Synthesis of Biodiesel from Palm Oil Using a Hybrid Mechanical Stirring and High Frequency Ultrasonic-Assisted Enzyme-Catalysed Process

Wen Chun Siaw[a], Lian See Tan[a,*], Peck Loo Kiew[a], Tomoya Tsuji[a], Ghulam Murshid[b]

[a]Malaysia-Japan International Institute of Technology, Universiti Teknologi Malaysia, Jalan Sultan Yahya Petra, 54100 Kuala Lumpur, Malaysia
[b]Department of Petroleum & Chemical Engineering, Sultan Qaboos University, Muscat, Oman
 tan.liansee@utm.my

Transesterification process involves different immiscible substrates includes feedstock oil, alcohol and catalyst to produce fatty acid methyl esters, i.e. biodiesel. However, a poor mixing of the substrates in the transesterification process could lead to a low triglyceride conversion into biodiesel. Appropriate design for mixing is important to promote better surface contact between the substrate to enhance the transesterification reaction. In addition, mechanical assisted mixing system in the intensified process with ultrasonic could help to improve the conversion percentage. In this study, the effect of high frequency ultrasonic in enzymatic transesterification process was investigated. The biodiesel conversion percentage for the enzymatic transesterification of palm oil with methanol via 3 different mechanical mixing systems, i.e., conventional shaking method, high frequency ultrasonic method with and without mechanical stirring, were evaluated. The transesterification reaction was carried out for 12 h at 35 °C with an alcohol-to-oil molar ratio of 6:1, a 10 wt% of liquid enzyme solution, with addition of 16 wt% of water (relative to the weight of enzyme). The results from this study indicated that an enhancement of biodiesel conversion percentage was achieved via high frequency ultrasonic assisted enzymatic transesterification compared to conventional shaking method. The biodiesel conversion percentage obtained via conventional shaking method and high frequency ultrasonic-assisted transesterification processes were 29% and 66%. Meanwhile, the application of additional mechanical stirring into high frequency ultrasonic process further increased the conversion percentage from 66% to 89%. The findings for this study bring a better understanding in high frequency ultrasound assisted enzymatic transesterification. There is a potential reducing the reaction time for enzymatic transesterification with high frequency ultrasound assisted process. This could provide an alternative transesterification process with milder operating conditions.

1. Introduction

Challenges related to increasing energy demand are getting critical in global scale due to the population growth and the desire for a higher quality of living (Alhassan et al., 2019). To reduce dependency on the non-renewable fossil fuel, increasing efforts are in place to increase the source of renewable energy as it can play an important role in increasing the energy security. Biodiesel is a renewable energy which could be produced from vegetable oil, animal fats or waste oil and it has close similarity with the conventional fossil diesel in terms of chemical structure and energy content (Aghbashlo et al., 2016).

Biodiesel is commonly produced via the transesterification of plant oils and animal fats. In transesterification reaction, short-chain alcohol such as methanol or ethanol is to react with the lipid molecule with the presence of base, acid, or enzyme catalysts. Both basic and acidic catalysts could vary in the form of homogenous or heterogenous, while the enzymes would be in the form of free or immobilized (Nasreen et al., 2018). Homogenous base-catalysed transesterification is a commonly utilized technique for biodiesel production (Haghighi et al., 2021). It is widely applied in the industry due to its rapid reaction with high conversion, able to

operate under mild reaction condition, and abundance in potassium hydroxide (KOH) and sodium hydroxide (NaOH) catalysts (Moazeni et al., 2019). However, the main limitation of it is the production for soaps as by-product due to its sensitivity towards free fatty acids (FFA) and causing catalyst poisoning (Nasreen et al., 2018). As for the acidic catalyst, undergo saponification does not take part in transesterification reaction due to its insensitivity towards FFA. Besides, acid catalyst can catalyse the esterification and transesterification concurrently. However, it has several drawbacks, including high alcohol consumptions, water sensitive, and corrosive to equipment and pipelines (Tacias-Pascacio et al., 2018).

