Prediction Primary Available Blend Biodiesel of Waste Oil from *Aurantiochytrium* sp. for General Diesel Engines

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Abstract

Chemical and enzyme transesterification were compared by discussing preliminary transesterification of waste oil of *Aurantiochytrium* sp., which was then used in transesterification for the primary available blend biodiesel for a general diesel engine in this study. We made progress on the winterized characteristics of the waste oil's biodiesel of *Aurantiochytrium* sp. and its biodiesel, which included the reactivity parameters and properties. This approach led to the development of a novel idea for the evaluation of kinetic parameters of winterization, along with obtaining the suitable operation and storage conditions of biodiesel. Therefore, the waste oil of *Aurantiochytrium* sp. could be developed for biodiesel production and successfully made into a suitable blend diesel. Overall, we acquired the best condition of mixtures and the highly mixed rate of petrodiesel: biodiesel = 80 : 20 (activation energy of winterization 21.32 kJ/mol; onset temperature of winterization -4.15 ℃; heat of combustion 43.15 MJ/kg; kinematic viscosity 3.51 mm^2/s; flash point 67.5 °C), which was an appropriate blend biodiesel from the waste oil's biodiesel of *Aurantiochytrium* sp.

Keywords: waste oil, *Aurantiochytrium* sp., biodiesel, winterization, blend biodiesel

1. Introduction

This study focused on *Aurantiochytrium* sp., which is a type of microalgae, the oil of which is refined for docosahexaenoic acid (DHA) for incorporating into health food or food additives [1]. More than 20 mass% of the solid waste of *Aurantiochytrium* sp. is discarded as rubbish under current operating procedures in food factories. In this study, we implemented the preliminary transesterification of solid waste of *Aurantiochytrium* sp., to determine the advantages and disadvantages of chemical and enzyme transesterification. Chemical [1-7] and enzyme [8-15] transesterification were compared to discuss the preliminary transesterification of waste oil of *Aurantiochytrium* sp., which could then be beneficially used in the development of transesterification for an available mixture of biodiesel. The main stages of chemical transesterification are as follows [1-7]: (a) saponification fixes the fatty acids, which are used for enhancing the yield of the transesterification reaction and removing impurities, glycerol, and free fatty acid; (b) reduction reaction, which reverts the fatty acids for convenient converting to biodiesel; and (c) acid-catalysed reaction, which makes the fatty acids and methanol for fully transesterifying into the fatty acid methyl ester. The main stages of enzyme transesterification are as follows: (a) triglyceride hydrolysis by hydrolytic enzymes is

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used for enhancing the yield of the transesterification reaction and removing impurities, glycerol, and free fatty acid [14]; and (b) immobilized enzyme reacts with the fatty acid, which reverts the fatty acids for converting to biodiesel [15].

We also developed the winterized characteristics of the waste oil's biodiesel of *Aurantiochytrium* sp. and its biodiesel, which included the reactivity parameters and properties [16-18]. Differential scanning calorimetry (DSC) was used to obtain the winterization temperature and the other detailed enthalpy of the exothermicity of the biodiesel (B100) and the various proportion of biodiesel for mixing with petrodiesel (B0). The parameters and reactivity properties could be applied to the designs of the operation, application, and transportation safety conditions [1, 16-17]. It is an important project for developing high quality available blend biodiesel from the waste oil of *Aurantiochytrium* sp. An available blend of biodiesel has the appropriate heat of combustion, kinematic viscosity [16], and storage safety [18]. It is an important performance indicator of biodiesel for mixing with B0, which is concerned with effective fuel for diesel engines. Here, the heat of combustion, kinematic viscosity, and storage safety of blend biodiesel were tested by DSC [16-17], flash point tester [18], viscometer [19-20], and oxygen bomb calorimeter [21-23], and these were also compared to the various proportions of B100 for mixing with B0. Then, the best condition of mixtures was obtained as an available blend diesel of waste oil's biodiesel of *Aurantiochytrium* sp.

2. Experimental and Method

2.1. Samples

The waste oil of *Aurantiochytrium* sp., which was supplied directly from VEDAN Enterprise Corp. in Taiwan, was stored frozen at −20 ℃. The original fatty acid profile of waste oil was conducted by gas chromatography (GC) analysis [24].

