

# Review of microalgae growth in palm oil mill effluent for lipid production

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**Abstract** Wastewater treatment using microalgae is an eco-friendly process without secondary pollution. During the process, the wastewater produced is reused, which allows efficient nutrient recycling. This review provides constructive information to enable progress of competent technology for microalgae based productions in palm oil mill effluent (POME). The characteristics of POME that will be described in this paper would be a source of pollution in water if discharged directly. Since microalgae have great potential to be isolated and cultivated in POME, previous studies to improve POME based culture media are still limited. Microalgae are highly competent in diminishing CO<sub>2</sub> emissions and reducing the organic components in POME. In conclusion, biological treatments by using microalgae discussed in this paper and the lipid production from microalgae biomass can be used as an alternative for energy production. The POME treatment with microalgae may meet the standards or limits before being discharged into the water body.

**Keywords** POME · Microalgae · Lipid · Biofuel

## Introduction

The benefits of microalgae in producing alternative sources of biofuels, animal feed, fertilizer and biopolymer applications seem very promising (Gouveia 2011). Moreover, microalgae can be utilized for wastewater treatment in pilot scales (Abdel-Raouf et al. 2012). The real impact of discharging wastewater rich in natural mixture and inorganic chemicals, for example, is that phosphates and nitrates are for the most part eutrophication (de Godos et al. 2009). Wastewater is utilized for nourishing microalgae that expel excess nutrients and accumulate biomass (Chinnasamy et al. 2010). Great potential has been shown by microalgae to utilize the remaining organic compounds and also to generate microalgae biomass in POME (Lam and Lee 2011).

POME is a major producer of agriculture wastewater. In 2011, there were 426 palm oil mills in Malaysia (Malaysian Palm Oil Board 2012). Table 1 shows the characteristics of POME. The high concentrations of suspended solids and grease are the source of organic compounds in POME.

Around 44 million m<sup>3</sup> of POME were produced in year 2013 to yield 19.66 million tonnes of total crude palm oil (Malaysian Palm Oil Board 2014). Around 85 % of palm oil mills have treated raw POME using biological treatment (Tong and Jaafar 2004). The biological treatment of POME is a series of pond systems, including anaerobic, facultative, and aerobic pond systems (Yacob et al. 2009). However, the final treatment by aerobic pond system is struggling to achieve the discharge standards because of inefficient operational design (Parthasarathy et al. 2016). The final effluent of the treated POME must comply with the discharge standards set by the Department of Environment (DOE), Malaysia, as illustrated in Table 2, which

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**Table 1** Characteristics of POME (Gobi and Vadivelu 2013)

pH	BOD (mg/L)	COD (mg/L)	Oil and grease (mg/L)	Suspended solids (mg/L)	Nitrogen content (mg/L)	References
4.7	25,000	50,000	4000	18,000	750	Ahmad et al. (2003)
3.5–4.2	10,000–44,000	16,000–100,000	–	5000–54,000	–	Zhang et al. (2008)
4.15–4.45	21,500–28,500	45,500–65,000	1077–7582	15,660–23,560	300–410	Wong et al. (2009)
5.6	–	46,000	–	42,800	–	Damayanti et al. (2010)
5.5	–	35,000–50,000	–	35,000	–	Gobi et al. (2011)

<sup>a</sup> All characteristics analysis based on American Public Health and Association (APHA 1998)

**Table 2** Standards of effluent discharge for crude palm oil mills according to Environmental Quality Act, 1974, Revision 2005

Parameter	Parameter limit
Biological oxygen demand (BOD), 3 days @ 30 °C (mg/L)	100
Chemical oxygen demand (COD) (mg/L)	– <sup>a</sup>
Total solids (mg/L)	– <sup>a</sup>
Suspended solids (mg/L)	400
Oil and grease (mg/L)	50
Ammonical nitrogen (mg/L)	150 <sup>b</sup>
Total nitrogen (mg/L)	200 <sup>b</sup>
pH	5–9
Temperature (°C)	45

<sup>a</sup> No discharge standard after 1984

<sup>b</sup> Value of filtered sample

remains the standard for POME management. This standard is an action by the Malaysia government to limit the impact of high organic compounds of POME on the environment.

Using microalgae in POME treatment is a known concept (Vairappan and Yen 2008), and the microalgae biomass extraction for lipid can be used as biofuel. However, literature about this topic is still limited. Ponraj and Din (2013) investigated biomass and lipid production of *Chlorella pyrenoidosa* using POME. Kamyab et al. (2014) studied the effect of POME on lipid productivity of *C. pyrenoidosa* in hybrid photo bio reactors (HPBR). Ibrahim et al. (2015) reported that optimization of retention time and pH on POME for nutrient removal using microalgae were 15 days and 9.2, respectively. Ding et al. (2016) studied a newly isolate microalgae named *Chlamydomonas sp UKM 6* in POME and determined the potential of microalgae production and removal of nutrients.

This review offers constructive information to further progress of competent technology for microalgae-based productions in POME. Hence, the application of microalgae in removing organic components could result in clean and sustainable production of palm oil. This paper will present cultivation operation and also systems for microalgae

growth. Furthermore, the biofuel production from microalgae biomass that extracts for lipids will be discussed.

