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Development of kinetic model for biodiesel production using liquid lipase as a biocatalyst, esterification step

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ABSTRACT

Biodiesel can be produced from vegetable oils using different catalysts including enzymes. This publication presents the development of a mathematical model for biodiesel production using the liquid lipase Callera Trans L (CTL) and analyzes the first block of reactions: esterification of free fatty acids (FFA) in biodiesel and hydrolysis of the latter. The relevant rate constants were evaluated by changing water, methanol, FFA and enzyme concentrations. The results were compared to the immobilized catalyst Novozym 435 (Nvz). The intriguing difference was observed for the apparent equilibrium constants of CTL (high K_{eq}^{app}) and Nvz (low K_{eq}^{app}). This thermodynamic "inconsistency" was explained by absence or presence of the catalyst carrier. Nvz carrier particles apparently help to disperse water, increasing its surface and hydrolytic activity in comparison to CTL. Another reactant, methanol, had a dual effect acting as (i) a substrate and (ii) a solvent of water in oil phase. The latter effect added to hydrolytic activity and decreased K_{eq}^{app} at increasing methanol (0–0.5 M). Inhibition and inactivation of CTL by methanol (<8% v/v) were insignificant. FFA acted as both substrate and reversible inhibitor of the enzyme suppressing its activity to approximately 25% at FFA >1.5 M.

1. Introduction

Vegetable or plant oil is one of the most important renewable resources in food industry, where up to 80% of feedstock is used. Recently, oil became a source of the environment-friendly biofuel [1], which is not toxic and degrades in the nature much faster than petrodiesel [2].

The main component of plant oil is triglyceride. This is an ester of glycerol with three fatty acids of different lengths and various double-bond patterns [3]. Triglycerides have high viscosity and flash point, and they need chemical alteration to facilitate their burning at lower temperatures [3–5]. The necessary properties are achieved after the reaction of transesterification with methanol or ethanol, where simple esters are produced, such as fatty acid methyl ester (FAME) or fatty acid ethyl ester (FAEE). They have lower flash point and can be used instead of or together with petroleum products. Alcoholysis of oil involves the exchange of fatty acid of tri-acyl glycerol (TAG) with alcohol. If the feedstock mainly consists of TAG, the conversion takes place in a series of reversible reactions, where di-acyl glycerol (DAG) and mono-acyl glycerol (MAG) are formed as the intermediate products. The general scheme of the reaction is shown in Fig. 1.

Biodiesel can be produced by different processes including pyrolysis, microemulsion technique, transesterification in the presence of alkali or acid, as well as supercritical transesterification [2,4,6]. FAME or FAEE (compliant with the standards, e.g., EN 14214 or ASTM) can be used in blends of biodiesel/diesel up to 50% applied to unmodified diesel engines [7]. In United States, B20 blend (20% FAME mixed with 80% petro-diesel) is the most commonly used type of such bio-fuel [1].

Recently, application of enzymes in the biodiesel industry attracted much attention due to more affordable prices of these biocatalysts [8–12]. Enzymes have the unique functional groups within their active sites that mediate chemical conversion under mild reaction condition [13]. For example, lipases find numerous applications including refinement and remodeling of vegetable oils,

Abbreviations: B, biodiesel; C, CH_3OH (methanol); CTL, Callera Trans L; E, the enzyme; EX, the enzyme with conjugated fatty acid; F, free fatty acid (FFA); FAEE, fatty acid ethyl ester; FAME, fatty acid methyl ester; G, glycerol; Nvz, Novozym 435; RSD, relative standard deviation; TAG/DAG/MAG or T/D/M, tri/di/mono-acyl glycerol; W, water as a fine micelle; WW, large water droplets; ΣW, (total water (in W concentration units).

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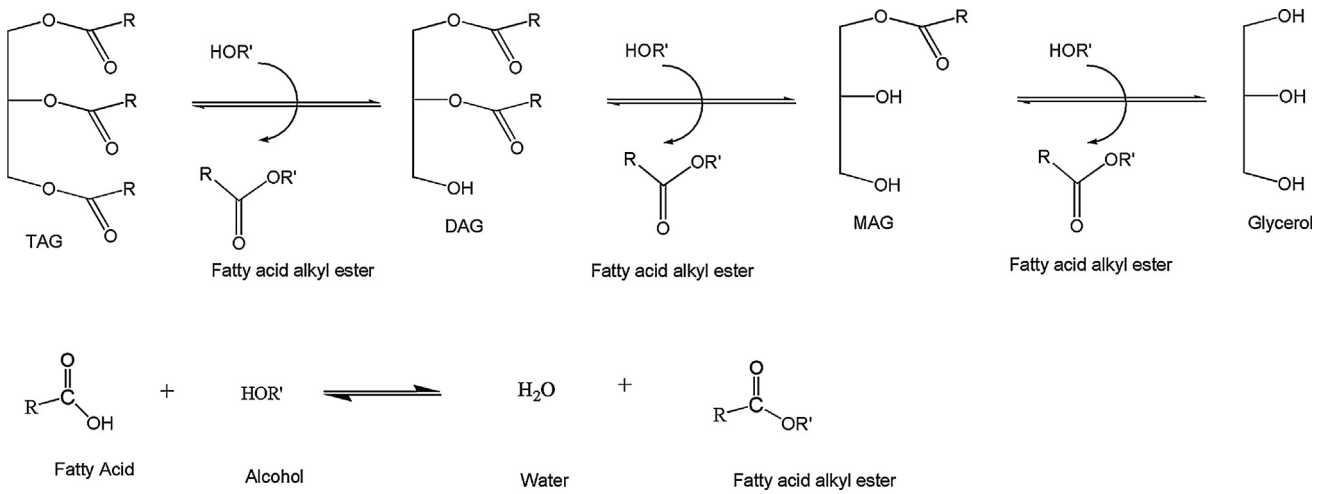

