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Evaluation of Microalgae *Chlorella vulgaris* and *Tetradesmus bernardii* for Cultivation and Nutrient Removal in Palm Oil Mill Effluent

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ABSTRACT

The palm oil industry is one of the key players in contributing to Malaysia's economy. Palm oil mill effluent (POME), a significant by-product of the oil extraction process, requires mandatory remediation to ensure proper treatment and disposal. Bioremediation using microalgae is a cost-effective and sustainable approach. This study aims to utilise pure and mixed microalgal species, *Chlorella vulgaris* and *Tetradesmus bernardii*, in phycoremediation and biomass production in different concentrations of POME (20%, 40%, 60%, and 80%). Cultivation of microalgae was carried out in 200 mL medium with pH 7–7.8, room temperature of 25±1°C for 21 days and continuous light illumination at 2000 lux. The highest biomass productivity was observed in 20% POME for mixed microalgae (mean = 0.1733 mg.mL^{-1} ± 0.0057), followed by *C. vulgaris* (0.1633 mg.mL^{-1} ± 0.0057) and *T. bernardii* (0.1603 mg.mL^{-1} ± 0.0020). Similarly, the highest nutrient removal was observed in 20% POME for mixed microalgae (COD:66.9801%, TN:86.9565%, TP:86.9655%), followed by *C. vulgaris* and *T. bernardii*. The results showed positive effects on growth, increased biomass production, and nutrient removal, with 20% POME being the optimal concentration for microalgae. Valuable by-products, such as high-quality pigments and biomass, are also generated by applying microalgae for remediation. Mixed microalgae are superior in the remediation of POME compared to single-culture algae. Treating wastewater through microalgal bioremediation is

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highly efficient in nutrient removal. This research has contributed towards the use of mixed microalgae to achieve effective nutrient removal and biomass for future industrial applications.

Keywords: Biomass, *Chlorella vulgaris*, growth, Malaysia, microalgae, palm oil mill effluent (POME), phycoremediation, *Tetradesmus bernardii*

INTRODUCTION

Malaysia is the second-largest producer and exporter of palm oil globally. (Khatun et al., 2017). Manufacturing of palm oil 2020 was 19.14 million tonnes, with the total export of palm products amounting to 26.73 million tonnes (Haryati et al., 2022). Palm oil manufacturing is expected to raise production by 25 million tonnes in 2035. According to the Food and Agriculture Organization (FAO), the demand for palm oil is anticipated to hit 156 million tonnes by 2050 (Chew et al., 2021). The palm oil expansion has contributed to a large portion of the country's Gross National Income (GNI), contributing towards 47% of global production (Rowland et al., 2022).

The palm oil industry by-products include empty fruit bunches, mesocarps, fibres, shells, and palm oil mill effluent (POME) (Singh et al., 2010). Manufacturing palm oil requires an extensive amount of water. One tonne of palm oil requires 5–7 tonnes of water for processing, with 2.5 m^3 of POME produced (Ratnasari et al., 2021). Wastewater produced by POME contains various dissolved and suspended contaminants consisting of 95%–96% water, 4%–5% total solids, and 0.6%–0.7% oil (Kamyab et al., 2015). POME consists of a high level of nutrients such as nitrogen, phosphorus, and ammonia; exceeding the level of these nutrients will cause contamination of the aquatic zone (Karim et al., 2021). The production of huge volumes of by-products during oil extraction may contaminate water bodies (Ahmad et al., 2016). Furthermore, the release of untreated POME can have a crucial impact on the diversity of phytoplankton, a disorder in the aquatic ecosystems' reproductive and physiological systems of fishes (Bala et al., 2015).

The conventional way of removing nutrients from palm oil mill effluent include using an open pond, phytoremediation, supercritical water gasification, aerobic-anaerobic process, coagulation-flocculation, and another option is using electrochemical advanced oxidation process (Kamyab et al., 2022). This paper focuses on the use of microalgae for the remediation and nutrient removal of palm oil mill effluent. Valuable by-products, such as high-quality pigments and biomass, are also generated by applying microalgae for remediation.

Several unconventional methods exist for POME treatment but are not without problems. These include adsorption, membrane filtration and electrochemical oxidation. The adsorption technique uses adsorbing materials such as activated carbon, clay, or other

porous substances with a high surface area that can attract and trap pollutants through physical and chemical interactions. However, the adsorbent has a limited capacity to adsorb contaminants. Once saturated, they need to be replaced or regenerated, which can be costly and require additional energy input, impacting the overall environmental footprint of the treatment process. The adsorption selection and specific contaminant removal can complicate the process (Mohammed, 2013).

Membrane filtration is a separation process that uses a semipermeable membrane to remove suspended solids, oils, and other impurities from the effluent. The membrane can become fouled or clogged over time, reducing their efficiency. Pre-treatment, regular cleaning, and maintenance have caused the elevation of the operational cost for the membrane filtration method (Azmi & Yunos, 2014; Udaiyappan et al., 2021). Electrochemical advanced oxidation processes (EAOPs) are innovative techniques that harness the in situ electro-generation of reactive oxygen species (ROS) and other powerful oxidant species, enabling the efficient mineralisation of organic compounds into carbon dioxide CO_2, water, and essential inorganic ions (Kamyab et al., 2021). The choice of (EAOPs) and the system's complexity should be carefully considered in terms of energy consumption, cost, and expertise.

Biological treatment, especially microalgal treatment methods, offers unique advantages compared to other methods. Microalgae are highly efficient at removing nutrients such as nitrogen and phosphorous from POME. They can reduce nutrient content to a level that meets environmental discharge standards, potentially facilitating nutrient recovery to reuse (Khalid et al., 2016). Microalgae can rapidly grow and multiply in POME, leading to biomass production; during the process, it can sequestrate the CO_2. Microalgae are adaptable to a wide range of environmental conditions and can thrive in different POME compositions, making them a versatile option for treatment (Hazman et al., 2018). The microalgae-based treatment of POME offers unique benefits related to nutrient removal, biomass production, carbon capture, and resource recovery. It represents a promising and environmentally sustainable approach for POME treatment, especially. In the context of sustainability and circular economy goals (Anto et al., 2020; Cheah et al., 2018).

Microalgae serve as multifunctional agents in wastewater treatment, with capabilities encompassing the efficient removal of excess nutrients, reduction of chemical oxygen demand (COD), and the accumulation of heavy metals through biosorption. These microorganisms also effectively inhibit harmful pathogen growth and, notably, enhance the visual appeal of treated water. Their comprehensive role is pivotal in preventing eutrophication, enhancing water quality, and beautifying treated water, underscoring their significance in sustainable wastewater remediation. Studies used microalgae species, *Chlorella vulgaris*, *Characium* sp., and *Scenedesmus* sp. for remediation of POME, municipal and polluted surface water (Talib et al., 2023).

Microalgae effectively capture carbon dioxide (CO_2) during photosynthesis and store it as biomass in the form of complex carbohydrates and lipids. Lipids can be used for biofuel applications, such as biodiesel, offering a renewable energy source. Among the microalgal species, *Chlorella* sp., *Chlamydomonas* sp., and *Nannochloropsis* were used in remediating palm oil mill effluent (POME), CO_2 sequestration, and biomass production (Ding et al., 2016; Hariz et al., 2018; Resdi et al., 2021). *Haematococcus pluvialis* and *Chromochloris zofingiensis* were used for phycoremediation of POME and producing valuable by-product, pigment astaxanthin (Fernando et al., 2021). Applications of astaxanthin can be used in diverse industries such as healthcare, cosmetics, and aquaculture.

Microalgae Roles in Wastewater Treatment and Bioremediation

***Chlorella vulgaris/Chlorella* sp.** *Chlorella vulgaris* has been widely studied due to its potential in various industries, such as supplementary food that provides essential nutrients (Orusmurzaeva et al., 2022). Mass cultivation of *Chlorella* sp. has produced a large amount of biomass that has been incorporated into various products such as animal feed (Medvedeva et al., 2022), aquaculture (Ahmad et al., 2020), cosmetics (Morais et al., 2020) and pharmaceutical as it contains a significant concentration of carotenoids that associated with anti-inflammatory and antioxidant properties (Velmurugan & Muthukaliannan, 2022). Another sector that has benefited from this alga is wastewater treatment and bioremediation. Studies have been conducted to assimilate them in heavy metal treatment of aqueous environment, such as removing cadmium and zinc (Al-Khiat et al., 2023; Ahmad et al., 2017).

In Malaysia, the palm oil industry has contributed to a large amount of wastewater from palm oil mill effluent's by-products. *Chlorella* sp. has been proven to have tolerance towards high pollutants and nutrients from the POME and managed to sequester the carbon content through the carbon fixation process (Hariz et al., 2019), apart from nutrient removal such as total nitrogen (TN) and total phosphorus (TP) along with biological oxygen demand (BOD) and chemical oxygen demand (COD) (Ahmad et al., 2017; Kamarudin et al., 2013; Tan et al., 2022).