## Characteristics of algae for POME treatment

Photosynthetic organisms (including macroalgae and microalgae) need sunlight, water, and carbon dioxide (CO<sub>2</sub>) to produce biomass (Barber 2009). Macroalgae (algae up to 60 m in length) can grow fast in marine and freshwater environments; such algae are also called “seaweed.” Some macroscopic algae grow on rocks (Graham et al. 2009), while some are photosynthetic microorganisms. Unicellular and multicellular microalgae fall into both general categories of organisms, which are either prokaryotic or eukaryotic (Wang et al. 2015). As primitive plants (thallophytes), microalgae have fewer roots, stems, and leaves and lack a sterile layer of cells around reproductive cells (Cho et al. 2011). Due to the lipid content in microalgae, they can float on the surface of water (phytoplankton). The survival of microalgae in various environmental conditions from aquatic to terrestrial habitats 1000 years ago had made them the Earth’s oldest living microorganism (Mata et al. 2010).

## Classification of microalgae

The number of microalgae species in the world is estimated at around 50,000, of which 30,000 have been categorized (Kim et al. 2010). Depending on their pigmentation, life cycle, and basic cell structure, they are classified into several major groups (Mata et al. 2010).

- Bacillariophyceae class, the diatoms: Marine phytoplanktons are dominated by these microalgae, and some may be present in fresh and brackish water environments. The number of species is around 100,000. Their cell walls contain polymerized silica (Si) and various forms of carbon. Another form of carbon is present in natural oils or carbohydrate polymers (chrysolaminarin).
- Chlorophyceae class, the green microalgae: Around 8000 species live in fresh water as single cells or in colonies. Higher plants, which are called “vascular plants,” evolved from these green microalgae. Starch and oil are the main storage compounds that can be generated under certain conditions.
- Cyanophyceae class, the blue green microalgae: They are not true microalgae, having no nucleus (structure that encloses the DNA) or chloroplast (structure that encloses the photosynthetic membranes) (Avagyan 2011). They play the role of fixing nitrogen in the atmosphere; their structure resembles that of bacteria. Around 8000 species live in various niches.
- Chrysophyceae class, the golden microalgae: Around 1000 species live in fresh water. They are similar to diatoms but have more complex pigment systems (yellow, brown or orange). Golden microalgae can produce natural oils and starch.
- Prymnesiophyceae class: Around 500 species are present in the ocean. Like diatoms, they are brown, and their main components are lipids and chrysolaminarin.
- Eustigmatophyceae class: These are the main “picoplankton,” which in diameter is around 2–4  $\mu\text{m}$ . *Nannochloropsis* sp. is a common genus of this microalgae in the ocean.

Microalgae can be cultivated in open pond systems and in closed photobioreactor systems under controlled environments (Graham et al. 2009), but during cultivation operations, other microorganisms can dominate these systems (Schenk et al. 2008). Moreover, POME contains high nutrient values of BOD and COD, which can cause contamination. Four types of carbon metabolism mechanism are undertaken by microalgae: autotrophic, mixotrophic, heterotrophic, and photoheterotrophic conditions. Some microalgae can use either autotrophic or heterotrophic carbon, such as *Chlorella protothecoides*, depending on the culture conditions (Lam and Lee 2011). Under environmental flux, they can shift this mechanism, especially due to carbon and light flux (Chen et al. 2011).

## Microalgae constituents

The main chemical components in microalgae are  $\text{C}_{0.48}\text{H}_{1.83}\text{N}_{0.11}\text{P}_{0.01}$  (Sialve et al. 2009). The major biochemical compounds of microalgae are proteins, carbohydrates, lipids, and nucleic acids. The proportion for each component varies with different species and environments (Markou and Georgakakis 2011). Sialve et al. (2009) reported that the main biochemical compositions in microalgae such as proteins, carbohydrates, and lipids were around 6–52, 5–23, and 7–23 %, respectively.

In the palm oil industry, Yacob et al. (2006) estimated that 1 tonne of POME can produce 12.36 kg of methane, which is emitted from the anaerobic pond. Methane gas can be used as biogas; for example, methanogenesis of rye straw for improving methane yield was done by (MarouŁek et al. 2012), and this improvement can be applied to a POME anaerobic pond. The increase in anthropogenic green house gasses (GHGs) discharges and exhausting fossil stores have sparked a lot of research on renewable energy, especially biofuel (Singh et al. 2011). The harvested microalgae can be further processed for lipids. Compared to other crops, the rapid production of microalgae produces higher lipid content (Huang et al. 2009). Microalgae from different taxonomic groups and varied growth environments will contribute different total lipid content and fatty acids (Pratoomyot et al. 2005). The accumulation of lipid content or fatty acid in microalgae biomass is correlated with the growth stage of microalgae (Laurens and Wolfrum 2011). The production of fatty acid is higher in the stationary phase than the exponential phase (Mata et al. 2010). During the stationary phase, the microalgae utilized all the nutrients in the cultivation growth media. This situation creates a stressed environment for microalgae, causing the rate of cell division to decrease. It will lead to the accumulation of lipid content in the microalgae (Mansour et al. 2005).

Many factors contribute to lipid content accumulation in microalgae by extending the available nutrients for microalgae growth (Liu et al. 2011). From the literature reported by (Stephenson et al. 2010), the alteration of nutrients for microalgae growth in cultivation affects lipid content accumulation in microalgae. The nutrients in media correlated with any changes in lipid content from free fatty acid compounds to triacylglyceride (TAG) compounds. Modification of the nutrient profile can be achieved by manipulating the N, Si, Fe content, and  $\text{CO}_2$  supply during cultivation (Amaro et al. 2011).

A long carbon chain molecule of lipid functions as the structural component of the microalgae membrane. As the lipid content increases, the specific gravity will decrease. This criterion makes algal cell buoy on water surface for solar light source (Shen et al. 2009). Manipulating the

specific species and factors affecting the microalgae results in different biomass lipids, proteins, and carbohydrate content (Shen et al. 2009). Examples of cultivation environments are the velocity of process, growth duration, and nutrient content in media (Adams et al. 2009).

