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Microalgae cultivation in palm oil mill effluent (POME) for lipid production and pollutants removal

Wai Yan Cheah[a,b], Pau Loke Show[c,*], Joon Ching Juan[d,e], Jo-Shu Chang[f,g], Tau Chuan Ling[a,*]

[a] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] Department of Environmental Health, Faculty of Health & Sport Sciences, MAHSA University, 42610 Jenjarom, Selangor, Malaysia
[c] Bioseparation Research Group, Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia
[d] Nanotechnology & Catalysis Research Centre (NANOCAT), University of Malaya, 50603 Kuala Lumpur, Malaysia
[e] School of Science, Monash University, Sunway Campus, 46150 Sunway, Malaysia
[f] Department of Chemical Engineering, National Cheng Kung University, Tainan 701, Taiwan
[g] Research Center for Energy Technology and Strategy, National Cheng Kung University, Tainan 701, Taiwan

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ABSTRACT

Microalgae cultivation in wastewaters has been identified as the solution for economical microalgae cultivation. This study investigated the feasibility of using POME for *Chlorella* sp. cultivation to yield biomass and lipids, as it generates massively in Malaysia which ranked world palm oil exporter. The optimal POME concentration and pretreatment strategy were applied to promote biomass and lipid productivities. *Chlorella sorokiniana* CY-1 attained maximal of 11.21% of lipid content with 2.12 g L^{-1} of biomass concentration when cultivation in acid-heat pretreated 30% (v/v) POME. This provides relatively higher yield than those reported values on POME. Pretreatment was found effective to enhance biomass productions, as it converts lignin in POME into reducing sugars to serve as the supplement. The pollutants removal efficiencies were 62.07% for TN, 47.09% for COD, and 30.77% for TP. This contributes towards greater feasibility in microalgae cultivation for biofuel productions and as well towards environmental sustainability.

1. Introduction

The atmospheric carbon dioxide (CO_2) concentration has risen 32% since industrial revolution [1]. This environmental damage has resulted tremendous effort exerted on finding the alternatives to substitute fossil fuels consumption [2,3]. Microalga is well-known as potential feedstock to produce biomass-based renewable energy, offering profitable bioethanol, biogas and bioproducts productions [4–6]. *Scenedesmus obliquus*, *Botryococcus braunii, Nannochloropsis* sp. and *Chlorella* sp. are well-known species for biofuel productions [2,7]. The lipid content of microalgae species can reach up to 80% per cell weight [8]. Till date, the major challenge impeded in microalgae-based biodiesel commercialisation would be the cost, which could be partially resolved by cultivating microalgae in wastewater. For instances, *Botryococcus braunii* LEM 14 yielded 36% lipid content and removed 80% nitrogen and 100% phosphorus, while grown in domestic wastewater [9]. Less freshwater would be consumed and cost reduction is thus possible to eliminate nutrients cost as microalgae assimilating pollutants from the wastewater.

Malaysia, being the world major exporter of palm oil, generates massive amount of Palm oil mill effluent (POME) daily [10]. The typical POME treatment process applied now is conventional treatment like open pond. *Characium* sp. shown removal of 21.5%, 80.0%, and 89.9% of chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP), respectively from POME [11]. This provides the ideal solution towards cost-effective commercial scale microalgae cultivation and wastewater nutrients remediation. Municipal, aquaculture and industrial wastewaters have been used for microalgae cultivation, but POME containing high amount of nutrients is also viable to serve this purpose [12–15]. To our best knowledge, there is only little study specifically on using POME for microalgae cultivation for lipid production and simultaneously nutrient remediation. Raw POME contains high organics and turbidity which hinder light penetration, and posing inhibitory effects on microalgae growth [16]. Mixotrophic cultivation can provide solution towards light penetration constraint. Higher growth and lipid productivity were attained on *Chlorella* sp. grown in 20% POME with urea and triple sugar phosphate additions [17]. *Chlorella vulgaris* exhibited higher lipid productivity, with additions of

* Corresponding author.
E-mail addresses: PauLoke.Show@nottingham.edu.my (P.L. Show), tcling@um.edu.my (T.C. Ling).

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glucose and glycerol [18]. These researches reported on optimisation of microalgae growth using supplements, however the study on POME pretreatment for microalgae cultivation is still lacking till date. If nutrients addition is encouraged, the original purpose to utilise POME for microalgae growth for cost reduction would be defeated. Also, the pretreatment is vital to be studied so as to simulate the condition in wastewater treatment plant. The appropriate primary or secondary treatment and the optimised POME parameters could be identified before incorporating microalgae cultivation in tertiary treatment.

The principal aims of the present work here were (i) to investigate the effectiveness of *Chlorella vulgaris* ESP-31 and *Chlorella sorokiniana* CY-1 to grow in POME for biomass and lipid (ii) to determine the optimal POME concentration for best yields (iii) to determine the effects of pretreatment strategy on biomass and lipid yields (iv) to examine the nutrients bioremediation. This study serves as a platform for those who are trying to cultivate microalgae in commercial scale as this contributes towards economical microalgae cultivation and POME bioremediation.