2.2. Transesterification

First, the fixed fatty acid composition was saponified with the three equivalents of sodium hydroxide under ca. 90 ℃ for three hours. Then, the impurities, the free fatty acids, glycerol, and microalgae cell wall were removed to form the waste oil soap. The oil soap was included in the further transesterification. We then obtained a high purity fatty acid for the next step of the reduction reaction. Second, the reduction reaction was mixed with sulphuric acid to form fatty acids under 85 ℃ for three hours. Finally, the acid-catalysed reactions were mixed with dil. sulphuric acid (less than 0.02 mass% ratio of fatty acid) and methanol (fatty acids: methanol = 1:5, an amount of methanol more than five-times that of the fatty acids is better) under reflux overnight [1].

For preparation of the 0.1 M of disodium hydrogen phosphate-sodium dihydrogen phosphate buffer solution, the buffer solution was maintained at pH 7. *Candida rugose* is a type of hydrolytic enzyme of lipase, which showed the preferred results of the hydrolysis reaction under a pH 7 buffer solution [8-15]. A 0.1 mass % of *C. rugose* was mixed with solid waste oil of *Aurantiochytrium* sp. (triglycerides), and then the mixture was conducted for 260 rpm and stirring for 12 hours under isothermal 35 ℃. From the above results, we removed the solid product and the oil layer of the upper layer (fatty acids), and then mixed this with *C. antarctica* (lipase acrylic resin of immobilized enzyme) (less than 2 mass% ratio of fatty acid) [15], methanol (fatty acids : methanol = 1:1), and isooctane (fatty acids: isooctane = 2:1) using a reflux apparatus under the isothermal conditions of 35 ℃ for 260 rpm and stirred for 16 hours.

2.3. Gas chromatography (GC) analysis

Gas chromatography (GC) analysis Fatty acid methyl esters (FAMEs) were analysed by gas chromatograph Agilent 6890N (Agilent Technologies, USA) equipped with a flame ionization detector (GC-FID) and a Restek Rt-2340 NB Cap. column (105 m ×

0.25 mm × 0.20 μm). The oven temperature was initially set at 170 °C and then programmed to 250 °C using helium as the carrier gas, at a flow rate of 1.1 mL/min and held for 30 min. The injector and detector temperatures were 250 °C. The split ratio was 1:80. Fatty acids were identified by comparing the retention time of FAME peaks with Supelco 37 FAME mixture standards (Sigma) [1, 16, 24].

2.4. Differential scanning calorimetry (DSC) tests

The temperature–programmed screening experiments were performed with DSC TA Q20-RCS90 (TA Instruments, USA). For the DSC analysis on the samples sealed in 20 μL aluminium pans, the lid was pressed onto the crucible using the pressure of a heavy mechanical force, and the seal tightened the crucible; the test cell was then sealed manually by a special tool equipped with TA's DSC [1, 16]. Approximately 1.7 mg to 2.5 mg of the sample was used for obtaining the experimental data. The B0 and B100 of the dynamic tests of the scanning rate selected for the programmed temperature ramp were 2, 4, 6, and 8 °C/min and B2-B50. The scanning rate selected for comparing the programmed temperature ramp was 4 °C/min for the range of temperatures cooling down from 30 °C to -40 °C for each for each winterization phase behaviour experiment. In all of studies with the DSC thermal analysis, high purity nitrogen was the purge gas, and the flow rate was 50 mL/min.

2.5. Diesel, biodiesel, and blend biodiesel winterization kinetic evaluation

Here, the proto-kinetic equation was applied to evaluate the winterization of the phase transfer for the exothermic reaction as follows [17]:

$$r_i = k_0 e^{\frac{-E_a}{RT}} \alpha^{n_1} (1-\alpha)^{n_2} \tag{1}$$

where E_a is the activation energy, k_0 is the pre-exponential factor, R is the ideal gas constant, α is the degree of conversion of a reaction or stage, and n_1 and n_2 are the reaction order of the phase transfer of the winterized exothermic reaction [17].

2.6. Biodiesel and blend Biodiesel kinematic viscosity measurement

The kinematic viscosity measurement is important for diesel engine fuel, and it is also an important indicator for biodiesel. To charge the sample into the viscometer, we placed the viscometer into the holder for fixing and inserted it into the constant temperature bath. The viscometer holder fit a Cannon-Fenske Routine viscometer 75 U534 (CANNON Instrument Company, USA) [16, 19]. We aligned the viscometer vertically in the bath by means of a small plumb bob in the tube. Approximately 10 minutes was allowed for the sample to come to the bath temperature of 40 °C. We repeated this process three times for each sample to measure the efflux time and check the run.

