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Optimization of In-situ Transesterification Process for Biodiesel Production from High Free Fatty Acid Feedstock by Semi-continuous Column Reactor

Chantra Tongcumpou
ERIC: Chulalongkorn University Environmental Research Institute

Nattapong Tuntiwiwattanapun (nattapong.t@chula.ac.th)
ERIC: Chulalongkorn University Environmental Research Institute https://orcid.org/0000-0003-0644-3382

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Abstract

An *in-situ* transesterification (*in-situ* TE) process can reduce the multiple steps of biodiesel production by merging vegetable oil extraction and biodiesel synthesis into a single step. However, high free fatty acid, generally found in waste feedstock, dramatically reduce both yield and quality of biodiesel. In this work the new concept of a semi-continuous column reactor was introduced to mitigate the negative effect of high free fatty acid found in spent coffee grounds (SCGs). The potassium methoxide solution, as reactive solvent, was pumped through the series of column reactors. At the beginning of process free fatty acid in SCGs was promptly extracted in form of soap as a pretreatment fraction. Then, the residual mono-, di- and triglyceride in SCGs matrix were covert to fatty acid methyl ester (FAME) biodiesel and simultaneously extracted. A liquid fraction analysis was conducted to distinguish the pretreatment fraction from biodiesel. A central composited rotatable design was applied to determine optimal process conditions of potassium methoxide concentration and solid retention time at 30°C. Under the optimal conditions, up to 88% FAME yield was observed. The residual triglyceride in defatted SCGs was less than 1.5%mass. Compared to batch reactor type, the semi-continuous column reactor used only half of the required methanol for *in-situ* TE by recirculating the reagent in the series of reactors. This could dramatically reduce the energy usage and environmental impact of FAME production from high free fatty acid feedstock via *in-situ* TE.

1. Introduction

In-situ transesterification (*in-situ* TE) is an alternative biodiesel production process. This method involves fewer production steps than conventional biodiesel production processes as it combines oil extraction from oil-bearing biomass and TE into a single step. In conventional biodiesel production, several complex steps must be involved, which are multiple oilseed conditioning steps, vegetable oil extraction, refining steps of extracted oil, biodiesel synthesis and purification steps. On the other hands, *in-situ* TE process used only alcohol with catalyst as the reactive solvent. The oil in oil-bearing biomass could be converted to biodiesel and simultaneously extracted by catalyzed alcohol. Several types of oil-bearing biomass, including agricultural biomass and residues [1–7], microbial biomass [8], and microalgae [9, 10] and biological refuse [11], have been successfully used to produce biodiesel via *in-situ* TE [12].

Batch reactors are commonly used for *in-situ* TE due to their large solid loading capacity and simplicity of operation. Nevertheless, when using such reactors, separating the liquid product from solid residues is inconvenient and low concentration of the final product are obtained. Moreover, batch reactors are not suitable for oil-bearing biomass with high free fatty acid (FFA) and moisture contents, since its biodiesel production involves multiple steps (Fig. 1A). A two-step acid-base catalyzed process is recommended to achieve better biodiesel yields from oil-bearing biomass with high FFA and water contents [3]. Recently, several works introduced co-solvent with acid catalyst, which could reduce the multiple steps of *in-situ* TE for high FFA and water content biodiesel feedstock [13, 14].

A semi-continuous column reactor can eliminate the requirement of multiple steps in two-step *in-situ* TE (Fig. 1). Several studies have implemented such an operating system under supercritical and subcritical conditions of alcohol without catalysts [1, 11, 15]. Under these conditions the entrapped oil and FFA in oil bearing biomass were converted to biodiesel and simultaneously extracted. However, such biodiesel production process must operate under high temperature and pressure. In addition, the large volume of alcohol is required to ensure good biodiesel yield and quality.

In this work, a potassium methoxide (MeOK) solution was fed into the semi-continuous column reactor contained SCGs under 30°C and 0.1 MPa, instead of supercritical methanol. Additional of potassium catalyst in methanol could dramatically reduce the reaction temperature and pressure in biodiesel synthesis. Due to the counter current extraction of this reactor type, the recovery yield of biodiesel could be improved compared to batch reactor type.