2. Materials and methods

2.1. Wastewater - palm oil mill effluent (POME)

POME was obtained from Seri Ulu Langat Palm Oil Mill, located in Peninsular Malaysia. POME was filtered using 0.45 μm pore size membrane to eliminate suspended solids. Growth medium BG11 was diluted using POME to prepare POME-BG11 medium with POME spike ratio of 5%, 10% and 20% (v/v). Some studies shown dilution of wastewaters with distilled water without sterilisation to avoid additional cost imposed [19]. Yet, the aim of this study also include investigation on the potential of high oil-yielding *Chlorella* sp. for higher biomass and lipids yields. Therefore, BG11 was diluted using POME. The pH of culture medium was set at about 7.5, adjusted using NaOH [5]. The microalga was then further cultivated in higher concentrations of spike ratios POME. The wastewater characteristics were determined according to the American Public Health Association on Standard Methods for water and wastewater study [20]. The analysis of COD, TN and TP of wastewater were carried out via spectrophotometer model DR 2800 and Hach standard reagent.

2.2. The selection of microalgae strains and the medium compositions

Two microalgae species namely *Chlorella vulgaris* ESP-31 and *Chlorella sorokiniana* CY-1, were used in this study. *Chlorella* sp. is commonly inhabited and distributed, making it becoming the typical model algae for research [21]. Initially, microalgae preculture was carried out for five days, using BG11 medium, and supply of 2.5% CO_2 constantly into the medium. The compositions of BG11 medium was shown in Table 1. The pre-cultured microalga was then being added into the medium with inoculum size of about 0.1 g L^{-1}.

2.3. Microalgal cultivation conditions

Microalgae species were cultivated using 1 L glass type vessel flask. The microalgal culture was irradiated, at all time, using 220–240 V fluorescent lamps mounted on both sides of flask. The light intensity was maintained at 8000 lx, measured at the flask wall. The culture worked at temperature 25 °C with 300 rpm agitation continuously. The CO_2 concentration supplied was 2.5% mixture with atmospheric air in 0.1 vvm CO_2 aeration. Gas was filtered using 0.22 μm pore size filter prior to gas supply into the medium. The liquid samples were collected daily, for analysis of biomass concentration and residual nitrogen source concentration; whereas sampling at set interval for lipid content determination.

Table 1
Compositions of BG11 medium.

Components	Concentrations (g L^{-1})
$NaNO_3$	1.5
K_2HPO_4	0.04
$MgSO_4{\cdot}7H_2O$	0.075
$CaCl_2{\cdot}2H_2O$	0.036
Citric acid	0.006
Na_2CO_3	0.02
Ammonium ferric citrate	0.006
EDTA 2Na	0.001
H_3BO_3	0.00286
$ZnSO_4{\cdot}7H_2O$	0.00022
$MnCl_2{\cdot}4H_2O$	0.00181
$Na_2MoO_4{\cdot}2H_2O$	0.00039
$CuSO_4{\cdot}5H_2O$	0.000079
$Co(NO_3)_2{\cdot}6H_2O$	0.000049

2.4. Biomass concentration determination

The biomass concentration was measured using spectrophotometer, at optical density of 680 nm. The microalgae biomass dry cell weight was determined by filtering 5 ml aliquots of culture using cellulose acetate membrane filter (0.45 mm pore size, 25 mm in diameter). The filter was then dried at 105 °C. The dry weight of blank filter was subtracted from the loaded and dried filter, so as to determine the microalgae dry cell weight. The biomass concentration can be calculated from OD_{680} readings, via calibration between OD_{680} and dry cell weight data. (1 OD_{680nm} is equivalent to 0.19 g L^{-1} DCW).

2.5. Residual nitrate concentration determination

Nitrate concentration was obtained in accordance to the method reported by Cataldo et al. [22]. Calibration of $NaNO_3$ was conducted using range of 50–250 mg L^{-1} with $R^2 = 0.9990$. The correlation obtained was $y = 0.0224 + 0.008x$. The y and x represent absorbance at 410 nm and $NaNO_3$ concentration, respectively.

2.6. Oil/lipids content determination

The lipid content of microalgae sp. was obtained as fatty acid methyl esters (FAMEs) after the *in-situ* transesterification process [23]. The samples were tested by gas chromatography (GC-2014, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID). Samples were injected into a 30 m long capillary column (Type no. 260M143P, Thermo Fisher Scientific, Waltham, MA, USA) with an internal diameter of 0.32 mm. The carrier gas used was Helium, with a flow rate of 1.3 ml/min. The temperature of injector was set at 250 °C, whereas the detector was at 280 °C. Initially, the oven temperature was set at 110 °C, subsequently increased from 150 to 180 °C at a rate of 10 °C/min, 180 to 220 °C at a rate of 1.5 °C/min, 220 to 260 °C at a rate of 30 °C/min and lastly held at 260 °C for 5 min.

2.7. Pretreatment study

Acid pretreatment was carried out on the raw POME. The POME was added with concentrated 1 M H_2SO_4 reaching pH 1–2 and stirred under constant speed for 30 min [24]. The medium was subsequently autoclaved as to provide thermal pretreatment and sterilisation [25,26]. Then, the same procedure as described in Section 2.1 was carried out prior to cultivation. The reducing sugar was determined using phenol-sulphuric acid assays developed by Dubois et al. [27].

Table 2
Characteristics of POME used in the study and the regulatory discharged standards.

Parameter	Concentration	Regulatory discharge standards[a]
pH	4.3	5.0–9.0
COD (mg L^{-1})	27,700	–
NH_3-N (mg L^{-1})	172	150
TN (mg L^{-1})	1100	200
TP (mg L^{-1})	180	–

[a] Environmental Quality (Prescribed Premises) (Crude Palm Oil) Regulations 1977.

3. Results and discussion

3.1. Effectiveness of using POME as medium for microalgae cultivation

Table 2 shows the pollutants concentrations present in POME together with the regulatory discharge standards. Several parameters were determined namely pH, COD, TN and TP. The results were shown non-compliance to the POME discharged limits. The POME has high concentration of nitrogen and phosphorus sources which can serve as the important nutrients required for microalgae growth [28]. POME treatment is in necessity before discharge and this could be achieved by microalgae cultivation.

