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Membrane reactors for biodiesel production

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DOI: 10.1533/9780857098658.2.122

Abstract: Over the last few decades, research into an alternative fuel for diesel engines has acquired great importance due to both the reduction of oil feedstock and the environmental pollution caused by the emissions of greenhouse gases. Biodiesel, a mixture of mono-alkyl esters obtained from vegetable oils, is a good candidate. This chapter presents a short overview of the exploitation of membrane bioreactors for biodiesel production, analyzing the enzymatic transesterification of glycerides in more detail. It will also look at problems that need to be overcome to exploit this process on an industrial scale, such as enzyme recycling, enzyme stability and optimal operating conditions.

Key words: biofuels, biomass, bioenergy, bioreactors, modeling.

5.1 Introduction

Before the advent of the economy based on fossil fuels, biomass was the main source of energy. The energy efficiency of biomass conversion was, however, very low. Fossil fuel energy therefore represented a more economical alternative for the developing society of the nineteenth century. However, energy demands have now reached a stage where fossil fuel energy is unable to meet the required level for sustainable growth of the world economy. The recognition that global crude oil reserves are finite and that crude oil depletion is occurring much faster than previously predicted has determined a new interest in biomass, which is considered as one of the few current sustainable resources available for the production of renewable energy (Klass, 1998). Moreover, the environmental deterioration resulting from overconsumption of petroleum-derived products is a serious menace to the sustainability of human society. As an example, the European Union has set a target of 10% for domestic production of biofuels. However, the actual feedstock supply is insufficient to meet the current demand, and the land requirement for biofuel production in order to meet this target would be higher than the amount of arable land potentially available for bioenergy crops. Extensive plantation, pressure for change in land use and an increase in cultivated fields could lead to

competition for land and loss of biodiversity, due to the felling of existing forests and the utilization of areas of ecological importance (Renewable Fuel Agency, 2008).

Second generation biofuels offer a possible solution to the current problems since they contribute to a reduction in land requirements, both because of their presumed higher energy yields per hectare and due to the fact that they do not require the use of agricultural land. Processes with high biomass-to-energy conversion efficiency are therefore required. In this context, the use of (bio)engineering is crucial to make the transition from a fossil fuel economy to a biomass-based economy a reality. However, there are currently no obvious routes to achieve this.

The following section will provide a short overview of some of the current results and issues in the development of biodiesel production, including the use of process engineering. The chapter will then move on to look at biocatalyst immobilization, discussing supports for lipase immobilization in more detail. The final section of the chapter will examine in detail membrane bioreactors for biodiesel production, covering different types of system including continuous separation, hollow fiber membranes and continuous stirred tank reactors.

5.1.1 Biodiesel production

Biodiesel has gained greater and greater importance as a viable substitute for fossil fuels, which are currently expected to run out within a century. Combustion of fossil fuels has created environmental issues related to the emission of exhaust gases, mainly CO_2. Growing awareness of these issues has encouraged the utilization of biodiesel, which can be considered a carbon-neutral fuel since the carbon present in the exhaust was originally fixed from the atmosphere. Biodiesel is a mixture of mono-alkyl esters that can be obtained either from vegetable oils or from other sources such as animal fat, waste cooking oil, greases and algae (Peterson, 1986; Ranganathan *et al.*, 2008). The utilization of vegetable oils as biodiesel is achieved by blending the oils with traditional diesel in a suitable ratio, although these ester blends are only stable for a short period. However, direct use of blended oils obtained in this way is not sustainable in the long term in commercial diesel engines due to high viscosity, acid contamination, carbon deposition, free fatty acid formation and polymerization (Ma and Hanna, 1999). This is especially the case for the most recent generation of diesel engines. Vegetable oils require further processing to attain properties similar to those of conventional diesel fuel so that they can be directly used in the available diesel engines. However, in order to become a viable alternative fuel and to survive in the market, biodiesel

must compete economically with diesel. Additionally, biodiesel needs to have lower environmental impacts than existing fuels, while ensuring the same level of performance (Mata *et al.*, 2010). The end cost of biodiesel largely depends on the price of the feedstock, which accounts for 60–75% of the total cost (Canakci and Sanli, 2008). In order to avoid competition with edible vegetable oils, low-cost and profitable biodiesel should be produced from low-cost feedstocks such as non-edible oils, used frying oils, animal fats, soap-stocks and greases.

Three different techniques are exploited to convert the vegetable oils into fuel form: pyrolysis, micro-emulsification and transesterification (Ranganathan *et al.*, 2008). The latter is definitely the most widespread process on an industrial scale and represents the alcoholysis of triglyceric esters resulting in a mixture of mono-alkyl esters and glycerol.

The general transesterification reaction scheme can be summarized as follows:

$$\text{Triglyceride} + \text{Alcohol} \leftrightarrow \text{Diglyceride} + \text{Ester}$$

$$\text{Diglyceride} + \text{Alcohol} \leftrightarrow \text{Monoglyceride} + \text{Ester}$$

$$\text{Monoglyceride} + \text{Alcohol} \leftrightarrow \text{Glycerol} + \text{Ester}$$

This reaction scheme can be also represented as follows (Freedman *et al.*, 1984):

$$\text{Trigyceride}(\text{TG}) + \text{R}'\text{OH} \underset{k_4}{\overset{k_1}{\rightleftharpoons}} \text{Diglyceride}(\text{DG}) + \text{R}'\text{COOR}_1$$

$$\text{Diglyceride}(\text{DG}) + \text{R}'\text{OH} \underset{k_5}{\overset{k_2}{\rightleftharpoons}} \text{Monoglyceride}(\text{MG}) + \text{R}'\text{COOR}_2$$

