Enzymatic Synthesis of Monoglycerides in a Membrane Bioreactor with an In-Line Adsorption Column

A. van der Padt*, J.T.F. Keurentjes[1], J.J.W. Sewalt, E.M. van Dam, L.J. van Dorp and K. van 't Riet
Wageningen Agricultural University, Food and Bioprocess Engineering Group, Department of Food Science, Biotechnion, 6700 EV Wageningen, The Netherlands

The chemical synthesis of monoglycerides requires high temperatures, which may lead to the polymerization of unsaturated fatty acids. The enzymatic synthesis of these esters is performed at moderate temperatures and, hence, polymerization is avoided. However, enzymatic processes often end up with a mixture of the product, by-product, substrate and enzyme. An alternative process is an immobilized enzyme membrane reactor equipped with an in-line adsorption column to adsorb the monoglycerides, preferentially onto the adsorbate. A silica 60 column has shown preferential adsorption of monocaprinate. The adsorption of a mixture of decanoic acid, mono- and diglycerides is based on two different mechanisms. The decanoic acid will interact with hydroxyl groups at the silica gel surface, which results in a noncompetitive decanoic acid adsorption onto 25% of the silica gel surface. On the remaining part of the silica gel surface, mono- and diglycerides adsorb competitively. When a mild eluant is used, such as 5% ethanol in hexane, only the competitively adsorbed molecules are desorbed. This results in a purification factor of approximately 90% after desorption. The column can be desorbed off-line in a continuous membrane/repeated batch column process. This results in an estimated production of monoglycerides of 60 mol (15 kg) of monoester per gram enzyme.

KEY WORDS: Adsorption, esterification, lipase, membrane reactor, monoacylglycerol, monoglyceride.

Monoglycerides (monoacylglycerols) are used as emulsifiers in food and in cosmetics. The chemical production of these compounds involves an inorganic catalyst and is performed at high temperatures. The major disadvantage of the chemical synthesis is polymerization of unsaturated fatty acids at high temperatures (1). Therefore, the chemical production of monoglycerides is limited to the incorporation of saturated fatty acids.

Enzymatic synthesis of monoesters overcomes the problem mentioned above. Moreover, when natural substrates are used, the enzymatically synthesized ester usually can be qualified as a natural ingredient for cosmetic and food products. To achieve enzymatic monoglyceride production, three routes have been presented in the literature (2–4). One way is to hydrolyze triglycerides with a 1,3-specific lipase. Holmberg and Osterberg (2) have reported hydrolysis in a micro-emulsion system, and yields up to 80% were obtained. Downstream processing of the monoesters, however, is troublesome due to the mixed surfactant system.

Another method is the alcoholysis of a triglyceride and glycerol (3). In this case a mixture of glycerol, a trace of water and triglycerides are emulsified and lipase is added. The reaction is started at 45°C and, after a while, the temperature is lowered, allowing the monoglycerides to precipitate (3). In the end, a solid fat-enzyme phase is obtained with a monoglyceride concentration larger than 90% (w/w). A disadvantage of this process is that efficient methods are not available to separate the enzyme and the monoglyceride while maintaining enzyme activity.

A third way to produce monoesters is to perform esterification of glycerol and fatty acid (4). Monoesters are the first product of the reaction chain, but esterification will proceed, and monoglycerides will be converted into diglycerides. In case a lipase without positional specificity is used, the diesters are subsequently converted into triglycerides. Weiss (4) has presented esterification in a system in which a solid fatty acid phase is dispersed in a glycerol phase. The enzymatic conversion of the fatty acids is over 85%. The formation of di- and triesters can be minimized by using an organic solvent to extract the monoglyceride. A 100% monooleate ester yield has been reported with dichloromethane as a solvent (5). The concentration in the extraction phase is approximately 2% w/w, and the monoester easily can be obtained by evaporation of the solvent. Miller and co-workers (6) have published a 100% monoester yield by using a derivatized glycerol, in which two of the three hydroxyl positions have been blocked by acetone. Once the esterification is completed, the blocking group can be removed by mild acid treatment.

The esterification of a long-chain fatty acid and glycerol is discussed in this paper. The enzymatic reaction requires a two-phase reaction system. This can be either an emulsion system or a membrane reactor. In a previous paper we have described a membrane two-phase reactor for the production of glycerides (7). In this reactor, the oil and water phases are kept separated. This allows a simple, in-line removal of monoglycerides by placing an adsorption column in the oil phase of the reactor system (Fig. 1). The produced monoglycerides should adsorb onto the column. This results in a low monoglyceride concentration in the oil phase, and represses further esterification to di- and triglycerides. When the adsorption column is saturated with monoglycerides, the column can be replaced and can be eluted off-line. This study deals with the development of a silica gel-based adsorption column for preferential adsorption of monoglycerides.

