

# Optimization of consortia *Chlorella vulgaris* and *Nannochloropsis oculata* in remediation of palm oil mill effluent

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**Abstract.** The palm oil industry in Indonesia produces abundant production waste, including solid, liquid, and gas forms. The management of palm oil mill wastewater (POME) is still not optimal. The purpose of this study was to analyze the remediation of palm oil wastewater using a consortium of *Chlorella vulgaris* and *Nannochloropsis oculata*. The study analyzed (COD), total suspended solids (TSS), pH, salinity, and microalgae cell density at various POME concentrations (0%, 25%, 50%, 75%, and 100%). The results showed that a POME concentration of 50% produced the highest total microalgae cell density at  $774 \times 10^3$  cells/mL. A significant decrease in salinity (18%) was observed at a POME concentration of 75% with a microalgae consortium. The effective pH for the microalgae consortium is between 8-9. In addition, the microalgae consortium effectively reduced COD by 70.3% and reduced TSS by 46.74%. Therefore, the consortium of *Chlorella vulgaris* and *Nannochloropsis oculata* has potential in the remediation of POME waste.

## 1 Introduction

One of the plantation commodities that has a strategic role in the economy in Indonesia is palm oil. The palm oil industry has provided employment for 16 million workers, both directly and indirectly. Palm oil and palm kernel production in February 2023 (3,883 thousand tons) consisting of 3,514 thousand tons of crude palm oil (CPO) and 369 thousand tons of palm kernel oil (PKO) [1]. It is hoped that the contribution of the plantation subsector to the national economy will increase and strengthen overall plantation development. The palm oil industry in Indonesia was built with an approach that prioritizes balance between social, economic and environmental aspects [2].

The existence of the palm oil industry will of course have the potential to produce large amounts of palm oil waste. This large amount of palm oil waste has not been utilized properly, even though the potential of this enormous palm oil waste can provide added value to the Indonesian palm oil industry if utilized properly [3]. Palm oil waste can be in the form of solid waste, liquid waste and gas waste [4]. The factory waste is produced from the remainder of the processing process from fresh fruit bunches. This waste is divided into two

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types, namely liquid waste and solid waste. Solid waste comes from solid materials, such as coir, shells and empty leaves [21-23]. The average empty fruit bunches of oil palm is around 20% to 35% of the total fresh fruit bunches processed [5]. The large volume of waste will pollute the environment. Liquid waste comes from liquid materials, such as boiling condensate and calcium carbonate solution. Liquid waste produced from processing is in the form of water and sludge [6]. The amount of waste water depends on the processing system, processing capacity, and state of the clarification equipment.

The palm oil industry wastewater treatment process that is widely carried out now is by using anaerobic ponds [7]. Several palm oil processing factories already use fairly good wastewater treatment systems. The processing system consists of a cooling process, processing in an anaerobic pond, aerobic processing and settling/stabilization. However, anaerobic ponds also have several disadvantages, for example: they cause odor problems and groundwater contamination in the area around the pond, have toxic methane gas by-products, require periodic maintenance, and require quite a long hydraulic holding time [8-9]. Therefore, a palm oil factory wastewater treatment system is needed using biological methods, namely bioremediation.

Bioremediation is the process of cleaning the environment from biological pollutants using organisms, both in-situ and ex-situ [10] [12-13]. Bioremediation is a technology that is currently developing, therefore research must continue to be carried out and developed to overcome increasingly complex environmental problems [10-11]. Some microalgae that have been widely studied for bioremediation are *Chlorella vulgaris*, *Dunaliella salina*, *Tetraselmis chuii*, *Spirulina platensis*, *Nannochloropsis oculata* and others.

The potential of microalgae as bioremediation has been widely explored, but it is still single, so there is no information on the most reliable microalgae species for bioremediation. This research must be carried out immediately to overcome the problem of liquid palm oil waste which continues to overflow and pollute the waters in the surrounding environment. Even though physical, chemical and biological processing has been carried out, the concentration is often still high. Utilization of microalgae in one of the biological ponds at the Waste Water Treatment Plant (IPAL) can result in a decrease in BOD, COD, TSS parameters.

