



PERFORMANCE OF MICROALGAE CONSORTIA IN PALM OIL MILL EFFLUENT REMEDIATION: AN ECO-FRIENDLY STRATEGY

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ABSTRACT

Palm oil production continues to grow in line with global market demand; however, production waste is also expected to increase. The treatment of Palm Oil Mill Effluent (POME) remains a critical environmental challenge due to its high organic load and complex pollutant composition, thereby requiring serious management. This study investigates the potential of four microalgal consortia in remediating POME. A quantitative experimental approach was employed to evaluate the efficiency of the microalgae consortium exposed to different POME concentrations (25%, 50%, and 75%) over 14 days. The results revealed that the three-species consortium CNT (*Chlorella vulgaris*, *Nannochloropsis oculata*, and *Tetraselmis chuii*) exhibited superior performance in reducing COD, BOD, and TSS, with removal efficiencies of 77.55%, 72.73%, and 49.7%, respectively, compared to the two-species consortium. The optimal physicochemical parameters supporting CNT growth were recorded at pH 9.19-9.6, 29.1-30.6 °C, and adaptive salinity up to 41 ppt. The biomass yield of the CNT consortium (3.862 g L⁻¹ at 25% POME) was lower than that of NT (4.671 g L⁻¹ at 25% POME), indicating that CNT allocated more energy to detoxifying and remediating POME than to biomass accumulation. Future research should focus on optimizing response surface methodology and scaling up photobioreactor systems through metagenomic and transcriptomic analyses to simultaneously maximize remediation efficiency and biomass productivity of the CNT consortium. These findings provide strategic opportunities to harness polyculture microalgae consortium as a sustainable, scalable, and economically viable technology for POME remediation.

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Keywords: bioremediation; microalgae consortium; POME; biomass productivity

INTRODUCTION

One of the most important economic sectors in the Tropics is the palm oil industry. Countries in Southeast Asia that dominate this industry are Indonesia and Malaysia, which are used in the food, cosmetics, and biofuel industries (Emparan et al., 2020). The palm oil industry has provided jobs directly and indirectly. Initially, the development of the palm oil industry prioritized social, economic, and environmental aspects in its implementation (Khatiwada et al., 2021). Ho-

wever, as the palm oil industry develops, it turns out to cause serious environmental problems. This is related to the liquid waste produced, namely Palm Oil Mill Effluent (POME). POME waste is produced as a by-product in large quantities during the palm oil extraction process (Mahidin et al., 2020). POME is a deep brown liquid waste with high temperatures and contains very high concentrations of COD and BOD, accompanied by high levels of suspended solids (TSS) and nutrients such as nitrogen and phosphorus (Kacaribu et al., 2025). If discharged into the environment without proper treatment, POME has the potential to cause serious water pollution, dis-

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rupt aquatic life, and promote eutrophication and odor pollution (Choong et al., 2018).

Conventional POME waste treatment methods require large land areas, take a long time to remove pollutants, and often still leave environmental problems (Soo et al., 2022). Serious environmental problems require the development of efficient, environmentally friendly POME waste treatment technologies. This development aims to eliminate pollutants and recover resources or energy derived from waste, in accordance with the principles of the circular economy (Siagian et al., 2024). One environmentally friendly waste treatment approach is bioremediation. Bioremediation is a process of removing pollutants that utilizes the activities of microorganisms, both in situ and ex situ, to degrade pollutants biologically and has economic value. Bioremediation degrades harmful substances such as heavy metals, hydrocarbons, polycyclic aromatics, and other toxic compounds. The advantages of bioremediation include its natural approach, relatively low operational costs, minimal disturbance to surrounding species during waste treatment, and potential for resource recovery (Almomani & Bohsale, 2021).

One of the unique and interesting bioremediation agents to research is microalgae (Kaloudas et al., 2021). Microalgae are photosynthetic organisms that can grow rapidly in various types of liquid waste, including POMEs, by utilizing nutrients (nitrogen and phosphorus) as well as carbon dioxide as their growth sources. In addition, microalgae used for waste remediation can be reused as fertilizer or animal feed (Devi et al., 2023). Microalgae can accumulate biomass rich in proteins, lipids (oils), carbohydrates, and pigments, making them potential feedstocks for various applications, such as biofuels (biodiesel), animal feed, biofertilizers, and other high-value products (Rashid et al., 2019; Chhandama et al., 2021). The microalgae species that have been widely researched for bioremediation are *Spirulina platensis*, *Chlorella* sp, *Tetraselmis chuii*, *Nannochloropsis oculata*, *Dunaliella salina*, etc.

Single-cultured microalgae for remediation have been extensively researched, but microalgae consortia remain under-researched. A consortium comprises two or more distinct species of microalgae with complementary properties and functions. The specific goal of microalgae consortia is to improve the efficiency of more complex biological processes compared to using a single species of microalgae. These cultivations can interact competitively or cooperatively (Rashid et al., 2019). The use of a consortium of microalgae is often superior to monoculture (one

species). Microalgae consortia improve waste treatment efficiency, enhance tolerance to variations in sewage conditions, and increase system stability (Gururani et al., 2022).

The application of microalgae consortia for POME waste bioremediation poses an interesting challenge due to its highly concentrated, cloudy, and high-temperature nature. This necessitates optimal adaptation of the microalgae consortium first (Raza et al., 2024). The POME waste to be applied also needs to be analyzed for several important parameters, such as pH, aeration, light intensity, salinity, BOD, COD, TSS, and temperature, so that it can achieve efficient waste treatment efficiency and optimal microalgae biomass production (Sari et al., 2022; Mohd et al., 2024). The parameters of palm oil mill effluent are adjusted in accordance with the Minister of Environment Regulation No. 5 of 2014 concerning the quality standards for palm oil industrial wastewater. Further research is needed to identify and isolate the consortium of local microalgae that are most suitable and resilient to the characteristics of POME. Species that are efficient in nutrient absorption and resistant to inhibition by toxic compounds in POME are key to the success of large-scale applications (Ahmad et al., 2022). The integration of microalgae bioremediation systems with other technologies, such as POME pretreatment or efficient biomass harvesting, can improve the economic feasibility and sustainability of this approach. The use of microalgae biomass as a value-added product can compensate for the cost of waste treatment (Liu & Hong, 2021; Sengupta et al., 2023; Uma et al., 2023). Therefore, this study aims to explore the potential of microalgae consortia in POME bioremediation as a sustainable wastewater treatment approach. The focus of this study is the efficiency of pollutant removal and the potential for microalgae biomass production from processed POME. The results of this study are expected to provide a deeper understanding of the mechanism of POME bioremediation by microalgae consortia, as well as to serve as the basis for the development of more innovative, efficient, and environmentally friendly POME processing technologies, supporting the sustainability of the palm oil industry in the future.

