

Review

Microalgae Cultivation in Palm Oil Mill Effluent (POME) Treatment and Biofuel Production

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Abstract: Palm oil mill effluent (POME) is the wastewater produced during the palm oil sterilization process, which contains substantial amounts of nutrients and phosphorous that are harmful to the environment. High BOD and COD of POME are as high as 100,000 mg/L, which endanger the environment. Effective pre-treatment of POME is required before disposal. As microalgae have the ability of biosorption on nutrients and phosphorous to perform photosynthesis, they can be utilized as a sustainable POME treatment operation, which contributes to effective biofuel production. Microalgae species *C. pyrenoidosa* has shown to achieve 68% lipid production along with 71% nutrient reduction in POME. In this study, a brief discussion about the impacts of POME that will affect the environment is presented. Additionally, the potential of microalgae in treating POME is evaluated along with its benefits. Furthermore, the condition of microalgae growth in the POME is also assessed to study the suitable condition for microalgae to be cultivated in. Moreover, experimental studies on characteristics and performance of microalgae are being evaluated for their feasibility. One of the profitable applications of POME treatment using microalgae is biofuel production, which will be discussed in this review. However, with the advantages brought from cultivating microalgae in POME, there are also some concerns, as microalgae will cause pollution if they are not handled well, as discussed in the last section of this paper.

Keywords: palm oil mill effluent (POME); microalgae; green approach; effluent treatment; bio-fuel production

1. Introduction

The global palm oil market demand stood at 71.48 million tons in 2019, and the growth rate is estimated to be 2.3% from 2020 to 2027 [1]. Palm oil is able to decrease cholesterol

level, boost brain health, reduce oxidative stress, slow the progression of health disease, increase vitamin A status, and improve skin and hair health [2]. The demand for palm oil is increasing constantly because of the increasing rate of consumer awareness regarding healthy products. Palm oil has been reported to be used in 50% of all consumers product sold daily. With the high demand for palm oil, the amount of biomass produced will continuously increase. Palm oil mill effluent (POME) is one of the primary biomasses that are produced in the palm oil mill in the form of wastewater produced from sterilization, extraction, and clarification processes in a palm oil mill. The wastewaters that are produced contain 90% of water, 0.6–0.7% of residual oil, 2–4% of suspended solids, and 4–5% soil particles [3]. A normal palm oil mill plant schematic diagram with biomass palm oil mill effluent is as shown in Figure 1.

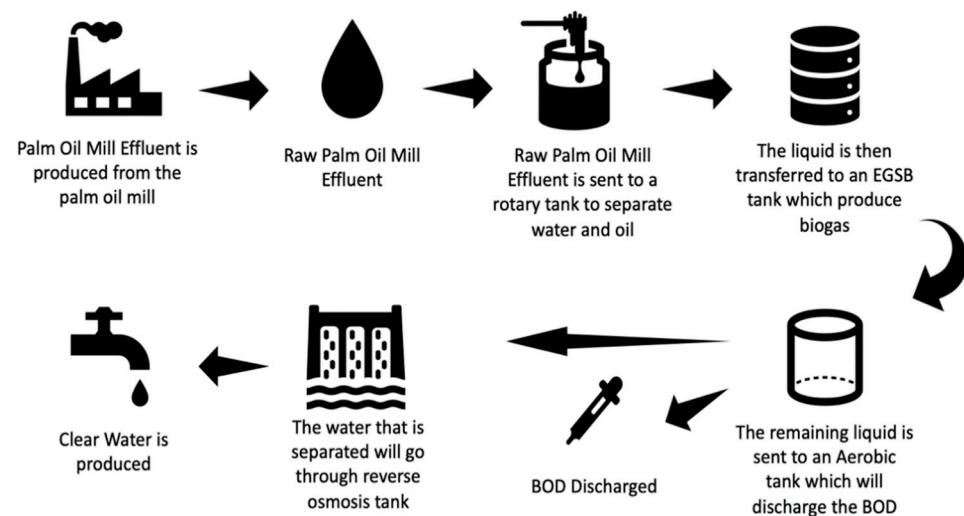


Figure 1. Schematic diagram of POME treatment.

In a commercial industry for palm oil processing, every ton of fresh fruit and palm oil will produce 0.5 and 2.5 ton of effluent, respectively [4]. Due to the acidic nature of the contaminant and very high level of biological oxygen demand (BOD), POME cannot be disposed without specific treatment due to the pollution threat to the environment [5]. Treating POME before disposal is one of the concerns and necessities for every palm oil manufacturer. POME treatment is a standard requirement that needs to be followed in order to persist in the manufacturing industry. Most of the POME treatments lead to biological handling due to its high lipids, nitrogenous compound, and protein and minerals content [6]. There are three main methods that are commonly preferred by the palm oil manufacturer, which are anaerobic digestion, feedstock for biodiesel, and composting method. Some of the manufacturers convert the POME produced from their plant into biodiesel by dark fermentation process, whereas some use treated POME on land application of compost production to act as fertilizer of the palm oil trees [7]. However, most of the manufacturers prefer anaerobic digestion, as it is cost friendly and can reduce the BOD and chemical oxygen demand (COD) [8]. Additionally, the biogas produced can be utilized as electricity generation to be supplied back to the palm oil mill.

