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by Muhamad Maulana Azimatun Nur

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RESEARCH ARTICLE

Utilization of Microalgae Cultivated in Palm Oil Mill Wastewater to Produce Lipid and Carbohydrate by Employing Microwave-Assisted Irradiation

M.M. Azimatun Nur[1,*], Dedy Kristanto[2] Tutik Muji Setyoningrum[1] and I.G.S Budiaman[1]

[1]*Chemical Engineering Department, Faculty of Industrial Technology, Universitas Pembangunan Nasional "Veteran" Yogyakarta. Jl. SWK 104 Condongcatur, Sleman, Yogyakarta 55281, Indonesia and* [2]*Petroleum Engineering Department, Faculty of Mineral Technology, Universitas Pembangunan Nasional "Veteran" Yogyakarta. Jl. SWK 104 Condongcatur, Sleman, Yogyakarta 55281, Indonesia*

Abstract: ***Background:*** Palm oil mill effluent (POME) is dominant agroindustrial wastewater in Indonesia and Malaysia. Some kind of microalgae can utilize the wastewater as media of cultivation, and produce value-added compounds. However, the production of lipid and carbohydrate from microalgae cultivated on untreated POME medium were not clearly reported.

Objective: to cultivate *Chlorella vulgaris*, *Dunaliella salina*, and *Spirulina platensis* on a media containing the different concentration of POME to produce lipid and carbohydrate by employing a microwave assisted method.

Methods: microalgae were cultivated on different POME concentration (10-30% v/v) to replace synthetic medium at 13 days of cultivation. The microwave-irradiation was employed on lipid extraction, carbohydrate hydrolysis, and fatty acid methyl ester (FAME) formation. GC/MS was employed to analyze the fatty acid and hydrocarbon compound on the lipid. Chemical oxygen demand (COD) on the medium was analyzed before and after the cultivation.

Results: Growth rate of the microalgae were decreased along with the increasing of POME addition except for *C.vulgaris*. The lipid and carbohydrate content were influenced by POME except for *D. salina*. The microwave-assisted method successfully enhanced carbohydrate yield in the hydrolysis process. The highest productivity was found on *C.vulgaris* with 12.60 mg/L/d lipid, and 11.22 mg/L/d carbohydrate, and remove 74% COD content. The highest FAME content was recorded from *S. platensis*.

Conclusion: In summary, the microalgae can utilize POME wastewater in low concentration under mixotrophic condition. The microwave-assisted method seems promising in the integrated biorefinery process of producing value-added compound from microalgae.

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1. INTRODUCTION

Biomass from microalgae is a well-known source for producing food, feed, and biofuel in the last decade. Several microalgae contain high amounts of lipid, protein and carbohydrate, which the production is focused in one of them. Biofuels from microalgae still appear not feasible and costly due to the process and investment [1]. However, the cost could be reduced by using the wastewater as media cultivation, and lowering the power consumption by employing a biorefinery processes [2, 3].

*Address correspondence to this author at the Chemical Engineering Department, Faculty of Industrial Technology, Universitas Pembangunan Nasional "Veteran" Yogyakarta. Jl. SWK 104 Condongcatur, Sleman, Yogyakarta 55281, Indonesia; E-mail: lanaazim@upnyk.ac.id

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Palm oil mill effluent (POME) is a dominant agroindustrial wastewater in Indonesia and Malaysia. It contains high organic content and micronutrient, and can causes eutrophication for the environment. Several researchers reported that the wastewater can be used as the media of mixotrophic cultivation condition for microalgae biofuel due to the organic content [4, 5]. Some types of microalgae can utilize the nutrient and organic carbon from the wastewater. It also produces lipid and or carbohydrate in stress condition as accumulation of energy stock [6]. Thus, selection of microalgae strain is also important as purpose to lower pollutant and also produce biofuel. *Spirulina platensis* and *Chlorella vulgaris* are two main commercial microalgae that can be cultivated in POME medium [4, 7]. However, last research was not focused on the production of lipid and carbohydrate from the biomass. Moreover, *Dunaliella salina* was not clearly reported to treat the POME wastewater.

