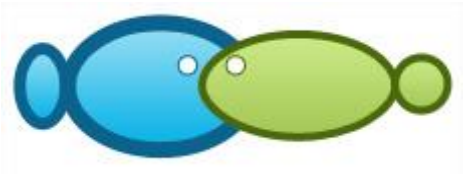

Growth optimization of marine protist *Thraustochytrium multirudimentale* MAST-1 and characterization of fatty acids using FTIR spectroscopy and gas chromatography

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Abstract. Microalgae and marine protists, including thraustochytrids synthesize essential fatty acids in their cells. Hence, they have become important subjects for research exploration. This research aims to investigate the optimum conditions for the growth of marine protist *Thraustochytrium multirudimentale* MAST-1, isolated from mangrove areas in Banda Aceh, Indonesia. Investigation of microbial oil from this strain has rarely been studied and reported. The results showed that the best carbon and nitrogen sources were glucose and yeast extract, respectively. By using this medium, the culture growth at 25-26°C showed an exponential phase starting from day 3 until day 6, followed by the stationary phase lasting until day 8. Increasing the optical density (OD) values were in line with the increase of biomass. *T. multirudimentale* grown for 8 days produced a biomass of 7.7 g L^{-1} (dry weight) and high amounts of total lipids (74%). The FTIR spectral analysis revealed that the thraustochytrid strain contained polyunsaturated fatty acids (PUFAs). The GC-MS results confirmed that the majority of unsaturated fatty acids in *T. multirudimentale* oil were oleic and linoleic acids. Further optimization of the growth condition, particularly for the growth at a low temperature and higher C/N ratio are promising areas to be explored in order to improve the strain's ability to accumulate omega-3 PUFAs in their cells.
Key Words: biomass, carbon sources fatty acids, microbial oil, thraustochytrids.

Introduction. Omega-3 polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid (DHA; C22:6) and eicosapentaenoic acid (EPA; C20:5) have been intensively studied for their benefit to human health. These PUFAs proved to have the ability to reduce the risk of Alzheimer's, type 2 diabetes, heart disease, bipolar disorder, and schizophrenia (Qawasmi et al 2013). Common sources of omega-3 PUFAs are marine fish and seafood. However, the issues of high demand and low supply, overfishing, as well as mercury contamination have forced researchers to look for new sources of omega-3 fatty acids. Moreover, current research reported that the availability of DHA in fish will be reduced by 10-58% by 2100 due to global warming (Schade et al 2020).

In recent years, oleaginous microorganisms, such as bacteria, fungi, and microalgae have been explored as alternative sources of omega-3 fatty acids. Biotechnology and genetic engineering have brought these microorganisms into industrial scale production of microbial oils. One of the first oleaginous microorganism to be cultivated for the commercial production of GLA-rich oil was *Mucor circinelloides* (Ratledge & Lippmeier 2017). Microalgae have been the subjects of studies in the laboratory and have also been scaled up to an industrial scale production in many countries. However, their dependence on photoautotrophic growth conditions limits their productivity. *Crypthecodinium cohnii* is an example of microalga able to synthesize PUFAs under heterotrophic conditions and proved to be a potential commercial DHA producer. In order to minimize the dependency on the photoautotrophic growth conditions, researchers are now attempting to find other microbial strains, which can be cultivated under heterotrophic conditions, but at the same time exhibit higher biomass and lipid productivity. This is part of some efforts to identify other

microbial candidates, which are able to be cultivated at low costs and based on renewable organic carbon sources (Patel et al 2021). Marine protists appear as potential candidates for commercial scale producers of single-cells oils containing EPA and DHA (Raghukumar 2008; Leyland et al 2017; Xiao 2018).

Marine protists, microalgae, fungi, bacilli, and yeasts are examples of marine microorganisms that can be found in mangrove ecosystems, particularly in mangrove plants, water, leaves and sediments. These microorganisms can act as a buffer zone, biogeochemically recycling marine waste into nutrient-rich depositions for marine and terrestrial species. They play an important ecological role in mangroves by decomposing decaying matter and aiding nutrient cycling (Morabito et al 2019). They also produce significant amounts of biomass, from which essential oils containing PUFAs can be extracted. Marine protists such as thraustochytrids were studied in many researches because they are capable of accumulating high amounts of lipids (Nham Tran et al 2020).

Thraustochytrids are non-photosynthetic marine microorganisms (Patel et al 2021), which are a heterotrophic fungus-like clade of the Stramenopiles group. Thraustochytrids are heterotrophic protists from the Labyrinthulomycetes class, but they have lost their plastids through evolution (Leyland et al 2017). There are four main genera of thraustochytrids well known for their commercial potential, namely *Aurantiochytrium*, *Schizotrichium*, *Thraustrochytrium*, and *Ulkenia*. These thraustochytrids are explored because of their high lipid content and particularly notable high DHA content (Chang et al 2014). It is reported that the life stages of thraustochytrids are highly nutritional dependent, and with cultivation in artificial media containing yeast extract and peptone, they can produce high amounts of biomass and accumulate a high amount of lipids (Morabito et al 2019). However, the lipid production is dependent on the medium composition, particularly the C:N ratio. Carbon is needed by heterotrophic cells to synthesize fatty acids and lipids. The limitation of nitrogen can cause the absence of cell division and redirect the triglycerides (TAG) production (Simionato et al 2013).

