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Research Article

Immobilised *Chlorella vulgaris* as An Alternative for The Enhancement of Microalgae Oil and Biodiesel Production

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Abstract

Microalgae are a promising alternative for biodiesel production and a valuable source of fatty acid methyl ester (FAME). In this research, *Chlorella vulgaris* has been chosen as the suitable microalgae because this species was able to produce highest oils for biodiesel processing. Previously, sodium alginate (SA) was used to entrap the microalgae in the culturing process due to its low toxicity and high transparency. However, SA have some disadvantages such as bead disruption which leading to the loss of microalgae cell. Therefore, this research has been conducted to evaluate the oil production of immobilised *Chlorella vulgaris* using different matric systems at different ratios which are 0.3:1, 1:1 and 2:1. Currently, six matric systems have been developed, they are SA as a control, a combination of SA and chitosan (SA+CT), SA and carrageenan (SA+CR), SA and gelatin (SA+GT), SA and calcium alginate (SA+CA), and SA and sodium carboxymethylcellulose (SA+CMC). The microalgae was first cultivated, harvested and extracted to produce oil, prior to use in the transesterification process. The SA+GT showed the highest oil yield with 59.14% and a total FAME of 0.56 mg/g. The FAME profile of oil extracted microalgae showed high potential for biodiesel production as it consisted of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). The results proved that the combination of SA+GT had improved the oil yield and fatty acid composition as compared to the other matric systems, which may have useful application for the biodiesel industry.

Keywords: *Chlorella vulgaris*; Immobilised; Oil yield; Matric systems; Biodiesel; Microalgae

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1. Introduction

In the current situation, air pollution are the biggest challenge due to the consumption of fossil fuels. Reducing the use of fossil fuels would reduce the amount of carbon dioxide and other pollutants being produced [1,2]. Renewable energy is a promising alternative solution because it can fix CO_2 in the atmosphere through photosynthesis [3-5]. Biodiesel production have become one of the alternative source of renewable energy due to the lubricating nature and eco-friendly fuel produced from various feedstock [6,7]. Based on the fuel problem scenario, aquatic microorganisms such as microalgae have been suggested as an alternative feedstock for bio-

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diesel production, as microalgae contain oil which is suitable for transesterification process for biodiesel production [8,9]. The specialty of microalgae not only to produce high oil contents, they also able to produce other valuable compounds such as proteins and carbohydrates, that can be used in various industries like pharmaceutical, cosmetic, and food industries [10-12]. In an environmental aspect, microalgae are able to mitigate CO_2 by biological fixation through photosynthesis with efficiency of 10–50 times greater than land plants for biomass production on a long-term basis [3,10]. In addition, these microorganism are being used for wastewater treatment [13].

Chlorella sp., is one of the common microalgae used due to the high oil content ranging between 20 and 70 % of dry weight [14]. *Chlorella vulgaris* has been considered as a suitable species for commercial production of biodiesel. The selection of the *C. vulgaris* as a strain is based on growth rate and capabilities to accumulate high amount of oil under stress conditions within short periods [15,16]. Furthermore, the *C. vulgaris* has been recognized as a potential species for producing biodiesel in the industry because of its extraordinary high protein content, around 50% used in biofuel area and human food, and its ability to grow in different culture medium [17,18].

A method of harvesting the microalgae has been conducted to recover the biomass and oil such as centrifugation and filtration. Centrifugation may not suitable for the recovery of fragile cells such as microalgae because this method is too expensive and need higher maintenance [19]. The filtration method may also unsatisfactory for the recovery of smaller size of microalgae cells because low concentration of microalgae and not suitable for small size of microalgae [9]. Small size of microalgae implies a problem in the application of biotechnology process to those organism. The immobilisation method have been developed in order to solve the problem. The immobilisation method of microalgae is one of the unusual techniques in biotechnology that has a lot of advantages *i.e.* it needs lesser energy and is easier to handle [20]. Application of the immobilisation technique is widely used in diverse, metal removal, toxicity measurement, including as an organic pollutants removal. This method is highly regarded as a potential for biodiesel production as it can extract high oil content from microalgae [21]. Although immobilized cells were the easiest method to harvest the cells, it also have some disadvantages such as beads rupture due to the insufficient divalent ions. The formation of biofilm is to protect the microalgae layer cells and the interruption of the nutrients transportation and carbon sources is causing the mass transfer limitation to occur [22].

Usually, sodium alginate is chosen to be as a matric in the immobilisation technique due to its low toxicity and high transparency. The function of the sodium alginate is to limit the free movement of the microalgae. The matric of alginate gel is widely used in the immobilisation of microalgae. The previous researcher has used several matric systems for microalgae cultivation and the production of biodiesel. This is because the selection of matric systems is important to produce the maximum oil yield. Previously, sodium alginate was usually used to entrap the microalgae in the culturing process. However, sodium alginate may not be fully effective to produce the highest oil yield because there are certain limitations such as bead disruption that lead to the loss of the microalgae cells and space limitation for the microalgae growth in the matric beads [22,23].