2.7. Biodiesel and blend Biodiesel heat of combustion measurement

The heat of combustion analysis of the samples involved a Parr 1341 oxygen bomb calorimeter instrument (Parr Instrument Company, USA) [16, 21-22]. We did a heat of combustion analysis for all of the samples as follows. To prepare, the water temperature was approximately 1.5 °C below room temperature, for which it was not necessary to use exactly 2 kg, but the amount selected must be duplicated within ±0.5 g for each experiment. We opened the filling connection control valve slowly and watched the gauge as the bomb pressure rose to the desired filling pressure (usually 30 bar, but never more than 40 bar). Then, we closed the control valve. Instead of weighing the bucket, it was filled from an automatic pipette or from any other volumetric device if the repeatability of the filling system was within +/− 0.5 mL and the water temperature was held within a 1 °C range. We

let the stirrer run for 5 minutes to reach equilibrium before starting a measured run [16, 21-22]. Approximately 0.5 g of all of the samples was used for acquiring the experimental data. At the end of this period, we recorded the time on the timer of the Parr 6775 digital thermometer and read the temperature.

2.8. Flash point measurement

The flash point was determined by HFP 360 Pensky-Marten Flash Point Tester (Walter Herzog GmbH, Germany), which met the requirements of the ASTM D93B standard [23, 25], and it was used to estimate the flash points of the B0, B100, and the various proportions of B100 for mixing with B0. The ASTM D93 test method was applied to measure the flash points for each sample [23, 25]. The tester amalgamated control devices programmed the instrument to heat the sample at a specific designated rate within a temperature range close to the expected flash point. The flash point was automatically tested by using an igniter at specified temperature test intervals.

3. Results and Discussion

3.1. The results of transesterification

From the sequences of the saponification, reduction reaction, and acid-catalysed reactions, which were conducted for the full process of transesterification, and excluding the impurities, the free fatty acids, and glycerol, we successfully obtained the biodiesel of waste oil of *Aurantiochytrium* sp. [1, 16]. Fig. 1(a) shows the fatty acid profile of waste oil from GC analysis, which verified the fatty acid composition of the waste oil and confirmed that the amount of palmitic acid and DHA was more than 75 mass% of total fatty acids [1]. In addition, from Fig. 1(b), the C14-C24 of the fatty acids were more than 92 mass% of the total fatty acid methyl ester profile by chemical transesterification, but via the enzyme transesterification, they were only 60 mass%.

(a) Fatty acid composition of waste oil   (b) Fatty acid methyl ester composition of waste oil
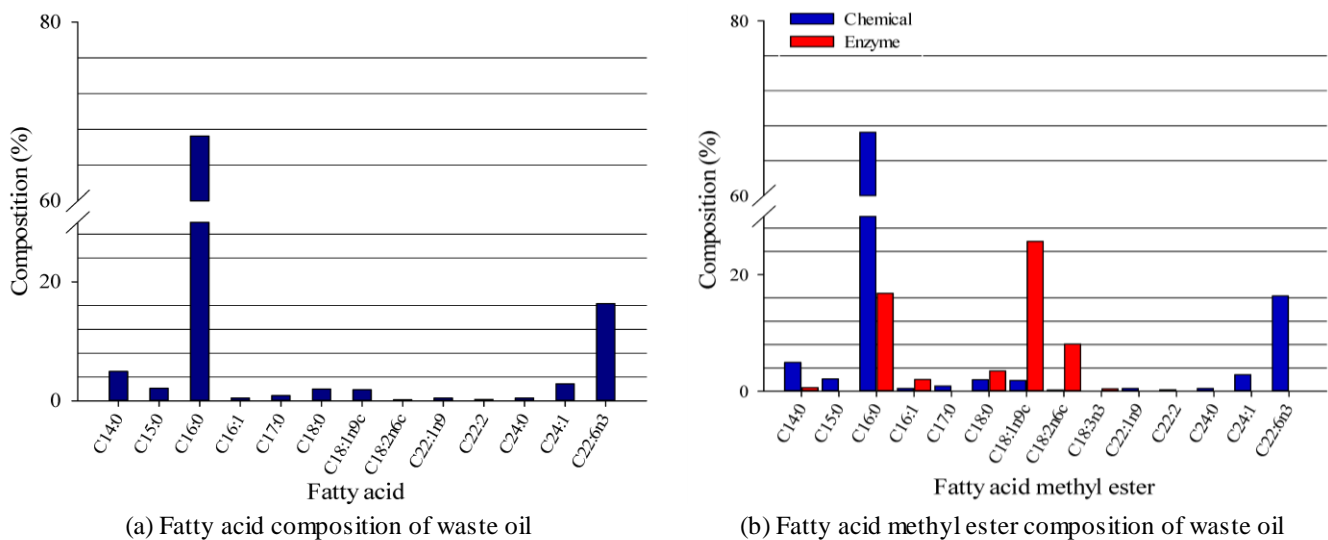
Fig. 1 Fatty acid and fatty acid methyl ester composition of waste oil of *Aurantiochytrium* sp