Tetradesmus bernardii

Tetradesmus bernardii is another species of green algae of Phylum Chlorophyta, classified under Chlorophyceae. This species is the basionym of an earlier identified species, *Scenedesmus bernardii* (Wynne & Hallan, 2015). This genus has previously been studied as one of the bioindicators of water quality assessment (El-Din et al., 2022), as different water quality levels impacted the size and shape of the cells and organelles (Bauer et al., 2012) and due to its rich components of lipids, protein, and carbohydrates made them suitable to be incorporated as the raw materials for bioplastics production (Song et al.,

2022). The potential of *Tetradesmus* sp. for the application of wastewater treatment and bioremediation has also been studied extensively. A study conducted to assess the capacity for nutrient removal from hydroponic greenhouse wastewater has discovered this genus to acquire a complete removal of N and T from the medium (Salazar et al., 2023). With a similar capability as the *Chlorella*, it has also been applied to include this alga in the studies of nutrient removal in POME (Kamarudin et al., 2013; Tan et al., 2022).

Most research focuses on using unialgal cultures for the bioremediation of wastewater. Limited work was done on using mixed algal species for remediation. In this study, we are testing the effects of using unialgal cultures of microalgae versus a mixed culture of microalgae. We hypothesised that using a mixture of two microalgae species will benefit wastewater remediation (POME). Unialgal cultures, *C. vulgaris* and *T. bernardii*, were isolated from the remediation ponds and will be tested for remediation as unialgal cultures versus a mixed culture. The cultures were tested towards different concentrations of POME to evaluate the efficiency of microalgae growth, biomass accumulation, and nutrient removal.

MATERIALS AND METHODS

Experimental Design

All the experiments in this research study were conducted in the plant physiology laboratory, Department of Biology, University Putra Malaysia (UPM). Microalgae *C. vulgaris* and *T. bernardii*, collected from the remediation ponds, were isolated into pure cultures. The cultures were grown in a 250 mL conical flask with a light intensity of 2000 lux, pH 7-7.8, and room temperature of 25±1°C for 21 days in triplicates. Palm oil mill effluents (POME) were collected in two 5 L plastic containers from the aerobic pond Bell Sri Lingga palm oil mill industry, Melaka (2°22'40.3" N 101°59'10.4" E). The POME was transferred to the Plant Physiology lab, Department of Biology, University Putra Malaysia and stored at 4°C to avoid microbial degradation. Mixed microalgae *C. vulgaris* and *T. bernardii* were cultivated in different concentrations of POME (20%, 40%, 60%, and 80%). The concentrations were based on preliminary research that included 100% POME concentration; however, microalgae cultivation was unsuccessful. The POME was prepared at different concentrations by dilution with autoclaved distilled water.

Purification and Identification of Microalgae

The microalgal cultures are from the Plant Physiology Lab UPM. The cultures were purified on agar plates to obtain single colonies. Isolated microalgal cultures were subjected to DNA extraction using DNeasy PowerSoil pro kit Qiagen (Qiagen GmbH, Qiagen Strasse 1, 40724 Hilden, Germany). The eukaryotic

primer for V8 forward 5'-ATAACAGGTCTGTGATGCCCT-3', and for V9 reverse 5'-CCTTCYGCAGGTTCACCTAC-3' were used to amplify the 18S ribosomal RNA genes (Bradley et al., 2016). The primer synthesis and PCR product sequencing were carried out by Apical Scientific (Seri Kembangan, Selangor). The 18S sequences were compared against the National Centre for Biotechnology Information (NCBI) BLAST database.

Growth Rate of Microalgae (Optical Density)

The growth rate of mixed microalgae (*C. vulgaris* and *T. bernardii*), *C. vulgaris*, and *T. bernardii* were measured in different concentrations of POME (20%, 40%, 60%, 80%) in triplicates by using optical density (OD) spectrophotometer (HITACHI U-1900). The OD was measured at three different wavelengths (680 nm, 685 nm, and 700 nm). The 680 nm was chosen as the optimum wavelength for this study (Hazman et al., 2018; Jasni et al., 2020). Cultivation of microalgae was carried out in 200 mL medium with pH 7–7.8, room temperature of 25±1°C for 21 days and continuous light illumination at 2000 lux.

Biomass of Microalgae (Dry Weight)

The biomass, in terms of dry weight, of microalgae was quantified on both day zero and day 21 of the study. Initially, the palm oil mill effluent (POME) underwent a filtration process employing 150 µm filters to eliminate undesired particles. Subsequently, the filtrate was subjected to a secondary filtration step utilising 0.45 µm autoclaved glass membrane filters (Schleicher & Schuell, Germany) to capture the microalgal cells. These samples were then transferred to sterile petri dishes and subjected to desiccation in an oven set at 60°C for 24 hours. The filter papers were weighed using an analytical balance with 0.1 mg sensitivity (AL204, Mettler Toledo) (Ding et al., 2016; Hazman et al., 2018). The biomass (dry weight) of microalgae was calculated in gL^{-1} as in the following Equation 1:

$$\text{DCW } (gL^{-1}) = \frac{(WF+s)-Wf)}{V} \qquad [1]$$

where WF+s is the weight of the filter plus sample, Wf is the weight of the filter, and V is the volume of the sample collected.

Pigment Content of Microalgae

Microalgal chlorophyll *a* and carotenoid were determined following trichromatic spectrophotometry methods with some modifications (Johan et al., 2014). Samples (25 mL) were centrifuged at 4000 rpm for 10 minutes. Then, 5 mL of 99% acetone was added to the samples and ground by mortar and pestle. Three mL of samples were transferred to a glass cuvette, and the chlorophyll *a* was measured using a spectrophotometer (HITACHI U-1900) (Aminot & Rey, 2000; Johan et al., 2014). Chlorophyll *a* was measured at three

wavelengths (630, 647, 664 nm) and (452 nm) for carotenoids. Chlorophyll *a* and catenoid were calculated in mgL^{-1} as in Equations 2, 3 and 4.

$$\text{Chlorophyll-}a = (mgm^{-3}) \frac{(Ca)*(Va)}{Va} \quad [2]$$

where, Ca= (11.6*OD664) – (1.31*OD647) – (0.14*OD630); Va= Volume of acetone used for extraction (mL); and Vc= Volume of algal culture filtered (mL)

$$\text{Chlorophyll-}a\ (mgL^{-1}) = \frac{\text{Chl a } (mgm^{-3})}{1000} \quad [3]$$

$$\text{Carotenoid } (mgL^{-1}) = \frac{(OD452)*(3.86)*(Va)}{Vb} \quad [4]$$

where, Va = Volume of acetone used for extraction (mL); and Vb = Volume of algal culture filtered (mL)

Nutrient Removal

The chemical parameter measurements of palm oil mill effluent (POME), including nutrients such as total nitrogen (TN), total phosphorous (TP) and chemical oxygen demand (COD), were measured in the laboratory at day 0 and day 21 by using Hach Multiparameter Portable Colorimeter (DR900, HACH). The chemical profiles of the sample collected were assessed based on the instructions: TN (Persulfate digestion method – 10072), TP (Molybdovanadate with Persulfate digestion acid method – 10127), COD (Reactor digestion method – 8000).

Statistical Analysis

Statistical analysis was performed using Statistical Packages for the Social Sciences (SPSS) software (version 21.0, SPSS Inc., Chicago, IL). The data was assessed using One-way ANOVA to determine the differences in the mean value of the dependent variable associated with the effect of the controlled independent variable. The standard deviation was used to measure variability, and post-hoc analysis (Duncan) was performed to determine the significant difference between species of algae in different concentrations of POME with the significant level for the p-value ($p<0.05$).

RESULTS

Isolation and Identification

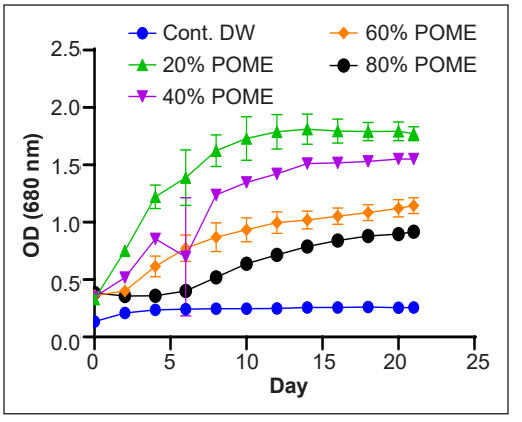
Unialgal cultures were identified as *C. vulgaris* and *T. bernardii* species, with 100% similarity. The gene sequences have been deposited in the NCBI GenBank with accession numbers (ON158767) for *C. vulgaris* and (OP804515) for *T. bernardii* (Figure S1).

Growth Rate, Optical Density (OD) of Microalgae in Different Concentrations of POME

The microalgae cultured in treatment batches showed relatively similar growth patterns for microalgae. Figure 1 indicates the growth performance of mixed microalgae, *C. vulgaris*, and *T. bernardii* in various concentrations of POME. It was shown that the highest growth rate was observed in 20% POME for mixed microalgae, followed by *C. vulgaris* and *T. bernardii*. The lowest growth was found in 80% POME for *T. bernardii*, followed by *C. vulgaris* and mixed microalgae, respectively. The trend showed that the reduction of POME concentration increases the growth rate of mixed microalgae, *C. vulgaris,* and *T. bernardii*, with 20% POME being the optimal concentration.