In addition, alginate cell has low stability of the alginate gel, low porosity of the lattice structure and biocompability due to the small amount of polyphenol that is contained in the alginate gel that can harm the sensitive cells [24]. The study of Abu Sepian *et al*. [25] have focused to figure out the suitable combinations of natural matrices to simplify the harvesting process. Abu Sepian *et al*. [25] have used three combinations of matrices in order to compose calcium alginate (CA) and sodium alginate (SA), sodium carboxymethylcellulose (CMC) and SA, and mixed matrices (SA, CA, and CMC).

Thus, the limitation can be improved by choosing new combinations of matric systems. Notably, there is no study of matric systems between these combinations such as sodium alginate with gelatin, sodium alginate with carrageenan and sodium alginate with chitosan. Therefore, the different matrices were studied in order to obtain the maximum production of oil recovery using the microalgae immobilisation method. Immobilisation can be the alternative method for harvesting microalgae where the maximum amount of microalgae oil can be obtained through the selection of matric systems. Hence, the present study aimed to evaluate the oil production of immobilised freshwater *Chlorella vulgaris* using different matric systems at different ratios of 0.3:1, 1:1 and 2:1, for biodiesel production.

2. Materials and Methods

2.1 Materials

The *Chlorella vulgaris* strain used in this research was obtained from the Culture Collection of Algae and Protozoa (CCAP), Scottish Marine Institute, United Kingdom. The *C. vulgaris* was cultured in Bold Basal Medium (BBM) with 3-fold Nitrogen and Vitamins. The BBM consisted of four types- BBM (I), BBM (II), BBM (III) and BBM (IV). The chemical compounds of BBM (I) contained of 75.0 g of $NaNO_3$, 2.50 g of $CaCl_2.2H_2O$, 7.50 g of $MgSO_4.7H_2O$, 7.5 g of $K_2HPO_4.3H_2O$, 17.5 g of KH_2PO_4, and 2.5 g of NaCl. The chemical compounds of BBM (II) consisted of 0.75 g of Na_2EDTA, 97.0 mg of $FeCl_3.6H_2O$, 41.0 mg of $MnCl_2.4H_2O$, 5.0 mg of $ZnCl_2$, 2.0 mg of $CoCl_2.6H_2O$, and 4.0 mg of $NaMoO_4.2H_2O$. BBM (III) and BBM (IV) contained the chemical compounds of 0.12 g of Vitamin B_1 (Thiamin hydrochloride) and 0.10 g of Vitamin B_{12} (Cyanocobalamin), respectively.

Four chemicals were used for oil extraction and the transesterification process, they were methanol (CH_3OH), chloroform ($CHCl_3$), hexane (C_6H_{14}) and hydrochloric acid (HCl), while the chemicals used for the immobilisation method were calcium chloride ($CaCl_2$) sodium anhydrous carbonate (Na_2CO_3), sodium alginate, calcium alginate and sodium carboxymethylcellulose. The chemicals were purchased from Merck (Germany), Sigma-Aldrich (USA), Oxoid (UK) and R&M Chemical (UK). Overall, the experimental setup for the biodiesel production was carried out from *C. vulgaris* as described in Figure 1.

2.2 Media Preparation

The same procedure for Modified Bold Basal Medium (BBM) was reported by Abdullah *et al.* [27] and Culture Collection of Algae and Protozoa. [26] have four types of BBM which is BBM (I), BBM(II), BBM(III) and BBM (IV). BBM (I) and BBM (II) were autoclaved for 15 min at 120 °C to avoid any contamination. BBM (IV) was the most concentrated solution. Therefore, 1 mL of the concentrated solution was added to 99 mL of sterilised distilled water to make 100 mL of new BBM (IV). BBM (III) and BBM (IV) were then transferred slowly into 250 mL of schott bottle using a sterilised syringe and filter membrane. After that, adding 10.0 mL of BBM (I), 6.0 mL of BBM (II), and 1.0 mL to each of BBM (III) and BBM (IV) to 1 L of steri-

