Green integrated continuous process for biodiesel production

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1. Introduction

The world is facing a major energy crisis. Given the increase in world population, the exponential development of underdeveloped areas and estimated current oil reserves, the price of crude is expected to rise above sustainable levels. Renewable energies represent a viable alternative to support our ever increasing energy demand. At the current rate of increase in population in developing countries, it is estimated that existing oil reserves will be exhausted in the next 40 to 100 years.[1] The use of biofuels, such as biodiesel, can help mitigate this problem.

Biodiesel is a mixture of alkyl esters of long-chain fatty acids derived from renewable feedstocks, such as vegetable oils or animal fats, after the latter have undergone a transesterification reaction with an alcohol (methanolysis) and made fit for use in compression-ignition diesel engines [2-3]. The conventional biodiesel production process via methanolysis of cooking oil with NaOH as catalyst raises environmental concerns, due to the large amount of waste produced [4-8].

Here, we describe an integrated process for the continuous production of biodiesel, via enzyme catalyzed transesterification of sunflower oil with methanol in supercritical carbon dioxide (Sc-CO_2).

Enzymes have proven to be able to overcome some of the problems of the conventional process for the production of biodiesel, namely, the formation of soap and the ability to convert to biodiesel both free fatty acids and triglycerides [9-11]. ScCO_2 solubilises both oil and methanol (scCO_2 can also be used to extract oil from several solid matrices), carrying them trough an enzymatic packed-bed reactor where the transesterification reaction takes place. By varying the pressure and temperature conditions of the outlet stream of the reactor, the reaction products can be fractionated and recovered. Conversions of >98 % were achieved with Lipozyme TL IM, at 313 K and 20 MPa, with a residence time < 1 min. Product separation was carried out at pressures between 10 and 15 MPa, and temperatures between 313 and 333 K, which allowed for a high enrichment of the gas phase in biodiesel.

2. Materials and Methods

2.1 Materials

The edible oil used in all experiments was virgin sunflower oil from FULA® . The acrylic resin immobilized Lipozyme TL IM® (*Thermomyces. lanuginosus* lipase) was acquired by Novozyms A/S, Bagsvaerd, Denmark. Methanol with a purity of 99.9% and Heptadecane with a purity of >98% were purchase from Sigma-aldrich. CO_2 was purchase to Air Liquide and had a purity of >99.98%.

2.2 Apparatus

A schematic diagram of the apparatus is shown in Figure 1. The apparatus for continuous biodiesel production consists of two main sections, namely a reaction section comprising a high pressure packed-bed enzymatic tubular stainless steel reactor and a separation section.

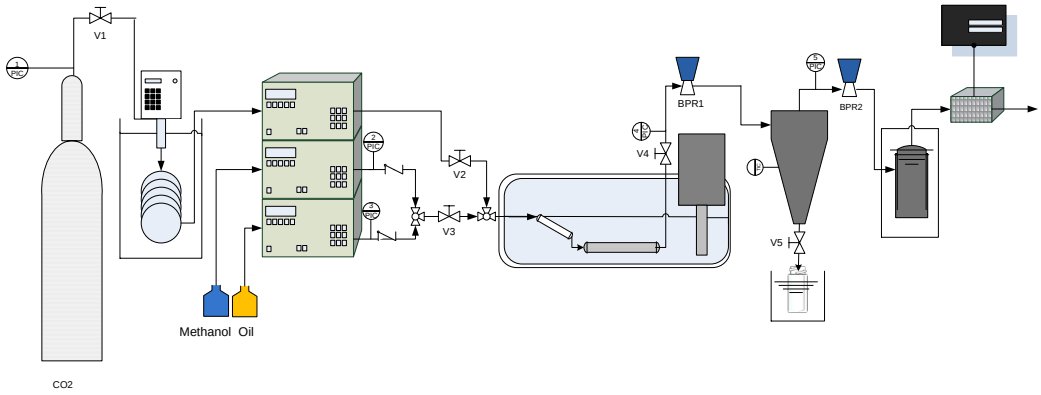

Figure 1: Experimental apparatus.

This apparatus is composed by a CO_2 vessel followed by a water/ ethylene glicol refrigeration system to liquefy the carbon dioxide and allow it to be pumped by an HPLC pump.