In recent years, as concerns regarding the feeding of these chemical-typed catalysts issues continue to grow, the enzymatic transesterification process has gain increasing attention as an eminent technique with high sustainability value. Lipase enzyme, being a biocatalyst, could improve downstream processing issues in chemical transesterification (Lam et al., 2010). Enzymatic catalysts have better product recovery, greater regenerative ability, able to operate in milder reaction conditions with less by-products produce despite being costly. They are also tolerable to low-quality oil with high FFA (Moazeni et al., 2019). Lipase enzyme can catalysed both the hydrolysis and the formation of long-chain fatty acid alkyl esters in biodiesel transesterification (Pourzolfaghar et al., 2016). Nevertheless, this process has two limitations, i.e., the triglyceride conversion into fatty acids alkyl esters is relatively slow and the reaction period for biodiesel production is relatively long (Patchimpet et al., 2020). Therefore, it is important to improve the enzymatic transesterification by incorporating alternative techniques to overcome the challenges (Wang et al., 2020).

Ultrasonic irradiation is one of the techniques which are applied to enhance the transesterification process. Low frequency ultrasonic irradiation generates the cavitation of micro bubbles in the reaction mediums, namely oil and alcohol (Amin and Yusup, 2015). Ultrasound-generated energy not only provides the mechanical energy for mixing but also the activation energy (E_a) required for the transesterification reaction. Furthermore, this technology contributes to chemical effects, since fatty acid will break down into free radicals during the cavitation of microbubbles (Tan et al., 2019a).

The combination of ultrasound and enzymatic catalyst in transesterification shows great potential for biodiesel production. However, most of these works has been researched in low frequency ultrasound at a range of (25 to 50 kHz). Recently, there is application of high frequency ultrasonic in alkaline catalysed transesterification process (Pâmella et al., 2021), but to date, the effect of high frequency ultrasound irradiation in enzymatic transesterification is not clearly understood. Hence, this study aims to investigate the impact of incorporating high frequency ultrasound (1.7 MHz) to the enzymatic transesterification process. Furthermore, this method is compared with the mechanical shaking technique and a combination of mechanical stirring with high frequency ultrasound to evaluate the best technique to improve the biodiesel conversion.

2. Materials and method

2.1 Materials

The lipase enzyme used in this work was Eversa® Transform 2.0 from Novozymes Malaysia Sdn. Bhd. Refined palm oil (Vesawit, Malaysia) was purchased from a local supermarket. A 99.9 % of analytical grade methanol purchased from Sigma Aldrich (Germany) was used for transesterification process. A 2-propanol obtained from Sigma Aldrich (Germany) and phenolphthalein obtained from Merck (United States) were used as titration medium and indicator.

2.2 Transesterification Method

A mixture of refined palm oil with methanol at 6:1 molar ratio, 5 wt% enzyme catalyst and water (1.6 wt% based on oil weight) were added into the reactor for transesterification process (Pâmella et al., 2021). In this study, three modes of transesterification process, i.e., mechanical shaking mode, high frequency ultrasonic irradiation mode, and high frequency ultrasonic irradiation coupled with mechanical stirring mode, were conducted and the product was compared in terms of conversion percentage. For mechanical shaking, a mixture of refined palm oil, methanol and enzyme catalyst was prepared in 250 mL conical flask. The mixture was transferred to incubated orbital shaker (Benctop N-Biotek, Korea) set at 35 °C and 200 rpm shaking speed for 12 h. Meanwhile, the transesterification process with high frequency ultrasonic irradiation was carried out in a 125 mL capacity jacketed stainless steel reactor. An ultrasonic set-up with 1.65 MHz frequency, equipped with 4 sonodiscs of 0.8 cm diameter each, was installed at the bottom of the reactor. The transesterification process was performed at 30 W ultrasonic power, 35 °C reaction temperature for 12 h. To evaluate the effect of high frequency ultrasound on the enzymatic transesterification, ultrasound treatment was carried out with and without conventional mechanical stirring method under 200 rpm stirring speed at similar condition as per mechanical shaking mode. After the enzymatic transesterification reaction was completed, the mixture was expected to contain three major components which are biodiesel, glycerine and the enzyme catalyst. The mixture was centrifuged at 10000 rpm for 30 min by using refrigerated centrifuge (SIGMA 3-18KS). The upper layer was

expected as the product. The residual was transferred to rotary evaporator under the condition of 115 °C for 10 min to remove the excess methanol and water present in the residual. The pure biodiesel was sent for 1H Nuclear Magnetic Resonance analysis for quantification purpose.