Adsorbent. For a porous adsorbent, the specific surface area, as well as the pore size distribution, is of importance, because this affects the capacity of the adsorbent in two ways. First, pores must be large enough to allow entrance of adsorbate molecules. Second, the adsorbate molecule can only enter a pore when it is filled with the adsorbate-containing phase. Whether a pore is filled is related to the pore size, the surface tension of the liquid and the wetting capacities of the adsorbent. For nonwetting conditions, a pore is filled when the applied pressure exceeds the Laplace pressure, which is a measure for the pressure difference at the two sides of the curved gas-liquid interface (8). In this way, the available area for adsorption can be calculated when the pore size distribution is measured and the physical properties of both the liquid and the adsorbent are known.

*To whom correspondence should be addressed at Wageningen Agricultural University, Dept. of Food Science, P.O. Box 8129, 6700 EV Wageningen, The Netherlands.
[1]Present address: Akzo Company, ARLA/CRP, P.O. Box 9300, 6800 SB Arnhem, The Netherlands.

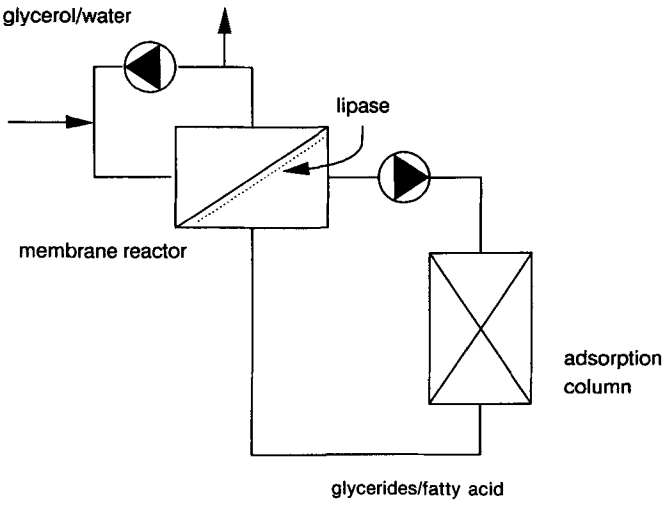

FIG. 1. The membrane bioreactor system combined with an in-line adsorption column.

Multicomponent adsorption. Assuming reversible adsorption at local adsorption sites and neglecting lateral interactions between adsorbate molecules, a monolayer is created. The adsorption equilibrium can then be described with the Langmuir equation (9). The Langmuir equation has been adapted for a bisolute system by Butler and Ockrent (10). One of the Langmuir premises is that the adsorption energy has the same value at all the adsorption sites. However, silanol groups are present at the silica gel surface. These groups can adsorb molecules by H-bond interaction, which results in a relatively high adsorption energy (11). Therefore, two different types of adsorption are possible—adsorption onto the silanol groups, and adsorption onto the silanol-free sites. The different mechanisms can cause a difference in competitive character of the two sites. Jain and Snoeyink (12) have extended the bisolute Langmuir model for this case. The adsorbent area is divided into a competitive part, where the components i and j compete for adsorption, and a noncompetitive part, where only component i will adsorb. When one component adsorbs noncompetitively and competitive multicomponent adsorption takes place for another three components, the amount adsorbed can be calculated as:

$$\Gamma_i = (\Gamma_{max1} - \Gamma_{max2}) \cdot b_i \cdot C_i/(1 + b_i \cdot C_i) + \Gamma_{max2} \cdot b_i \cdot C_i/(1 + b_i \cdot C_i + b_j \cdot C_j + b_k \cdot C_k + b_l \cdot C_l) \quad [1a]$$

$$\Gamma_n = \Gamma_{max2} \cdot b_n \cdot C_n/(1 + b_i \cdot C_i + b_j \cdot C_j + b_k \cdot C_k + b_l \cdot C_l) \quad [1b]$$

for $n = j$ or $n = k$ or $n = l$; where $b_{i,j,k,l}$, affinity constant ($m^{+3} \cdot mol^{-1}$); $C_{i,j,k,l}$, equilibrium concentration ($mol \cdot m^{-3}$); $\Gamma_{i,j,k,l}$, amount adsorbed ($mol \cdot kg^{-1}$); Γ_{max1}, maximum attainable amount adsorbed of component i ($mol \cdot kg^{-1}$); and Γ_{max2} maximum attainable amount adsorbed of component n ($mol \cdot kg^{-1}$).

The first term on the right-hand side of the adsorption equation of component i (Eq. [1a]) is the Langmuir expression for noncompetitive adsorption. The second term of this equation is the amount of component i at the adsorbent surface that is adsorbed competitively with components j, k and l. If Γ_{max1} equals Γ_{max2}, the equation changes into the competitive multicomponent Langmuir model.

