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Double step method in lipid extraction from biomass *Aurantiochytrium sp* powder

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ABSTRACT

Aurantiochytrium sp is a marine microalgae that is rich in lipids. Extraction of lipids from microalgae requires effort to select a suitable solvent and extraction method. This research used a double-step extraction method to study a mixture of n-hexane and methanol as a solvent. The variables studied were stirring time, the n-hexane to methanol (H/M) mixture ratio, and the solvent-to-biomass ratio (S/B). This research concluded that an optimum stirring time was 30 min, and a mixture of n-hexane and methanol solvents with a volume ratio of 1:1 is optimum. The optimum solvent-to-biomass ratio was S/B=20 mL/g dry microalgae. Under these conditions, the yield of oil was 83.88%. Double-step extraction can increase the yield by 10-40%.

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1. INTRODUCTION

Along with increasing public awareness about the importance of maintaining health after the COVID-19 pandemic, the need for raw materials for supplements has also increased. The production of Omega 3 (docosahexaenoic acid (DHA)) and squalene compounds from *Aurantiochytrium sp* microalgae has attracted the attention of researchers and industrial practitioners because of its several advantages compared to other sources. *Aurantiochytrium's* advantages include high lipid productivity, fast cell growth, non-fish raw materials, high purity, and not containing heavy metals. *Aurantiochytrium* microalgae can be used as a source of squalene raw materials and biofuels [1], [2]. *Aurantiochytrium sp* microalgae are abundant in mangrove forests [3], [4]. Internationally, studies on applying *Aurantiochytrium sp.*, including fish and livestock feed, cosmetics, antioxidants, and biofuels, to the COVID-19 vaccine adjuvant [5]. Unfortunately, even though Indonesia is known to have the largest mangrove forest in the world, studies on the production and application of products derived from *Aurantiochytrium sp* microalgae are still minimal in Indonesia [6], [7].

Biocomponents such as DHA and squalene are components contained in lipids found in *Aurantiochytrium sp*. The biocomponents (lipids) extraction from microalgae is challenging because microalgae have strong cell walls [8]. There are some efforts to extract lipids from microalgae to disrupt the cell wall, including hydrodynamic cavitation [8], stirring (homogenization), ultrasonication, microwave [9], and high-shear mixer [10].

The mixing (stirring) method is easiest to do on an industrial scale because this method is the simplest. Hexane is the cheapest solvent most widely used commercially [11], [12]. However, conducting various experiments using solvents (n-hexane and methanol) is necessary to obtain high results. The obstacle in the extraction process is the mass transfer of the solvent into the cells because it must break down the cell walls first. Therefore, conducting a study using relatively inexpensive and safe solvents is necessary. The one-step extraction process generally leaves lipids in the dregs (biomass residue), so it is necessary to study a two-step extraction. This research aimed to obtain information on the optimum use of n-hexane and methanol solvents in the lipid extraction process from dry biomass of *Aurantiochytrium sp*. The variables studied were stirring time, the ratio of n-hexane to methanol (H/M) mixture, and the ratio of the amount of solvent to the biomass (S/B).

2. RESEARCH METHOD

Lipid (oil) extraction from dry biomass of *Aurantiochytrium sp* was carried out at the Bioprocess Laboratory of the Chemical Engineering Study Program, Universitas Ahmad Dahlan, Yogyakarta. The material used was *Aurantiochytrium sp* powder (purchased from Xi'an Taian Biotechnology Co., Ltd.) with a moisture content of 6.03% and ash of 3.54%. The solvents used were n-hexane (technical grade, density 0.670-0.683 g/mL) and methanol (technical grade, density 0.77 g/mL). Both solvents were purchased from PT. Brataco, Yogyakarta). The equipment used includes a magnetic stirrer (Ika C-Mag HS7, 250 rpm), centrifuge (DLab, 2400 rpm), and a series of distillation apparatus. Two grams of dry microalgae were put into a 250 mL Erlenmeyer flask with n-hexane and methanol as solvents. Magnetic stirring was carried out at 40 °C for 30 minutes at a stirring speed of 250 rpm. Subsequently, the mixture was transferred into a centrifuge tube and centrifuged for 15 minutes at 2400 rpm. After centrifugation, the mixture was separated between the supernatant and residue. The supernatant was transferred into a distillation flask for the distillation process to be carried out at a temperature of 69-72 °C until all the solvent was vaporized. The oil remaining in the flask is weighed (W1) using an analytical balance (Ohaus). The same procedure was carried out for the biomass residue to increase the yield of lipids, as shown in Figure 1. The total lipid obtained was the sum of the lipids (oil) yields from the first (W1) and second extractions (W2). Some of the obtained oil samples were analyzed for fatty acid content using the gas chromatography-mass spectrometry (GCMS) and Fourier transform infrared (FTIR) methods.