Table 3 shows the constituents of various basic microalgae species. *Botryococcus braunii* have the highest lipid content around 33–86 %. Ibrahim et al. (2015) optimized POME treatment by using *Botryococcus braunii*, resulting in  $\text{NH}_3\text{-N}$  and  $\text{PO}_4^{3-}$ , removal rates of 92.857 and 56.5 %, respectively. However, they did not focus on microalgae growth and lipid production.

All the constituents shown in Table 3 change by relying on the media and conditions of the environments producing the microalgae biomass (Becker 2007) and will be discussed further in next section in this review. The end product of biofuel can be produced from lipid extraction in microalgae (Shen et al. 2009). The new oil technology that can boost oil yields without surpassing the temperature limit that can degrade oil quality already has been applied to grape seed (Maroušek et al. 2015a). This technology may be applied to lipid production from microalgae, which usually undergoes extraction using chemicals. The remaining compounds such as carbohydrates and proteins are left after lipid extraction. However, the leftover carbohydrates and proteins can be fermented for methane or ethanol, and solid residue after the fermentation process can be pyrolyzed for biogas carbon powder (Maroušek et al. 2015b). These researchers used solid pulp from fermentation of maize silage for biogas carbon powder, which can be an alternative for charcoal or costly wood. Meanwhile, the fermentation of carbohydrates will be producing ethanol (Coppola et al. 2009). Then the ethanol itself can be a key component of the transesterification process for biodiesel production, which serves as a catalyst. The reaction of ethanol with sodium produces sodium

ethanolate, which can also be a catalyst. Both these catalysts may produce biodiesel and glycerol after the reaction takes place (Coppola et al. 2009).

Protein is built up by one or more peptides and folded into a solid or fiber form. In several studies on high nutritional quality in microalgae, protein was compared with the protein found in vegetable crops. Humans consume macroalgae or seaweed most often as health food, pharmaceutical and animal feeds produced by using microalgae (Adams et al. 2009). Biogas (hydrogen and methane) for transportation can be produced from microalgae. In industry, domestic, and agriculture, microalgae can assimilate the N and P in effluent or wastewater and sequester  $\text{CO}_2$  derives from industrial flue gas (Clarens et al. 2011).

### Operation condition for microalgae growth

Generally, abiotic and biotic factors affect microalgae growth. Abiotic factors comprise light, temperature, nutrients,  $\text{O}_2$  concentration,  $\text{CO}_2$  concentration, pH, and salinity (Kamarudin et al. 2015). Rapid algal growth can be indicated by the increase of pH (Moheimani 2005). Toxic chemicals influence the growth of microalgae (Yang et al. 2015). Meanwhile, the biotic factor consists of pathogens due to competition with other species of microalgae in cultivation systems (Mata et al. 2010). Operation factors during cultivation such as mixing and aeration go under biotic factors to homogenize microalgae cell distributions, heat and nutrient and to encourage the movement of gas in the culture media (Kamarudin et al. 2015). The optimum condition for microalgae cultivation is summarized in Table 4.

These optimum conditions can be used as basic instructions for microalgae cultivation. If these microalgae

**Table 3** Constituents of microalgae (% of dry matter)

Microalgae	Lipid	Protein	Carbohydrate	References
<i>Botryococcus braunii</i>	33–86	4–40	20	Sydney et al. (2010)
<i>Chlamydomonas reinhardtii</i>	18–22	46–48	17	Kebelmann et al. (2013)
<i>Chlorella ellipsoidea</i>	10–30	34–45	24–51	Han et al. (2012)
<i>Chlorella pyrenoidosa</i>	8–35	31–47	20–57	Han et al. (2012)
<i>Chlorella vulgaris</i>	10–50	29–58	12–17	Xu et al. (2011)
<i>Dunaliella tertiolecta</i>	3–13	26–61	22	Shuping et al. (2010)
<i>Euglena gracilis</i>	11	29	32	Yadavalli et al. (2014)
<i>Nannocloropsis oculata</i>	20	39	17	Du et al. (2012)
<i>Nannocloropsis oceanic</i>	24.8	19.1	22.7	Cheng et al. (2014)
<i>Prymnesium cruentum</i>	22–38	30–45	25–33	Becker (2007)
<i>Scenedesmus obliquus</i>	9–12	–	6–16	Ho et al. (2012)
<i>Spirulina platensis</i>	4–13	42–63	8–30	Jena and Das (2011)
<i>Synechocystis</i> sp.	2.8	53.7	40.5	Wagner et al. (2016)

**Table 4** Optimum conditions for microalgae cultivation (Perumal et al. 2015)

Parameter	Range	Optimum
Temperature (°C)	16–27	18–24
Salinity (g/L) <sup>a</sup>	12–40	20–24
Light intensity (lx)	1000–10,000 (depend on volume and density)	2500–5000
Photoperiod (light:dark, h)	–	16:8 (minimum) 24 h (maximum)
pH	7–9	8.2–8.7
CO <sub>2</sub> rate	1–4 %	1 % of the volume of air
Nutrients	–	N:P (16:1) and silicon <sup>a</sup> (Alabi et al. 2009)

<sup>a</sup> For marine microalgae

optimal conditions are applied in POME treatment, the organic compounds (N and P) available in POME that do not contain heavy metals and radioisotopes can be utilized.