Fig. 2 shows that the DSC tests programmed temperature ramps were 2, 4, 6, and 8 ℃/min for the range of temperatures cooling from 30 to -40 ℃ for each B0 and B100 experiment, respectively. Moreover, Figs. 2 (a) and 2 (b) show the onset temperature of ca. -6 ℃ and 20 ℃ for the winterization of B0 and B100, respectively. B100 was the onset temperature (ca. 20 ℃) of winterization slightly below room temperature, which would clog the oil lines and affect the diesel engine performance at the low ambient temperature.
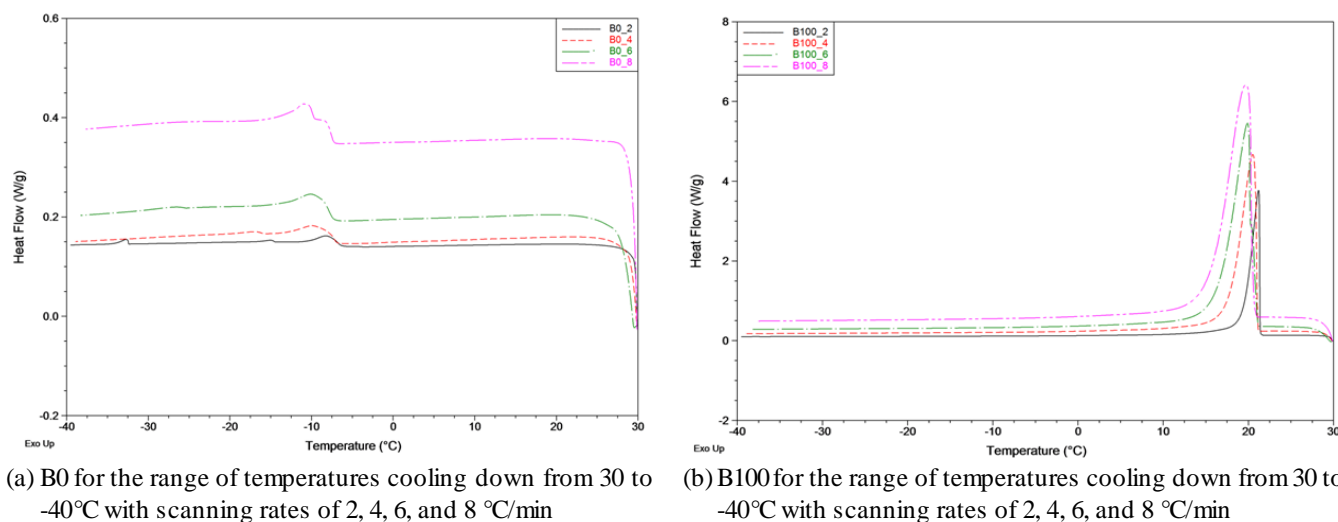
(a) B0 for the range of temperatures cooling down from 30 to -40℃ with scanning rates of 2, 4, 6, and 8 ℃/min

(b) B100 for the range of temperatures cooling down from 30 to -40℃ with scanning rates of 2, 4, 6, and 8 ℃/min

Fig. 2 DSC thermal curves of heat flow versus temperature

3.2. Kinetic parameter of winterization

Table 1 Comparisons of the B0 and B100 kinetic parameters for the evaluation with scanning rates of 2, 4, 6, and 8 ℃/min

