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Research Article

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Isolation and characterization of microalgae isolated from palm oil mill effluent (POME) for biodiesel feed stocks with β-carotene as co-product

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ABSTRACT

In this study, three species were isolated from Palm Oil Mill Effluent (POME). The feasibility of the microalgae as feedstock for biodiesel production was evaluated. The species were identified and characterized consisting of bimolecular analysis, determination of biomass content including lipid accumulation as well as fatty acid identification. Optimization of culture condition by combining nitrogen deficiency and rice husk (RH) extract addition as nutrient supply was performed to enhance lipid yield. Betha carotene content as co-product was also determined. Sequence analysis of 18 S rDNA gene revealed that the three species were Micractiniumspehime, Micractinium sp. CCAP211/92 and Mychonastesrotundus. Biomass productivity of the three strains based on the individual optimum growth rate and dry biomass weight were 57.22 mg/L d, 57.53 mg/L d and 59.98 mg/L d respectively. While lipid content of respective species under normal nutrient conditionwere9.96 ± 0.76 %, 26.65 ± 0.98 % and 27.67 ± 1.28%.Optimization for lipid accumulation was only performed to the two species with highest lipid content (Micractinium sp CCAP211/92 and Mychonastesrotundus) which reached lipid content up to 43.42 % and 37.92 % with lipid productivity of 20.9 mg/L d and 15.8 mg/L d respectively. Fatty Acid Methyl Ester (FAME) analysis showed that palmitic acid (C16:0) is the predominant fatty acid followed by oleic, linoleic and linolenic acids. Betha carotene identification demonstrated that Mychonastesrotundus has the highest β-carotene content among the three species tested with percentage of 0.77 % of dry cell weight (dwc). The significance of lipid accumulation with suitable lipid profile and the presence of β-carotene reveal that microalgae strains isolated from palm oil mill effluent (POME) have the potential as feedstock for bioproducts especially biodiesel.

Keywords: Microalgae, Lipid Accumulation, β-carotene, Bimolecular Identification, SEM, GC-MS, HPLC, POME (Palm Oil Mill Effluent), Rice Husk Extract

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INTRODUCTION

Energy crisis and population growth are two main challenges that put tremendous pressure on global oil production. Due to the concerns over depletion of fossil fuels, there have been intensive and worldwide researches on the potential of microalgae as a renewable source of biofuels, especially biodiesel. Most of biodiesel which is currently produced from plants and animal oil seem to be replaced by microalgae biodiesel in the near future, as microalgae could serve as an interesting raw material source with high growth rate, high lipid content and smaller cultivation area [1].

Microalgae have a wide range of species. Different species and strain of microalgae has different lipid content. Many microalgae have the ability to produce substantial amounts (20-50% dry cell weight) of triacylglycerol (TAG) as storage lipid[2]. For large scale of biodiesel production, microalgae with high lipid content are desirable. However microalgae have certain growth patterns. Under optimal growth condition, large amount of algae biomass is produced, but with relatively low lipid content. While species with high lipid content are typically slow growing[3]. Some researchers have focused on increasing lipid content of microalgae using various approaches, one of which is through the induction of lipid biosynthesis, e.g. by environmental stress. In this case, some microalgae

have the ability to modify lipid metabolism in response to changes in environmental condition. Several lipid induction techniques which can be applied are the use of nutrient stress, including nitrogen and/or phosphorus starvation, light irradiation, pH, temperature, heavy metals and other chemicals. One common treatment is lipid induction of nitrogen deficiency. Under nitrogen deficiency, microalgae could increase cellular lipid level from 20-30% to 60-70%[4].

Besides being feedstock for biodiesel production, other important applications of microalgae are their function for nutritional and nutraceutical supplements. Many bioactive compounds such as pigments, antioxidants, eicosapentanoic acid, docosahexaenoic acid and biofertilizer, have been reported to have the potential as valuable co-products [5]. Carotenoids are of lipid soluble pigments that naturally exist in phytoplankton, marine algae, plants and limited species of bacteria [6]. Carotenoids contain high reactive conjuncted double bonds which act as free radical traps and function as antioxidants through playing important role in quenching of toxic radical[7]. The average concentration of carotenoid in most algae is 0,1 – 2% of total dry cell weight, but under special condition some microalgae demonstrate the increase in carotenoids content up to 14% for *Dunaliella*and 3 % for *Haemotococcus*[8], both of which are well known as microalgae species for β-carotene and astaxanthin productions, respectively. Some studies have successfully identified and characterized various carotenoids in some microalgaes[9-11]. The most widespread and important carotenoids is β-carotene which found abundantly in some plants [12]. It serves as dietary source of vitamine A (retinol), an essential micronutrientfor human and other mammalian species which cannot be synthesized within the body[13]. It can alsofunction as coloring additives and antioxidant[14].

Microalgae live in a wide range of ecological habitats.They are able to live well either in fresh water, brackish, marine or even in extreme environmental condition. Interestingly many species of microalgae have been reported to be capable to make use of nutrients from wastewater for the growth while accumulating significant lipid body [15-17].

