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Batch production of FAEE-biodiesel using a liquid lipase formulation

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ABSTRACT

The application of lipase catalysis to the production of biodiesel has received much interest during the past several years. Although most of the previous work has involved the use of immobilized enzyme, more recent work has indicated that liquid formulations of lipase can provide a highly competitive option for the conversion of oils and fats to biodiesel. This study investigates the impact of several process parameters on the production of fatty acid ethyl esters from rapeseed oil in a pure batch process on the liquid lipase formulation Callera™ Trans L. Oil conversion in excess of 98% was achieved by combining a 50% stoichiometric excess of ethanol (1.5 equivalents) with 20% (w/w) water relative to the oil. The rate of reaction was directly proportional to the amount of lipase added in this system (500–2000 LU per gram oil). Addition of glycerol to the initial reaction mixture reduced the initial reaction rate, but also improved the final yield of biodiesel by suppressing hydrolysis.

1. Introduction

In the scientific literature today there are an increasing number of reports about the use of lipases (EC 3.1.1.3) to catalyze the conversion of triglycerides in vegetable and animal oils and fats, to complement the first generation methods for producing biodiesel (fatty acid alkyl esters) using either acid or more commonly alkali catalysis [1,2].

The production of biodiesel from vegetable oils and fats takes place in a complicated reaction system that incorporates both transesterification and esterification reactions involving several compounds. The transesterification of glycerides is a multistep reaction. Ester bonds attaching the fatty acids (FFAs) on the triglyceride (TAG) are split one at a time, converting the TAG initially to diglyceride (DAG) and subsequently to monoglyceride (MAG) and finally to glycerol. Each step releases either an alkyl ester (biodiesel) or a fatty acid, dependent upon whether alcohol or water is used as the acyl acceptor (i.e. either transesterification or hydrolysis, respectively). In parallel with these reactions, the lipase also catalyzes the esterification of free fatty acid and alcohol to biodiesel. The lipase therefore converts several different substrates during the course of the reaction.

The ability of lipases to catalyze the different reactions varies between particular enzymes. The transesterification reaction can be done most successfully using *Thermomyces lanuginosus* lipase (TLL; formerly *Humicola lanuginosa*) [3]. On the other hand, esterification is more effectively carried out by *Candida antarctica* lipase B (CALB) [4,5]. This makes the latter enzyme useful for esterifying free fatty acids in either the feed oil (to prepare the oil for subsequent alkaline transesterification) or alternatively to 'polish' the biodiesel (following transesterification) such that it can meet commercial specification [5–7].

The main limiting factor for implementation of lipase catalysis in biodiesel production has been the cost of the enzyme. Most of the work to date has been focused on immobilized preparations such as Novozym® 435 (which is based on CALB) from Novozymes A/S [3–5]. The cost of such preparations makes it necessary to reuse them many times for the process to be economically feasible. The immobilized formulations enable effective recycle via simple filtration and also allow operation in a packed bed, in cases where plug-flow hydrodynamics are favored by the kinetics [5,8]. However, maintaining activity for a long period of time is a challenge under the conditions used in the biodiesel process [9]. Recently, it was suggested that the process could be simplified further and made more robust by the use of a liquid lipase formulation, Callera™ Trans L [10]. Removing the added cost for immobilization of the catalyst drastically reduces the required number of catalyst reuses, and hence the required biocatalyst stability [11,12].

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Using a liquid formulation of lipase instead of an immobilized one changes the reaction system in several ways. The most immediate is that water is introduced which promotes hydrolysis, meaning that the product will contain some FFA that will need to be removed post-reaction. However the introduction of water also has the benefit that the alcohol in the system will be diluted, thereby moderating its effect on the enzyme. Likewise, the presence of water in the system leads to the formation of a second liquid phase, creating an interface that is known to activate many lipases, including TLL [13–15]. The performance of the lipase in this system is thus determined by the interaction of several process parameters, including interfacial area (and by extension the volumetric ratio of oil to polar phase, and mixing), enzyme concentration and alcohol concentration. Increasing the amount of alcohol in the system pushes the equilibrium toward the formation of biodiesel, and also increases the reaction rate to some extent. However, beyond a certain point a further increase in alcohol content will mainly add to the operating cost involved in separating and recirculating the excess alcohol. Furthermore, damage to the lipase is likely at higher alcohol concentrations [3,16].

It has been suggested to replace the methanol that is used in the traditional process with other alcohols such as ethanol or butanol. This is both to improve the 'sustainability profile' of the produced biodiesel, and because these alcohols are expected to be less aggressive to the enzymes [17]. It should be noted that these alcohols are more expensive – especially per alcohol functionality (rather than weight) – than methanol. The previously published work on CalT clearly indicated the potential of this catalyst for the production of biodiesel. However, the reaction has not been investigated in a system that maximizes the interfacial area available to the enzyme. In this work, we have therefore studied the CalT-catalyzed transesterification of rapeseed oil in a system with high water-to-oil ratio and more efficient overhead mixing in a baffled vessel. The reaction was set up as a pure batch reaction system (i.e. with all components present from the start of the reaction) to avoid limitations in terms of substrate supply. Ethanol, rather than methanol, was used in the interest of investigating the possibility of making a fully renewable biodiesel, making the product fatty acid ethyl ester (FAEE). The study assesses the impact of water content and two types of mixing on the performance of the catalyst together with the impact of the catalyst concentration. Finally, the impact of adding glycerol to the initial reaction mixture has been studied, partially to investigate the effect on the extent of hydrolysis. The results of the experiments are then used to assess the feasibility of recycling the polar phase from one batch to the next as a potential method of reusing the biocatalyst.

