# Enzymatic production of ceramide from sphingomyelin

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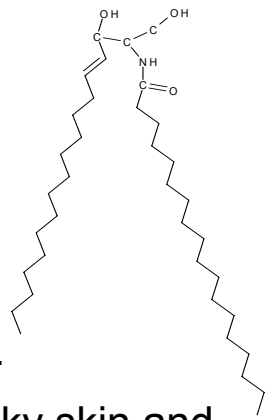
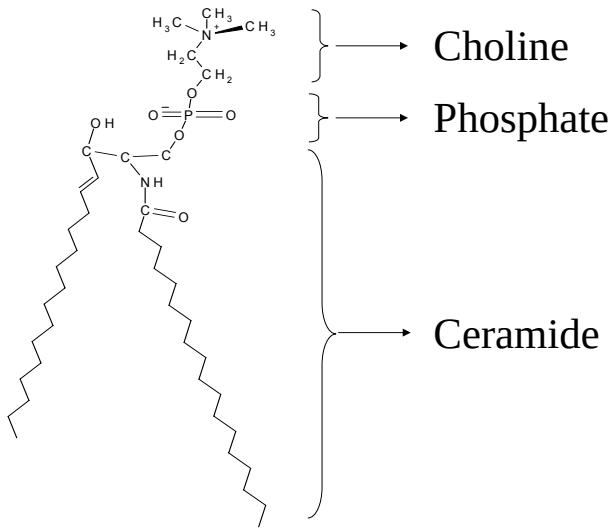
## Ceramide, an N-acyl sphingosine

Ceramide application can:



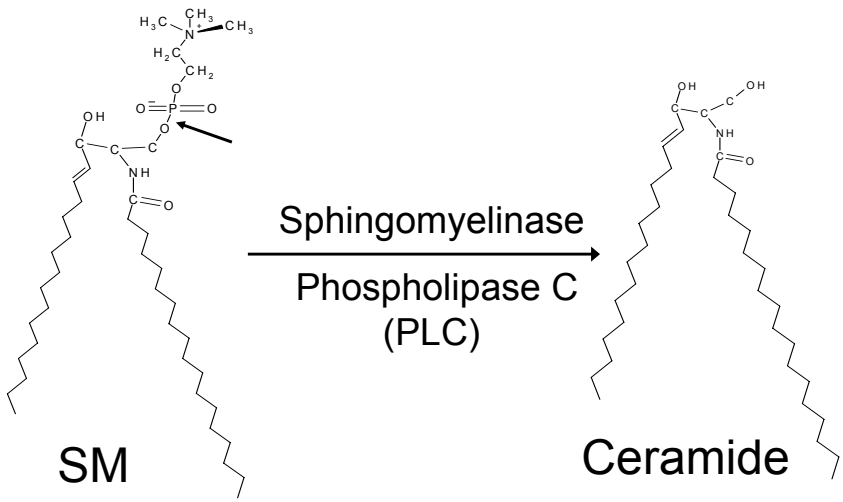
- Dramatic increase skin's hydration level.
- Noticeable reduction of the appearance of fine lines.
- Repair the cutaneous barrier.
- Contribute to reducing dry flaky skin and aged appearance

## Sphingomyelin (SM), a ubiquitous component of animal cell membranes.

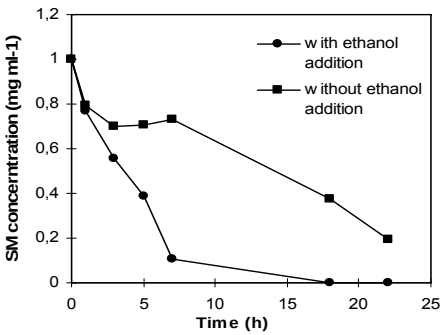


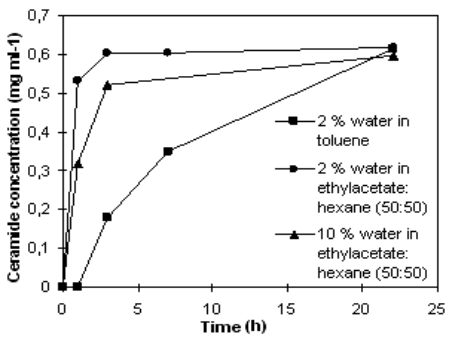
# Hydrolysis reaction:

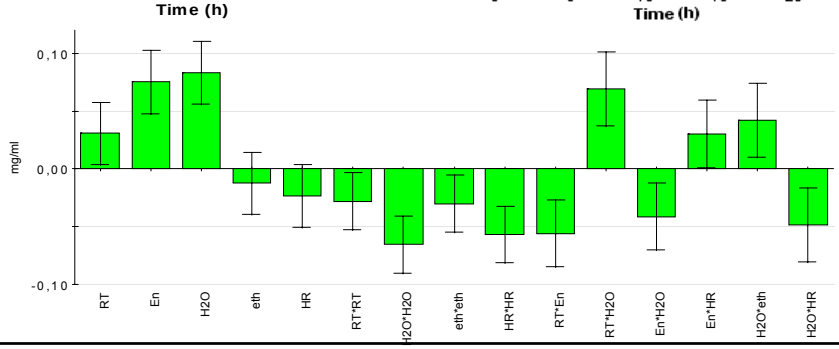
## SM + $H_2O \rightarrow$ ceramide + phosphorylcholine




# Basic reaction characteristics



## Optimal conditions with RSM for basic reaction system

General optimal conditions:

- 75 min reaction time
- 3 U $ml^{-1}$ enzyme amount
- 6 % water amount
- 1.8 % ethanol amount
- 46 % hexane in organic solvent.

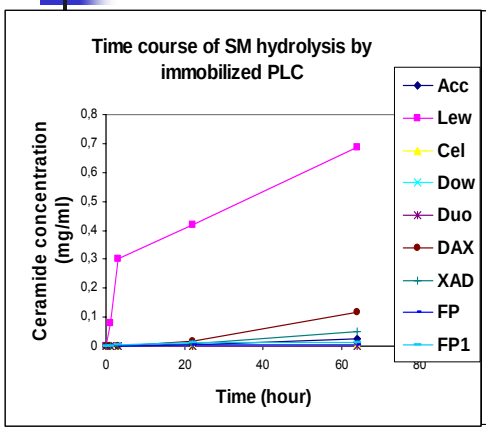
## Enzyme immobilization on carrier

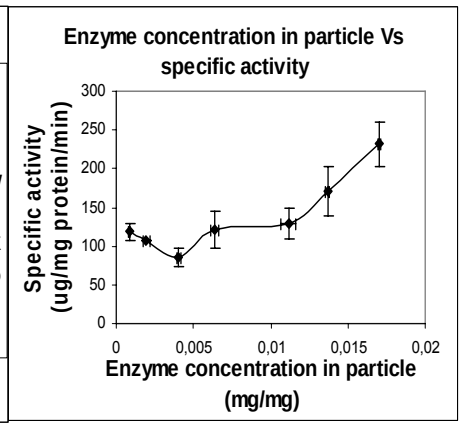
- Enzymes are expensive
- Reuse is difficult
- Reaction system is limited

On the other hand

- Little information available about immobilization

# Immobilization process

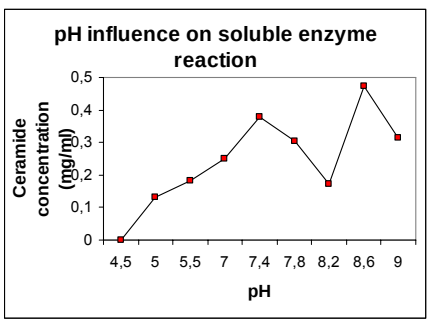


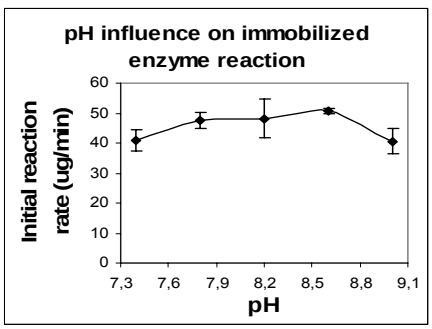




The enzyme immobilized on Lewatit VP OC 1600 (Bayer AG) (Divinylbenzene cross-linked polymer) retain the highest catalytic activity.

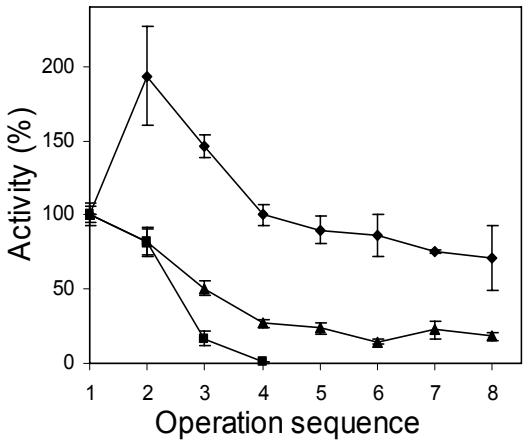
# Optimization of immobilized PLC

- Immobilization shift optimal temperature of the hydrolysis from 37°C to 46°C
- Optimal water amount is changed from 6 to 3.5%
- pH