Kinetic Parameter	Sample	Condition[a]			
		2	4	6	8
$\ln(k_0)/\ln(1/s)$[b]		7.6886	7.0606	6.8166	8.0858
E_a[c]		26.7237	23.8218	22.5479	24.1341
n_1[d]	B0	1.1179	1.1126	1.0977	1.1352
n_2[e]		1.0108	1.2552	1.1630	2.7679
$\underline{\Delta H}$[f]		5.1302	7.6498	7.0392	11.0410
$\ln(k_0)/\ln(1/s)$		4.1213	4.5116	4.4545	4.7674
E_a		14.8082	14.2822	14.2331	14.6001
n_1	B100	1.1501	1.1493	1.1126	1.1045
n_2		2.0334	2.5039	2.0072	1.9112
ΔH		169.0000	179.6522	162.9779	168.3870

The B0 and B100's kinetic parameters of winterization were evaluated as listed in Table 1. From Table 1, we compared the E_a and of winterization, B0 and B100 ca. 24 kJ/mol and 14 kJ/mol, respectively. We found that the simulation method of applying the proto-kinetic equation could be appropriately used in the exothermic reaction for winterization of waste oil's biodiesel of *Aurantiochytrium* sp. Moreover, from Table 1, the results of the parameter evaluation for proto-kinetic simulation demonstrated that the model provided much more consistent results for B0 and B100. Therefore, while analysing the B0 and B100 kinetic parameters of winterization, we obtained a better model by proto-kinetic simulation in this study.

Similar to the various proportions of B100 for mixing with B0 of B2-B50 blend biodiesel, Table 2 and Fig. 3 show that the DSC onset temperature, peak maximum temperature and enthalpy measured for the B2-B50 clearly discriminated the differences. Fig. 3 shows that there was an onset temperature from ca. -6 ℃ to 6 ℃ for the winterization of B2-B50 by DSC analyses. An important characteristic of a good blend biodiesel is the ability to avoid the winterization phenomenon under the low ambient temperature. Therefore, while at the ambient low temperature, a reliable blend biodiesel cannot be solidified in the pipeline and tank, which could cause engine failure [1, 16]. In contrast to Tables 1 and 2, we observed the results of the proto-kinetic simulation for B0–B100, in which the kinetic parameters provided clear and specific results. The E_a values of B0-B100 were in the range of 23.82–14.28 kJ/mol. Moreover, Table 2 shows that the result was explicit: B0-B100 of E_a, along with the higher proportion of B100, became smaller. From the above results of the kinetic parameter evaluation of winterization, we obtained the highly proportional mixture of B100, which was easy for winterizing the blend biodiesel.

Table 2 Results of DSC test and proto-kinetic equation simulation from B2 to B50
kinetic parameters for the evaluation at a scanning rate of 4 ℃/min

| Sample	B2	B5	B8	B10	B20	B30	B50
Mass[a]	2.0	2.0	2.0	2.0	2.0	1.9	2.0
$ExoT_o$[b]	-6.22	-4.85	-5.71	-5.34	-4.15	-1.03	6.09
$ExoT_p$[c]	-7.31	-14.54	-14.50	-12.66	-7.67	-1.78	5.70
$Exo\Delta H$[d]	10.40	13.50	19.00	22.70	34.20	46.90	81.10
$\ln(k_0)/\ln(1/s)$[e]	7.9899	6.9724	6.5301	6.3565	6.6144	6.3312	5.2386
E_a[f]	26.8404	23.6787	23.0120	22.4650	21.3152	21.3428	18.7669
n_1[g]	1.0718	1.1073	1.0886	1.0935	1.1686	1.1335	1.1055
n_2[h]	0.6631	1.8153	0.9095	1.0190	1.6253	1.4865	2.2704
ΔH[i]	11.2995	18.4967	19.3829	23.1463	34.9629	47.6906	82.7576
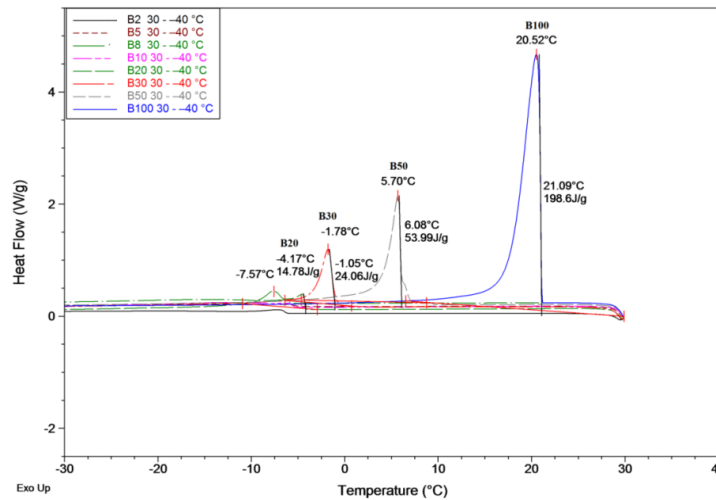
Fig. 3 DSC thermal curves of heat flow versus temperature for the B2-B100 for the range of temperatures cooling down from 30 to -40 ℃ with a scanning rate of 4 ℃/min

