

Principal Component Analysis (PCA) for Evaluation of Fatty Acid Monoalkyl Ester (FAME) Quality towards Sustainable Biodiesel Production from Indonesian Microalgae Strains

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ABSTRACT

Biodiesel production from sustainably cultivated plant sources holds extremely high promise globally [6]. Microalgae have been intensely explored as a next-generation source for transport fuel production [9], as they combine attractive characteristics: rapid growth, high lipid content, and environmental benefits. Nevertheless, technical challenges abound regarding the feedstock potential, cultivation process, and its fatty acid mono-alkyl ester (FAME) properties. Performance evaluations for specific microalgal strains [2, 4, 10, 15] are thus of particular interest. The case of Indonesia is particularly significant due to the country's large size, population, biodiversity of terrestrial and marine plant species, and the variety of microalgae that can be harvested and used on an industrial scale for biodiesel production, especially in different media and cultivation methods. Many strains, such as *Botryococcus braunii*, *Chlorella* sp., *Chlamydomonas* sp., and *Nannochloropsis* sp., have a high identified potential [1, 5]. This paper presents the implementation of an established statistical methodology (Principal Component Analysis, PCA) to evaluate the potential of some nitrogen sources for sustainable biodiesel production from Indonesian microalgae strains.

Keywords: Biodiesel, Indonesia, Microalgae, Nitrogen, Principal Component Analysis

INTRODUCTION

Biodiesel is an alternative fuel supply for diesel engines composed of FAME derived from renewable feedstock. Biodiesel has received much scientific attention since it offers comparable fuel characteristics [3, 5]. It is generally produced from a feedstock of vegetable oils, animal fats, and any renewable stock through a chemical reaction called transesterification [1]. Compared to the conservative fuels, biodiesel is more manageable, storable, and transportable due to its biodegradability, high flash point, improved cetane number, and reduced exhaust emission [3, 4]. Nonetheless, the production of biodiesel from second-generation feedstock has led to major conversation concerning the competition between food and fuel. Conversely, the third generation of biodiesel derived from microalgae does not vie with food crops for arable land and water resources.

Microalgae like *Botryococcus braunii*, *Chlorella* sp., *Chlamydomonas* sp., and *Nannochloropsis* sp., which are the most potential strains that are typically found in Indonesian freshwater, marine water, and brackish water systems, are one of the most promising feedstocks for biodiesel production due to their strong adaptability to a wide range of conditions and their characteristics of rapid growth, high oil content, and especially sequestration of atmospheric CO₂ (**Figure 1**) [2, 4, 9]. Microalgae are predicted to produce biodiesel with 15–300 times more oil and 10 times more per unit area of land than a typical terrestrial oleaginous crop used in the first and second generations of biodiesel [2, 9, 10, 14].



Figure 1. Geographical Representation of Microalgae Biodiversity Research Across Indonesia [3].

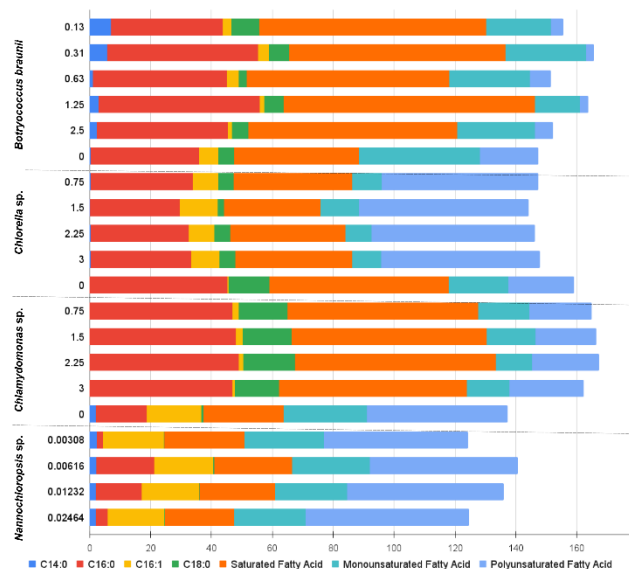


Figure 2. Fatty acid profiles across nitrogen concentration on *Botryococcus braunii*, *Chlorella* sp., *Chlamydomonas* sp., *Nannochloropsis* sp. [7, 16, 17].

