Supporting Information

Real-time monitoring of transesterification by ^{1}H NMR spectroscopy: catalyst comparison and improved calculation for biodiesel conversion

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Reagents

Anhydrous methyl alcohol (99.8%) with Acroseal™ and olive oil (purified/refined) were purchased from Acros Organics (Morris Plains, NJ). 1,3-diolein was purchased from MP biomedical (Solon, OH). 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) was purchased from Sigma-Aldrich (St. Louis, MO). Deuterated chloroform ($CDCl_3$) with tetramethylsilane (TMS, v/v 0.05%) was purchased from Cambridge Isotope Laboratories (Andover, MA). Reagent grade CH_3OH was purchased from Fisher Scientific (Fairlawn, NJ). Commercial biodiesel composed of fatty acid methyl esters was obtained from a gas station in Woodland, CA. Standard 5 mm NMR tubes (Wilmad Labglass, Vineland, NJ) were used for NMR spectroscopy experiments. 4 mL or 20 mL glass vials were used for bench scale reactions. Anhydrous methanol was used, except where the use of reagent-grade (i.e. non-anhydrous) methanol is indicated.

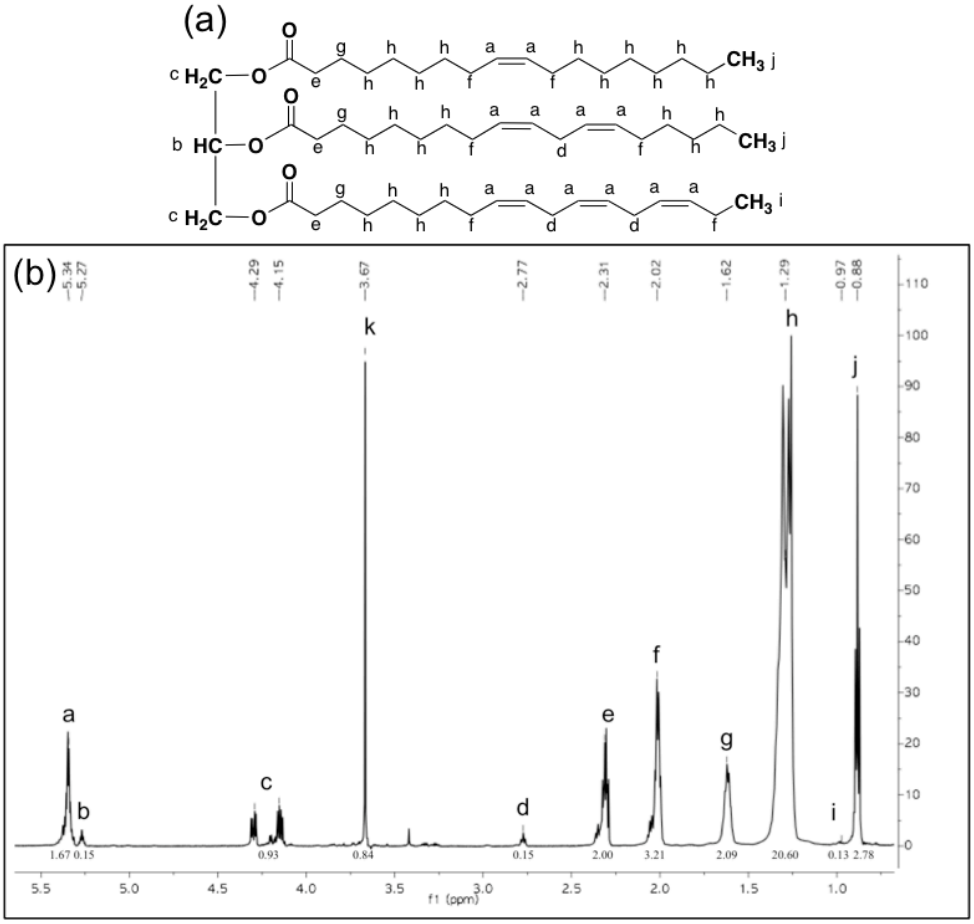


Figure S1. (a) Structure of TAG with common fatty acids oleic, linoleic, and linolenic acid (chemical shift assignments correspond to Table S1) and (b) ^{1}H NMR spectra (600 MHz, 16 scans, 45 degrees, 1 s relaxation delay, $CDCl_3$) of partially converted biodiesel. Letters correspond to Table S1.