POME treatment using microalgae to produce biofuel appears to be a highly competitive tool in recent years due to the global energy security issue [9,10]. Improvements via biomodifications can achieve economics of biodiesel production [11]. Successful biomethane, biohydrogen, and bioethanol can be produced via POME treatment using microalgae [12]. This paper presents a brief review of biofuel production from POME treatment using microalgae. The current study discusses the application of microalgae in POME treatment from a unique perspective, focusing on reviewing microalgae and POME by employing an interrelationship approach. The understanding of the interrelationship is crucial for exploring microalgae's role in POME treatment, where the biofuel production

is of interest. The paper first examines the potential of microalgae in POME treatment and discusses the biofuel production from POME treatment in second part. The future perspectives and challenges of microalgae usage in POME treatment are also discussed in this paper.

2. Microalgae as a Potential Tool in POME Treatment

2.1. Microalgae Cultivation in POME and Benefits of Treating POME with Microalgae

Microalgae are a group of various unicellular microorganisms such as blue-green algae, eukaryotic protists, and prokaryotic cyanobacteria. These microorganisms have a lot of good characteristics that bring benefits to various types of biotechnological processes. Microalgae can be grown through photosynthesis and use light, carbon dioxide, nutrients, and water to turn into energy for cell development [13]. Microalgae mainly consist of lipids, proteins, and carbohydrates as their major chemical component [14]. The composition of the components varies for different cells. Microalgae are very flexible and adaptable to grow in various types of environments. They are flexible and can be cultivated in both open ponds and closed photobioreactors [15,16]. Microalgae constituents of different species are listed in Table 1 below.

Table 1. Constituents of different microalgae species.

Microalgae	Lipid	Protein	Carbohydrate
<i>B. braunii</i> [17]	33–86	4–40	20
<i>C. reinhardtii</i> [18]	18–22	46–48	17
<i>C. ellipsoidea</i> [19]	10–30	34–35	24–51
<i>C. pyrenoidosa</i> [19]	8–35	31–47	20–57
<i>C. vulgaris</i> [20]	10–50	29–58	12–17
<i>S. platensis</i> [21]	4–13	42–63	8–30
<i>D. tertiolecta</i> [22]	3–13	26–61	22
<i>E. gracilis</i> [23]	11	29	32

Wastewater treatment using microalgae is an eco-friendly process that can eliminate pollution [24]. Microalgae have potential in treating POME, because algae grow using solar energy via photosynthesis, and converting them into biomass can bring profit by utilizing nitrogen and phosphorous compound that are present in POME. The growth rate of microalgae is very fast due to their very high photosynthetic efficiency, which is 10 times faster than terrestrial plants. Besides that, using microalgae to treat POME is very economically friendly, as biomass of microalgae growth in POME can potentially be a major source of biofuel [25–27]. Another advantage of using microalgae is that they can be cultivated on non-agricultural land [28]. Using microalgae to treat POME can also help by lowering down the BOD and COD, which can help in preventing pollution [29].

The possibility of using POME for microalgae cultivation is very wide and beneficial. This is due to the fact that not only can using microalgae to treat POME reduce the nutrient level contained in it, but the biomass produced can also be utilized as bulk biomass or value-added product. Contaminant in cultivated microalgae on POME is rich in lipids, proteins, and carbohydrates, which are mainly focused on animal feed and fuel. On the other hand, microalgae produce high value biofuel and bioactive compounds, which are beneficial for energy and pharmaceutical industry, respectively. Several experiments had been done by researchers with different types of microalgae, and the outcome of the studies is as shown in Table 2.

Table 2. Microalgae performance cultivated in POME.

Microalgae Species	Nutrient Reduction (%)	Lipid Production (%)	Growth Rate (1/day)	Biomass Productivity (g/L/d)	Duration (d)
<i>Chlamydomonas</i> [30]	68	60	1.2	0.08	7
<i>Chlorella</i> sp. [31]	-	-	0.066	0.058	15
<i>S. plantensis</i> [21]	-	-	-	9.8	13
<i>C. pyrenoidosa</i> [32]	71.16	68	1.8	0.13	10
<i>T. suecica</i> [33]	95	27	-	0.211	7
<i>N. oculata</i> [34]	93.6	39	-	0.52	7

Treating POME with microalgae is a better way compared to the conventional method such as anaerobic digestion, as the latter is unable to fulfil the requirements set by Department of Environment, as the BOD level exceeds 100 mg/L before release to the aquatic environment [35]. Treating POME with microalgae is very beneficial for all the palm oil manufacturers due to its environmental-friendliness and economical-friendliness [24]. In addition, one of the benefits of microalgae usage is that it can absorb carbon dioxide from the atmosphere and release oxygen into the system, which can lead to higher oxygen concentration in the system [15].

