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RESEARCH ARTICLE

Enhanced Mixing Towards the Production of Fatty Acid Methyl Esters by In Situ Transesterification of *Eucheuma Cottonii*: Experimental and Computational Fluid Dynamics (CFD) Analysis

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Abstract Biodiesel is one of the alternative replacements for the conventional fossil fuel-based diesel as the demand for energy is increasing with the increasing world population. The production of biodiesel (also known as fatty acid methyl esters (FAME)) from macroalgae (*E. cottonii*) is the focus in this study. Conventionally, production of FAME from macroalgae will be carried out through the two-step transesterification processes, which consists of extraction of algal oil (lipids and free fatty acids) and subsequent transesterification step. However, the two-step transesterification method is time and energy consuming processes and thus ways to enhance the production yield of the biodiesel produced are being studied. This paper concentrates on utilization of enhanced mixing technique via in situ transesterification (ISTE) process in FAME production which elucidate the effects of reaction time and mixing intensity. The ISTE reaction was carried out at the ratio of biomass: Methanol (MeOH): Hydrochloric Acid (HCl) (w/v/v) of 1:20:5 and a reaction temperature of 60 °C. The total reaction time for the reaction was 90 minutes, where samples were collected at 30 minutes interval. The increase in reaction time and mixing intensity gave a significant positive impact on the production of FAME. At 90 minutes of reaction time and impeller rotational speed of 900rpm, the maximum amount of methyl palmitate and methyl stearate produced were 0.5729 wt% and 0.0559 wt% respectively. The results of contour of volume fraction (VF) of palmitic acid obtained from Computational Fluid Dynamics (CFD) analysis from the Aspen Fluent Software is in good agreement to the experimental results of the study.

Keywords: Biodiesel, In Situ Transesterification, Fatty Acid Methyl Ester (FAME), Seaweed, Modelling, *Euchema cottonii.*

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Introduction

Various anthropogenic emissions have caused gradual increase in concentration of CO_2 in the atmosphere, and it has disturbed the global carbon cycle. Mitigation of CO_2 by biological means have become one of the most feasible, sustainable, and efficient solution [1]. During the growth of biomass, they consume CO_2 in the air through photosynthesis. Since the growth rate of terrestrial plants are quite slow, the indirect CO_2 capture by plants is considered an inadequate solution. Besides, the capture of CO_2 in agriculture only contributes about 3-6% of fossil fuel emissions approximately [2]. On the other

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hand, microalgae, a group of rapid growing unicellular microorganism has been widely studied [3] as it holds the potential solution due to its abundance and fast growth rate. It can capture solar energy and CO_2 with approximately 50 times greater efficiency compared to the terrestrial plants due to its simpler structure [4]. Like microalgae, macroalgae (also known as seaweed) are multicellular, large-size algae, visible with the naked eye [5] share the similar advantages and it is found that the potential of capturing carbon by cultivated seaweed can exceed that of terrestrial farmed crops, with minimally intensive agricultural practices and nutrient requirements [6]. Macroalgae can produce more biomass per acre in offshore marine farms compared to terrestrial crop. It can be harvested sustainably as it does not require the usage of valuable arable land. It is also considered as a better option as no unsustainable nutrients are required. Besides capturing CO2, the biochemical composition of macroalgae adds its advantages to be used as a promising feedstock for the production of biofuels [6]. Hence, macroalgae can serve dual purpose by mitigating global warming through fixation of carbon dioxide and as a promising feedstock for renewable biofuels to satisfy the increasing energy demand.

Biofuel from macroalgae appears to be a promising replacement of conventional petroleum-based fuels. One of the most promising forms of biofuel that could replace diesel would be biodiesel. Biodiesel consists of long-chain alkyl (methyl, propyl or ethyl) esters and usually made from vegetable oil or animal fats. Biodiesel is produced chemically by reacting lipids (e.g. vegetable oil, animal fat (tallow)) with an alcohol producing fatty acid esters. As such it is a renewable fuel with added advantages such as low toxicity, high oxygen content, low emission of carbon monoxide and increased biodegradation [7]. Biodiesel burns cleaner and can be used in any diesel engine with only little or no modification [8].

Biodiesel can be synthesized from various sources. Biodiesel that are produced from edible vegetable oil such as rapeseed and palm oil is called first generation biodiesel. Biodiesel from non-edible oils such as Jatropha is termed as second generation and those from algae and waste oils are the third-generation biodiesel [9]. However, limited availability and high cost of the first- and second-generation feeds make the third-generation feedstock as the most suitable feedstock for production of biodiesel. In this paper, a red macroalgae (*Eucheuma cottonii*) will be used as feedstock to synthesize biodiesel as it does not require special conditions to grow, and thus does not directly compete with food crops for arable land. It was abundance in the region of Southeast Asia where Indonesia and Malaysia are major producers of this type of algae. Figure 1 shows the visual appearance of *Eucheuma cottonii*.

Figure 1. Dried *Eucheuma cottonii*

Biodiesel has been produced conventionally using two-step transesterification process where the first step is the extraction of algal oil using a suitable solvent and its subsequent solvent separation. In the second step the purified algal oil undergoes catalyst assisted reaction with low chain alcohol where fatty acids constituents in algal oil and its triglycerides esters undergoes esterification and transesterification processes respectively to produce biodiesel (FAME). The produced biodiesel later being purified from catalyst and the excess alcohol. This approach needs higher capital cost as well as operational cost compared to direct transesterification method or also known as In situ Transesterification (ISTE) method. In the ISTE, the alcohol used for the transesterification of lipids in algal biomass acts simultaneously as both extractive solvent and reactants in the combined extractive reaction processes when converting the algal oil into FAME thus eliminating the need to use lipid extraction procedure [10].