Considering microalgae's potential and impact on the environment, numerous investigations have explored efforts to develop biodiesel production research, such as the different nitrogen sources' application impacts in microalgae cultivation [2, 4, 10, 15]. Nitrogen (N) is an essential core for microalgae's growth; it is a precipitant in all the structural and functional proteins such as chlorophylls, peptides, enzymes, energy transfer molecules, and genetic materials in algal cells. Nitrogen concentration in a culture medium strongly affects both cellular biochemical compositions and growth rate in microalgae, such as decreasing cell growth rate and raising the lipid or carbohydrate content, as well as reducing protein synthesis. It is shown in **Figure 2** that different nitrogen

sources and concentrations significantly influenced the FAME of all four microalgal strains, although the proportions and trend of the response were highly dependent on the strain [7, 16, 17]. Changes were observed not only in the total fatty accumulation but also in the relative distribution of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA). These results demonstrate that nitrogen restriction systematically influences lipid metabolism; nevertheless, the consequent FAME profiles are specified by variation in species strategies, which underscores the value of aligning nitrogen use with the microalgae selection to achieve ideal lipid compositions.

In terms of using variations of microalgae, there are several nitrogen sources that can be used in the cultivation stage of biodiesel production, such as KNO_3 , NaNO_3 , $\text{CO}(\text{NH}_2)_2$, NH_4HCO_3 , $(\text{NH}_4)_2\text{CO}_3$, NH_4NO_3 , NH_4Cl , and $(\text{NH}_4)_2\text{SO}_4$. Different ways to choose nitrogen sources have different effects on the biodiesel production performance; for example, ammonium (NH_4^+) and nitrate (NO_3^-) with either limitation or starvation conditions have been reported as the critical culturing conditions affecting lipid accumulation in many microalgae [15].

The research on variable nitrogen supplementation in microalgae cultivation has increasingly understood how different nitrogen sources might influence biodiesel production and analyzed it based on the FAME properties they have to find out the biodiesel productivity and its overall lipid quality.

The study stated that nitrogen does not simply affect biomass accumulation but also drives substantial shifts in the FAME profile, which then ultimately determines the fuel performance, stability, and suitability for the large-scale application. Therefore, to accurately characterize these shifts and to identify which nitrogen sources offer the most favorable outcomes for sustainable biodiesel generation, a thorough study of statistical processing methodology using principal component analysis (PCA) is needed to pinpoint the most promising potential nitrogen inputs for sustainable biodiesel production from diverse microalgae strains [3].

Various PCA models concerning FAME characteristics are prevalent in **Figure 3**. This study investigated four strains: *Botryococcus braunii*, *Chlorella* sp., *Chlamydomonas* sp., and *Nannochloropsis* sp., which are the most promising for high lipid and oil production in Indonesia, based on several studies about Advanced Biodiesel Production, as shown in **Table 1**. PCA works as a multivariate statistical method that combines the information from several variables and reduces them in order to focus on the essential features and information needed, the indicators with maximum variables [6].

METHODOLOGY

The PCA models we use are scree plot and biplot models. The scree plot model visualizes how much information each PC contributes to the dataset and plots it on the x-axis and the explained variance ratio on the y-axis; it helps us determine how many components should be retained from the graph. The scree plot in **Figure 4** showed us the amount of variances each principal component (PC) explains across all four microalgae strains. It is clear that PC1 and PC2 together explained most of the variance, which is sufficient for interpreting 85.9% of the overall data. The PCA biplot serves as a scatter plot to visualize the relationship between the zoriginal data and principal components.

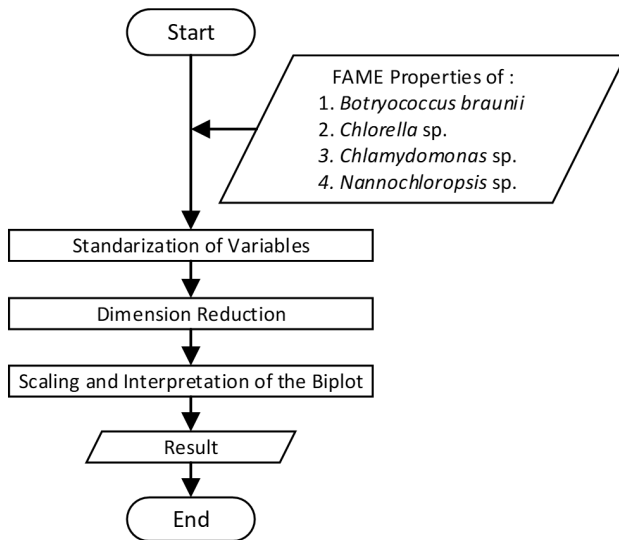


Figure 3. PCA Workflow on Different Nutrient Sources' Effects on Biodiesel Production from Indonesian Microalgae Strains [6].