METHODS

Sampling and Characterization of POME

Palm Oil Mill Effluent (POME) was obtained from a palm oil industry in South Sulawesi, Indonesia. The POME sample was collected

from the wastewater treatment facility (IPAL) at sediment pond no. 13. For experimental purposes, a total of one gallon (15 L) of POME was collected and subsequently analyzed at the Universitas Negeri Semarang laboratory. The composition of POME in the treatment pond is presented in Table 1.

Table 1. Initial POME Waste Test on Pond No. 13

Parameters	Unit	Result	Method
BOD	mg/L	788	5210 B #)
COD	mg/L	2382	SNI 6989 2 2019
TSS	mg/L	1758	2504 D #)
Oils & fats	mg/L	< 2.7	SNI 6989 10 2011
Total N	mg/L	41,2	4500-NH3 C#)
pH		7,87	4500-H*B #)

Microalgae Cultivation

Three species of microalgae were used in this study: *Chlorella vulgaris*, *Nannochloropsis oculata*, and *Tetraselmis chuii*. These microalgae were obtained from the Brackish Water Aquaculture Research Institute (BBPAP) in Jepara, Indonesia. Prior to experimentation, each species was cultured as stock according to research requirements (Figure 1). The culture medium used was F/2 Guillard, prepared at a ratio of 1 mL of stock solution per 1 L of microalgae culture. Each species was cultivated in five separate jars, for a total of 15. Optical Density (OD) measurements were performed using a spectrophotometer at 680 nm. Microalgae selected for consortium preparation had an OD of approximately 0.300 ± 0.300 .



Figure 1. Microalgae Cultivation for POME Remediation

Phytoremediation Procedure of POME

When microalgae cultures reached the optimal OD ($\pm 0.300 \text{ \AA}$), a microalgae consortium was prepared at a 1:1 ratio for each treatment. Four types of consortiums were tested: *Chlorella vulgaris* with *Nannochloropsis oculata* (CN), *Chlo-*

rella vulgaris with *Tetraselmis chuii* (CT), *Nannochloropsis oculata* with *Tetraselmis chuii* (NT), and *Chlorella vulgaris* with *Nannochloropsis oculata* and *Tetraselmis chuii* (CNT). The consortium cultures were monitored for 14 days to achieve optimal OD for remediation. In addition, the microalgae consortium culture in POME remediation was repeated 3 times.

Subsequently, the consortium (CN, CT, NT, and CNT) with the optimal OD was exposed to POME at 25%, 50%, and 75% for 14 days. For instance, CN 25 refers to the CN consortium treated with 25% POME. The same coding system applies to other treatments. POME concentrations of 0% (consortium only) and 100% (without consortium) were used as controls to establish baseline conditions. Cell growth, pH, temperature, and salinity were monitored daily for 14 days. Cell growth was measured by OD using a UV-Vis spectrophotometer, while environmental parameters (pH, temperature, salinity) were measured using a pH meter, a thermometer, and a salinity refractometer. COD, BOD, TSS, and the morphology of the microalgae consortium was analyzed after 14 days of incubation.

Measurement of Chemical Oxygen Demand (COD)

COD was determined using the closed reflux spectrophotometric method. Both samples and blanks (distilled water) were prepared for COD measurement. A 10 mL sample was placed in a 50 mL Erlenmeyer flask and diluted to 30 mL. Then, 2.5 mL of the sample was transferred into a test tube containing 1.5 mL of $\text{K}_2\text{Cr}_2\text{O}_7$ and 3.5 mL of H_2SO_4 . The same procedure was applied to the blank. Samples and blanks were placed in a COD reactor at 150°C for 2 hours, then cooled to room temperature. COD absorbance was measured using a UV-VIS spectrophotometer (Thermo Scientific Genesys 10S UV-Vis). COD values were recorded in mg/L.

Measurement of Biological Oxygen Demand (BOD)

BOD was measured using the BOD_5 (5-day BOD test) method. Distilled water (2 L) was aerated for 1 hour and supplemented with nutrients, including phosphate buffer, CaCl_2 , FeCl_3 , and MgSO_4 , each at 1 mL. Two 100 mL bottles were prepared: the first for an initial DO measurement, and the second for a DO measurement after 5 days of incubation (DO_5).

For the initial DO measurement, 2 mL of sample was placed in a test tube and diluted to 60 mL with nutrient-enriched distilled water. Then, 1 mL each of alkali, iodine, and MnSO_4

was added, and the mixture was left for 15 minutes. Subsequently, 1 mL of H_2SO_4 was added, and the mixture was shaken until the precipitate dissolved. A clear sample (50 mL) was taken, 4–5 drops of starch indicator were added, and titration was performed using sodium thiosulfate until the solution became clear. The result was recorded. DO_5 was measured using the same procedure as the initial DO , but the sample was incubated at $20^\circ C$ for 5 days in the dark.

$$BOD \left(\frac{mg}{L} \right) = \frac{(D_i - D_f) \times P}{V}$$

Where:

- D_i : initial dissolved oxygen (mg/L)
 D_f : dissolved oxygen after 5 days of incubation (DO_5 , mg/L)
 P : the dilution factor
 V : sample volume (mL)

Measurement of Total Suspended Solids (TSS)

TSS was measured using the gravimetric method. Whatman filter paper was placed into a filter funnel. A total of 10 mL of distilled water was filtered through the paper, then dried until no water droplets remained. The filter paper was subsequently placed in an oven at $105^\circ C$ for 60 minutes, then cooled in a desiccator for 15 minutes. The filter paper was weighed using an analytical balance and recorded as the filter weight.

Next, 10 mL of the POME sample was prepared. The same procedure described above was applied to the sample until the final weight of the filter paper containing the sample residue was obtained. TSS was calculated using the following formula:

$$TSS \left(\frac{mg}{L} \right) = \frac{(W_f - W_i) \times 1000}{V}$$

Where:

- W_f : final weight of filter paper after sample filtration (mg)

W_i : initial weight of filter paper (mg)

V : volume of sample filtered (mL)

Measurement of microalgal consortium biomass

The microalgae consortium culture was centrifuged at 3000 rpm for 7 minutes, followed by filtration to determine the sample's wet weight. The resulting pellet was dried in an oven at $70^\circ C$ for 24 hours and subsequently weighed on an analytical balance (PX224e) to determine the sample's dry weight. Biomass was calculated using the following formula:

$$\text{Biomass concentration: } C \text{ (g L}^{-1}\text{)} = DW/V$$

$$\text{Biomass productivity: } p \text{ (g L}^{-1}\text{ day)} = \frac{X_2 - X_1}{t_2 - t_1}$$

$$\text{Specific growth rate: } \mu \text{ (day)} = \frac{\ln X_2 - \ln X_1}{t_2 - t_1}$$

Where:

- DW : dry weight of the sample (g)
 V : sample volume (L)
 X_1 : initial dry weight of the sample (g)
 X_2 : final dry weight of the sample (g)
 t_1 : initial observation time (days)
 t_2 : final observation time (days)

Statistical Analysis

The experiment was repeated three times. Differences in growth rates during the culture of microalgae consortia in POME were statistically analyzed using SPSS version 25 and Microsoft Excel. All data are presented as mean \pm standard error (SE), and a 5% significance level was used for statistical significance.

