Enzyme Engineering

6. Enzyme Reactions in Non-Conventional Media

6.1 Enzyme Reactions in Organic Solvents

6.2 Enzyme Reactions in Non-Conventional Media

Enzyme Reactions in Non-Conventional Media

-In organic solvent

-In supercritical liquid

-In ionic liquid

-In high salt condition

Background

- Enzymes in vivo – not aqueous always
- Enzymes in vitro

- Steroid bioconversion

-1st stage – cell culture (intracellular enzyme)

-Permeabilization of cell wall

-2nd stage – 2-phase reaction

* Solubility of substrate, product in aqueous solution - low

Background

- Equilibrium Shift in organic solvent

* Why equilibrium shift?

- Lipase : polyester
- Laccase : urushiol polymer (Polycardinol / Polyphenol / Polyaniline)
- HRP(Horseredish peroxidase) : polymer formation
- Protease : peptide degradation

- Product separation in situ
  - Good for product inhibition kinetics
- Selectivity change
  - Chiral compounds

Prof. Martinek(Moscow State Univ.)

→ Prof. Klivanov(MIT)

→ Prof. Dordick(PRI)

6.1 Enzyme Reactions in Organic Solvents

Organic Solvent System

- 2-Phase reaction - Water-immiscible solvent

  e.g. steroid bioconversion

- Nearly anhydrous

  e.g. lipase

- Water – miscible solvent

- **Advantages :** new reaction, less contamination

- **Disadvantages :** solvent is toxic to human and enzyme, low stability of enzyme

Enzyme Activity in Solvent System

- **Activity vs. log P**

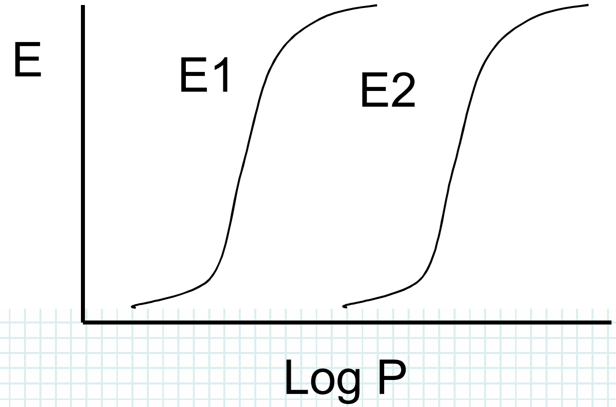
(log P : log of partition coefficient in (octanol / water)

Activity vs. solubility parameter

Activity vs. dielectric constant

Activity vs. dipole moment

→ **no correlation**



* High enzyme activity at hydrophobic condition

Enzyme Activity in Solvent System

- **NMR study :** changes of enzyme structure is negligible

  Solvent molecule in enzyme – affect flexibility

- **Activity vs. water activity**

- Nearly anhydrous organic solvent system

- Water activity → equilibrium constant

* Proposed by Prof. S.B.Lee (POSTECH)

- **pH memory**

pH of enzyme buffer solution(aqueous)

= pH characters of enzyme after resuspension in organic solvent

- **Essential water layer**

How to Increase the Efficiency of Enzyme Reaction in Organic Solvent?

1) Solvent engineering

- Selection of solvent(mixture), concentration
- Trial and error

2) Enzyme engineering

- More hydrophobic residues at the surface
- More hydrogen bonds at the surface

3) IME

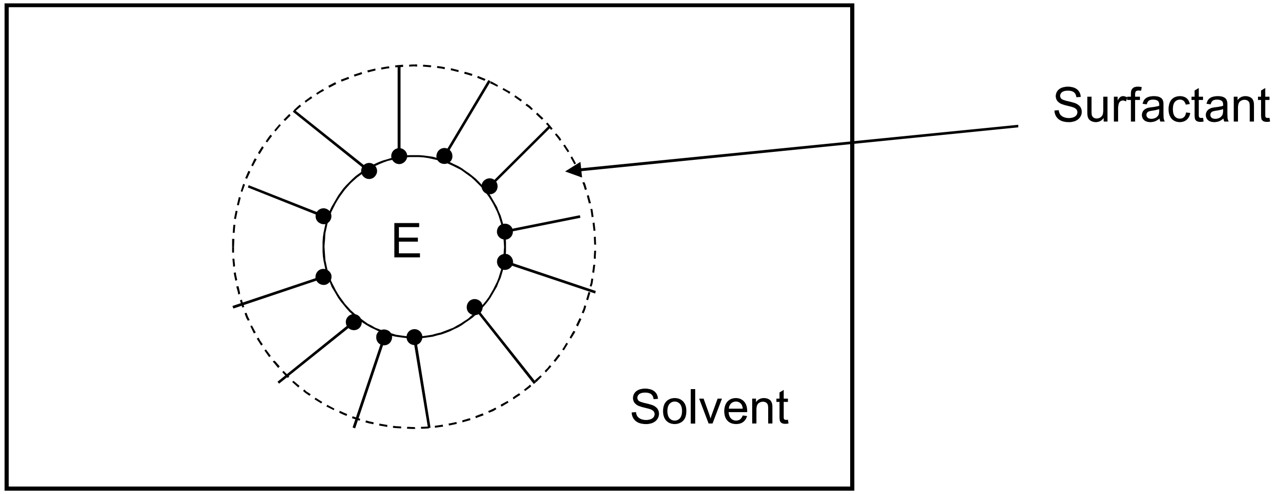
4) PEG modification

- Support bead → hydrophobicity / hydrophilicity

How to Increase the Efficiency of Enzyme Reaction in Organic Solvent?