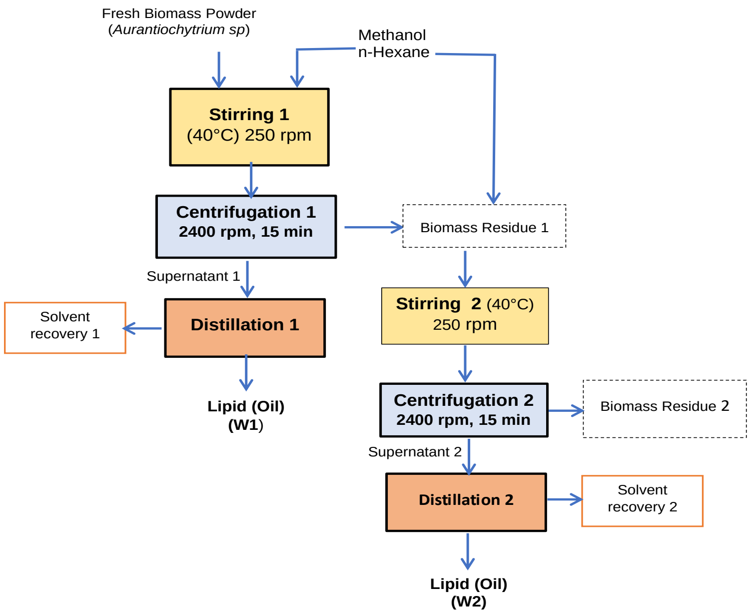


Figure 1. Experimental procedure of lipid extraction from *Aurantiochytrium sp*

3. RESULTS AND DISCUSSION

3.1. Effect of stirring time

Extraction is carried out with a solvent ratio of (hexane/methanol)=1. The effect of stirring time on the total oil yield of extraction can be seen in Figure 2. The longer the extraction time, the higher the yield, but after 30 minutes, the yield decreased. It may be due to the excessive evaporation of components in the biomass during the distillation process because n-hexane is highly volatile [12].

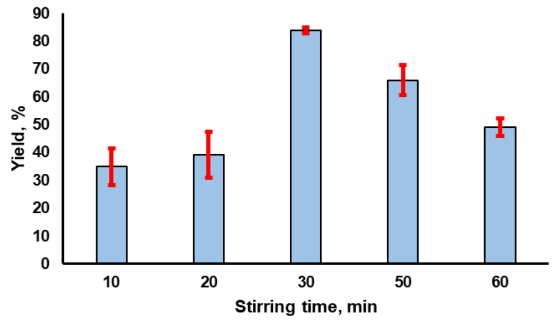


Figure 2. The effect of stirring time on the total yield of oil from *Aurantiochytrium sp*

3.2. Effect of solvent mixture

Extraction used a solvent mixture of n-hexane and methanol with various volume ratios (i.e., H/M=0.33; 0.5; 1; 3). The stirring time was 30 minutes, and the solvent volume ratio to biomass (S/B) was 8-20 (mL/g). The research results can be seen in Figure 3, especially in Figures 3(a)-(d).

From Figure 3, overall, it can be seen that double-step extraction can increase oil yield. Figure 3(a) explains that at n-hexane/methanol 0.33 v/v, the second step gets more oil than the first. It might happen because the first extraction was not optimal, so the amount of oil remaining in the residue was sufficient, and it could be recovered in the second extraction. Although double-step extraction was carried out, using a single solvent, n-hexane only, or methanol alone is not better than mixed solvents. It can be seen in Figure 4. Figure 4(a) shows the oil yield on the double step and the total yield shown in Figure 4(b).

The type of solvent used influences the yield. Lipid extraction from the microalga *Chlorella sp* with a mixed solvent of n-hexane/methanol (1:2 v/v) yielded 4.06% [9]. The results of this study were better, getting a yield of 26%, because extraction was carried out in double steps. Figure 4 shows that the ratio of hexane to methanol (H/M)=1 produces the highest yield.