Column characteristics. The amount adsorbed in an adsorption column is not only determined by the adsorbent properties, but also is related to the column characteristics, such as flow conditions. The major problem in column operation is the occurrence of channelling. If channelling can be avoided, an estimation of the adsorption capacity can be made. In a system where intraparticle diffusion can be neglected, the rate-determining step is diffusion of the adsorbate molecule from the bulk liquid through the stagnant film to the adsorbent surface. An approximation of the film mass transfer coefficient can be made by using the Chilton-Colburn factor. This factor is a function of the bed porosity, throughput, liquid viscosity, particle diameter and diffusion coefficient of the adsorbate molecules (13). Once the transfer coefficient is known, the mass transfer zone in a column can be estimated (14), which leads to the adsorbent load. This estimation refers to the column load under ideal conditions. However, it can occur that a load of 50% of the calculated load is measured, due to channelling (13).

Assuming laminar flow and neglecting intraparticle diffusion, the diffusion rate is determined only by the diffusion of the adsorbate through the stagnant film layer. This diffusion rate can be calculated from the Sherwood number that is known for a packed bed (13). Once the mass transfer coefficient is known, the mass transfer zone shape can be calculated as shown by Beverloo *et al.* (14) for the case of high affinity adsorption, the adsorption can be described by the saturation equation:

$$\Gamma_n = \Gamma_{max2} \text{ for } C_n > 0 \text{ or } C_n = 0 \text{ for } \Gamma_n < \Gamma_{max2} \quad [2]$$

When the adsorbate particles are taken as rigid spheres, they have shown that the concentration in the mass transfer zone is described by:

$$C_n(z) = C_{n,in} \cdot e^{(3 \cdot k_f (z_{av} - z)/(r_p \cdot u \cdot (1 - \varepsilon_b)) - 1)} \quad [3]$$

in which $C_n(z)$ adsorbate concentration at place z ($mol \cdot m^{-3}$); $C_{n,in}$, adsorbate concentration at the inlet ($mol \cdot m^{-3}$); k_f, film mass transfer coefficient ($m \cdot s^{-1}$); r_p, particle radius (m); u, superficial velocity ($m \cdot s^{-1}$); z, axial length coordinate (m); z_{av}, axial average position of the mass transfer zone (m); and ε_b, bed porosity (—).

When breakthrough occurs, z equals H, and in this case the axial average position z_{av} can be estimated from:

$$z_{av} = H + r_p \cdot u \cdot (1 - \varepsilon_b) \cdot (1 + \ln(C_{n,out}/C_{n,in}))/(3 \cdot k_f) \quad [4]$$

where H, column height (m) and $C_{n,out}$, adsorbate concentration at the outlet ($mol \cdot m^{-3}$). From this equation the breakthrough time can be calculated. Eq. [3] can be integrated from the start of the mass transfer zone (z=0) to the height of the column (z=H), which results in the average concentration in the mass transfer zone. At a given outlet concentration, both the average adsorbate concentration in the column, $C_{n,av}$ ($mol \cdot m^{-3}$), as well as the amount of adsorbate that has passed the column, M_{passed} (mol), can be calculated:

$$C_{n,av} = C_{n,in} \cdot (3 \cdot k_f \cdot z_{av} - r_p \cdot u \cdot (1 - \varepsilon_b) \cdot e^{1-N})/(3 \cdot k_f \cdot H) \quad [5]$$

$$M_{passed} = C_{n,in} \cdot \pi R^2 \cdot r_p \cdot u \cdot (1 - \varepsilon_b) \cdot e^{1-N}/(3 \cdot k_f \cdot H) \quad [6]$$

and

$$N = 3 \cdot k_f \cdot (H - z_{av})/(r_p \cdot u \cdot (1-\varepsilon_b)) \quad [7]$$

Where N (—) is the number of transfer units between z_{av} and H, and R is the radius of the packed bed. When the column load is known, the amount adsorbed onto the adsorbent can be calculated from the mass balance.

EXPERIMENTAL PROCEDURES

Materials. Decanoic acid (95% pure) was obtained from Unichema (Emmerich, Germany). Ethanol (100%), formic acid, hexadecane, phenolphthalein, silica 60 (0.063–0.200 mm) and sodium hydroxide (0.1N) were from Merck (Darmstadt, Germany). Glycerol (99+%) came from Janssen (Beerse, Belgium), and hexane was from Rathburn (Walkerburn, U.K.). Lipase of *Candida rugosa* was from Meito Sangyo (Tokyo, Japan), and the membrane module (Andante) was purchased from Organon (Boxtel, The Netherlands). Monocaprinate (99%) came from Sigma Chemical Co. (St. Louis, MO) and the silica gel No. 1 was obtained from Crosfield Chemicals (Warrington, U.K.).

