

INTER AND TRANSESTERIFICATION REACTIONS UNDER HIGH PRESSURE OF CO₂ USING IONIC LIQUIDS: PERSPECTIVE FOR BIODIESEL PRODUCTION

L. Schrive^{1*}, A. Margoto¹, K.H. Dang², A. Finiels², Mike Robitzer², C. Moreau², F. Quignard^{2*}

¹ CEA - DEN/DTCD/SPDE/LFSM - BP 111 - F 26702 PIERRELATTE Cedex

² ICG/Laboratoire Matériaux Avancés pour la Catalyse et la Santé ; UMR 5253 CNRS-ENSCM-UM2-UMI, Ecole Nationale Supérieure de Chimie, 8, Rue de l'Ecole Normale, F 34296 MONTPELLIER

luc.schrive@cea.fr; quignard@enscm.fr

ABSTRACT

Because of fossil resource scarceness, biodiesel production from spent or native biomass (vegetable oil) is a promising possibility. New routes to achieve trans or inter-esterification are under progress using different catalysts as enzymes or ionic liquids (ILs). Associating these routes with pressurised CO₂ is potentially beneficial because of viscosity reduction of the oil/CO₂ mixture leading to overcome mass transfer limitations. But as previous works showed that supported lipase (Novozym 435) is CO₂ sensitive, the objective of this work was to demonstrate that trans or inter-esterification can be improved using a complex mixture of (oil/CO₂/IL/enzyme). Reaction rates were evaluated with lipase alone, IL alone or a mixture of enzymes/ IL.

Results showed that :

- The more acidic IL leads to better reaction rates ;
 - Enzyme is not clearly stabilized by IL ;
 - Transesterification carried out with (IL + CO₂) was faster than with (enzyme+CO₂);
 - Transesterification carried out with (IL + CO₂) was around 6 times faster than with IL alone.
- New investigations must determine the importance of IL purity regarding the catalytic effect. IL behaviour must be studied in relation with CO₂ pressure and viscosity mixture dependence. Because of low IL vapour tension, the chemical equilibrium displacement by continuous ester extraction should be assessed in the future.

1 - INTRODUCTION

Vegetable oils or animal fats, mainly composed of triglycerides, reacted with an alcohol usually ethanol or methanol, produce glycerol and methyl or ethyl-esters. Compared to triglycerides, the lower molecular mass of the products (about 1/3 of original triglyceride) makes a viscosity decrease and volatility increase useful for engine working. Typically, the reaction between an ester (triglycerides are glycerol's esters) and an alcohol is called transesterification, see Figure 1 a. Another type of reaction is interesterification, characterized as a reaction between two different esters exchanging their alkyl chains (Figure 1 b).

In common vegetable oils, it is possible thus to enrich ω₃ esters for nutraceutic interest.

New routes to achieve transesterification or interesterification reactions are under progress using different catalysts as enzymes or ionic liquids, or unconventional solvents as supercritical fluids (SFC). Using SC CO₂ in place of usual organic solvents makes the process environmentally acceptable and helps to overcome diffusive limitations by viscosity lowering due to CO₂ solubilization [1].

