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## A Review Study of Biofilm Bacteria and Microalgae Bioremediation for Palm Oil Mill Effluent: Possible Approach

S K Al-Amshawee<sup>1</sup>, M Y Yunus<sup>1,2</sup>, A A Azoddein<sup>1</sup>

<sup>1</sup>Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Gambang, 26300 Gambang, Pahang, Malaysia.

<sup>2</sup>Earth Resource and Sustainability Centre (ERAS), Universiti Malaysia Pahang, Gambang, 26300 Gambang, Pahang, Malaysia.

Corresponding e-mail: yusri@ump.edu.my

Corresponding address: Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Gambang, 26300 Gambang, Pahang, Malaysia.

Abstract. It was known where water is, there is a life, but presently, water is the primary source of diseases, viruses, and microbes. Before the industrial revolution, freshwater was available in vast quantities and everywhere, but the unwell treatments of wastewater have contaminated our fresh water. The palm oil industries discharge palm oil mill effluent (POME) under the forced standards, but it still pollutes the freshwater because it streams contaminated water, and not freshwater. There are many methods for wastewater treatment, but most of it reached its maximum effort, for example, physical technologies probably can give 90% removal of total pollutants with high capital cost. Hence, industries are trying to evolve biological treatments such as microalgae, and biofilm because of being friendly, and cost-efficient. This article reviews microalgae and biofilm bacteria ability for POME processing, and what possible advantages or valuable byproducts can produce. It concluded that uniting both treatments can lead to outstanding performance defeating withdraws and limitations.

#### Introduction 1.

Palm tree requires tropical areas to grow which made Malaysia and Indonesia are appropriate places for palm tree evolution. Its originated from the west of Africa, and it is called Elaeis guineens. Indonesia and Malaysia produce about 85% of the worldwide palm oil production. Over the years, Malaysian production of palm oil had been developing quickly. So, Malaysia is turned into the prime producer and exporter of palm oil and its derivatives. For instance, in the year 1960, palm oil mills were around 10 units, while it had increased to 410 units in the year 2008. Since palm oil industry is agriculture based production, so more than 3.79 million hectares were employed for cultivation purposes which its equal to 11% of the total Malaysian land area, and more than one-third of the total cultivated land area in the year 2003 [1]. The records show that palm oil production in Malaysia was around 16,044,874 and 17,734,441 ton in the year 2009 and 2008, respectively (Malaysian Palm Oil Board 2008, 2009).

Palm oil industries need 5 to 7.5 ton of water to produce one ton of crude palm oil (CPO), associated with 2.5 to 3.5 ton of palm oil mill effluent (POME) generation [2][3]. POME can be defined as a thick brownish viscous liquid having high concentration of chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), and turbidity [4]. In Malaysia, about 44 million ton of POME was produced in the year 2008, while 20 million ton of POME was generated in the year

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1993 [5]. Discharging unwell treated POME to the environment leads to severe aquatic issues because it contains a wide range of pollutants (e.g., 19,020 mg/l of suspended solids (SS), 43,635 mg/l of total solids, 8370 mg/l of oil and grease, 53,630 mg/l of COD, 25,000 mg/l of BOD) [6][7]. Although, daily streaming of effluents which is poorly treated are discharged into rivers and waterways in Malaysia. The Malaysian Department of Environment (DOE) had set a discharging regulation and rules to secure the environment, and the watercourses. The 1977 regulation stated to limit the wastewater discharge to range 100 mg/l of BOD3 concentration, 5 to 9 of pH, 200 mg/l of total nitrogen, 50 mg/l of oil and grease, and 400 mg/l of suspended solids [8][9][10]. Therefore, the treatment system should be designed to meet the streaming standards, and to control the environmental pollution.

