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Circular economy approach to fatty acid production using aurantiochytrium microalgae and industrial wastes

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ABSTRACT

This study investigates the sustainable production of fatty acids, specifically docosahexaenoic acid (DHA omega-3), which is essential for the nutrition, cosmetics, and pharmaceutical sectors. The research investigation evaluates the potential of utilizing low-cost substrates in a circular economy framework, employing Aurantiochytrium microalgae, a species recognized for its elevated DHA content and lack of heavy metal contamination. The cultivation process employed three substrates: glycerol, molasses, and fruit waste. The microalgae were cultivated on these substrates, subsequently undergoing sonication to improve emulsion stability. Fatty acid profiles were analyzed using GC-MS to assess DHA yields and the efficiency of biomass production. The findings suggested that glycerol served as the most effective substrate, producing the highest DHA content (54.88%) and wet biomass (53 g). Molasses and fruit waste exhibited moderate efficiency, presenting viable and cost-effective alternatives. Furthermore, glycerol yielded the most uniform emulsion particles (1,874 nm, PI 0.02677), suggesting enhanced substrate compatibility. The findings highlight the capability of Aurantiochytrium-based bioprocesses for the sustainable production of high-value fatty acids. This approach leverages industrial and organic waste materials, enhancing environmental sustainability and economic viability while fostering innovation in microalgae biotechnology.

Keywords: Aurantiochytrium, circular economy, fatty acids, microalgae, omega-3.

INTRODUCTION

Fatty acids have long been recognized as an essential source of nutrition, crucial for the health of both humans and other living organisms (Behrens & Kyle, 1996; Harayama & Shimizu, 2020). These compounds play vital roles in supporting critical bodily functions, including heart, brain, and immune system health (Sakhi Ghelichi et al., 2021). As awareness of their benefits grows, the demand for essential fatty acids, particularly in the food, health, and animal feed sectors, continues to rise. Additionally, industrial trends indicate increasing demand for fatty acid-based products in pharmaceutical, cosmetic, and bioenergy applications, solidifying their status as economically valuable commodities (Shahidi & Ambigaipalan, 2018).

One of the most well-known fatty acids is omega-3, specifically docosahexaenoic acid (DHA), which is essential for human health as it cannot be synthesized by the body and must be obtained from dietary intake. However, the current primary source of DHA, derived mostly from fish oil, faces

significant challenges in terms of sustainability and environmental impact. Overfishing threatens marine ecosystems, while marine pollution—contaminated with heavy metals and microplastics—further compromises the quality of fish oil as a DHA source. These issues underscore the urgent need for more sustainable and environmentally friendly alternatives (Ji & Huang, 2019; Oliver et al., 2020; Patel, Karageorgou, et al., 2021).

A promising solution lies in microbial biotechnology, particularly through bioprocessing marine microalgae such as Aurantiochytrium, a species known for its high omega-3 content (Alhattab & Puri, 2024). This microalga, commonly isolated from mangrove ecosystems, has been proven to be a rich source of DHA, free from heavy metal and microplastic contamination(Swetha & Mathanghi, 2024). As the country with the largest mangrove ecosystems in the world, Indonesia holds significant potential for leveraging Aurantiochytrium as a sustainable source of high-value fatty acids.

Previous studies have demonstrated the efficacy of Aurantiochytrium as an efficient producer of DHA (Russo, Langellotti, Sacchi, et al., 2022). However, its exploration in Indonesia remains limited, despite the country's extensive mangrove biodiversity. This gap highlights the need for further research to uncover the potential of Aurantiochytrium in Indonesia and to develop technologies that utilize this resource effectively, thereby promoting local biodiversity and its applications.

One of the major challenges in large-scale production of this microalga is the high operational cost, particularly for cultivation substrates (Russo, Langellotti, Verardo, et al., 2022). Addressing this challenge requires a low-cost substrate approach, such as the use of fruit waste or industrial organic waste (Nazir, Halim, et al., 2020; Patel, Sarkar, et al., 2021). This strategy not only reduces production costs but also aligns with the principles of a circular economy, utilizing waste as a valuable input. Such an approach has the potential to generate economic value for local communities and regions by optimizing available resources.

For these reasons, this study aims to explore the sustainable production of economically valuable fatty acids using cost-effective substrates. This approach seeks to support the implementation of a circular economy that leverages local biodiversity while generating significant economic benefits for coastal communities in Indonesia. This paper provides a novel initiative for developing sustainable, locally adapted microalgae-based technologies.

METHODOLOGY

Cultivation process

Cultivation was conducted using a pure isolate obtained from mangrove forests in Bunaken, North Sulawesi. The center point of Bunaken Island is located at coordinates 1°36'59.99" N and 124°44'59.99" E. The formulation and the cultivation stagewise used in this study have been verified previously to produce consistent microalgae biomass (Suhendra et al., 2024). Biomass production was carried out in three stages: standing culture (SC), pre-culture (PC), and main culture (MC). The composition of the media varied across the standing culture and pre-culture stages. The formulations of the media are presented in Table 1 and Table 2. The main culture cultivation was performed over four days. The progression of cultivation was observed at the end of the second, third, and fourth days through micrograph observation.