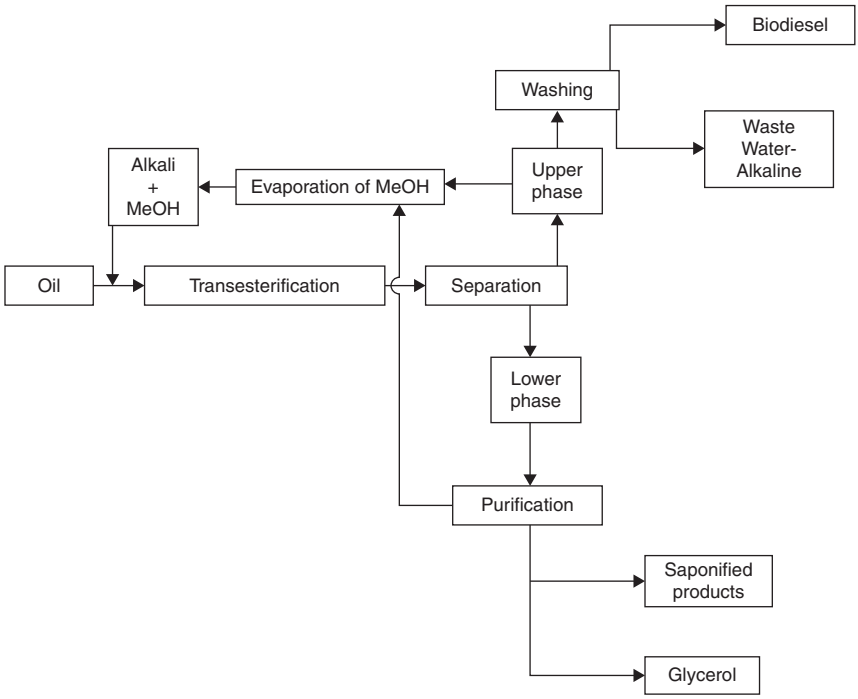
$$\text{Monoglyceride}(\text{MG}) + \text{R}'\text{OH} \underset{k_6}{\overset{k_3}{\rightleftharpoons}} \text{Glycerol}(\text{GL}) + \text{R}'\text{COOR}_3$$

The high-viscosity compound glycerol is separated and removed so as to achieve a low-viscosity final product similar to conventional diesel fuel; the mixture of these mono-alkyl esters does indeed represent a good substitute for fossil fuels. The transesterification process can be performed in different ways, namely by an alkaline catalyst, by an acid catalyst or by a biocatalyst that could be immobilized in a proper support such as a membrane. In the alkaline process either sodium hydroxide or potassium hydroxide

is employed as a catalyst together with methanol, ethanol, isopropanol or butanol, although methanol is the most common due to its low cost and availability. During the process, alcoxy is formed by the reaction between the catalyst and the alcohol, then the alcoxy is reacted with any vegetable oil to form biodiesel and glycerol. Glycerol and biodiesel are eventually separated in a decanter by exploiting their different densities (Barnwal and Sharma, 2005). This process is actually very efficient and is characterized by a high reaction rate even if the operating temperature is rather high, for example, 333 K (Fukuda *et al.*, 2001).

A reasonable alternative to the alkaline process is represented by the utilization of an acid catalyst instead of a base. Any mineral acid can be used to catalyze the process, but the most common acids are actually sulfuric acid or sulfonic acid. The biodiesel yield from this process is high, however the acids may cause damage to the equipment (Freedman *et al.*, 1984).

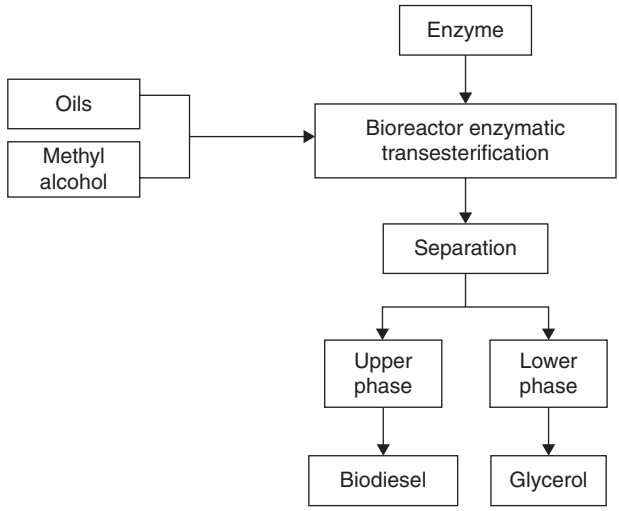
Compared to both alkaline and acid catalysts, the utilization of enzymes such as lipase, obtained from micro-organisms like *Mucormiehei*, *Rhizopusoryzae*, *Candida antarctica* and *Pseudomonas cepacia*, offers several advantages that may allow for the design of more rational transesterification processes (Watanabe *et al.*, 2000; Noureddini *et al.*, 2001; Hama *et al.*, 2007; Ricca *et al.*, 2009). Enzymes can be immobilized, thus allowing the re-utilization of the biocatalyst without any additional separation; immobilized biocatalysts also apply to the transesterification of waste oils (De Paola *et al.*, 2009). Moreover, the operating temperature of the process is lower (up to 323 K) compared to other techniques; finally, it is not necessary to make use of any strong alkaline or acid compound that will eventually need to be disposed of. However, enzymatic processes also have some disadvantages in terms of the inhibitory effects observed when alcohol is added to the mixture (Calabrò *et al.*, 2010), the decay of enzyme activity and the high cost of enzymes. Research has mostly focused on ways to overcome these problems and on modeling, designing and controlling efficient membrane bioreactors with immobilized enzymes in which to perform biodiesel production on an industrial scale. At present, of all of the above-mentioned processes, only the alkaline route is performed on an industrial scale due to its cost effectiveness and higher efficiency. However, downstream processing is complicated when using this method, since the separation of both catalyst and unreacted methanol from biodiesel is actually quite difficult. The removal of the catalyst involves many problems as the biodiesel has to be repeatedly washed so as to achieve the required purity. Figures 5.1 and 5.2 compare alkaline and enzymatic processes in terms of downstream operations. The production of biodiesel using a biocatalytic process reduces most of the disadvantages characterizing the alkaline process and allows the user to obtain a very high purity final product with fewer or no downstream operations (Ranganathan *et al.*, 2008).