### Light intensity and cell density

The important metabolic process for microalgae growth is carried out by the primary energy source, which is light. Light intensity, quality of spectral wavelength, and photoperiod should be taken into account (Perumal et al. 2015). The source of light may be solar or artificial (fluorescent tube). Table 4 shows the optimum intensity. Photo inhibition and overheating may be caused by too high light intensity (Alabi et al. 2009). The most preferred light spectrum, which is blue or red, is suitable for photosynthesis. Turbidity in the culture that reduces photosynthesis will occur if the cell density is high (Chen et al. 2011). Kaewpintong (2004) reported that the increment of cell density and specific growth should be sensible with light intensity up to certain limit. Furthermore, the abundant solar light in Malaysia and other tropical countries is suitable for microalgae cultivation (Rajkumar et al. 2013).

### Temperature

The optimal temperature for microalgae cultures may differ depending on the culture media and species. Temperatures of less than 16 °C will decrease microalgae growth, while high temperatures of more than 35 °C are harmful for various species (Perumal et al. 2015). High temperature will cause degradation and inactivation of enzymes involved in the photosynthetic process (Zuppini et al. 2007). Countries in the tropical regions like Malaysia do not have four seasons which are warm throughout the year. Solar radiation is abundant in Malaysia which is closed to equator and the daylight around 6 h per day. Rate of evaporation is affected by cloudiness and temperature. Cloudiness will result in less solar radiation and lower temperature (Singh et al. 2013). During hot days,

overheating will occur in closed culture systems, and the temperature in reactors will reach 55 °C. Economical evaporative water cooling systems can be used to reduce the temperature to around 20–26 °C (Moheimani 2005).

### Nutrients

Organic carbons (sugars, proteins, and fats), vitamins, ionic salts, and other nutrients (N and P) are essential for the growth of microalgae (Mata et al. 2010). The density of microalgae growth in cultures is greater than that in nature because the cultures are enriched with nutrients. Richmond (2008) describes various specific recipes for algal culture media. Macronutrients needed for microalgae are N, P (in an approximate ratio of 6:1), and Si. Diatoms microalgae need Si for development of protective shells (Perumal et al. 2015). Various trace metals and Vitamins B<sub>1</sub>, B<sub>12</sub>, and biotin are called micronutrients. Walne media and Guillard's F/2 are suitable for the growth of most microalgae. Alternative enrichment media that are suitable for mass production of microalgae in large-scale extensive systems contain only the most essential nutrients and are composed of agriculture grade rather than laboratory grade fertilizers (Perumal et al. 2015).

### pH

Failure to maintain the pH will completely break down the culture system. Since POME pH is acidic, there is a need to recheck the pH value after POME dilution for microalgae culture. Moreover, optimum pH can be achieved by using aeration in the culture. In high concentrations of microalgae cell in culture, the pH tends to increase up to pH 9, but the addition of CO<sub>2</sub> solves this problem. The CO<sub>2</sub> addition acts as a pH buffer in terms of CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> balance (Perumal et al. 2015). Weissman and Goebel (1987) explained that the assimilation of CO<sub>2</sub> into high pH water is accelerated by one of two main reaction paths (without a catalyst), which is direct reaction of CO<sub>2</sub> with the hydroxyl

ion,  $H^+$  to form bicarbonate,  $HCO_3^-$ . This  $CO_2/HCO_3^-$  balance reaction occurs rapidly at pH values below 8 and above 10.

### Mixing

Ambient air contains 0.03 %  $CO_2$  for photosynthesis. This condition will limit microalgae growth in culture. Concentrated  $CO_2$  may be supplied during cultivation (Alabi et al. 2009). Microalgae sediment can be prevented by mixing, which allows light, gas exchange or nutrients to be uniformly exposed and then can prevent thermal stratification, especially in outdoor cultivation (Kaewpintong 2004). Mixing can be done by stirring manually (test tubes, Erlenmeyer), aerating (bags, tanks), or using paddle wheels and jet pumps (ponds), depending on the culture system (Perumal et al. 2015). Turbulence mixing in certain limit large-scale microalgae productions is vital to promote cell circulation from dark to light regime (Barbosa 2003). Only certain microalgae species can endure rapid mixing (Perumal et al. 2015). The optimum mixing rate should be researched to prevent any decline in microalgae productivity (Barbosa 2003).

### Cultivation systems for microalgae growth

Phototrophic cultivation is a condition in which light is used by microalgae as the only source of energy under photosynthesis, the chemical energy being converted (Brennan and Owende 2010). Microalgae can be grown in open pond systems or photobioreactors (PBRs) (Menetrez 2012). Algal cultivation units can be operated in batch or in

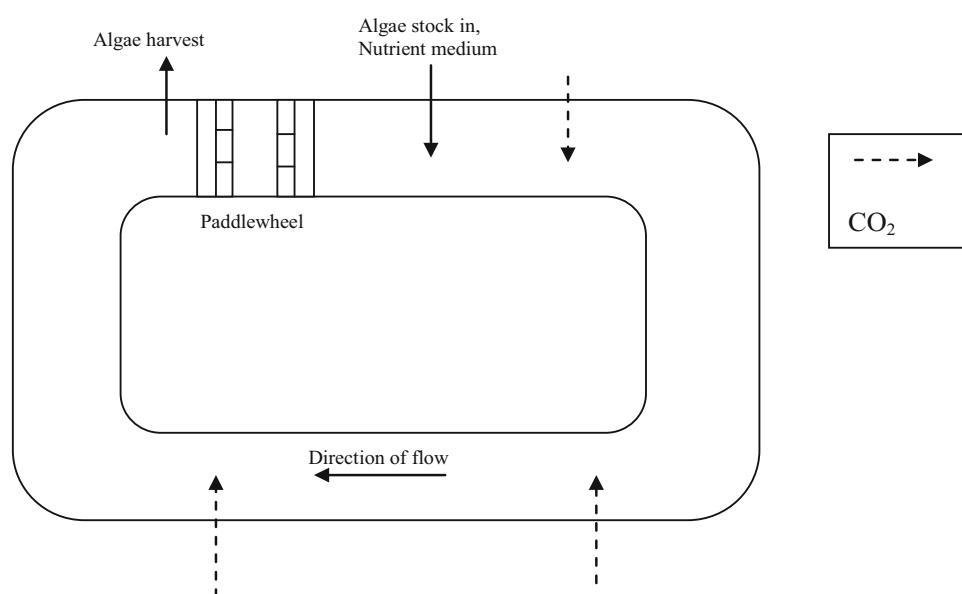
continuous mode, and the production units can be open or closed systems. This depends on the microalgae species selected, expected environmental conditions, availability of nutrients, and even possibility of combining microalgae growth with a pollution control strategy of another industry, for example, for the removal of  $CO_2$  from flue gas emissions or removal of N and P from a wastewater effluent (Mata et al. 2010). Strains of microalgae that produce high lipid yields are usually cultivated in open pond systems (González-Fernández et al. 2011). PBRs are closed, controlled systems with equipment that provides an ideal environment for high productivity (González-Fernández et al. 2011).