Isolation and identification of thraustochytrids has been carried out worldwide and the results are sometimes specific according to the region and climate where they were isolated. In Aceh Province, Indonesia, isolation of microorganisms from mangrove leaves and sediments had morphologically identified two strains (Anwar et al 2013). One of the isolates that has been identified genetically is *Thraustochytrium multirudimentale* MAST-1 (Anwar et al 2021). Investigation of microbial oil from *T. multirudimentale* has rarely been reported in the literature.

Several methods have been reported for extracting oil from microorganisms, each with advantages and limitations. The extraction methods include osmotic shock, pulsed electric field technology (PEF), enzymatic extractions, ultrasonic-assisted extraction, supercritical fluid extraction, homogenization, mechanical pressing and solvent extraction (Mercer & Armenta 2011). The principle of the solvent extraction method is the degradation of microorganism cell walls and extraction of oil based on oil solubility in organic solvent. Some effective organic solvents are benzene, cyclohexane, hexane, acetone and chloroform. Hexane is reported as the most common solvent used to extract microbial oil and it is also efficient and cheap. To increase extraction efficiency, the solvent must fully penetrate into the biomass and match the polarity of the targeted compound (Cooney et al 2009).

The characterization of microbial oil can be examined using FTIR spectroscopy. This technology works in an advanced, but non-destructive method reported to be rapid and less expensive to determine the fatty acid profile and peroxide value (PV) of oil (Maggio et al 2009). Basic principle behind spectroscopy assists the understanding of how the infrared (IR) technology can be applied in a fatty acids analysis. IR spectra are produced when molecules absorb IR radiation and the changes in absorption are recorded. The molecules undergo mechanical motions, mainly vibrational and rotational modes, due to the absorption of energy (Guillen & Cabo 1997). In this research, the examination of fatty acids composition in microbial oil was also evaluated by another analytical method using Gas Chromatography-Mass Spectrometry (GC-MS). This research aimed to optimize the growth of *T. multirudimentale* MAST-1 based on the carbon and nitrogen sources, with the biomass and the microbial oil produced being examined.

Materials and Method

Materials. The research materials used were: the axenic culture of *T. multirudimentale* MAST-1, natural sea water, distilled water, yeast extract-peptone-glucose-seawater (YPGS) media (consist of yeast extract (Merck), mycological peptone (Sigma), glucose (Difco), and bacto agar (Difco), microbiological agar (Oxoid), ammonium nitrate, monosodium glutamate, antibiotics (penicillin and streptomycin), Lugol staining, FAME standards for GC (FAME Mix C8-C24), methyl tricosanoate in toluene, fatty acid standards for FTIR (cis-4,7,10,13,16,19-DHA methyl ester ≥98%, cis-5,8,11,14,17-eicosapentaenoic acid ≥85%, liquid), butylated hydroxytoluene (BHT), boron trifluoride, methanol, chloroform, and hexane.

Experimental methods. The experiments were divided into five stages: 1) the growth refreshment of the *T. multirudimentale* MAST-1 on solid media; 2) the optimization growth based on different nitrogen sources (yeast extract, sodium nitrate and monosodium glutamate); 3) the optimization growth based on different carbon sources (glucose and lactose); 4) the oil extraction from dried biomass; and 5) the characterization of the fatty acids using Fourier Transform Infrared (FTIR) spectroscopy and GC-MS.

Inoculum preparation and optimization of nitrogen sources. Inoculum preparations for starter culture, yeast extract-peptone-glucose (YEPG) liquid media and culture cultivation in an orbital shaker followed the procedure from our previous investigation (Anwar et al 2022). The optimization of nitrogen sources was conducted according to the method written in Anwar et al (2018). The YEPG added with 1.5% microbiological agar was then used to refresh the isolate, observe the colony as well as the cell morphology of *T. multirudimentale* MAST-1 in order to make sure that the isolate was axenic.

Optimization of media compositions. In the second step of this research, the medium was optimized based on nitrogen sources, which were yeast extract, sodium nitrate and monosodium glutamate. The medium preparation followed the determination made by Fan et al (2002), with 10 g nitrogen source, 1 g peptone and 10 g glucose in 1 L of 15‰ natural seawater. In the third step of this research, the medium was optimized based on the carbon sources, which were glucose and lactose. The medium used for the growth of *T. multirudimentale* MAST-1 consisted of 1 g nitrogen source, 15 g peptone and 20 g carbon source glucose in 1 L of 15‰ natural seawater (Vongsvivut et al 2013). In each case, the culture was incubated for 8 days, in an orbital shaker with a speed of 130 rpm, at temperature of ±25-26°C.

