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Ultrasound-assisted production of biodiesel FAME from rapeseed oil in a novel two-compartment reactor

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Abstract

BACKGROUND: Ultrasonication has been proposed as a promising technique for enzymatic transesterification. In contrast, excess ultrasonication causes an enzyme inactivation. This paper presents enzymatic transesterification to produce fatty acid methyl ester (FAME) from

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rapeseed oil using Callera Trans LTM using a an original two-compartment reactor. The reactor was composed of a mechanically stirred compartment (ST) and ultrasound irradiation compartment (US). The reaction solution was recirculated between the ST and the US. The enzyme was only exposed by ultrasonication in the US. The reactor system has the option to control the direct irradiation period of ultrasonication to soluble enzyme, regulated by the mean residence time in the US.

RESULTS: The production of FAME with ultrasound irradiation gave a final yield of 91wt% after 15hours. The reaction rate was enhanced up to 2-fold through the use of the two-compartment reactor compared with liquid lipase catalyzed transesterification without any ultrasound treatment. The V_{max} with the ultrasound irradiation was 2.3-fold higher than that of the ultrasound free system, while the K_m remained at almost the same level. The reaction rate and the conversion increased with a shorter mean residence time in the US.

CONCLUSION: The excellent advantages of the two-compartment reactor were presented to produce biodiesel (FAME) resulting in acceleration of the enzyme reaction by ultrasound irradiation. Especially, reaction enhancement was maximally obtained using a separate compartment of the reactor. A shorter mean residence time of reaction solution in the US and higher ultrasound power successfully realized a higher production rate of FAME.

Keywords: Biodiesel, Lipase, Ultrasound, Transesterification, Rapeseed oil

INTRODUCTION

Biodiesel has emerged as an environmentally-friendly and renewable alternative fuel. The estimated global production of Biodiesel in 2015 was 3.1×10^{10} L yr⁻¹. Biodiesel production is estimated to increase to 11×10^{10} L yr⁻¹ by 2020.¹ Biodiesel is produced from triglycerides by transesterification with short chain alcohols (Figure 1).^{2, 3} Alkaline catalysis has often been employed in the industrial production of biodiesel, although it requires excess alkaline loading and results in environmental problems involving waste disposal.⁴⁻⁶

In the last decade an alternative enzymatic process using a lipase enzyme has been suggested to catalyze the transesterification because it has the advantage of being able to convert low-grade feed-stocks with high free fatty acid (FFA) content, resulting in significant economic as well as environmental benefits.^{7, 8} In order to keep the cost of the enzyme low enough at first researchers attempted to recycle the enzyme, aided by immobilization.⁹⁻¹² Nevertheless, despite some success it has subsequently become clear that the use of soluble (liquid) enzyme comes at a lower cost contribution to the final product and results in

significant process simplification. Today the use of liquid lipase for the transesterification in the presence of free fatty acids has been reported scientifically and commercially demonstrated.¹³⁻¹⁵

Several scientific publications attest to the clear advantages of such a system catalyzed by Callera Trans L™, a liquid lipase formulation from *Thermomyces lanuginosus* (Novozymes A/S, Denmark).¹⁶

From previous studies it is known that the lipase from *Thermomyces lanuginosus* has high catalytic activity for the transesterification reaction.¹⁷⁻¹⁹ Conventionally, in order to make use of this enzyme activity, and since the enzyme is interfacially-activated the aqueous-organic interface has been made as large as possible by mechanical stirring, using a well agitated stirred tank. This is a considerable cost for a commercial process, even at moderate power inputs per unit volume of reaction solution.

More recently ultrasound irradiation has been proposed as a useful technique to accelerate transesterification reactions.²⁰⁻²⁵ Reports suggest that using ultrasound energy can considerably intensify the process by generating cavitation in the reaction liquid phase.^{26, 27} Cavities subsequently grow and finally collapsed, releasing a large amount of energy in a small volume of solution.²⁸ As a result, the very high density of energy influences mass transfer between different phases²⁹, and potentially the enzyme also. Previous investigations on the use of ultrasound energy for biodiesel production with enzymes has been published. Table 1 lists other types of lipase used in enzymatic transesterification assisted by ultrasound

irradiation.³⁰⁻³⁵ The results indicate that ultrasound irradiation would create an effective micro-scaled interfacial area where lipid and lipase react in a biodiesel process.