The primary advantage of these approaches is the identification of the most effective nitrogen source for strain cultivation in biodiesel production. As shown in **Figure 5**, the PCA requires several steps to get the suitable and compatible variables for making the biplot: standardization of the variables, dimension reduction, scaling and interpreting the biplot, optional de-emphasizing of cases or variables in the biplot, and optional adding of supplementary variables to the biplot. Biplot in **Figure 5** demonstrates that microalgal species are clearly different from each other based on the FAME composition. PC1 displays the condition of lipid saturation very clearly, while PC2 illustrates the length of the chain and how it changes with monounsaturation. These distinctions show that each strain has its own technique of producing lipids and adjusting to its surroundings.

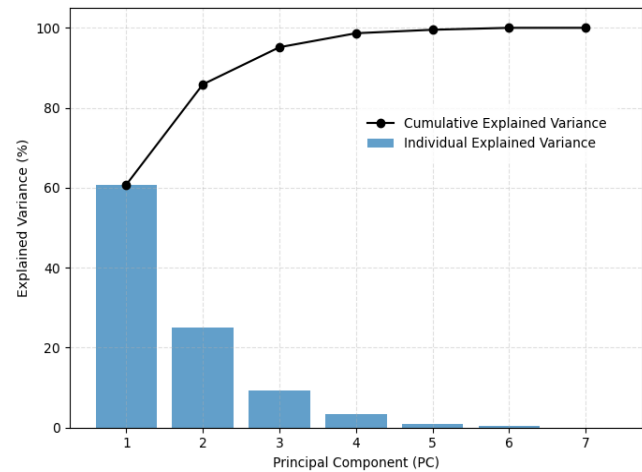


Figure 4. PCA scree plot highlighting Nitrogen concentration relationships between *Botryococcus braunii*, *Chlorella* sp., *Chlamydomonas* sp., and *Nannochloropsis* sp.

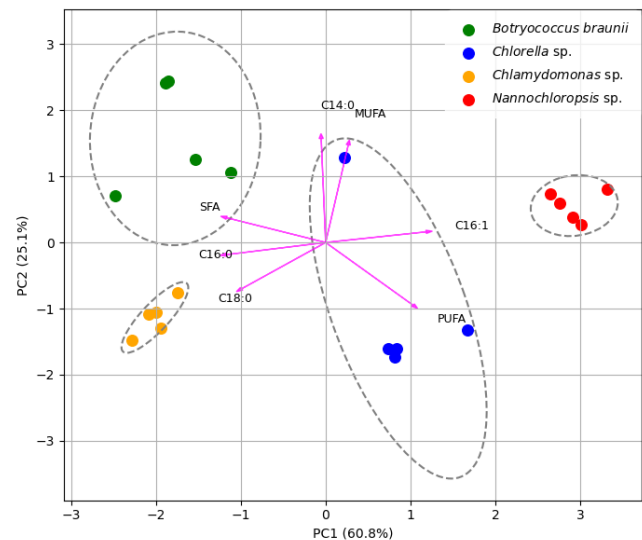


Figure 5. PCA biplot illustrating the relationships among *Botryococcus braunii*, *Chlorella* sp., *Chlamydomonas* sp., and *Nannochloropsis* sp. under different nitrogen concentrations, based on their FAME profiles.

This research focuses on the impact of nitrogen concentration and its linearity to the type of nitrogen used on Indonesian microalgae strains for bioethanol production. Therefore, the workflow we use reached the analytical point with the dataset we use from **Table 1** using the same scree plot and biplot method. In **Figure 6**, we use a similar scree plot to understand how much information we can gain from each dataset; we observe that PC1 explains the most variance, approximately 34.9%, indicating it captures the dominant pattern in the data. On the other hand, PC2 and PC3 make a big difference and contribute substantially with 26.1% and 19%. The explained variance therefore drops more gradually

after PC3 until it's almost negligible. There is a clear elbow around PC3 and PC4, which means the first two PCs are sufficient to represent most of the meaningful structure in the data.