Analysis of cell growth. Measurement of the biomass was carried out by measuring the turbidity of culture suspension according to Li et al (2008). The culture medium (1.5 mL) was collected from each flask of growth from nitrogen source experiments, every day during the incubation period (8 days), and the absorbance was measured at 600 nm. The OD value accepted was ≤0.4, because if the OD value is too high, then it does not correlate well with cell densities. The values that were ≥0.4 (OD_{undil}) were diluted at first and the absorbances were read as OD_{dil}. The value of OD_{dil} was then divided by its dilution factor and written as OD_{corr}. The final OD of microalgae cells' densities was recorded as the mean of the OD_{corr}.

Biomass collection and drying. The biomass was collected by the centrifugation method. Every 10 mL of the culture medium, which contained biomass, were placed into the centrifugation tube and centrifuged for 10 minutes at 5000 rpm. If the supernatant was cloudy, then centrifugation was repeated. Once the supernatant was clear, it was separated from the microalgae biomass (as sediment in the tube). The wet biomass was weighed and dried in the oven at 40°C for 12 hours. The dried biomass was collected and weighed.

Oil extraction from dried biomass. Oil extraction from dried biomass was conducted with the solvent extraction method. Briefly, the dried biomass (1 g) was wrapped carefully in a Whatman filter paper and place into the Soxhlet tube. Hexane was used as solvent and extraction was carried out for 6 hours. The solvent was then separated from the oil using a rotary evaporator at 50°C. The microbial oil was collected and weighed.

Characterization of fatty acids in microbial oil using FTIR spectroscopy. All spectral analyses were performed with a FTIR spectrometer (IRPrestige-21 Shimadzu, Japan) equipped with a horizontal type attenuated total reflectance (ATR) sampling accessory with a zinc selenide (ZnSe) prism. FTIR spectra were acquired at 32 scans, resolution of 4 cm^{-1} and region of 4000–600 cm^{-1}, at ambient temperature. A few mL of sample was placed on the ATR surface, then the spectra of samples were subtracted against the background of air spectrum. The ATR plate was carefully cleaned using soft tissue soaked in 70% of ethanol to remove any residues. All spectra were recorded as absorbance units at each data point in triplicate.

Fatty acids analysis using gas chromatography. Sample preparation for esterification followed the AOAC Official Method 991.39. The conversion of carboxylic acids into ester began with mixing 25 mg of microalgae oil into a glass tube, then 1-2 mg of margaric acid (C17) internal standard was added, followed by an addition of a mixture of NaOH and methanol. The tube was closed tightly, vortexed for 10 min and heated in a water bath at 80°C for 5 min followed by addition of BF_3 in methanol and vortexing was continued. The mixture once again was heated in a water bath at 80°C for 25 min. Into the tube, hexane was added then vortexed. Saturated NaCl was also added, vortexing continued for 1 min. The mixture formed two layers in which the upper part (hexane phase) was separated and transferred into a clean bottle, stored at -25°C and ready for GC injection.

GC analysis was conducted using GC with Flame Ionization Detector (FID) (Agilent Technology, USA) with capillary column (J&W Agilent DB 23, 30 m x 0.25 mm x 0.25 µm). Typical run conditions were: injector temperature of 250°C, detector 260°C, hydrogen carrier gas at 2.5 mL min^{-1}. The initial oven temperature was set at 120°C and held for 6 min, increased to 230°C and held for 25 min.

Data analysis. All measurements were carried out in duplicate, and, from these results, mean values and standard deviation were calculated. The quantification of microbial oil was calculated gravimetrically.

Results

Optimization of nitrogen sources. Nitrogen is considered to be one of the main essential nutrients for thraustochytrids growth and is consumed in the form of nitrate, nitrite, ammonia and urea (Selmani et al 2013). Figure 1 shows the distinct differences in the growth of *T. multirudimentale* MAST-1. When yeast extract was used as a nitrogen source, the OD values increased significantly from day 0 up to day 4. However, the OD values only rose slightly if sodium nitrate and MSG were added in the culture medium. The grey straight line from day 0 to day 5 informed that MSG was least favored by *T. multirudimentale* MAST-1 during its cultivation.