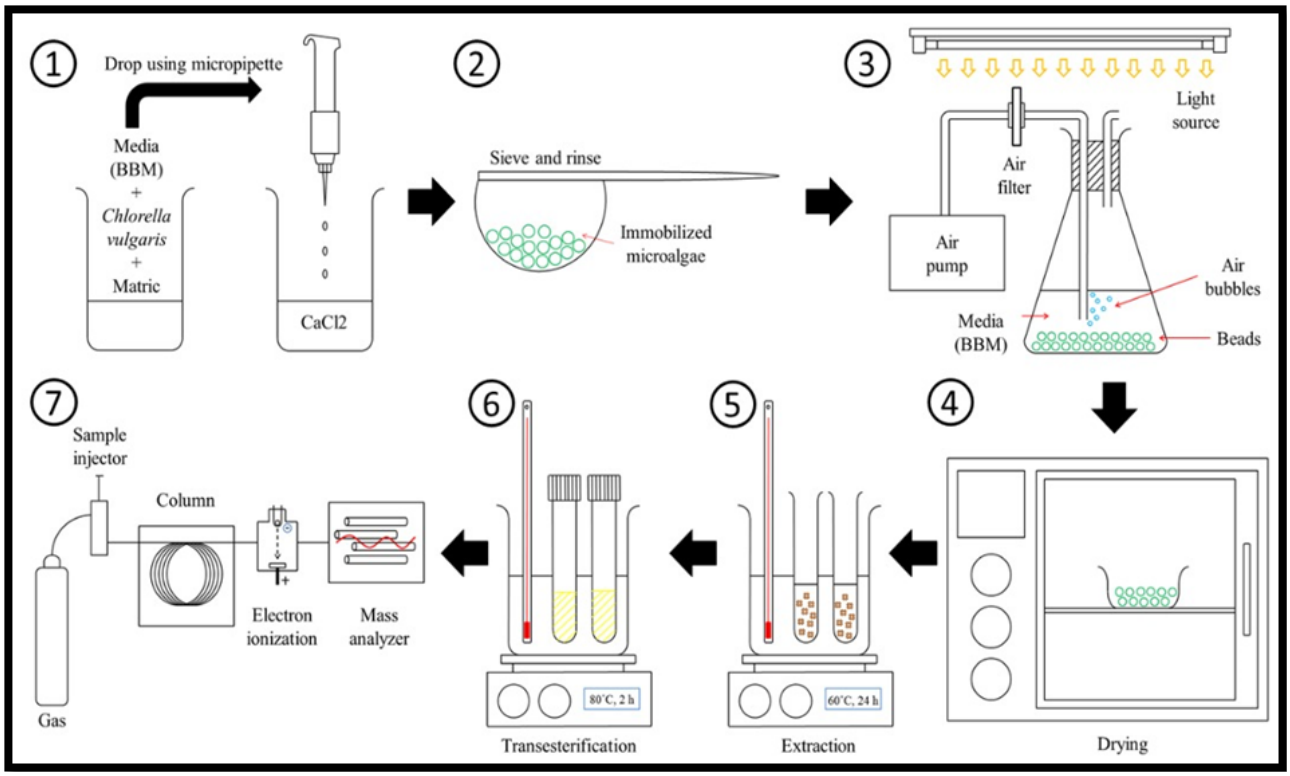

Figure 1. Schematic diagramm of the main experimental setup of biodiesel production consisted of (1) preparation of the beads, (2) Sieve and rinse the beads, (3) Immobilisation culture of *Chlorella vulgaris*, (4) Drying the microalgae biomass, (5) Extraction process, (6) Transesterification process, (7) Analysis of fatty acid methyl ester (FAME).

lised distilled water to prepare the culture medium solution.

2.3 Preparation of Free Cell Culture for *Chlorella vulgaris*

The *C. vulgaris* was seeded into 2 L schott bottle filled with sterilised distilled water that contained BBM (I) until BBM (IV) at a temperature of 25 °C. Two fluorescent lamps (Philip TL-D 36W/865, light output 3050 lm) were used to expose the culture for continuous illumination and the culture was continuously aerated with bubbling air (3.5 L/min) within 11 days to provide constant air pressure [27].

2.4 Preparation of Immobilised Microalgae Beads

2.5 mL of BBM (I), 1.5 mL of BBM (II), and 0.25 mL for each BBM (III) and BBM (IV) were added in a conical flask filled with 250 mL of sterilized distilled water (DW) for the preparation of the immobilised beads. Sodium alginate (SA) solution of 2% (w/v) was prepared as a control. The solution was mixed with 10 mL of microalgae solution at volumetric ratio of 1:1. The mixture was stirred until all SA was completely dissolved. After that, 2 w/v% of $CaCl_2$ solution was prepared by adding 0.6 g of $CaCl_2$ into 30 mL of sterilised distilled water. The immobilised beads were then prepared by slowly dripping the microalgae solution into the $CaCl_2$ solution. Beads were formed directly and left for 1 h at room temperature to harden and stabilised the beads formation. Then, the beads were filtered and rinsed 3 times with sterilised distilled water. The hardened beads were placed in 250 mL of conical flask filled with 250 mL of growth medium for 11 days of cultivation. The culture was continuously aerated by bubbling air through it at a constant pressure. The above methods were repeated with different combinations of matric systems. The combinations resulted from a 2% (w/v) of each matric systems comprising the 1:1 ratio of SA and calcium alginate (CA), 1:1 of SA and sodium carboxymethylcellulose (CMC), 1:1 of SA and gelatin (GT), 1:1 of SA and chitosan (CT) and 1:1 of SA and carrageenan (CR). The experiment was continued using the best matric systems at different volumetric ratio of 0.3:1 and 2:1 [25]. The preparation of immobilised beads was described in details as shown in Figure 2.