In this paper, we present a two-compartment reactor system using ultrasound irradiation to enhance the enzyme reactivity. The two reactor compartments are connected by a recirculation loop and are organized to allow the benefit of ultrasound irradiation on productivity of enzyme reaction, while minimizing exposure.

EXPERIMENTAL

Materials

Rapeseed oil was obtained from Emmelev A/S (Otterup, Denmark). Methanol (99.8%, technical grade) was purchased from VWR Bie & Berntsen A/S (Herlev, Denmark). Table 2 shows the properties of refined oil used in this study. Acetic acid (99%), n-heptane (99%), isopropanol (99%), and tert-butyl methyl ether (99.8%) were obtained from Sigma-Aldrich A/S (Brøndby, Denmark). Enzymatic reactions were carried out using soluble lipase (Callera LTM), which was kindly donated by Novozymes A/S (Bagsværd, Denmark). The activity of the enzyme was reported as approximately 1×10^5 U/g-original liq., where 1U was defined as the activity required to produce 1 μ mol butyric acid from the hydrolysis of tributyrin under standard conditions (pH 7.5, 0.2M substrate).³⁶

Recycle mode

Figure 2 presents a schematic diagram of the two-compartment reactor. One compartment is termed the “stirred compartment (ST)”, and the other termed the “ultrasound compartment (US)”. The ultrasonic horn was not installed at the deepest location in the US cell, since the glass made bottom of the US cell was mechanically damaged by direct irradiation of ultrasonication. Therefore, the ultrasonic horn was installed at the 2cm depth from the top of the liquid level for safety reactions. The reaction fluid was then introduced to the top of the US cell in which nearby the ultrasonic horn was placed, as illustrated in Figure 2.

The ST compartment was made of a cylindrical glass. The initial reaction volume was 267mL while rapeseed oil 220mL, initial methanol 25mL, and enzyme solution 22mL. The volume of recycle loop was in total 40mL. The inside diameter of ST compartment was 5.5cm. Mean depth of reaction solution in ST compartment at steady state was 5.4cm. Two baffle plates (1cm width) were attached on inside lateral of ST compartment. A 2.4cm diameter six-blade rushton turbine impeller was installed in the ST compartment and spun at 1400min^{-1} . Two baffles (1cm width) were attached on the inside lateral of the ST compartment. The ST compartment was immersed in a water bath (308K). The temperature was controlled by equipment from Julabo Labor-technik GmbH; (Seelbach, Germany).

The effective liquid volume in US compartment (glass flow cell) in steady state was 80mL. the ultrasound irradiation device was inserted inside this and supplied by GD14K (Hielscher Ultrasonic GmbH, Germany). Ultrasonic irradiation at a frequency of 24kHz was directly provided into reaction solution using a horn-shaped cylindrical device UP200S. The diameter

ϕ of the device was 14mm. The cross sectional area of the ultrasonic horn was 153mm². The guaranteed high surface power density was 1.25W mm⁻². That can be controlled at 20, 40, and 60% of maximum. The applied ultrasound output was therefore calculated as 38, 77 and 115W, respectively. The temperature in the US compartment was maintained at 308K by a water jacket. It was regulated by a Thermo DC10 (Thermo HAAKE, USA).

The two compartments (ST and US) were connected by a recirculation flow that was adjusted using a Watson MARLOW 520S. The recirculation flow rate (F [mL s⁻¹]) varied from 0.17 to 1.67mL s⁻¹. The mean residence time of the reaction solution in the US (θ [s]) calculated by the volume of US reactor vessel (V_U [mL]), which was 80mL, divided by the recirculation flow rate as Eq. (1).

$$\theta = \frac{V_U}{F} \quad (1)$$

The volume of the connected recirculation loop (V_L [mL]) between the ST and the US was 40mL. The initial volume of ST solution (V_S [mL]) was 267mL. Therefore, the overall circulation time (Θ [s]) throughout US, ST and recirculation loop was calculated by Eq. (2).

$$\Theta = \frac{V_U + V_S + V_L}{F} \quad (2)$$

Table 3 presents details of recirculation conditions in the reactor system. In this work, the ratio of residence time of US to the overall reaction line (θ/Θ) was constant at 0.21.

