SUPERCRITICAL CARBON DIOXIDE ASSISTED REACTION AND CROSS-FLOW FILTRATION OF VEGETABLE OIL ON AN ENZYMATIC MEMBRANE

E. Pomier[1,2,3*], G.M. Rios[1,3], S. Sarrade[2,3], D. Paolucci-Jeanjean[1,3], M. Pina[4]

[1]- European Institute of Membranes – UMR CNRS 5635 – UMII, CC 047, 2 pl. Eugène Bataillon - 34095 Montpellier, France

[2]- Supercritical Fluids and Membranes Laboratory – CEA VALRHO, DEN/DTE/SLP, BP 111 - 26702 Pierrelatte, France

[3]- Joint research team «Supercritical Fluids and Porous Media » (CEA/CNRS/UMII/ENSCM)

[4]- International Cooperation Centre in Agronomic Search for the development– 73 av. JF Breton – 34398 Montpellier, France

* Corresponding author : tel : +(33) 5 75 50 43 99
fax : +(33) 5 75 50 43 42
e-mail address : emeline.pomier@cea.fr

Abstract : This study deals with the biotransformation of vegetable oils by reaction. The interesterification of castor oil is realized by coupling the fluidification/filtration process assisted by supercritical carbon dioxide (SC CO_2) and an enzymatic reaction. An enzyme-immobilized membrane reactor has been developed and the main objective of our study is to characterize its behaviour and its performances. The reaction was preliminary tested in a batch reactor and the feasibility was proved on a cross-flow filtration pilot plant with the oil/oleate system, in the presence of SC CO_2.

Keywords : vegetable oil, fluidification by supercritical carbon dioxide, interesterification, enzymatic membrane, cross-flow filtration

INTRODUCTION

Viscosity of highly viscous compounds is a limiting factor for their filtration on membrane in terms of fluxes. Several years ago, a few attempts were made to decrease viscosity by either using high temperatures or adding chemicals. A new process has been now developed to reduce liquid viscosity by injecting SC CO_2 within sticky fluids. The main advantages over the previous attempts are to work at room temperature (interesting for thermosensitive products) and not to use solvents which have to be separated afterwards.
Coupling fluidification by injection of pressurized CO_2 and filtration was performed for the polyethyleneglycol and mineral oils (Ph D thesis of D. Gourgouillon, 1996-1999 [1]). The application of this process for recycling used motor oils was also studied (Ph D thesis of C. Rodriguez, 1999-2002 [2]).

In the continuity of these conclusive studies, it was attractive to work on vegetable oils which represent very important stakes for food and health industry. We proposed to transform fluidified oils by reaction on an enzymatic membrane in order to obtain high-added value products. The enzymatic membranes are elaborated according to an original method developed since a few years [3], [4].

Because of the expansion of biotechnologies, it was interesting to choose an enzymatic reaction. In comparison with chemical reactions, they have the advantages to be less polluting (environmental interest), more specific and could be more easily used in bioprocessing and consequently for vegetable oils.

Recently, a few studies have been carried out on enzymatic reactions catalysed by lipases in supercritical fluids [5]. They proved that these fluids are good reaction media [6]. Indeed, they present interesting properties such as low viscosity and high diffusivity in comparison with usual organic solvents. Among supercritical fluids, SC CO_2 is ideal for heat-sensitive substances treatment and biocatalytic conversions because of its relatively mild critical temperature (31°C) and pressure (74 bar). In addition, it is non-toxic, nonflammable and inexpensive. It is environmentally harmless because it is easily separated from the products and recycled. SC CO_2 is frequently used for applications in the food industry and pharmacy.

Our study is based on these three previous results : The coupling of fluidification by SC CO_2 and filtration of viscous fluids [7] [8], the elaboration of enzymatic membranes and the enzymatic reaction in a supercritical fluid medium [9].
This paper presents the feasibility of the system on the membrane reactor : coupling a SC CO_2-assisted interesterification and a cross-flow filtration of vegetable oil on an enzymatic membrane.

I-MATERIALS AND METHODS

I-1 Enzymes and substrates

Enzymes

The enzyme we used is a lipase issued from *Candida antarctica* (B lipase), supplied by Novo Nordisk.
In the case of the batch reactor attempts, the reaction is catalysed by Novozym 435, an immobilized lipase on a macroporous acrylic resin.
The experiments on the pilot plant were carried out on an enzymatic membrane where Novozym 525L was immobilized.