Biorefinery is a technique that integrates cell cultivation, biomass conversion, and separation, in which the focus is to obtain several products. Microwave-assisted is a well-known technique to disrupt cell wall and potential for commercial scale [8]. It can increase extraction of the lipid [9], and trans-esterification of FAME from microalgae [10]. However, only few researchers reported the use of microwave to enhance glucose yield in acid hydrolysis from the biomass. The purpose of this research was to evaluate the cultivation of three different microalgae, *Chlorella vulgaris*, *Dunaliella salina*, and *Spirulina platensis*, in the media containing different concentrations of untreated POME for producing lipid and carbohydrate by employing microwave-assisted method.

2. MATERIAL AND METHODS

2.1. POME Effluent Characterization

POME was collected from traditional palm oil factory located in Lampung, Sumatra. The factory employs biological method to treat the wastewater. The effluent is processed in a traditional anaerobic-aerobic lagoon before being released to the river. The source of POME in this study was from 2nd traditional lagoon containing activated sludge. POME was filtered using 40 mesh filter cloth to reduce total suspended solid. POME in this research contained COD 1520 mg/L and total nitrogen 254 mg/L.

2.2. Microalgae Strain and Cultivation Condition

Three selected microalgae strains, *C. vulgaris*, *S. platensis*, and *D. salina*, were purchased from BBPAP Jepara. The cultures were maintained in Bold basal medium except for *S. platensis* which was grown in Zarrouk medium. All the media were modified by adding inorganic carbon source 1 g $L^{-1}NaHCO_3$ resulting autotrophic condition.

Cultivation was carried in 2 L flask Erlenmeyer. Each medium consists of 30% v/v inoculum that equals to 0.06 g/L dry weight. POME concentration was varied to replace synthetic medium (10, 30, and 50% v/v). All of the media were diluted using distilled water. Cultivation was operated in 13 days. Fluorescent lamp was employed to obtain the light intensity at 4000 lux. The temperature was set to 26-28°C. The pH was maintained at 6.8-7.2, except for *S. platensis* which was maintained at pH=9-10. Salinity was adjusted for 1 ppt, except for *D. salina* which used 5 ppt of NaCl.

Every 24 hours, the microalgae was measured using Spectrophotometry to obtain the optical density. Both *S. platensis* and *C.vulgaris* were recorded at 680nm wavelength absorbance. For *D. salina*, the wavelength was 630nm.

The specific growth rate (μ, day^{-1}), lipid productivity (P_l, $mg.L^{-1}.day^{-1}$), and carbohydrate productivity (P_c, $mg.L^{-1}.day^{-1}$) were calculated by Eqs. (1), (2), and (3), respectively.

$$\mu = \ln (X_t/X_o)/t \quad (1)$$

where X_t is the concentration of cells at specific time, X_0 is the concentration of cells at the beginning of batch run, and t is the time of cultivation.

$$P_{lipid} = \mu . X . L \quad (2)$$

$$P_{carbohydrate} = \mu . X . C \quad (3)$$

Where L and C are lipid and carbohydrate content at the end of cultivation (%), respectively.

Cells were harvested by autoflocculation. NaOH 0.5 M was added to each of the culture medium until the pH reach 11. Then it was gently

stirred at 300 rpm for 2 hours. Cells were settled overnight to collect the broth. Wet biomass was filtered by using pre-weighed Whatman filters GF/F type, 25 mm in diameter, and dried in 60°C until reaching a constant dry weight (X, g/L). The filtrate was stored for further analysis.

2.3. Carbohydrate hydrolysis and analysis

Carbohydrate hydrolysis was employed with a modification method [11]. Suspension of dried algae in water (0.1 g/10 mL or 10%) was mixed at 100 rpm stirrer bar to homogenize the suspension. H_2SO_4 1.1% v/v was added to the sample as a catalyst. For a conventional hydrolysis method, the mixture was heated at 115°C and mixed at 300 rpm. For microwave assisted method, the mixture was processed in 70% power (equal to 630 W) with frequency 2450 Mhz and output 900 W. The sample was collected each 2 min with final process 10 min. Phenol sulphuric acid by Dubois *et al*.[12] (1956) was employed to analyze the total carbohydrate.