Several POME concentrations were used for microalgae growth and lipid production. The spike ratios of POME used were 5%, 10% and 20% (v/v), to cultivate *Chlorella vulgaris* ESP-31 and *Chlorella sorokiniana* CY-1. Fig. 1(a) and (b) showed the time course growth profile of *Chlorella vulgaris* ESP-31 and *Chlorella sorokiniana* CY-1 grown in POME at three concentration levels. Under varied POME spike ratio, 5% and 10% (v/v) POME have shown maximal biomass concentration for ESP-31 (3.46 g L^{-1}) and CY-1 (3.30 g L^{-1}), respectively. ESP-31 has shown slight difference only in terms of biomass concentration under zero spike ratio and 5% (v/v) spike ratio. However, high concentration of POME did not support microalgae growth. The biomass concentrations for ESP-31 was only reaching maximal of 1.16 g L^{-1} throughout the 15th days of culture, under spike ratio of 20% (v/v) POME. CY-1 has also revealed maximal growth when cultivated under 10% (v/v) of POME which was higher than solely cultivated in BG11 medium. Growth of both species were inhibited in high POME spike ratios medium due to the characteristics of POME which is in black liquor form. There is shading effect exerted as light penetration though medium is blocked. This is the same as reported by Nur [29], whereby high POME concentration inhibit growth rate and take longer time in lag phase for adaptation due to limited light penetration. Longer lag phase may results longer exponential and stationary phase, thus lowering the overall cell growth. Despite this, to compare between both species, the results shown that CY-1 exhibited higher tolerance level towards high spike ratio of POME as ESP-31 shown poor growth in 20% (v/v) POME (1.16 g L^{-1}) as compared to CY-1 (2.52 g L^{-1}). This means that CY-1 could exhibit higher feasibility to grow in higher POME concentration as compared to ESP-31, together with the better tolerance level shown in CY-1. The same as reported by Putri et al. [18] who worked on five microalgae strains namely *Chlorella vulgaris*, *Chlorella sorokiniana*, *Chlorella pyrenoidosa*, *Botryococcus sudeticus* and *tetraselmis* sp., were grown in 250 mg L^{-1} POME with BBM medium; and *Chlorella sorokiniana* outcompeted the other species on its biomass growth rate and productivity.

The effectiveness of using POME for microalgae cultivation was not only determined by biomass concentration but also the lipid content. Fig. 2(a) and (b) shown the lipid content of *Chlorella vulgaris* ESP-31 and *Chlorella sorokiniana* CY-1 obtained from cultivation at varied concentration levels. As shown in Fig. 2(a) and (b), higher lipid contents were obtained for both strains cultivated under 5% (v/v) POME when compared to 10% (v/v) POME. The highest lipid content for ESP-31 and

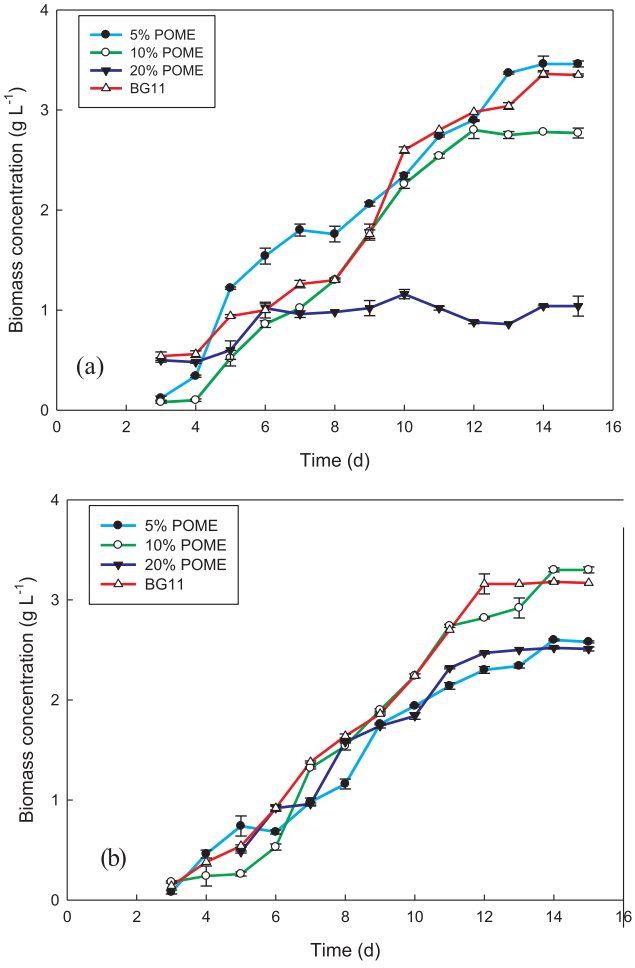

Fig. 1. Growth curves of *Chlorella vulgaris* ESP-31 (a) and *Chlorella sorokiniana* CY-1 (b) grown in POME at different concentration levels.