Morphology of Microalgal Consortium

Cell morphology of the microalgae consortium was observed using a Scanning Electron Microscope (SEM) to identify structural characteristics, cell integrity, and potential morphological changes during the remediation process.

The research flowchart is illustrated in Figure 2.

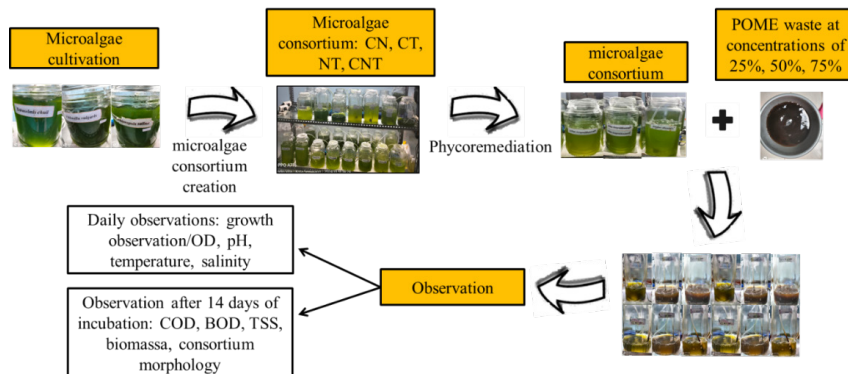


Figure 2. Research Flowchart

RESULTS AND DISCUSSION

COD removal efficiency

A significant increase in COD removal was observed from day 7 to day 14 of cultivation, indicating that a longer cultivation period enables the microalgal consortium to achieve its optimal capacity for mineralizing and metabolizing complex organic compounds in POME. On day 14, the CNT consortium demonstrated superior performance, achieving the highest COD removal efficiency of 77.55% in 50% POME, followed by 74.95% in 25% POME, and 64.74% in 75% POME. In contrast, the CT consortium exhibited the lowest performance, with a maximum efficiency of only 52.09% in 25% POME.

The CNT consortium demonstrated the best COD removal performance due to functional complementarity and metabolic diversity

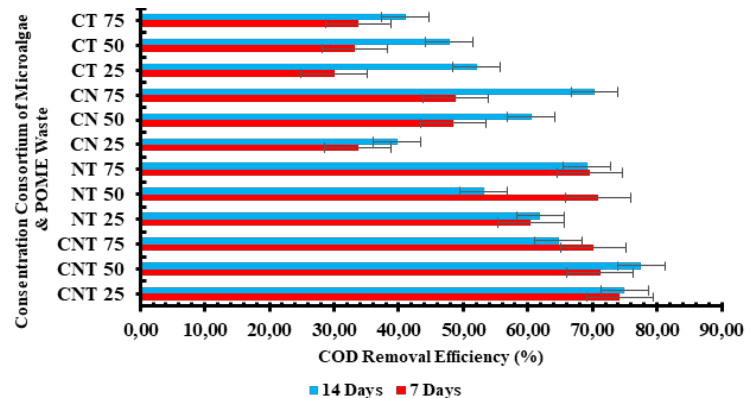


Figure 3. COD Removal Efficiency in Each Microalgal Consortium with Various POME Waste Variations In 7-Day And 14-Day Cultivation

The mechanism of COD removal by CNT is presumed to involve a series of biochemical processes activating both autotrophic and heterotrophic degradation pathways. The autotrophic pathway begins with the uptake of inorganic nutrients (N, P, K) from POME by all three microalgal species. *Chlorella vulgaris* acts as the primary nutrient accumulator, while *Nannochloropsis* and *Tetraselmis* assimilate specific minerals according to their niche preferences. During photosynthesis, all three species generate oxygen, which is subsequently utilized for the oxidative degradation of organic compounds bound in POME. *Tetraselmis* exploits the heterotrophic pathway, using dissolved organic carbon as an energy source, while the other two species perform photorespiration simultaneously (Putra et al., 2023).

Additionally, the consortium's production of exopolysaccharides (EPS) serves as a biofilm matrix, enhancing surface area and supporting enzyme-catalyzed degradation. Intermediate products excreted by one species serve as substrates for the others, preventing the accumulation of inhibitory intermediates and maximizing the

not found in a dual-species consortium. *Chlorella vulgaris* plays a role in rapid nutrient uptake and utilization of dissolved organic compounds; *Nannochloropsis oculata* contributes to osmoregulation and produces accessory pigments that enhance light-harvesting efficiency; while *Tetraselmis chuii* exhibits heterotrophic growth under dark conditions, adapts to high-COD POME with elevated salinity, and maintains metabolic stability. This synergistic combination enables CNT to degrade a broader spectrum of organic compounds in POME. In contrast, dual-species consortia such as CN or CT rely primarily on one species as the main degrader of organic compounds, while the other species plays only a limited complementary role, resulting in incomplete mineralization of organics in POME (Cheah et al., 2018; Hasnain et al., 2023).

complete oxidation of COD into CO_2 and H_2O (Alazaiza et al., 2023). The advantage of CNT lies in the ability of these three species to simultaneously execute multiple degradation pathways without inhibitory competition, resulting in a cumulative COD reduction efficiency of 77.55%, significantly higher than that of a dual-species consortium that activates only one or two limited pathways.

This three-species consortium aligns with findings from the past five years, emphasizing the importance of species diversity. The consortium of *Chlorella vulgaris* and *Nannochloropsis oculata* reduced COD in POME to 70.3% at 75% concentration under laboratory-ale conditions (Amalia et al., 2025). Moreover, a semi-continuous system based on a natural microalgal consortium achieved an efficiency of 81.3% after 12 days of cultivation (Nur et al., 2019).

BOD removal efficiency

One of the critical parameters of wastewater quality is Biological Oxygen Demand (BOD). The initial BOD content in POME was recorded

at 788 mg/L, which is attributed to the high levels of suspended solids, lipids, and carbohydrates resulting from the palm oil extraction process. According to Indonesian regulations, the permissible threshold for BOD discharge into the environment is 100 mg/L, whereas Malaysia imposes

a stricter limit of 20 mg/L for POME effluent. The BOD parameter is directly associated with the risk of eutrophication; therefore, monitoring BOD is essential in the remediation of POME using a microalgae consortium.