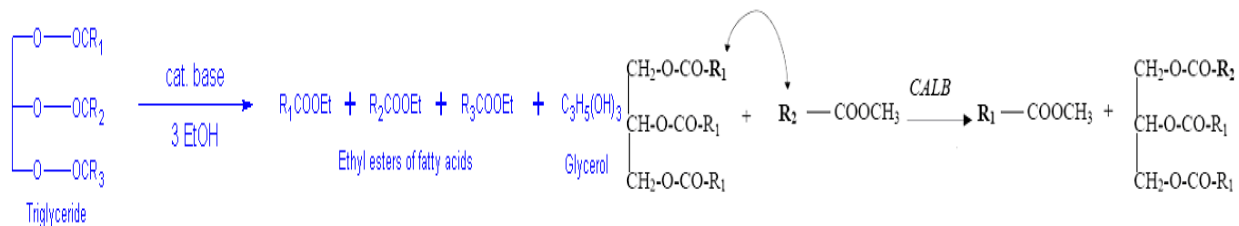


Figure 1 : a) Transesterification reaction

b) Interesterification reaction

However, lipase enzyme showed some limitations under CO₂ pressure [2]. Other works demonstrated a negative effect of mechanical pressure on enzymatic reaction potentially due to convective limitation in stirred reactor [3]. It has been also demonstrated that the enzyme can be negatively pressure dependent under CO₂ atmosphere due to the solvated state of closed molecules [4]. But other works showed that electrophilic characteristic of CO₂ can positively stabilized the complex lipase/substrate [5]. Or, depending on pressure range, a mitigated effect was also shown [6].

Ionic Liquids are described as ionic molten salts composed of an organic cation and a mineral anion. Common cations are pyridinium, dialkylimidazolium, ammonium and phosphonium, while anions can be PF₆⁻, BF₄⁻, or other strong acids conjugated base.

In non conventional media or extreme conditions, some authors showed a beneficial role of ionic liquids that apparently could stabilized the enzyme activity [7]. Last but not least, it could be interesting to continuously extract the light products by means of SC CO₂ in order to displace the kinetic equilibrium. Thus using immobilized enzyme or low partial pressure compound as IL could be of great interest. The objective of this work is to evaluate transesterification optimization using a complex mixture of (oil/CO₂/IL/enzyme).

2 - MATERIAL AND METHODS

2-1 Interesterification reaction and analysis

C12 ester (methyl laurate) was selected to react with a common sunflower cooking oil (mainly composed of C18 chains, particularly C18-2). Mole fraction of substrate and reactive species (respectively sun flower oil and methyl laurate) was 20/1.

The lipase B *Candida Antarctica* was used as a catalyst, either in supported form (Novo Nordisk “Novozym 435”, acrylic resin ball, average size : 0.5 mm, active layer thickness : 100 μm, pore diameter around 50 nm), or as free non porous cross-linked enzyme aggregates (CLEA, Fluka).

Three different ionic liquids were used (see Figure 2):

1-ethyl-3-methylimidazolium hexafluorophosphate (EMIm-PF₆, Fluka)

1-ethyl-3-methylimidazolium tetrafluoroborate (EMIm-BF₄, Fluka)

1-(4-sulfonic acid) butylpyridinium hydrogen sulfate (BpyrSO₃-HSO₄) was lab-synthesised using the reported recipe [8].

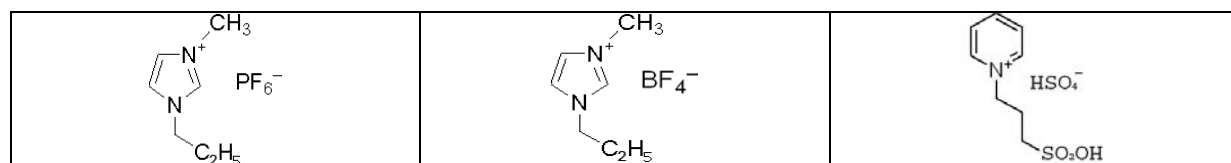


Figure 2 : IL liquid structure, respectively EMIm-PF₆, EMIm-BF₄ et BpyrSO₃-HSO₄

Results were based upon C12 ester disappearance. GPC was used (Agilent DB85 column, FID detector, internal calibration method with C11 ester) for comparing the added C11 to the C12 residual amount. The conversion rate X was defined as :
$$X = \left(1 - \frac{\text{AreaC11}/\text{AreaC12}_{\text{final}}}{\text{AreaC11}/\text{AreaC12}_{\text{initial}}}\right) \times 100$$

2.2 Transesterification reaction and analysis

Transesterification was carried out with methanol (using the C18-1 rich rapeseed oil at low pressure) or with ethanol (using C18-2 rich sunflower oil at high pressure). Reaction products (a mixture of C18-1, C18-2 and C18-3 esters) were analysed by HPL or GP chromatography. For HPLC analysis, an Agilent Technologies chromatograph was used, fit out with a RID detector and a C18 column Eclipse XDB. Analysis conditions were : eluent 60/40 v/v methanol/isopropanol, program flow of 0.4 ml/min from 0 to 15 min, 2 ml/min from 16 min to 35 min. Octadecane was employed as internal standard.

