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Membranes in the enzymatic synthesis of biotensides from renewable sources

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Abstract

A novel integrated process of enzymatic synthesis of sugar fatty acid esters from renewable sources was proposed for the system oleic acid/α-methylglucoside focussing on the application of different membrane techniques. The operational parameters were studied and optimised carrying out the reaction in an enzymatic membrane reactor (EMR) where the catalyst remained retained by means of ultrafiltration. A pervaporation unit coupled to the EMR was applied for by-product removal (water). A proper product separation and isolation was achieved applying combined techniques including filtration, evaporation, extraction and alternatively stepwise elution chromatography or dialysis.

Keywords: Biotensides; Enzymatic synthesis; Enzymatic membrane reactor; Pervaporation; Dialysis

1. Introduction

Biotensides, in particular sugar fatty acid esters (SFAEs), find nowadays a wide range of applications in the pharmaceutical, personal-care and food industry because of their biodegradability, biocompatibility and special surfactant properties [1]. In 1995 the worldwide production of biosurfactants exceeded 3 million tonnes per year (at an estimated value of 4 billion US$), rising to over 4 million tonnes by the end of last century [2]. Nowadays the consumption of biosurfactants as household/laundry detergents accounts for 54% of the total output with only 32% destined for industrial use.

There is a considerable interest in the development of novel methods for producing surfactants, specially those using two renewable, inexpensive and easily accessible raw materials: sugar and fat/oil [3]. These products would qualify for the generally accepted 'natural' label, assuming that methods used for their production are natural, i.e. enzymatic [4].

The enzymatic synthesis of SFAEs is achieved by coupling a sugar and a fatty acid (FA) using an enzyme (lipase) as biocatalyst. The reaction system usually consists of insoluble substrates mixed together with the lipase in presence of a solvent. The substrates remain mainly in the solid phase as substrate reservoir. As the reaction proceeds, the substrates dissolved in the catalytic phase are transformed into products that can sometimes crystallise/precipitate [1]. The esterification involves the formation of water as a by-product that must be continuously removed from the solution in order to avoid the reverse reaction and to shift the reaction to the products.

Main goal of this work was the development of an integrated process establishing the application of membranes in the enzymatic synthesis of sugar fatty acid esters from renewable sources. Aspects derived from the use of organic solvents, the proper mixing of the educts and the separation and isolation of the products were taken into account [5].

2. Experimental methods and materials

Free and immobilised commercial lipases from *Candida antarctica* (CAL-B), *Rhizomucor miehei* (both Novo Nordisk, Denmark) and also new screened ones (from *Trichosporon beigilie*) were applied in the reaction between different sugars (glucose, α-methylglucoside) and fatty acids (oleic and stearic acid) using *n*-hexane, acetone or *tert*-

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butanol as solvent. The mixing grade of the initial reaction system was increased by coupling stirring and ultrasounds.

The synthesis of SFAEs took place in a enzymatic membrane reactor (Bioengineering AG, Switzerland) where the catalyst remained retained by means of ultrafiltration (membranes from Nadir Filtration GmbH, Germany).

The separation of water from the rest of the components was achieved by using alternatively a molecular sieve fixed bed (Riedel-de Haen, Germany) or a pervaporation unit (membranes from SULZER CHEMTECH GmbH, Germany and CM-CELFA Membrantrenntechnik AG, Switzerland) coupled to the reactor. A Karl Fischer titration method (Mettler Toledo, Germany) was applied in both cases in order to determine the water content.

In order to separate the esters (monomer and dimer) from the non-reacted educts and the organic solvent, an entire downstream process was developed, including both mechanical and thermal techniques. The final step involves alternatively a dialysis or a stepwise elution chromatography method for separating the fatty acid from the product. Fig. 4 shows a scheme of the entire production process.

The progress of each step was qualitatively followed by Thin Layer Chromatography on silica plates (MERCK, Germany) with a mobile phase consisting of chloroform/methanol/acetic acid/water (70:20:8:2, v/v). The quantitative analysis was carried out by High Pressure Liquid Chromatography (HPLC) using a C18-column, LiChrosphere® (MERCK, Germany) and operating under isocratic conditions with a mobile phase consisting of acetonitrile/*tert*-butanol/water (90:5:5, v/v) at 1.6 mL/min and 40 °C.

