Physical chemistry and process engineering of an emulsion/membrane bioreactor

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Physical chemistry and process engineering of an emulsion/membrane bioreactor

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STELLINGEN

1. Door voorbij te gaan aan het effect van spreiding van water aan het eind van poriën komt Vaidya tot de foutieve conclusie dat alleen de grensvlakspanning tussen olie- en waterfase bepalend is voor doorbraak van water door een hydrofoob membraan.

   Vaidya A.M., G. Bell, P.J. Halling (1992) Aqueous-organic membrane bioreactors. Part I. A guide to membrane selection. Journal of Membrane Science 71: 139.

2. Paradoxaal genoeg zijn hydrofobe oppervlakken die bedekt zijn met blok-copolymeer moleculen hydrofoob dankzij de aanwezigheid van grote hydrofiele groepen in deze moleculen (Hoofdstuk 4 van dit proefschrift).

3. Het meten van randhoeken aan oppervlakken met geadsorbeerde (blok co)polymeren is geen goede indicatie voor het al dan niet optreden van eiwitadsorptie omdat daarbij voorbij gegaan wordt aan het optreden van sterische hindering door de geadsorbeerde polymeermolekulen.

   Absolom, D.R., A.W. Neumann (1988) Modification of Substrate Surface Properties through Protein Adsorption. Colloids Surfaces 30: 25; **Absolom, D.R., C.J. Van Oss, W. Zingg, A.W. Neumann** (1981) Determination of Surface Tensions of Proteins. II. Surface Tension of Serum Albumin, altered at the Protein-Air Interface. Biochimica et Biophysica Acta 670: 74.

4. Eiwitadsorptie aan met blok-copolymeren bedekte hydrofobe oppervlakken wordt voorkomen door sterische hindering en niet door een toename in hydrofiliciteit.

   Halperin, A. and P.G. de Gennes (1986) Wetting of Polymer Covered Surfaces. Journal de Physique, 47: 1243; **Brink, L.E.S. and D.J. Romijn** (1990) Reducing the Protein Fouling of Polysulfone Surfaces and Polysulfone Ultrafiltration Membranes: Optimization of the Type of Presorbed Layer. Desalination, 78: 209.

5. Gezien het sportaanbod op de Nederlandse TV is het verbazingwekkend dat cyclobal (fietsvoetbal) geen geliefde sport is.

6. Een softbal is bij uitstek geschikt om "hardbal" te spelen.

7. De algehele aversie tegen onnodige toevoeging van kleurstoffen verdwijnt als sneeuw voor de zon zodra het om oranje gaat.

8. Gebrek aan ruggegraat en flexibiliteit kunnen gevaarlijk dicht bij elkaar liggen.

9. Door aan mensen die net een rijbewijs hebben een jaar lang geen auto te verhuren werken garages de verkeersonveiligheid in de hand.

10. Om bondig te schrijven moeten veel woorden gebruikt worden.

11. Gezien het beeld dat in films van wetenschappers geschapen wordt is het erg verwonderlijk dat het "mad scientist syndrome" niet verder verspreid is.

Stellingen behorend bij het proefschrift: " Physical chemistry and process engineering of an emulsion/membrane bioreactor."

Karin Schroën, 3 maart 1995

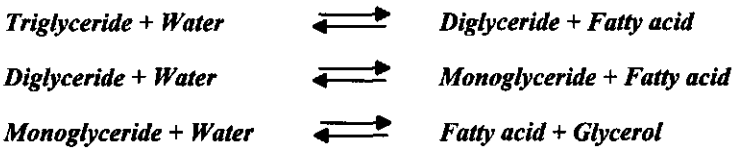
Contents

Chapter 1

INTRODUCTION

I. HYDROLYSIS TRIGLYCERIDES

Vegetable and animal fats and oils consist of mainly triglycerides (official name triacylglycerols). A triglyceride molecule is an ester of glycerol and three fatty acids. Triglycerides can be hydrolysed into fatty acids and diglycerides, monoglycerides or glycerol. The reaction scheme is as follows:



Each of the products (glycerol, fatty acids, diglycerides and monoglycerides) can be used in different applications. Except for glycerol, the properties, and therewith, the possible applications depend on the length and saturation of the fatty acids. Diacylglycerols can be used in e.g. liquid crystals, monoglycerides are mostly used as emulsifying agents, fatty acids are a bulk chemical for the soap and paint industries and glycerol finds its application in cosmetics, detergents and plastics (overview in [1]). The hydrolysis reaction can be carried out by either a chemical or an enzymatic process. This chapter discusses the pro's and cons of both types of processes.

I.a Chemical hydrolysis

Nearly 5000 years ago soap was produced "chemically". Animal fat, water and wood ashes (which contain caustic metal oxydes) were mixed and boiled for several days and a

soap solution was obtained in which the fatty acids are present in deprotonated form (soaps) [2].

Around 1800, a new process was developed in which hydrolysis of fats was carried out by steam injection at elevated temperature and pressure. Under the process conditions the solubility of water in fat is enhanced. Because the reaction rate depends on both the concentration of dissolved water and the temperature also the reaction rate is enhanced [3,4].

At the end of the nineteenth century, Twitchell discovered a catalyst for this process. The catalyst, a sulfonated aromatic compound, has two effects. Firstly, hydrogen ions are formed that catalyse the reaction and, secondly, the solubility of water in the fat phase is increased. The Twitchell catalyst greatly enhances the reaction rate [5]. The process (catalyst) has even been so successful that it is still in use in the U.S.A. although only on a small scale [4].

In the 1990's, chemical hydrolysis of triacylglycerols is carried out in continuous counter current splitting columns at a pressure of 50-60x10^5 Pa and a temperature of 240-260 °C. There are some restrictions to the application of the process, the most important being that it is not possible to hydrolyse oils with a high amount of (poly)unsaturated fatty acids. At the process conditions in the column, the unsaturated fatty acids will polymerise. The double bonds of the fatty acids will conjugate and form dimers, trimers and so on. For the hydrolysis of these oils new processes were developed which operated at a somewhat lower temperature (200-240 °C). However, also at these conditions polymerisation cannot be prevented and, therefore, only a small part of the variety of fats and oils that nature offers can be hydrolysed without destruction of the double bonds [6].

I.b Enzymatic hydrolysis

The fact that enzymes can break down ester bonds was first demonstrated by Bernard in 1856 for lipase from pancreatic juice [7]. At the beginning of the 20th century enzymatic

hydrolysis of triacylglycerols was applied on a fairly large industrial scale. This process got into dis-use after the development of steam hydrolysis which was economically more favourable [6].

During the last decades (1970-94) lipase catalysed reactions are regaining interest because of the mild reaction conditions under which the catalyst can carry out the reaction. The enzyme lipase (EC 3.1.1.3) can either break or form ester bonds at 20-70 °C. Polymerisation reactions of e.g. unsaturated fatty acids, which form a problem in continuous splitting columns, do not take place [6].

Lipase will catalyse hydrolysis reactions only if the enzyme is present at an interface or surface [8]. Therefore, it is necessary to create a large oil/water interface in order to obtain a high volumetric activity [9,10]. The enzyme should also preferably have a high stability. This can be achieved in e.g. emulsion and membrane reactors. The activity and stability of the enzyme depend on the immobilisation material (membrane or other carrier material) and the physical conditions such as pH, temperature and the concentration of glycerol (membrane reactors [9,11-13]; emulsion [14-18]). These factors have to be considered for an appropriate reactor design. In the next paragraph only the membrane bioreactor (extensively reviewed by Malcata [19]) and the emulsion bioreactor are discussed.

II. BIOREACTOR CONCEPTS

II.a Membrane bioreactors

Two types of membrane bioreactors can be distinguished: the *permeation* [11] and the *diffusion* type [20]. In the *permeation* type membrane bioreactor, oil and water are forced through a membrane onto which lipase is immobilised [11]. The reaction takes place whenever both substrates (water and oil) are available to the enzyme. The membrane is preferentially wetted by either the water phase or the oil phase, therefore, the bioreactor activity is limited by the transport of the other substrate toward the enzyme [19]. The

activity of such a bioreactor is relatively low due to the mass transport limitations. The stability of the enzyme depends on the type of membrane onto which the enzyme is immobilized [11] and, of course, on the physical conditions.

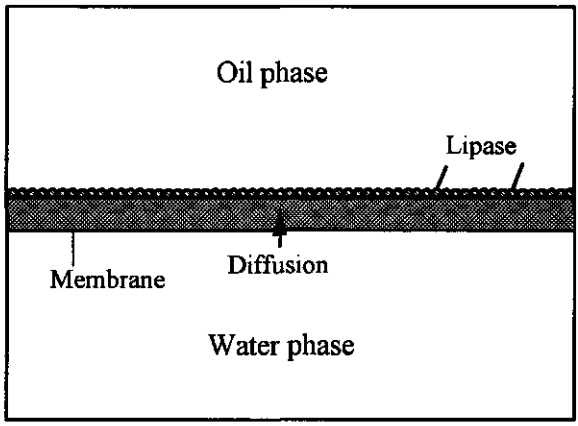


Figure 1. Schematic representation of a (hydrophilic) membrane bioreactor of the diffusion type.

In the *diffusion* type of bioreactor the enzyme is immobilised onto the membrane, but contrary to the permeation type bioreactor, the membrane is used to keep the oil phase and the water phase separated [19-24]. One of the substrates diffuses through the membrane toward the lipase where the reaction takes place and the products diffuse back into either the oil phase (fatty acid, acylglycerol) or the water phase (glycerol). Because the products are kept separated, downstream processing is relatively easy.

Hoq [21-23] reports that the transmembrane pressure in a polypropylene hydrophobic *diffusion* type membrane reactor should not exceed 0.04×10^5 Pa otherwise the membrane cannot keep the phases separated. For large scale operations the transmembrane pressure cannot be controlled within 0.04×10^5 Pa, therefore, the application of the polypropylene bioreactor is very limited. Contrary to this reactor a cellulose based hydrophilic membrane

bioreactor described by Pronk [20] can withstand a transmembrane pressure of $1x10^5$ Pa. This is due to the pore radius of the membranes used. The pores of the hydrophilic cellulose membrane used by Pronk are nanometer size, the hydrophobic polypropylene membrane used by Hoq has an average pore size of 0.4 μm. The minimum transmembrane pressure at which one of the phases can break through the membrane is reversely proportional to the pore size of the biggest pore, r_{max} (Laplace law). Therefore, membranes with small pores should be chosen for large scale application of *diffusion* type membrane reactors [25].

Although the cellulose membrane bioreactor is not limited by a low minimum breakthrough pressure and the enzyme stability is high enough to make continuous operation possible, large scale operation for fatty acid production is not (yet) feasable because the membranes are too expensive for the production of bulk chemicals. For specialty products with a high added value the membrane bioreactor can be an alternative for "classical" production methods [26].

The reaction rate of the hydrophilic membrane bioreactor described by Pronk is limited by the maximum amount of lipase that can be immobilized per m^3 reactor. The reaction rate of the reactor can be enhanced by using a hollow fibre membrane unit with a small fibre diameter. However, the diameter of the fibres of the membrane used by Pronk (0.2 mm) is already very small. And although the surface roughness may further enhance the reaction rate of the reactor, the effect is expected to be relatively small. Therefore, it is interesting to work with a reactor type in which more lipase per volume substrate can be used. This can be achieved in an emulsion reactor.

II.b Emulsion bioreactors

In an emulsion reactor oil, water and lipase are mixed in a stirred vessel. Because of the high costs, the enzyme has to be re-used in order to make a process economically

attractive [26]. The removal of lipase from an emulsion can be facilitated by immobilisation of the lipase on e.g. particles or fibres [11,13,19,27-32]. However, the carrier material will be wetted preferentially by either the oil phase or the water phase. Therefore, the bioreactor with immobilized lipase will have a low activity because one of the substrates is not readily available to the enzyme [19]. Immobilisation of the lipase does generally not result in a high volumetric activity of a two phase reactor.

If the enzyme is used in its free form the lipase will adsorb onto the oil/water interface where both substrates are available and a high volumetric activity can be achieved. The enzyme does not necessarily have be immobilized in order to make re-use of the enzyme possible. Emulsion reactors with free enzyme are known from literature [9,14,33]. Bühler [14] separates the emulsion by centrifugation into three phases: oil, water, and a lipase rich middle phase (concentrated emulsion). By using two mixer/settler systems, 90% of both the fat phase and the water phase is separated from the emulsion. During continuous operation 90% of the lipase is recycled to the mixers. The rest of the lipase is present in the water phase and removed together with this phase from the reactor. The volumetric activity of the lipase in the vessel is high but the stability of the enzyme is low as compared to the hydrophilic membrane bioreactor. Moreover, 10% of the enzyme is removed with the water phase and hence the enzyme costs are relatively high. Also, the separation step with centrifuges consumes a lot of energy. Therefore, the reactor concept of Bühler is not economically feasible for the bulk production of fatty acids. To prevent loss of enzyme it is necessary to select a different separation method that is preferably also less energy consuming. Membrane separation might be a good alternative.

In the emulsion/membrane bioreactor [10] the oil phase and the water phase are selectively removed by means of a hydrophobic and a hydrophilic membrane in series (see figure 2). The enzyme is retained within the reactor because the pore size of the hydrophilic membrane is such that the enzyme cannot permeate through it. Lipase is not soluble in the oil phase and will, therefore, not permeate through the hydrophobic

membrane since only the oil phase permeates through it. Although a high volumetric activity (10-fold higher than the hydrophilic membrane reactor [20]) is achieved still some problems have to be solved in order to make application of the emulsion/membrane bioreactor possible.

Both membranes are severely fouled (1000-fold flux decrease). Consequently, the membrane surface area required for the separation is too large. Hence, the advantage of the decrease in membrane costs (as compared to the hydrophilic membrane reactor [9]) is nullified. This can only be overcome if fouling is decreased or even prevented. Also small amounts of water permeating through the hydrophobic membrane are reported [10]. This problem should also be overcome, before the reactor can be used in a continuous system.

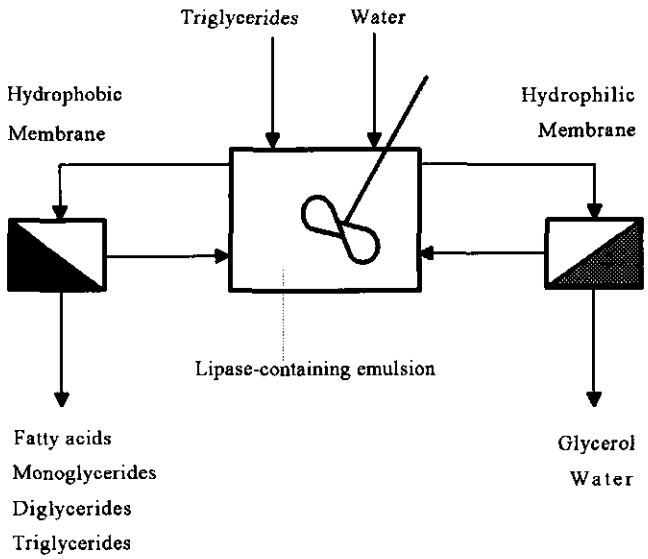


Figure 2. Schematic representation of the emulsion/membrane bioreactor.

III. PROBLEM DEFINITION

As described previously, the emulsion/membrane bioreactor potentially has a high volumetric activity. If the process conditions are chosen properly, a high enzyme stability is possible too. However, fouling of the membranes (mainly caused by protein adsorption) has to be reduced and preferably prevented in order to make application of the emulsion/membrane bioreactor economically attractive.

Fouling of the membranes can be influenced by either adjusting the process conditions (e.g. flow velocity and transmembrane pressure) and/or modifiying the membrane properties (e.g. surface tension and charge of the membrane). In this thesis mainly the last option, adjustment of the membrane properties is considered. The hydrophobic membrane is modified with a block copolymer in order to influence protein adsorption.

The aims of this thesis are: -Firstly, to determine which block copolymer is best suited for reduction of protein adsorption at the *hydrophobic* membrane and to determine the mechanism behind this reduction of protein adsorption by block copolymers. -Secondly, to study the process conditions under which fouling of the *hydrophilic* membrane is within acceptable proportions. -Thirdly, to identify and model the engineering phenomena that are important for the continuous hydrolysis of triacylglycerols in the emulsion/membrane bioreactor.

IV. OUTLINE OF THIS THESIS

This thesis discusses the lipase-catalysed hydrolysis of triacylglycerols in an emulsion/membrane bioreactor. Special attention is paid to modification of the hydrophobic membrane in order to prevent protein adsorption.

Chapter 2 presents the emulsion/membrane bioreactor. The problem of water permeation through the hydrophobic membrane at low transmembrane pressure is pointed out and the hypothesis that protein adsorption is responsible for water permeation is presented.

Membrane modification with a tri-block copolymer (F108) is presented as a solution to overcome water permeation. This block copolymer consists of two poly(ethylene oxide) end blocks and one poly(propylene oxide) middle block. The block lengths of the PEO blocks turns out to be important.

In order to verify whether the low transmembrane pressure thershold for water permeation through a hydrophobic membrane is caused by lipase adsorption, chapter 3 discusses the transmembrane pressure at which water can permeate through a hydrophobic membrane and the theoretical relation to wettability changes caused by protein adsorption. The theoretical discussion is strengthened by various experimental results. The transmembrane pressure at which water can permeate through an F108-modified membrane is constant in time which proves that no protein adsorption takes place at this membrane.

In chapter 4 the influence of pre-adsorbed block copolymers on the wettability of a hydrophobic surface is discussed. It was found that the wettability is mainly ruled by the surface/oil interactions and hardly influenced by the presence of the large hydrophilic blocks of the copolymers. The membrane remains hydrophobic upon treatment with block copolymer. Hence, in contrast to widespread belief the suppression of protein adsorption by the block copolymer is not due to an increase in hydrophilicity.

The mechanism behind prevention of protein adsorption by block copolymers is elucidated in chapter 5. The influence of the surface properties, length of the hydrophilic groups and the surface coverage with block copolymer is studied systematically. It could be concluded that the configuration of the adsorbed block copolymer molecule and the length of the hydrophilic groups determine whether prevention of protein adsorption is possible. The mechanism behind prevention of protein adsorption by F108 is steric hindrance of attachment to the surface. An unsaturated layer F108 hinders the attachment also severely.

In chapter 6 the results of continuous experiments with the emulsion/membrane bioreactor are discussed. From literature, a model is adapted for sunflower oil hydrolysis and verified using an independent set of measurements. With the model, the performance of reactors in series are evaluated. The model shows that in a co-current feed and bleed system an acceptable production per gram added enzyme can be reached. The reactor volume is such that the process is economically feasable.

Finally, in chapter 7, the emulsion/membrane bioreactor is optimized. Some results on the esterification of oleic acid with glycerol in the emulsion/membrane bioreactor are also shown. New application possibilities for the block copolymer modified membranes such as filtration of aqueous protein solutions and separation of emulsions with micro-organisms are presented.

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Chapter 2

MEMBRANE MODIFICATION TO AVOID WETTABILITY CHANGES DUE TO PROTEIN ADSORPTION IN AN EMULSION/MEMBRANE BIOREACTOR

ABSTRACT

This study addresses problems encountered with an emulsion/membrane bioreactor. In this reactor, enzyme- (lipase) catalysed hydrolysis in an emulsion was combined with two in-line separation steps. One is carried out with a hydrophilic membrane, to separate the water phase, the other with a hydrophobic membrane, to separate the oil phase. In the absence of enzyme, sunflower oil/water emulsions with an oil fraction between 0.3 and 0.7 could be separated with both membranes operating simultaneously. However, two problems arose with emulsions containing lipase. First, the flux through both the hydrophilic and the hydrophobic membranes decreased with exposure to the enzyme. Second, the hydrophobic membrane showed a loss of selectivity demonstrated by permeation of both the oil phase and the water phase through the hydrophobic membrane at low transmembrane pressure.

This chapter has been published as:

Membrane modification to avoid wettability changes due to protein adsorption in an emulsion membrane bioreactor

C.G.P.H. Schroën, M.C. Wijers, M.A. Cohen Stuart, A. Van der Padt, K. Van 't Riet (1993)

Journal of Membrane Science, **80**: 265-274

These phenomena can be explained by protein (i.e. lipase) adsorption to the polymer surface within the pores of the membrane. It was proven that lipase was present at the hydrophilic membrane and that this, in part, explains the flux decrease of the hydrophilic membrane.

To prevent the observed loss of selectivity with exposure to protein, the hydrophobic polypropylene membrane (Enka) was modified with block copolymers of propylene oxide (PO) and ethylene oxide (EO). These block copolymers act as a steric hindrance for proteins that come near the surface. The modification was successful: After 10 days of continuous operation the minimum transmembrane pressure at which water could permeate through an F108-modified membrane was 0.5×10^5 Pa, the same value as that observed in the beginning of the experiment. This indicates that loss of selectivity due to protein adsorption is prevented by the modification of the membrane.

INTRODUCTION

One of the major problems in membrane separations is the occurrence of fouling due to protein adsorption. As a protein solution is filtered through a membrane the protein comes into contact with the surface of the membrane, it will also come into contact with the pore walls. Protein adsorption onto the membrane surface and particularly onto the pore walls leads to a dramatic decrease of the flux.

One possible consequence of protein adsorption is a change in wettability of the membrane. Absolom *et al.* [2,3] reported that a hydrophobic surface will display more hydrophilic properties after adsorption of a protein. A change in wettability, resulting from protein adsorption can also exert great influence on the selectivity of the membrane. Keurentjes *et al.* [4] found that selectivity was lost (both the oil phase and water phase permeate through the membrane) as soon as oleate adsorbs onto a hydrophobic membrane.

In order to prevent such a change in wettability it is necessary to prevent protein adsorption. Many physicochemical parameters have been reported to affect adsorption of proteins, including solution properties such as pH and ionic strength and surface properties such as surface charge and surface tension [5,6]. Although protein adsorption can be influenced by these parameters it cannot be prevented totally by controlling them.

Recently, Lee *et al.* [7] and Tan and Martic [8] reported that protein adsorption to latex particles can be prevented by adsorbing block copolymers onto the surface of the particles. The block copolymers consisted of three parts, two hydrophilic buoy groups and one hydrophobic anchor group (figure 1). Such block copolymers provide the surface with a steric hindrance for proteins that come near the surface.

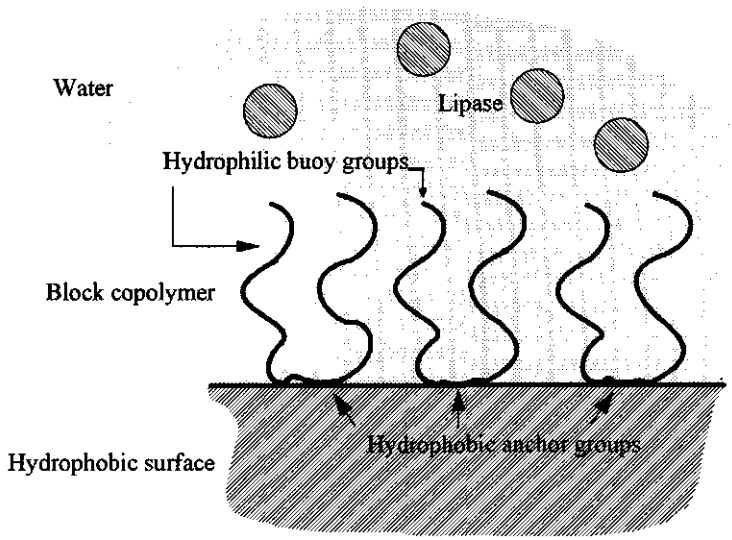

Figure 1. Block copolymers adsorbed at a hydrophobic surface.

According to Lee *et al.* [7] and Tan and Martic [8], adsorption of several proteins could be prevented by appropriate choice of the length of the buoy groups to provide the necessary steric hindrance.

The aim of this study was to demonstrate that the concept of preventing protein adsorption by modification with block copolymers could be extended to membranes. The effectiveness of the steric hindrance provided by block copolymer adsorption depends on the length of the polyethylene oxide (buoy) part of the block copolymer [9,10]. Therefore, three different block copolymers (F108, P75 and L92), with approximately the same length of the polypropylene oxide anchor group, were used. Lee *et al.*, and Tan and Martic [7,8] stated that F108 block copolymer (large buoy groups) adsorbed onto polystyrene latex prevented protein adsorption. Since P75 (medium length buoy groups) did not prevent protein adsorption, we expect that L92 (shorter buoy groups than P75) will also be incapable of preventing protein adsorption. The effect of modification on protein adsorption can be detected by monitoring water permeation through both modified and unmodified hydrophobic membranes.

EXPERIMENTAL

In the bioreactor studied here triglycerides are hydrolysed into diglycerides, monoglycerides, fatty acids and glycerol by means of a lipase catalysed reaction. Because the lipase is active only if both substrates (glyceride and water) are present, the reaction takes place at the oil/water interface.

The hydrolysis reaction can be carried out in several reactors, e.g. a membrane reactor [11-13] or an emulsion reactor [14]. The subject of this research is the emulsion/membrane bioreactor first described by Pronk *et al.* [1]. The emulsion/ membrane bioreactor is given in figure 2.

In this reactor, the large specific surface area of an emulsion is combined with a membrane-based separation step. The advantage of using an emulsion is that a large surface area is created in a relatively small volume, resulting in a large volumetric productivity. The emulsion is separated continuously into its constituent phases by passing it over hydrophilic and hydrophobic membranes.

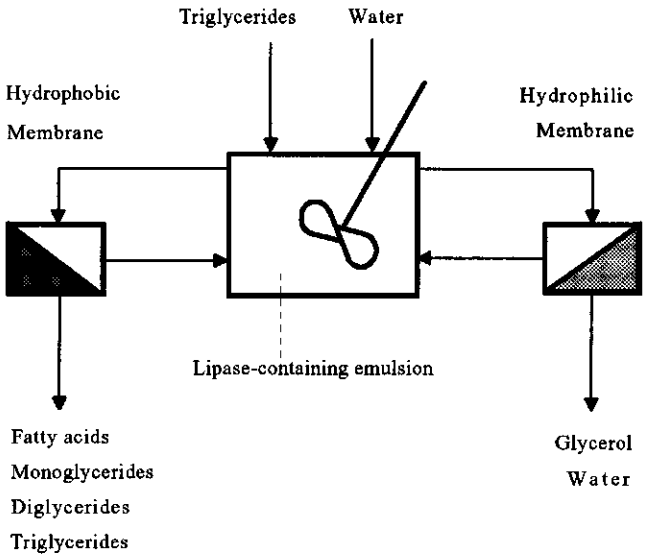

Figure 2. The emulsion/membrane bioreactor

The hydrophilic membrane is wetted by the water phase of the emulsion, and the water phase permeates through it. The hydrophobic membrane is wetted by the oil phase (which contains fatty acids, monoglycerides, diglycerides and triglycerides), and this phase permeates through it. The enzyme is present at the oil/water interface and in the water phase. To obtain a continuous process, the enzyme must be retained by the membranes. Therefore the molecular weight cut-off of the hydrophilic membrane must be smaller than the molecular weight of the lipase.

MATERIALS

Sunflower oil of edible quality (esters of glycerol and fatty acids of which over 95% is C16 and C18 acids) was purchased from SMILFOOD B.V. (Heerenveen, the Netherlands). The enzyme, Lipase B, was obtained from Biocatalysts and originated

from the yeast *Candida rugosa* (formerly called *Candida cylindracea*). Hexadecane, fuming nitric acid (100%), methylene blue and Sudan VII b were all analytical grade and purchased from Merck (Germany). Doubly distilled water was used throughout. In order to prevent microbial growth 0.01 % sodium azide was added to the emulsions.

All block copolymers used were gifts from ICI (Rotterdam, the Netherlands) and are summarised in table 1. The block copolymers consist of three blocks, one polypropylene oxide (PPO) anchor part and two polyethylene oxide (PEO) buoy parts. The anchor parts are similar in molecular weight while the buoy parts vary considerably. The polyethylene oxide was purchased from Aldrich Chem. Comp.

The hydrophilic membrane used was a cellulose (Cuprophan) hollow fibre device from Organon Technika (Boxtel, The Netherlands) with a surface area of 0.77 m^2, a membrane wall thickness of $8x10^{-6}$ m and a nominal molecular weight cut-off value of 5,000 Dalton.

Table 1. Polymers used in this study

Trade name	Total molecular weight (Da)	M.W. PEO (Buoy) (Da)	M.W. PPO (Anchor) (Da)
L92	3,450	345	2,760
P75	4,150	1,075	2,000
F108	14,000	5,600	2,800
Poly(ethylene oxide)	900,000	-------	-------

A flat sheet polypropylene membrane, provided by Enka (Wuppertal, Germany), with a mean pore size of $0.1x10^{-6}$ m was chosen to be the hydrophobic membrane. The flat sheet membrane was used in a Megaflow module (type TM 100, effective surface area $64x10^{-4}$ m^2) from New Brunswick Scientific (Edison, USA). A hollow-fibre polypropylene membrane with a mean pore size of $0.2x10^{-6}$ m and a surface area of 0.07 m^2 (Organon Technika) was employed in those experiments in which technical difficulties with the

module prevented the use of a flat sheet membrane. This was necessary only in two membrane modification experiments (modification with polyethylene oxide and modification with F108).

METHODS

Determination of the continuous phase in the emulsion

The nature of the emulsion (oil in water or water in oil) was inferred from conductivity measurements and by adding colouring agents to the emulsions. Conductivity measurements were performed using a 400 Hz AC current in order to avoid electrophoresis. In some colouring experiments, a solution of Sudan VII b in sunflower oil was placed on top of the emulsion. In others, emulsion was added to a solution of methylene blue in water. Depending on which phase was the continuous phase in the emulsion, a red or a blue colour diffused into the emulsion. In order to exclude demixing of the emulsion within the time of the experiment, other experiments were performed in which a coloured phase was mixed with a non-coloured phase to see whether the coloured phase was present as droplets or as a continuous phase.

Flux measurements

For all membrane experiments the pump delivery was 1.67×10^{-6} m^3 per second. For both the cellulose and the flat sheet polypropylene membrane, the permeate fluxes were measured as a function of the oil content in the emulsion. The emulsion contained no lipase. The pressure was kept at a constant value of 0.1×10^5 Pa for the hydrophobic membrane and at 0.15×10^5 Pa for the hydrophilic membrane. Sunflower oil and water (varying composition with a total volume of 500×10^{-6} m^3) were emulsified in a stirred vessel, containing 4 baffles (12×10^{-4} m^2 each). A four bladed standard turbine stirrer (diameter 4.5×10^{-2} m) was used at 450 rpm. The emulsion was led over the membrane at 1.67×10^{-6} m^3 per second and the volume of the permeate was determined as a function of time and pressure. Both permeate and retentate were recycled to the emulsion vessel.

Membrane modification with block copolymers

Membrane modification was carried out as follows:

- pre-wetting the membrane by rinsing the membrane with hexadecane for 30 minutes; hexadecane permeated through the membrane
- modification with an emulsion (1:2 v/v hexadecane in water) containing 6 gram block copolymer per litre of emulsion. This emulsion was pumped along the retentate side of the hydrophobic membrane for 15 minutes. Only hexadecane permeated through the membrane. Both the permeate and the retentate were recycled to the stirred emulsion vessel.
- rinsing with water for 15 minutes; water did not permeate through the membrane
- rinsing with a sunflower oil/water emulsion (1:1 v/v) for 15 minutes, sunflower oil permeated through the membrane

For all the rinsing steps the respective liquid was pumped over the membrane at the retentate side.

Membrane modification with polyethylene oxide

The influence of a modification with only poly(ethylene oxide) was tested in a hollow fibre membrane. A hollow fibre was chosen because of the practical impossibility to pretreat a flat sheet membrane with fuming nitric acid. (The module would have been destroyed by the acid.) The pretreatment is necessary to improve the binding of polyethylene oxide to the membrane. The pretreatment and modification were carried out as follows:

- leading the vapour of fuming nitric acid at 40 °C through the hollow fibre membrane for 1 minute
- rinsing the membrane with demineralized water for 5 minutes
- rinsing a polymer solution containing 6 grams polyethylene oxide per litre over the membrane for 30 minutes

- removing the polymer solution from the fibres by replacement with a 1:1 v/v sunflower oil in water emulsion

Addition of lipase

Fluxes were determined for the F108-modified hydrophobic (both the flat sheet and the hollow fibre module), unmodified hydrophobic (flat sheet) and the hydrophilic membrane with an emulsion of sunflower oil and water (1:1 v/v) containing 3 g lipase per litre of emulsion. The emulsion was mixed in a vessel as described before. The crude lipase preparation was dissolved in water and centrifuged at 13.000 rpm (Heraus, biofuge A). The sediment was discarded; only the supernatant was used in experiments.

Determination of maximum transmembrane pressure

The maximum transmembrane pressure without water permeation was determined for the hydrophobic membrane with an emulsion (1:1 v/v) in which 95% of the oil phase consisted of fatty acids and no lipase was present. The maximum transmembrane pressure without water permeation was also measured for both modified and unmodified membranes with an emulsion (1:1 v/v) containing 3 grams lipase per litre of emulsion. The maximum transmembrane pressure without water permeation for the F108 modified (flat sheet) membrane was determined as follows. First, the transmembrane pressure was gradually increased from 0.06×10^5 Pa to a maximum of 0.5×10^5 Pa. The transmembrane pressure was then kept constant at 0.5×10^5 Pa for 10-15 minutes. It was noted whether water permeated through the membrane. Subsequently the transmembrane pressure was decreased to 0.06×10^5 Pa. Every other day such an experiment was performed with the F108 modified membrane. For the unmodified membrane, the L92 modified and the P75 modified membrane, the transmembrane pressure was kept as low as possible during the experiments, about 0.03×10^5 Pa, to prevent water permeation at an early stage of the experiment. At the time indicated in table 2 (see results section) the transmembrane was

gradually increased. The transmembrane pressures indicated in table 2 are those at which the water phase started to permeate through the membrane.

Determination of degree of hydrolysis

The degree of hydrolysis in the emulsion/membrane bioreactor was determined by dissolving a sample of approximately 1 g oil phase in $20x10^{-3}$ l of a solution of phenolphthalein in ethanol and than titrating with 0.1 N NaOH. Viscosity measurements were performed with a Ubelohde capillary viscometer. The temperature during all experiments was kept at 30 °C.

RESULTS AND DISCUSSION

In colouring experiments, a phase inversion was observed at 0.7 volume fraction sunflower oil in the emulsion. A finite conductivity was measured in emulsions with an oil fraction up to 0.7 volume fraction oil. Both types of experiments indicated that at oil fractions 0-0.7 water is present as the continuous phase and at higher oil fractions water is present as the dispersed phase.

Hydrophilic membrane

Figure 3 shows the flux through the hydrophilic membrane as a function of the oil fraction in the emulsion at a constant pressure value of $0.15x10^{5}$ Pa. Three regions can be distinguished. At oil volume fractions of 0-0.7 apparently the oil droplets within the continuous phase do not influence the flux since it is approximately equal to the flux of the water alone. From 0.7-1.0 volume fraction oil in the emulsion, water is the dispersed phase. This was also confirmed for the membrane experiment. In both the inlet stream and the retentate, the oil phase is the continuous phase and demixing of the emulsion in its constituent phases in the module, can be excluded. In order to contribute to the flux, the water droplets have to be transported to the membrane and then coalesce at the membrane surface. At oil volume fraction of 0.7-0.8, the flux with the emulsion is

comparable with the clean water flux, indicating that both transport of the emulsion droplets towards the membrane and coalescence at the membrane still take place. These results indicate that it is possible to remove not only a continuous phase, but, under these conditions, also a dispersed phase can be removed. For emulsions with oil volume fractions of 0.8-1.0 no flux is found. Apparently, transport and/or coalescence of emulsion droplets do not take place. It is still unclear what mechanism is responsible for the abrupt change in flux.

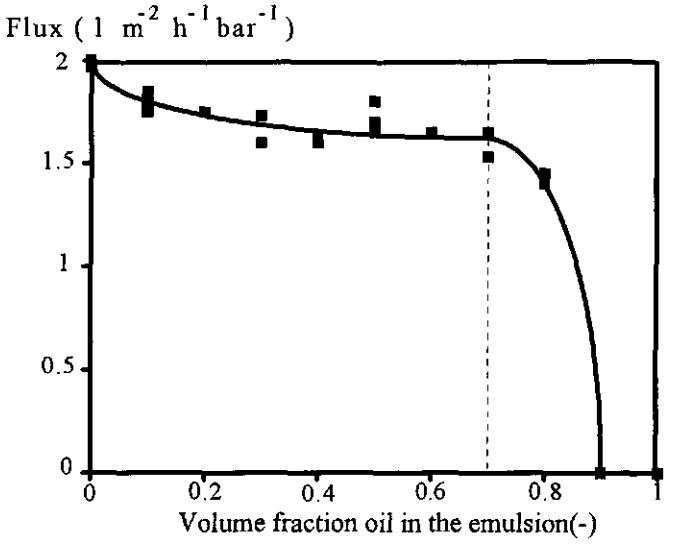

Figure 3. Flux through the hydrophilic cellulose membrane as a function of the volume fraction of sunflower oil in the emulsion. The dashed line indicates the phase inversion concentration as detected by colouring and conductivity experiments.

Hydrophobic membrane

Figure 4 shows the flux through the hydrophobic polypropylene membrane as a function of the volume fraction of sunflower oil in the emulsion at a constant pressure value of 0.1×10^5 Pa. Again, three regions can be distinguished. In the region 0.7-1 volume fraction oil, the oil is present as the continuous phase and a constant value for the flux is

found. In the region 0.3-0.7 volume fraction oil, in which water is present as a continuous phase in the emulsion, also a constant flux value is found. Apparently, transport of oil droplets toward the hydrophobic membrane is sufficient to maintain a high flux value. For emulsions that contain between 0 and 0.3 volume fraction oil the flux is zero. As with the hydrophilic membrane, it appears that the dispersed phase can permeate, but it is still unclear how this is precisely related to the properties of the emulsion. Nevertheless, we can draw the practical conclusion from figures 3 and 4 that it is possible to separate emulsions with an oil volume fraction between 0.3 and 0.7 using the cellulose and the polypropylene membrane operating simultaneously. Therefore, an emulsion with an oil volume fraction of 0.5 is chosen for all the other experiments.

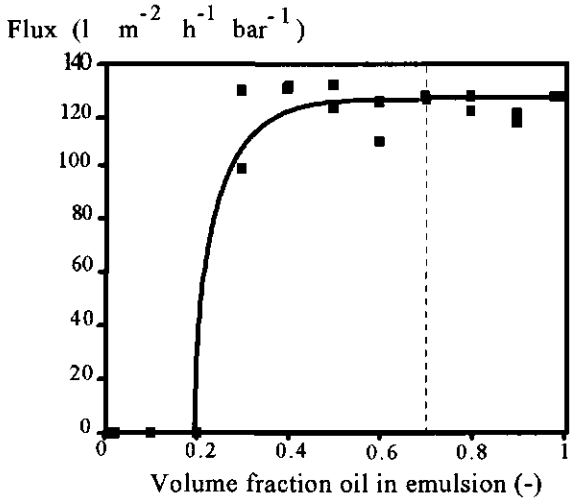

Figure 4. Flux through the polypropylene membrane as a function of the volume fraction of oil. The dashed line indicates the phase-inversion concentration.