2.3 NMR analysis

In this work, the methyl ester composition in the collected product was quantified by 1H NMR. The samples were dissolved in methanol D4 before analysis via 300 MHz NMR spectrometer (model VNMRS, Varian Instruments, Germany). The conversion percentage was calculated by Eq(1), using the relationship between the areas of the methyl ester hydrogen signal ($I_{3.7}$ = CH_3OCO – δ_H 3.7 ppm) and of the alpha-carbonyl methylene hydrogen signal ($I_{2.2-2.4}$ = $OCOCH_2$ – δ_H 2.2–2.4 ppm). The 2/3 factor is defined as the different numbers of hydrogen atoms presence in methyl and methylene groups (de Jesus et al., 2015).

$$C_{ME}(\%) = \left[\frac{2(I_{TAG+ME})}{3(I_{\alpha CH_2})} \right] \times 100\% \tag{1}$$

The terms I_{TAG+ME} represent the signal area of the hydrogens in the -O-CH_3 group which only represent in the methyl ester products while the term $I_{\alpha CH_2}$ represents the signal area of the unreacted triglycerides, corresponding to the two carbonyl α hydrogen CH_2-COOR. Table 1 shows the description of the peaks in NMR spectroscopy for biodiesel sample (Moawia et al., 2019).

Table 1: NMR peaks description for biodiesel

Chemical shift, δ_H (ppm)	Proton (s)	Descriptions
0.80 – 1.00	CH_3–C	Terminal methyl group
1.28	–$(CH_2)_n$–	Backbone CH_2
1.62	CH_2–CH_2–COOR	All carbonyl β H
2.05	–CH_2–CH=CH–	All alkyl H
2.30	CH_2–COOR	All carbonyl α H
2.78	CH=CH–CH_2–CH=CH	Linoleic
3.67	CH_3–OCOR	Methyl esters
4.09 – 4.34	–CH_2OCOR	Methylene group (C_1 and C_3) of glyceride
5.35	–CH=CH–	All olefins

2.4 Determination of FFA

Acid-base titration method was performed to determine the FFA percentage in refined palm oil and biodiesel. The oil sample was mixed with 10 mL of 2-propanol solution, while phenolphthalein solution was few dop to the solution as a color changes indicator. The solution was titrated against 0.1 N KOH solution to neutralize the acid in the solution. The titration was terminated when pale pink color was observed in the solution. The value of neutralized acid obtained in the solution represents the sample of acid number whereas the acid number is proportionate to the free fatty acid (FFA) content as shown in Eq(2).

$$FFA\ (\%) = \frac{(Titrant\ Volume, ml - Volume\ of\ Blank, ml) \times Normality, N \times 28.2}{Weight\ of\ Oil\ Sample, g} \times 100\ \% \tag{2}$$

3. Result and discussion

3.1 Effect of ultrasonic irradiation and mechanical stirring

Figure 1 shows the biodiesel conversion percentage obtained at three different modes, i.e., mechanical shaking, high frequency ultrasonic irradiation, and high frequency ultrasonic irradiation coupled with mechanical stirring. In this study, the highest biodiesel conversion percentage was achieved using ultrasonic irradiation coupled with mechanical stirring, with conversion of 89.63 %, followed by ultrasonic irradiation without stirring with conversion of 66.53 %, whereas mechanical shaking only produced biodiesel conversion of 27.59 %. In mechanical shaking process, mixing intensity played an important role to increase surface contact between triglyceride and alcohol. However, due the limited contact area between the reactants via this method, the diffusion of triglyceride molecules from triglyceride-rich phase towards the methanol interface is affected. Therefore, the ineffective mass transfer at their phase boundary would result in low reaction rate (Tan et al., 2019b). The high frequency ultrasonic generated a micro-turbulence in the solutions during the rapid formation of cavitation bubbles (Amin and Yusup, 2015). This process enhanced the miscibility of the reactants and improved the surface contact

between the reactants. Hence, reaction rate was increased and shifted the equilibrium towards the product side, resulting in a higher conversion percentage of triglyceride into biodiesel.