There are many kinds of POME treatments, such as an aerated lagoon, anaerobic digester, decanter, and holding system, but ponding system is the most common method for POME treatment [11][12]. On the other hand, the open ponding releases greenhouse gases (e.g., Co<sub>2</sub> and CH<sub>4</sub>) into the atmosphere. Sustainable treatment of POME is achievable by creating an efficient treatment system to guarantee excellent discharge quality of POME and securing waterways from harmful nutrients and heavy metals. Chin et al. (2013) estimated the conceivable electricity generation from biogas generation from POME treatment which can be equal to 3.8 million ringgit per year for milling 60 tons/hr of palm oil [13]. In the year 2011, 92.9 million ton of oil palm got processed which makes Malaysia able to save equal to 671.65 million ringgits yearly. However, this biogas is still harmful because it produces CO<sub>2</sub> once its converted into energy. Finally, attentions have been made to seek and develop a sustainable method like microalgae and biofilm treatment to treat POME achieving zero waste energy [13].

#### 2. Biological Treatments For POME

There are a lot of biological mechanisms and methods to polish POME, but microalgae and biofilm bacteria are novel ways and still in their earlier stages. Besides, POME is a suitable and appropriate medium for the growth of algae and bacteria. Biological treatment occurs in one of the most common POME treatments, known as ponding system. It utilises bacteria to decompose large organic molecules into smaller, while algae perform photosynthesis consuming dissolved  $CO_2$  and producing oxygen under the sunlight availability. It is considered a low-cost operation barely needs management. In contrast, some researchers reported that biological treatment is not entirely efficient because it is slow, and hard to control. In conclusion, biological POME treatments still require a lot of research and development to attain the optimal conditions and production quality [14].

Bacteria in POME has a decent desire to seek surfaces to attach and start their life cycle, and it is called immobilisation or granulation process. Yu, Mu, and Zhang found biofilm bacteria acting fast to form and develop biofilms under aerobic circumstances, but it takes an extended period for granulation process [15][16]. It was occurred based on temperature, pH, nutrients availability, surrounding environment, and biofilm carrier physical and chemical compositions [16].

During 1950's, microalgae were identified as a potential food source for animals and humans [17][18]. Now, microalgae are highly preferred than other wastewater treatments because it can treat high organic load POME and convert the dissolved CO<sub>2</sub> into oxygen. Besides, algae seek a medium like POME having high nutrient content to grow and develop. Biofilm bacteria and microalgae approaches are reviewed in separated subjects in below, to show their details, latest developments and to examine their promising future towards wastewater treatment.

#### 2.1 Biofilm Bacteria

Biofilms are complicated matrix of microorganisms that are assembled in a unique structure with the help of extracellular polymeric substance (EPS). These communities of microorganisms attach to various kinds of surfaces [19][20][21]. The development of biofilms start by single kind or different sorts of microorganisms that have potential to attach and develop on biotic, and abiotic surface [19][22][23]. The biofilm growth can be summarised in few steps, starting with initial attachment, then maturation, and development, and finally detachment from the surface [19][20][24]. Biofilm bacteria produce EPS as an adhesive substance to start building the colonies at the selected surface. Figure 1 shows Biofilm bacteria process.

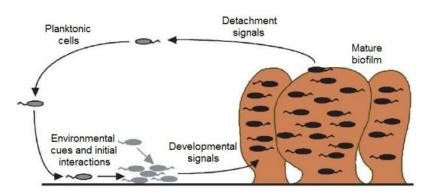


Figure 1. Biofilm bacteria life cycle

Watnick and Kolter (2000) reported that the human community built is like the bacterial biofilm formation [25]. Also, these bacteria have enough potential to attach and get densely on surfaces ranging from plastics to metals [26]. The structural integrity is variant based on many factors, such as temperature, nutrients amount, and physical and physiological circumstances [27]. The complex bacterial communities consist of surrounding immediate detritus, particulate material, metabolites, absorbed nutrients, and polymers, and the bacterial EPS stability is affected by the flow velocity, the shear rate, and the environmental hydrodynamic conditions [28]. Biofilm matrix protects and secures the community from any harmful effect like environmental distress [29]. The negative impact of severe changes in toxic materials, temperature, and pH can be minimised by the bacterial EPS, according to Wingender [30]. Usually, biofilms bacteria require an extended period to complete process POME, but they produce poor amount of biomass.

