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Opportunities and Challenges of Microalgal Cultivation on Wastewater, with Special Focus on Palm Oil Mill Effluent and the Production of High Value Compounds

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Abstract

South East Asia dominates the production of palm oil worldwide. During the traditional wet processing, palm oil mill effluent (POME) wastewater is generated which poses serious environmental problems. Wastewater treatment using microalgae was initiated recently because of the advantages to lower nutrient content efficiently while the biomass can be utilized as bulk biomass or value added product. In the present review the utilization of wastewater for microalgal cultivation is discussed with particular attention to the feasibility of utilizing POME as microalgal growth medium. Whereas much recent research was focused on the production of bulk biomass, the potential for the production of value-added compounds has not often been addressed. Various strategies of obtaining high-value products are discussed. These include cultivation systems, algal species selection as well as and their growth strategies (autotrophic, heterotrophic, mixotrophic). In addition, potential problems associated with microalgal cultivation on POME will be evaluated. Finally, the concept of using stepwise strategies to obtain high value added product will be proposed.

Keywords Palm oil mill effluent · Wastewater · Microalgae · Value added compound · Cultivation

Introduction

South East Asia is the region with the highest production of palm oil worldwide. Based on FAOSTAT [39], South East Asia coconut palm oil (CPO) production shares 88.6% of the total world production of 54.38 million ton, increasing from 40.33 to 48.12 million ton between 2010 and 2013. Within this region, Indonesia is currently known as the largest CPO producer, followed by Malaysia and Thailand [39]. Badan Pusat Statistik (BPS) [17] recorded that CPO production in Indonesia rose at 30.14% between 2010 and 2014.

During CPO production, a high amount of wastewater is produced, which poses a challenging environmental

problem. Palm oil milling by wet processing is commonly used in Indonesia and Malaysia [61, 165]. About 1 ton of so-called fresh fruit bunch (FFB) produces 0.66 ton palm oil mill effluent [63]. Upon discharge, the raw POME is a brownish liquid with temperatures ranging between 80 and 90 °C. Further characteristics include: pH ranging from 4.0 to 5.0, biological oxygen demand (BOD) and chemical oxygen demand (COD) ranging from 15,000 to 30,000 mg/L and 40,000 to 90,000 mg/L respectively, high total suspended solids (TSS) between 20,000 and 40,000 mg/L, total dissolved solid (TDS) between 15,000 and 30,000 mg/L, and volatile suspended solids (VSS) between 15,000 and 35,000 mg/L (Table 1). It is suggested that further treatment should be done to meet standard regulations before POME can be released into the environment (i.e. rivers, lakes) [128, 133, 145].

To treat POME, several mechanical, chemical or biological methods have been developed, as reviewed by Rupani et al. [128] and Liew et al. [83]. These methods include filtration, sedimentation and flocculation. However, to date, the conventional ponding system is the most common method for treating POME [83, 165]. In Malaysia, more than 85% of palm oil mills have employed the ponding system for

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Table 1 POME characteristic before and after treatment and standard discharge

Parameters	Cooling pond ^a	Aerobic pond ^a	Standard discharge ^b
Temperature (°C)	70–80	30–40	–
pH	4.0–5.0	7.0	5.5–9.0
Total COD (mg/L)	40,000–90,000	350–1300	100
Total BOD ₅ (mg/L)	15,000–30,000	100–700	50
TSS (mg/L)	20,000–40,000	700	400
TDS (mg/L)	15,000–30,000	–	–
VSS (mg/L)	15,000–35,000	–	50
Total nitrogen (TN) (mg/L)	1494.66	456–750	150
Total phosphorus (PO ₄ -P) (TP) (mg/L)	315.36	68.40–180	–
Kalium (mg/L)	1000–2500	110–924	–
Magnesium (mg/L)	250–1000	17–152	–

^aPOME characteristics [133]^bStandard discharge [31]

POME treatment due to its low capital cost [149]. This system employs a series of anaerobic, facultative anaerobic, and aerobic ponds in an open lagoon [75]. Nevertheless, the conventional ponding system has various limitations such as a long hydraulic retention time (HRT), a strong smell, greenhouse gas (GHG) emission, and the large area required for treatment [63, 145]. Moreover, the POME treated within these ponding systems still does not meet the quality requirements upon standard discharge (Table 1).

Clearly, both POME wastewater production and treatment pose a serious environmental problem. Thus, even if the wastewater quality could be lowered down to its standard regulation, the community near the factory would likely still complain when it is discharged into the environment due to its smell and dark color. Sasongko et al. [133] reported, based on a palm mill with a capacity of 45 tons FFB/h, located in a 20,000 ha plantation, 360,000 m³/year of POME is produced. Furthermore, wastewater treatment plants at two particular ponding sites (PTPN V Riau province, and PTPN VII Lampung province, Indonesia) had an average production of 21,454 m³/month, and an effluent rate of 20,000 m³/month.

In recent years, the concepts of utilizing waste into more useful products are increasing to meet industrial demands. Valorization is the process of converting waste materials into valuable products. Several researchers reported the valorization of POME into bioenergy, yeast, and enzymes. Louhasakul et al. [86] reported the fermentation by yeast of POME medium thereby accumulating > 33% of lipid. Iwuagwu and Ugwuanyi [64] utilized POME as a source of carbon and nitrogen for food grade yeast biomass production. Hasanudin et al. [56] reported that the utilization of treated POME as liquid fertilizer increased fresh fruit bunch (FFB) production up to 13%, while Md-Din et al. [94] showed that POME can be utilized as substrate for bacteria to produce poly-β-hydroxyalkanoates. Finally, POME was found to be

suitable substrate for bacteria to produce biomethane [8]. However, based on the recent literature, the utilization of POME by microalgae was mainly focused on lipid production, bulk biomass and wastewater treatment. The present review focuses on the utilization of POME for the production of valuable resources by microalgae. The most recent cultivation strategies, based on different types of wastewater are discussed, whereas strategies to enhance the value added product yield from microalgae growing on POME medium will be proposed.

Microalgae

Microalgae (including diatoms) are sunlight-driven cell factories that convert carbon into potential biofuels, foods, feeds and high-value bioactives. They can produce valuable lipids, carbohydrates, proteins, pigments, vitamins and other value added products that are promising for present and future feedstocks and pharmaceutical applications in an integrated approach [91, 137]. Microalgae do not require arable land for growth. In addition, they have been shown to be sustained on several types of wastewater [41, 72]. Researchers are interested in applying microalgae within wastewater treatment systems because they can exhibit high production rates all year round, they can efficiently remove nutrients and heavy metals, while simultaneously utilizing CO₂ in large amounts [138].

Microalgal cultivation on wastewater effluents has received much attention in recent years, due to their applications and tolerance to a wide variety of nutrient sources [1]. Microalgae are a diverse group of organisms occurring in various natural habitats. While most microalgae are photoautotrophic, some of them are known to grow under mixotrophic or heterotrophic conditions [114]. Environmental growth conditions such as irradiance, temperature, carbon, and macronutrient (nitrogen, phosphorus, silicon)

and micronutrient (a.o. Fe, Mn) availability influence microalgal biochemical composition. Thus, the productivity and biochemical composition of microalgae depend strongly on the mode of cultivation, medium composition, and nutrient profile [48].

Microalgae Cultivation Conditions

Microalgae commonly grow under photo-autotrophic conditions. Several species are able to switch between photo-autotrophic and heterotrophic growth, while in a mixotrophic cultivation both metabolic life styles occur at the same time [114]. Cultivation of microalgae on POME is challenging since the chemical oxygen demand (COD), biological oxygen demand (BOD) and macronutrient levels are high. In addition, the often dark color of POME inhibit light penetration causing light limitation for microalgal photosynthesis and growth [106].