### Phototrophic cultivation in open system

Phototrophic cultivation in open ponds has been used since the 1950s and can be categorized as stagnant water (lagoons, lakes, and small ponds) and tanks or containers. Shallow circular ponds and tanks (Fig. 1) or parallel raceway ponds (PRPs) are also open systems (González-Fernández et al. 2011).

The main problem of open systems is contamination by other microorganisms (González-Fernández et al. 2011). The nature of open ponds and their weakness in handling contamination have restricted cultivation of *Spirulina* sp., *Dunaliella* sp., and *Chlorella* sp. as reported by Huntley and Redalje (2007). Dealing with contamination involves genetic modification of microalgae (Spolaore et al. 2006). Processes using genetically modified microalgae are currently being developed by many biofuel industry participants (Raja et al. 2008).

**Fig. 1** Plan view of a raceway open pond adapted from Chisti (2007)



### Phototrophic cultivation in closed/photobioreactors system

Many studies have attempted to increase microalgal biomass production rates by developing closed photobioreactors (PBRs) system (Chisti 2007). Indeed, this system may cost ten times more than open systems (Mata et al. 2010). Table 5 summarizes the advantages and disadvantages of both systems. It seems that open systems are more suitable for POME treatment. The advantage of microalgae approach in POME treatment is readily available facilities; for example, ponds and tanks could reduce the cost of coupling microalgae treatment with conventional treatment (Kamarudin et al. 2015).

PBRs can facilitate good control for culture environment and meet the optimal growth requirements like the concentration of carbon dioxide, temperature, light intensity, mixing, density of culture, and pH (Mussgnug et al. 2007). The requirements for specific growth are internally maintained in a closed system. The PBRs illustration is seen in Fig. 2.

High density cultures of microalgae contained within bioreactors may limit light intensity (Mussgnug et al. 2007). The exposed 7.6–10 cm of light penetration because of high turbidity is caused by microalgae growth and

culture media (Mussgnug et al. 2007). The helical tubular shape PBRs designed by Briassoulis et al. (2010) have been applied to limit growth optimization for nonstop production. The following are conditions optimizations in this PBRs design:

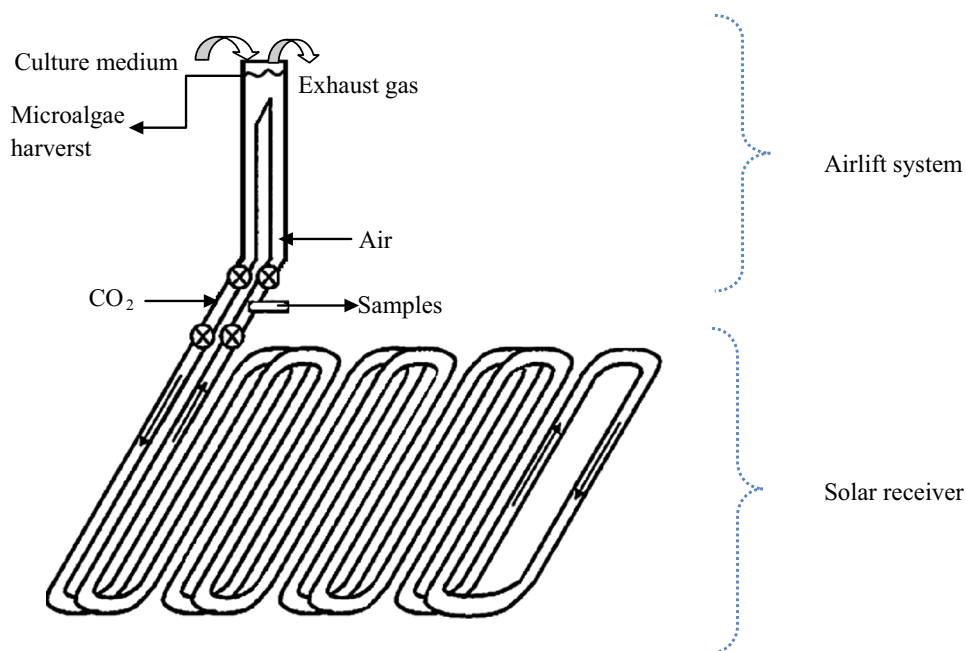
- the size of volume to surface area ratio;
- inhibition for temperature fluctuation and contamination;
- spatial distribution of fresh ambient air and CO<sub>2</sub>;
- CO<sub>2</sub> exchange rate; and
- automated flow through sensors.