Biofilm process is accessible by utilizing planktonic microorganisms in a high surface area reactor. They have high chance to adapt and survive even in unfavourable conditions. It receives massive attention compared to chemical and physical treatments because of being efficient and economical [31]. Decho et al. (2000) found the biofilm treatment safe, having good potential, and capable for high organic load [29]. Biodegradation process by biofilm microorganisms means bacterial enzymes decompose various elements and matters, while biosorption refers to converting elements from the liquid phase to solids phase. Also, biofilm bacteria can remove dyes and achieve decolourisation since they possess biosorption and degradation mechanism [25]. Hence, researchers look to develop biofilm treatment to achieve tremendous bioremediation for contaminations removal from wastewater. There are a lot of technologies use biofilm treatment as a significant factor for wastewater treatment. Table 1 Presents various technologies that involve biofilm bacteria in their processing.

In POME treatment, microorganisms utilise nutrients of POME as a source of energy for their growth and development. Also, it decomposes harmful elements (e.g., heavy metals) and compounds [36]. Microorganisms continue to grow and mature the complicated matrix till it reaches its maximum thickness. Then, it can be considered stable, thin, and active to offer numerous benefits to process POME [27]. It is highly recommended to optimise the startup time, and growth speed of biofilm treatment to avoid any required extra maintenance, and biofilm clog [37]. Therefore, biofilm bioremediation process is appropriate method to process POME by removing suspended solids, turbidity, COD, BOD, and nutrients. Biofilm reactors are classified into fixed biofilm bed, and unfixed biofilm bed.

The unfixed biofilm reactor is the most widely used compared to the fixed film bed reactor. Its involved in many technologies such as internal circulation reactors, biofilm airlift suspension (BAS), expanded granular sludge blanket (EGSB), fluidised biofilm bed, and up-flow sludge blanket [38]. The influent rate and active biomass concentration decide the bioremediation performance and effluent quality [39][40]. Also, a successful active control on biofilm thickness might lead to efficient, stable treatment and prevent biofilms from washout [37].

Method	Operation	Capability	Ref.
Integrated anaerobic-aerobic fluidised bed reactor	Comprises from cylindrical fluidised bed and pulverised pumice-stone as carrier for planktic microorganism's attachment and growth	Able to remove nitrogen, and organic carbon.	[32]
Anaerobic-aerobic fixed film bioreactor	Arranged media combined with two fixed film bioreactors	Able to treat high levels of oil and grease	[33]
Anaerobic-aerobic granular biofilm bioreactor	biofilms present mineralisation process for various pollutants inside up-flow anaerobic sludge bed (UASB) reactor	Able to remediate chlorinated contaminates	[34]
Rotating biological contactors	Includes entirely or partially submerged sequential disc configuration, which supports and gives a good chance for biofilm attachment	Able to handle COD range 12,000 mg l <sup>-1</sup>	[24]
Aerobic membrane bioreactor	Biodegradation process occurrs along with membrane filtration which leads to retaining the large molecules in the reactor, while the small gets pass through the membrane layer	Able to process high strength wastewater	[35]

Table 1. Biofilm Technologies for Wastewater Remediation

The fixed biofilm reactor is a very suitable process for a wastewater having extreme contamination (e.g., high biomass concentrations). It involves an immobilised inert medium inside the reactor to increase the chances of biofilm bacteria attachment on the medium surfaces [41].