Heterotrophic Growth

Heterotrophic growth is a process where microalgae utilize organic substrates through aerobic respiration thereby generating energy without light. Under heterotrophic conditions, the cultivation of microalgae has been shown to be successful for commercialization of high-value chemicals, such as cosmetics, pharmaceuticals and food supplements [114]. The economic advantages of heterotrophic growth over photo-autotrophic growth using large-scale microalgae cultivation were summarized a few years ago by Chen [22] and Borowitzka [14]. They reported that high cell population and biomass densities (between 20 and 100 g/L) can be achieved in darkness under heterotrophic cultivation in fermenters. In addition, compared to photo-autotrophic conditions, heterotrophic conditions showed enhanced ammonium and phosphate uptake in synthetic wastewater by *Chlorella vulgaris* [113]. Moreover, Ummalyma and Sukumaran [150] reported increased lipid production by *Chlorococcum* sp. when cultivated on dairy effluent wastewater under heterotrophic compared to mixotrophic conditions. The utilization of POME as the growth medium for heterotrophic microalgae is not clear. Several studies reported the cultivation of microalgae on photo-heterotrophic conditions during which microalgae utilize light and organic carbon as the source of energy. Also, Nur and Hadiyanto [104] reported that *C. vulgaris* biomass was enhanced during photo-heterotrophic cultivation by adding D-glucose to POME medium as a co-organic substrate that was mixed with air, containing CO₂. Sukumaran et al. [144] reported that the addition of 4% raw POME to the medium of *Spirulina platensis* changed the condition into heterotrophic growth due to the dark color that inhibited irradiance exposure. However, specific growth rate, pigments, and biomass were higher during heterotrophic growth

compared to autotrophic cultivation that utilized commercial fertilizer without POME addition. It was also demonstrated that micronutrients of POME promoted microalgae growth.

Mixotrophic Growth

Mixotrophic growth is the process where microalgae use inorganic as well as organic carbon sources in the presence of light. Under this condition, photo-autotrophic and heterotrophic processes may occur simultaneously [70, 154]. Inorganic carbon (i.e. CO₂, HCO₃³⁻) and macronutrients are utilized through photosynthesis, which is influenced by irradiance quality, quantity and dynamics, while organic compounds (i.e. glucose, carboxylic acid, glycerol) are assimilated through aerobic respiration, which is affected by the availability and type of organic substrate.

Mixotrophic conditions for wastewater treatment were described by Salla et al. [132], and Li et al. [82], showing increased biomass, carbohydrates, lipids, and growth rate for *Spirulina platensis* and *Chodatella* sp.. The utilization of wastewater by microalgae under mixotrophic conditions was reviewed by Wang et al. [154]. Overall, the efficiency of nutrient removal (N, P) from wastewater by microalgae seems higher under heterotrophic and mixotrophic conditions than under photo-autotrophic conditions [82, 115]. Nur and Hadiyanto [104] documented that the biomass and growth rate of *C. vulgaris* were lower when POME was added to the medium as organic carbon source and NaHCO₃ as inorganic carbon source compared to the addition of organic carbon (i.e. D-glucose and glycerol). Furthermore, Sukumaran et al. [144] stated that specific growth rate, biomass, and pigment content of *S. platensis* were higher under mixotrophic conditions compared to heterotrophic conditions.

Apart from the cultivation under mixotrophic or heterotrophic conditions, microalgal growth, biomass composition, production, and nutrient uptake using wastewater are also influenced by biotic factors such as the presence of competitors (zooplankton, bacteria, viruses) [103]. Finally, cultivation strategies of microalgae (batch, fed-batch, and continuous culturing) also affect growth, biomass accumulation, composition and nutrient uptake as reported by several researchers [28, 46, 74]. Table 2 summarizes studies focusing on the production of value-added products by microalgae cultivated on wastewater using the different growth strategies described above.

Microalgae Cultivation Mode

Fed-Batch Cultivation Mode

The fed-batch cultivation mode is common in the bioprocess industry. This semi-batch strategy is used to avoid the

Table 2 Summary of microalgae production under different operational and cultivation

Mode	Wastewater source	Microalgae	Biomass (mg/L/day)	Nutrient uptake	Product (mg/L/day)	References
Continuous	Digested anaerobic starch	<i>Chlorella pyrenoidosa</i>	342.6	61.9% TOC; 78.7% TN 97.2% TP	Lipid (43.37)	Chu et al. [27]
	Urban wastewater	<i>Scenedesmus obliquus</i>	380	86.8% TN; 97.7% TP	Lipid (105)	Ruiz et al. [126]
	Diluted municipal wastewater	<i>Scenedesmus</i> sp. AMDD	300	–	Carbohydrate (130) Protein (120)	Dickinson et al. [33]
	Industrial wastewater	<i>Scenedesmus</i> sp.	900	34.6 mg N/L/day 12.7 mg PO ₄ ³⁻ /L/day	Lipid (133.2) ^b	Jebali et al. [65]
	Aquaculture wastewater	<i>C. vulgaris</i>	42.6	TN 86.1%, TP 82.7%	–	Gao et al. [42]
	Municipal wastewater	<i>Scenedesmus</i> sp.	234–267	TN 99% TP 99%	–	McGinn et al. [93]
	Digested POME	<i>S. platensis</i>	267	24% BOD	–	Suharyanto et al. [143]
Fed-batch	Soybean processing	<i>C. pyrenoidosa</i>	1070	77.8% COD; 88.8% TN 7 Gao 0.3% TP	Lipid (400)	Hongyang et al. [60]
	Digested piggery manure	<i>Desmodesmus</i> sp.	25 ^a	94.2% TN; 88.7% PO ₄ -P	Lipid (6.525) ^b	Ji et al. [66]
	Poultry litter	<i>S. platensis</i>	126.5 ^a	NH ₄ -N > 99% PO ₄ -P > 99%	Protein (66.53) ^b Phycocyanin (15.3) ^b	Markou [92]
		<i>Chorella vulgaris</i>	127 ^a	NH ₄ -N > 99% PO ₄ -P > 99%	Lipid (53) ^b	
	Whey permeate	<i>Chlorella protothecoides</i>	1720 ^b	–	Lipid (352.6) ^b	Espinosa-Gonzalez et al. [37]
	Blended wastewater	<i>Micractinium inermum</i>	951	–	–	Park et al. [112]

^aCalculated from biomass production (mg/L) and total cultivation time (days)

^bCalculated from biomass productivity (mg/L/day) and value added compound (%)

limitation or inhibition of substrate and the accumulation of toxic compounds (catabolic respiration) during cultivation. The medium is replaced periodically during the process after which the biomass is harvested at the end of the process.

Several researchers reported the advantages of the fed-batch mode in microalgal cultivation. Xie et al. [167] and García-Cañedo et al. [43] reported that by applying autotrophic cultivation, the fed-batch cultivation mode increased pigment content of *S. platensis* and *Scenedesmus incrassatulus* as influenced by nutrient addition. Under mixotrophic fed-batch conditions, cellular contents of the long chain polyunsaturated fatty acid EPA was enhanced [168]. Moreover, under heterotrophic fed-batch mode, carbohydrate and protein contents were found to be higher for *Neochloris oleoabundans* as compared to batch mode [99].