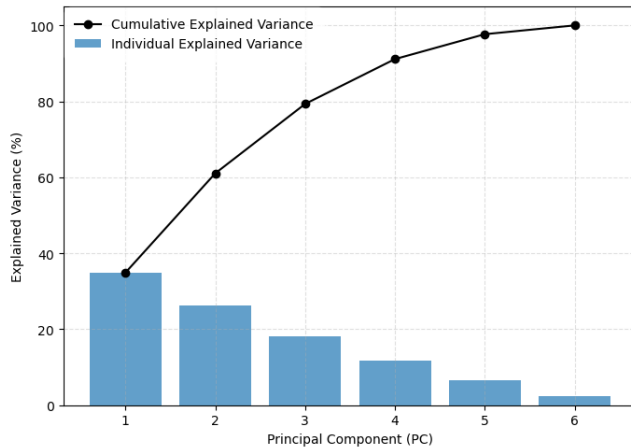


Figure 6. PCA scree plot showing the nitrogen source relationships among *Botryococcus braunii*, *Chlorella* sp., *Chlamydomonas* sp., and *Nannochloropsis* sp.

The FAME data from each strain included different FAME properties, including lauric acid (C12:0), tridecanoic acid (C13:0), tetradecanoic acid (C14:0), myristoleic acid (C14:1), trans-myristoleic acid (C14:1T), cis-myristoleic acid (C14:1C), pentadecanoic acid (C15:0), methyl pentadecanoic (C15:1), palmitic acid (C16:0), palmitoleic acid (C16:1), hexadecadienoic acid (C16:2), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), stearic acid (C18:0), elaidic acid (C18:1n9t), and oleic acid (C18:1n9c), as shown in **Table 1**. The main focus of this research is 4 different selected variables: lauric acid, tetradecanoic acid, palmitic acid, palmitoleic acid, stearic acid, and oleic acid. The lauric acid and tetradecanoic acid are medium-chain saturated fatty acids that increase biodiesel's cetane number and its combustion quality. Palmitic and stearic acid are long-chain saturated fatty acids that strongly increase the cetane number but significantly worsen the cold-flow behavior, such as higher cloud point, pour point, and Cold Filter Plugging Point (CFPP). Palmitoleic acid enhances cold-flow properties relative to saturated fats, while oleic acid is regarded as the optimal biodiesel component due to its strong balance between good cetane number, excellent cold-flow characteristics, and huge oxidative stability [11].

RESULTS & DISCUSSION

Using the scree plot and biplot, we have visualized clear patterns in how four microalgae strains responded to different nitrogen sources. The PCA results showed clear separation patterns driven by both strain

differences and the nitrogen source effect in **Figure 6** and **Figure 7**. The first principal component (PC1) shows that all four microalgae strains formed distinct clusters, indicating that the strains' identity is the dominant factor influencing the fatty acid composition. *Chlamydomonas* sp. was placed at the upper-right region of the score plot and reflected a slightly different behavior from the other strains, while the *Nannochloropsis* sp. formed a separate cluster in the upper-middle area. *Chlorella* sp. shows a tighter grouping in the lower-left area, which means that the lipid profile is more stable across different levels of nitrogen. On the other hand, *Botryococcus braunii* shows the most significant area along PC1, especially when $\text{CO}(\text{NH}_2)_2$ is present. The spread in this case means strain metabolism changes a lot depending on the nitrogen supply employed in the study.

The second main component (PC2) from **Table 1** data shows how different nitrogen sources are making any impact. Nitrogen sources like NH_4NO_3 and NH_4Cl made the PC2 trend in *Chlamydomonas* sp. increase significantly, showing how much they affect this strain's lipid metabolism. On the other hand, NaNO_3 circumstances tended to diminish PC2 values in many species. **Figure 7**'s biplot analysis showed that saturated fatty acids like C12:0, C13:0, C16:0, and C18:0 were the main factors that caused separation along PC1. This supports their significance in telling species apart. At the same time, fatty acids like C17:1 and C15:0 had greater loadings on PC2, which shows that they are sensitive to changes in nitrogen.