Figure 2 shows the mechanism of ISTE process between triglyceride (TG) and methanol. Generally, 1 mole of TG (vegetable oil or animal fats) reacts reversibly with 3 moles of alcohol to produce 3 moles of alkyl esters (biodiesel) and 1 mole of glycerol, a co-product [11]. The alkyl ester produced depends on the type of mono-alcohol being used. Fatty acid methyl ester (FAME) is produced when methanol is used while fatty acid ethyl ester (FAEE) is produced when ethanol is used as the alcohol.

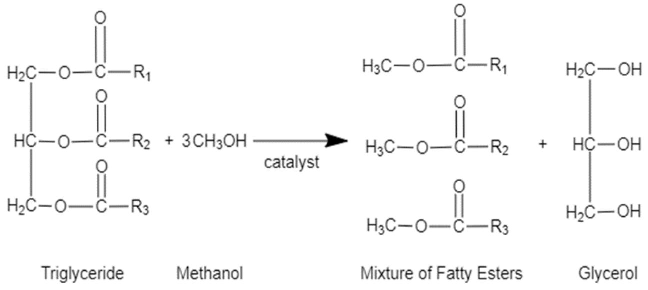

Figure 2. A typical transesterification process

During the FAME production, there are several reaction parameters that need to be controlled, including methanol amount, concentration of catalyst (either acid or base catalyst), reaction time, and reaction temperature [12]. There are also certain studies found that stirring can give positive influence towards FAME production through ISTE [13]. However, it can be said that various research is being conducted on biodiesel synthesis, but there is not much work has been conducted on large scale ISTE of the macroalgae to produce biodiesel [14]. Most research that has been carried out for the production of FAME are in small laboratory scale, mostly only involving simple laboratory apparatus. In this work, the use of a real continuous stirred tank reactor (BR) will be a better approach for scaling-up purposes. However, currently, due to biodiesel's high cost of production, more study need to explored to lower the cost. Hence, this issue is tackled with some economical approaches for the biodiesel production in this paper. A single step direct ISTE of macroalgae biomass is used to synthesize biodiesel. The oil extraction and transesterification happen at a time with the aid of an impeller of 6 blades in a BR. The higher rate of mixing provided by BR is expected to increase the conversion and yield of FAME produced. The mixing properties in the BR also has to be studied in order to enhance the FAME production.

Materials and Methods

Experimental

Figure 3 shows the glass Batch Reactor used in this study. The BR is connected to a DAQ link to the MATLAB (Simulink) Software. There are two water tanks and a heater connected the reactor which act as the heating medium for the reaction. The biomass used in this study was *E. cottonii*. The seaweed was initially dried in an oven at 50 °C for 8 h. The dried *E.cottonii* was then grinded into smaller sizes in a grinder. Then, the raw material was stored in a container and ready to be used in the experiment. Then, 50g of dried *E.cottonii* was weighed. 1000ml of MeOH and 50ml of acid catalyst was measured. The acid catalyst used in the experiment was hydrochloric acid (HCl). The ratio of biomass:MeOH:HCl (w/v/v) was 1:20:5. This ratio was kept constant for all runs in the experiment. The flow of heating medium was started and MeOH and HCl were mixed in the BR followed by dried *E.cottonii*. The impeller rotational speed was varied at three different speeds: 300, 600 and 900rpm. The reactor temperature was set at 60 °C for the whole duration of experiment. It is because 60 °C is the optimum temperature for the best transesterification process [10,11]. The total duration of each experiment was 90 min, where samples were collected every 30 min. The samples collected at 30 min intervals contains the unreacted reactants, the catalyst, FAME and side product (glycerol). FAME must be separated prior to being analyzed on gas chromatograph. The samples were put into the separating funnels and kept for 24 h to settle the biodiesel and sediment layers clearly. After the gravity settling, two major layers were formed: FAME at the top and mixture of methanol and glycerol at the bottom [15]. Glycerol was discarded, MeOH was evaporated and distilled water was used to wash the FAME 2-3 times and swirled gently. This step was done to remove any residual soap or catalyst in the biodiesel layer. Then, the FAME layer was collected.

The FAME Mix RM-5 standard was injected into the gas chromatograph to obtain the specific retention times for the FAME. The FAME samples dissolved in n-hexane solution was analyzed by the same gas chromatograph (Shimadzu GC-2010). The GC was equipped with flame ionization detector (FID) and capillary column BPX70 (30 m × 0.32 mm × 0.25 µm). The column temperature was set at 120 °C initially and increased at 3 °C/min up to 250 °C. Different compounds were separated at specific retention time, while the peak areas in the chromatogram represent the concentrations of the compounds in the samples injected.