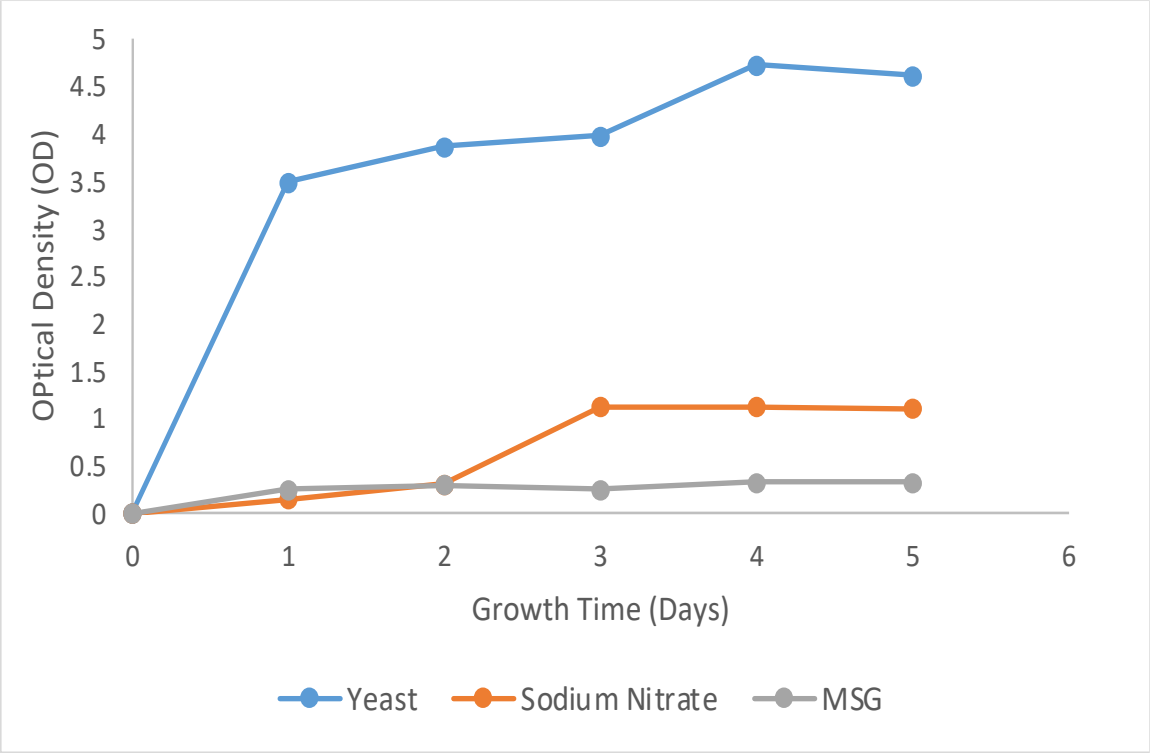

Figure 1. Growth curves of *Thraustochytrium multirudimentale* MAST-1 in 100 mL flask shaker incubator with three different nitrogen sources.

Optimization of carbon sources. The results indicate that when glucose was used as the carbon source, the OD number increased and was in line with the increasing weight of dried biomass (Figure 2a). However, a different trend was observed for the strain growth using lactose. The OD number and weight of dried biomass reached maximum at day 5 then started to decrease (Figure 2b). The OD numbers were also much higher in glucose medium than in lactose medium.

Oil extraction from biomass. The accumulation of wet biomass of *T. multirudimentale* was dried and the oil was extracted from the dried biomass using solvent. Due to low productivity of the strain grown in medium containing lactose, the *T. multirudimentale* oil was extracted only from the biomass obtained from the strain that grew in the glucose medium. The total lipids obtained were 5.7 g L^{-1} of liquid culture or 74% lipids in dry biomass.

FTIR spectra of microbial oils. For this experiment, we examined the *T. multirudimentale* oil as well as a commercial sample of menhaden (clupeiformes) fish oil. Fish oil was chosen because it is well known for its composition of PUFAs such as ALA, EPA and DHA. The FTIR spectra of *T. multirudimentale* and fish oils is in the range of 2800 – 3050 cm^{-1}. The peak representing PUFA was observed at 3018 cm^{-1} on the fish oil spectrum as the C-H stretching band of cis-olefinic group =CH- due to its high degree of PUFA, but this peak did not appear on *T. multirudimentale* oil spectra (Figures 3 and 4).