For GPC analysis, the same method than for interesterification reaction was used, based only upon the main C18-2 product.

2.3 Reactors

2.3.1 Low pressure reactors used with IL

Transesterification reactions with ILs were performed in thermostatic glass round bottom flasks at low temperature (340 K) and in a cylindrical glass reactor (Büchi, Figure 3) at higher temperatures (483 K). The molar ratios oil/methanol/ionic liquid were the following : 12/1/0.3 at low temperature and 12/1/0.057 at high temperature. Both reactor volumes were 100mL.

2.3.2 High pressure reactor

The set up was already described [3], consisting in a 40 mL pressurised sapphire vessel (Figure 4). Operating conditions are described bellow.



Figure 3 : low pressure reactor used for transesterification with IL

- (1) Sapphire vessel
- (2) Magnetic stirrer
- (3) CO₂ inlet
- (4) CO₂ outlet
- (5) Sampling system

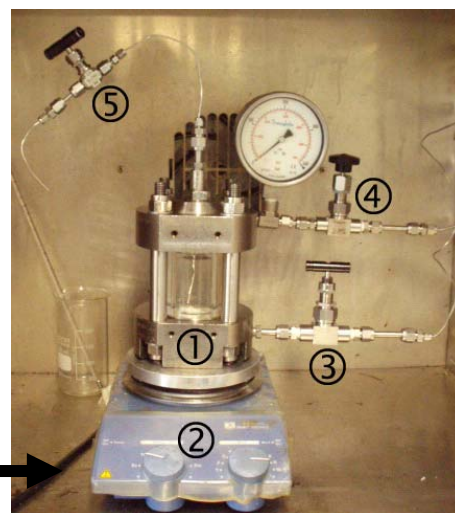


Figure 4 : High pressure sapphire reactor

High pressure Interesterification without IL

CO₂ atmosphere between 0-12 MPa ; temperature : 328 K ; reaction time = 1 h ; Enzyme mass : 0.5 % either Novozym or CLEA.

Triplicates were sampled at the end of each run after degassing. Each sample was weighted, then mixed with a few droplets of acetone (in order to inactivate enzymes which could be incidentally captured in the aliquot), and conserved in a freezer for a few days for analysis.

High pressure Interesterification with IL

P = 8 MPa of CO₂, T = 333 K, reaction time = 1 h, Novozym mass = 0.5 %, IL mass = 1 %.

High pressure Transesterification with IL

P = 8 MPa of CO₂ ; T = 333 K for IL or T = 318 K for enzyme trials; reaction time = 6 h ; Novozym mass = 0.5 % ; IL mass = 1 % ; alcohol/oil ratio (mol/mol) = 12/1.

3 - RESULTS

3.1 Interesterification reaction with enzyme

First, high pressure experiments were carried out to check the reliability of previous kinetic results [3]. Once more, it was demonstrated that conversion rates were accelerated during the first 10 minutes either under CO₂ pressure or helium pressure compared to atmospheric experiments (Figure 5). In a second time, the slopes of both CO₂ pressurised or atmospheric experiments were the same, while the slope of helium pressurised experiments was decreasing.

In a second time, experiments were carried out for 60 minutes at increasing pressure, both under CO₂ and He pressure with Novozym balls, or under CO₂ pressure with CLEA enzyme (Figure 6). The conversion gain with Novozym appears at low pressure increase (< 2 MPa) both under CO₂ or He atmosphere.

Thus the CO₂ induced viscosity reduction just compensates the negative mechanical effect upon Novozym. Secondly, comparing Novozym and CLEA behaviours under pressure (Figure) proves once again [3] that, in one hand, the pressure gain is mainly due to fact that the active sites accessible in the Novozym surrounding layer depth are more numerous, and in the other hand, as the number of active sites remains constant in the non porous CLEA material, conversion rates remain constant. Then, the CALB lipase is negatively CO₂ sensitive, and would be advantageously used under CO₂ atmosphere if stabilized by IL.