3. Results and discussion

3.1. Reaction parameters

The desired product of the enzymatic reaction between oleic acid and α-methylglucoside was the dimer (α-methylglucosidedioleate), which can be found in a number of cosmetic formulations. In order to get this compound as main product (85%), the initial molar substrate ratio (fatty acid:sugar) was set to be 2:1. Although the initial idea was to develop the reaction in a solvent-free system, the poor mixing grade of the educts made necessary the use of an organic solvent. The selection of this substance must consider not only the solubility but also the ecological and toxicological aspects regarding the later product application in the personal-care. These requirements were fulfilled best by using *tert*-butanol in comparison to *n*-hexane and acetone. This solvent did not affect negatively the water activity level (around 2%) required for the lipase to be functional in the reaction system considered. The optimal esterification reaction conditions were found at 55 °C and pH 6.5 with a solvent proportion of 125% (w/w) substrates [6].

The experimental data were confirmed by the simulation (MATLAB/SIMULINK) of the reaction progress during 40 h considering the kinetic to be described by the Ping-Pong-Model [7].

Table 1
Catalyst selection for the synthesis of α-methylglucosidedioleate in *tert*-butanol media

Catalyst	Diester yield [%]
Lipase from *Trichosporon beigilie*	12
Lipase from *Rhizomucor miehei* (Lypozyme RM IM)	27
Lipase B from *Candida antarctica* (Novozym 435)	54
Lipase B from *Candida antarctica* (NS-40013)[a]	72

[a] Commercial free lipase aqueous solution, 110 U/mg.

Table 1 shows the α-methylglucosidedioleate yields achieved for the reaction in *tert*-butanol with different catalysts. According to the results, the free CAL-B was considered to be the proper lipase for posterior studies.

3.2. Dispersion of the initial reaction system

In order to increase the initial mixing grade of the educts the coupling of different mixing techniques such as stirring and ultrasounds was studied. Different sedimentation experiments were developed for three sugar fraction sizes, regarding the progression of the boundary layer as key parameter in order to describe the stability of the mixture. The highest mixture stability was achieved for sugar particles below 63 μm at 55 °C, after 10 h mixing at 1000 rpm stirring speed and ultrasound frequency of 50 kHz [8].

With respect to the lipase stability the experiments showed a slight increase (8%) on the enzyme activity when adding the catalyst to the educts/solvent mixture 5–10 min before feeding to the membrane reactor.

3.3. Enzymatic membrane reactor

Fig. 1 shows a scheme of the enzymatic membrane reactor used. The reaction volume including magnetic stirrer

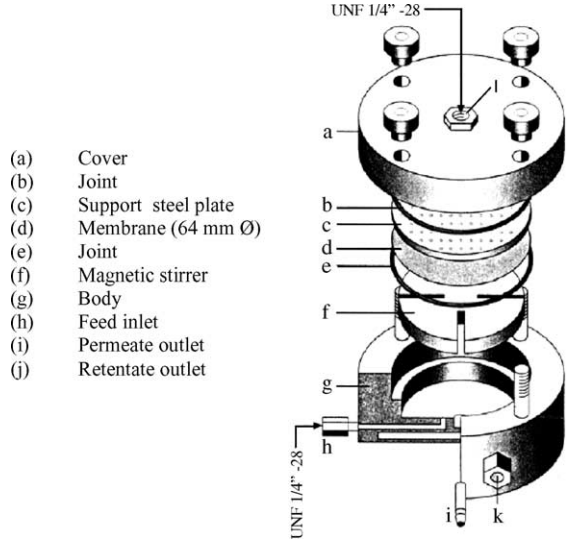

Fig. 1. Enzymatic membrane reactor.

Table 2
CAL-B average retention for different UF-membrane materials

Organic solvent	Cellulose 5 kDa-MWCO [%]	Polypropylene 5 kDa-MWCO [%]	Polyethersulfone 20 kDa-MWCO [%]
tert-Butanol	98	96.9	11.8
Acetone	98.4	97	12.6
n-Hexane	97.8	97	12

and membrane was 10 mL and the total volume from the substrate inlet to the products outlet amounted to 12 mL. The reactor behaviour was studied by conductivity measurements, providing hydrodynamic characteristics corresponding to those of a stirred tank. In order to maintain the catalyst retained the application of different ultrafiltration membranes was studied (Table 2). The phenomenon of concentration polarisation was minimised by feeding the reactor tangentially to the membrane. The experimentation with *tert*-butanol showed for the 5 kDa-molecular weight cut off (MWCO) cellulose membrane a permeability of 2.14×10^{-11} m/s Pa and an average lifetime over 300 h (1.75×10^{-11} m/s Pa and 230 h, respectively with the 5 kDa-MWCO polyethersulfone membrane) [6].