The type of substrate was a primary variable in the cultivation process. The substrates used included glycerol, molasses, and fruit waste. Food-grade glycerol was sourced from PT. Brataco, Indonesia. This product is commonly used for wide application in including food industries and is expected to become readily available in the future as a by-product of the biodiesel industry. Molasses was obtained as a by-product from the sugar industry in Madukismo, Yogyakarta, Indonesia.

Table 1. Substrates formulation for standing culture (SC) and pre-culture (PC)

Substrates	Standing culture (SC)		Pre-culture (PC)	
	Mass (g)	Water (mL)	Mass (g)	Water (mL)
Glucose	0,75	20	1,5	40
Reef salt	0,36	10	0,72	20
Yeast extract	0,25	20	0,5	40

Table 2. Substrates formulation for main culture (MC)

Substrates	Main culture 1 (with glycerol)		Main culture 2 (with molasse)		Main culture 3 (with glycerol)	
	Mass (g)	Water (mL)	Mass (g)	Water (mL)	Mass (g)	Water (mL)
Glycerol	80	400	-	-	-	-
Molasse	-	-	80	400	-	-
Fruit waste	-	-	-	-	80	400
MSG	18	400	18	400	18	400
Reef salt	7.2	200	7.2	200	7.2	200
Total volume		1000		1000		1000

The fruit waste materials consisted of orange peels (50 grams), mango peels (50 grams), and tomatoes (50 grams). The three components were blended together with 50 cc of coconut water and then subjected to sonication. Sonication was performed using a probe sonicator with an ultrasonic frequency of 25 kHz and a power output of 200 W to homogenize the mixture. The amplitude was set at 60 µm, and the sonication duration was 10 minutes with 30-second intervals to prevent overheating. A 500 mL mixture was placed in a borosilicate container, and an active cooling system was utilized to maintain the temperature below 50 °C. During the process, a titanium alloy probe was directly immersed in the mixture to ensure efficient energy transfer, resulting in optimal homogenization. The resulting liquid was subsequently subjected to filtration.

Particle size analysis of emulsion

Particle size analysis was performed using a particle size analyzer (PSA) and the Zeta Potential instrument Malvern Zetasizer Pro (ZSU 3200). This instrument is used to measure the particle size distribution in a suspension. This instrument utilizes the scattering behavior of light when a laser beam passes through a group of particles. The size distribution is calculated by measuring the angles of light scattered by particles of varying sizes.

Fatty acid analysis using GC-MS

The common method for isolating bioactive compounds entails a two-phase process utilizing n-hexane and methanol as solvents (Sulistiawati et al., 2023). However, the components produced are not ideal for extracting DHA. Therefore, this study presents a novel solvent type modification based on multiple prior researches (Bratu et al., 2013; Ichihara & Fukubayashi, 2010; Suhendra et al., 2024; Yi et al., 2014).

The preparation process of biomass analysis begins with the separation of microalgal biomass from the supernatant using centrifugation. The obtained biomass is then rinsed twice with water to ensure sample cleanliness. Subsequently, 1 ml of microalgal culture with a maximum biomass concentration of 10 g/L is taken for analysis. The biomass is resuspended in a preparation solution to ensure optimal conditions for DHA analysis.

The first step involves adding 500 µl of anhydrous methanol to the biomass, which is then transferred to an acid-resistant sealed bottle with a capacity of approximately 15 ml. Next, 2 ml of a freshly prepared mixture of anhydrous methanol and acetyl chloride (10:1 ratio) is added. The sample in the bottle is incubated at 50 °C for 16 hours using a water bath or dry bath. This incubation ensures optimal lipid transesterification, enabling DHA detection.

After incubation, methyl ester extraction is performed by adding 5 ml of n-hexane to the solution. The mixture is shaken using a rotary shaker for 15 minutes to ensure phase separation. The upper layer (organic phase) containing the methyl ester is transferred to a 50 ml round-bottom flask. This process is repeated twice to ensure efficient extraction, and all extracts are collected in the same flask.

The extract is then evaporated using a rotary evaporator at 45 °C for approximately 5 minutes until all n-hexane solvent has evaporated, leaving only residue at the bottom of the flask. This residue is redissolved in 1.5 ml of n-hexane and vortexed for at least 20 seconds to achieve homogenization. The final solution is transferred to a 1.5 ml GC vial for fatty acid profile analysis using Gas Chromatography-Mass Spectrometry (GC-MS).