The flux is measured as a function of time for both the hydrophobic and the hydrophilic membrane using an emulsion containing 3 g of lipase per litre of emulsion. For the hydrophilic membrane a constant value of 1.5 l $m^{-2} h^{-1} bar^{-1}$ was measured. This value is slightly lower than the flux obtained without lipase, a decrease observed in a number of

experiments. Probably, this is due to adsorption or concentration polarisation of lipase or another component of the crude lipase preparation. The presence of lipase at the membrane surface was demonstrated as follows. First, the emulsion was removed from the lumen of the membrane fibres by flushing with sunflower oil. At the same time, water was pumped through the shell side of the membrane. By prolonging these operations the situation approximates the membrane reactor reported by Pronk *et al.* [11]. Because oil was converted into fatty acids we conclude that lipase was present at the membrane surface. The activity of the reactor (mole fatty acid released per second and per square meter membrane surface area) was a factor 5 lower than for the reactor described by Pronk.

Modified membranes

For the hydrophobic membrane, the flux decreased very rapidly (figure 5) and both oil and water permeated through the membrane after 50 hours. The decrease in flux and the permeation of water are probably due to protein adsorption. Therefore the membrane had to be modified with block copolymers. These polymers provide the membrane with a steric hindrance for proteins that come near to the surface, this is discussed in more detail in the introduction.

In table 2 the results for unmodified membranes and membranes modified with different block copolymers are summarised in terms of the time at which water permeation first occurred. Initially, the maximum transmembrane pressure without water permeation for a F108 modified (flat sheet) membrane is 0.5×10^5 Pa. Permeation of water was observed with a membrane modified with L92 after 2 days at a transmembrane pressure of 0.04×10^5 Pa. Permeation of water occurred for P75 modified membranes after 5 days at a transmembrane pressure of 0.08×10^5 Pa. Apparently the steric hindrance provided by P75 or L92 is not enough to prevent protein adsorption; however, the P75 modified membrane does show a better performance than the unmodified membrane. Probably

protein adsorption was slowed down somewhat by the P75 modification. This is in agreement with the results of Lee *et al.* [7] and Tan and Martic [8].

Table 2. Permeation of water through membranes

Modification with	M.W. buoy ($g\ mol^{-1}$)	Time (days)	Maximum pressure without water permeation (10^5 Pa)
F108	5,600	>10	>0.5
P75	1,075	5	0.08
L92	475	2	0.04
Unmodified	-------	2	0.02

For a membrane (flat sheet) modified with F108 interesting characteristics are found. No water permeated through the membrane. On raising the transmembrane pressure, water started to permeate at $0.5x10^5$ Pa, which is a value equal to that found for the emulsion without the enzyme. This indicates that the membrane surface retained its hydrophobic properties unlike the unmodified membrane which allowed water permeation at low transmembrane pressure when protein adsorption presumably made the surface more hydrophilic. Therefore we suppose that no protein adsorption has taken place at the F108 modified membrane and specifically the F108 block copolymer prevented lipase adsorption.

When a hollow fibre polypropylene membrane was modified with polyethylene oxide only, the oil flux through the membrane decreased from 120 $l\ m^{-2}\ h^{-1}\ bar^{-1}$ to 50 $l\ m^{-2}\ h^{-1}\ bar^{-1}$ upon modification, indicating that polyethylene oxide was deposited on/in the membrane. The polyethylene oxide-modified membrane lost its selectivity after 5 days at a transmembrane pressure of $0.05x10^5$ Pa. This suggests that it is not only the polyethylene oxide in the block copolymer that is responsible for the favourable properties of the F108 modified membrane but the specific form of the block copolymer.

A hollow fibre polypropylene membrane was also modified with F108. A flux curve comparable to figure 5 was found and also the transmembrane pressure at which water started to permeate through the membrane remained constant. It can therefore be concluded that the difference in the results between the F108 modified membrane and the polyethylene oxide-modified membrane is not caused by the difference in the module design but due to the effectiveness of the steric hindrance provided by F108.

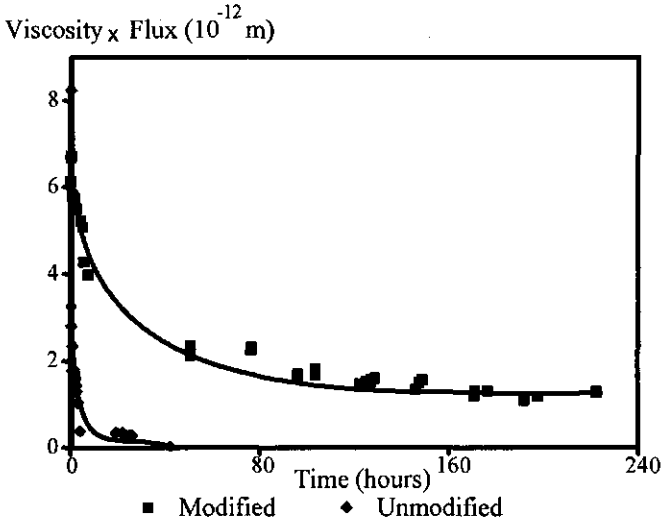

Figure 5. Viscosity corrected volume fluxes through a modified and unmodified membrane as a function of time.

The initial flux for sunflower oil through the F108 modified (flat sheet) membrane is lower than the flux found for the unmodified membrane, (40-60 l m^{-2} h^{-1} bar^{-1} for the modified membrane as compared to 80-120 l m^{-2} h^{-1} bar^{-1} for the unmodified membrane). This shows that an additional resistance due to block copolymer adsorption is present at the membrane surface or in the pores of the membrane. The flux is measured as a function of time during enzyme catalysed hydrolysis. Initially, the flux through the modified membrane increased by a factor of two. This effect can entirely be attributed to

a decrease in viscosity of the oil phase upon hydrolysis. Sunflower oil with a viscosity of $33x10^{-3}$ Pa s is converted into an oil phase containing 90-95% fatty acids with a viscosity of $15.5x10^{-3}$ Pa s within 2 hours. In order to account for changes in viscosity, and compare the resistance of modified and unmodified membranes, the flux is multiplied by the viscosity of the permeate and the product is plotted in figure 5.

After 4 hours, the initially constant flux starts to decrease slowly and a value of $1x10^{-12}$ m is obtained after 10 days. There are several possible explanations for this decrease in flux. First, further refinement of the emulsion may have occurred during the first day resulting in a more stable emulsion [15]. Second, as reported by Graham and Philips [17-19] inactivation of lipase in the emulsion [16] can influence the elasticity of the protein film at the oil/water interface, and this may affect the flux. Third, a minor component in the oil (e.g. phospholipids) may be blocking membrane pores.

Although the flux decreases by a factor 5, it is still very acceptable, given that the selectivity of the F108 modified membrane was maintained, i.e. no water permeated through the membrane. Therefore, we conclude that no protein adsorption takes place at the F108 modified membrane, and that the F108 modified membrane is suitable for application in the emulsion/membrane bioreactor. However, membranes modified with the P75 and L92 block copolymer could not be used because those copolymers apparently did not prevent protein adsorption.

CONCLUSIONS

It is possible to separate a sunflower oil/water emulsion with a volume fraction oil between 0.3 and 0.7 by simultaneous use of a hydrophilic cellulose membrane and a hydrophobic polypropylene membrane as long as no lipase is present in the emulsion.

The presence of lipase in the emulsion has a distinct influence on the flux and on the selectivity of the hydrophobic membrane. For the hydrophilic membrane a slightly lower

flux value is found with lipase present. Lipase was shown to be adsorbed to the membrane.

The behaviour of the hydrophobic membrane is more complex. Initially, only oil permeates through the hydrophobic membrane. After 2 days, water is also capable of permeating through the hydrophobic membrane, even at low transmembrane pressure. Protein adsorption onto the hydrophobic membrane probably makes it more hydrophilic, enabling water to permeate through that membrane. This loss in selectivity of the unmodified membrane makes it impossible to run the emulsion/membrane bioreactor in a continuous mode.

Modification of the hydrophobic membrane with an F108 block copolymer apparently prevents protein adsorption. After 10 days, the transmembrane pressure at which water permeates through the membrane is still equal to the value at the start of the filtration. Thus, an emulsion/membrane bioreactor containing the F108 modified membrane is suitable for use in a continuous mode.

ACKNOWLEDGEMENT

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Chapter 3

MINIMUM BREAKTHROUGH PRESSURE AS A MEASURE FOR WETTABILITY CHANGES CAUSED BY PROTEIN ADSORPTION AT HYDROPHOBIC MEMBRANES

ABSTRACT

In this article, ΔP_{BT}^{min}, the minimum breakthrough pressure of water through a hydrophobic membrane, is used as a measure for wettability changes caused by surface active agents like e.g. proteins. Wettability experiments showed that a hydrophobic surface is wetted by oil. However, the surface is no longer wetted by oil after protein (lipase) adsorption, oil droplets even detach from the surface. This implies that the surface is hydrophilic after lipase adsorption. It was found that the ΔP_{BT}^{min}-value of a hydrophobic membrane decreases likewise upon addition of lipase to a sunflower oil-in-water emulsion. It was shown in theory and by experiments that the decrease in ΔP_{BT}^{min} is caused by protein adsorption and not by, e.g., the reaction products formed by lipase.

This chapter has been published as:

Minimum breakthrough pressure as a measure for wettability changes caused by protein adsorption at hydrophobic membranes

C.G.P.H. Schroën, M.A. Cohen Stuart, A. Van der Padt, K. Van 't Riet (1994)

Bioseparation **4**: 151-163

Breakthrough pressure

Adsorption measurements were carried out with hydrophobic powder modified with block copolymers. It was found that no lipase adsorption takes place at powder modified with block copolymer F108. For a membrane modified with F108, no decrease in ΔP_{BT}^{min} takes place in the course of an experiment of 14 days. This indicates that protein adsorption on the membrane is prevented by pre-adsorbed F108.

INTRODUCTION

Protein adsorption influences the properties of surfaces and therewith their wettability. Absolom and co-workers [1,2] quantified this effect by measuring contact angles. High contact angles were measured for water on a hydrophobic surface without an adsorbed protein layer. However, the same authors found low contact angles for water droplets on the same surface, but now with an adsorbed protein layer. This indicates that the surface properties had changed upon protein adsorption, and that the surface had obtained hydrophilic properties.

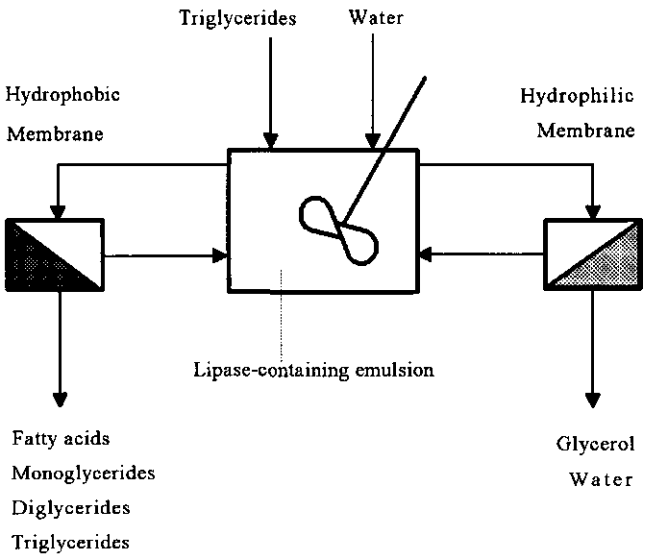


Figure 1. The emulsion/membrane bioreactor.

For emulsion separation with membranes, it is important that the membranes retain their wettability by either the water phase or the oil phase. A hydrophilic membrane is wetted by the water phase of the emulsion and only this phase should permeate through the membrane. A hydrophobic membrane is wetted by the oil phase and only this phase should permeate through the membrane. However, upon protein adsorption the hydrophobic membrane will change from an oil-phase-wetted to a water-phase-wetted membrane.

Adsorption phenomena can be important when operating the emulsion/membrane bioreactor (figure 1). A protein-containing-emulsion is continuously split into its constituent phases by passing the emulsion over both a hydrophobic and a hydrophilic membrane [3]. If the wettability of the hydrophobic membrane changes, phase separation and consequently, continuous operation is impossible [4]. Therefore, protein adsorption has to be prevented.

Protein adsorption is in many cases an entropy-driven process. The unfavourable interactions between water and a (hydrophobic) surface are replaced by protein/surface interactions. Although the protein loses entropy upon adsorption, the entropy gain of the water is large enough for spontaneous adsorption to take place. The conformation of the molecule often changes upon adsorption in order to optimise its attractive contacts with the surface. For hydrophobic surfaces, generally, high adsorbed amounts of proteins are reported [5,6]. Desorption of proteins is difficult [7-9].

Lee [10] and Tan [11] have successfully attempted to prevent protein adsorption by means of steric hindrance by pre-adsorbed block copolymers. It is reported that adsorption of several proteins could be prevented if a hydrophobic polystyrene latex was covered with F108, a tri-block copolymer. Appropriate block copolymers have distinct regions with hydrophilic and with hydrophobic properties. The tri-block copolymers used by Lee and Tan and in this study consist of one hydrophobic poly(propylene oxide) (PPO) anchor group (situated in the middle of the molecule) and two hydrophilic poly(ethylene oxide) (PEO) buoy groups (situated on both ends of the molecule). In general, it can be

concluded that this type of tri-block copolymer adsorbs onto hydrophobic surfaces in a brush conformation [12-14], the poly(propylene oxide) part adsorbs onto the surface, the poly(ethylene oxide) parts are extended into the liquid (see figure 2). The conformation of the thus formed layer is different from that formed by homopolymers having loops and trains at the surface.

The aim of this article is to explain and quantify the effect of protein adsorption on ΔP_{BT}^{min}, the minimum transmembrane pressure at which the water phase breaks through a hydrophobic membrane. The influence of proteins and reaction products on ΔP_{BT}^{min} for unmodified and block copolymer modified membranes will be discussed.

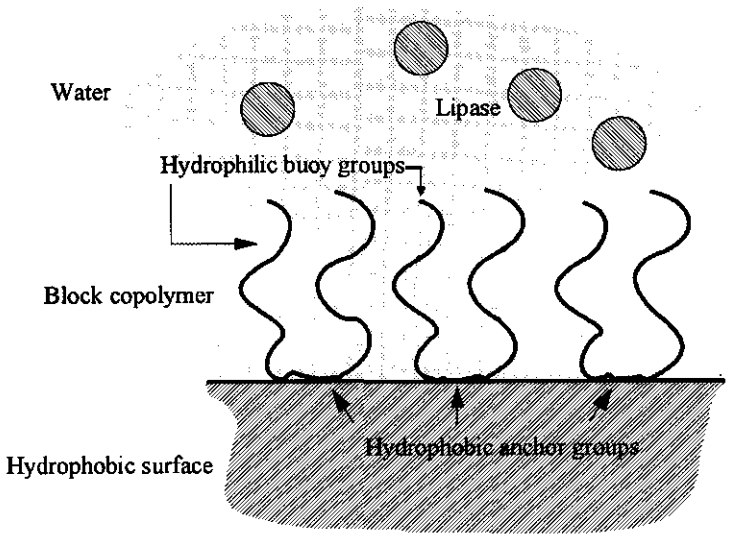


Figure 2. Block copolymers adsorbed onto a hydrophobic surface

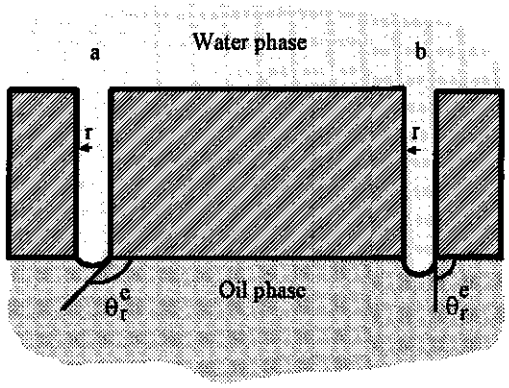
THEORY

Emulsions can be separated by membranes, namely when either the oil phase or the water phase preferentially wets the membrane and passes through it. The other phase will be

rejected. However, if the transmembrane pressure exceeds a certain value, also the non-wetting phase can permeate through the membrane. The minimum pressure at which the water phase can permeate through a hydrophobic membrane will be called the minimum breakthrough pressure, ΔP_{BT}^{min} (Pa). The situation at breakthrough is pictured in figure 3a, for the case of uniform pores. ΔP_{BT}^{min} is equal to the Laplace pressure (ΔP_{lap}), corresponding to R (m), the curvature of the oil/water interface inside the pore, and γ_{ow} the surface tension between oil phase and water phase, (N m^{-1})

$$\Delta P_{BT}^{\min} = \Delta P_{lap} = \frac{2 \cdot \gamma_{ow}}{R} \qquad 1$$

Usually, membranes have a pore size distribution and the largest pore with a radius $r = r_{max}$ determines the Laplace pressure. For cylindrical pores R is equal $r_{max} / sin\ \theta_r^e$, with θ_r^e the receding contact angle of the oil phase on the pore end with respect to the plane of the membrane (see also figure 3a) [15].



$\theta_r^e > 90$: ΔP_{cap}

Figure 3a. Capillary pressure

$\theta_r^e = 90$: $\Delta P_{cap} + \Delta P_{c.a.}$

Figure 3b. Contact angle = 90°; Minimum breakthrough pressure in case of no spreading

In all situations discussed below a membrane is simplified to an array of uniform, cylindrical capillaries. For the purpose of comparison between different wettabilities, this is acceptable. The surface tension between oil phase and water phase is a constant, therefore the Laplace pressure is determined by the capillary radius and the contact angle. The contact angle is in all cases the receding contact angle of the oil phase on the capillary end, θ_r^e. Equation 1 can be rewritten to:

$$\Delta P_{BT}^{\min} = \frac{2 \cdot \gamma_{ow}}{r_{max}} \cdot \sin \theta_r^e \qquad 2$$

In the context of breakthrough, it is the situation at the exit of the pore that determines the value of the minimum breakthrough pressure. Several situations can be distinguished (see figure 3 a-d). At the start, the oil/water interface will be at the pore entrance. When the transmembrane pressure is increased, the oil/water interface is shifted from the entrance of the pore to the exit. This situation is given in figure 3a, the pressure equals the value predicted by equation 2. If no spreading occurs at the end of the capillary then the maximum value of the minimum breakthrough pressure is obtained at $\theta_r^e = 90°$ (figure 3b) and is equal to $(2\ \gamma_{ow} / r_{max})$ [16]. ΔP_{BT}^{min} at 90° is equal to the sum of ΔP_{cap} and $\Delta P_{c.a.}$ ΔP_{cap} is the pressure at a certain contact angle, which is just $(2\ \gamma_{ow}/r_{max})$ x $(\sin \theta_r^e)$ (figure 3a). $\Delta P_{c.a.}$ is the pressure necessary to decrease the contact angle (c.a.) at the capillary end to 90°, in this case equal to $(2\ \gamma_{ow} / r_{max})$ x $(1 - \sin \theta_r^e)$; (figure 3b). When this value is reached the water bubble will grow spontaneously, no extra pressure is required (figure 3c). ΔP_{BT}^{min} is in this case equal to $2\ \gamma_{ow} / r_{max}$.

However, if the liquid spreads at the capillary end (figure 3d, $R_s >> r$), no extra pressure is needed to curve the interface and the value of ΔP_{BT}^{min} is equal to the value needed to press the liquid through the capillary i.e. equal to ΔP_{cap}. The value of ΔP_{cap} can be calculated using equation 2. Keeping in mind that $90° < \theta_r^e < 180°$, equation 2 can be rewritten to

$$\Delta P_{BT}^{\min} = \frac{2 \cdot \gamma_{ow}}{r_{\max}} \cdot (1 - \cos^2 \theta_r^e)^{0.5} \qquad 3$$

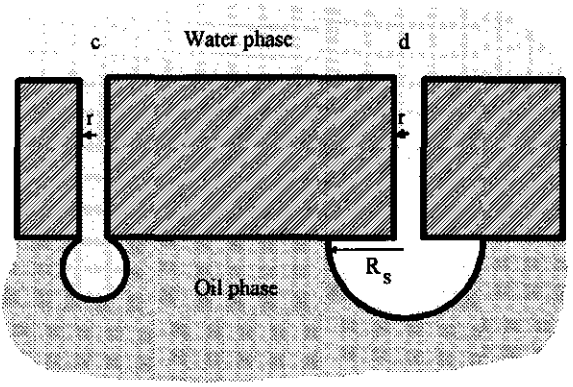
For $\cos \theta_r^e$ Young's law is valid:

$$\cos \theta_r^e = \frac{\gamma_{ws} - \gamma_{os}}{\gamma_{ow}} \qquad 4$$

γ_{ws} is the surface tension between water phase and surface (N m^{-1}) and γ_{os} is the surface tension between oil phase and surface (N m^{-1}). Combining equations 3 and 4 gives:

$$\Delta P_{BT}^{min} = \frac{2}{r_{max}} \cdot [\gamma_{ow}^2 - (\gamma_{ws} - \gamma_{os})^2]^{0.5} \qquad 5$$

Equation 5 is only relevant in case $90° < \theta_r^e < 180°$. θ_r^e can only be lower than 90° after the minimum breakthrough pressure is exceeded (figure 3c), the minimum breakthrough pressure is in this case equal to the value reached at 90°. This also implies that no spreading has taken place otherwise the contact angle could not be decreased to 90°. If θ_r^e is equal to 90° than the situation given in figure 3b is reached, the breakthrough pressure is equal to $2\gamma_{ow}/r_{max}$. If θ_r^e is 180°, the oil phase does not wet the capillary end and the water phase will spread spontaneously and the breakthrough pressure is zero according to equation 2.



$\theta_r^e < 90$: spontaneous growth $\qquad R_s >> r$: spreading

Figure 3c. Spontaneous growth of water droplet

Figure 3d. Spreading

Because $90° < \theta_r^e < 180°$ (spreading) $-\gamma_{ow} < (\gamma_{ws} - \gamma_{os}) < 0$ is also valid. ΔP_{BT}^{min} will have a lower value as compared to the non-spreading case because under non-spreading equation conditions $\Delta P_{BT}^{min} = 2\gamma_{ow}/r_{max}$. In the case of spreading at the capillary end the minimum breakthrough pressure is determined not only by the properties of the emulsion (γ_{ow}) and

the radius (r_{max}) of the capillary but also those of the surface (γ_{ws} and γ_{os}). The normalised minimum breakthrough pressure, ΔP_N, defined as $\Delta P_{BT}^{min}/(2\ \gamma_{ow} / r_{max})$, is given as a function of the receding contact angle in figure 4. As discussed before, there are two regions. For $0° < \theta_r^e < 90°$ the normalised pressure, $\Delta P_N = 1$; for $90° < \theta_r^e < 180°$, $\Delta P_N = sin\ \theta_r^e$. Protein adsorption can cause spreading at the capillary end, therefore, a lower breakthrough pressure as compared to the protein free case is expected.

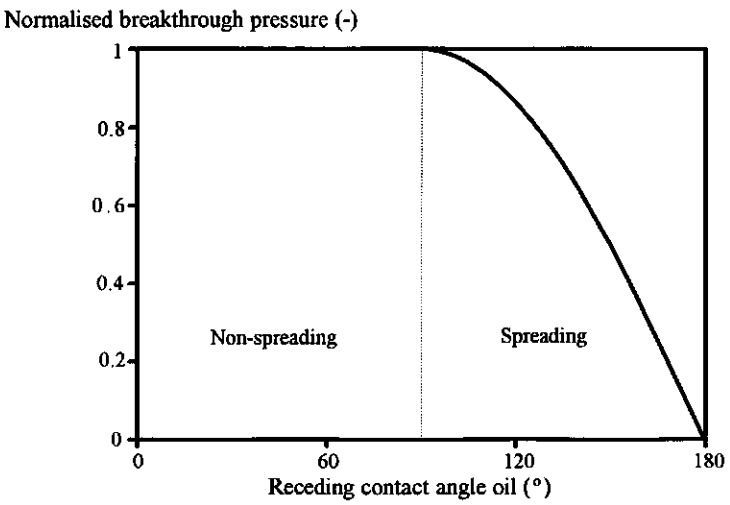


Figure 4. ΔP_{BT}^{min} as a function of the receding contact angle of the oil phase

Although this theory is given here for the idealised case of cylindrical capillaries/pores, similar equations can be derived for tapering capillaries/pores [17]. Also in this case two regions can be distinguished. For the non-spreading region the surface tension between the oil phase and the water phase of the emulsion, γ_{ow}, and the capillary/pore radius determine the minimum breakthrough pressure. In the case of spreading the minimum breakthrough pressure will not only be determined by γ_{ow} and r_{max} but also by the surface properties γ_{os} and γ_{ws}.

MATERIALS

The block copolymers were gifts from ICI (Rotterdam, the Netherlands) and are summarised in table 1. The block copolymers consist of three blocks, one poly(propylene oxide) (PPO) anchor part and two poly(ethylene oxide) (PEO) buoy parts. The anchor parts are similar in molecular weight while the buoy parts vary considerably.

Table 1. Block copolymers used in this study

Trade name	Total molecular weight (Da)	M.W. PEO (buoy) (Da)	M.W. PPO (Anchor) (Da)
L92	3,450	345	2,760
P75	4,150	1,075	2,000
F108	14,000	5,600	2,800

A flat sheet polypropylene membrane (trade name Accurel), provided by Enka (Wuppertal, Germany), with a mean pore radius of 0.05×10^{-6} m (radius largest pores 0.15×10^{-6} m) was chosen as the hydrophobic membrane. The membrane is symmetric and has a nodular structure. The flat sheet membrane was used in a Megaflow module (type TM 100, effective surface area 64×10^{-4} m^2) from New Brunswick Scientific (Edison, USA). The channel height is 1×10^{-3} m.

Sunflower oil of edible quality (tri-esters of glycerol and fatty acids of which over 95% is C16 and C18 acids) was purchased from Smilfood B.V. (Heerenveen, the Netherlands). The enzyme, Lipase B, was obtained from Biocatalysts and originated from the yeast *Candida rugosa* formerly called *Candida cylindracea*). Hexadecane (reagent grade was obtained from Merck (Germany). The polypropylene powder originated from Aldrich (Belgium). The specific surface area of the polypropylene powder is 16 ± 2 m^2 g^{-1}. This value was determined by nitrogen adsorption/ desorption, the powder was not porous. Doubly distilled water was used throughout. Sodium azide, 0.01% (Merck, reagent grade was added to the emulsions in order to prevent microbial growth.

Silicon wafers originated from Wacker Chemitronic GmbH (Germany). Toluene (reagent grade) was obtained from Janssen Chimica (Belgium), dimethyldichlorosilane (reagent grade) was purchased from Merck (Germany).

METHODS

All experiments were carried out at 30 °C.

Wettability experiments

The oxidised silicon plates were derivatised by submerging them in a 1% (w/w) dimethyldichlorosilane solution in toluene during 5 minutes. Subsequently the plates were rinsed with pure toluene. The surface tension of the silicon plates thus treated (27 mN m^{-1}; [18]) is approximately equal to the surface tension of polypropylene, 29 mN m^{-1} [19]. For lipase adsorption at hydrophobic surfaces mainly the hydrophobic interactions determine the adsorbed amount [9]. Polypropylene powder is used in the adsorption experiment, polypropylene membranes are used in all membrane experiments.

The plates were used either directly after derivatisation or after modification with an F108 solution. For modification, the plates were submerged in an aqueous F108-solution of 0.5×10^3 g m^{-3}. After 1 hour the plates were rinsed with water. A modified or unmodified plate was submerged in a horizontal position in either water or a lipase solution with concentration 3×10^3 g m^{-3}. A droplet of sunflower oil (or hydrolysed oil, i.e., the oil phase of the reference emulsion; see membrane experiments paragraph d.) was placed on the upper surface of the submerged plate. Two cases could be distinguished: the droplet of sunflower oil (or hydrolysed oil) wetted the surface or detached from the surface.

Adsorption experiments

Adsorption of lipase was measured by means of a depletion experiment. Polypropylene powder was added to a lipase solution. After 24 hours the remaining concentration of lipase in the solution was determined with a modified Lowry method [20].

Adsorption at modified surfaces was measured by first adding polypropylene powder to a block copolymer solution ($3x10^3$ g m^{-3}). After 24 hours the polymer solution was replaced by a lipase solution. The polypropylene powder was rinsed before bringing it into contact with the lipase solution. After another 24 hours the remaining lipase concentration was determined with the modified Lowry method.

Membrane experiments

I. Emulsion/membrane bioreactor

a. Reaction vessel

Sunflower oil and water with a total volume of $0.5x10^{-3}$ m^3 were emulsified in a stirred vessel (diameter $10x10^{-2}$ m, containing 4 baffles ($12x10^{-4}$ m^2 each). A four bladed standard turbine stirrer (diameter $4.5x10^{-2}$ m) was used at 450 rpm. The emulsion was led over the membrane at $1.67x10^{-6}$ m^3 s^{-1} and the volume of the permeate was determined as a function of time and pressure. Both permeate and retentate were recycled to the emulsion vessel.

b. Addition of lipase

An emulsion of sunflower oil and water (1:1 v/v) was mixed in a vessel as described above. The crude lipase preparation was dissolved in water and centrifuged at 13,000 rpm (Heraus, biofuge A). The sediment was discarded; only the supernatant was used in experiments. The emulsions used in the ΔP_{BT}^{min} experiments contained $3x10^3$ g (lipase) m^{-3}.

c. Membrane modification with block copolymers

Membrane modification was carried out as follows: the membrane was pre-wetted by rinsing with hexadecane for 30 minutes; hexadecane permeated through the membrane. It was then treated with an emulsion (1:2 v/v hexadecane in water) containing $6x10^3$ gram block copolymer per m^3 of emulsion. This emulsion was pumped along the retentate side of the hydrophobic membrane for 15 minutes. Only hexadecane permeated through the membrane. Both the permeate and the retentate were recycled to the stirred emulsion

vessel. It was subsequently rinsed with water for 15 minutes; water did not permeate through the membrane. Finally the membrane was rinsed with a sunflower oil/water emulsion (1:1 v/v) for 15 minutes, during which sunflower oil permeated through the membrane. For all the rinsing steps the respective liquid was pumped over the membrane at the retentate side, the pump capacity was $1.67 \times 10^{-6} m^3 s^{-1}$.

d. Determination of the minimum breakthrough pressure for emulsions without lipase

The minimum breakthrough pressure of the hydrophobic membrane was determined with an emulsion (1:1 v/v) in which 95% of the oil phase consisted of fatty acids and no lipase was present. This emulsion was prepared as follows: Lipase was added to a sunflower oil in water emulsion in the stirred vessel. The lipase starts hydrolysing the sunflower oil. After 2 hours an equilibrium composition is reached in the emulsion. The oil phase now consists of 95% fatty acids and small amounts of mono-, di- and triglycerides. The water phase consists of water and approximately 10% (w/w) glycerol.

The thus formed emulsion is separated with a Sorval RC 5B Superspeed Centrifuge (15,000 rpm). The oil phase was put in a stirred vessel together with a water/glycerol mixture. The composition of this emulsion was equal to the equilibrium composition of the emulsion with lipase, however, now the lipase is no longer present. This emulsion is called the reference emulsion. The emulsion was led over an unmodified membrane and the transmembrane pressure was increased gradually. When water permeated through the membrane the ΔP_{BT}^{min} was reached. After ΔP_{BT}^{min} was reached the permeate turned opalescent, the water content was approximately 1% (w/w). The maximum water solubility of sunflower oil is 0.2% (w/w). After 5 minutes at ΔP_{BT}^{min} visible (by eye) water droplets permeated through the membrane.

For the reference emulsion a ΔP_{BT}^{min} of 0.5×10^5 Pa was measured during 3 consecutive days. The same experiment was carried out with an F108-modified membrane. Again 0.5×10^5 Pa was found for ΔP_{BT}^{min}.

e. Determination of minimum breakthrough pressure for lipase-containing emulsions

As mentioned before, the minimum breakthrough pressure for the reference emulsion was $0.5x10^5$ Pa. The minimum breakthrough pressure was also measured as a function of time with an emulsion (1:1 v/v) containing $3x10^3$ grams lipase per m^3 of emulsion for both modified and unmodified membranes. The minimum breakthrough pressure for the F108-modified membrane was determined as follows. First, the transmembrane pressure was gradually increased from $0.06x10^5$ Pa up to a maximum of $0.5x10^5$ Pa. The transmembrane pressure was then kept constant at $0.5x10^5$ Pa for 10-15 minutes. It was noted whether or not the water permeated through the membrane. Subsequently the transmembrane pressure was decreased to $0.06x10^5$ Pa. Every other day such an experiment was performed with the F108-modified membrane. For the unmodified membrane, the L92-modified and the P75-modified membrane, the transmembrane pressure was kept as low as possible during the experiments, $0.03x10^5 \pm 2x10^2$ Pa, to prevent water permeation at an early stage of the experiment. At the time given in table 3, the transmembrane pressure was gradually increased. The transmembrane pressures indicated in table 3 are those at which the water phase was observed to start permeating through the membrane.

II. Membrane bioreactor

The membrane bioreactor used in this study is comparable to the reactor described by Hoq [21]. Lipase is immobilised onto a hydrophobic membrane. A polypropylene membrane with an average pore radius of $0.05x10^{-6}$ m (Enka, Accurel) is used. On one side of the membrane an oil phase is circulated, on the other side a water phase is circulated. The oil phase consists of triglycerides, diglycerides, monoglycerides and fatty acids. The water phase contains water and glycerol (See also figure 7).

ANALYSIS

a. Determination of degree of hydrolysis

The degree of hydrolysis in the oil phase of the emulsion was determined by dissolving a sample of approximately 1 g oil phase in 20×10^{-6} m^3 of a phenolphthalein in ethanol solution followed by titration with 0.1×10^3 mol m^{-3} NaOH.

b. Measurement of γ_{ow}

An overflowing cylinder device [22] was used to measure the surface tension between the oil phase and the water phase. A layer of sunflower oil was put on top of the water phase already present in the device. A Wilhelmy plate was brought into contact with the oil/water interface and the surface tension was measured as a function of time in both the absence and presence of lipase. The interface was not expanded in case lipase was present.

c. Determination of water content permeate

The water content of the permeate was determined by Karl Fisher titration.

RESULTS AND DISCUSSION

Wettability experiments

The wettability of derivatised silicon plates was tested qualitatively after they had been in contact with water or lipase solution. A distinction was made between cases where an oil droplet wetted (W) the surface or detached (D) from the surface. The oil droplets were either sunflower oil or hydrolysed oil containing 95% (w/w) fatty acids. The results are given in table 2.

As expected, a hydrophobic surface is wetted by oil as well as by hydrolysed oil. However, after lipase adsorption a hydrophobic surface is neither wetted by oil nor by hydrolysed oil. Obviously, the adsorbed lipase makes the surface hydrophilic and therefore the sunflower oil droplets and the hydrolysed oil droplets detach from the surface. This is

a clear indication that the influence of lipase adsorption is more important than the presence of reaction products.

Table 2. Wettability of hydrophobic surfaces, (W) = Wetting; (D) = Detachment

	Bare surface without lipase	Bare surface plus lipase
Sunflower oil	W	D
Hydrolysed oil	W	D
	F108-modified surface without lipase	**F108-modified surface plus lipase**
Sunflower oil	W	W
Hydrolysed oil	W	W

F108-modified surfaces are wetted by sunflower oil and hydrolysed oil in both the presence and the absence of lipase. The contact angle for hydrolysed oil at a bare surface is only slightly (2°) higher than at the bare surface. This is also the case for sunflower oil. This proves that the wettability of block copolymer coated surfaces is hardly influenced by the presence of the block copolymers. Due to the enzymatic hydrolysis, the sunflower oil will be converted into hydrolysed oil, hence, the contact angle decreases to the contact angle for hydrolysed oil.

Adsorption experiments

Adsorption of lipase was measured onto block-copolymer-modified and unmodified surfaces in order to select suitable block copolymers. The block copolymers form a

monolayer on the surface (Schroën, to be published). In figure 5 the adsorbed amount of lipase is given as a function of the equilibrium concentration of lipase in solution.

At an unmodified (polypropylene) surface lipase adsorption exceeds several times monolayer coverage, which is about $4x10^{-3}$ g crude lipase per m^2 polypropylene. For the block- copolymer-coated polypropylene powder the adsorbed amount is less. Apparently, the block copolymer hinders protein adsorption. For the P75-modified powder an adsorbed amount approximately corresponding to that of monolayer coverage is found. At the F108-modified powder adsorption is nearly absent. This is in agreement with the findings of Lee [10] and Tan [11] for human serum albumin, fibrinogen, immunoglobulin and whole plasma. The explanation might be that the buoy groups of F108 cause a steric hindrance that keeps lipase away from the surface. The steric hindrance provided by the smaller buoy groups of P75 is apparently not strong enough. Only F108 can be of use for the prevention of lipase adsorption.

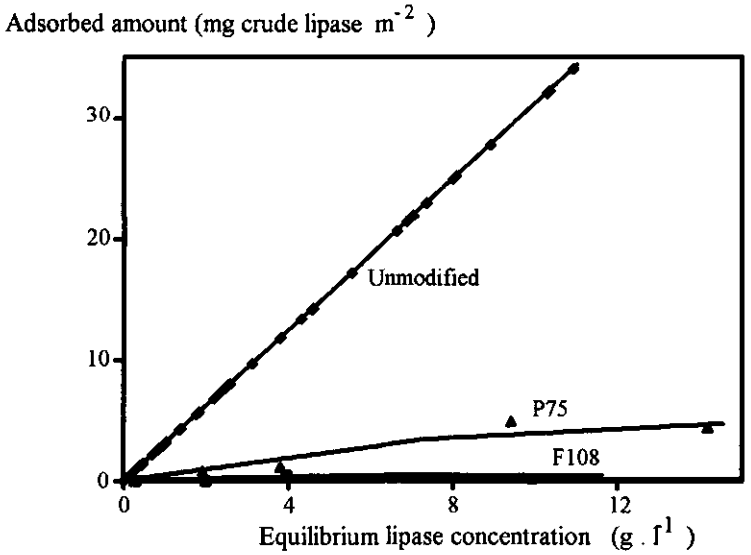


Figure 5. Adsorption of lipase onto modified and unmodified polypropylene powder.

Minimum breakthrough pressure for emulsions without lipase

When the reference emulsion, containing 95% fatty acids in the oil phase and no lipase, was used the minimum breakthrough pressure was determined to be $0.5x10^5$ Pa. The theoretical minimum breakthrough pressure in case of no spreading can be calculated with equation 1. The surface tension between the oil phase and the water phase, γ_{ow}, in the reference emulsion is $4.5x10^{-3}$ N m^{-1}. The radius of the biggest pore is $0.15x10^{-6}$ m (manufacturer data) and is in this case equal to 90°. This leads to a value of $0.6x10^5$ Pa for ΔP_{BT}^{min}. The theoretically calculated and the measured minimum breakthrough pressure are quit similar. The 20% difference might be explained by a non-cylindrical pore shape or a slightly bigger maximum pore radius of the membrane. It is important to notice that the presence of reaction products (although these lower γ_{ow} substantially) does not result in wetting of the membrane. If wetting would occur, the minimum breakthrough pressure would decrease dramatically.

