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Enzymatic esterification of acid oil from soapstocks obtained in vegetable oil refining: Effect of enzyme concentration

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ABSTRACT

The enzymatic esterification of an acid waste oil was investigated using a commercial lipase (*Thermomyces lanuginosus*) in batch reactors, under the following reaction conditions: temperature of 35 °C, molar ratio of acid:alcohol of 1:1.5, vigorous magnetic stirring and enzyme concentration from 2 to 5 wt.%. The reaction progressed during 24 h. The acid oil obtained from soapstock of vegetable oil refining had an acidity of 65.5 wt.% and a very high sulphur content of 10 400 mg/kg. Thus, a pretreatment to reduce the mineral acidity before enzymatic esterification was necessary. The selected pretreatment consisted of one wash with 1:1 V/V oil:NaOH solution followed by two washings with 1:1 V/V oil:-distilled water. The results from the esterification of the pretreated oil showed a clear influence of enzyme concentration in the reduction of the acidity, most of which was achieved in the first 7 h. The amount and type of alcohol had minor influence in the reaction conversion and the fractionated addition of methanol had only expressive effect for lower catalyst concentrations, with final conversions being still unsatisfactory. The best conditions found were 4 wt.% of enzyme, 35 °C, 24 h, and 1:1.5 molar ratio of acid:alcohol, which afforded an 80% reduction of acidity.

1. Introduction

The world economy, and particularly the transport sector, is heavily dependent on fossil fuels. The massive use of petroleum products in this sector results in high emissions of carbon dioxide (CO_2), carbon monoxide (CO), nitrogen oxides (NO_X) and sulphur dioxide (SO_2), with significant effects on the environment, in particular the greenhouse effect and the production of acid rain [1,2]. Such facts incentivated countries to look at alternative fuels, and, consequently, biofuels are seen as a key element for the national energy strategy.

Biofuels are a real alternative to fossil fuels, due to their also high energy content but significantly less CO_2 emissions associated with their use [3]. Biodiesel is a biofuel that can replace fossil diesel, known for having low toxicity, high biodegradability and less emissions of most pollutants to the atmosphere resulting from its combustion. In addition, compared to fossil diesel, it has a significant added value due to its greater lubrication capacity, higher flash point and lower aromatic content. In agreement, it can extend the engine life and reduce vehicle maintenance costs [3]. As disadvantages, biodiesel presents a slightly lower calorific value as well as less oxidation stability, higher cold filter plugging point and less compatibility with some polymeric materials [4,5].

Biodiesel is conventionally a mixture of fatty acid methyl esters obtained by transesterification of triglycerides found in vegetable oils and animal fats. The homogeneous alkaline transesterification is widely used in the biodiesel industry (e.g. sodium hydroxide and methoxide), being the most economic and proven solution [6]. However, when the raw material has a high free fatty acids (FFA) content, this route is not feasible as it leads to catalyst consumption, soaps production and yield reduction [7].

In order to make biodiesel more competitive and environment friendly, low cost raw materials such as non-food oil, waste frying oils, animal fats and acid waste oils from different sources might be used [8], therefore enabling the application of alternative energy solutions to current practice.

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The soapstock is a residue from the chemical process used in the refining of vegetable oils, resulting from neutralizing the FFA. Usually, it is acidified with a strong mineral acid, such as sulfuric or hydrochloric acid (which enables the destruction of the emulsion), to produce an acid oil that consists essentially of FFA; also, it contains other lipids, such as triglycerides, diglycerides, monoglycerides and some other minor components such as phospholipids, sterols, tocopherols and pigments [9]. Soapstock is generated at a rate of about 6 %Vol. of refined oil, and its market price is about one tenth of the crude vegetable oil [10]. Given the low cost of this raw material and its high acidity, such acid waste oil seems to be a promising and sustainable raw material for biodiesel production and its study is of high relevance in the context of biomass conversion. However, due to its high FFA content, alternative biodiesel production routes are required, due to the reasons previously mentioned [7].

Esterification, whereby FFA react with an alcohol, in a presence of a catalyst, to produce fatty acid esters (biodiesel) and water, is conventionally used for FFA reduction and biodiesel production [11]. The homogeneous acid catalysts are mostly used but significant disadvantages are associated with the process, such as the high corrosivity caused by H_2SO_4 (most used catalyst) and operational costs (high amount of alcohol and higher temperature) as well as high volume of generated wastewater [6,12].