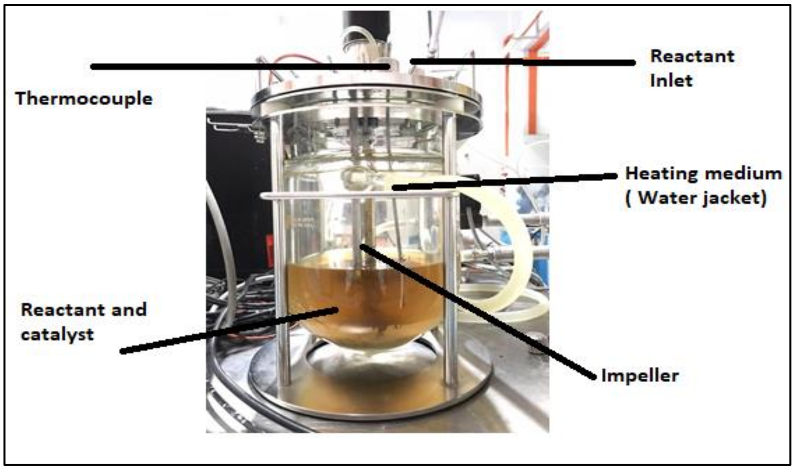

Figure 3. Batch Reactor (BR) used for the FAME production

Computational Fluid Dynamics (CFD) Analysis

The CFD simulations for the BR used in the experiment was conducted with the ANSYS Fluent (Fluid Simulation Software) version ANSYS 2019 R3. The geometry of the BR consists of a stirred vessel and an impeller with 6 blades and was drawn by using the Design Modeller. Prior to conducting the simulation, a mesh sensitivity analysis (Grid-independent test) was conducted. The main purpose of this analysis was to verify the minimum grid resolution required to develop a solution and tetrahedrons method was used. The built model was meshed using the ICEM software and imported to ANSYS 2019 R3, where the simulation was performed. Properties of MeOH is obtained from the readily available ANSYS database. However, the properties of methyl palmitate were entered manually [16]. The Euler-Euler multiphase model was chosen and the reactor was filled with MeOH initially. The impeller was set to rotate at 300rpm. The changes in volume fraction of triglycerides are going to be determined. Since the simulation is done for immiscible fluids, the Volume of Fraction (VOF) multiphase model is used. The step size used in for the calculation is 0.01 s. A total iteration of 500 was set to run. The contour of volume of fraction profile of the methyl palmitate obtained for 300, 600 and 900 rpm were discussed.

AutoDock Vina program was used to run docking simulation. A total of 15 polyphenol compounds from *A. occidentale* were docked with AG and DPP-4 enzymes at the binding site. Then, as a control, acarbose and sitagliptin each was docked to AG and DPP-4 enzymes respectively. The docking coordinate and parameter file for proteins and ligands were prepared by using Autodock 4.2 version. The preparation of the coordinate file included the addition of hydrogen atoms, Kollman charges and Gasteiger charge to the protein or target receptor [28]. Then, the output file for the protein was saved in PDBQT file format. Next, the grid boxes with the size 20x24x24 and 24x20x26 were set using AutoGrid on AG and DPP-4 enzymes respectively, which covered all the targeted binding site regions which the ligand was supposed to bind. The grid spacing was 1.000 Å and the search was based on the Lamarckian Genetic Algorithm to search the best conformer [28]. As for ligands, all compounds were assigned with Gasteiger charges and saved as PDBQT file format [14]. As all the coordinate files for protein and ligands were prepared, the docking simulation was run using AutoDock Vina program which the protein was treated as a rigid body. In contrast, the ligand was flexible during the docking process. Based on the docking result, the binding energy of the protein-ligand complexes was evaluated.

Results and Discussion

Analysis on the Synthesis of FAME

The experiment on the synthesis of FAME from *E. cottonii* has been carried out by varying the impeller rotational speed at 300, 600 and 900 rpm. For each speed, three samples were collected at every 30 min of reaction time and the sample was taken twice each time to get an accurate result. It was found that the most abundant FAME produced from *E. cottonii* was methyl palmitate (C16:0) (scientific name: hexadecanoic acid methyl ester). From the chromatogram analysis, there was another component which had same retention time as the component found in FAME standard which is methyl stearate (C18:0) (scientific name: methyl octadecenoate). Hence, these are two of the many FAME components found from *E. cottonii*.

Table 1. FAME produced from ISTE of *E. cottonii* at 300 rpm

FAME produced	30 min		
	Run 1 wt (%)	Run 2 wt(%)	Average wt (%)
C16:0	0.1580	0.1460	0.1522
C18:0	0.0350	0.0350	0.0348
	60 min		
C16:0	0.1690	0.1550	0.1622
C18:0	0.0470	0.0420	0.0444
	90 min		
C16:0	0.2430	0.2220	0.2324
C18:0	0.0480	0.0470	0.0474

Table 2. FAME produced from ISTE of *E. cottonii* at 600 rpm

FAME produced	30 mins		
	Run 1 wt (%)	Run 2 wt (%)	Average wt (%)
C16:0	0.1870	0.1420	0.1643
C18:0	0.0350	0.0410	0.0377
	60 min		
C16:0	0.2070	0.2190	0.2129
C18:0	0.0470	0.0480	0.0473
	90 min		
C16:0	0.2380	0.2950	0.2666
C18:0	0.0490	0.0510	0.0499

Table 3. FAME produced from ISTE of *E. cottonii* at 900 rpm

FAME produced	30 min		
	Run 1 wt (%)	Run 2 wt (%)	Average wt (%)
C16:0	0.305	0.303	0.3043
C18:0	0.062	0.035	0.0482
	60 min		
C16:0	0.504	0.376	0.4402
C18:0	0.067	0.040	0.0534
	90 min		
C16:0	0.651	0.495	0.5729
C18:0	0.068	0.044	0.0559

The weight percentage (wt%) of the methyl esters identified are calculated from their respective peak areas in the chromatogram analysis. The weight percent of methyl palmitate and methyl stearate obtained from the experiments are calculated and shown in Table 1, 2 and 3 for 300, 600 and 900rpm respectively. Methyl palmitate is produced abundantly from the ISTE of *E. cottonii* as its fatty acid profile show high percentage of palmitic acid of the total fatty acids [17]. This finding is in line with another research where one of the most significant FAME produced from *E. cottonii* was n-hexadecanoic acid methyl ester [18]. Meanwhile, the low percentage of stearic acid found in *E. cottonii* explains the reason of obtaining smaller amount of methyl stearate [17].