Biomass of Microalgae (Dry Weight)

Figure 2(a) shows biomass accumulation in different concentrations of POME for mixed microalgae, *C. vulgaris*, and *T. bernardii.* The microalgal species biomass accumulation cultivated in POME in different concentrations was described by mean biomass (dry weight). The result obtained from dry-weight cells of microalgae in different concentrations of POME indicates that the highest biomass was significantly observed in 20% POME for mixed microalgae ($p < 0.05$). At the same time, unialgal cultures of *C. vulgaris* and *T. bernardii* were not significant. The lowest biomass was found in 80% POME for all groups between the treatment groups. It



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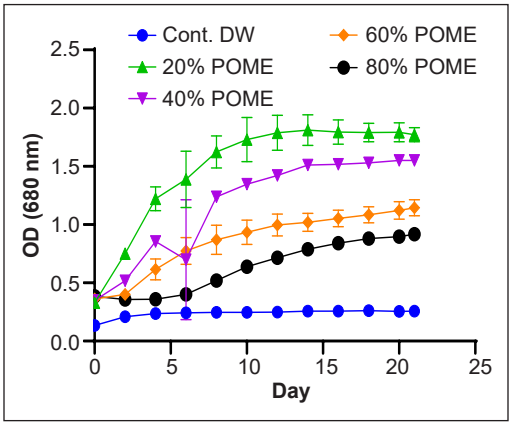

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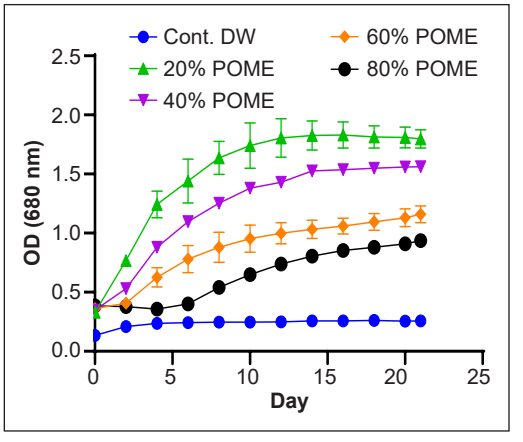


(c)

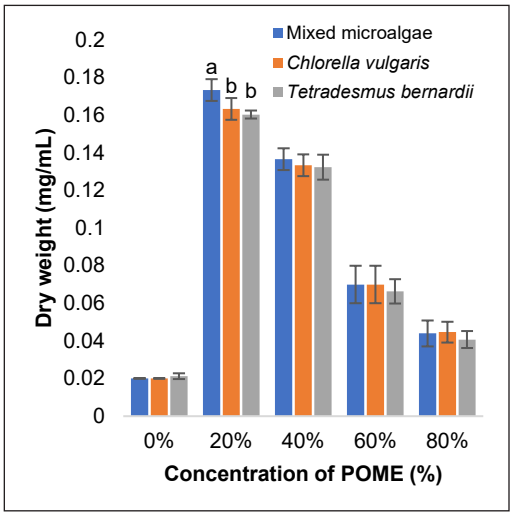
Figure 1. The growth of (a) mixed microalgae, (b) unialgal cultures of *Chlorella vulgaris*, and (c) unialgal cultures of *Tetradesmus bernardii* in different POME concentrations

was shown that by decreasing POME concentration, the biomass accumulation of mixed microalgae, *C. vulgaris* and *T. bernardii* increased, with the optimal concentration of 20% POME for all microalgae species.

Microalgae Pigments (Chlorophyll *a* and Carotenoids)

The productivity of algal pigments such as chlorophyll *a* and carotenoids was also noted in Figure 2(b); the highest chlorophyll *a* was recorded in mixed microalgae culture and *C. vulgaris* at 20% POME concentrations. The lowest chlorophyll *a* was recorded in 80% POME concentration.

As for the carotenoids Figure 2(c), mixed microalgae show the highest concentration of carotenoids, followed by *C. vulgaris and T. bernardii* ($p < 0.05$) at 20% POME. The lowest carotenoids were found in 80% POME. Cultures of 20% POME were found to have the highest chlorophyll *a* and carotenoid levels of mixed microalgae, *C. vulgaris,* and *T. bernardii*.



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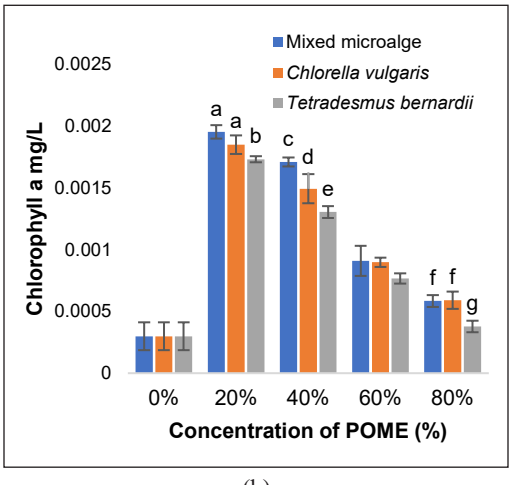

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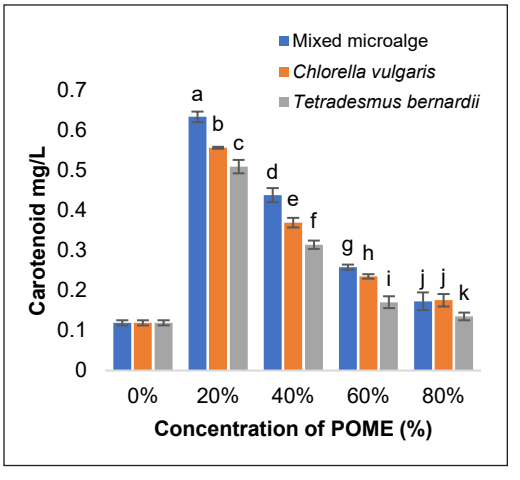


(c)

Figure 2. (a) Biomass, (b) chlorophyll *a*, and (c) carotenoids of mixed microalgae, unialgal cultures of *Chlorella vulgaris*, and *Tetradesmus bernardii* in different POME concentrations. Values are presented as average ± standard deviation (n = 3). Lowercase letters indicate significant differences between mixed microalgae, *C. vulgaris* and *T. bernardii* (One-way ANOVA, $p < 0.05$, Duncan test). Error bars without lowercase letters indicate no significant difference

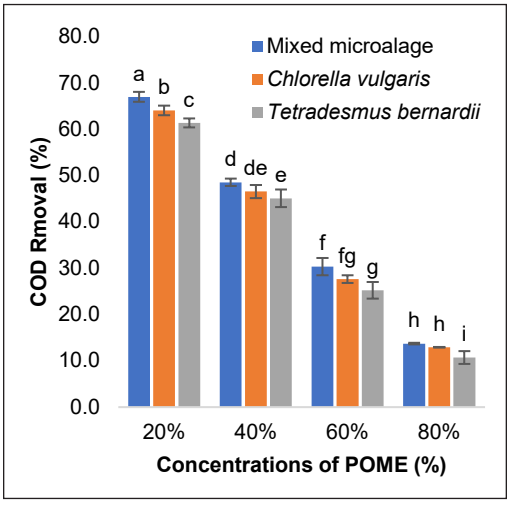
Nutrient Removal

Figure 3(a) shows the nutrient removal from POME in different concentrations of POME by mixed microalgae, *C. vulgaris*, and *T. bernardii*. Mixed microalgae showed significantly the highest COD removal percentage ($p < 0.05$) in comparison to unialgal cultures. All treatments found the lowest growth in 80% POME concentration. At 20% POME concentration, mixed microalgae were most efficient at removing COD.

Mixed microalgae were also found to be efficient at removing total nitrogen at 20% POME concentration ($p < 0.05$) in comparison to the two unialgal cultures of *C. vulgaris* and *T. bernardii* (Figure 3(b)).

For total phosphorus, the mixed microalgae cultures were also found to be significantly higher in removing total phosphorus ($p < 0.05$), while the unialgal cultures were not significantly different (Figure 3(c)).

Table 1 describes the nutrient removal of POME using various microalgae species. From this research, mixed microalgae removed 66% of COD, 86% of TN, and 68%



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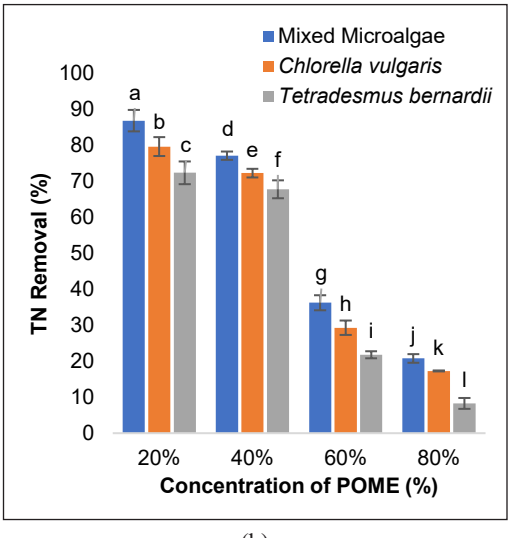

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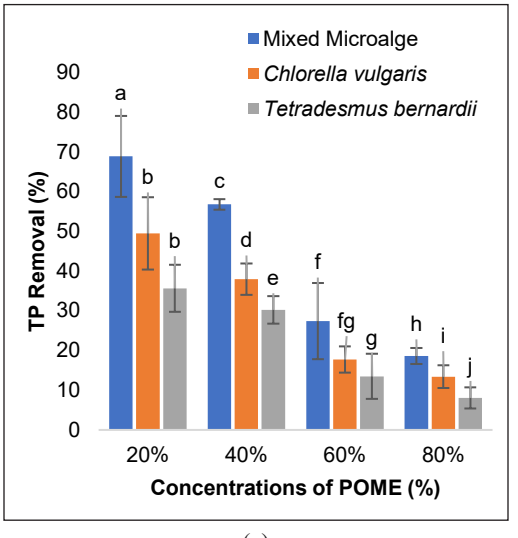