Influence of lipase on the minimum breakthrough pressure for unmodified membranes

For lipase-containing emulsions completely different results were obtained. After 2 days both the oil phase and the water phase could permeate through the membrane even at a transmembrane pressure of $0.03x10^5$ Pa.

The surface tension of a sunflower oil-in-water emulsion without lipase is equal to 23 mN m^{-1}. Two hours after addition of lipase when 95% of the oil phase consists of fatty acids the surface tension, γ_{ow} is reduced to 4.5 mN m^{-1}. Because the value of this γ_{ow} is equal to the γ_{ow} of the reference emulsion (without lipase) it can be concluded that γ_{ow} is not directly influenced by the presence of lipase but by the reaction products formed by lipase. Therefore, the value of ΔP_{BT}^{min} should be equal to ΔP_{BT}^{min} of the reference emulsion. This means that the presence of lipase must have caused the decrease in breakthrough pressure. The mechanism can be that the wettability of the membrane changed as a consequence of

protein adsorption resulting in a decrease in minimum breakthrough pressure. Such a change in wettability was indeed observed in the wettability experiments.

It takes about 1 day after lipase addition, before water permeation through the membrane was observed. Initially, lipase is not present at the permeate side of the membrane and in the pores. It takes some time before the lipase can reach the permeate side of the membrane to cause spreading of the water phase resulting in the decrease in minimum breakthrough pressure. A tentative explanation is the following.

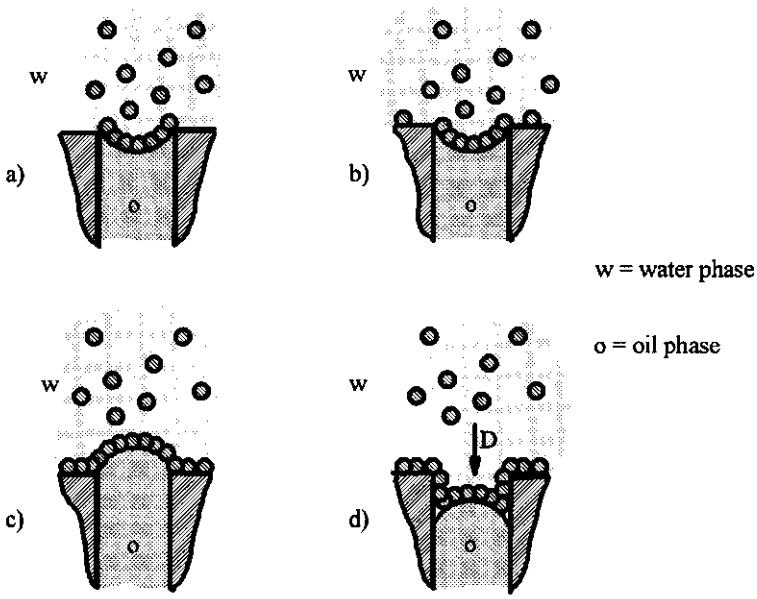


Figure 6. Adsorption lipase in pores

Lipase is normally only present at the oil/water interface and in the water phase. Because water is the continuous phase in the emulsion, lipase can freely come into contact with the upper surface of the membrane (figure 6a+b). When the membrane is covered with lipase

(figure 6c) the water phase will wet the protein covered surface. (See also wettability experiments.) The water phase starts to penetrate into the pore, however, the oil phase is still present there (figure 6c). The pore surface onto which no lipase adsorption has taken place yet, is wetted by the oil phase and cannot be replaced by the water phase unless lipase adsorption takes place.

If lipase adsorbs at the 3-phase contact line as indicated in figure 6d than the contact angle will increase and consequently the oil phase will recede. The velocity at which the receding takes place depends on the transport rate of the lipase to the 3-phase contact line and the adsorption rate onto the pore surface.

For lipase it is known that adsorption takes place rapidly [9], therefore, only transport by diffusion will be considered. The transport is diffusion limited, therefore, a time can be estimated in which the lipase will reach the permeate side of the membrane. In equation 6 Fick's law is given.

$$J = D \cdot \frac{dC}{dx} \approx D \cdot \frac{\Delta C}{\Delta x} \qquad 6$$

J is the flux of lipase through a plane perpendicular to the direction of diffusion (mol m^{-2} s^{-1}), D is the Fickian diffusion coefficient (m^2 s^{-1}), ΔC the concentration difference (mol m^{-3}) and Δx the distance over which the concentration difference exists (m).

Assuming an adsorbed amount of 4×10^{-3} g m^{-2}, a pore radius (r_{max}) of 0.15×10^{-6} m, a pore tortuosity of 4, a diffusion coefficient of 10^{-10} m^2 s^{-1}, a concentration difference (ΔC) of 1.5×10^{2} g m^{-3} (lipase content of the crude lipase preparation is 5%; manufacturer data) and a membrane thickness of 100×10^{-6} m (manufacturer data) a "breakthrough time" for lipase can be calculated. A value of 16 hours is found. This value is in the same order of magnitude as the breakthrough time which was measured during the membrane experiments. Although both values are in the same order of magnitude, the calculated value has to be treated with care. The diffusion coefficient of lipase is an approximated value and the tortuosity is not known for this membrane, although a value of 4 seems reasonable. Furthermore, the lipase concentration profile is considered to be linear,

therefore, the calculated time is only an indication of the time scale of the diffusion process.

It seems reasonable to presume that diffusion of lipase is the rate limiting step for breakthrough of the water phase. Hence, the minimum breakthrough pressure of a membrane covered with lipase has to be very low from the start of the experiment. From literature a comparable situation is known, a membrane bioreactor where the lipase is immobilised onto the membrane. When a microfiltration membrane is used in the bioreactor, the lipase is present in the membrane pores and also on both sides of the membrane. On one side of the membrane a water phase is circulated on the other side an oil phase is circulated (figure 7) [21]. The oil phase in the membrane reactor consist of fatty acids, monoglycerides, diglycerides and triglycerides, the water phase consists of water and glycerol. The composition of oil phase and water phase is comparable to the composition of the oil phase and the water phase in the emulsion/membrane bioreactor.

Hoq [21] reported that for hydrolysis of olive oil in a membrane bioreactor the transmembrane pressure over the polypropylene membrane ($0.2x10^{-6}$ m average pore radius) should not exceed $0.02x10^{5}$ Pa otherwise the oil phase and the water phase could not be kept separated. This value is comparable to the minimum breakthrough pressure found in this study for separation of an emulsion. In case of a contact angle of 90° for the membrane used by Hoq a minimum breakthrough pressure of $0.4x10^{5}$ Pa can be calculated.

The polypropylene membrane (used also in the emulsion separation experiments) was used as an immobilised enzyme membrane bioreactor for the hydrolysis of sunflower oil. Lipase was immobilised onto the membrane by rinsing a lipase solution through the membrane. After 15 minutes the lipase solution was replaced by sunflower oil at one side of the membrane and water at the other side of the membrane (figure 7). The transmembrane pressure had to be kept low ($0.02x10^{5}$ Pa) as soon as the lipase solution was replaced. This is in accordance with the assumption that the lipase is responsible for

the breakthrough of the phases at a low transmembrane pressure. In case of the immobilised enzyme membrane bioreactor diffusion is not needed and an instantaneous effect can be expected. Vaidya [17] reported that membrane selection is important for successful operation of membrane reactors. It was stated that the reaction products determine the breakthrough pressure or in our case the minimum breakthrough pressure. This is in contrast to our results. For the reference emulsion without enzyme (γ_{ow} = 4.5 mN m^{-1}) a ΔP_{BT}^{min} of 0.5×10^5 Pa is found. For emulsions with enzyme (γ_{ow} = again 4.5 mN m^{-1}) a lower ΔP_{BT}^{min} is found, indicating that the enzyme and not the reaction product is responsible for the decrease in ΔP_{BT}^{min}.

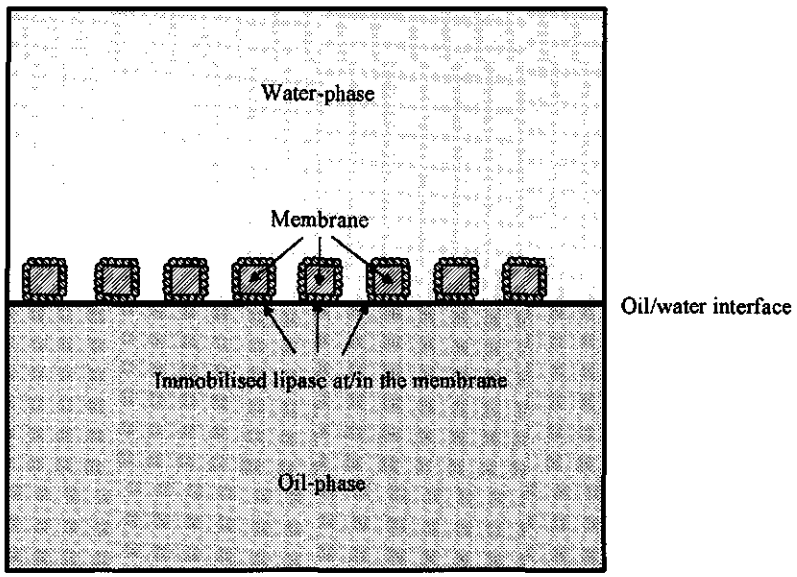


Figure 7. Membrane bioreactor

Vaidya [17] states that ultrafiltration membranes have higher ΔP_{BT}^{min} values than microfiltration membranes because of their pore size. From the results reported here, it is mainly the presence of an adsorbed protein layer that influences the wettability of the

membrane, and therewith ΔP_{BT}^{min}. Yet, the pore size can be important because it can influence the wettability in the following way: Proteins cannot permeate through most ultrafiltration membranes, thus they will not adsorb to the pore surface and would be expected to have no affect on either the wettability or ΔP_{BT}^{min}. If no wettability change takes place equation 2 is valid at $\theta_r^e = 90°$ and therefore, for ultrafiltration membranes only the pore radius and the surface tension between oil phase and water phase are important. However, this in only valid if the proteins cannot permeate through the membrane.

If other surface active substances (molecular weight below cut-off value), that influence the wettability of the membrane are present in an emulsion, than again a lower ΔP_{BT}^{min} is expected. Keurentjes [23] shows that sodium oleate (soap) adsorption can lead to very low ΔP_{BT}^{min} values. Only if this adsorption is prevented than a high value for ΔP_{BT}^{min} was found. Keurentjes [23] avoided sodium oleate adsorption by adjusting the surface tension of the membrane.

Minimum breakthrough pressure for modified membranes

The hydrophobic membrane was modified with three block copolymers (see table 1). The length of the anchor blocks is comparable, the buoy groups differ considerably. All block copolymers adsorb in a monolayer fashion onto hydrophobic surfaces. It was previously shown that only F108 (largest buoy groups) is capable of preventing protein adsorption. It might be expected that P75 and L92, both having smaller buoy groups, are not capable of preventing protein adsorption because the steric hindrance is presumably not big enough.

In table 3 the results for several modified membranes are summarised in terms of the time and the pressure at which both the water phase and the oil phase could permeate through the membrane.

Water can permeate through the L92-modified and the P75-modified membrane at the indicated time and pressure. Apparently, protein adsorption could not be prevented by these block copolymers. It should be noted that the P75-modified membrane shows an

enhanced performance as compared to the unmodified membrane but yet showed permeation after 5 days. The F108-modified membrane had a minimum breakthrough pressure that was larger than 0.5×10^5 Pa (see methods) even after 14 days of continuous operation. ΔP_{BT}^{min} was measured as a function of time, the results are given in figure 8.

Table 3. Minimum breakthrough pressure of membranes.

Membrane	Time (days)	Minimum breakthrough pressure (10^5 Pa)
Unmodified	2	0.03
With L92	2	0.04
With P75	5	0.08
With F108	> 14	>0.5

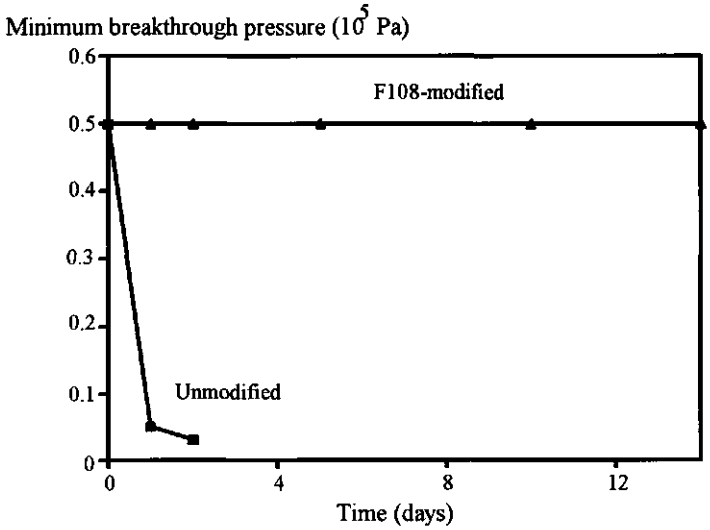


Figure 8. ΔP_{BT}^{min} as a function of time for an F108-modified and unmodified membrane

ΔP_{BT}^{min} did not change during the experiment. Therefore, it can be assumed that no protein adsorption has taken place, otherwise water would have been capable of permeating through the membrane already at a lower transmembrane pressure. This in accordance with the data found in the adsorption experiments (figure 5). The F108-modified membrane is suitable for application in the emulsion/membrane bioreactor.

CONCLUSIONS

The minimum breakthrough pressure of a membrane, ΔP_{BT}^{min}, is the minimum pressure at which the water phase of an emulsion can permeate through a hydrophobic membrane. The value of ΔP_{BT}^{min} is determined by the surface tension between the oil phase and the water phase of the emulsion (γ_{ow}), the pore radius (r) and the receding contact angle of oil at the end of the pore (θ_r^e). Two cases can be distinguished:

$$\Delta P_{BT}^{\min} = 2 \cdot \frac{\gamma_{ow}}{r_{\max}} \qquad 0 < \theta_r^e < 90^o \qquad \text{Non-spreading}$$

$$\Delta P_{BT}^{\min} = 2 \cdot \frac{\gamma_{ow}}{r_{\max}} \cdot \sin\theta_r^e \qquad 90^o < \theta_r^e < 180^o \qquad \text{Spreading}$$

With lipase-free emulsions, ΔP_{BT}^{min} for polypropylene membranes was always in the non-spreading range (0.5×10^5 Pa). Lipase adsorption influences the wettability of hydrophobic surfaces and membranes. This was supported by two findings: (i) a derivatised silicon wafer was not wetted by oil after lipase adsorption had taken place. (ii) after addition of lipase ΔP_{BT}^{min} of the polypropylene membrane decreased to 0.03×10^5 Pa. It was proven that lipase adsorption was responsible for the observed decrease in ΔP_{BT}^{min} and not the reaction products.

The effect of lipase adsorption onto the membrane was suppressed when the membrane was modified by block copolymers. From direct adsorption experiments it could be concluded that the F108-modified polypropylene powder had no/little lipase adsorption. For a membrane modified with block copolymer F108 a constant value for ΔP_{BT}^{min} was

found during an experiment lasting 14 days. This indicates that the wettability of this membrane did not change during the experiment. It was concluded that no/little protein adsorption takes place at the F108-modified membrane during the experiment.

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LIST OF SYMBOLS AND ABBREVIATIONS

o	: Oil phase	
r	: Pore radius(m)	
r_{max}	: Radius of largest pore	(m)
w	: Water phase	
D	: Diffusion coefficient	($m^2\ s^{-1}$)
J	: Lipase flux	(mol $m^{-2}\ s^{-1}$)
R	: Curvature	(m)
R_s	: Curvature for spreading conditions	(m)
γ_{os}	: Surface tension between oil phase and surface	(N m^{-1})
γ_{ow}	: Surface tension between oil phase and water phase	(N m^{-1})
γ_{ws}	: Surface tension between water phase and surface	(N m^{-1})
θ_r^e	: Receding contact angle	(°)
ΔC	: Concentration difference	(mol m^{-3})
ΔP_{BT}^{min}	: Minimum breakthrough pressure	(Pa)
$\Delta P_{c.a.}$	: Pressure necessary to reach contact angle of 90°	(Pa)
ΔP_{cap}	: Pressure at a certain contact angle	(Pa)
ΔP_{lap}	: Laplace pressure	(Pa)
ΔP_N	: Normalised breakthrough pressure	(-)
Δx	: Diffusion distance	(m)

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Chapter 4

WETTABILITY OF TRI-BLOCK COPOLYMER COATED HYDROPHOBIC SURFACES

-Predictions and measurements-

ABSTRACT

Hydrophobic surfaces with adsorbed tri-block copolymers are wetted by oil in spite of the hydrophilic buoy groups of the block copolymer that are present near the surface. The effect of the buoy group length of the adsorbed molecules on the wettability of hydrophobic surfaces is studied by contact angle measurement and by computer modelling.

The computer model predicts an increase in interfacial free energy with increasing buoy group length for equilibrium adsorption of block copolymer from water. Molecules with large buoy groups occupy more lateral space; therefore the "bare" surface gets more exposed and the anchor groups contribute less to the interfacial free energy which thus increases with the buoy group length.

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Wettability of tri-block copolymer coated hydrophobic surfaces - Predictions and Measurements -

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Colloids and Surfaces A. Physiochemical and Engineering Aspects, 90: 235.

The calculations showed that the variation of the interaction parameter between solvent and buoy group hardly influences the interfacial free energy. In contrast the interaction parameter between solvent and surface influences the interfacial free energy to a large extent because the oil/surface interactions have a lower energetic value as compared to water/surface and therefore the interfacial free energy is lower than in water. The interfacial free energy varies slightly with increasing buoy group length, depending on the value chosen for the solvent/ surface interaction parameter.

Advancing and receding contact angles of hexadecane, sunflower oil and hydrolysate (partly hydrolysed sunflower oil) were measured on hydrophobic surfaces. All oil/water contact angles were small, indicating a hydrophobic apolar surface character. It was found that, for oils with a "good" interaction with the surface (hexadecane and sunflower oil), the contact angle has a minimum value at a certain buoy group length. For hydrolysate (less strong interaction with the surface) the contact angle decreases monotonically with increasing buoy group length. The results for hexadecane, sunflower oil and hydrolysate are in reasonable agreement with the model predictions. The effect of increasing buoy group length is weak; both decreasing and increasing angles are found, depending on the type of oil used.

INTRODUCTION

Hydrophobic membranes can be used for selective separation of oil from an emulsion [1, 2]. If an oil droplet comes into contact with the membrane it will easily permeate, provided it wets the membrane. If the membrane is hydrophobic, only the oil phase will permeate through the membrane provided the transmembrane pressure does not exceed the Laplace pressure associated with the membrane [3].

If proteins (e.g., lipase) adsorb onto the hydrophobic membrane, a hydrophilic protein layer is formed on the surface [4]. The oil droplets cannot wet such a hydrophilic layer.

As a consequence the membrane will become water permeable, so that the selectivity on which separation is based is lost.

In a preceding study we found that a hydrophobic polypropylene membrane can be modified with a suitable block copolymer in such a way that protein adsorption, and the associated wettability change are suppressed [3,5]. This block copolymer consists of one hydrophobic, poly(propylene oxide) (PPO), anchor group and two hydrophilic, poly(ethylene oxide) (PEO), buoy groups. Suppression of (protein) adsorption by the same polymer is also found by Lee [6] and Tan [7], for several proteins on polystyrene latex.

The effectiveness with which protein adsorption is suppressed increases with buoy group length [3]. Halperin and de Gennes [8] have calculated the effect of a brush on wettability. Their results suggest that a hydrophobic surface with either adsorbed or grafted hydrophilic polymer will become hydrophilic. It is known from various publications that proteins adsorb less on hydrophilic surfaces and this may seem to explain the suppression of protein adsorption [4]. However, in contradiction to this, the block copolymer modified hydrophobic membrane remains oil wetted. In spite of the presence of the hydrophilic groups of the polymer, the membrane is still hydrophobic.

The aim of this article is to explore the influence of adsorbed block copolymers on the wettability of a hydrophobic surface, with special attention to the buoy group length. This was done experimentally by contact angle measurements for three different oils and buoy group lengths of 0, 8, 21 and 128 monomers. Also theoretical calculations were made using a self-consistent field theory for polymer adsorption [9-12], the buoy group length being varied between 0 and 150 monomers.

THEORY

Contact angle and spreading tension

The equilibrium contact angle, $\theta_{1,surf}$ (°), between a liquid/liquid interface (formed by phases 1 and 2) and a solid surface (figure 1) satisfies Young's law

$$\cos\theta_{1,surf} = \frac{\gamma_{2,surf} - \gamma_{1,surf}}{\gamma_{1,2}} \qquad (1)$$

where, $\gamma_{1,surf}$ and $\gamma_{2,surf}$ (N m^{-1}) are the interfacial surface tensions between the solid surface and phases 1 and 2 respectively, and $\gamma_{1,2}$ (N m^{-1}) is the interfacial tension between phase 1 and 2 [13].

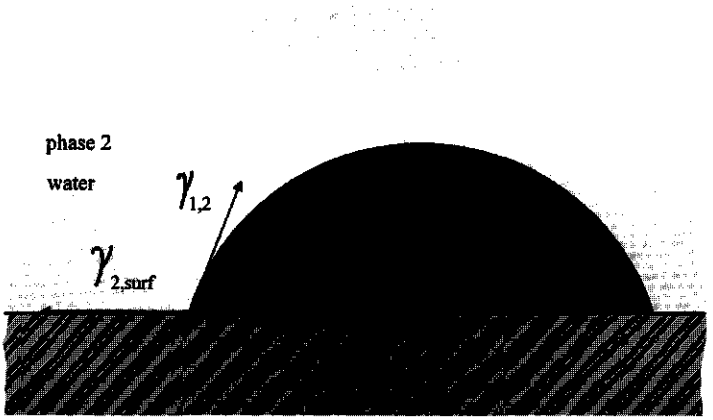

Fig 1. Interfacial free energies at the three phase contact point

The spreading tension, S (N m^{-1}), is a parameter which is often used to characterise wettability of a surface. It is defined as:

$$S = \gamma_{2,surf} - \gamma_{1,surf} - \gamma_{1,2} \qquad (2)$$

For a system of one solid and two liquids, three cases can be distinguished. (i) If $S \geq 0$, phase 1 will spread over the surface, (ii) if $-1 < S < 0$, there is a finite contact angle and (iii) if $S \leq -1$, phase 2 will spread [13].

Block copolymer molecules at the solid surface will influence $\gamma_{1,surf}$ and $\gamma_{2,surf}$. Therefore, an influence on the contact angle and spreading tension is expected. A theoretical evaluation of the effect of adsorbed block copolymers on $\gamma_{1,surf}$ and $\gamma_{2,surf}$ is made in the computer calculations section.

Halperin and de Gennes [8] have discussed some aspects of the wettability of polymer-covered surfaces. Phase 1 is in their case air, phase 2 is a solvent for the polymer. The polymers (denoted by subscript p) were either grafted (denoted by superscript g; see equations 3 and 4) or physically adsorbed (denoted by superscript f, see equations 5 and 6). For the grafted case they find:

$$S^{g}_{2,\,surf} = S^{g}_{0} + k \cdot T \cdot \Gamma \cdot [\,(1-\chi) - \frac{1}{2} \cdot \phi \cdot (1-2\chi) + ...] \qquad (3)$$

with

$$S^{g}_{0} = \gamma_{p,2} - \gamma_{1,2} + (\gamma_{surf,\,p} - \gamma_{surf,\,1}) \cdot (1-\sigma) \qquad (4)$$

where, χ is the dimensionless Flory-Huggins interaction parameter between polymer and solvent, ϕ the monomer volume fraction (-), k the Boltzmann constant (J K^{-1}), T the temperature (K), Γ the total number of monomers per unit area (m^{-2}), γ the surface tension between the two components denoted in the subscript (N m^{-1}) and (1-σ) is a factor describing the reduction in solid/liquid interactions due to the grafted sites (-). For physisorbed polymers Halperin and de Gennes [8] propose

$$S^{f}_{2,\,surf} = S^{f}_{0} + \frac{k \cdot T}{D^{2}} \qquad (5)$$

with

$$S^{f}_{0} = \Gamma \cdot a^{2} \cdot (\gamma_{surf,\,p} + \gamma_{p,\,2} - \gamma_{surf,\,2}) + \gamma_{surf,\,2} - \gamma_{surf,\,1} - \gamma_{1,2} \qquad (6)$$

with D the average distance between adsorbed polymers (m) and a the mesh size of the appropriate Flory-Huggins lattice (m).

If the chains are made longer (while the grafting density, 1-σ, is constant), Γ increases proportionally. Equation 3 predicts that in this case $S^{f}_{2,surf}$ increases as $kT\Gamma$ (1-χ) (if ϕ is small), i.e., linearly with chain length. This results in a higher spreading tension for phase 2 and eventually spreading of this phase. For strong adsorption Halperin and de Gennes state that $S^{f}_{1,surf}$ is dominated by the kT/D^2 term. D increases with increasing chain length, and therefore an increase in $S^{f}_{2,surf}$ is expected; the situation is comparable to that for grafted molecules. For weak adsorption, Halperin finds $S^{f}_{2,surf}$ S^{f}_{0}. In that case, $S^{f}_{2,surf}$ increases linearly with Γ [8]. Scheutjens finds that Γ increases with the chain length [14]; it can therefore be expected that $S^{f}_{2,surf}$ also increases with the chain length.

Block copolymers constitute a different case. The number of adsorbed chains may vary with chain length, but the role of the adsorbing anchor block should also be considered. This can be done by using more detailed numerical theory as discussed in the computer calculations section.

For both grafting and physical adsorption as described by Halperin and De Gennes [8] the first phase is air. Air is a 'poor solvent' for the polymer, and therefore the polymer layer will collapse onto the surface if the polymer is not in contact with the liquid phase. This effect might also be important if the second phase is a liquid non-solvent instead of air. Depending on the solvent quality the polymer will swell or collapse. This effect can be simulated using the numerical theory mentioned in the computer calculations paragraph.

Gibbs equation

The Gibbs adsorption equation relates changes in the interfacial free energy ($d\gamma$, kT per site) to the excess number of moles (n; mol) and changes in the chemical potentials ($d\mu$, kT per mole) of the components in the interface [13].

$$\Sigma n_i \cdot d\mu_i + A \cdot d\gamma = 0 \qquad (7)$$

It is convenient to divide equation 7 by the interfacial area A (m^2) and replace n_i/A by Γ_i, the adsorbed amount (mol m^{-2}). Let us now consider a two-component system. By choosing a reference plane in such a way that the surface excess of one component (say, 1) becomes zero, equation 7 can be replaced by [13]

$$d\gamma = -\Gamma_2 \cdot d\mu_2 \qquad (8)$$

To predict the influence of the buoy group length l of adsorbed block copolymers we need to know $d\gamma / dl$. We can rewrite this as $(d\gamma / d\mu_2) \cdot (d\mu_2 / dl)$. This can be rewritten with equation 8 as

$$\frac{d\gamma}{dl} = -\Gamma_2(l) \cdot \frac{d\mu_2}{dl} \qquad (9)$$

Where $\Gamma_2(l)$ is always positive (adsorption) although decreasing with l. Hence, the sign of $d\gamma / dl$ will be determined by $d\mu_2 / dl$. This factor can be either positive or negative, depending on the case considered. For simple homopolymers at fixed concentration it will be positive: longer chains are less soluble. For block copolymers, however, it can be negative: a longer soluble block enhances the solubility.

MATERIALS

The block copolymers were gifts from ICI (Rotterdam, the Netherlands and Everberg, Belgium) and are summarised in table 1. The block copolymers consist of three blocks, one poly(propylene oxide) (PPO) anchor part and two poly(ethylene oxide) (PEO) buoy parts. The anchor parts of L92, P94 and F108 are similar in molecular weight while the buoy parts vary considerably. PPO with molecular weights of 2,000 and 3,000 was purchased from Janssen Chimica (Beerse, Belgium). Some data on the polymers are given in table 1.

Table 1. Polymers used in this study

Polymer	Total M.W. (Da)	M.W. PPO (Da)	M.W. PEO (one buoy group) (Da)
PPO	2,000	2,000	------
PPO	3,000	3,000	------
L92	3,450	2,760	345
P94	4,600	2,760	920
F108	14,000	2,800	5,600

Sunflower oil of edible quality (triesters of glycerol and fatty acids of which over 95% is C16 and C18 acids) was purchased from Smilfood B.V. (Heerenveen, the Netherlands). Hexadecane (analytical grade) originated from Merck (Germany). Demineralised water was used throughout.

The enzyme, Lipase B, was obtained from Biocatalysts and originated from the yeast Candida rugosa (formerly called Candida cylindracea). The enzyme was first dissolved in water and subsequently centrifuged at 13,000 rpm (Heraus, Biofuge A). Only the supernatant was used in the experiments; the sediment was discarded.

Highly pure silicon wafers of the Czochralsky-type were purchased from Wacker Chemitronic GmbH (Germany). Toluene (analytical reagent grade) was obtained from Janssen Chimica (Belgium), dimethyldichlorosilane (analytical reagent grade) was purchased from Merck (Germany).

METHODS

The experiments were performed at 20 ± 2 °C.

Preparation of hydrolysate

Lipase was added to a sunflower oil in water emulsion (1:1 v/v) in a stirred vessel. The lipase catalyses the hydrolysis of sunflower oil into fatty acids and glycerol. After 2

hours equilibrium is reached in the emulsion. At equilibrium, the oil phase consists of 95% fatty acids and 5% of a mixture of monoglycerides, diglycerides and triglycerides. The aqueous phase contains 10 wt% glycerol. The emulsion thus formed is separated with a Sorval RC 5B Superspeed Centrifuge (15,000 rpm). The oil phase was separated and is further referred to as hydrolysate. No lipase is present in the hydrolysate.

Preparation of surfaces and contact angle measurements

A. Hydrophobisation of surfaces

Microscope glass plates were first cut into smaller pieces and submerged in ethanol (analytical reagent grade, Merck, Germany) for 15 minutes to clean them. After drying at ambient temperature the plates were submerged in toluene. After 20 hours the plates were removed from the toluene and dried at ambient temperature. Subsequently the plates were submerged for 5 minutes in a 1 wt% dimethyldichlorosilane solution in toluene. Finally the plates were rinsed with pure toluene.

The oxidised silicon wafers were also hydrophobised by submerging them during 5 minutes in a 1% (w/w) dimethyldichlorosilane solution in toluene. Subsequently they were rinsed with pure toluene. The surface tension of the silicon wafers thus treated (27 mN m^{-1}, [15]) is approximately equal to the surface tension of the polypropylene membrane, 29 mN m^{-1} [16].

B. Contact angles at surfaces without polymer

Hydrophobised silicon wafers and glass plates were submerged in water in a horizontal position and a droplet of oil was placed on the upper surface of the plates. The advancing contact angle was measured using a microscope with a goniometric eyepiece (Krüss GmbH, Hamburg, Germany). For the determination of the receding contact angle, liquid was removed from the droplet with a Pasteur pipette. After the oil/surface contact line

started receding no more oil was removed and the contact angle was measured. After 5 minutes the contact angle was measured again in order to check whether it was constant.

The contact angles were compared and it was found that the contact angles at the hydrophobised glass plates were a little higher (approximately 3°) than those measured at hydrophobised silicon wafers, which have a very high purity and smoothness. For both the silicon wafers and the glass plates the measured contact angles were reproducible. Therefore it can be concluded that the surface tension of the hydrophobised glass plates is comparable to the surface tension of the hydrophobised silicon wafers.

C. Preparation of surfaces with adsorbed polymer

The glass plates were used either directly after hydrophobisation or after modification with a polymer solution. For modification, the plates were submerged in an aqueous polymer solution. The concentrations of the solutions were 0.3; <0.01; 0.825; 2.8 and 1.5 kg m^{-3} for PPO 2000, PPO 3000, L92, P94 and F108 respectively. PPO 3000 is hardly soluble in water [17]. The PPO 3000 solution was prepared as follows. A total amount of 0.1 gram PPO 3000 was added to one litre of water and mixed. A small droplet of PPO 3000 remained at the bottom of the flask. After 20 hours the clear PPO 3000 solution was decanted, the droplet remaining in the flask. The decanted solution was used in the experiments; the concentration of PPO 3000 is not known exactly but probably much lower than 0.01 kg m^{-3}. The glass plates were submerged in one of the polymer solutions for 5 minutes. This time is sufficient to obtain a saturated adsorption layer. Subsequently, the plates were rinsed with water.

D. Contact angle measurement at polymer covered surfaces

A polymer covered glass plate was submerged in a horizontal position in water and the contact angle was measured. The same procedure as described for surfaces without polymer was used. The values for the contact angle given in figures 10, 11 and 12 are average values of at least six independent measurements. The statistical 95% confidence

interval is calculated for all contact angles measured on one type of surface (F108-modified etc.). All calculated intervals are smaller than 3°, most of the intervals are even smaller than 1°.

Measurement of $\gamma_{o,w}$

An overflowing cylinder device [18] was used under flowing conditions to measure the surface tension between sunflower oil and water. The device was used in non-flowing conditions to measure the surface tension between hydrolysate and water (under flowing conditions an emulsion was formed). A layer of sunflower oil or hydrolysate was put on top of the water phase already present in the device. A Wilhelmy plate was brought into contact with the oil/water interface and the surface tension was measured as a function of time.

Computer calculations

A. Introduction

A computer programme, GOLIATH (see acknowledgement), was used to predict the interfacial properties of a surface with adsorbed block copolymers [12]. The programme [10,11] is based on Evers' extension of the Scheutjens-Fleer theory for polymer adsorption and calculates the free energy and equilibrium configuration of an adsorbed block copolymer (using Flory-Huggins interaction parameters). This theory is based on a lattice model for the solution adjacent to the interface and calculates the volume fractions of each component in each of the lattice layers parallel to the surface. The programme can be used in either of two modes: (i) at fixed chemical potential (bulk concentration) or (ii) at fixed coverage of the adsorbing species.

Assume the interfacial free energy for adsorption of block copolymers from water to be $\gamma_{2,surf}$ (see equation 1) and that for other solvents ("oil") as $\gamma_{1,surf}$. As explained in the theory, both these interfacial tensions have to be considered separately. For each case we

have three interaction parameters in solution corresponding to solvent/anchor, solvent/buoy and anchor/buoy contacts, respectively. In addition, we must specify interaction parameters of each component with the solid substrate. With the programme GOLIATH the influence of the interaction parameter between solvent/buoy group and between solvent/surface on the interfacial free energy is calculated. By varying the interaction parameters between solvent/buoy group and solvent/surface over a large range, the wettability of a block copolymer covered surface can be evaluated for a large variety of "oils". The calculated trends rather than the absolute values are compared to measured contact angles. This is because absolute values for the Flory-Huggins parameters are not known.

B. Input parameters

In the calculations we attempted to mimick the experimental situation as much as possible. First, adsorption of polymer from water was considered ($\chi_{water,ethylene\ oxide}$ = 0.48; [19]). The tri-block copolymer used in the calculations consists of a "hydrophobic" ($\chi_{water,propylene\ oxide}$ = 0.7) middle block and two "hydrophilic" buoy groups. The length of the "hydrophobic" (representing PPO) block is always 48 units. The buoy group length varies between 0 and 150 units. The other parameters are given in table 2.

Table 2. Model parameters

Parameter	Value for simulation of water	Values for simulation of solvents
$\chi_{solvent,\ surface}$	10	10; 5; 2; 0; -2; - 5
$\chi_{solvent,\ propylene\ oxide}$	0.7	0.7
$\chi_{solvent,\ ethylene\ oxide}$	0.48	0.4; 0.48; 0.6; 0.8
$\chi_{surface\ ,\ propylene\ oxide}$	-5	-5
$\chi_{surface,\ ethylene\ oxide}$	0	0
$\chi_{ethylene\ oxide,\ propylene\ oxide}$	0	0
ϕ_{bulk}	$1x10^{-4}$	0

A diamond lattice between two parallel plates is used so that the number of possible conformations of the various molecules is finite. The lattice constant λ_l is 3/12 and the lattice consists of 100 equidistant layers (one lattice site has an area of 9×10^{-20} m^2).

C. Procedure to evaluate $\gamma_{surf,sol}$

For each buoy group length the equilibrium adsorbed amount from water is first calculated. Subsequently the adsorbed amount is fixed at this value and the bulk concentration is reduced to zero (situation after rinsing with water). The adsorbed amount is kept constant for each buoy group length but one has to keep in mind that the adsorbed amount may vary with buoy group length.

The influence of the interaction between buoy group and solvent on the interfacial free energy ($\gamma_{surf,sol}$) is evaluated, by varying $\chi_{sol,EO}$ from 0.4 (good solvent) to 0.8 (poor solvent). Also, the influence of the interaction parameter between solvent and surface was calculated by varying $\chi_{sol,surf}$ between -5 and 10.

RESULTS AND DISCUSSION

Evaluation of $\gamma_{surf,sol}$ for surfaces with adsorbed polymer

A. Modelling block copolymer adsorption from water

GOLIATH is used to calculate the interfacial free energy of a surface with adsorbed block copolymer and the chemical potential of the molecules. As a starting point, adsorption from water is chosen: $\chi_{sol,EO} = 0.48$ [19], the PPO blocks adsorb strongly. An example of the segment density distribution over the lattice is given in figure 2.

The propylene oxide is mainly situated in the first layer of the lattice. This is to be expected because of the high surface affinity of propylene oxide. The ethylene oxide segments cannot adsorb because of the higher surface affinity of propylene oxide. Hence,

the ethylene oxide groups are situated in the layers beyond the first one, thus forming a brush.

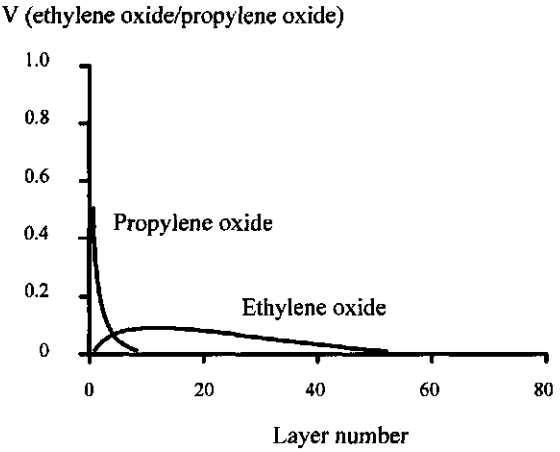

Figure 2. Volume fraction (V) ethylene oxide and propylene oxide in lattice layers (0 = surface). (Solvent + ethylene oxide + propylene oxide = 1 in every layer).