3.4. Water removal: pervaporation

A zeolite (2 mm diameter and 0.4 nm pore diameter) fixed bed (0.9 mm inner diameter and 19 cm height) allowed the batch by-product removal of almost 99% of the water formed. In order to develop the semi-batch process, the coupling of the EMR to a pervaporation unit was considered. The equipment used was similarly designed to the EMR (Fig. 1). Experimentation with different polyvinyl alcohol (PVA) and polysulfone (PS) membranes supplied (PERVAP® 2510 and PERVAP® 2216, both from SULZER CHEMTECH GmbH) provided non proper results when working with the entire reaction system including educts, catalyst, solvent and products due to irreversible damage of the membrane surface after few hours of operation (due to the fatty acid and organic solvent concentration applied, higher than recommended for those membranes).

Better results were achieved with a CMC-CF-02 membrane (CM-CELFA Membrantrenntechnik AG). The application of a sugar fraction size below 63 μm minimized the accumulation of this compound on the membrane surface, a phenomenon that negatively affects the pervaporation process. The average operating time of the membrane was determined to be 350 working hours considering the experimentation time (esterification reaction) and the membrane regeneration time.

The effect of the downstream pressure in the pervaporation process was studied developing different assays in the range of 13–20 mbar (Fig. 2). Within the applied range of pressure this had apparently no effect on the water removal process. The permeation flux varied between 0.12 and 0.15 kg/m^2 h. Notice that the difference between both

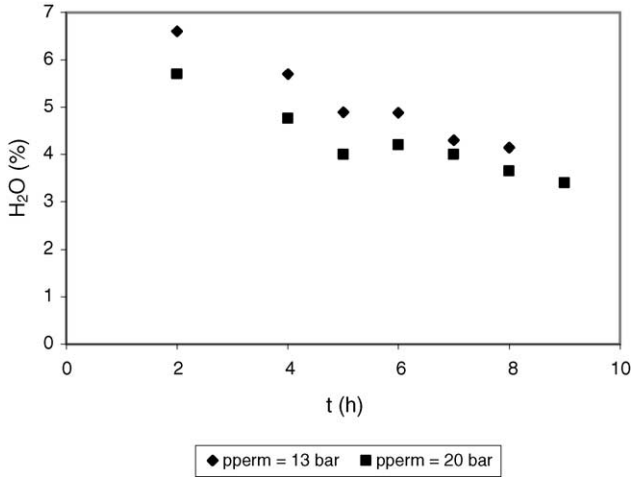

Fig. 2. Pervaporation: effect of downstream pressure on water contain at 60 °C.

experiments can be explained by the lower initial water concentration after 2 h (conditioning time) but the tendency is the same.

During the development of the assays with esterification coupled to pervaporation carried out for 20 h, the plot of the experimental data was similar to the one shown in Fig. 3 (experiment at 13 mbar and 3 mL/min flow rate). There is an initial period, extending from A to B, when the esterification has not taken place yet and only water remaining from previous washing is removed (the application of the organic solvent itself as cleaning agent provided dissatisfactory results). Only pervaporation occurs during this stage. Then, the water concentration reaches a maximum (C) that can be explained by two competing effects: the water formation in the membrane reactor due to esterification that tends to increase the overall water content and the water removal by means of pervaporation that tends to lower the water content in the reaction mixture. During the early period of this stage, the esterification yield is relatively low and so is the water concentration and the amount of water removed from the

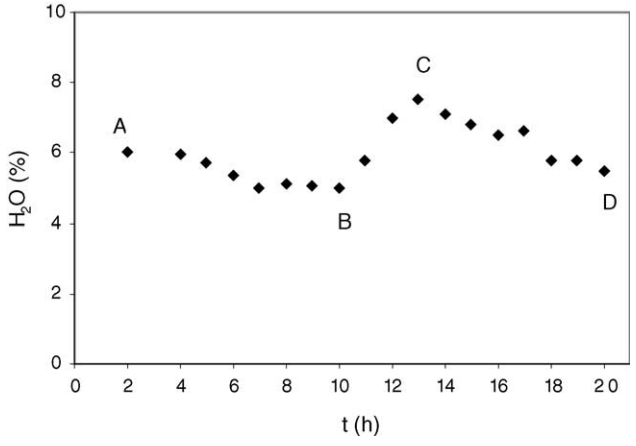

Fig. 3. Coupling EMR-pervaporation: water removal at 13 mbar and 55 °C.