Similar trend of findings was also found by Patchimpet et al. (2021) when the transesterification process was conducted at high frequency ultrasonic using alkaline catalyst. High conversion was achieved with high frequency ultrasonic coupled with stirring due to the generation of cavitation which provides high availability of the interfacial area for mass transfer and it promotes better surface contact between the catalyst and reactants (Ho et al., 2016). In alcoholysis process, ultrasonic irradiations improved the transesterification reaction as it enhanced the transfer rate between triglycerides from the oil toward the methanol interface (Noureddini and Zhu, 1997). Besides, circulating enzyme in vertical movement via high frequency ultrasonic provided a more evenly spread dispersion in the reaction medium and increased the surface contact between substrate and the enzyme, resulting in higher biodiesel conversion (Aghbashlo et al., 2016).
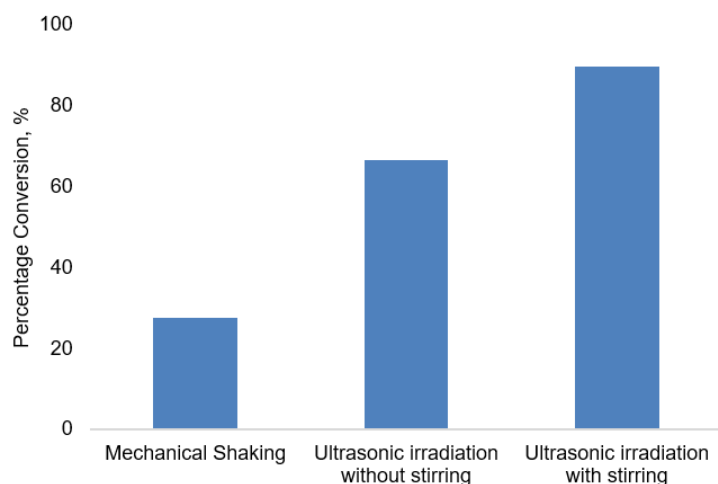

Figure 1: Comparison of biodiesel production via 3 different methods

3.2 Figures Analysis of NMR results and FFA determination

Biodiesel analysis by 1H NMR had been reported for the quantification of fatty acid in palm oil and biodiesel (Jitendra et al., 2009). Figures 2(a) and 2(b) depict the 1H NMR spectra of palm oil and biodiesel product obtained from this study, respectively. The descriptions of 1H NMR peaks were listed in Table 1. Before transesterification process, 1H NMR spectrum of palm oil at -CH_2OCOR region (4.08–4.30 ppm) showed a double quartet signal pattern which corresponding to glyceride. During the transesterification of glyceride, the intensity of the triplet signals at 4.08–4.30 ppm decreased and that of the methyl ester (3.6–3.7 ppm) increased with the reaction time. Meanwhile, 1H NMR spectrum in the R-CH_2 region (2.1–2.4 ppm) showed an unmerged quadruplet pattern corresponding to fatty acid as shown in Figure 3(a). As the reaction progressed, the intensity of the triplet signals as to fatty acid decreased and that of the ester increased with the reaction time. Finally, at the end of 12 h, the peaks corresponding to fatty acid had almost disappeared, and only a triplet corresponding to that of the ester (biodiesel) was detected as depicted in Figure 3(b).
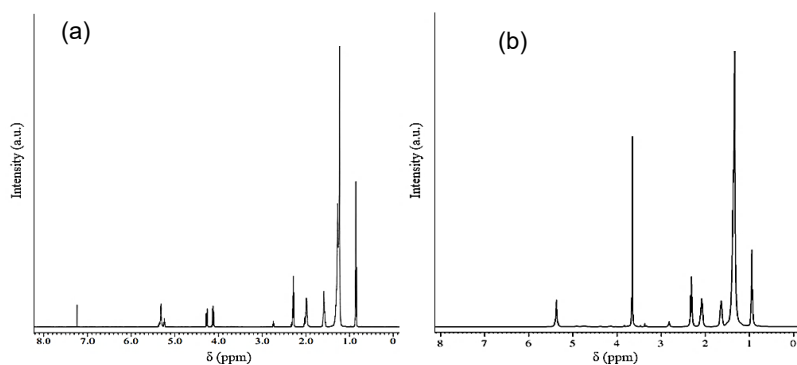

Figure 2: 1H NMR spectra of (a) palm oil (b) biodiesel
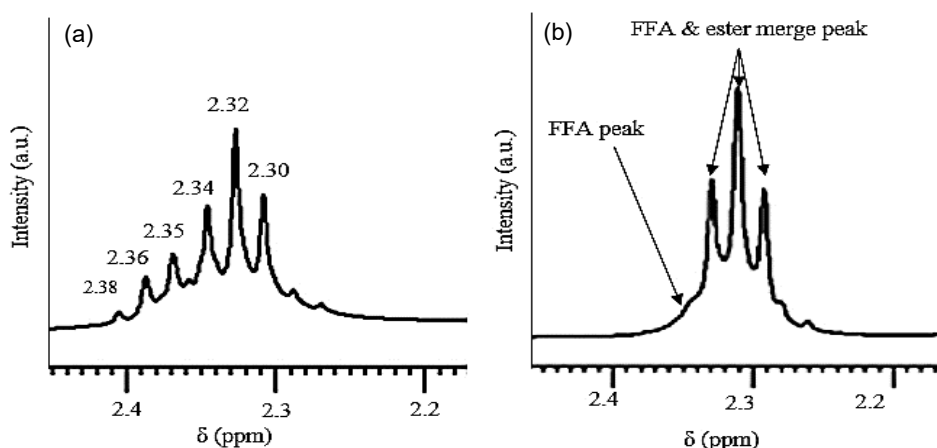
Figure 3: 1H NMR spectra in α–CH₂ region for (a) palm oil (b) biodiesel