In figure 3 the interfacial free energy ($\gamma_{w,\ surf}$) is given as a function of the buoy group length. The interfacial free energy increases with increasing buoy group length. This seems somehow contradictive because longer hydrophilic PEO groups are present near the surface and a lower interfacial free energy seems more logical. However, another effect plays an important role.

The interfacial free energy is mainly determined by the surface and the molecules in the first layer of the lattice. PEO does not adsorb onto the surface, the buoy groups are extended into the solvent (water). Therefore, only PO/surface and solvent/surface interactions contribute to the calculated interfacial free energy. An increase in the number of PO/surface interactions results in a decrease in interfacial free energy. PPO molecules can adsorb relatively close to each other, the molecules do not repel each other too much, and therefore a high packing density can be achieved. However, if block copolymers are

adsorbed, $\gamma_{w,surf}$ increases. From equation 9 we see that both Γ and ($d\mu$ /dl) play a role. With increasing buoy group length Γ decreases owing to steric hindrance of the buoy groups and ($d\mu$ /d l) increases as a result of the enhanced solubility of the PEO/PPO block copolymers compared with the PPO block alone. As a result, $\gamma_{w,surf}$ increases with respect to the value at zero buoy length.

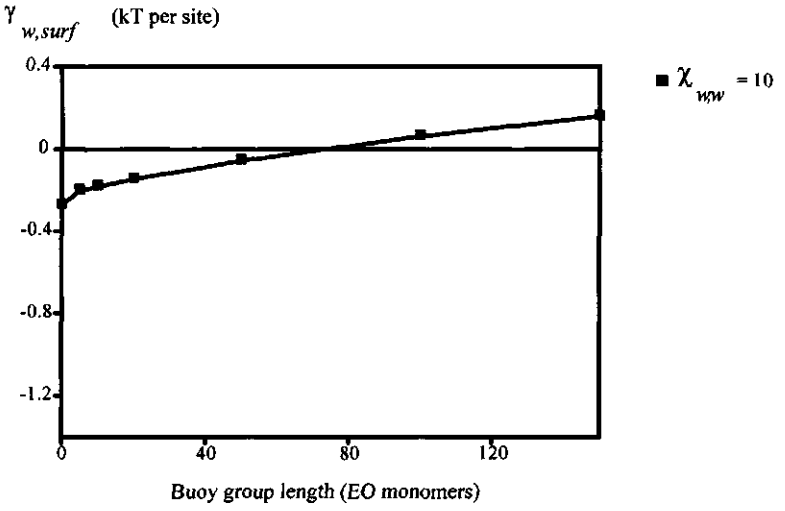

Figure 3. Prediction of the interfacial free energy ($\gamma_{w,surf}$) as a function of the buoy group length for adsorption from water; $\chi_{water,EO} = 0.48$.

B. Influence of buoy group solvency

In order to obtain more insight in the effect of solvent quality, $\chi_{sol,EO}$ is varied from 0.4 to 0.8. The results are given in figure 4. For all solvent qualities an increase in interfacial free energy is found as a function of the buoy group length. Because all the lines are very near to each other it can be concluded that the solvent quality for PEO hardly influences the interfacial free energy for $\chi_{sol,surf} = 10$. This result differs from that of Halperin and de

Gennes for polymers; they predict a strong influence of the solvent quality [8], this point will be discussed further below (general discussion).

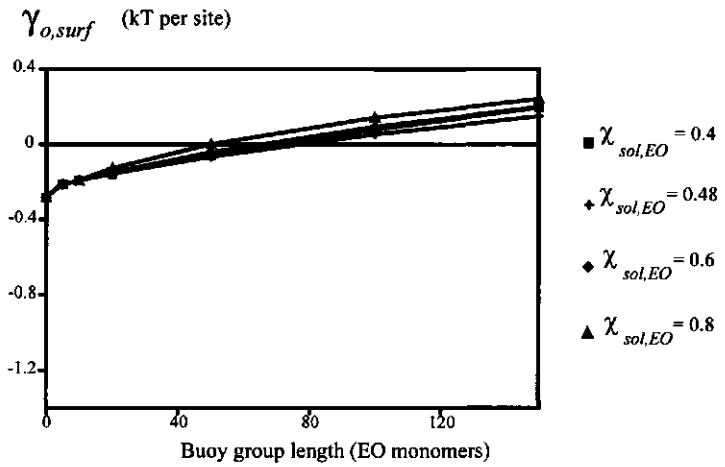

Fig 4. Prediction for the interfacial free energy ($\gamma_{o,surf}$) for several $\chi_{sol,EO}$ values; $\chi_{sol,surf}$ = 10

C. Influence of solvent/surface interaction

For water $\chi_{sol,surf}$ is taken equal to 10 kT per site. If the solvent is oil, which is expected to have a much better interaction with the surface, a lower $\chi_{sol,surf}$ should be used. Therefore, $\chi_{sol,surf}$ is decreased stepwise from 10 (water) to -5 (equal to interaction between PO and surface). In figures 5, 6 and 7 the results are given for $\chi_{sol,EO}$ values of 0.4, 0.48 and 0.8 respectively.

The interaction parameter between solvent and surface influences the interfacial free energy much more than the solvent quality ($\chi_{sol,EO}$) alone. As discussed before, the surface/solvent interactions contribute much to the interfacial free energy. Since the interaction parameter between surface and solvent is lowered, a decrease in interfacial free energy is inevitable.

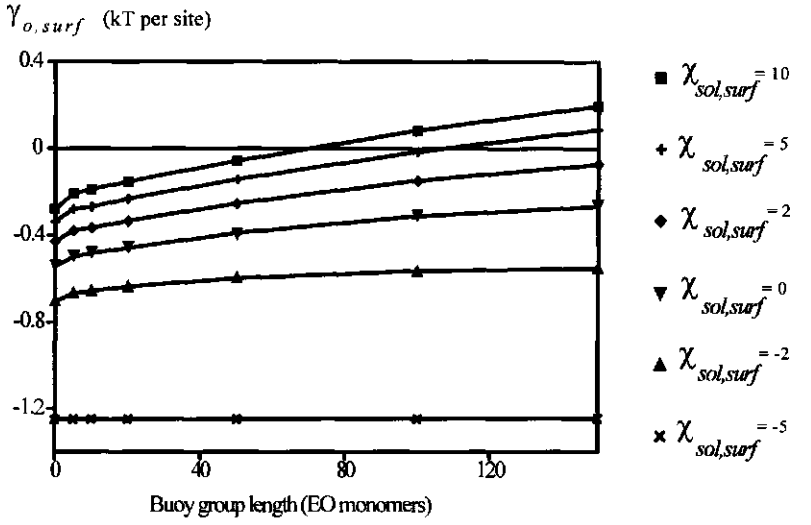

Fig 5. Prediction for the interfacial free energy ($\gamma_{o,surf}$) for several $\chi_{sol,surf}$ values; $\chi_{sol,EO} = 0.4$

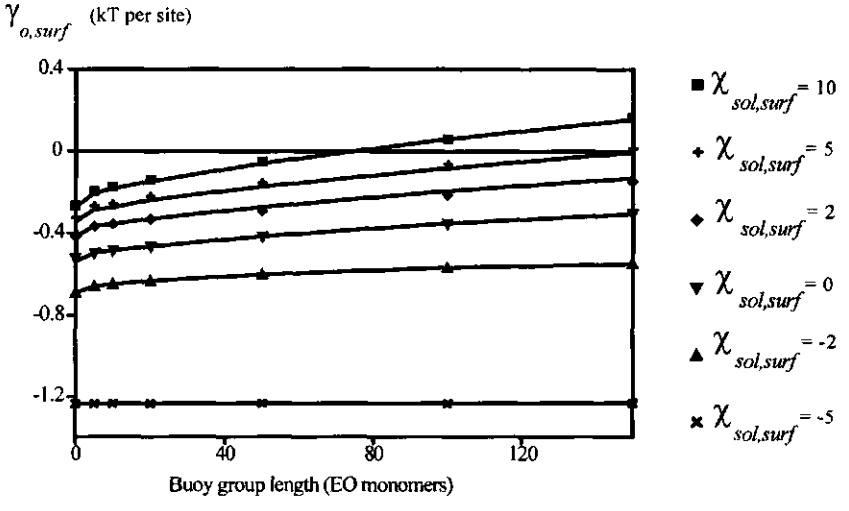

Fig 6. Prediction for the interfacial free energy ($\gamma_{o,surf}$) for several $\chi_{sol,surf}$ values; $\chi_{sol,EO} = 0.48$

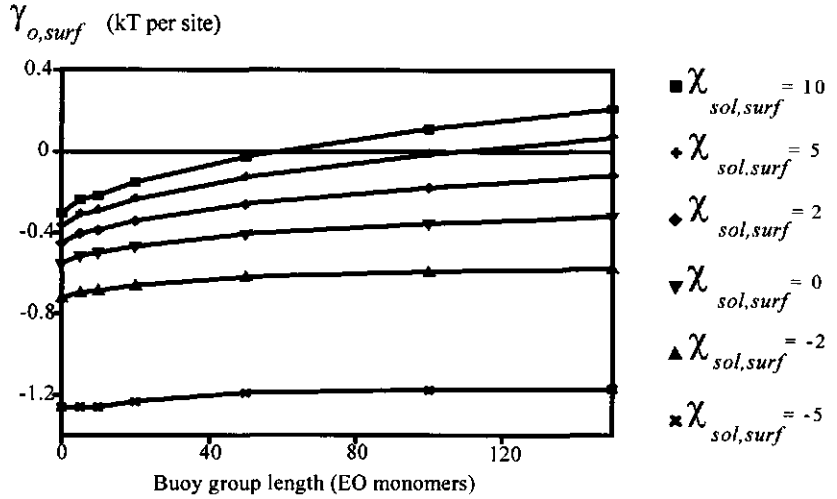

Fig 7. Prediction for the interfacial free energy ($\gamma_{o,surf}$) for several $\chi_{sol,surf}$ values; $\chi_{sol,EO}$ = 0.8

Whatever the buoy solvent interaction, for $\chi_{sol,surf}$ values between 10 and -2 an increase in interfacial free energy is found as a function of the buoy group length (analogous to the predicted curve for water). Only if $\chi_{sol,surf}$ is equal to -5 ($\chi_{PO,surf}$ also equals -5) a constant value for the interfacial free energy is found for $\chi_{sol,EO}$ = 0.4 or 0.48. This is due to the fact that in case of $\chi_{sol,surf} = \chi_{sol,EO}$ the surface free energy of a completely PO covered surface equals that of a "bare" surface. For $\chi_{sol,EO}$ = 0.8 an increase in interfacial free energy is found. The increase is due to the collapse of the buoy groups onto the surface as a consequence of the poor solvent quality. From the calculated segment density profile it could be concluded that EO units accumulate at the surface (results not shown), so that the interfacial free energy has to increase. The results in figures 5-7 are in contradiction with the theory of Halperin in which a small influence of the surface properties is predicted for adsorbed and grafted polymers.

Although the interaction parameter between surface and solvent influences the interfacial free energy more than the solvent/EO interaction parameter, the influence of the latter is

discussed first in more detail. For buoy groups smaller than 20 units, the interfacial free energy is constant for all $\chi_{sol,EO}$ values (results not shown).

For block copolymers with larger buoy groups the differences become more pronounced; the interfacial free energy increases for both an increase and a decrease in solvent quality (compared to water $\chi_{sol,EO}$ = 0.48). In figure 8 the interfacial free energy is given as a function of $\chi_{sol,EO}$ for a buoy group length of 150.

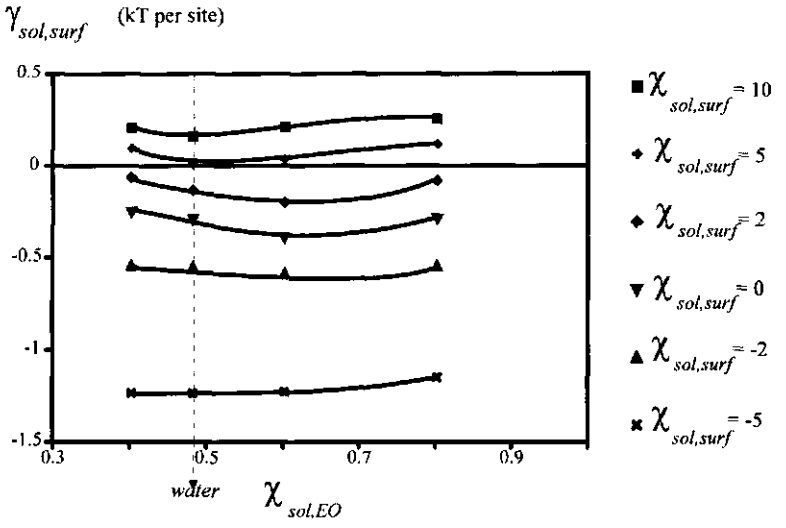

Fig 8. Prediction for the interfacial free energy ($\gamma_{sol,surf}$) as a function of $\chi_{sol,EO}$ (buoy group length of 150).

One would expect an increase in interfacial free energy for better solvents and a decrease for worse solvents. In a good solvent the PEO chains will swell and "pull" PO units from the surface, fewer PO/surface and more solvent/surface interactions will be formed, resulting in an increase of the interfacial free energy. However, also for poor solvents ($\chi_{sol,EO}$ = 0.8) the interfacial free energy increases compared with that for water ($\chi_{sol,EO}$ = 0.48). An increase in interfacial free energy is only possible if the number of PO/surface interactions decreases. In a poor solvent the EO/solvent contacts are not favourable, the

EO groups collapse. In order to keep the molecule adsorbed fewer PO/surface interactions are necessary to counteract the swelling of the EO groups; the molecule will gain configurational freedom as the number of PO/surface contacts decreases. This explains the increase in interfacial free energy.

D. Prediction of $\gamma_{w,surf} - \gamma_{o,surf}$

Let us assume that a solvent influences both the solvent/surface interaction parameter and the EO/solvent interaction parameter. In that case the lines in figures 5, 6 and 7 with exception of the line given in figure 3 are predictions for the interfacial free energy for other solvents than water. All the lines are predictions for $\gamma_{o,surf}$ as a function of the buoy group length.

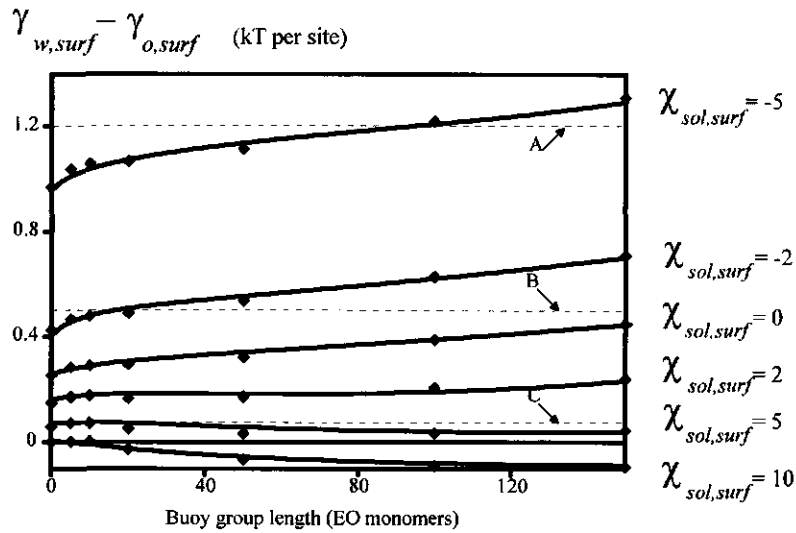

Fig 9. Prediction for the interfacial free energy difference ($\gamma_{w,surf} - \gamma_{o,surf}$) for water and a poor solvent as a function of the buoy group length. The dashed lines indicate γ_{ow} of hexadecane (A), sunflower oil (B), hydrolysate (C).

The difference between these lines and the line in figure 3 is a prediction for the interfacial free energy difference between water and oil, $\gamma_{w,surf} - \gamma_{o,surf}$. The difference in interfacial free energy between water and a poor solvent ($\chi_{sol,EO} = 0.8$) is given in figure 9. Since $\chi_{sol,EO}$ does not influence the interfacial free energy too much (figure 8), $\gamma_{w,surf} - \gamma_{o,surf}$ is also insensitive to the solvent quality; the trends in figure 9 are valid for both good and poor solvents for poly(ethylene oxide).

If the interaction of solvent/surface is better than water/surface, a positive value for $\gamma_{w,surf} - \gamma_{o,surf}$ is found. For solvents with a good interaction with the surface ($\chi_{sol,surf} < 0$) a continuously rising line is found. For solvents with $\chi_{sol,surf}$ between 0 and 5 the curve has an optimum. Only if the interaction parameter between solvent and surface is equal to the interaction parameter between water and surface (both bad) negative values are predicted for $\gamma_{w,surf} - \gamma_{o,surf}$. In that case, the surface is wetted preferentially by water. In all other cases the surface is wetted preferentially by the solvent. From the $\gamma_{w,surf} - \gamma_{o,surf}$ curves in figure 9 it can be concluded that $d(\gamma_{w,surf} - \gamma_{o,surf})/ dl$ is small, much smaller than the effects predicted by the theory of Halperin and de Gennes [8]. This point is discussed further in the general discussion section. In the next two paragraphs the relation between the predictions and the contact angle measurements is discussed.

Measured contact angles

In figure 10 the cosine of the advancing and receding contact angles of hexadecane is given as a function of the buoy group length (number of EO monomers). The cosine of the advancing contact angle of hexadecane at the "bare" surface approximates 0.78. Upon adsorption of PPO 3000 the cosine of the contact angle decreases. This is expected because PPO 3000 is less hydrophobic than the surface. Upon adsorption of PPO 2000 the cosine of the advancing contact angle increases. This can only be explained if PPO 2000 desorbs from the surface and adsorbs onto the hexadecane/water interface and thus lowers the surface tension, $\gamma_{o,w}$. For hexadecane, PPO 2000 covered surfaces will not further be discussed.

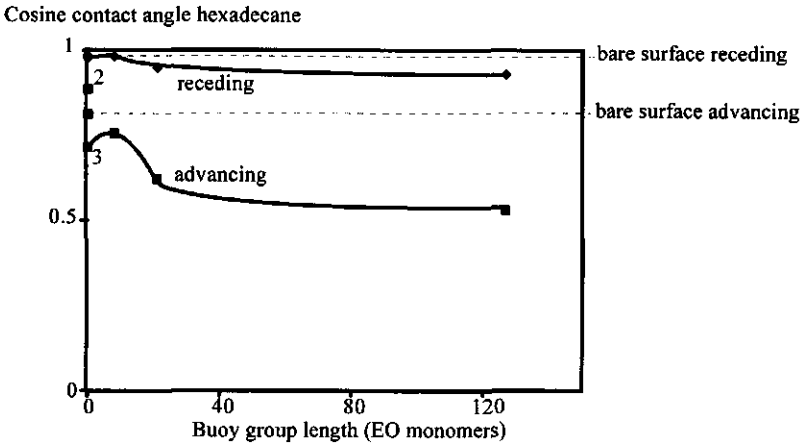

Fig 10. Advancing and receding contact angle for hexadecane, the broken lines indicate the values for the "bare" surface, (2) corresponds to a PPO 2000 covered surface, (3) corresponds to a PPO 3000 covered surface.

Upon increasing the buoy group length the cosine of the contact angle first increases as compared with the PPO 3000 covered surface and subsequently decreases again for longer buoy groups. The cosine of the receding contact angle of hexadecane is higher than the cosine of the advancing contact angle for all surfaces studied. The difference between the two corresponds to a difference in contact angle of 30°. The shape of the plot of the cosine of the receding contact angle as a function of the buoy group length is comparable to that of the plot of the cosine of the advancing contact angle.

The cosine of the advancing and receding contact angles of sunflower oil as a function of the buoy group length is given in figure 11. Again, upon adsorption of PPO 3000 or PPO 2000 the cosine of the advancing contact angle decreases as compared with the "bare" surface, the advancing contact angles for PPO 2000 and 3000 being comparable. With increasing buoy group length, the cosine of the advancing contact angle first increases compared with the PPO-covered surfaces and decreases again for long buoy groups,

although the decrease is less pronounced as compared with hexadecane. For sunflower oil the cosines of the receding contact angles are higher than the cosines of the advancing contact angles; the difference between both values is comparable to the difference found for hexadecane (30°).

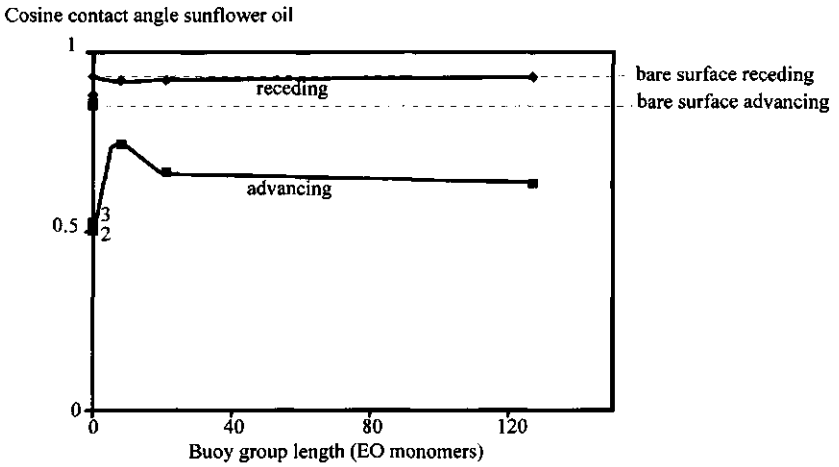

Fig 11. Advancing and receding contact angle for sunflower oil, thebroken lines indicate the values for the "bare" surface, (2) corresponds to a PPO 2000 covered surface, (3) corresponds to a PPO 3000 covered surface.

In figure 12 the cosine of the advancing and receding contact angles of hydrolysate are given. Compared with the "bare" surface the cosine of the advancing contact angle decreases upon adsorption of PPO 2000 and 3000. Both values are comparable. For increasing buoy group length the cosine of the advancing contact angle increases monotonically. For the cosine of the receding contact angle higher values are found and the shape of the cosine of the receding contact angle as a function of the buoy group length is comparable to that of the cosine of the advancing contact angle.

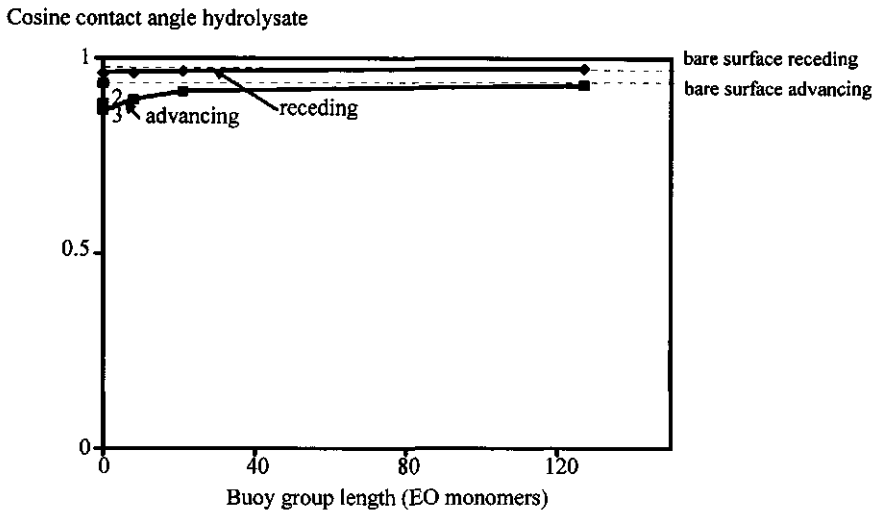

Fig 12. Advancing and receding contact angle for hydrolysate, the broken lines indicate the values for the "bare" surface, (2) corresponds to a PPO 2000 covered surface, (3) corresponds to a PPO 3000 covered surface.

Comparison predicted and measured contact angles

Hexadecane has a high value for $\gamma_{o,w}$, therefore, a high value for the contact angle is expected at the "bare" surface. Both sunflower oil and hydrolysate have lower $\gamma_{o,w}$ values; therefore lower contact angles are expected. This is in agreement with the measured contact angles at "bare" surfaces (indicated by the dotted lines in figures 10-12), hexadecane has the highest advancing contact angle followed by sunflower oil and hydrolysate.

The cosine of the contact angle is obtained from Young's law: $\cos\theta = (\gamma_{w,surf} - \gamma_{o,surf}) / \gamma_{ow}$. Here $\gamma_{o,w}$ does not depend on the substrate. Therefore, the effect of polymer structure on the shape of the curves in figure 9 should be comparable with the experimental data (cos θ as a function of the buoy group length). Because finite contact angles were measured,

the cosine of the contact angle has a value between -1 and 1, as a consequence, the interfacial free energy difference will have a value between $-\gamma_{o,w}$ and $\gamma_{o,w}$. The $\gamma_{o,w}$ for hexadecane, sunflower oil and hydrolysate is equal to 55 [19], 23 and 4.5 mN m^{-1}, respectively. This is indicated by the three dotted horizontal lines in figure 9. All the curves below the horizontal lines are predictions for the cosine of the contact angle.

It thus turns out that our experimental finding that block copolymer-coated hydrophobic surfaces retain their hydrophobic, oil-wettable character corroborates the theoretical calculations. Most of the predicted $\gamma_{w,surf}$-$\gamma_{o,surf}$ values are positive, so that the corresponding oil/water contact angles are smaller than 90°. Also the effect of making the hydrophilic buoy groups longer is very small; theory predicts a slight increase in contact angle, whereas the experimental data show nearly constant contact angles as a function of the buoy block length, or even a shallow minimum. The analysis presented by Halperin and de Gennes led to the quite different conclusion that longer chains would make the surface more hydrophilic. The reason why this does *not* occur seems to be that when the buoy blocks become longer, fewer chains can adsorb, and this effect compensates for the increased hydrophilicity of the chains.

Even though the general picture seems to be clear, we note that there are minor discrepancies between theory and experiment. The monotonic increase in contact angle that was predicted was not found experimentally for hexadecane and for sunflower oil. For hydrolysate the situation is the reverse: a minimum is predicted, but a monotonic increase is found. We tend to attribute these (small) discrepancies to differences in anchor block solvency between water and oil, that were ignored in the calculations. Such differences manifest themselves most strongly for short buoy blocks, where the anchor density on the surface is relatively high, and may well lead to the observed effects. Indeed, when appropriate variations in the PPO-solvent χ parameter are studied (e.g, 0.6 and 0.8), we find small changes in the interfacial free energy of the surface with anchor block only (-0.16 and -0.40 respectively for $\chi_{sol,EO} = 0.48$; $\chi_{sol,surf} = 10$). However, because

of the lack of reliable solubility data for all the oils used in this study, we do not pursue this point any further.

General discussion

The fact that the block copolymer coated surfaces are wetted by all three oils under study is a result of a combined effect of the surface tension between oil and water, γ_{ow}, and the interfacial free energy difference, $\gamma_{w,surf}$ - $\gamma_{o,surf}$. For hexadecane, γ_{ow} is high and the interfacial free energy difference is big. Hydrolysate on the other hand has a low γ_{ow} and a small interfacial free energy difference (see figure 13). Both situations can result in comparable values for the contact angle.

In the literature, adsorbed block copolymers are often regarded as grafted polymers. The main difference is that a grafted polymer is chemically attached to the surface with one of its monomers while a block copolymer is attached with the anchor group. For the interfacial free energy of a surface it makes a difference whether polymers are adsorbed or grafted.

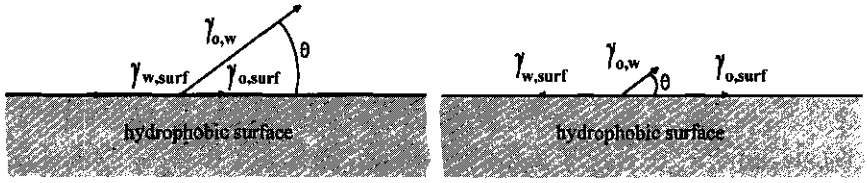

Fig 13. Contact angle for different interfacial free energies

Halperin et al. [8] give a theory to predict the influence of adsorbed and grafted polymers on the spreading coefficient (see equation 3-6). They show that the kT/D^2 term (eq. 5) dominates spreading, S_0 is not so important. The spreading is mainly ruled by entropy of dissolution. This is because their solvent (air) is so poor that the polymer molecules

collapse onto the surface. Hence, every EO/surface interaction that is replaced with a solvent 2/surface interaction is favourable. The spreading will be dominated by dissolution of the polymer. However, in the solvents used in this study we do not expect the buoy groups to collapse entirely onto the surface. Therefore, the spreading is mainly ruled by the surface itself (the S_0 term in equation 5). In order to have an oil-wetted and protein repelling surface, the grafting density must be kept relatively low (and uniform), and the grafted chains must be sufficiently, long. Controlling this by chemical reactions is quite difficult.

In a previous study, membrane surfaces are modified with block copolymer to prevent protein adsorption. It is important that the surface remains hydrophobic upon adsorption of block copolymers because an oil phase has to wet the membrane and subsequently has to permeate through the membrane. In addition, the surface properties should be uniform, otherwise only part of the membrane is effectively used. Both pre-requisites are "automatically" met with adsorbed block copolymers, so their application for modification purposes is more promising.

Because the wettability of the membranes is hardly influenced by the adsorbed block copolymers, it can be stated that suppression of protein adsorption is not due to a change in hydrophobicity, but to steric hindrance by the buoy groups. Hence, for (block co)polymer-covered surfaces, the contact angle is not a reliable indicator for the possibility of protein adsorption [4,21].

CONCLUSIONS

The influence of adsorbed polymer molecules on the wettability of a hydrophobic surface is studied by contact angle measurement and computer modelling. The interfacial free energy of a block copolymer coated surface in water and in oil has been calculated theoretically. In water, the interfacial free energy increases with increasing buoy group length. Steric hindrance by the buoy groups results in fewer adsorbed molecules per

surface area. Therefore, the "bare" surface contributes more to the interfacial free energy for molecules with large buoy groups, resulting in an increase in interfacial free energy with increasing buoy group length.

The interaction parameter between solvent and buoy groups does not influence the interfacial free energy too much. Only minor effects were found for block copolymers with large buoy groups. However, the interfacial free energy decreases strongly upon an improvement of the interaction between solvent and surface. The shape of the curves is comparable to the shape of the curve predicted for water.

The model predicts that a block copolymer-coated surface is wetted by the oil phase in practically all cases considered. Only if the interaction parameter between solvent and surface is equal to the interaction parameter between water and surface, will water wet the surface. Contact angle measurements with hexadecane, sunflower oil and hydrolysate confirmed that block copolymer-coated hydrophobic surfaces are wetted by a broad variety of oils (contact angles below 90^0 were measured). There are small discrepancies between theory and experiment but the effects are only of minor importance.

ACKNOWLEDGEMENT

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LIST OF SYMBOLS AND ABBREVIATIONS

a	: Mesh size of lattice	(m)
f	: Physisorbed polymer	
g	: Grafted polymer	
i	: Component i	

k	: Boltzmann constant	($J K^{-1}$)
l	: Length of EO group	(-)
n	: Excess number of moles	(mol)
o	: Oil phase	
p	: Polymer	
sol	: Solvent	
surf	: Surface	
w	: Water phase	
A	: Interfacial area	(m^2)
D	: Average distance between polymers	(m)
EO	: Ethylene oxide	
PO	: Propylene oxide	
S	: Spreading tension	($N m^{-1}$)
S_0	: Spreading tension surface without polymer	($N m^{-1}$)
T	: Temperature	(K)
χ	: Flory-Huggins interaction parameter	(-)
γ	: Interfacial surface tension	($N m^{-1}$)
$d\gamma$	: Interfacial surface tension difference	($kT m^{-2}$)
λ_1	: Lattice constant	(-)
$d\mu$	: Chemical potential difference	($N m^{-1}$)
θ	: Equilibrium contact angle	(°)
σ	: Fraction of grafted sites at surface	(-)
Φ	: Bulk monomer fraction	(-)
Φ_{bulk}	: Bulk polymer fraction	(-)
Γ	: Total number of monomers per unit area	(m^{-2})
Γ_i	: Adsorbed amount of component i	($mol m^{-2}$)
1	: Phase 1	
2	: Phase 2	

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Chapter 5

INFLUENCE OF PRE-ADSORBED BLOCK COPOLYMERS ON PROTEIN ADSORPTION

-surface properties, layer thickness and surface coverage-

ABSTRACT

In this article the influence of pre-adsorbed block copolymers on lipase adsorption is studied. The Pluronic tri-block copolymers used in this study (P75 and F108, respectively) both have one hydrophobic (polypropylene oxide) block in the middle and two hydrophilic (polyethylene oxide) blocks at the ends of the molecules. It was concluded that block copolymers adsorb onto a hydrophobic surface with the middle block, the buoy groups are extended into the water, thus forming a brush. The layer thickness of F108 is 10 nm. At a hydrophilic surface the buoy groups adsorb and a flat pancake configuration is formed. The layer thickness is 1 nm.

This chapter has been submitted as:

Influence of pre-adsorbed block copolymers on protein adsorption - surface properties, layer thickness and surface coverage

C.G.P.H. Schroën, K. van der Voort Maarschalk, M.A. Cohen Stuart, A. van der Padt and K. van 't Riet

Protein (lipase and Bovine Serum Albumin) adsorption is prevented by F108, provided this is adsorbed in a brush configuration; a pancake configuration is not effective. Prevention of protein adsorption is not solely caused by the presence of F108 at the surface, above that the configuration of the adsorbed molecule is essential. The steric repulsion caused by a brush is stronger than that caused by a pancake.

The effect of brush density on protein adsorption has been systematically studied for the F108/lipase system. Both the protein adsorption rate and the final adsorption level were measured as a function of the amount of pre-adsorbed F108. It is found that small amounts of adsorbed F108 (10% saturation of the surface) reduce the initial adsorption rate of lipase severely (approximately 20 fold). The maximum value of the adsorbed amount at such a surface is 3 times lower as compared to a "bare" surface. It can therefore also be concluded that protein binding to the surface is already hindered by low levels of pre-adsorbed block copolymer. In the case of a saturated F108-layer no protein adsorption takes place.

INTRODUCTION.

From literature it is known that poly(ethylene oxide) (co-)polymers reduce protein adsorption at solid surfaces. Lee [1] and Tan [2] found suppression of adsorption of several proteins in case a PEO-PPO-PEO block copolymer (F108) was pre-adsorbed at a *hydrophobic* polystyrene latex. It was found for *hydrophobic* polypropylene membranes (surface tension 29.5 mN m^{-1} [3]) that the adsorbed amount of protein decreases with increasing molecular weight of the PEO group of the block copolymer [4]. Protein adsorption was prevented at a membrane modified with F108 [5]. A comparable effect was found by Gölander [6] for protein adsorption at PEO hydrogels. The adsorbed amount of protein decreases with increasing molecular weight of PEO. For *hydrophilic* surfaces, e.g., polysulfone membranes (surface tension 60-70 mN m^{-1} [3]), de Roo and co-workers [7] reported an optimum buoy group length at which protein fouling was minimal compared to both longer and shorter buoy groups. In their case the adsorbed

amount of protein at block copolymer coated (hydrophilic) membranes is smaller than, but still of the order of magnitude of the adsorbed amount at an untreated membrane (less than a factor of 3 difference).

Although a lot of experimental data are available, the underlying mechanism is not understood. Halperin and De Gennes [8] suggest that the excess free energy of a solid/sovent interface will decrease upon coating with a soluble polymer. This implies that a *hydrophobic* surface with either adsorbed or grafted hydrophilic polymer will become hydrophilic. It is known from various publications [9-15] that proteins adsorb less on hydrophilic surfaces and this may seem to explain the suppression of protein adsorption. However, in contradiction to this it was found that polypropylene membranes remain oil-wetted after adsorption of PEO-PPO-PEO tri-block copolymers [4]. In spite of the presence of the hydrophilic groups of the polymer, the membrane is still hydrophobic. Therefore, the reduction in protein adsorption cannot be ascribed to increased hydrophilicity [16].

A steric mechanism seems more appropriate since adsorbed (or grafted) polymer molecules will hinder other molecules that approach the surface. Jeon *et al.* [17,18] made a theoretical evaluation of the steric hindrance of terminally grafted PEO polymers. They found an "optimum" surface (grafting) density at which resistance against protein adsorption is strongest. However, from the predictions of Jeon *et al.* it could not be concluded to what extent protein adsorption is influenced.

If a steric mechanism is responsible for the observed effects on protein adsorption than the conformation of the poly(ethylene oxide) molecule and the number of ethylene oxide monomers per surface area will be of influence. If (PEO-PPO-PEO) block copolymer molecules adsorb, the conformation of the block copolymer molecule will depend on the properties of the surface and the quality of the solvent [19-22]. For block copolymer adsorption from water onto a hydrophobic surface, the propylene oxide block acts as the anchor, being mainly situated near the surface because of its high surface affinity. The

ethylene oxide segments were found not to adsorb; they have no affinity for the surface. A brush conformation is the result (figure 1). The thickness of a (saturated) brush layer depends mainly on the length of the ethylene oxide groups [23]. For a hydrophilic substrate like silica the situation is different. Ethylene oxide has the highest surface affinity and will adsorb preferentially, thus forming a pancake conformation (figure 1). Compared to a saturated brush layer the pancake layer is in general less thick and is expected to give less steric hindrance to proteins that approach the surface.

The aim of this article is to investigate the effect of steric hindrance on protein adsorption systematically. Firstly, the influence of the conformation of the adsorbed block copolymer molecules was evaluated by measuring protein adsorption at hydrophilised (pancake conformation) and hydrophobised silica (brush conformation). Secondly, the effect of brush density on protein adsorption was studied. This was done by varying the adsorbed amount of block copolymer (between bare and saturated surface) and subsequently studying the protein adsorption as a function of time. The results were compared with the theoretical evaluation of Jeon *et al.* [17,18].

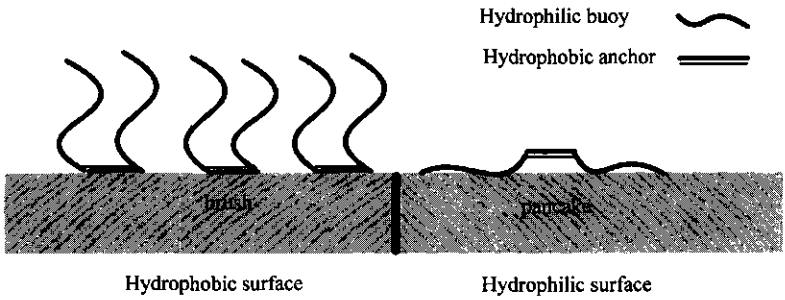

Figure 1. Schematic representation of block copolymers adsorbed at a hydrophobic and a hydrophilic surface.