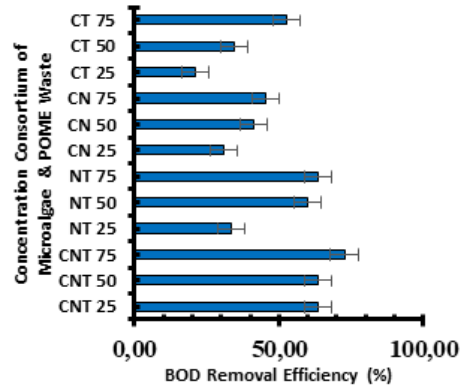


Figure 4. BOD Removal Efficiency of Each Microalgae Consortium with Various Variations of POME Waste

Based on Figure 4, the three-species microalgae consortium CNT at a 75% POME concentration exhibited the highest BOD reduction efficiency, achieving 72.73%. Among the two-species consortia, NT demonstrated relatively superior performance with BOD removal efficiencies ranging from 33.33% to 63.64% across all POME concentrations. In contrast, the CT consortium showed lower performance, with the lowest BOD removal efficiencies ranging from 21.21% to 52.73%.

The superior BOD removal capacity of the three-species consortium can be attributed to synergistic interactions among species within complex metabolic processes, where each species plays a unique role in utilizing different organic substrates. POME concentrations of 50–75% provided optimal nutrient availability to support the growth of the microalgae consortium. Previous studies have reported that microalgae consortium

achieved bio removal efficiencies of 57.8–88% in POME treatment due to the availability of nitrogen and phosphorus elements in microalgae metabolism (Sari et al., 2022)

TSS removal efficiency

The measurement of Total Suspended Solids (TSS) over 14 days of cultivation revealed that the highest TSS removal efficiency was achieved by the CNT consortium at 49.66%, followed by NT (47.95%), CN (42.72%), and the lowest performance was observed in CT with a reduction efficiency of 33.62% (Figure 6). All these results were obtained at 75% POME concentration. Across all concentration levels, the TSS removal efficiency of the four consortia ranged from 11.43% to 49.66%, indicating that higher POME concentrations supplied to the microalgae consortium resulted in lower TSS levels.

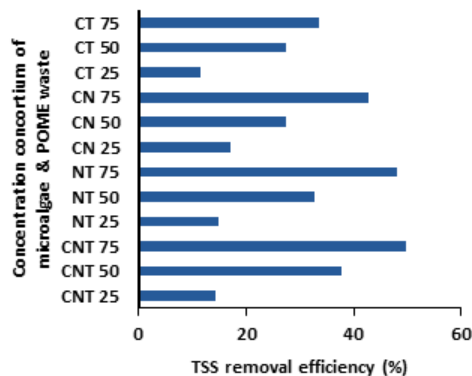


Figure 5. Total Suspended Solid (TSS) Removal Efficiency in Each Microalgae Consortium on Day 14

The superiority of the CNT consortium lies in the synergistic interactions among microalgae. *Chlorella vulgaris* contributes to high biomass production and efficient organic nutrient uptake (Kandimalla et al., 2016; Sidabutar et al., 2024), *Nannochloropsis oculata* plays a role in lipid optimization and salinity tolerance, and can remove most nitrogen and phosphate (Minturo et al., 2022), while *Tetraselmis chuii* enhances system stability (de Alva & Pabello, 2021). The three-species consortium creates high functional diversity, thereby reducing the risk of system failure if one species experience severe environmental stress. This consortium demonstrates greater stability than monocultures and exhibits high adaptability to variations in POME concentration.

Previous studies have reported that microalgae consortium efficiently reduced TSS levels by 80–95% through auto-flocculation, bio sedimentation, and biological adsorption (Nur et al., 2019). In auto flocculation and bio sedimentation, microalgae cells naturally aggregate to form flocs that trap pollutant particles, resulting in cells that contain absorbed TSS particles. These cells subsequently settle at the bottom of the medium (bio sedimentation) due to increased weight. In

biosorption, negatively charged cell walls bind and retain TSS and other organic pollutants through physicochemical interactions. In certain systems, microalgae may also form biofilms with bacteria or fungi, within which fine particles can be physically entrapped (Low et al., 2021).

Physicochemical parameters during the remediation process

An appropriate pH condition fosters optimal growth of the microalgae consortium, as pH directly influences cellular metabolism by affecting enzymatic activity. As shown in Figure 6, all consortia operated within the pH range of 8.4–9.75 during POME remediation. Increasing POME concentrations consistently elevated the medium's pH across all the consortia.

The pH tended to rise slightly over time, then stabilized toward the end of the 14-day observation period. This increase was attributed to microalgal photosynthetic activity, in which inorganic carbon (typically as CO_2 or HCO_3^-) was assimilated from the medium and converted into biomass. The uptake of inorganic carbon reduced hydrogen ion concentrations, thereby shifting the medium toward a more alkaline state.

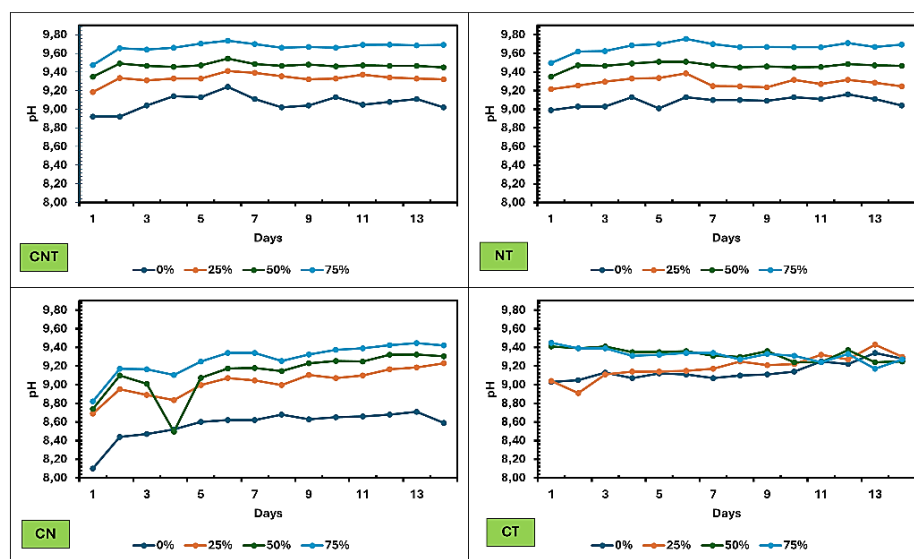


Figure 6. pH in a Microalgae Consortium with A Concentration of POME Waste Of 0%, 25%, 50%, and 75%

Consortium paired with *Tetraselmis chuii* (NT, CT, CNT) generally exhibited higher average pH values, whereas the CN consortium—without *T. chuii*—maintained lower pH levels. *Tetraselmis chuii* is recognized as a microalgae species tolerant to environmental variability, including pH fluctuations. Previous studies have reported that *Chlorella vulgaris* grows optimally at pH 7-7.5,

Nannochloropsis oculata at pH 8, and *Tetraselmis chuii* at pH 7-8.5 (Amalia et al., 2025). Research on another species within the genus, *Tetraselmis striata*, further confirmed that pH 8 supports optimal growth and biomass productivity (Patrinou et al., 2022). Daily monitoring of pH stability is therefore essential to maximize microalgal biomass productivity and to prevent oxidative stress.