Generally, PRPs (open pond) are cheaper than PBRs because of low costs for construction materials and operation, but low biomass productivity will result (Hu et al. 2008). PBRs are expensive, but they can produce more microalgae biomass for lipid contents (40–55 %) (Chisti 2007). The production of biodiesel from microalgae in an open system costs around US \$9–25 per gallon, whereas in a closed system, it will cost US \$15–40 per gallon (Amaro et al. 2011). The pros and cons of both systems associated with a promised hybrid culture system made as the best practice (Hu et al. 2008). Since POME utilization using microalgae is still in the research phase and not commercialized, there is a need to study the potential of microalgae

**Table 5** Comparison of closed and open systems for microalgae cultivation (Pulz 2001)

System	Closed systems (tubular reactor and PBRs)	Open systems (circular stirred and PRPs)
Contamination control	Easy	Difficult
Contamination risk	Reduced	High
Sterility	Achievable	None
Process control	Easy	Difficult
Species control	Easy	Difficult
Mixing	Uniform	Very poor
Operation regime	Batch or semi-continuous	Batch or semi-continuous
Area/volume ration	High (20–200 m <sup>-1</sup> )	Low (5–10 m <sup>-1</sup> )
Population (microalgae cell) density	High	Low
Investment	High	Low
Operation cost	High	Low
Capital/operating cost ponds	Ponds 3–10 times lower cost	PBRs >ponds
Light utilization efficiency	High	Poor
Temperature control	More uniform temperature	Difficult
Productivity	3–5 times more productivity	Low
Hydrodynamic stress on microalgae	Low–high	Very low
Evaporation of growth media	Low	High
Gas transfer control	High	Low
CO <sub>2</sub> losses	Depends on pH, alkalinity, etc.	PBRs CO <sub>2</sub> is same as occurs in ponds
O <sub>2</sub> inhibition	Greater problem in PBRs	PBRs >ponds
Biomass concentration	3–5 times in PBRs	PBRs >ponds
Scale up	Difficult	Difficult

**Fig. 2** Basic design of a horizontal tubular PBRs adapted from Becker (1994)



selection, type of treatment reactor, bioprocess optimization, carbon dioxide sequestration by microalgae, and potential of microalgae biomass recovery as a biofuel resource.

### Heterotrophic cultivation

Heterotrophic cultivation is a condition in which organic carbon sources are used by microalgae as carbon and energy sources in dark conditions (Chojnacka and Marquez-Rocha 2004). Limited light in high density microalgae usually occurs in PBRs under phototrophic cultivation, which can be solved under heterotrophic cultivation (Huang et al. 2010). High microalgae biomass and high lipid content (40 % increase) under heterotrophic cultivation using *Chlorella protothecoides* was investigated by Xu et al. (2006). Heterotrophic microalgae can utilize various organic carbon sources (i.e., glucose, acetate, glycerol, fructose, sucrose, lactose, galactose, and mannose) for growth (Liang et al. 2009). However, organic carbon sources used in heterotrophic cultivation face the contamination problem (Ogbonna et al. 1997).

### Mixotrophic cultivation

The microalgae that can grow in mixotrophic cultivation uses light as the main source of energy for photosynthesis. Essential energy sources from organic carbon and CO<sub>2</sub> are necessary for microalgae. The organisms can live either autotrophically or heterotrophically (Graham and Wilcox 2000). This ability is called amphitrophy (subtype of mixotrophy), and it depends on the availability of either

organic carbon or light (Chojnacka and Marquez-Rocha 2004). Compared with phototrophic and heterotrophic cultivations, mixotrophic cultivation is rarely used in microalgae lipid production because only a few microalgae species are mixotrophic (Liang et al. 2009).

### Photoheterotrophic cultivation

To use organic carbon source to produce energy, a light source is required for photoheterotrophic cultivation (Mata et al. 2010). This mechanism is known as photoorganitrophy, photo assimilation, or photo metabolism. The photoheterotrophic and mixotrophic metabolisms have no differences according to different sources of carbon used to produce energy for growth and specific metabolite production. This type of cultivation requires organic carbon like sugar and light simultaneously (Chojnacka and Marquez-Rocha 2004). Although the production of some light regulated useful metabolites can be enhanced using photoheterotrophic cultivation, this approach to producing biofuel is very rarely used, as in the case with mixotrophic cultivation (Ogbonna et al. 2002).

### POME as alternative media for microalgae cultivation

Microalgae cultivation in POME offers an alternative to conventional forms of tertiary wastewater treatments and spontaneously utilizes organic compounds in POME to generate microalgae biomass for lipid production (Lam and Lee 2011). The next section will review the current