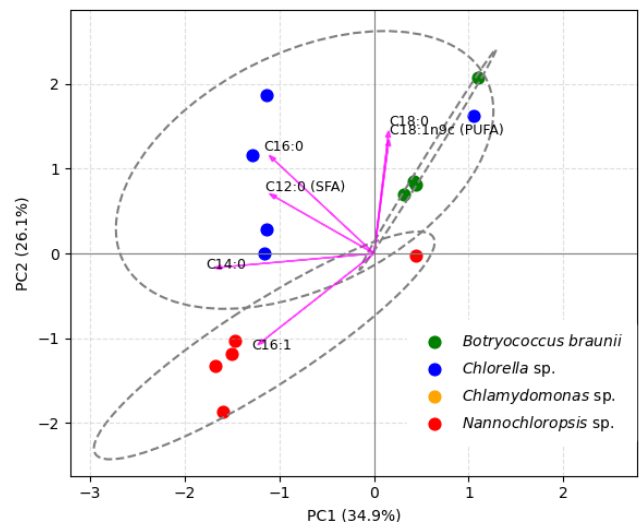


Figure 7. PCA biplot demonstrating the correlation among *Botryococcus braunii*, *Chlorella* sp., *Chlamydomonas* sp., and *Nannochloropsis* sp. under C12:0, C14:0, C16:1, C18:0, and C18:1N9C FAME properties.

The discussion in **Figure 6** and **Figure 7** analyzes all

Table 1. FAME profiles of selected Indonesian microalgae strains cultivated with various nitrogen sources. *[a] C12:0, [b] C13:0, [c] C14:0, [d] C14:1. [e] C14:1t, [f] C14:1c, [g] C15:0, [h] C15:1, [i] C16:0, [j] C16:1, [k] C17:0, [l] C17:1, [m] C18:0, [n] C18:1n9t, and [o] C18:1n9c.

Strains	Nitrogen Source	a*	b*	c*	d*	e*	f*	g*	h*
<i>Botry-ococcus braunii</i>	KNO ₃	6.96	6.71	1.41	1.92			0.5	0.24
	NaNO ₃	6.87	5.3	1.45	3.31			0.53	0.41
	CO(NH ₂) ₂	6.18	5.97	1.24	2.42			0.42	1.14
	NH ₄ HCO ₃	4.56	3.44	1.81	3.37			0.67	0.62
<i>Chlorella sp.</i>	NaNO ₃	14.3		5					
	CO(NH ₂) ₂	3.5		2.6					
	(NH ₄) ₂ CO ₃	21.4		4.2					
	KNO ₃	24.4		4.5					
<i>Chla-mydo-monas sp.</i>	NH ₄ NO ₃	23.1		4.9					
	NH ₄ NO ₃	0.31		0.4	0.05				
	NH ₄ Cl	0.29		0.32	0.05				
	KNO ₃	0.29		0.4	0.07				
<i>Nanno-chlo-ropsis sp.</i>	NH ₄ HCO ₃	0.2		5			0.9		
	(NH ₄) ₂ SO ₄			5.6			0.9		
	NaNO ₃			3.8			0.7		
	NH ₄ Cl	0.5		4.4		3.6			
	NH ₄ NO ₃	0		5.1			0.6		

Strains	Nitrogen Source	i*	j*	k*	l*	m*	n*	o*	Ref.
<i>Botry-ococcus braunii</i>	KNO ₃	43.25	4.17	3.62	0.12	0.8	0.01	17.8	[4]
	NaNO ₃	43.48	6.54	0.03	0.03	1.02	0.25	16.64	
	CO(NH ₂) ₂	32.88	4.78	9.93		13.23	0.17	10.3	
	NH ₄ HCO ₃	39.44	8.77	8.44		5.81	0.14	11.18	
<i>Chlorella sp.</i>	NaNO ₃	41	7.8			7.6		16.8	[15]
	CO(NH ₂) ₂	26.3	4.8	6.5				36.4	
	(NH ₄) ₂ CO ₃	39.7	7.6			.4.4		11.5	
	KNO ₃	28	7.5					7	
<i>Chla-mydo-monas sp.</i>	NH ₄ NO ₃	27.6	6			3.1		3.7	[2]
	NH ₄ NO ₃	10.2	2.91			1.89		4.95	
	NH ₄ Cl	9.7	3.6			1.6		4.61	
	KNO ₃	12.1	3.76			2.64		5.76	
<i>Nanno-chlo-ropsis sp.</i>	NH ₄ HCO ₃	38.2	32	0.2	0.3	1.7		9.6	[14]
	(NH ₄) ₂ SO ₄	26	29.7	0.6	1	0.2		6.1	
	NaNO ₃	33.8	0.7	0.4		2.3		9.6	
	NH ₄ Cl	34.8	29.2	0.2	0.3	2.2		6.8	
	NH ₄ NO ₃	32.2	30.7		0.5	1.6		6.9	