BET and pore size distribution. Nitrogen adsorption is measured at different pressures at 77K. When the adsorbed volume of N_2 (STP) is known in relation to the relative pressure, the specific surface can be calculated (8). The relative pressure P/P_o is the actual pressure over the saturated vapor pressure. The BET adsorption and desorption isotherms may differ for a porous adsorbent. This so-called hysteresis loop only appears if mesopores (2 nm $< r_p <$ 20 nm) exist. Assuming only cylindrical pores exist, it is possible to estimate the pore size distribution (6). The smallest detectable radius is 1.6 nm, which is three to four times the diameter of the nitrogen molecule.

Adsorption measurements. Silica gel is washed with hexane and dried under vacuum. The adsorption isotherms for decanoic acid and monocaprinate, respectively, are determined by depletion measurements from a hexadecane solution. Multicomponent depletion measurements are performed by adding different amounts of silica gel to a mixture of decanoic acid, mono-, di- and tricaprinate in hexadecane. Concentrations are determined by gas chromatographic analysis and fatty acid titration.

Desorption measurements. The adsorbed column is rinsed with hexane to remove glycerides and fatty acids present in the void volume. Afterwards the column is eluted with different solvents.

Adsorption membrane bioreactor system. The membrane bioreactor consists of a cellulose hollow-fiber membrane module, an internal oil circuit (*circa* 80 mL) and an external glycerol-water circuit. The glycerol concentration is kept constantly making use of a feed and bleed system. The oil circuit is operated batch wise. The conversion takes place at 25°C. The biocatalyst is adsorbed at the inner fiber side (7). The membrane unit contains 6000 fibers with an internal diameter of 0.2 mm and a wall thickness of 8 μm. The total membrane surface is 0.77 m^2. Monoglyceride production is started with 50% w/w decanoic acid in hexadecane. At the start of the experiment, an adsorption column is placed in the oil circuit. Samples are taken at the outlet of the column.

Concentration measurements. The composition of the oil is determined by gas chromatography. Each sample is diluted (400 times) in hexane, 1 μL is injected cold on a 5-m CP-Sil-5-CB column (Chrompack, The Netherlands). The oven temperature of the Carlo Erba system (Milano, Rome) is 80°C at the moment of injection. After one minute the temperature is increased at Middleburg, 20°C/min^{-1} to 320°C. The FID detection of decanoic acid, mono-, di- and tricaprinate occurs at 370°C. He is chosen (4 mL/min) as carrier gas. The concentration of decanoic acid also can be measured by dilution with ethanol, followed by titration of the acid with sodium hydroxide against a phenolphthalein indicator.

RESULTS AND DISCUSSION

Specific surface area of silica gel. The specific surface area of silica 60 is calculated according to the BET equation from the N_2 desorption isotherm and is approximately 500×10^3 $m^2 \cdot kg^{-1}$. The pore size distribution has been derived and shows that the surface area belonging to macropores approximates 115×10^3 $m^2 \cdot kg^{-1}$. The smallest pore detected has a pore diameter of 3.2 nm. When this value is compared to the estimated tail length of the decanoic acid molecule [1.3 nm, calculated from bond length date (15)], it can be concluded that the adsorbate molecules fit even in the smallest pores.

To calculate the smallest pore that will be filled with liquid, the Laplace pressure has to be compared to the applied pressure. The surface tension of an oil is approximately (16) 30×10^{-3} $N \cdot m^{-1}$ and the contact angle (17) with silica gel is about 32°. When a static pressure of 0.5×10^5 Pa is applied, the radius of the smallest pore filled with the oil phase will be 0.5 μm. This value suggests that no pore will be filled at all, although all pores are large enough to allow passage of adsorbate molecules. Only a part of the surface area belonging to the macropores (115×10^3 $m^2 \cdot kg^{-1}$) is available for adsorption.

The specific surface area available for adsorption also can be derived from adsorption isotherms. For both decanoic acid and monocaprinate in hexadecane the adsorbed amounts *vs.* the equilibrium concentrations are given in Figure 2. The maximum attainable amounts adsorbed are 1.2 ± 0.1 $mol \cdot kg^{-1}$. The fact that these values are equal for both components is in agreement with their molecular sizes, which are approximately the same. In a vertical, head-down position, estimates for the molecular surface (18) are between 0.17 and 0.24 nm^2, resulting in a specific surface for silica 60 of 120×10^3 to $170 \times 10^3 \cdot m^2$ kg^{-1}. This value is in good agreement with the measured specific surface of the macropores from the gas desorption data. In the following paragraphs a value of 120×10^3 $m^2 \cdot kg^{-1}$ will be used.

