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Enzymatic conversion of coconut oil for biodiesel production

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ABSTRACT

The kinetics of the enzymatic conversion of coconut oil to biodiesel (fatty acid alkyl esters) have been investigated using ethanol and 1% (w/v) lipase at 50 °C. Coconut oil is being evaluated for biofuel production and an enzymatic process was selected to minimize side reactions (such as saponification) which can occur with the alkali-catalysed route if free fatty acid (FFA) concentration is significant. Rate data comparison showed that the NaOH-promoted conversion was about 2 orders of magnitude faster than the lipase-catalysed system but resulted in saponification/partial solidification of the reaction mixture. Analysis also revealed that the ratios of the kinetic constants for ester:glycerol (1:1.12) and that for ester:ethanol (1:3.11) during enzymatic transesterification are in agreement with reaction stoichiometry. Additionally, FFA esterification rates were higher (1.5 to 2.5) than triglyceride transesterification rates under similar conditions. Supplementation with ultrasonics at 43 kHz also permitted nearly 20-fold improvement in conversion rate. The study also demonstrated that with *Saccharomyces cerevisiae*, the growth yield on glycerol of 0.77 g g^{-1} is greater than that usually found for growth of yeast on glucose (viz. 0.4–0.45 g g^{-1}). Thus, opportunities for process enhancement exist through the use of increased enzyme concentrations, ultrasound techniques and growth of yeast on residual by-product glycerol.

1. Introduction

With the continuing high prices of imported liquid fuels, as well as environmental concerns over the increasing use of fossil fuels, the opportunities exist in a number of countries for the production of biofuel blends which use locally produced oils such as those from palm kernels, coconuts, soybean, canola and jatropha [1–5]. To produce such blends, transesterification of the triglycerides in these oils into esters is necessary (see Fig. 1A). This is achieved through either an alkali or enzyme catalytic conversion process, the latter usually with lipase [6,7] although the use of inorganic catalysts have also been reported [8]. Esterification of the FFAs to esters occurs also in these catalytic conversion processes (Fig. 1B). However, where the coconut oil contains relatively high concentrations of FFAs (up to 12% w/v), saponification or gelling can occur, and in such cases a two stage acid/alkali process has been used to minimize these effects and thereby produce a high quality biodiesel [9]. Engine studies have also been reported for such biodiesel blends [3,10] with particular emphasis on meeting standards such as the ASTM 7467-10 specification for biodiesel B6–B20 blends.

In the present investigation, the enzymatic (lipase) conversion of coconut oil for biodiesel production has been evaluated as well as the use of by-product glycerol for fodder yeast production. The advantages of such enzymatic processes are that lower reaction temperatures (e.g. 30–50 °C) are usually optimal, no side reactions such as saponification occur, water management in the reaction is not a problem and the potential exists for process enhancement through novel reactor design and enzyme immobilization and/or recycling [11,12]. However, issues such as enzyme cost, relatively slow reaction rates and possible enzyme inhibition effects all need to be addressed for comparison with alkali and/or acid/alkali catalytic processes [13,14]. This study has particular relevance to Pacific island countries where the potential exists for both increased coconut oil production [15,16] and the establishment of economically-viable biofuel processes with associated valuable by-products.

2. Materials and methods

2.1. Coconut oil characteristics

Punjas coconut cooking oil purchased locally in Australia was used in the present study. As reported previously [17], the main components of coconut oil are triglycerides, and up to 99.8% of these triglycerides can be fractionated into 13 groups based on carbon numbers from 28 to 52 (even numbers). From the % composition of these triglycerides and their molecular weights, an overall molecular weight (MW) of 648 g mol^{-1} for the coconut oil can be estimated. Coconut oil also contains a small percentage of low molecular weight free fatty acids (FFAs) with the most common being identified as lauric acid

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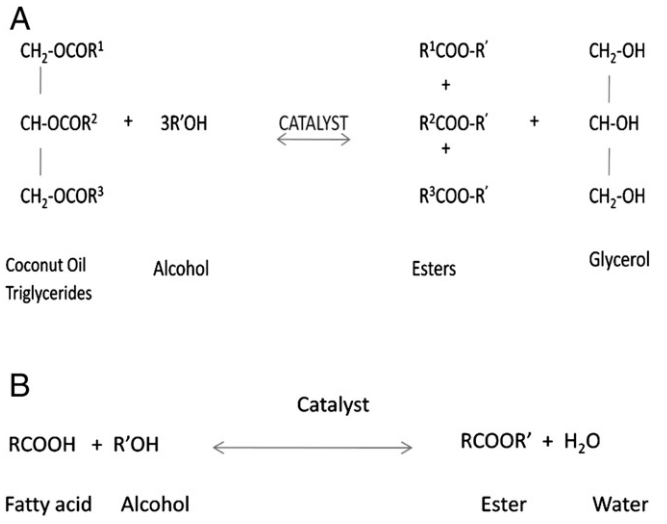

Fig. 1. (A) Transesterification process for the catalytic conversion of the triglycerides in coconut oil to esters, and (B) esterification of free fatty acids (FFAs) to esters.