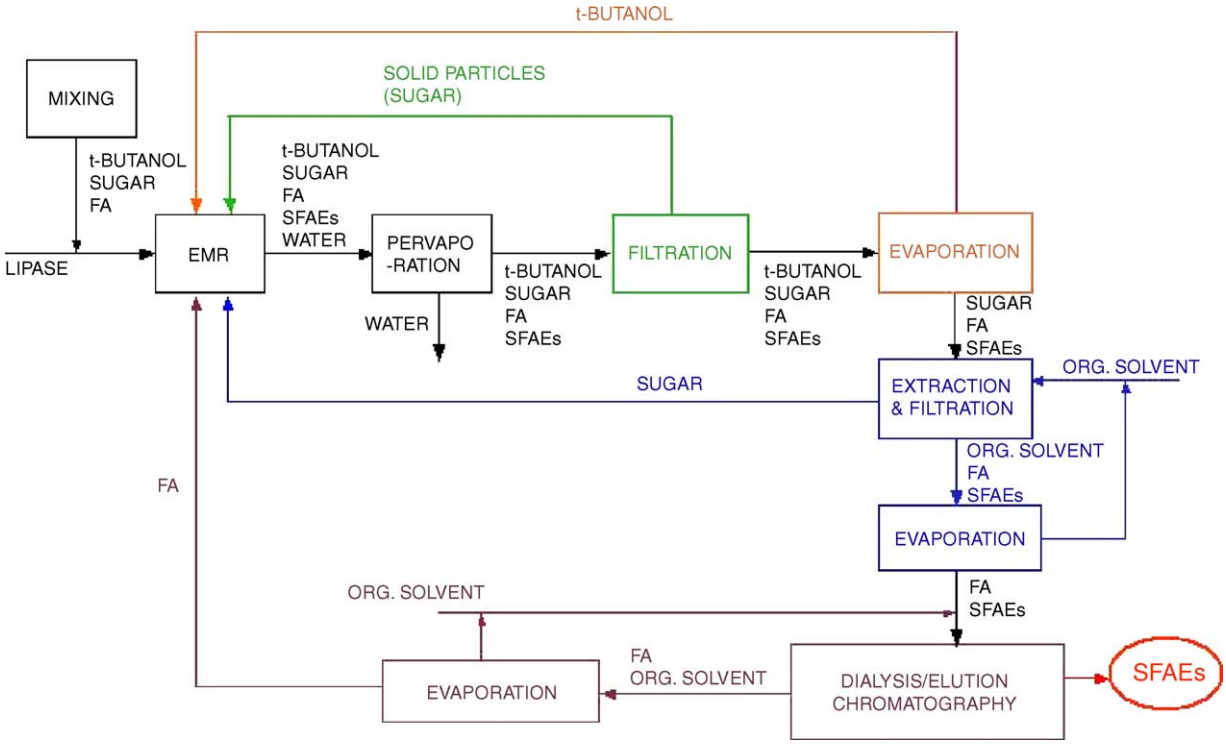

Fig. 4. Integrated process for the enzymatic synthesis of sugar fatty acid esters.

reactor. The by-product content gradually increases until it reaches a maximum when the formation and removal rates become equal (97% water removal). Thereafter the water removal is faster than its formation, reducing the presence of water in the reactor (C–D) due to the fact that no fresh substrates are added.

3.5. Global separation and purification process

Fig. 4 shows the integrated process for the enzymatic synthesis of biosurfactants including the main stages of the separation and purification procedure after reaction and by-product removal. Standard separation techniques such as filtration, evaporation and extraction were applied in order to recover the organic solvent and the non-reacted sugar. The results obtained showed a 97% of *tert*-butanol recovery by means of evaporation under vacuum (0.1 bar, 70 °C) and 96% of α-methylglucoside recovery by extraction with warm *n*-hexane (55 °C) followed by filtration. The experimental data validated the simulation results (ASPEN PLUS 11.1) for the considered steps. Focus was on the purification of the SFAEs and on the recovery of the non-reacted fatty acid by means of stepwise elution chromatography or, alternatively, by dialysis [8].