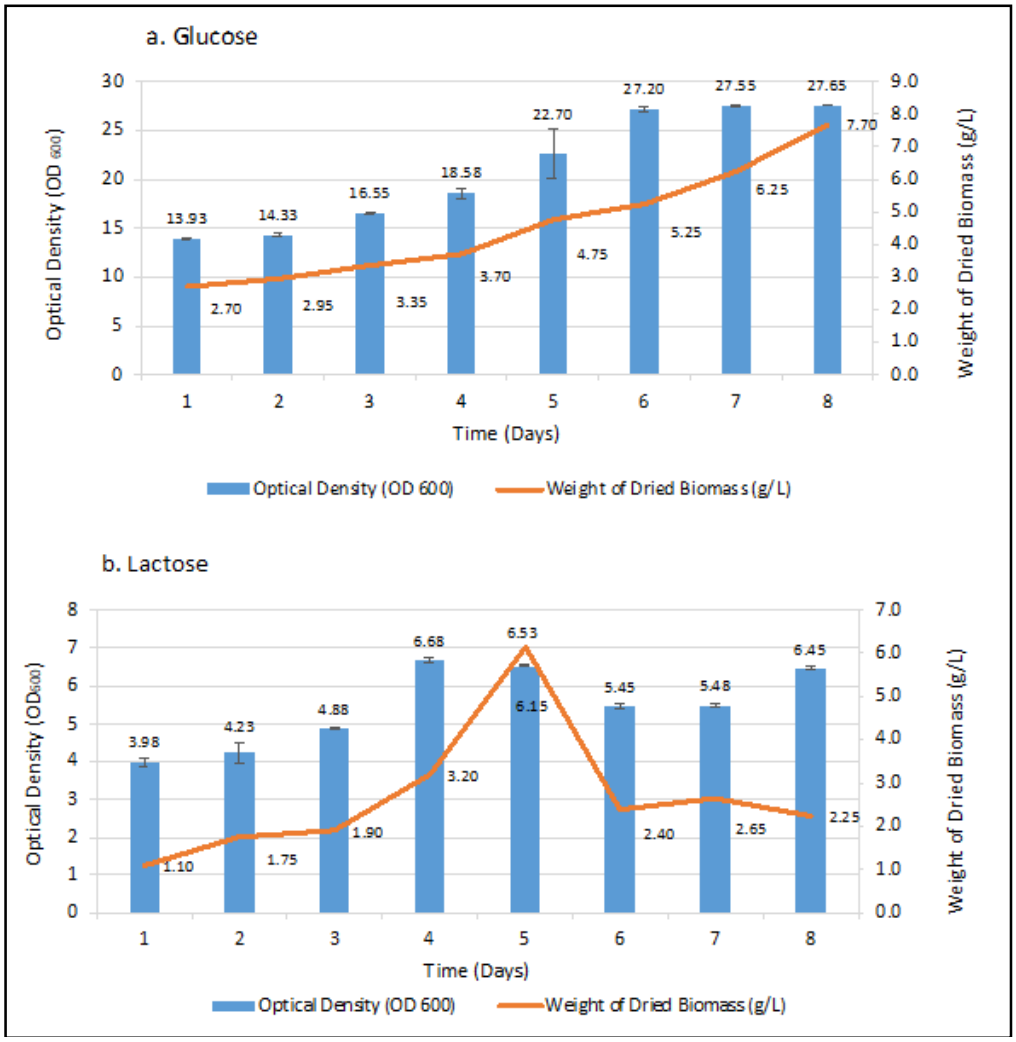

Figure 2. Growth curves of *Thraustochytrium multirudimentale* MAST-1 incubated in 100 mL flask shaker incubator with yeast extract as nitrogen source and: a) glucose as carbon source, b) lactose as carbon source.

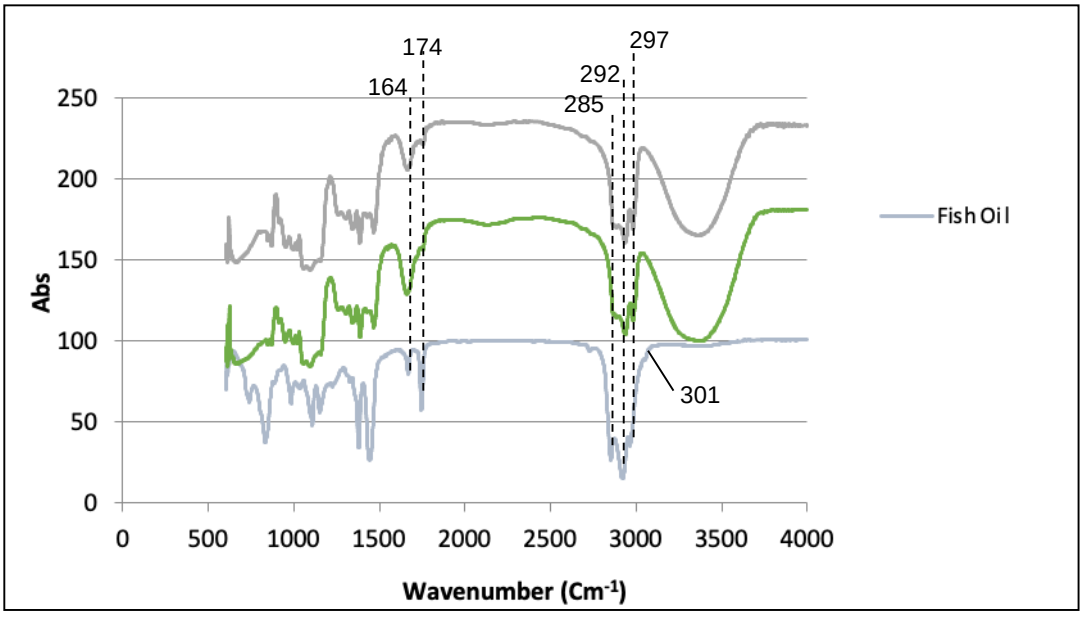

Figure 3. The FTIR spectra of *Thraustochytrium multirudimentale* oil and fish oil.