## 2 Materials and Methods

The research was carried out in January-July 2024 at the UNDIP Biology Laboratory and the UNNES Environmental Laboratory. The materials used in this research were: *Chlorella vulgaris* culture, *Nannochloropsis oculata* culture, liquid palm oil waste, RO water, blue grease brand synthetic salt, F/2 Guillard medium, plastic wrap, jar, distilled water, 500 ppm KHP solution, acid solution concentrated sulfate, low digestion solution, filter paper, litmus paper. The tools used in this research include: UV-VIS spectrophotometer, pH meter, thermometer, analytical balance, oven, COD reactor, TDS meter.

The independent variable in this research is the percentage concentration of liquid palm oil waste (POME waste) taken from PT Mandiri Palmera Agrindo, South Sulawesi. The dependent variable in this research is the growth of microalgae as a bioremediator agent. The research plan procedures are as follows:

1. Preparation of raw materials for liquid palm oil waste  
Palm oil liquid waste is taken from PT Mandiri Palmera Agrindo
2. Preparation of inoculum/microalgae seeds  
Preparing the microalgae *Chlorella vulgaris* and *Nannochloropsis oculata*. *Spirulina* seeds were obtained from BBPAP Jepara. Initial populations of *Chlorella vulgaris* and *Nannochloropsis oculata*. as inoculum is 10,000 cells/ml.
3. Test the characteristics of microalgae as a bioremediation agent

The process of inoculating the *Chlorella vulgaris* and *Nannochloropsis oculata* consortium into liquid palm oil waste is by adding microalgae to liquid palm oil waste according to the microalgae/waste volume ratio (v/v): A (4 : 0), B (3 : 1), C (2 : 2), D (1 : 3), E (0 : 4). Providing nutrition at the beginning of treatment. Next, we observed the parameters of cell density (Optical Density), BOD, COD, TSS, Salinity and pH as well as the morphology of the microalgae at the beginning and at the end. Growth measurement and monitoring: Monitoring of algae growth was done daily for two weeks. A certain volume was collected from each culture vessel to determine the concentration based growth responses optically using absorbance 680 nm.

4. Quality standards are determined based on Minister of Environment and Forestry Regulation No. 5 of 2014 concerning waste water quality standards can be seen in the Table 1.

**Table 1.** Wastewater Quality Standards for Palm Oil Industry Businesses and/or Activities

Parameter	Concentration of Quality Standard	Unit
COD	350	mg/L
TSS	250	mg/L
pH	6-9	

### 3 Result and Discussion

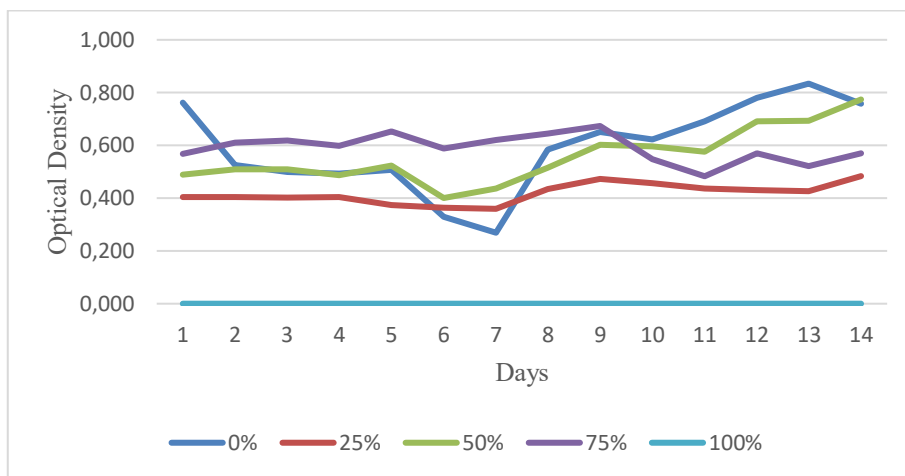
This research was carried out in several research stages to produce the potential for a consortium of *Chlorella vulgaris* and *Nannochloropsis oculata* in remediating palm oil liquid waste. There are 5 variations in concentration, namely:

- a. Treatment A (control): 100% microalgae consortium
- b. Treatment B: 75% microalgae consortium and 25% POME waste
- c. Treatment C: 50% microalgae consortium and 50% POME waste
- d. Treatment D: 25% microalgae consortium and 75% POME waste
- e. Treatment E: 100% POME waste

The parameters used to determine the effectiveness of the *Chlorella vulgaris* and *Nannochloropsis oculata* consortium for processing palm oil liquid waste are as follows:

#### 3.1 Density of Microalgae Consortium Cell Numbers in POME Waste Remediation

*Chlorella vulgaris* and *Nannochloropsis oculata* are types of microalgae that have a fairly short life cycle. Generally spirulina will complete one life cycle within 14-20 days. Therefore, it is necessary to know the highest growing period so that you can get the most abundant biomass harvest. Therefore, optical density standards are used to know for sure when the culture can be harvested. Optical density measurements can be carried out using a spectrophotometer with a wavelength of  $\lambda = 680$  nm, so that the growth data obtained for *Chlorella vulgaris* and *Nannochloropsis oculata* are shown in Fig. 1.



**Fig.1.** Growth of *Chlorella vulgaris* and *Nannochloropsis oculata* in 14 Days on Various Treatments

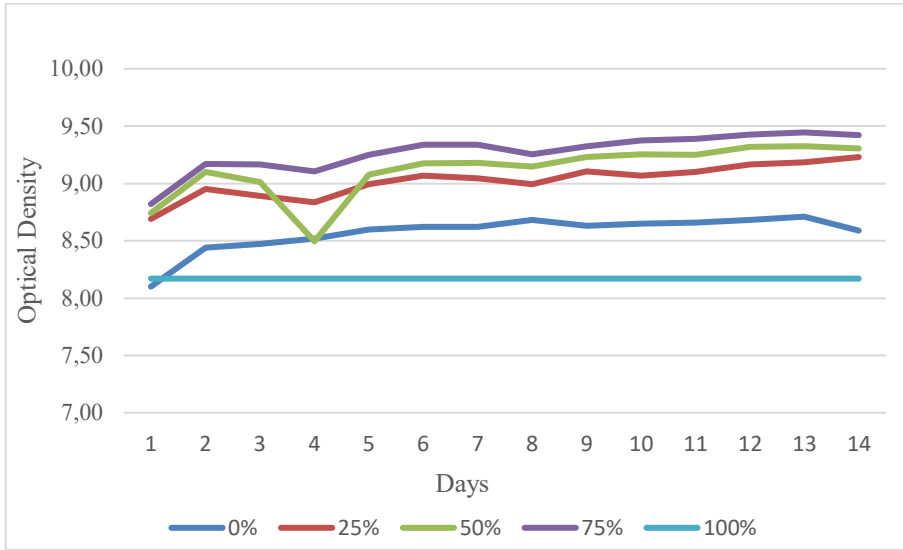
Based on the research results, it shows that the growth of the *Chlorella vulgaris* and *Nannochloropsis oculata* consortium up to day 14 experienced a significant increase in all treatments at concentrations of 25%, 50% or 75%. A concentration of 50% shows that the peak cell density in the *Chlorella vulgaris* and *Nannochloropsis oculata* consortium reaches  $774 \times 10^3$  cells/mL. This is in contrast to the control treatment which contained a consortium of *Chlorella vulgaris* and *Nannochloropsis oculata* which experienced a decrease on the 14th day.

The condition of the *Chlorella vulgaris* and *Nannochloropsis oculata* consortium experienced a log phase starting on day 7 as did all other treatments. This occurs because at the beginning of the treatment each microalgae is still adapting to the new environment, which is different from cultivating a single microalgae in 7 days, the increase is actually on day 4-5. The adaptability of the *Chlorella vulgaris* and *Nannochloropsis oculata* consortia is indeed slower than the respective microalgae cultivation. Even though the adaptation was slow, growth in both control and waste treatment actually showed a sharp increase in cell growth. The growth rate of free suspended cells is slightly slower during the first 3 days (lag phase) compared to immobile cells.