Microalgae are flexible to grow in any suitable environment, and therefore do not cost anything. POME is one of the most suitable places for microalgae growth, as it has all the elements that are needed for microalgae to grow, which are light, carbon dioxide, nutrients, water, and phosphorous [36]. Thus, microalgae can form naturally in POME pond. Additionally, pre-treated POME can be used to produce biohydrogen using microalgae [37]. Furthermore, algae can also grow in the presence of heavy metals [14]. This means that microalgae not only can remove organic compound, but also are capable of removing heavy metals [38].

Microalgae have been examined as a potential target to extract lipid for biofuel production due to their economical-friendliness and environmental-friendliness [39,40]. Furthermore, recent studies have been shown to enhance microalgae for further biofuel production [41–43]. Figure 2 shows the bioethanol production using bioengineered microalgae *Synechocystis* sp. PCC 6803, with a theoretical yield of 0.696 g ethanol/g CO₂ [44]. Protein removal in algae solution has been studied by using membrane chromatography technique [45]. Lipid content in microalgae is very much related to the performance of microalgae in the wastewater system [43]. Lipid is made up of a functionally diverse group of compounds, meaning different types of microalgae will have various lipid content. Thus, microalgae *C. vulgaris* (CV) and *C. pyrenoidosa* (CP) were cultivated in a medium containing POME with concentration of 10:90, 50:50, and 95:5 (raw POME: algae ratio) to evaluate its lipid production [46]. Both microalgae are able to adapt to POME wastewater, and CV has better growth rate and acclimatization compared to CP in POME for 20 days. They also tended to stabilize themselves, as shown by the constant absorbance value after day 14. Both microalgae attained their optimum growth under POME: algae ratio of 95:5 and concentration ratio of 10:90 are unsuitable for microalgae growth. Despite the better growth of CP than CV, CP still produced more lipid content than CV at ratio 95:5. Both CP and CV were found to grow well in POME, but CP dominated in POME. This shows POME, as a carbon-enriched medium, can enhance microalgae growth to reduce pollution in wastewater. On the other hand, oleaginous microalgae can produce oil efficiently in the presence of sunlight due to their high lipid content (30–70%) [47].

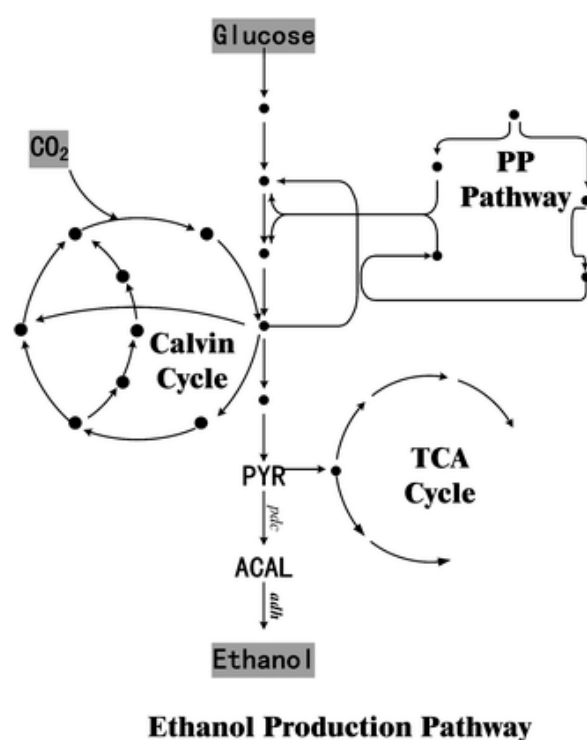


Figure 2. Bioethanol production pathway using *Synechocystis* sp. PCC 6803. Reproduced from [44] with permission from The Royal Society of Chemistry.

Furthermore, biomass formed from microalgae is very valuable, as it can be used to produce many useful applications. Utilizing microalgae biomass can be very profitable for palm oil manufacturers, as the products that are produced from microalgae biomass are high end products such as food colorant, vitamins, cosmetics, and pharmaceutical applications [48,49]. These applications are very profitable value products. Also due to the richness in protein of microalgae biomass, it can also be used as ruminant feed for animals [50,51]. Microalgae are also a potential biofuel feedstock material due to their capable characteristics [52]. It was found that using POME to cultivated microalgae *Spirulina platensis* contains 1.8 g/L biomass and 0.216 g/L phycocyanin, which can be utilized for the products aforementioned [53]. Moreover, microalgae have high level of polyunsaturated fatty acids, which are highly comparable with the fish fatty acids and are able to replace them. Different algae have their own ability to be utilized on different products [54]. The residual biomass of green algae that are extracted from wastewater can be further processed into many applications such as livestock feed, organic fertilizer, and biostimulants and used for energy cogeneration to produce electricity and heat energy.