MATERIALS

Doubly distilled water was used throughout. Lipase B was obtained from Biocatalysts and originated from the yeast *Candida rugosa* (formerly called *Candida cylindracea*). The molecular weight is 60,000 D according to the manufacturer. The crude lipase preparation (lipase content is approximately 5%) was dissolved in water and centrifuged at 13,000 rpm (Hereaus, biofuge A). The sediment was discarded; only the supernatant was used in experiments. Bovine Serum Albumin was obtained from Boehringer (Mannheim, Germany). The purity is over 95% and the molecular weight is 68,000 D. The Bovine Serum Albumin was dissolved in water and used without further treatment.

The block copolymers were gifts from ICI (Rotterdam, the Netherlands) and their characteristics are summarised in table 1. The block copolymers consist of three blocks, one polypropylene oxide (PPO) anchor part and two polyethylene oxide (PEO) buoy parts. The anchor parts are similar in molecular weight while the buoy parts vary considerably.

Table 1. Block copolymers used in this study.

Trade name	M.W. PPO (anchor) (Da)	M.W. PEO (buoy) (Da)	Total M.W. (Da)
P75	2,000	1,075	4,150
F108	2,800	5,600	14,000

Highly pure silicon wafers of the Czochralsky-type were supplied by Wacker Chemitronic GmbH (Germany).

Dimethyldichlorosilane, nitric acid, potassiumdichromate, sulphuric acid, sodium chloride and ethanol (all reagent grade) were purchased from Merck (Germany), toluene (99%+ pure) originated from Janssen Chimica (Belgium).

METHODS

All experiments were carried out at 20 °C.

Reflectometry [24, 25]

A. Preparation of substrates (surfaces)

The silicon wafers were oxidised in an oven in ambient air at 1000 °C for 1 hour. After oxidisation the wafers were cut into strips of 1.5 cm wide. Gloves were used to avoid contamination of the surfaces. The strips were initially cleaned by red glowing in a natural gas flame for about 5 s. Later a milder UV/ozon treatment was used for cleaning. Both methods led to very similar and reproducible results [24]. After cleaning the strips were used in an experiment or hydrophobised.

The oxidised silicon plates were submerged in a 1% (w/w) dimethyldichlorosilane solution in toluene during 5 minutes and subsequently rinsed with pure toluene. The surface tension of the silicon plates thus treated is 27 mN m^{-1} [26]. The plates were used immediately after hydrophobisation.

B. Experimental set-up [24]

The experimental set-up for reflectometry (figure 2) is very similar to the one described by Dijt [24]. A polarised He/Ne laser beam is reflected on the substrate. For the detection the beam is split into its parallel and perpendicular components (with respect to the plane of incidence) by means of a polarising beamsplitter cube. Both polarisation components are detected by photodiodes and their ratio is taken by an analog divider. This gives the output signal S, defined in equation (1). Under the experimental conditions the angle of incidence is 70° (near the H_2O/SiO_2 Brewster angle), the wavelength of the laser is 632.8 nm and the thickness of the oxide layer is 92 nm. Either oxidised silicon strips or hydrophobised silicon strips were used as substrates.

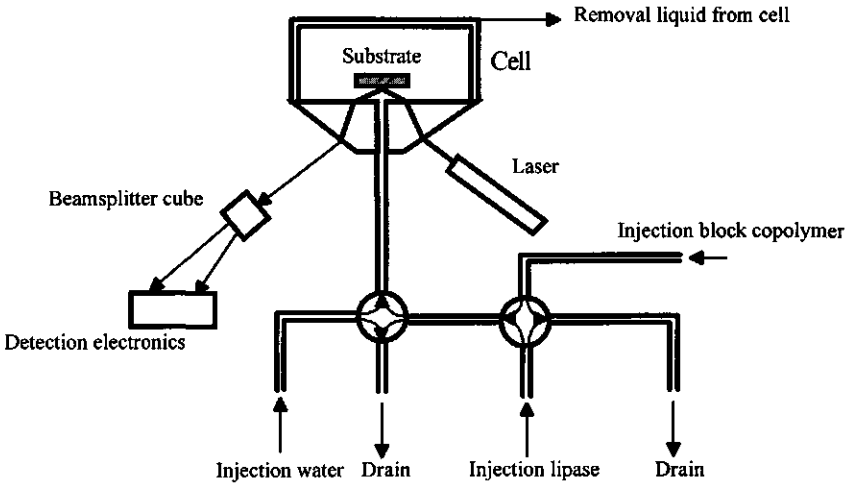

Figure 2. Experimental set-up reflectometry.

Water, polymer solution or protein solution was pumped into the cell with the substrate. With the help of 2 two-way valves it is possible to switch from water to polymer solution or to protein solution or visa versa.

C. Determination of the adsorbed amount by reflectometry [24]

The technique of reflectometry makes use of the fact that the adsorption of polymer causes a change in the reflectivity of the substrate. The reflected intensities of the perpendicular (I_s) and parallel (I_p) polarisation components are continuously measured and electronically combined to give the output signal S defined as:

$$S = \frac{I_p}{I_s} \tag{1}$$

The reflected intensities I_s and I_p can be expressed in the incoming light intensities I_s^0 and I_p^0, the reflectivity's R_s and R_p of the substrate, and loss factors f_s and f_p, respectively:

$$I_s = f_s \cdot R_s \cdot I_s^0 \tag{2}$$

$$I_p = f_p \cdot R_p \cdot I_p^0 \tag{3}$$

The factors f_s and f_p account for losses at the reflecting surfaces of prisms and beamsplitter. Combining equations 1, 2 and 3 gives:

$$S = f \cdot \frac{R_p}{R_s} \tag{4}$$

Where $f = f_p I_p^0 / f_s I_s^0$. The factor f depends on I_s^0 and I_p^0, i.e., on the (adjustable) polarisation angle of the laser. For a given value of this angle, f is a constant which can be determined by a suitable calibration method as described by Dijt [24].

The change in signal caused by adsorption, ΔS^*, is by definition equal to:

$$\Delta S^* = \frac{S_{aa} - S_{ba}}{S_{ba}} \tag{5}$$

Where S_{aa} is the signal after adsorption and S_{ba} the signal before adsorption. It can be shown that under appropriate conditions, ΔS^* is to a very good approximation proportional to Γ

$$\Gamma = a \cdot \Delta S^* \tag{6}$$

Where a (mg m^{-2}) is the sensitivity of the reflectometer (constant). Conditions for optimum sensitivity and linearity have been extensively discussed by Dijt *et al.* [24,25]. The sensitivity of the reflectometer, $dS/d\Gamma$, depends on $dR_s/d\Gamma$, $dR_p/d\Gamma$ and the constant f. In order to obtain $dR_s/d\Gamma$ and $dR_p/d\Gamma$, the reflectivity of the surface with adsorbed block copolymer or protein is numerically calculated using Hansen's method based on the exact matrix formalism of Abeles. The reflection surface is optically modelled as a set of flat, parallel layers of uniform refractive index [24]. For the block copolymer and the protein the values of the constant a (equation 6) are 41.86 and 36.58 mg m^{-2}, respectively.

D. Adsorption measurements

The procedure for the adsorption measurements was as follows:

- First the substrate was exposed to either block copolymer solution or protein solution (step 1).
- Subsequently, the substrate was exposed to water in order to remove the surplus of block copolymer or protein (step 2).
- The substrate is exposed to protein solution in the experiments concerning protein adsorption at block copolymer covered surfaces (step 3).
- Subsequently the substrate is again exposed to water (step 4).
- In some cases step 3 and 4 were repeated (step 5 and 6).

Streaming potential measurement

The hydrodynamic layer thickness of adsorbed block copolymers was studied by means of streaming potentials in glass capillaries as described by Dijt *et al.* [27]. Glass capillaries with an internal diameter of 0.3 mm were cleaned before use by placing them in chromic acid (potassiumdichromate dissolved in 50% sulphuric acid in a total concentration of 37.5 g $K_2Cr_2O_7$ per litre) for at least one week. Subsequently, the capillaries were placed in nitric acid for 3 days. After rinsing with water the capillaries were either used directly or hydrophobised by immersing them in a 1% (v/v) solution of dimethyldichlorosilane in toluene for 20 minutes. The capillaries were rinsed once with toluene, several times with ethanol, and finally with deionised water. After hydrophobisation the capillaries were used as soon as possible in the experiments.

The streaming potential was measured with reversible Ag/AgCl electrodes and a high impedance mV-meter. Solutions of 1, 10, 100 or 1000 mg l^{-1} block-copolymer in electrolyte were used. The electrolyte concentration was always $5x10^{-4}$ mol l^{-1} sodium chloride. In each experiment the streaming potential is followed in time until a constant value is obtained. From this constant value, the effective thickness of the layer is calculated [27]. Then the capillary is flushed with electrolyte solution after which the streaming potential and corresponding layer thickness is measured again.

RESULTS AND DISCUSSION

Layer thickness determined by streaming potential measurement

The layer thickness of F108 has been measured in a *hydrophobic* capillary. For (finite) polymer bulk concentrations of 1-1000 mg l^{-1} a value of 12 nm has been found. After rinsing with electrolyte the layer thickness decreases to a lower, constant value of 10 nm. This is consistent with the observation to be discussed that the adsorbed amount also decreases slightly after rinsing with water (see figure 4). Both values for the layer thickness are in good agreement with results from the literature. Lee [1] measured a layer thickness of 11.8 ± 1.5 nm (dynamic light scattering) for F108 adsorbed on polystyrene latex particles. Kayes [28] reports values of 13.5 ± 2.0 nm (micro-electrophoresis) and 13.4 ± 4.5 nm (dynamic light scattering) for F108 adsorbed at polystyrene latex.

For P75 the layer thickness in a *hydrophobic* capillary is 2.5 nm for polymer concentrations of 1-1000 mg l^{-1}. Upon rinsing with electrolyte this value decreases to 1.5 nm. These values are comparable to the layer thickness (1.4 ± 0.5 nm) reported by Lee [1] for P105, a block copolymer with buoy groups of comparable length to those of P75. Hence, the layer thickness in a hydrophobic capillary increases with the length of the buoy groups. This, together with the fact that the layer thickness is much bigger than could be expected from the dimensions of the PO block, confirms that the block copolymers are adsorbed in a brush configuration at the hydrophobic surface. The results are in agreement with those of Cohen Stuart [29] who reported an increase in layer thickness with increasing length of the ethylene oxide block for di-block copolymer (synperonic NPE) adsorption at a hydrophobic capillary. These results imply that EO blocks do not adsorb from water on the hydrophobic surface. This is consistent with the finding that PEO at water/air interfaces lowers the surface tension to about 60 mN m^{-1} ; the surfaces considered here have a tension well below that so that PEO cannot adsorb on them.

Completely different results were obtained for F108 and P75 adsorbed at a *hydrophilic* capillary. The layer thickness is approximately 1 nm for both block copolymers at polymer concentrations ranging form 1-1000 mg l^{-1}. This indicates that the block copolymers adsorb in a flat configuration (pancake). Because the layer is only 1 nm thick, it is very unlikely that it is a brush, and therefore it is probably less effective against protein adsorption.

Adsorption of P75 and F108 onto *hydrophobic* surfaces

The adsorbed amount of P75 and F108 at hydrophobic substrates has been determined with reflectometry. First, the substrate is exposed to a block copolymer solution of 100 mg l^{-1} till the signal is constant (figure 3, step 1). The substrate is subsequently rinsed with water (step 2). The signal first decreases slightly and then levels off again. Apparently, some molecules are removed from the polymer layer but the remaining molecules adhere strongly to the surface: the signal is constant for more than 1 hour.

The adsorbed amounts, after rinsing with water, are 1.0 and 1.8 mg m^{-2} for P75 and F108, respectively. The adsorbed amount of P75 is in good agreement with the value of 0.9 mg per m^2 reported by Tadros [22] for depletion experiments. Kayes [28] reported an adsorbed amount of 1.2 mg F108 per m^2 for polystyrene latex; this value is somewhat lower than the value reported here.

If both F108 and P75 form a brush, one expects that the adsorbed amount (g m^{-2}) of F108 is higher than that of P75 because the buoy groups of F108 (127 monomers) are longer than those of P75 (24 monomers). If P75 and F108 molecules would occupy the same lateral space, the adsorbed amount F108 should be 3 to 4 times as high as for P75. However, the difference is less than a factor of 2. Obviously, F108 molecules adsorb less densely due to steric hindrance of the buoy groups.

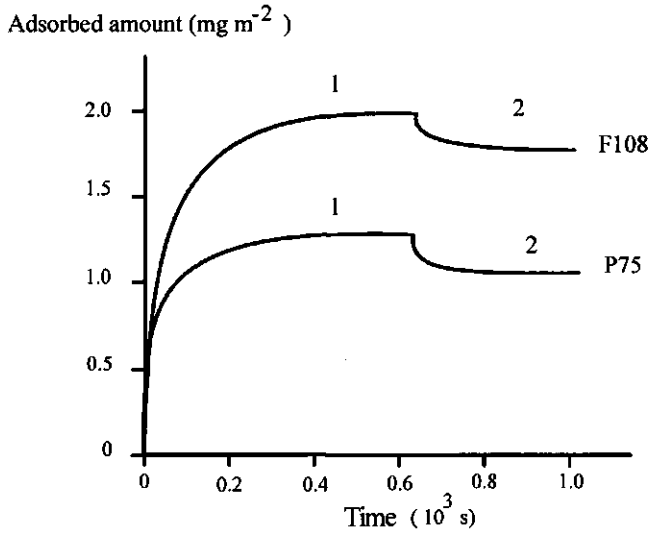

Figure 3. Adsorbed amount of F108 and P75 at a hydrophobic surface.

Adsorption of P75 and F108 onto *hydrophilic* surfaces

First, the substrate is exposed to a block copolymer solution of 100 mg l^{-1} till the signal is constant (figure 4, step 1). The substrate is subsequently rinsed with water (step 2). After rinsing with water the adsorbed amount of P75 and F108 at hydrophilic surfaces is only 0.4 and 0.5 mg m^{-2}, respectively (figure 4), which is considerably less than what was found for hydrophobic surfaces (figure 3). Obviously, molecules adsorbed in a flat pancake configuration occupy more space than molecules adsorbed in a brush configuration and the effect of molecular weight is expected to be weak. Indeed we find that the relative difference of the adsorbed amounts of P75 and F108 at hydrophilic substrates is smaller than that on hydrophobic surfaces. Moreover, from the layer thickness measurement, it was concluded that both block copolymers adsorb on hydrophilic surfaces in a relatively flat conformation.

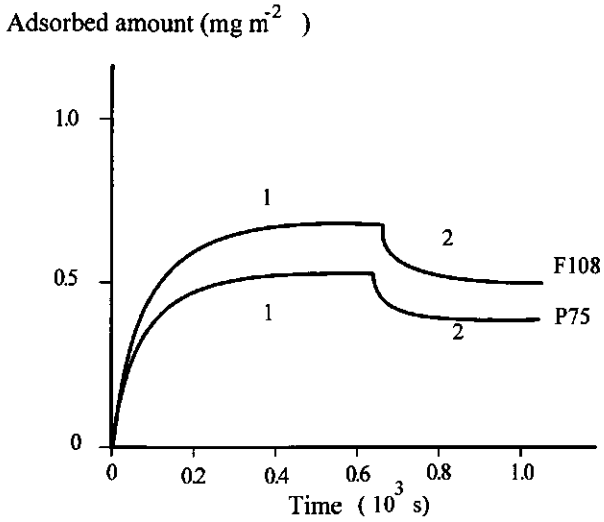

Figure 4. Adsorbed amount of F108 and P75 at a hydrophilic surface.

Adsorption of BSA and lipase onto hydrophobic and hydrophilic surfaces

For all experiments in figures 5-7 the concentrations of lipase and BSA were prepared as 0.5 mg l^{-1}. (The lipase contains 9.5 mg l^{-1} solute, mainly glucose which does not adsorb. For BSA and lipase basically the same results have been obtained (fig 5-7). Therefore, only the results for lipase will be discussed. Lipase adsorbs strongly onto both "bare" hydrophobic surfaces and hydrophilic surfaces. A gradually increasing adsorbed amount is found, no real plateau value is reached within 1 hour. The adsorbed amount of lipase after one hour is approximately 0.5 mg m^{-2} for both types of surfaces. After rinsing with water, the adsorbed amount on a hydrophobic surface decreases to 0.4 mg m^{-2}, indicating that the lipase is tightly attached to the surface. For a hydrophilic surface the adsorbed amount decreases to 0.25 mg m^{-2}, which indicates that the protein molecules are less strongly adsorbed at the hydrophilic surface. This can be explained when hydrophobic interactions (both between hydrophobic parts of the protein and the surface as well as

dehydration of the surface) play an important role in adsorption onto hydrophobic surfaces. For hydrophilic surfaces these interactions are less important resulting in weaker binding of the protein to the surface.

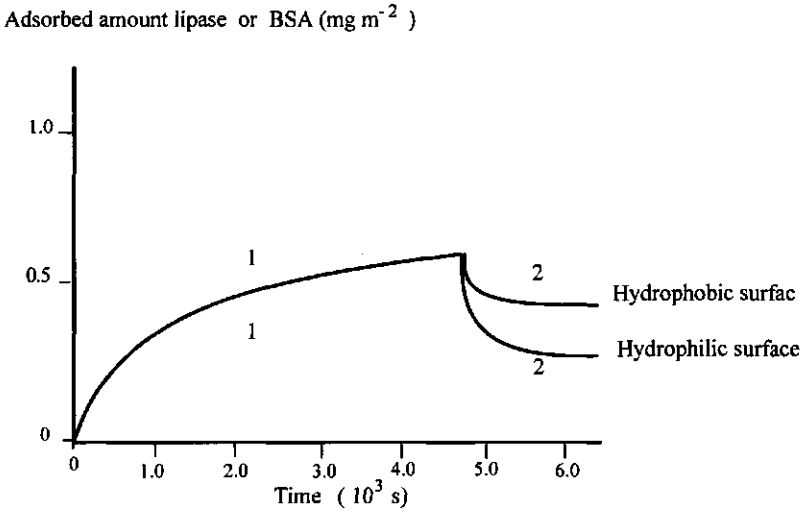

Figure 5. Adsorbed amount of lipase on a hydrophobic and a hydrophilic surface.

Geluk [30] reported adsorbed amounts (after 16 hours of equilibration) of lipase at hydrophobic polystyrene powder (0.8 mg m^{-2}) and hydrophilic cellulose (0.2 mg m^{-2}). The adsorbed amounts reported here are in the same order of magnitude. Our results are also in agreement with those reported by Brink [31] who reported an adsorbed amount of 0.4 mg m^{-2} for BSA on polysulfone membranes.

The initial adsorption rate of lipase (~16.0 μg m^{-2} s^{-1}) is equal for a hydrophobic and a hydrophilic surface. This suggests that transport of lipase towards the surface (convection diffusion) and not binding is the rate limiting step for adsorption to the surface. Indeed is the experimental rate close to that estimated for the impinging jet [27]. If binding would have been the rate limiting step then a difference in initial adsorption rate between both

surfaces would have been expected. It is remarkable however that the rate of adsorption beyond the initial stage is also independent of the nature of the surface.

Influence of block copolymer conformation on protein adsorption

A. Hydrophilic surfaces

From literature [1,2,4,5] it could be concluded that only F108 could be of use for complete prevention of protein adsorption. Therefore, only F108 was studied in combination with protein adsorption. These experiments were carried out sequentially as follows (see figure 6). First F108 is adsorbed until saturation has been reached (step 1). Then, the surface is rinsed with water (step 2); 0.5 mg m^{-2} remains adsorbed at the hydrophilic substrate. Next, lipase is allowed to adsorb (step 3) after which the surface is again rinsed with water (step 4). The increase in adsorbed amount (indicated by Δ) is measured. The results are given in figure 6: Δ= 0.1 mg l^{-1}.

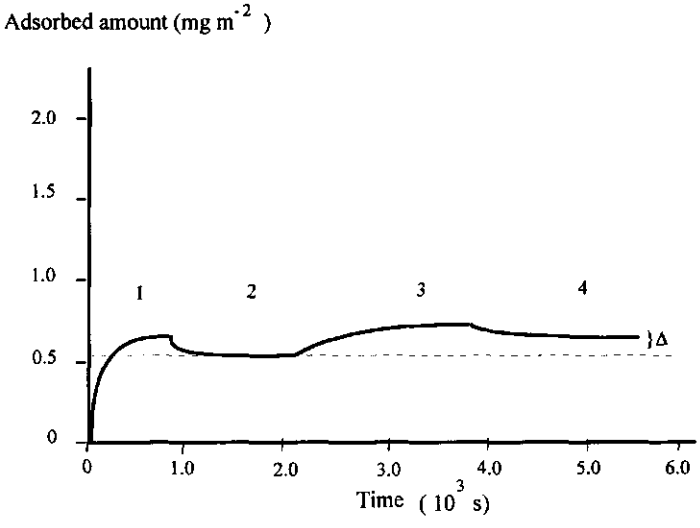

Figure 6. Adsorbed amount of lipase on a hydrophilic substrate with pre-adsorbed F108.

Because it is impossible to distinguish between the signal of block copolymer and the signal of lipase/BSA it is only possible to conclude that at the F108-coated hydrophilic surface an additional adsorbed amount of 0.1 mg per m^2 is present. If the block copolymer is not replaced by lipase than this will also be the absolute adsorbed amount of lipase. Because the adsorbed amount of lipase at a bare surface is 0.25 mg m^{-2} (figure 5) it can be concluded that at least 0.35 mg m^{-2} F108 remains adsorbed. A more important conclusion is that a saturated layer of F108 adsorbed onto a hydrophilic surface is not capable of preventing protein adsorption, although it reduces it.

B. Hydrophobic surfaces

For hydrophobic surfaces, similar sequential adsorption experiments were carried out. First, block copolymer F108 was adsorbed (figure 7, step 1). Subsequently, the surface was rinsed with water (step 2), followed by adsorption of lipase (step 3). The surface was again rinsed with water (step 4). Finally, steps 3 and 4 were repeated (steps 5 and 6).

The adsorbed amount of F108 after rinsing with water (step 2) is 1.8 mg m^{-2}. After rinsing with lipase solution (step 3) a small increase in signal is found. The increase is caused by an experimental problem: a small volume of block copolymer solution still present in the tubing between the 2 two-way valves. First the adsorbed amount of F108 will increase but as soon as the F108 in the tubing is replaced by lipase solution the signal decreases again to a level as found after step 2 which indicates that no protein adsorption has taken place. After step 4, rinsing with water, the signal remains constant. Repeated rinsing with lipase solution (step 5) does not result in an increase in adsorbed amount. This indicates that on this surface with a saturated F108-layer no protein adsorption takes place.

From figures 6 and 7 it can be concluded that F108 prevents protein adsorption only if it is adsorbed onto a hydrophobic surface. The mere presence of F108 is not enough for prevention of protein adsorption: the conformation of the adsorbed block copolymer molecules is important. Because comparable results were obtained for BSA, explanations

invoking a specific repulsive interaction between block copolymer and lipase molecules cannot be accepted.

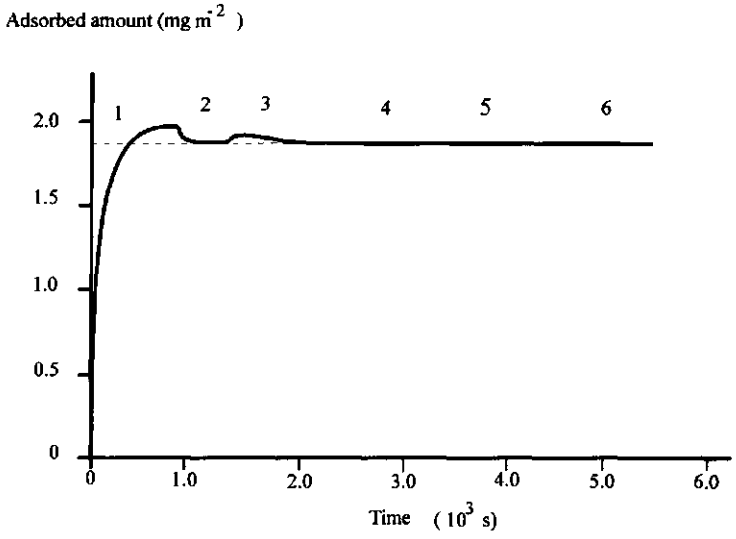

Figure 7. Adsorption of lipase at an F108-coated hydrophobic surface.

A remaining question is whether the block copolymer layer has to be saturated for the successful suppression of protein adsorption or whether lower coverages can also produce this effect. This question is investigated in the following section.

C. Effect of adsorbed amount F108 on lipase adsorption

The same procedure was followed as for protein adsorption at a saturated block copolymer layer but now supply of F108 was interrupted at lower levels of coverage. After rinsing with water, the substrate was exposed to a lipase solution (10 mg l^{-1} ; step 3), rinsed with water (step 4) and exposed again to lipase solution (step 5). The adsorbed amount was then measured as a function of time. Lipase still adsorbs for F108 surface coverages between 10 and 70% of saturation. The adsorbed amount increases linearly in

time. The remarkable finding, however, is that it does so very slowly (step 5). The plateau value for the adsorbed amount of lipase is not reached within 5 hours. In figure 8 the relative initial adsorption rate (with respect to the initial adsorption rate of lipase at a "bare" hydrophobic surface of 16 $\mu g\ m^{-2}\ s^{-1}$) is given as a function of the percentage saturation of the block copolymer layer.

Figure 8 shows that at 70-100% saturation with F108 no measurable adsorption takes place. If the adsorbed amount of block copolymer is decreased below this level the lipase adsorption rate begins to increase, but remains very low even for 10% coverage with F108. Apparently, protein molecules can begin to penetrate into the F108-layer when the coverage falls to below 70% saturation. However, the number of 'holes' in the F108-layer remains very low down to 10% saturation.

In this context it may be useful to make an estimate of the amount of surface area left open at low block copolymer coverages. The average distance between two F108 molecules at 10% saturation is about 11 nm. Each F108 molecule carries two PEO blocks of MW 5700 each, which have an estimated radius of gyration of 2.5 nm [32]. This would leave about 40 nm^2 uncovered area per F108 molecule. This corresponds roughly to the area required for one protein molecule to fit in so that one would expect an adsorbed amount of protein of 0.8 $mg\ m^{-2}$ if all 'holes' were rapidly filled. Clearly, this is at variance with our experimental result, which shows both a lower total coverage and a strongly reduced adsorption rate (see figure 9).

For terminally attached PEO chains Jeon *et al.* [17,18] predict an optimum grafting density at which protein resistance is largest. For proteins with a radius of 2 nm (the radius of lipase is approximately 3 nm [30]) and PEO-chains with a length of 120 monomers (F108 has 127 monomers) they predict an optimum grafting distance of 0.9-1.1 nm. If we consider adsorbed F108 to behave as 2 grafted chains then the average distance between two PEO groups will be 2.5 nm which is a factor 2 higher compared to the predicted optimum surface density of Jeon *et al* [17,18]. For lower surface densities

Jeon *et al.* predict the protein resistance to be less which was confirmed by our experiments. At unsaturated F108 layers the initial adsorption rate increases with decreasing saturation percentage. Unfortunately, the surface density of F108 could not be increased to values at which the distance between the PEO chains decreases below 0.9 nm. Therefore, the theory of Jeon could not be tested completely.

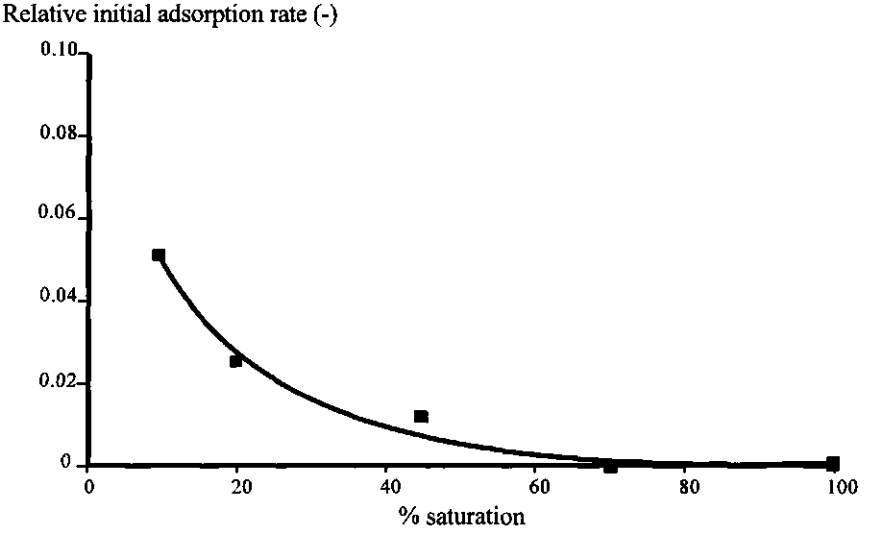

Figure 8. Relative adsorption rate of lipase (10 mg l^{-1}) at block copolymer layers with various degree of saturation.

Adsorption (of lipase) at a "bare" surface is a 2-step process determined by transfer of lipase toward the surface and attachment to the surface [27]. In equation 7 the adsorption rate $d\Gamma / dt$ (mol m^{-2} s^{-1}) is given as a function of the diffusion resistance k^{-1} (m s^{-1}), the attachment resistance K^{-1} (m s^{-1}) and the bulk concentration c_b (mol m^{-3}).

$$\frac{d\Gamma}{dt} = \left(\frac{1}{k^{-1} + K^{-1}}\right) \cdot c_b \qquad (7)$$

For adsorption at a "bare" surface (figure 8) K^{-1} is zero, the initial adsorption rate is determined by k^{-1}. For surfaces with pre-adsorbed block copolymer k^{-1} remains constant. The decrease in initial adsorbed amount for block copolymer covered surfaces is caused by an increase in attachment resistance. If the block copolymer is not replaced by the lipase (which would cause an additional resistance) then the initial adsorption rate will increase linearly with the bulk concentration. This was tested by measuring lipase adsorption at a 10% saturated surface for (crude) lipase concentrations between 25 and 250 mg l^{-1} (figure 9).

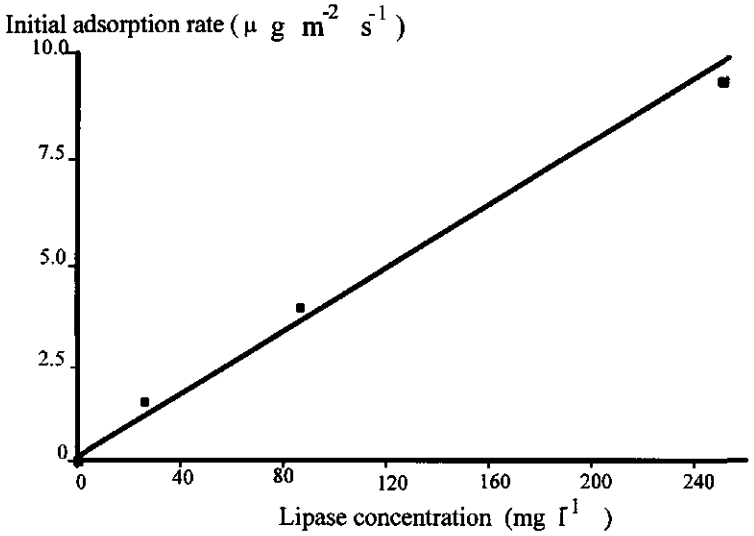

Figure 9. Initial adsorption rate of lipase at a hydrophobic surface at 10% saturation with block copolymer.

The initial lipase adsorption rate increases (practically) linearly with the lipase concentration, which is an indication that the block copolymer is not replaced by lipase. The maximum adsorbed amount of lipase could be determined and was fairly constant for all lipase concentrations but approximately 3 times lower as compared to a surface without F108. For all lipase concentrations the available space between the block

copolymer molecules is apparently filled with lipase. The block copolymers are not removed from the surface; otherwise an adsorbed amount comparable to that on a "bare" surface would have been expected. This confirms that F108 hinders attachment to the surface.

In conclusion, it can be stated that adsorbed block copolymers hinder attachment of lipase to the surface, even if the adsorbed amount of block copolymer is of the order of 10% of a saturated layer. At such a layer the lipase adsorbs without displacing the block copolymer. In the case of a fully saturated F108-layer hindrance of attachment is clearly thus severe that no protein adsorption at all takes place.

CONCLUSIONS

The conformation of adsorbed F108 (block copolymer) and therewith the layer thickness depends on the substrate properties. At a hydrophobic surface the molecules adsorb in a brush configuration resulting in a layer of 10 nm thickness. At a hydrophilic surface a mushroom configuration is formed corresponding with a layer thickness of 1 nm. Only F108-molecules adsorbed in the brush configuration prevents lipase adsorption if the block copolymer layer is saturated.

At unsaturated block copolymer layers lipase adsorption takes place. However, the adsorption rate is low, at 10% saturated surface this value is in the order of 5% of the adsorption rate at a "bare" hydrophobic surface. It can be concluded that attachment of lipase is hindered by the block copolymer molecules.

The initial adsorption rate increases linearly with the lipase concentration for 10% F108-saturated surfaces. The maximum adsorbed amount of lipase at such a surface is 3 times lower as compared to a "bare" surface, therefore, it can be concluded that the block copolymers are not replaced by lipase. In case of a fully saturated F108-layer, hindrance of attachment is clearly thus severe that no protein adsorption takes place.

ACKNOWLEDGEMENT

The authors wish to thank The Dutch Programme Committee for Membrane Research (PCM) and The Technology Foundation (STW) for their financial support.

LIST OF SYMBOLS AND ABBREVIATIONS

a	: Sensitivity reflectometer	(g m^{-2})
aa	: After adsorption	
ba	: Before adsorption	
c_b	: Bulk concentration	(mol m^{-3})
f	: Loss factor	(-)
k^{-1}	: Diffusion resistance	(m s^{-1})
p	: Parallel	
s	: Perpendicular	
t	: Time	(s)
I	: Reflected intensities	(cd)
I_0	: Incoming light intensities	(cd)
K^{-1}	: Attachment resistance	(m s^{-1})
PEO	: Poly(ethylene oxide)	
PPO	: Poly(propylene oxide)	
R	: Reflectivity	(-)
S	: Signal	(-)
Δ	: Increase in adsorbed amount	(g m^{-2})
Γ	: Adsorbed amount	(g m^{-2})

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Chapter 6

DYNAMIC MODELLING OF THE ENZYMATIC HYDROLYSIS OF TRIGLYCERIDES IN AN EMULSION/MEMBRANE BIOREACTOR

ABSTRACT

This article describes the hydrolysis of triglycerides in an emulsion/membrane bioreactor. This reactor consists of a stirred vessel in which fatty acids and glycerol are produced and two membrane separation steps removing the products from the emulsion in the stirred vessel.

The inactivation constant of lipase present at the oil/water interface is measured to be $4.6x10^{-3}$ h^{-1}. It is found that inactive enzyme at the oil/water interface is replaced by active enzyme, therefore, a constant fatty acid production rate can be obtained during a longer period of time if the reactor is operated at an enzyme concentration beyond the saturation concentration of the oil/water surface area.

This chapter has been submitted as:

Dynamic modelling of the enzymatic hydrolysis of triglycerides in an emulsion/membrane bioreactor

C.G.P.H. Schroën, P.E.A. Smulders, S. Van Hoof, A. van der Padt and K. van 't Riet

The fatty acid production rate equation for the emulsion/membrane bioreactor is derived from the work of Pronk [1,2]. This equation gives the production rate as a function of the glycerol mole fraction, the fatty acid mole fraction and the initial enzyme activity. The fatty acid and glycerol production in the reactor are predicted for variable net oil and water flows with the reaction rate equation and a model for enzyme inactivation. The model predictions are in good agreement with the experimental data. Subsequently, the model is used to evaluate reactors in series. The produced amount of fatty acid per gram added enzyme and the volume of the reactors are thus that the production of fatty acids in a co-current series of reactors should be feasible.

INTRODUCTION

Chemical hydrolysis of triglycerides (official name triacylglycerols which are the main constituent of edible oils and fats) for the production of fatty acids is carried out in continuous counter current columns at 240-260 °C and 50-60 bar pressure. Due to the high temperature polymerisation of unsaturated fatty acids takes place [3,4]. Therefore, the variety of fats and oils that can be hydrolysed in this way is limited. Alternative methods are developed for oils with a higher (but still low) amount of unsaturated fatty acids. Although these alternative processes are carried out at 200-240 °C [5], polymerisation products are formed and have to be removed in order to obtain an acceptable product [4]. Polymerisation does not take place if the reaction is carried out enzymatically at for example 30 °C [6,7]. The enzyme, lipase, catalyses the following hydrolysis reactions:

Triglyceride + Water	⇌	***Diglyceride + Fatty acid***
Diglyceride + Water	⇌	***Monoglyceride + Fatty acid***
Monoglyceride + Water	⇌	***Fatty acid + Glycerol***

The enzyme will catalyse the reactions if it is present in its active conformation at the oil/water interface [8]. In order to obtain a high volumetric activity the oil/water surface

area in the reactor should be high. For economic and product purity reasons the products and the enzyme should be separable. Several reactor concepts are proposed in literature which all have their own specific advantages and disadvantages.

In e.g. membrane reactors [9,13], or packed bed reactors [14,15] the enzyme is immobilised in order to facilitate product separation. If a suitable immobilisation material is chosen, the enzyme is stabilised. However, the immobilisation material is wetted preferentially by either one of the substrates and therefore diffusion limitation can occur. In addition, the surface area per unit of reactor volume is relatively low compared to an emulsion reactor and, consequently, the volumetric reactor activity will also be relatively low. An extensive literature overview on immobilised enzyme reactors is given by Malcata [16].

A high volumetric reactor activity can be achieved by using the enzyme in its free form in emulsions [17,19]. However, product separation is more difficult as compared to reactors with immobilised enzyme because the emulsion is stabilised by the enzyme. Bühler [17] uses centrifuges to separate the emulsion. The disadvantage of this method is that 10% of the enzyme is lost during the separation process.

This problem does not occur in case of an emulsion/membrane bioreactor. The reactor concept is given in figure 1. Triglycerides and water are mixed in a stirred vessel in the presence of lipase and an emulsion is formed. The emulsion is subsequently separated with a hydrophilic cellulose membrane (cut-off value 6,000) and a modified hydrophobic polypropylene membrane (pore size 0.1 μm) (see materials section). The hydrophilic membrane is not permeable for the enzyme due to its pore size. The enzyme does also not permeate through the modified hydrophobic membrane since the enzyme is not soluble in the permeating oil phase. The enzyme is retained within the system and the emulsion can be separated with both membranes working simultaneously [19].

The aim of this article is to show that the emulsion/membrane bioreactor can be used for the continuous hydrolysis of triacylglycerols and to develop a model based on a reaction

rate equation. The reaction rate equation accounts for the effect of glycerol concentration, fatty acid mole fraction, temperature and inactivation of the enzyme. The model is adapted from literature, and measured enzyme inactivation in an emulsion system is added. The model is validated with experimental data and used to calculate fatty acid production in one and in a series of reactors, respectively.