2.4. Total Lipid Extraction and Analysis

For total lipid analysis, dry-algae was extracted by microwave aqueous extracted method. A modified Folch method, consists of 2:1 chloroform and methanol with total volume 10 ml, was used to extract 100 mg of dry biomass [9]. The process was carried out in a three-neck flask equipped with a modified condenser, and then irradiated using 30% power (equal to 300 W) for 5 min with frequency 2450 Mhz and output 900 W. Sample was cooled and centrifuged at 1000 rpm for 20 min. At the center of layers, chloroform containing lipid was gently pipetted out. The mixture was evaporated until a constant weight was obtained.

2.5. In Situ Transesterification

The microwave was employed for in situ transesterification of microalgae with a modified method [10]. One gram dry algae was homogenized with 6 ml methanol and 6 ml n-hexane, and 2% H_2SO_4 was used as the catalyst. The mixture was placed in the three-neck flask equipped with the modified condenser, and then irradiated using 30% power for 6 min. After that, sample was immediately cooled and water was added in the sample to separate the layer between methanol and n-hexane. Sample was centrifuged at 3000 rpm for 10 min. The upper layer containing n-hexane, FAME, and algal hydrocarbon was collected for GC/MS analysis.

2.6. FAME and Hydrocarbon Analysis

FAME and hydrocarbon were analyzed by GC-MS (QP-2010 Shimadzu, Japan) using silica capillary column (30 m x 0.25 mm, 25 μm) with flow rate of 1 ml/min. Helium was used as carrier. FAME was diluted with pure n-hexane before injected into the column. For FAME analysis, temperature was programmed at 130°C to 280°C at a rate of 3°C/min. For hydrocarbon analysis, temperature was initiated from 40°C to 300 min at a rate of 3°C/min The samples were identified by Mass Spectrometer according to previous research [13].

2.6. COD Analysis

Standard National Indonesia (SNI) protocol No. 06-6989.22-2004 was used to determine the COD content of POME before and after used as medium of cultivation. Organic content was expressed from the amount of $KMnO_4$ consumed for the reaction [14].

2.7. Statistical Analysis

All experiments were performed in two replications except for lipid, FAME and carbohydrate analysis. Data were analyzed with Minitab Statistics software version 6.0. Significant effects ($p < 0.05$) of the interaction were determined using one-way analysis of variances (one-way-ANOVA). Data were reported as mean values with standard deviation ($\pm SD$).

3. RESULTS AND DISCUSSION

3.1. Effect POME on Growth Rate

Fig. **1** and Table **1** show the logarithmic growth of microalgae. The highest growth was found on the control medium. On 10% and 30% v/v POME, *C. vulgaris* and *S. platensis* can maintain the production as high as the control medium. Moreover, the medium can generate in mixotrophic cultivation condition. Azimatun Nur and Hadiyanto [4] stated that *C. vulgaris* grew well on POME wastewater under mixotrophic condition. However, the addition of 50% v/v