Crude Palm Oil(CPO) wastewater which is also known as palm oil mill effluent (POME) is rich in organic matter[18].Since biochemical and physiological changes in microalgae cells can be affected by culture media, growing condition and nutrient compositions[19], it is expected that microalgae being isolated from POME would demonstrate better characteristics, especially in lipid content. The objective of the research was to evaluate whether microalgae isolated from POME could serve as potential source for biodiesel feedstocks with β-carotene as co-product.

EXPERIMENTAL SECTION

Sampling and isolation

Palm Oil Mill Effluent (POME) as water sample was collected from MutiaraAgam Company, Agam Regency, West Sumatera, Indonesia. Isolation were carried out using serial dilution method in which 1 mL of sample were diluted by adding 9 mL of BBM medium, starting from dilution of 10^{-1} to 10^{-10}. The cultures were incubated at room temperature under sunlight intensity captured inside the laboratory for approximately 4 weeks. The purities of culture were ensured by repeated subculturing and regular observation under an optical microscope. The pure cultures obtained were cultivated in conical flask containing sterile BBM with bubbling air from aerator to maintain the stirring of the culture. Illumination was performed under the same condition as isolation step. Regular subculturing was carried out after every 21 days.These cultures were subjected for further identification and characterization.

Morphology Identification

To identify the morphology of the isolates, optical microscopy analysis was performed followed by scanning electron microscopy (SEM) analysis. Optical microscope gave the morphological features of microalgae cell, and more absolute morphological study was determined by SEM instrument. For SEM analysis, the preparation was carried out by fixing the sample with buffer aldehyde, then fixing it in Osmium tetraoxide followed by dehydrating it in ethanol. Then continued by drying it with air dryer, mounting it on a specimen stub and coating with carbon, than examining under SEM instrument.

Biomolecular Identification

This step was started by extracting Genomic DNA from microalgae species using DNeasy Plant Mini Kit (Qiagen) based on the instruction of manufacturer. The process was continued with electrophoresis in BIO-RAD Electrophoresis chamber and Power Pac apparatus. The 18S rDNA was amplified by PCR with universal primer: forward5'-CCTGGTTAGTCCTGCCAG-3' and reverse 5'-TTGATCCTTCTGCAGGTTCA-3'. PCR reaction consisted of forward and reverse primer (0,4 µM s each), 1x Go Taq Green PCR master mix (Promega), 50 ng

microalgal genomic DNA as a DNA template and nuclease-free water was added to get 25 µL final volume. The reaction was performed in PCR BIO-RAD C1000 Thermal Cycler apparatus. The PCR condition was set up which consisted of preheating at 95^{o}C for 5 min, 35 cycles of DNA denaturation for 30 s at 95^{o}C which followed by annealing for 30 s at 56,3^{o}C and extension for 2 min at 72^{o}C, then final extension for 7 min at 72^{o}C prior to a 4^{o}C hold. A 5 µL of PCR products was electrophoresized using 1,5% agarose gel, stained with GelRed and visualized in BIORAD Molecular Imager Doe XR+ Imaging System. The remaining PCR products were purified based on Na-Acetate method. The purified products were then sent to Macrogen, South Korea for sequencing. Geneious 7.0.6 software was used to edit the sequencing data. The 18S rDNA sequences of the isolates were compared with the sequences in NCBI database using BLAST. Phylogenetic tree was then constructed using Geneious Tree Builder according to Neighbor-Joining (NJ) method.

Growth Evaluation

Cell growths of isolates were determined indirectly by daily measuring the optical density at 680 nm (OD_{680}) using spectrophotometer. The data obtained were used to determine dry biomass weight. A set of culture with different concentration was measured by spectrophotometer followed by determining individual dry biomass weight. Based on the data, standard curve was set up. Optimum dry biomass per liter culture was then calculated. Biomass productivity was also determined.

Analysis of Biomass Content

The content of biomass which mainly consists of carbohydrate, protein and lipid were evaluated. Determination of carbohydrate was performed based on the procedure of fenol-sulphuric acid method. Protein was analyzed using Lowry method. While total lipid content was determined according to the procedure of Bligh and Dryer method with little modification from procedure reported by Cheng Yuan et al [20]. A 40 mg of dry biomass in 10 ml test tube was prepared and added by 4 mL chloroform and 2 mL methanol. The mixture was then incubated for 12 h at room temperature while shaking at 200 rpm/min. Supernatant was separated from biomass by centrifugation. The collected supernatant was transferred into other test tube, then added by 2 mL methanol and 3,6 mL deionized water. The mixture was shaken and separated by centrifugation. The chloroform layer was removed and transferred into a pre-weight tube and dried for about 1 h in an oven at temperature of 50 ^{o}C until constant weight was obtained.