The CFD simulations for the BR used in the experiment was conducted with the ANSYS Fluent (Fluid Simulation Software) version ANSYS 2019 R3. The geometry of the BR consists of a stirred vessel and an impeller with 6 blades and was drawn by using the Design Modeller. Prior to conducting the simulation, a mesh sensitivity analysis (Grid-independent test) was conducted. The main purpose of this analysis was to verify the minimum grid resolution required to develop a solution and tetrahedrons method was used. The built model was meshed using the ICEM software and imported to ANSYS 2019 R3, where the simulation was performed. Properties of MeOH is obtained from the readily available ANSYS database. However, the properties of methyl palmitate were entered manually [16]. The Euler-Euler multiphase model was chosen and the reactor was filled with MeOH initially. The impeller was set to rotate at 300rpm. The changes in volume fraction of triglycerides are going to be determined. Since the simulation is done for immiscible fluids, the Volume of Fraction (VOF) multiphase model is used. The step size used in for the calculation is 0.01s. A total iteration of 500 was set to run. The contour of volume of fraction profile of the methyl palmitate obtained for 300, 600 and 900rpm were discussed.

The Effect of Mixing Towards the Synthesis of FAME

The effects of mixing in the synthesis of FAME are studied through three different impeller rotational speed, which are 300, 600 and 900 rpms. The BR used for this experiment was equipped with a 6 bladed impeller, totally immersed in the reaction solution. Figures 4 and 5 are the graphs of weight percentage of FAME produced under different impeller rotational speed obtained from the result of sample analysis in GC. The weight percentage of FAME was taken at the reaction time of 90 min as highest amount of FAME is produced at that time. From Figure 4 and 5, it clearly seen that the weight percentage of methyl palmitate and methyl stearate produced increases with the increasing impeller rotation speed from 300 to 900 rpm. However, the increment of weight percentage from 300 to 600 rpm is not very significant as compared to the increment of weight percentage when speed is increased from 600 to 900 rpm.

From Figure 4, the weight percentage of methyl palmitate obtained in 90 min of reaction under 300, 600 and 900 rpm is 0.2324, 0.2666 and 0.5729 wt% respectively. The increment of weight percentage from 300 to 600 rpm is only 0.0342 but a huge increment of 0.3063 is obtained when the impeller rotational speed is increase to 900 rpm. The maximum amount of methyl palmitate obtained from this experiment is 0.5729 wt%, under the ratio of biomass:MeOH:HCl (w/v/v) of 1:20:5, reaction temperature of 60°C, 90 min of reaction and 900 rpm. Based on Figure 5, the amount of methyl stearate produced from ISTE reaction is 0.0599 wt% at 90 min of reaction time and 900 rpm. The increments of weight percentage of methyl stearate from 300 to 600 and 600 to 900 rpm are 0.0025 and 0.0060 wt% respectively.

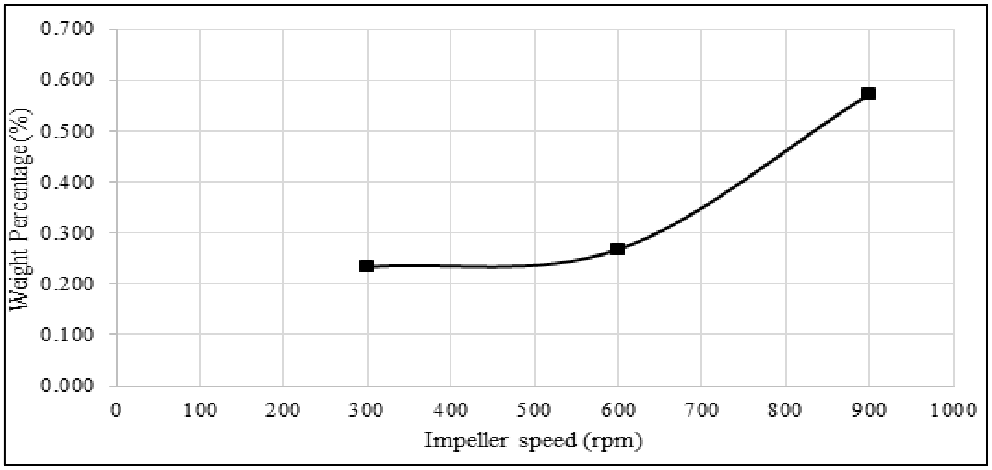

Figure 4. Weight percentage of Methyl palmitate produced under different impeller rotational speed