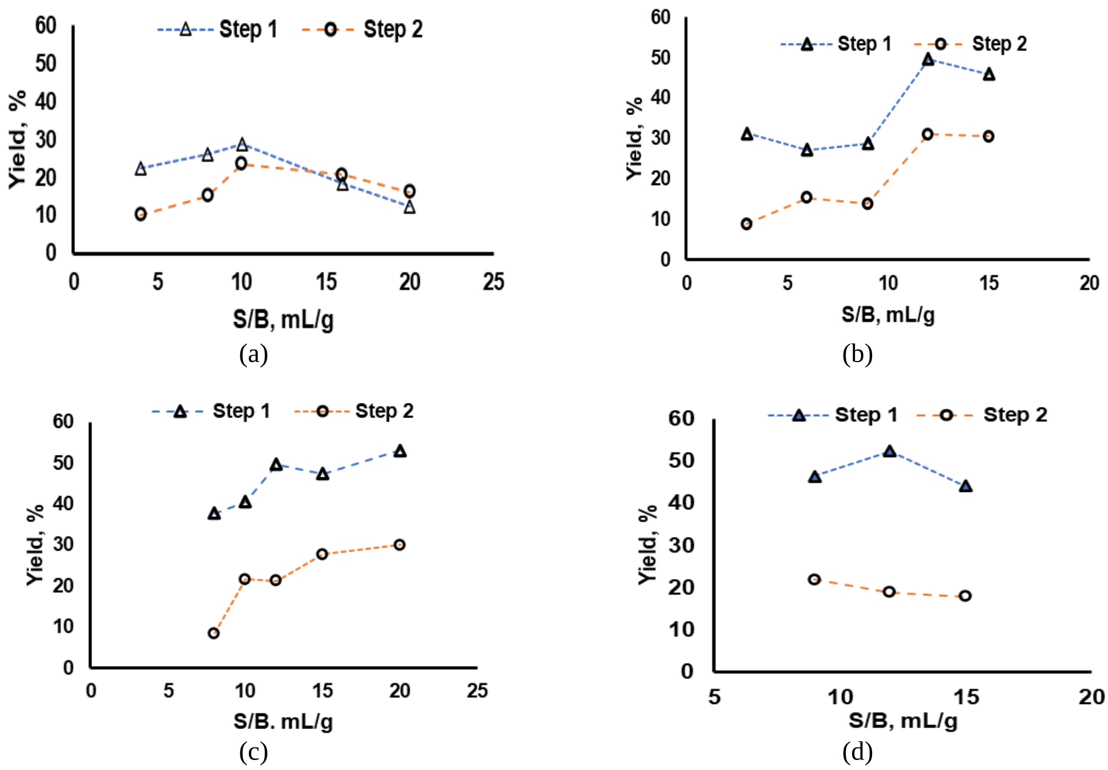
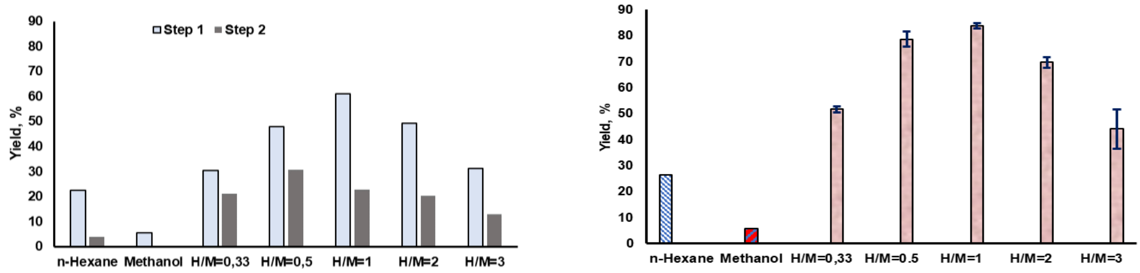


Figure 3. The impact of (solvent/biomass) on yield at (a) H/M=0.33, (b) H/M=0.5, (c) H/M=1, and (d) H/M=3



(a) (b)

Figure 4. The oil yield was obtained using the solvent n-hexane, methanol, and a mixture of hexane and methanol solvents: (a) double step and (b) total yield

3.3. Effect of solvent-to-biomass ratio

The effect of solvent-to-biomass ratio on lipid yield is presented in Figure 5. At the same solvent composition H/M, the greater the solvent-to-biomass (S/B) ratio, the greater the yield. However, after reaching S/B 20 mL/g, the lipid extracted is the same. It may be due to the conditions the extraction has been approaching its mass balance, i.e., the mass concentration of oil outside has been balanced with the concentration of oil in the cell.