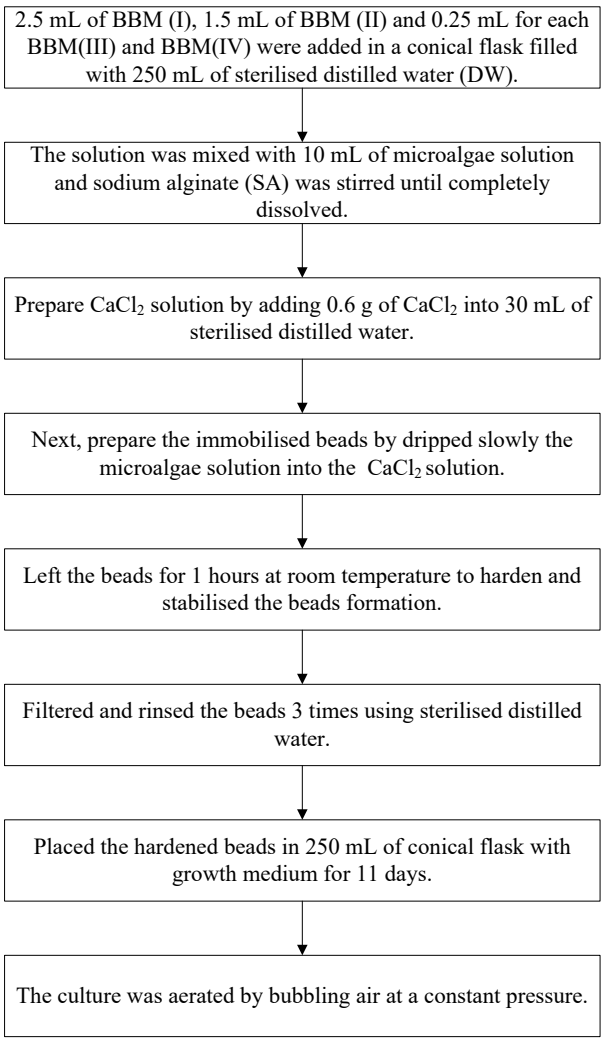

Figure 2. Experimental protocol for preparation of immobilised microalgae beads.

2.5 Determination of Microalgae Biomass

Beads of good condition were selected and each of 5 immobilised microalgae beads were solubilised in 2 mL of sodium carbonate anhydrous solution and dried in an oven at 80 °C for 24 h. After that, the dried weight of biomass (DW) was collected for oil extraction [25].

2.6 Oil Extraction

The oil extraction from the microalgae biomass was conducted using the solvent extraction method. The microalgae biomass (0.07 g) from the immobilisation methods was extracted to determine the oil contents of microalgae. The biomass was mixed with 5.5 mL of distilled water and sonicates (sonicator Fisher brand FB15051) for 5 min to lyse the cell. 8 mL of methanol and 4 mL of chloroform were added to the microalgae biomass for an extraction process at 65 °C for 24 h. After the extraction process, the mixture was centrifuged at 4000 rpm for 2 min and the bottom layer was collect-

ed. Vacuum oven was used to evaporate the solvent and the oil were left. The weight of the oil was measured gravimetrically [25]. The extraction of oil was carried out according to the procedure described in Figure 3.

2.7 Transesterification Method

The mixture consisted of oil with 4 mL of hexane, 4.25 mL of methanol and 0.215 mL of hydrochloric acid. The solution was heated on the hot plate at 85 °C. After 2 h of transesterification process, the solution was centrifuged at 4000 rpm for 2 min [25]. Then, two layers were formed where the upper layer consisted of fatty acid methyl ester and the lower layer contained glycerol.

2.8 Analysis of Fatty Acid Methyl Ester

Fatty acid methyl ester (FAMEs) analysis was performed using a gas chromatography mass spectrometry (GC-MS Agilent 7890 A). The samples were injected with the intial oven temperature at 40 °C. It is vaporised and carried onto the capillary column which is 30 m length and 0.25 m. Then it was held for 5 min and raised to 300 °C at a ramping rate of 10 °C/min. Then, the injector temperature was set at 260 °C for 3 min [25]. The fatty acid composition in the microalgae oil was observed and the result was recorded.