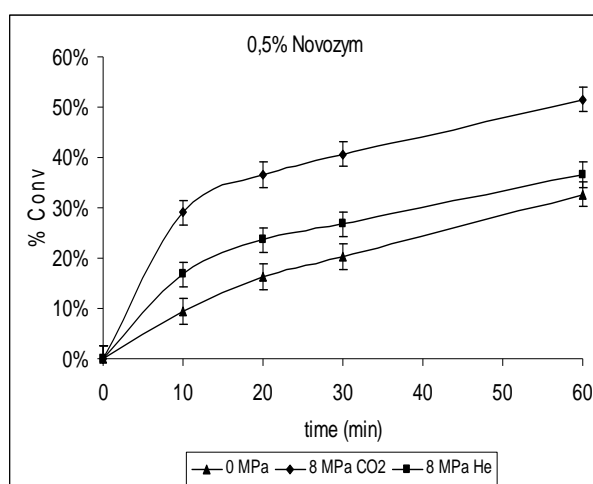


Figure 5 : Interesterification kinetics with 0.5% Novozym respectively under atmospheric pressure, 8 MPa He and 8 MPa CO₂

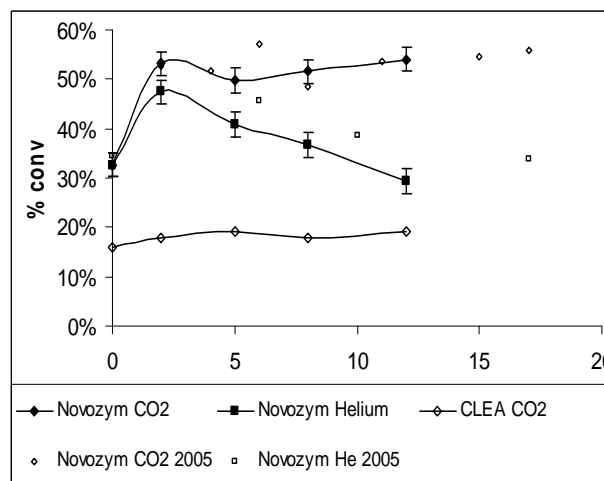


Figure 6 : Interesterification : Effects of He or CO₂ pressure experiments on CALB lipase either with 0.5% Novozym (55°C) or CLEA (40°C) ; constant time experiment : 60 min

3.2 Interesterification reaction with IL

Figure 7 shows results concerning CALB lipase alone, ionic liquids alone and a combination of both when changing the IL. No clear advantage can be found when combining IL and enzyme for interesterification reaction.

Among ILs, BpyrSO₃-HSO₄ is the more efficient when combined with enzyme. The possible reason could be the hydrophobic characteristics of the mixture, contrary of transesterification reaction where alcohol presents hydrophilic properties.

The enzymatic results are coherent with other bibliographic results [9, 10].

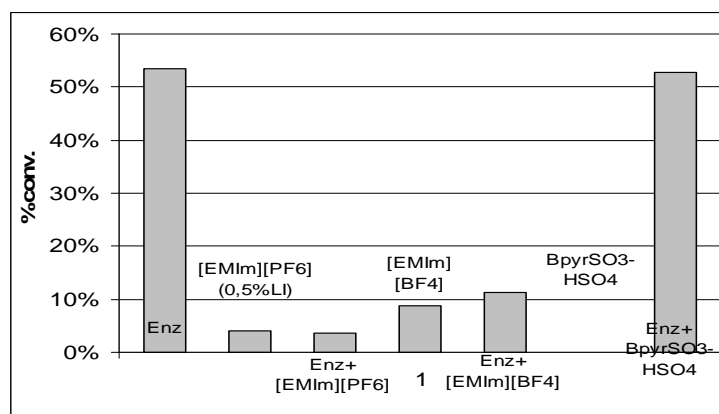


Figure 7 : Pressurized interesterification conversion rates combining Lipase CALB alone, different IL alone or combining them (8 MPa, CO₂, 333 K, 0.5 % Novozym, 1 or 0.5 % IL, 1h).