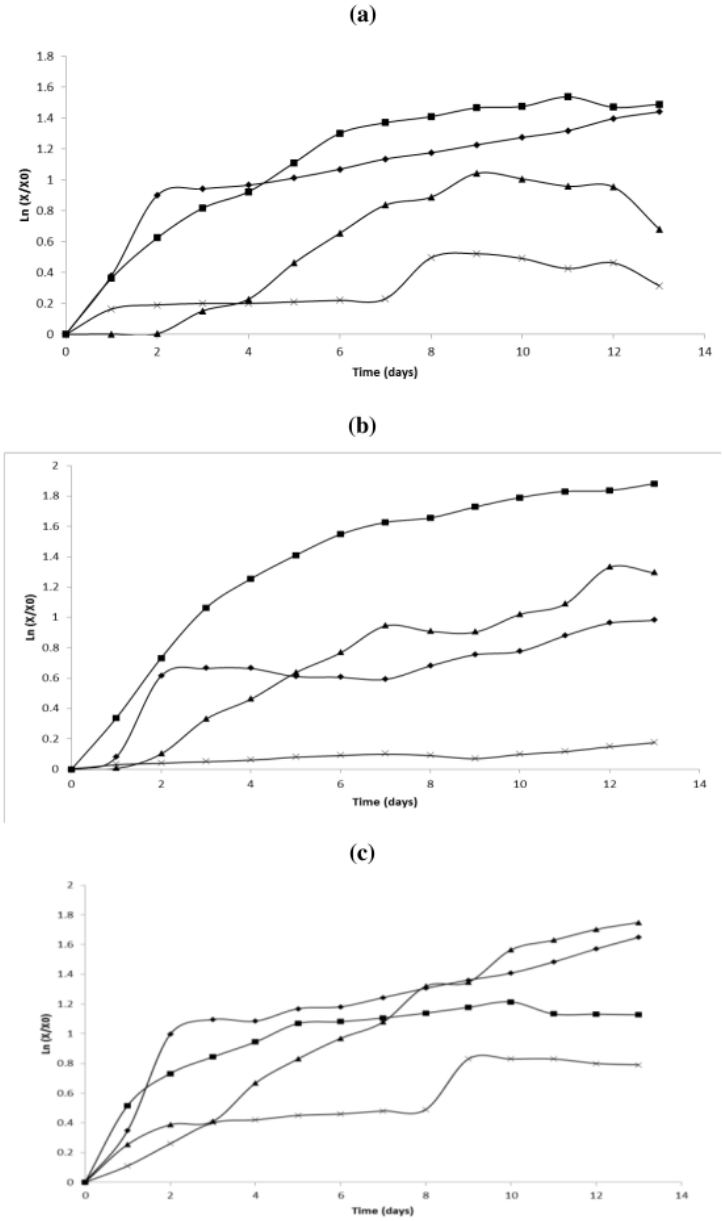

Fig. (1). Logarithmic growth profile of (a) *S. platensis* (b) *D. salina* and (c) *C. vulgaris* under different concentration of synthetic medium (■), 10% v/v POME (▲), 30% v/v POME (♦) and 50% v/v POME (×).

of POME drastically decreased the growth. It indicated that POME can serve as the inhibitor for microalgae due to the lower light penetration, and the high content of organic carbon. It was found that the growth of *D. salina* significantly decreased with the increasing of POME. It seems that the medium was not suitable for saline microalgae which need high salinity and high light intensity. This finding was in agreement with Takriff *et al*. who cultivated *D. salina* in treated anaerobic POME [15]. However, POME was not treated in our experiment. The bacteria activity may also inhibit the microalgae growth.

For Table **1**, specific growth rate decreased along with the increasing of POME concentration except for *C. vulgaris*. It seems that micronutrient in 10 and 30% v/v POME was suitable for *C. vulgaris* to maintain the growth, in spite of its mixo-

Table 1. Parameter of microalgae in different POME concentration.

Medium	*Chlorella vulgaris*				*Dunaliella salina*				*Spirulina platensis*			
	μ	X	L	C	μ	X	L	C	μ	X	L	C
Control	0.152	272 ±1 .3	30.26	32.76	0.210	254±8.4	22.37	42.29	0.167	266 ± 4 .3	24.54	20.90
10% POME	0.160	213±7.3	12.93	34.82	0.110	225±7.7	31.42	39.47	0.134	214 ± 3 .1	24.43	26.52
30% POME	0.154	203±2.1	37.30	33.15	0.092	182±8.4	34,98	38.73	0.123	219 ± 5 .7	12.36	30.17
50% POME	0.079	158±5.3	11.44	28.98	0.049	124±7.7	15.64	28.91	0.109	165 ± 2 .4	10.68	32.41

μ= specific growth rate (day^{-1}), X = dry biomass ($mg.L^{-1}$), L = lipid content (%), C= carbohydrate content (%)

Table 2. COD removal.