Table S1. Comparing known percentages of 1,3-diolein (as a representative DAG) in DAG/FAME mixtures using ^{1}H NMR spectroscopy with 1 second or 10 second relaxation delay for 4 scans or 1 scan. Three replicates of each mixture were prepared individually from a 100 mg/mL stock solution of either DAG or FAME in $CDCl_3$. Percent DAG by known is based on mass. Percent DAG calculated by integrating 1,3-diolein methine (4.09 ppm, 1H) and FAME methyl (3.67 ppm, 9H). Data points represent an average of two replicates with standard deviation. Acquired with 600 MHz at 23 ˚C in $CDCl_3$. It was determined, based on these experiments, that 4 scans with 1 second relaxation delay allows for representative quantification of DAG components.

	10% (2 mg)	**5% (1 mg)**	**1% (0.2 mg)**
1 second (4 scans)	13.0	7.0 ± 0.0	2.5 ± 0.7
1 second (1 scan)	13.5 ± 0.7	6.5 ± 0.7	1.5 ± 0.7
10 second (1 scan)	14.5 ± 0.7	4.5 ± 2.1	1.5 ± 2.1

Table S2. Assignment of chemical shifts and integration notations in the ^{1}H NMR spectra of TAGs and FAMEs.

	Protons	**Chemical shift (splitting)[a]**
a	olefinic methine	5.30-5.43 (m)
b	glyceryl methine	5.23-5.30 (quintet)
c	glyceryl methylenes	4.27-4.33 (dd) and 4.11-4.17 (dd)
d	diallylic methylenes	2.74-2.79 (t)
e	methylenes α to the carbonyl	2.27-2.34 (t)
f	allylic methylenes	1.98-2.04 (m)
g	methylenes β to the carbonyl	1.58-1.67 (m)
h	all other methylenes	1.21-1.43 (m)
i	n3 methyls	0.96-1.00 (t)
j	all other methyls	0.85-0.93 (t)
k	methyl esters	3.66-3.68 (s)
[a] s = singlet, t = triplet, m = multiplet, dd = doublet of doublets		

Table S3. Selected comparison of incorrect (C_K)[1] and new conversion calculation (C_F) reported here. Values for transesterification reaction with 25 mol% of 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) shown.

Time (minutes)	**C_K, Incorrect conversion calculation (%)**	**C_F, New conversion calculation (%)**	**Difference in calculations (%)**
0	42	13	29
4	57	21	36
9	70	32	38
13	73	35	38
18	74	37	38
31	75	38	37