(c)

Figure 3. (a) COD, (b) TN, and (c) TP removal efficiency of mixed microalgae, unialgal cultures of *Chlorella vulgaris*, and *Tetradesmus bernardii* in different POME concentrations. Values are presented as average ± standard deviation (n = 3). Lowercase letters indicate significant differences between mixed microalgae, *C. vulgaris* and *T. bernardii* (One-way ANOVA, $p < 0.05$, Duncan test)

Table 1
Comparison of nutrient removal of POME using various microalgal species

Microalgae	COD (%)	TN (%)	TP (%)	Description	References
Mixed Microalgae	66	86	68	This study	
Chlorella vulgaris	61	80	56	This study	
Tetradesmus bernardii	64	79	49	This study	
Scenedesmus sp. *strain UKM9*	57	100	91	Cultivation and application for nutrient removal	Udaiyappan et al., 2021
Haematococcus pluvialis and *Chromochloris zofingiensis*	50.9%	49.3	3.95	Phycoremediation and astaxanthin production	Fernando et al., 2021
Nannochloropsis sp.	71% 48%	-	-	Cultivation and application for nutrient removal with and without beads	Emparan et al., 2020)
Chlorella sorokiniana sp.	47.09%	62.07	30.77	Cultivation and application for nutrient removal for lipid production	Cheah et al., 2018
Chlamydomonas sp. UKM6	15-20%	-	5-20	Microalgae-bacteria interaction in palm oil mill effluent treatment	Udaiyappan et al., 2020
Chlorella sp.	-	80.9	-	CO_2 sequestration by using microalgae sustainable strategies for environmental protection	Hariz et al., 2018
Chlamydomonas sp UKM6, Chlorella soroliniana	56%	65	34	CO_2 fixation capability of *Chlorella* sp. and treating POME	Hazman et al., 2018
Chlamydomonas sp. UKM6	8.59-29.13%	43.5-72.97	58.58-100	Cultivation and application for nutrient removal and biomass production	Ding et al., 2016
Chlamydomonas	67.35%	-	-	Efficiency of microalgae in nutrient removal from POME	Kamyab et al., 2015
Micro and macroalgae	71	-	-	Micro-macroalgae mixture as a promising agent for treating POME	Kamyab et al., 2014

of TP. The nutrient removal efficiency of POME by mixed microalgae was higher than *C. vulgaris* or *T. bernardii* unialgal cultures (see supplementary data Tables S1, S2, and S3). The comparison of other research using microalgae is also shown in Table 1.

DISCUSSION

Growth Rate

Wastewater originates from a range of agriculture, domestic, and industrial water activities, and it encompasses contaminants along with inorganic and organic compounds (Ahmed

et al., 2022). Treating wastewater before releasing it into the environment is essential to prevent contamination from entering natural water systems and harming the environment (Otondo et al., 2018). The utilisation of microalgae in biological wastewater treatment is widely applied due to their environmental advantages and cost-effectiveness.

Particulate and suspension substances of POME affect microalgae growth and significantly decrease the growth rate. In this study, by increasing the concentration of POME, the growth of microalgae is limited and influenced by the physical characteristics of POME. High suspensions of POME gradually limited light penetration for microalgae growth. The dark colour of POME also inhibits the light intensity from the bottom of the culture flasks. As a result, it disrupts photosynthesis and limits algal growth (Talib et al., 2023).

Microalgae cultures cultivated in highly concentrated wastewater POME require a long time to reach the stationary phase because the growth is slower than in low concentrations. The dark colour of POME affected the growth rate of microalgae. *Coelastrella* sp., *Chlamydomonas* sp., and *Scenedesmus* sp. cultures showed a low growth rate in high concentrations, resulting in algae cells staying longer in the adaptation phase (Ding et al., 2016; Udaiyappan et al., 2020). Diluting POME in different concentrations reduces the effect of higher concentrations of POME in the culture media, limiting the growth rate and reducing the lag phase for the algae (Cheah et al., 2018; Khalid et al., 2016). Diluted palm oil effluent (POME) effectively grew microalgae *Characium* sp. (Khalid et al., 2016).

POME is rich in organic compounds such as nitrogen, phosphorus, and ammonia, with high levels of chemical oxygen demand (COD) and low levels of biological oxygen demand (BOD) (Fernando et al., 2021). On the other hand, these organic compounds are still essential for microalgae growth at a certain concentration range (Emparan et al., 2020).

In conclusion, the results indicate that the highest growth was for 20% concentration for mixed microalgae. As the concentration of POME decreases, the growth rate increases. It suggests that POME can be cost-effective and is readily available for microalgae cultivation. However, the physical characteristics of POME, such as dark colour and high levels of suspended solids, have negatively affected microalgae growth by limiting light penetration and inhibiting photosynthesis. Diluting POME in different concentrations can mitigate these effects and improve the growth profile of microalgae in wastewater treatment applications.

Biomass

For this study, low concentrations of POME for microalgae revealed a significantly higher biomass production because of adequate light intensity for the cell to speed up growth and biomass accumulation. In other studies, low concentrations of POME also showed higher biomass production in microalgae *Chlamydomonas*, whereas high concentrations of POME (100% and 50%) limit biomass accumulation (Ding et al., 2016).

Meanwhile, high concentrations of POME indicate less biomass accumulation, which may be due to the dark colour of POME and inadequate light intensity at the bottom of culture flasks. Additionally, the high number of cells prevents light penetration for other cells. Excessive number of algal cells causes self-shading on the cells. Therefore, it causes low light intensity for algal growth and biomass production. Furthermore, it will reduce photosynthesis, resulting in less biomass production (Kumaran et al., 2023). It is possible to correlate the CO_2 amount with biomass production. Higher biomass production results from high CO_2 fixation in low POME concentrations, and less biomass accumulation is due to poor CO_2 absorption (Hariz et al., 2018).

Microalgal biomass cultivated in POME has much potential to be utilised in various applications. Microalgal biomass has the potential to produce biofuel because of its low lignin content, a higher growth rate than plants and a high range of nutrient absorption from wastewater (Ahmad et al., 2016). Microalgae are emerging as a highly promising option for biofuel production, mainly attributable to their exceptional photosynthetic efficiency, which enables efficient CO_2 sequestration. They also exhibit remarkable attributes such as elevated biomass yields, significant lipid and carbohydrate accumulation, robust adaptability to various environmental conditions, resistance to contamination, and the presence of valuable components that hold significant potential for the development of non-fuel bioproduct (Cheah et al., 2016; Saidu et al., 2017).

Numerous species of algae are cultivated as human food sources and used as a biofertiliser, animal feed, and pharmaceuticals (Michalak et al., 2019). Consequently, less growth and biomass yield in higher concentrations of POME directly affected the amount of chlorophyll *a* and carotenoid in culture batches (Hazman et al., 2018). POME may contain various nutrients, including nitrogen and phosphorus compounds essential for microalgae growth. However, the specific impact on microalgae growth can depend on the composition and concentration of nutrients within the POME (Hadiyanto & Nur, 2014).

Microalgal Pigments (Chlorophyll *a* and Carotenoids)

Microalgal biomass contains essential pigments such as chlorophyll *a*, carotenoid, and other components, which might be useful for various industrial applications (Aburai et al., 2013). The chlorophyll *a* concentration is a significant indicator for assessing the nutritional state of water (Johan et al., 2014). Accurate measuring of algal chlorophyll *a* is crucial to predict the biomass production and the photosynthetic rate of algae (Simon & Helliwell., 1998). Chlorophyll *a* is an important element indicating the photosynthetic level in microalgae (Hariz et al., 2018). The concentration of chlorophyll *a* and other pigments available in algae implies the algal growth and biomass production (Aminot & Rey, 2000).

Photosynthetic organisms such as plants and algae utilise carotenoids within the photosynthesis light-harvesting complex. They serve as supplementary pigments and

are recognised for safeguarding photosystems against oxidative harm, acting as a photoprotective shield (Aburai et al., 2013). Microalgae contain several types of carotenoids that act as free-radical foragers or antioxidants (Nobre et al., 2013; Takaichi., 2011). Various colourants derived from microalgae, such as phycocyanin (the blue pigment from *Spirulina*), β- carotene (the yellow pigment from *Dunaliella*), and astaxanthin (ranging from yellow to red, obtained from *Haematococcus*), are increasingly valued over synthetic alternatives due to their non-toxic and non-carcinogenic properties (Begum et al., 2016).

Microalgae grown in wastewater were used for the production of astaxanthin from *H. pluvialis* and *C. zofingiensis* (Fernando et al., 2021), phycocyanins from *Nostoc* sp., and *Arthrospira platensis;* as well as phycoerythrin from *Porphyridium purpureum* (Arashiro et al., 2020).

Nutrient Removal

A comparison of nutrient removal efficiencies of microalgae in POME is shown in Table 1. From this study, we found that mixed microalgae removed the highest COD (66%), followed by *C. vulgaris* (64%) and *T. bernardii (*61%) in concentrations of 20% POME. Other studies have reported COD percentage removal of 15%–71%. As indicated in Table 1, various microalgae species perform differently when removing COD (Ding et al., 2016; Emparan et al., 2020; Kamyab et al., 2014).