**Table 6** Growth infrastructure for microalgae in POME

Source of POME/concentration	Microalgae	Growth infrastructure	References
POME collected from pond with no dilution	<i>Chlorella pyrenoidosa</i>	3 L PBR system Temperature: 24–26 °C Mixing 60 rpm Lighting 8 h:16 h L:D pH 6.5–7.5 Light intensity of 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Ponraj and Din (2013)
Fresh POME with dilution 50 % + 1 g/L urea	<i>Chlorella</i> sp.	1 L glass flask disk Light intensity 3000 lx pH 6.8–7.2 Temperature 28 °C Mixing using aeration aquarium air pump	Hadiyanto et al. (2012)
POME from anaerobic pond with 40 % dilution	<i>Spirulina plantesis</i>	1 L glass flask disk Mixing using aeration aquarium air pump pH 9–10.5 Light intensity, 4000–6000 lx	Hadiyanto and Hartanto (2012)
POME collected from pond with concentration 250 mg COD/L	<i>Chlorella sorokiniana</i>	Room temperature Light intensity-continuous illumination at intensity of $\pm 15 \mu\text{mol m}^{-2} \text{s}^{-1}$	Putri et al. (2011)
POME collected from pond with concentration 250 mg COD/L	<i>Chlorella pyrenoidosa</i>	5 L HPBR reactor with turbine impeller Temperature, 30 °C Light intensity, illuminated by four 32 W white fluorescence light continues lighting (24 h) (Philip, Germany) C:N, 100:6 OLR, 36 kg COD $\text{m}^{-3} \text{d}^{-1}$	Kamyab et al. (2014)
POME collected from pond with concentration 250 mg COD/L	<i>Chlamydomonas incerta</i>	250 mL Erlenmeyer flask Temperature, 30 °C Light intensity, 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$ C:N, 100:7 OLR, 36 kg COD $\text{m}^{-3} \text{d}^{-1}$	Kamyab et al. (2015)
Fresh POME with dilution of 500 mL of POME in 400 mL deionized waster	<i>Chlorella</i> sp.	1 L conical flask Pressure regulators bring down the pressure of both CO <sub>2</sub> and compressed air to two bars before entering their flow meters. The sparging tube of a flask culture was placed at the bottom of the flask. Light intensity, 10,000 lx CO <sub>2</sub> concentration (% v/v), 16 % Sparging rate (vvm), 0.8 vvm	Ahmad et al. (2015)
Fresh POME with dilution of 1 %	<i>Arthrospira plantesis</i>	10 L of culture media in 20 L tank Outdoor	Sukumaran et al. (2014)

microalgae growth infrastructure and the potential of using POME as alternative media for microalgae biomass production.

### POME concentration for microalgae growth and infrastructure

Various environmental and operational factors affect microalgae growth to make cultivation fruitful. The natural

effluent discarded from POME might be colloidal, dark, and viscous, which should be considered prior to media preparation for microalgae culture. Microalgae cannot tolerate the high ammonium concentration in POME (Bello et al. 2013). Vairappan and Yen (2008) found that for the marine *Isochrysis* sp., the concentration of POME at 5 % dilution is the best concentration for culture media, due to properties of POME shown in Table 1. This dilution procedure will enhance light penetration into media (Olguín et al. 2003).

**Table 7** Growth infrastructure for microalgae in industrial wastewater

Source from industrial wastewater	Microalgae	Growth infrastructure	References
Swine wastewater (no pretreatment) with dilution of 5–15 %	<i>Micractinium inermum</i> <i>NLP-F014</i>	<i>Seed culture condition:</i> 150 mL volume in a 250-mL Erlenmeyer flask Temperature, 25 °C Duration, 7 days Mixing, 70 rpm Light intensity, 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ <i>Preculture condition:</i> 0.8 L volume in air-lift column PBR (HxD:66 × 5 cm) Temperature, 25 ± 2 °C White fluorescent lamps Light intensity, 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ CO <sub>2</sub> , 2 % vv <sup>-1</sup> Flow rate, 0.4 L min <sup>-1</sup>	Park et al. (2015)
Starch wastewater	<i>Scenedesmus</i> sp.	100 mL closed reactor Duration 120 h Temperature, 35 ± 1 °C Light intensity, 70.4 mEm <sup>-2</sup> s <sup>-1</sup> continuously	Ren et al. (2015)
Domestic wastewater and pre treated oil refinery effluent	<i>Chlamydomonas debaryana</i> , <i>C. luteoviridis</i> , <i>Desmodesmus subspicatus</i> , <i>Hindakia tetrachotoma</i> & <i>Parachlorella hussii</i>	<i>Laboratory microalgae cultivation</i> 250 mL flasks Mixing, 2 Hz Temperature, 22 °C Photo period, 16 h light: 8 h dark Light intensity, 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ <i>Open pond microalgae cultivation</i> 150 L tank (depth 10 cm) No artificial lighting Flow rate, 2000 L h <sup>-1</sup>	Osundeko and Pittman (2014)
Slaughterhouse wastewater	<i>Chlamydomonas subcaudata</i> , <i>Anabaena</i> sp. and <i>Nitzschia</i> sp.	75 L indoor reactor (LxWxH:1.25 × 0.6 × 0.18 m) Mixing, 15 rpm Liquid velocity, 31 cm/s Light intensity, 4500 ± 150 lx around 12 h Temperature, 25 ± 2 °C	Hernández et al. (2016)
Marine aquaculture wastewater	<i>Platymonas subcordiformis</i>	Temperature, 25 °C pH 8.0 Light intensity of 50 $\mu\text{E}/(\text{m}^2 \text{s})$ Photo period, 14 h:10 h light: dark cycle	Guo et al. (2013)
Industrial tofu wastewater	<i>Chlorella vulgaris</i>	In a 18 L PBR Light intensity, 5000 lx Pressure, 1 atm Temperature, 28 °C Aeration of CO <sub>2</sub> and air 5 L/min	Rizkytata et al. (2014)

Table 6 shows that limited growth infrastructures were done on palm oil mill effluent, whereas Table 7 shows recent and evolved growth of infrastructures using various industrial wastewaters. Microalgae biomass productivity and biomass composition will be affected by these factors (Markou and Georgakakis 2011).

Most of the microalgae species used in POME treatment as shown in Table 6 were *Chlorella* sp., *Spirulina* sp., and *Arthrospira* sp., which seems limited in the literature compared to other microalgae species. Most studies use PBRs systems and focus on lab scale microalgae cultivation for POME, which could be a

challenge in real POME treatment aimed at commercialization.