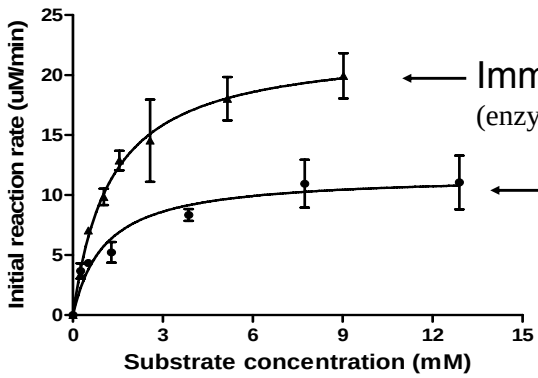



# Reuse of immobilized PLC




Legend:
■ soluble enzyme reaction
♦ IE reaction at 37 °C
▲ IE reaction at 46 °C

# Kinetic study

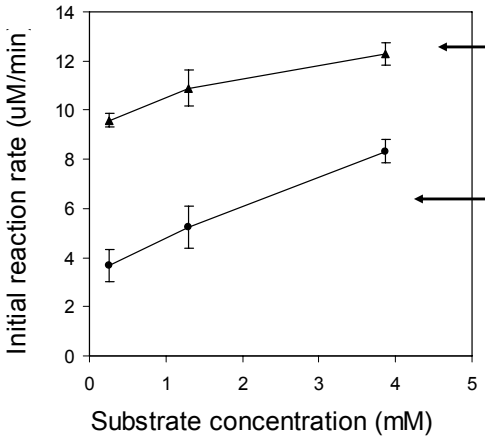



Immobilized enzyme
(enzyme amount 12.5 μg)

Soluble enzyme
(enzyme amount 0.31 μg)

| Enzyme type | $K_m$ (mM) | $V_{max}$ (μM/μg enzyme.min) |
|---|---|---|
| Soluble enzyme | 1.07±0.32 | 37.68±2.91 |
| Immobilized enzyme | 1.26±0.19 | 1.80±0.09 |

# Ceramide activation

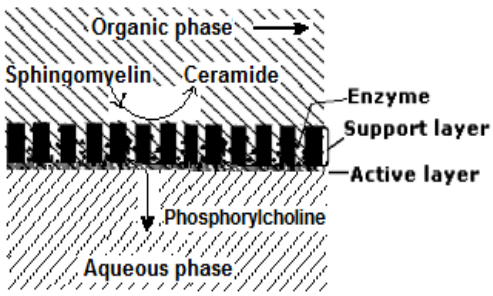


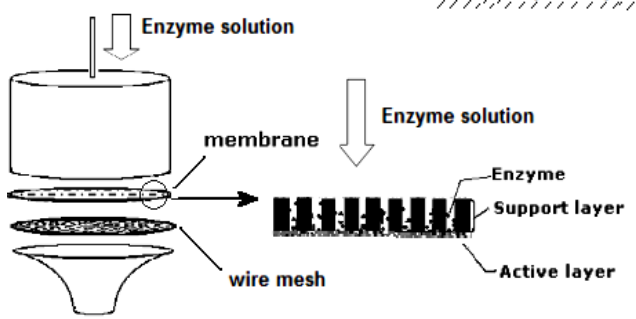

Ceramide was added (0.33 mM) before the reaction

Without addition of ceramide

# Membrane Reactor

Membrane reactor can combine bioconversion and product separation together




Immobilization of the enzyme by filtration

# Membrane Reactor

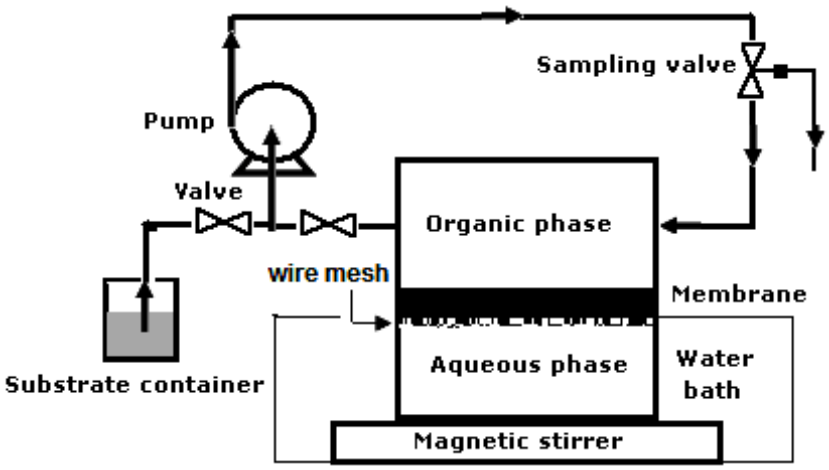



Table 2. Comparison of different membranes used in phospholipase C immobilization