RESULTS

The resulting biomass

Based on micrograph analysis, the cells of the microalga Aurantiochytrium exhibited a spherical shape, a characteristic feature of this species. Figure 1 shows micrograph of cell growth from aurantiochytrium microalgae. The clearly defined cell morphology indicates that the microalga successfully grew on the various tested substrates, namely glycerol, molasses, and fruit waste. No significant contamination was observed in any of the cultures, suggesting that the fermentation media with these substrates can support the growth of pure microalgal colonies.

Cell diameter analysis revealed an increase in size over time across all three substrates. On glycerol substrate, the average cell diameter increased from approximately 3.14 µm on day 2 to

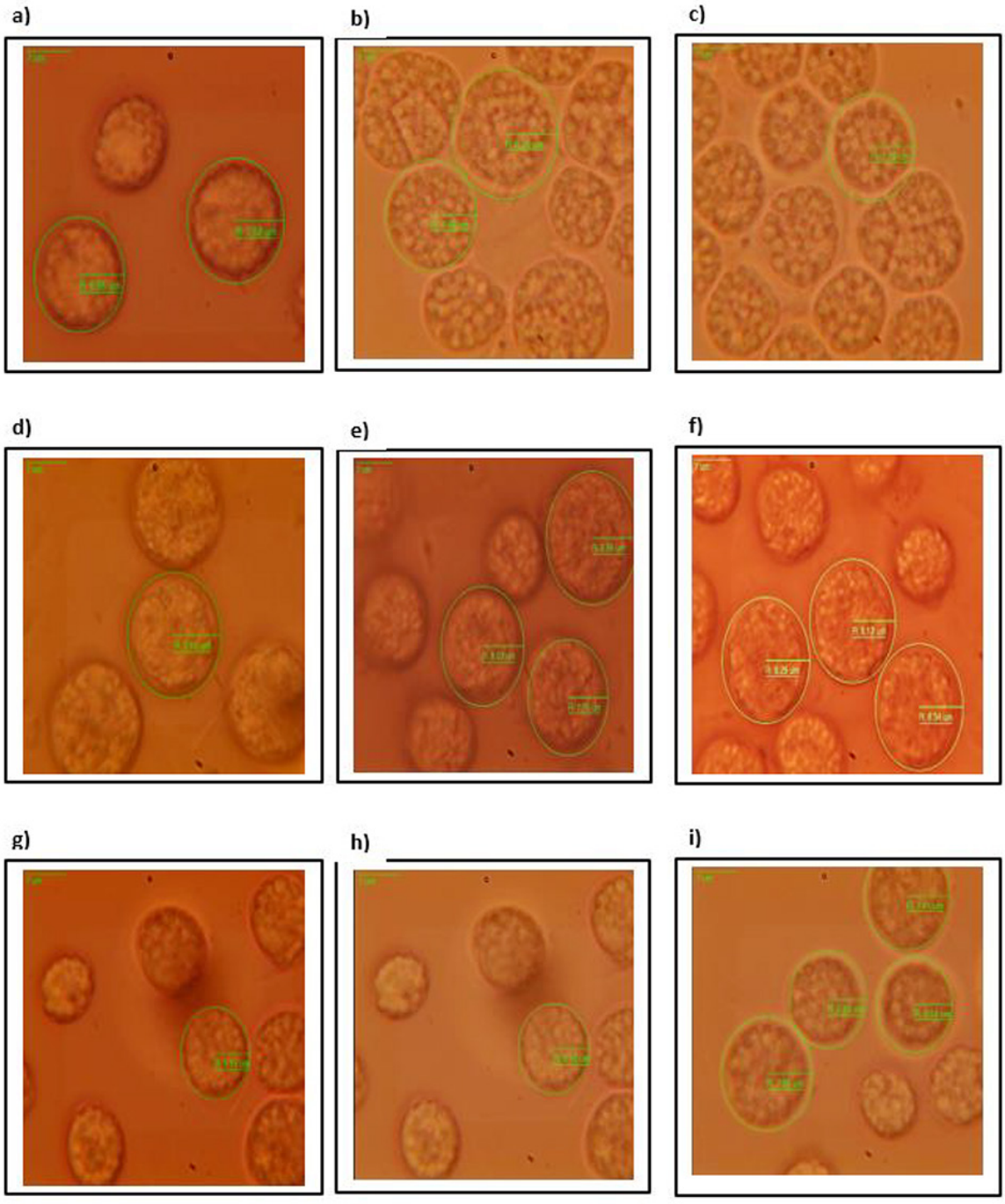

Figure 1. Micrograph of cell growth from *Aurantiochytrium* microalgae. a – c: using glycerol substrate; d – f: using molasses substrate; g – i: using fruit waste substrate, at day 1,2, and 3 respectively

3.92 µm on day 4. Molasses substrate resulted in smaller average cell diameters, starting at 2.76 µm on day 2 and increasing to 3.29 µm on day 4. Similarly, on fruit waste substrate, the average cell diameter grew from 2.71 µm on day 2 to 3.34 µm on day 4. This growth indicates the microalga's ability to proliferate and develop on all three substrates.