Substrates

With regard to the vegetable oil, we had to make a compromise between its viscosity and its melting point. Indeed, the oil had to be liquid at room temperature (to be easily poured in the tank) and enough viscous too. We chose castor oil, obtained by Novance. It is a high viscous liquid at room temperature (640 mPa.s at T=25°C).
The co-reagent is an ester, the methyl oleate, supplied by Novance too.
SC CO_2 from Carboxique society, (purity of 99.9%) was used as a fluidifying agent.

I-2 Enzymatic membrane preparation

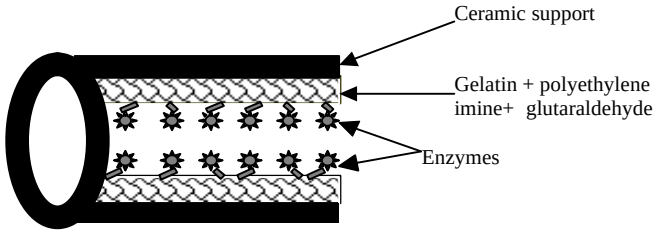


Figure 1 : An enzymatic membrane

The membrane material is a ceramic support (α-alumina covered with a zircon layer, 1.4µm of pore diameter). Enzymes are immobilized chemically onto this support : A mixed gelatin/polyethyleneimine solution is deposited by adsorption on the membrane. Then, glutaraldehyde, a cross-linking agent, is fixed by imine linkage on the polymers (gelatin + polyethyleneimine). Finally, enzymes are immobilized by imine linkage to glutaraldehyde. The more detailed elaboration procedure is described by Lozano [10].

I-3 The reaction

The *Candida antarctica* lipase B catalyses the interesterification between castor oil triglycerides (TG) and methyl oleate with SC CO_2 :

$$\text{Castor oil TG + Methyl oleate} \xrightarrow[SC\ CO_2]{Lipase} \text{Methyl ricinoleate + TG}$$

A specific analytic method has been set up : samples of permeate are taken and reaction products are separated by planar chromatography. When the reaction occurs, we can visualize the methyl oleate disappearance and the formation of methyl ricinoleate.

I-4 The process and the pilot plant

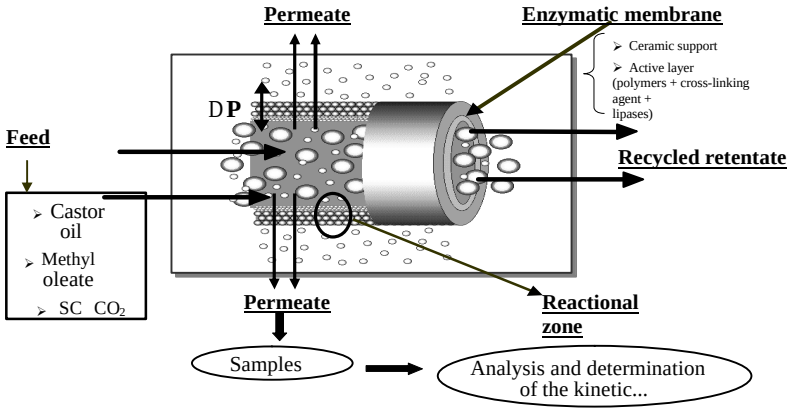


Figure 2 : The process principle

The membrane feed is composed by the reagents of the interesterification and SC CO_2, solubilized in the mixture. This mixture is conveyed through the enzymatic membrane. Due to a transmembrane pressure, a part of this feed crosses trough the membrane and constitutes the permeate, the retentate is recycled. The reactional zone is located on the internal surface of the membrane and in the pores. Some samples of permeate are taken in order to be analysed.