3.3. The results of kinematic viscosity measurement

From Fig. 4, we obtained the B0-B100 of the kinematic viscosity average values range from 3.14 mm²/s to 4.35 mm²/s. Generally, the biodiesel would be a fuel for a diesel engine. The kinematic viscosity range followed the ASTM D6751 and EN 14214 specification standards [23, 24], from 1.9 mm²/s to 6 mm²/s and from 3.5 mm²/s to 5.0 mm²/s, respectively. It was too thin for B2-B10 as a blend biodiesel for EN 14214 specification standards, and it could not provide the appropriate lubrication of diesel engines. We also observed that B20-B100 (3.51-4.35 mm²/s), whose values of kinematic viscosity were included in a suitable range, was also concerned with the effective fuel for diesel engine, such as the winterization, the heat of combustion, the flash point, and the storage conditions of blend biodiesel (see Fig. 4). Then, the best conditions of the mixtures were obtained as an available blend biodiesel in this study.

3.4. Heat of combustion measurement

We obtained the best condition of mixtures and the maximum proportion of biodiesel as a good blend biodiesel of waste oil's biodiesel. In this study, for the heat of combustion of blend biodiesel, the basic value was B0 (43.98 kJ/g), and this value was used as a comparison benchmark for other diesels. From Fig. 4, we obtained the heat of combustion and showed that B100, B50, and B30 were less than the others, which also showed that they were unsuitable for a blend biodiesel. From the comparisons of all samples heat of combustion, the mixed ratio of blend biodiesel smaller than B20 was acquired. This was the fuel that was suitable for use in compression ignition diesel engines in this study. Meanwhile, from Fig. 4, we observed the B2-B20 (43.30-43.15 MJ/kg), whose values of heat of combustion were included in the suitable range, and this also showed that the highly mixed ratio of blend biodiesel was B20 (43.15 MJ/kg) in this study.
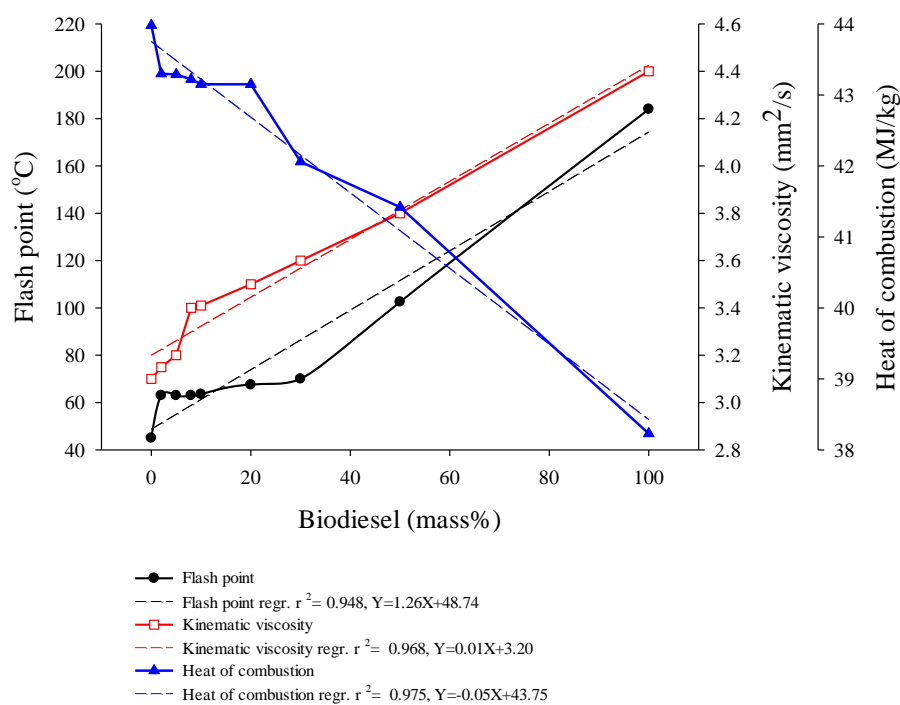
Fig. 4 Heat of combustion, kinematic viscosity, and flash points of various proportions of B0 for mixing with B100