Among the substrates, glycerol demonstrated the best performance in supporting microalgal cell growth, both in terms of cell diameter and colony uniformity. This was followed by molasses and fruit waste, which also supported biomass production, albeit with slightly smaller cell sizes. These findings highlight that different substrates influence the growth rate of microalgae, with glycerol providing the most optimal environment for cell development.

Overall, the results demonstrate that all three substrates are capable of supporting the cultivation of Aurantiochytrium microalgae, making them promising options for biomass production. However, glycerol stands out as the superior substrate, offering enhanced performance compared to molasses and fruit waste in promoting maximal cell size growth.

Produced biomass

Figure 2 shows the produced wet biomass of Aurantiochytrium microalgae during cultivation on days 2, 3, and 4. The graph illustrates a steady increase in biomass across all evaluated substrates: glycerol, molasses, and fruit waste. The data demonstrate that all three substrates may enable microalgal biomass growth, though with varying productivity levels.

The glycerol substrate produced the highest biomass growth among the substrates tested. The wet biomass rose from 37 g on day 2 to 45 g on day 3, ultimately reaching 53 g on day 4. This substrate demonstrated the highest efficacy in supporting microalgal growth. Additionally, the biomass generated using molasses substrate exhibited notable growth, increasing from 32 g on day 2 to 42 g on day 3, and reaching 47 g on day 4. Despite having a marginally lower productivity than glycerol, molasses offered a conducive environment for microalgal cultivation.

The fruit waste substrate exhibited the lowest biomass productivity among the three substrates, with an increase from 29 g on day 2 to 38 g on day 3 and reaching 45 g on day 4. Nevertheless, these results suggest that fruit waste can still serve as a viable alternative substrate with potential for microalgal cultivation.

Overall, the graph illustrates a significant upward trend in biomass growth over time for all substrates. Glycerol demonstrated the best performance, followed by molasses and fruit waste, establishing itself as the optimal choice for supporting the biomass production of Aurantiochytrium microalgae.

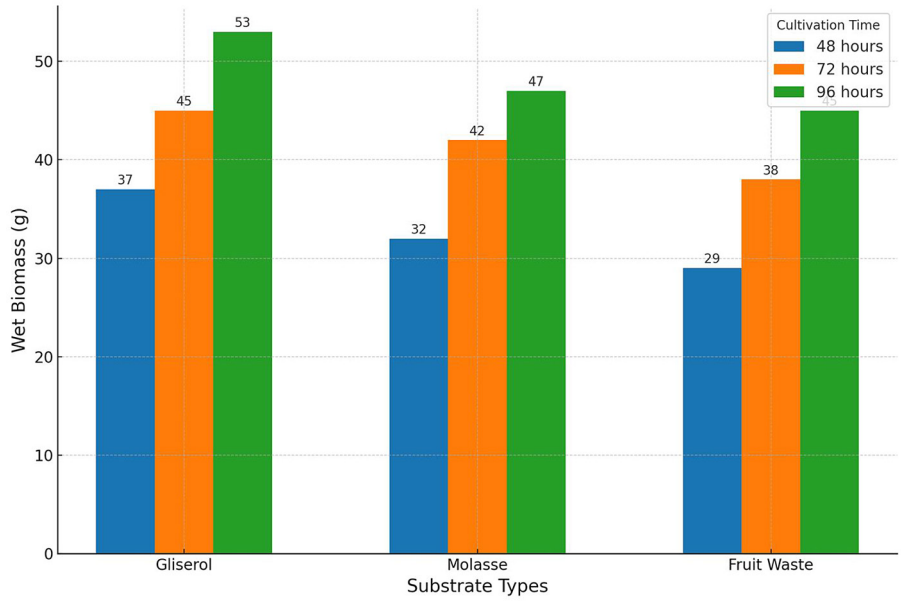

Figure 2. Wet biomass generated from the cultivation by substrate types and cultivation time

Particle size analysis

The analysis of emulsion particle size derived from Aurantiochytrium microalgal biomass revealed that the substrate type significantly influences the average particle size, homogeneity, and emulsion quality. Figure 3 shows particle size distribution of emulsion derived from different substrates: glycerol, molasses, and fruit waste. The substrates tested—glycerol, molasses, and fruit waste—produced distinct results, highlighting the impact of substrate selection on the final properties of the emulsion.

Table 3 shows the resume of particle size analysis of the biomass. The biomass derived from the glycerol substrate resulted in an average emulsion particle size of 1874 nm, accompanied by a Polydispersity Index (PI) of 0.02677, which signifies a notably high degree of homogeneity. The uniform particle size distribution indicates that glycerol serves as the most effective substrate for minimizing particle size while generating a highly stable emulsion. Glycerol serves as an excellent substrate for facilitating microalgal biomass-based applications.