REACTION SECTION

The liquid mixture (oil and methanol) is pumped through a HPLC pump system. To ensure a homogeneous mixture, both liquid and gas are mixed in a static mixer before passing through the packed-bed enzymatic reactor already field with enzyme. The static mixer and the reactor are immersed in a water bath in which temperature is controlled by the controller (minimum measurement interval of 1ºC). The packed bed reactor consists of a tubular stainless steel vessel .The reaction pressure is controlled with a back pressure regulator value and measured by a digital pressure meter with an interval of 1 bar.

SEPARATION SECTION

The products/ CO_2 mixture obtained after the reactor which until now has always been in the one phase region, is decompressed to a specific pressure and temperature in the separator C01. The separation pressure is controlled with a back pressure regulator and is measured with an analytic pressure meter with a measurement interval of 1 bar.
The separation temperature is measured in-line with a digital pressure meter and a NI-CrNi thermocouple and controlled by the temperature controller with an interval of 1ºC.
After the first separator, the gas phase containing the biodiesel, passes through a second separator at room pressure and cooled in ice recovering the final product. The CO_2 flow is measured at the end by passing through a mass flow meter with a capacity between 0,002 – 0,04 kg/min.

2.3 Methods

FAME, triglycerides, di- and mono-glycerides, and by-products were measured by GC analysis performed with a Trace 2000 Series Unicam gas chromatograph with on-column injection, equipped with a 10 m x 0.32 mm i.d. column coated with a 0.10 μm thickness film of 5 %-phenyl and 95 %-dimethylpolysiloxane, from Zebron (ZB-5HT Inferno column). The method used was ASTM D6584, with the operating conditions set as follows: the carrier-gas was helium at 0.1 ml/min for 1 min, followed by a 1.5 ml/min ramp up to 1.1 ml/min, holding until the end of the program; the oven temperature program was 50-80 ºC at 15 ºC/min, followed by a 7 ºC/min ramp up to 230 ºC, and a 30 ºC/min ramp up to 380 ºC, with a holding time of 10 min. A flame ionization detector was used, set at 250ºC. Peak identification was carried out using known standards (FAME mixture, Sigma-Aldrich). Heptadecane was used as the internal standard. The data were processed with software *Excalibur*. No products were detected in assays carried out without enzyme. All the results presented are the average of at least two experiments.

3. Results

The transesterification reaction was studied using sunflower oil and methanol for the production of fatty acid methyl esters (**FAME**).
The parameters that influence both reaction and separation step were optimized. An optimization of temperature, residence time a oil: methanol molar ratio was carried for the continuous process. For the separation step the parameters optimized were temperature and pressure.

3.1. Enzymatic transesterification in supercritical CO_2

3.1.1. Temperature effect

The determination of the optimal temperature for the enzymatic reactions in SCFs it is very important since it influences enzyme activity. Enzyme activity must be optimized taken into account the activity decrease due to the thermal deactivation with increasing temperature and the physical properties of the solvent which may be favorable at higher temperatures due to lower mass transfer limitations and viscosity, surface tension and solving power.
The transesterification reaction was catalyzed by Lipozyme TL IM® at 20MPa, using a loading of $0.7 g_{Enzyme}/ml_{reactor}$, a CO_2 flow rate of 13ml/min, which represents a residence time of 20s, an oil to methanol molar ratio of 1:29 and temperatures between 30-50ºC (Table 1).

Table 1: Temperature effect in sunflower oil conversion into biodiesel with Lipozyme TL IM®.

TEMPERATURE (°C)	REACTION CONVERSION (%)
30	22
35	67
40	99
45	89
50	45

The reaction conversion increases up to 98.6 % as temperature increases to 40 ºC. The effect of thermal denaturation of the enzyme start to be noticeable at higher temperatures by a decrease in conversion.

3.1.2. Residence time effect

In a continuous process, solvent flow rate and residence time are very important parameters in the design of the reactor. Smaller residence times are more convenient for implementation at industrial scale, since they signify smaller reactor volumes and lower equipment costs.

The transesterification reaction was catalyzed by Lipozyme TL IM® at 20MPa and 313.15K, using a loading of $0.7 g_{Enzyme}/ml_{reactor}$, an oil to methanol molar ratio of 1:29. Different CO_2 flow rates were studied representing residence times from 14 to 28s (Table 2).

Table 2: Residence time effect in sunflower oil conversion into biodiesel with Lipozyme TL IM®.

RESIDENCE TIME (s)	REACTION CONVERSION (%)
28	96
20	99
17	81
15	56
14	46

As expected, reaction conversion increases as the residence time increases approaching then the equilibrium. The value obtained for reaction conversion for a residence time of 20s is already so high that no additional gains are anticipated by further increasing residence time.

