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Analysis of biodiesel conversion using thin layer chromatography and nonlinear calibration curves

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ABSTRACT

Biodiesel (BD) is a fuel produced by the (trans)esterification reaction between the components of vegetable oil (or animal fat) and an alcohol. The presence of several substrates complicates analytical separation of the mixture, yet understanding of the complex reaction kinetics requires acquisition of a large body of data. The two well-established methods of gas chromatography (GC) and HPLC are time consuming and expensive when analyzing multiple samples. Additionally, it is not always possible to record all the reactants on one elution profile. We examined applicability of thin layer chromatography (TLC) for this purpose, where the detection was based on either flame ionization detector (FID) or a modified staining procedure. The suggested staining method gave no background and appeared well suited for quantitative analysis. The relevant calibrations are presented, and the general principles of analysis of nonlinear responses are discussed. Several experimental samples were produced by enzymatic conversion of rapeseed oil to BD. One reaction step resulted in 85–95% conversion (6 h). The second step (after removal of glycerol and water) increased the yield to 97–98%. All components of the mixtures were separated and quantified. Relation of the BD contents measured by TLC and GC gave the values of 1.03 ± 0.07 (TLC-staining) and 0.95 ± 0.04 (TLC–FID), indicating applicability of the TLC-methods.

1. Introduction

Biodiesel (BD) is a fuel produced in the (trans)esterification reaction between components of a vegetable oil and an alcohol (typically methanol or ethanol) [1–3]. The substrates in oil are triglycerides (TG), diglycerides (DG), monoglycerides (MG) and free fatty acids (FA). The products obtained are BD, glycerol and water. An efficient conversion requires application of a catalyst, where KOH, $NaOCH_3$, H_2SO_4, etc. are among the most commonly used compounds [1]. Recently, enzymatic production of BD has attracted attention as an environmentally advantageous alternative to chemical conversion [2–4]. Several preparations of immobilized lipases are currently under examination as potential candidates for the industrial application [2–4]. Yet, presence of multiple substrates and products requires a thorough knowledge of the enzymatic reaction kinetics.

Examination of oil and BD samples is often based on gas chromatography (GC) or HPLC [5–9]. Both methods are well established but have a few disadvantages including a relatively long time of analysis (approximately 30 min per sample), high operational costs and difficulties in getting all relevant compounds on one profile. At the same time, monitoring of all reactants over the time is important for a thorough kinetic description of such process, where numerous measurements are required. The two methods discussed below appear, in fact, better suited for this purpose. These are thin layer chromatography (TLC) on chromarods assisted by flame ionization detector (FID) and TLC on plates, where the spots are visualized by staining.

TLC–FID method is described in the literature; though, the results are somewhat contradictory [10–12]. For instance, both linear [12] and nonlinear dependencies [10,11] of the signal on the loaded mass are described, even if the detector settings were the same. Interpretation of nonlinear calibration curves is, however, complicated because a twofold change in the mass gives disproportional signals from different compounds, each of them following its own curve. Likewise, a change in the signal (caused by technical reasons but not the mass) introduces disproportion to the apparent composition of the mixture unless the signal is scaled to the original calibration. Application of an internal standard compensates this error, but the mass of the standard must be exactly the same under all measurements. This approach is not always possi-

Abbreviations: BD, biodiesel; FAEE, fatty acid ethyl ester; FAME, fatty acid methyl ester; FA, fatty acid (free); GC, gas chromatography; m/m, mass per mass; m/v, mass per volume; OA, oleic acid; TG/DG/MG, tri-/di-/mono-glyceride; TO/DO/MO, tri-/di-/mono-oleine; TLC–FID, thin layer chromatography assisted by flame ionization detector.

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Table 1
Composition of the oil samples used to produce calibrations.

Major component	Component BD, %	Component TG, %	Component FA, %	Component DG, %	Component MG, %
BD (rapeseed)	96	0	1	1	2
TG (rapeseed)	0	95	2	2	1
FA (OA)	0	0	98	1	1
FA (mix)	0	29	46	20	5
DG (sunflower)	0	5	1	72	22
MG (OG)	0	2	1	2	95

ble, especially when the standard and the analyte partially overlap. As a partial solution, some authors recommend application of different combinations of linear response factors to different parts of the nonlinear dependencies [11]. All the above issues were not adequately covered in the literature and require a more general approach.

Separation of lipids on TLC plates followed by staining is another potentially convenient procedure [13,14]. The staining is often based on $KMnO_4$ oxidation, yet the described method gives a strong violet background with irregular patterns, which precludes any reliable quantitative analysis [14]. Additionally, the produced spots are not stable and fade over the time. A proper modification is required to combine a low cost and simplicity of this method with the sufficient reliability.

In the current publication we present a modification of the TLC-staining which avoids the problems of background and fading of the spots. A comparison of several methods was carried out, where the oil components under enzymatic biodiesel conversion were separated and quantified. Correct application of the nonlinear calibration curves, scaled with help of the total sample mass, is discussed.