Central composite rotatable design (CCRD), a statistical tool, was applied to optimize the operating conditions, which were MeOK concentration and solid retention time (SRT). The fatty acid methyl ester (FAME), which is the main component of biodiesel in this work was quantified by high performance liquid chromatography with evaporative light scattering as a detector (HPLC-ELSD).

2. Materials And Methods

2.1 Materials

Analytical grade methanol, n-hexane, and KOH were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). HPLC grade methanol and isopropanol were obtained from Fisher Chemical (Leicestershire, UK).

The stock solution of MeOK (10 g in 100 mL methanol) was prepared by dissolving 10 g of KOH in methanol and adjusting volume to 100 mL. This stock solution was kept in brown bottle and store in room temperature.

Spent coffee grounds (SCGs) were collected from 16 coffee cafés located in Chulalongkorn University (Bangkok, Thailand). The collected SCGs were dried at 105 ºC overnight, after which they were ground and stored in plastic bags at 4 ºC. The particle sizes of the SCGs were fractionated to be <0.25 mm using a metal sieve (US mesh no 60). Further, through Soxhlet n-hexane extraction and HPLC-ELSD, the total triglyceride (TG) content was 12.89±0.12 %mass with acid value of 10.51±0.09 mg KOH g^{-1} oil. The extracted SCGs oil was analyzed for its fatty acid profile by gas chromatography with flame-ionization detector at The Halal Science Center (Chulalongkorn University, Thailand). Linoleic acid (42%) and palmitic acid (34%) are the predominant fatty acid found in SCGs extracted oil.

2.2 *In-situ* transesterification using a semi-continuous column reactor

Four glass column reactors (diameter x height: 2 cm x 17 cm) were connected using a silicon tube (inner diameter 0.2 cm) and polypropylene plastic valve. (Fig.1B). The volumetric capacity of each of these glass column reactors is 80 mL, and 15 g of SCGs was loaded into each reactor. Glass wool was placed at the bottom of each column to separate the SCGs from the MeOK solution, which was prepared by dissolving KOH in methanol. Two peristaltic pumps were used to feed this MeOK solution into the first column, and pump the liquid fraction (LF) out of the fourth column at a flow rate of 5.25 mL min^{-1}. The temperature and pressure were fixed at ambient levels (30 °C and 0.1 MPa). After completion of the process, the residual MeOK solution in the columns was drawn out using a vacuum pump at 6.0 – 6.2 kPa, and pooled with the LF. The LF was separated into pretreatment and biodiesel fractions based on its product composition, including soap, MeOK, FAME, and TG, determined via HPLC-ELSD.

The defatted SCGs in the column were removed and residual methanol was evaporated at 105 ºC overnight. Subsequently, the residual TG content of the dried defatted SCGs was evaluated via Soxhlet n-hexane extraction and HPLC-ELSD.

2.3 Analysis of product composition in liquid fraction by HPLC-ELSD

The product composition (soap, MeOK, biodiesel, and TG) of the LF obtained was directly analyzed through HPLC (Shimadzu-10Avp, Japan) with ELSD (Sedex 75, France). The instruments and analytical conditions used are described in our previous work [5]. Briefly, 10 µL of LF was injected into the C18 column with 5 µm, 4.6 x 250 mm (Inertsil® ODS-3, Japan). The temperature of column was set at 70 °C. The mixture of methanol and isopropanol was selected as mobile phase (gradient elution: starting at 100% methanol and ending at 15% methanol after 30 min) with flow rate of 0.75 cm^3 min^{-1}. The temperature of ELSD chamber was 40 °C with pressure of 210-220 kPa.

2.4 Evaluating biodiesel yield and residual TG

The SCGs oil from Soxhlet n-hexane extraction was used to synthesize FAME following the procedure reported in the literature [16], and adopted as the SCGs FAME standard. The FAME yield was calculated using Eq.1.

$$\text{FAME Yield (\%)} = \frac{\textbf{Recovered FAME (g)}}{\textbf{Theoretical FAME in SCGs (g)}} \times 100 \qquad \textbf{(1)}$$

where *Recovered* FAME was calculated from the FAME content in LF multiplied by volume of LF. Theoretical FAME in SCGs was calculated by multiplying total TG in SCGs with conversion factor. The conversion factor for converting TG mass to FAME was equal to 1.00 based on their molecular weight [6].