The highest removal of total nitrogen (TN) using mixed microalgae was 86%. Other studies have reported between 43.5 % (*Chlamydomonas* sp. *UKM6*) (Ding et al., 2016) to 100% (*Scenedesmus sp. UKM9*) (Udaiyappan et al., 2021). The highest removal of total phosphorus (TP) in this study was mixed microalgae at 68%. Other studies have reported between 3.95% (*H. pluvialis and C. zofingiensis*) (Fernando et al., 2021) to 100% (*Chlamydomonas* sp. *UKM6*) (Ding et al., 2016). In *Nannochloropsis*, 60% POME and Walnes medium effectively removed 62% COD (Resdi et al., 2021).

Nitrogen is a necessary compound for algal growth and metabolism. POME contains high amounts of nutrients, which are viable for algal growth. In this study, microalgae exhibit substantial nitrogen removal efficiency when exposed to low concentrations of POME. In contrast, their nitrogen removal capacity decreased as the POME concentration increased, in alignment with prior research findings on mono species of *H. pluvialis* and *C. zofingiensis* (Fernando et al., 2021), as well as on mono species of *Chlorella UKM8* and *Chlamydomonas UKM6* (Hazman et al., 2018).

Microalgae assimilate phosphorus as phosphate for algal growth, biomass production, and metabolism (Goh et al., 2022; Nakarmi et al., 2023). Microalgae can thrive in lower concentrations of POME because nitrogen and phosphorus, which are vital nutrients for their growth, may be scarce. In such conditions, microalgae can efficiently absorb and use these limited nutrients since they are not in surplus, facilitating their growth (Dominic

& Baidurah., 2022). The high concentrations of wastewater and POME can encompass diverse organic compounds and heavy metals, including recalcitrant and potentially harmful organic substances (Al-Amshawee et al., 2020). These compounds possess the capacity to impede the growth of microalgae and their uptake of nutrients.

Toxic compounds have the potential to disrupt the microalgal capacity to efficiently assimilate and utilise nutrients and heavy metals (Zhao et al., 2023). Microalgae are capable of environmental adaptation; for instance, of lower POME concentrations, they demonstrate the ability to acclimate by adjusting their physiological processes and nutrient uptake mechanisms, thereby optimising nutrient utilisation. Conversely, when confronted with higher POME concentrations, microalgae may encounter challenges adapting to fluctuating and potentially more hostile conditions (Saidu et al., 2017).

Consortium species might have superior nitrogen removal than the mono species as different microalgae species can have varying growth rates and responses to environmental conditions (Fallahi et al., 2020). If one species faces unfavourable conditions, the other species may continue to thrive and contribute to nutrient removal. Mixed microalgae are more effective at removing nutrients due to the diverse nutrient needs of each species and can compensate for the nutrient loss of another. If one species is highly efficient at utilising nitrogen but less so at using phosphorus, and another species is the opposite (efficient at phosphorus but not nitrogen), they can complement each other's nutrient demand. It means that mixed microalgae can effectively utilise the available nutrients in the POME, leading to higher overall nutrient removal and reduction of COD. Higher nutrient removal and COD reduction in agricultural wastewater using mixed microalgae were due to microalgal selection, medium composition, and physicochemical variables (Qin et al., 2016).

The research findings reveal the effectiveness of microalgae-based treatment as a highly efficient biological method for substantially removing nutrients and organic loads. The variability in bioremediation of palm oil mill effluent (POME) efficiency using microalgae is affected by several factors: (1) POME composition, (2) microalgal selection, (3) inoculum size, (4) POME physicochemical state after collection and treatment, and (5) duration of the remediation.

In terms of POME composition, the starting material for POME was different for each experiment (for example, 2.5%–100% for anaerobic ponds and 20%–80% for aerobic ponds). POME composition indicates diverse nutrient levels in different ponds (cooling, anaerobic and aerobic) as well as sampling time (wet or dry season). The source of palm oil and the condition of the trees and fruits during the oil extraction and residue can directly affect the viability of the nutrients in POME.

The selection of microalgae significantly affects the bioremediation of POME due to their capacity for nutrient absorption, tolerance towards adverse seasons (dry and wet) and toxic substances in POME. Inoculum size (the number of microalgae introduced

into the culture media) can also affect the bioremediation of POME. A higher amount of inoculum is essential for acclimatising the culture that undergoes the lag phase after some time (Khalid et al., 2016). A high amount of inoculum also contributes to controlling the cultivation and remediation of POME by microalgae (Lau et al., 1995).

The physicochemical state of POME after collection and treatment differs in terms of pH, temperature, suspended solids, and heavy metals concentration. Raw POME samples were collected from the cooling pond, and the treated POME samples were collected from anaerobic and aerobic ponds. Untreated POME from cooling ponds is highly acidic and contains particles (sand, wood, residue from extraction of oil) that can cause a reduction in microalgal growth and nutrient removal. The experimental design and duration in each research differed in terms of nutrient removal, growth, and biomass accumulation. These factors make comparing the current study findings with previous research difficult.

CONCLUSION

Mixed microalgae are superior in the remediation of POME compared to single-culture algae. This research reports for the first time a mixed microalgae combination of *C. vulgaris* and *T. bernardii* in bioremediating wastewater from the palm oil mill effluent. Mixed microalgae have the benefit of fulfilling each other's specific nutrient demands for growth. This research found a significant reduction in COD, TN and TP levels from the palm oil mill effluent. Based on the data, the optimal concentration for effective nutrient reduction was 20% of POME.

The research was conducted indoors in controlled lab conditions. The results may vary if conducted on a large scale outdoors. When evaluating the performance of microalgae in nutrient removal efficiencies, the parameters are usually different in terms of the growth parameters, algae selection and cultivation or time of remediation.

Treating wastewater through microalgal bioremediation is effective at nutrient removal and environmentally friendly. This research has contributed towards the use of mixed microalgae to achieve effective nutrient removal and biomass for future industrial applications. Further research is needed on lower concentrations of POME as well as different microalgae consortia on effective palm oil waste effluent wastewater remediation.

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REFERENCES

Aburai, N., Ohkubo, S., Miyashita, H., & Abe, K. (2013). Composition of carotenoids and identification of aerial microalgae isolated from the surface of rocks in mountainous districts of Japan. *Algal Research*, *2*(3), 237-243. https://doi.org/10.1016/j.algal.2013.03.001

Ahmad, A., Bhat, A. H., & Buang, A. (2017). Immobilized *Chlorella vulgaris* for efficient palm oil mill effluent treatment and heavy metals removal. *Desalination and Water Treatment, 81*, 105-117. https://doi.org/10.5004/dwt.2017.20991

Ahmad, A., Buang, A., & Bhat, A. H. (2016). Renewable and sustainable bioenergy production from microalgal co-cultivation with palm oil mill effluent (POME): A review. *Renewable and Sustainable Energy Reviews*, *65*, 214–234. https://doi.org/10.1016/j.rser.2016.06.084

Ahmad, M. T., Shariff, M., Yusoff, F. M., Goh, Y. M., & Banerjee, S. (2020). Applications of microalga *Chlorella vulgaris* in aquaculture. *Reviews in Aquaculture*, *12*(1), 328-346. https://doi.org/10.1111/raq.12320

Ahmed, S. F., Mofijur, M., Parisa, T. A., Islam, N., Kusumo, F., Inayat, A., Van, G. L., Badruddin, I. A., Khan, T. M. Y., & Ong, H. C. (2022). Progress and challenges of contaminate removal from wastewater using microalgae biomass. *Chemosphere*, *286*(1), Article 131656. https://doi.org/10.1016/j.chemosphere.2021.131656

Al-Amshawee, S. K., Yunus, M. Y., & Azoddein, A. A. (2020). A review study of biofilm bacteria and microalgae bioremediation for palm oil mill effluent: Possible approach. *IOP Conference Series: Materials Science and Engineering 736*(2), Article 02203. https://doi.org/10.1088/1757-899X/736/2/022034

Al-Khiat, S. H., Bukhari, N. A., Ameen, F., & Abdel-Raouf, N. (2023). Comparison of the microalgae *Phormidium tenue* and *Chlorella vulgaris* as biosorbents of Cd and Zn from aqueous environments. *Environmental Research*, *235*, Article 116675. https://doi.org/10.1016/j.envres.2023.116675

Aminot, A., & Rey, F. (2000). Standard procedure for the determination of chlorophyll *a* by spectroscopic methods. *International Council for the Exploration of the Sea*, *112*, Article 25.