With respect to wastewater treatment, Ji et al. [66] reported that nutrient removal from wastewater by *Desmodesmus* sp. was higher in fed-batch compared to batch mode, lowering total nitrogen (TN) by 94.2 and

PO₄-P 88.7%, while generating 25 mg/L/day biomass and 6.525 mg/L/day lipid for 40 days. Under this condition, the alga was cultivated on digested pig manure that contained high levels of ammonium which could negatively impact algal growth. Similar results were found by Markou [92] who reported that ammonia and phosphorus removal were more than 95% in anaerobically digested poultry litter that contained high ammonia levels. When cultivated in fed-batch mode, *S. platensis* produced 126.5 mg/L/day biomass, 66.53 mg/L/day protein and 15.3 mg/L/day phycocyanin. By employing fed-batch cultivation, the inhibition by excess nutrients in the wastewater could therefore remain limited.

Hongyang et al. [60] reported that the biomass and lipid production of *C. pyrenoidosa* on soybean wastewater that contained high amounts of COD could be enhanced by applying fed-batch mode, resulting in 1070 mg/L/day biomass accumulation, and 400 mg/L/day of lipid productivity. At the same time, the nutrient content was lowered by 77.8% for COD, 88.8% for TN, and 70.3% for TP.

Espinosa-Gonzalez et al. [37] found that the utilization of high concentrations of glucose and galactose from dairy industry wastewater could be optimized by applying fed-batch cultivation, which resulted in 1720 mg/L/day of biomass and 352.6 mg/L/day of lipid. While Park et al. [112] found that the biomass productivity of *M. inermum* was higher in fed-batch cultivation (950 mg/L), which was supplemented with external nutrient at day 2, compared to batch control (800 mg/L). The cultivation was run in an airlift photobioreactor by using a mixture of digested pig wastewater as well as domestic wastewater. This study demonstrated that the fed-batch process may avoid high COD and strong color that could otherwise limit the light penetration in the wastewater medium.

Continuous Cultivation Mode

In continuous mode, the outflow of harvested biomass and inflow of the fresh medium are maintained in an equal, steady state. Typically, this mode is started after a batch cultivation phase, to obtain a certain cell density. Several researchers employed a continuous cultivation mode to increase the chosen value added product. Sloth et al. [141] and Van-Wagenen et al. [153] employed continuous culturing under mixotrophic conditions to enhance lutein yield. The phycocyanin content of *Galdieria sulphuraria* was higher in a continuous culture set-up compared to a fed-batch set-up [46]. Coelho et al. [28] compared cultivation modes of *Chlorella* sp. and found a higher biomass accumulation rate in continuous compared to fed-batch mode. Furthermore, Kumar et al. [74] found a higher biomass and lipid productivity in continuous cultures of *Chlorella sorokiniana* as compared with fed-batch mode. Similarly, carbohydrate productivity was found to be high under mixotrophic conditions using continuous culturing [Wang et al. 158] and EPA yield was found to be higher during continuous culturing [161] as compared with the fed-batch mode [168]. Beta carotene yield was also increased in continuous mode [175] compared to fed-batch [169] when growing *Dunaliella salina* under autotrophic conditions.

With respect to wastewater treatment applications, recent studies showed that continuous cultivation can be used to increase the value added content in microalgae while simultaneously lowering the nutrient content in wastewater. Dickinson et al. [33] utilized *Scenedesmus* sp. grown in municipal wastewater to produce carbohydrate (130 mg/L/day) and protein (120 mg/L/day). Furthermore, Jebali et al. [65] utilized industrial wastewater for *Scenedesmus* sp. cultivation, resulting in 506.7 mg/L/day carbohydrate and 171.9 mg/L/day protein, while the nitrogen and phosphorus were removed at a rate of 34.6 and 12.7 mg/L/day, respectively. In addition, Ruiz et al. [126] applied the same species and cultivation system on urban wastewater, resulting

in 105 mg/L/day lipid accumulation rate, removing 86.8% TN, and 97.7% TP from the wastewater. McGinn et al. [93] found that biomass productivity from the algae cultivated on municipal wastewater was almost twofold greater in continuous mode compared to batch mode, while nutrients could be lowered up to 99% for both TN and TP. The product accumulation rates of *C. pyrenoidosa* growing on digested anaerobic starch, which contained high levels of organic carbon and a strong white color, was also enhanced by applying continuous cultivation: the microalga produced 43.37 mg/L/day lipid, and lowered organic carbon content by 61.9% and total nitrogen and phosphorus by 78.7 and 97.2% respectively [27]. Similarly, Gao et al. [42] found that the biomass productivity of *C. vulgaris* cultivated in a continuous cultivation on nutrient rich aquaculture wastewater, was 5.8-fold higher compared to batch cultivation. The microalgae produced 42.6 mg/L/day biomass and removed 86.1% TN and 82.7% TP. Furthermore, Suharyanto et al. [143] cultivated *S. platensis* on POME medium to produce bulk biomass by applying continuous cultivation. It was reported that 24% BOD was removed and 0.267 g/L/day biomass was produced in this cultivation mode, being higher compared to batch mode.

Overall, continuous microalgal cultivation may have several advantages, but at the same time, it faces multiple challenges during the process as reviewed by Fernandes et al. [40]. However, continuous cultivation strategies seem to be promising in wastewater treatment, since the inhibition and limitation of substrates from wastewater can be avoided. Clearly, biomass and high value product yield benefit from a continuous supply of relatively low levels of nutrients but relatively high, constant irradiance levels, as compared with fed batch mode.

Environmental Factors Affecting Microalgal Cultivation

Several environmental factors influence microalgal cultivation. In large scale cultivation systems, these factors are ideally manipulated and controlled to obtain the desired product in the most optimal way,

Light

Photoautotrophic microalgae utilize light as the energy source but during the photosynthetic process 50% of the energy may be lost in the conversion of solar energy to chemical energy [116]. Algae containing chlorophyll *a* and *b*, which are the major light harvesting pigments for green algae, primarily absorb blue and red light. Therefore, green algae are found to grow better in blue and red light [140].

Light energy can be stored in the form of carbohydrates or lipids [21]. Several researchers demonstrated the light dependency of carbohydrate storage in algal cells [20, 58].

Subramanian et al. [142] found that storing energy as carbohydrates under high light is energetically less favorable than triacylglycerol (TAG) on a per carbon basis. He et al. [57] also reported that under high light (400 $\mu\text{mol photon/m}^2/\text{s}$), carbohydrate decreased along with an increasing lipid content.

Under nutrient saturated conditions, light is the critical factor for photosynthetic activity. Microalgae require a specific light level to reach their maximum growth rate, which is referred to as the saturating light level. If the light intensity is far above the saturation level, it will inhibit growth (photo-inhibition). On the other hand, if the light intensity is far below the saturation level, growth will be light limited (light-limitation) [76]. Lee and Lee [77] found that the specific removal rate of organic carbon from artificial wastewater under light–dark cycles was higher than under continuous illumination. Yan et al. [170] reported that the nutrient removal from domestic wastewater by *C. vulgaris* was influenced by irradiance wavelength as well as irradiance intensity when applying a light emitting diode (LED). In addition, the dependency of the irradiance responses to remove nutrients from wastewater varied with irradiance level and irradiance period depending on the algal strain [44, 81]. Furthermore, Olgúin et al. [110] found that light intensity influenced both the lipid accumulation rate and the lipid profile of *S. platensis* grown on digested pig waste. Marcilhac et al. [89] found that light intensity affected microalgae–bacteria interactions when grown on urban wastewater. In addition, Jiang et al. [67] reported that different light intensities influenced lipid, protein, and carbohydrate content of *C. vulgaris* cultivated on monosodium glutamate wastewater. Several researchers reported the cultivation of microalgae on POME medium by varying light parameters to obtain optimal yields of the desired products. Kamyab et al. [69] stated that the light cycle plays an important role in the lipid production of *C. pyrenoidosa*, cultivated on diluted POME, and continuous irradiance exposure gave the highest growth rate and lipid content compared to exposure to light dark cycles. Furthermore, regular dilution was employed to enhance irradiance in the cultures when cell densities were getting high, as reported by Hadiyanto and Nur [54]. In addition, Takriff et al. [147] increased light penetration by adding activated carbon as adsorbent on the POME pre-treatment, thereby decreasing light attenuation.