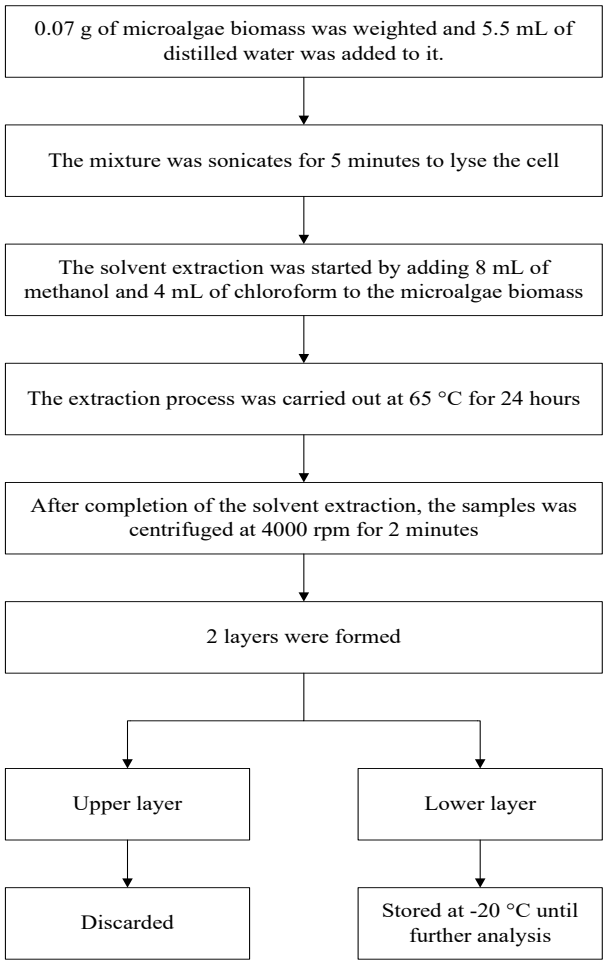

Figure 3. Experimental protocol for oil extraction of *Chlorella vulgaris* biomass.

2.9 Scanning Electron Microscopy (SEM)

In this study, a total of 6 beads immobilisation of microalgae for each combination of matrix systems prepared in Section 2.4 were scanned for the element study. The surface morphologies of the beads microalgae was examine by Carl Zeiss Supra 35VP SEM (Germany). All the sample was flushed with a platinum coating by JEOL Auto Fine Coater JFC-1600 to avoid any charge build-up by the electron beam which was often called space charge effect during surface imaging.

3. Results and Discussion

3.1 Comparison of Pore Immobilised Microalgae Beads

The comparison of pore immobilised microalgae beads at different matric systems was performed to investigate the presence of pores on the surface of beads using scan electron microscopy (SEM) analysis. Figure 4(a) shows the SEM image of pore immobilised microalgae using SA. As is shown, the outer membrane layer beads using a single matric of SA has a flat surface structure with regular pores. This phenomenon might have occurred due to the formation of beads having small pore size that can cause hindrance to enter the media in the beads [28]. The surface beads of Figure 4(b) show that the combination of SA+GT is more compact and has irregular size of pore with a rough surface. The combination of SA+GT seems to increase the porosity of the beads and is made up of a large number of pores with biggest pore size. Since sodium alginate beads have scaffold porosity, when a matric of SA and GT combine it forms many pores on the surface of the beads, which helps the mass transfer of nutrients and CO_2 between the outer and inner part of the microalgae beads [29]. Figure 4(c) shows the surface morphology images of the outer membrane layer of beads using SA+CA where the beads have large and irregular pores, whereas the structure of bead for Figure 4(d, e & f) were approximately the same with only a few number of pores existing on the surface beads. The combination of SA with CMC, CR and CT could be not suitable matrices due