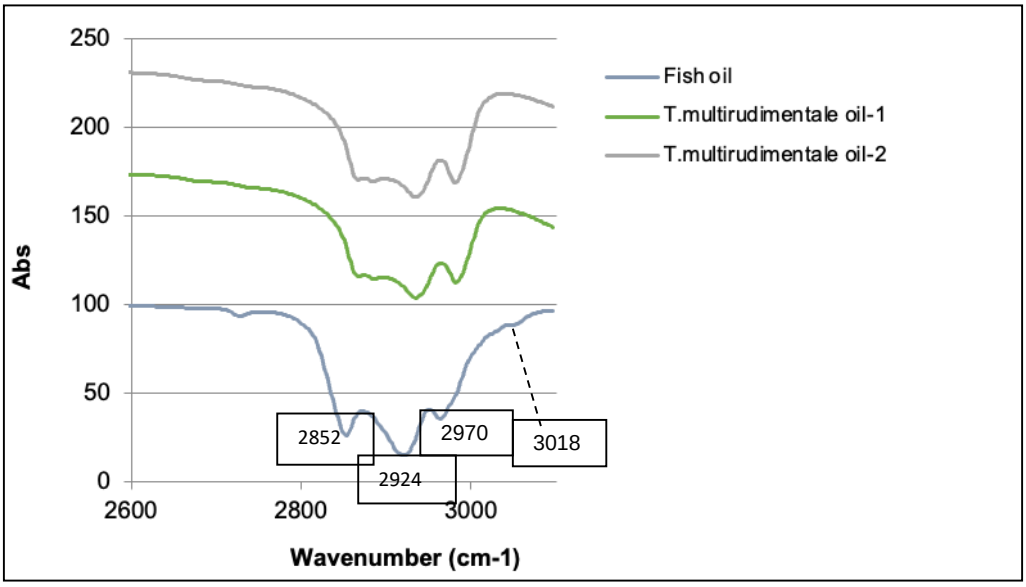

Figure 4. FTIR spectra of *Thraustochytrium multirudimentale* oil and fish oil at C-H stretching region of lipids.

Fatty acid profiling of microbial oil. Based on GC analysis, the result showed that the thraustochytrid oil contains several fatty acids, as listed in Table 1.

Table 1

The profile of fatty acids of *Thraustochytrium multirudimentale* MAST-1

No	*Systematic name*	*Trivial name*	*Lipid numbers*	*Amount (mg FA 100 g^{-1})*
1	Hexadecanoic acid	Palmitic acid	16:0	339.14
2	Octadecanoic acid	Stearic acid	18:0	96.28
3	9-hexadecenoic acid	Palmitoleic acid	16:1	41.98
4	9-octadecenoic acid	Oleic acid	18:1	471.02
5	9,12-octadecadienoic acid	Linoleic acid	18:2	157.32

Discussion. Figure 1 shows that the growth of *T. multirudimentale* MAST-1 with yeast extract was higher than that in sodium nitrate and MSG. This can also be seen from the broth turbidity, where broth with MSG as nitrogen source was less cloudy indicating that the growth of *T. multirudimentale* MAST-1 was not optimal yet. Compared to our previous research (Anwar et al 2018), the use of MSG was similar to that of yeast extract. However, in this study, the growth increased significantly when the yeast extract was used, slightly increased when sodium nitrate was used, and was very low when MSG was used, remaining stable. The low absorbance in MSG at day 5 incubation was expected because the ability of the strain to absorb nitrogen from MSG was very low at this time. According to Li et al (2008), marine protists such as microalgae use nitrogen to form chlorophyl until the nitrogen is diminished. By this time, microalga start to convert nitrogen-rich compounds to become proteins, nucleic acids, and cell wall, which will help the cell growth. Chlorophyl is an important component involved in photosynthesis process and functions as an absorber of light and CO_2 and finally helps cell growth. Thus, the faster nitrogen is absorbed, the better the growth of the microorganisms is. This study utilized complex nitrogen i.e., yeast, which had multiple benefits, not only as a nitrogen source but also to provide proteins, free nucleic acids, sugars, fat and vitamins. Yeast consists of many essential nutrients for microbial growth (Tao et al 2023). Although the effect of various nitrogen sources is different across different species, it was reported that MSG and yeast were the best

nitrogen sources for the Thraustochytriaceae family to produce dried biomass (Chen et al 2010).

Figure 2 shows the growth of *T. multirudimentale* MAST-1 using different carbon sources (glucose and lactose). Glucose is reported to be the best and most commonly used carbon source for thraustochytrid growth in heterotrophic cultures. This is because glucose possesses more energy content per mol (2.8 kJ mol^{-1} of energy) compared with other substrates such as acetate, which has only 0.8 kJ mol^{-1} (Boyle & Morgan 2009). Glucose promotes physiological changes in certain species of microalgae. These changes strongly affect the metabolic pathways of carbon assimilation, volume densities of storage materials (such as starch, lipids, protein, chlorophyll and vitamin), as well as the size of the cells (Martinez et al 1991).