5) Reversed micelle

- Separate the enzyme from the solvent



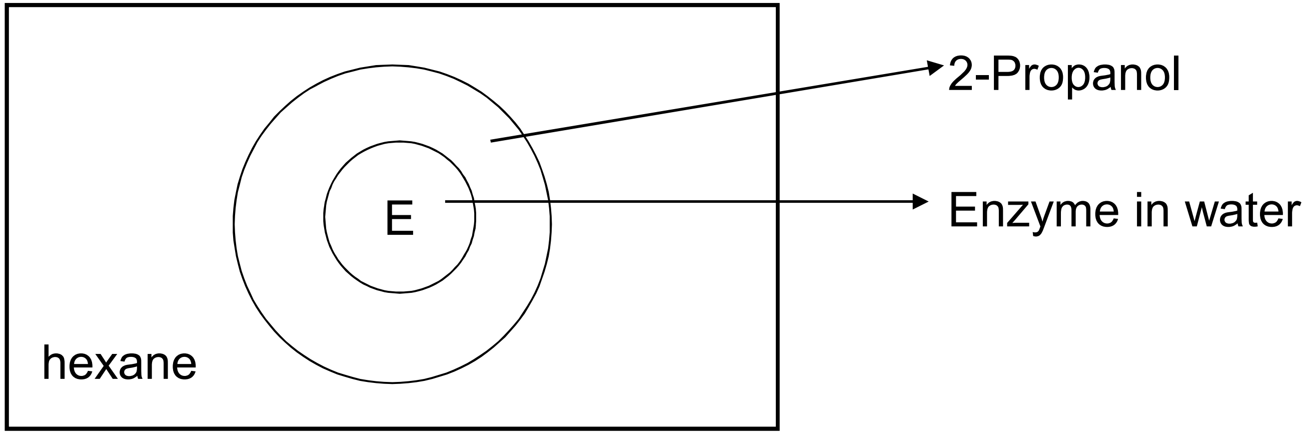
Advantages : activity, stability

Disadvantages : mass transfer, residual surfactant should be removed

How to Increase the Efficiency of Enzyme Reaction in Organic Solvent?

6) Microemulsion system

- Hexane / 2-propanol / water



No surfactant is used

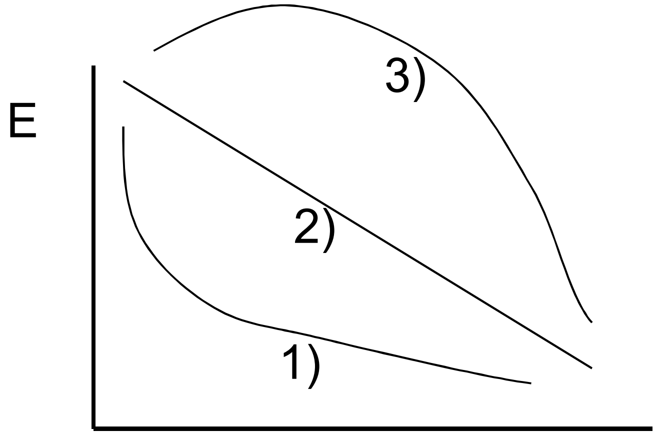
* High RPM → affect water layer

Solvent Selection

Not to destruct hydration shell of enzyme by solvent

(how to maintain hydration shell?)

1) Water, glycerol, EG, formamide
2) Methyl formamide, dimethyl formate
3) Methanol, ethanol, toluene



•**Polyol**

- High viscocity - not good for mixing

- Low polarity – low substrate solubility

→ **not much used**

Enzyme Engineering: Research Activity at SNU

- **Increase activity and stability in organic solvent**
- Examples from directed evolution; hydrogen bond become strengthened