Direct contact between suspended free cells and POME can exert physical and chemical stress on microalgae cells, resulting in longer cell adaptation to the POME substrate [14]. This statement is also in accordance with the results of previous research that *Chlorella* sp was able to survive in sago liquid waste with a peak cell density of  $233.33 \times 10^3$  cells/mL after 9 days [15]. Apart from that, research is related to the effectiveness of *Nannochloropsis oculata* on POME waste.

### 3.2 pH and salinity of the Microalgae Consortium in POME Waste Remediation

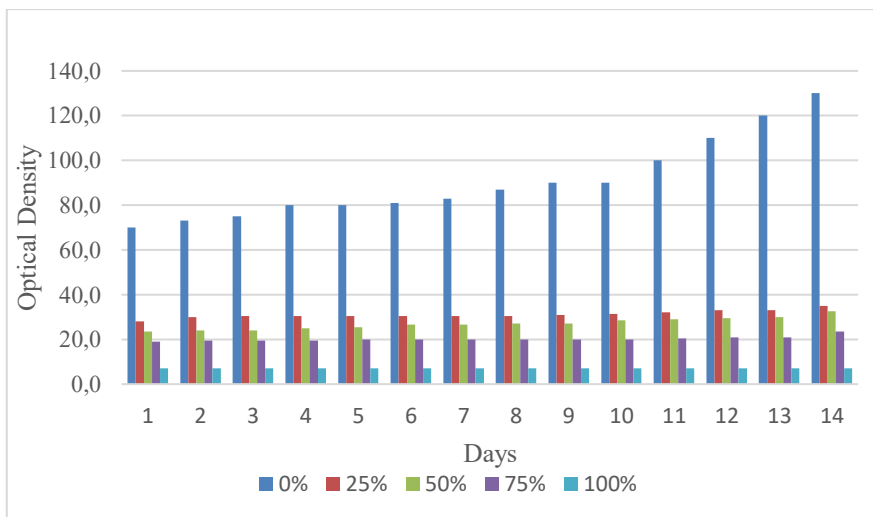
Environmental conditions influence the growth and activity of microalgae. The parameters measured are pH, temperature, salinity, light intensity and nutrient concentration which greatly influence the efficiency of the microalgae consortium in remediation of POME waste. Based on the research results obtained, it can be seen in Fig. 2.



**Fig. 2.** Average pH of *Chlorella vulgaris* and *Nannochloropsis oculata* in 14 Days in Various Treatments

Based on the research results, it shows that the average consortium of *Chlorella vulgaris* and *Nannochloropsis oculata* up to day 14 in various treatments was between 8-9. The average pH in the 25%, 50% and 75% treatments increased from the beginning of the treatment to the end of the treatment, whereas the control treatment without POME waste showed a decrease in pH at the end of the treatment.

This is in accordance with previous research [16] that *Chlorella vulgaris* experienced a decrease in the pH value of the control treatment (0%) at the end of the study in laundry waste remediation. Although there were subsequent increases, at 5%, 10%, 20%, 30% and 40% and 50% at the end of the study. Another parameter observed in this research was the salinity of the microalgae consortium. The results obtained in this research can be seen in Fig. 3.