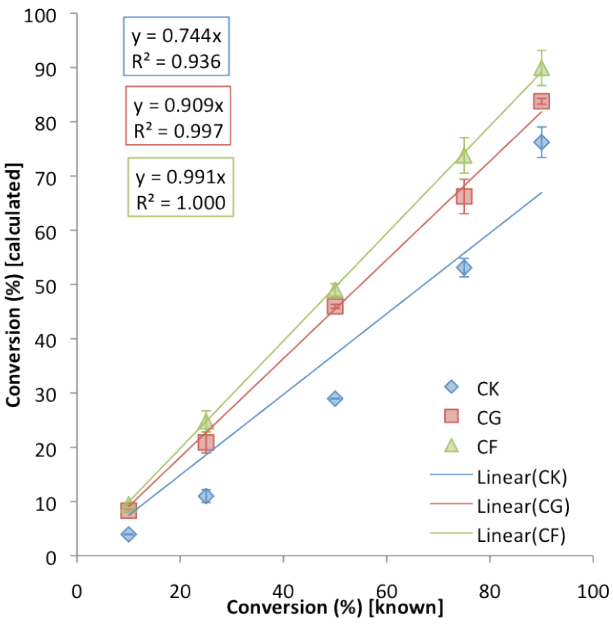


Figure S2. Comparison of calculations for biodiesel conversion using ^{1}H NMR spectroscopy for known mixtures of biodiesel and olive oil. Three replicates of each mixture were prepared individually from a 100 mg/mL stock solution of either biodiesel or olive oil in $CDCl_3$. Conversion by known is based on mass per 20 mg (biodiesel:olive oil mixtures = 10:90, 25:75, 50:50, 75:25, 90:10). Data points represent an average of three replicates with error bars for standard deviation. Acquired with 600 MHz, 4 scans, 1 s relaxation delay at 23 °C in $CDCl_3$. Equation (C_F) shows excellent correlation (R^2 = 1.000) and good linearity (slope = 0.991).

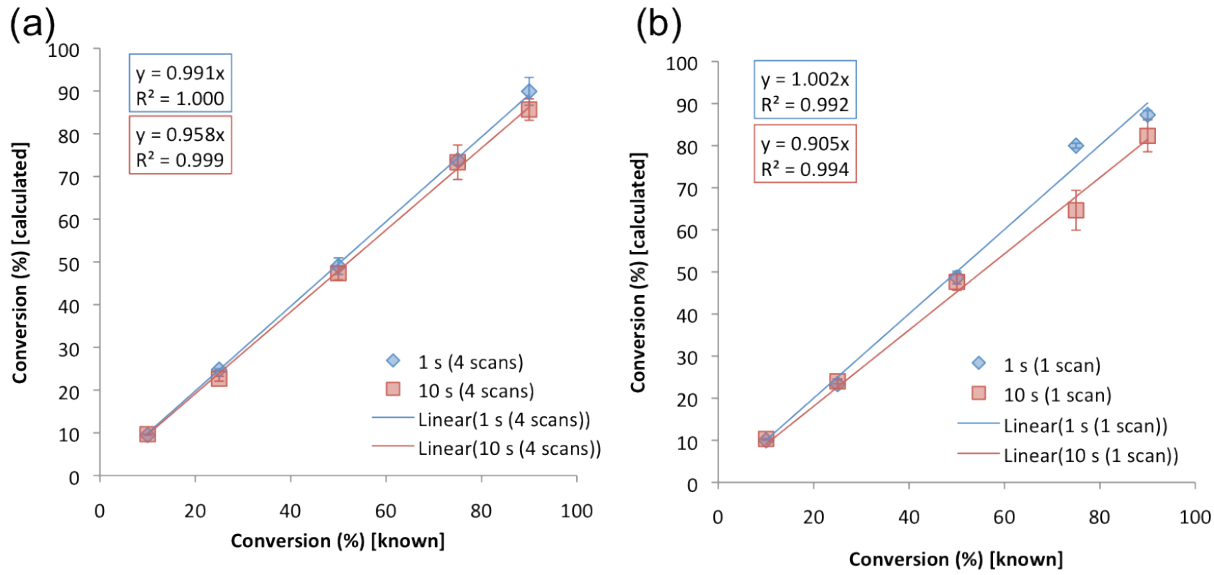


Figure S3. Conversion of biodiesel and olive oil mixtures based on ^{1}H NMR spectroscopy comparing 1 second and 10 second relaxation delay for a) 4 scans and b) 1 scan. Three replicates of each mixture were prepared individually from a 100 mg/mL stock solution of either biodiesel or olive oil in $CDCl_3$. Conversion was determined based on comparison to samples of known biodiesel/olive oil mixtures (10:90, 25:75, 50:50, 75:25, and 90:10). Conversion calculated by Eq. 3 (C_F). Data points represent an average of three replicates with error bars for standard deviation. Acquired with 600 MHz at 23 ˚C in $CDCl_3$. Based on these experiments, it was determined that 4 scans with 1 second relaxation delay allows for representative and optimal quantification for biodiesel conversion.