Fatty Acids Analysis

Fatty acid composition of the isolates were analysed using Gas Chromatograph Mass Spectrometer (GCMS-QP2010 Ultra Shimadzu) equipped with a DB-WAX Capillary Column(30 m x 0,25 mm x 0,25 πm). Preparation of FAME (Fatty Acid Methyl Esther) was carried out using the method proposed by Lievo M.L Laurenset al [21] with slight modification. Briefly, a small quantity of dried biomass (8-9g) was prepared. Lipids present in the algal cell were solubilized in 0,2 mL chloroform/methanol (1 :1, v/v), then trans esterified with 0,3 mL of HCl/methanol (5 %, V/V), followed by heating for 1 h 20 min at 82^{0}C in the present of 200µL nonadecanoic acid methyl ester (C19-FAME) as internal standard. FAME resulted were extracted with 1 mL hexane at room temperature for 1 h and a dilution of extract in hexane with ratio of 1: 3 was quantified by GC-MS. The conditions of GC-MS were as follows, the column temperature was maintained at 70^{o}C for 1 min then increased to 240^{o}C at a rate of 3^{o}C/min, and the injector temperature was set at 260^{o}C.

Optimation of Culture Condition for Lipid Accumulation

Optimization of culture media was carried out to enhance lipid accumulation. Microalgae isolated were cultured in the modified media by reducing nitrogen content (ten times lower than that of normal BBM media) and supplemented by rice husk(RH) extract as nutrient supply with RH extract concentration of 1 g/L, 2 g/L, 3 g/L and 4 g/L.

Identification of β-carotene by HPLC analysis

HPLC analysis was performed with ShimadzuSPD-20A/SPD-20AV prominence with UV-VIS detector and operated by LC-Solutions software. Stock solution of β-carotene standard was prepared by taking 6 mg in 100 mL mobile phase of solvent system consisting of dichloromethane, acetonitrile and methanol with the ratio of 20:70:10 (v/v/v). Stock solution was diluted to different known concentrations. Extractions of pigment from biomass of the isolates were carried out using pure acetone. As much as 100 mg of dried biomass sample was extracted with acetone until all the pigment extracted and the biomass colorless. The solution was centrifuged by 4000 rpm for 3 minutes to separate cell debri. The solvent was dried up and the dried carotenoid samples were dissolved in 100 µL mobile phase. Beta carotene standard and sample solutions were injected into HPLC system equipped with Shim-pack VP-ODS, LUNA C18 or equivalent ODS column (150 x 4.6 mm i.d., 5µm)and measured at 450 nm.

RESULTS AND DISCUSSION

Isolation and Morphology Identification

In general, under optical microscopy observation these isolates demonstrated similar features with little different in size. The cells are green, unicellular and round in shape. Further morphological features examination under SEM instrument showed the structures of the cell wall surface. *Mycractiniumspehime IPOME-1 and Mycractiniumsp CCAP211/92IPOME-2* demonstrated a quite similar pattern with little difference in which *Mycractiniumspehime IPOME-1* looks more compact and rigid compared to that of *Mycractinium sp CCAP211/92IPOME-2*. While *Mychonastesrotundus IPOME-3* has a layer like surface cell wall which indicates similarity to that reported by Cheng Yuan et al [20] in which both belong to the same genus.

Figure 1 shows morphology features of the strains which were observed under optical microscope and SEM analysis. The three species are *Mycractiniumspehime IPOME-1*,*Mycractiniumsp CCAP211/92IPOME-2*, and *Mychonastesrotundus IPOME-3*.

