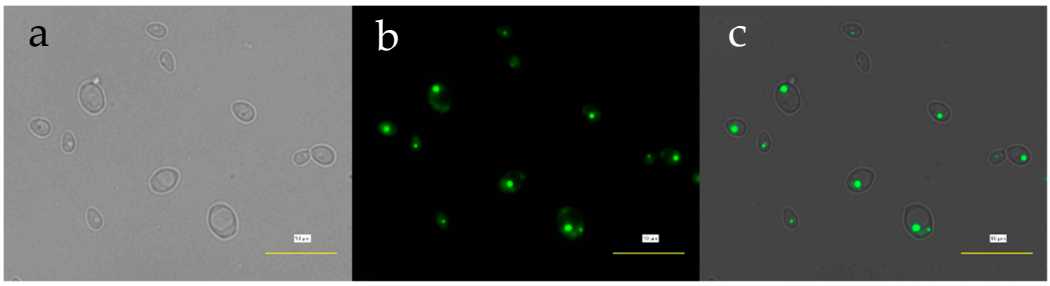
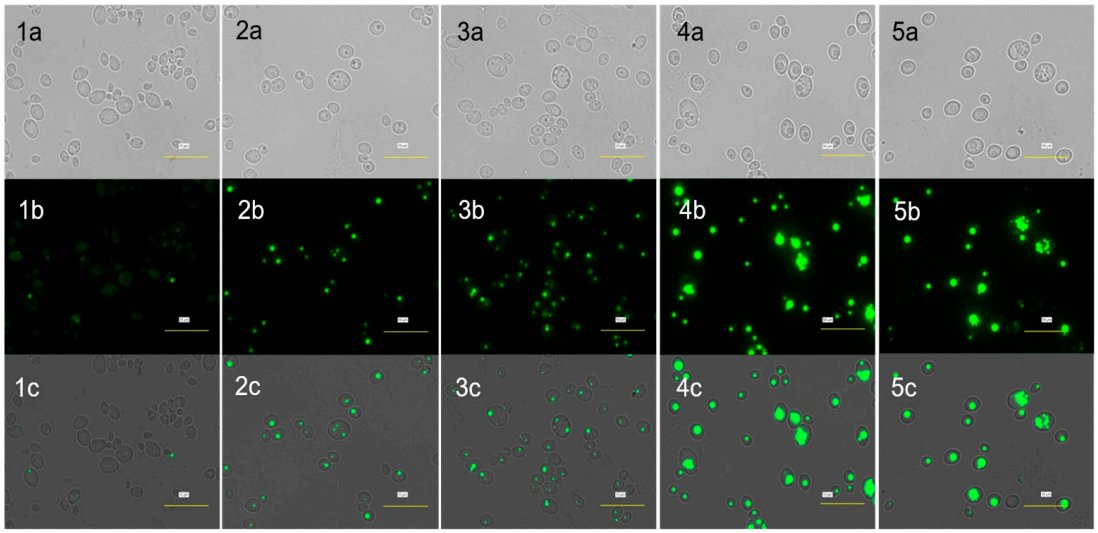


Figure 4. Microscopic images of BODIPY-stained cells from one of the fermentation cultures acquired with brightfield (**a**), fluorescence (**b**), and overlap image (**c**) at the beginning of fermentation at Day 0. Scale bar: 10 µm.