5.1 Production of biodiesel by the alkaline process. (*Source*: Readapted from Ranganathan *et al.*, 2008.)

5.1.2 Process engineering as a viable tool for biodiesel production

The large number of existing and semi-developed technologies for the production of biofuels makes it necessary to employ process engineering tools to deal with some of the intrinsic problems encountered when developing a new technology. Process engineering can be used to design innovative processes that can help to reduce production costs and improve sustainability. However, this requires the process engineer to determine a suitable process configuration for converting the raw materials into the desired biofuel(s) within the given specifications. This task requires the generation and assessment of several alternative process flow sheets, in order to determine the configuration with the best performance indicators. In this way, the impact of specific technologies over the global process and the production costs can be elucidated. This is called process synthesis. During the next step, process analysis, the structure of the synthesized flowsheets is established in order to improve the process by providing a more detailed insight. In both the above-mentioned steps, process modeling and simulation play a significant



5.2 Production of biodiesel by the enzymatic process. (*Source*: Readapted from Ranganathan *et al.*, 2008.)

role in the successful design of alternative configurations for biofuels production. This is particularly true in the case of continuous processes where a proper dynamic analysis is crucial for an appropriate design.

Process integration is also essential to the design of innovative and cost-effective processes. In the case of biofuel production, process integration aims to integrate all of the single unit operations involved in the process, by developing integrated bioprocesses that combine several steps in one. Thus, reaction–separation integration by removing, for instance, the obtained product(s) from the zone where the biotransformation takes place, offers several opportunities for increasing process yield and consequently reducing the product costs. Process integration is attracting increasing interest due to its potential to reduce energy costs, decrease both the size and number of process units and intensify the biological and the downstream processes. Process optimization is another essential tool used in process design. In the case of second generation biofuels, it is believed that some current technologies have actually reached their inherent limits. The development of novel and less expensive alternatives could therefore allow for new parameters in process optimization.

A detailed analysis of all the above-mentioned techniques is far beyond the scope of the present contribution. The following section will provide a short overview of the most important engineering aspects, with reference to the characterization and improvement of the performance of membrane bioreactors.

5.2 Biocatalyst immobilization

Enzyme immobilization was originally conceived as a stabilization technique. Subsequently, other characteristics of immobilized enzymes made this technique more promising, including the possibility of separating the biocatalyst from the reaction products and reusing it and the possibility of running continuous and easily controlled processes (Katchalski-Katzir, 1993). In lipase-catalyzed reactions, immobilization can help to provide the non-aqueous conditions necessary for ester synthesis and inter-esterification (Christensen *et al*., 2003).

The methods used for enzyme immobilization fall into four main categories: physical adsorption onto an inert carrier, inclusion in the lattices of a polymerized gel, cross-linking of the protein with a bifunctional reagent and covalent binding to a reactive insoluble support, as shown in Table 5.1.

Physical adsorption of enzymes on solid surfaces includes different steps. Salis and collaborators (Salis *et al*., 2003) reported them with respect to lipase immobilization: (1) lipase molecules in solution are transferred to the solid surface by diffusion; (2) lipase is adsorbed onto the solid surface and (3) lipase undergoes structural rearrangements. Worsfold (1995) reported that:

There are three important aspects of the immobilization procedure that must be specified in detail, independently on the exploited immobilization technique:

1. The properties of the free enzyme
2. The type of support used
3. The methods of support activation and enzyme attachment…

When specifying the properties of the original enzyme, its working name as well as its systematic name and associated code number must be stated. In addition, the source of the enzyme, the physical form of the enzyme (e.g., lyophilized), its purity (and method of purification), its catalytic activity and details of other constituents must be also given.

Table 5.1 Comparison of immobilization methods

Method	Advantages	Disadvantages
Adsorption	Cheap, easy, no enzyme disruption	Desorption, a specific adsorption
Occlusion	No enzyme disruption	Not suitable for enzyme acting on macromolecular substances
Cross-linking	Low desorption	Expensive, low activity
Covalent binding	Low desorption	Limited reagents

The above information permits direct comparison of enzymes of different sources…

The support material can have a critical effect on the stability of the enzyme and on the efficiency of enzyme immobilization, although it is difficult to predict in advance which support will be most suitable for a particular enzyme; the type of support can, however, be conveniently classified into one of three categories:

1. Hydrophilic biopolymers based on natural polysaccharides such as agarose, dextran and cellulose;
2. Lipophilic synthetic organic polymers (membranes) such as polyacrylamide, polystyrene and nylon;
3. Inorganic materials such as controlled pore glass and iron oxide…

The most important requirements for a support material are that it must be insoluble in water, have a high capacity to bind enzyme, be chemically inert with respect to the substrate(s) and the product(s) of the reaction and be mechanically stable.