Fig. 1. The overall scheme of transesterification, where TAG, DAG, MAG and FFA are converted to fatty acid alkyl esters.

hydrolysis and glycerolysis of oils under mild conditions, incorporation of atypical compounds into lipid molecules etc [8]. Recently, lipases were successfully used for production of biodiesel with high yields and rates [6,10–16]. In some cases, the reaction rate was increased by using a mixture of two lipases with different substrate specificities targeted toward either FFA and partial glycerides (e.g., *Candida antarctica* B-lipase) or TAG (e.g., *Thermomyces lanuginosus* lipase) [16].

Yet, the enzymatic catalysts are still rather expensive and sensitive to the reaction conditions in comparison to the simple chemical catalysts. Partial stabilization of the enzymatic activity can be achieved by immobilization of the protein on a solid carrier [10,13,17–20]. In addition, application of a carrier material simplifies the recovery of the catalyst for repeated reactions. Among the popular preparations of immobilized enzymes are Novozym 435 (Nvz) and Lipozyme TL HC, which contain the lipases from *C. antarctica* and *T. lanuginosus*, respectively. Both enzymes are adsorbed on hydrophobic beads of high physical stability. The first catalyst (Nvz) was a subject of an extensive kinetic investigation [21,22]. It showed that the optimal field of application for Nvz is decrease of FFA, MAG and DAG in oil and biodiesel. On the other hand, conversion of TAG was very slow, hindering application of Nvz to oils rich in TAG. The second catalyst (Lipozyme TL HC) demonstrated an excellent performance in the test reactions under conversion of rapeseed oil to biodiesel [21], but it was too expensive for such type of reactions. The economic issue is a general problem for all immobilized enzymes used in the biodiesel industry, because the bearing material and the immobilization procedure significantly increase the price in comparison to the original "free enzyme". A large initial investment is a serious obstacle under manufacturing of a "low price" product biodiesel, especially because alcohols can easily deteriorate the sensitive biocatalyst.

This publication investigates the esterification of free fatty acids (FFA) using the liquid enzyme Callera Trans L (CTL). One of the major concerns about the application of liquid enzymes is addition of a considerable amount of water, necessary to preserve the catalytic activity of the enzyme under exposure to methanol. Presence of water in the reaction mixture is expected to cause the proportional increase in FFA due to accelerated hydrolysis. Therefore, we put here an accent on examination of FFA ↔ biodiesel conversions to assess feasibility of CTL application for synthesis of biodiesel. The current work describes a detailed analysis of the reaction kinetics and the thermodynamic equilibriums and confirms feasibility of the method. This block of reactions will become a part of a general mathematical model of CTL with more substrates and products included.

2. Materials and methods

2.1. Materials

All salts and solutions were purchased from Sigma–Aldrich. Biodiesel of 96–97% purity was prepared as described below using rapeseed oil from Danish supermarket. Liquid enzyme Callera Trans L and immobilized lipase Novozym 435 (≥10,000 of propyl laurate units per g, 30 °C) were kindly provided by Novozymes (Denmark). Oleic acid (98%) was purchased from Sigma–Aldrich. The concentration of enzyme in Callera Trans L was assumed as 500 μM according to the information provided by the manufacturer. The preparation also contained 75% of water and 25% of propylene glycol.

2.2. Preparative synthesis of biodiesel

Biodiesel was synthesized enzymatically on the preparative scale to be used as a substrate–solvent in the current kinetic study. The reaction was conducted in closed shake flasks containing 1 L of rapeseed oil and 5% (m/v) Novozyme 435 (37 °C, 200 rpm in shake incubator, other conditions are also possible). Three portions of MeOH (1/3, 1/3 and 1/2 equivalents of a 100% conversion) were added at 0 h, 24 h and 48 h of the reaction. After 72 h, glycerol was partially removed by precipitation, whereupon another 1/3 equivalent of MeOH was added. The reaction was continued overnight, and then glycerol and the remaining enzyme particles were removed by settling. The rest of MeOH was evaporated under heating at low vacuum. Separation of an additional small portion of glycerol took place under this procedure. Purity of freshly prepared biodiesel (96–97%) was assessed by chromatographic methods described earlier [23,24].

2.3. Analytical hydrolysis and esterification of biodiesel

The substrate mixtures (biodiesel, deionized water) were placed into 20 mL glass bottles, each equipped with a cap containing silicone septum. The total volume was regulated by adding different quantities of biodiesel, which became thereby connected to water by material balance. The solutions were pre-warmed to 35 °C. Before the reaction was started, the samples were vigor-

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