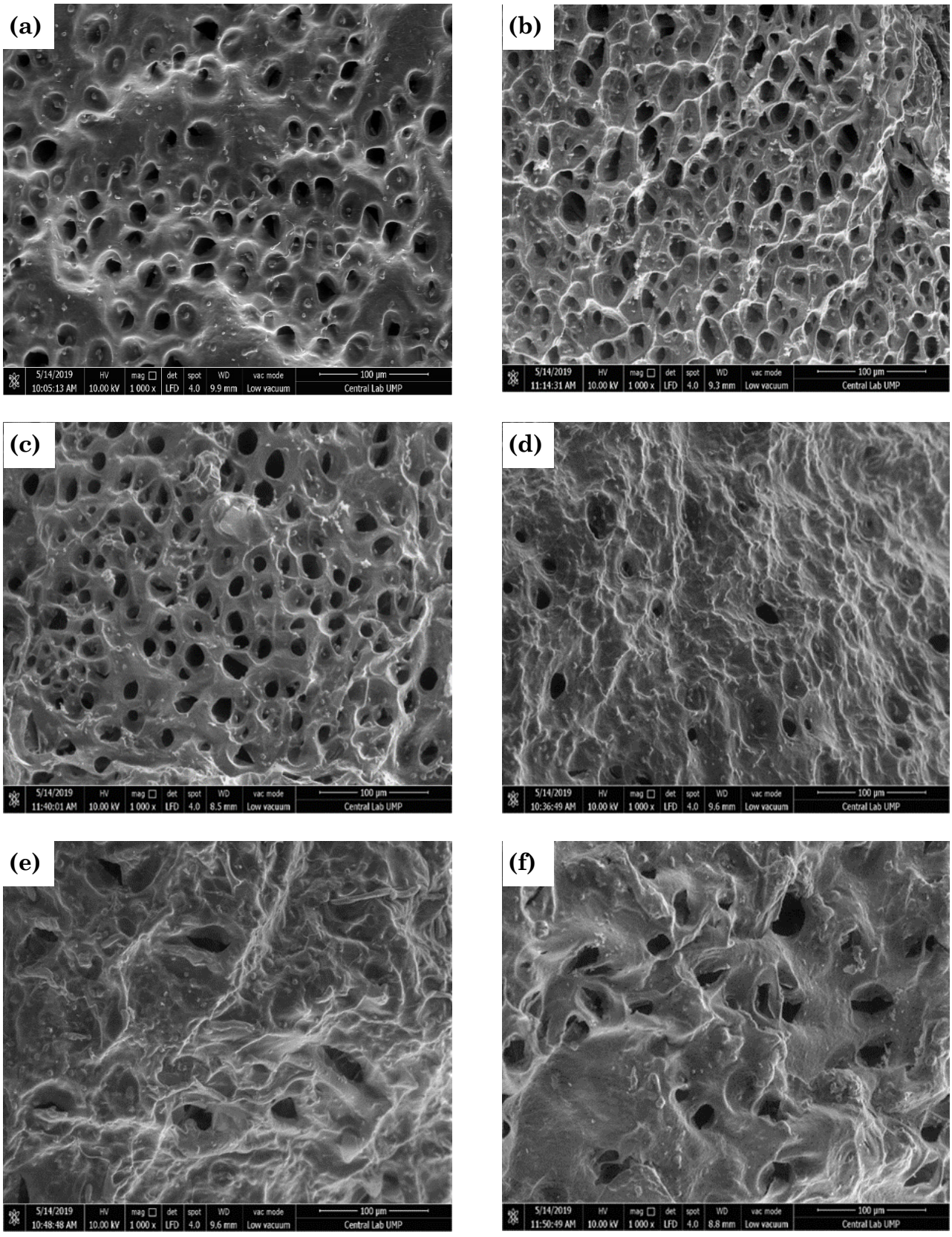

Figure 4. SEM images of a pore immobilised microalgae beads using matric systems of (a) SA, (b) SA+GT, (c) SA+CA, (d) SA+CMC, (e) SA+CR and (f) SA+CT with 1000× magnification.

to the weakest interaction between the combinations of matrices. Only the least number of pores were formed on the surface and this phenomena may reduce the flow of nutrient in and out of the beads. Therefore, it is undeniable that the pore size of each bead will be different based on the different matric systems used, since the characteristic of each matric were different.

3.2 Effect of Different Matric Systems on Oil Production of Immobilised Cell

In the production of oil, the selection of matric systems was important because it may effects the immobilised performance of beads. Previously, sodium alginate (SA) was used in entrapment of immobilised microalgae and use as a single matric. However, SA have some limitation such as limited space for microalgae to growth and easy to rupture. Then, the combination of matric systems was proposed to study the effect of matric systems on the oil production. As shown in Figure 5, there was a different amount of oil that had been obtained from the combination of matrices and single matric of SA at the volume ratio of 1:1. The highest production of oil extraction from *Chlorella vulgaris* biomass that was using the matric systems was SA+GT which is 59.14% at the volume ratio of 1:1, followed by the combination of SA+CA (56.86%), SA+CT (43.14%), SA+CR (24.29%), SA+CMC (32.29%) and SA (29.43%). Among the results, the immobilised beads by single matric of SA that gave the lowest yield of oil was 29.43%.

Oil extraction of immobilised microalgae using a combination matric system of SA and GT at the volume ratio of 1:1 was found to be the most effective matric compared to other combination of matric systems and single matric of SA. In this study, solvent extraction was used as this method was efficient techniques to extract oil from microalgae biomass. The cell of *C. vulgaris* biomass can be easily extracted into the solvent as this method was high solubility towards lipid which has a capability to extract the intracellular lipid that lies between the cell walls of microalgae [25]. Adding to it, at the temperature of 65 °C, the cell of *C. vulgaris* was break and the weak interaction affect the cell wall. As a result, *C. vulgaris* biomass can be easily extracted into the solvents.

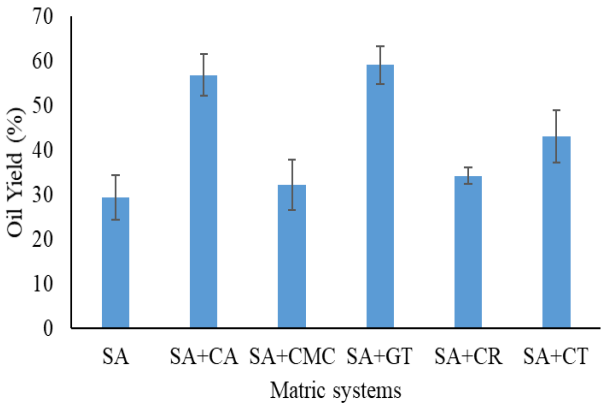

Figure 5. Oil production of *Chlorella vulgaris* with different matric systems at 1:1 ratio.