After methanol evaporation, the defatted SCGs from *in-situ* TE process was evaluated the residual TG content. Ten grams of defatted SCGs was put in a cellulose thimble and refluxed by 100 mL of n-hexane for 5 h in Soxhlet apparatus. The temperature of heating mantel was set at 70 °C. The rotary evaporation (Heidolph model Heizbad HB digit, Germany) was applied to evaporate n-hexane from extracted sample

under vacuum pressure of 6.0-6.2 kPa at 70 °C. Then, isopropanol was added to dissolve the extracted sample and adjusted the volume to 25 mL. Finally, the TG content in isopropanol was evaluated by HPLC-ELSD and calculated the residual TG in defatted SCGs.

2.5 Statistical analysis

An ANOVA test and non-linear regression analysis were performed using the STATISTICA 10 program (StatSoft, Tulsa, OK, USA) at 95% confidence level. CCRD was applied to optimize the process conditions for maximizing FAME yield. In this approach, quadratic model with 2 levels was selected to generate the prediction model of FAME yield and residual TG in defatted SCGs. It should be noted that the predicted values were only valid withing the experimental range used in this study at 95% confidence level. All experiments were conducted with three replications, excepted fraction analysis.

3. Results And Discussion

3.1 Effect of MeOK concentration on product composition of the liquid fraction

The advantage of using a feeding pump in a semi-continuous reactor is that it allows fractional analyses for different operation durations or volumes. Such data could be used to design suitable conditions for optimization of *in-situ* TE. The effect of MeOK concentration (1.0 – 2.5 g KOH in 100 mL methanol) on LF composition was investigated to determine the boundary between the pretreatment and biodiesel fractions. The total mass of SCGs was 60 g and MeOK loading volume was 240 mL. The presence of MeOK, soap, FAME, and TG in 25 mL aliquots of the LF were examined via HPLC-ELSD as shown in Fig.2. The composition of the first and second aliquots of the LF could not be analyzed because the soap signal was very broad and overlapped with the biodiesel signal. Further, only 175 mL of the LF could be collected because some part of the LF was absorbed by the SCGs, and therefore could not be drawn out under vacuum pressure (6.0–6.2 kPa).

As demonstrated by the results obtained, most of the MeOK catalyst was consumed during saponification with FFA in the early stage of *in-situ* TE (Fig.2A), resulting in the formation of soap (Fig.2B). Moreover, the higher the MeOK concentration, the higher amount of soap formed, with highest soap formation recorded at 2.50 g KOH in 100 mL methanol. This could reduce biodiesel yield remarkably (Fig.2C) because at high MeOK concentrations, saponification is enhanced and TG is converted to soap instead of FAME. Further, high soap content in the final biodiesel product could lead to difficulties during biodiesel purification by water washing, which in turn can result in a significant loss of the biodiesel product.

Low concentrations of MeOK were beneficial in reducing soap formation. However, it was not sufficient for FAME synthesis, thereby leading to low FAME yield and high TG content in the LF (Fig.2C and 2D). If there are residual TG in the LF, the final biodiesel product does not satisfy the biodiesel standard. Therefore, it is essential to select a suitable boundary to separate the pretreatment fraction (i.e.,

LF with high soap content) from the FAME fraction. Further, a MeOK concentration at which the highest FAME yield can be achieved with the lowest soap and TG contents in the LF needs to be identified.

Based on these results, the first 50 mL of the LF (i.e., the first and second 25 mL aliquots) was classified as the pretreatment fraction. MeOK concentration between 1.0–1.5 g KOH in 100 mL methanol was set as the experimental boundary, which is to be optimized in the subsequent step.

3.2 Optimizing MeOK concentration and SRT

In a previous study [17], we found that *in-situ* TE could have two reactive extraction rates based on the location of the oil. Oil located on the surface was rapidly converted to FAME and simultaneously extracted to the LF at a high rate. However, it took much more time for alkaline methanol to diffuse through the SCGs matrix via small porous channels and reach oil trapped within the matrix. This could severely reduce the performance of the type of semi-continuous reactor used in this study compared to the performance achieved in our previous study.

Insufficient mixing is one of disadvantages of using a semi-continuous reactor because this reactor does not have a mixing instrument. In this study, gravity and the pressure exerted by the peristaltic pump were sufficient for the MeOK solution to diffuse through the SCGs in the column. This implies that solid retention time (SRT), which is the time that SCGs is submerged in the solution, is a crucial parameter and therefore this parameter was optimized in addition to MeOK concentration. The SRT of this study was adjusted by the feeding rate of the peristaltic pump.