Rapeseed oil (220mL) was used as a substrate for the reaction. 2g of lipase solution was dissolved in 20g of distilled water. The concentration of enzyme based on the mass fraction is therefore 0.09 [g-enzyme • g⁻¹ - (enzyme +water)] and used throughout this paper. n-Heptane

was employed as an inert component in the organic phase to prepare the desired concentration of substrate. The substrate concentration varied from 0.23 to 0.9 [g-oil $\cdot$ g⁻¹ - (oil +n-heptane +methanol)].

The US was initially empty. Methanol (25mL) was initially added to the rapeseed oil in the ST before the reaction. The enzyme aqueous solution (22mL) was then added to the rapeseed oil and methanol mixture. The reaction was initiated by adding enzyme, and the recirculation flow between the ST and the US was simultaneously started.

After the initiation of the reaction, methanol was continuously fed (50 μ L s⁻¹) into the ST. Methanol addition was a useful technique to obtain a higher yield of biodiesel according to our previous experience. The methanol was introduced using a KNF STEPODS. 03 pump (KNF Neuberger AB, Stockholm. Sweden).

Ultrasound irradiation was initiated when the volume of reaction solution in the US became stable at 80mL at the steady state. The recirculation flow rate was monitored and regulated to keep the volume of reaction solution in the US at 80mL throughout the reaction.

Sample preparation and analysis by high-performance liquid chromatography

50 μ L samples were periodically taken from the ST and then mixed with 500 μ L solvent A (99.6% (v/v) n-heptane and 0.4% (v/v) acetic acid). The mixed sample was centrifuged at 14500 rpm for 5 min, and then 10 μ L of supernatant was mixed with 990 μ L of solvent A before high-performance liquid chromatography (HPLC) analysis.

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40 μ L of the above sample was injected into an HPLC system (Ultimate 3000, Dionex A/S, Hvidovre, Denmark). The HPLC was employed to measure the concentration of triglyceride (TAG), diglyceride (DAG), monoglyceride (MAG), free fatty acid (FFA), and fatty acid methyl ester (FAME). These five compounds were separated using a cyanopropyl column (0.25 $\times$ 0.004m) (Discovery[®], Cyano, Sigma Aldrich A/S, Brøndby, Denmark), a U3000 auto sampler, a TCC-3000SD column oven, U3400A quaternary pump modules, and a CoronaR Charged Aerosol Detector (Thermo Scientific Dionex, Chelmsford, MA, USA).

A binary gradient program was employed to separate the five different compounds using solvent A, solvent B (99.6% (v/v) tert-butyl methyl ether and 0.4% (v/v) acetic acid), and solvent C (iso-propanol). The compounds were detected after separation with the column using a CoronaR Charged Aerosol Detector from Thermo Scientific Dionex (Chelmsford, MA, USA) with nitrogen gas at a pressure of 241kPa.

RESULTS and DISCUSSION

Effect of ultrasound on production of FAME

In this work, fatty acid methyl ester (FAME) was measured as a main component of biodiesel. In Table 4, molecular weight and density of the component reaction fluid was presented. The mass fraction of FAME in the reaction solution was employed as an indication of the reaction progress of biodiesel production.

$$\text{Name of component [Mass\%]} = \frac{\text{Mass of each compartment at desired running time [g]}}{\text{Total mass of each component at the initial condition (TAG[g]+DAG[g]+MAG[g]+FFA[g]+FAME[g])}} \times 100 \quad (3)$$

Figure 3 depicts the time course a typical transesterification reaction using liquid lipase. The concentration of TAG, DAG, MAG, and FAME monitored during 24h of the reaction. FAME was produced by transesterification of TAG with methanol with the aid of lipase. The transesterification is a sequence of three consecutive steps. In the first step, TAG is converted to DAG. In the second step, DAG is converted to MAG. In the third step, MAG is converted to FFA. Each conversion step yields one FAME molecule, given a total of three FAME molecules per one TAG molecule. Fig. 3(a) shows the effect of ultrasound irradiation on the time course of the concentration of TAG. The concentration of TAG decreased evidently faster especially in the initial 1h by ultrasound irradiation. In the case of intermediates (DAG and MAG) in Fig. 3(b) and (c), the peak time of DAG and MAG was also accelerated by ultrasound. In this manuscript, the purity of FAME was indicated as the mass fraction of FAME in reaction fluid. The final mass fraction of FAME achieved over 90wt% at 15h for ultrasound treatment (Fig.3 (d)). In contrast, 24h was needed in the system without any ultrasound treatment to give the same yield. Accelerated production was evidently realized by ultrasonic treatment compare with no treatment. The cause of reaction enhancement by ultrasound irradiation is speculated to be that cavitation caused by the ultrasound induced micro-scale turbulence and that the mass transfer resistance was eliminated.³⁷⁻³⁹ Previous reports have suggested that ultrasound could cause the enzyme structure to become flexible;