Quantification of the FFA content in palm oil and biodiesel was based on the appearance of R-CH₂ peaks as FFA and R-CH₃ peaks as methyl esters. The difference in chemical shift (between the acid and ester) is due to the greater deshielding effect of the carboxylic group compared to the ester group. One of the peaks represented FFA (at 2.36 ppm in Figure 3a) shifts out of the R-CH₂ region of the ester, and the other two peaks (2.34 and 2.30 ppm) are merged with those due to the ester at 2.35 and 2.31 ppm. In other words, a sample containing FFA and ester shows a triplet spectral pattern in the R-CH₂ region of the 1H NMR spectrum (Figure 3b) with the intensity ratio of 1:2:1. Besides, the unmerged peak of the FFA triplet can be used to determine the FFA content in palm oil and biodiesel (Figure 3b). The total area corresponding to R-CH₂ of both FFA and ester can be determined by integrating the spectral region 2.20–2.40 ppm. The concentration of FFA (wt%) in oil or biodiesel was calculated by Eq(3):

$$FFA\ (\%) = \frac{Area\ of\ R-CH_2\ triplet\ of\ FFA}{Total\ Area\ of\ R-CH_2\ triplet\ of\ FFA\ and\ ester} \tag{3}$$

For validation of this method, a titration method was performed for palm oil and biodiesel to determine FFA content with Eq(2). Based on the tabulated result shown in Table 2, value wise, the estimation of FFA based on NMR results was relatively close to results obtained through titration method, with error of 12% for both oil and biodiesel. Nevertheless, the trend of FFA content based on 1H NMR analysis, before and after the transesterification process, did not show an increase as per the trend based on titration analysis method. The slight increment of FFA content in biodiesel after the transesterification process, detected via titration method, could be due the addition of water for enzymatic transesterification. It is deduced to contribute to the hydrolysis reaction which hydrolysed triglyceride into FFA (Pietro et al., 2020). The slight inconsistency of FFA results trend obtained by these 2 methods, despite the emerging usage of 1H NMR for quantification of FFA content in oil indicated that further studies are required to reaffirm the reliability of 1H NMR for the purpose.

Table 2: FFA content of oil and biodiesel

Sample	FFA content (%)	
	Titration	1H NMR
Oil	0.495	0.557
Biodiesel	0.615	0.537

4. Conclusions

High frequency ultrasonic, coupled with mechanical stirring was found to be effective in enhancing enzyme-catalyzed transesterification process of palm oil and methanol compared to the mechanical shaking method. Under the conditions of 1.65 MHz ultrasonic frequency, 30 W irradiation power supply, 200 rpm stirring speed, methanol to oil molar ratio of 6:1, 5 wt% of enzyme, and temperature of 35 °C, 89.63 % conversion was achieved after a 12 h process. 1H NMR was also found to be a simple and fast analysis method for quantification of free fatty acids in palm oil and biodiesel. Nevertheless, more studies are required to reaffirm the reliability of 1H NMR for the purpose.

Acknowledgments

References

Batistella L., Lerin L.A., Brugnerotto P., Danielli A.J., Trentin C.M., Popiolski A., Treichel H., Oliveira J.V., Dde Oliveira, 2012, Ultrasound-assisted lipase-catalyzed transesterifcation of soy bean oil in organic solvent system, Ultrasonics Sonochemistry, 19, 452–458.

Demisu D.G., 2021, Factors affecting biodiesel production from non-edible vegetable oil via base-catalyzed transesterification process: synthesis, International Journal Sustainable and Green Energy, 10, 85–91.

Ho W.W.S., Ng H.K., Gan S., 2016, Advances in ultrasound-assisted transesterification for biodiesel production, Applied Thermal Engineering, 100, 553–563.