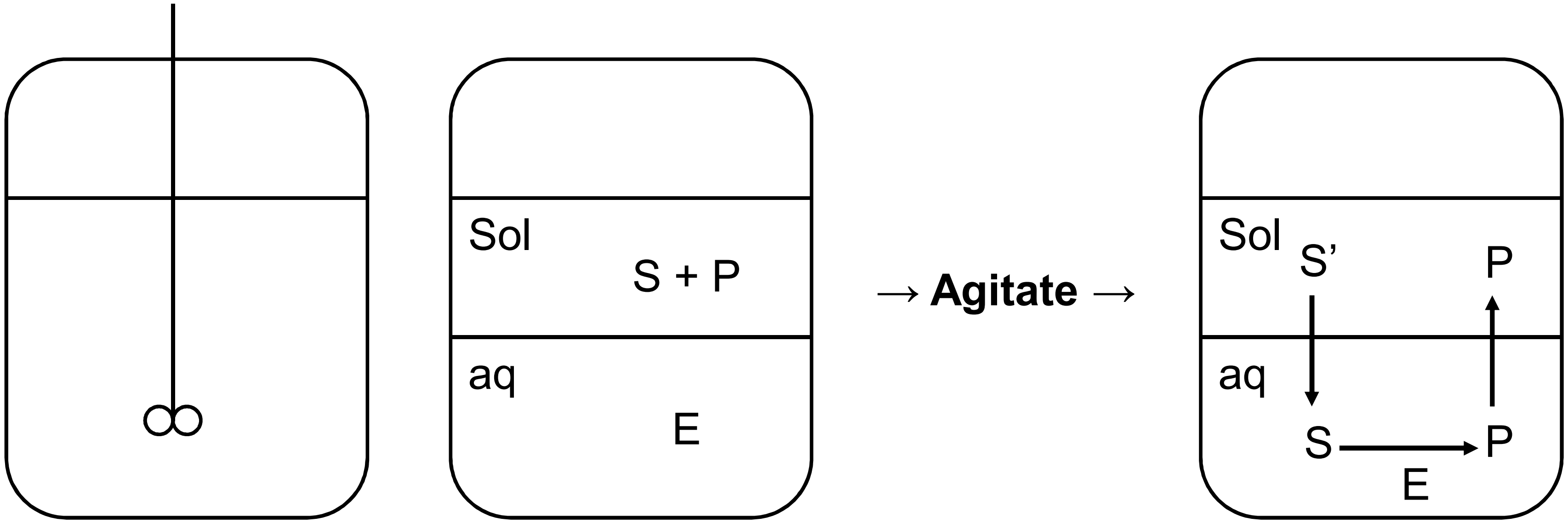
- Solvent molecule in enzyme; change residues to reduce the space for solvent

- Increase flexibility

*** Lipase as a model enzyme**

Reaction System

1) Biphase system



Sol

S + P

aq

E

→ **Agitate** →

Sol

S'