CY-1 were 17.5% (on 14th days) and 20.9% (on 12th days), respectively. ESP-31 though exhibited higher biomass production than CY-1, yet the maximal lipid content attained was lesser than the latter. CY-1 showed higher lipid content than ESP-31 for every sample taken. This phenomenon is similar as reported in literature, whereby high biomass growth did not translate to high lipid accumulation as energy is diverted for cell growth rather than lipid accumulation [30]. CY-1 in 10% (v/v) POME has exhibited maximal biomass concentration which was 3.30 g L^{-1} with the lipid content accumulated was 20.3%. This lipid content attained was 0.6% lower than cultivated in 5% (v/v) POME with maximal biomass concentration of 2.60 g L^{-1}. The little difference in lipid content and significant difference in biomass concentration were observed between 5% and 10% (v/v) of POME. High spike ratio encourages better feasibility and more economical for commercial scale microalgae cultivation in POME. Therefore, after the thorough comparison between the microalgae strains, *Chlorella sorokiniana* CY-1 was selected for the subsequent experiment.

3.2. Effectiveness of Chlorella sorokiniana CY-1 grown in varied POME concentrations

The enhancement on higher spike ratio of POME is desirable so as to improve the feasibility of using POME as culture medium. The growth of CY-1 was investigated at spike ratios of 20%, 30%, 50% and 75% (v/v) for its biomass and lipid production. As shown in Fig. 3, the maximal biomass production was achieved on 15th days of cultivation, which was 2.02 g L^{-1} and 1.68 g L^{-1} in 20% and 30% (v/v) POME, respectively. The maximal biomass productivities for 20% and 30% (v/v)

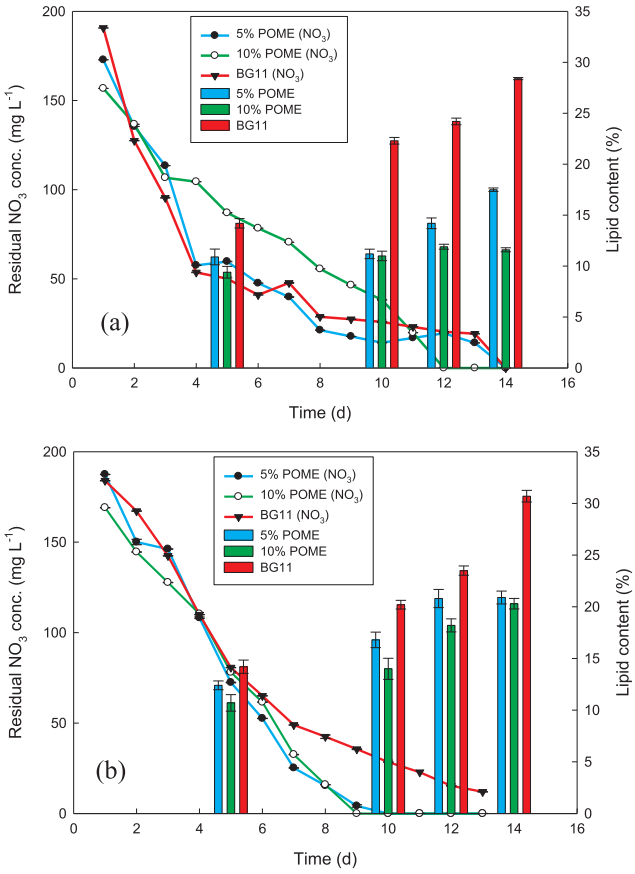

Fig. 2. The lipid content versus time of *Chlorella vulgaris* ESP-31 (a) and *Chlorella sorokiniana* CY-1 (b) obtained from cultivation at varied concentration levels (bar) with the residual NO_3 concentration (line).

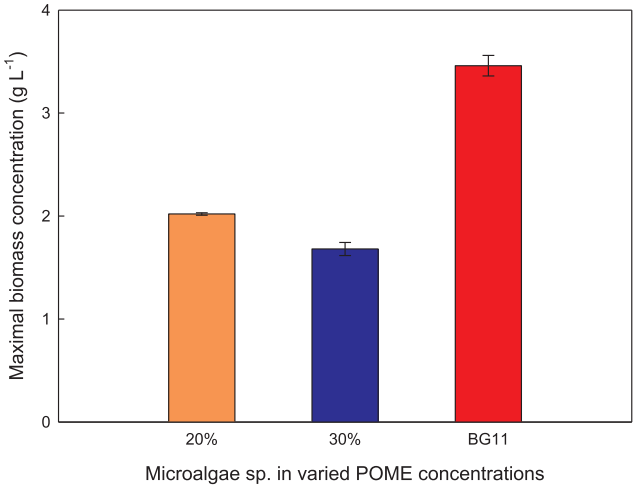

Fig. 3. The maximal biomass concentration of *Chlorella sorokiniana* CY-1 grown at varied POME concentrations.

were 0. 15 $g\,L^{-1}\,d^{-1}$ and 0.11 $g\,L^{-1}\,d^{-1}$, respectively. The maximal biomass productions of CY-1 cultivated in 20% and 30% (v/v) POME have brought considerably significant amount of biomass production, but the spike ratio of higher than 30% (v/v) POME was unable to support microalgae growth (data not shown). The biomass concentration and biomass productivities would decrease along with increase in POME concentration. This condition is similar as reported by Kamyab et al. [28], whereby *Chlamydomonas incerta* as well shown highest growth in the absence of POME, and reacted slow growth along with increase in POME concentration. CY-1 could be considered as compelling species, eliminating the natural substances available in POME at higher spike ratios. Organics naturally present in POME is difficult to be decomposed under natural conditions. Vairappan and Yen [31] revealed that the best concentration to cultivate *Isochyrisis* sp. is only 5% (v/v) POME due to the polluting characteristics of POME. Dilution is thus required to evaluate the feasibility of microalgae cultivation in POME, and optimization strategy to be carried out in the subsequent stage. The feasibility of using POME for cost effective and large scale microalgae cultivation would only be achieved if higher concentration of POME used as cultivation medium. Thus, 30% (v/v) POME was the concentration chosen for the subsequent pretreatment study.

