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Enzymatic transesterification of waste vegetable oil to produce biodiesel

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ABSTRACT

An experimental study on enzymatic transesterification was performed to produce biodiesel from waste vegetable oils.

Lipase from *Pseudomonas cepacia* was covalently immobilized on a epoxy–acrylic resin support. The immobilized enzyme exhibited high catalytic specific surface and allowed an easy recovery, regeneration and reutilisation of biocatalyst.

Waste vegetable oils – such as frying oils, considered not competitive with food applications and wastes to be treated – were used as a source of glycerides. Ethanol was used as a short chain alcohol and was added in three steps with the aim to reduce its inhibitory effect on lipase activity.

The effect of biocatalyst/substrate feed mass ratios and the waste oil quality have been investigated in order to estimate the process performances. Biocatalyst recovery and reuse have been also studied with the aim to verify the stability of the biocatalyst for its application in industrial scale.

1. Introduction

Biodiesel is a mixture of alkyl esters, produced by catalytic transesterification of glycerides with short chain alcohols (Fukuda et al., 2001). Transesterification is the alcoholysis of triglycerides resulting in a mixture of mono-alkyl esters and glycerol, which is separated and removed to achieve a low-viscosity product similar to conventional diesel fuel. Biodiesel shows a lower viscosity as compared to petro-diesel and it is less polluting since it allows to save 2.4–3.2 kg of CO_2 per kg of fuel. Furthermore, it is biodegradable and, during the combustion, a reduced level of particulate, carbon monoxide and nitrogen oxides is produced (Ma and Hanna, 1999).

Waste or low quality vegetable oils should be used as substrate for the production of biodiesel, so that the transesterification allows their valorisation and determines significantly reduced post-treatments aimed to a proper disposal. The amount of waste oils is over 15 million tons per year, which, if converted to biodiesel, satisfy the European demand, estimated at 10 million tons (2010). Furthermore, waste vegetable oils as substrate permit to overcome the competition with the food field: the area of different crops that are needed to meet the 50% of the demand for diesel in the United States of America is estimated in 0.265 billion m^3 per year. Since triglycerides represent the most relevant cost of the biodiesel production (almost 71% when fresh oils are used), economic feasibility of the process is obtained by using waste oils (Chisti, 2007).

The transesterification process can be performed by different catalysts: alkaline, acid or biological catalysts. The enzymatic process has some advantages, such as a higher yield in esters and a better glycerol recovery, as well as the possibility of using, in the reaction mixture, free fatty acids oils without saponification products (Formo, 1954; Freedman et al., 1986; Srivastava and Prasad, 2000). Moreover, the process is performed at lower temperatures (up to 323 K), lipase can esterify free fatty acids (Ban et al., 2001, 2002).

The reaction pattern of bio-catalytic transesterification of triolein in presence of ethanol was described as a sequence of three reactions in series (Al-Zuhair et al., 2007), leading to the production of one mole of ester in each step and of glycerol in the last step, according to the following scheme:

Triolein + ethanol ↔ diolein + ethyloleate

Diolein + ethanol ↔ monolein + ethyloleate

Monolein + ethanol ↔ glycerol + ethyloleate

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Table 1
Commercial vegetable oil based on sunflower and fractionated vegetable oils: fatty acids components.

Sunflower fatty acid composition	wt%
Palmitoleic (C16:1)	6.08
Stearic (C18:0)	3.26
Oleic (C18:1)	16.93
Linoleic (C18:2)	73.73

In a previous paper, Calabrò et al. (2009) showed that immobilized-lipase catalysed transesterification could reliably be described by a Ping–Pong bi–bi kinetic mechanism, with ethanol inhibition. Even if an excess of alcohol promotes the transesterification reaction (Ban et al., 2002), it has an inhibitory effect on enzymatic activity. Some studies (Kaieda et al., 1999; Samukawa et al., 2000; Shimada et al., 2000; Ban et al., 2002; Shimada et al., 2002), carried out with methanol as alcohol, showed the possibility to introduce alcohol step by step. Among alcohols, ethanol is less used than methanol, but it lead to high conversions; moreover, it has been also demonstrated that the lipase prefers to exert its activity on long chains of alcohol as compared to short ones (Nelson et al., 1996; Shimada et al., 1997; Mittelbach and Enzelsberger, 1999; Kaieda et al., 2001).

As a consequence of the previous observations, an experimental study has been carried out in order to investigate the effect of a key parameter such as the mass ratio biocatalyst/oil, as well as the quality of oil, in a batch bioreactor. In this study, ethanol was used with the aim to realise a completely green bio-process. Ethanol was added step by step and the stoichiometric molar ratio oil/ethanol was reached in three steps. Since the main disadvantage associated with the use of biocatalysts is their high cost, the possibility of reuse them for multiple reaction cycles can be a solution. Nevertheless, it needs to analyse how enzymatic activity varies with time, because a lot of factors can lead to enzyme degradation, such as prolonged use, contact with ethanol and washing procedure. For this reason, stability of biocatalyst, reused for more cycles, was verified in order to set the bases of a proper design of bioreactors, by analysing the effects of process and operating conditions on system performances.