P

aq

S

P

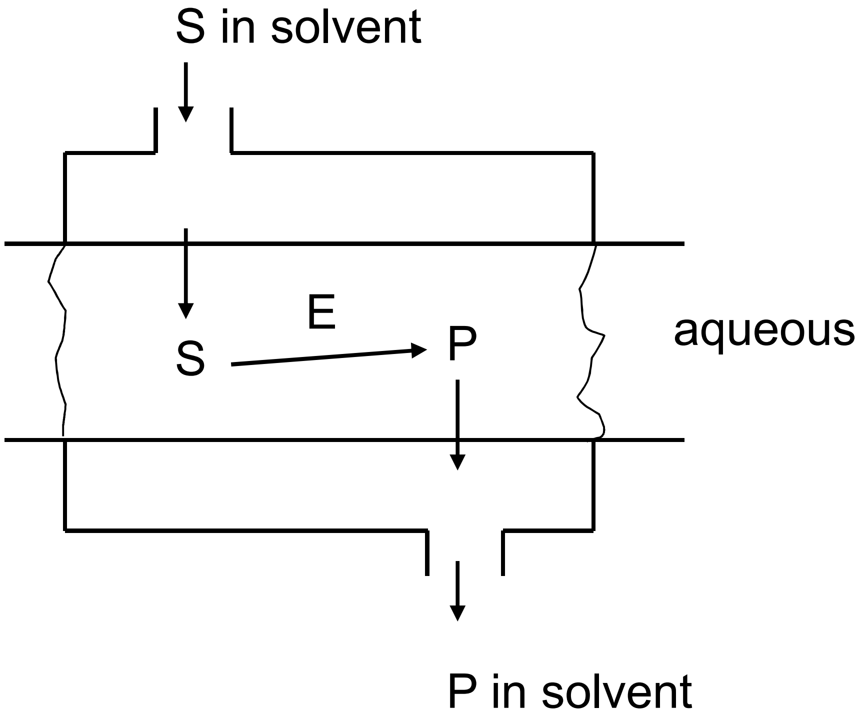
E

Reaction

Phase separation

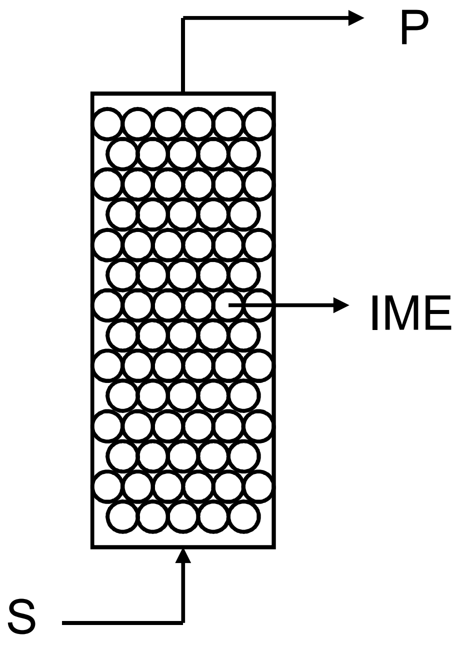
Reaction System

2) Membrane bioreactor



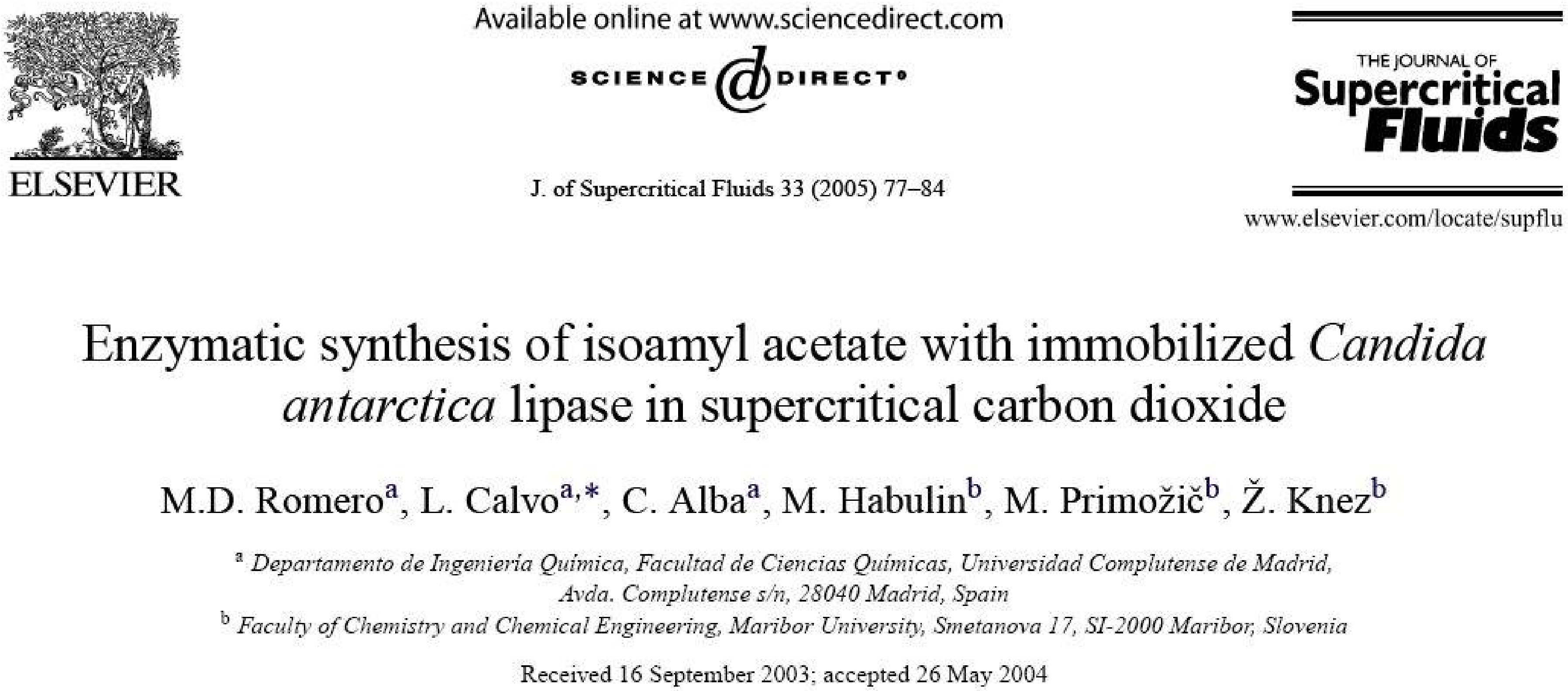
3) IME in solvent

- Enzyme is more stable



6.2 Enzyme Reactions in Non-Conventional Media

(1) Supercritical Fluid



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Enzymatic synthesis of isoamyl acetate with immobilized *Candida antarctica* lipase in supercritical carbon dioxide

M.D. Romero[a], L. Calvo[a,*], C. Alba[a], M. Habulin[b], M. Primožič[b], Ž. Knez[b]

[a] *Departamento de Ingeniería Química, Facultad de Ciencias Químicas, Universidad Complutense de Madrid,*
Avda. Complutense s/n, 28040 Madrid, Spain
[b] *Faculty of Chemistry and Chemical Engineering, Maribor University, Smetanova 17, SI-2000 Maribor, Slovenia*

