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Kinetic model of biodiesel production using immobilized lipase Candida antarctica lipase B

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ABSTRACT

We have designed a kinetic model of biodiesel production using Novozym 435 (Nz435) with immobilized Candida antarctica lipase B (CALB) as a catalyst. The scheme assumed reversibility of all reaction steps and imitated phase effects by introducing various molecular species of water and methanol. The global model was assembled from separate reaction blocks analyzed independently. Computer simulations helped to explore behavior of the reaction system under different conditions. It was found that methanolysis of refined oil by CALB is slow, because triglycerides (T) are the least reactive substrates. Conversion to 95% requires 1.5–6 days of incubation depending on the temperature, enzyme concentration, glycerol inhibition, etc. Other substrates, free fatty acids (F), diglycerides (D) and monoglycerides (M), are utilized much faster (1–2 h). This means that waste oil is a better feedstock for CALB. Residual enzymatic activity in biodiesel of standard quality causes increase of D above its specification level because of the reaction 2M ↔ D + G. Filtration or alkaline treatment of the product prior to storage resolves this problem. The optimal field of Nz435 application appears to be decrease of F, M, D in waste oil before the conventional alkaline conversion. Up to 30-fold reduction of F-content can be achieved in 1–2 h, and the residual enzyme (if any) does not survive the following alkaline treatment.

1. Introduction

Biodiesel (B) is a fuel produced from plant oils in the reaction of (trans)esterification, where glycerides and free fatty acids (F) interact with an alcohol (typically methanol, MeOH) [1–6]. Use of alkaline catalysts (KOH, NaOH, methoxide, etc.) puts strict requirements to the content of F in the feedstock to prevent neutralization of alkali and formation of soaps [3,5]. The alternative acid conversion is insensitive to F, but this process is abandoned in many countries because of its high corrosivity [2,3,5]. Recently, several formulations of lipases were introduced to the market as perspective enzymatic catalysts. They worked well with feedstocks of different quality and did not require high temperature and excessive alcohol [1,3,4]. Additionally, application of enzymes facilitated recovery of the catalyst and significantly improved quality of the raw byproduct glycerol (G) [1,3,4].

Efficiency of lipases in biodiesel production was demonstrated, at least on the laboratory scale, e.g. for Candida antarctica lipase B (CALB) [7–11] and Thermomyces lanuginosus lipase (TLL) [9,12,13]. The obtained yields of 90–95% were quite impressive, though the final products did not comply with the international standards EN 14214 and ASTM D-6751 [3]. The reported incubation times of 10–50 h were relatively long, and lipases were sensitive to an excessive concentration of short-chain alcohols [7,11,12]. Comparison of CALB and TLL indicated a higher resistance of the first enzyme toward inactivation by alcohol, yet methanolysis of oil by CALB was seemingly slower than conversion performed by TLL [7,12,13]. Another problem, when working with immobilized lipases, concerned adsorption of glycerol (G) on the carrier particles [13,14]. It caused clogging of the enzyme, especially if working with silica-based carriers [13,14]. All the above facts call for a careful experimental design, and the current publication intends to explore novel approaches to optimization of the enzymatic method.

Abbreviations: B, biodiesel (FAME); CALB, Candida antarctica lipase B; C, CN, species of CH_3OH with different chemical activity; D, diglyceride; EtOH, ethanol; E, enzyme; EX, enzyme with attached fatty acid; FAEE, fatty acid ethyl ester; FAME, fatty acid methyl ester (the same as B); F, free fatty acid; *FR*, fluorescence ratio F_{630}/F_{570}; γ, substrate ratio c_0/m_0; M, monoglyceride; m/m, mass per mass; m/v, mass per volume; MeOH, methanol; T, triglyceride; TLC-FID, thin layer chromatography assisted by flame ionization detector; v/v, volume per volume; W, WW, WN, species of water with different chemical activity.

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$$E \overset{+S_1}{\leftrightarrow} E\left[\frac{S_1}{X \cdot P_1}\right] \overset{-P_1}{\leftrightarrow} EX \overset{+S_2}{\leftrightarrow} E\left[\frac{X \cdot S_2}{P_2}\right] \overset{-P_2}{\leftrightarrow} E;$$

Scheme 1.

$$E \underset{-P_1}{\overset{+S_1}{\leftrightarrow}} EX \underset{-P_2}{\overset{+S_2}{\leftrightarrow}} E$$

Scheme 2.

Attempts to improve the reaction conditions typically pursue three below strategies: (1) trials and errors [7–11]; (2) interpolational search for the maximal yield in randomized experiments [15,16]; and (3) analysis of the reaction kinetics by the appropriate velocity equations [17–20]. The third approach has a great potential, because a correct computer model can promptly simulate the process under different conditions. On the other hand, quantification of the necessary kinetic constants is problematic when working with complex reaction. For example, the complete set of substrates and products in oil–biodiesel mixtures includes tri-, di- and monoglycerides (T, D, M), free fatty acids (F), water (W), glycerol (G), alcohol (C) and biodiesel (B). The situation is complicated by involvement of several reactants in phase transitions and inhibition of the enzyme.