Production of biomass in this study was lower when glucose was replaced by lactose. These occurrences were also observed by many others. Nagano et al (2009) used various carbon sources for the growth of a marine thraustochytrid *Aurantiochytrium limacinum* strain mh0186. Measurement of the cells' growth proved that when glucose was used, the OD660 was 2.8. The replacement of lactose decreased the cell growth and the OD660 was only 0.8. Lactose, sucrose, lactate, and ethanol have also been tested under heterotrophic microalgae cultures with negative results in growth and metabolite production (Wang & Peng 2008). In another study, the growth of three different species of marine microalgae, *Nannochloropsis salina*, *Dunaliella tertiolecta* and *Tetraselmis suecica* were examined using glucose, fructose, sucrose, lactose and galactose. Among the carbon sources tested, these species showed a higher growth and higher lipid production in the order of glucose, sucrose, fructose, galactose and lactose treated culture (Velu et al 2015). Lactose is a disaccharide which must be hydrolyzed into galactose and glucose before it is ready to be assimilated by microorganisms. Some microalgae do not have invertase to assimilate disaccharides (Perez-Garcia et al 2011). It was reported that disaccharides connected to carbon 1 or carbon 4 are not transported and, as a consequence, the disaccharides uptake is poor (Komor et al 1985). Nevertheless, the carbon sources uptake during microbial growth can be different depending on the type of microorganism that utilizes them and can also be determined by the enzymatic complexes (Fortes 2016).

The percentage of lipids in this study was higher than our earlier research (Anwar et al 2022) and higher than in various other studies: 20.3% in *Thraustochytrium aureum* ATCC 34304 (Bajpai et al 1991), 25% in *Thraustochytrium roseum* ATCC 28210 (Singh & Ward 1997), and 57.8% in thraustochytrid strain 12B (Perveen et al 2006). The highest result of 81.7% total lipids was reported by Burja et al (2006), who extracted the biomass from *Thraustochytrium striatum* ONC-T18. The production of oil is associated with the presence of nitrogen in the medium. Lipid production in the cells of the microorganism is correlated well with nutrient availability, particularly nitrogen (in the form of an ammonium salt) and a surfeit of carbon contained in the medium growth (Wynn & Ratledge 2005). The lipid accumulation in microbial cells starts once the nitrogen availability is exhausted in the first 24 hours of growth (Figure 5).

Figure 5 explains how the cells are stimulated to produce lipids, according to Wynn & Ratledge (2005). It shows the relationship between nutrient availability in culture media with the formation of biomass and lipids during cultivation time (hours). As a consequence of the nutrient exhaustion, the cells continue to take up the sugar present in the medium, but they can no longer grow and multiply. This surplus sugar becomes the source of carbon for lipid biosynthesis. Absorption of glucose occurs over a longer period of time (70 h) while the nitrogen is absorbed faster and eventually finishes at 20 h of cultivation. Biomass and lipid production are stable for the first 20 h and then start to increase significantly afterward. At the time when nitrogen in the cultivation media is over then the cells start to accumulate lipids. The carbon supply, which is commonly in the form of glucose, also starts to decrease. Depletion of nutrients in liquid media causes discontinuation of cell growth and division. At this time, the conversion of glucose into lipids takes place. Although nitrogen is diminished, the cells are still able to absorb glucose. These situations stimulate lipid biosynthesis in the cells of the microorganism. Li et al (2008) underlined that when the external nitrogen in media ceased, then nitrogen rich substances are converted into

protein, nucleic acids, and cell walls, which can help the growth process. In addition, the biomass concentration is also increased.

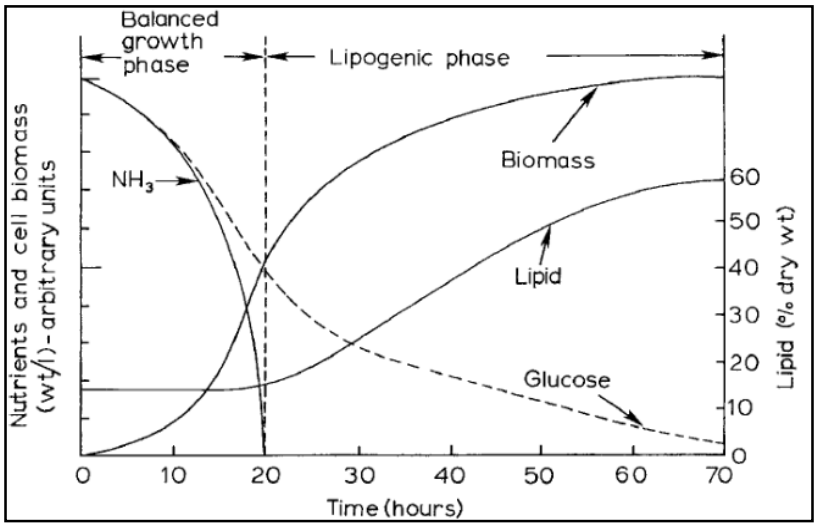

Figure 5. Nutrient availability during cultivation of oleaginous microorganisms according to Wynn & Ratledge (2005).