Temperature

The effect of temperature on microalgal growth makes it one of the most important environmental factors influencing growth rate and biochemical composition of algae. In one study [123], optimal growth rates for mesophilic species growing outdoors in a wastewater treatment plant were found between 20 and 25 °C, increasing up to 40 °C for

thermophilic strains (*Chaetoceros* sp., *Anacystis nidulans*) or decreasing to 17 °C for psychrophilic strains (*Asterionella formosa*). Furthermore, Delgadillo-Mirquez et al. [30] found optimum conditions for *Chlorella* cultivated on wastewater at 25 °C. Zhang et al. [173] reported optimal temperatures between 18 and 25 °C for *Chlorella* sp. to produce biodiesel in a wastewater treatment plant.

Decreasing the temperature below the optimal level may increase the unsaturation of lipids as reported by Wang et al. [157]. *C. sorokiniana* LS-2 grown at a suboptimal temperature of 18 °C showed enhanced lipid content and mainly contained unsaturated fatty acids. However, lipid productivity was decreased since lower temperatures prolonged the exponential phase and decreased growth rate compared to 26 °C. In addition, sub optimal cultivation temperatures may affect pigments and growth rate. The total carotenoid content of *Chlorococcum* sp. almost doubled when growth temperature increased from 20 to 35 °C [84]. Also, astaxanthin production in *Haematococcus* sp. increased threefold when growth temperature increased from 20 to 30 °C [148]. Vairappan and Yen [151] reported that growth rate and biomass of *Nannochloropsis* sp grown on POME medium was affected by cultivation temperature and light. The culture conditions were compared using a 1 L photobioreactor (Temp: 23 °C, illumination: 180–200 $\mu\text{mol photons/m}^2/\text{s}$) and a 10 L outdoor system (Temp: 26–29 °C, illumination: 50–180 $\mu\text{mol photons/m}^2/\text{s}$). Growth rates were higher in the photobioreactor but biomass production was higher in the outdoor culture.

Carbon

For autotrophic growth the supply of inorganic carbon (CO_2 and HCO_3^{3-}) is most important. The $\text{CO}_2 \rightarrow \text{CO}_3^{3-} \rightarrow \text{HCO}_3^{3-} \rightarrow \text{CO}_3(2-)$ system serves as important buffer in freshwater cultivation systems and may control and maintain specific pH levels that are suitable for large scale cultivation [47]. Under mixotrophic and heterotrophic cultivation conditions, organic carbon plays an important role in microalgal growth, final yield and lipid accumulation. In general, energy storage molecules, such as lipids and carbohydrates (starch and glycogen) are accumulated under heterotrophic and mixotrophic conditions; therefore, the cellular content of these compounds may be higher than under photo-autotrophic conditions [26].

Various types of wastewater already contain carbon that can be utilized by microalgae [1]. However, a high organic content may limit the growth of certain microalgae in raw wastewater, resulting in a higher retention time [104]. Several researchers reported the supplementation of external carbon in mixotrophic and heterotrophic wastewater cultivation systems to increase biomass, growth rate and nutrient uptake. Perez-Garcia [115] found the highest growth rate

and ammonium uptake of *C. vulgaris* growing on sterilized municipal wastewater when adding sodium acetate under heterotrophic conditions, while the absence of external carbon supplementation resulted in reduced growth. Gupta et al. [49] reported that the addition of glycerol enhanced biomass productivity and nutrient uptake of *C. vulgaris* and *Nannochloropsis oculata*, when cultivated on municipal wastewater. Similar results were reported by Ma et al. [87] who reported that nutrient uptake and lipid yield was increased when synthetic wastewater was supplemented with waste glycerol. The addition of inorganic carbon (CO₂) could also improve the lipid accumulation of microalgae cultivated on domestic wastewater under mixotrophic conditions [32].

Raw POME contains 12.75% crude protein, 10.21% crude lipid, and 29.55% carbohydrate [50]. In most cases, researchers utilized the wastewater at low concentrations to avoid growth inhibition due to toxic levels of a variety of components. Shah et al. [134] utilized 1–20% v/v of raw wastewater, which contained 148–2833 mg/L COD, to promote the growth of *Isochrysis galbana* during which 76.4–74.8% of the COD could be removed. In addition, Sukumaran et al. [144] utilized 1–4% v/v fresh raw POME in commercial fertilizer by applying fed-batch cultivation to avoid inhibition during the cultivation of *S. platensis*.

It is possible to lower the organic carbon of raw POME by applying anaerobic fermentation processes. During this process, complex carbohydrates, lipids and proteins are degraded. This degradation leads to the formation of CH₄, CO₂ and carboxylic acid (i.e. acetic acid, propionic acid and butyric acid) [100]. It is reported that digested POME contains acetic acid at concentration ranging between 1170 and 3540 mg/L (Poh et al. 2010). Several researchers reported that microalgae can be cultivated on higher concentrations of digested POME as compared with undigested POME. Zainal et al. [171] utilized 100% v/v of anaerobically digested POME, which was demonstrated to be rich in organic acid, as growth medium for *S. platensis*. Here, 90% of COD was removed. Rajkumar and Takriff, [121] also reported that *S. platensis* and *Scenedesmus dimorphus* could be cultivated on 90% v/v anaerobically digested POME in an open pond system.

Nitrogen

In general, microalgae have a limited ability to accumulate nitrogen storage materials when growing under nitrogen-sufficient conditions. Conversely, cyanobacteria produce sufficient nitrogen storage under high nitrogen concentrations in the form of pigments such as phycocyanin pigments [16]. Recently, growth and biomass yield of microalgae were found to be influenced by the supplementation of different nitrogen compounds and concentrations [122]. Beuckels et al. [12] showed that nitrogen availability affected

phosphorus removal of microalgae during wastewater treatment.

It is reported that digested POME contains high total Kjeldahl nitrogen (TKN), but low concentrations of inorganic nitrogen, indicating that it contains high levels of organic nitrogen [133; Poh et al. 2010]. Therefore addition of external inorganic nitrogen is recommended to support algal growth, of which the recommended inorganic N:P ratio should be around 6.8–10:1 [109]. Mutanda et al. [101] reported that the addition of 5 mM NaNO₃ on post-chlorinated wastewater could increase the biomass productivity of *Chlorella* sp. Hadiyanto et al. [53] supplemented digested POME wastewater with urea fertilizer in order to obtain higher growth rates of *Chlorella* sp by modifying the nitrogen to phosphorus ratio. Furthermore, Halim et al. [55] reported that the addition of sodium nitrate and dihydrogen phosphate to digested POME, which resulted in 10:1 N (NaNO₃)/P (NaH₂PO₄) molar ratio, could increase the biomass production of *Nannochloropsis* sp. and remove 90% TN and 83% TP, compared to the control digested POME medium without external nutrient addition. The nutrient removal was higher compared to the study by Shah et al. [135], who reported that *N. oculata* removed 64–75% of total nitrogen (TN) from raw POME at low concentrations (1–15%).