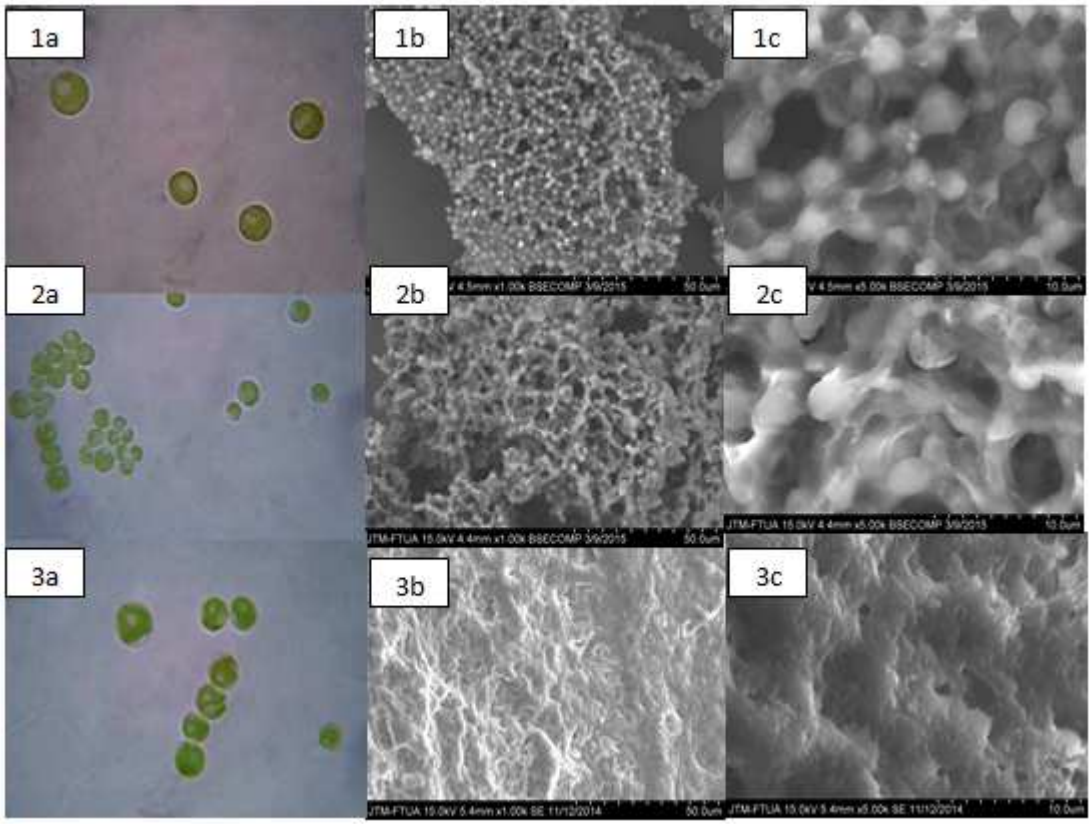


Figure 1. Morphology features of strains(1)*Mycractiniumsp.ehime IPOME-1* (2) *Mycractiniumsp CCAP211/92IPOME-2*, and (3) *Mychonastesrotundus IPOME-3* each under(a) optical microscopic identification at magnitudes of 1000x,(b)SEM analysis at magnitudes of 1000x and (c)SEM analysis at magnitudes of 5000x

Phylogenetic Analysis

Figure 2 shows the phylogenetic trees which demonstrate the relationship of the isolated strains with other species of the same genus and other species.

The BLAST analysis of the three species revealed that IPOME-1 showed the highest percentage identity of 100% and quary cover of 100% with *Mycractiniumspehime* (GenBank accession numberJX889639.1), IPOME-2 had highest percentage identity of 99% and quary cover of 100% with *Mycractiniumsp CCAP211/92* (GenBank accession numberJX889639.1). It can be inferred that IPOME-1 IPOME-2 belongs to the same genus. While IPOME-3 showed the similarity with *Mychonastesrotundus strain CCAP 260/14* with percentage identity of 100% and query cover of 100 %. The three isolates have been deposited into the National Center for Biotechnology Information (NCBI) under accession numbers of KT030770, KR936170 and KR936171 for IPOME-1, IPOME-2 and IPOME-3 respectively.