The mass transfer phenomenon in biocomponents (lipids) from inside the cell to the broken cell wall, then extracted by the solvent, will reach a maximum at the state mass balance [13], [14]. It follows several other studies on extraction, which explain that the more solvent used, the greater the extract (substance dissolved in the solvent) obtained [14], [15].

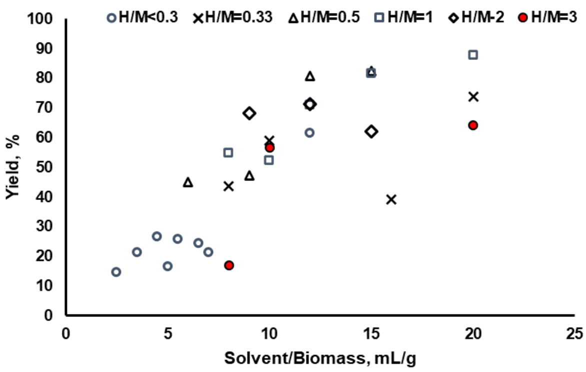


Figure 5. The effect of solvent-to-biomass ratio on lipid yield

3.4. Comparison of single and double-step extraction

The performance of single-step and double-step extraction can be seen in Table 1. Table 1 shows that at a low H/M ratio (0.08-0.25) and S/B less than 8, the yield obtained is also low, but double-step extraction can increase the yield by almost 100% (from the first extraction). The highest total yield was at a ratio of hexane to methanol (H/M)=1 and the ratio of solvent to biomass (S/B)=20, with yields from the first and second extractions, 60.96% and 22.92%, respectively, or total yield 0f 83.88%. However, this result could not reach 90% yield as done by Kwak *et al*. [10] because they used a high shear mixer with a speed of 7000 rpm and was treated with acid degumming treatment, whereas this research only worked at 250 rpm without the same treatment.

3.5. Total fatty acid component by gas chromatography-mass spectrometry

The essential fatty acid components in the oil from *Aurantiochytrium sp* were palmitic acid (22.54%), linoleic acid (20.53%), lauric acid (19.84%), linolenic acid (13.54%), myristic acid (10.32%), oleic acid (6.61%), and pentadecanoic acid (1.4%). Other fatty acid components were below 1%. A comparison of the other researchers is expressed in Table 2.

DHA, as a high-value component, was not detected in this study, while Chauhan *et al*. [16] obtained 31.41%, Abdel-Wahab *et al*. [17] 4.89%, Dong *et al*. [18] 14.45%, and Yoneda *et al*. [19] 45.0%, respectively. All PUFAs are susceptible to oxidative damage, so special treatment is required during processing and storage to minimize exposure to air, light, and heat. Peroxidation reaction in the product also causes oil damage, so antioxidants must be added [11]. Adding the enzyme sesamol during the *Aurantiochytrium* cultivation period can increase DHA levels by 90% [20]. Uses of *Ananas comosus* MD2 extract can also increase DHA levels during cultivation [21].

Table 1. The performance of single-step and double-step extraction (stirring time 30 min, 40 °C, 250 rpm)

H/M, V/V	S/B, mL/g	Step 1, (yield, %)	Step 2 (yield, %)	Total yield, %
0.25	5	10.32	6.24	16.56
0.17	7	11.14	10.06	21.20
0.25	2.5	9.48	5.10	14.58
0.17	3.5	13.54	7.62	21.16
0.13	4.5	8.62	17.86	26.48
0.10	5.5	13.82	11.96	25.78
0.08	6.5	17.18	7.28	24.46
0.20	12	40.86	20.78	61.64
0.33	8	27.30	16.46	43.76
0.33	10	31.80	27.30	59.10
0.33	16	18.45	20.75	39.20
0.33	20	34.90	38.90	73.80
0.50	6	28.18	16.98	45.16
0.50	9	31.05	16.10	47.15
0.50	12	49.65	31.05	80.70
0.50	15	48.35	34.15	82.50
1.00	8	34.16	20.74	54.90
1.00	10	43.55	8.70	52.25
1.00	12	49.75	21.30	71.05
1.00	15	50.55	31.00	81.55
1.00	**20**	**60.96**	**22.92**	**83.88**
2.00	9	46.35	21.95	68.30
2.00	12	52.35	18.85	71.20
2.00	15	44.05	17.95	62.00
3.00	8	5.10	11.90	17.00
3.00	10	31.30	25.20	56.50
3.00	20	44.35	19.95	64.30

