APPLICATION OF STABLE EMULSIONS TO LIPASE IMMOBILISED MEMBRANE REACTORS FOR KINETIC RESOLUTION OF RACEMIC ESTERS

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ABSTRACT

The paper discusses the use of stable emulsion, prepared by membrane emulsification technology, to improve the enantiocatalytic performance of immobilised lipase in multiphasic membrane reactors. The production of optical pure (S)-naproxen from racemic naproxen methyl ester has been used as model reaction system.
The enzyme was immobilised in the sponge layer (shell side) of capillary polyamide membrane with 50 kDa cut-off. The O/W emulsion, containing the substrate in the organic dispersed phase, was fed to the enzyme membrane reactor from shell-to-lumen.
The results evidenced that lipase maintained stable activity during all the operation time (more than 250 hours), showing an enantiomeric excess (96 ±2%) comparable to the free enzyme (98 ± 1%) and much higher compared to similar lipase-loaded membrane reactors used in two-separate phase systems (90%).
The study showed that immobilised enzymes can achieve high stability as well as high catalytic activity and enantioselectivity.

INTRODUCTION

Lipases (triacylglycerol acyl hydrolases, EC 3.1.1.3) are frequently utilised in kinetic resolutions of racemic mixtures because of their capability to discriminate between enantiomers.[1-2] One of the most important characteristics of lipases is activation by organic/aqueous biphasic interface (interface activation).[3-5]
Due to these peculiar characteristics, the lipases are feasible to be used as immobilised in multiphase reactors, such as biphasic organic/aqueous membrane reactors.
A key parameter of these type of systems is the realisation of the water/organic interface at the same level where the lipase is immobilised. In fact, this condition regulates the interfacial activation of the enzyme, promotes the contact between the enzyme and the substrate, and favours the extraction of the product into the aqueous phase as it is formed.
Furthermore, it is generally observed that immobilised enzymes increase the stability while decrease the catalytic activity and selectivity. This might be not considered as a general rule, but as the observed result of conditions that favour the increase of molecular rigidity (which favours the increase of stability) at the expenses of molecular flexibility (which guarantees a high selectivity). When the proper compromise between rigidity and flexibility is achieved, immobilised enzyme can exhibit high stability as well as high activity and selectivity.
We have tried to demonstrate this challenge by combining enzyme membrane reactor with the characteristics of large interfacial area of emulsion within the porous structure of the membrane where the enzyme is immobilised. In other words, by using a lipase immobilised in the sponge layer of an asymmetric hydrophilic membrane and feeding it with an oil-in-water emulsion as reaction mixture.[6]

RESULTS AND DISCUSSION

Membrane emulsification: Pa 10 kDa membrane from Berghof were used. They were first pretreated with water/isopropanol, ipopropanol, isopropanol/isooctane and isooctane to remove water from the pores and substitute it with isoctane. This procedure allows the non-polar solvent to permeate through the hydrophilic ultrafiltration polymeric membrane.[7]

Oil-in-water (O/W) emulsion was prepared using isoctane containing naproxen methyl ester (as oil dispersed phase), 50 mM phosphate buffer pH 7.00 (as continuous phase), sodium dodecil sulphate (SDS) and polyvinyl alcohol (PVA) as emulsifier and stabiliser, respectively.

In membrane emulsification, droplets are formed at the pore mouth of a membrane by forcing the dispersed phase to permeate through the membrane and stripping the droplets from the pore into the continuous phase by action of the axial velocity. The emulsions were prepared by applying a transmembrane pressure from the organic to the aqueous phase of about 80 kPa and an axial velocity of the aqueous phase along the lumen side of about 1.5 m/s.

The droplets size were measured by optical microscopy (furthermore, random control was also made by light-scattering measurements). Emulsion with narrow droplet size (about 1.5 μm) could be prepared. After preparation, the emulsion was kept at 20 (±2) °C, and the droplet's size and density was measured as a function of time. The emulsion stability did not show any decrease for at least three months.

Emulsion enzyme membrane reactor: the membrane used to immobilise lipase were made of polyamide with 50 kDa NMWCO; 1.2/2.4mm inner/outer diameter. The lab-made membrane modules were prepared by assembling 4 capillary membranes inside a Pyrex glass cylinder of 1.2 cm I.D., 20 cm long. The external membrane area was of 57.38 cm^2.