2. Materials and methods

2.1. Materials

Rapeseed (canola) oil, kindly donated by Emmelev A/S (Otterup, Denmark), was used as the substrate in the transesterification reactions. The oil contained 0.82 wt.% free fatty acids (ISO 660), 0.06 wt.% water (ISO 662) and 71 mg/kg phosphorus (ISO 10540 3). A volume of 96% (v/v) ethanol (Kemetyl A/S, Køge, Denmark) was used as the acyl acceptor. HPLC calibration standards, methyl oleate (≥99%), 1-monooleoyl-*rac*-glycerol (≥99%) and dioleoylglycerol (≥99%, a mixture of 1.3- and 1.2-isomers), and HPLC grade solvents for the analyses, *n*-heptane (≥99%) and *t*-butyl methyl ether (≥99.8%), were purchased from Sigma-Aldrich. All reactions were carried out using a soluble lipase (Callera™ Trans L) provided by Novozymes A/S (Bagsværd, Denmark). The activity of the enzyme formulation was approx. 100,000 LU/g, where 1 LU is defined as the activity required to produce 1 μmol butyric acid in the hydrolysis of tributyrin under standard conditions (pH 7.5, 0.2 M substrate) [18].

2.2. Methods

2.2.1. Batch experiments

Experiments were carried out in two different setups (referred to as Setup 1 and Setup 2) each with three separate reactors, connected to a common stirrer motor (Shin Myung Servo Co. Ltd., Incheon, Korea). Each setup consisted of three 250 mL glass reactors equipped with baffles and overhead stirring. The reactor vessels were immersed in a water bath with temperature control (Julabo Labortechnik GmbH, Seelbach, Germany). The temperature in all experiments was maintained at 35 °C. Setup 1 was equipped with a four-blade marine propeller and used a stirring speed of 1200 rpm, whereas Setup 2 employed a Rushton turbine (blade-width/diameter = 1/5) and a stirring speed of 1400 rpm. Details of the reactor system dimensions can be found in Table 2.

In all batch experiments 100 g (Setup 1) or 70 g (Setup 2) of rapeseed oil was mixed with predefined amounts of alcohol, glycerol, lipase solution, and demineralized water. The amount of alcohol used was defined in units of equivalents (eq.), where 1 equivalent corresponds to the stoichiometric amount of alcohol needed to convert all fatty acid residues in the oil to biodiesel (i.e. 1 equivalence = 3 moles of alcohol per mole of TAG). Water and lipase solution were added in percent (w/w) relative to the mass of oil used in each experiment, with the lipase solution contributing to the water content (e.g. 1% lipase solution also contributed 1% of water). In experiments with glycerol addition, water was substituted by glycerol on a weight basis.

2.2.2. Sample preparation

A volume of 0.5 mL of the reaction mixture was centrifuged at 14,500 rpm for 5 min to separate the oil and aqueous phase, thereby terminating the reaction. Fifty five microliters of the oil phase was pipetted into 500 μL *n*-heptane. The sample was then further diluted 100-fold with *n*-heptane. A volume of 40 μL of this solution was injected for analysis on the HPLC.

2.2.3. High-performance liquid chromatography

The content of triglycerides (TAGs), diglycerides (DAGs), monoglycerides (MAGs), free fatty acids (FFAs), and fatty acid ethyl esters (FAEE) in the oil phase were analyzed by HPLC (Ultimate 3000, Dionex A/S, Hvidovre, Denmark) equipped with a cyanopropyl column (0.25 × 0.004 m) (Discovery®, Cyano, Sigma Aldrich A/S, Brøndby, Denmark), U3000 auto-sampler, TCC-3000SD column oven, U3400A quaternary pump modules and a Corona® Charged Aerosol Detector (Thermo Scientific Dionex, Chelmsford, MA, USA). The detector was supplied with nitrogen at 241 kPa (35.0 psi). A binary gradient program was applied using phase A: 99.6% *n*-heptane, 0.4% acetic acid and phase B: 99.6% methyl tertiary butyl ether, 0.4% acetic acid, as described previously [5]. The chemicals were acquired from Sigma Aldrich A/S (Brøndby, Denmark). The composition of the reaction samples are reported on a mass percentage basis, relative to the sum of quantified masses of the five analyzed components (TAG, DAG, MAG, FFA, FAEE).

3. Results and discussion

The course of a typical transesterification reaction using soluble lipase is presented in Fig. 1, where the concentration of FFA, FAEE, TAG, DAG, and MAG have been monitored during 24 h of reaction. The reaction mixture was composed of rapeseed oil with 0.5% lipase solution and 20% water (including the mass of the enzyme solution) relative to the oil, together with 1.5 eq. ethanol. The reaction was biphasic in all experiments presented in this study, consisting of