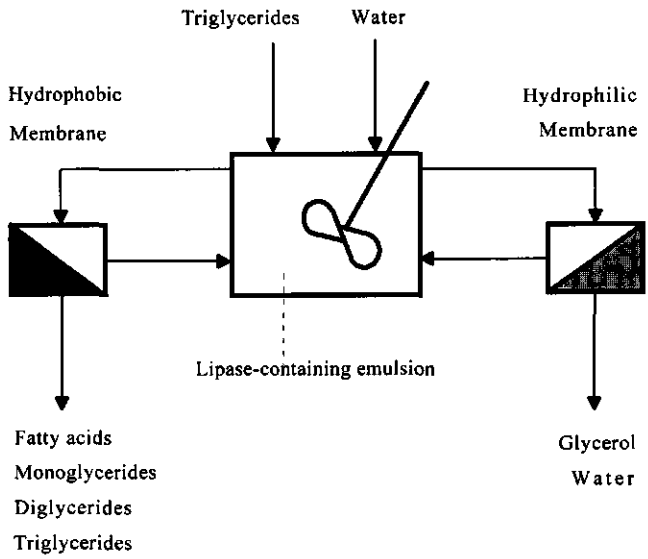

Figure 1. The emulsion/membrane bioreactor.

THEORY

It is assumed that the hydrolysis reactions can be described by one overall-reaction in which triglycerides react with water to form fatty acids and glycerol.

$$\textit{1 Triglyceride} + \textit{3 Water} \rightleftharpoons \textit{3 Fatty acids} + \textit{1 Glycerol}$$

The actual reaction rate of the reactor can be calculated from mass balance equations.

$$\frac{dn_i}{dt} = \phi_{i,in} - \phi_{i,out} + r_i \cdot M \qquad (1)$$

In which n is the number of moles of component i (mol), ϕ is the number of moles that is pumped into or removed from the reactor per unit of time (mol h^{-1}), r is the volumetric reaction rate (mol i mol^{-1} (oil phase or water phase) h^{-1}), M the total number of moles of oil phase or water phase in the emulsion (mol) and t is the time (h). The subscripts *in*, *out* and *prod* denote incoming, outgoing and produced.

Pronk [1,2] gives two equations with which the fatty acid and glycerol production rate in a batch membrane bioreactor can be predicted. These equations can be adjusted for the emulsion reactor by addition of the E_0-term which accounts for the amount of enzyme initially present in the emulsion. This results in:

$$r_F = a_1 \cdot E_0 \cdot e^{-k_d \cdot t} \cdot (1 - a_2 \cdot X_G) \cdot e^{\frac{-\Delta E_h}{R} \cdot (\frac{1}{T} - \frac{1}{298})} \cdot (X_F^{eq} - X_F)^{n^*} \qquad (2)$$

In this equation r_F is the production rate of fatty acid (mol (fatty acid) h^{-1} mol^{-1} (oil phase)), a_1 is a fitparameter (h^{-1}), E_0 is the initial enzyme concentration (g l^{-1} (emulsion)), k_d is the inactivation constant (h^{-1}), t is the time (h), a_2 is a fitparameter (-), ΔE_h is the activation energy of the hydrolysis reaction (12.3 kJ mol^{-1}; [1]), R is the gas constant (kJ mol^{-1} K^{-1}), T is the temperature (K), X^{eq} is the equilibrium mole fraction (mol mol^{-1} (oil phase)) and n^* is the order of the reaction (-). The subscripts F and G relate the quantity to fatty acid or glycerol. For the integration of equation 1 the fourth and fifth order Runge-Kutta algorithm is used. The production rate of glycerol, r_G, is related to the fatty acid production rate as

$$r_G = \frac{1}{3} r_F \qquad (3)$$

MATERIALS

Chemicals

Sunflower oil of edible quality (esters of glycerol and fatty acids of which over 95% are C16 and C18 acids) was purchased from Remia (Den Dolder, the Netherlands). The enzyme, Lipase B, was obtained from Biocatalyst and originated from the yeast *Candida*

rugosa (formerly called *Candida cylindracea*). Hexadecane (analytical grade) was purchased from Merck (Germany). Glycerol (>99.5% pure) was obtained from Janssen Chimica (Belgium). Sodium hydroxide (titrisol, analytical grade) and phenolphthalein (analytical grade) for the fatty acid titration and the maleic acid (analytical grade) and the arabic gum for the tributyrine assay originated from Merck (Germany). Tributyrine (analytical grade) was obtained from Serva (Germany). Distilled water was used throughout.

F108 block copolymer was a gift from ICI (Rotterdam, the Netherlands). The block copolymer consists of three blocks, two poly(ethylene oxide) (PEO) blocks at both ends of the molecule and one poly(propylene oxide) (PPO) middle block. The total molecular weight of F108 is 14,000 g mol^{-1}, the molecular weight of the middle block is 2,400 g mol^{-1}.

Membranes

The hydrophilic membrane used was a cellulose (Cuprophan) hollow fibre device from Organon Technika (Boxtel, The Netherlands) with a surface area of 0.77 m^2, a membrane wall thickness of 8 μm and a nominal molecular weight cut-off value of 6,000 g mol^{-1}.

For the hydrophobic membrane both flat sheet and hollow fibre polypropylene membranes were used. The flat sheet polypropylene membrane was provided by Enka (Wuppertal, Germany), and has a mean pore size of 0.1 μm. The flat sheet membrane was used in a Megaflow module (type TM 100, effective surface area $64x10^{-4}$ m^2) from New Brunswick Scientific (Edison, USA) with a channel height of 2 mm. The hollow fibre polypropylene device originated from Mycrodyne (Germany) and has a total surface area of 0.1 m^2. The length of the fibres is 0.5 m, the diameter is 1.8 mm and the average pore size is 0.2 μm.

Chapter 6

METHODS

The temperature during all experiments was kept at 30 ^{0}C.

Continuous reactor experiments

For the continuous reactor experiments a stirred vessel of 1000 ml is used. Sunflower oil and water (1:1 v/v) with a total volume of 900 ml were emulsified in a stirred vessel (diameter 0.1 m), containing 4 baffles (12cm x 1cm) with four-bladed turbine stirrers (diameter 4.5 cm, blade width 11 mm) at a stirrer speed of 450 rpm. Two stirrers were placed at 4 cm distance from each other on one axis, the blades of the two stirrers being in one line. The lower stirrer was placed at a distance equal to the stirrer diameter from the bottom of the vessel.

The reactor was operated at a water/oil phase volume ratio of 1:1. The volume ratio was checked each day and adjusted if necessary. In case the volume of the reactor exceeded 900 ml, a pump removed the excess amount of emulsion from the stirred vessel in order to prevent overflow of the reactor.

The transmembrane pressure over both membranes was not constant and, therefore, the transmembrane flux also was not constant. In order to make continuous operation possible the permeate was collected in over-flow vessels. At set times a certain volume of oil or water phase was removed from these vessels and the same volume of oil or water was added to the reactor. The surplus of permeate was directly recycled to the reaction vessel.

The fatty acid content in the reactor was determined by titrating either a sample of emulsion or a sample of the permeate of the hydrophobic membrane. The glycerol concentration was determined in the permeate of the hydrophilic membrane (see analysis section).

Reaction rate

a. Continuous experiment

The incoming and outgoing mass values were measured (and adjusted if necessary) during the continuous experiment. The reaction rates of all the components in the reactor (fatty acids, glycerol, triglycerides and water) were calculated from balance equations (see equation 1). The fatty acid concentration in the reactor was measured analogous to the continuous reactor experiment by titrating (i) a sample of the emulsion or (ii) a sample of the permeate of the hydrophobic membrane. The glycerol concentration was determined in the permeate of the hydrophilic membrane (see analysis section).

b. Batch experiment

For the batch experiments a stirred vessel as described for the continuous reactor experiments was used. Lipase was added to the emulsion in a concentration range of 0.05 to 8 g per litre of emulsion. Samples of approximately 0.5 g were taken from the emulsion and the fatty acid concentration was determined by titration (see analysis section).

The calculated concentration using eq. 1 (ϕ_{in}= ϕ_{out}= 0) and 2 was fitted to the (seven) measured curves by minimising the residual sum of squares. The fitparameters a_2 and n^* were fitted for all the curves simultaneously. Fitparameter a_1 was fitted separately, hence, for all the curves the value of a_1 is different (see results section). It was assumed that inactivation of the enzyme did not take place during the experiment.

Enzyme inactivation

a. Continuous experiments with the emulsion/membrane bioreactor

The reaction rate during a continuous experiment (r_F) was measured using the balance equation (equation 1). This reaction rate includes the effect of the inactivation of the enzyme. By taking the e^{-kt} term equal to 1 (see equation 2), a theoretical reaction rate

without enzyme inactivation ($r_{F,\ theory}$) was calculated for a given fatty acid and glycerol concentration in the stirred vessel. For first order inactivation the logarithm of the quotient of r_F and $r_{F,\ theory}$ versus time should render a straight line with slope k_d.

b. Batch experiment (tributyrine standard method)

Sunflower oil and water (1:1 v/v) with a total volume of 450 ml were emulsified in a stirred vessel (diameter 0.1 m), containing 4 baffles (12 cm^2 each). A four-bladed standard turbine stirrer (diameter 4.5 cm, blade width 11 mm) was used at 450 rpm. The stirrer was placed at one stirrer diameter from the bottom of the vessel. To the emulsion lipase was added in a concentration range of 0.5 to 5 g per litre of emulsion. From the emulsion samples were taken and the enzyme activity of the sample was measured using the tributyrine standard method (see analysis section).

Addition of lipase

The crude lipase preparation was dissolved in water and centrifuged at 13.000 rpm (Hereaus, biofuge A). The sediment was discarded; only the supernatant was used in experiments. The enzyme concentration is expressed as grams of crude enzyme preparation.

Membrane modification with block copolymers

The membrane was pre-wetted by rinsing the membrane with hexadecane along the retentate side for 30 minutes; hexadecane permeated through the membrane. Subsequently the membrane was modified with an emulsion (1:2 v/v hexadecane in water) containing 6 gram block copolymer per litre of emulsion. This emulsion was pumped from the stirred vessel along the retentate side of the hydrophobic membrane for 15 minutes. Only hexadecane permeated through the membrane. Both the permeate and the retentate were recycled to the stirred vessel. The emulsion was removed from the system by rinsing the retentate side with water for 15 minutes; water did not permeate

through the membrane. After this the membrane was used in the emulsion/membrane bioreactor.

ANALYSIS

Activity of lipase

Lipase activity was tested in a separate tributyrine assay at 30 °C. The assay liquid contained 1 ml of tributyrine and 50 ml of a solution containing 0.15 (w/v) Arabic gum and 2 mM maleic acid (pH = 6.7). The liquid was emulsified 3 times for 30 seconds with a sonifier (Kinematica AG, Switzerland). The emulsion was stirred in a vessel thermostated to 30 °C. The enzyme solution was added and the pH was kept at 6.1 by addition of sodium hydroxide (0.01 mol l^{-1}). One unit lipase activity was defined here as the amount of lipase releasing 1 μmole of butyric acid per minute under these conditions. The amount of active enzyme added to the assay emulsion was kept constant by varying the volume of the samples. The surface area in the tributyrine assay was thus that the amount of active enzyme determined the measured activity.

Composition oil phase

Samples of the emulsion were directly added to 20 ml of ethanol (which contains 0.02% w/w phenolphthalein, Merck) in order to inactivate the enzyme. The fatty acid content was measured by titration with 0.1 mol l^{-1} NaOH (titrisol, Merck). The samples of the permeate of the hydrophobic membrane were also added to ethanol and subsequently titrated.

Glycerol concentration

The refractive index of the water phase was determined at 30 °C. The correlation between refractive index and glycerol concentration is given in Weast [20].

RESULTS AND DISCUSSION

Membrane performance

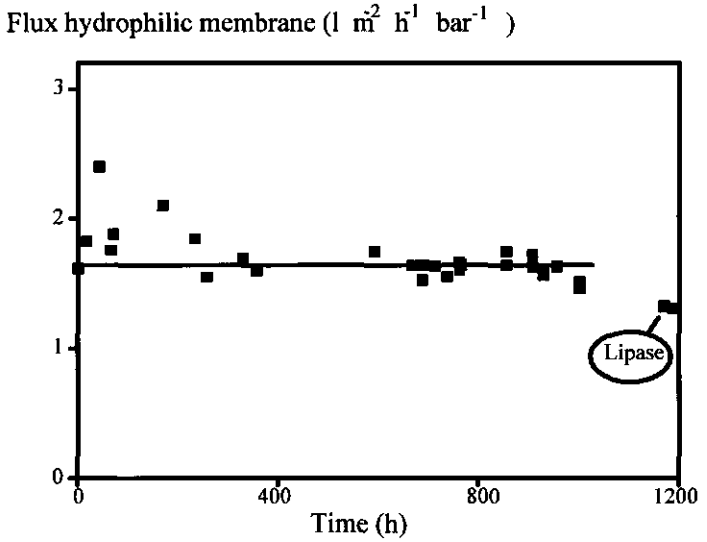

Figure 2. Flux through the hydrophilic membrane as a function of time; at t =1180 hours 0.8 gram lipase is added.

The membranes are tested during a 1200 hours lasting experiment with the continuous reactor. The net oil and water flow through the reactor are 18 and 40 ml h^{-1}, respectively. The enzyme concentration is 0.9 g l^{-1}. The flux through both membranes is measured as a function of time. Figure 2 shows that the flux through the hydrophilic membrane remains fairly constant at 1.7 (l m^{-2} h^{-1} bar^{-1}) over 1000 hours. At 1180 hours extra lipase (0.8 g) is added to the emulsion. The flux decreases to 1.4 (l m^{-2} h^{-1} bar^{-1}) and remains constant for 8 hours. Apparently, this flux decrease is caused by lipase adsorption. The extent to which the flux decreases is comparable to previously obtained results [19]. Because the membrane flux remains constant after the initial flux decrease, it is concluded that the cellulose membrane is suitable for application in the emulsion/membrane bioreactor.

The flux through the F108-modified hydrophobic membrane is also measured as a function of time and the permeate is monitored for water permeation. Water permeation (loss of selectivity for the oil phase) is an indication for protein adsorption at the hydrophobic membrane, which can be prevented by pre-adsorbed block copolymer F108 [21,23]. If during the experiment water permeation takes place it can be concluded that the modification is not effective [24]. During the experiment no water permeation takes place which indicates that the modification with block copolymer remains effective during the 1200 hours of operation. The block copolymers are not replaced by lipase which is conform previous findings [22]. However note that membrane modification is essential otherwise water permeation would certainly have taken place [22,24].

The flux through the membrane varies with e.g. the transmembrane pressure and the viscosity of the permeate [25] and the composition of the emulsion. Because these factors vary during the experiment the flux (l m^{-2} h^{-1} bar^{-1}) varies likewise. In spite of all this, the flux remains between 15 and 30 (l m^{-2} h^{-1} bar^{-1}) during the experiment which is an acceptable level.

Reaction rate in batch experiments

The equilibrium fatty acid mole fraction, X_F^{eq}, is measured and a value of 0.995 is found. This value is used in the fitting procedure. The model (equation 2 with $k_d = 0$) is fitted to the fatty acid concentration in emulsions with a lipase concentration between 0.05 and 8 g enzyme per litre of emulsion using the procedure described in the methods section. Figure 3 shows a typical example for an enzyme concentration of 0.9 g l^{-1} emulsion; the fit and the measured points agree well. Also for the other enzyme concentrations the agreement between experimental data and fit is good; the standard deviation ($(\Sigma(\text{measured value - predicted value})^2$ / number of datum points$)^{0.5}$) is for all enzyme concentrations < 0.01. Parameter a_2 is 3.0 for all enzyme concentrations; which indicates that the reaction rate is hardly influenced by the glycerol concentration. The order of the reaction, n^*, is 1.58 near to the value of 1.75 reported by Pronk [1,2].

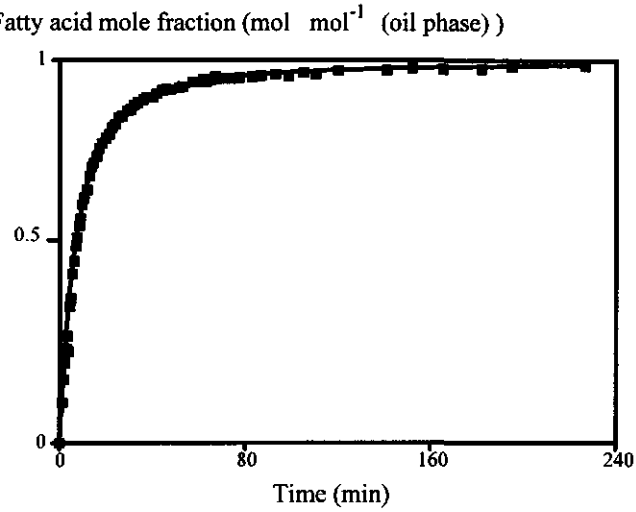

Figure 3. The fatty acid mole fraction as a function of time in an emulsion with 0.9 g lipase per litre of emulsion; the line gives the best fit to the measured values, the symbols represent the measurements. For the fit the following parameters are used; E=0.94, a_1 = 6.57; a_2=3.0, n^*=1.58 and X_F^{eq}=0.995.

Fitparameter a_1, which is an indication for the initial reaction rate, decreases with increasing enzyme concentration (see figure 4). This indicates that the reaction rate of the enzyme decreases with increasing enzyme concentration. This effect becomes even more pronounced if the reaction rate per gram enzyme (mol mol^{-1} (oil phase) h^{-1} g^{-1} (enzyme)) at a fatty acid mole fraction of 0.5 is plotted as a function of the enzyme concentration (see figure 5). A fatty acid mole fraction of 0.5 is chosen because the initial reaction rate can only be determined ambiguously. If the reaction rate per gram enzyme was independent of the enzyme concentration than a horizontal straight line would be obtained. Obviously, this is not the case.

For enzyme concentrations <1.2 g l^{-1} the fatty acid production rate (mol (fatty acid) mol^{-1} (oil phase) h^{-1}) increases strongly with increasing enzyme concentration (results not shown). At low enzyme concentration the amount of enzyme determines the fatty acid

production rate, and therewith, the strong increase in production rate can be understood. For high enzyme concentrations (>1.2 g l^{-1}) it is expected that the oil/water surface area will be fully occupied with enzyme and the surplus of lipase will be present in the water phase of the emulsion thus not contributing to the fatty acid production rate. If the surface area in all the emulsions is equal than it is expected that the fatty acid production rate remains constant at high enzyme concentrations. This is not the case, for enzyme concentrations >1.2 g l^{-1} the fatty acid production rate increases further although only slightly. This can be explained by the emulsifying effect of the enzyme which results in a larger surface area in the emulsion and thus in a (slightly) increasing fatty acid production rate at increasing enzyme concentration.

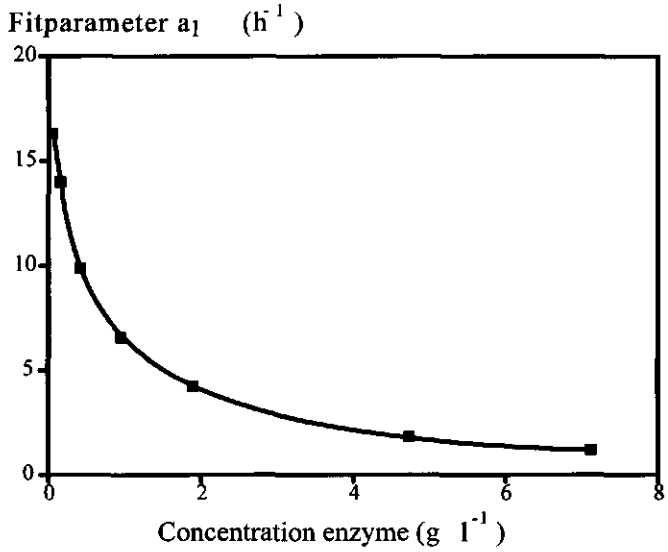

Figure 4. Fitparameter a_1 as a function of the enzyme concentration in the emulsion.

The reaction rate (activity) of the enzyme decreases with increasing enzyme concentration (see also figure 4) also for low enzyme concentrations. The reason for the decreasing activity at increasing degrees of occupancy may be related to the influence of lateral interactions between the lipase molecules or to a decreased accessibility of the

substrate due to crowding of the oil/water interface [26]. In order to take the effect of the decreasing activity at high enzyme concentrations into account a_l from equation 2 is replaced by (see line in figure 4)

$$a_1 = \frac{1}{0.056 + 0.11 \cdot E_0} \qquad (4)$$

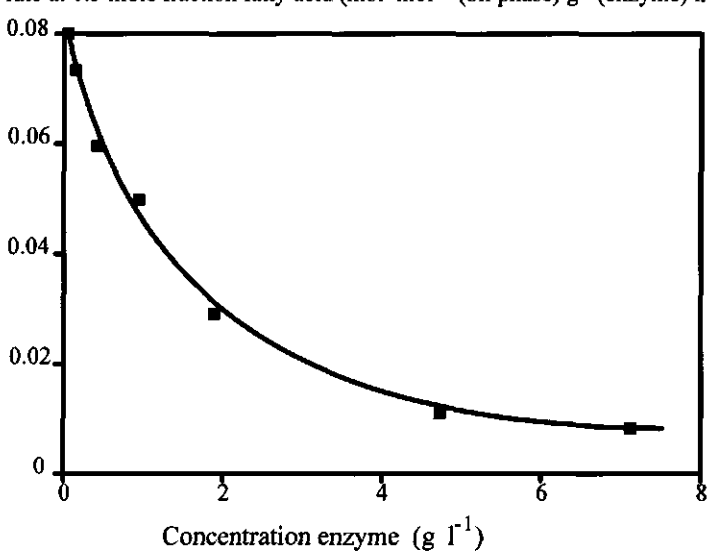

Figure 5. The reaction rate per gram enzyme at 0.5 mole fraction fatty acid as a function of the enzyme concentration in the emulsion.

Enzyme inactivation in the emulsion/membrane bioreactor

For the batch reaction rate experiments, as described above, it is assumed that inactivation of the enzyme could be neglected. In order to check this assumption a continuous experiment is performed with the emulsion/membrane bioreactor.

The enzyme concentration in the reactor is 0.9 g l^{-1} emulsion. This concentration is used because in this case the reaction rate is limited by the amount of enzyme and not by the surface area of the emulsion as discussed previously. Consequently, enzyme inactivation

has to result in a change in reaction rate. In figure 6 the natural logarithm of the actual over the theoretical reaction rate is plotted as a function of time (see methods section; enzyme inactivation). During the experiment the water flow rate is kept at 40 ml h^{-1} and the oil flow rate at 18 ml h^{-1}.

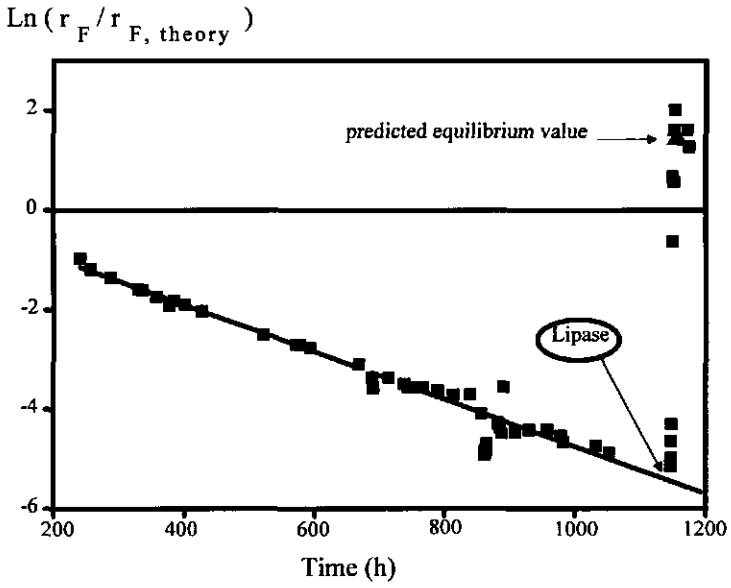

Figure 6. Natural logarithm of the actual and theoretical reaction rate as a function of time; at 1180 hours 0.8 gram enzyme is added to the emulsion.

During the first 200 hours the reactor is not controlled properly and emulsion is removed from the reactor by the overflow device, therefore, these data are not given in figure 5. Between 200 and 1100 hours the reactor is stable and these data are used for the determination of the inactivation constant in the reactor. Linear regression of these data points renders an inactivation constant, k_d, of 4.6×10^{-3} h^{-1}. This value is in good agreement with the value of 5×10^{-3} h^{-1} found in a batch inactivation experiment in which the activity of lipase (0.3 g l^{-1}) is determined with the tributyrine standard method.

Extra enzyme (0.8 g) is added to the emulsion after 1180 hours, when approximately 10% of the enzyme is still active, in order to check whether active lipase is hindered by inactive enzyme. It is expected that the inactive enzyme either leaves the oil/water interface upon inactivation or is replaced by the active enzyme. This should result in an increase in the fatty acid production, which is found to be the case (see figure 6; t=1180 h). The reaction rate after addition of the new lipase is comparable to the reaction rate predicted by equations 2 and 3 for emulsions without inactive lipase. This implies that the reactor can be operated at a constant reaction rate (without changing the oil or water flow rate) for a longer period of time if "high" enzyme concentrations are used. However, the enzyme will also inactivate in the bulk water phase without contributing to the fatty acid production, therefore, the enzyme load has to be optimised for continuous reactor operation.

Enzyme inactivation in batch experiment

Let us assume that the active (not inactivated) enzyme in the emulsion is present either in the bulk water phase or at the oil/water interface.

$$E_a = V \cdot c_e + A \cdot \Gamma \tag{5}$$

In this equation E_a is the total amount of active lipase present in the emulsion (units), V is the volume of the emulsion (m^3), c_e is the concentration of active enzyme in the bulk water phase (units m^{-3} (emulsion)), A is the surface area in the emulsion (m^2) and Γ is the adsorbed amount of lipase (units m^{-2}). If the enzyme inactivates both at the oil/water interface and in the bulk water phase and active enzyme replaces inactive enzyme at the oil/water interface (as shown in figure 6) then the concentration active enzyme in the bulk water phase decreases as (see also figure 7)

$$\left.\frac{d\,c_e(t)}{dt}\right|_{c_e>0} = -\frac{\Gamma^* \cdot A}{V} \cdot k_{d,surf} - c_e(t) \cdot k_{d,bulk} \tag{6}$$

With t the time (h), Γ^* the maximum adsorbed amount of lipase at the oil/water interface (units m^{-2}) and $k_{d,surf}$ and $k_{d,bulk}$ the inactivation constants at the oil/water interface and in

the bulk water phase respectively. If c_e has a value above 0 then equations 5 and 6 can be rewritten for the change in activity.

$$\left.\frac{dE_a(t)}{dt}\right|_{c_e>0} = V.\frac{dc_e(t)}{dt} \tag{7}$$

If c_e becomes equal to zero then equations 4 and 5 become

$$\left.\frac{dE_a(t)}{dt}\right|_{c_e=0} = -\Gamma(t)\cdot A\cdot k_{d,surf} \tag{8}$$

It is assumed that the oil/water surface area is constant during the experiment. This is a reasonable assumption since the composition of the emulsion only changes during the first two hours of the experiment.

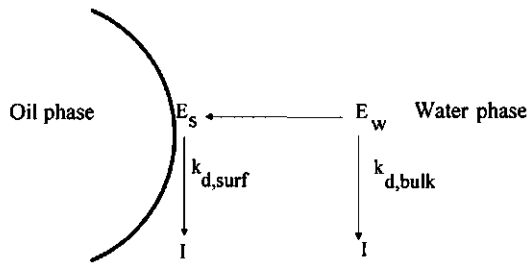

Figure 7. Schematic representation of inactivation in an emulsion, E_s is active enzyme at the oil/water interface, E_w is active enzyme in the bulk water phase, I is inactive enzyme.

As discussed before, in emulsions with an enzyme concentration >1.2 g l^{-1} the fatty acid production rate increases only slightly with increasing enzyme concentration. Hence, for emulsions containing 3.0 g (enzyme) l^{-1} it can safely be assumed that part of the enzyme is present in the water phase. The inactivation constants at the oil/water interface in an emulsion and in water are measured in independent batch inactivation experiments for an enzyme concentration of 0.3 g l^{-1}. Values of $5x10^{-3}$ h^{-1} (see inactivation in continuous emulsion reactor) and $1x10^{-3}$ h^{-1} (measured in water with 5% glycerol; equilibrium

glycerol concentration in batch experiments) are found for $k_{d,surf}$ and $k_{d,bulk}$, respectively. Subsequently, a batch inactivation experiment is performed with an emulsion with 3 g lipase per litre of emulsion. It is assumed that 40% (1.2 grams of the total of 3 grams) of the enzyme contributes to the fatty acid production (see also previous paragraphs). Hence, the product of A and Γ^* should be equal to 40% of the added amount of enzyme for an enzyme concentration of 3 g l^{-1}. The absolute values of A and Γ^* are not important. The logarithm of the quotient of the enzyme activity (E_a) and the initial enzyme activity ($E_a(0)$) is plotted as a function of time in figure 8. The line in figure 8 gives the model prediction of equations 6, 7 and 8 for $k_{d,surf} = 5x10^{-3}$ h^{-1}, $k_{d,bulk} = 1x10^{-3}$ h^{-1}, $V = 0.5x10^{-3}$ m^3, $A = 9$ m^2 (Van der Padt, 1993), $E_a(0) = 80.000$ units, $\Gamma^* = 1.900$ units m^{-2} [27]).

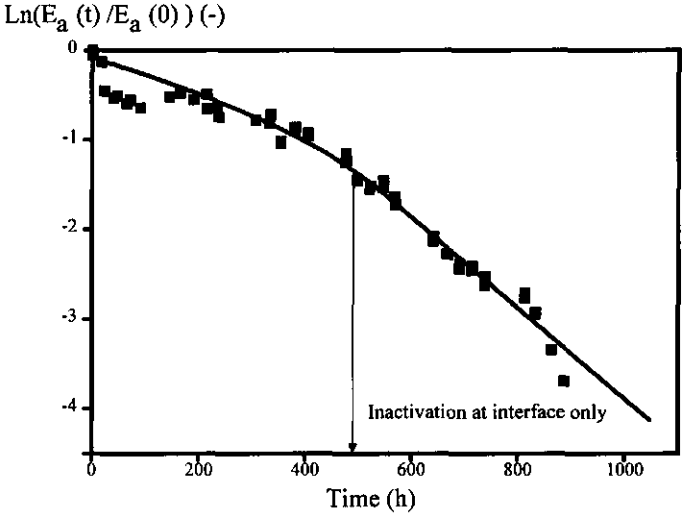

Figure 8. Residual activity as a function of time for an enzyme concentration of 3 g l^{-1}.

The model predicts the data rather well especially if it is taken into consideration that only parameters are used that are experimentally determined in separate experiments. The model strengthens our points that (i) inactivation takes place both at the oil/water

interface and in the bulk water phase and (ii) the inactive enzyme is replaced by active enzyme at the oil/water interface. However, note that during the first 200 hours the prediction and the measured data vary considerably. The reason for the discrepancy is not yet understood but is not the result of an experimental error. Pronk and co-authors [18] report for inactivation of *Candida rugosa* (Biocatalysts) lipase in a soybean oil in water emulsion the same initial discrepancy while the other experimental data obtained by Pronk support the previously proposed model.

Variation of net flow oil and water in the emulsion/membrane bioreactor

Now that the parameters for the reaction rate and enzyme inactivation are determined these parameters are combined in a dynamic model. With this model the fatty acid and glycerol concentration in the emulsion/membrane bioreactor can be predicted for varying inflows of oil and water. However, experimental problems occur during the continuous experiment; the tubes in the gear pumps wear off resulting in partial blockage of the tubes. By adjusting the pump velocity the net oil and water flow rate is kept constant during the daytime. However, during the night overflow of the reactor occurs. From the volume of emulsion that is removed from the reactor during the entire experiment it is concluded that a total of 45% of the enzyme is lost. The volume of emulsion lost per night is rather constant. This can be corrected for by adding and extra "inactivation" term to equation 2, in which a_i is replaced by equation 4. An overall loss of 45% corresponds to a $k_{d,loss}$ of 3.5×10^{-3} h^{-1}. In figures 9 and 10 the lines represent the predictions of the adjusted dynamic model for the oil and water flows indicated in table 1.

The measured fatty acid mole fraction and the glycerol concentration during the continuous experiment are given in figures 9 and 10. At t=0 hours 0.285 g l^{-1} enzyme is added to the reactor. During the first 2.5 hours the experiment runs in a batch mode. After 2.5 hours the water and oil phase are continuously removed from the reactor and water and oil is pumped into the reactor with a net water flow rate of 31 ml h^{-1}, and a net oil flow rate of 24 ml h^{-1}, respectively. The fatty acid mole fraction and the glycerol

concentration decrease slightly between 2.5 and 93.5 hours of operation as a consequence of enzyme inactivation.

Table 1. Water and oil flows during a continuous experiment with the emulsion/membrane bioreactor.

Time (h)	Water flow rate (ml h^{-1})	Oil flow rate (ml h^{-1})
0	0	0
2.5	31	24
93.5	30	123
125.5	30	63
170	40	63
192	79	63

At t=93.5 hours the oil flow rate is increased to 123 ml h^{-1}. The fatty acid mole fraction decreases as a consequence of the shorter residence time in the reactor. Because the fatty acid reaction rate increases with decreasing fatty acid mole fraction the glycerol concentration increases. After continuous operation for 125.5 hours 70% of oil is present in the emulsion which causes the emulsion to change from an oil in water to a water in oil emulsion. The viscosity of this emulsion is thus that the pressure drop over the membrane module, and therewith the transmembrane pressure, becomes unacceptably high, and therefore, the oil flow rate is decreased to 63 ml h^{-1} and the oil/water volume ratio in the emulsion is adjusted. Upon this decrease in oil flow the fatty acid mole fraction increases as a consequence of the longer residence time and the glycerol concentration decreases as a consequence of the lower fatty acid production rate at high fatty acid mole fractions.

At t=170 hours the water flow rate is increased to 40 ml h^{-1}. The fatty acid mole fraction is hardly influenced by this change in flow which is also expected because the reaction rate is hardly influenced by the glycerol concentration (see equation 2). The glycerol concentration decreases as a consequence of the shorter residence time. At t=192 hours the water flow rate is further increased to 79 ml h^{-1}, which leads to a further decrease of the glycerol concentration, while the fatty acid concentration is hardly influenced.

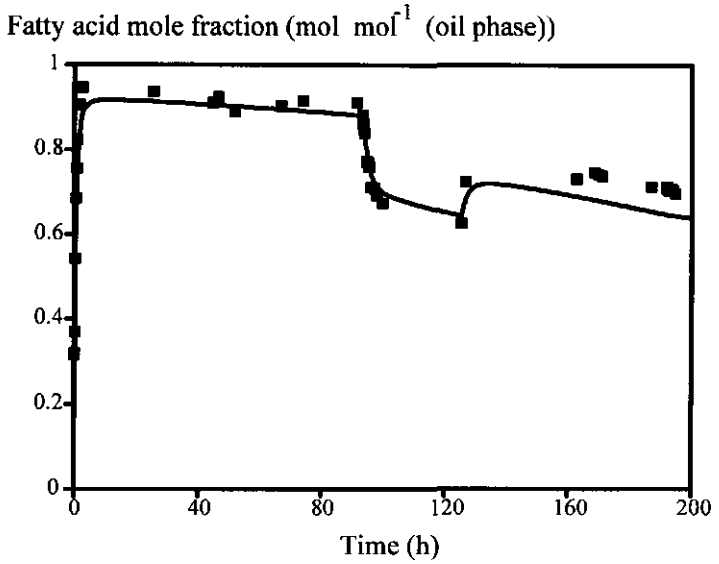

Figure 9. Fatty acid mole fraction during continuous experiment with varying oil and water flows.

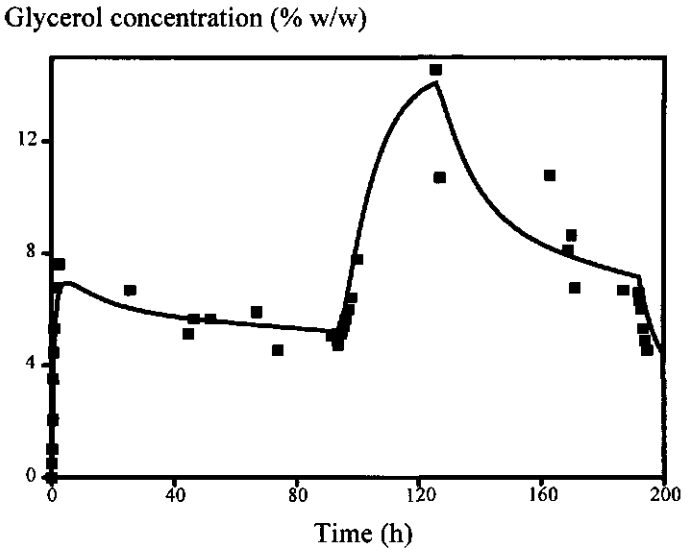

Figure 10. Glycerol concentration during continuous experiment with varying oil and water flows.

Unfortunately the reactor overflows overnight as a consequence of blockage of the tube with which the oil phase is removed from the reactor and the experiment is stopped at this point.

Figures 9 and 10 show that the predictions are in agreement with the measurements. The model predicts a strong influence of the oil flow rate on the fatty acid mole fraction and the glycerol concentration which is confirmed by the measurements. Also the predicted weak influence of the water flow rate on the fatty acid concentration is confirmed by the measurements.

In 1992 Pronk [18] has shown that the concept of the emulsion/membrane bioreactor could work, now we improved the performance of the reactor. Mainly the membrane performance has been improved. For the hydrophobic polypropylene membrane a flux of 25 (l m^{-2} h^{-1} bar^{-1}) is found while Pronk reports a flux of 0.5 (l m^{-2} h^{-1} bar^{-1}). The difference between both values is caused by membrane fouling by lipase, which is prevented by the block copolymers used in this study. For the hydrophilic cellulose membrane the difference in flux is also remarkable: 1.5 (l m^{-2} h^{-1} bar^{-1}) compared to 0.05 (l m^{-2} h^{-1} bar^{-1}). This difference is mainly caused by the high transmembrane pressure which enhances membrane fouling at the cross-flow velocities used in the study of Pronk.

The measured inactivation constant of the enzyme ($k_{d,surf}$ =4.6x10^{-3} h^{-1}) is comparable to the value reported by Pronk (4.7x10^{-3} h^{-1}; [18]). The stability of lipase in emulsions is lower than the stability of the membrane reactor of Pronk ([13]; k_d of 6.8x10^{-4} h^{-1}). However, it has to be noted that the stability of the emulsion reactor is not necessarily a function of the enzyme stability. Because active enzyme adsorbs preferentially at the oil/water interface the apparent stability of the reactor will be (much) higher then the stability of the enzyme if the concentration of lipase is beyond the saturation concentration of the oil/water interface. The volumetric activity of the emulsion/membrane bioreactor is at least a factor of 20 higher than that of a membrane reactor [18], therefore, the emulsion reactor should be preferred to the membrane reactor

if the membrane costs are the limiting factor for application of a bioreactor. However, if the enzyme costs are the limiting factor then one should reconsider the immobilised enzyme membrane reactor.