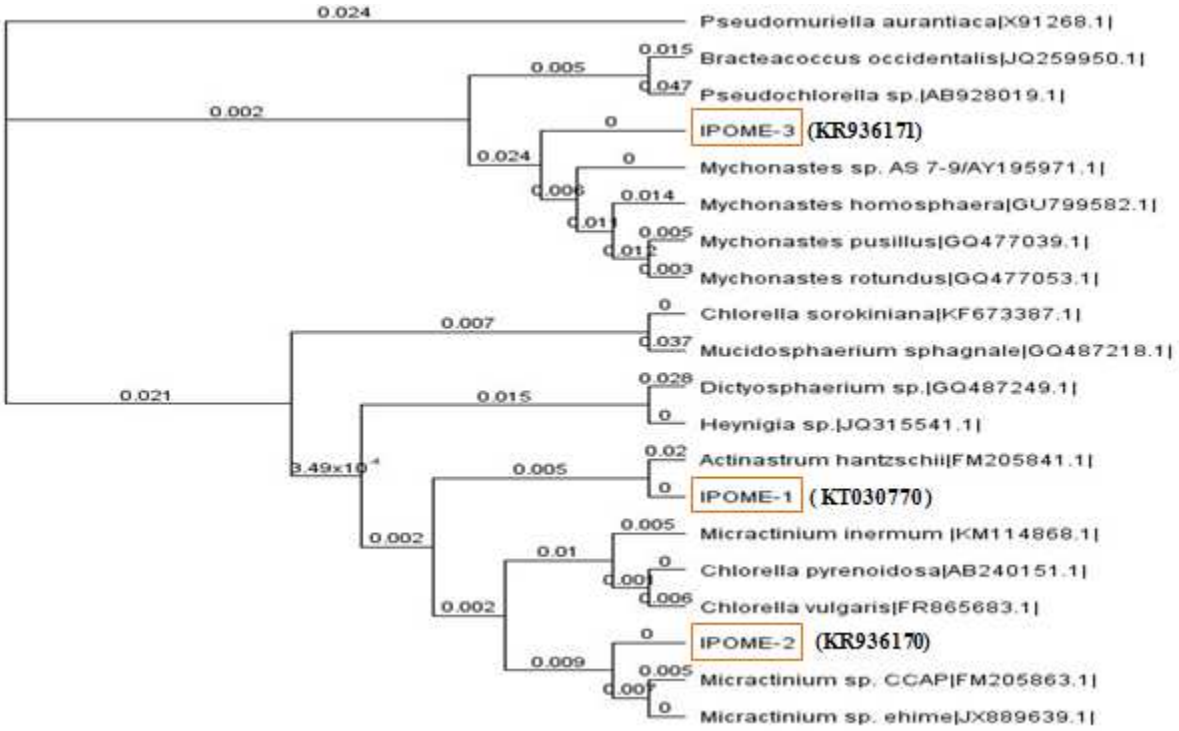


Figure 2.Phylogenetic tree

Microalgae Growth Rate and Dry Biomassa Value

The curve pattern indicates that all the species reached optimum growth at almost the same time. *Mycractiniumspehime IPOME-1* reached optimum growth at day 19, while *Mycractiniumsp CCAP211/92IPOME-2* at day 21, and *Mychonastesrotundus IPOME-3* attained optimum growth at the day 18. Dry biomass determination based on standard curve obtained from set of dry biomass values at OD_{680} for individual microalgae strain are 1.0872 g/L, 1.2082 g/L and 1.0797 g/L for *Mycractiniumspehime IPOME-1*, *Mycractiniumspsp CCAP211/92IPOME-2* and *Mychonastesrotundus IPOME-3* respectively. Biomass productivity of the three strains based on the individual optimum growth rate and dry biomass weight are 57.22 mg/L d, 57.53 mg/L d and 59.98 mg/L d respectively. It can be concluded that *Mychonastesrotundus IPOME-3* has a slight higher biomass productivity compared to that of the two others.

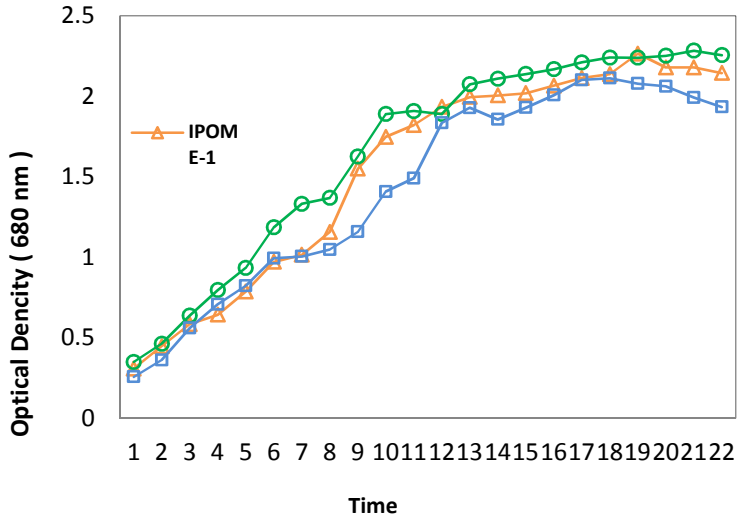


Figure 3.Growth Curve of the *Mycractiniumspehime IPOME-1,Mycractiniumsp CCAP211/92IPOME-2*, and *Mychonastesrotundus IPOME-3* under normal BBM media

Biomass Content

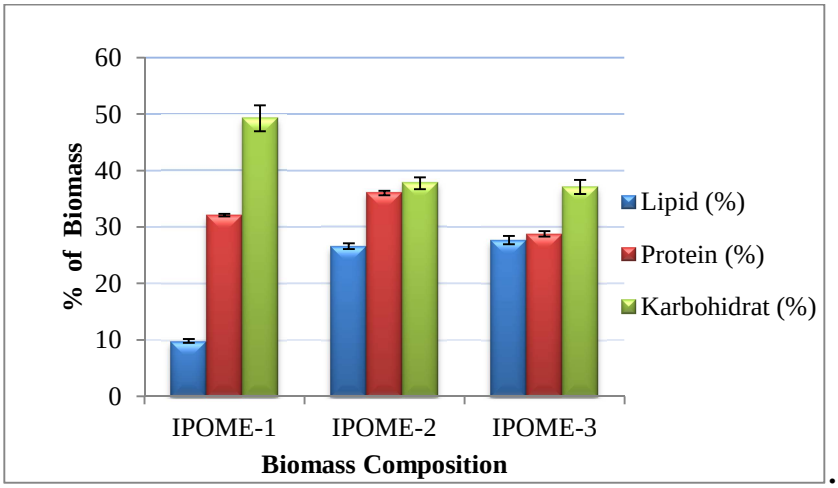


Figure 4. Biomass composition of *Mycractiniumspehime IPOME-1*,*Mycractiniumsp CCAP211/92IPOME-2*, and *Mychonastesrotundus IPOME-3* under normal BBM media

The determination of charbohydrate content through phenolic-sulphuricacid method showed that *Mycractiniumspehime IPOME-1*has the highest content of charbohydrate which accounts for49,261 ± 2.275 % of total biomass, whereas the contents in *Mycractiniumsp CCAP211/92IPOME-2* and *Mychonastesrotundus IPOME-3*are 34.989 ± 1.060 % and 37.113 ± 1.243 % respectively. This result revealed that *Mycractiniumspehime IPOME-1* should be further studied for ethanol production due to relatively high charbohydrate content.Significant charbohydrate content in microalgae could be a potential source for bioethanol production [22].