thus, the enzyme might shift into its active configuration.⁴⁰

In order to understand further the origin of this enhancement a study of reaction kinetics was undertaken. Figure 4 presents the results of a kinetic experiment measuring reaction rate as a function of substrate concentration ranging from 0.23 to 0.9 [g-oil • g⁻¹ - (oil+ n-heptane + methanol)]. In this study, n-heptane was employed as an inert solvent because of the need to evaluate the process at different substrate concentrations. As expected, the initial reaction rate increased with increasing substrate concentration.

The kinetic parameters were conveniently determined by a Hanes Woolf plot (Figure 5). The maximum reaction rate V_{max} with the ultrasound irradiation system was 2.3-fold higher than that of the ultrasound free system, but interestingly the K_m remained at almost the same level (Table 5). An increase in V_{max} seems to indicate that a considerable movement of reactants to the active site of the enzyme and the reaction products to the medium were achieved under the influence of the ultrasonic field.⁴¹

Changing ultrasound power

Figure 6 shows the effect of ultrasound power on the initial reaction rate, which increased with increasing ultrasound power. This clearly demonstrates the positive effect of ultrasound irradiation in accelerating the reaction. With an increase in the ultrasound power, the number of cavitation bubbles also increases giving strong effect by cavitation.^{42,43} The initial reaction rate of mean residence time of 470s showed that the downward trend after over 77W. It is

important to note here that further increase in treatment time caused harmful effects, as continuous exposure to cavitating conditions for prolonged time led to degradation of the amino acid residues which contributes to the substrate binding domain or catalytic domain of the enzyme molecules resulting in decrease in enzyme stability.⁴⁴ This is the real advantage of the two compartment reactor to control the exposure time to ultrasound.

The ultrasonic irradiation for a reaction solution in short period of 48s and 96s was effective to enhance enzymatic initial reaction rate even in the higher power case of 115W. In contrast, longer continuous irradiation of ultrasonic power even in the lower ultrasonic power caused unexpected damage of enzyme activity. In our results, the irradiation period 470s resulted lower initial reaction rate than that of other shorter period cases (48s and 96s) in our experimental range of ultrasound power. The cavitation during ultrasound irradiation induced oscillation by stable cavitation bubbles, that changes the spatial conformation of enzyme.⁴⁵

Even if same accumulated time of ultrasonic irradiation, periodic shorter intermittent residence in the US compartment was strategically excellent mode to enhance initial reaction rate and to minimize damage of enzyme activity, as illustrated in Figure 7. Molecular structure damaged of enzyme well recovered in isolated period just after exposure period in the US. Longer period of exposure of high intensity ultrasound resulted in an unexpected inhibition to the catalytic activity of enzyme. On the other hands, shorter exposure of ultrasound attractively increased the activities of enzymes.⁴⁶ It can be inferred that ultrasound brings the conformational change of enzyme. Higher oscillated frequency of irradiation and

isolation periods, illustrated in Fig. 7 (b), proposed the high frequent opportunity for enzyme re-activation and refolding of molecular structure of enzyme. Further detail investigation needed to establish optimal irradiation manner for enzyme.

Figure 8 shows the effect of ultrasound power on the produced mass fraction of FAME with different mean residence time in the US. For mean residence time of 48s and 96s, the maximum mass fraction of FAME appeared at 77W. The mass fraction of FAME obtained at 24h indicated the maximum at 77W for residence time 48s and 96s. It was lower at power inputs above 77W regardless of higher initial reaction rate. Ultrasound irradiation, under these extreme conditions, could cause great damage to polypeptide chains, leading to inactivation of the enzyme.⁴⁷ In the case of residence time of 470s, it was slightly decayed with increasing ultrasound power. According to our results, residence time less than 96s and ultrasonic irradiation power indicated an optimal condition for high initial reaction rate and mass fraction of FAME at 24h in reaction solution.

The ultrasound energy applied to the enzyme seemed too large to disrupt the function of enzyme.⁴⁸⁻⁵⁰ Further investigation on the change of molecular structure especially nearby active site of the enzyme during ultrasound irradiation was necessary to determine an optimal residence time in US in conjunction with the irradiation power of ultrasound.