POME concentration (%)	COD loading rate (mg/L)	*Chlorella vulgaris*		*Dunaliella salina*		*Spirulina platensis*	
		COD out (mg/L)	Efficiencies (%)	COD out (mg/L)	Efficiencies (%)	COD out (mg/L)	Efficiencies (%)
10	150±2	41±5	73	54±2	66	65±2	58
30	450±4	114 ±10	74	131±19	70	157 ±39	65
50	750±3	206±17	71	265±21	63	401±12	46

trophic condition. Abreu *et al*. recorded that *C. vulgaris* in mixotrophic condition gave higher growth rate than autotrophic condition [16]. However, the growth decreased when 50% v/v POME was added. It seems that high organic content inhibited the growth of microalgae. Kamyab *et al*. found that above COD content 500 mg/L of POME, the biomass production of microalgae was decreased significantly [17, 18].

3.2. Effect Microwave on Carbohydrate Hydrolysis

For Fig. **2**, the microwave-assisted method was successfully increasing glucose yield. Comparing to the conventional method, the microwave gave shorter time to reach optimum yield. Hermiati *et al*. reported that the microwave influenced glucose yield in acid hydrolysis for starch polymer [19]. While Zhao and Monteiro reported that microwave radiation was useful method to enhance hydrolysis of bacterial cell wall into carbohydrate [20].

Based on three microalgae, the optimum glucose yield of *S. platensis* was recorded at 6 min, while *C. vulgaris* and *D. salina* at 8 min. Most of microalgae species have a tough cell wall, which needs to be disrupted before the product is extracted, and results in the increasing of energy consumption [3]. It is well known that the cell wall of green algae, such as *Chlorella* and *Dunaliella*, contain cellulose that were not easily rigid [21]. While *S. platensis* cell wall lacks of lignin, hemicellulose and even cellulose [22]. The lack of lignin in microalgae results in lower demand for harsh pre-treatments to release the biodegradable organic matter, as the case for lignocellulosic biomass [23]. Fig. **2** shows that glucose yield of *C. vulgaris* was the lowest in the conventional hydrolysis, while the addition of microwave irradiation gave better result.

3.3. Effect POME on Microalgae Lipid and Carbohydrate

The addition of POME concentration could serve as mixotrophic condition and influence carbon to nitrogen (C/N) ratio, forming nitrogen starvation [4;7]. This condition influenced microalgae to accumulate lipid or carbohydrate as the stock of energy [24, 25]. According to Table **1**., the addition of POME up to 30% v/v did not influence lipid and carbohydraye content for *C. vulgaris* indirectly ($P>0.05$). However, the highest carbohydrate was recorded in 10% v/v, and the highest li-

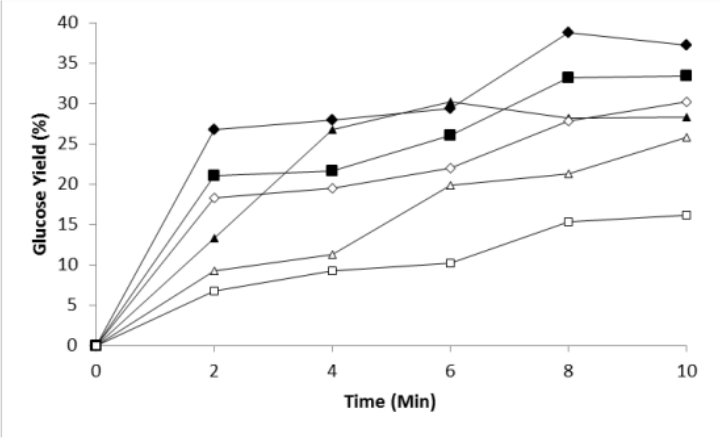

Fig. (2). Glucose yield versus different time on 70% power microwave assisted acid hydrolysis of *D. salina* (◆), *C. vulgaris* (■), *S. platensis* (▲), and conventional hydrolysis of *D. salina* (◊), *C. vulgaris* (□), *S. platensis* (Δ).

pid was found in 30% v/v POME addition. It seems that the light intensity and organic carbon substrate influenced *C. vulgaris* to accumulate energy into lipid and or carbohydrate [4,23].

This result was also found in *S. platensis* that accumulated energy into carbohydrate rather than lipid which its content decreased when the POME wastewater was added ($P<0.05$). The condition was in line with Salla *et al*. who used whey protein concentrate as the media cultivation of *S. platensis* in mixotrophic condition. And the result showed that the carbohydrate was higher compared to the control medium [26].