The biomass derived from the molasses substrate exhibited an average particle size of 2427 nm, which is greater than that recorded with glycerol. The substrate exhibited a moderate level of homogeneity, indicated by a PI value of 0.1394. In comparison to glycerol, molasses generated emulsions characterized by larger particle sizes and a less uniform distribution. These results indicate a notable enhancement compared to the fruit waste substrate.

Conversely, when utilizing fruit waste as the substrate, the average emulsion particle size measured 2782 nm, accompanied by a PI value of 0.1725. The level of homogeneity was moderate, yet it was lower than that observed in molasses and glycerol. This suggests that fruit waste produced larger particles with a less uniform distribution. This substrate possesses potential, primarily due to its abundant availability and low cost.

The data indicate that glycerol yields superior performance in the production of emulsions characterized by smaller particle sizes and enhanced homogeneity. Molasses represents a superior alternative, providing enhanced quality relative to fruit waste. Fruit waste presents a feasible alternative substrate for cost-effective applications, though it yields suboptimal results relative to the other two substrates. The selection of substrate is largely contingent upon the specific application and production criteria.

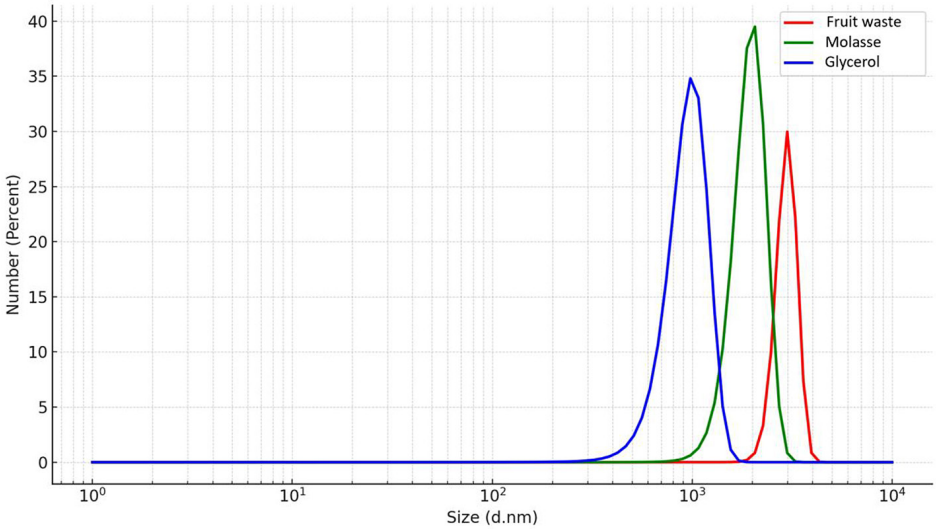

Figure 3. Particle size distribution of emulsion derived from different substrates: glycerol, molasses, and fruit waste

Table 3. Resume of particle size analysis of biomass emulsion

Types of substrate	Average diameter (nm)	Polydispersity index	Homogeneity
Glycerol	1874	0.02677	Very high (highly homogeneous)
Molasses	2427	0.1394	Moderate
Fruit waste	2782	0.1725	Less homogen

Analysis of fatty acids compounds

The GC-MS analysis of biomass derived from glycerol substrate identified three primary lipid components. Table 4, 5 and 6 reveals the results of GC-MS analysis. The predominant compound was 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, referred to as DHA. DHA is a vital fatty acid and a principal constituent of omega-3 fatty acids present in neural tissues. The second principal component was methyl stearate, with the molecular formula C19H38O2, commonly referred to as stearic acid. Stearic acid is extensively utilized in cosmetic formulations and acts as a surfactant, facilitating skin cleansing by emulsifying oil, water, and debris. The third notable lipid component was hexadecanoic acid methyl ester, with the molecular formula C17H34O2, generally known as palmitic acid. Palmitic acid is prevalent in palm oil and is extensively employed in the cosmetic industry as a surfactant, emulsifier, and skin-conditioning agent.

Table 4. GC-MS analysis of lipid components from produced biomass using glycerol substrate

Compounds name	% Area
Furan, tetrahydro-3-methyl	1.93
Methyl tetradecanoate	6.08
Pentadecanoic acid, methyl ester	3.29
Hexadecanoic acid, methyl ester	10.11
Heptadecanoic acid, methyl ester	1.73
Methyl octadeca	0.53
9,12,15-octadecatrienoic acid, methyl ester	0.73
Methyl stearate	6.53
Methyl 4,7,10,13,16-docosapentaenoate	1.51
Methyl eicosa-5,8,11,14,17-pentaenoate	2.07
8,11,14-Eicosatrienoic acid, methyl ester	0.9
omega-3 Arachidonic Acid methyl ester	1.56
Methyl 18-methylnonadecanoate	1.06
4,7,10,13,16,19-Docosahexaenoic acid, methyl ester	54.88
Methyl 4,7,10,13,16-docosapentaenoate	0.92
Methyl 4,7,10,13-hexadecatetraenoate	0.79
Docosanoic acid, methyl ester	1.47
Tetracosanoic acid, methyl ester	0.68
Hexacosanoic acid, methyl ester	2.51
Cholesterol	0.72