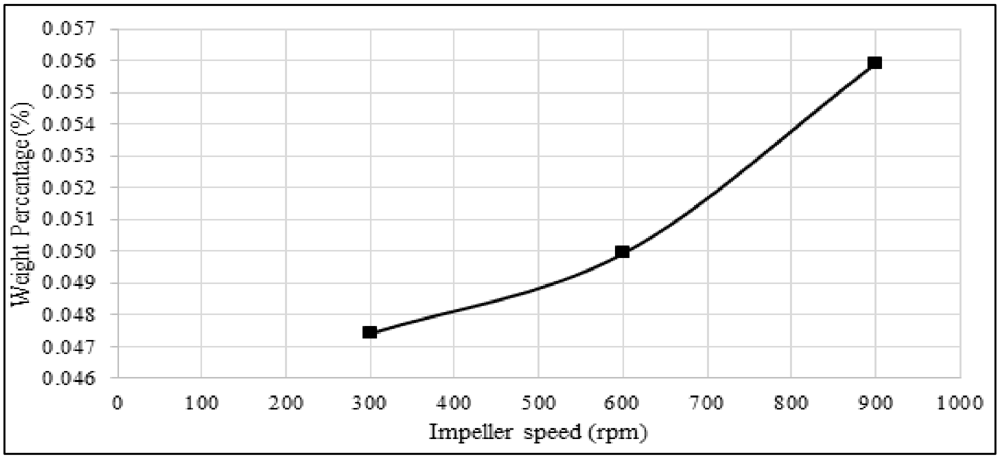

Figure 5. Weight percentage of Methyl stearate produced under different impeller rotational speed

In this experiment, MeOH is used as an extractant to extract oil from the biomass (*E. cottonii*) by disrupting their cell walls and also as a reactant to produce FAME. One of the mechanisms affecting the ISTE reaction is the mass transfer. The oil and TG extracted from *E. cottonii* and MeOH has poor miscibility and will form two layers. Hence, mixing at higher speed and operating at higher temperature will overcome the immiscibility and increase the mass transfer rate and collision rate between the reactants. Since the elevated operating temperature is kept constant, when the reactants are mixed at high speed, the surface area in contact between them increases as the methanol will be dispersed as fine droplets into the TG phase, instead of forming two layers and only reacting on the surface [19], [20]. In short, impeller rotational speed has a significant positive effect on the production FAME. From this study, the highest weight percentage of FAME was produced when the highest speed of impeller rotation (900 rpm) was set. However, the impeller speed of 300 and 600 rpm has no significant difference in terms of weight percentage of FAME produced. This may be due to insufficient mixing introduced to the reactants which only improved the miscibility among them to a certain extent.

Computational Fluid Dynamics Analysis of Mixing in ISTE

The computational fluid dynamics analysis of the mixing property in the bench scale ISTE used for the experiment has been studied by using ANSYS Fluent Software. It is assumed that only palmitic acid is reacting with methanol in this simulation where the study just to proof the mixing property of the ISTE is actually promoting the effectiveness of the extraction and reaction in the reactor.

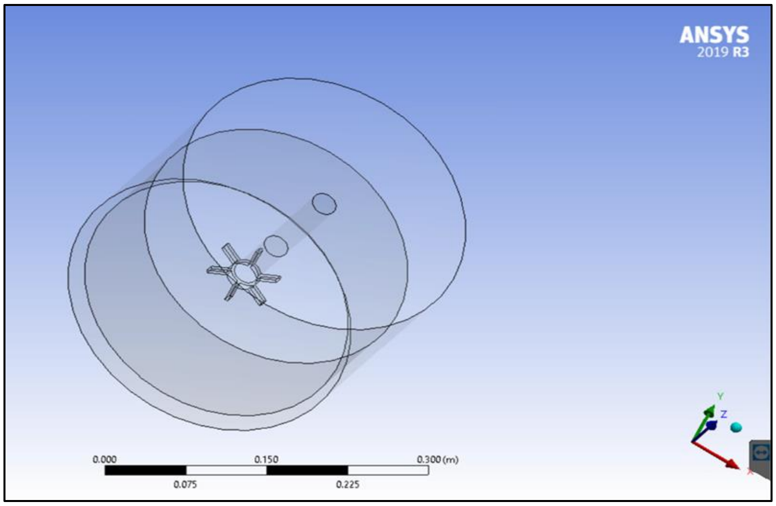

Figure 6. Computational domain of BR in XYZ plane

The bench scale BR used for the FAME production was created with the exact dimensions as shown in Figure 6. The outer domain of the BR is the tank wall while the inner domain is where the fluid will be mixed with the aid of the impeller. Table 4 shows the dimensions of the main parts of BR.

Table 4. Dimensions of main parts of BR

Parts of BR	Dimension (mm)	Direction of plant
BR Body	Radius: 145	x-direction
	Height: 250	z-direction
Impeller Shaft	Radius: 12	x-direction
	Height: 180	z-direction
Impeller Blade	Length: 23.25	x-direction
	Width: 3	y-direction
	Height: 10	z-direction

The process of creating a mesh or grid cells overlaying the geometry of the whole domain is called meshing. In order to simulate the flow properties, the whole domain has to be discretized into several smaller subdomains, which are non-overlapping. Among the many types of 3D mesh elements, tetrahedral is chosen as it suits almost all of the 3D volumes. Figure 7 shows the computational mesh of BR by using tetrahedral mesh element in (a) top view (XY plane) and (b) bottom view (XYZ plane).