(47%), myristic acid (17%) and palmitic acid (9%) [18]. The FFA content of coconut oil will depend on the method of its extraction and the duration of its storage with oils having an FFA content above 3% w/v being considered lower in quality. In the present enzyme-based study the FFA content of the oil was estimated at 4.5% w/v.

2.2. Transesterification of triglycerides in coconut oil

The transesterification experiment was conducted in a shaking incubator at 30 °C and 350 rpm in a 500 ml conical flask using a commercial lipase (Novozyme 435) donated by Novozyme Australia P/L. To give a 1:3 molar ratio of coconut oil triglycerides to ethanol, 300 ml of coconut oil and 75 ml of absolute ethanol were added to the flask. For the molar ratio calculation, the MW and density of coconut oil were taken as 648 g mol^{-1} (see above) and 0.92 g ml^{-1} respectively while those for ethanol were 46 g mol^{-1} and 0.78 g ml^{-1} respectively.

The mixture was agitated at 350 rpm for at least 30 min to ensure adequate homogenization before adding 1% (w/v) lipase to initiate the reaction. Samples were taken at specified intervals until the reaction was completed. The mixture was then left overnight at room temperature to achieve effective separation of the ester-containing phase from the aqueous glycerol phase.

The concentrations of the triglycerides and ethyl esters in the coconut oil phase were determined by gas liquid chromatography (GLC) using the method reported previously [19]. The glycerol and ethanol concentrations in the aqueous phase were determined by high pressure liquid chromatography (HPLC) [20].

2.3. Esterification of major free fatty acids

The kinetics of esterification of the three major FFAs in coconut oil viz. lauric (C_{12}), myristic (C_{14}) and palmitic (C_{16}), to their esters were determined using the same experimental conditions as for the transesterification of the triglycerides. Samples were taken at regular intervals for GLC determination of FFA and ester concentrations.

2.4. Ultrasonic-assisted transesterification

An ultrasonic cleaner Model D80H was used for this experiment, with an operating frequency of 43 kHz, and output power of 80 W. A 1:3 molar ratio of coconut oil:ethanol was again used and the conversion (%) of triglycerides to esters was determined as a function of time.

Table 1
Properties of by-product glycerol.

Properties	% (w/v)
Glycerol	78
Esters	10.7
Monoglycerides	0.78
Di-glycerides	7.01
Triglycerides	1.04
FFA	2.43

2.5. Yeast growth on by-product glycerol

Baker's yeast *Saccharomyces cerevisiae* ATCC 26603 was used in these growth studies. The media for yeast growth contained 20 g l^{-1} glycerol and 10 g l^{-1} yeast extract, 0.2 g l^{-1} $MgSO_4 \cdot 7H_2O$, 1.25 g l^{-1} KH_2PO_4 and 1 g l^{-1} urea. The composition of the glycerol by-product following phase separation is shown in Table 1 and when using glycerol derived from the enzyme reaction, the medium was filtered through a 0.45 μm syringe filter to remove the enzyme beads. Prior to the growth studies, the media were autoclaved at 121 °C for 20 min. The batch culture experiments were carried out in a 3 l Braun Fermentor using 1 l of culture medium. Temperature was controlled at 30 °C and the pH at 5.0 by addition of 3 M sodium hydroxide. The dissolved oxygen concentration was maintained at 20% air saturation. During yeast growth, samples were taken at various times for the determination of cell biomass and glycerol concentrations; the former by optical density (OD) measurements at 600 nm and use of an OD vs dry weight calibration curve, and the latter by HPLC.

3. Results and discussion

3.1. Alkali-catalysed runs

For comparison with the subsequent enzyme studies, an initial evaluation was carried out of the traditional alkali-catalysed alcoholysis of the triglycerides in coconut oil to esters. This was carried out at pilot-scale (200 l) at the Scientific Research Organization of Samoa (SROS) and involved the addition of 0.5% (w/v) NaOH to a 1:3 molar ratio of coconut oil:ethanol. The initial FFA content of the coconut oil was 3% (w/v). The reactants at 50 °C were mixed with a high speed circulating pump and the products were allowed to separate overnight with esters predominantly in the oil phase and glycerol in the aqueous phase. As shown in Fig. 2, relatively rapid conversion occurred in the alkali catalysed process with 91% conversion of triglycerides to esters in about 30 min.

The ester product concentration transient profile suggests that the direct ethanolysis of the triglyceride molecule did not produce any

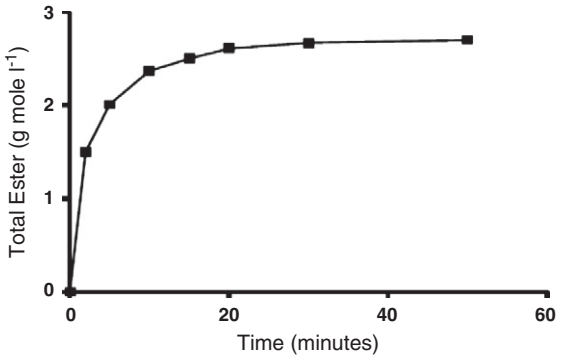

Fig. 2. Conversion of triglycerides in coconut oil to esters in a 200 L reactor using a 1:3 molar ratio of coconut oil:methanol at 50 °C catalysed by 0.5% sodium hydroxide.