Anto, S., Mukherjee, S. S., Muthappa, R., Mathimani, T., Deviram, G., Kumar, S. S., Verma, T. N., & Pugazhendhi, A. (2020). Algae as green energy reserve: Technological outlook on biofuel production. *Chemosphere*, *242*, Article 125079. https://doi.org/10.1016/j.chemosphere.2019.125079

Arashiro, L. T., Boto-Ordóñez, M., Van Hulle, S. W., Ferrer, I., Garfí, M., & Rousseau, D. P. (2020). Natural pigments from microalgae grown in industrial wastewater. *Bioresource Technology*, *303*, Article 122894. https://doi.org/10.1016/j.biortech.2020.122894

Azmi, N. S., & Yunos, K. F. M. (2014). Wastewater treatment of palm oil mill effluent (POME) by ultrafiltration membrane separation technique coupled with adsorption treatment as pre-treatment. *Agriculture and Agricultural Science Procedia*, *2*, 257-264. https://doi.org/10.1016/j.aaspro.2014.11.037

Bala, J. D., Lalung, J., & Ismail, N. (2015). Studies on the reduction of organic load from palm oil mill effluent (POME) by bacterial strains. *International Journal of Recycling of Organic Waste in Agriculture*, *4*, 1-10. https://doi.org/10.1007/s40093-014-0079-6

Bauer, D. E., Conforti, V., Ruiz, L., & Gómez, N. (2012). An in-situ test to explore the responses of *Scenedesmus acutus* and *Lepocinclis acus* as indicators of the changes in water quality in lowland streams. *Ecotoxicology and Environmental Safety*, *77*, 71-78. https://doi.org/10.1016/j.ecoenv.2011.10.021

Begum, H., Yusoff, F. M., Banerjee, S., Khatoon, H., & Shariff, M. (2016). Availability and utilization of pigments from microalgae. *Critical Reviews in Food Science and Nutrition*, *56*(13), 2209-2222. https://doi.org/10.1080/10408398.2013.764841

Bradley, I. M., Pinto, A. J., & Guest, J. S. (2016). Design and evaluation of Illumina MiSeq-compatible, 18S rRNA gene-specific primers for improved characterization of mixed phototrophic communities. *Applied and Environmental Microbiology*, *82*(19), 5878-5891. https://doi.org/10.1128/AEM.01630-16

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. https://doi.org/10.1016/j.enconman.2018.08.057

Chew, C. L., Ng, C. Y., Hong, W. O., Wu, T. Y., Lee, Y. Y., Low, L. E., Kong, P. S., & Chan, E. S. (2021). Improving sustainability of palm oil production by increasing oil extraction rate: A review. *Food and Bioprocess Technology, 14*, 573-586. https://doi.org/10.1007/s11947-020-02555-1

Ding, G. T., Yaakob, Z., Takriff, M. S., Salihon, J., & Rahaman, M. S. A. (2016). Biomass production and nutrients removal by a newly-isolated microalgal strain *Chlamydomonas* sp. in palm oil mill effluent (POME). *International Journal of Hydrogen Energy*, *41*(8), 4888-4895. https://doi.org/10.1016/j.ijhydene.2015.12.010

Dominic, D., & Baidurah, S. (2022). Recent developments in biological processing technology for palm oil mill effluent treatment. *Biology*, *11*(4), Article 525. https://doi.org/10.3390/biology11040525

El-Din, N. G. S., El-Sheekh, M. M., El-Kassas, H. Y., Essa, D. I., & El-Sherbiny, B. A. (2022). Biological indicators as tools for monitoring water quality of a hot spot area on the Egyptian Mediterranean Coast. *Arabian Journal of Geosciences*, *15*(18), Article 1485. https://doi.org/10.1007/s12517-022-10701-6

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*, Article 101043. https://doi.org/10.1016/j.jwpe.2019.101043

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), Article e2586. https://doi.org/10.30498/IJB.2020.2586

Fernando, J. S. R., Premaratne, M., Dinalankara, D. M. S. D., Perera, G. L. N. J., & Ariyadasa, T. U. (2021). Cultivation of microalgae in palm oil mill effluent (POME) for astaxanthin production and simultaneous phycoremediation. *Journal of Environmental Chemical Engineering*, *9*(4), Article 105375. https://doi.org/10.1016/j.jece.2021.105375

Goh, P. S., Lau, W. J., Ismail, A. F., Samawati, Z., Liang, Y. Y., & Kanakaraju, D. (2022). Microalgae-enabled wastewater treatment: A sustainable strategy for bioremediation of pesticides. *Water*, *15*(1), Article 70. https://doi.org/10.3390/w15010070

Hadiyanto, & Nur, M. M. A. (2014). Lipid extraction of microalga *Chlorella* sp. cultivated in palm oil mill effluent (POME) medium. *World Applied Sciences Journal*, *31*(5), 959-967. https://doi.org/10.5829/idosi.wasj.2014.31.05.2006

Hariz, H. B., Takriff, M. S., Ba-Abbad, M. M., Yasin, N. H. M., & Hakim, N. I. N. M. (2018). CO^2 fixation capability of *Chlorella* sp. and its use in treating agricultural wastewater. *Journal of Applied Phycology*, *30*, 3017-3027. https://doi.org/10.1007/s10811-018-1488-0

Hariz, H. B., Takriff, M. S., Yasin, N. H. M., Ba-Abbad, M. M., & Hakimi, N. I. N. M. (2019). Potential of the microalgae-based integrated wastewater treatment and CO_2 fixation system to treat palm oil mill effluent (POME) by indigenous microalgae; *Scenedesmus* sp. and *Chlorella* sp. *Journal of Water Process Engineering*, *32*, Article 100907. https://doi.org/10.1016/j.jwpe.2019.100907

Haryati, Z., Subramaniam, V., Noor, Z. Z., Hashim, Z., Loh, S. K., & Aziz, A. A. (2022). Social life cycle assessment of crude palm oil production in Malaysia. *Sustainable Production and Consumption*, *29*, 90-99. https://doi.org/10.1016/j.spc.2021.10.002

Hazman, N. A. S., Yasin, N. H. M., Takriff, M. S., Hasan, H. A., Kamarudin, K. F., & Hakimi, N. I. N. M. (2018). Integrated palm oil mill effluent treatment and CO_2 sequestration by microalgae. *Sains Malaysiana*, *47*(7), 1455-1464. https://doi.org/10.17576/jsm-2018-4707-13

Jasni, J., Arisht, S. N., Yasin, N. H. M., Abdul, P. M., Lin, S. K., Liu, C. M., & Takriff, M. S. (2020). Comparative toxicity effect of organic and inorganic substances in palm oil mill effluent (POME) using native microalgae species. *Journal of Water Process Engineering*, *34*, Article 101165. https://doi.org/10.1016/j.jwpe.2020.101165

Johan, F., Jafri, M. Z., Lim, H. S., & Maznah, W. W. (2014, December 9-12). *Laboratory measurement: Chlorophyll-a concentration measurement with acetone method using spectrophotometer*. [Paper presentation]. IEEE International Conference on Industrial Engineering and Engineering Management, Selangor, Malaysia. https://doi.org/10.1109/IEEM.2014.7058737

Justine, I., Chin, G. J. W. L., Yong, W. T. L., & Misson, M. (2023). Characterization and optimization of *Rhodotorula toruloides* and *Ankistrodesmus falcatus* co-culture in palm oil mill effluent for efficient COD removal and lipid production. *Biocatalysis and Agricultural Biotechnology, 51*, Article 102782. https://doi.org/10.1016/j.bcab.2023.102782

Kamarudin, K. F., Yaakob, Z., Rajkumar, R., & Tasirin, S. M. (2013). Bioremediation of palm oil mill effluents (POME) using *Scenedesmus dimorphus* and *Chlorella vulgaris*. *Advanced Science Letters, 19*(10), 2914-2918. https://doi.org/10.1166/asl.2013.5044

Kamyab, H., Din, M. F. M., Keyvanfar, A., Majid, M. Z. A., Talaiekhozani, A., Shafaghat, A., & Ismail, H. H. (2015). Efficiency of microalgae *Chlamydomonas* on the removal of pollutants from palm oil mill effluent (POME). *Energy Procedia*, *75*, 2400-2408. https://doi.org/10.1016/j.egypro.2015.07.190

Kamyab, H., Fadhil, M., Lee, C., Ponraj, M., Soltani, M., & Eva, S. (2014). Micro-macro algal mixture as a promising agent for treating POME discharge and its potential use as animal feed stock enhancer. *Jurnal Teknologi*, *68*(5), 1-4.

Kamyab, H., Yuzir, M. A., Riyadi, F. A., Ostadrahimi, A., Khademi, T., Ghfar, A. A., & Kirpichnikova, I. (2022). Electrochemical oxidation of palm oil mill effluent using platinum as anode: Optimization using response surface methodology. *Environmental Research*, *214*(3), Article 113993. https://doi.org/10.1016/j.envres.2022.113993

Karim, A., Islam, M. A., Khalid, Z. B., Yousuf, A., Khan, M. M. R., & Faizal, C. K. M. (2021). Microbial lipid accumulation through bioremediation of palm oil mill effluent using a yeast-bacteria co-culture. *Renewable Energy*, *176*, 106-114. https://doi.org/10.1016/j.renene.2021.05.055

Khalid, A. A. H., Yaakob, Z., Abdullah, S. R. S., & Takriff, M. S. (2016). Enhanced growth and nutrients removal efficiency of *Characium* sp. cultured in agricultural wastewater via acclimatized inoculum and effluent recycling. *Journal of Environmental Chemical Engineering*, *4*(3), 3426-3432. https://doi.org/10.1016/j.jece.2016.07.020

Khatun, R., Reza, M. I. H., Moniruzzaman, M., & Yaakob, Z. (2017). Sustainable oil palm industry: The possibilities. *Renewable and Sustainable Energy Reviews*, *76*, 608-619. https://doi.org/10.1016/j.rser.2017.03.077