3.3. Effects of POME pretreatment for Chlorella sorokiniana CY-1 cultivation

Palm oil mill generate massive amount of POME after the process of milling, fresh fruits bunch sterilisation and after the digestion process [10]. There is composition of lignin present in POME, originated from plant component which results to its dark color. Black liquor composition consists of dissolved lignin products along with cellulosic, hemicellulosic hexose and pentose [32]. Kamal et al. [24] pretreated POME before using it as medium for biohydrogen production by *Clostridium butyricum*. The hydrogen yield was found increased as carbohydrate contents in POME have been broken up by using alkaline-heat pretreatment into simple sugars prior fermentation. Organic solvent is the powerful agent used for pretreatment. Lignin can be broken down into hemicellulose, cellulose and glucose after solvent pretreatment either by using alcohol, acid, alkali or enzyme [33,34]. Lignin-containing black liquor is hydrophilic and very soluble in strong acidic solution like pH 2 [35]. Sari et al. [36] documented that acidification at pH 2, 70 °C is effective to remove black liquor from paper making industry.

POME pretreatment strategy was therefore applied using the concept of acidification to produce reducing sugars prior to investigate its effects towards CY-1 biomass and lipid productions. The method as described in Section 2.7 was employed to quantify simple sugars concentration. The correlation obtained was $y = 0.0662x - 0.1046$; $R^2 = 0.9996$. The y and x represent absorbance at 485 nm and reducing sugar concentration, respectively. This method used was similar as reported by Saha et al. [37], in the study of pretreatment of pulp mill waste sludge to produce soluble sugars. Evstigneev [38] studied the effect of temperature on lignin solubility in aqueous alkali solution, claimed that lignin fully dissolved in 100 °C. Pretreatment in the study is considered as acid-heat pretreatment, as autoclaving involves temperature of 121 °C [25,26]. The reducing sugars concentration of pretreated POME showed higher than the non-pretreated POME by 3.06% ($P = 0.045$ which was $P < 0.05$). The fact that lignin degradation is supported here with the increase in reducing sugar concentration.

As shown in Fig. 4(a) and (b), it was interesting to note that there was increase in biomass concentration and productivity of CY-1 when it was grown in pretreated POME. The maximal biomass concentration and productivity of pretreated POME were 0.26 $g\,L^{-1}$ and 0.03 $g\,L^{-1}\,d^{-1}$ higher than the non-pretreated POME, respectively. This has revealed that POME pretreatment is contributing to enhance microalgae growth as it brought more sugars into the medium. Reducing sugars uptake were observed in Fig. 4(c). The lipid content versus time of CY-1 cultivated in pretreated and non-pretreated 30% (v/v) POME were as well shown in Fig. 4(c). The effectiveness of acid-heat pretreatment also justified based on lipid production. The maximal lipid content obtained from CY-1 grown in pretreated POME was 11.21%, which was higher than non-pretreated POME by 1.24%. We hypothesised that higher lipid contents are influenced by cultivation in pretreated growth medium. ANOVA analysis has shown that microalgae lipid contents were significantly influenced by pretreatment ($p < 0.05$). Glucose has been widely used as optimization strategy to

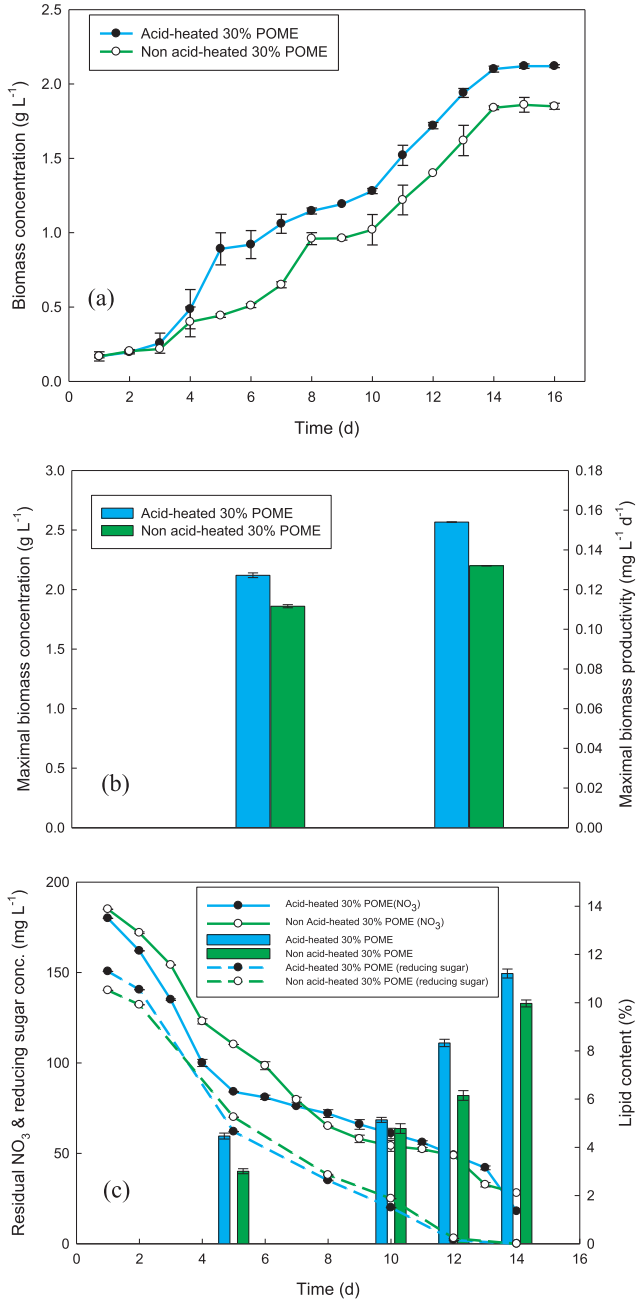

Fig. 4. Time course profile of the biomass concentration (a); maximal biomass concentration (left bar) and productivities (right bar) (b); and the lipid content versus time of *Chlorella sorokiniana* CY-1 cultivated in pretreated and non-pretreated 30% (v/v) POME with the residual NO_3 and reducing sugar concentrations (c).