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1. Reactants (acetic acid, acetic anhydride 등)
2. Two different immobilized lipases
3. Esterification extent of 100%
4. CO_2/substrates molar ratio
5. CO_2 vs. Conventional organic solvent(n-hexane)

Isoamyl alcohol → Isoamyl acetate

Impact of acyl donor

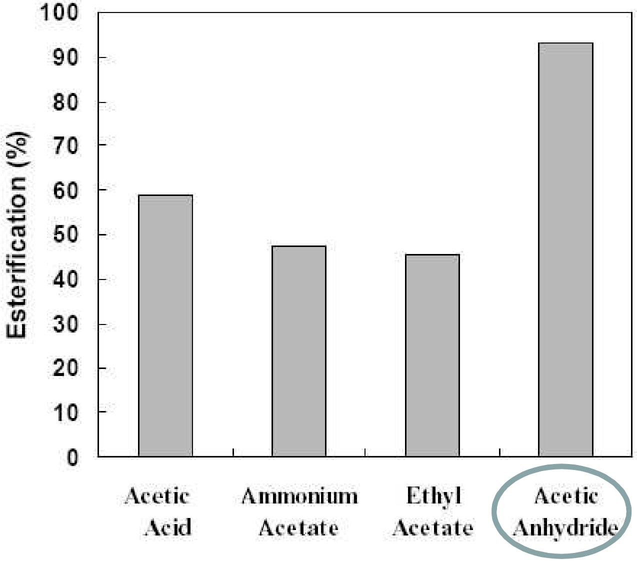


Fig. 4. Impact of the type of acyl donor on the production of isoamyl acetate from isoamyl alcohol using Novozym 435 as enzyme in supercritical CO_2 at 2 h reaction time. Operating conditions: 15 MPa and 313 K.

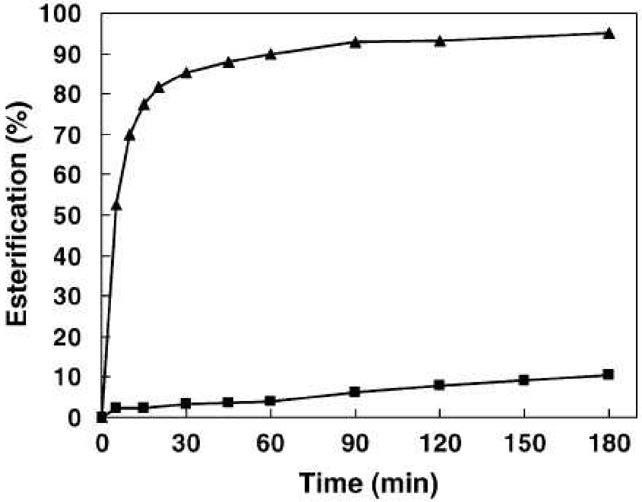


Fig. 5. Comparison of acetic acid (■) and acetic anhydride (▲) as acyl donors at a concentration of 0.8 M on the production of isoamyl acetate from isoamyl alcohol using Novozym 435 as enzyme in supercritical CO_2. Operating conditions: 15 MPa and 313 K.

Impact of the type of immobilized enzyme

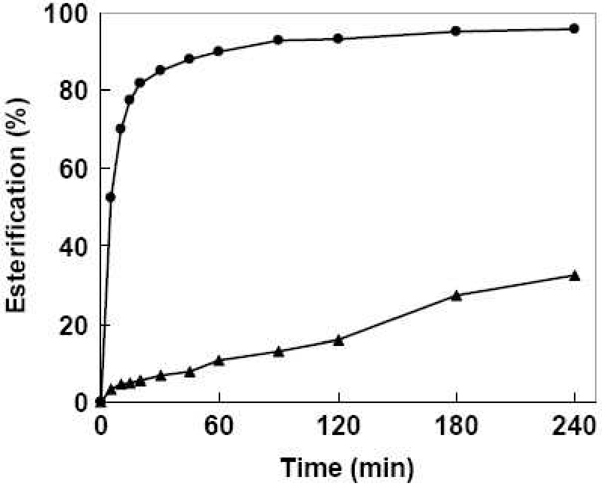


Fig. 6. Impact of the type of immobilized enzyme on the production of isoamyl acetate from isoamyl alcohol and acetic anhydride (0.8 M) in supercritical CO_2: (●) Novozym 435; (▲) Lipozyme RM-IM. Operating conditions: 15 MPa and 313 K.