Table S4. Comparing known percentages of biodiesel (FAME) in biodiesel/olive oil mixtures based on ^{1}H NMR spectroscopy using 1 second or 10 second relaxation delay for 4 scans or 1 scan. Three replicates of each mixture were prepared individually from a 100 mg/mL stock solution of either biodiesel or olive oil in $CDCl_3$. Conversion was determined based on comparison to samples of known mass per 20 mg. Percent FAME calculated by Eq. 3 (C_F). Data points represent an average of three replicates with standard deviation. Acquired with 600 MHz at 23 ˚C in $CDCl_3$. Based on these experiments, it was determined that 4 scans with 1 second relaxation delay allows for representative and optimal quantification for biodiesel conversion in the widest range of mixtures. A 10 second relaxation delay may be better for mixtures containing larger amounts of biodiesel as the T1 is longer. For mixtures of smaller quantities of biodiesel (larger quantities of acylglycerols), a shorter relaxation delay may be better.

	10% (2 mg)	**25% (5 mg)**	**50% (10 mg)**	**75% (15 mg)**	**90% (18 mg)**
1 second (4 scans)	9.5 ± 0.2	24.8 ± 1.9	49.0 ± 1.1	73.8 ± 3.3	89.9 ± 3.2
10 second (4 scans)	9.9 ± 0.4	23.0 ± 1.5	47.3 ± 4.0	73.6 ± 2.6	85.7 ± 2.2
1 second (1 scan)	9.9 ± 0.8	23.4 ± 1.6	48.5 ± 0.9	80.0 ± 1.3	87.3 ± 5.9
10 second (1 scan)	10.3 ± 0.7	24.0 ± 1.9	47.6 ± 4.9	64.6 ± 3.8	82.4 ± 1.5

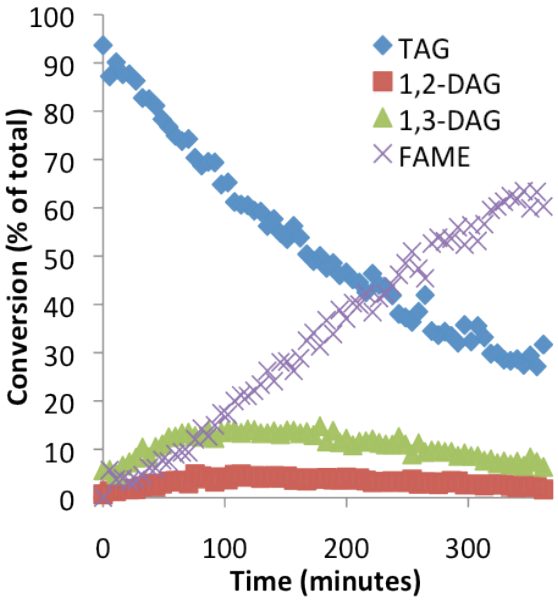


Figure S4. Data points for reaction profile of transesterification with 0.6 M H_2SO_4 in fresh reagent-grade methanol (30:1 methanol/oil ratio), performed at 60 ˚C in $CDCl_3$. Data were acquired on a 600 MHz instrument with acquisitions taken every 5 min (8 scans, 45 degrees, 1 s relaxation delay). Conversions are calculated by integration of acylglycerol methine (TAG = 5.27 ppm, 1,3-DAG = 4.09 ppm, 1,2-DAG = 5.08 ppm) and FAME methyl (3.67 ppm).