The CCRD was applied to optimize the conditions for *in-situ* TE in a semi-continuous column reactor in terms of MeOK concentration and SRT. The design of experimental conditions based on CCRD, and the corresponding biodiesel yields and residual TG contents of defatted SCGs are presented in Table 1. Results of ANOVA indicated that both MeOK concentration and SRT significantly affected FAME yield and residual TG level in defatted SCGs (p-value <0.01). However, at the 95% confidence level, no effect of MeOK concentration and SRT on FAME yield and residual TG level in defatted SCGs was observed. Moreover, as revealed by HPLC-ELSD results, TG was not detected in the product fraction at a detection limit of 100 ppm; only FAME was detected.

Table 1 Experimental design based on CCRD to identify optimal MeOK concentration and SRT for *in-situ* TE, as determined based on FAME yields and residual TG levels in defatted SCGs.

Treatment	[MeOK] (g KOH in 100 mL methanol)	SRT (min)	FAME Yield (%)		Residual TG Level in Defatted SCGs (%mass)	
			Experimental	Predicted	Experimental	Predicted
1	1.00	30.48	57.16±0.71	57.37	5.64±0.03	5.25
2	1.00	60.95	63.81±0.89	68.46	4.95±0.16	4.20
3	1.50	30.48	77.10±1.42	75.68	3.00±0.54	3.18
4	1.50	60.95	84.46±0.09	87.47	1.64±0.18	1.46
5	0.90	45.71	64.46±1.12	61.70	4.25±0.03	4.94
6	1.60	45.71	88.55±1.59	88.09	1.66±0.19	1.54
7	1.25	24.16	59.98±2.34	61.51	4.75±0.46	4.78
8	1.25	67.26	82.43±0.70	77.68	2.29±0.03	2.83
9	1.25	45.71	83.27±0.92	83.84	3.34±0.02	3.13
10	1.25	45.71	82.72±0.74	83.84	2.91±0.04	3.13

In terms of FAME yield, optimal MeOK concentration and SRT can be determined based on a quadratic model with two levels, as shown in Eq.2 (adjusted R^2 0.92). The predicted value of residual TG level in the defatted SCGs was calculated based on Eq.3 (adjusted R^2 0.88). Both equations for predicting FAME yield and residual TG level in defatted SCGs were passed through a goodness-of-fit test (*p*-value <0.05). The predicted values (Table 1) were used to generate contour graphs for FAME yields and residual TG in the defatted SCGs, which are shown in Fig.3A and Fig.3B, respectively. It should be noted that the predicted values are valid only the experimental range used in this study.

$$\text{FAME Yield (\%)} = -153.16 + 214X_1 - 71.52X_2^2 + 3.12X_2 - 0.03X_2^2 + 0.05X_1X_2 \quad \textbf{(2)}$$

$$\text{Residual TG in Defatted SCGs (\% mass)} = 12.15 - 5.05X_1 + 0.90X_1^2 - 0.12X_2 - 0.04X_1X_2 \quad \textbf{(3)}$$

where X_1 is the MeOK concentration (g KOH in 100 mL methanol) and X_2 is the SRT (min).

According to model prediction, the optimal conditions were MOK concentration of 1.5 g KOH in 100 mL methanol and SRT of 52.15 min, with MeOK loading of 4 mL g^{-1} SCGs and feeding rate of 5.25 mL min^{-1} at 30 ˚C. Under these optimal conditions, biodiesel yield of 88.1% (11.4 g FAME per 100 g SCGs) was achieved. Further, analysis of the product fraction by HPLC-ELSD at a detection limit of 100 ppm did not reveal the presence of TG under these conditions.

The average residual TG content in the defatted SCGs from all four column reactors was 1.5 %mass. The defatted SCGs from the first column reactor had the lowest residual TG content (0.7 %mass), followed by those from the second (0.8 %mass), third (1.4 %mass), and fourth (3.1 %mass) column reactors.