Protein analysis of the three isolates indicated that *Mycractiniumsp CCAP211/92IPOME-2* has the highest protein content with the content of 36.015 ± 0.373 % of total dry biomass, followed by *Mycractiniumspehime IPOME-1* and *Mychonastesrotundus IPOME-3*which accounts for32.119 ± 0.215 % and 28.772 ± 0.481 % of respective dry biomass. Analysis of lipid accumulation under normal nutrient condition showed that lipid content in *Mycractiniumsp CCAP211/92IPOME-2* and *Mychonastesrotundus IPOME-3* are comparable, constitutes of 26.65 ± 0.98% and 27.65 ± 1.28% (dcw) respectively. While lipid yields in *Mycractiniumspehime IPOME-1*was very low of only 9.6 ± 0.76 % (dcw). Further analysis on optimization of lipid accumulation, *Mycractiniumspehime IPOME-1* would not be included in this study.

Fatty Acid Identification

Fatty acid composition of oil plays important role in the performance of biodiesel in diesel engine which is characterized by significant amount of saturated and monounsaturated fatty acids [23, 24]. In this study gas chromatographic analysis demonstrated the relative percentage of various fatty acid methyl esters contained in the transesterified oil of microalgae. Table 1 shows FAME Composition of the *Mycractiniumsp CCAP211/92IPOME-2 and Mychonastesrotundus IPOME-3.*

Table1. FAME Composition of the *Mycractiniumsp. CCAP211/92IPOME-2 and Mychonastesrotundus IPOME-3*

No	NAME	Structure	% of Total FAME	
			IPOME-2	IPOME-3
1	MirysticAcid	C14:0	-	0.44
2	Palmitic Acid	C16:0	31.07	34.90
3	Palmitoleic acid	C16:1	3.37	-
4	Hexadecadienoic acid	C16:2	3.36	4.31
5	StrearicAcid	C18:0	1.08	4.83
6	Oleic Acid	C18:1	16.75	25.89
7	Linoleic Acid	C18:2	21.97	23.69
8	LinolenicAcid	C18:3	21.80	5.94

It is observed that palmitic acid (C16:0) is the predominant fatty acid for both isolates accounting for 31.07 % and 34.90 % of the total fatty acid respectively. Second major fatty acids of Mycractiniumsp CCAP211/92 IPOME-2 are linoleic and linolenic acids with repective content of 21.97 % and 21.80 %. While oleic acid as the next major fatty acid accounted for 16.75 % of the total FAME. On the other hand, *Mychonastesrotundus IPOME-3* showed

different fatty acid profile in which oleic acid emerge as the second major fatty acid with content up to 25.89 %, and followed by linoleic acid which accounted for 23.69 % It was also identified that other fatty acids existed in small quantities. Due to significant saturated fatty acid content, in this case showed by relatively abundance palmitic acid content, both strains are desirable for good quality of biodiesel. Saturated fatty acid methyl ester offersoxidative stability to the fuelas these increase the cloud point, cetane number and stabilityof biodiesel[25].Cetane number is a measure of fuel's ignition delay, the higher the number the shorter the time between the injection of the fuel and its ignition, resulting in lower starting temperature for engines [26]. In addition saturated fatty acid is a balance to polyunsaturated fatty acids which naturally exist in microalgae oil. Polyunsaturated fatty acid tend to undergo oxidation during storage[1],which can reducethe feasibility of microalgae application forbiodiesel. Furthermore, oleic acid is another determining factor for the quality of biodiesel as oleic acid is the most important monounsaturated fatty acid. Together with palmitic acid it improves the performance of biodiesel in engine diesel. The result revealed that in case of fatty acid profile, *Mychonastesrotundus IPOME-3* has better fatty acid composition compared to that of *Mycractiniumsp CCAP211/92IPOME-2*. However lipid content for both strains are comparable. It can be concluded that *Mycractiniumsp CCAP211/92IPOME-2* and *Mychonastesrotundus IPOME-3* can be applied as feedstock for biodiesel production.

Optimization for Lipid Accumulation

Figure 5 shows lipid content of *Mycractiniumsp CCAP211/92IPOME-2* and *Mychonastesrotundus IPOME-3* strains under optimization culture condition. Lipid yield for both isolates under nitrogen depletion with addition of rice husk (RH) extract in several different concentration was compared to that of nitrogen depletion culture medium without rice husk supplement (0 g/L RH).

Culturing time of 21 day as also implemented to normal nutrient culture was applied to the culture with this nitrogen starvation condition (0g/L RH). However, this period of incubation was not suitable to the culture with RH medium due to longer incubation time could cause damage to microalgae cells as it was proved in the initial assessment prior to preparation for culturing. Hence, the incubation period was set up for 14 days.