2. Materials and methods

2.1. Materials

All salts and solutions were purchased from Sigma–Aldrich. Chromarods S III were form SES GmbH-Analysesystem (Germany). TLC plates Polygram Sil G 20 cm × 20 cm (gel 0.2 mm) were from Macherey–Nagel (Germany). Lipid standards of high purity (methyl oleate, ethyl oleate, triolein, diolein, monoolein, oleic acid) were from Sigma–Aldrich. Rapeseed oil was from a Danish supermarket. Preparations of MG, DG and FA were from Danisco (Denmark), see Table 1 for details. Immobilized enzyme preparations of lypozyme TL HC and Novozym 435 were kindly provided by Novozymes (Denmark).

2.2. Methods

2.2.1. Enzymatic preparation of the calibration mixtures

An FA-enriched mixture was prepared in the following way. Rapeseed oil was incubated with 20% (v/v) water and 4% (m/v) Lypozyme TL HC for 3 h (35 °C, 200 rpm). The contents of FA after hydrolysis was determined by titration.

BD samples of 96% purity (FAME or FAEE) were prepared from rapeseed oil and MeOH or anhydrous EtOH as described below for "98%" analytical sample except for the absence of molecular sieves at the second step and the incubation time shortened to 12 h.

The above samples were used either separately or in the mixture with commercial preparations of TG, DG and MG.

2.2.2. Enzymatic preparation of BD-containing samples

The test mixtures were notated according to the detected level of BD, e.g. "24%". They were produced by incubation of the below components with 3% (m/v) of Lypozyme TL HC at 35 °C, 200 rpm (all compounds in relative volumes v): (1) 0.92 v oil, 0.04 v water, 0.04 v ethanol, incubated for 6 h ("24%" sample); (2) 0.88 v oil, 0.04 v water, 0.12 v ethanol, incubated for 6 h ("65%" sample); (3) 0.82 v oil, 0.007 v water, 0.17 v ethanol added in two steps at 0 h and 3 h over 6 h ("85%" sample). Two more samples "97%" and "98%" were produced according to a separate procedure, where two incubation steps were involved. Step 1 generally followed the method for "85%" sample except for supply of 96% ethanol added at 0 h, 2 h, 4 h as three portions (each of 0.09 v). The process ended by removal of enzyme glycerol, water and ethanol. Enzyme particles were separated by filtration, glycerol was settled as a separate phase by gravity (2 h), and excessive ethanol and water were evaporated under vacuum (1 mbar, 1 h). The obtained product was subjected to the second reaction (step 2) with 4% (m/v) Novozym 435, 8% (v/v) anhydrous ethanol, and 8% (m/v) of molecular sieves. The conversion was continued for 21 h ("98%" sample) and 90 h ("97%" sample) at 35 °C, 200 rpm.

The more detailed kinetic records of the reaction were produced for step 1 with 1 v of oil and 5% (m/v) of Lypozyme TL HC (35 °C, 200 rpm, 6 h). The supply of 96% ethanol was as follows: 0.045 v was added at the beginning of reaction, whereupon 0.2–0.23 v was continuously added over 1.5–4 h (see Section 3.7). The reaction was continued for 6 h, and small samples were collected at time intervals. They were centrifuged to precipitate glycerol and after evaporation of ethanol and water subjected to GC or TLC analysis.

2.2.3. GC-analysis

Quantification of FAEE (%, m/m) was performed with methyl heptadecanoate as internal standard according to the EN14103 standard method on a Varian Chrompack CP-3800 gas chromatograph (GC) equipped with a Varian "Select Biodiesel for FAME" (30 m, 0.32 i.d.) column.

2.2.4. TLC–FID analysis

The general experimental procedure followed the method described earlier [11,12]. Separation was carried out on Chromarods S III cleaned by two sequential runs on Iatroscan (see below). The lipid samples were diluted in hexane and loaded on each rod at the total mass of 1–15 μg. The developing mixture of 16% diethyl ether and 0.04% formic acid in hexane was used and provided a good separation of all components (Fig. 1). The rods were dried at 120 °C for 3 min before scanning. Detection was performed on Iatroscan MK 6 s using the below settings: air flow 2 L/min, hydrogen flow 160 mL/min, scan speed 30 s per rod. The recorded profiles were analyzed by Crom-Star 6.0 program, see an example in Fig. 1. The full experimental proceeding of 10 samples on one chromarod frame took approximately 2 h.

2.2.5. Separation on TLC-plates and the staining procedure

Silica-gel TLC-plates (Polygram Sil G) on plastic support were used. Aluminum support is not recommended because it crucially hinders soaking in water at the later step. The size of the plates was 20 cm × 20 cm, 10 cm × 20 cm or 10 cm × 10 cm (height × width). The lipid samples (0.1–4 μL per lane) were loaded without dilution at a distance of 1.8–2.5 cm from each other and 1–2 cm from the bottom. The development was carried out in either 10% ethyl acetate in hexane (20 cm × 20 cm plates, 1.5 h) or 15% ethyl acetate