Table 5. GC-MS analysis of lipid components from produced biomass using molasses substrate

Compounds name	% Area
2-Ethyl-oxetane	8.43
Pentane, 3-methyl-	47.18
Methyl tetradecanoate	0.82
Pentadecanoic acid, methyl ester	0.41
Hexadecanoic acid, methyl ester	40.87
Methyl stearate	1.08
Cyclononasiloxane, octadecamethyl	0.09
Methyl 4,7,10,13,16-docosapentaenoate	0.32
4,7,10,13,16,19-Docosahexaenoic acid, methyl ester	0.72
Cyclononasiloxane, octadecamethyl	0.08

Table 6. GC-MS analysis of lipid components from produced biomass using fruit waste substrate

Compounds name	% Area
2-Ethyl-oxetane	19.59
Methyl tetradecanoate	5.71
Pentadecanoic acid, methyl ester	2.28
Hexadecanoic acid, methyl ester	33.89
Hexadecanoic acid, methyl ester	24.97
Heptadecanoic acid, methyl ester	0.94
Methyl stearate	4.88
Methyl 4,7,10,13,16-docosapentaenoate	2.22
4,7,10,13,16,19-Docosahexaenoic acid, methyl ester	4.76
Hexacosanoic acid, methyl ester	0.76

The GC-MS investigation on biomass derived from molasses substrate revealed various lipid constituents, including 3-methylpentane, methyl hexadecanoate, and methyl tetradecanoate. 3-Methylpentane (C6H14) functions as a solvent in chemical synthesis, a lubricant, and a precursor for carbon generation. Hexadecanoic acid methyl ester (palmitic acid, C17H34O2) was also identified and has widespread applications in cosmetics as a surfactant, emulsifier, and skin-conditioning agent. The third component was methyl tetradecanoate (myristic acid, C15H30O2), typically located in nutmeg seeds (Myristica fragrans Houtt). Myristic acid serves as an anti-stress element in animal feed and as an aromatherapy component in medications.

The GC-MS analysis of the fruit waste substrate identified two principal lipid constituents: hexadecanoic acid methyl ester (palmitic acid) and methyl tetradecanoate (myristic acid). Methyl hexadecanoate (C17H34O2) is prevalent in palm oil and is employed in the cosmetic sector as a surfactant, emulsifier, and skin-conditioning agent. Methyl tetradecanoate (C15H30O2) serves analogous purposes as an anti-stress addition in

animal feed and as an aromatherapy component in pharmaceutical formulations. The findings emphasize the distinctive lipid profiles of Aurantiochytrium cultivated using different substrates, each exhibiting particular advantages for industrial applications. The glycerol substrate is distinguished by its elevated DHA content, rendering it appropriate for use in nutritional and health goods. Simultaneously, the molasses and fruit waste substrates exhibited promise as economical substitutes for generating valuable lipids, such as palmitic and myristic acids, which have extensive applications in the cosmetics, pharmaceuticals, and animal feed sectors.

DISCUSSION

Performance of biomass production

The findings demonstrate that glycerol serves as the most effective substrate for promoting biomass growth in Aurantiochytrium microalgae, in comparison to molasses and fruit waste. On day 4, biomass growth on the glycerol substrate attained 53 g, surpassing that of molasses at 47 g and fruit waste at 45 g. This indicates that glycerol offers the most favorable conditions for microalgal metabolism and growth, considering both carbon availability and compatibility with microalgal demands.

Molasses and fruit waste demonstrated potential in promoting biomass growth. Molasses yielded moderate results with a stable growth pattern, whereas fruit waste, despite demonstrating the lowest productivity, is a viable option due to its availability and cost-effectiveness. The increase in biomass across all substrates demonstrates that Aurantiochytrium is capable of utilizing different carbon substrates for its cultivation.

The findings highlight the tactical significance of utilizing glycerol as a substrate, especially for industrial applications targeting high biomass yield within a specified time frame. The better development associated with glycerol is a result of its straightforward carbon structure (C3), which promotes effective metabolic uptake and swift incorporation into cellular pathways (Abdel-Wahab et al., 2022; Nazir, Kaid, et al., 2020). This efficiency highlights glycerol's potential as a favored carbon source for versatile microalgae-based bioprocesses. The performance of molasses is moderate, indicating its durability as a substrate. The composition, characterized by simple sugars and trace minerals, supports a consistent growth rate, rendering it a favorable choice for scenarios where process consistency is prioritized over maximum yield. Moreover, molasses serves as a by-product of sugar production, aligning with principles of the circular economy and providing an economically sustainable alternative.Fruit waste, although producing restricted biomass, serves a resource with considerable environmental and economic importance (W.K. Park et al., 2018). The utilization of this strategy reduces organic waste and supports cost-effective cultivation strategies. The variability in composition and the necessity for pre-treatment indicate potential areas for optimization, implying that specific processing methods may enhance productivity from this substrate.