Phosphorus

Phosphorus is another major macronutrient that influences cellular metabolic processes by forming structural and functional components required for maintenance, growth and survival [38]. Most algal species show a rather consistent phosphorus content, averaging 0.03–3% of their dry weight [124]. This implies that limiting phosphorus concentrations in the medium results in the repression of photosynthesis [11]. However, previous research reported that microalgae can only utilize phosphate as an inorganic phosphorus source, while phosphite and organic phosphate cannot be used [85]. Therefore, supplementation of phosphate in lagoon wastewater increased the chlorophyll-a content and lipid productivity of consortium microalgae, while alteration of the nitrogen to phosphorus ratio also increased nutrient removal efficiency [78]. In addition, Zhang et al. [172] reported that the supplementation of phosphorus and iron positively influenced biomass yield, lipid yield, and nutrient uptake of *S. obliquus* cultivated on municipal wastewater.

Poh et al. (2010) showed that digested POME contains a high total phosphorus load, mostly consisting of phospholipids and other forms (i.e. methaphosphate, orthophosphate, etc). Several researchers reported the reclamation of phosphate from POME by employing microalgae. Rajkumar and Takriff [121] documented a 96.8% removal of total phosphorus from digested POME by *S. platensis*. In addition,

Halim et al. [55] reported that optimizing the nitrogen to phosphorus ratio by adding external nitrogen and phosphorus to a N (NaNO_3)/P (NaH_2PO_4) molar ratio of 10:1 could improve biomass accumulation of *C. vulgaris* up to 1.5 g/L. This was confirmed by a 88% total nitrogen and 91% total phosphorus removal by using the algae cultivated on 50% v/v digested anaerobic POME wastewater.

Silicon

Especially for diatoms, silicon is an important macronutrient. Brzezinski [18] reported that marine diatoms, mainly from the genera *Thalassiosira* and *Chaetoceros*, have a silicon to nitrogen atomic ratio around 1. Silicate plays a vital role in diatom cell wall formation and deoxyribonucleic acid (DNA) synthesis during the metabolic process [29]. Roessler [125] reported that silicon deficiencies influenced the accumulation of neutral lipids in *Cyclotella cryptica*, and silicon limitation also resulted in a 5% shift in lipid chain length, from C18 to C16 fatty acids [6]. In addition, lipid accumulation was found to be influenced by silicate depletion both in autotroph [129, 163] and heterotroph cultures [160]. Graham et al. [45] concluded that the presence of silicon was important when freshwater diatoms (genera *Cyclotella*, *Aulacoseira*, *Fragilaria*, *Synedra*) were cultivated on wastewater. The amount of silicon present or added to the medium for diatoms should be 17.5 times the mass of phosphorus for optimal utilization of phosphorus and nitrogen in wastewater medium.

Micronutrients

Several micronutrients are important for microalgal growth and composition. Iron plays a vital role in cellular biochemical composition because of its redox properties and implications in fundamental processes such as photosynthesis, respiration, nitrogen assimilation and DNA synthesis [88]. To date, Fe is supplied in mass cultivation systems in a chelated form such as ferric citrate ($\text{C}_6\text{H}_5\text{FeO}_7$), ferric EDTA (FeCl_3 -EDTA) or ferrous ascorbate [15]. Several researchers reported the effect of iron on microalgal pigments such as astaxanthin, beta-carotene, and fucoxanthin [159, 19, 97, 35]. Recent studies showed that the addition of iron in wastewater enhanced biomass yield and nutrient uptake of *Chlorella* sp [174]. Similar results were obtained by Zhang et al. [172] who found that the addition of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ to municipal wastewater influenced biomass yield, lipid yield, and nutrient uptake of *S. obliquus*. However, Habib et al. [51] measured the micronutrient concentration of digested POME and concluded that POME contains sufficient levels of micronutrients such as potassium (963 mg/L), calcium (531 mg/L), aluminium (136 mg/L), magnesium (87 mg/L),

iron (79 mg/L), sodium (69 mg/L), and zinc (48 mg/L). These micronutrients influenced the polyunsaturated fatty acid (PUFA) profile of *C. vulgaris* growing on different POME concentration.

Salinity

Salinity affects the production of pigments in microalgae. For example, highest chlorophyll-a and total carotenoids productivity was found at a salinity of 2 ppt for *Dunaliella viridis* [62]. Xia et al. [166] compared four types of salts (NaCl , NaHCO_3 , $\text{Na}_2\text{S}_2\text{O}_3$ and Na-Acetic acid) with respect to lipid production in *Desmodesmus abundans*, and the highest production was obtained for NaCl . In addition, in *Dunaliella tertiolecta* ATCC 30,929, a high salinity increased lipid content up to 70% [146]. In the freshwater alga *Scenedesmus* sp., lipid production was stimulated by NaCl [130]. However, excess salinity stress in the cultivation medium inhibits photosynthesis which further reduces the biomass and net lipid productivity. As discussed by Minhas et al. [96], salinity stress tends to remarkably affect the fatty acid profile of microalgae.

With respect to wastewater utilization, Salama et al. [131] documented that *Chlamydomonas mexicana* exhibited a higher specific growth rate when 100 and 200 mmol/L NaCl was supplemented to municipal wastewater, whereas nitrogen was completely removed and 38% of lipid was obtained when 400 mmol/L NaCl was added.

pH

The efficient growth of microalgae in wastewater depends on critical variables, including pH, temperature, and the availability of nutrients [117]. It is well known that digested POME and other wastewaters contain nitrogen mostly in the form of ammonia [92, 133, Poh et al. 2010, 92]. Yet, the equilibrium between ammonia (NH_3) and ammonium (NH_4^+) is depended on pH and temperature [73]. It is reported that the ratio of ammonia to ammonium increases 10-fold for each unit increase in pH [36].

Some researchers reported the negative effects of ammonia on the growth of microalgae related with pH. Abeliovich and Azov [3] found that ammonia at a concentration over 2 mM and a pH over 8 in a high-rate sewage oxidation pond inhibited the growth of *S. obliquus*. Furthermore, Belkin and Boussiba [10] reported that the growth of *Arthrospira platensis* was inhibited by 50% due to high ammonia concentrations up to 140 mg/L at a pH of 10. Khoo et al. [71] reported that the growth of *C. vulgaris*, which was cultivated on municipal wastewater, was highly influenced by initial pH. Under acidic (pH 2) or alkaline (9 or 11) pH, growth rates were low. In contrast, highest biomass productivity and nutrient removal efficiency were recorded at pH

3. Furthermore, Hodaifa et al. [59] reported that the specific growth rate, protein, and chlorophyll content of *S. obliquus* were high when the medium (olive-mill wastewater) was maintained at a constant pH value of 7.0. Yet, PUFAs and essential fatty acids increased when the pH was set at nine.

High-Value Products from Microalgae

Generally, microalgae are cultivated on wastewater to obtain carbohydrates, lipids, and proteins which are mainly focused on fuel and animal feed. However, microalgae are known to produce high value bioactive compounds, which may have different pharmaceutical and cosmetic applications. Table 3 summarizes the presently available studies of microalgal cultivation on POME resulting in value-added products.

Pigments obtained from microalgae could be useful for food colorants, vitamins, cosmetics and pharmaceutical applications [9]. Sukumaran et al. [144] found 1.8 g/L biomass and 0.216 g/L phycocyanin from *S. platensis* cultivated after the addition of 1% raw POME using fed-batch mode. The cultivation was carried out in an outdoor open-pond system and therefore temperature and light intensity were variable at 25–40 °C, 800–1600 $\mu\text{mol photons/m}^2/\text{s}$, respectively.