The oil production was higher for microalgae beads that used the combination matric systems of SA+CA, SA+CMC, SA+CT and SA+CR compared to SA. This result was similar to the research done by Abu Sepian *et al.* [25], where the higher oil yield of immobilisation microalgae was achieved when the combination matric was used compared to single matric. This observation might have happened due to the physical characteristics of SA which disturbed the microalgae ability to extract the oil. The mass transfer occurrences were obstructed in the sodium alginate beads because of scaffold porosity [29]. The gelatin used as a combination matric with SA in the immobilised cells produced a higher oil yield, this might be due to its characteristic which is easily obtained with high porosity and good mechanical strength [30]. This finding was parallel with the surface morphology microalgae beads of SA+GT as is shown in Figure 4(b) where the beads surface is porous and is made up of a large number of pores with the biggest size of pores. In addition, the gelatin itself may facilitate great diffusion of the mass transfer of media into the beads [31].

Correspondingly, the combination of matric systems in this study has shown higher production of oil yield, although the observation from Lam and Lee [21] have shown contradictory result. Lam and Lee [21] reported that when the SA was combined, the yield of oil extraction had become low while oil recovery was higher in the single matric. The observation was different in the study of Abu Sepian *et al.* [25] and Rushan *et al.* [32], which stated that the amounts of oil yield obtained from combination of matrices with SA had a significant difference amount of oil. The previous study was in line with this current study where the combination matric systems showed a high percentage of oil yield compared to SA. However, the single matric of SA can still be considered to be used as a matric as long as it is applied in appropriate proportions in order to

achieve a high yield of oil extraction. In addition, SA is nontoxic and permeable, so it is still suitable to be used as a matric system for immobilisation method [22]. From the result in this research, it has been proven that a combination of matric systems gives a huge impact to increase the oil yield compared to the single matric. Thus, SA+GT was chosen as the best matric systems to be used for the next stage of study in Section 3.3.

3.3 Effect of Volumetric Ratio of Sodium Alginate and Gelatine to Microalgae

The common ratio of 0.3:1 might be undergo beads instability and beads leakage. Therefore, a suitable matric to microalgae of volumetric ratio need to be determine to obtain the maximum oil yield. The study was important because the volumetric ratio may affect the performance of immobilised beads on the oil production. Figure 6 shows the effects of the combination matric system of SA and GT on the oil yield production of immobilisation microalgae with different volumetric ratio. The ratio of matrices to microalgae were manipulated at 0.3:1, 1:1 and 2:1. In this study, the microalgae strain that was used was kept constant at 10 mL while the amount of matric used was different based on the ratio. As in Figure 6, microalgae beads that are synthesized with a combination of SA and GT at a ratio of 1:1 have exhibited the highest production of oil which is 59.14%. The result was followed by the volumetric ratio of 2:1 with a percentage of oil yield at 33.29% whereas the lowest oil yield was obtained using the volumetric ratio of 0.3:1 which was 25.52%.

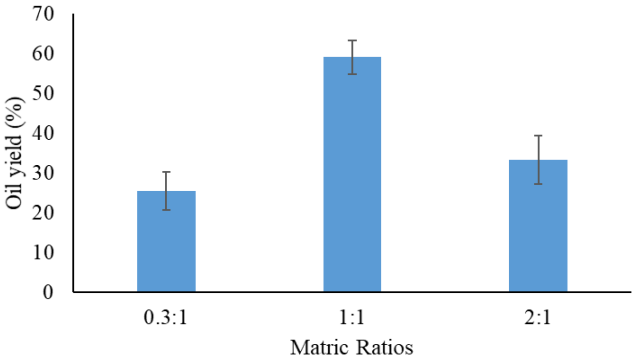

Figure 6. Oil production with different matric ratio.

Microalgae beads with a volumetric ratio of 0.3:1 might improve the mass transfer of nutrients and CO_2 into the beads due to the thin matric layer of biofilm formed. However, the thin biofilm formed could be unstable and could easily shrink. Therefore the volumetric ratio of 0.3:1 was not suitable to culture microalgae for long durations. The production of oil was increased when the volumetric ratio of matric to microalgae increased from 0.3:1 (25.52%) to 1:1 (59.14%). This might be due to the microalgae beads which did not shrink and had the largest pore in the surface of the beads. However, further increase of volumetric ratio at 2:1 showed reduction in oil production, this may happen because mass transfer of nutrients and CO_2 was insufficient uptake into the microalgae bead, so it led to the reduced production of oil yield. In addition, these results were in agreement with Lam and Lee [12] when the volumetric ratio of matric to microalgae increase, the oil production was reduced. This was because of the increasing thickness of the matric layer biofilm. Furthermore, both sodium alginate and gelatin were natural carriers with hydrophilicity and biocompatibility as the characteristic advantages [14]. Thus, the microalgae with the volumetric ratio of 1:1 were chosen as the best volumetric ratio.