A researcher reported using two staged anaerobic biofilm treatment involving one methanogenic and one acidogenic reactor for food waste-recycling wastewater. It achieved stable process with COD removal range 73 to 85.9% [42]. Wang et al. (2010) confirmed the functional stability of bioremediation process by using full-scale two-staged wastewater system [43]. Moreover, tt was reported that biofilm treatment could deliver maximum effort under temperature equal to 44.5 °C, and 5.7 of pH.

Westerholm et al. (2010) reported the segregation of one bacterium from numerous microorganisms in the mesophilic methanogenic digester, known as Syntrophaceticus schinkii [44]. It showed the capability to consume lactate, betaine, and ethanol as carbon sources and grows under pH range 6 to 8, and 25 to 40 °C of temperature range. Another bacterium got isolated named as Comamonas sp., and it showed ability to grow on consuming n-butyric, propionic, and acetic acids with exceptional potential to form poly hydroxy alkanoates [45]. The methanogenic population involves 2, 14, 84% of Methanobacterials, Methanosarcinales, and Methanomicrobials, respectively [46]. Chemicals have been found effecting wastewater microbial population, for instance, Methylocystis sp. dominates in high ammonium circumstances, while Methylomicrobium album dominates in high nitrite mediums [47]. Hence, biogas production quantity and quality is based on the dominant producer activity [48]. Zhou et al. (2010) used Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), triphenyl tetrazolium chloride (TTC) dehydrogenase activity, and gas chromatograph (GC) analysis to examine microbial quantity, structure, and activity [49][50]. Other researcher used PCR-DGGE to detect UASB microbial population [58]. On the other hand, there are some limitations have led to restricting biofilm treatment such as clogging, costly liquid distributors, uncontrollable thickness, overgrowth, long startup time, and challenges in handling large amounts of POME having high organic loading rate [38].

#### 2.2 Microalgae

Among numerous types of significant microorganisms, a unique organism has been versatile, and functional to survive and develop under different circumstances known as algae. It exists in marine and aquatic environments. Algae is classified based on the size into macroalgae and microalgae which both are available in POME. Microalgae species have different quantities of protein, starch, and lipid (fat) [51]. They are classified up to their chemical properties of stored products, cell wall ingredients, and pigment sort, such as red algae (Rhodophyta), brown algae (Phaeophyta), golden algae (Chrysophyta),

blue-green algae (cyanobacteria), green algae (Chlorophyta), and Diatoms (Bacillariophyta) [52]. Microalgae possess unique feature known as photosynthesis, which allows algae to grow and develop under sunlight availability. Precisely, microalgae seize light energy for cell generation and producing simple sugar as an energy source by converting inorganic molecules via cell metabolism [53]. Microalgae can deliver various metabolism, like photoheterotrophic, mixotrophic, heterotrophic, and autotrophic [54][55][56]. Previous studies reported that blue-green algae, like Prochloron, is considered cyanobacteria, because it is unlike eukaryotes by having single circular chromosome and lack of membrane-bound organelles [57]. Cyanobacteria and microalgae offer high potential towards nutrients decomposition of wastewater by performing photosynthesis with different approaches [57]. Table 2 presents various strains of microalgae with their characteristics.

In the current wastewater treatments, open ponds are made for microalgae growth. It requires 3 to 4 meters of pond depth to ensure light penetration during the photosynthesis process. Other factors can cause significant impacts on microalgae treatment quality such as thermal stratification, and mechanical mixing. If the apropriate conditions are not made, the photosynthesis would completely shut off.