Various types of industrial wastewater used in the studies mentioned in Table 7 can successfully produce different types of microalgae. However, most of the studies were done on a laboratory scale. Osundeko and Pittman (2014) conducted an experiment in an open system using a 150-L tank (depth 10 cm) without artificial lighting and a flow rate around 2000 L h<sup>-1</sup> which are possible if applied in POME treatment.

### Microalgae performance cultivate in POME

The concentrated nutrients (i.e., C, N, P, carbohydrates, lipids, proteins, and minerals) in POME are highly applied in biotechnology studies (Kamyab et al. 2014). As mentioned earlier, the concentration range of POME in different receiving water bodies may have a high impact on the aquatic environments if the discharge exceeds the limit of standards of the Malaysia Environmental Quality Act. Various species of microalgae present in freshwater, seawater, or brackish water make it possible to grow them in large scale reactors on unfertile lands. Hence, microalgae cultivation would not compete with other food crops (Brennan and Owende 2010). The use of microalgae in the utilization or remediation of excess nutrients and CO<sub>2</sub> present in natural water resources, lagoons, and ponds is phycoremediation (Olgúí 2003). The biological treatment was introduced about 40 years ago in places that usually used tertiary wastewater treatment (Rawat et al. 2011). Previously, it was found that microalgae could be cultivated very well in metal concentrated water environment (Parameswari et al. 2010).

The harvested microalgae can be further processed for various applications, including biofuel production and energy production. Microalgae can be extracted for lipid, and this lipid can be used as biofuel (Pokoo-Aikins et al. 2010). Many autotrophic microalgae such as *Chlorella vulgaris*, *Isochrysis galbana*, *Botryococcus braunii*, *Navicula pell iculosa*, *Scenedsmus acutus*, *Cryptocodinium*

*cohnii*, *Dunaliella primolecta*, *Monallanthus salina*, *Neochloris oleoabundans*, *Phaeodactylum tricornutum*, *Chlamydomonas reinhardtii*, and *Tetraselmis sueica* can produce lipid (Picardo et al. 2013). Species of microalgae used for biofuel production must meet these criteria:

- high microalgae specific growth rate;
- high lipid content;
- can survive in stressful environments caused by temperature, nutrient input, light intensity and contamination from bacteria;
- easy harvesting process;
- further lipid extraction method; and
- potential to be applied in food formulation, cosmetics, and pharmaceutical production.

The specific growth rate ( $\mu$ ) and biomass productivity for microalgae adapted from Converti et al. (2009) are calculated by Eq. (1).

$$\mu = \frac{1}{tx \ln\left(\frac{X_m}{X_0}\right)} \quad (1)$$

where  $X_m$  is the concentration of biomass at the end of the batch run,  $X_0$  is the concentration of biomass at the beginning of the batch run, and  $t$  is the duration of the batch run (h, d). In addition, lipid productivity is achieved through Eq. 2:

$$P_{\text{lipid}} = \frac{C_l}{t} \quad (2)$$

where  $C_l$  is the concentration of lipids at the end of the batch run and  $t$  is the duration of the run (h, d).

The production process consists of four stages: microalgae cultivation, dry weight biomass (DWB) separation, lipid extraction, and transesterification. The performance of many microalgae cultivated in POME is reported in works cited in Table 8.

Kamyab et al. (2014) focussed on nutrients reduction in POME, lipid production, and microalgae growth. Other researchers were not focussed on nutrients reduction. Nutrients reduction in POME is important to relate with

**Table 8** Microalgae performance cultivated in POME

Microalgae	Nutrient reduction (%)	Lipid production (%)	Growth rate (d <sup>-1</sup> )	Biomass productivity (g/L/d)	Duration (d)	References
<i>Chlorella pyrenoidosa</i>	–	42	–	2.19	18	Ponraj and Din (2013)
<i>Chlorella</i> sp.	–	–	0.066	0.058	15	Hadiyanto et al.(2012)
<i>Spirulina plantesis</i>	–	–	–	9.8	13	Hadiyanto and Hartanto (2012)
<i>Chlorella sorokiniana</i>	–	28.27	0.099	8.0	20	Putri et al. (2011)
<i>Chlorella pyrenoidosa</i>	COD, 71.16 %	68	1.8	0.13	10	Kamyab et al. (2014)
<i>Chlorella</i> sp.	–	–	–	1.562	7	Ahmad et al. (2015)
<i>Arthrospira plantesis</i>	–	–	–	0.211	7	(Sukumaran et al. 2014)

microalgae growth. However, excess nutrients also can inhibit microalgae growth, and not all nutrients can be utilized by microalgae. Then excess nutrients will be converted to toxic compounds that can inhibit microalgae growth (Munn et al. 1989). Optimum POME concentration mixed with synthetic nutrient for *Spirulina* sp. cultivation was done by Azimatun and Hadiyanto (2014) to obtain optimum microalgae biomass.

## Conclusion

In this review, integration of POME with microalgae cultivation has provided a sustainable way to reduce pollutants in wastewater or final effluents from POME. There is a need to study this potential to contribute to alternative culture medium for microalgae growth. The open system is relevant for pilot scale POME treatment using high lipid content microalgae such as *Botryococcus braunii*, *Nannochloropsis* sp., and *Chlamydomonas* sp. Various aspects need to be studied for microalgae cultivation in POME, especially to control contamination. Moreover, the location of Malaysia on the Earth's equator makes microalgae cultivation potentially productive. Other aspects that should be considered include the concentration of POME used as media, isolation/selection of microalgae strains, reactor/system selection and optimization of growth operating conditions for microalgae biomass to be extracted for lipids as an alternative biofuel resource.

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