Observations regarding presence of water in reagent grade methanol on the rate of conversion and intermediate formation

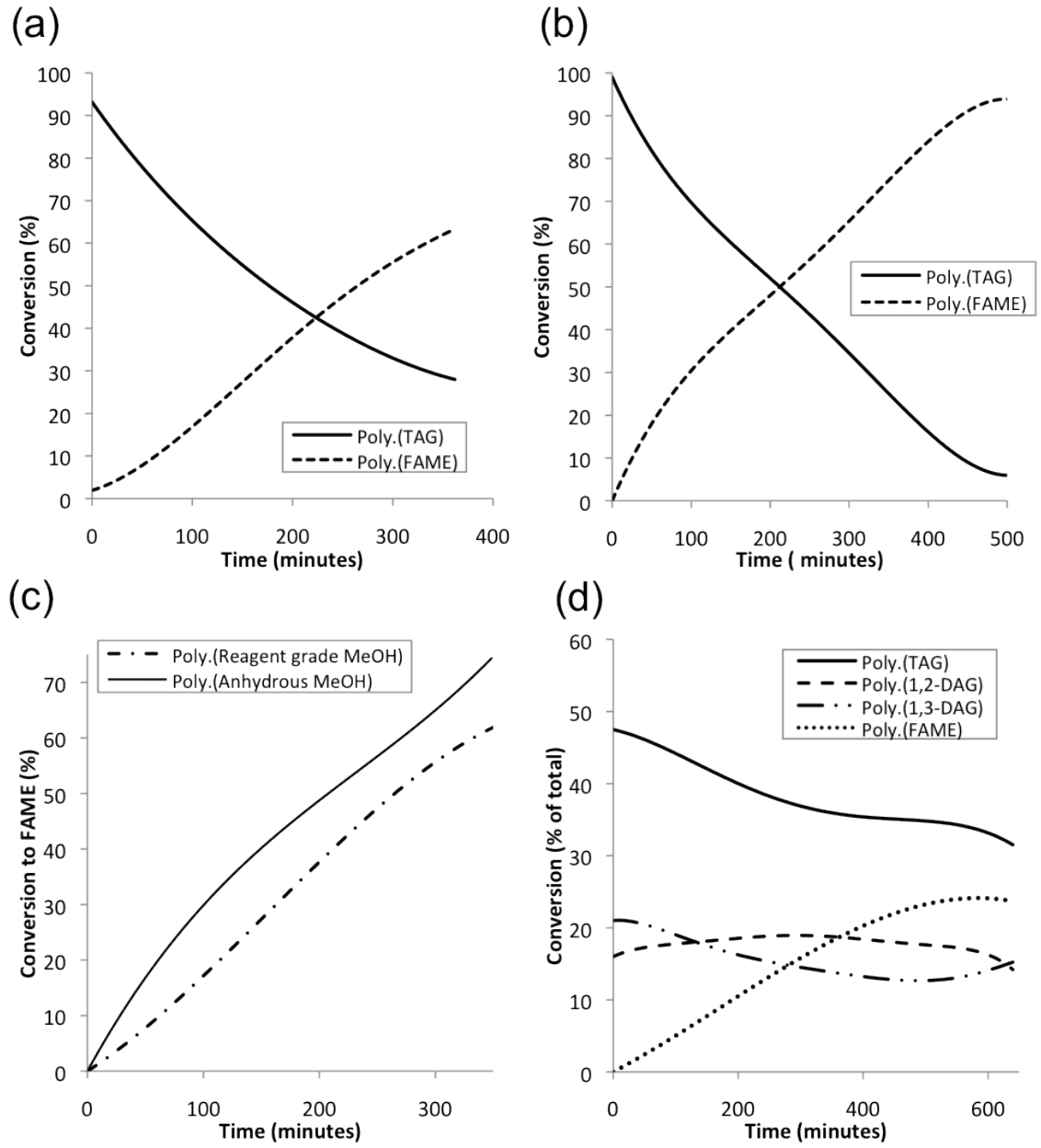
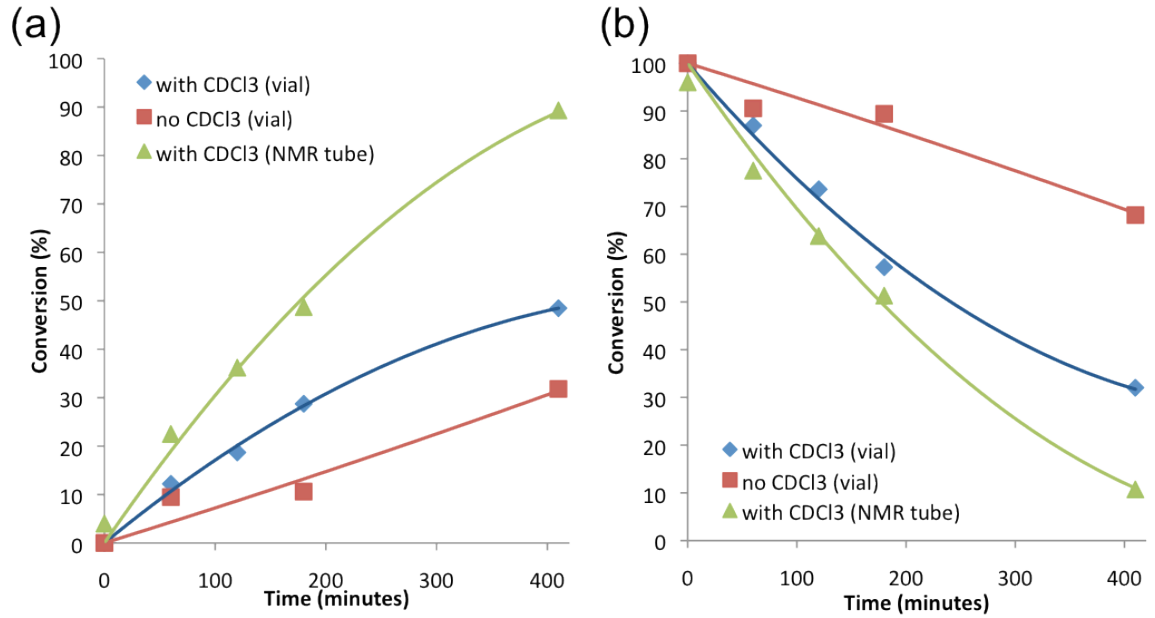