Table 3 outlines the annual biodiesel production, yield, and land required for different source types [55]. High biofuel yield and low land requirement of microalgae point towards the positive usage of microalgae as biofuel producer. Additionally, biofuel production using microalgae does not compete with production of food for humans, and therefore does not possess food crisis in the long run. Thus, cultivating microalgae in POME not only can remove the pollutant material in the palm oil mill, but also produce valuable biomass by-product. Thorough analysis on experimental and modelling results indicates that microalgae are capable of aiding in global warming prevention and can be used as an alternative energy source [56]. Besides that, microalgae do not require fertile land to grow, as they can grow in wastelands such as brackish, wastewater, or ocean [50,57]. This shows that the cultivation of microalgae does not compete with resources for food production such as rice that require big piece of land to grow. Moreover, microalgae will be more environmentally sustainable with respect to extensive cultivation of crops.

Table 3. Annual biodiesel production, yield, and land required for different source type [55].

Source Type	Yield (L oil/ha Year)	Land Required (m ² /kg Biodiesel Year)	Biodiesel Production (kg/ha Year)
Corn	172	66	152
Hemp	363	31	321
Soybean	636	18	562
Jatropha	741	15	656
Camelina	915	12	809
Rapeseed	974	12	862
Sunflower	1070	11	946
Castor	1307	9	1156
Palm oil	5366	2	4747
Microalgae	58,700–136,900	0.1–0.2	51,927–121,104

2.2. Condition of Growth of Microalgae in POME

Microalgae can grow in any medium that has all the nutrients necessary for algae growth. The nutrients that are necessary for algae growth include nitrogen, phosphorus, and carbon, which can be found in POME pond [58]. The growth rate and lipids produced from microalgae can be increased with light intensity, glucose, and carbon dioxide concentration [59–61]. From the experiment, it was found out that higher concentration of carbon dioxide increases the microalgae growth rate. POME is a suitable medium for growth of microalgae, as it is rich in all the nutrients necessary for algae growth and sufficient amount of sunlight, as it is an open pond medium. Table 4 shows the POME constituents, which are suitable for microalgae growth.

Table 4. Constituents of POME [62,63].

Parameter	Mean	Range
pH	4.2	3.4–5.2
BOD (mg/L)	25,000	10,250–43,750
COD (mg/L)	51,000	15,000–100,000
Total solids (mg/L)	40,000	11,500–79,000
Suspended solids (mg/L)	18,000	5000–54,000
Volatile solids (mg/L)	34,000	9000–72,000
Oil and grease (mg/L)	6000	130–18,000
Ammoniacal nitrogen (mg/L)	35	4–80
Total nitrogen (mg/L)	750	180–1400

For microalgae growth, there are several variables that play a crucial role to meet the condition for growth. Microalgae mainly undergo photosynthesis for growing; thus, light intensity and concentration of carbon dioxide will be a major component that can directly affect the growth of microalgae. Effect of light intensity and concentration of carbon dioxide was carried out to evaluate the on the growth of microalgae, as shown in Figure 3 [64]. In this study, microalgae growth is directly proportional to light intensity, which is also supported by another study [65]. High concentration of carbon dioxide will lead the medium to be acidic, which significantly decreases the photosynthetic activity and slows down the growth rate. Optimal CO₂ concentration was found to be 12.5% [66]. On the other hand, maximum lipid production of *Ettlia* sp. YC001 was found to be under 0.05% CO₂ concentration and light intensity of 400 µE/m²/s [67]. Additionally, highest photosynthetic activity and maximum oxygen production rate were found to be with green light, at different microalgae loadings [68].

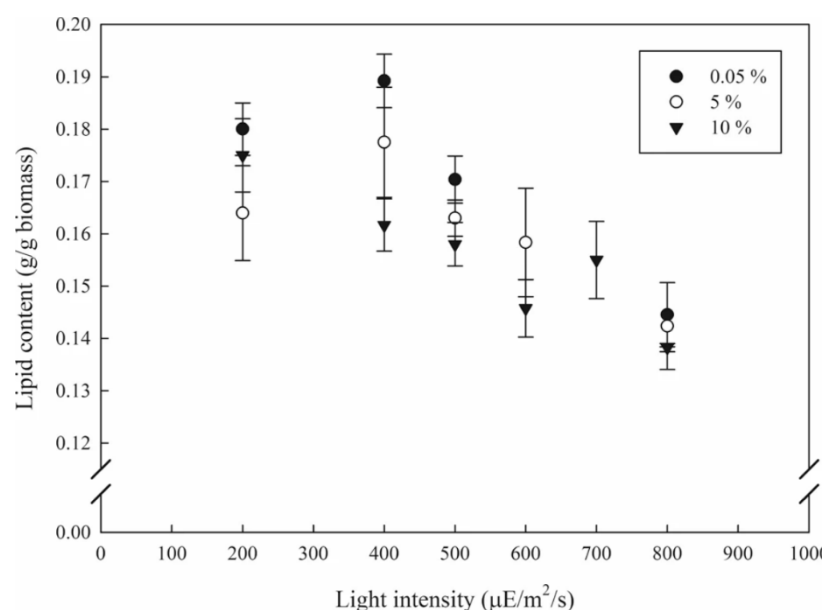


Figure 3. Lipid production of microalgae with different light intensities and CO₂ concentrations. Reproduced from [67] with permission from Springer Nature.