Day 2



Day 4

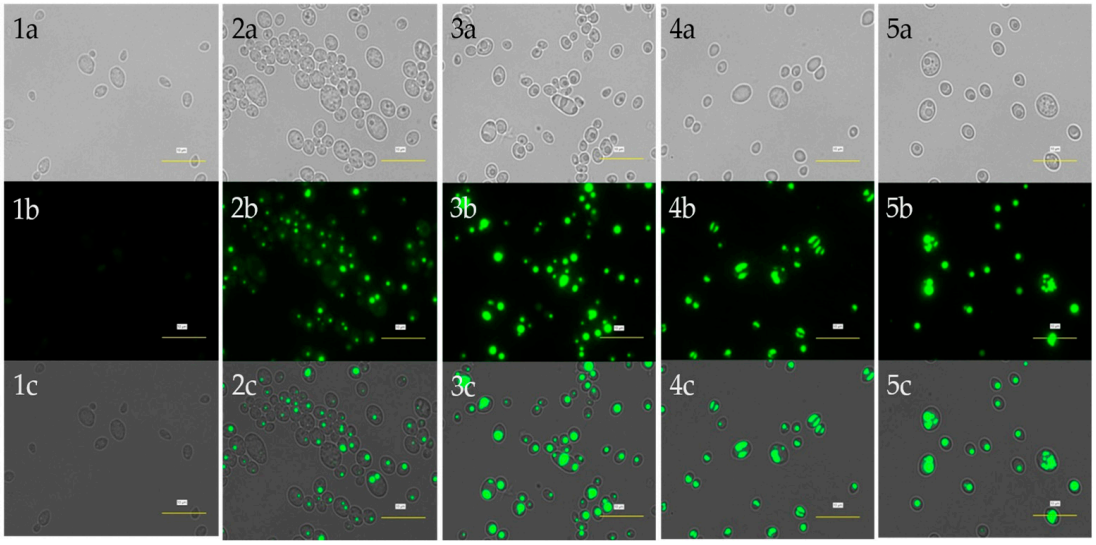


Figure 5. Microscopic images of BODIPY-stained cells cultivated in 0% (*w*/*v*) PAO (1), 1% (*w*/*v*) PAO (2), 4% (*w*/*v*) PAO (3), 6% (*w*/*v*) PAO (4), and 10% (*w*/*v*) PAO (5) acquired with brightfield (first row; a), fluorescence (middle row; b), and overlap images (last row; c). Scale bar: 10 µm.

On the other hand, with higher concentrations of PAO (6% and 10%, *w*/*v*), the cells appeared to accumulate more lipids after 2 days of cultivation (Figure 5). In this regard, it is suggested that lipid biosynthesis is activated under carbon-rich conditions. Notably, cells cultivated with 6% (*w*/*v*) and 10%(*w*/*v*) of PAO displayed multiple smaller lipid bodies rather than a single, larger lipid body. This phenomenon may also be associated with the development of peroxisomes, which are typically induced when the yeast is grown in an environment or medium containing high fatty acids or alkanes [40,41]. The fatty acids from the PAO are taken up by the cells and metabolized into acetyl-CoA, leading to intracellular lipid synthesis within the respective peroxisomes formed. Comparing lipid accumulation across the sample in Figure 5, an increase in fluorescence intensity is observed with higher PAO concentrations. Thus, this suggests that when more carbon sources are available for the cells, cellular uptake and subsequent lipid synthesis are elevated, contributing to higher lipid accumulation.

Gene expression analysis was performed to investigate the potential association between the formation of multiple lipid bodies and peroxisomal function. The housekeeping gene *TUB1* was selected for the reference gene expression analysis. *TUB1* encodes α-tubulin, a structural protein critical for cytoskeletal functions. *TUB1* was chosen due to its stable expression across different conditions in yeast [42]. Peroxisomal biogenesis factors, essential for the assembly and maintenance of peroxisomes, are encoded by *PEX* genes. Among all the genes, the *PEX14* gene, which encodes a peroxisomal membrane protein, was analyzed and discussed. *PEX14* is an essential component of the peroxisomal membrane and serves as a docking site for *PEX5*, the receptor responsible for importing cargo proteins into the peroxisome [43]. As illustrated in Figure 6, the expression of *PEX14* increases with a higher concentration of PAO. In the absence of PAO as a carbon source, the relative expression of *PEX14* was 5.3, while at a high concentration of 10% (*w*/*v*) PAO, the expression significantly increased to 52.7. These results suggested that a higher concentration of PAO may induce the upregulation of the *PEX14* gene, potentially resulting in increased formation of peroxisomes within the cell. Thus, peroxisome formation could explain the presence of multiple lipid bodies in cells cultured with high PAO concentrations.

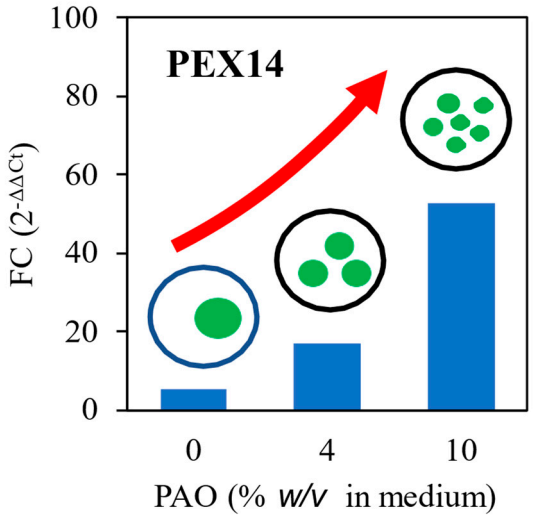


Figure 6. Expression of *PEX14* gene in *M. guilliermondii* PK01 and the hypothetical relationship between the concentration of PAO and the formation of multiple lipid bodies. FC: fold change in *PEX14* expression relative to *TUB1* used as a housekeeping gene.

3.4. Analysis of Fatty Acid Composition

The lipid extracted from the cells grown in different concentrations of PAO was transmethylated and analyzed by GC-MS. Most lipids produced were long-chain unsaturated fatty acids containing 16-carbon and 18-carbon fatty acid derivatives. Identified fatty acids include palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) (Figure 7). The observed fatty acid composition closely resembles that of plant-derived oils, specifically that of palm oil [44].