improve microalgae biomass and lipid productions. Optimisation using pre-treatment can also be applied rather than supplement additions. This could be practical for large scale cultivation as microalgae cultivation usually take place in tertiary treatment. Primary treatment in wastewater treatment plant typically equipped with neutralisation will ease POME acidification. Therefore, acid-heat pretreatment is workable in treatment plant in addition to the high temperature and highly acidic of the generated POME.

The comparative studies on microalgae cultivated in POME were summarised in Table 3. Overall, our CY-1 cultivation in POME has attained the best performance in terms of biomass growth if were to compare among all studies. When referring to Table 3, *Botryococcus sudeticus* and *Chlorella* sp. were cultivated in 20–60% (v/v) diluted POME exhibited only about 0.5–1.5 g L^{-1} biomass growth, with the additions of synthetic nutrients, glucose, urea and glycerol [17,29,39]. Our findings in both acid-heat pretreated and non-pretreated 30% (v/v) POME achieved better maximal biomass growth, without any addition of supplement. Ponraj and Din [40] have reported high biomass concentration up to 39.41 g L^{-1} due to the difference in PBR system, which was programmable controlled reactor tank, applied for cultivation. In view of lipid content, some studies found that the lipid contents of *Chlorella* sp. were less than 10%, though they were cultivated in diluted POME added with supplements [17,29]. Our findings attained were higher in lipid content in both pretreated and non-pretreated POME without supplement addition. Putri et al. [18] reported higher lipid content of *Botryococcus sudeticus* and *Chlorella* sp. with up to 30%. It should be noted that there were different methods applied, whereby lipid extraction used was Bligh and Dyer method followed by gravimetric measurement on extracted lipids, whereas we applied transesterification with lipid/FAME quantification.

The microalgal-based lipid was converted to FAME through transesterification, subsequently the FAME compositions were quantified using gas chromatography. The FAME profiling of CY-1 cultivated in pretreated and non-pretreated 30% (v/v) POME were summarised in Fig. 5. C16:0, C18:0 and C18:1 contribute to increase kinetic viscosity, while C18:2 and C18:3 are desired for greater fuel-air mixing and thus enhancing combustion [43]. In view of comparison between the FAME compositions of CY-1 cultivated in both medium, balanced FAME profile were exhibited. A balanced proportion could produce good quality of biodiesel [3,44]. Nevertheless, higher total lipid contents were attained in 30% (v/v) pretreated POME medium. The percentage of C16 and C18 combination obtained in the study has revealed the potential in biodiesel production. Additionally, there was also polyunsaturated fatty acids (PUFA) obtained (C20:5), indicated its potential of towards bioproducts productions [45,46].

3.4. Nutrients removal of POME

The parameters of COD, TN and TP were tested as described in Section 2.1. Reduction in the pollutants were observed along the cultivation cycle (Fig. 6). The highest removal efficiencies obtained were 62.07% for TN, 47.09% for COD and 30.77% for TP, attained in pretreated 30% (v/v) POME. These results shown better pollutant removal performance than in non-pretreated 30% (v/v) POME. The pollutant removal efficiencies of organics though was lower than nitrogen, however in view of COD concentration, it has been degraded for 3800 mg L^{-1} in COD along the cultivation cycle. This was in accordance to Redfield C:N:P ratio of 106:16:1 on composition of microalgae cell, which represents the ratios of essential elements required in the culture medium, to ensure effective growth performance [47–49]. Nitrogen appeared to be favourable to be assimilated compared to phosphorus in the study. Nitrogen source is vital towards microalgae growth and lipid regulation, whereas and phosphorus is important for ribosomal RNA synthesis [50].

4. The potential – implications in industrial wastewater treatment plant

The major challenge hindered on microalgal-biofuel application, till date, would be the high cost incurred in upstream and downstream processing, making the overall process not reaching the state of economical viable and feasible. Monero-Garcia et al. [51] claimed that 1443 m^3 of wastewater was treated during production of 1 tonne of *Chlorella vulgaris*. The wastewater credit earned has reduced the cost of biomass from $808.79 to $231.59. Microalgae cultivation has to be cost-effective in larger scale cultivation as medium costs would be one great fraction of total cost [9]. Microalgae cultivation is economical favourable when incorporated with wastewater treatment [52–54]. Adesanya et al. [55] reported that the water footprint was 1700 m^3 per

Table 3
The biomass and lipid yields of microalgae species grown in POME.