Figure S5. Reaction profile of transesterification with 0.6 M H_2SO_4 in methanol (30:1 methanol:oil ratio), performed at 60 ˚C in $CDCl_3$ a) reagent grade methanol; b) anhydrous methanol; c) FAME conversion comparison with reagent grade or anhydrous methanol; and d) reaction profile of transesterification with 0.6 M H_2SO_4 in reagent grade methanol (30:1 methanol:oil ratio), performed at 60 ˚C in $CDCl_3$. Data were acquired on a 600 MHz instrument with acquisitions taken every 5 min (4 scans, 45 degrees, 1 s relaxation delay). Conversions are calculated by integration of TAG methine (5.27 ppm, 1H) and FAME methyl (3.67 ppm, 9H). Because of methanol's hygroscopic nature, it is important to assess the effects of reagent grade methanol (previously opened and amount of water undetermined) versus anhydrous methanol on the formation of intermediates and rate of reaction. Upon comparing the effect of reagent-grade methanol and anhydrous methanol, we observe an increase reaction rate with anhydrous methanol. For all experiments, when the type of methanol is not indicated, reagent grade methanol was used.

Figure S6. Reaction profile for transesterification of NMR scale reaction (20 mg) vs. benchtop reaction (500 mg) in vial, with and without $CDCl_3$, using 0.6 M H_2SO_4 at 60 ˚C: (a) production of FAME; and (b) conversion/consumption of TAG. Conversion calculated based on integration of TAG methine (5.27 ppm, 1H) and FAME methyl (3.67 ppm, 9H). Acquired with 600 MHz, 4 scans, 45 degrees, 1 s relaxation delay in $CDCl_3$.