Other researchers have found microalgae as a source of biofuel and biomass, such as biodiesel, fertilizer, medicines, bioethanol, jet fuel, nutritional compounds, animal feed, food, omega-3 fatty acids, vaccines, pharmaceuticals, recombinant proteins, biodegradable plastics, gasoline, aviation gas, and various pigments [59][60]. Also, algal biofuel has many advantages compared to fossil-based fuel, such as carbon neutral to the environment, non-toxic, and clean burning [61]. Hence, studies had improved biomass and bioenergy generation of wastewater treatment by microalgae. On the other hand, microalgae have drawbacks presented as a costly growth medium, and complicated methods of cultivation. Although, POME can be used as an appropriate growth medium, with no demand for expensive means of cultivation. Also, it has been suggested to use a hybrid system consisting of the open and closed system, which they are an open pond and closed photobioreactor [57][62][63]. Researchers and wastewater industries prefer using an open system because of less supervision requirement, low maintenance, durability, low cost, and simplicity. Raceway ponds with an endless loop, circular ponds, and inclined ponds are different types of algae cultivation ponds which had been structured and functioned on a large scale.

The inclined system had been built in Western Australia for chlorella cultivation. Richmond et al. (1999) reported about the inclined ponds capability to deliver adoption of less than one-centimetre thin culture to operate maximum cell concentration up to 10 gm/l by using gravity and pumping flow to generate high turbulent flow [64].

In Earthrise Farms in California and Hawaii, many companies and researchers applied raceway ponds for cyanobacteria cultivation with capacity 150,000 m<sup>2</sup> and 75,000 m<sup>2</sup>, respectively. Raceway pond is highly proposed because of being capable of substantial scales, easy to maintain, economical, and simple operation [64]. Paddlewheel inside raceway ponds provides force to distribute and keep microalgae suspended in the growth medium.

Agitation occurs in circular ponds by using long rotating mixing arm. Circular ponds are not commercially proposed for being employed at large scales for microalgae growth. There are several limitations, and disadvantages impact raceway ponds efficiency like other microorganism's contaminations, lack of temperature control, and direct exposure to sunlight, while circular ponds face different issues like mixing energy, and high capital cost [51][65]. Despite circular pond's limitations, it is still widely employed to cultivate chlorella for biomass production in Asia, mostly in Taiwan, and Japan [66]. Also, there are some negative impacts of using inclined ponds such as pumping energy demand and cells sedimentation process, and generally, the open pond system causes CO<sub>2</sub> emission, contaminations in the microalgae cultivation process. The tubular reactor, the vertical photobioreactor, and the flat plate reactor are examples of the advancements on the closed photobioreactor design [67][68][69][70][71]. The closed system had overcome open ponds disadvantages and can deliver contamination removal, controllable temperature, high productivity, high light utilisation, clean microalgae culture, but its costly [72].

Group	Strain	Habitat	Lipid content	Lipid productivity	Biomass productivity
			(%	(mg/L/day)	(g/L/day)
			biomass)	(iiig, <u>_</u> , auj)	(8, 2, 00))
Red algae	Porphyridiumcruentum	Marine	9.5	34.8	0.37
Prymnesiophytes	Pavlovasalina CS 49	Marine	30.9	49.4	0.16
	Pavlovalutheri CS 182	Marine	35.5	50.2	0.14
Eustigmatophytes	Nannochloropsis sp. F&M-M26	Marine	29.6	61.0	0.21
	Nannochloropsis sp. F&M-M27	Marine	24.4	48.2	0.20
	Nannochloropsis sp. F&M-M24	Marine	30.9	54.8	0.18
	Nannochloropsis sp. F&M-M29	Marine	21.6	37.6	0.17
	Nannochloropsis sp. F&M-M28	Marine	35.7	60.9	0.17
	Isochrysissp(T-ISO) CS 177	Marine	22.4	37.7	0.17
	Isochrysissp F&M-M37	Marine	27.4	37.8	0.14
Green algae	Chlorococcum sp. UMACC 112	Freshwater	19.3	53.7	0.28
U	Scenedemusquadricauda	Freshwater	18.4	35.1	0.19
	Scenedemus F&M-M19	Freshwater	19.6	40.8	0.21
	Scenedemus sp.DM	Freshwater	21.1	53.9	0.26
	T.suecica F&M-M33	Marine	8.5	27.0	0.32
	Tetraselmis sp. F&M-M34	Marine	14.7	43.4	0.30
	T.suecica F&M-M35	Marine	12.9	36.4	0.28
	Ellipsoidion sp. F&M-M31	Marine	27.4	47.3	0.17
	Monodussubterraneus UTEX 151	Freshwater	16.1	30.4	0.19
	Nannochloropsis sp. CS 246	Marine	29.2	49.7	0.17
Diatoms	Chaetocerosmuelleri F&M-M43	Marine	33.6	21.8	0.07
	Chaetoceroscalcitrans CS 178	Marine	39.8	17.6	0.04
	P. tricornutum F&M M40	Marine	18.7	44.8	0.24
	Skeletonomacostatum CS 181	Marine	21.0	17.4	0.08
	Skeletonoma sp. CS 252	Marine	31.8	27.3	0.09
	Thalassioriapseudonana CS 173	Marine	20.6	17.4	0.08
	Chlorella sp. F&M-M48	Freshwater	18.7	42.1	0.23
	Chlorella sorokiniana IAM-212	Freshwater	19.3	44.7	0.23
	<i>Chlorella vulgaris</i> CCAP 211/11b	Freshwater	19.2	32.6	0.17
	Chlorella vulgaris F&M-M49	Freshwater	18.4	36.9	0.20