The adaptability of Aurantiochytrium for different carbon sources is a significant finding. This indicates a wide applicability for the microalga, enabling the incorporation of diverse substrates based on availability, cost, and sustainability objectives of the production system. Future research may concentrate on improving the productivity of molasses and fruit waste substrates via nutrient supplementation or pre-treatment methods, thereby increasing their comparative advantage with glycerol in particular for industrial use.

The emulsion particle size of produced microalgae biomass indicated that glycerol resulted in the smallest particle size (1874 nm) and the lowest Polydispersity Index (PI) of 0.02677, demonstrating a high degree of homogeneity. The substrate demonstrated the highest stability and uniformity in emulsion formation, rendering it a superior option for industrial applications.

Conversely, molasses resulted in larger particles (2427 nm) and exhibited a higher polydispersity index (0.1394), suggesting moderate homogeneity. The fruit waste exhibited the largest particle size at 2782 nm, accompanied by a polydispersity index of 0.1725, indicating a less uniform particle size distribution. Therefore, both substrates continue to be viable, particularly because of their economic potential.

Analysis of substrates for microalgae production

Glycerol serves as a carbon source in the production of Aurantiochytrium microalgae biomass. Glycerol, a carbon compound with the chemical formula $C_3H_8O_3$, exhibits high carbon efficiency. Microalgae metabolize glycerol through the

glycerol kinase pathway, resulting in the direct production of phosphoglyceraldehyde, which serves as an intermediate in glycolysis (Kim et al., 2015). Aurantiochytrium microalgae possess the capability to effectively convert carbon from glycerol into lipid biosynthesis, particularly omega-3 fatty acids (DHA) (Yu et al., 2016). Glycerol presents certain limitations, including the absence of supplementary nutrients, such as nitrogen or minerals, necessitating a balanced media formulation to facilitate microalgal growth. The glycerol fermentation process is generally slower than that of sugar-based carbon sources, presenting a challenge for large-scale production (Patel et al., 2019).

Molasses represents a substrate with significant potential for microalgae fermentation. Molasses, a mixture abundant in simple sugars such as glucose, fructose, and sucrose ($C_{12}H_{22}O_{11}$), supplies readily metabolizable carbon for microorganisms through glycolysis. Molasses is not only carbon-rich but also contains minerals like potassium, magnesium, and calcium, as well as trace amounts of nitrogen, which provide supplementary benefits for microalgal growth without the need for extensive media supplementation. The primary advantage is its high fermentation efficiency, with microalgae rapidly metabolizing simple sugars. Molasses presents certain disadvantages, including the presence of non-sugar compounds that may marginally impede fermentation and its low pH, which could necessitate additional buffering. Molasses is an effective option for economical and rapid fermentation, especially in biomass production (Abad & Turon, 2015).

Fruit waste represents an economical and sustainable substrate alternative. The composition includes simple sugars such as glucose, fructose, and sucrose, alongside complex carbon compounds including pectin, hemicellulose, and lignin (Park et al., 2018). The variation in carbon content enhances the potential for microalgal growth, as fruit waste is also rich in vitamins, minerals, and other organic compounds. The utilization of this waste-based material is environmentally sustainable and promotes circular economies through the repurposing of discarded materials. Nonetheless, the intricate carbon present in fruit waste is not entirely bioavailable to microalgae without undergoing pre-treatment methods, including thermal or enzymatic hydrolysis. The chemical composition of fruit waste varies according to the type and condition of the fruit, which presents challenges in achieving consistent results (Nazir, Kaid, et al., 2020).

Each analyzed substrate possesses unique strengths and limitations. Table 7 shows the evaluation of substrate selection for the cultivation of Aurantiochytrium microalgae. Glycerol is effective for the production of high lipid content, including DHA, though it necessitates media supplementation. Molasses provides efficient fermentation and contains additional nutrients, rendering it a beneficial choice for biomass production. Fruit waste offers a cost-effective and sustainable solution; however, pre-treatment is necessary to improve carbon availability. Choosing the suitable substrate and refining the fermentation process can enhance microalgae production regarding efficiency and sustainability (Guo et al., 2022).