Aghbashlo M., Tabatababei M., Hosseinpour S., Hosseini S.S., Ghaffari A., Khounani Z., Mohammadi P., 2016, Development and evaluation of a novel low power, high frequency piezoelectric-based ultrasonic reactor for intensifiying the transesterification reaction, Biofuel Research Journal, 12, 528–535.

Alhassan K.A., Abdullahi B.T., Shah M.M., 2019, A review on biogas production as the alternative source of fuel, Journal of Applied and Advanced Research, 4, 61–65.

Amin M.M, Yusup, S., 2015, Transesterification via direct-ultrasonic irradiation of rubber seed oil with waste materials based heterogeneous catalyst, Chemical Engineering Transactions, 45, 1267 – 1272.

Haghighi S.F.M., Parvasi P., Jokar S.M., Basile A.B., 2021, Investigation the effects of ultrasonic frequency and membrance technology on biodiesel production form chicken waste, Energies, 14, 1–21.

Jitendra K., Satyarthi, Srinivas D., Ratnasamy P., 2009, Estimation of free fatty acid content in oils, fats, and biodiesel by 1H NMR spectroscopy, Energy & Fuels, 23, 2273–2277.

Lam M.K., Lee K.T., Mohamed A.R., 2010, Homogenous, heterogenous and enzymatic catalysis for transesterification of high free fatty acid oil (waste cooking oil) to biodiesel: A review, Biotechnology Advances, 28, 500–518.

Pietro M. E. D., Mannu A., Mele A., 2020, NMR determination of free fatty acids in vegetable oils, Processes, 8, 410–425.

Moawia R.M., Nasef M.M., Mohamed N.H., Ripin A., Farag H., 2019, Production of biodiesel from cottonseed oil over aminated flax fibres catalyst: kinetic and thermodynamic behaviour and biodiesel properties. Advances in Chemical Engineering and Science, 9(4), 281–298.

Moazeni F., Chen Y., Zhang G., 2019, Enzymatic transesterification for biodiesel production from used cooing oil, A review, Journal of Clean Production, 216, 117–128.

Nasreen S., Nafees M., Qureshi L.A., Asad M.S., Sadiq A., Ali S.D., 2018, Review of catalytic transesterification methods for biodiesel production, Biofuels, 1, 93–110.

Noureddini H, Zhu D, 1997, Kinetics of transesterification of soy bean oil, Journal of American Oil Chemists' Society, 74, 1457–1463.

Pâmella A.O., Raphaela M.B., Gabriel C.M., André V.A., Costa-Félix R.P.B., 2021, Ultrasound-assisted transesterification of soybean oil using low power and high frequency and no external heating source, Ultrasonics Sonochemistry, 78, 105709.

Patchimpet J., Sangkharak K., Klomklao S., 2020, Optimization of process variables for the production of biodiesel by transesterifcation of used cooking oil using lipase from Nile tilapia viscera, Renewable Energy, 153, 861–869.

Pourzolfaghar H., Abnisa F., Daud W.M.A.W., Aroua M.K., 2016, A review of the enzymatic hydroesterification process for biodiesel production, Renewable and Sustainable Energy, 61, 245–257.

Tacias-Pascacio V.G., Torrestiana-Sánchez B., Dal Magro L., Virgen-Ortiz J., Suárez-Ruiz F.J., Rodrigues R.C., Fernandez-Lafuente R., 2018, Comparison of acid, basic and enzymatic catlysiscatalysis on the production of biodiesel after RSM optimization, Renewable Energy, 135, 1–19.

Tan S.X., Lim S., Ong H.C., Pang Y.L., 2019a, State of the art review on development of ultrasound-assisted catalytic transesterification process for biodiesel production, Fuel, 235, 886–907.

Tan S.X., Lim S., Ong H.C., Pang Y.L., Kusumo F., Goh B.H.H., Chong C.T., 2019b, Two-step catalytic reactive extraction and transesterification process via ultrasonic irradiation for biodiesel production from solid Jatropha oil seeds, Chemical Engineering and Processing-Process Intensification, 146, 107687–107724.

Wang X., Xu X., Wang Q., Huang Z., He J., Qiu T., 2020, Fatty acid methyl ester synthesis through transesterifcation of palm oil with methanol in microchannels: Flow pattern and reaction kinetics, Energy & Fuels, 34, 3628–3639.