Kumaran, M., Palanisamy, K. M., Bhuyar, P., Maniam, G. P., Rahim, M. H. A., & Govindan, N. (2023). Agriculture of microalgae *Chlorella vulgaris* for polyunsaturated fatty acids (PUFAs) production employing palm oil mill effluents (POME) for future food, wastewater, and energy nexus. *Energy Nexus*, *9*, Article 100169. https://doi.org/10.1016/j.nexus.2022.100169

Lau, P. S., Tam, N. F. Y., & Wong, Y. S. (1995). Effect of algal density on nutrient removal from primary settled wastewater. *Environmental Pollution*, *89*(1), 59-66. https://doi.org/10.1016/0269-7491(94)00044-E

Medvedeva, L. N., Zorkina, O. V., & Moskovets, M. V. (2022). Use of *Chlorella vulgaris* as a dietary supplement for quails bred at private farms. *Journal of Agronomy and Animal Industries*, 17(4), 499-513. https://doi.org/10.22363/2312-797X-2022-17-4-499-513

Michalak, I., Baśladyńska, S., Mokrzycki, J., & Rutkowski, P. (2019). Biochar from a freshwater macroalga as a potential biosorbent for wastewater treatment. *Water*, *11*(7), Article 1390. https://doi.org/10.3390/w11071390

Mohammed, R. R. (2013). Decolourisation of biologically treated palm oil mill effluent (POME) using adsorption technique. *International Refereed Journal of Engineering and Science*, *2*(10), 1-11.

Morais, F. P., Simões, R. M. S., & Curto, J. M. R. (2020). Biopolymeric delivery systems for cosmetic applications using *Chlorella vulgaris* algae and tea tree essential oil. *Polymers*, *12*(11), Article 2689. https://doi.org/10.3390/polym12112689

Nakarmi, K. J., Daneshvar, E., Mänttäri, M., & Bhatnagar, A. (2023). Removal and recovery of nutrients from septic tank wastewater using microalgae: Key factors and practical implications. *Journal of Environmental Management*, *345*, Article 118922. https://doi.org/10.1016/j.jenvman.2023.118922

Nobre, B. P., Villalobos, F., Barragan, B. E., Oliveira, A. C., Batista, A. P., Marques, P. A. S. S., Mendes, R. L., Savova, H., Palavra, A. F., & Gouveia, L. (2013). A biorefinery from *Nannochloropsis* sp. microalga–extraction of oils and pigments. Production of biohydrogen from the leftover biomass. *Bioresource Technology*, *135*, 128-136. https://doi.org/10.1016/j.biortech.2012.11.084

Orusmurzaeva, Z., Maslova, A., Tambieva, Z., Sadykova, E., Askhadova, P., Umarova, K., Merzhoeva, A., Albogachieva, K., Ulikhanyan, K., & Povetkin, S. (2022). Investigation of the chemical composition and physicochemical properties of *Chlorella vulgaris* biomass treated with pulsed discharges technology for potential use in the food industry. *Slovak Journal of Food Sciences*, *16,* 777-789.

Otondo, A., Kokabian, B., Stuart-Dahl, S., & Gude, V. G. (2018). Energetic evaluation of wastewater treatment using microalgae, *Chlorella vulgaris*. *Journal of Environmental Chemical Engineering*, *6*(2), 3213-3222. https://doi.org/10.1016/j.jece.2018.04.064

Qin, L., Wang, Z., Sun, Y., Shu, Q., Feng, P., Zhu, L., & Yuan, Z. (2016). Microalgae consortia cultivation in dairy wastewater to improve the potential of nutrient removal and biodiesel feedstock production. *Environmental Science and Pollution Research*, *23*, 8379-8387. https://doi.org/10.1007/s11356-015-6004-3

Ratnasari, A., Zaidi, N. S., Syafiuddin, A., Boopathy, R., Kueh, A. B. H., Amalia, R., & Prasetyo, D. D. (2021). Prospective biodegradation of organic and nitrogenous pollutants from palm oil mill effluent by acidophilic bacteria and archaea. *Bioresource Technology Reports*, *15*, Article 100809. https://doi.org/10.1016/j.biteb.2021.100809

Resdi, R., Lim, J. S., & Idris, A. (2021). Batch kinetics of nutrients removal for palm oil mill effluent and recovery of lipid by *Nannochloropsis* sp. *Journal of Water Process Engineering, 40*, Article 101767. https://doi.org/10.1016/j.jwpe.2020.101767

Rowland, D., Zanello, G., Waliyo, E., & Ickowitz, A. (2022). Oil palm and gendered time use: A mixed-methods case study from West Kalimantan, Indonesia. *Forest Policy and Economics*, *137*, Article 102682. https://doi.org/10.1016/j.forpol.2021.102682

Saidu, H., Jamaluddin, H., & Mohamad, S. E. (2017). Nutrient removal and biokinetic study of freshwater microalgae in palm oil mill effluent (POME). *Indian Journal of Science and Technology*, *10*(24), 1-10. https://doi.org/10.17485/ijst/2017/v10i24/114584

Salazar, J., Santana-Sánchez, A., Näkkilä, J., Sirin, S., & Allahverdiyeva, Y. (2023). Complete N and P removal from hydroponic greenhouse wastewater by *Tetradesmus obliquus*: A strategy for algal bioremediation and cultivation in Nordic countries. *Algal Research*, *70*, Article 102988. https://doi.org/10.1016/j.algal.2023.102988

Simon, D., & Helliwell, S. (1998). Extraction and quantification of chlorophyll *a* from freshwater green algae. *Water Research, 32*(7), 2220-2223. https://doi.org/10.1016/S0043-1354(97)00452-1

Singh, R. P., Ibrahim, M. H., Esa, N., & Iliyana, M. S. (2010). Composting of waste from palm oil mill: A sustainable waste management practice. *Reviews in Environmental Science and Biotechnology*, *9*(4), 331–344. https://doi.org/10.1007/s11157-010-9199-2

Song, X., Bo, Y., Feng, Y., Tan, Y., Zhou, C., Yan, X., Ruan, R., Xu, Q., & Cheng, P. (2022). Potential applications for multifunctional microalgae in soil improvement. In *Frontiers in Environmental Science*, *10*, Article 1035332. https://doi.org/10.3389/fenvs.2022.1035332

Takaichi, S. (2011). Carotenoids in algae: Distributions, biosynthesis and functions. *Marine Drugs*, *9*(6), 1101-1118. https://doi.org/10.3390/md9061101

Talib, S. L. A., Yasin, N. H. M., Takriff, M. S., & Japar, A. S. (2023). Comparative studies on phycoremediation efficiency of different water samples by microalgae. *Journal of Water Process Engineering*, *52*, Article 103584. https://doi.org/10.1016/j.jwpe.2023.103584

Tan, K. A., Lalung, J., Wijaya, D., Ismail, N., Omar, W. M. W., Wabaidur, S. M., Siddiqui, M. R., Alam, M., & Rafatullah, M. (2022). Removal of nutrients by using green microalgae from lab-scale treated palm oil mill effluent. *Fermentation*, *8*(11), Article 658. https://doi.org/10.3390/fermentation8110658

Udaiyappan, A. F. M., Hasan, H. A., Takriff, M. S., Abdullah, S. R. S., Yasin, N. H. M., & Ji, B. (2021). Cultivation and application of *Scenedesmus* sp. strain UKM9 in palm oil mill effluent treatment for enhanced nutrient removal. *Journal of Cleaner Production*, *294*, Article 126295. https://doi.org/10.1016/j.jclepro.2021.126295

Udaiyappan, A. F., Hasan, H. A., Takriff, M. S., Abdullah, S. R. S., Maeda, T., Mustapha, N. A., Mohd Yasin, N. H., & Hakimi, N. I. N. M. (2020). Microalgae-bacteria interaction in palm oil mill effluent treatment. *Journal of Water Process Engineering*, *35*, Article 101203. https://doi.org/10.1016/j.jwpe.2020.101203

Velmurugan, A., & Muthukaliannan, G. K. (2022). Genetic manipulation for carotenoid production in microalgae an overview. *Current Research in Biotechnology*, *4*, 221-228. https://doi.org/10.1016/j.crbiot.2022.03.005

Wynne, M. J., & Hallan, J. K. (2015). Reinstatement of *Tetradesmus* GM Smith (Sphaeropleales, Chlorophyta). *Feddes Repertorium*, *126*(3-4), 83-86. https://doi.org/10.1002/fedr.201500021

Zhao, D., Cheah, W. Y., Lai, S. H., Ng, E. P., Khoo, K. S., Show, P. L., & Ling, T. C. (2023). Symbiosis of microalgae and bacteria consortium for heavy metal remediation in wastewater. *Journal of Environmental Chemical Engineering, 11*(3), Article 109943. https://doi.org/10.1016/j.jece.2023.109943

Table S1
Nutrient removal efficiency in different dilutions of POME by mixed microalgae