Adsorption measurements. Figure 2 shows that both decanoic acid and monocaprinate isotherms appear to be high-affinity isotherms. The affinity constant b cannot be determined from these one-component adsorption isotherms, due to the fact that the smallest detectable equilibrium concentration still yields the maximum attainable adsorption of 1.2 $mol \cdot kg^{-1}$. To obtain the affinity constants, multicomponent experiments are required. These multicomponent adsorption measurements are performed with a mixture of decanoic acid, mono-, di- and tricaprinate, dissolved in hexadecane. This mixture is brought into contact with different amounts of silica gel. As an example, the mono- and dicaprinate adsorption is

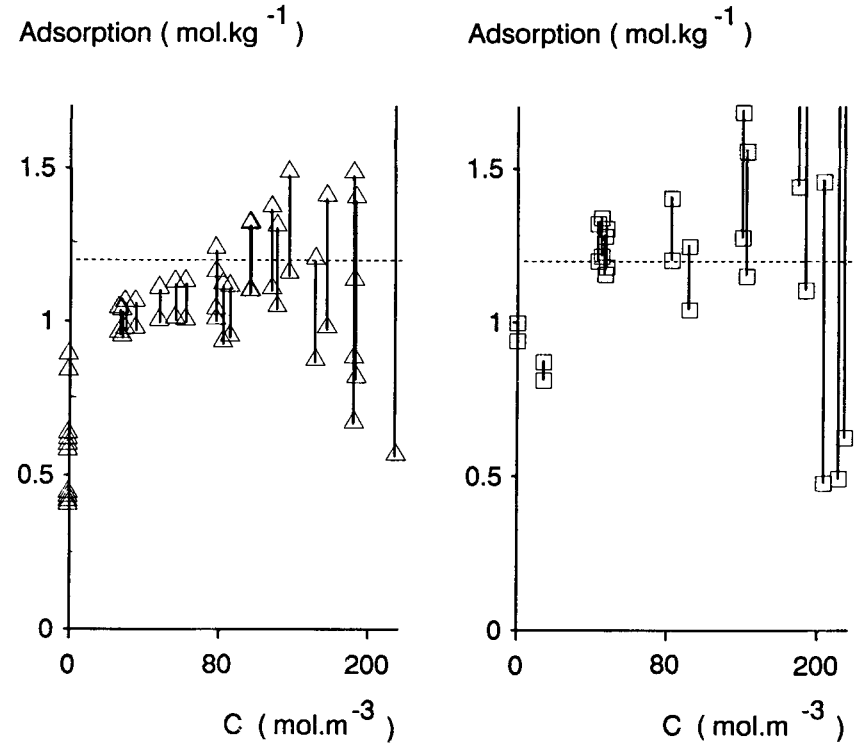

FIG. 2. Adsorption isotherm of decanoic acid (Δ) and monocaprinate (□) on silica 60.

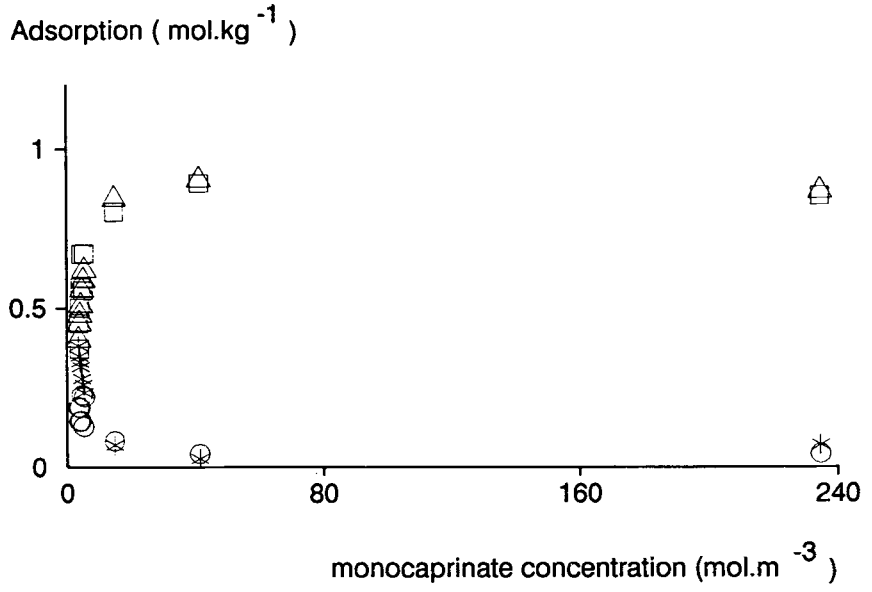

FIG. 3. Adsorption of monocaprinate (□) and dicaprinate (○) from different oil mixtures onto silica gel *vs.* the equilibrium monocaprinate concentration and the calculated non-competitive and competitive adsorption for monocaprinate (△) and dicaprinate (✕), respectively.