In addition to pigments, microalgae may contain high levels of (Long Chain) Poly Unsaturated Fatty Acids (PUFA), which are suitable as a replacement source of fish fatty acids [79]. Vairappan and Yen [151] cultivated *Isochrysis* sp on 5% digested POME supplemented with external nutrient in an outdoor batch cultivation at 26–29 °C and 50–180 $\mu\text{mol photons/m}^2/\text{s}$ intensity system for 7 days. The algae produced 91.7, 48.41, and 23.84 mg/m²/day of biomass, lipids and PUFAs, respectively. Nur et al. [105] documented that *C. vulgaris* was more suitable to be cultivated on digested POME wastewater compared to *S. platensis* and *D. salina*. The algae were cultured for 13 days on 10–30% digested POME in laboratory batch cultivation mode, under 24 h continuous light irradiation (4000 lx), and the temperature was set to 26–28 °C. *C. vulgaris* produced 12.6 and 11.22 mg/L/day of lipids and carbohydrates, respectively. On the other hand, *S. platensis* could produce 100% fatty acid methyl ester (FAME) which is suitable as a source of biodiesel, while the FAME in *D. salina* and *C. vulgaris* was 14.77 and 37.38%, respectively. Nur et al. [106] also recorded that *B. braunii* could produce 17.63 and 18.11 mg/L/day of lipid and carbohydrate with the addition of 250 mg/L COD substrate from digested POME. The cultivation was maintained at 5000 lx (24 h duration), 28 °C temperature, 0.15M salinity, and pH 6.8–7.2. The lipid contained 4.25, 37.29, and 2.1% of C16, C23, and C18, respectively.

Shah et al. [134] cultivated *I. galbana* and *Pavlova lutheri* on raw POME, diluted with different fractions of filtered sea

water, showing that the POME fraction influenced the lipid content of the algae.

Furthermore, *N. oculata* and *T. suecica* on raw POME, diluted with different fractions of filtered sea water, showing that the POME fraction influenced the lipid content of the algae. Under optimal conditions (10% POME), the PUFA content was 9.07 and 8.88% for the two species, while the biomass produced was 0.15 and 0.14 g/L/day respectively [135].

Habib et al. [51] revealed that the addition of 10% digested POME positively influenced the PUFA composition of *C. vulgaris* as compared with commercial medium due to the high micronutrient content of the wastewater. They also reported that 985 g/L biomass could be produced under these conditions, consisting of 18.3% lipids, 28.7% proteins, 26.1% carbohydrates and 15% linolenic acid. Although several studies have focused on high value compound production using POME wastewater, more work is urgently needed, especially utilizing species from other taxonomic groups. For example, the application of diatoms in cultivation studies using POME has not been addressed so far. Diatoms may contain much higher levels of the fish fatty acids (LC-PUFAs) EPA and DHA as compared with the most common species used for cultivation (as given in Table 3) Boelen et al. [13] reported that EPA and DHA production rates were highly species specific. The highest EPA productivity was found in the diatom *Chaetoceros brevis* (174 $\mu\text{g/L/day}$), while DHA productivity was highest in *Emiliania huxleyi* (164 $\mu\text{g/L/day}$). Moreover, diatoms contain high levels of the carotenoid fucoxanthin, which may have significant health benefits. In general, a limited number of species (Table 3) were grown on POME medium with the aim to produce pigments and other valuable compounds. Since the type and level of high value compounds produced are highly dependent on the species cultivated, more studies are therefore required to focus on species-specific differences in POME cultivation success related with value added product yield.

Pretreatment of Wastewater for Microalgal Growth

To optimize the utilization of wastewater for microalgal growth, several pretreatment methods have been proposed. These pretreatments were done to lower COD, BOD, turbidity, suspended solids, and microorganisms. Table 4 summarizes several pretreatment methods using thermal, chemical, mechanical, and biological processes. Hadiyanto et al. [52] utilized water lily in the process to lower COD and BOD content from POME as the medium for *S. platensis*. Takriff et al. [147] reported an increase in *S. dimorphus* biomass in pretreated POME medium by using activated carbon to increase the light penetration in the medium. Nwuche

Table 3 Summary of POME medium for microalgae growth and production

Medium	Process/method	Time (days)	Product/output	Microalga	References
5% POME (anaerobic digested) + 0.075% NPK + sea water	Open photobioreactor outdoor; batch cultivation; (26–29 °C); light intensity (50~180 μmol photons/m ² /s), pH = 8.3–8.5, salinity 30 ppt	7	91.7 mg/m ² /day biomass; 52.8% lipid; fatty acid 104 mg/g; PUFA 26% higher than indoor	<i>Isochrysis</i> sp.	Vairappan and Yen [151]
1% fresh raw POME + commercial nutrient	Addition of urea by fed-batch up to day 3; outdoor cultivation; 25–40 °C, 800~1600 μmol photons/m ² /s	7	1.8 g/L biomass; 12.013% phycocyanin, 0.573% carotenoid, 1.045% chlorophyll	<i>S. platensis</i>	Sukumaran et al. [144]
10% digested POME + water	Laboratory culture flask batch cultivation; 24 h light 10 k lux	–	0.985 g/L biomass; 18.3% lipid; 28.7% protein; 26.1% carbohydrate; 15% linolenic acid	<i>C. vulgaris</i>	Habib et al. [51]
20% digested POME + 40% synthetic nutrient + water	Laboratory culture flask; batch cultivation; 24 h light illumination, 4–6 Klux, pH=6.8–7.2, 26–28 °C	7	34 mg/L/day lipid productivity; dominant saturated fatty acid	<i>Chlorella</i> sp.	Hadiyanto and Nur [54]
10% raw POME + sea water	Laboratory culture flask batch cultivation; 12:2 (L:D); 7–8, 90 mol photons/m ² /s, 28 ± 2 °C, pH=8	16	39% lipid; SFA 29.24%, PUFA 9.07%, COD removal 93.6%	<i>N. oculata</i>	Shah et al. [135]
150 mg/L COD of POME	Laboratory culture flask, batch, 5000 lx, 13 days, 24:0 h (L:D), pH = 7.2–6.8, 24 h illumination	16	27% lipid; SFA 68.74%, PUFA 8.88%, COD removal 95%	<i>Tetraselmis suecica</i>	
250 mg/L COD of POME	Laboratory culture flask, 6 days, 5000 lx, 24:0 h (L:D), 28 °C temperature, 0.15 M salinity, and pH 6.8–7.2	13	12.6 mg/L/day lipid, 11.22 mg/L/day carbohydrate, 74% COD	<i>C. vulgaris</i>	Nur et al. [105]
		6	17.63 mg/L/day lipid, 18.11 mg/L/day carbohydrate	<i>Borriyococcus braunii</i>	Nur et al. [106]