four strains with the specific chosen FAME properties, such C12:0, C14:0, C16:0, C16:1, C18:0, SFA, and PUFA. These FAME properties are explicitly showing us the lipid quality and having the majority of the data from all 4 strains analyzed. The scree plot in **Figure 6** shows that the first two principal components (PC1 and PC2) account for 61.0% of total variances, which means these two components sufficiently summarize the major

variability in Table 1 and justify the use of PC1 and PC2 plots for interpretation. The score plot above demonstrated that PC1 primarily separated all four strains' variables based on the inherent species-level differences. *Chlamydomonas sp.* formed a distinct cluster on the positive side of PC1, while *Nannochloropsis sp.* was very close to the negative side. The gap between the strains shows that there are significant differences in the fatty

acid properties. In contrast, *Botryococcus braunii* showed minimal dispersion, which means its fatty acid profile was relatively stable across the nitrogen treatments. On the other hand, *Chlorella* sp. indicated a steadier movement and metabolic response to different variants of nitrogen.

The biplot in **Figure 7** showed us further insight into the variables after integrating both sample positioning and contribution of fatty acids. C18:1n9c and C18:0 aligned strongly with the positive PC1 axis, indicating that these components were elevated particularly in *Chlamydomonas* sp. However, *Nannochloropsis* sp. seemed to be related with negative PC1 values, which were linked to greater amounts of shorter-chain fatty acids such C14:0 and C16:1. Along PC2, saturated fatty acids such as C12:0, C16:0, and partially C18:0, were positively aligned, reflecting the upward distribution of *Chlorella* sp., which means that these nitrogen sources caused more saturated lipids in this strain.

The combined PCA value shows that strains identity is the main thing that causes variance (PC1), though PC2 may reflect variation associated with the nitrogen treatments we used, as evidenced by the differential movement of all four strains along this axis. The results also point out the significant uniqueness of biochemical responses of each strain, *Chlamydomonas* sp. is characterized by longer-chain fatty acids, *Nannochloropsis* sp. by shorter-chain and monounsaturated fatty acids, and *Chlorella* sp. shows a deep sensitivity to nitrogen form through heightened saturated fatty acid accumulation. This multivariate method clearly shows how the amount of nitrogen changes the lipid makeup in different species.

CONCLUSIONS

PCA on this research effectively shows the kind of strain and nitrogen source affect FAME in Indonesian microalgae relevant to the biodiesel production. While PC1 stipulated that essential strain-level differences were the major causes of variance, PC2 captured additional variability that may be associated with different nitrogen source effects on lipid metabolism. *Botryococcus braunii* exhibited minimal dispersion which indicates a steady fatty acid profile across nitrogen treatments. *Chlamydomonas* sp. was characterized by longer-chain fatty acids, *Nannochloropsis* sp. by shorter-chain and monounsaturated fatty acids, and *Chlorella* sp. shows sensitivity to nitrogen form through heightened saturated fatty acid accumulation. These strain-specific lipid characteristics are relevant to biodiesel quality parameters such as oxidative stability and fuel performance, it concluded that all four Indonesian strains have great potential to be used in biodiesel through an appropriate nitrogen treatment.