The enzyme was immobilised by cross-flow ultrafiltration from shell to lumen; therefore, the oil-in-water emulsion was also fed from shell to lumen. The emulsion enzyme membrane reactor was operated in concentration mode (no aqueous phase was added as it permeated) or in semi-continuous mode (the aqueous phase volume was maintained constant by adding new phase as it permeated through the membrane).

The experiments were carried out maintaining constant permeate flux as a function of time, so that the residence time of flowing solution was constant and results of different experiments were not affected by product extraction rate.

Samples were collected from permeate side continuously and were also taken from the emulsion side at intervals of time for the analysis of the concentration of (S)- and (R)-naproxen by HPLC.

The performance of free lipase, with and without emulsifier, was investigated in stirred tank reactors to compare it with the immobilised lipase in membrane reactor. In the stirred tank reactor with the presence of PVA as emulsifier, the production, was one time higher compared to that without PVA. The results are related to the function of PVA as emulsifier of o/w mixture solution. The results indicate that PVA is a good surfactant for the lipase-catalyzed resolution reaction. For both reactors, after a few hours of reaction, the production did not increase significantly any more. This is basically due to the deactivation of lipase by shear stress of stirring process and product inhibition.

The organic/aqueous interface played an important role in lipase enantiocatalytic hydrolysis of racemic naproxen ester both in stirred tank reactor and in lipase-immobilised membrane reactor. In stirred tank reactor, enzyme selectivity was very good (about 98%) but stability was low (about 15 hours of half-life time). As a comparison, lipase immobilised in membrane reactor showed stable activity and selectivity during 250 hours of reaction process. By combining the advantage of emulsion in its large interfacial area and the advantage of membrane reactor for stable enzyme activity and simultaneous separation of product from reaction miroenvironment, the emulsion enzyme membrane reactor in the present study showed good performance in lipase enantiocatalytic reaction measured by production, enantiomeric excess and enantioselectivity. Higher production, enantiomeric excess [96 %] and enantioselectivity (50) were obtained compared to two-separate phase enzyme membrane reactor (90% and 14, respectively). Table 1 summarises the results for different multiphasic membrane reactors.

Table 1. Comparison between various types of multiphasic enzyme membrane reactors.[6, 8-9]

Lipase	Membrane	Reactor	Immob. Site	Lipase amount (mg)	Ee_p (%)	E (-)	Activity (μmol/h g)
Crude	PA 10 kDa	B-EMR	Thin	2.3	77	7.8	1.9
Crude	PA 50 kDa	B-EMR	Thin	5.5	90	20	6.36
Crude	PA 50 kDa	B-EMR	Sponge	8.2	76	6.2	_
Crude	PA 50 kDa	E-EMR (emulsion by stirred method)	Sponge	2.5	82	10	_
Pure	PA 50 kDa	B-EMR	Sponge	2.4	89	17	_
Crude	PA 50 kDa	E-EMR (emulsion by membrane)	Sponge	2.6 <1	94 96	30 50	14.5

REFERENCES

1. Faber K, Riva S. 1992. Synthesis. 895-910.
2. Bornscheuer UT, Kazlauskas RJ. 1999. Hydrolases in Organic Synthesis. Weinheim Wiley-VCH.
3. Verger R. 1980. Enzyme kinetics of lipolysis. In: Colowick SP, Kaplan NO, editors. Methods in Enzymology, Vol. 64B. New York: Academic Press, pp 340-92.
4. Derewenda U, Brzozowski AM, Lawson DM, Derewenda ZS. 1992. Catalysis at the interface: the anatomy of a conformational change in a triglyceride lipase. Biochemistry 31:1532-1541.
5. Theil F. 2000. Lipases as a tool for the synthesis of chiral intermediates. Chemistry Today, 61-64.

6. Giorno L, Li N, Drioli E. 2003, Use of stable emulsion to improve stability, activity and enantioselectivity of lipase immobilised in a membrane reactor, Biotech & Bioeng, submitted.
7. Giorno L, Li N, Drioli E. 2003. Preparation of oil-in-water emulsion using polyamide 10 kDa hollow fibre membrane. J Membr Sci. 217:173-180.
8. Sakaki K., Giorno L., Drioli E. 2001. Lipase-catalyzed optical resolution of racemic naproxen in biphasic enzyme membrane reactors, J. Membrane Sci. 184:27-38.
9. Li N, Giorno L, Drioli E. 2003. Effect of Immobilization Site and Membrane Materials on Multiphasic Enantiocatalytic Enzyme Membrane Reactors. Annals of the New York Academy of Science.984:436-452.

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