A comparison of the advantages of using MeOK solution in a semi-continuous column reactor and those of using other types of reactor and operating conditions for *in-situ* TE is presented in Table 2. A semi-continuous column reactor has a superior in lowering the methanol/ethanol usage, compared to batch reactor type. This is due to the multistage countercurrent extraction found in continuous and semi-continuous reactor types. The fresh solvent contacts the first oil-bearing material in first column, and the extracted liquid is fed to the subsequence columns. Such operation conditions provide the most dilute extract contacts the oil-bearing material with the least amount of residual oil which enhances the diffusive flux by the difference in concentration gradient of oil between solvent and oil-bearing material [18].

Table 2 A comparison of *in-situ* TE processes performed with different operating systems and under different conditions.

Feedstock (mass per batch)	Reactor	Alcohol, Catalyst, and Co-solvent	Temperature (°C)	Time (h)	Yield (%)
Rice Bran (10 g)	Batch	Step 1: Methanol (15 mL g^{-1}), H_2SO_4 (18 %mass of biomass)	60	0.2	96.8 – 97.4 (FAME Yield)
[3]	Step 2: Methanol (0.8 mL g^{-1}), Sodium hydroxide (5.0 M)	60	0.8		
Jatropha (20 g)	Batch	Step 1: Ethanol (10.5 mL g^{-1}), H_2SO_4 (7.5 %mass of biomass)	Microwave	0.6	97.2 (Fatty acid ethyl ester Yield)
[19]	Step 2: Ethanol (4.1 mL g^{-1}), KOH (5.0 M)	Microwave	0.2		
SCGs (4 kg)	Batch	Step 1: Methanol (3.3 mL g^{-1})	45	1.0	81.8 (Biodiesel Yield)
[5]	Step 2: Methanol (2.9 mL g^{-1}), KOH (0.225 M)	60	3.0		
SCGs (1 g) [4]	Semi-continuous (single state)	Supercritical Methanol (5 mL g^{-1}), water (60 %mass of biomass) under 9 MPa	270	0.3	86.3 (FAME Yield)
SCGs (15 g)	Semi-continuous (single state)	Step 1: Methanol (3.7 mL g^{-1})	30	1.0	88.8 (FAME Yield)
[6]		Step 2: Methanol (2.0 mL g^{-1}), KOH (0.446 M), Isopropanol (0.7 mL g^{-1}) as co-solvent	30	2.0	
SCGs (60 g) This study	Semi-continuous (multistate)	Methanol (3.5 mL g^{-1}), KOH (0.268 M) under 0.1 MPa	30	0.9	88.1 (FAME Yield)

Tuntiwiwattanapun *et al.* [20] compared the canola oil extraction performance between single stage extraction and triple stage extraction using aqueous surfactant solution. The results showed that the triple stage extraction had higher oil extraction yield than single stage by 50%. Co-solvent such as isopropanol could compensate the lower FAME yield from single stage extraction. Our previous study [6]

showed that using a single column in semi-continuous column reactor achieved the FAME yield only 60.9%. Additional of isopropanol at 25% volume in methanol could improve FAME yield to 88.8%, in which FAME yield was similar to multistage semi-continuous column reactors applied in this work (Table 2).

As MeOK solution was reused in this study, the ratio of alcohol to oil-bearing biomass is obviously lower than that reported in other studies. Compared to our previous study with a batch reactor [5], the semi-continuous column reactor used in this study could reduce the amount of MeOK solution required by half. In a case of SCGs, the pH of MeOK solution after *in-situ* TE was reduced, especially in low concentration of MeOK [6]. This might be due to the high lignin content in SCGs. KOH catalyst reacts with lignin, which similar to kraft reaction in paper pulp process. The insufficient KOH concentration led to low FAME recovery in *in-situ* TE, especially in batch reactor type. For semicontinuous reactor, this problem could be minimized due to the multistage counter current extraction which provides the fresh MeOK solution into the system. In addition, the dissolved lignin by KOH could be separated as the pretreatment fraction with the soap formation.

Therefore, applying semi-continuous column reactor could markedly reduce the energy requirement for methanol recovery, which has been identified as the most energy intensive step of *in-situ* TE based on a life cycle assessment [5]. Beside catalyzed alcohol, the other alternative reagent such as ionic liquid and deep eutectic solvent could be used in this reactor [21-23].