Table 2. Microalgal strains with their content and productivity of lipid, and biomass productivity [58]

The algal potential for wastewater remediation was discovered and introduced in 1940's by Caldwell [73][74][75]. After 10 years, it has been utilised to treat wastewater and produce biomass. Since then, researchers have examined algal treatment for various kinds of wastewater, such as soybean processing [76][77], piggery [78], textile [79], steel plant [80], and municipal [71]. The goal of making examinations and studies on microalgae treatment is to reach a sustainable treatment with no harm, or pollution effect on the waterways and the microalgae treatment is a promising method and eco-friendly. It was suggested to use microalgae as a tertiary stage to treat domestic and municipal wastewater removing nutrients (e.g., phosphorus and nitrogen) [81]. Also, various researches had declared microalgae potential towards nutrients removal which leads to a reduction in COD, and BOD concentrations [82]. A significant removal in BOD and COD had been showed on olive farming, piggery, and textile wastewater by using microalgae application in laboratory scale [83]. A previous study reported that employing chlorella vulgaris microalgae can achieve 50% reduction in COD concentration. Another researcher utilised Scenedesmus obliquus microalgae to polish urban wastewater. It achieved 97, and 100% removal of phosphorus, and ammonium concentrations, respectively [84].

Method	Wastewater	Characteristics (All in mg/l, except pH)	Results (All in mg/l, except pH)	Ref.
Biofilm airlift loop bioreactor (ALR)	high ammonia wastewater	pH 7.8, COD 655, BOD 312, NH4 <sup>+</sup> -N 365, total nitrogen (TN) 395, SS 122	pH 7.5, COD 73.1, BOD 20, NH4 <sup>+</sup> -N 4.5, TN 183, SS 26	[95]
Moving bed biofilm reactor (MBBR)	coking wastewater	2020±5 of COD	COD removal at carrier filling ratio are 357±4 at 20%, 287±4 at 30%, 229±3 at 40%, 210±3 at 50%, 230±4 at 60%	[96]
Anaerobic biofilm reactor (Up-flow)	Whey wastewater	-	COD removal is 91% by using Polyethylene/clay packing media	[97]
Anaerobic biofilm reactor (Up-flow)	Synthetic dairy wastewater	-	COD removal is 97.9–98.8% by using PVC rings	[97]
Fluidized bed membrane bio electrochemical reactor	cheese factory wastewater	-	87.1% of COD removal at 24 h of HRT, and 56% of COD reduction at 12 h of HRT	[98]