Medium infrared spectroscopy has been used to identify organic compounds because some functional groups of atoms in material show vibrational characteristics in the infrared region. Polyunsaturated fatty acids (PUFA) have been identified in some regions of infrared around 3000 cm^{-1} characterized by the C-H stretching vibration (Kiefer et al 2010). This is in agreement with the results of this study where the FTIR spectra of *T. multirudimentale* and fish oils are in the range of 2800–3050 cm^{-1}, which characterize C-H stretching of lipids (-CH_2, -CH_3). The bands at 2970 cm^{-1} were attributable to asymmetric C–H stretching vibrations of –CH_3 and 2924 and 2852 cm^{-1} were asymmetric/symmetric aliphatic –CH_2 functional groups of lipids, indicating that *T. multirudimentale* oil contains PUFA. Maurer (2012) used spectral differences at 3010 cm^{-1} (cis olefinic =CH double bonds), 2950 and 2845 cm^{-1} (asymmetric/symmetric –CH2 stretching) to show the degree of PUFA in flax and sacha inchi oils. The peak at 1745 cm^{-1} showed different intensity of the band that is assignable to C=O stretching of ester groups from triglycerides and fatty acids, and represents total lipid. The intensity suggested that *T. multirudimentale* oil contains lower amounts of total lipids than fish oil.

The profile of fatty acids of *T. multirudimentale* MAST-1 (Table 1) shows that unsaturated fatty acids were more dominant than saturated fatty acids with different quantities. The quantities of fatty acids are as follows: oleic acid (471.02 mg 100 g^{-1}), palmitic acid (339.14 mg 100 g^{-1}), linoleic acid (157.32 mg 100 g^{-1}), stearic acid (96.28 mg 100 g^{-1}) and palmitoleic acid (41.98 mg 100 g^{-1}). The unsaturated fatty acids with double bonds that were found were palmitoleic acid (16:1ω–7), oleic acid (18:1ω–9) and linoleic acid (18:2ω–6). Other polyunsaturated fatty acids such as linolenic acid, EPA, and DHA were not detected. The reasons could be related to the condition of the culture growth, mainly the percentage of carbon used, the carbon/nitrogen ratio, the pH and temperature. The most important parameter that determines the profile of fatty acid in thraustochytrids is temperature (Caamaño et al 2017). This research optimized the growth of *T. multirudimentale* at ±25-26°C. According to the literature, at this temperature range, production of polyunsaturated fatty acids such as linolenic acid, EPA, and DHA are limited or very low, but the biomass production is high. This could be the reason for the profile of fatty acids identified in this study. Caamaño et al (2017) investigated the growth of *Thraustochytrium kinnei* at 25°C and found that the species produced more biomass than at 10°C. They also proved that an increase in temperature produced an increase of stearic acid (C18:0) and at the same time decreased the total content of unsaturated fatty acids. 10°C was the most favorable condition for the synthesis of unsaturated fatty acids,

including omega-3 fatty acid such as DHA and EPA, but minor biomass (Singh & Ward 1997). At high temperatures, the carbon is not completely used, but the nitrogen is diminished, and the biomass production is maximum. In addition, at 25°C, the enzyme Δ9-fatty acid desaturase, which determines the transformation of stearic acid into oleic acid, decreased its activity (Caamaño et al 2017).

Fatty acid composition is this study is similar with that in previous studies. Irmak & Arzu (2020) reported that the highest content of fatty acids found in microalgae were for palmitic and oleic acids. Fathurohman et al (2021) and Mir et al (2020) also highlighted that the highest amount of fatty acid present in microalgae was for oleic acid. Differences among these studies can be influenced by several factors, such as the species of thraustochytrids, genotype of the microorganisms, culture conditions or substrate used (Ratledge & Lippmeier 2017). It is important to note that it is possible for *T. multirudimentale* to produce omega-3 PUFAs via modification of the culture conditions and this will be our focus for future work.

Conclusions. The growth of *T. multirudimentale* strain MAST-1 has been optimized using glucose as the carbon source and the yeast extract as the nitrogen source, at temperature of ±25-26°C. At this condition, it produced 7.7 g L^{-1} of dry biomass and a high amount of total lipids (74%). The FTIR spectra revealed that the thraustochytrid oil contained polyunsaturated fatty acids (PUFAs). The PUFA detected were dominated by oleic acid (471.02 mg FA 100 g^{-1}) and linoleic acid (157.32 mg FA 100 g^{-1}). This study suggests that *T. multirudimentale* MAST-1 is a promising strain to produce essential oil and further optimization of the growth conditions is needed.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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