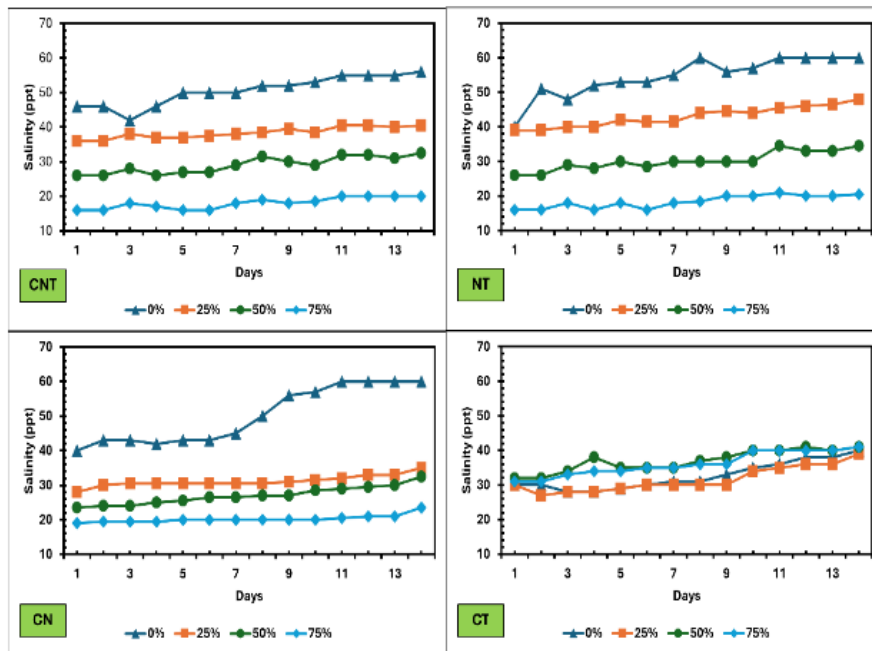


Figure 7. Salinity In Microalgae Consortia with POME Waste Concentrations Of 0%, 25%, 50% And 75%

As illustrated in Figure 7, each microalgae consortium demonstrates distinct salinity fluctuation patterns in response to varying concentrations of POME. Under the 0% POME control, the three consortia (CNT, NT, CN) consistently maintained high, stable salinity levels (CNT: 42-56 ppt; NT: 40-60 ppt; CN: 40-60 ppt), reflecting their intrinsic osmotic resilience in the absence of POME. Increasing POME concentrations led to a marked decline in salinity, which subsequently stabilized at lower levels. In contrast, the CT consortium exhibited a unique profile, maintaining relatively constant salinity (20-42 ppt) across both the control and all POME concentrations. This observation suggests that the CT consortium possesses comparatively limited osmoregulatory adaptation relative to the other consortium.

Salinity plays a pivotal role in regulating gas and nutrient solubility within the medium, thereby influencing metabolic activity and modulating other physicochemical parameters such as pH and temperature. Optimal salinity conditions are essential for sustaining microalgal growth, ensuring osmoregulatory balance, and enhancing photosynthetic efficiency. Microalgae consortium

employs several mechanisms to regulate salinity, including selective ion uptake from POME, metabolite excretion, and pH modulation (Ahmad et al., 2022; Muthukumaran et al., 2024; Kacaribu et al., 2025).

Cell density of the microalgae consortium

Cell density data in Figure 10 showed highly variable growth patterns among the four microalgae consortia at different POME concentrations. The peak cell density of the consortium (exponential phase) occurred between days 4 and 8 of remediation, followed by a stationary phase and, by day 14, a decline toward cell death. The CNT consortium exhibited the best performance, with the highest cell density and the most consistent growth under all waste conditions. At a 75% POME concentration, CNT reached a peak of 0.811 Å on day 8 of remediation, and its cell density remained stably high, with OD ranging from 0.656 to 0.798 Å until the end of the period. In contrast, the CT consortium showed the lowest cell density. Its peak cell density occurred on day 6 of remediation (OD ranging from 0.406 to 0.480 Å), then declined to the end of the period.

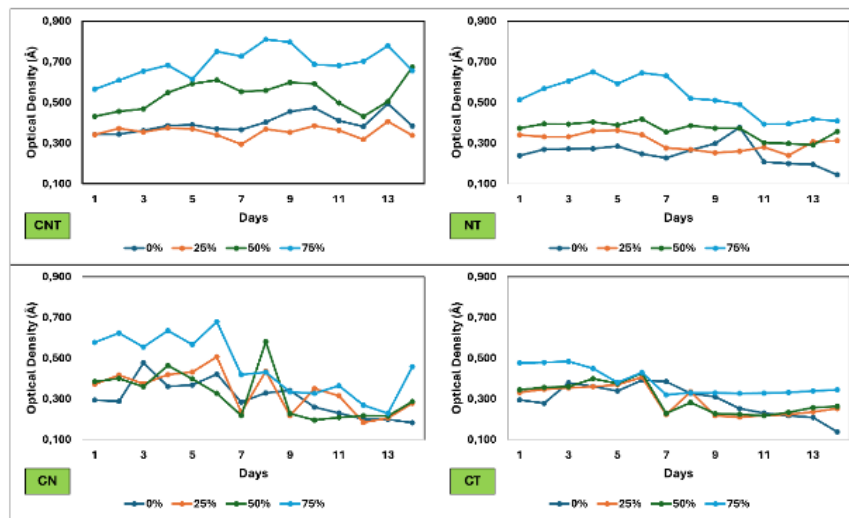


Figure 8. Optical Density in Each Microalgae Consortium in POME Waste Remediation For 14 Days

Cell density reflects the accumulation of microalgae biomass that produces metabolic enzymes for photosynthesis and organic matter degradation (Huang et al., 2024; Kong et al., 2024). In the CNT consortium, photosynthesis is presumed to be highly effective because the three species use different photosynthetic pigments to enhance efficiency. High cellular respiration in CNT also enables substantial ATP production for cell division and protein synthesis. This leads to rapid increases in microalgae biomass, thereby accelerating organic matter degradation. Consequently, CNT achieved higher COD, BOD, and TSS removal efficiencies in POME remediation compared to other consortia (Abate et al., 2024).