The enzyme binding capacity is determined by the available surface area, both internal (pore size) and external (bead size or tube diameter, depending on the support configuration), the ease with which the support can be activated and the resultant density of enzyme binding sites.

The inertness refers to the degree of non specific adsorption and pH, pressure and temperature stability. In addition, the surface charge and hydrophilicity must be considered…

An activated support is defined herein as a material having an enzyme reactive functional group covalently attached to an otherwise inert surface. The stability of the resulting bond between the enzyme and the support, the local environment of the enzyme and the potential loss of activity due to immobilization must all be considered (Worsfold, 1995).

The apparent activity of the immobilized enzyme will also depend upon the bulk mass transfer and local diffusion properties of the system. Limitations to the rate of the enzymatic reaction include diffusion of the substrate from the bulk solution to the micro-environment of an immobilized enzyme. The thickness of the diffusion film depends upon rate at which the substrate passes over the insoluble particle. This in turn determines the substrate concentration in the vicinity of the enzyme and so affects the rate of reaction.

The molecular weight of the substrate can also have a significant effect on the rate of reaction. Steric hindrance in the matrix limits the diffusion

of large molecules, which can be observed in the enzyme activity. The relative activity of bound enzymes towards high molecular weight substances has generally been found to be lower than bound enzyme activity towards low molecular weight substrates. However, in some cases this may have the advantage of protecting the immobilized enzymes from attack by large inhibitor molecules.

5.2.1 Supports for immobilizing lipases for transesterification and esterification reactions

As already discussed, the catalysts used for the transesterification and esterification of triglycerides are classified as alkali, acid and enzymatic. Although chemical transesterification (and esterification) using a chemical (alkaline oracid) catalyst produces a high conversion rate to esters in a short reaction time, the reaction has several drawbacks. Not only is it energy intensive, but the recovery of glycerol is difficult: the acidic or alkaline catalyst has to be removed, alkaline and acidic waste water requires treatment and free fatty acids and water interfere with the reaction (Meher *et al*., 2006).

Enzymatic catalysts such as lipases are able to effectively catalyze the transesterification of glycerides, overcoming the problems mentioned above. In particular, glycerol as a by-product can be easily removed and free fatty acids contained in waste oils and fats can be completely converted into alkyl esters. On the other hand, the production costs of a lipase catalyst are, in general, significantly greater than that of an alkaline one. This drawback can be overcome, however, by immobilizing the enzyme and reusing it in many reaction cycles.

Lipase is generally used in its immobilized form and supports can be classified as either organic or inorganic, as shown in Tables 5.2 and 5.3.

A brief discussion of membrane bioreactors designed to perform the transesterification of glycerides will now be presented.

Table 5.2 Classification of organic supports for lipase immobilization

	Micro-organism	Support
Vinylic polymers	*Candida rugosa*	Accurel® EP100
	Mucor javanicus	Accurel® MP1004
	Various micro-organisms	Membrane reactors
Ionic exchange resins	*(Rhizo)Mucor miehei*	Duolite® A568: ionic exchange resin
Acrylic resins	*Candida antarctica*	Amberlyst®

Table 5.3 Classification of inorganic supports for lipase immobilization

	Micro-organism	Support
Silicates	*Thermomyces lanuginosa*	Silica gel
	Pseudomonas fluorescens, Pseudomonas cepacia, M. javanicus, Candida rugosa, Rubus niveus	Kaolinite
	P. cepacia	Ceramic particles
	M. javanicus	SBA-15®
Diatomaceous earth	*P. cepacia*	Diatomaceous earth

5.3 Membrane bioreactors

Different configurations of membrane bioreactors can be operated to perform the enzymatic transesterification of triglycerides. For the sake of brevity in the present chapter, attention will be focused on the limited number of bioreactors that have a real significance due to their possible exploitation on a pilot/industrial scale:

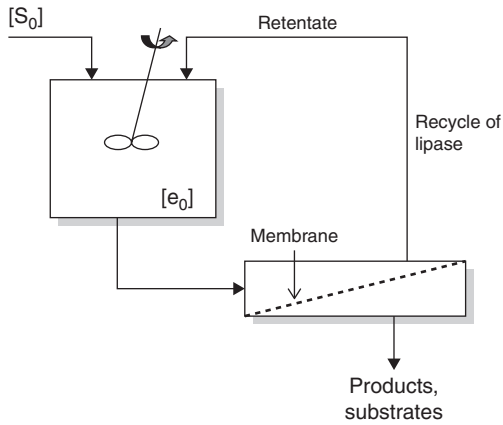
1. a system in which the biocatalyst is continuously separated by a membrane system and then recycled back to the reaction tank;
2. a continuous hollow fiber bioreactor in which the biocatalyst is immobilized/entrapped within the porous structure of an ultrafiltration (UF) membrane;
3. a well-mixed bioreactor in which the biocatalyst is immobilized on the surface of a membrane by means of a covalent binding.

In particular, it will be shown, especially in case (3), that a proper combination of experimental analysis and kinetic modeling is helpful in characterizing the actual behavior of a membrane bioreactor, thus determining how to operate the system, improve its performance and maximize its productivity.

5.3.1 Biocatalyst continuously separated by a membrane system and recirculated into the reaction tank

This type of bioreactor couples a continuously stirred tank reactor in which a known amount of enzyme has been already loaded to a cross-flow filtration unit, as shown in Fig. 5.3.

Lipase is retained by the membrane and continuously recirculated to the reaction tank where the optimal reaction occurs. Depending on the



5.3 Schematic of membrane bioreactor with continuous biocatalyst recirculation.

membrane properties and substrate characteristics, either partial or total substrate rejection can be achieved.