3.1.3. Oil to methanol molar ratio effect

According to the transesterification reaction stequiometry, 1 oil mole reacts with 3 mole of MeOH. However, in order to achieve a high yield the molar ratio oil: methanol must be higher than 1:3. By increasing this ratio the reaction equilibrium will be shift to the direction of the product, nevertheless the excess of methanol may cause a decrease in the conversion due to enzyme denaturation.

The transesterification reaction was studied with Lipozyme TL IM® at 20MPa and 313K, using a loading of $0.7 g_{Enzyme}/ml_{reactor}$, a residence time of 28s and for the following molar ratios: 1:36; 1:29; 1:24; 1:18; 1:12 (Table 3).

Table 3: Molar ratio oil to methanol effect in sunflower oil conversion into biodiesel with Lipozyme TL IM®.

OIL:METHANOL MOLAR RATIO	REACTION CONVERSION (%)
1/36	53
1/29	96
1/24	97
1/18	84
1/12	78

The optimum molar ratio oil: methanol is 1:24 achieving a reaction yield of **97%.** Increasing the amount added of methanol can cause the denaturation of the enzyme resulting in a decrease of reaction yield. On the other hand, molar ratios lower than 1:24, the quantity of methanol added does not promote the direct reaction in order to achieve a good yield.

3.2. Product fractionation

Although, it was already shown that the enzymatic transesterification of sunflower oil under supercritical CO_2 is able to yield a high purity biodiesel (>98.5%), for an industrial application of this process, it is necessary to separate all the reaction products, namely, biodiesel, glycerol, the excess methanol as well as any unreacted mono-, di- and triglyceride in order to obtain biodiesel able to be sold in the European market.
FAME must be recovered from the reaction mixture through a post-reaction fractionation stage in which $ScCO_2$ solvation properties are manipulated through temperature and pressure variation which enable the selective precipitation of dissolved species. In case of lower reaction conversions, it is also possible to increase the purity of the biodiesel obtained by using the separation step. This may happen for example in cases where the enzyme is losing its activity and changing the enzyme is not economically viable.

A pressure range of 12-14 MPa and a temperature range of 40-60 ºC were tested in this work. The reaction conditions were previously selected achieving biodiesel yields of 55% in order to obtain a significant amount of FAME, triglycerides, mono and diglycerides to study the separation efficiency.

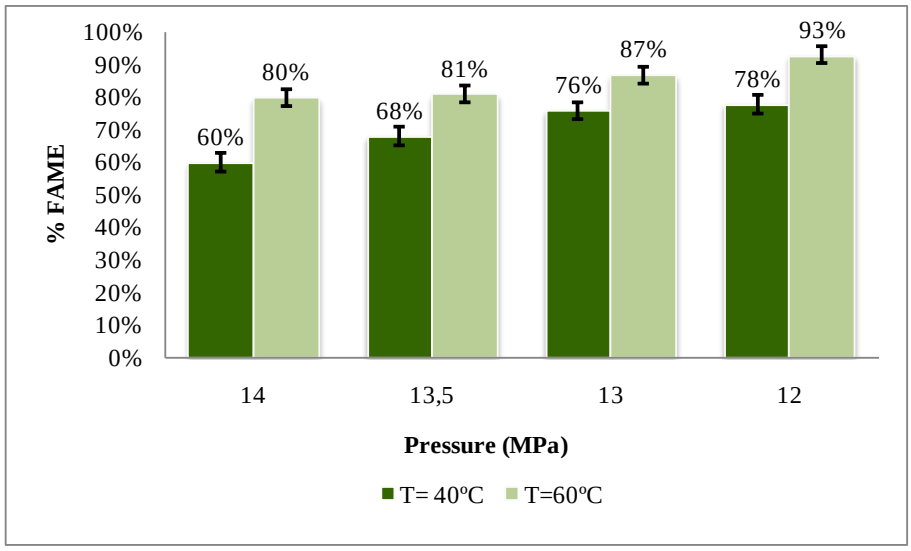

Figure 2: Effect of the temperature of operation of the first separator, kept at P = 12 MPa, on the recovery of FAME. The bars represent the mass fraction of FAME in the liquid phase recovered at the bottom of the second separator (Section 2.2).