Productivity of microalgae consortium

The productivity of the microalgae consortium in POME remediation can be measured by biomass, biomass productivity, and specific growth rate (Table 2). At 25% POME concentration, the NT consortium achieved the highest biomass and biomass productivity, with values of 4.671 g/L and 0.244 g/L, respectively.

For the specific growth rate, the CN consortium showed the highest value of 0.111 μ /d at 25% POME. Previous studies have reported that mixed microalgae with equal proportions can more effectively exploit organic waste compounds, completely removing nitrogen and phosphate, compared to monocultures (Fallahi et al., 2020).

Table 2. Productivity Of Microalgae Consortium in POME Wastewater

Sample	Biomass Concentration (g L ⁻¹)	Biomass Productivity (g L ⁻¹ d ⁻¹)	Specific Growth Rate (μ d ⁻¹)
CNT 25	3,862	0,102	0,032
CNT 50	3,456	0,070	0,024
CNT 75	2,887	0,027	0,010
NT 25	4,671	0,244	0,088
NT 50	3,788	0,176	0,072
NT 75	1,681	0,014	0,009
CN 25	3,343	0,197	0,111
CN 50	2,793	0,154	0,098
CN 75	2,659	0,144	0,094
CT 25	3,230	0,169	0,088
CT 50	2,845	0,140	0,078
CT 75	2,624	0,123	0,072

Increasing POME concentration from 25% to 75% significantly reduced biomass productivity and growth across all microalgae consortia. This decline was likely caused by several factors, including the accumulation of toxic compounds in high-concentration POME that potentially inhibited photosynthesis and cellular metabolism; increased osmotic stress from POME exceeding the osmoregulatory capacity of each consortium, leading to cell plasmolysis; and the limitation of specific nutrients required by microalgae, particularly nitrogen and phosphorus, which were bound in complex forms and thus difficult to access.

Interestingly, the CNT consortium displayed a distinct pattern. While CNT showed the best performance in reducing COD, BOD, and TSS in POME, its biomass productivity and specific growth rate were lower than those of the dual-species consortium. Energy allocation in CNT was likely prioritized for detoxification and remediation of complex POME rather than for growth. Similar patterns have been reported in

other studies. A consortium combining *Scenedesmus* and *Tribonema* successfully reduced COD by up to 86.7%, but the species exhibited different biomass productivity levels (Gupta & Marchetti, 2024). Another study reported that *Chlorella*, *Scenedesmus*, and *Parachlorella* formed a less effective composition, resulting in decreased biomass productivity (Cho et al., 2020). Optimal bicultures are often easier to regulate and tend to show higher productivity compared to monocultures or polyspecific cultures (La Bella et al., 2023; Liu et al., 2024).

Salinity and pH affect the biomass produced by microalgae consortia. Salinity above 60 ppt causes microalgae death, and microalgae consortia are only effective in remediating POME between 40-50 ppt. Another environmental parameter, pH, also affects the biomass produced by microalgae consortia. The optimal pH for microalgae cultivation in POME is between 9 and 9.5.

Morphology of microalgae consortium

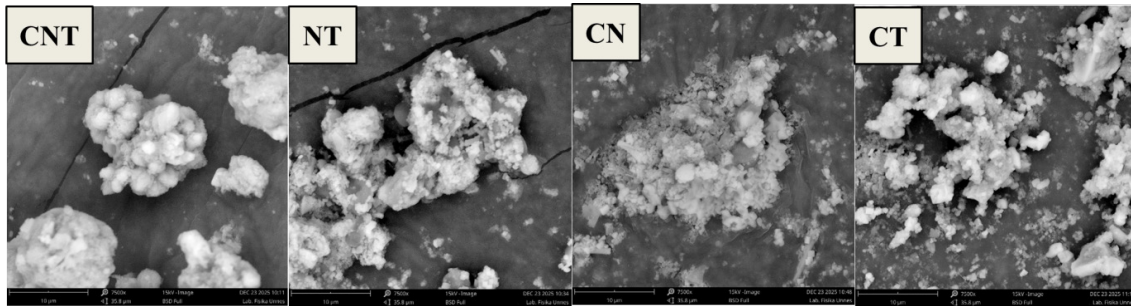


Figure 9. A Microalgae Consortium Observed Using a Scanning Electron Microscope (7500x)

Based on Figure 9, the CNT consortium exhibited well-ordered and organized cell aggregation with relatively clear boundaries between microalgae clusters. This indicates preservation of cellular membrane integrity. In contrast, the NT consortium displayed more loosely arranged aggregation patterns, though cellular structures remained intact. The CN consortium showed early signs of cellular damage with visible degradation at cell boundaries. The most significant damage was observed in the CT consortium, where SEM imagery revealed severe morphological disruption with collapsed cell structures and clearly damaged cell walls, indicating severe oxidative stress during POME remediation. These differences in morphological viability positively correlated with COD, BOD, and TSS removal efficiency: CNT and NT, which demonstrated structural preservation, achieved superior removal, while CT, with severe cellular damage, exhibited the lowest performance (Mathew et al., 2022). The superior surface morphology of

CNT cells are likely due to their greater capacity to mitigate oxidative stress during POME remediation. Enzymatic and non-enzymatic mechanisms in CNT operate more synergistically and powerfully in a multi-species system than in a dual-species consortium.

CONCLUSION

A three-species CNT microalgae consortium (*Chlorella vulgaris*, *Nannochloropsis oculata*, and *Tetraselmis chuii*) demonstrated superior performance in COD, BOD, and TSS removal compared to a two-species consortium. Optimal physicochemical parameters supporting CNT growth were recorded at pH 9.19-9.6, temperatures of 29.1–30.6 °C, and adaptive salinity up to 41 ppt. However, the biomass yield of the CNT consortium (3,862 g L⁻¹ at 25% POME) was lower than that of NT (4,671 g L⁻¹ at 25% POME), indicating that CNTs allocated more energy to POME detoxification and remediation

than biomass accumulation. The microalgae consortium demonstrated enhanced nutrient uptake and greater environmental resilience, making it a promising candidate for large-scale applications to validate its effectiveness and develop environmentally friendly strategies for addressing POME treatment.