Reactant, enzyme 결정 → economical point of view

g enzyme/mol alcohol

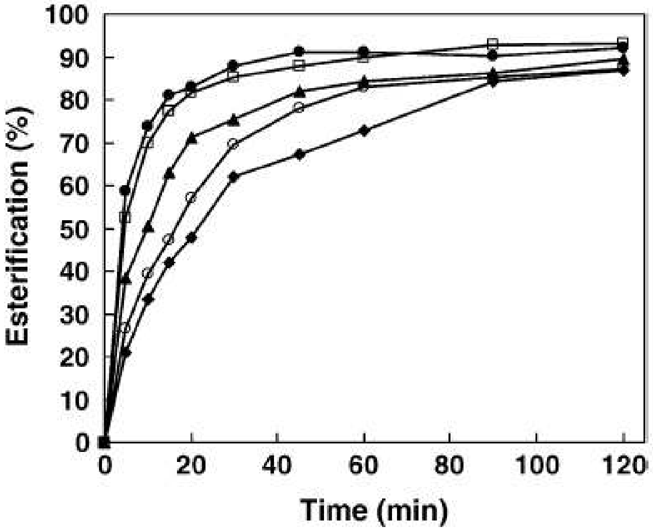


Fig. 7. Effect of added amount of Novozym 435 (g enzyme/mol alcohol) on the production of isoamyl acetate from isoamyl alcohol in supercritical CO_2: (●) 12.5 g/mol; (□) 6.25 g/mol; (▲) 5.0 g/mol; (○) 3.2 g/mol; (♦) 1.5 g/mol alcohol.

CO_2/substrates molar ratio

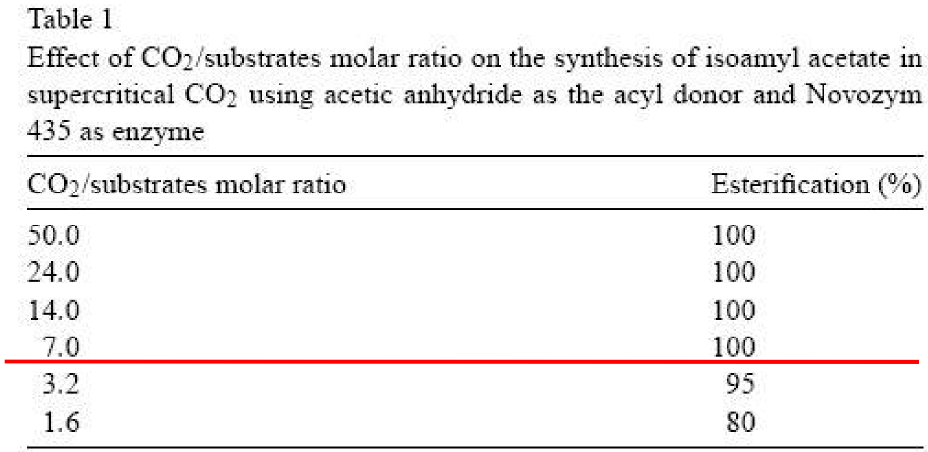


Table 1
Effect of CO_2/substrates molar ratio on the synthesis of isoamyl acetate in supercritical CO_2 using acetic anhydride as the acyl donor and Novozym 435 as enzyme

CO_2/substrates molar ratio	Esterification (%)
50.0	100
24.0	100
14.0	100
7.0	100
3.2	95
1.6	80

Reactant, enzyme 결정 → economical point of view → operating condition

Effect of temperature

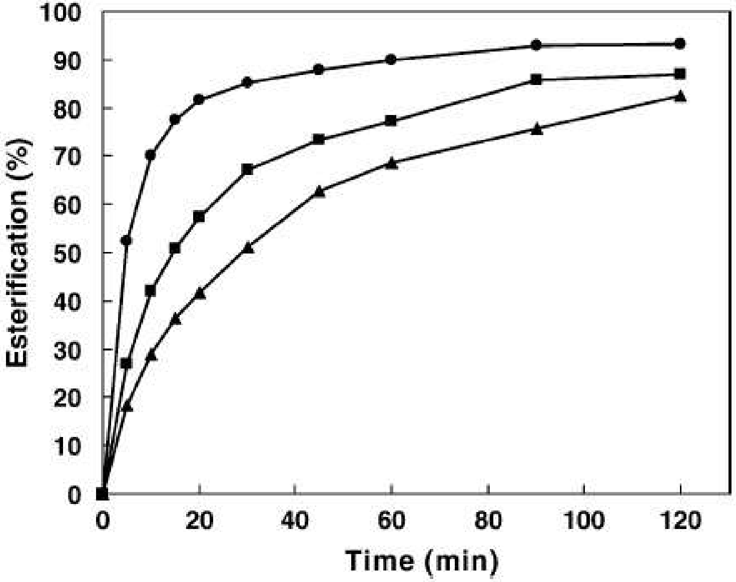


Fig. 9. Effect of temperature on isoamyl acetate synthesis using acetic anhydride as acyl donor and Novozym 435 as enzyme in supercritical CO_2 at 10 MPa: (●) 313 K; (■) 323 K; and (▲) 333 K.