In order to study the mixing behavior, the pressure-based solver was used. Since palmitic acid and methanol are two immiscible liquid, the volume of fluid multiphase model and k-omega viscous model was selected. After setting the boundary conditions, the impeller was set to rotate at 300, 600 and 900 rpm. The contours of the volume fraction profile of palmitic acid at 300, 600 and 900 rpm obtained are shown in Figure 8, 9 and 10 respectively. By comparing Figures 8 to 10, it can be seen that there is a huge difference in the volume fraction (VF) contour of palmitic acid. Under impeller rotational speed of 300 rpm, the VF contour of palmitic acid has no further changes after 17s. Similarly, VF contour of palmitic acid under 600 and 900rpm became constant after 35 and 40s respectively. The color gradient shows the VF of palmitic acid where red is maximum and blue is minimum (absence of palmitic acid in that region). The VF increases as it moves from blue to red region as shown in the legend of the figures below.

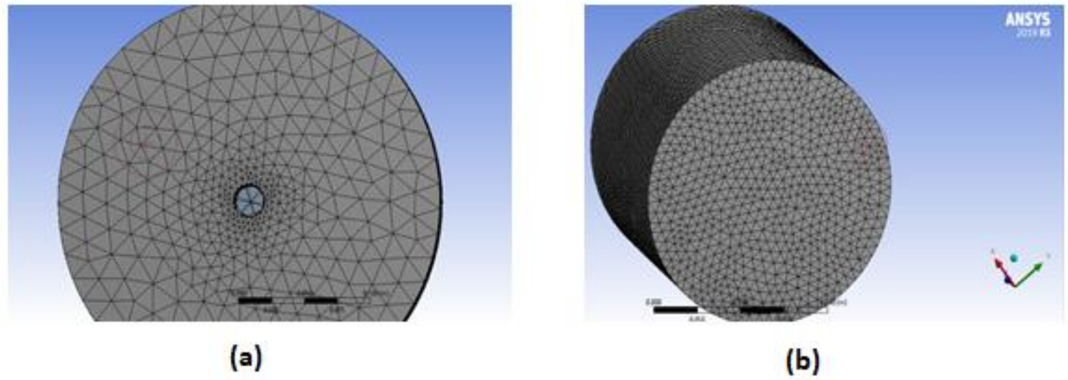

Figure 7. (a) Computational mesh (top view) (b) Computational mesh (bottom view)

From the figures, it clearly seen that the palmitic acid is not dispersed in the tank under rotational speed of 300 rpm. The blue region and green region are more visible than red region which means the volume fraction of palmitic acid is not spread evenly. However, there is a slight improvement in the VF under impeller rotational speed of 600 rpm. The palmitic acid is spread evenly in the tank as the impeller speed is increased up to 900 rpm. The contour shows high fraction of palmitic acid throughout the tank which means the palmitic acid and methanol are mixed vigorously in the tank.