3.5. Flash point measurement

Here, the results of the flash point tests of B0, B100, and the various proportions of B100 for mixing with B0 also followed the U.S. Department of Transportation (DOT 49 CFR 173.120) regulations [18]. From Fig. 4, we obtained the B0-B100 of the flash point temperature range from 45 °C to 184 °C, individually. From Fig. 4, we also observed the B2-B100 (63-184 °C) of flash points were all above 60.5 °C. This temperature of flash points exceeded the safety condition that was related to a suitable fuel for diesel engine. This study also showed that adding the waste oil's biodiesel of *Aurantiochytrium* sp. could increase the storage and transport safety of diesel. The above transesterified results of fatty acid via GC analyses and DSC tests repeatedly corroborated the accuracy of the transesterification and the specific characteristic, proving that waste solid byproducts of *Aurantiochytrium* sp. oil formed biodiesel. Then, by conducting the DSC, bomb calorimeter, viscometer, and flash point tester, we compared the winterization, kinematic viscosity, and heat of combustion of the various proportions of B100 for mixing with B0, which could establish the best conditions of the mixture B20 as an available blend biodiesel.

4. Conclusions

The waste oil of *Aurantiochytrium* sp. can be developed for biodiesel production and successfully made into a suitable blend biodiesel. We obtained the best conditions of mixtures and the highly mixed rate of B20 (E_a of winterization 21.32 kJ/mol at DSC scanning rate 4 °C/min; onset temperature of winterization -4.15 °C at DSC scanning rate 4 °C/min; heat of combustion 43.15 MJ/kg; kinematic viscosity 3.51 mm^2/s; flash point 67.5 °C) as an appropriate blend biodiesel from the waste oil's biodiesel of *Aurantiochytrium* sp. Thus, from a green energy perspective, this project addressed the problem of "will not be occupied" arable land, ease of management, and ease of extensive cultivation for the production of biomass. We will enhance the transesterification technology for the waste oil of *Aurantiochytrium* sp. to find improved energy efficiency, a less polluting method, and appropriate chemical and physical stabilities, which will be used in the mass production of green energy in future work.

Acknowledgements

References

[1] S. Y. Tsai, H. Y. Lin, G. Y. Lu, and C. P. Lin, "Solid byproducts of Aurantiochytrium sp. oil made into the biodiesel oil made into the biodiesel," Journal of Thermal Analysis and Calorimetry, vol. 120, no. 1, pp. 563-572, April 2015.

[2] J. Wang, L. Cao, and S. Han, "Effect of polymeric cold flow improvers on flow properties of biodiesel from waste cooking oil," Fuel, vol. 117, pp. 876-881, January 2014.

[3] A. Hayyan, M. A. Hashim, M. E. Mirghani, M. Hayyan, and I. M. Alnashef, "Esterification of sludge palm oil using trifluoromethane sulfonic acid for preparation of biodiesel fuel," Korean Journal of Chemical Engineering, vol. 30, no. 6, pp. 1229-1234, June 2013.

[4] S. K. Hoekman, A. Broch, C. Robbins, E. Ceniceros, and M. Natarajan, "Review of biodiesel composition, properties, and specifications," Renewable and Sustainable Energy Reviews, vol. 16, no. 1, pp. 143-169, January 2012.

[5] X. Meng, J. Yang, X. Xu, L. Zhang, Q. Nie, and M. Xian, "Biodiesel production from oleaginous microorganisms," Renewable Energy, vol. 34, no. 1, pp. 1-5, January 2009.

[6] J. V. Gerpen, "Biodiesel processing and production," Fuel Processing Technology, vol. 86, no. 10, pp. 1097-1107, June 2005.