Stability of Novozym 435

30일 동안 100% 수율 유지

Effect of solvent

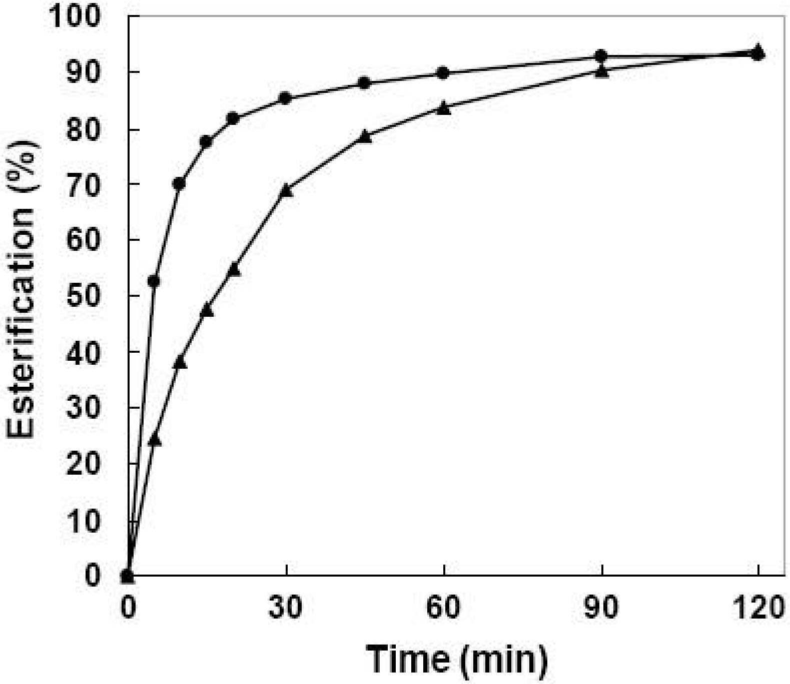


Fig. 10. Effect of solvent nature on isoamyl acetate synthesis using acetic anhydride as acyl donor and Novozym 435 as enzyme at 313 K: (●) in supercritical CO_2 at 14 MPa; (▲) in *n*-hexane at atmospheric pressure.

Conclusions

Novozym 435, was very efficient in catalysing the esterification of isoamyl alcohol.

Its catalytic activity was maximum at 313K.

Enzyme/substrate ratio of 6.25 g/mol alcohol was the optimum for maximizing reaction rate and percentage of esterification.

Acetic anhydride resulted to be better acyl donor than acetic acid.

Enzyme deactivation by acetic anhydride and/or produced acetic acid limited the solvent/substrates molar ratio to a maximum of 7.0.

The esterification extent achieved in *n-hexane* and supercritical carbon dioxide were similar but the initial reaction ratewas higher in SC-CO2 probably due to improved diffusivity of the reactants in this medium.

(2) Biocatalytic Transformation in Ionic Liquid

Solvent Properties of Ionic Liquids

- Ionic liquids
  - Highly polar solvents
  - ex) [BMIm][BF4], [MMIm][MeSO4]
  - Miscibility of ionic liquids varies widely and unpredictably

Ionic Liquids and Enzymes

- Tolerance of lipases
  Higher activity → [HMIm][PF6]
  Not active → methylsulfate, nitrate, lactate anion

- Ionic liquids seem to affect enzymes in much the same way that conventional organic solvents do

Ionic Liquids and Enzymes

- Higher thermal stability

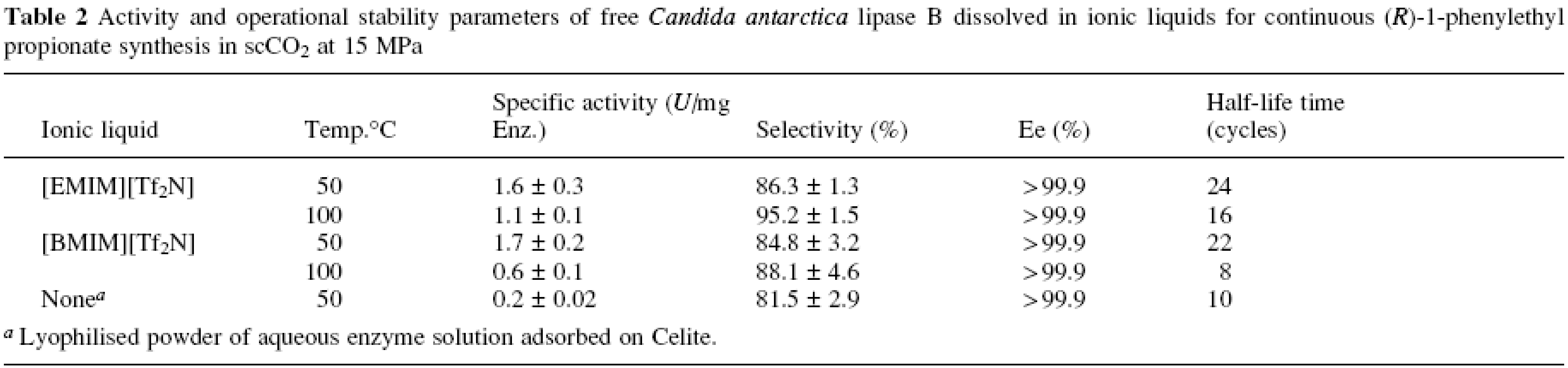


Table 2 Activity and operational stability parameters of free *Candida antarctica* lipase B dissolved in ionic liquids for continuous (*R*)-1-phenylethyl propionate synthesis in $scCO_2$ at 15 MPa

