# ENZYMES AS BIOCATALYST

BY

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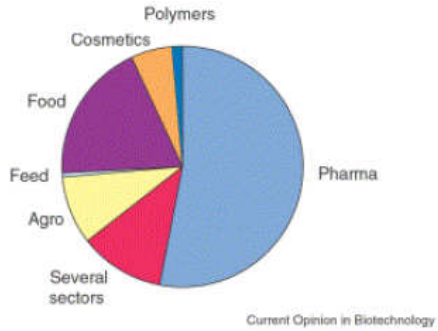
advanced enzymes

Where ENZYME is Life

# Bio catalysis in Pharmaceutical Industry

- Chiral compounds production has become more and more important, especially in pharmaceutical industry.
- Application of bio catalysis in pharmaceutical industry is the major part among industrial bio catalysis.
- Even so, in many cases, bio catalysis is still under-utilized. It is used only when chemical methods are failed.
- There is increasing number of bio catalysis used in industrial scale over the past decades.



**Biocatalyst** (biochemical catalyst)- A Biological substances that initiates or modifies the rate of chemical reaction in a living organism, required for their survival and reproduction, without itself being affected.

- **Enzymes ( proteins in nature)**

**Enzyme Technology** – a sub-field of biotechnology- new process utilizing Enzymes as Biocatalysts to meet with various human needs.

**Biotechnology** – " Biotechnology is the integration of natural sciences and engineering sciences in order to achieve the application of organisms, cells, parts thereof and molecular analogues for products and services."
( EFB,1989)

**Enzyme Technology** utilizing biocatalysts to manufacture both Bulk and High value added products:

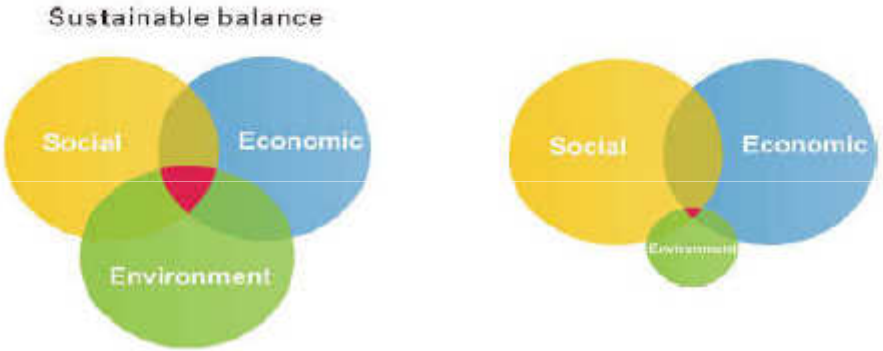
- **Food** ( e.g. Bread, Cheese, Beer, Sweeteners, Vinegar)
- **Animal Feed**
- **Fine Chemicals**
- **Pharmaceuticals** and their Intermediates

**And to provide services:**

- **Housework** ( e.g. Laundry, detergent)
- **Industry** (Textile, Paper)
- **Environmental Technologies**
- **Analytical purposes**
- **Diagnostics**

# Goals of Enzyme Technology

- Development of **New and Better** products, process and services.
- Design Innovative products and processes that are not only competitive but also meet criteria of **sustainability**