REFERENCES

1. Aletheia SP, Syauqi A, Kelvin , Khaira K, Rafi MM. Techno-economic analysis of biodiesel and bioethanol production from chlorella sp. algae biomass. E3S Web Conf. 503:02004 (2024). <https://doi.org/10.1051/e3sconf/202450302004>
2. Amin NF, et al. Effect of Some Nitrogen Sources on Growth and Lipid of Microalgae *Chlorella* sp. For Biodiesel Production. *Journal of Applied Sciences Research* 9(8): 4845-4855 (2013).
3. Andriani F, Hendrayanti D, Yasman Y. Exploring microalgae diversity in indonesia: harnessing potential for lead bioremediation. BES 8:26-42 (2024). <https://doi.org/10.22236/jbes/13225>
4. Ashokkumar V, et al. Optimization and characterization of biodiesel production from microalgae *Botryococcus* grown at semi-continuous system. *Energy Conversion and Management* 88: 936-946 (2014). <http://dx.doi.org/10.1016/j.enconman.2014.09.019>
5. Deshmukh S, Kumar R, Bala K. Microalgae biodiesel: a review on oil extraction, fatty acid composition, properties and effect on engine performance and emissions. *Fuel Processing Technology* 191:232-247 (2019). <https://doi.org/10.1016/j.fuproc.2019.03.013>
6. Greenacre M, et al. Principal Component Analysis. *Economic Working Series* 1856. Nature Reviews Methods Primers 2(1) (2022). <https://doi.org/2010.1038/s43586-022-00184-w>
7. Huang X, Huang Z, Wen W, Yan J. Effects of nitrogen supplementation of the culture medium on the growth, total lipid content and fatty acid profiles of three microalgae (*tetraselmis subcordiformis*, *nannochloropsis oculata* and *pavlova viridis*). *J Appl Phycol* 25:129-137 (2012). <https://doi.org/10.1007/s10811-012-9846-9>
8. Huber GW, Iborra S, Corma A. Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering. *Chem. Rev.* 106:4044-4098 (2006). <https://doi.org/10.1021/cr068360d>
9. . World energy outlook 2022. OECD (2022). <https://doi.org/10.1787/3a469970-en>
10. Jahirul MI, Rasul MG, Brown RJ, Senadeera W, Hosen MA, Haque R, Saha SC, Mahlia TMI. Investigation of correlation between chemical composition and properties of biodiesel using principal component analysis (PCA) and artificial neural network (ANN). *Renewable Energy* 168:632-646 (2021). <https://doi.org/10.1016/j.renene.2020.12.078>
11. Kasmin H, Lazim AM, Awang R. Determination of fatty acid composition and quality characteristics of oils from palm fruits using solvent extraction. AIP

Conference Proceedings 1678:050002 (2015).

<https://doi.org/10.1063/1.4931281>

12. Lee THW, Show PL, Ong HC, Ling TC, Lan JCW, Chang JS. Microalgae isolation and cultivation technology for mass production. Handbook of Biorefinery Research and Technology :1-29 (2022).
https://doi.org/10.1007/978-94-007-6724-9_10-1
13. Lertwattanasakul N, Rodrussamee N, Kumakiri I, Pattanakittivorakul S, Yamada M. Potential of thermo-tolerant microorganisms for production of cellulosic bioethanol. Handbook of Biorefinery Research and Technology: Production of Biofuels and Biochemicals :33-62 (2024).
https://doi.org/10.1007/978-981-97-7586-6_35
14. Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. Renewable and Sustainable Energy Reviews 14:217-232 (2010).
<https://doi.org/10.1016/j.rser.2009.07.020>
15. An M, Wang Y, Liu F, Qi X, Zheng Z, Ye N, Sun C, Miao J. Biomass, nutrient uptake and fatty acid composition of chlamydomonas sp. ICE-L in response to different nitrogen sources. Acta Oceanol. Sin. 36:105-110 (2017).
<https://doi.org/10.1007/s13131-017-0984-4>
16. Ruangsomboon S. Effects of different media and nitrogen sources and levels on growth and lipid of green microalgae *Botryococcus braunii* KMITL and its biodiesel properties based on fatty acid composition. *Bioresource Technology* 191: 377-384 (2015).
<http://dx.doi.org/10.1016/j.biortech.2015.01.091>
17. Sivaramakrishnan R, Incharoensakdi A. Enhancement of total lipid yield by nitrogen, carbon, and iron supplementation in isolated microalgae. *Journal of Phycology* 53:855-868 (2017). <https://doi.org/10.1111/jpy.12549>

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