It is supposed that the reaction products are contained only in the permeate (this can be achieved by a proper choice of membrane characteristics), which is continuously removed from the system; this configuration, therefore, is particularly useful in those cases in which the biocatalytic reaction is product-inhibited. Moreover, compared to a classical enzyme membrane reactor composed of a stirred tank equipped with a UF membrane, this bioreactor exhibits a much lower occurrence of concentration polarization phenomena, which significantly limit the process efficiency. Finally, this type of bioreactor is characterized by a rather large surface-to-volume ratio and by a high level of compactness, making the system more suitable for large-scale operations, as it is required in most industrial applications.

A proper theoretical analysis of this reactor may provide useful indications about bioreactor performance. One of the most important engineering parameters that can be introduced is bioreactor productivity, Θ, which at a generic time, t, is defined as the ratio between the total amount of product formed at time t and the total amount of enzyme fed to the bioreactor. It has been shown (Curcio, 2011) that productivity can be expressed as a function of the degree of conversion, ψ, and that it is a linear function of dimensionless time t/τ_R, that is, the ratio between the process time t and the residence time in bioreactor, τ_R. Productivity also depends on the initial values of both substrate concentration, $[S_0]$, and enzyme concentration, $[e_0]$:

$$\Theta = \frac{\psi \cdot [S_0]}{[e_0]} \cdot \frac{t}{\tau_R} \Theta = \frac{\psi \cdot [S_0]}{[e_0]} \cdot \frac{t}{\tau_R} \qquad [5.1]$$

With reference to the biocatalytic reactions involved in biodiesel production, Equation [5.1] is very useful in determining bioreactor behavior, since it can be used to determine the set of operating conditions that must be exploited to maximize the conversion of glycerides.

5.3.2 Hollow fiber membrane bioreactor in which unreacted substrate is recycled

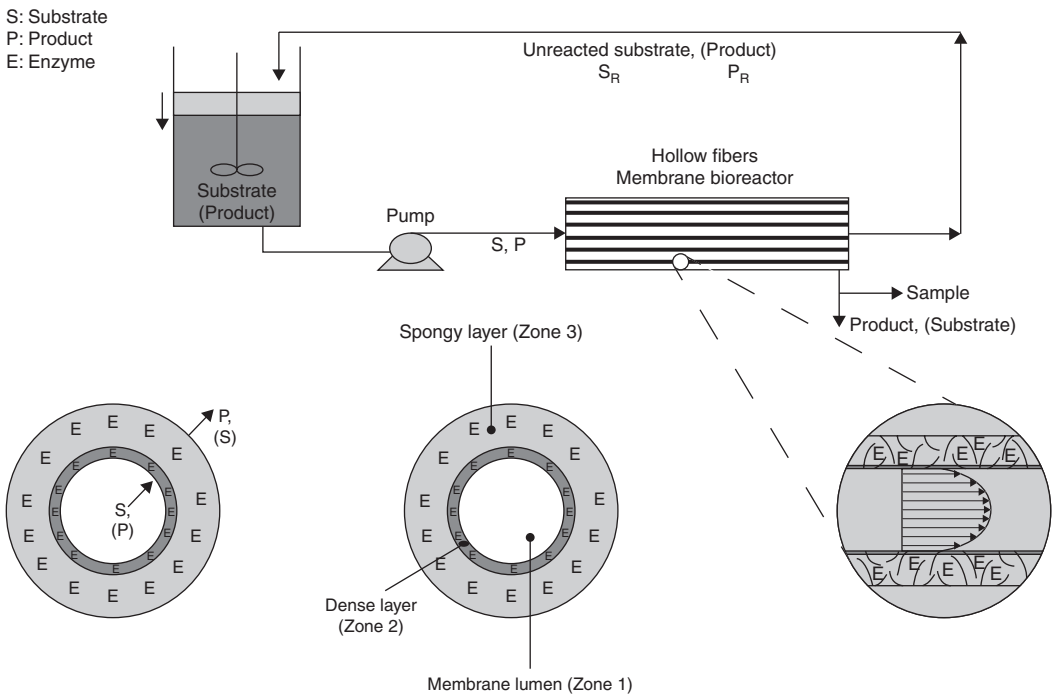
This type of membrane bioreactor consists of a bundle of UF hollow fibers assembled in a cylindrical cartridge in a tube and shell configuration (Fig. 5.4).

The fibers are asymmetric membranes in which lipase has previously been immobilized or entrapped; the membrane is composed of a thin skin supported on a porous matrix, which provides the fibers with their structural integrity. The UF process forces the substrate to permeate through the membrane wall, where it comes into contact and reacts with the immobilized enzyme. As the biocatalytic reaction occurs, products and unconverted substrate are continuously removed in a radial direction from the permeate stream, while the retentate stream, still containing substrate and a certain amount of product, is instead recycled back to the feed tank. Within the hollow fibers, three regions can be distinguished: the membrane lumen (region 1), where the substrate continuously flows, mainly in the axial direction and partially in the radial direction; the membrane dense layer or 'skin' (region 2) and the membrane spongy layer (region 3). It is supposed that the enzyme molecules or the whole cells containing the lipase are actually confined only in regions 2 and 3.