Economic evaluation

The fatty acid and the glycerol production can be predicted using equation 2, therefore, a general economic evaluation is possible. It is assumed that the process is carried out at a constant active enzyme concentration and that no enzyme is lost during the process. Kloosterman [28] states that conditions such as the produced amount of fatty acid per gram added enzyme and the degree of conversion should meet minimum values for an economic process. Although Kloosterman makes the evaluation for membrane reactors the same principles can be applied for the emulsion/membrane reactor.

Table 2. Economic evaluation of the emulsion/membrane bioreactor.

	Targets	Calculated values Enzyme concentration (g l^{-1})	
		0.1	3
Production			
(kmol kg^{-1} (enzyme))	>0.2	120	1
(mmol m^{-2} (membrane area) h^{-1})	>10	13.5	13.5
Degree of conversion			
(mol (fatty acid) mol^{-1} (oil phase))	≥0.9	0.9	0.9
Volume (m^3)	<200	560	110

The economic targets are given in table 2 together with the calculated values for the emulsion/membrane bioreactor for a fatty acid mole fraction of 0.9 and a glycerol mole fraction of 0.03. It is assumed that the enzyme concentration is constantly adjusted to 0.1 or 3 g l^{-1}. No active enzyme is removed from the reactor. The calculated value for the production per m^2 membrane is based on a flux of 35 (l m^{-2} h^{-1} bar^{-1}) and a transmembrane pressure of 0.2 bar for the hydrophobic membrane. The flux of the hydrophilic membrane

is assumed to be 1.5 (l m^{-2} h^{-1} bar^{-1}) and is to be operated at a transmembrane pressure of 0.3 bar (experimental data from the continuous experiment with the emulsion/membrane bioreactor). The costs of the hydrophilic membrane are a factor 25 less then those of the hydrophobic membrane according to the manufacturer. The volume of the reactor is calculated for a production of 10 tons fatty acid per hour. The calculations have been made for enzyme concentrations of 0.1 and 3.0 g l^{-1}.

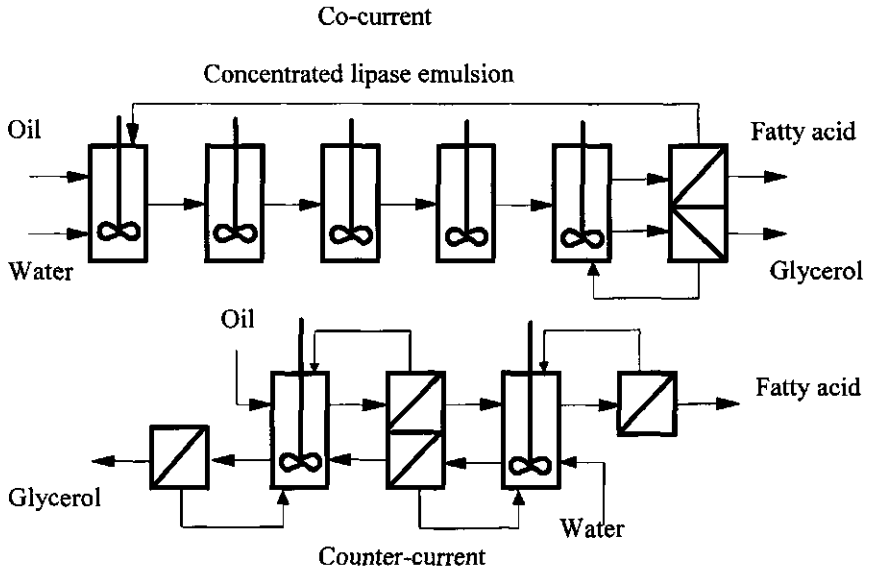

Figure 11. Schematic representation of series of emulsion/membrane bioreactors in co-current and counter-current configuration.

Most targets can be met by the emulsion/membrane bioreactor. For a high production per gram enzyme the reactor should be operated at a low enzyme concentration. Kloosterman [28] states that 75% of the process costs of a membrane reactor are determined by the enzyme costs, therefore, it is economically attractive to work at low enzyme concentrations. However, a low enzyme concentration also results in a large reactor

volume, which is not practically feasible. Of course a higher enzyme concentration can be used but this will reduce the production per gram enzyme.

Enzyme amount can be optimised by optimisation of the reactor configuration. Without detailed analysis it is clear that production in a counter-current configuration will only be slightly better than production in a co-current configuration because of the fact that the glycerol concentration (0-15% w/w) hardly influences the reaction rate. On the other hand, the membrane surface area required for counter-current processing increases linearly with the number of stirred vessels (see figure 11). Because the production per m^2 membrane is just high enough to meet the prerequisite it is not desirable to increase the membrane surface area, therefore, only co-current processing is considered and the total volume of the stirred vessels is calculated (see figure 12).

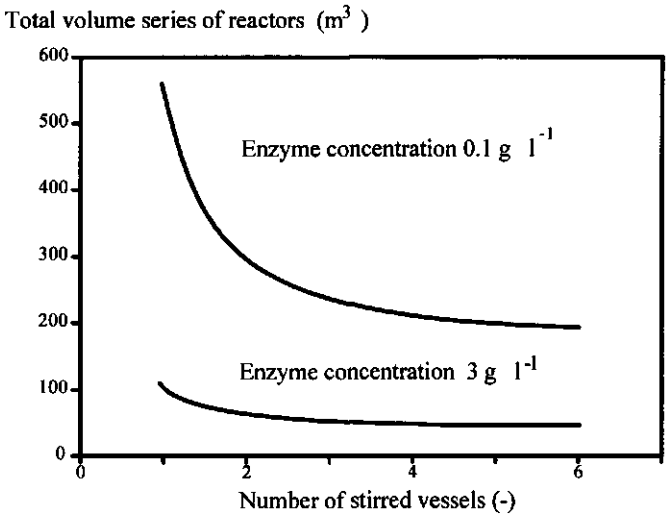

Figure 12. The total volume of a series of fed-enzyme emulsion/membrane bioreactors in co-current configuration for a production of 10 tons of fatty acid per hour. It is assumed that the active enzyme concentration is constant and no enzyme is lost during operation.

The total volume of a series of reactor decreases drastically upon increasing the numbers of reactors. The total volume of 560 m^3 for one reactor at an enzyme concentration of 0.1 g l^{-1} decreases to a total volume of approximately 200 m^3 which corresponds to a volume of 40 m^3 per stirred vessel for a series of 5. This value is well within practical limits. For an enzyme concentration of 3 g l^{-1} the volume of the series of reactors is lower as compared to the values found for an enzyme concentration of 0.1 g l^{-1}, as low as 8 m^3 for 5 tanks in series. Therefore, the reactor volume at this concentration is not a problem. It can be concluded that, in principle, enzymatic hydrolysis of triglycerides is feasible in a series of co-current emulsion/ membrane bioreactors. However, further evaluation is necessary to obtain a complete overview of all the costs connected with the process.

CONCLUSIONS

The fatty acid and glycerol production in the emulsion/membrane bioreactor is predicted with a reaction rate equation in which the effect of glycerol mole fraction, the fatty acid mole fraction and the enzyme activity is accounted for. The model predictions and the experimental data for continuous experiments are in good agreement.

The inactivation constant of lipase present at the oil/water interface is measured to be 4.6×10^{-3} h^{-1}. It is found that the active enzyme is not hindered by inactive enzyme, which indicates that a constant fatty acid production rate can be obtained during a longer period of time if the reactor is operated at an enzyme concentration beyond the saturation concentration of the oil/water surface area. The fluxes of the hydrophilic and the modified hydrophobic membrane remain fairly constant during a 1200 hours lasting experiment, thus making continuous production possible.

From a brief economic evaluation of the emulsion/membrane bioreactor it can be concluded that hydrolysis of triglycerides is, in principle, feasible if a co-current series of reactors is used.

ACKNOWLEDGEMENT

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LIST OF SYMBOLS AND ABBREVIATIONS

A	Surface area in the emulsion	(m^2)
c_e	Concentration active enzyme bulk water phase	(units m^{-3} (emulsion))
E_a	Total lipase activity in the emulsion	(units)
E_0	Initial enzyme concentration	(g l^{-1} (emulsion))
R	Gas constant	(J mol^{-1} K^{-1})
T	Temperature	(K)
V	Volume of the emulsion	(m^3)
X	Mole fraction of a component (eq denotes equilibrium)	(mol mol^{-1} (oil or water phase))
a_1	Fitparameter	(h^{-1})
a_2	Fitparameter	(-)
k_d	Inactivation constant	(h^{-1})
n	Number of moles	(mol)
n^*	Order of the reaction	(-)
r	Reaction rate	(mol (fatty acid) h^{-1} mol^{-1} (oil phase))
t	Time	(h)
ΔE_h	Activation energy hydrolysis reaction	(kJ mol^{-1})
Γ	Adsorbed amount of lipase	(units m^{-2})
Γ^*	Maximum adsorbed amount lipase at the oil/water interface	(units m^{-2})
ϕ	Mass flow rate	(mol h^{-1})

Superscripts and subscripts

F	Fatty acid
G	Glycerol
bulk	Water phase
i	Component i
in	Incoming
loss	Loss of enzyme caused by overflow reactor
out	Outgoing
prod	Produced
surf	Oil/water interface

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CHAPTER 7

GENERAL DISCUSSION

-reactor configurations, economic evaluation and possible applications-

INTRODUCTION

This thesis presents the emulsion/membrane bioreactor for the hydrolysis of triglycerides (figure 1 chapter 2). Chapters 2 & 6 show the actual flux of the membranes and a first optimisation. In section A1 of this general discussion the emulsion/membrane bioreactor is compared with a flat sheet and a hollow fibre immobilised enzyme membrane reactor. Further an economical evaluation, based on the dynamic model given in chapter 6, is carried out in section A2. The evaluation is made for a flow-adjusted and a fed-enzyme system using the produced amount of fatty acid per gram of enzyme and the volume of the reaction vessel as criteria.

A fundamental study of the modified hydrophobic membrane (chapters 3-5) leads to the following conclusions (i) protein adsorption at a hydrophobic polypropylene membrane will be prevented if block copolymer F108 is pre-adsorbed at the membrane and (ii) the wettability of an F108-coated hydrophobic membrane is hardly influenced by the presence of F108 and, therefore, the surface can be wetted by a large variety of organic solvents (oils). These conclusions indicate that application of the emulsion/membrane bioreactor is not limited to hydrolysis of triacylglycerols only. Therefore, two other reaction systems are considered in this chapter. Firstly, esterification of oleic acid and glycerol is studied in section B1 and downstream processing is discussed. Secondly, the

separation of an emulsion of dodecane in water containing baker's yeast is discussed in section B2.

The finding that no protein adsorption takes place at the block copolymer-modified *hydrophobic* membrane (chapters 2, 3 and 5) is in contradiction with the general opinion that less protein adsorption takes place at *hydrophilic* membranes as compared with hydrophobic ones (overview in [1,2]). Although several surface modification methods for hydrophilic membranes are proposed in literature none of these methods results in prevention of protein adsorption, therefore, use of the modified hydrophobic membrane becomes interesting if it can be made permeable for water. Section B3 shows a possible route to achieve this.

A. EVALUATION EMULSION/MEMBRANE BIOREACTOR

A1. Membrane bioreactor

For lipase catalysed reactions, the reaction rate is related to the surface area onto which the enzyme is adsorbed. Therefore, the volumetric activity i.e. the activity per m^3 reactor, should be a function of the specific area. For emulsion reactors this is the oil/water interfacial area in the emulsion (chapter 6). For immobilised enzyme membrane reactors this is the membrane surface area, and therefore, hollow fibre reactors are considered to be preferable to flat sheet reactors because of their favourable surface to volume ratio.

For the experiments cellulose membranes with a cut-off value of 6,000 and a thickness of 10 μm are used (Akzo, Germany). The flat sheet membrane is mounted in a Megaflow module (New Brunswick Scientific) with a surface area of 64×10^{-4} m^2 and a retentate channel height of 2 mm. The hollow fibre membrane has a surface area of 0.77 m^2. For batch reactor operation the method of Pronk [3] is used.

Surprisingly enough, a discrepancy in initial interfacial reaction rate (factor of 50 difference) is found (see figure 1) when comparing batch experiments in a hollow fibre

and a flat sheet membrane bioreactor. It is known from literature that the immobilisation material can influence the activity of an enzyme to a very large extent [4]. However, the membranes used are both made of regenerated cellulose obtained from Akzo (Germany), therefore, the difference in fatty acid production cannot be explained by the influence of the immobilisation material on the activity of the enzyme. For both reactors the membrane surface is saturated with lipase and a difference in the immobilised amount of enzyme per m^2 membrane is not likely since the interfacial activity is expected to be equal.

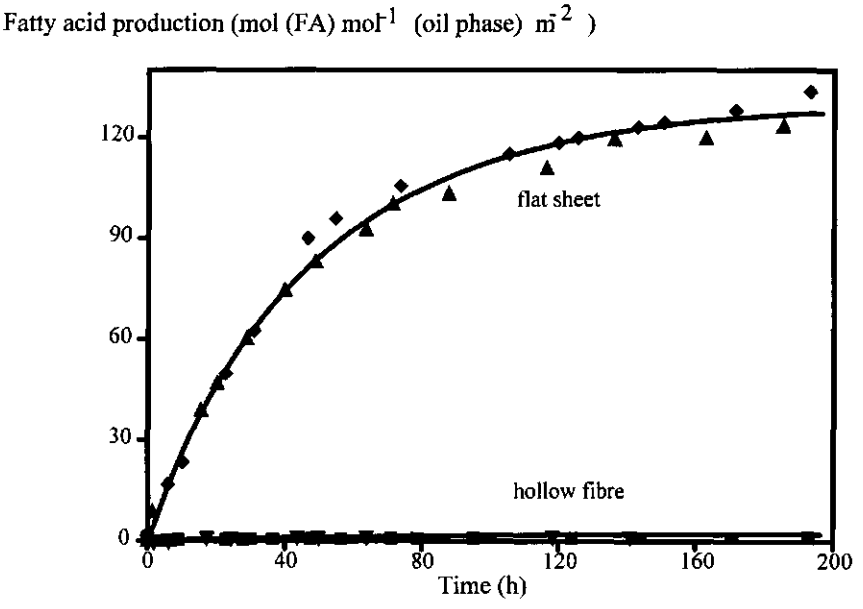

Figure 1. Comparison fatty acid production in hollow fibre and flat sheet membrane bioreactors. For both reactor types the results of two independent measurements based on the method of Pronk [3] are shown.

In the hollow fibre reactor the pressure difference between the inlet and outlet of the oil phase increased during the experiment. This is remarkable because the viscosity of the oil phase decreases with increasing fatty acid concentration, hence, a decrease in pressure is

expected. The observed effect can only be explained by hindrance of the liquid flow through the membrane module. From literature it is known that part (up to 90%) of the fibres in hollow fibre dialysis membrane can get blocked [5,6] and, therewith, part of the difference in fatty acid production can be explained.

A general comparison is possible between an immobilised enzyme membrane reactor and an emulsion/membrane bioreactor, based on the production per m^2 of membrane surface area. The following example shows this procedure. Let us assume that the mole fraction fatty acid in the oil phase is required to be 0.7, the flux of the hydrophobic membrane is 35 l m^{-2} h^{-1} bar^{-1} and it is operated at 0.1 bar (data from chapter 6). The production per m^2 hydrophobic membrane is calculated to be approximately 5 mol (fatty acid) m^{-2} h^{-1}. Besides the hydrophobic membrane surface area also the hydrophilic membrane has to be taken into account. The flux of this membrane is 1.5 l m^{-2} h^{-1} bar^{-1} under the assumption that the membrane is operated at 0.1 bar (data from chapter 6). At 15% (w/w) glycerol in the water phase it can be concluded that the membrane surface area of this membrane should be 50 times larger than that of the hydrophobic one. The production per m^2 total membrane area in the reactor is thus approximately 0.1 mol (fatty acid) m^{-2} h^{-1}. Of course, in practice the hydrophilic membrane will be operated at a higher transmembrane pressure which will result in a higher production per m^2 membrane surface area. Also the use of a high flux membrane will increase the production per m^2 membrane surface area. Further optimisation of the hydrophilic membrane is necessary for optimal use of the membrane surface area.

For a batch operated immobilised enzyme membrane reactor the following comparison can be made. For the flat sheet reactor the slope of the curve in figure 1 shows that at the time at which a fatty acid mole fraction of 0.7 is reached is 120 h. The total number of moles of oil phase is at that time 62 mmol and it can be calculated that the average production is approximately 0.8×10^{-3} mol (fatty acid) m^{-2} h^{-1}. This value is more then a factor 100 lower as compared with the emulsion/membrane bioreactor. Because the fatty acid production in immobilised enzyme membrane reactors is kinetically controlled the

production per m^2 of membrane surface area can not exceed the previously mentioned value. The membranes are more effectively used in the emulsion reactor because they are only used as a separation device and not as the immobilisation carrier.

A.2. Optimisation of the emulsion/membrane bioreactor

As a starting point for optimisation of emulsion reactors for hydrolysis of triglycerides the reaction rate equation from chapter 6 is taken which is based on results of Pronk [7-8]. For continuous operation at a constant temperature, fatty acid mole fraction, glycerol mole fraction and equilibrium fatty acid mole fraction, this equation can be rewritten to

$$r_F = \frac{E \cdot r'_F}{0.056 + 0.11 \cdot E} \qquad (1)$$

In which r_F is the fatty acid production rate (mol (fatty acid) mol^{-1} (oil phase) h^{-1}) and E the concentration active enzyme (g l^{-1}). Note that the fatty acid production rate increases with increasing amount of enzyme but does so less than proportionally. The enzyme is more active at low concentrations (see also chapter 6). In equation 1, r'_F (mol (fatty acid) mol (oil phase)$^{-1}$ h^{-1}) is the fatty acid production rate per gram enzyme at a certain temperature, T (K), glycerol mole fraction, X_G (-), fatty acid mole fraction, X_F (-) and equilibrium fatty acid mole fraction, X_F^{eq} (-). In equation 2 a relation for r'_F is given.

$$r'_F = e^{-k_d \cdot t} \cdot (1 - a_2 \cdot X_G) \cdot e^{\frac{-\Delta E_h}{R} \cdot (\frac{1}{T} - \frac{1}{298})} \cdot (X_F^{eq} - X_F)^{n^*} \qquad 2$$

In which k_d is the inactivation constant (h^{-1}), t is the time (h), a_2 is a fitparameter (-) (see chapter 6), ΔE_h is the activation energy of the hydrolysis reaction (12.3 kJ mol^{-1}; Pronk, 1991), R is the gas constant (kJ mol^{-1} K^{-1}), and n^* is the order of the reaction (-). Note that r'_F is a constant for set product requirements.

The fatty acid product concentration can be kept constant by: (i) continuous addition of enzyme to the reactor (from now on called fed-enzyme system; the active enzyme is not hindered by the inactive enzyme; see chapter 6) or (ii) adjustment of the net oil and water

inflow of the reactor (flow-adjusted system) and thus make use of the increase in activity at low enzyme concentrations. The main advantage of the flow-adjusted system is that the enzyme is used effectively, and therefore, a higher production per gram enzyme is expected. However, an intrinsic disadvantage of this reactor is that the required permeate volume flow decreases in time, which might make continuous operation difficult if not impossible. This not the case for fed-enzyme operation, no flow adjustment is necessary, but the enzyme use is less effective as compared to the flow-adjusted system.

In the following paragraph the fatty acid production in a flow-adjusted and in a fed-enzyme reactor is evaluated. As optimisation criteria are taken:

- the production per gram enzyme, r_F^E (figure 2)
- the production per hour, r_F^V
- and derived from this criterion the volume of a reactor, $V_{10\,000}$, with which a production of 10 tons of fatty acid per hour is possible (figure 3)

Of course, the actual reactor design is determined by the production conditions, hence, by r_F'.

A.2.1. Production per gram enzyme

The costs of the enzyme determine 75% of the total costs of a membrane bioreactor [9]. Hence, it is important to optimise the produced amount of fatty acid per unit added enzyme. For the calculations the (initial) enzyme concentration in both reactor types, is varied between 0.1 and 7.5 g l^{-1} (within these limits the model is valid; see chapter 6).

For the flow-adjusted system the reactor is assumed to be ineffective as soon as an active enzyme concentration of 0.05 g l^{-1} is reached, this value coincides with the lower limit of the model but has no actual relevance. Subsequently, the produced amount of fatty acid per gram added enzyme, r_F^E, is calculated. The amount of added enzyme on hourly basis is calculated as the initial enzyme concentration divided by the time at which a concentration of 0.05 g l^{-1} is reached.

For the fed-enzyme system the amount of enzyme necessary to compensate for enzyme inactivation is calculated. With this value r_F^E can be calculated for a reactor of one litre containing 1.6 mole oil phase as: r_F x 1.6 over the amount of enzyme added on hourly basis. For both reactors it is assumed that the reactors produce continuously without any down-time. The calculated production per gram enzyme, r_F^E, divided by r_F' (see equation 1) is given as a function of the amount of enzyme added per hour in figure 2.

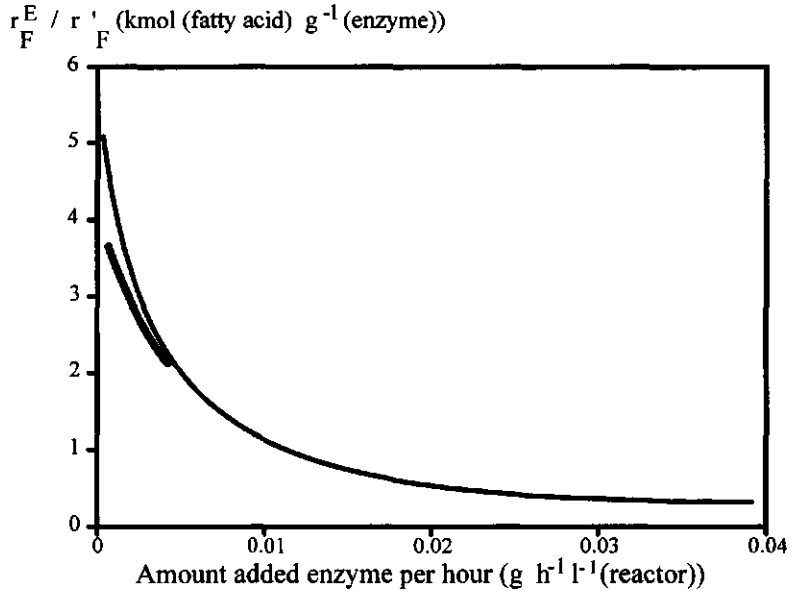

Figure 2. The production per gram added enzyme per litre reactor as a function of the amount of added enzyme on hourly basis; thin line fed-enzyme system, thick line flow-adjusted system. The amount of enzyme added on hourly basis corresponds with enzyme concentrations between 0.01 and 7.5 g l^{-1} for both reactors.

From figure 2 can be concluded that the produced amount of fatty acid increases with decreasing amount added enzyme for both reactors. This effect is caused by the increase in activity with decreasing enzyme concentration as described by equation 1. At low amounts added enzyme per hour the production in the fed-enzyme system exceeds that of

the flow-adjusted system although the difference is not very pronounced. The explanation for this finding is the following, in the fed-enzyme system the enzyme carries out the reaction at a low concentration, which is favourable from a view point of activity per gram enzyme. In the flow-adjusted system the reaction is initially carried out at a higher enzyme concentration if the same hourly added amount of enzyme is chosen as for the fed-enzyme system. Therefore, the production per gram enzyme will be higher in the fed-enzyme system. Specially at low enzyme concentrations this effect will become more pronounced as is shown in figure 2.

If the produced amount of fatty acid per gram of enzyme is the deciding criterion then the fed-enzyme system should be chosen and operated at low enzyme concentrations. In paragraph A.2.6. the actual produced amount per gram enzyme is evaluated for various fatty acid concentrations.

A.2.2. Production per hour

The production per hour r_F^V for the fed-enzyme system containing 1.6 mol of oil phase can be calculated as r_F x 1.6 (see section A.2.1.), For the flow-adjusted system the averaged amount of fatty acid produced per hour over the period till an active enzyme concentration of 0.05 g l^{-1} is reached is the total amount divided by the time at which this concentration is reached.

For both reactors r_F^V increases with increasing amount of added enzyme as predicted by equation 1. The production in a flow-adjusted system is always (slightly) lower as compared with the fed-enzyme system. The explanation for this phenomenon is analogous to the explanation for the difference in production per gram enzyme as shown in figure 2 (see previous paragraph).

At high amounts of added enzyme the production in a fed-enzyme system of one litre can be as high as 13 mol h^{-1}. If the volume of the reactor is the determining factor than high

concentrations of enzyme, addition rates above 0.005 g h^{-1} l^{-1}, should be used in a fed-enzyme reactor.

A.2.3. Reactor volume as a function of r_F'

Next step is to evaluate the volume of a reaction vessel for a production of 10,000 kg fatty acid per hour. This value, $V_{10,000}$, is calculated by dividing the required production by $r_F{}^V$. Because the volume of the reaction vessel differs with the reaction conditions the volume is multiplied by r_F'. This product is plotted as a function of the amount of added enzyme per hour and per litre reactor in figure 3.

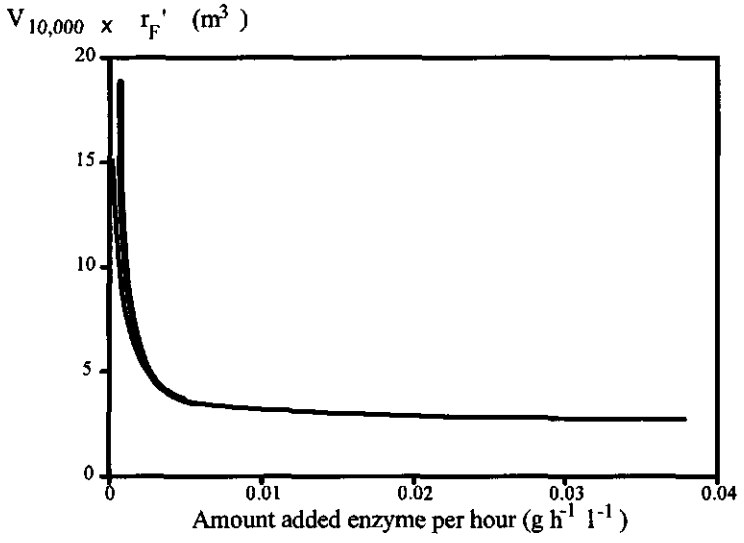

Figure 3. The volume of a reactor for a production of 10.000 kg fatty acid per hour as a function of the amount of added enzyme per hour and per litre reactor; thin line fed-enzyme system, thick line flow-adjusted system.

The volume of both types of reactors decreases with increasing amount added enzyme because of the increasing production per hour at increasing enzyme concentration

(section A.2.2.). The volume of the flow-adjusted system is always slightly higher then that of the fed-enzyme system, which is a result of the difference in r_F^V of both reactors.

The actual reactor volume is a function of the reaction conditions, therefore, r_F' is evaluated in the next section. Because the volume of both reactor types is almost equal only the fed-enzyme system is considered further because it has the smallest volume of both reactor types.

A.2.4. Evaluation of r'_F-values and actual reactor volume

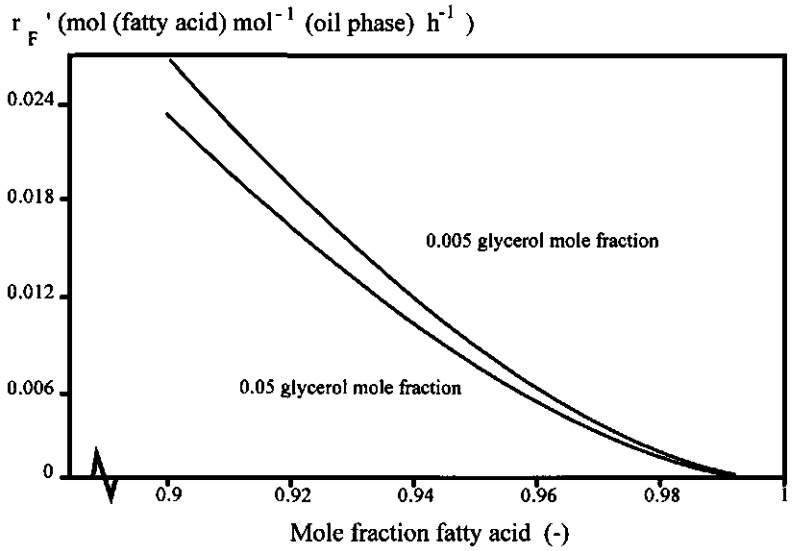

Figure 4. r_F' as a function of the fatty acid mole fraction and the glycerol mole fraction.

For fatty acid mole fractions ranging from 0.9 to 0.992 and glycerol mole fractions between 0.005 and 0.05, r'_F is calculated (see figure 4). From this figure can be concluded that the mole fraction fatty acid has a strong influence on r'_F while the influence of the glycerol mole fraction is only weak. For a mole fraction fatty acid of 0.9 an average r'_F of 2.4×10^{-2} mol (fatty acid) mol^{-1} oil phase h^{-1} is found which decreases to 1×10^{-4} mol (fatty

acid) mol^{-1} (oil phase) h^{-1} for a fatty acid mole fraction of 0.992; which is very close to the equilibrium mole fraction of 0.995 at a glycerol mole fraction of 0.03.

The values for r'_F imply that for a fatty acid mole fraction of 0.9 the volume of the reaction vessel is between 110 and 600 m^3 for the fed-enzyme system (see also figure 3). For a fatty acid mole fraction of 0.992 this volume increases to a value between 26,000 and 150,000 m^3. The calculated volumes are thus that the process is not practically feasible for large conversion values. The volume of the reaction vessel can be decreased if (i) the reaction is carried out at elevated temperature, (ii) the reaction is carried out at a lower fatty acid mole fraction or (iii) more stirred vessels are put in series.

An increase in reaction temperature of 5 degrees will result in an increase in reaction rate of a factor of three [7] and thus in a decrease in reactor volume of a factor of three. As a result the volume of the fed-enzyme reactor will be practically feasible for high enzyme concentrations if the required fatty acid mole fraction is not too high. However, an elevated reaction temperature also results in stronger inactivation of the enzyme. For a proper evaluation of the influence of reaction temperature on the inactivation of the enzyme an extra correction term [7] can be incorporated in equation 1 or 2.

At a lower fatty acid mole fraction the reaction rate will increase drastically. For a fatty acid mole fraction of 0.8, r_F' increases to 0.07. The volume of the reactor decreases with a factor of 3.5 compared to the situation at a fatty acid mole fraction of 0.9 to a volume of approximately 30 m^3 (for a fatty acid concentration of 0.8), which is a reasonable value for a stirred vessel. However, the lower fatty acid concentration will hamper downstream processing. Therefore, production in a series of reactors, in which high fatty acid mole fractions can be achieved, is favourable to a one stage production at low fatty acid concentration.

A.2.5. Series of reactors

A series of reactors can be placed in co-current and counter-current configuration (see figure 5). Because the influence of the glycerol concentration on the fatty acid production rate in the range of operation is small (see figure 4), the total volume for both configurations will be comparable which is in agreement with the findings of Pronk [14] for a series of membrane bioreactors. The total membrane surface area will not be comparable. In the counter-current configuration it is necessary to separate the emulsion after every stirred vessel, therefore, the membrane surface area increases linearly with the number of stirred vessels in the series. For co-current operation only one separation step is required. Because the membrane costs are up to 15% of the total process costs [9], extra membrane separation steps should be avoided, and therefore, only the co-current configuration for the fed-enzyme system is considered further.

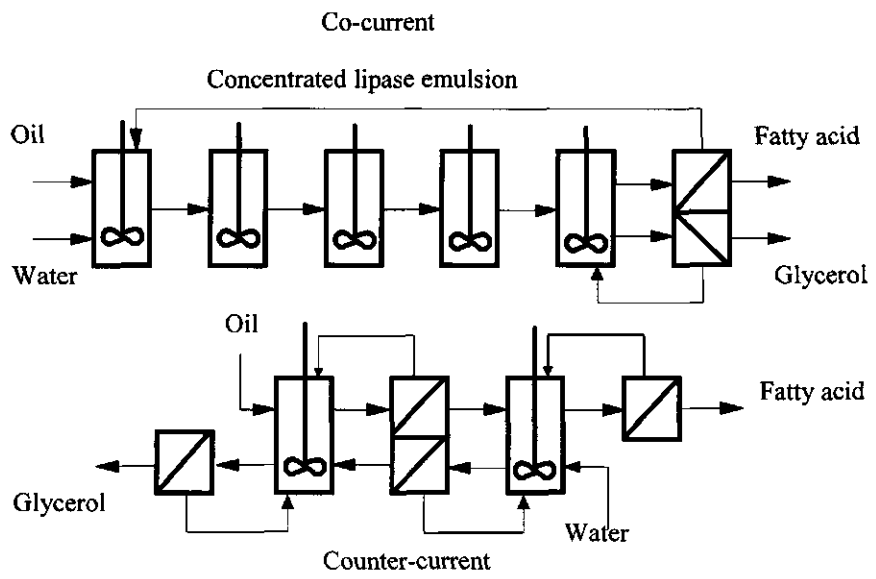

Figure 5. Schematic representation of reactors in series in co-current and counter-current configuration.

It is assumed that all the stirred vessels placed in series have the same volume and that the outgoing oil phase of the cascade contains 0.9 mole fraction fatty acid. The total volume of the series of reactors is given as a function of the number of stirred vessel in figure 6 for enzyme concentrations of 0.1 and 3 g l^{-1}, corresponding to hourly added amounts of enzyme of 0.5 and 15 mg h^{-1} l^{-1} (emulsion), respectively.

Going from one to two stirred vessels the volume of the cascade of stirred vessels decreases drastically (figure 6). Increasing the number of stirred vessel further leads to a decrease of the total volume till it goes down to a value of 200 m^3 and 40 m^3 for an enzyme concentration of 0.1 g l^{-1} or 3 g l^{-1}, respectively. The volume of each of the vessels in a series of 6 is then 40 and 8 m^3, respectively, which are practically feasible values.

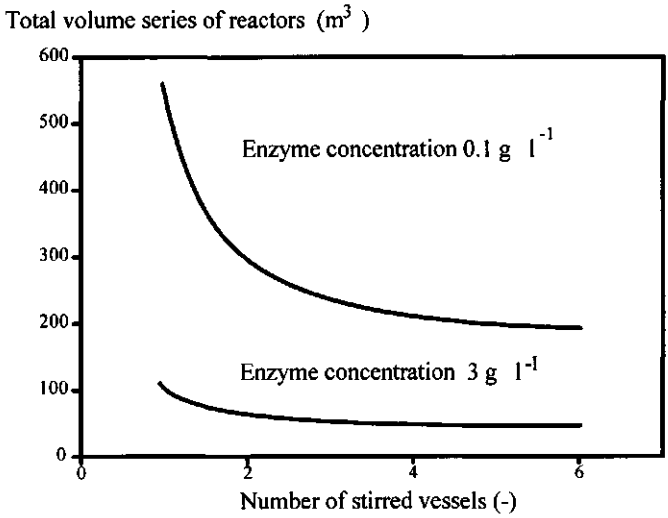

Figure 6. The total volume of stirred vessels in series as a function of the number of equivolumetric stirred vessels; mole fraction fatty acid in the oil phase is 0.9, temperature is 30 °C.

Because the volumes of the stirred vessels in series for an enzyme concentration of 3 g l^{-1} are small also higher fatty acid mole fractions can be considered. In figure 7 the volumes of series of co-current reactors are given for fatty acid mole fractions of 0.95, 0.97 and 0.99. For a fatty acid mole fraction of 0.99 further down-stream processing of the fatty acid product stream becomes superfluous.

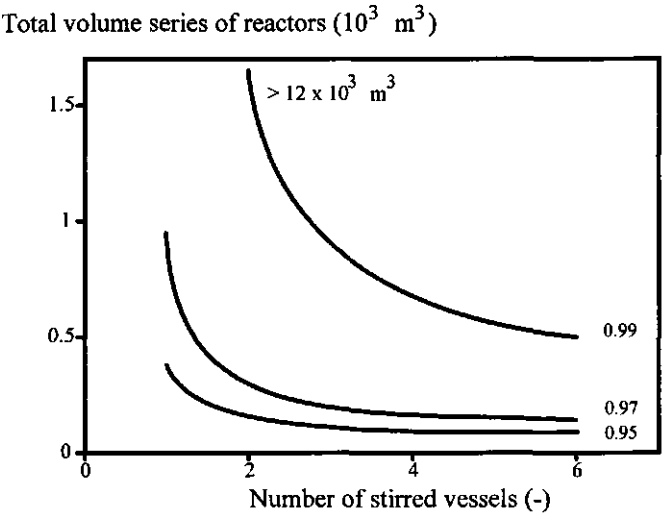

Figure 7. The total volume of stirred vessels in series as a function of the number of equivolumetric stirred vessels of a mole fractions fatty acid in the oil phase of 0.95, 0.97 and 0.99; the temperature is 30 °C.

The total volume increases dramatically with increasing fatty acid mole fraction up to a volume of 12,000 m^3 for a fatty acid mole fraction of 0.99 if only one stirred vessel is used. For six stirred vessels in series the volume of each of the vessels decreases to a maximum of 85 m^3 for a fatty acid mole fraction of 0.99. For lower fatty acid concentrations this volume decreases to 23 and 13 m^3 for fatty acid mole fractions of 0.97 and 0.95, respectively. These values indicate that it is possible to obtain higher fatty acid product concentrations for an enzyme concentration of 3 g l^{-1} without having to use unfeasibly large stirred vessels. Further economic evaluation has to decide on the

optimum number of vessels to be placed in series and on which fatty acid concentration they should be operated.

A.2.6. Comparison obtained values with literature data

Kloosterman [9] carried out an economical evaluation for membrane reactors and states that 90% of the substrate has to be converted into the desired product otherwise down-stream processing becomes to costly. In all calculations a mole fraction fatty acid of at least 0.9 is used.

Kloosterman [9] also concludes that the production per gram enzyme should exceed 0.2 kmol fatty acid per kg of added enzyme in order to make a process economically feasible. From figure 5 the absolute values for the produced amount of fatty acid per gram added enzyme in the emulsion/membrane bioreactor can be calculated for various reaction conditions and values between 1 and 120 kmol fatty acid per kg enzyme are found for a fatty acid mole fraction 0.90 in a fed-enzyme reactor. If a higher fatty acid mole fraction is chosen, e.g. 0.992, than the produced amount of fatty acid per gram added enzyme decreases to between 0.5 and 5 kmol fatty acid per kg enzyme for the fed-enzyme reactor. All these values meet the economic targets of Kloosterman.