2. Experimental: materials and methods

2.1. Chemicals

Commercial vegetable oil based on sunflower and fractionated vegetable oils was used to perform the experimental tests, whose composition is reported in Table 1 in terms of esters of fatty acid. Two kinds of pre-treatments were performed, in order to obtain waste vegetable oils, simulating cooking and frying process: Waste 1 was the commercial oil fried at 190 °C for 30 min, Waste 2 was the vegetable oil baked at 250 °C for 2 h. In Fig.1 chromatograms (HPLC) of all kinds of oils are reported.

Ethanol (99.8% grade) from Fluka was used as substrate. HPLC grade acetone and acetonitrile were supplied from Fluka and used as mobile phase in HPLC analysis. Triolein, diolein, monolein and ethyloleate were supplied from SIGMA and used as standard components in HPLC analysis.

2.2. Biocatalyst

Epobond *Pseudomonas cepacia* kindly supplied from SPRIN Technologies (Trieste, Italy) was used as biocatalyst. This lipase from Pseudomanas cepacia was covalently immobilized on a support consisting of an epoxy–acrylic resin, with particle size of 200–500 μm. The activity of the biocatalyst was 161 U/g_{DRY} (unit: 1-phenylethyl acetate).

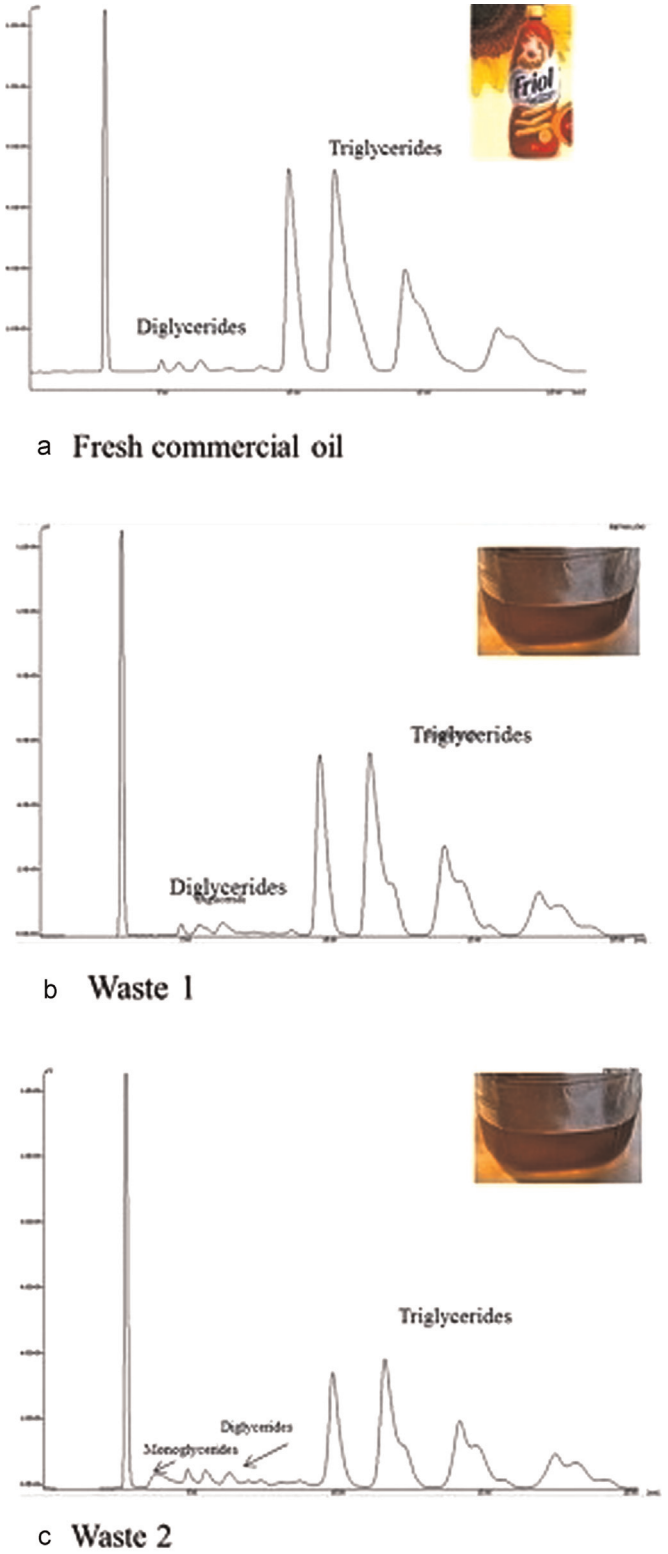

Fig. 1. Chromatograms of commercial and waste oils used during the experimental activity. (a) Fresh commercial oil, (b) Waste 1: commercial oil fried at 190 °C for 30 min, and (c) Waste 2: commercial oil baked at 250 °C for 2 h.

2.3. Experimental protocol

Tests were performed at 37 °C and neutral pH in a mixed batch