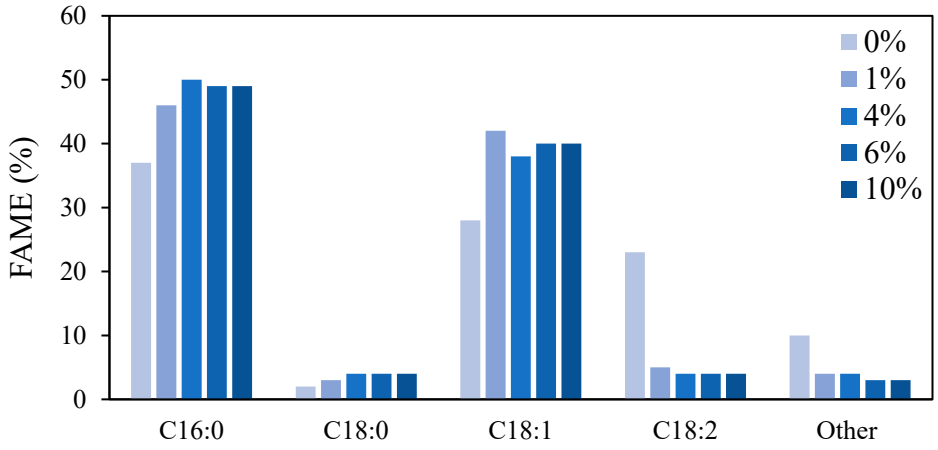


Figure 7. The composition of fatty acids (FAME, methyl ester form) produced in the *M. guilliermondii* PK01 grown in YP medium containing PAO (%, *w*/*v*) at 0, 1, 4, 6, and 10.

There are notable differences between the composition of fatty acids and lipids produced by *M. guilliermondii* when cultivated in the presence or absence of a carbon source. In general, the fatty acid composition produced in the yeast cells is highly dependent on the medium of cultivation and fermentation conditions [45]. The type of carbon source used contributes to differing fatty acid profiles by activating specific metabolic pathways and key genes involved in lipid biosynthesis [46]. This is likely the primary factor contributing to the observed differences in fatty acid profiles when *M. guilliermondii* is grown with or without PAO, as well as when glucose is used as the feedstock (Figure S1), where the fatty acid compositions of the lipid are almost similar to the lipid produced in the presence of PAO.

Among the fatty acids detected, C16:0 and C18:1 together accounted for more than 80% of the total fatty acids content. Fatty acid composition of palm oil consisted of approximately 35.8% of C16:0 and 43.8% of C18:1 [38], which closely resembles the fatty acid profile of the lipids produced in this study. Therefore, yeast-derived oil shows potential as a sustainable alternative to plant-derived oil for producing value-added products such as biodiesel. Palm oil has been successfully converted into biodiesel, also referred to as palm biodiesel, or blended with petroleum diesel [47]. Palm biodiesel generally exhibited fuel properties comparable to those of petroleum diesel [48].

The utilization of PAO, a byproduct of the palm oil industry, as a substrate for lipid production offers significant environmental and economic advantages. Valorizing PAO contributes to waste reduction and supports the principles of a circular bioeconomy [49]. By employing this waste material, the need for agricultural resources dedicated to lipid production is minimized, reducing land use and environmental impacts. Unlike plant-based oils, microbial oil production does not require huge land cultivation. Moreover, microbial oils can help mitigate the rising global price of plant-derived oils caused by the competing demand for edible oils and biofuel production [50].

Economically, PAO represents a cost-effective and readily available feedstock compared to refined feedstocks, making it a sustainable alternative. Additionally, the robust yeast *M. guilliermondii* demonstrates shortened fermentation times, potentially reducing production cost. The resulting microbial lipids have broad industrial applications highlighting their market relevance. Although this study did not perform a full life-cycle analysis and cost analysis, future work could quantitatively evaluate the environmental benefits and economic feasibility of this approach. By focusing on the underutilized potential of *M. guilliermondii*, this work advances the understanding of its role as an oleaginous yeast and highlights its potential in waste valorization for sustainable lipid production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr13020311/s1, Figure S1: Fatty acids composition of lipid produced by *Meyerozyma guilliermondii* PK01 grown in YPD supplemented with 50% of glucose as the carbon source. The fermentation was conducted for 4 days; Table S1: Primer list for qPCR analysis. The PCR product of each gene was designed to be 100 bps. TUB1 was used as a housekeeping gene; Table S2: Sequence of genes for qPCR analysis.

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Abbreviations

The following abbreviations are used in this manuscript:

POME	Palm oil mill effluent
PAO	Palm acid oil
COD	Chemical oxygen demand
BOD	Biological oxygen demand
SCO	Single-celled oil
TAGs	Triacylglycerols
FFAs	Free fatty acids

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