3.5.1. Purification of the SFAEs and recovery of the non-reacted fatty acid by elution chromatography

The chromatography column height was 35 cm with an internal diameter of 2 cm using different silica particles as adsorbent (packing of 24–25 cm by means of nitrogen). Best results were achieved with regular silica 60 (0.015–0.04 mm, MERCK, Germany).

Thin layer chromatography was used to determine the most appropriate solvent system. Ethyl acetate was chosen

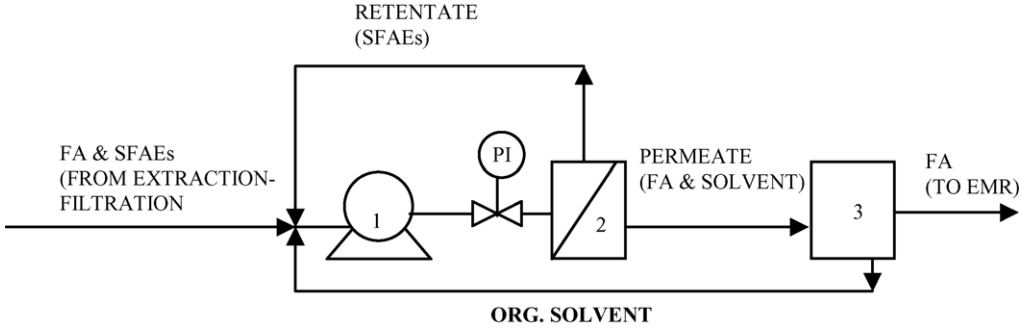

Fig. 5. Schema of the dialysis process for the recovery of non-reacted fatty acid: (1) feed pump; (2) dialysis unit and (3) evaporation unit.

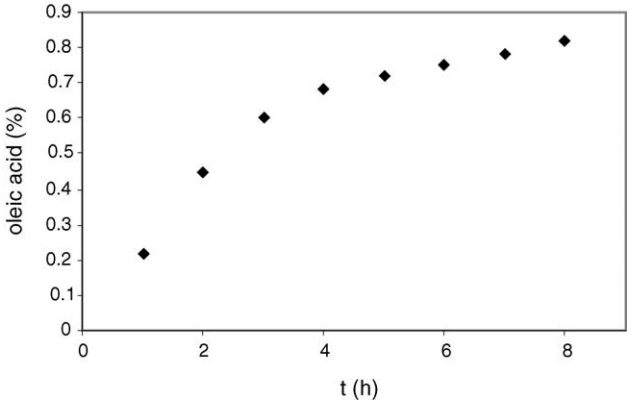

Fig. 6. Non-reacted oleic acid recovery by dialysis with *n*-hexane at 25 °C.

as elution system to separate SFAEs from fatty acids because of its low boiling point and consequently its easy removal after product caption, result that coincides with former studies [1].

The ester and fatty acid separation consisted of two isocratic elution steps (2.2 mL/min, 1.2 bar and 45 °C). In the first one, ethyl acetate was used as mobile phase to elute oleic acid, while the α-methylglucosiddioleate – that moves slower – remained in the column. Once the oleic acid had been eluted, the eluent strength was increased stepwise using a mixture of ethyl acetate/methanol (90:10, v/v) to facilitate the elution of the α-methylglucosiddioleate. 91% of the ester was recovered with a purity of 93% quantified by HPLC.

3.5.2. Purification of the SFAEs and recovery of the non-reacted fatty acid by means of dialysis

The dialysis step was performed at 25 °C in a discontinuous mode (Fig. 5) using a latex membrane with an effective area of 35 cm^2, previously conditioned with ethyl ether. Fundamental of this separation technique was the formation of micelles of the ester molecules due to the change in solvent polarity. Tetrahydrofuran, *n*-hexane and 2-methy-2-butanol were applied, whereas best results were obtained using *n*-hexane with 80% of the non-reacted oleic acid recovered (Fig. 6). The ester (90% isolated) presented a purity of 89% determined by HPLC.

4. Conclusions

A small-scale process for the enzymatic production of sugar fatty acid esters from renewable sources was developed and optimised. The problem of poor mixing grade of the initial reaction system was overcome by coupling stirring and ultrasound techniques. An enzymatic membrane reactor where the lipase remained retained by means of ultrafiltration allowed a semi-batch process coupled to a pervaporation unit for by-product removal. For downstream processing combined steps including filtration, evaporation, extraction and alternatively a dialysis or an elution chromatography method showed good results in order to isolate and purify the product.

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