Microalgae are metabolically flexible and have a short doubling time. There are many species of microalgae, in which most of the microalgae are photoautotrophs. Many species undergo metabolic shift to heterotroph in different type of conditions and environments. Microalgae carry out photosynthesis as the main energy source and utilize carbon dioxide and organic molecules for carbon source. Besides that, POME pond is in stagnant condition, as the wastewater is disposed into a pond without any flow. This will help in growth of microalgae, as water movement will disrupt the growth of microalgae, as they can only grow in a calm stream.

The range and optimum conditions for microalgae growth in POME for lipid production are shown below in Table 5.

Table 5. Conditions of microalgae growth in POME [69].

Parameter	Range	Optimum
Temperature (°C)	16–27	18–24
Salinity (g/L)	12–40	20–24
Light Intensity	1000–10,000	2500–5000
Photoperiod (light:dark)	-	16:8 (Minimum) 24 h (Maximum)
pH	7–9	8.2–8.7
CO ₂ rate	1–4%	1% of volume in air
Nutrients	-	N:P (16:1) and Silicon

2.3. Pre-Treatment of POME for Microalgae Growth

There are several processes to pre-treat POME in order to maintain a healthy growth rate of microalgae in the system. Many types of pre-treatment, such as thermal, chemical, mechanical, and biological processes, are ways to treat the wastewater before cultivation. Pre-treatment of POME, such as coagulation process and absorption, can be done in order to improve the penetration of light during culturing process of microalgae in POME system [70]. The coagulation process was done by using rice starch and tapioca starch. On the other hand, the absorption process was done by using activated carbon from other biomass such as palm kernel shell, which makes it a good way to utilize the biomasses produced in the mill. The pre-treatment was evaluated by optimizing several different parameters which are pH, particle size, stirring speed, and dosage. The pre-treatment

methods were performed with culturing of *S. dimorphus*, *C. vulgaris*, and *D. salina*. Absorption process using activated carbon from palm kernel shells achieved better reduction in turbidity (83.33%), COD (83.91%), and suspended solids (92.30%) than coagulation using rice and tapioca starch. However, the absorption process takes a much longer time than coagulation [70].

Another pre-treatment of POME is acid–heat treatment. POME contains lignin from the plant component, which results in its dark colour. The dark coloured liquid contains lignin, cellulosic, hemicellulosic, hexose, and pentose [71]. By using acid–heat pre-treatment method, the hydrogen yield was found to increase as the contents of carbohydrate in POME broke down into glucose. Lignin in POME is very soluble in acidic solution, which will be removed by the pre-treatment process [72]. This pre-treatment will help in growth of microalgae, as it brings more sugar into the medium. Additionally, this pre-treatment clears up the dark coloured lignin, which will enable more light to enter the medium for microalgae growth.

In order to optimize the usage of microalgae for POME, pre-treatment of POME should be done to lower the BOD, COD, turbidity, suspended solids, and microorganism content. One of the researchers used water lily to lower the content of COD and BOD in POME, as the medium for microalgae *S. platensis* [73]. Another researcher used filtration method to remove contaminants from POME, which resulted in the increase of lipid productivity [74]. A new method of autoclaving was applied to treat the wastewater, which turned into an increase in microalgal biomass yield and productivity [75], whereas a similar treatment combining autoclaving, centrifugation, and filtration was applied to treat the wastewater [76]. In these treatments, microalgae were found to accumulate high lipid levels when cultivated in POME medium. Moreover, pre-treatment of POME before cultivation of microalgae can also be done by adding chemical agent or activated carbon to coagulate and absorb the colour. With the addition of activated carbon to the wastewater, the turbidity of wastewater will be lowered, which will help the microalgae to perform photosynthesis more efficiently. A study shows that adding activated carbon to the medium have no negative impact on microalgae [77].

3. Biofuel Production from POME Treatment Using Microalgae

3.1. Characteristics of Algae for POME Treatment

Microalgae bring a lot of advantages to POME treatment due to their unique characteristics and show multifaceted roles in wastewater treatment [9]. Many algae characteristics are suitable to treat wastewater such as POME. One of microalgae's characteristics is that they can grow using photosynthesis [58]. This characteristic can fix the problem of a high amount of carbon dioxide in the system, by decreasing the carbon dioxide level in the system, as microalgae use it for photosynthesis [78]. Microalgae also support aerobic bacterial oxidation of organic matter, producing oxygen by photosynthesis, which aids in POME treatment [29]. This characteristic of algae will help to solve the problem of high BOD and COD that occurs in POME [79]. In addition, photosynthesis will generate nutrients and carbon dioxide, which will be used for growth of algal biomass; for example, nitrogen in the algal cell bound to proteins tends to be composed of 45–60% of dry weight, and phosphorus is essential for synthesis of nucleic acids, phospholipids, and phosphate esters [80].