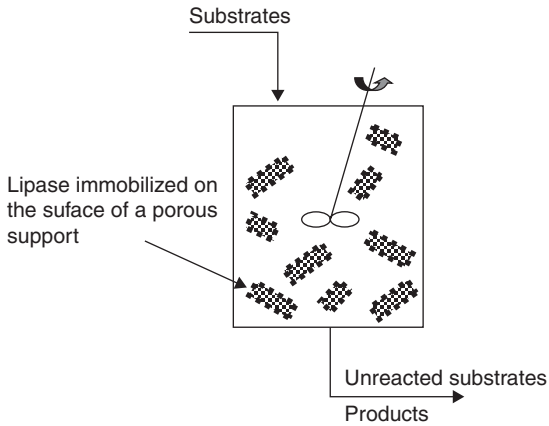
5.3.3 Continuously stirred tank reactor with biocatalyst immobilized on the membrane surface (CSTMB)

Figure 5.5 shows a schematic of the system under investigation. It consists of a bioreactor continuously fed by a stream with a triglyceride concentration equal to S_0. This membrane bioreactor, both on a lab-scale and on a pilot-scale, exhibited interesting and promising performance expressed in terms of biodiesel productivity, and is therefore worth a more detailed analysis from both a kinetic and an experimental point of view.

Small pieces of flat-sheet membrane with a biocatalyst immobilized on the external surfaces are uniformly distributed in the tank. The geometrical characteristics of the pellets are not actually significant in this case, since only the external mass transfer resistance is taken into consideration. The behavior of the CSTMB, or of the corresponding mixed batch reactor in which no material is supplied to or withdrawn from the reactor during the



5.4 Schematic of a hollow fiber membrane bioreactor in recycle configuration.



5.5 Schematic of a continuous stirred membrane bioreactor in which biocatalyst is immobilized on the membrane surface.

reaction, strongly depends on the actual reaction rate, which therefore has to be determined in an accurate way.

The reaction pattern of biocatalytic transesterification of triolein (assumed to be representative of any triglyceride) in the presence of ethanol has already been described in a previous paper (Calabrò *et al.*, 2010) as a sequence of three reactions in series, leading to the formation of one mole of ester for each step and the obtainment of glycerol only at the third step:

$$\mathrm{Triolein(T)+Ethanol(Et)\leftrightarrow Diolein(D)+Ethyl\,oleate(EO)}$$

$$\mathrm{Diolein(D)+Ethanol(Et)\leftrightarrow Monolein(M)+Ethyl\,oleate(EO)}$$

$$\mathrm{Monolein(M)+Ethanol(Et)\leftrightarrow Glycerol(G)+Ethyl\,oleate(EO)}$$

The proposed mechanism was revised and simplified, using triolein and ethanol as the substrates and ethyloleate, glycerol and the other glycerides (monolein and diolein) as the products. These glycerides are found in the reaction mixture at the end of the biocatalytic process. The complex kinetic mechanism was eventually described by a Ping-Pong Bi-Bi mechanism with ethanol inhibition and the King-Altman kinetics method, based on singling out geometrical rules that permitted evaluation of the enzyme concentration in all its complexes ([*E*], [*e*], [ES], [EP], etc.), was adopted. By considering the actual rate of each elementary reaction, it was possible to

formulate the overall kinetic rate equation, expressed as the disappearance of triolein as follows:

$$-\frac{\mathrm{d}[T]}{\mathrm{d}t}=\frac{K_1[T][\mathrm{Et}]-K_2[P][\mathrm{EO}]}{\begin{array}{c}K_3[T]+K_4[\mathrm{Et}]+K_5[T][\mathrm{Et}]+K_6[P]+K_7[\mathrm{EO}]+K_8[P][\mathrm{EO}]\\+K_9[T][P]+K_{10}[\mathrm{Et}][\mathrm{EO}]+K_{11}[\mathrm{Et}]^2+K_{12}[\mathrm{Et}][P]\end{array}}\cdot[e_0] \quad [5.2]$$

where $[T]$ represents the triolein concentration (mol m^{-3}); [Et] is the ethanol concentration (mol m^{-3}); $[P]$ the overall concentration of glycerol, monolein and diolein (mol m^{-3}); [EO] is the ethyloleate concentration (mol m^{-3}); $[e_0]$ the lipase concentration (mol m^{-3}) and K_i ($i = 1, \ldots, 12$) the kinetic constant to be estimated.

The original expression for the reaction rate (Equation [5.2]) was simplified by analyzing a set of experimental data (Calabrò *et al.*, 2010):

$$-\frac{\mathrm{d}[T]}{\mathrm{d}t}=\frac{\alpha\cdot[T]\cdot[\mathrm{Et}]-\beta\cdot[P]\cdot[\mathrm{EO}]}{[T]^2+\delta[T]+\varepsilon} \quad [5.3]$$

where $\alpha, \beta, \delta, \varepsilon$ are kinetic constants.

Using reaction stoichiometry and semi-empirical correlations as a basis, the concentration of products and ethanol was then expressed as a function of the actual triolein concentration $[T]$ and of the initial substrate concentrations $[T_0]$ and $[\mathrm{Et}_0]$:

$$\delta=\delta_1\cdot[\mathrm{Et}_0]+\delta_0 \qquad \varepsilon=\varepsilon_2{}^2\cdot[\mathrm{Et}_0]^2+[\mathrm{Et}_0]+\varepsilon_0 \quad [5.4]$$

$$[\mathrm{Et}]=2.25\cdot([T]-[T_0])+[\mathrm{Et}_0] \quad [5.5a]$$

$$[\mathrm{EO}]=-2.25\cdot([T]-[T_0]) \quad [5.5b]$$

$$[P]=[T_0]-[T] \quad [5.5c]$$

The linearity shown in Equations [5.5a]–[5.5c] could be justified according to the following considerations: (a) A highly specific 1,3-lipase, such as that exploited to perform the kinetic study, led to an ethyloleate/reacted triolein ratio of 2 to which an additional contribution of 0.25, due to acyl migration, was to be added. (b) The fitting of experimental data, as performed in the

Table 5.4 Values of kinetic parameters as calculated by Calabrò *et al.*, 2010

Kinetic parameter	Estimated numeric value
δ_1	−1.85
δ_0	0.618
ε_2	2.84
ε_1	−3.34
ε_0	1.11
α	0.00387
β	0.000162

range of tested operating conditions, was in agreement with this assumption. (c) The reliability of the predictions of the complete theoretical model, as given by Equation [5.3] and Equations [5.5a]–[5.5c], has been demonstrated in previous works at different enzyme/substrate ratios and substrate/alcohol molar ratios.