Ionic liquid	Temp.°C	Specific activity (*U*/mg Enz.)	Selectivity (%)	Ee (%)	Half-life time (cycles)
$[EMIM][Tf_2N]$	50	1.6 ± 0.3	86.3 ± 1.3	>99.9	24
	100	1.1 ± 0.1	95.2 ± 1.5	>99.9	16
$[BMIM][Tf_2N]$	50	1.7 ± 0.2	84.8 ± 3.2	>99.9	22
	100	0.6 ± 0.1	88.1 ± 4.6	>99.9	8
None[a]	50	0.2 ± 0.02	81.5 ± 2.9	>99.9	10

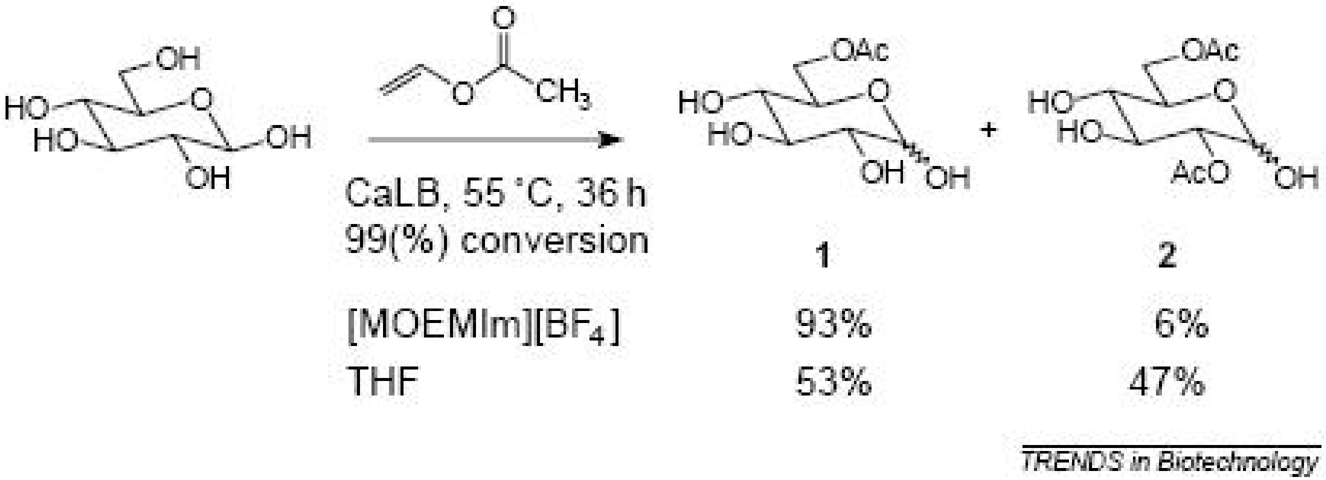
[a] Lyophilised powder of aqueous enzyme solution adsorbed on Celite.

* Continuous green biocatalytic processes using ionic liquids and supercritical carbon dic

Biotransformations in Ionic Liquid Medium

- Enantioselective acylation

  → One of major industrial applications of lipases



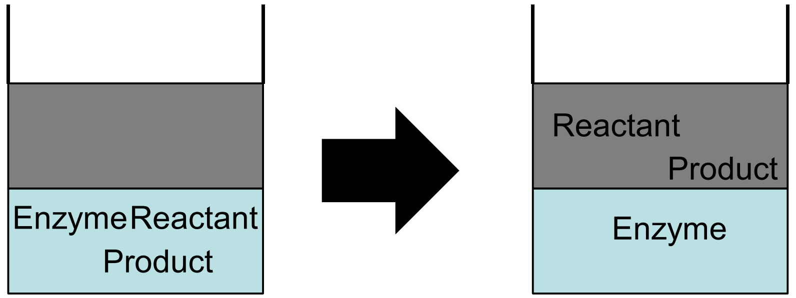
CaLB, 55 °C, 36 h
99(%) conversion

	1	2
[MOEMIm][BF_4]	93%	6%
THF	53%	47%

TRENDS in Biotechnology

Reaction Systems

- Catalyst recycling



- Product evaporation
  → Ionic liquids lack a vapor pressure
- Two-phase system with supercritical CO_2
- Two-phase aqueous systems

Conclusion

- Activities are generally comparable with or higher than conventional organic solvents
- Enhanced thermal and operational stabilities
- Higher enantioselectivities