[7] P. Sivakumar, K. Anbarasu, and S. Renganathan, "Bio-diesel production by alkali catalyzed transesterification of dairy waste scum," Fuel, vol. 90, no. 1, pp. 147-151, January 2011.

[8] M. Xiao, S. Mathew, and J. P. Obbard, "Biodiesel fuel production via transesterification of oils using lipase biocatalyst," Global Change Biology Bioenergy, vol. 1, no. 2, pp. 115-125, April 2009.

[9] N. Sangaletti, M. Cea, M. A. B. Regitano-d'Arce, T. M. F de Souza Vieirac, and R. Navia, "Enzymatic transesterification of soybean ethanolic miscella for biodiesel production," Journal of Chemical Technology and Biotechnology, vol. 88, no. 11, pp. 2098-2106, November 2013.

[10] F. Hasan, A. A. Shah, and A. Hameed, "Industrial applications of microbial lipases," Enzyme and Microbial Technology, vol. 39, no. 2, pp. 235-251, June 2006.

[11] M. Aarthya, P. Saravananb, M. K. Gowthamana, C. Rosea, N. R. Kaminia, "Enzymatic transesterification for production of biodiesel using yeast lipases: an overview," Chemical Engineering Research and Design, vol. 92, no. 8, pp. 1591-1601, August 2014.

[12] R. Abdulla, and P. Ravindra "Immobilized Burkholderia cepacia lipase for biodiesel production from crude Jatrophacurcas L. oil," Biomass and Bioenergy, vol. 56, pp. 8-13, September 2013.

[13] A. Guldhe, B. Singh, T. Mutanda, K. Permaul, and F. Bux, "Advances in synthesis of biodiesel via enzyme catalysis: Novel and sustainable approaches," Renewable and Sustainable Energy Reviews, vol. 41, pp. 1447-1464, January 2015.

[14] T. C. Kuo, J. F. Shaw, and G. C. Lee, "Conversion of crude Jatropha curcas seed oil into biodiesel using liquid recombinant Candida rugosa lipase isozymes," Bioresource Technology, vol. 192, pp. 54-59, September 2015.

[15] X. Fang, Y. Zhan, J. Yang, and D. Yu, "A concentration-dependent effect of methanol on Candida antarctica lipase B in aqueous phase," Journal of Molecular Catalysis B: Enzymatic, vol. 104, pp. 1-7, June 2014.

[16] S. Y. Tsai, W. S. Tseng, C. T. Wu, and C. P. Lin, "Dwarf-castor oil made into a suitable biodiesel," Procedia Engineering, vol. 84, pp. 940-947, 2014.

[17] C. P. Lin and J. M. Tseng, "Green technology for improving process manufacturing design and storage management of organic peroxide," Chemical Engineering Journal, vol. 180, pp. 284-292, January 2012.

[18] G. B. Wang, C. C. Chen, H. J. Liaw, Y. J. Tsai, "Prediction of flash points of organosilicon compounds by structure group contribution approach," Industrial & Engineering Chemistry Research, vol. 50, no. 22, pp 12790-12796, September 2011.

[19] Cannon-Fenske Opaque Viscometers, CANNON Instrument Company, 2014, www.cannoninstrument.com.

[20] ASTM D6751, Standard specification for biodiesel fuel blend stock (B100) for middle distillate fuels, ASTM International, West Conshohocken, PA, 2015, www.astm.org.

[21] Parr Bulletin 1341, Plain jacket calorimeter, Parr Instrument Co., Moline, IL, 1977.

[22] A. M. Halpern, G. C. McBane, Experimental physical chemistry. 3rd ed., New York: Freeman, W. H., pp. 5.1-5.15, 2006.

[23] A. S. Gundogar and M. V. Kok, "Thermal characterization, combustion and kinetics of different origin crude oils," Fuel, vol. 123, pp. 59-65, May 2014.

[24] F. Gasparini, J. R. de O. Lima, Y. A. Ghani, R. R. Hatanaka, R. Sequinel, D. L. Flumignan, and J. E. de Oliveira, "EN 14103 adjustments for biodiesel analysis from different raw materials, including animal tallow containing C17," World Renewable Energy Congress 2011, Bioenergy Technology, May, 2011, pp. 101-108.

[25] G. Knothe, "Analyzing biodiesel: standards and other methods," Journal of the American Oil Chemists' Society, vol. 83, no. 10, pp 823-833, October 2006.