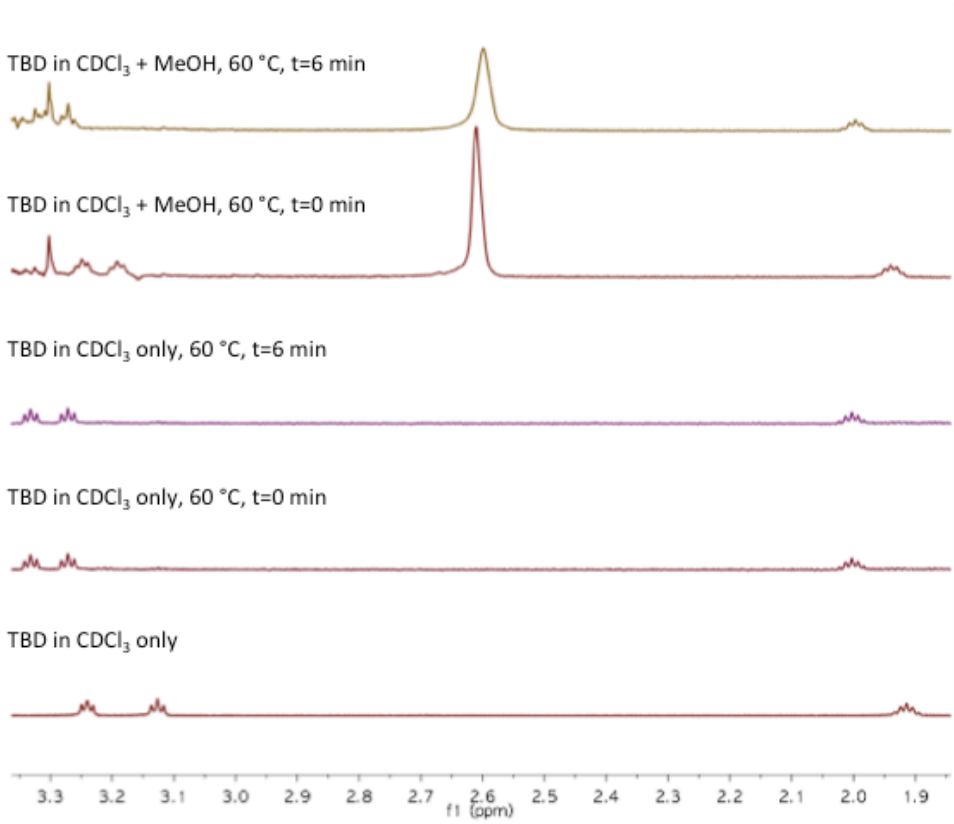


Figure S7. Comparison of ^{1}H NMR spectra for 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) in $CDCl_3$ and/or MeOH (non-anhydrous) with or without heat (expansion of 1.85 - 3.35 ppm region). Acquired on a Varian 600 MHz spectrometer, 16 scans, 45 degrees, 1 s relaxation delay.