Despite increased lipid or carbohydrate accumulation, energy accumulation of *D. salina* was not significantly affected by POME concentration ($P>0.05$). It could be *D. salina* accumulates protein content when the media was in mixotrophic condition as stated by Kadkhodaei *et al*. [27]. However, it needs more attention in the further research.

3.4. COD Removal by Microalgae

Table **2** indicates that organic carbon in POME wastewater was consumed during cultivation. The highest COD removal efficiency was found in *C. vulgaris* (74%). However, *D. salina* and *S. platensis* gave higher efficiencies when POME concentration was added in low concentration (10-30%v/v). It seems that the microalgae need more time to consume organic carbon, in spite of the growth rate inhibited by POME wastewater as seen in Fig. **1**.

However, in this experiment, POME medium was not treated to lower the bacterial activity. It seems that the growth-symbiosis of the algae-bacteria activity could also possible to lower COD content during the cultivation. Further investigation should be addressed to this result.

3.5. Effect of Organic Content on Carbohydrate and Lipid Production

From Fig. **3**, the highest carbohydrate and lipid productivity were found in *C. vulgaris*, followed by *D. salina* and *S. platensis*. The productivity was influenced by POME concentration with different COD contents ($P<0.05$), except for the carbohydrate productivity of *S. platensis* and the lipid productivity of *C. vulgaris*. It indicates that the microalgae constantly accumulate energy only in low POME concentration.

3.6. FAME and Hydrocarbon Profile in Microalgae Cultivated in 30% v/v POME

Table **3** shows that the highest FAME content was recorded from *S. platensis*, followed by *C. vulgaris* and *D.salina*. The highest methyl palmitate and methyl linolenate were found in *S. platensis*. It indicates that *S. platensis* was the most suitable strain for biodiesel production.

However, in our result, *D. salina* contained high amount of hydrocarbon. This finding was in agreement with Park *et al*. who stated that the hydrocarbon of *D. salina* was similar to *B. braunii*, which is suitable for liquid fuel production [28]. Furthermore, the hydrocarbon found in *C. vulgaris* contained long