given as a function of the monocaprinate equilibrium concentration (Fig. 3). Of course, the adsorption is not only a function of the monocaprinate concentration, but also of the decanoic acid, di- and tricaprinate concentration. Figure 2 shows that for monosolute adsorption the maximum attainable amount is 1.2 mol·kg^{-1}. Figure 3 shows, contrary to this result, that the maximum amount of ester adsorbed at multicomponent conditions is 0.9 mol·kg^{-1}. Thus, only 75% of the available adsorption area is occupied with esters, despite the high-affinity character of both components. This can be explained with the formation of H-bonds between decanoic acid and the silanol groups of the silica. Glycerides are incapable of forming H-bonds. On this thesis we can assume that decanoic acid

is noncompetitively adsorbed onto that part of the silica gel surface having the silanol groups ($\Gamma_{max1}-\Gamma_{max2}$ = 0.3 mol·kg^{-1}), while the remaining surface (Γ_{max2} = 0.9 mol·kg^{-1}) is available for competitive adsorption. In all our experiments the esters have been adsorbed onto a column loaded with decanoic acid at solute concentrations below the detection limit. This means that the affinity of decanoic acid is orders of magnitude lower than that of the glycerides. In terms of Eq. [1], this means that $b_a \cdot C_a << b_m \cdot C_m + b_d \cdot C_d + b_t \cdot C_t$. Now Eq. [1] can be rewritten as:

$$\Gamma_a = \Gamma_{max1} - \Gamma_m - \Gamma_d - \Gamma_t \quad [8a]$$

$$\Gamma_n = \Gamma_{max2} \cdot b_n \cdot C_n / (1 + b_m \cdot C_m + b_d \cdot C_d + b_t \cdot C_t) \quad [8b]$$

$$\text{for } n = m \text{ or } n = d \text{ or } n = t.$$

Based on Eqs. [8a] and [8b] and the measured multicomponent adsorption data, the affinity constants for mono- di- and tricaprinate are calculated as 5.31, 0.35 and 0.07 m^3·mol^{-1}, respectively. All adsorption data can thus be estimated by using the last set of affinity constants (Fig. 3). In this Figure, the calculated points are given because at all points the decanoic acid, di- and triester vary. Therefore, it is not possible to interpolate.

Desorption. Several batches of pre-adsorbed silica gel are brought into contact with different solvents to elute the adsorbed compounds. With water as elution solvent, this efficiently removes adsorbed fatty materials from silica gel. However, it cannot be used without drying of the silica gel afterwards. Once silica gel is adsorbed with water, no adsorption of fatty materials will occur at all. When hexadecane is used as eluant, esters are found in the eluate phase. The monoester concentration in hexadecane, however, does not exceed 200 mol·m^{-3} (6%, w/w), which approximates saturation. The hexadecane elution shows that the adsorption is a reversible process, which is one of the Langmuir premises.

A homologous series of alcohols also is tested as eluant. Good results have been obtained with ethanol. All the adsorbed compounds are desorbed in a 100% ethanol solution. This technique is used for repeated use of the adsorbent. With a decreasing alcohol in hexane concentration, the desorption of decanoic acid becomes less complete. At 5% ethanol in hexane solution, decanoic acid is only partially removed from the adsorbent surface. We assume that only the competitively adsorbed amount of decanoic acid is removed, while the solvent silica gel interactions are too weak to break the H-bonds between decanoic acid and silica gel. The partial desorption of decanoic acid is favorable as it provides the opportunity to increase product concentration. Desorption as described above is measured as a function of the volume of eluant that has passed the column (Fig. 4). The maximum monocaprinate concentration equals 7.5% w/w in the eluate phase, which approximates saturation in this solvent. The purification factor in this particular fraction (20- to 60 mL) is over 98% as compared to the remaining decanoic acid concentration in the eluate, the average purification factor of all the fractions is 95%, and the average concentration is about 2.6% w/w.

Membrane bioreactor with an in-line adsorption column. To recover the monoesters produced, a silica 60 column is placed in the oil phase of a membrane reactor. Initially, decanoic acid adsorbs onto the column and no decanoic acid is measured at the outlet of the column. Within a few minutes, the column is saturated with decanoic acid and the acid concentration increases to 2.5 kmol·m^{-3}. During