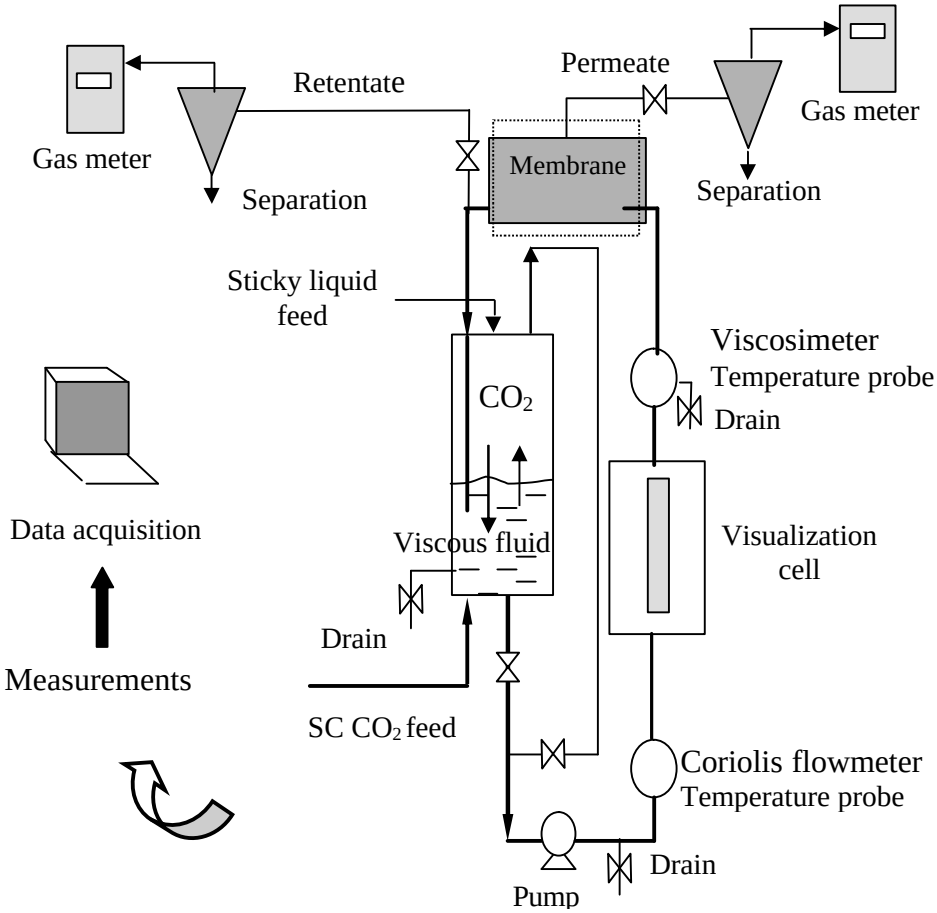


Figure 3 : Filtration experimental set- up

The filtration loop presented on figure 3 is composed by a tank, a circulation pump, a Coriolis flowmeter, a visualization cell, a vibrating rod viscosimeter and a membrane. When there is no transmembrane pressure, all the fluid returns to the tank by a diving tube. When a transmembrane pressure is applied, permeate goes through a cyclone separator. Different sensors allow to control temperature and pressure in the pilot.

Viscous fluid and SC CO_2 are introduced in the tank. Temperature and pressure are fixed. Solubilisation of CO_2 is monitored by the recording of viscosity and density. When viscosity and density are constant, the equilibrium is reached and then we are able to make a measurement : the objective is to apply a permanent transmembrane pressure and to recover different permeate samples in order to determine the reaction kinetic.

I-5 The operating conditions

The batch reactor experiments were carried out on 20mL reactional volume, with 4% w/w of enzymes and a molar ratio equal to 20/1 (oil/oleate). There were no carbon dioxide, no pressure and the temperature was equal to 55°C.

In the case of the attempts on the enzymatic membrane, the pressure was equal to 120 bar, the temperature to 55°C. We worked on a reagent mixture of 0.5L and the molar ratio was the same as the batch reactor attempts.