4. Conclusions

Semi-continuous reactor was successfully introduced in *in-situ* TE for SCGs FAME production. There were two advantages of using this reactor compared to the batch type. First, the pretreatment and biodiesel fraction could be simply separated based on product composition, which could reduce FAME production steps of high FFA biodiesel feedstock. Second, the chemical reagent could be recirculated in the series of reactors. Under the optimal conditions, a semi-continuous column reactor uses only half the volume of MeOK solution required for *in-situ* TE by a batch reactor, while still maintaining a similar FAME yield up to 88%. This could dramatically reduce the energy usage and environmental impact of biodiesel production via *in-situ* TE.

Declarations

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Availability of data and materials

The data that support the findings of this manuscript are openly available in Research Square.

Authors' contributions

Chantra Toncumpou (First author)

- Supervising the scope and finding of this work.
- Verifying the analytical method.
- Discussing the results and contributing to the final manuscript

Nattapong Tuntiwiwattanapun (Correspondent author)

- Developing the scope of experiment to prove the hypothesis.
- Performing the experiment and analyzing the data
- Discussing the results and contributing to the final manuscript

Ethics approval and consent to participate

This manuscript does not involve human/animal participants, human/animal data, human/animal tissue.

Consent for publication

This manuscript does not contain any individual person's data in any form.

Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

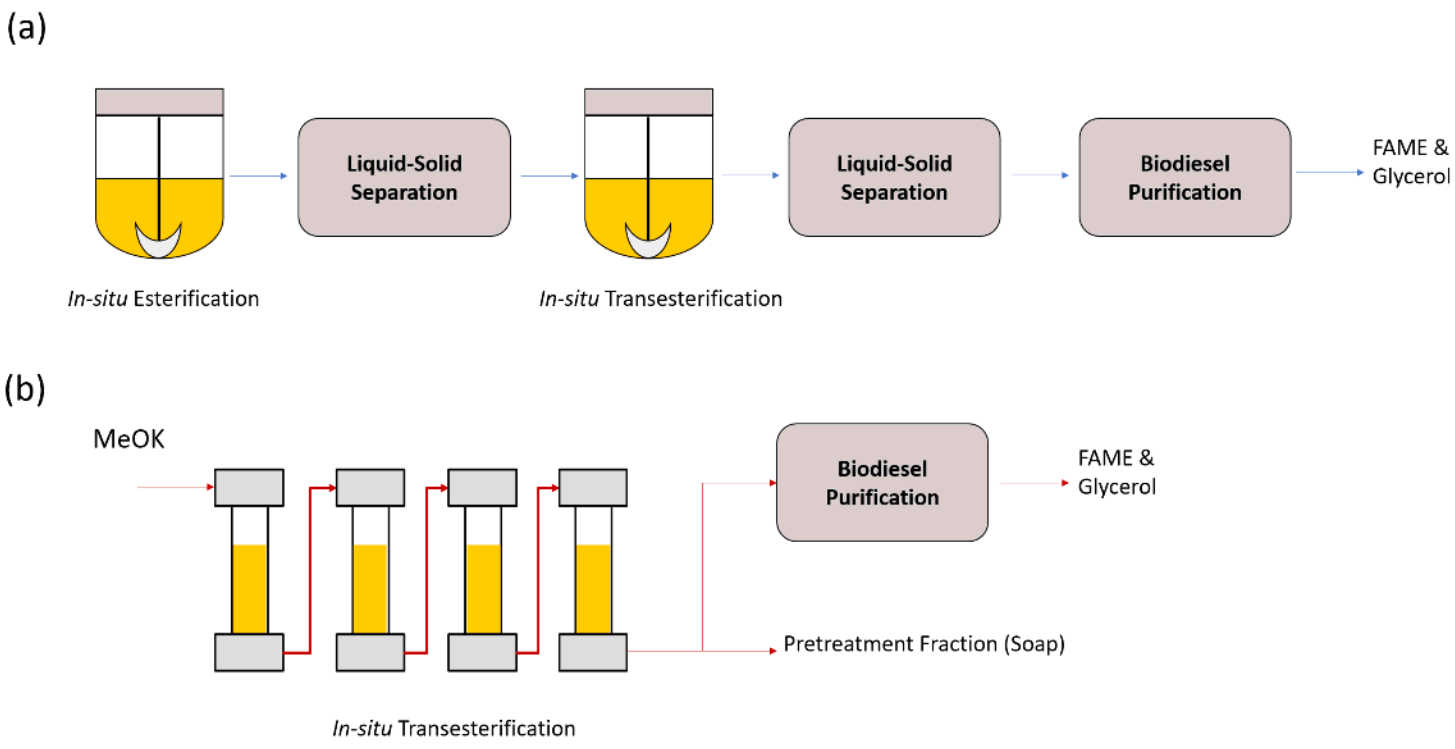

Figure 1

A comparison of the processing steps involved in two-step in-situ TE of feedstock with high FFA content using a batch reactor (A) and the semi-continuous reactor of this study (B).