The values of δ_i and ε_i and of kinetic constants α and β depend on the particular reacting system under consideration and can be estimated by performing a proper experimental analysis of the transesterification reaction carried out by immobilized lipase. In the case presented by Calabrò *et al.* (2010), these values are listed in Table 5.4.

According to Equations [5.4] and [5.5a]–[5.5c], and after some rearrangement, the reaction rate (Equation [5.3]) can be written in terms of the kinetic parameters δ_i and ε_i and of the initial concentrations of both triolein $[T_0]$ and ethanol $[\mathrm{Et}_0]$:

$$-\frac{\mathrm{d}[T]}{\mathrm{d}t}=\frac{\alpha[T]\cdot[\mathrm{Et}_0]+2.25\cdot\alpha\cdot[T]\cdot([T]-[T_0])+2.25\cdot\beta\cdot([T_0]-[T])^2}{\varepsilon_2^2\cdot[\mathrm{Et}_0]^2+(\varepsilon_1+\delta_1\cdot[T])\cdot[\mathrm{Et}_0]+[T]^2+\delta_0\cdot[T]+\varepsilon_0}\cdot[e_0] \quad [5.6]$$

A relationship presenting the actual reaction rate as a function of a set of kinetic parameters and of the concentration of the species involved in the transesterification reaction definitely represents the basis for any theoretical modeling of the behavior of either a CSTMB or of the corresponding well-mixed batch configuration.

It is worthwhile noting that, due to the non-linear form of Equation [5.6], the resulting theoretical model cannot be solved analytically, only by using proper numerical algorithms. Besides theoretical modeling, a proper experimental analysis performed on lab-scale systems may provide a useful indication of the actual behavior of a membrane bioreactor designed for biodiesel production. Next, we present some of the most interesting results collected

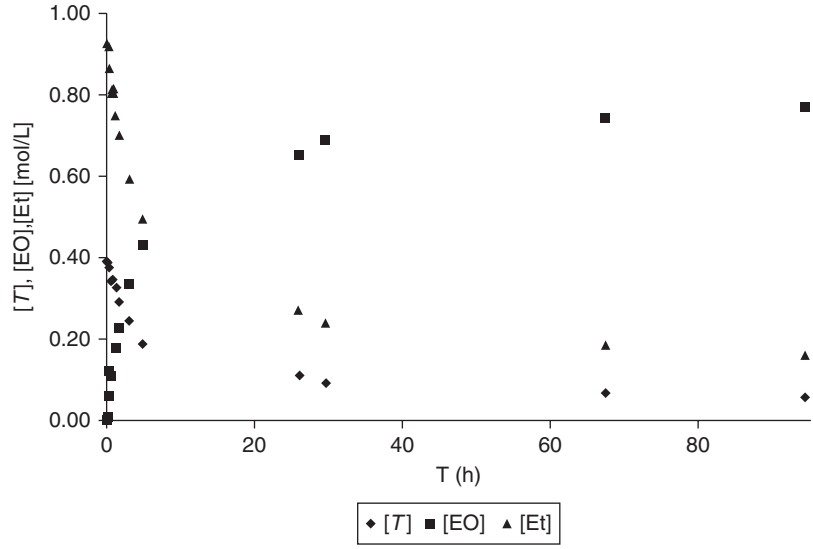
from a well-mixed batch reactor with a volume of 125 mL. The enzyme, lipase from *Mucormiehei* immobilized on the external surface of a porous support, was uniformly distributed in the tank.

Simulating oil with a 60% (w/w) of pure triolein was used to perform the kinetic analysis and then to analyze the bioreactor behavior. The remaining 40% of the mixture included fatty acid or mono and di-glycerides. Some experimental runs were also performed using very low quality olive husk oil whose triolein content (most relevant triglycerides in the oil) was equal to 60%. Ethanol (99.8% grade) from Fluka was used as the secondary substrate.