No.	Microalgae strain	Culture medium	Maximal biomass concentration ($g\,L^{-1}$)	Growth rate ($g\,L^{-1}$)[a] Biomass productivity ($g\,L^{-1}\,d^{-1}$)	Lipid content (%)	Lipid productivity ($g\,L^{-1}\,d^{-1}$)	Nutrients reduction	References
1	***Chlorella sorokiniana* CY-1**	**30% (v/v) POME**	**1.86**	**0.13**	**9.97**	**0.08**	**Removal of 45.05% COD, 54.23% TN, 29.20% TP**	**This study**
2	***Chlorella sorokiniana* CY-1**	**Pretreated 30% (v/v) POME**	**2.12**	**0.15**	**11.21**	**0.07**	**Removal of 47.09% COD, 62.07% TN, 30.77% TP**	**This study**
3	*Chlorella vulgaris*	POME + 60 $mg\,L^{-1}$ urea	1.07	0.08	–	0.01	Removal of 45.08% COD	[41]
4	*Chlorella* sp.	50% (v/v) POME + 1 $g\,L^{-1}$ urea	–	0.06	–	–	–	[42]
5	*Chlorella pyrenoidosa*	10% (v/v) POME	39.41	0.04	–	–	–	[40]
6	*Botryococcus sudeticus*	POME	1.03	–	26.6	–	–	[39]
7	*Chlorella vulgaris*	POME	1.47	–	15.1	–	–	[39]
8	*Botryococcus sudeticus*	250 $mg\,L^{-1}$ POME in BBM + 1 ml Chloramphenicol	–	0.005	30.83	–	–	[18]
9	*Chlorella pyrenoidosa*	250 $mg\,L^{-1}$ POME in BBM + 1 ml Chloramphenicol	–	0.003	21.51	–	–	[18]
10	*Chlorella sorokiniana*	250 $mg\,L^{-1}$ POME in BBM + 1 ml Chloramphenicol	–	0.008	28.27	–	–	[18]
11	*Chlorella vulgaris*	250 $mg\,L^{-1}$ POME in BBM + 1 ml Chloramphenicol	–	0.006	21.34	–	–	[18]
12	*Tetraselmis* sp.	250 $mg\,L^{-1}$ POME in BBM + 1 ml Chloramphenicol	–	0.004	25.69	–	–	[18]
13	*Chlorella vulgaris*	40% (v/v) POME + D-glucose	1.43	–	9.7	0.20	–	[17]
14	*Chlorella vulgaris*	40% (v/v) POME + glycerol	0.98	–	7.3	0.02	–	[17]
15	*Chlorella* sp.	20% (v/v) POME + 40% synthetic nutrients	0.73	0.749[a]	6.9	0.04	–	[29]
16	*Chlorella* sp.	40% (v/v) POME + 60% synthetic nutrients	0.69	0.531[a]	7.3	0.03	–	[29]
17	*Chlorella* sp.	60% (v/v) POME + 80% synthetic nutrients	0.59	0.269[a]	7.6	0.01	–	[29]

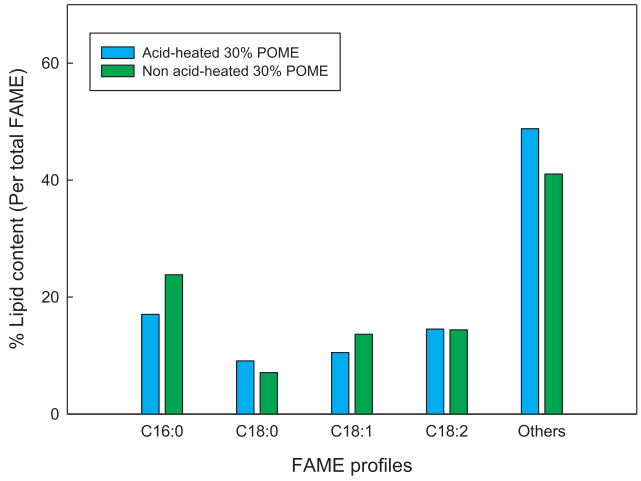

Fig. 5. FAME profiling of CY-1 cultivated in 30% (v/v) pretreated and non-pretreated POME.

ton biodiesel produced, used for cultivation, in their PBR (58.89 m^3) and pond (392 m^3). Significant water demand could be offset by using wastewater cultivation. Jayakumar et al. [56] stated that the elimination of water and nutrients supply alone saves $2342.8/ha for 109 MT/ha/year microalgae output. Cost of raw materials accounts 60–75% of total cost for biodiesel production [57]. The savings of using waste nutrients could be about 1–6.5% of total biomass cost [58].

Additionally, credits can be earned via wastewater treatment. Most technologies is efficient in removing pollutants from POME, however its application was unsuccessful due to financial infeasibility. This makes most palm oil millers in Malaysia still using ponding system [5]. Nutrients N and P are removed conventionally usually by nitrification-denitrification and precipitation with metal salts, respectively. In microalgae nutrients remediation, the process is simplified to a single step, as both nutrients are essential for microalgae growth thus degrade them [59]. This allows nutrient remediation together with treatment cost reduction as less land space is required to construct the pond or tank and thus less operating cost incurred. Together with the credits earned in wastewater treatment, Van Wagenen et al. [58] claimed that the revenue generated by waste treatment could be enough to cover some of the microalgae cultivation cost. Energy production from biomass cultivated in urban wastewater together with energy savings due to simplified water treatment step, outweighed energy cost of cultivation and harvesting [60].