Valuable fatty acids

GC-MS analysis results suggested that the glycerol substrate produced the highest percentage of 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester (DHA methyl ester) at 54.88%. DHA is a vital omega-3 fatty acid essential to the health of neural tissue. Other notable components comprised methyl stearate (6.53%), pertinent in the cosmetics industry, and hexadecanoic acid methyl

Table 7. Evaluation of substrate selection for microalgae biomass production

Criteria	Glycerol	Molasses	Fruit waste
Carbon content	High (simple C3)	High (simple C6 & C12)	Variable (C6 & complex)
Carbon efficiency	Very high	High	Moderate (with pre-treatment)
Additional nutritional potential	None	Present (minerals and small amounts of N)	Present (vitamins and minerals)
Fermentation rate	Moderate	Fast	Moderate (with pre-treatment)
Lipid production	Excellent	Good	Fair
Cost suitability	Moderate	Low	Very low
Carbon content	High (simple C3)	High (simple C6 & C12)	Variable (C6 & complex)

ester (10.11%), recognized as palmitic acid, commonly used as a surfactant and emulsifier. In the molasses substrate, hexadecanoic acid methyl ester constituted 40.87% of the lipid composition, whereas DHA methyl ester was present at a mere 0.72%. The fruit waste substrate exhibited a predominance of hexadecanoic acid methyl ester at 33.89%, while the proportion of DHA methyl ester was 4.76%. The results demonstrate that the glycerol substrate is more effective in enhancing DHA production relative to the other substrates.

DHA undergoes derivatization to form its methyl ester for analysis via GC-MS (Bratu et al., 2013; Yi et al., 2014). The native form of DHA is polar and possesses a high boiling point, making it difficult to vaporize and thus unsuitable for analysis. The derivatization process involving methanol and an acid catalyst, such as HCl or BF_3, transforms the carboxyl (-COOH) group into an ester ($-COOCH_3$), resulting in the formation of docosahexaenoic acid methyl ester, characterized by increased stability, non-polarity, and volatility.

GC-MS analysis frequently utilizes methyl ester standards for the comparison of retention times and fragmentation patterns. The alignment of the sample with the standard confirms the successful derivatization and identification of DHA in its methyl ester form. This form enables precise and effective analysis (Ichihara & Fukubayashi, 2010). The derivatization process facilitates the precise detection of DHA via GC-MS, allowing for accurate identification and quantification of fatty acid components. This step is crucial in lipid analysis for the effective characterization of fatty acids.

This study demonstrates that glycerol serves as the optimal substrate for enhancing microalgae biomass growth, yielding more uniform emulsions and facilitating the most efficient production of DHA. Molasse and fruit waste exhibit potential as alternative substrates, offering advantages in economic feasibility and abundant availability. Further optimization is necessary to improve the yield of high-value lipid products from these two substrates. The findings advance the development of microalgae-based technologies for applications in food, pharmaceuticals, and industry.

Circular economy potential

The integration of Aurantiochytrium-based bioprocess technologies into a circular economy framework presents a significant opportunity for sustainable production of omega-3 fatty acids, particularly DHA. By utilizing organic waste materials such as glycerol, molasses, and fruit waste as substrates, this approach transforms low-cost or waste inputs into high-value outputs. Aurantiochytrium, isolated from mangrove ecosystems, is a unique microalga capable of producing DHA without concerns about heavy metal or microplastic contamination. This positions it as an environmentally friendly and scalable alternative to traditional fish oil-based sources of omega-3 fatty acids, which face sustainability and environmental challenges (Monteiro et al., 2024).

Moreover, incorporating waste-derived substrates not only reduces production costs but also aligns with the principles of circular economy, minimizing waste and creating economic value for local communities. This sustainable approach supports the dual objectives of reducing environmental impact and fostering economic development. The ability to efficiently convert diverse substrates into DHA and other valuable fatty acids underscores the potential of Aurantiochytrium as a pivotal organism in achieving sustainable and economically viable fatty acid production systems.

Utilizing green extraction

Previous research has been conducted on the extraction of fatty acids from the biomass of Aurantiochytrium microalgae (Sulistiawati et al., 2023). Commonly utilized solvents comprise n-hexane and methanol. Emerging trends indicate a shift towards the adoption of more sustainable extraction techniques. One eco-friendly extraction technology utilizes ionic liquids (Eppink et al., 2021). The purification stage utilizes two-phase extraction with semi-hydrophobic natural eutectic ionic liquids. This method improves separation efficiency, lowers operational costs, and preserves quality.

CONCLUSIONS

This study demonstrated the significant potential of Aurantiochytrium microalgae in producing high-value fatty acids, particularly DHA, using sustainable and cost-effective substrates. Among the tested substrates, glycerol proved to be the most effective, yielding the highest biomass and DHA content while producing highly stable emulsions with the smallest particle size. Molasses and fruit waste, while less efficient in

lipid production, showed strong potential as economical and sustainable alternatives, especially in circular economy applications. The ability of Aurantiochytrium to adapt to diverse substrates highlights its versatility and suitability for industrial-scale production of omega-3 fatty acids.

By integrating industrial by-products such as glycerol from the biodiesel industry and agricultural waste like molasses and fruit waste, this study aligns with the principles of a circular economy. This approach not only reduces costs but also minimizes environmental impact by converting waste into valuable resources. The findings underscore the feasibility of employing Aurantiochytrium-based bioprocesses for sustainable fatty acid production, offering innovative solutions for the food, pharmaceutical, and cosmetic industries while promoting environmental sustainability and economic development.

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