Anaerobic granular sludge (AGS)	Municipal wastewater	COD 722, BOD 386, SS 317, NH <sub>3</sub> -N 35	BOD 15, SS 10, NH <sub>3</sub> -N 2	[99]
UASB	Natural rubber processing wastewater	-	Total removal efficiency (%) is about $55.6\pm16.6$ for COD and $77.8\pm10.3$ for BOD	[100]
Anaerobic baffled reactor (ABR) – Algal Tank	Natural rubber processing wastewater	5.5 of pH, 3,700 of total chemical oxygen demand (TCOD), 3,450 of total biochemical oxygen demand (TBOD), 200 of TSS, 220 of TN, 108 of ammonia	8.1 of pH, 222 of TCOD, 92 of TBOD, 126 of TSS, 97 of TN, 77 of ammonia	[100]
Hybrid membrane- aerated biofilm reactor (MABR)	Oil-field wastewater	480 of COD, 22.4 of oil content, 5.3 of NH4 <sup>+</sup> -N, 31 of TN	85 of COD, 3.2 of Oil content, 3.6 of NH4 <sup>+</sup> -N, 8.7 of TN	[101]
Anoxic moving bed biofilm reactor (ANMBBR)	Coal gasification wastewater	COD 138.0, total organic carbon (TOC) 60.0, NO <sup>-</sup> 2-N 0.8, NH4 <sup>+-</sup> N 32.0, TN 49.0, total phosphorus (TP) 30.0, BOD5 5.5	COD 52.1, TOC 27.2, NO <sup>-</sup> 2-N 0.6, NH4 <sup>+</sup> -N 15.4, TN 15, TP 9.1, BOD5 17.7	[102]
Chlorella vulgaris + activated sludge	Synthetic Wastewater	-	83.6% COD, 89.4% Nitrogen, 91.4% Phosphorus	[103] [104]
Chlorella. sorokiniana + aerobic sludge	Swine wastewaters	-	62.3% COD, 82.7% Nitrogen, 58.0% Phosphorus	[103] [105]
Scenedesmus sp. ZTY2	Domestic wastewater	Dissolved organic carbon (DOC) 42.7 ± 0.60, COD 142.0 ± 0.00, TN 27.7 ± 0.11, TP 1.59 ± 0.03	DOC (%) 63.4±0.02, COD (%) 40.8±0.05, TN (%) 14.0±0.01, TP (%) 35.4 ± 0.04	[106]
Scenedesmus sp. ZTY3	Domestic wastewater	DOC 42.7 $\pm$ 0.60, COD 142.0 $\pm$ 0.00, TN 27.7 $\pm$ 0.11, TP 1.59 $\pm$ 0.03	DOC (%) 52.9 ± 0.07, COD (%) 39.4±0.04, TN (%) 9.8±0.01, TP (%) 32.7±0.06	[106]
Chlorella sp. ZTY4	Domestic wastewater	DOC 42.7 ± 0.60, COD 142.0 ± 0.00, TN 27.7 ± 0.11, TP 1.59 ± 0.03	DOC (%) 64.4±0.01, COD (%) 40.8±0.02, TN (%) 15.8±0.01, TP (%) 49.9±0.05	[106]
Pilot high-rate algal ponds (HRAPs)	Urban Wastewater	T (°C) 16±1, DO 7±2, pH 8±1, COD 342±107, NH4N 19±4	T (°C) 13±1, DO 12±2, pH 9±1, COD 52±7 (88%), NH4-N 0.4±0.1 (98%), TSS 149±15, Biomass production 6±0.3	[107]

Table 3. Latest empirical works on wastewater bioremediation by bacteria and microalgae