Microalgae have high capacity for inorganic nutrient uptake, as they use nitrogen and phosphorous to grow, because both components are elements that provide nutrition to algae. They are able to rearrange the molecules to make use of nitrogen and phosphorous to produce food and energy for growth. Nutrient load in the wastewater will be decreased with algae, consuming nitrogen and phosphorous for growth. One of the microalgae *C. Vulgaris* displayed nutrient removal efficiency of 86% and 78% for inorganic nitrogen and phosphorous, respectively [81].

On the other hand, microalgae can be utilized to remove heavy metals present in POME [82,83]. Heavy metals that are present in POME can be detoxified, transformed,

and volatilized by microalgae metabolism. Due to the characteristic of microalgae being non-pathogenic, gases released will not harm the atmosphere. Microalgae can utilize the waste as a nutrition source for growth and consume the pollutants. Biosorption of microalgae is the adsorption process of heavy metals on their surfaces. In addition, using microalgae as biosorbents for biosorption process to remove heavy metals in the wastewater is also feasible [84,85]. Microalgae have high metal binding capabilities due to their characteristics of having properties such as lipids, polysaccharides, and protein on the surface of microalgae cell wall [86]. These properties on the cell wall contain some functional groups such as amino, hydroxyl, carboxyl, and sulphate that can act as binding sites for the heavy metals. This concludes that microalgae are suitable and reliable for removing heavy metals from the POME system.

The performance of microalgae in treating POME is mainly based on the ability to remove the undesired products in the medium. There are many undesired products contained in POME wastewater that are also pollutants that can harm the environment, which must be treated in order to prevent pollution from POME. The undesired products contained in POME are nitrogen, phosphorus, BOD, COD, heavy metals, and many more, which can be eliminated by the cultivation of microalgae due to their unique characteristics, which utilize the undesired products for growth.

There are many types of microalgae that can be used to treat POME, though getting the most efficient microalgae is very important to get maximum work done [50]. Much research was carried out to remove nitrogen and phosphorous to treat POME with various types of microalgae. Comparing all the microalgae used, *Chlamydomonas* was found to be the most efficient microalgae, as it has the fastest growing rate among all the microalgae, along with the ability to remove pollutants in POME [30]. *Chlamydomonas* consumes COD as a growing medium and uptakes it for itself. COD consumption rate of *Chlamydomonas* was found to be 58%. *Chlamydomonas* is optimum in removing pollutant substances and consumed most COD with the concentration of 250 and 1000 mg/L of POME, respectively.

An experiment was carried out to evaluate the efficiency of microalgae to remove these undesired products such as COD and nutrients consisting of ammonium and phosphorus. This experiment was done with two types of microalgae, *C. vulgaris* and *A. brasilense*, cultivated in different conditions. Microalgae are shown to be able to remove ammonium, phosphorous, and COD in both types of cultivation condition, agitation and aeration. However, microalgae performance in removing the components may vary in different types of cultivation condition, where agitation cultivation is more suitable in removing phosphorus and COD, while aeration is suitable to remove all components generally [87].

Another experiment was carried out on removal of COD and total nitrogen from POME [88]. There are two variables that are manipulating the removal percentage of nitrogen, which is the Na-alginate concentration percentage and the time of microalgae in contact with POME. Higher Na-alginate concentration shows better removal of nitrogen, while POME with blank beads shows no improvement in removing nitrogen over time, as there were no cells to remove the nitrogen. This proves that higher concentration of microalgae will perform better in removing nitrogen in POME medium. On the other hand, the time in contact of POME and the microalgae is also affecting the removal percentage of nitrogen. This trend shows that the removal percentage of nitrogen increased over time with microalgae in contact with POME, as longer treatment time is allowed to increase the removal efficiency. The trend of COD removal is similar to that of nitrogen removal as the removal efficiency of COD increased over time. The nitrogen and COD removal percentage was 62% and 65%, respectively, for concentration of 8%.

The experiments above proved that microalgae are able to remove undesired product such as COD, phosphorous, nitrogen, ammonia, nutrients, etc., providing a cost effective and sustainable solution for POME treatment.

3.2. Increased Production of Biodiesel Feedstock Using Microalgae from POME Treatment

There are many benefits of treating POME with microalgae. This method of treatment for POME not only can reduce the pollutants contained in the wastewater, but also creates a new opportunity for other applications. Microalgae that are cultivated in POME can be used as feedstock for the production of biofuels. This is because microalgae have high biomass yield, and they utilize smaller land areas that are not suitable for food production [57]. Besides that, the ability of microalgae to utilize emitted carbon dioxide for photosynthesis will offer a carbon neutral biofuel [89].