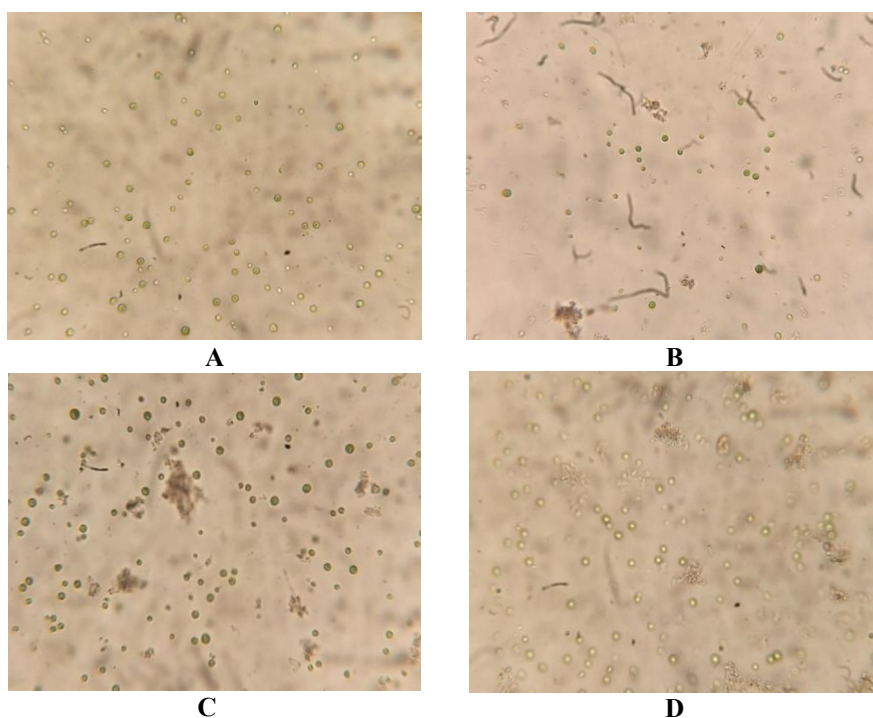


**Fig. 3.** Average Salinity of *Chlorella vulgaris* and *Nannochloropsis oculata* in 14 Days in Various Treatments

The initial conditions of the microalgae consortium before treatment showed very high salinity compared to other treatments. Over time until the last day of treatment, salinity showed a very significant decrease, namely a decrease of 18% in treatment D (25% microalgae consortium and 75% POME waste). This shows that the provision of a microalgae consortium is able to reduce salinity levels in POME waste. The salinity in the treatment is influenced by the volume of POME waste added, the more waste added, the smaller the salinity will be. This is in accordance with previous research which showed that the growth of *Nannochloropsis oculata* (on single microalgae) had salinity levels between 25-35 ppt [17]. Conditions of salinity that are too high cause a decrease in cell growth, inhibit photosynthesis and the growth of microalgae cells [18]. Salinity influences the maintenance of environmental osmotic pressure.

### 3.3 Microscopic morphology of the Microalgae Consortium in POME Waste Remediation

The microscopic morphology of the microalgae consortium in various treatments needs to be observed to determine the movement or activity of the microalgae consortium in the remediation of POME waste. Fig. 4 is a picture of the *Chlorella vulgaris* and *Nannochloropsis oculata* consortium in POME waste.



**Fig. 4.** Morphology of the *Chlorella vulgaris* and *Nannochloropsis oculata* consortium on day 14 (Treatments A, B, C, and D)

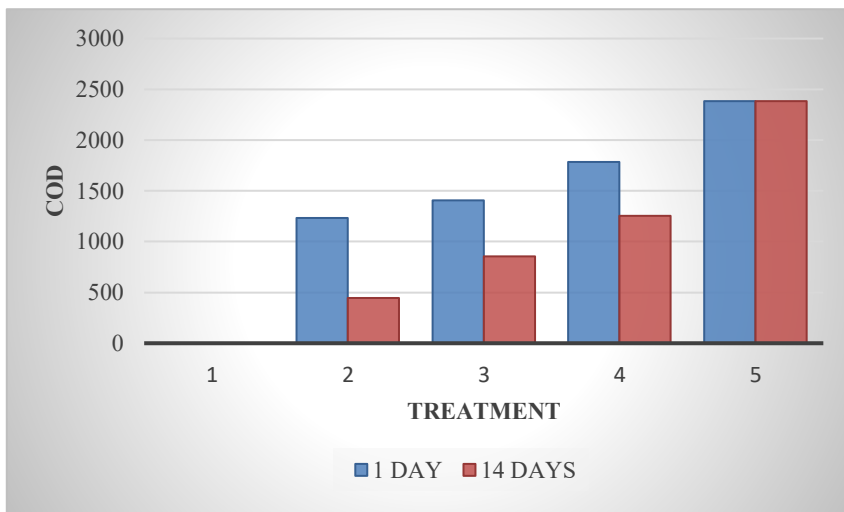
Based on the research results, it shows that the morphology of the *Chlorella vulgaris* and *Nannochloropsis oculata* consortium without the addition of POME waste is less than with the addition of POME waste at concentrations of 50% and 75% on day 14. The results of this study mean that POME waste is suitable for use for nutrition of the *Chlorella vulgaris* consortium and *Nannochloropsis oculata* and can be used as a remediating agent. In addition,

based on microscopic observations, the density of *Chlorella vulgaris* increased compared to *Nannochloropsis oculata* as the concentration of POME waste added to it increased.