Changing flow rate

Figure 9 shows the effect of the space velocity of the reaction solution in the ultrasound

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compartment. The space velocity is the reciprocal of the mean residence time (θ [s]).

$$\text{space velocity [s}^{-1}\text{]} = \frac{1}{\theta} \quad (4)$$

As seen in the figure, the initial reaction rate increased with increasing space velocity under constant ultrasound power (38W). It is speculated that the attractive association between enzyme and substrate was facilitated by micro-scaled cavitation caused by ultrasound irradiation. Enhancement of the reaction rate was previously reported for lipase reactions.⁵¹⁻⁵³

Figure 10 indicates the effect of exposing the enzyme to ultrasound energy on the initial reaction rate. The ultrasound energy is calculated by the following equation.

$$\text{ultrasound energy exposed to enzyme [J]} = \text{irradiated ultrasound power [W]} \times \text{mean residence time in ultrasound compartment [s]} \quad (5)$$

The initial reaction rate decreased as -0.3 power of the ultrasound energy exposed to enzyme.

It is speculated that this occurred because the ultrasound irradiation accelerated the biodiesel reaction and maintained the enzyme activity due to the higher circulation flow rate.

CONCLUSIONS

Biodiesel (FAME) was successfully produced using a two-compartment reactor with optimal exposure to ultrasound balancing enhancement with suppression of enzyme damage. Enzymatic production of biodiesel by ultrasound irradiation was achieved with the reactor. The reaction rate was increased by ultrasound irradiation. The FAME yield was over 90wt%. In particular reactions with ultrasound treatment reached equilibrium faster than with no treatment. A shorter mean residence time and higher ultrasound power in the ultrasound

compartment realized higher initial reaction rate of FAME production.

Nomenclature

F : volumetric circulating flow rate [mL s⁻¹]

K_m : Michaelis constant [g-oil g⁻¹-(oil+n-heptane+methanol)]

[S]: substrate concentration [g-oil g⁻¹-(oil+n-heptane+methanol)]

V_i : initial reaction rate [g-FAME (g-(oil+n-heptane+methanol) · s)⁻¹]

V_L : volume of the connected recirculation loop 40mL

V_{max} : maximum reaction rate [g-FAME (g-(oil+n-heptane+methanol) · s)⁻¹]

V_S : initial volume of ST solution 267mL

V_U : volume of US reactor vessel 80mL

Greek symbols

θ : mean residence time of the reaction solution in the US [s]

θ^{-1} : space velocity of the reaction solution in the US [s⁻¹]

Θ : overall circulation time of reaction line (US+ ST+ recirculation loop) [s]

φ : diameter of ultrasonic device (cylindrical shape) [mm]

Abbreviations

DAG: diacylglyceride

FAME: fatty acid methyl ester, biodiesel

FFA: free fatty acid

MeOH: methanol

ST: stirred compartment

TAG: triacylglyceride

US: ultrasound irradiation compartment

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Caption of Figures

Figure 1 Consecutive reaction processes for fatty acid methyl ester (FAME) production by lipase with alcohol.

Figure 2 Schematic diagram of the circulation two-compartment reactor.

Figure 3 Change of mass fraction of components of transesterification reactions. (a) TAG. (b) DAG. (c) MAG. (d) FAME.

Figure 4 Reaction enhancement of ultrasound irradiation on FAME production with ultrasound irradiation in US compartment (38W). Enzyme concentration was set at $0.09 \text{ [g-enzyme g}^{-1} - (\text{enzyme} + \text{water})]$.

Figure 5 Hanes-Woolf plot for reaction parameters FAME production by soluble lipase (Callera LTM) donated by Novozymes A/S (Bagsværd, Denmark). Substrate was a rapeseed oil obtained from Emmelev A/S (Otterup, Denmark).

Figure 6 Effect of ultrasound power on initial reaction rate involved with mean

residence time in the US compartment.

Figure 7 Different mean residence time in the US compartment of reaction fluid.

(a) Periodic longer intermittent residence in the US. Ultrasonic irradiation to enzyme caused unexpected damage of enzyme. Enzyme hardly recovered its reactivity. (b) Periodic shorter intermittent residence in the US. Damage of enzyme by short ultrasonic irradiation was quickly recovered during isolated period in the ST just after exposure in the US.