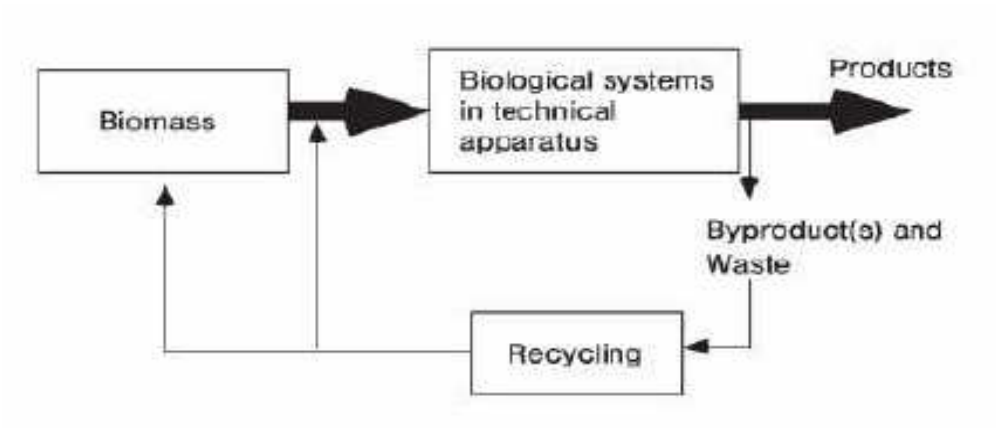



**Concept of Sustainability-** The aim is to promote a necessary "…..

Development that meets the needs of the present without compromising the ability of future generations to meet their own needs"

**Sustainable processes** must be designed to :

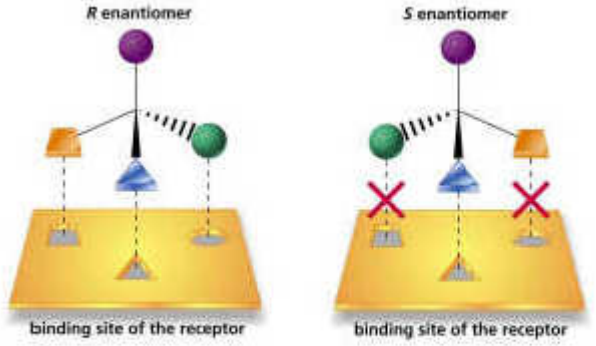
- Reduce **Consumption** of resources ( e.g. Raw materials, energy, air, water)
- Reduce **Waste** production
- Reduce **Environmental impact**
- Increase the **recycling of the waste**
- Increase the use of **Renewable raw material**- Biomass



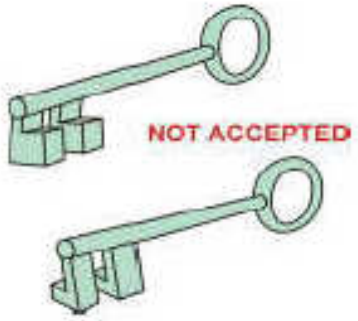
# Enzyme function and Mechanism

- Enzyme Function – reaction in which enzyme acts as catalysts
- Numerous Theories of Enzyme mechanism developed

- Lock and Key model ( Fischer 1894)
- Induced fit ( Koshland, 1960)

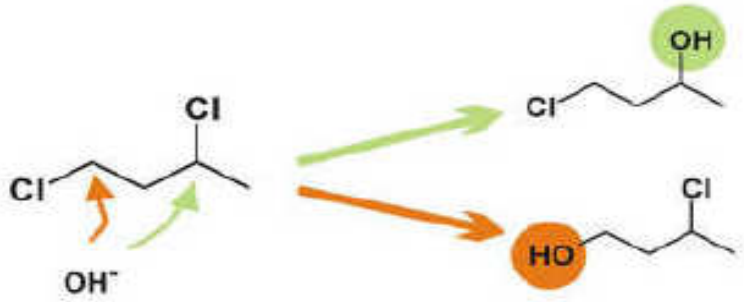
# Enzyme function and Mechanism

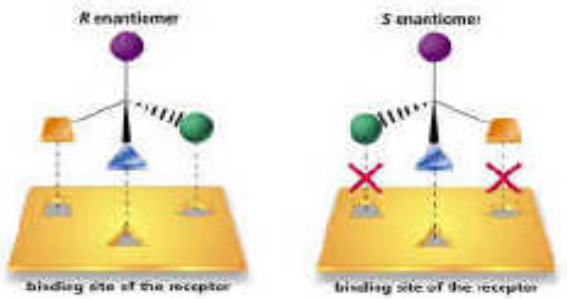





# Enzyme Selectivity / Specificity

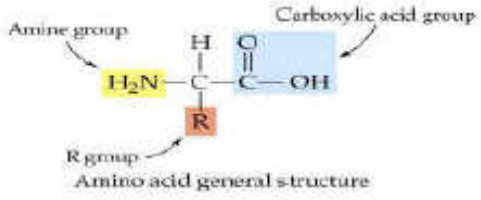
- **Functional Group Selectivity ( Chemoselectivity)** – ability to act on one functional group selective when other groups may be more chemically reactive.
- **Substrate Specificity** – ability to distinguish a particular compound from among a mixture of chemically related compounds.
- **Regioselectivity** – ability to act on one location in a molecule
- **Stereoselectivity** – ability to act on a substrate or produce a product of one enantiometric or diastereometric form