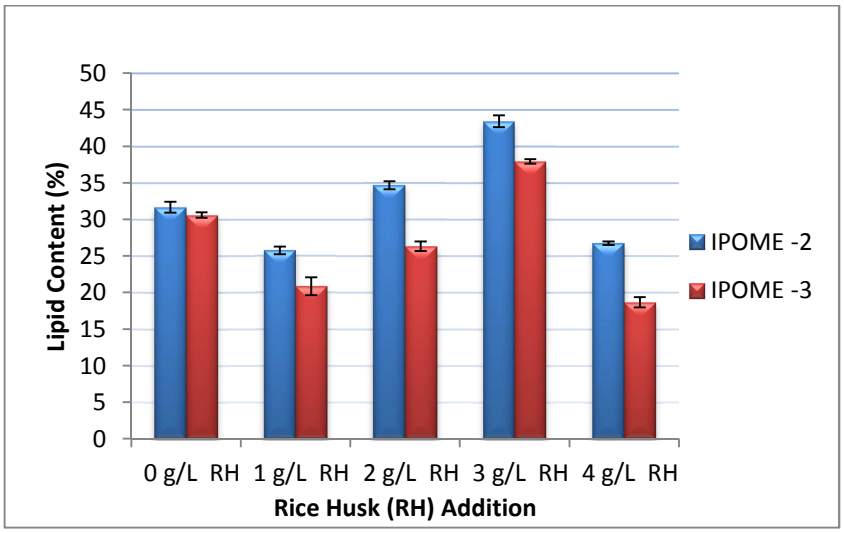


Figure 5.Lipid content of *Mycractiniumsp CCAP211/92IPOME-2 and Mychonastesrotundus IPOME-3* under different concentration of rice husk (RH) extract

The result of the studies showed that highest lipid content for *Mycractiniumsp CCAP211/92IPOME-2*was up to 43.42 % (dcw) which was achieved under 3 g/L RH culture condition. The similar result was also recorded for *Mychonastesrotundus IPOME-3* with lipid content of 37.92% (dcw) also under the same culture condition.

Introduction of rice husk extract to the culture medium was intended to overcome low lipid productivity which occurredas a consequence of nitrogen stresscondition in an effort to enhance lipid content.Lipid productivity is an expression of combination of lipid content and biomass yield, is a more meaningful index rather than single lipid content to indicate the ability of lipid production of microalgae[27]. Several studies have reported the strategies to enhancelipid productivity.Some of them are the treatment of the culture with macro and micronutrients[28], regulation of light intensity [29] and by applyingcollective effects of nutrient stress (nitrogen, phosphorous and iron)[30]. Rice husk extract was used in this study based on the fact that rice husk is a lignocellulosic biomass which contains 75-90 % organic matter such as cellulose, lignin and the rest of mineral componentssuch as silica, alkalis

and trace elements,and due to high lignin content which composts very slowly, it can be converted into fertilizer[31]. As rice husk is an agricultural waste, it is expected making use of it would reduce production cost of microalga in a large scaleof cultivation.

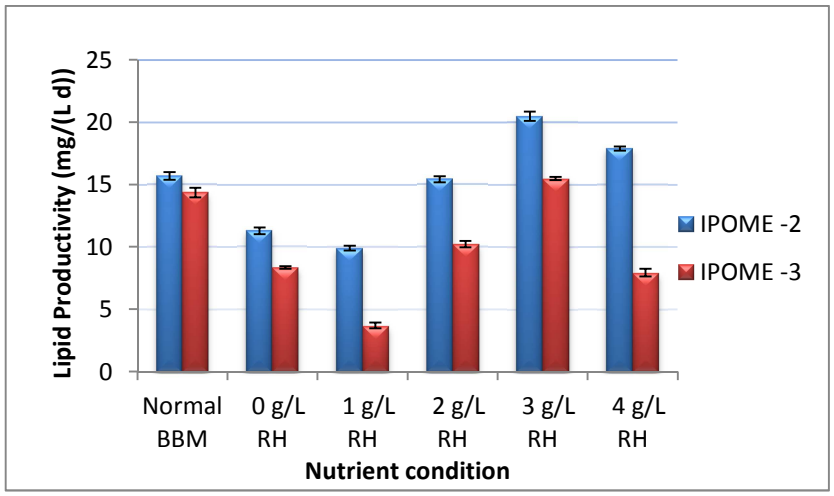
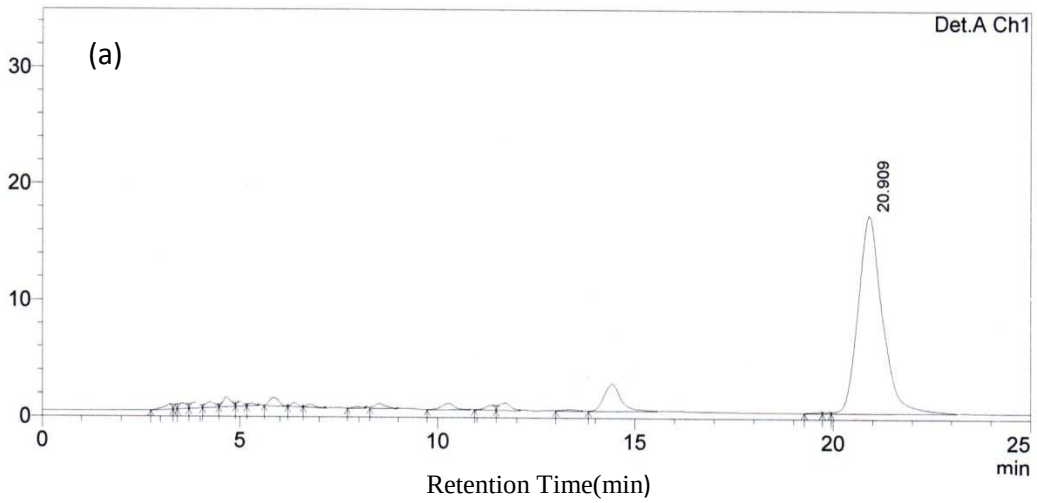