3.2 Transesterification

3.2.1 Low pressure ILs experiments

Experiments were carried out with methanol. Figure 8 illustrates the effect of increasing reaction time and temperature in the transesterification of rapeseed oil in the presence of BpyrSO₃-HSO₄ as catalyst; both effects lead to an increase in ester production.

3.2.2 High pressure experiments

These trials were carried out with ethanol (producing ethyl esters). The objective was to assess ethyl linoleate production which was (in this case) the only detected ester by GPC analysis.

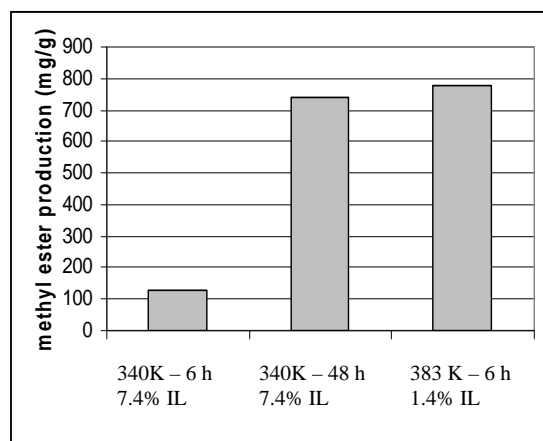


Figure 8 : Low pressure trans-esterification ; Total methyl ester production vs time, temperature and IL ratio

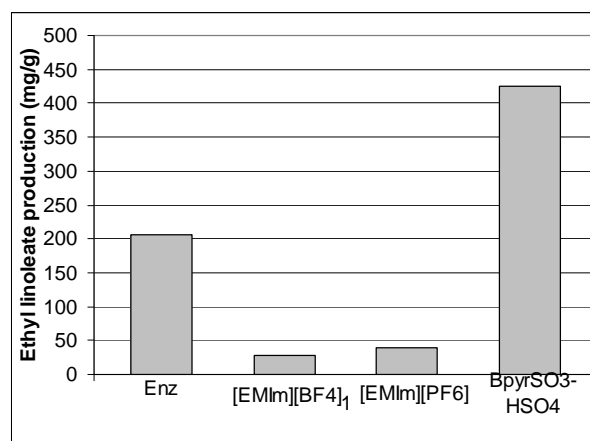


Figure 9 : High CO₂ pressure Transesterification : Linoleate-ester production combining CALB Lipase alone, different IL alone (8 MPa, 333 K, 0,5 % Novozym, 1 % IL, 6h)

It can be seen (Figure 9) that BpyrSO₃-HSO₄ IL is more efficient than other catalysts. Its strong acidic anion HSO₄⁻ is probably advantageously active compared to the other ILs used. Finally, a remarkable and encouraging feature resulting of combining BpyrSO₃-HSO₄ IL catalysts and SC CO₂ is the positive effect due to the presence of CO₂ as illustrated in figures 8 and 9. For the experiments conducted at around 333 K and 6 h of reaction time, raw data give an increase by a factor of 4 for the ester production. Refining these data by taking into account that Figures 8 and 9 give respectively the production for the whole methyl esters (C18-1, C18-2 and C18-3) or for the only C18-2 ethyl ester (that counts just for 48 to 70 % of the total esters), it is possible to assume a 6 times higher ester production for CO₂ pressurised experiments.

CONCLUSION

In order to improve conversion rates of inter or transesterification reactions in oils, CO₂ was used as a fluidizing agent in order to lower the diffusive limitations.

As CALB lipase seems negatively affected by CO₂ pressure, different ILs were tested to check enzyme stabilization. No clear advantages could be assessed when combining both.

However for interesterification as well as for transesterification reactions, the more acidic IL gives some interesting results.

Finally, high pressure reaction under CO₂ atmosphere is about 6 times faster than low autogenic pressurized experiments.

New investigations must determine the importance of IL purity regarding the catalytic effect. ILs behaviour must be studied regarding CO₂ pressure, temperature and viscosity dependence. Because of low IL vapour tension, the chemical equilibrium displacement by continuous ester extraction should be assessed in the future.

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