Table 2. Fatty acid composition of the *Aurantiochytrium* (%)

Fatty acid	Name	This study	*Aurantiochytrium sp.* from glucose 40 g/L [16]	*Aurantiochytrium* YB-05 [17]	*Schizochytrium* salinity 20g/L [18]
C11:0	Undecanoic acid	-	-	1.30	-
C12:0	Lauric acid	19.84	-	6.70	-
C14:0	Myristic acid	10.32	0.51	0.89	2.75
C15:0	Pentadecanoic acid	1.40	26.13	3.10	1.26
C16:0	Palmitic acid	22.54	27.50	24.02	8.52
C16:1ω7	Palmitoleic acid	0.29	0.96	0.69	2.15
C17:0	Heptadecanoic acid	-	5.18	1.41	0.39
C18:0	Stearic acid	0.29	-	6.96	0.48
C18:1ω9	Oleic acid	6.61	1.20	25.01	1.10
C18:1ω7	Vaccenic acid	0.89	-	1.17	-
C18:2ω6	Linoleic acid	20.53	-	20.72	-
C18:3ω3	Linolenic acid	13.54	-	1.40	-
C20:0	Arachidic acid	-	-	0.50	-
C20:1ω9	Gondoic acid	-	-	0.60	-
C20:4ω6	Arachidonic acid (ARA)	-	0.60	-	-
C20:5ω3	Eicosapentaenoic acid (EPA)	0.38	0.61	-	0.20
C22:5ω6	Docosapentaenoic acid (DPA)	-	0.85	0.64	4.38
C22:6ω3	DHA	-	31.41	4.89	14.45
Total saturated fatty acids (SFA)		**52.70**	**59.32**	**44.88**	**13.40**
Total monounsaturated fatty acids (MUFA)		**6.61**	**2.16**	**27.47**	**3.25**
Total polyunsaturated fatty acids (PUFA)		**34.07**	**33.47**	**27.65**	**19.45**

3.6. Fourier transform infrared results

The analysis results using the FTIR for *Aurantiochytrium sp* powder are shown in Figure 6. In contrast, the extracted oil samples compared to shark liver oil and the raw microalgae (*Aurantiochytrium sp* powder) are expressed in Figure 7 and Table 3. *Aurantiochytrium sp* microalgae powder has a broad FTIR spectrum at wave numbers 3293.1 cm^{-1}, indicating the presence of single bonds (O-H, C-H, or hydroxy group) [22], [23]. *Aurantiochytrium* powder in this research contains lipids and a small amount of water (6.03%). In Figure 7 the four treatments show similar spectra, in the wave number 2500-3000 cm^{-1} which indicates the presence of single bonds, and in the range 1500-2000 cm^{-1} which indicates the presence of double bonds [22], and a strong peak at 1600-1700 cm^{-1} indicates the presence of the carbonyl group (C=O) [24]. The spectral region of the fingerprint (500-1500 cm^{-1}) needs to be studied further [22], [25].