REFERENCES

- Abate, R., Oon, Y. S., Oon, Y. L., & Bi, Y. (2024). Microalgae-bacteria nexus for environmental remediation and renewable energy resources: Advances, mechanisms and biotechnological applications. *Heliyon*, *10*(10).
- Ahmad, A., Banat, F., Alsafar, H., & Hasan, S. W. (2022). Algae biotechnology for industrial wastewater treatment, bioenergy production, and high-value bioproducts. *Science of the Total Environment*, *806*, 150585.
- Alazaiza, M. Y., He, S., Su, D., Abu Amr, S. S., Toh, P. Y., & Bashir, M. J. (2023). Sewage water treatment using *Chlorella vulgaris* microalgae for simultaneous nutrient separation and biomass production. *Separations*, *10*(4), 229.
- Ali, A., Khalid, Z., & Ajarem, J. S. (2023). Wastewater treatment by using microalgae: insights into fate, transport, and associated challenges. *Chemosphere*, *338*, 139501.
- Almomani, F., & Bhosale, R. R. (2021). Bio-sorption of toxic metals from industrial wastewater by algae strains *Spirulina platensis* and *Chlorella vulgaris*: Application of isotherm, kinetic models and process optimization. *Science of the Total Environment*, *755*, 142654.
- Amalia, A. V., Soeprbowati, T. R., & Christwardana, M. (2025). Optimization of consortia *Chlorella vulgaris* and *Nannochloropsis oculata* in remediation of palm oil mill effluent. In *E3S Web of Conferences* (Vol. 605, p. 03030). EDP Sciences.
- Azubuike, C. C., Chikere, C. B., & Okpokwasili, G. C. (2016). Bioremediation techniques—classification based on site of application: principles, advantages, limitations and prospects. *World Journal of Microbiology and Biotechnology*, *32*(11), 180.
- Cheah, W. Y., Show, P. L., Juan, J. C., Chang, J. S., & Ling, T. C. (2018). Microalgae cultivation in palm oil mill effluent (POME) for lipid production and pollutants removal. *Energy Conversion and Management*, *174*, 430-438.
- Chhandama, M. V. L., Satyan, K. B., Changmai, B., Vanlalveni, C., & Rokhum, S. L. (2021). Microalgae as a feedstock for the production of biodiesel: A review. *Bioresource Technology Reports*, *15*, 100771.
- Cho, D. H., Cho, K., Heo, J., Kim, U., Lee, Y. J., Choi, D. Y., Yoo, C., Kim, H. S., & Bae, S. (2020). High-throughput in vitro screening of changed algal community structure using the photobio-box. *Journal of Microbiology and Biotechnology*, *30*(11), 1–7.
- Choong, Y. Y., Chou, K. W., & Norli, I. (2018). Strategies for improving biogas production of palm oil mill effluent (POME) anaerobic digestion: A critical review. *Renewable and Sustainable Energy Reviews*, *82*, 2993-3006.
- de Alva, M. S., & Pabello, V. M. L. (2021). Phycoremediation by simulating marine aquaculture effluent using *Tetraselmis* sp. and the potential use of the resulting biomass. *Journal of Water Process Engineering*, *41*, 102071.
- Devi, A., Verma, M., Saratale, G. D., Saratale, R. G., Ferreira, L. F. R., Mulla, S. I., & Bharagava, R. N. (2023). Microalgae: a green eco-friendly agents for bioremediation of tannery wastewater with simultaneous production of value-added products. *Chemosphere*, *336*, 139192.
- Emparan, Q., Jye, Y. S., Danquah, M. K., & Harun, R. (2020). Cultivation of *Nannochloropsis* sp. microalgae in palm oil mill effluent (POME) media for phycoremediation and biomass production: Effect of microalgae cells with and without beads. *Journal of Water Process Engineering*, *33*, 101043.
- Fallahi, A., Hajinajaf, N., Tavakoli, O., & Sarrafzadeh, M. H. (2020). Cultivation of mixed microalgae using municipal wastewater: Biomass productivity, nutrient removal, and biochemical content. *Iranian Journal of Biotechnology*, *18*(4), 88–97.
- Goswami, R. K., Mehariya, S., & Verma, P. (2024). Sub-pilot scale sequential microalgal consortium-based cultivation for treatment of municipal wastewater and biomass production. *Environmental Pollution*, *348*, 123796.
- Gupta, S., & Marchetti, J. M. (2024). Co-cultivation of high-value microalgae species with filamentous microalgae for dairy wastewater treatment. *Npj Clean Water*, *7*(1).
- Gururani, P., Bhatnagar, P., Kumar, V., Vlaskin, M. S., & Grigorenko, A. V. (2022). Algal consortiums: a novel and integrated approach for wastewater treatment. *Water*, *14*(22), 3784.
- Hasnain, M., Abideen, Z., Hashmi, S., Naz, S., & Munir, N. (2023). Assessing the potential of nutrient deficiency for enhancement of biodiesel production in algal resources. *Biofuels*, *14*(1), 1–34.
- Huang, F., Liu, Z., Luo, D., Xu, Z., Wei, K., He, N., & Sun, X. (2024). Microalgae-bacterial consortiums for enhanced degradation of nonylphenol: Biodegradability and kinetic analysis. *Journal of Environmental Management*, *370*.
- Kacaribu, A. A., Aisyah, Y., & Febriani, F. (2025). Development of wastewater treatment methods for palm oil mill effluent (POME): A comprehensive review. *Resources Chemicals and Materials*, 100130.
- Kaloudas, D., Pavlova, N., & Penchovsky, R. (2021). Phycoremediation of wastewater by microalgae: a review. *Environmental Chemistry Letters*, *19*(4), 2905-2920.