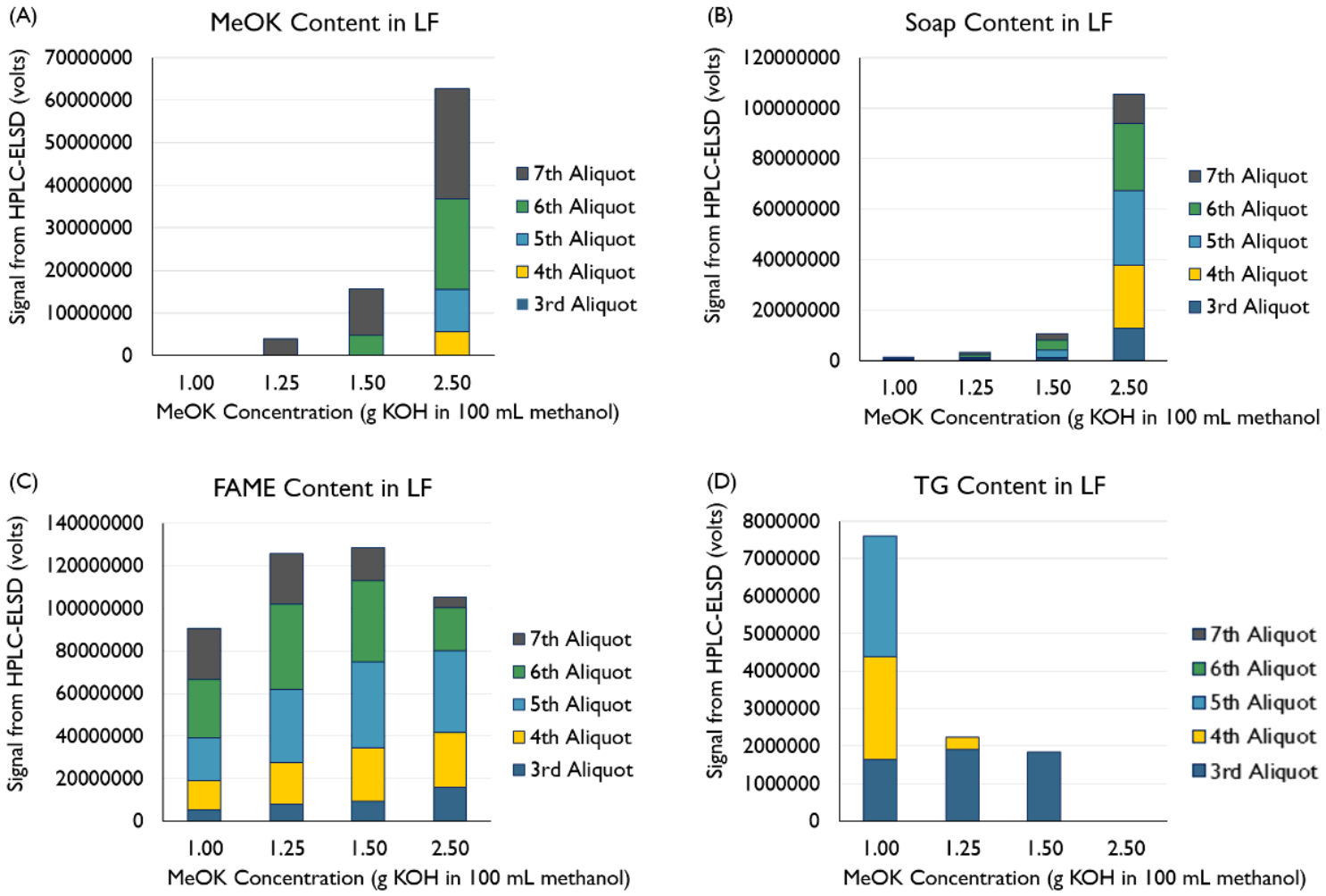

Figure 2

Product composition of each 25 mL liquid fraction at different MeOK concentrations (g KOH in 100 mL methanol).