| Membrane | Active layer | Support layer | Fixation level (%) | Specific activity (μg/(mg enzyme min)) | SM conversion (%)[a] | MWCO |
|---|---|---|---|---|---|---|
| ETNA 01PP | Hydrophilic coated PVDF | PP | 12.2 | 14.8 | 52.0 | 1.000 |
| ETNA 10PP | Hydrophilic coated PVDF | PP | 62.0 | 6.7 | 49.8 | 10.000 |
| ETNA 20A | Hydrophilic coated PVDF | PP | 24.6 | 20.0 | 33.4 | 20.000 |
| GR 90PP | PSf | PP | 20.3 | 25.3 | 80.8 | 2.000 |
| GR 81PP | PES | PP | 27.1 | 7.9 | 58.2 | 10.000 |
| E1005/Job Tex | N.A. | Woven PP | 61.7 | 6.1 | 65.2 | N.A. |
| E1004/TX | ePTFE by Pristync® | Woven PP | 4.6 | 93.1 | 75.5 | N.A. |
| Hekla 10A | Hydrophilic coated PES | PP | 14.7 | 51.1 | 81.1 | 10.000 |
| RC 70PP | Regenerated cellulose acetate | PP | 9.4 | 138.4 | 78.8 | 10.000 |
| FS 61PP | PVDF | PP | 22.9 | 22.9 | 58.9 | 20.000 |

[a] SM conversion was calculated at the reaction time 54 h.

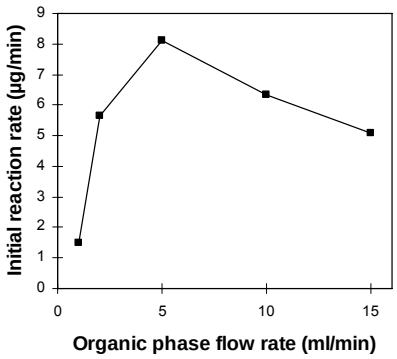
[b] MWCO: Molecular weight cut-off values reported by supplier.

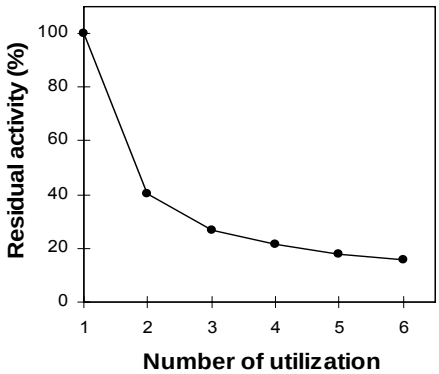
Abbreviations: PVDF, polyvinylidenefluoride; PP, polypropylene; PSf, polysulfone; PES, polyethersulphone; ePTFE, expanded polytetrafluoroethylene; N.A., not available.

Comparison of different methods for phospholipase C immobilization in the membrane

| Membrane | Immobilization method | Fixation level (%) | Specific activity (μg/(mg enzyme min)) | Sphingomyelin conversion (%)[a] |
|---|---|---|---|---|
| RC 70PP | Filtration | 9.4 | 138.4 | 78.8 |
| | Covalent binding | 71.7 | 28.3 | 9.6 |
| GR 90PP | Filtration | 20.3 | 25.3 | 80.8 |
| | Cross-linking | 100 | 10.2 | 13.7 |
| E1004/TX | Filtration | 4.6 | 93.1 | 75.5 |
| | Cross-linking | 95.5 | 14.1 | 12.9 |

[a] Sphingomyelin conversion was calculated at the reaction time 54 h.




# Summary

- SM hydrolysis reaction has been improved through system evaluation and the optimization of several important factors.
- *C. perfringens* PLC immobilized on Lewatit retain the highest activity. After seven cycles (20 min reaction time), immobilized enzyme retain around 70% of its initial activity.
- The hydrolysis reactions catalyzed by the soluble and immobilized enzyme follow Michaelis-Menten kinetics. Ceramide activates the hydrolysis.
- The enzyme immobilized in membrane RC 70PP had low immobilization efficiency, but retained the highest catalytic activity. The immobilized enzyme retained 16 % of the original activity after 5 cycles (24-h intervals).

## Acknowledgment

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