Table 1. FAME composition (mg/g DW) of *Chlorella vulgaris* for different matric systems at volumetric ratio of 1:1.

Fatty acid methyl ester (FAME)	Number of carbon atom	SA	SA + CA	SA +CMC	SA + GT	SA + CR	SA + CT
Palmitic acid	(C16:0)	0.19	0.17	0.18	0.33	0.24	0.34
Stearic acid	(C18:0)	0.12	0.09	< 0.01	0.18	< 0.01	0.17
Oleic acid	(C18:1)	0.08	0.07	0.01	0.03	0.04	0.02
Linoleic acid	(C18:2)	0.02	0.05	0.03	0.01	0.02	0.01
Linolenic acid	(C18:3)	0.01	0.05	0.02	0.01	< 0.01	< 0.01
Total SFA		0.31	0.26	0.18	0.51	0.24	0.51
Total UFA		0.11	0.17	0.06	0.05	0.06	0.03
Total FAME		0.42	0.43	0.24	0.56	0.30	0.54

3.4 Fatty Acid Methyl Ester (FAME) Composition

The composition of fatty acid methyl ester (FAME) for the *C. vulgaris* was analysed using gas chromatography mass spectrometry (GC-MS Agilent 7890 N). FAME that containing carbon atom (16-18) was considered for producing biodiesel [33]. Table 1 shows the FAME profile in *C. vulgaris* cells which consisted of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). It was found that oil extracted from immobilised microalgae biomass has high potential for biodiesel production due to the similar fatty acid methyl ester profile as other oil bearing crops [34]. Based on Table 1, the fatty acid profiles were mainly composed of palmitic acid and stearic acid. The results from the previous study of Linares *et al.* [35] was in line with the current study that palmitic acid was the main FAME in all cultures and strains, along with the stearic acid (C18:0), they were the single two unsaturated accumulated FAME. Sharma *et al.* [36] reported that highly saturated fatty acid give an excellent cetane number and oxidative stability to biodiesel. Biodiesel quality is directly related to cetane number which shows ignition quality in engine. From Table 1, the oleic acid was increased, being the third FAME that was more produced. A good quality biodiesel should also have a high content of oleic acid which increases the oxidative stability for a longer storage of fuel [37].

The length of the carbon chain and degree of unsaturation of FAME are the important characteristics of good quality biodiesel. From Table 1, the fatty acid profile of this microalgae comprises of saturated fatty acid (SFA) which represented stearic acid (C18:0) and palmitic acid (C16:0) and unsaturated fatty acid (UFA) with the presence of linolenic acid (C18:3), oleic acid (C18:1) and linoleic acid (C18:2). The results of the total FAME show the highest amount for SA+GT (0.56 mg/g). This was followed by 0.54 mg/g (SA+CT), 0.43 mg/g (SA+CA), 0.42 mg/g DW (SA), 0.30 mg/g DW (SA+CR) and 0.24 mg/g (SA+CMC). Since the matric systems of SA+GT gave the highest production of oil extraction, this result proved that the total FAME of *C. vulgaris* increased as the oil yield increased. According to Yeh and Chang [38] the chain length of microalgae oil and saturated fatty acid contents may cause significant changes in the biodiesel properties.

According to the European regulation (EN 14213 and EN 14214), more than 12% of linolenic acid (C18:3) was not favorable for a good quality of biodiesel. Based on the results obtained, linolenic acid was represented as 2.38 % (SA) followed by (SA+CA), (SA+CMC) and (SA+GT) with 11.63%, 8.33% and 1.79% respectively. Whereas the linolenic acid produced were less than 1% for SA+CR and SA+CT. Since, the composition of fatty acid of linolenic acid for all microalgae biomass that used matric systems was less than 12%, the fatty acid methyl ester had meet the requirement of the European Standard EN 14214. Thus, immobilised biomass from immobilised cells can be a good potential candidate for biodiesel production since the FAME compositions were able to meet the standard of European regulation for transportation uses.

4. Conclusions

Each combination of matric systems had formed its own structure and membrane layer which affected the bead structure and the transfer of nutrients between the outer and inner part of the membrane. In this study, the combination matric of SA and GT at the volumetric ratio of 1:1 was successfully carried out to enhance the production of oil compared to the single matric of SA. It has been proven that oil extracted from immobilised *C. vulgaris* biomass has high potential for biodiesel production due to its similarity in fatty acid methyl ester profile compared to other prominent oil crops.

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