The use of heterogeneous catalysts, such as biological catalysts (biocatalysts), is an appealing alternative, and enzymes seem promising taking into account the mild reaction conditions that could be implemented and the possibility of enzyme reuse. Additionally, the lipase ability to catalyze both the esterification of FFA and the transesterification of triglycerides is recognized [13]. The biocatalysts also show important features, such as versatility, selectivity for the substrate, by-products of higher quality (cleaner glycerol) and environmental acceptance (limited use of hazardous reagents) [14]. Considering the enzyme application to waste raw material pre-treatment, its use has, furthermore, as advantage, the fact that it enables waste recovery, contributing to the reduction of the related environmental impacts.

The use of acid oil from soapstock for biofuels production is still poorly studied [15–18], especially concerning the use of biocatalysts for its esterification [15–17]. Most studies reported on the use of such raw materials towards biofuel production, evaluated chemical conversion processes [15,16,18]. One previous study evaluated the enzymatic production of fatty acid methyl esters from acid oil using *Candida rugosa* and *Candida antarctica* lipase by hydrolysis followed by esterification [17] with good results being obtained. However, the influence of acid oil pre-treatment and the effect of different reaction conditions, such as enzyme concentration and fractionated addition of alcohol towards the direct esterification of such acid oil was not studied.

In agreement, the present study evaluated the use of enzymatic esterification for biomass conversion towards biodiesel production from an acid waste oil resulting from soapstocks of vegetable oil refining. The main objectives were to: i) characterize the acid waste oil aiming enzymatic esterification and to evaluate the need for pretreatment; ii) study the effect of enzyme concentration during esterification using selected reaction conditions; and, iii) perform complementary studies for assessing the effect of other key variables.

2. Material and methods

2.1. Material

Novozym 40116, a liquid formulation of *Thermomyces lanuginosus* lipase, was provided by Novozymes.

The acid waste oil from soapstock of vegetable oil refining (mixture of seeds) was provided by the company Nature Light, S. A.

Methanol (Fischer Scientific $\geq$ 99%) and ethanol (Panreac $\geq$ 99.8%) were used as the acyl acceptor. All the other reagents were of analytical grade.

2.2. Characterization of the raw material

The physico-chemical properties determined in the raw material were acidity, water content and sulphur content.

The acidity (FFA content) was determined from the acid value, by volumetric titration, using a potassium hydroxide solution (0.1 mol L^{-1}), according to NP EN ISO 660 (2009); the amount of sample used depended on the expected acid value of the raw material and the results are expressed as the weight percentage in relation to oleic acid (molar mass 282 g mol^{-1}).

Due to the interference of water in the enzymes activity, determination of moisture in the feedstock was considered relevant. Taking into account the expected values for water content, it was done by determining samples weight loss at T = 105 °C ± 2 °C (oven method), until constant weight, according to EN 12880 (2000); results are expressed as weight percentage, in wet basis. The sulphur content was obtained according to ASTM D129 (2013) by sample (0.6 g each) combustion in oxygen pressurized atmosphere (Parr bomb, model 1672) followed by gravimetric determination of the precipitated barium sulphate, after precipitation using barium chloride.

2.3. Soapstock oil pretreatment

The neutralization of the mineral acidity is a key issue before starting the enzymatic processes, in order to avoid inhibition of the enzyme by the low pH.

The neutralization was performed by alkaline wash (250–750 ppm NaOH solutions) followed by double washing with warm distilled water in a separatory funnel using 1:1 V/V of oil:-washing solution per wash.

2.4. Enzymatic esterification

Esterification reactions were carried out under batch conditions using 100 mL Erlenmeyer flasks at 35 °C in an incubator (Brand Laviband) with a stirring plate (Aqualytic model AL 353) to ensure a constant vigorous magnetic stirring in all samples (plate regulated to “high stirring”). The reaction was monitored for 24 h, based on the literature [15,17]. Samples of 0.5 mL were collected in different time periods (0 h, 7 h and 24 h) to measure the FFA content. The number of samples was minimized in agreement with the amount of raw material available and to ensure that less than 5% of reaction mixture was taken during the trial. The reaction periods of 7 and 24 h were fixed based on the literature [17,19,20] and preliminary evaluation.

The effectiveness of the pretreatment was evaluated after esterification under the following standard reaction conditions: molar ratio of acid:methanol 1:1.5 and 5 wt.% of enzyme. Following, to assess the effect of enzyme concentration, in the range 2–5 wt.%, the raw material was neutralized with the 750 ppm of NaOH solution. The effect of the stepwise addition of methanol in aliquots of the total amount (eg. using 3 portions, each third was added in 3 different periods) was also verified.

The efficiency of the esterification was expressed by acidity reduction, according to Equation (1).

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