- Kandimalla, P., Desi, S., & Vurimindi, H. (2016). Mixotrophic cultivation of microalgae using industrial flue gases for biodiesel production. *Environmental Science and Pollution Research*, 23(10), 9345–9354.
- Khawiwada, D., Palmén, C., & Silveira, S. (2021). Evaluating the palm oil demand in Indonesia: production trends, yields, and emerging issues. *Biofuels*, 12(2), 135–147.
- Kong, T., Li, A., & Zhang, L. (2025). Microalgae-based wastewater treatment: mechanisms, strategies, and the role of biochemical composition. *Journal of Environmental Chemical Engineering*, 118131.
- Kong, W., Kong, J., Feng, S., Yang, T., Xu, L., Shen, B., ... & Lyu, H. (2024). Cultivation of microalgae–bacteria consortium by waste gas–waste water to achieve CO₂ fixation, wastewater purification and bioproducts production. *Biotechnology for biofuels and bioproducts*, 17(1), 26.
- Kurniawan, T. A., Ali, M., Mohyuddin, A., Haider, A., Othman, M. H. D., Anouzla, A., ... & Al-sultan, G. A. (2025). Innovative transformation of palm oil biomass waste into sustainable biofuel: Technological breakthroughs and future prospects. *Process Safety and Environmental Protection*, 193, 643–664.
- La Bella, E., Occhipinti, P. S., Puglisi, I., Fragalà, F., Saccone, R., Russo, N., ... & Baglieri, A. (2023). Comparative phycoremediation performance of three microalgae species in two different magnitude of pollutants in wastewater from farmhouse. *Sustainability*, 15(15), 11644.
- Liu, W., Shang, Q., Fu, D., Shi, W., & Singh, R. P. (2024). Development of microalgal dual-cultures for enhanced productivity in sewage: An experimental study. *Biomass and Bioenergy*, 180.
- Liu, X. Y., & Hong, Y. (2021). Microalgae-based wastewater treatment and recovery with biomass and value-added products: a brief review. *Current Pollution Reports*, 7(2), 227–245.
- Low, S. S., Bong, K. X., Mubashir, M., Cheng, C. K., Lam, M. K., Lim, J. W., ... & Show, P. L. (2021). Microalgae cultivation in palm oil mill effluent (POME) treatment and biofuel production. *Sustainability*, 13(6), 3247.
- Mahidin, S., Erdiwansyah, H., Hisbullah, H., Zhafran, M., Sidiq, M. A., Rinaldi, A., ... & Bindar, Y. (2020). Analysis of power from palm oil solid waste for biomass power plants: A case study in Aceh Province. *Chemosphere*, 253, 126714.
- Mandal, S., & Corcoran, A. A. (2022). A novel approach to build algal consortia for sustainable biomass production. *Algal Research*, 65.
- Mathew, M. M., Khatana, K., Vats, V., Dhanker, R., Kumar, R., Dahms, H. U., & Hwang, J. S. (2022). Biological approaches integrating algae and bacteria for the degradation of wastewater contaminants—A review. *Frontiers in Microbiology*, 12, 801051.
- Minturo, G. J., Noorain, R., Hitam, S. M. S., Shoiful, A., Azni, M. E., Ernawati, L., ... & Mohamad, R. (2022, April). Immobilized Nannochloropsis oculata in a down-flow hanging sponge (DHS) reactor for the treatment of palm oil mill effluent (POME). In *IOP Conference Series: Earth and Environmental Science* (Vol. 1017, No. 1, p. 012024). IOP Publishing.
- Mohd, N., Yasin, N. H. M., Osman, W. H. W., & Takriff, M. S. (2024). Unlocking the potential of Chlamydomonas sp. for sustainable nutrient removal from POME: A biokinetic investigation. *Journal of Water Process Engineering*, 57, 104590.
- Muthukumar, M., Rawindran, H., Noorjahan, A., Parveen, M., Barasarathi, J., Blessie, J. J., ... & Balakumar, B. S. (2024). Microalgae-based solutions for palm oil mill effluent management: Integrating phycoremediation, biomass and biodiesel production for a greener future. *Biomass and Bioenergy*, 191, 107445.
- Nur, M. M. A., & Buma, A. G. (2019). Opportunities and challenges of microalgal cultivation on wastewater, with special focus on palm oil mill effluent and the production of high value compounds. *Waste and Biomass Valorization*, 10(8), 2079–2097.
- Nur, M. A., Garcia, G. M., Boelen, P., & Buma, A. G. J. (2019). Enhancement of C-phycocyanin productivity by Arthrospira platensis when growing on palm oil mill effluent in a two-stage semi-continuous cultivation mode. *Journal of Applied Phycology*, 31(5), 2855–2867.
- Parveen, R. R., & Selvasembian, R. (2025). Harnessing microalgal consortia potential for the tannery effluent treatment and sustainable valorization of algal biomass. *Journal of Environmental Chemical Engineering*, 13(4), 117088.
- Patrinou, V., Daskalaki, A., Kampantais, D., Kanakis, D. C., Economou, C. N., Bokas, D., Kotzamanis, Y., Aggelis, G., Vayenas, D. V., & Tekerlekopoulou, A. G. (2022). Optimization of Cultivation Conditions for Tetraselmis striata and Biomass Quality Evaluation for Fish Feed Production. *Water (Switzerland)*, 14(19).
- Putra, S. E., Marten, T. W., Yuniarti, E., & Handayani, L. (2023). Potential of Chlorella sp As A Bioremediation Agent in Tofu Liquid Waste: Literature Review. *Jurnal Biologi Tropis*, 23(1), 140–146.
- Rashid, N., Ryu, A. J., Jeong, K. J., Lee, B., & Chang, Y. K. (2019). Co-cultivation of two freshwater microalgae species to improve biomass productivity and biodiesel production. *Energy Conversion and Management*, 196, 640–648.
- Raza, N., Rizwan, M., & Mujtaba, G. (2024). Bioremediation of real textile wastewater with a microalgal-bacterial consortium: an eco-friendly strategy. *Biomass Conversion and Biorefinery*, 14(6), 7359–7371.
- Sari, F. Y. A., Suryajaya, I. M. A., Christwardana, M., & Hadiyanto, H. (2022). Cultivation of Microalgae Spirulina platensis in Palm Oil Mill Effluent (POME) Media with Variations of

- POME Concentration and Nutrient Composition. *Journal of Bioresources and Environmental Sciences*, 1(2), 57–62.
- Sengupta, S. L., Chaudhuri, R. G., & Dutta, S. (2023). A critical review on phycoremediation of pollutants from wastewater—a novel algae-based secondary treatment with the opportunities of production of value-added products. *Environmental Science and Pollution Research*, 30(54), 114844-114872.
- Siagian, U. W. R., Wenten, I. G., & Khoiruddin, K. (2024). Circular economy approaches in the palm oil industry: Enhancing profitability through waste reduction and product diversification. *Journal of Engineering and Technological Sciences*, 56(1), 25-49.
- Sidabutar, R., Trisakti, B., Bani, O., Nasution, J. A., Khodijah, P., Alexander, V., ... & Takriff, M. S. (2024). Treatment of effluent from the up-flow anaerobic sludge blanket-hollow centered packed bed fermentor by utilizing *Chlorella vulgaris* in a fed-batch system. *Case Studies in Chemical and Environmental Engineering*, 9, 100756.
- Soo, P. L., Bashir, M. J., & Wong, L. P. (2022). Recent advancements in the treatment of palm oil mill effluent (POME) using anaerobic biofilm reactors: Challenges and future perspectives. *Journal of Environmental Management*, 320, 115750.
- Suparmaniam, U., Lam, M. K., Lim, J. W., Tan, I. S., Chin, B. L. F., Shuit, S. H., ... & Kiew, P. L. (2024). Abiotic stress as a dynamic strategy for enhancing high value phytochemicals in microalgae: Critical insights, challenges and future prospects. *Biotechnology Advances*, 70, 108280.
- Uma, V. S., Usmani, Z., Sharma, M., Diwan, D., Sharma, M., Guo, M., ... & Gupta, V. K. (2023). Valorisation of algal biomass to value-added metabolites: emerging trends and opportunities. *Phytochemistry Reviews*, 22(4), 1015-1040.