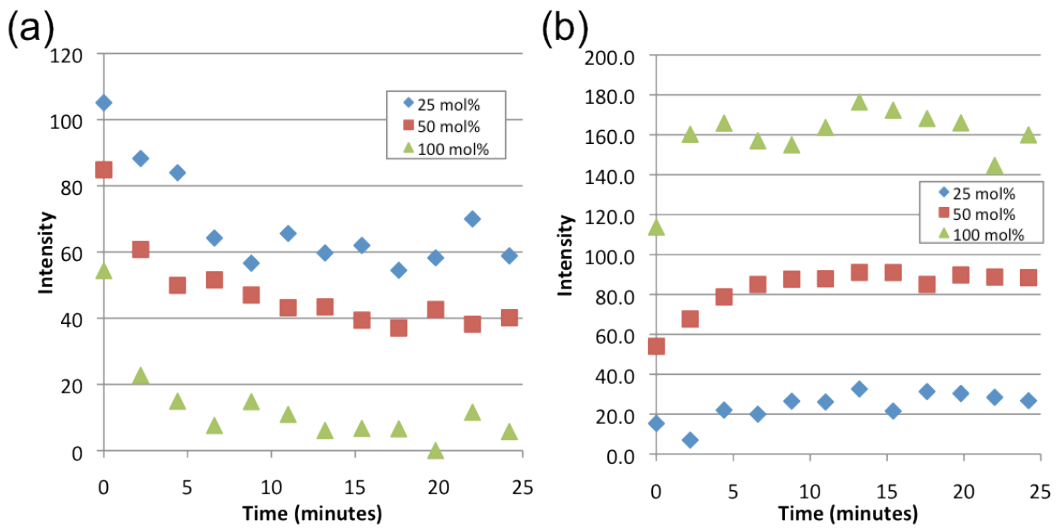


Figure S8. Reaction profile of transesterification with 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) mol % in methanol (non-anhydrous) (30:1 methanol/oil ratio) on (a) TAG depletion and (b) FAME formation. Reaction conditions: olive oil (20 mg) with TBD in methanol, performed at 60 ˚C in $CDCl_3$. Data were acquired on a Varian 600 MHz with acquisitions taken every 2 min (4 scans, 45 degrees, 1 s relaxation delay). The integrated glyceryl methine represents the conversion TAG = 5.27 ppm and the integrated methyl ester represents the FAME = 3.67 ppm.

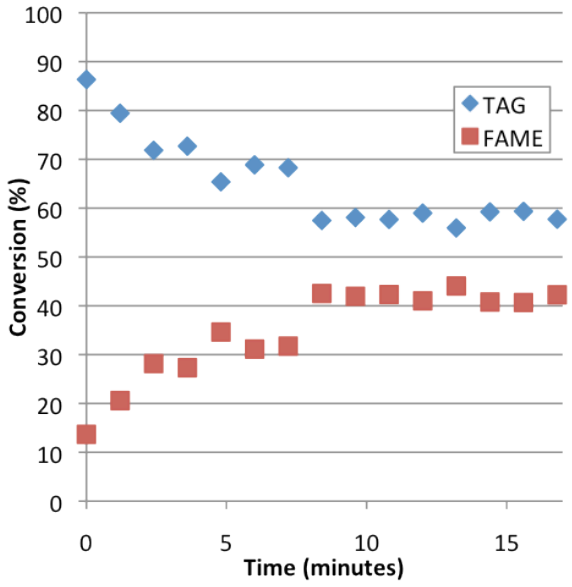


Figure S9. Reaction profile of transesterification with 1% (w/w) NaOH in methanol (non-anhydrous) (30:1 methanol/oil ratio), performed at 23 ˚C in $CDCl_3$. Data were acquired on a 600 MHz instrument with acquisitions taken every 1 min (4 scans, 45 degrees, 1 s relaxation delay). The integrated glyceryl methine represents the conversion of acylglycerols (TAG = 5.27 ppm) and the integrated methyl ester represents the FAME (3.67 ppm product). 1% (w/w) NaOH at 60 °C converted to >80% FAME within 5 minutes by the first NMR acquisition and the reaction was not monitored further.

References

1) Knothe, G., Monitoring a progressing transesterification reaction by fiber-optic near infrared spectroscopy with correlation to ^{1}H nuclear magnetic resonance spectroscopy. *J. Am. Oil Chem. Soc.* **2000,** *77* (5), 489-493.

2) Gelbard, G.; Brès, O.; Vargas, R.; Vielfaure, F.; Schuchardt, U., ^{1}H nuclear magnetic resonance determination of the yield of the transesterification of rapeseed oil with methanol. *J. Am. Oil Chem. Soc.* **1995,** *72* (10), 1239-1241.