Figure 8 Lipase productivity on FAME at 24h enhanced by ultrasonic irradiation.

Figure 9 Effect of space velocity in the US compartment on initial reaction rate under the ultrasound power 38W. Initial reaction rate was remarkably improved in higher space velocity in the US compartment.

Figure 10 Logarithmic correlation of initial reaction rate and ultrasound energy employed. Higher initial reaction rate was successfully attained in higher space velocity consisted with minimalized enzyme damage in US compartment even if high irradiation energy of ultrasound.

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Overview of experimental apparatus

ST

US

Figure 2

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Figure 3

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Figure 9

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Table 1 Previous articles for production of biodiesel from natural resources with ultrasound irradiation.

Author	Year	Ultrasound reactor type	Ultrasound power [W] / frequency [kHz]	Feedstock	Catalyst	Solvent	Yield	Reference
Subhedar, P.B. and Gogate P.R.	2016	Direct (horn)	80W / 20kHz	Waste cooking oil	Enzyme (Lipozyme RM IM)	Methyl acetate	96.1%	[43]
Adewale, P. et al.	2015	Direct (horn)	500W / 20kHz	Waste tallow	Enzyme (Candida antartctica lipaseB)	Methanol	85.6%	[38]
Subhedar, P.B. et al.	2015	Indirect (bath)	120W / 45kHz	Sunflower oil	Enzyme (Lipozyme RM IM)	Methanol	96%	[39]
Michelin, S. et al.	2015	Indirect (bath)	132W / 40kHz	Macauba coconut oil	Enzyme (Novozyme 435)	Ethanol	70%	[30]
Gharat, N. and Rathod, V.K.	2013	Indirect (bath)	200W / 25kHz	Waste cooking oil	Enzyme (Novozyme 435)	Methanol	87%	[31]
Tupufia, S.C et al.	2013	Indirect (bath)	80W / 43kHz	Coconut oil	Enzyme (Novozyme 435)	Ethanol	91%	[32]
Batistella, L. et al.	2012	Indirect (bath)	100W / 37kHz	Soybean oil	Enzyme (Novozyme 435, Lipozyme RM IM)	Ethanol	90%	[33]
Kumar, G. et al.	2011	Direct (horn)	100W / 24kHz	<i>Jatropha curcas</i> oil	Enzyme (immobilized lipase from <i>Enterobacter aerogenes</i>)	Methanol	84.5%	[34]
Yu, D et al.	2010	Indirect (bath)	250W / 40kHz	Soybean oil	Enzyme (Novozyme 435)	Methanol	96%	[53]

Table 2 Typical component of refined rapeseed oil.

Properties		Refined rapeseed oil [%]
Myristic acid	C14:0	0.1
Palmitic acid	C16:0	4.8
Palmitoleic acid	C16:1	0.3
Stearic acid	C18:0	1.8
Oleic acid	C18:1	63.7
Linoleic acid	C18:2	18.8
Linolenic acid	C18:3	7.9
Arachidic acid	C20:0	0.6
Gadoleic acid	C20:1	1.1
Behenic acid	C22:0	0.3
Erucic acid	C22:1	0.1

Table 3 Details of recirculation conditions in the two-compartment reactor.

Volume of US: V_U [mL]	Volume of ST: V_S [mL]	Volume of connected recirculation loop between ST and US: V_L [mL]	Flow rate: F [mL s ⁻¹]	Mean residence time in US: θ [s]	Overall circulation time of reaction line : Θ [s]	θ / Θ [-]
80	267	40	0.17	470	2276	0.21
			0.50	160	774	0.21
			0.83	96	466	0.21
			1.67	48	231	0.21

$$\theta = \frac{V_U}{F}$$

$$\Theta = \frac{V_U + V_S + V_L}{F}$$

Table 4 Properties of rapeseed oil.

	Molecular Weight	Density [kg m ⁻³]
FAME	295	-
FFA	282	895
TAG	881	920
DAG	618	-
MAG	355	-
Rapeseed oil	-	910

Table 5 Kinetic parameters of transesterification of rapeseed oil in two-compartment reactor.

Ultrasound irradiation	V_{max} [g-FAME (g-(oil+n-heptane+methanol) • s) ⁻¹]	K_m [g-oil g ⁻¹ -(oil+n-heptane+methanol)]
Free	2.65×10^{-5}	0.23
38W	6.07×10^{-5}	0.27

Figure 10

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