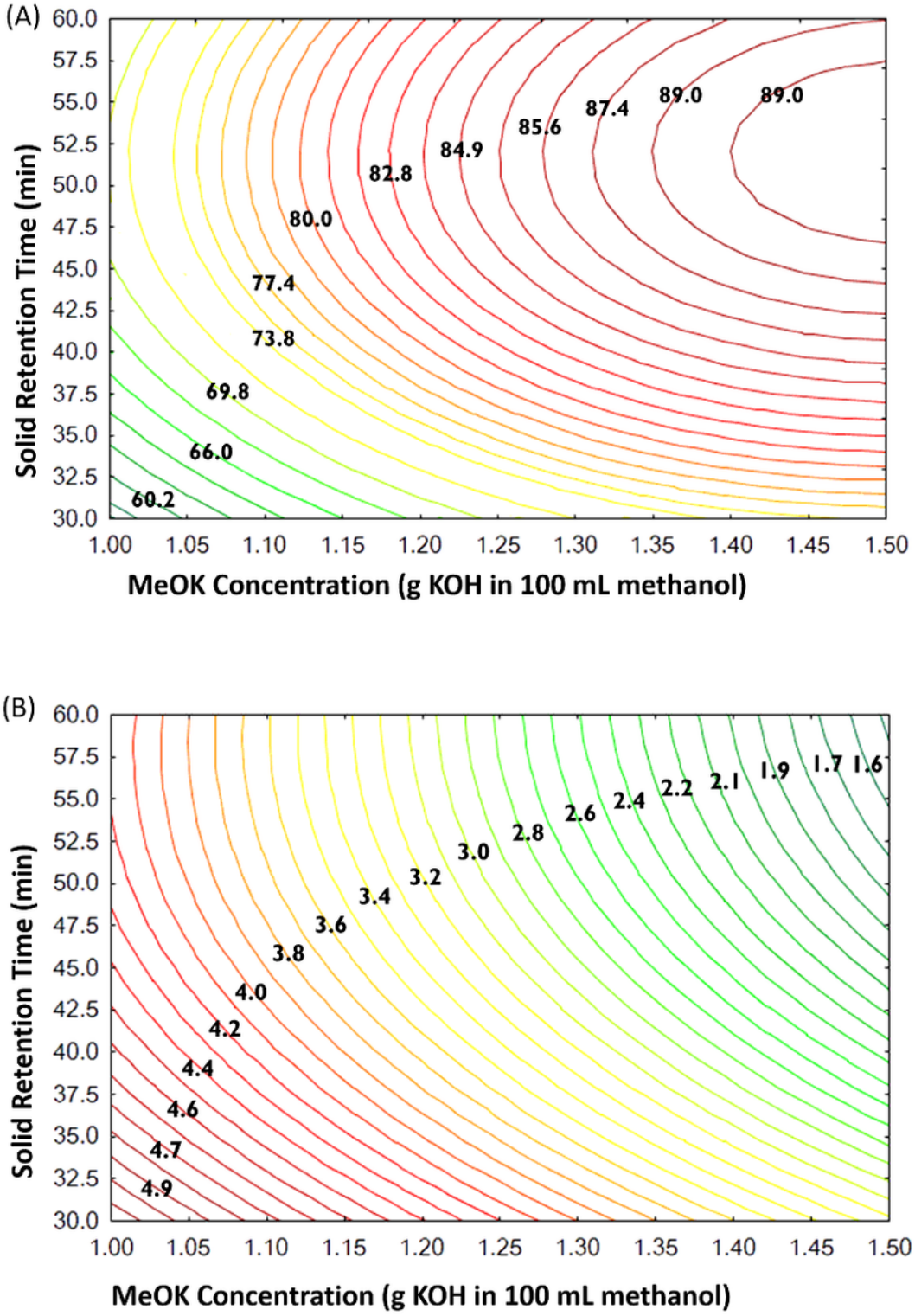

Figure 3

Predicted values of FAME yield (%) (A) and residual TG in the defatted SCGs (%mass) (B) from in-situ TE calculated using Eq. 2 and Eq. 3, respectively.