Figure 6. Comparison of lipid productivity of *Mycractiniumsp CCAP211/92IPOME-2 and Mychonastesrotundus IPOME-3*under various culture conditions

In case of lipid productivity, figure 6 shows the similar trend to that of lipid content in figure 5. Optimal lipid productivity resulted in this study was 20.9 mg/L d and 15.8 mg/L d under the culture with concentration of 3 g/L RH for *Mycractiniumsp CCAP211/92IPOME-2* and *Mychonastesrotundus IPOME-3* respectively, while three other tested culture conditions gave lower lipid productivity. Thus, it can be concluded that culturing *Mycractiniumsp CCAP211/92IPOME-2* and *Mychonastesrotundus IPOME-3* under culture condition of 3 g/L rice husk additionis asuitable approach to enhance lipid content as well as lipid productivity.

B-Carotene Identification

The results of HPLC analysis revealed that *Mycractiniumspehime IPOME-1*, *Mycractiniumsp CCAP211/92IPOME-2*and *MychonastesrotundusIPOME-3* had peaks at almost the same retention time as β-carotene standard.It confirmed that all the species tested contain β-Carotene. Calculation of β-Carotene in samples was based on the concentration of β-carotene standard and HPLC peak area. The results obtained were 0.009%, 0.003% and 0.77 % of dry cell weight) for *Mycractiniumspehime IPOME-1*, *Mycractiniumsp CCAP211/92IPOME-2* and *Mychonastesrotundus IPOME-3*repectively. Figure 7 shows HPLC chromatogram of*Mychonastesrotundus IPOME-3*compared to that of standard solution. Due to low β-carotene concentration in the two other strains, their chromatograms were not shown.



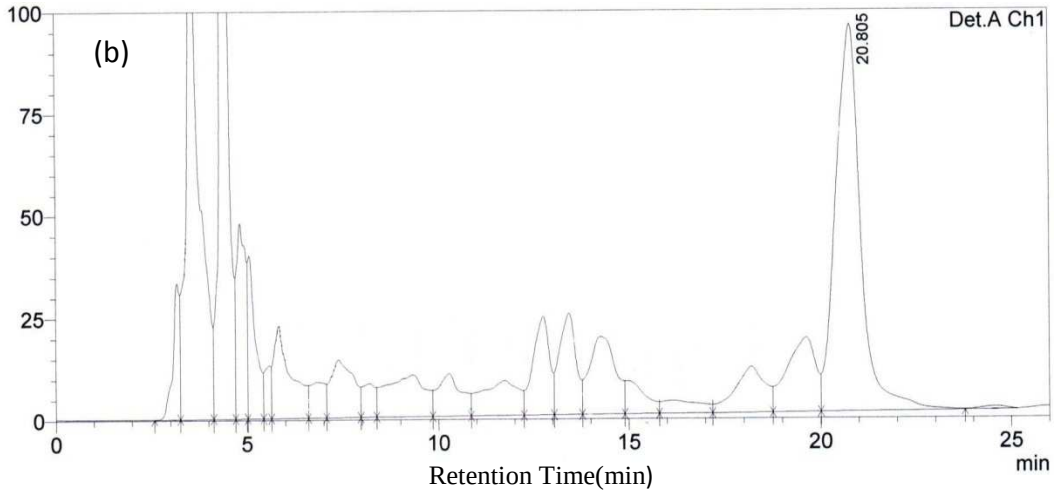


Figure 7. HPLC chromatograms of β-Carotene, for standard solution (a) and *Mychonastesrotundus IPOME-3*(b)

It can be concluded that β-Carotene content in *Mychonastesrotundus IPOME-3* is quite significant while the content *in Mycractiniumspehime IPOME-1* and *Mycractiniumsp CCAP211/92IPOME-2* were too low.In order to increase β-Carotene content, optimization should be carried out either by nutrient limitation, light regulation or any other approaches. In the chromatogram above, it was also detected that there were other peaks higher than that of β-Carotene, suggesting further study should be performed to explore other carotenoids for valuable products.

CONCLUSION

Mycractiniumsp CCAP211/92IPOME-2 and *Mychonastesrotundus IPOME-3* isolated from POME were found to be good candidate for biodiesel production. With lipid content of 43.42 % and 37.92 % for respective strains and supported by good profile of fatty acid components, it is feasible for both strains to be applied in a large scale. In addition, the use of rice husk can be applied to increase lipid content which at the same time enables it to reduce nutrient cost as rice husk exist abundantly as agricultural waste in the paddy field.This study also revealedthat *Mychonastesrotundus IPOME-3* can be explored for β-Carotene production, making these microalgae more valuable as feedstocks for bioproducts.

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