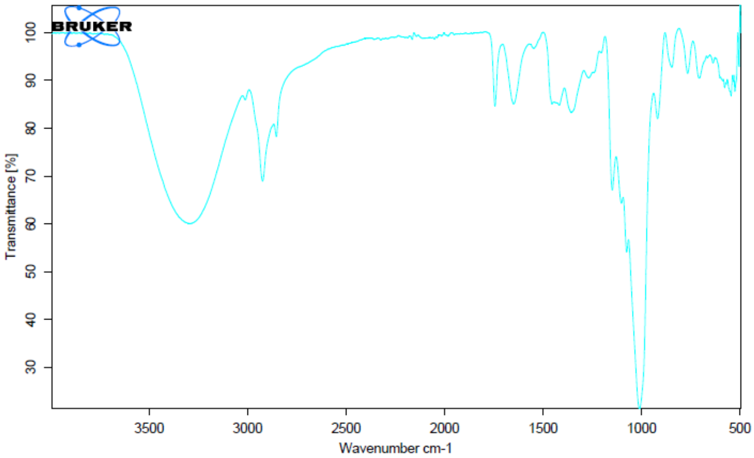


Figure 6. FTIR analysis of *Aurantiochytrium* sp powder

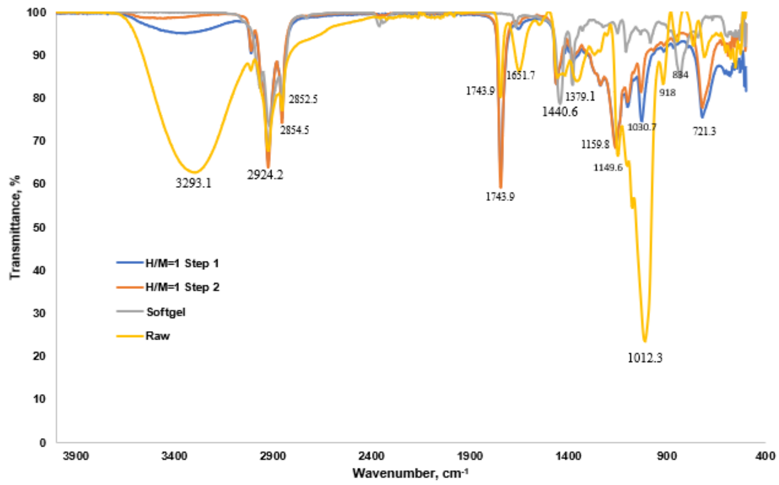


Figure 7. FTIR results of lipid samples

Table 3. FTIR data of raw materials, extraction oil from *Aurantiochytrium sp*, and shark liver oil (soft gel)

Raw (*Aurantio-chytrium sp*)	Oil (H/M=1, Step 1)	Oil (H/M=1, Step 2)	Softgel (shark liver oil)	Literature wavenumber (cm^{-1})	Functional groups
-	721.3	721.3	-	750-720	-$(CH_2)_n$- rocking (n≥3) [22]
918.0	-	-	834.0	1300-700	Skeletal C=C vibrations [22]
1012.3	1028.7	1030.7	-	1300-700	Skeletal C=C vibrations [22]
1149.6	1098.4	1098.3	-	1150-1050	C-O stretch [22]
-	1159,8	1159.8	-	1159-1164	C-O stretch [22]
-	-	-	1379.1	1380-1370	C-H bend [22]
-	1459.1	1461.1	1440.6	1485-1445	C-H bend [22], [26]
1651.7	-	-	-	1680-1620	C=C stretch [22]
1743.9	1743.9	1743.9	-	1750-1725	C=C stretch, Ester [22]
2854.5	2852.5	2852.5	2852.5	2880-2860	C-H bending, sharp [22], [26]
2924.2	2924.2	2924.2	2914.0	2935-2915	C-H stretch, sharp [22], [26]
3293.1	-	-	-	3400-3200	-OH stretching, broad [22], [26]

4. CONCLUSION

Double-step extraction can increase oil yield, especially at low solvent-to-biomass ratios. A better hexane-to-methanol ratio was at 1:2 to 2:1 but optimum at 1:1 v/v. Under optimum conditions (H/M=1, S/B=20), the yield obtained from the double-step extraction could increase by almost 23%, and the highest total yield was 83.88%. The double-step method can increase oil yield by 10-40% for overall conditions.

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REFERENCES

[1] A. Patel, U. Rova, P. Christakopoulos, and L. Matsakas, "Simultaneous production of DHA and squalene from Aurantiochytrium sp. grown on forest biomass hydrolysates," *Biotechnology for Biofuels*, vol. 12, no. 1, p. 255, Dec. 2019, doi: 10.1186/s13068-019-1593-6.

[2] A. Patel, S. Liefeldt, U. Rova, P. Christakopoulos, and L. Matsakas, "Co-production of DHA and squalene by thraustochytrid from forest biomass," *Scientific Reports*, vol. 10, no. 1, p. 1992, Feb. 2020, doi: 10.1038/s41598-020-58728-7.