Previous research also showed that at a waste concentration of 30% POME was effectively used as nutrition for *Chlorella* sp [19]. Therefore, at a concentration of 50-75% in this study it also showed an increase in cell density in the *Chlorella vulgaris* and *Nannochloropsis oculata* consortium. Previous research also showed that at a waste concentration of 30% POME was effectively used as nutrition for *Chlorella* sp [19]. Therefore, at a concentration of 50-75% in this study it also showed an increase in cell density in the *Chlorella vulgaris* and *Nannochloropsis oculata* consortium.

### 3.4 Chemical Oxygen Demand (COD) and Total Suspended Solids (TSS) Levels in the Microalgae Consortium in POME Waste Remediation

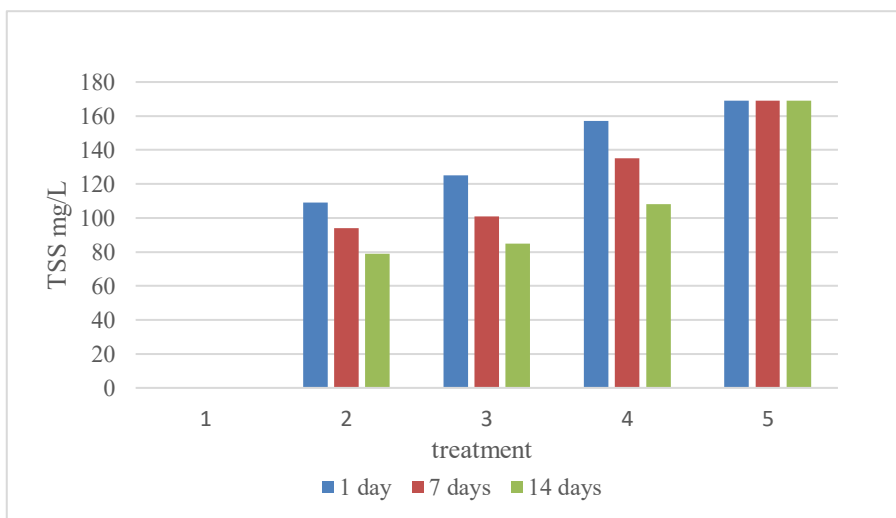
COD is a measure of the amount of oxygen required to oxidize organic matter in water. In other words, the more liquid waste that is added, the more organic matter and pollutants there are in the water, requiring more oxygen to decompose it. Therefore, an increase in the volume of liquid waste will usually cause an increase in COD values, indicating that the water is more polluted and requires greater effort to treat it.



**Fig. 5.** COD concentration in *Chlorella vulgaris* and *Nannochloropsis oculata* consortium on day 1 and day 14 (Treatment B, C, D, and E)

Based on the research results shown in Fig. 5, it shows that COD levels in the *Chlorella vulgaris* and *Nannochloropsis oculata* consortium are decreasing day by day. The microalgae consortium was proven to reduce COD levels to a percentage of 70.3% in treatment D, namely with a waste volume of 75% with the addition of a consortium of *Chlorella vulgaris* and *Nannochloropsis oculata* of 25%. The reduction in COD levels in this treatment was due to the microalgae consortium being able to absorb organic and inorganic compounds contained in POME waste. These organic compounds are nutrients needed for growth. During treatment, organic and inorganic compounds will decompose into proteins and amino acids naturally by bacterial activity which breaks down complex compounds into simple ions which are ready to be used as nutrients for microalgae. During the nutrient absorption process, microalgae need oxygen to absorb organic compounds in POME waste as well as waste reduction efforts.