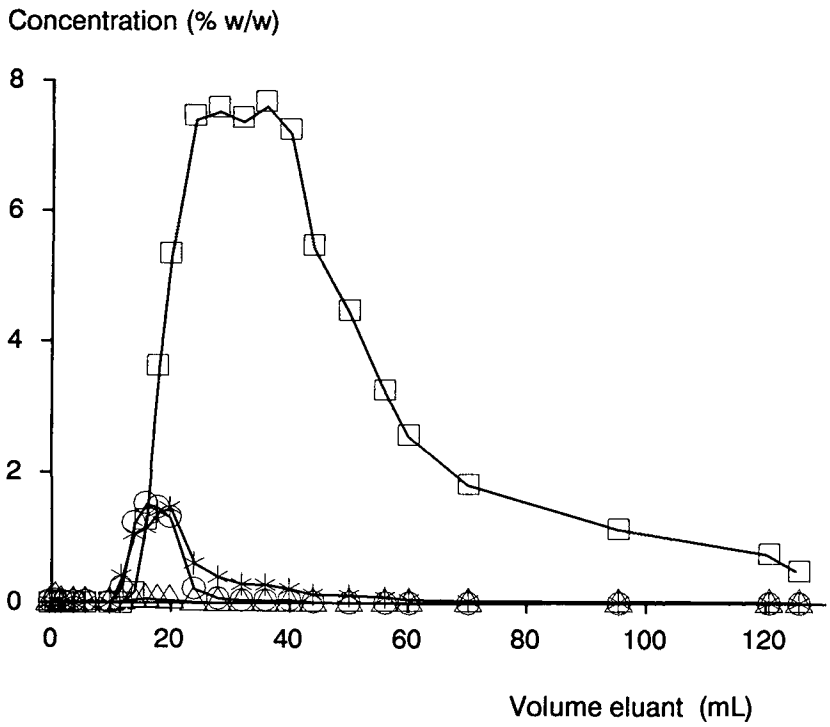

FIG. 4. Concentration of the components in 5% ethanol in hexane during desorption of decanoic acid (△), mono- (□), di- (○) and tricaprinate (✕) from a silica gel column.

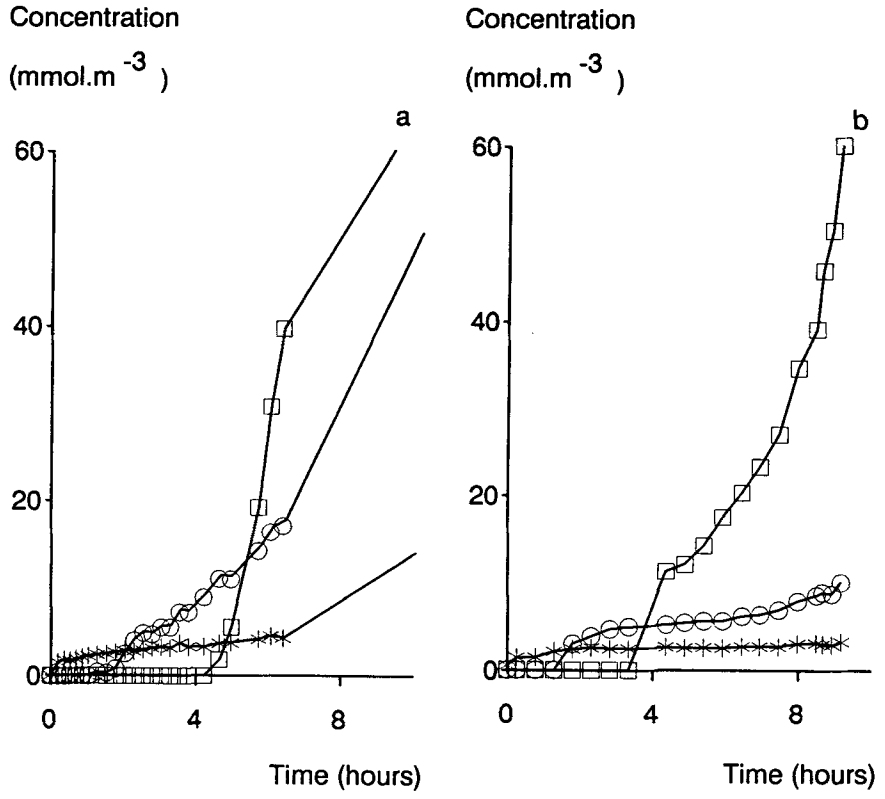

FIG. 5. The mono- (□), di- (○) and tricaprinate (✕) concentration at the outlet of a silica gel column in an adsorption membrane bioreactor system.

the first two hours of the enzymatic process, only triglycerides are measured at the outlet of the column (Fig. 5). This indicates that the produced mono- and diglycerides do adsorb onto the silica. After 2 h dicaprinate is measured, and after 4.5 h, monocaprinate also is measured. As soon as monoesters are detected in the column outlet flow, the column is removed. At this moment, the monoester production rate in the membrane reactor is measured as 1.9 μmol•s^{-1}. Assuming that this production rate occurs during the adsorption, the adsorbed amount of monoester should be 0.56 mol•kg^{-1} silica gel.

At the moment the column was removed from the oil phase, close examination of the column revealed that about three-quarters of the material was wetted by the oil phase. A completely wetted sample from the column is desorbed with ethanol as eluent to determine the adsorbed amounts. Values are given in Table 1. The remaining silica gel is eluted with ethanol, for which the desorption data are given in Table 1. The total amount of monocaprinate in the eluate is 32 mmol, which equals 0.57 mol•kg^{-1}. This value agrees with the measured production rate of 0.56 mol•kg^{-1}.