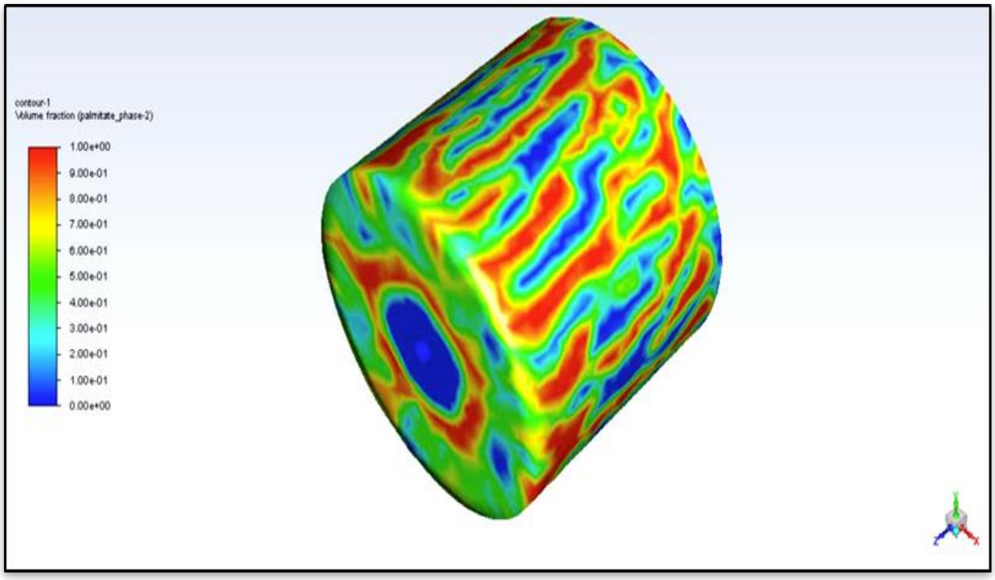

Figure 8. The contour of VF of Palmitic acid under impeller speed of 300 rpm

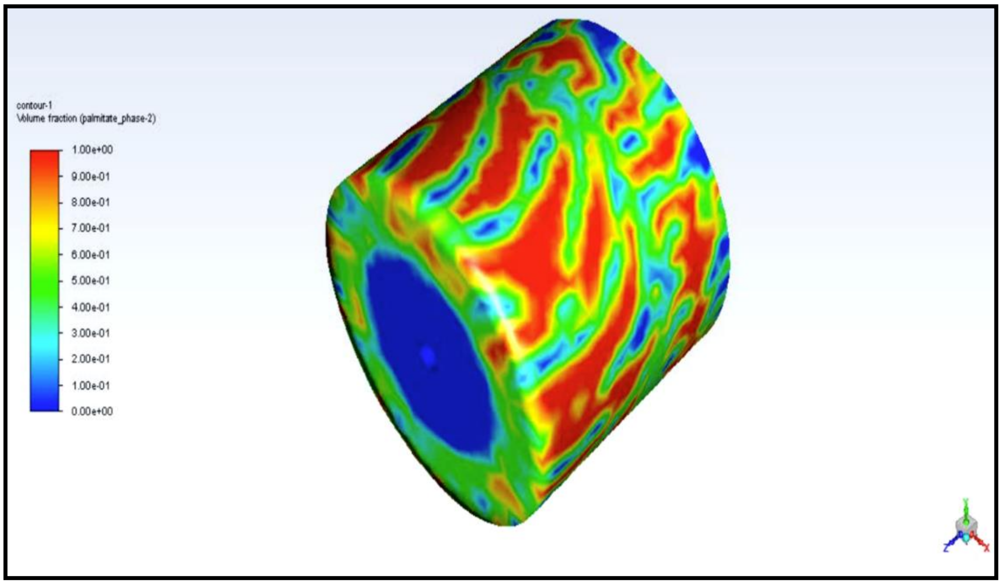

Figure 9. The contour of VF of Palmitic acid under impeller speed of 600 rpm

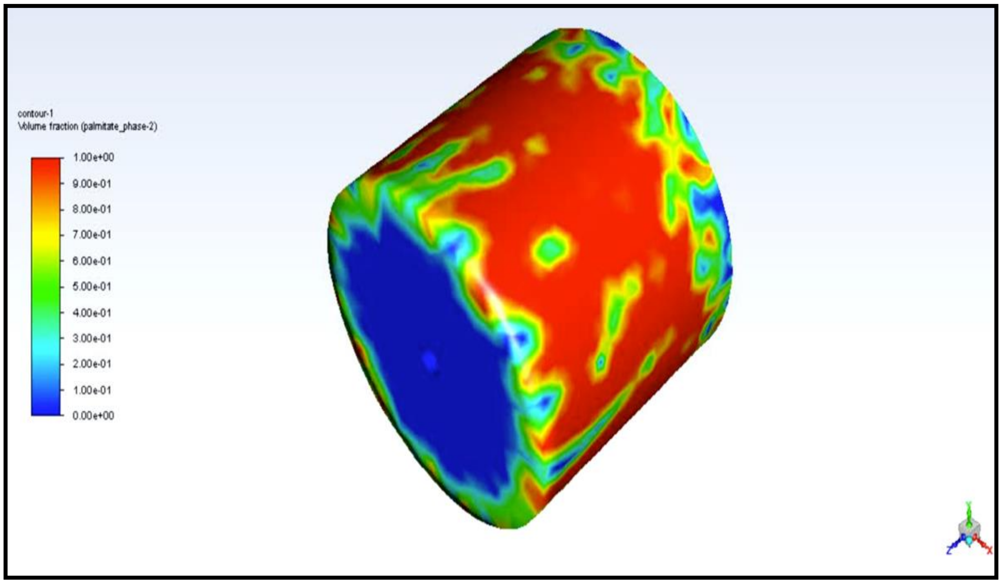

Figure 10. The contour of VF of Palmitic acid under impeller speed of 900 rpm

The VF contours results of palmitic acid clearly show that the contact between methanol and palmitic acid in the tank is improved significantly when the impeller rotational speed is increased from 300 to 900rpm. When the impeller speed is higher, the mass transfer barrier of the immiscible reactants can be overcome, and thus the rate of reaction becomes higher. The findings of the CFD analysis are found to be in good agreement with the experimental data obtained in section 3.1 and 3.2. From the experiment, the weight percentage of FAME produced from of *E. cottonii* was highest (0.5729 wt% for methyl palmitate) when the rotational speed was at its' highest, which is 900rpm. Hence, it is proof that mixing has significant positive effect in the production of FAME. Better mixing enhances the rate of reaction and favors the formation of FAME under suitable operating conditions.

Conclusions

In this study, it is found that from the ISTE of *E. cottonii*, methyl palmitate and methyl stearate are two of the many FAME produced. Methyl palmitate is produced abundantly as the palmitic acid content in *E. cottonii* is high. Besides operating temperature, biomass to alcohol (MeOH) ratio and biomass to catalyst (HCl) ratio, the reaction time and mixing intensity has great significant positive impact towards the for the production of FAME. In this study, the ISTE reaction was carried out at the ratio of biomass: MeOH:HCl (w/v/v) of 1:20:5 and a reaction temperature of 60°C. The total reaction time for the reaction was 90 min, where samples were collected at 30 min interval. The rotational speed of impeller was varied from 300 to 900 rpm.

The weight percentage of FAME produced increases significantly when the reaction time increases. This is because when the reaction time is longer, more oil is being extracted from *E. cottonii* and reacted with MeOH. When more oil is being reacted, higher amount of FAME will be produced as the time increases. At the reaction time of 30 min, the amount of methyl palmitate obtained is relatively low (0.1522 wt% at 300rpm, 0.1643 wt% at 600rpm and 0.3034 wt% at 900rpm) as compared to the reaction time of 90 min (0.2324 wt% at 300 rpm, 0.2666 wt% at 600rpm and 0.5729 wt% at 900 rpm). Similar trend is observed for the production of methyl stearate, where highest weight percentage of FAME produced was at 90 min (0.0474 wt% at 300 rpm, 0.0499 wt% at 600 rpm and 0.0559 wt% for 900 rpm). The idea of mixing the reactants in the BR at a constant speed enhances the production of FAME through ISTE process. The highest amount of methyl palmitate (0.5729 wt%) produced was when highest impeller rotational speed was set, at 900rpm. The highest amount of methyl stearate produced was 0.0559 wt%, also when the impeller rotational speed was set at 900rpm. The findings of the CFD analysis on the mixing behavior in the bench scale BR was in good agreement to the experimental results obtained. The higher the impeller rotational speed, the better the mixing between the two immiscible reactants, the higher the rate of reaction to produce FAME.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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References

[1] Kondili, E. M. and J. K. Kaldellis. (2007). Biofuel implementation in East Europe: Current status and future prospects. *Renewable and Sustainable Energy Reviews, 11*(9), 2137-2151. https://doi.org/10.1016/j.rser.2006.05.001.