Table 4 Pretreatment methods in wastewater for microalgae medium

Input media	Pretreatment method	Microalga	Yield or Productivity	Final effluent	References
Digested POME	Water lily (8 days)	<i>S. platensis</i>	0.945 g/L biomass	96.9% COD; 80% TN	Hadiyanto et al. [52]
Digested POME	Addition of activated carbon	<i>S. dimorphus</i>	0.17 g/L biomass	–	Takriff et al. [147]
Raw POME	Filtration, centrifugation and autoclave	<i>I. galbana</i>	0.142 mg/L/day biomass (26.3% lipid)	77.4% COD; 58.8% TOC; 57.4% TN	Shah et al. [134]
		<i>Pavlova lutheri</i>	0.130 mg/L/day biomass (34.5% lipid)	80.1% COD; 62.3% TOC; 61.3% TN	
Digested POME	Filtration (0.22 µm)	<i>C. sorokiniana</i>	12.4 mg/L/day lipid 0.134 mg/L/day chlorophyll	63% COD	Nwuche et al. [107]
Centrate wastewater	Autoclave (121 °C)	<i>Chlorella</i> sp.	1.175 g/L biomass	90.3% COD, 89.9% total nitrogen	Li et al. [80]
Secondary municipal wastewater	Filtration (0.2 µm)	<i>Chlorella</i> sp.	22.9 mg/L/day lipid	92%TN 86% TP	Cho et al. [25]
	UV-B irradiation (3 min)		13 mg/L/day lipid	75% TN 84% TP	
Diluted synthetic wastewater	Electrolysis (2 h)	<i>C. vulgaris</i>	1.087 g/L biomass	83.7% TOC; 99.3% NH ₄ -N	Wang et al. [156]
Diluted dairy manure	Centrifuge (10,000 rpm 15 min)	<i>C. vulgaris</i>	20.6 mg/L/day biomass	–	Shi et al. [136]
	Autoclave (121 °C)		47.2 mg/L/day biomass	–	
Dairy manure	Chemical addition NaOCl (30 ppm)	<i>C. vulgaris</i>	44 mg/L/day lipid	63.1% COD; 77.8% TKN; 99.5% TP	Qin et al. [120]
	UV irradiation (15 min)		31.5 mg/L/day lipid	74% COD; 95.4% TKN; 94.8% TP	
Diluted olive oil mill effluent	Chemical addition NaOCl (12.5 g/L)	<i>S. platensis</i>	1.69 g/L biomass	65.53% COD; 100% Phenol; 100% TP	Markou et al. [90]
Domestic wastewater	Chemical addition NaOCl (0.4 mg/L)	<i>Chlorella</i> sp.	93.8 mg/L biomass	–	Mutanda et al. [101]
Piggery wastewater	Bubbled with air (5% CO ₂ 100 ml/min)	<i>Desmodesmus</i> sp. CHX1	118.2 mg/L/day lipid	87.3% TN; 93.1% TP	Cheng et al. [23]
Digested Poultry manure effluent	Electrolysis (+2 g/L NaCl; 15 V; 2 h)	<i>C. vulgaris</i>	0.53 g/L biomass	99.7% NH ₄ -N 91.6% TP	Wang et al. [155]
	Centrifugation (8000 rpm 15 min)		0.56 g/L biomass	99.5% NH ₄ -N 99.3% TP	
	Autoclave (120 °C 30 min)		0.48 g/L biomass	98.3% NH ₄ -N 98.3% TP	

et al. [107] and Cho et al. [25] used a filtration method to remove contaminants from wastewater which resulted in the increase of lipid productivity in *Chlorella* sp.. Autoclaving was applied by Shi et al. [136] and Li et al. [80]; this resulted in an increase in microalgal biomass yield and productivity. In contrast, Wang et al. [155] obtained a lower microalgal biomass when the wastewater was pretreated using autoclaving. Here, autoclaving was not found to be effective for lowering the turbidity in the medium. A similar pretreatment was employed by Shah et al. [134] by combining autoclaving, centrifugation and filtration. Following this procedure, marine microalgae were found to accumulate high lipid levels when cultivated on the treated POME. Other

pretreatment processes include electrolysis [156], centrifugation [136, 155] and sparging with air [23].

Researchers also reported the pretreatment process by adding chemical agent or activated carbon to coagulate and adsorb the color. Activated carbon addition was found to be more effective to adsorb the dark color compared to the addition of starch and rice powder as a coagulator. Mutanda et al. [101] described the application of NaOCl in wastewater medium to lower turbidity. While Markou et al. [90] utilized 12.5 g/L NaOCl as pretreatment of olive oil mill effluent, which resulted in no negative impact on microalgae. Nevertheless, Qin et al. [120] showed that the addition of 30 ppm NaOCl gave a better pretreatment for

dairy manure rather than UV irradiation. Overall, any pretreatment process of wastewater is highly recommended to enhance microalgal growth and production.

Future Prospects

Microalgae have a great potential to produce food, feed, fuel, fine chemicals and fertilizers on a commercial scale, in spite of facing several issues [41, 95, 127]. Apart from biofuel production, microalgae may serve as a potential renewable source for other commercial applications [95]: (a) Environmental applications such as wastewater treatment and CO₂ mitigation; (b) Human nutrition; (c) Animal and aquatic feed; (d) Cosmetics production; (e) High-value compounds such as fatty acids; (f) Pigments such as beta-carotene, astaxanthin, fucoxanthin, phycobiliproteins; (g) Stable biochemicals; (h) Biofertilizer; (i) Drug synthesis for antimicrobial, antiviral and anticancer treatments.

To date, the value added production by microalgae still faces high production costs compared to other sources [162]. For example, Molina-Grima et al. [98] reported that the production of high-purity Eicosapentaenoic Acid (EPA) from the diatom *Phaeodactylum tricornerutum* requires a total production cost of US\$ 4602/kg, with 60% of the costs arising from the recovery process and 40% from the biomass processing. These costs should be reduced by 80% to be economically viable. However, the worldwide demand of EPA is 300 ton per annum [139].

Microalgae are considered to be promising for future raw feedstock because of their potency to produce derivative products in biorefinery processes by utilizing wastewater [2]. Biorefinery techniques are necessary to exploit all products from microalgae after cultivation. The main problem is to separate the different fractions without damaging one or more of the product fractions [152]. Microalgae are mainly composed of carbohydrates, lipids and proteins, and other components such as pigments. The combination of the biorefinery concept in downstream processing and utilization of wastewater for microalgal growth could reduce the production costs in the microalgae industry. Eventually, the whole cell could be utilized for food, feed, fuel, and fertilizer product, and wastewater could replace the (environmental) costs of synthetic nutrient additions. In addition, screening of microalgae based on the biochemical composition for efficient biomass production from wastewater treatment also plays a vital role in commercial applications [4].

Despite the high nutrient levels, the utilization of POME as growth medium for microalgae at industrial scale is still challenging. First, the high organic compounds, consisting of tannins, lignin, and phenolic compounds could negatively affect growth [51, 102, 105]. The dark coloration due to high

concentrations of suspended solids could inhibit light penetration, which is a critical factor for photosynthetic growth [34, 106, 111].

Moreover, the presence of heterotrophic bacteria may affect biomass productivity [24, 80]. The low pH and salinity of the wastewater needs to be adjusted before it can be used as growth medium for alkaline microalgae such as *S. platensis* or marine microalgae, such as *P. tricornerutum* which contains high levels of bioactive compounds. In addition, in some cases, the heavy metals in POME could prevent utilization of the bioactive compounds from the algae as pharmaceutical, cosmetic enhanced palm oil mill effluent or human consumption unless the metals are carefully removed [7].

However, the conditions as mentioned above could be prevented by employing some pretreatment process to lower COD, color, and heavy metals from the POME as described previously in Sect. 3. To increase the salinity, the cultivation might be relocated to seashore areas. Furthermore, the wastewater could be blended with hypersaline wastewater generated from industrial activities, such as chemical manufacturing, and oil production [164], to make the cultivation become more feasible for marine microalgae.