[3] M. A. Abdel-Wahab, A. E.-R. M. A. El-Samawaty, A. M. Elgorban, and A. H. Bahkali, "Fatty acid production of thraustochytrids from Saudi Arabian mangroves," *Saudi Journal of Biological Sciences*, vol. 28, no. 1, pp. 855–864, Jan. 2021, doi: 10.1016/j.sjbs.2020.11.024.

[4] M. A. Abdel-Wahab, A. M. Elgorban, and A. H. Bahkali, "Single cell oil of oleaginous marine microbes from Saudi Arabian mangroves as a potential feedstock for biodiesel production," *Journal of King Saud University - Science*, vol. 35, no. 4, p. 102615, May 2023, doi: 10.1016/j.jksus.2023.102615.

[5] A. Gupta, C. J. Barrow, and M. Puri, "Multiproduct biorefinery from marine thraustochytrids towards a circular bioeconomy," *Trends in Biotechnology*, vol. 40, no. 4, pp. 448–462, Apr. 2022, doi: 10.1016/j.tibtech.2021.09.003.

[6] S. Suhendra, E. Sulistiawati, R. T. Evitasari, T. R. Ariandi, L. Septianingsih, and A. Hutari, "Bioprocess potentials of Aurantiochytrium microalgae from Kulonprogo mangrove forest Yogyakarta, Indonesia," in *AIP Conference Proceedings*, 2023, vol. 2667, p. 070004, doi: 10.1063/5.0112298.

[7] S. Suhendra, T. Pantoiyo, S. Fazlia, E. Sulistiawati, and R. T. Evitasari, "Bioprocess potentials of squalene from thraustochytrids microalgae for nutraceuticals in new normal era isolated from Indonesian mangroves: a review," *CHEMICA: Jurnal Teknik Kimia*, vol. 8, no. 1, p. 18, Jun. 2021, doi: 10.26555/chemica.v8i1.19121.

[8] M. Setyawan, P. Mulyono, S. Sutijan, Y. S. Pradana, L. Prasakti, and A. Budiman, "Effect of devices and driving pressures on energy requirements and mass transfer coefficient on microalgae lipid extraction assisted by hydrodynamic cavitation," *International Journal of Renewable Energy Development*, vol. 9, no. 3, pp. 467–473, Oct. 2020, doi: 10.14710/ijred.2020.26773.

[9] M. Tantichantakarun, P. Chetpattananondh, and S. Ratanawilai, "Chlorella sp. Extraction and estimation of fuel properties of lipids derived from FFA profiles," *Engineering and Applied Science Research*, vol. 46, no. 2, pp. 106–113, 2019.

[10] M. Kwak, S. Roh, A. Yang, H. Lee, and Y. K. Chang, "High shear-assisted solvent extraction of lipid from wet biomass of Aurantiochytrium sp. KRS101," *Separation and Purification Technology*, vol. 227, p. 115666, Nov. 2019, doi: 10.1016/j.seppur.2019.06.004.

[11] G. Chi *et al.*, "Production of polyunsaturated fatty acids by Schizochytrium (Aurantiochytrium) spp.," *Biotechnology Advances*, vol. 55, p. 107897, Mar. 2022, doi: 10.1016/j.biotechadv.2021.107897.

[12] U. Manikrao Ingle, P. R. Pawar, and G. Prakash, "Acid-assisted oil extraction directly from thraustochytrids fermentation broth and its energy assessment for docosahexaenoic acid-enriched oil production," *Bioresource Technology*, vol. 367, p. 128272, Jan. 2023, doi: 10.1016/j.biortech.2022.128272.

[13] P. R. Dewati, Rochmadi, A. Rohman, and A. Budiman, "A Preliminary study of extraction and purification processes of astaxanthin from haematococcus pluvialisas a natural antioxidant," *IOP Conference Series: Materials Science and Engineering*, vol. 778, no. 1, p. 012032, Apr. 2020, doi: 10.1088/1757-899X/778/1/012032.

[14] E. Sulistiawati, R. Rochmadi, M. Hidayat, and A. Budiman, "Enhancement of phycocyanin extraction from dry spirulina platensis powder by freezing-thawing pre-treatment," *International Journal of Technology*, vol. 14, no. 4, p. 780, Jun. 2023, doi: 10.14716/ijtech.v14i4.5169.