Column characteristics. The mass transfer coefficient k_f for this system is calculated to be between 8.5 and 9.5 $\times$ 10^{-6} m.s.$^{-1}$. This range is caused by variations in flux (Table 2). The calculated affinity constants and concentrations in the experiment given in Figure 3 show that we can assume a high-affinity adsorption of monocaprinate. Thus, Eqs. [4–7] can be applied and both the adsorption of monocaprinate onto the column and the breakthrough time of the column can be calculated. Parameter values are given in Table 2. The breakthrough time is calculated to be 8.3 h (Eq. [4]) and average monocaprinate adsorption equals 0.81 mol•kg^{-1} (Eqs. [5–7]). The experimentally determined breakthrough time of 4.5 h is 55% of the calculated value. The adsorption is about equal to the amount determined for the fully wetted sample.

The reduction in breakthrough time can be caused by channelling in the column. Assuming that channelling results in a decrease of the column radius, the apparent column radius at which the calculated breakthrough time equals the experimentally measured time can be estimated. This apparent radius equals 0.01 $\pm$ 0.001 m; in this case the wetted amount of silica gel is 0.6–0.7 of the silica present in the column. This apparent radius is chosen to calculate the amount of ester that is adsorbed onto the wetted part of the column, and this results in an adsorption of 0.42 to 0.53 mol•kg^{-1}. After correcting for channelling, the adsorbed amounts do correspond with the measured values of the thoroughly wetted silica gel sample (Table 1). It can be concluded that the low adsorption is caused by channelling, and adsorption onto the thoroughly wetted silica gel can be described with the adsorption model.

Monocaprinate production. The immobilized nonspecific lipase catalyzes not only the production of monoesters, but also diesters and triesters are obtained. When no adsorption column is used, the monoglyceride production is about 18% w/w (7). If a silica gel adsorption column is placed in the oil circuit of the membrane bioreactor, the produced monoesters are preferentially adsorbed onto the silica gel. The downstream processing of the adsorbed

TABLE 1

Desorption Data of the Silica 60 Column Placed in the Oil Phase of a Membrane Reactor After 4.5 h of Adsorption

Compound	Column average ($mol \cdot kg^{-1}$)	Correction for channeling ($mol \cdot kg^{-1}$)	Thoroughly wetted sample ($mol \cdot kg^{-1}$)
Decanoic acid	0.19	0.29	0.26
Monocaprinate	0.57	0.87	0.79
Dicaprinate	0.05	0.08	0.07
Tricaprinate	0.01	0.02	0.01
Amount silica-gel (kg)	55.8×10^{-3}	36.3×10^{-3}	

TABLE 2

Column Parameters

Column porosity	ε_b	0.780	(−)
Particle radius	r_p	0.13×10^{-3}	(m)
Kinematic viscosity	ν	0.36×10^{-6}	$(m \cdot s^{-2})$
Diffusion coefficient	D	10^{-10}	$(m^2 \cdot s^{-1})$
Column radius	R	0.013	(m)
Column height	H	0.21	(m)
Specific surface	A_{sp}	120×10^3	$(m^2 \cdot kg^{-1})$
Column load		628	$(kg \cdot m^{-3})$
Maximum attainable adsorption	Γ_{max2}	0.9	$(mol \cdot kg^{-1})$
Inlet concentration monoester	$C_{m,in}$	12.3	$(mol \cdot m^{-3})$
Flux	Φ	0.16×10^{-6}	$(m^3 \cdot s^{-1})$

monoesters is handled off-line. This implies that the membrane reactor can be used to produce monoesters in a continuous process, which then adsorb onto a sequence of colunns. A half-life time of the immobilized enzyme of 50 d should be possible (19) and a rough estimation of the production capacity then is 100 mol (15 kg) of monoester for a gram of enzyme.

The purification factor of 90% based on the competitive adsorbed compounds is about the same as presented in the literature (2-6). However, in our case the eluate phase contains only mono-, diesters and fatty acids, and neither enzyme nor other emulsifiers are present. Downstream processing of the eluate only includes saponification of the fatty acids and evaporation of the solvent. Holmberg and Osterberg (2) present a microemulsion system to produce monoglycerides. This system will end up with a mixture of nonionic emulsifier, enzyme, solvent, fatty acid and esters. Downstream processing involves several extraction steps and, unfortunately, denaturation of the enzyme takes place. Therefore, the enzyme can be used for one batch only, which results in a production of 45 mmol monoester per gram enzyme. Downstream processing of the solid fat system as presented by McNeill and Yamane (3) and Weiss (4) involves a heating step to melt the monoglycerides produced, which also results in inactivating the enzyme. The extraction system of Graille (5) resembles our system in one way—no enzyme is present in the organic phase. Downstream processing of the organic phase is analogous to downstream processing of the eluate of the membrane system. The enzyme preparation can be used 20 times and production becomes 220 mmol per gram enzyme. The process developed by Miller and co-workers (6) cannot be compared with the other systems because no data are available.

The purification factor of the membrane bioreactor with in-line adsorption equals those of the systems shown in the literature, while enzyme-based productivity in continuous production is at least 250 times the productivity shown in the literature. This high productivity combined with off-line downstream processing of the monoesters in the major advantage of this system.

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