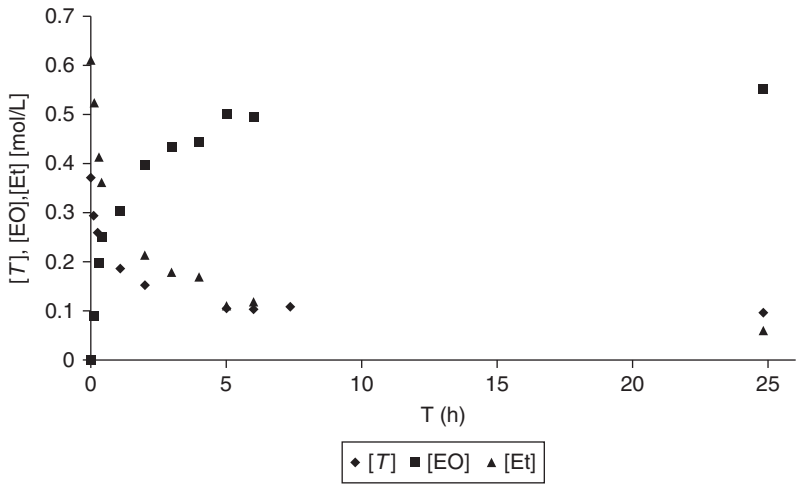
All the experiments were performed at an operating temperature of 37°C and neutral pH; the reaction mixture was prepared according to the procedure reported by Calabrò *et al*. (2010) in order to guarantee good mixing conditions. Reaction samples of 200 μL were collected, ensuring that there was no catalyst present in the sample and avoiding collecting samples that totaled more than 5% of the total volume. The mass ratios of enzyme/triolein $[e_0/T_0]$ fed to the bioreactor were 1:8, 1:20, 1:30; the reactants molar ratios of ethanol/triolein $[\mathrm{Et}_0/T_0]$ were 2:1, 2.5:1 and 3:1 in anhydrous conditions. In order to verify the possibility of recovering and reusing the enzyme after the reaction runs, a proper procedure was followed. After a first reaction run performed with fresh enzyme, the enzyme was recovered by filtration, washed three times with acetone, then dried at room temperature and reused for a new reaction run (Soumanou and Bornscheuer, 2003). The recovery/reuse procedure was carried out in two subsequent reaction runs.

The concentrations of reactants, for example, glycerides, and of product, that is, ethyloleate, were quantitatively measured using high performance liquid chromatography (HPLC) (JASCO instrumentation) under the following conditions: RI detector, eluent phase composition acetone/acetonitrile 70/30 v/v, flow rate 1 mL/min, internal normalization as integration method. The ethanol concentration was not directly measured, but obtained assuming a 1:1 stoichiometric ratio with ethyloleate. The HPLC column used was Alltech Adsorbosphere HS (C18) 5 μm with a length of 250 mm and an inlet diameter of 4.6 mm; the column was provided with a 7.5 × 4.6 mm Alltech pre-column.

Figures 5.6 and 5.7 show some typical results obtained from operating the well-mixed batch bioreactor, expressed as the time evolution of triolein, ethyloleate and ethanol concentrations. It is worth observing that the system performance remains good whether the bioreactor is fed with simulating oil or with real olive husk oil. Triolein concentration tends to decrease quite rapidly until a plateau value is reached. Correspondingly, the ethyloleate concentration increases, reaching a high value at the end of the experiment, indicating that a properly scaled-up and optimized bioreactor



5.6 Time evolution of triolein [*T*], ethyloleate, [EO] and ethanol [Et] concentrations during transesterification by immobilized lipase in a well-mixed batch reactor fed by a simulating oil mixture ($[e_0/T_0]$ = 1:8; $[Et_0/T_0]$ = 2:1).



5.7 Time evolution of triolein [*T*], ethyloleate [EO] and ethanol [Et] concentrations during transesterification by immobilized lipase in a well-mixed batch reactor fed by a real olive husk oil.

could effectively be exploited for biodiesel production on a pilot/industrial system.

5.4 Conclusion

Membrane science and technology offer a significant contribution to the development of biotechnology and, more specifically, to enzyme reactor engineering, which aims to realize efficient and innovative systems with which to perform the biocatalytic conversion of one or more substrate(s) into the desired product(s). The possibility of confining the biocatalyst in a defined region of space, for instance within or on the surface of a membrane where it can retain its catalytic activity and be repeatedly and continuously used, permits the realization of very efficient reactor systems. The confinement of the biocatalyst keeps the reactant/product and enzymes separate, allowing reuse of the enzyme and easy separation of the product from the reaction mixture. In addition, the stability of the biocatalyst can be enhanced, operating costs and enzyme consumption are significantly reduced and the process operates with a higher productivity. Membrane bioreactors offer the possibility of coupling a separation process to a (bio)chemical reaction, resulting in plant simplification and further cost reduction. The removal of a reaction product from the reaction environment could be easily achieved using selectively permeable membranes, and this would be of great advantage in thermodynamically unfavorable conditions such as reversible reactions or product-inhibited enzyme reactions. The behavior of membrane bioreactors aimed at biodiesel production using immobilized lipase can be elucidated by a proper combination of experimental analysis and kinetic modeling. This chapter has shown that feeding a membrane bioreactor system with either simulating oil or with real olive husk oil results in good performance. These results represent a promising basis for future scale-up and optimization of membrane reactors, which can also be used for biodiesel production on a pilot/industrial scale.

5.5 References

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5.6 Appendix: abbreviations and symbols

$[e_0]$	enzyme concentration (mol m^{-3})
[EO]	ethyloleate concentration (mol m^{-3})
[Et]	ethanol concentration (mol m^{-3})
$[Et_0]$	ethanol initial concentration (mol m^{-3})
$[P]$	overall concentration of glycerol, monolein and diolein (mol m^{-3})
$[S_0]$	substrate concentration (mol m^{-3})
$[T]$	triolein concentration (mol m^{-3})
$[T_0]$	triolein initial concentration (mol m^{-3})
K_i $(i = 1, \ldots, 12)$	kinetic constants
t	time (s)

Greek symbols

Θ	bioreactor productivity (dimensionless)
α	kinetic constant (Equation [5.3])
β	kinetic constant (Equation [5.3])
δ	kinetic constant (Equation [5.3])
δ_0	kinetic parameter (Equation [5.4])
δ_1	kinetic parameter (Equation [5.4])
ε	kinetic constant (Equation [5.3])
ε_0	kinetic parameter (Equation [5.4])
ε_1	kinetic parameter (Equation [5.4])
ε_2	kinetic parameter (Equation [5.4])
τ_R	residence time in bioreactor (s)
ψ	degree of conversion (dimensionless)