Down-stream processing is facilitated at high fatty acid mole fractions, therefore, it can be attractive to work at a relatively low production per gram enzyme. However, the reactor volume increases as a consequence of the low reaction rate at low fatty acid concentration. The volume of *one* vessel increases from approximately 100 m^3 for a mole fraction of 0.9 at an enzyme concentration of 3 g l^{-1} in a fed-enzyme system to 400 and 12,000 m^3 for fatty acid mole fractions of 0.95 and 0.99, respectively. Placing reactors in series will result in a decrease in the volume per reactor, for a mole fraction of 0.95 the volume of one of the vessels in series decreases to 70, 40 and 25 m^3 for a series of 2, 3 and 4 reactors, respectively. Evaluation of the equipment costs will determine whether processing at high fatty acid mole fractions is possible.

Besides enzyme costs Kloosterman [9] also considers the membrane costs, which contribute 15% to the total costs in a membrane reactor. The production per m^2 membrane in an emulsion reactor is related to the flux of the membrane, i.e. the transmembrane pressure, the cross-flow velocity etc. Now let us assume that the flux of the hydrophobic membrane is 35 l m^{-2} h^{-1} bar^{-1} and the membrane is operated at 0.2 bar (data from chapter 6), the production based on the flux of the hydrophobic membrane is then 21.6 mmol m^{-2} h^{-1}. If the transmembrane pressure or the cross flow velocity is increased then this value will increase. As mentioned in section A.1 mainly the surface area of the hydrophilic membrane has to be taken into account. During the continuous experiment the transmembrane pressure is kept at 0.3 bar and the flux of the hydrophilic membrane remained constant at 1.5 l m^{-2} h^{-1} bar^{-1}. If equally expensive membranes are used this leads to a decrease in production per m^2 membrane surface area of a factor of 16 for the emulsion/membrane bioreactor. This indicates that the lower limit for practical application, 10 mmol m^{-2} h^{-1} as reported by Kloosterman, is not reached. However, if a high flux membrane is chosen in stead of the low flux cellulose membrane then the production per m^2 membrane surface area will be determined by the hydrophobic membrane. As mentioned before, in that case a production of 21.6 mmol m^{-2} h^{-1} is expected which is approximately twice as high as the target formulated by Kloosterman.

It can be concluded that all considered targets: the production per gram enzyme, the volume of the stirred vessel(s) and the production per m^2 membrane can be met by the emulsion/membrane bioreactor. A complete analysis of the equipment costs (stirred vessels and down-stream processing equipment) needs to be carried out in order to determine the optimum process conditions.

B. OTHER APPLICATIONS

B.1. Esterification

B.1.1. Introduction

For esterification of oleic acid and glycerol in the emulsion/membrane bioreactor it is necessary that the volumetric reaction rate is high, the enzyme is sufficiently stable, and both the hydrophilic and the hydrophobic membrane remain selectively wetted and sufficiently permeable for one of the two phases of the emulsion. Whether this is the case is checked by (a) determining the reaction rate in emulsions (b) measuring inactivation of lipase in emulsions with different glycerol concentrations (c) measuring the flux through the hydrophilic membrane as a function of the glycerol concentration and (d) measuring the flux of both block copolymer-modified and unmodified hydrophobic membranes as a function of time. This leads to a process proposal and added to that some possible down-steam process routes are given.

B.1.2. Reaction rate

The fatty acid concentration in an emulsion with 36 vol% oleic acid and a water phase with 0.5 mole fraction of glycerol is measured in order to determine the reaction rate at 30 °C as a function of time. The emulsion is mixed in a vessel as described in chapter 6 (one stirrer) at a stirring speed of 800 rpm. Lipase is added to the emulsion with a total concentration of 3 g l^{-1}. The average droplet diameter is determined with a Coulter counter laser. The interfacial and volumetric reaction rates are given in table 1.

The specific surface area of the emulsions used by Van der Padt [10] is a factor of 10 lower. Since in this study the water phase is the continuous phase while in the work of Van der Padt the organic phase is the continuous phase, the difference in specific surface area is not surprising. The volumetric activities in both emulsions (see table 1) are comparable while the interfacial activities differ more then a factor of 10. In the work of Van der Padt an optimum enzyme load is used; increasing the enzyme concentration

results in a slight increase in volumetric activity (see also chapter 6). The enzyme concentration used in this study is the same as in Van der Padt's work. However, the specific surface area is a factor of 10 higher in this study, therefore, the interfacial and volumetric activity might have been a factor of 10 higher if an optimum enzyme load had been used. If this is the case, the interfacial activity at optimum enzyme load will be more or less the same as the value reported by Van der Padt while the volumetric activity will be a factor of ten higher then the activity reported by Van der Padt.

Table 1. Interfacial and volumetric activity in emulsions

	This work	Van der Padt [10]	
	emulsion	emulsion	membrane
Volumetric activity (mol m^{-3} s^{-1})			
10 min. after addition lipase	0.5		
20 min. after addition lipase	0.34	0.36 (0.097*)	0.07 (0.02*)
Interfacial activity (μmol m^{-2} s^{-1})	1.2	18.0 (4.1*)	13.0 (3.5*)
Specific surface area (m^2 m^{-3})	$2.8x10^5$	$0.2x10^5$	$5x10^3$

*Original values of Van der Padt (determined at 25 °C) are shown between brackets, other values are corrected for temperature (30 °C) using the Arrhenius equation as given in chapter 6.

If the activities in an emulsion are compared to those in a membrane reactor then it can be concluded that the interfacial activity of both systems is comparable but the volumetric activity is a factor of 5 (Van der Padt) or possibly 50 higher if an optimum enzyme load had been used in the previously described experiments. The activities might even be increased if the volume fraction of the organic phase is increased or the emulsion droplet diameter is reduced. A high volumetric activity results in a compact reactor which is advantageous. Since also the membrane surface area required for an emulsion reactor is lower than that required in a membrane reactor (as shown in paragraphs A.1. and A.2. of

this chapter for hydrolysis) the use of an emulsion reactor seems advantageous if the enzyme is sufficiently stable. Whether this is the case is demonstrated in the next section.

B.1.3. Enzyme inactivation

In the previously described stirred vessel oleic acid and water/glycerol (volume ratio 1:1) are mixed with a six-bladed turbine stirrer (stirrer diameter 3 cm) at 450 rpm. To the emulsion lipase is added in a total concentration of 3 grams per litre of emulsion and equilibrium is reached within 2 hours. The activity of lipase is measured separately with the tributyrine standard method (see chapter 6) as a function of time for initial glycerol mole fractions in the water phase of 0.55, 0.60 and 0.73 which correspond to glycerol weight percentages of 86, 88 and 93, respectively. The normalised activity, the quotient of the activity of lipase at a certain time and the activity of the lipase preparation before addition to the emulsion (approximately 60 units per mg enzyme), is plotted as a function of time in figure 8.

A model comparable with the one for enzyme inactivation given in chapter 6 can be used to describe the inactivation in this type of emulsion.During the first ten hours of the experiment the activity decreases very fast. After this time the activity still decreases but does so less rigorously. Also Van der Padt [11] reports a fast initial decrease in activity followed by a much slower decrease for lipase in glycerol/water mixtures. However, the long term inactivation in an emulsion cannot be predicted by his two-step inactivation model if the inactivation constants for glycerol/water mixtures are used. Because the results for inactivation in water/glycerol are accurately predicted by the model, the difference between these results and those for emulsions are most probably caused by either the presence of the oil/water interface which can stabilise the enzyme or by the presence of a small amount of oleic acid in the water/glycerol which stabilises the enzyme [12].

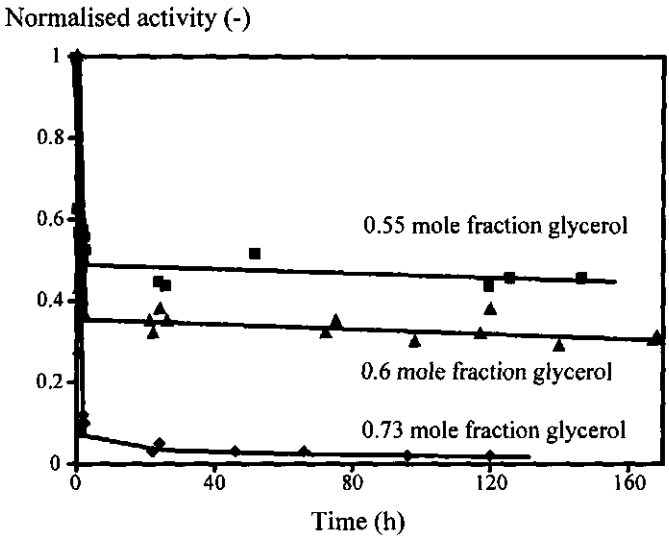

Figure 8. The normalised activity of lipase as a function of time in emulsions with different glycerol concentrations.

The data in figure 8 are analysed by determining the first order inactivation constant for the measurements during the first 10 hours of the experiment (initial $k_{d,i}$) and for the datum points after 10 hours (long term $k_{d,l}$). The obtained values are given in table 2. As an indication for the residual activity the long-term inactivation prediction is extrapolated to $t = 0$ and this value is given in table 2.

As stated before the initial inactivation is much stronger than the long term inactivation which results in a difference between the initial and the long term inactivation constant of a factor of 20 for a glycerol mole fraction of 0.73 up to a factor of 750 for a mole fraction of 0.55. Because the initial decrease in activity is so strong no distinction can be made between the initial inactivation constants for different glycerol mole fractions but the long term inactivation constants differ significantly. The lower the glycerol concentration the lower the inactivation constant, the long term inactivation constant for an initial glycerol mole fraction of 0.55 is even lower than the one found during hydrolysis in an

emulsion ($5x10^{-3}$ h^{-1}; see chapter 6) which indicates that the enzyme can be very stable in an esterifying emulsion. Because the residual activity in an emulsion with 0.55 mole fraction glycerol is acceptable it is expected that such an emulsion can be used in the emulsion/membrane bioreactor.

Table 2. Inactivation constants in emulsions (1:1 v/v) with a lipase concentration of 3 g l^{-1} emulsion.

Mole fraction glycerol	$k_{d,t}$	$k_{d,l}$	Initial activity based on long-term inactivation
(-)	(h^{-1})	(h^{-1})	(%)
0.55	0.15	$0.2x10^{-3}$	49
0.60	0.24	$1.1x10^{-3}$	37
0.73	0.40	$8.3x10^{-3}$	8

With an emulsion always a mixture of esters is produced (see table 3). The amount of triesters formed at equilibrium is constant for all initial glycerol concentrations. For high glycerol concentrations more monoglycerides and diglycerides are formed. For production of one of the esters combination of the emulsion/membrane bioreactor with a down-stream processing step is necessary (see B.1.7.). However, before considering this first the results on membrane performance are discussed.

Table 3. Measured equilibrium composition of esterifying emulsions (1:1 v/v)

Initial mole fraction	Equilibrium mole fraction				
Glycerol	Triglycerides	Diglycerides	Monoglycerides	Fatty acid	Glycerol
0.55	0.08	0.27	0.20	0.45	0.47
0.60	0.08	0.29	0.23	0.41	0.51
0.73	0.08	0.30	0.27	0.35	0.61

B.1.3. Flux hydrophilic membrane

For the hydrophilic membrane a cellulose hollow fibre device (Organon Technica, The Netherlands) with a surface area of 0.77 m^2 is chosen. This is the same membrane as used in the "hydrolysis" reactor (chapters 2 and 6). Because the pressure drop over the membrane becomes unacceptably high if a 1:1 (v/v) emulsion is used (the viscous oil phase is the continuous phase in such an emulsion) the flux measurements are performed with an emulsion with an initial oleic acid volume fraction of 0.36. (The inactivation constants of lipase in such an emulsion is measured to be equal to the inactivation constants in a 1:1 emulsion.)

The flux through the membrane is measured as a function of the glycerol concentration The flux is multiplied with the relative viscosity (as compared to water) of the permeate which is between 1 (-) for water and 53 (-) for 84w% glycerol [13]. The viscosity corrected flux decreases with increasing glycerol concentration (see figure 9). This indicates that the total resistance against permeation increases with increasing glycerol concentration. The total resistance consists of the membrane resistance, a concentration polarisation resistance and possibly a fouling resistance [14]. The flux remains constant with time at a constant glycerol concentration, therefore, fouling is not likely to be the explanation for the decrease in flux. The flux is also independent of the cross-flow velocity, therefore, concentration polarisation is probably not the reason for the flux decrease. An increase in the membrane resistance is possible, since the flux of cellulose membranes increases with increasing water content of the membrane [15]. Glycerol can "extract" water from the membrane due to its hygroscopic nature which results in lower fluxes [16]. If glycerol extracts water from the membrane then the flux value will restore to its original value if water is added to the emulsion. This is experimentally proven to be the case, therefore, it can be concluded that the flux decrease is caused by an increase in membrane resistance.

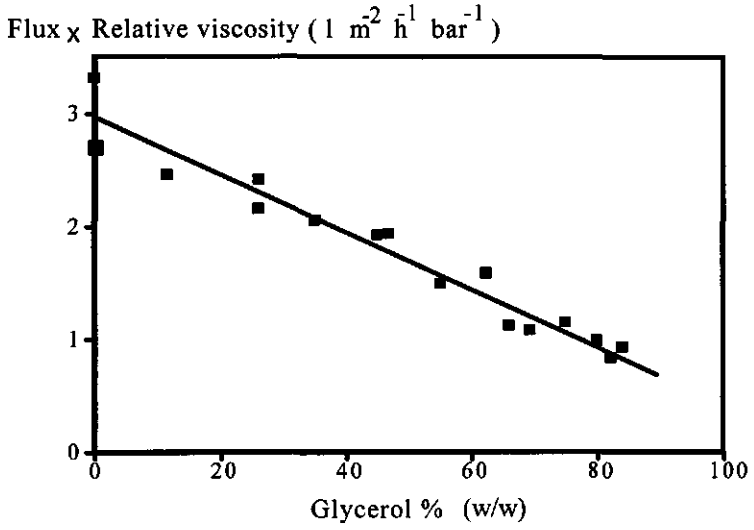

Figure 9. Flux through a hydrophilic membrane as a function of the glycerol concentration.

After testing the glycerol concentration an experiment is performed with 3 g lipase per litre of emulsion at a glycerol concentration of 80 w%. The flux multiplied by the relative viscosity remains constant for eight hours at approximately 1.0 l m^{-2} h^{-1} bar^{-1}, therefore, it can be concluded that the cellulose membrane is suitable for application in the emulsion/membrane bioreactor although the flux is rather low.

B.1.5. Flux hydrophobic membrane

A polypropylene flat sheet membrane with an average pore size of 0.1 μm and a surface area of 64 cm^2 is chosen and the membrane is modified with block copolymer F108 (see chapters 2, 3 and 6). An emulsion with initially 36 vol% oleic acid and 0.5 mole fraction glycerol in the water phase is used. To the emulsion 3 g l^{-1} lipase is added. Note that this system equals the batch emulsion/membrane bioreactor as described in chapter 2. The flux is measured as a function of time and corrected for the viscosity by multiplication

with the viscosity of the permeate, which increases from 20 mPa s for oleic acid to 50 mPa s for the oil phase at reaction equilibrium (see figure 10).

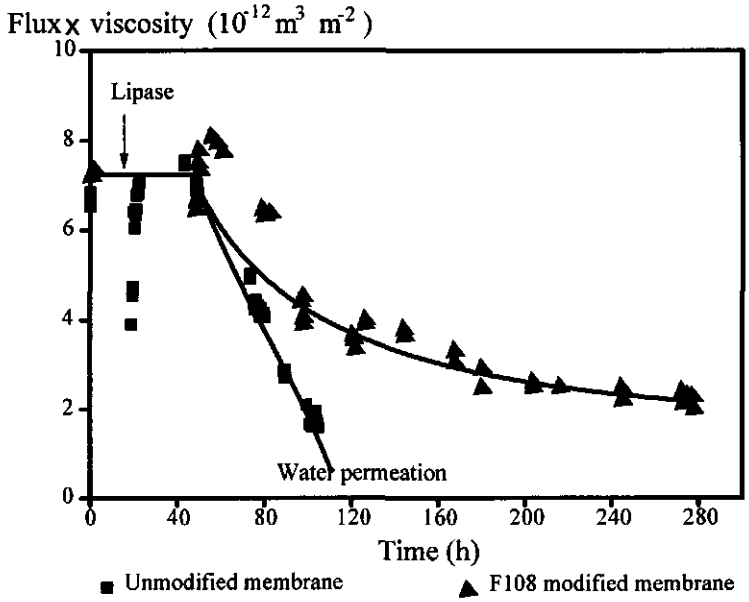

Figure 10. Viscosity corrected flux through an unmodified and an F108-modified membrane.

During the first 40 hours of the experiment with the unmodified membrane water evaporated from the emulsion and the flux dropped. After addition of water the flux restored to its original value. For both membranes the flux at 60 hours is equal to the initial flux, which is remarkable but can not be explained yet. The flux through the unmodified membrane decreases rapidly after 60 hours of operation but the decrease is not as fast as found for the unmodified membrane in the "hydrolysis" reactor (see chapter 2). After 100 hours water breaks through the membrane even at a relatively low pressure. In chapter 3 breakthrough of water is related to lipase adsorption and the diffusion coefficient is found to be the breakthrough determining factor. The diffusion coefficient of lipase in water will be higher than in a 84% w/w glycerol solution and, therefore, the

time at which breakthrough occurs will be longer. However, no quantitative data on the diffusion coefficient are available in literature, therefore, no estimate can be made for the influence of the glycerol concentration on the time of breakthrough.

The F108-modified membrane does not become water permeable. The flux of the membrane stabilises more or less to a value of $2x10^{-12}$ m^3 m^{-2} after 240 hours. This flux value is comparable to the flux found for the hydrolysis system. Also the initial fluxes are comparable. The reason for the flux decrease is still not elucidated. For esterification emulsions it is found that the emulsion droplets become more and more stable during the experiment. These emulsion droplets will coalesce less rapidly then the those present at the beginning of the experiment. However, to what extent this effect plays a role is not yet understood. What is clear is that the decrease in flux is completely reversible, if the emulsion is replaced with a new emulsion without lipase than the initial flux is found again. Hence, it can be concluded that the flux decrease is related to the emulsion and not caused by protein adsorption. Altogether, it can be stated that the F108-modified membrane can be used to separate "esterification" emulsions.

B.1.6. Conclusions for esterification process

All the subsystems of the emulsion/membrane bioreactor can be used for esterification reactions. The results are comparable to those found for the subsystems used in the hydrolysis emulsion/membrane bioreactor (see chapter 2 and 6). Because the hydrolysis reactor has been used successfully in continuous experiments, it can be expected that also for esterification a continuous experiment can be performed. However, this is beyond the scope of this thesis and is not carried out.

B.1.7. Down-stream processing for production of monoglycerides and triglycerides

For the specific production of monoglycerides or triglycerides the emulsion/membrane bioreactor should be combined with a down-stream processing step. Combination with an adsorption column [17] renders a system with which monoglycerides (emulsifying

agents) can be produced. Combination of the emulsion reactor with a pervaporation membrane unit [18] will render a system with which triglycerides can be produced.

B.2 Model system for reaction catalysed by micro-organisms

The principle of the emulsion/membrane bioreactor: catalysis in an emulsion and in-line separation with a sequence of a hydrophilic and a hydrophobic membrane can be used for any reaction in a two-phase system as long as the membranes don't get blocked and/or loose their selectivity. Separation of yeast with a hydrophilic membrane has been studied extensively and it has been found that appropriate hydrophilic membranes are available for this separation [19]. However, for hydrophobic membranes no data are available. Experiments are done with a dodecane in water emulsion (1:1 v/v) with yeast concentrations varying between 1 to 50 gram (wet weight yeast) per litre of emulsion. Separation is done with the F108-modified membrane at a transmembrane pressure of approximately 0.1 bar. Note that this emulsion is used only as a model system for extractive biocatalysis. In contradiction to most emulsions with micro-organisms, this emulsion is not very stable.

Only dodecane permeates through the modified membrane during a four hour lasting experiment. The flux of the membrane decreases slightly but remains at a high level (in the order of 100 l m^{-2} h^{-1} bar^{-1}) which makes application of the modified membrane in such a system possible. However, further tests under controlled conditions are necessary to study the influence of e.g. cross-flow velocity and transmembrane pressure on the flux.

B.3 Filtration aqueous protein solutions with modified membranes

The F108-modified membrane can be used for the separation of the oil phase from a protein containing emulsion. Protein adsorption at such a membrane is prevented by the block copolymer (F108-) molecules which provide a steric hindrance to protein molecules (see chapter 5 and [20-21]. It is interesting to investigate whether the same principle of steric hindrance can be used for water permeable "hydrophilic" membranes.

In chapter 5 it is shown that steric hindrance is only effective if the block copolymers are adsorbed at a hydrophobic surface. Therefore, a hydrophobic polypropylene membrane with an average pore size of 0.1 μm is used in the experiments. Because this membrane has to become water permeable the modification method is changed. Block copolymer F108 is dissolved in methanol (analytical grade) in a concentration of 4 g l^{-1} and the membrane is rinsed with this solution for 30 minutes. The methanol permeates through the membrane. Subsequently, the membrane is rinsed with doubly distilled water for half an hour (water permeated through the membrane) and the pure water flux is measured. After this measurement the water is replaced with a solution of 0.6 g l^{-1} Bovine Serum Albumin (95% pure; Boehringer Mannheim, Germany) in water and the flux is measured as a function of time. For the unmodified membrane the same procedure is followed with exception of the addition of F108 to the methanol. During the experiments the transmembrane pressure is kept extremely low at 400 Pa in order to prevent concentration polarisation.

The pure water fluxes are 0.0324 and 0.077 g s^{-1} for the F108-modified and unmodified membrane, respectively; which corresponds to approximately 4.5 and 10.8 m^3 m^{-2} h^{-1} bar^{-1} (see figure 11). Membrane modification results in a flux decrease of approximately 55% as indicated by the initial water flux. This is due to the adsorbed block copolymer which increases the total resistance against permeation. The flux through the unmodified membrane decreases instantaneously after BSA is filtered instead of pure water. This instantaneous effect is caused by protein adsorption. After the initial decrease in flux, the flux decreases further to approximately 40% of the initial value after 70 hours. This long-term decrease in flux is probably caused by internal fouling of the membrane and/or pore blocking.

The flux through the modified membrane does not decrease during the first 3 hours of the experiment which indicates that instantaneous protein adsorption is prevented by the block copolymers. The flux decreases only slightly to 92% after 100 hours. An explanation for this phenomenon has not been found. The performance of the modified

membrane is definitely better than that of the unmodified membrane but note that the volume fluxes at the end of the experiments are comparable for both membranes. The flux decrease caused by protein adsorption is comparable to that caused by block copolymer adsorption.

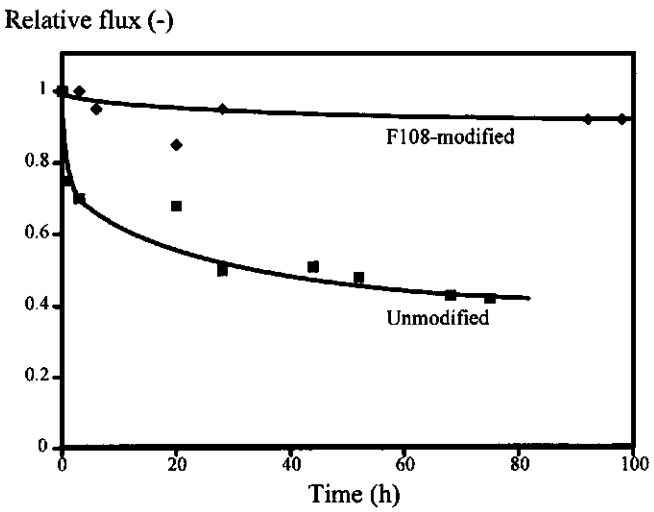

Figure 11. Water flux through unmodified and F108-modified membranes as a function of time for a transmembrane pressure of 400 Pa and a BSA concentration of 0.6 g l^{-1}. The initial pure water fluxes are 0.0324 and 0.077 g s^{-1} for the F108-modified and unmodified membrane, respectively.

In literature (e.g. [22]) it is often stated that the hydrophilicity of a membrane is the determining factor for protein adsorption/fouling. The adsorbed amount at hydrophobic surfaces is in general much higher then at hydrophilic surfaces [1-2]. Therefore, it seems very logical to look for hydrophilic membrane materials in order to control fouling of membranes. However, from the results in chapter 2-6 and from those given in figure 10 it can be concluded that hydrophobic membranes can be used very effectively, if they are protected against protein adsorption. It is clear that hydrophilicity is not the only factor that determines whether protein adsorption can take place or not. Steric hindrance is just

as important. It might even be that modification of *hydrophilic* membranes, in such a way that they also require a steric hindrance against protein adsorption, will yield membranes with a (drastically) improved performance.

CONCLUSIONS

This thesis contains engineering data for the emulsion/membrane bioreactor and data on fundamental aspects of block copolymers. In this chapter these data are used for aims beyond the scope of the previous chapters e.g. to compare the emulsion reactor with a membrane reactor, to make an economic evaluation of the process, to test the emulsion reactor for other reaction systems or to test the hydrophobic membrane for filtration of aqueous protein solutions.

The production per m^2 membrane surface area in a flat sheet reactor is found to be a factor of 50 higher then in a hollow fibre immobilised enzyme reactor, the difference is caused by blocking of the fibres in the hollow fibre module. When a flat sheet membrane reactor is compared with an emulsion reactor then the emulsion reactor is found to be a factor of 1000 more active because of the high volumetric activity that is achieved in this type of reactor.

An economic evaluation of the emulsion/membrane bioreactor is carried out and it is shown that the production per gram added enzyme, the production per m^2 membrane surface area and the volume of the emulsion vessels in a series of co-current reactors are thus that the process is feasible.

The emulsion/membrane bioreactor is tested for esterification of oleic acid and glycerol and it is shown that the long-term stability of the enzyme is high and both membranes remain sufficiently permeable and selectively wetted. The emulsion reactor can in principle be used for this reaction.

The modified hydrophobic membrane is successfully used for the separation of a dodecane in water emulsion containing up to 50 grams of yeast (wet weight) per litre of emulsion. The modified membrane can be made water permeable and is subsequently used for filtration of Bovine Serum Albumin in water solutions. The modification method is also successful for this application.

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LIST OF SYMBOLS AND ABBREVIATIONS

E	Concentration active enzyme	(g l^{-1})
R	Gas constant	(kJ mol^{-1} K^{-1})
T	Temperature	(K)
X_G	Glycerol mole fraction	(-)
X_F	Fatty acid mole fraction	(-)
X_F^{eq}	Equilibrium fatty acid mole fraction	(-)
a_2	Fitparameter	(-)
k_d	Inactivation constant	(h^{-1})
n^*	Order of the reaction	(-)
t	Time	(h)
r_F	Fatty acid production rate	(mol (fatty acid) mol^{-1} (oil phase) h^{-1})
r'_F	Fatty acid production rate per gram enzyme	(mol (fatty acid) mol^{-1} (oil phase) h^{-1})
ΔE_h	Activation energy hydrolysis reaction	(kJ mol^{-1})

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SUMMARY

Fatty acids (and glycerol) are produced by hydrolysis of fats and oils in counter-current fat-splitting columns which operate at a temperature of 200-240 °C and a pressure of 50-60 bar. Undesired side-products are formed during the process. These have to be removed in order to obtain an acceptable product. The side-reactions do not take place if the fatty acids are produced enzymatically at 30 °C.

The enzyme lipase catalyses the hydrolysis reaction at the oil/water interface. Therefore, a large oil/water interfacial area has to be available to the enzyme for a high volumetric reactor activity. A stirred vessel is used in which an emulsion is formed by thorough mixing of oil, water and enzyme. The products (fatty acid and glycerol) are separated from the emulsion in the stirred vessel by means of two membrane separation steps. A (modified) hydrophobic membrane is used to selectively separate the fatty acids from the protein-rich emulsion. With a hydrophilic membrane the water phase, which also contains the glycerol, is removed from the vessel. This reactor concept, the emulsion/membrane bioreactor, is the subject of this thesis.

The hydrophobic membrane can become permeable for water during the experiment. This effect is caused by enzyme adsorption at the membrane. The enzyme forms a hydrophilic layer on the membrane, in which case it can be preferentially be wetted by the water phase and eventually the water phase will permeate through the membrane (chapter 3).

For proper reactor operation, the hydrophobic membrane has to remain selectively wetted and sufficiently permeable for the fatty acids. Therefore, lipase adsorption has to be prevented. A membrane pre-treatment method with block copolymer F108 as shown in chapter 2 is an effective method to prevent lipase adsorption (see chapter 5), and, therewith, water permeation (see chapter 3). Block copolymer F108 consists of two

hydrophilic poly(ethylene oxide) blocks at both ends of the molecule and one hydrophobic poly(propylene oxide) block in the middle. If F108 is contacted with a hydrophobic surface then the middle block will adsorb at the surface and both hydrophilic groups will extend from the surface thus forming a so called "brush" configuration. The "brush" hinders enzyme molecules that approach the surface in such a way that they can not adsorb. The effectivity of hindrance at a *hydrophobic* surface is a function of the length of the poly(ethylene oxide) groups and the number of pre-adsorbed block copolymer molecules. It is concluded that steric repulsion is the mechanism behind prevention of protein adsorption (chapter 5).

If the block copolymer is adsorbed at a *hydrophilic* surface then the molecule will adsorb relatively flat onto the surface (pancake configuration). The block copolymer can not prevent enzyme adsorption in this configuration (chapter 5).

Pre-adsorbed block copolymer F108 prevents protein adsorption. However, the membrane hydrophilicity should not be changed (too much) by the presence of the block copolymers. Otherwise the membrane might become water permeable because of the presence of the block copolymers. In chapter 4 it is shown theoretically and experimentally that hydrophobic surfaces with pre-adsorbed block copolymers remain oil-wetted. The surface properties are hardly influenced by the presence of F108 and the surface can be wetted by a large variety of "oils".

The F108-modified membrane can be used for the continuous hydrolysis of oil in the emulsion/membrane bioreactor. From literature a model for the production of fatty acid and glycerol in a membrane reactor is adapted and extended with a model for enzyme inactivation in an emulsion. The model predictions are in agreement with the experimental data (chapter 6).

With the model an economic evaluation of the emulsion/membrane bioreactor is made in chapter 7. It is found that the production per gram enzyme, the concentrations of fatty acid and glycerol in the product streams and the production per m^2 membrane area is

within the limits for an economically feasible processes. If the reaction is carried out in a co-current series of reactors then also the volume of the stirred vessels is economically feasible.

A promising spin-off of the modification research is the use of the block copolymer for filtration of aqueous protein solutions (see chapter 7). It is shown that the flux of this membrane remains high during long-term operation.

SAMENVATTING

Vetzuren en glycerol worden geproduceerd door hydrolyse van vetten en oliën in tegenstroom-vetsplitsingskolommen. De hydrolyse wordt uitgevoerd bij een temperatuur van 200-240 °C en een druk van 50-60 bar. Tijdens dit proces worden nevenprodukten gevormd die verwijderd moeten worden om een acceptabel produkt te verkrijgen. De nevenreacties treden niet op als de vetzuren geproduceerd worden met behulp van een enzym bij 30 °C.

Aangezien het enzym de hydrolysereacties katalyseert aan het olie/water grensvlak moet er voor een hoge volumetrische reactoractiviteit veel olie/water grensvlak aanwezig zijn. Daarom wordt gebruik gemaakt van een geroerd vat waarin olie, water en lipase intensief gemengd worden. De produkten (vetzuur en glycerol) worden vervolgens afgescheiden met behulp van twee membraanscheidingsstappen. Een hydrofoob membraan wordt gebruikt om de vetzuren uit het reactiemengsel te verwijderen. Met een hydrofiel membraan wordt de waterfase, die ook glycerol bevat, afgescheiden. Dit reactor concept, de emulsie/membraan bioreactor is het onderwerp van dit proefschrift.

Het hydrofobe membraan kan permeabel voor water worden tijdens een experiment. Dit effect is niet gewenst en wordt veroorzaakt door enzymadsorptie aan het membraan. Het enzym vormt een hydrofiele laag op het membraan dat daarna preferent bevochtigd wordt door de waterfase. Uiteindelijk kan water door het membraan permeëren (hoofdstuk 3).

Voor een continu-proces is het nodig dat het hydrofobe membraan selectief doorlaatbaar blijft voor de vetzuren. Daarom moet enzymadsorptie verhinderd worden. In hoofdstuk 2 worden membranen voorbehandeld met blok-copolymeer F108 met als gevolg dat enzymadsorptie wordt voorkomen (hoofdstuk 5). Daardoor blijft het membraan alleen maar olie en geen water doorlaten (hoofdstuk 3). Blok-copolymeer F108 bestaat uit twee

hydrofiele polyethyleenoxide blokken aan beide uiteinden van het molekuul en een hydrofoob polypropyleenoxide middenblok. Als F108 adsorbeert aan een hydrofoob oppervlak dan adsorbeert alleen het middenstuk terwijl de uiteinden zich in de vloeistof bevinden. Op die manier wordt een "brush" (borstel) configuratie gevormd. De "brush" hindert enzymmolekulen die zich naar het oppervlak toe willen bewegen op een dusdanige manier dat adsorptie niet mogelijk is. De effectiviteit waarmee enzymadsorptie voorkomen wordt aan *hydrofobe* oppervlakken is een functie van de lengte van de polyethyleenoxide-groepen en het aantal geadsorbeerde blok-copolymeer molekulen. Er kan geconcludeerd worden dat het mechanisme achter voorkoming van eiwitadsorptie aan hydrofobe oppervlakken sterische repulsie is (hoofdstuk 5).

Als het blok-copolymeer op een *hydrofiel* oppervlak geadsorbeerd wordt dan adsorbeert het molekuul plat op het oppervlak ("pancake" ofwel pannekoek configuratie). Deze configuratie kan enzymadsorptie niet voorkomen (hoofdstuk 5).

Geadsorbeerd blok-copolymeer F108 voorkomt weliswaar enzymadsorptie maar het mag de eigenschappen van het membraan anderzijds niet te veel beïnvloeden. Anders kan het hydrofobe membraan waterpermeabel worden als gevolg van de aanwezigheid van de blok-copolymeren. In hoofdstuk 4 wordt theoretisch en experimenteel aangetoond dat hydrofobe oppervlakken met geadsorbeerd blok-copolymeer olie-bevochtigbaar blijven. De oppervlakte-eigenschappen van het membraan worden nauwelijks beïnvloed door de aanwezigheid van de blok-copolymeren en het membraan kan door een scala aan "oliën" bevochtigd worden.

Het met F108 gemodificeerde membraan kan gebruikt worden voor de continue hydrolyse van olie in de emulsie/membraan bioreactor. Uit de literatuur is een model voor de produktie van vetzuren en glycerol in een membraanreactor overgenomen en uitgebreid met een model voor enzyminactivatie in een emulsie. De voorspellingen van het model komen goed overeen met de experimenteel gevonden waarden (hoofdstuk 6).

Met het model is een economische evaluatie voor de emulsie/membraan bioreactor uitgevoerd (hoofstuk 7). De geproduceerde hoeveelheid vetzuur per gram toegevoegd enzym, de concentraties vetzuur en glycerol in de produktstromen en de geproduceerde hoeveelheid vetzuur per m^2 membraanoppervlak zijn allen dusdanig hoog dat het proces in principe economisch haalbaar is. Als de reactie uitgevoerd wordt in een serie van meestroomreactoren dan is ook het volume van de geroerde vaten dusdanig dat het proces practisch uitvoerbaar wordt.

Een interessante spin-off van het membraanmodificatieonderzoek is het gebruik van het met F108 gemodificeerde membraan voor filtratie van eiwitoplossingen (hoofdstuk 7). Er is aangetoond dat het membraan ook voor deze toepassing gedurende lange tijd een hoge flux houdt, dit in tegenstelling tot niet gemodificeerde membranen.

NAWOORD

De afgelopen vier jaar heb ik met veel plezier gewerkt aan het promotieonderzoek dat beschreven staat in dit proefschrift. Nu zou ik de indruk kunnen wekken dat ik het proefschrift helemaal alleen geschreven heb maar dat kon alleen maar dankzij de directe en indirecte hulp van een groot aantal mensen. Een aantal daarvan wil ik hierbij met name noemen.

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Het werk van veel afstudeerstudenten en een stagiaire staat beschreven in dit proefschrift. Christel Wijers heeft zich succesvol in het ondoordringbare woud van blok-copolymeren gewaagd; Paul Diepenmaat is tot een verantwoorde keuze voor het hydrofiele membraan gekomen; Ton Dings heeft enzymen op verschillende manieren geïnactiveerd; Jaap Oostra heeft grote verschillen tussen vlakke plaat en holle vezel membraanreactoren ontdekt; Hans Koenraad heeft na een sterke initiële inactivatie voldoende restactiviteit

overgehouden; Ine Smulders heeft modelmatig lijn gebracht in enzyminactivatie in emulsies; Kees van der Voort Maarschalk heeft laten zien dat marinades van grensvlakchemie en proceskunde erg smaakvol zijn; Valérie Jeanneau has been the first to show that the emulsion reactor can be used for esterification en Stephan van Hoof gaat waarschijnlijk nog eens een fabriekje beginnen op basis van zijn dynamisch model. Allen heel hartelijk dank voor jullie inzet en de prettige samenwerking.

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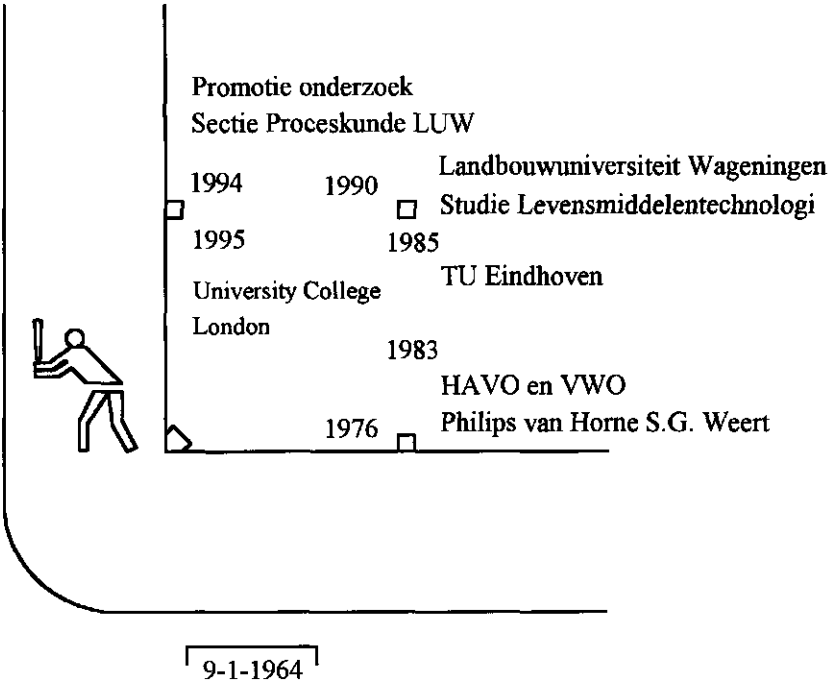
CURRICULUM VITAE



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Geboren te Nederweert