[15] E. Sulistiawati, M. Setyawan, Z. Abidin, M. Darmawan, H. A. Makasar, and T. W. Pamungkas, "Performance comparison of phycobiliprotein extraction from spirulina platensis through stirring and freezing-thawing," *Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan*, vol. 18, no. 1, p. 41, Jun. 2023, doi: 10.15578/jpbkp.v18i1.912.

[16] A. S. Chauhan *et al.*, "Enhanced production of high-value polyunsaturated fatty acids (PUFAs) from potential thraustochytrid Aurantiochytrium sp.," *Bioresource Technology*, vol. 370, p. 128536, Feb. 2023, doi: 10.1016/j.biortech.2022.128536.

[17] M. A. Abdel-Wahab, A. E.-R. M. A. El-Samawaty, A. M. Elgorban, and A. H. Bahkali, "Utilization of low-cost substrates for the production of high biomass, lipid and docosahexaenoic acid (DHA) using local native strain Aurantiochytrium sp. YB-05," *Journal of King Saud University - Science*, vol. 34, no. 7, p. 102224, Oct. 2022, doi: 10.1016/j.jksus.2022.102224.

[18] L. Dong, F. Wang, L. Chen, and W. Zhang, "Metabolomic analysis reveals the responses of docosahexaenoic-acid-producing Schizochytrium under hyposalinity conditions," *Algal Research*, vol. 70, p. 102987, Mar. 2023, doi: 10.1016/j.algal.2023.102987.

[19] K. Yoneda, Y. Ishibashi, M. Yoshida, M. M. Watanabe, M. Ito, and I. Suzuki, "Proteomic and lipidomic analyses of lipid droplets in Aurantiochytrium limacinum ATCC MYA-1381," *Algal Research*, vol. 67, p. 102844, Sep. 2022, doi: 10.1016/j.algal.2022.102844.

[20] Z. Bao *et al.*, "Enhancement of lipid accumulation and docosahexaenoic acid synthesis in Schizochytrium sp. H016 by exogenous supplementation of sesamol," *Bioresource Technology*, vol. 345, p. 126527, Feb. 2022, doi: 10.1016/j.biortech.2021.126527.

[21] Y. Nazir, H. Halim, N. K. N. Al-Shorgani, V. Manikan, A. A. Hamid, and Y. Song, "Efficient conversion of extracts from low-cost, rejected fruits for high-valued Docosahexaenoic acid production by Aurantiochytrium sp. SW1," *Algal Research*, vol. 50, p.

101977, Sep. 2020, doi: 10.1016/j.algal.2020.101977.
[22] A. B. D. Nandiyanto, R. Oktiani, and R. Ragadhita, "How to read and interpret FTIR spectroscope of organic material," *Indonesian Journal of Science and Technology*, vol. 4, no. 1, p. 97, Mar. 2019, doi: 10.17509/ijost.v4i1.15806.
[23] K. Wahyuningsih, S. Yuliani, and H. Hoerudin, "Characteristics of silica nanoparticles from rice husk as influenced by surface modification with used solvent containing silane," *Journal of Engineering and Technological Sciences*, vol. 53, no. 4, p. 210403, Aug. 2021, doi: 10.5614/j.eng.technol.sci.2021.53.4.3.
[24] N. Nurlela, N. Ariesta, D. S. Laksono, E. Santosa, and T. Muhandri, "Characterization of glucomannan extracted from fresh porang tubers using ethanol technical grade," *Molekul*, vol. 16, no. 1, p. 1, Mar. 2021, doi: 10.20884/1.jm.2021.16.1.632.
[25] J. Presson, Y. I. Kedang, M. L. Guterres, R. E. Y. Adu, E. Korbafo, and H. Suseno, "Synthesis of biodiesel from feun kase (thevetia peruviana) seed oil using NaOH catalyst," *Jurnal Kimia Sains dan Aplikasi*, vol. 25, no. 8, pp. 270–279, Sep. 2022, doi: 10.14710/jksa.25.8.270-279.
[26] S. D. Marliyana *et al.*, "Stigmasterol and stigmasterone from methanol extract of calophyllum soulattri burm. f. stem bark," *Jurnal Kimia Sains dan Aplikasi*, vol. 24, no. 4, pp. 108–113, Apr. 2021, doi: 10.14710/jksa.24.4.108-113.

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