At constant pressure, the density of $ScCO_2$ decreases as temperature increases. As it was expected, the enrichment of the gaseous phase increases with increasing temperature. Due to the decrease in the density of sc-CO_2 its solvent power decreases increasing its selectivity, in this case towards biodiesel. Therefore, as the temperature of the first separator increases at a constant pressure of 12 MPa, $ScCO_2$ becomes more selective for FAME enriching the gas phase formed in the first separator. As Figure 2 shows, at the lower pressure, when the temperature of the first separator is 40ºC, 78 % of the liquid obtained at the end of the process are FAME. If that temperature is 60 ºC, the purity of the FAME obtained at the end of the process is 93 %.

In Figure 3, we look at the effect of pressure on the separation and recovery of FAME. Several experiments were made keeping temperature constant at 60 ºC and varying pressure in the range 12-14 MPa. At constant temperature, with an increase of pressure, the density of $ScCO_2$ also increases resulting in a lower selectivity in the separation and a recovery of FAME with a lower degree of purity (93 % at 12 MPa vs. 79 % at 14 MPa).

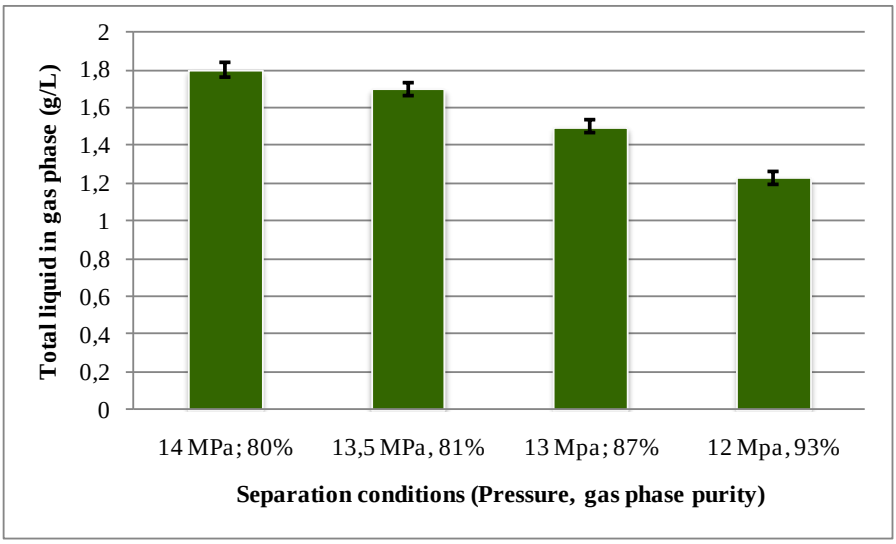

Figure 3: Effect of the pressure of operation of the first separator, kept at T = 60 ºC, on the recovery of FAME.

Another aspect to take into account is the loading of the gas phase. Less dense $ScCO_2$ is more selective towards FAME, but since it has lower solvation ability, the amount of FAME it can dissolve is lower too. Obviously, with a decrease in the solvent power of CO_2 the amount of liquid recovered in the gas phase will also decrease. Thus, when the first separator is kept at 12 MPa and 60 ºC, FAME are obtained with 93 % purity at 1,2 g/L, but when the first separator operates at 14MPa, more FAME are obtained per unit time (1,8 g/L) but with 80 % purity.

Taking advantage of the gathered separation data, judicious choice has to be made regarding separation conditions. In case of milder reaction conditions or lower enzyme activity, the separation step has to be more selective, meaning pressures near 12 MPa and temperatures in the range of 60ºC. On the other hand, in cases where only a small purification of the product stream is necessary the separator should be operated at pressures near the 14 MPa and temperatures of about 40ºC. In this case, the importance is given to the amount of product recovered in the gas stream of the separator.

4. Conclusions

We have looked at the production/recovery of biodiesel using a $ScCO_2$-based continuous, integrated process, comprising enzymatic synthesis via a methanolysis reaction followed by downstream separation. It was shown that conversions higher than 98 % can be achieved at 20 MPa, 40 ºC, a residence time of 20 s and an oil to methanol ratio of 1:24. It was concluded that for the separation of biodiesel from the other components a first separator should be operated at temperatures between 40ºC and 60 ºC and at pressures higher than 12 MPa.

All parameters studied must be taken into account when designing an industrial process. Reaction conditions, lifetime and deactivation rate of the enzyme, separation conditions, recycling rate of the liquid phase, recovering of glycerol and methanol in a second separator are critical points that must be studied in terms of energetic and economical costs to determined the viability of the process.

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