Microalgae use POME as a source of food to generate more cells because its full of nutrients. Hence, a significant reduction is approachable in COD, and BOD concentrations. For instance, K, Ca, Mg, P, Zn, and Fe are essential elements for microalgae growth, and entirely are available in POME. Some

researchers reported that COD, phosphorus, and nitrogen removal is achievable for POME by utilising Spirulina plantesis microalgae and other commercial strains [85][86]. CO<sub>2</sub> supply is vital to optimise microalgae growth rate, but most of the sparged CO<sub>2</sub> in POME escape with the bubbles into the atmosphere. Inorganic carbon source like CO<sub>2</sub> and organic carbon sources like fructose, acetate, and glucose are consumed by microalgae to generate and produce lipid [87]. Also, ammonium concentration has a negative impact on POME quality, but it is highly demanded by microalgae because it is considered the favoured sort of nitrogen source [88]. On the other hand, high range of ammonium concentration can prevent the active growth of microalgae, for instance, Spirulina platensis microalgae can grow and develop within 100 ppm (5.65  $\mu$ mol L<sup>-1</sup>) of ammonium level. Unfortunately, POME ammonium concentration is ranging 4 to 80 mg L<sup>-1</sup> (222.22 to 4,444.44  $\mu$ mol L<sup>-1</sup>), which means it is not a fanciful medium for microalgae growth [89]. Luckily, there are some methods can be used to decline ammonium concentration like diluting POME with another kind of wastewater, or increasing temperature and pH, which leads to ammonium volatilizing into the atmosphere [57][88].

Determining microalgae strain for POME treatment is a difficult selection because each species has different characteristics and tolerances to culture conditions (e.g., CO<sub>2</sub> range, ammonium concentration, light tense). A researcher achieved 67.35% of COD reduction by employing Chlorella incerta for POME treatment in 28 days. Another researcher found that Chlamydomonas microalgae are the best species for POME remediation because of its fast growth. However, some other studies suggest cyanobacteria because its inherent with high effective potential. A researcher studied Chlorella sp. growth in POME medium. POME having 6.8 to 7.2 of pH level, under 3000 to 6000 lux of fluorescence lamp, and 1.0 g/l of urea was employed for Chlorella sp. microalgae growth. The cultivation process delivered 0.066 per day of Chlorella sp. specific growth rate for 15 days. Meng et al. (2014) suggested using Green macroalgae Cladophora species for wastewater dyes degradation and biosorption process [90][91][92].

The biotreatment for POME can generate biomass, which can be utilised for commercial products, like biodiesel and biofertilizer [93]. Also, biomass production of POME treatment proves waste to wealth system is quite approachable by microalgae treatment. It can provide a vast revenue and welfares not just financially but also eco-friendly. Microalgae is highly capable of producing around 5,000 to 15,000 gallons per acre/year of biodiesel. Also, microalgae performance of oil production is 30 times more than terrestrial oilseed crops capability. Thus, it seems that microalgae are the tremendous option to replace fossil-based diesel production [53][94]. After reviewing microalgae and biofilm treatments, different findings are listed in Table 3 with details to show the real ability of biofilm bacteria and microalgae towards wastewater treatment

#### 3. Conclusion and Future studies

It is quite hard to prefer or compare between biofilm bacteria and microalgae because both have different lifecycle, and process. However, both treatments are promising methods towards sustainability and clean energy. However, the algal and bacterial treatments are in their earlier stages and researchers found limitations need to be solved. It is expected that fossil-based fuel will be replaced with biofuel. Thus, biotreatment will be a significant option for wastewater treatment to produce biomass and biofuel.

This review found using a hybrid system in treating wastewater is very efficient, easy to operate, high quality, and able to defeat numerous limitations of microalgae and biofilm treatments. Hence, microalgae are highly feasible and convenient from being integrated with biofilm bacteria inside one reactor. Although, it requires studies to find cultivation temperature, overgrowth, oxygen depletion, light tense, CO2 concentration, and sludge production. The facultative pond is an example of integrating microalgae with biofilm bacteria for wastewater bioremediation, where microalgae produce oxygen as by-product and consuming CO2, while biofilm bacteria generate CO2, and gather oxygen for the cell generation, growth, and development.

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