As stressed earlier, bioenergy and high-value biochemical production from the palm oil industry could benefit from the combination of wastewater treatment procedures [2]. Figure 1 shows the proposed concept for the utilization of POME wastewater as the growth medium for microalgae with the aim to obtain value added products and bioenergy feedstock. To optimize wastewater utilization, pretreatment processes of the raw source need to be employed. Several researchers reported that a high turbidity from the wastewater may induce heterotrophic or mixotrophic growth that benefits biomass production, lipid accumulation, and nutrient uptake [5, 49]. Furthermore, to provide CO₂ for mixotrophic cultivation, and lowering COD and BOD content in POME, the wastewater could be processed in an anaerobic fermentor, resulting in methane that could be used directly as an energy source. A previous report showed that the biomethane production could be enhanced by employing a co-culture of *Chlorella* sp coupled to palm oil empty fruit bunch. This resulted in a biogas production rate of 0.128–0.129 m³/kg COD/day and a biomethane yield of 5256.8–5295.8 mL/L POME/day. The methane yield was 1.4-fold higher compared to the process without microalgae [8].

Another proposed strategy is the cultivation of specific microalgae–bacteria consortia in the treatment of wastewater to enhance the bioactive compound (Fig. 1). Wastewater provides ideal media for bacterial growth and decomposition of organic matter in the presence of oxygen. However, only several types of bacteria can efficiently remove inorganic nutrients such as phosphorus, which is usually the main cause of eutrophication of freshwater ecosystems [111]. During this processes, microalgae provide oxygen for aerobic bacteria

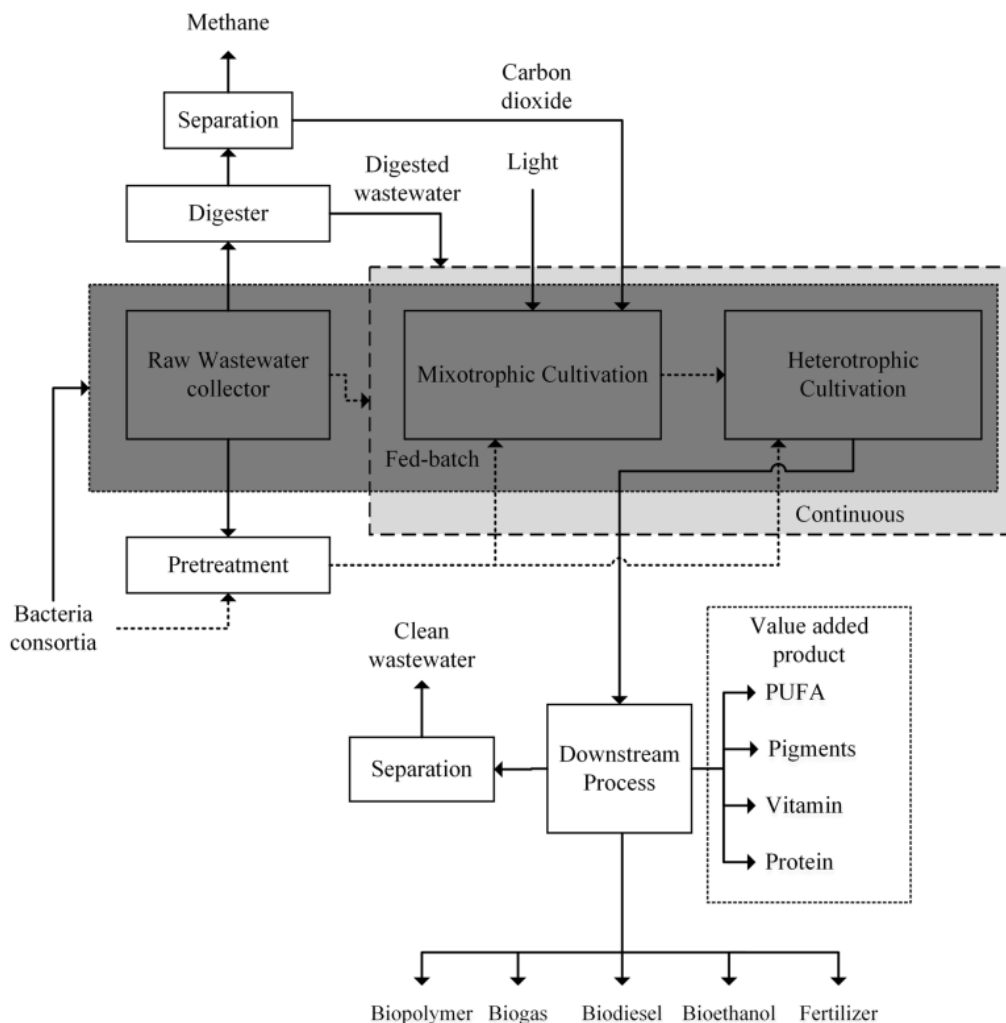


Fig. 1 Proposed concept for the utilization of wastewater for microalgal medium in order to produce value added compounds

to degrade organic matter and consume the carbon dioxide produced by bacterial respiration. Thus, these systems are an economical alternative to conventional aerobic treatments of wastewater [30, 119]. For example, Delgadillo-Mirquez et al. [30] demonstrated nitrogen and phosphate removal from wastewater using co-cultures of microalgal and bacterial consortia. However, bacteria may compete with microalgae for the available nutrients such as N and P, producing metabolites that are inhibitory to microalgal growth [68]. Furthermore, a strategy is proposed to utilize fed-batch or continuous cultivation modes to avoid inhibition by toxic levels of certain compounds in POME wastewater. Here, bacterial consortia could be injected in the fed-batch mode to increase COD and BOD efficiencies. As an alternative,

raw wastewater could be utilized in a continuous mode to increase biomass and products from microalgae.

To optimize value added product accumulation in microalgae, a two-step cultivation process seems promising. In the first step, microalgae could be cultivated under mixotrophic conditions to increase biomass productivity. In the second step, the culture could be transferred to heterotrophic conditions to increase compound yield. A similar strategy was proposed by Ogbonna et al. [108] who employed a two-step cultivation of autotrophic and heterotrophic conditions to increase the protein and chlorophyll content of *C. pyrenoidosa* C-212. With this system, the biomass concentration was recorded at 14 g/L containing 60.1% protein and 3.6% chlorophyll. Furthermore, Van-Wagenen et al. [153]

employed cyclic autotrophic/heterotrophic cultivation of *C. sorokiniana* with the addition of acetate as external substrate. This resulted in higher biomass productivity up to 1.2 g/L/day compared to the mixotrophic or autotrophic condition. Other solutions to enhance biomass and product yields might be to modify and/or control the environmental factors that were discussed in [Environmental factors affecting microalgal cultivation](#). Finally, it should be considered that the type of cultivation system such as open pond cultivation versus closed cultivation using photo-bioreactors could also influence the growth, biomass, and products. It may be clear that more research must be done to further optimize wastewater (including POME) utilization for high-value product generation by microalgae. However, it has become clear from the research executed over the past decennia, that optimization should be done based on a combination of approaches (choice of species, cultivation conditions, cultivation modes, nutrient ratio's, pretreatments etc.) before valorization could become economically feasible.

Concluding Remarks

Recent information on oil palm production faces serious environmental problems in the South East Asian region. In the wet processing system, vast quantities of POME wastewater are generated which may harm the environment. This study concludes that different cultivation modes, careful species selection and different conditions may improve growth, biomass yield, and productivity of several value added products generated by microalgae growing on wastewater. Other future research should highlight LC-PUFA production, fatty acid and pigments based on species which were not tested on POME before. Thus, more scientific work needs to be done to arrive at other value added products in feasible, sustainable, and economic cultivation systems.

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