The quality of biodiesel feedstock from conversion of microalgae is based on their high biomass content, lipid productivity, and suitable fatty acid compositions. One of the microalgae species *Chlorella* is the most common microalgae that has been used for treatment of POME, which is of major interest for production of biodiesel feedstock due to the ability to accumulate large amount of lipids under stress [90]. To further review the availability of *Chlorella* microalgae acting as a feedstock for biodiesel, an experiment was done based on the growth of *Chlorella* in different cultivation systems using POME as medium to evaluate the algae formed for biodiesel feedstocks. It was found that POME is rich in N and P nutrients, which are able to support photosynthesis and produce biomass such as lipid, protein, and carbohydrate that can be converted into products such as biodiesel [46,91,92]. *Chlorella* microalgae cultivated in medium containing POME has the highest growth rate evaluated by all three photobioreactors. The biomass and lipid production of *Chlorella* microalgae cultivated in POME was higher than other standard medium, demonstrating the high potential of microalgae in biodiesel production feedstock. With this treatment, not only POME can be treated, but additional profit can also be gained by utilizing the microalgae cultivated as biodiesel production feedstock. Thus, this experiment concludes that POME is a good medium to cultivate *Chlorella* microalgae. Additionally, lifecycle assessment has proven biodiesel production via microalgae to be highly feasible [93].

The biofuel production of microalgae is dominated by the lipid compounds. Bio-engineered methods within the cell are common effective techniques to enhance lipid production. In this sense, the understanding of the lipid metabolic pathway within the cell is important [94]. The net effect is light enters, leaving oxygen and chloroplast as end products.

4. Challenges and Future Perspectives

4.1. Will Microalgae-Based POME Treatment Cause Pollution?

Microalgae are well-known wastewater treatment component that remove the pollutants in the wastewater. However, mass cultivation of microalgae can cause pollution to the environment. Mass cultivation of microalgae, also known as controlled eutrophication process, should be managed well by harvesting the microalgae formed. This is because decomposition of dead algal biomass will consume oxygen from the water medium [95], which will defeat the purpose of using microalgae, as treating a component for POME affects the aquatic lives that require oxygen for respiration. The consequence of water pollution caused by POME is water and habitat contamination. The water that can be used becomes limited when it is polluted by the disposed wastewater. Release of large scale cultivation water medium to the environment will cause eutrophication and decrease the seagrass communities which are essential for stabilizing the ecosystem [96]. Besides that, natural habitats for animals and aquatic life will be destroyed, as the contaminants of wastewater are very harmful to the ecosystem of the aquatic life where they are cooped up. Next, another impact of disposing wastewater without treatment is soil degradation [5]. Untreated wastewater will tend to find way into the soil, which causes it to yield lesser crops at a slower rate. The crops that are affected will eventually pose a potential threat to human health and food safety.

The main challenge in global biofuel commercialization is its high production cost [58,97,98]. A lot of palm oil manufacturers prefer microalgae cultivation in open ponds because of the

lower cost compared to reactors. However, microalgae cultivation in open ponds will make them vulnerable to contamination. Ponds that are not properly managed and constructed will cause a threat and pollute the environment by the leaking of content of the pond into the ground. Although the contaminants of POME are non-toxic, they will lead to contamination of ground water. Pollution of groundwater will also affect other streams of water such as lakes, rivers, and oceans, as groundwater is connected to them. Nearly half of the rivers and lakes on earth are polluted [99], and palm oil mills have also been reported to be one of the largest contributors to the pollution of surface water, as a large amount of POME containing high amount of nutrients from the mills are being disposed without treatment [100].

In addition, using microalgae cultivation to treat POME will also cause impacts to terrestrial diversity. This is due to the fact that construction of ponds will lead to displacement and destruction of fauna, which will cause destruction of habitat. Environmental impact assessment shows that the level of impact for construction of large scale ponds for microalgae cultivation is high. The effects on terrestrial biodiversity from changing the landscape for construction of ponds will lead to habitat lost for wildlife. An example related to the impact of construction of large-scale microalgae cultivation ponds is the construction of reservoirs. An average size of a reservoir is around 12 km² area. Although a reservoir is bigger than an expected microalgae cultivation pond, it will give an idea of the impact that it poses. Building resources such as a reservoir will pose problems such as loss of woodland, flora, individual species on site, and most importantly destruction of habitat of wildlife.

It is clear that constructing microalgae cultivation ponds poses environmental threat to the aquatic life and land habitat. However, it also poses pollution to the atmosphere. The level of pollution is greatly dependent on the scale of cultivation medium. Large scale cultivation medium will potentially enhance the biological fixation of carbon dioxide from photosynthesis. The varying organisms contained in the medium will result in different uptake of carbon dioxide from the atmosphere. As an example, a researcher had conducted an experiment on the carbon dioxide uptake with diatom. *P. tricornutum* has a carbon uptake of 1.5 mg/min and microalgae *A. microcopia Nageli* with carbon uptake of 28 mg/min [15]. When the microalgae are cultivated at optimum conditions, the carbon dioxide uptake is significantly higher. This will require a large surface to remove the emissions: studies show that to remove 2.5% emissions of carbon dioxide requires 65,800 km² land, which is equivalent to 0.43% of land on earth. Carbon capture from microalgae is one of the methods to reduce emission of carbon. Microalgae tend to capture carbon to fertilize their growth through Calvin-cycle activity. This is possible because microalgae have active bicarbonate pumps and can concentrate bicarbonate in the cell [101]. With the capability of microalgae to capture the carbon emission, it can greatly reduce the costs needed to remove the emission of carbon.