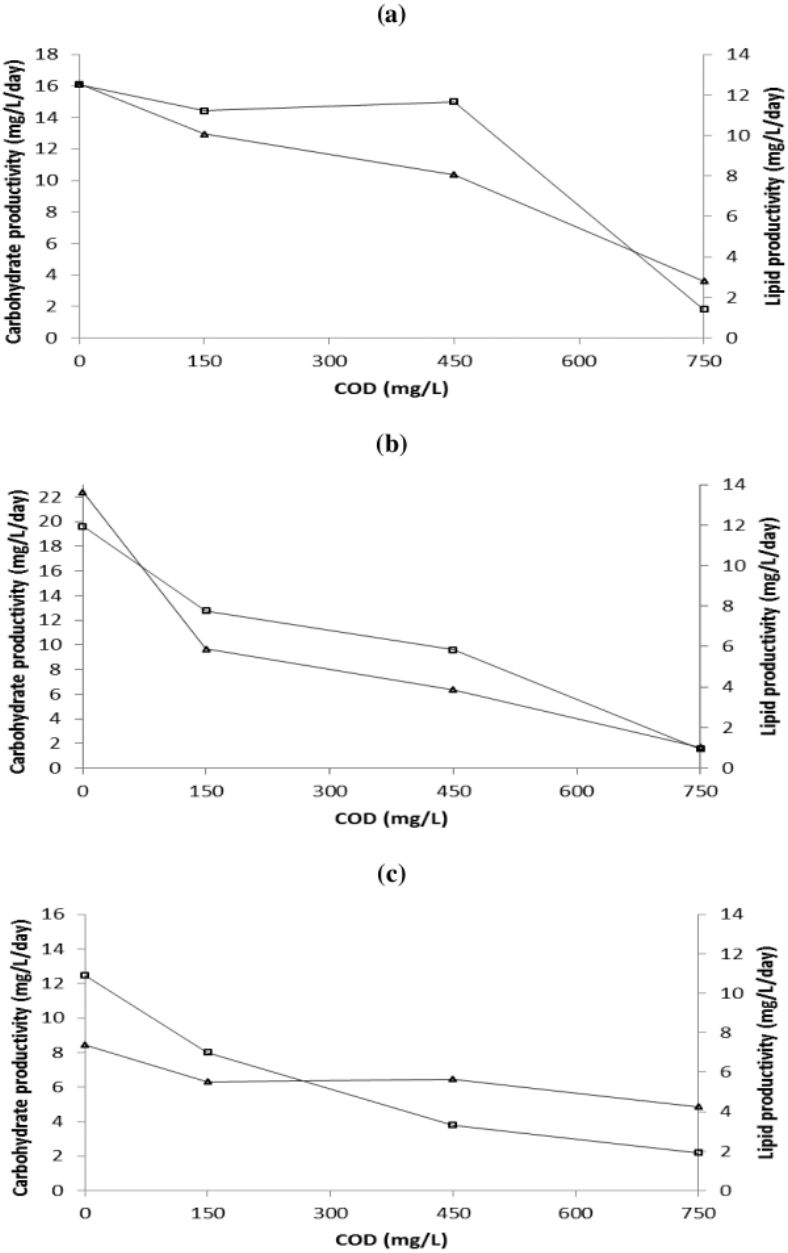

Fig. (3). Carbohydrate productivity (Δ), and lipid productivity (□) of (a) *C. vulgaris*, (b) *D. salina* (c) *S.platensis* using different COD in diluted POME.

chain carbon ranging from C14 to C29. This result was in line with Patterson [29].

4. CONCLUSION

In summary, results from this study showed that three selected strain of microalgae can utilize POME wastewater in low concentration. It was observed that the growth rate was decreased along with the increasing of POME, except for *C. vulgaris*. POME was favored resulting in mixotrophic condition. The COD content decreased along with organic carbon consumed as a source of energy. Lipid and carbohydrate content were also influenced by POME concentration except for *D. sa-*

Table 3. Fatty acid and hydrocarbon profile of microalgae cultivated under 30% v/v POME.

Fatty Acid Methyl Ester	GC/MS Area (%)		
	Dunaliella salina	*Spirulina platensis*	*Chlorella vulgaris*
4-Hexenoic acid, 2,2,5-trimethyl-, ethyl ester [C12 H24 O]	-	32,91	-
Methyl palmitate [C17 H34 O2]	11,53	35,97	12,16
Methyl linolenate [C19 H32 O2]	-	7,49	19,72
Methyl Stearate / Isostearate [C19H38O2]	3,24	-	5,5
Metyl oleate [C17 H32 O2]	-	17,07	-
Methyl Arachidat [C21 H42 O2]	-	6,57	-
Total Content	14,77	100,00	37,38
Hydrocarbon			
Tetrametyl [C14H30]	3.35	-	0,86
Pentadecane [C15H32]	2.77	-	-
n Hexadecane [C16H34]	6,96	-	7.16
n Heptadecane [C17H36]	4.3	-	4.82
n Octadecane [C18H38]	10.29	-	6.43
n Eicosane [C20H42]	3.35	-	5.86
n Pentadecane [C21H44]	7.4	-	6.33
n Docosane [C22H46]	5.89	-	-
Eicosane [C23H48]	-	-	6.08
n Tetracosane [C24H50]	-	-	6.62
n Tricosane [C23H48]	10,2	-	4,16
n Hexyleicosane [C26H54]	5.72	-	5.72
n Nonacosane [C29H60]	6.49	-	7.00
Nd	15,21	-	6.59
Total Content	85,23	0	62,62

lina. Microwave assisted method was successfully employed for lipid, carbohydrate, and FAME production from microalgae. The best candidate for biorefinery feedstock was *C. vulgaris* due to the carbohydrate and lipid productivity, and the FAME profile, with the addition of 10-30%v/v POME as replacing synthetic media.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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HIGHLIGHTS

- POME medium can replace synthetic medium in low concentration
- Low POME medium influence growth of *Dunaliella salina* and *Spirulina platensis*
- Microwave-assisted increase yield of glucose in acid hydrolysis of microalgae
- The highest lipid and carbohydrate productivity were found in *Chlorella vulgaris*

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