Only a few kinetic models of the enzymatic biodiesel production exist to date, and they are based on analysis of the straightforward conversion of oil, where all reactions take place simultaneously [17–19]. Determination of 15–30 rate constants in one experimental setup is not very realistic. To simplify the task, some models ignore "unimportant" reactants (e.g. F and W), and none of them considers reversibility of the process and phase separation effects. In this and previous publication [20], we explore another strategy and characterize separate blocks of the global model. These partial reactions include a limited number of compounds, making the velocity equations less complex and hence the analysis more reliable. We also use a truncated form of the lipase ping-pong mechanism reducing its full-length record in Scheme 1 to a less elaborate form in Scheme 2, as was discussed earlier [20]:

The simplified Scheme 2 ignores formation of the dissociable enzyme-ligand complexes, and presents the catalytic transformation as an efficient collision between lipase and its substrates. This approximation is valid if the enzyme has low affinity for the ligands, which appears to be plausible [19,20]. The reduced number of rate constants in Scheme 2 "frees space" and allows incorporation of full reversibility and phase effects into the model.

Steady state velocity equation for Scheme 2 can be written as follows:

$$\frac{v}{e_0} = \frac{k_{s1} \cdot s_1 \cdot k_{s2} \cdot s_2 - k_{p1} \cdot p_1 \cdot k_{p2} \cdot p_2}{k_{s1} \cdot s_1 + k_{s2} \cdot s_2 + k_{p1} \cdot p_1 + k_{p2} \cdot p_2} \quad (1)$$

where k_{s1}, k_{s2} and k_{p1}, k_{p2} coefficients refer to the forward and backward reactions, respectively. The derived expression provides a good imitation of the "full scale" ping-pong mechanism. For example, increase in the concentration of each substrate/product causes hyperbolic increase/decrease of velocity in Eq. (1) resembling saturation/inhibition of the enzyme. Haldane equilibrium ratios are identical in both schemes. Only simultaneous and proportional change of the two substrates (or the two products) in the absence of products (substrates) will cause noticeable deviation of Eq. (1) from the correct behavior when enzyme saturation exceeds ≈40%.

Earlier we have analyzed enzymatic esterification of fatty acids in biodiesel, employing a novel monitoring technique based on the calibrated fluorescence of Nile Red [20]. This compound is soluble in hydrophobic liquids and responds to alcohols, partial glycerides, free fatty acids, etc. dissolved in the organic phase [20–22]. This makes Nile Red well suited to follow the reaction progress, and here we continue exploration of its possibilities.

In the current publication we have accomplished construction of the full kinetic model of biodiesel production using CALB as a catalyst. The performed computer simulations indicated that high-quality oils are poor substrates for this lipase, because it converts triglyceride very slowly. On the contrary, CALB works much more efficiently with partial glycerides and free fatty acids. The enzyme is particularly suitable to decrease the acid value of waste oils, making these low-cost feedstocks applicable to the following alkaline conversion.

2. Experimental

2.1. Materials

All salts and solutions were purchased from Sigma–Aldrich. TLC-FID Chromarods S III were from SES GmbH – Analysesystem (Germany). TLC plates Polygram Sil G 20 cm × 20 cm (gel 0.2 mm) were from Macherey-Nagel (Germany). Preparations of monoglyceride based on oleic acid (95% purity), a diglyceride-containing mixture (D = 72%, M = 22%, T = 5%, F = 1%, m/m) and oleic acid (98% purity) were purchased from Danisco (Denmark). Refined rapeseed oil (T = 96.9%, D = 2%, M = 1%, F = 0.1%) was from Danish supermarket. Biodiesel of ≈96% purity was prepared as described in Section 2.2 using refined rapeseed oil. Some experiments involved crude rapeseed oil kindly provided by Emmelev A/S (Denmark). Novozym 435 was a generous gift from Novozymes A/S (Denmark).

2.2. Preparation of biodiesel

Biodiesel (B) on preparative scale was produced in closed shake flasks containing 1 L of rapeseed oil and 5% (m/v) Novozyme 435 (37 °C, 200 rpm in shake incubator). 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, then glycerol and the remaining enzyme particles were removed by centrifugation. Excessive alcohol was evaporated. Fresh biodiesel was of ≈98% (m/m) purity. During storage, the composition changed to B = 96%, T = 0.2%, D = 2%, M = 0.8%, F = 1% based on TLC or TLC-FID measurements [23]. This effect is discussed in Sections 4.12, 4.13 and 5.6 (Fig. 9B). The endogenous water in the stored preparation was ≈500 ppm according to Karl Fisher titration.

Conversion of rapeseed oil to biodiesel on the analytical scale was conducted in a similar manner in tightly closed 50 mL bottles (30 °C, 360 rpm in shake incubator or 15 rpm upside-down rotation). The samples of 100 μL were taken at time intervals for the following analysis.

2.3. Conditions of partial reactions

The substrate mixtures (e.g. M + C, F + C, etc.) were placed into 15 mL Falcon tubes or 20 mL bottles, where the total volume was regulated by adding different quantities of either B or T used as the reaction mediums. The solutions were pre-warmed to 30–45 °C. Before the reaction was started, the samples were vigorously shaken and aliquots of 0.6 mL were collected. Fresh enzyme beads were added (e.g. 800 mg of Novozym 435 per 20 mL, 4%, m/v), and the reaction was carried out in a shake incubator at 200–360 rpm. The samples were placed horizontally to improve suspension of the enzyme particles. An alternative setup used upside down rotation at 15 rpm. At the stipulated time intervals, the enzyme particles were allowed to settle for 15–20 s, and the samples of 0.6 mL were