Besides emission of carbon dioxide, cultivation of microalgae on large-scale will also pollute the atmosphere by emission of methane. Methane is a gas that has potential to cause global warming, which also affects the ozone layer that will eventually affect climate changes and air quality. Methane is produced from anaerobic decomposition by methanogenic bacteria. Microalgae cultivation ponds that are well managed will not have anaerobic conditions because of the constant air interaction with the medium. Studies show that methane is a very saturated gas above water surface with respect to the atmospheric levels. It was also found that methane is produced by water under toxic conditions [102]. Thus, any scale of microalgae cultivation medium will make a contribution to methane emission to the atmosphere. Application of anaerobic method to treat POME will release greenhouse gases such as carbon dioxide, methane, and hydrogen sulphide, which are the main gases that cause global warming [103]. Furthermore, POME will release a strong odor to the communities nearby the oil mills if it was not managed properly.

Moreover, microalgae cultivation will also emit nitrous oxide (N₂O). N₂O is potentially 264 times more powerful than carbon dioxide as a greenhouse gas in over 20 years'

time [104], which means that emission of N_2O must be controlled to prevent too much leakage of greenhouse gases. Some solutions are being proposed to reduce emission of nitrous oxide, which is cultivated under non-axenic condition. This cultivation can be done either from autotrophic bacteria with hydrogen or sulfur as an electron donor. On the other hand, cultivation under non-axenic condition can also be achieved by using heterotrophic denitrifiers, which use organic compound as electron donor.

4.2. Impact on Aquatic Organism

Apart from the wastewater that was generated, POME is one of the biomasses from palm oil production that have the highest pollution rate to the environment. POME is 100 times more polluting than the municipal sewage [30]. This is due to the fact that POME has high BOD, COD, low pH, and colloidal nature. The effluent also contains high concentration of organic nitrogen, phosphorous, and other types of supplementary substances. A palm oil processing plant that utilizes 10 tons of fresh fruit per hour would need a water treatment plant as big as what is required by half a million individuals. In a conventional palm oil mill, a range of 600–700 kg of POME is generated for every 1000 kg of processed fresh fruit bunch [35]. POME is often disposed in nearby waterways, which will greatly impact the environment by creating highly acidic fluid and BOD.

Bacteria utilize oxygen to consume BOD, which causes depletion of oxygen in the water, making it difficult for aquatic life to survive [105]. On the other hand, disposal of POME on land will threaten the soil properties, microbes, and plants [106]. Palm oil mills in present introduce anaerobic and facultative ponds as a conventional way to treat the wastewater biologically. However, during the rainy season, water pouring into the water treatment plant will tend to overflow, causing water pollution to nearby waterways.

In addition, POME does affect the growth of aquatic organism Nile tilapia at the early stages. Exposure of POME will bring negative impact to the hatching rate, survival rate, body length, and heart rate of Nile tilapia. This concludes that POME will bring harm to the aquatic life; thus, disposing it into the rivers and lake should be prohibited, as it will harm the aquatic life and affect the ecosystem [107,108]. Not only microalgae have been investigated under POME solution, but also under seafood, wet market wastewater, and paper industry effluent [40,109,110].

5. Conclusions

To sum up this review article, the demand of palm oil is increasing yearly, which directly affects the amount of generated POME. Based on all the research that is reviewed, microalgae are very highly potential component in treating POME due to their unique characteristics, which can remove all the pollutant components present in POME. However, some characteristics in POME might affect this treating method, such as high organic compounds consisting of tannis, lignin, and phenolic compound, which can affect the microalgae growth. Besides that, the suspended solids that cause POME to be dark in colour will also affect microalgae from performing photosynthesis, as it is difficult for light to penetrate through the water. Thus, pre-treatment of POME is still essential to obtaining optimum performance from microalgae treatment. In this review article, the experimental performance of microalgae on POME was reviewed. Results show that the removal percentage of pollutant materials such as COD, nitrogen, and phosphorous is significantly high and efficient. This shows the potential of microalgae in treating wastewater such as POME with low cost. The biomass produced from microalgae can also be used for various applications, from biofuel to cosmetics products. It is very valuable to make a profit out of the biomass of microalgae. Although treatment using microalgae is very beneficial, it will pose a negative impact if it is not handled well. Thus, correct operating procedure must be followed when utilizing microalgae for treatment of POME.

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