NASA has proposed Offshore Membrane Enclosures for Growing Algae (OMEGA), a floating PBR to cultivate microalgae in municipal wastewater [61]. Aquaflow Bionomic Corp., New Zealand has applied their microalgae technology in wastewater treatment [61]. We could observe the trend of development of microalgal-based biofuel is heading towards improved cultivation method to be applied using wastewater. Some researchers proposed co-location strategy on microalgae cultivation to industrial plant, so as to fully utilising wastewater, waste nutrients and waste flue gas, allowing overall cost reduction and environmental feasibility [15,51,55,62,63]. In this study, the biomass and lipid yields of CY-1 were found improved by cultivated in pretreated POME. Acid pretreatment can be carried out in the wastewater treatment plant easily as it equipped with neutralisation treatment. Raw olive mill wastewater was pretreated with nitric acid prior to cultivate *Scenedesmus dimorphus* and *Arthrospira platensis* [64]. Table 4 shown the energy balance for commercial scale lipid production in our study. Positive energy balance of 0.24 and 0.21 were attained from *Chlorella sorokiniana* CY-1 cultivated in pretreated and non-pretreated 30% (v/v) POME. Energy balance could be further improved by optimisation and incorporation of biogas production using the residual lipid-extracted biomass could be made [65]. Additionally, the optimal

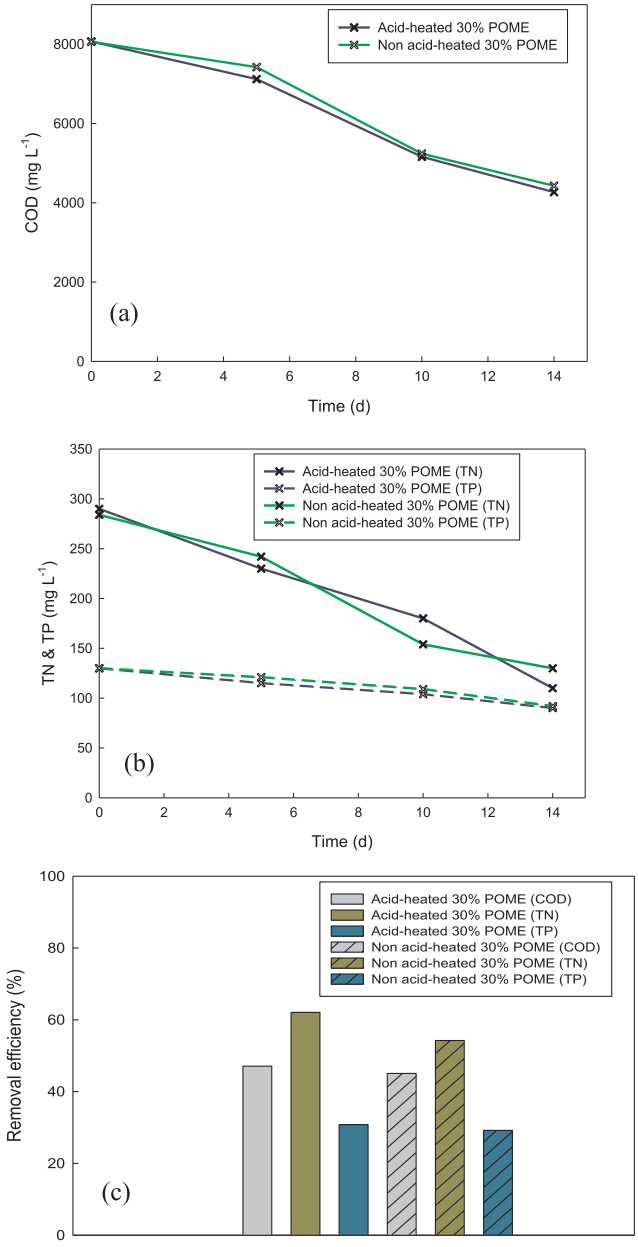

Fig. 6. Time course profile of COD (a), TN and TP (b) reduction by *Chlorella sorokiniana* CY-1 cultivated in pretreated and non-pretreated 30% (v/v) POME with the pollutants removal efficiency (%) (c).

condition for POME medium could be further studied for effective microalgae assimilation together with nutrients remediation. The approaches which promotes pollutants remediation can also be determined for environmental protection. Microalgae indeed has huge potential to serve as alternative energy source, and most importantly the research has to move on.

5. Conclusions

The study shown the potential to cultivate *Chlorella sorokiniana* CY-1 using 30% (v/v) pretreated POME. Pretreatment study has positively enhanced biomass and lipid productions without supplement addition. The biomass and lipid productions exerted are relatively higher and pollutants removal efficiencies were comparable to those reported values. Further research on cultivation strategy and pretreatment study prior microalgae cultivation ought to be carried out, to investigate on its effects in promoting greater feasibility in cultivating microalgae for

Table 4
Energy balance for commercial scale lipid production (for 100 tonne biomass per year production) [66].

Variables	Cultivation medium	
	Pretreated POME	Non-pretreated POME
Biomass concentration ($g\,L^{-1}$)	2.12	1.86
Biomass productivity ($g\,L^{-1}\,d^{-1}$)	0.15	0.13
[a]Volume of medium required (m^3)	1791	2060
Lipid content (%)	11.21	9.97
[b]Net lipid yield (m^3 per year)	12.46	11.08
[c]Estimated energy demand (GJ/kg biodiesel)	1824	1824
[d]Energy produced as lipid (GJ)	437.76	389.27
Net energy ratio	0.24	0.21

[a] Determined by dividing biomass production per year by biomass productivity.
[b] Determined by dividing product of biomass production per year and lipid content by lipid density ($0.9\,kg\,L^{-1}$).
[c] Estimated from [67], Energy demand is 18.24 MJ/kg biodiesel.
[d] Determined by product of net lipid yield and energy content of lipid ($35.133\,kJ\,L^{-1}$).

biofuel productions as well achieving environmental sustainability.

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Conflict of interest

The authors have declared no conflict of interest.

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