Apart from that, to see the effectiveness of the microalgae consortium in remediation of POME waste, it can be observed using the Total Suspended Solids (TSS) parameter. To find out the TSS levels in each treatment, you can see in Fig. 6.



**Fig. 6.** TSS concentration in *Chlorella vulgaris* and *Nannochloropsis oculata* consortium on days 0, 7 and 14 (Treatment B, C, D, and E)

Total Suspended Solids (TSS) is an important parameter used to measure the amount of suspended solid particles in wastewater. TSS includes all particles that are insoluble in water, such as mud, silt, clay, organic matter, and remains of microorganisms found in wastewater. These particles are usually more than 2 micrometers in size and can be removed through filtration or settling processes.

TSS measurements are important because suspended particles can cause various problems in water bodies, such as decreasing water quality, increasing turbidity, and disrupting the photosynthesis process of aquatic plants due to reduced light penetration. High TSS values in wastewater indicate a high level of pollution, which can have a negative impact on aquatic ecosystems if not treated properly.

## 4 Conclusion

The use of microalgae consortia in the remediation of POME waste has a very significant effect based on research results which show that the amount of biomass increases when 50% POME waste is given by  $774 \times 10^3$  cells/mL. reducing COD levels in POME waste by up to 70.3% when using a consortium of *Chlorella vulgaris* and *Nannochloropsis oculata*. The TSS concentration showed a decrease with the addition of the microalgae consortium with a reduction percentage reaching 46.74%. This shows that the *Chlorella vulgaris* and *Nannochloropsis oculata* consortium is effective in remediating POME waste.

## References

1. M. Sardjono, [www.gapki.id](http://www.gapki.id). (2023)
2. M.R.B. Kencana, [www.liputan6.com](http://www.liputan6.com) (2023)
3. M. A. Dirgantoro, R. Adawiyah, *Biowallacea*, **5** (2), 825-837 (2018)
4. A. Haryanti, N. Norsamsi, P.S.F. Sholiha, N. P. Putri, *Konversi*, **3**(2), 20-29 (2014)
5. M. Apriyanto, *J. Teknologi Pertanian*, **9**(2), 86-93 (2020)
6. E.A. Putra, *AGROTEKNOSE J Teknologi dan Enjiniring Pertanian* **4**(2), (2017)
7. R. Komala, S. Aziz, *J. Redoks*, **4**(2), 7-16 (2019)
8. O.S. Oktari Setiyorini, Doctoral dissertation, STIK Bina Husada Palembang, (2019)
9. N. Kamal, *Teknik Kimia*, ITENAS, Bandung (2012)
10. Y. Suryani, *J. Istek*, **5**(1-2), (2011)
11. A. Kurniawan, L.N.M. Salamah, A.A. Amin, A.T. Yanuar, Z. Pramudia, Y.A.D Susanti, ... & A. Rizkymaris, Universitas Brawijaya Press (2022)
12. V.M. Kensa, *I Control Pollution*, **27**(2), 161-168 (2011)
13. S. Das, H.R. Dash, *Microbial biodegradation and bioremediation* (pp.1-21), Elsevier (2014)
14. S. Elystia, R.S. Muria, L. Anggraini, Removal of COD and total nitrogen from palm oil mill effluent in flatphotobioreactor using immobilised microalgae *Chlorella* sp. (2018)
15. I. Effendi, M.N. Putri, U.L. Astika, R. Kurniawan, *Jurnal Natur Indonesia*, **21**(1), 55-62 (2023)
16. M.A. Akhere, H. Chukwuka, *Afr. Sci*, **24**, 175-182 (2023)
17. H. Hardiani, T. Kardiansyah, S. Sugesty, *Jurnal Selulosa*, **1** (01), (2016)
18. S. Murwani, *Jurnal Ilmiah Biologi Eksperimen dan Keanekaragaman Hayati*, **4**(1), 23-30 (2017)
19. W.Y. Cheah, P.L. Show, J.C. Juan, J.S. Chang, T.C. Ling, *Energy Conversion and Management*, **174**, 430-438 (2018)