II-RESULTS AND DISCUSSION

II-1 Rheology of castor oil with pressurized CO_2

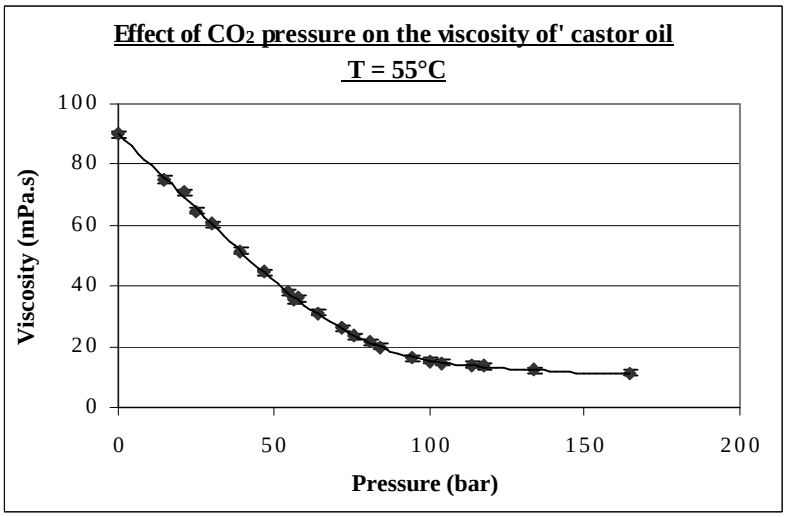


Figure 4 : Fluidification by pressurized CO_2

Figure 4 shows the viscosity reduction of the oil by injection of pressurized CO_2 (reduction of 90% from atmospheric pressure to P=170 bar ; T=55°C). These results were obtained on the experimental set up with the vibrating rod viscosimeter. The maximum of CO_2 solubility is nearly reached for P=100 bar. In supercritical conditions, the viscosity vary from 10 to 20 mPa.s instead of 100 mPa.s at room pressure. Due to this result, the reaction can perform in this range of viscosity. We chose P=120 bar.

II-2 The reaction

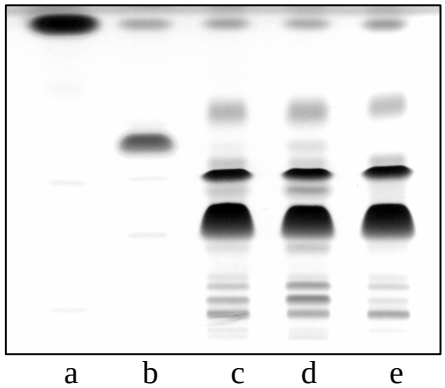
Preliminary experiments with castor oil, methyl oleate and lipases were realized in batch reactor at atmospheric pressure and without carbon dioxide (T = 55°C). They allowed to underscore the reaction feasibility. We determined the reaction kinetics and we obtained a bioconversion of about 90%.

Then, the reaction was performed on the pilot plant with the oil/oleate system in the presence of SC CO_2. A thin layer chromatography plate with different samples (retentate, permeate, initial reaction mixture) including reference solutions (methyl oleate and methyl ricinoleate) is presented on figure 5 :

Figure 5 : Chromatography plate

a : Reference solution of methyl oleate
b : Reference solution of methyl ricinoleate
c : Retentate (t = 2 days)
d : Permeate (t = 2 days, transmembrane pressure = 2.5 bar)
e : Initial reaction mixture (t=0)

c, d and e : equal quantity deposits



We can observe formation of products including methyl ricinoleate in the retentate and in the permeate. This plate shows the entire feasability of the reaction on the pilot plant. This prove that the reaction occurred and lipases are still active for the interesterification of castor oil in the applied conditions.
In addition, the plate shows that there is more methyl ricinoleate in the permeate than in the retentate. Therefore, the reaction happened mainly in the pores of the membrane where the contact enzyme/substrate is much more favourable than at the internal surface.
Thus, we prove the feasibility of our system : coupling a SC CO_2 assisted interesterification and a cross-flow filtration of vegetable oil on an enzymatic membrane.

CONCLUSION

These first experiments carried out on the supercritical installation with the enzymatic membrane are positive and validate the feasibility of the membrane reactor concept. The fluidification/filtration/reaction process is operating for the system chosen.
Work perspectives are to quantify the reaction by gas phase chromatography and to study the effects of the different operating conditions particularly transmembrane pressure which may be a very important parameter. These parameters will have to be optimised.

REFERENCES

[1] Gourgouillon D., Ph D thesis, University of Montpellier II, **1999**

[2] Rodriguez C., Ph D thesis, University of Montpellier II, **2002**

[3] Negrel J.L., Belleville M.P. and Rios G.M., Journal of Membrane Science, Vol. 134, **1997**, p.163

[4] Belleville M.P., Lozano P., Iborra J.L. and Rios G.M., Separation and Purification Technology, Vol. 25, **2001**, p.229

[5] Cernia E. and Palocci C., Methods in Enzymology, Vol. 286, **1997**, p.495

[6] Hartmann T., Schwabe E., Scheper T. and Combes D., Stereoselective Biocatalysis, **2000**, p.799

[7] Gourgouillon D., Schrive L., Sarrade S. and Rios G.M., Sep. Sci. Technol., Vol. 35, **2000**, p.2045

[8] Gourgouillon D., Schrive L., Sarrade S. and Rios G.M., Environmental Science and Technology, Vol.34, **2000**, p.3469

[9] Ballesteros and al., Biocatalysis and Biotransformation, Vol. 13, **1995**, p.1

[10] Lozano P., Pérez-Marin A.B., De Diego T., Gomez D., Paolucci-Jeanjean D., Belleville M.P., Rios G.M. and Iborra J.L., Journal of Membrane Science, Vol. 201, **2002**, p.55