	Parameter	20%	40%	60%	80%
COD	Initial value	319.0000 ± 1.0000	408.0000 ± 6.5574	579.6667 ± 4.0414	729.6667 ± 3.5118
	Terminal value	105.3333 ± 3.51188	210.0000 ± 3.0000	403.6667 ± 8.0208	629.6667 ± 4.0414
	Removal efficiency	66.98016%	48.52941%	30.36228%	13.52215%
TN	Initial value	69.0000 ± 1.0000	103.6667 ± 1.5275	119.3333 ± 1.5275	133.0000 ± 1.0000
	Terminal value	9.0000 ± 2.0000	23.6667 ± 1.5275	76.0000 ± 2.0000	105.3333 ± 2.0816
	Removal efficiency	86.95652%	75.88452%	36.31283%	20.80203%
TP	Initial value	0.9667 ± 0.0577	1.2867 ± 0.088	1.8000 ± 0.1000	2.5000 ± 0.1000
	Terminal value	0.3000 ± 0.1000	0.5567 ± 0.0513	1.3000 ± 0.1000	2.0367 ± 0.1305
	Removal efficiency	68.96553%	56.73574%	27.77778%	18.53332%
N	Initial value	16.3000 ± 0.2000	21.4000 ± 0.3000	28.7333 ± 0.2087	33.3667 ± 0.2516
	Terminal value	3.1000 ± 0.1000	6.1333 ± 0.3055	17.5333 ± 0.3511	23.4667 ± 0.2516
	Removal efficiency	80.9816%	71.33957%	38.97912%	29.67033%
P	Initial value	0.8267 ± 0.0404	1.1000 ± 0.2000	1.5700 ± 0.1212	2.2000 ± 0.1000
	Terminal value	0.2433 ± 0.0450	0.4500 ± 0.0458	1.1400 ± 0.0529	1.6767 ± 0.0305
	Removal efficiency	64.84017808%	81.84931102%	40.72163163%	31.37653211%

Table S2
Nutrient removal efficiency in different dilutions of POME by Chlorella vulgaris

	Parameter	20%	40%	60%	80%
COD	Initial value	319.0000 ± 1.0000	408.0000 ± 6.5574	579.6667 ± 4.0414	729.6667 ± 3.5118
	Terminal value	114.6667 ± 3.5118	218.0000 ± 3.0000	419.3333 ± 2.5166	635.3333 ± 3.5118
	Removal efficiency	64.05433%	46.56863%	27.65958%	12.92829%
TN	Initial value	69.0000 ± 1.0000	103.6667 ± 1.5275	119.3333 ± 1.5275	133.0000 ± 1.0000
	Terminal value	14.0000 ± 2.0000	28.6667 ± 1.5275	84.3333 ± 2.0816	110.0000 ± 1.0000
	Removal efficiency	79.71014%	72.34724%	29.32962%	17.29323%
TP	Initial value	0.9667 ± 0.0577	1.2867 ± 0.088	1.8000 ± 0.1000	2.5000 ± 0.1000
	Terminal value	0.4867 ± 0.0808	0.8000 ± 0.1000	1.4800 ± 0.0721	2.1667 ± 0.1527
	Removal efficiency	49.65516%	37.82385%	17.77778%	13.33332%
N	Initial value	16.3000 ± 0.2000	21.4000 ± 0.3000	28.7333 ± 0.2087	33.3667 ± 0.2516
	Terminal value	4.5000 ± 0.2000	7.3667 ± 0.2081	18.7667 ± 0.1527	24.5000 ± 0.2645
	Removal efficiency	72.39264%	65.57632%	34.68676%	26.57343%
P	Initial value	0.8267 ± 0.0404	1.1000 ± 0.2000	1.5700 ± 0.1212	2.2000 ± 0.1000
	Terminal value	0.3367 ± 0.0251	0.5533 ± 0.0251	1.1767 ± 0.0152	1.7800 ± 0.0360

Table S3
Nutrient removal efficiency in different dilutions of POME by Tetradesmus bernardii

	Parameter	20%	40%	60%	80%
COD	Initial value	319.0000 ± 1.0000	408.0000 ± 6.5574	579.6667 ± 4.0414	729.6667 ± 3.5118
	Terminal value	123.3333 ± 3.5118	224.0000 ± 5.0000	433.3333 ± 7.3711	651.6667 ± 7.5718
	Removal efficiency	61.33752%	45.52941%	30.36228%	13.52215%
TN	Initial value	69.0000 ± 1.0000	103.6667 ± 1.5275	119.3333 ± 1.5275	133.0000 ± 1.0000
	Terminal value	19.0000 ± 2.000	33.3333 ± 3.0550	93.3333 ± 2.0816	122.0000 ± 2.6445
	Removal efficiency	72.46377%	67.8457%	21.78772%	8.270677%
TP	Initial value	0.9667 ± 0.0577	1.2867 ± 0.088	1.8000 ± 0.1000	2.5000 ± 0.1000
	Terminal value	0.6200 ± 0.0200	0.9000 ± 0.1000	1.5533 ± 0.0152	2.2967 ± 0.0513
	Removal efficiency	35.86209%	30.05183%	13.88889%	8.13332%
N	Initial value	16.3000 ± 0.2000	21.4000 ± 0.3000	28.7333 ± 0.2087	33.3667 ± 0.2516
	Terminal value	6.1533 ± 0.0503	9.2667 ± 0.1527	19.4333 ± 0.3055	26.3000 ± 0.1000
	Removal efficiency	62.24949%	56.69782%	32.36659%	21.17883%
P	Initial value	0.8267 ± 0.0404	1.1000 ± 0.2000	1.5700 ± 0.1212	2.2000 ± 0.1000
	Terminal value	0.3833 ± 0.0152	0.6167 ± 0.0251	1.2533 ± 0.0152	1.8367 ± 0.0305
	Removal efficiency	53.62909%	43.93936%	20.16987%	17.19242%

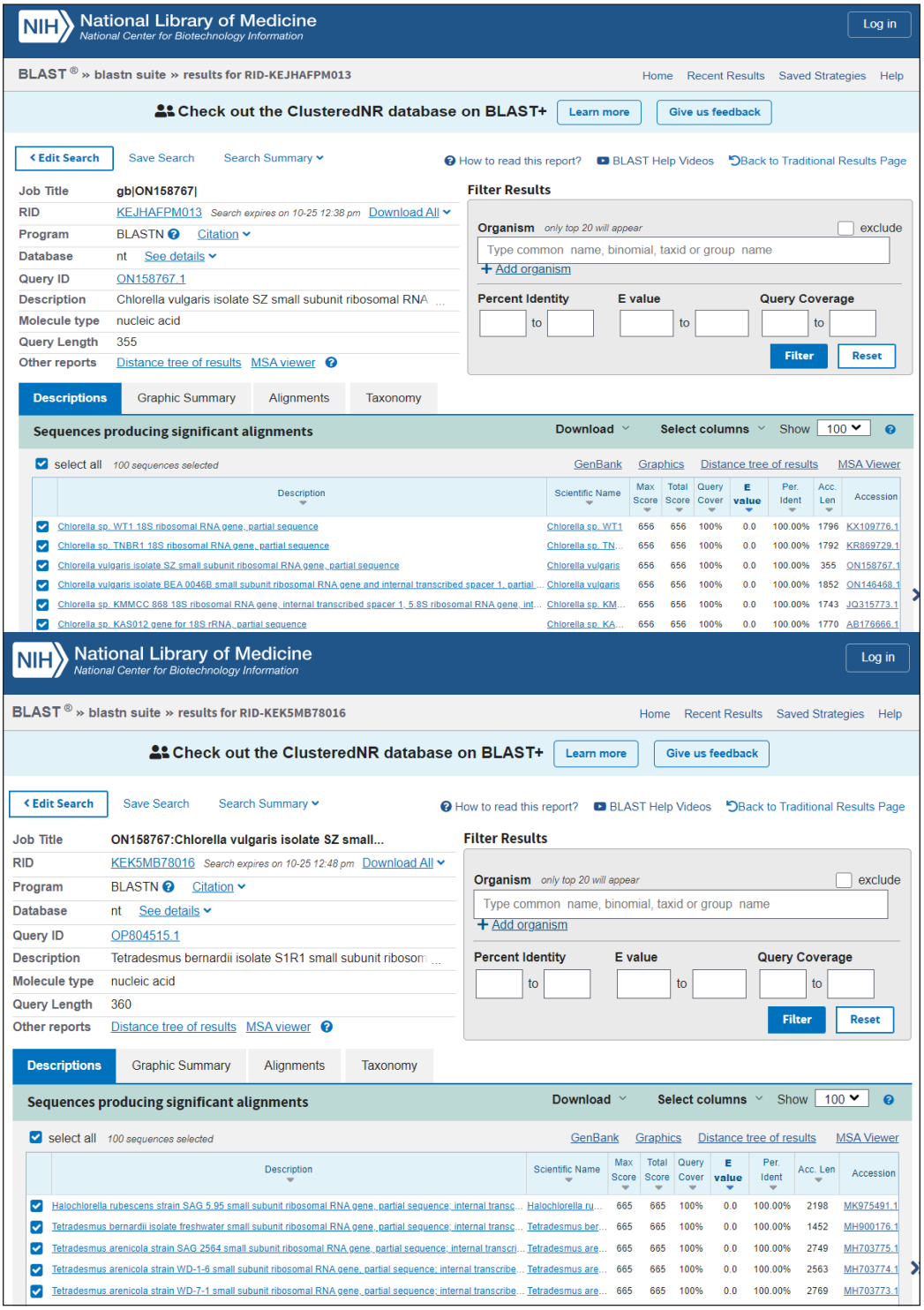

Figure S1. The NCBI blast results for *Chlorella vulgaris* (ON158767) and *Tetradesmus bernardii* (OP804515)