[2] Skjånes, K., P. Lindblad, and J. Muller. (2007). BioCO$_2$ – A multidisciplinary, biological approach using solar energy to capture CO$_2$ while producing H$_2$ and high value products. *Biomolecular Engineering, 24*(4), 405-413. https://doi.org/10.1016/j.bioeng.2007.06.002.

[3] Hossain, N., T. M. I. Mahlia, and R. Saidur. (2019). Latest development in microalgae-biofuel production with nano-additives. *Biotechnology for Biofuels, 12*(1). https://doi.org/10.1186/s13068-019-1465-0.

[4] Li, Y., *et al.* (2008). Biofuels from microalgae. *Biotechnology Progress, 24*(4), 815-820. https://doi.org/10.1021/bp.070371k.

[5] Khan, M. I., J. H. Shin, and J. D. Kim. (2018). The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial Cell Factories, 17*(1). https://doi.org/10.1186/s12934-018-0879-x.

[6] Laurens, L. M. L., M. Lane, and R. S. Nelson. (2020). Sustainable seaweed biotechnology solutions for carbon capture, composition, and deconstruction. *Trends in Biotechnology, 38*(11), 1232-1244.https://doi.org/10.1016/j.tibtech.2020.03.015.

[7] Chung, Z. L., *et al*. (2019). Life cycle assessment of waste cooking oil for biodiesel production using waste chicken eggshell derived CaO as catalyst via transesterification. *Biocatalysis and Agricultural Biotechnology,* 21. https://doi.org/10.1016/j.bcab.2019.101317.

[8] Gautam, R. and S. Kumar. (2020). Performance and combustion analysis of diesel and tallow biodiesel in CI engine. *Energy Reports*, *6*, 2785-2793. https://doi.org/10.1016/j.egyr.2020.09.039.

[9] Singh, D., *et al.* (2019). Chemical compositions, properties, and standards for different generation biodiesels: A review. *Fuel, 253*, 60-71. https://doi.org/10.1016/j.fuel.2019.04.174.

[10] Kasim, F. H., A. P. Harvey, and R. Zakaria. (2010). Biodiesel production by in situ transesterification. *Biofuels*, *1*(2), 355-365. https://doi.org/10.4155/bfs.10.6.

[11] Ejikeme, P. M., *et al.* (2010). Catalysis in biodiesel production by transesterification processes-An insight. *E-Journal of Chemistry*, *7*, 689051. https://doi.org/10.1155/2010/689051.

[12] Tang, Y., *et al.* (2016). Optimization of one-step in situ transesterification method for accurate quantification of epa in nannochloropsis gaditana. *Applied Sciences (Switzerland), 6*(11). https://doi.org/10.3390/app6110343.

[13] Suganya, T., R. Kasirajan, and S. Renganathan. (2014). Ultrasound-enhanced rapid in situ transesterification of marine macroalgae Enteromorpha compressa for biodiesel production. *Bioresource Technology*, *156*, 283-290. https://doi.org/10.1016/j.biortech.2014.01.050.

[14] Bošnjaković, M. and N. Sinaga. (2020). The perspective of large-scale production of algae biodiesel. *Applied Sciences (Switzerland)*, *10*(22), 1-26. https://doi.org/10.3390/app10228181.

[15] Ahmed, A. S., *et al.* (2010). Biodiesel production from macro algae as a green fuel for diesel engine. *Journal of Energy & Environment*, *2*(1), 5. http://journal.uniten.edu.my/ojs3/index.php/jee/article/view/265/101.

[16] Rasulov, S. M., I. A. Isaev, and S. M. Orakova. (2019). Viscosity and density of methyl palmitate. *High Temperature*, *57*(2), 269-271.https://doi.org/10.1134/S0018151X19020160.

[17] Matanjun, P., *et al*. (2009). Nutrient content of tropical edible seaweeds, Eucheuma cottonii, Caulerpa lentillifera and Sargassum polycystum. *Journal of Applied Phycology, 21*(1), 75-80. https://doi.org/10.1007/s10811-008-9326-4.

[18] Karpanaiselvan, B. (2016). Synthesis and characterization of biodiesel derived from marine algae and cotton seeds for application in diesel engines. Thesis in Faculty of Technology Anna University.http://hdl.handle.net/10603/181291.

[19] Helwani, Z., *et al*. (2009). Technologies for production of biodiesel focusing on green catalytic techniques: A review. *Fuel Processing Technology*, *90*(12), 1502-1514. https://doi.org/10.1016/j.fuproc.2009.07.016.

[20] Ehimen, E. A., Z.F. Sun, and C.G. Carrington. (2010). Variables affecting the in situ transesterification of microalgae lipids. *Fuel, 89*(3), 677-684. https://doi.org/10.1016/j.fuel.2009.10.011.