# Enzyme  Stereo Selectivity

- Enzymes – Chiral Biomolecules made from Amino acids
- Kinetic resolutions – Enzymes recognize chirality of substrate
- Asymmetric synthesis – Enzymes act asymmetrically with prochiral substrates




# Misconception about using Enzymes as Bio catalysis

- 'Enzymes are too unstable.'

  immobilized enzymes have shown stability toward reuse.

| Biocatalyst | Reported half-life |
|---|---|
| Aspartase | 6 months – 2 years |
| Fumarase | 180 days |
| Penicilin amidase | > 6 months |
| Lactase | 90 days |
| Protease | > 60 days |
| Tryptophan Synthase | 50 days |

- 'Enzymes act on natural substrate only.'

  Enzyme-substrate recognition's is not 'lock and key' but 'induced fit'.

# Misconception about using Enzymes as Bio catalysis

- 'Enzymes are too expensive.'

  The price of enzymes are about the same with other chiral catalyst.

| Enzyme | Approx. price in $/kg |
|---|---|
| Lactic dehydrogenase | 100, 000 |
| Porcine liver esterase | 15, 000 |
| Penicillin amidase | 10, 000 |
| Aspartase | 10, 000 |
| Trypsin | 5000 |
| Lipase | 5000 |
| Glucose isomerase | 500 |
| Detergent protease | 250 |
| Glucoamylase | 100 |

| Catalyst | Approx. price in $/kg |
|---|---|
| BINAP | 40, 000 |
| ChiraPhos | 10, 000 |
| Platinum | 12, 000 |
| Sharpless | 10, 000 |
| $Pd(Diphos)_2$ | 5000 |
| $Rh(PPh_3)_3Cl$ | 2000 |
| Jacobsen | 1000 |
| Chirald | 500 |
| Raney nickel | 30 |

- 'Productivity are too low'

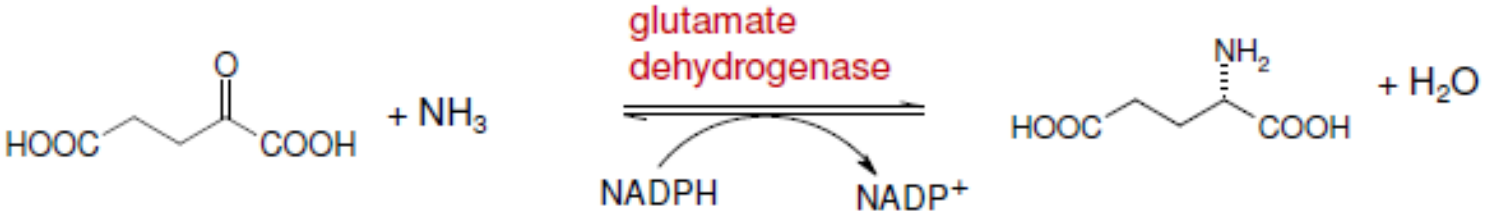
  Majority of industrial enzymes give products concentration of > 100 g/L, and productivity of 100 t/a to > 10,000 t/a

# Advantages of Biocatalysts

- ❑ Very **Efficient** catalysts
- ❑ High degree of **Selectivity**
- ❑ Environmentally **friendly**
- Sustainable
- Fully biodegradle
- No toxic metals required
- Operation at mild conditions ( 0-90 deg, pH 3-10)
- ❑ Catalyze **broad spectrum** of reactions
- ❑ **Compatibility** with each other
- ❑ Less **byproducts**
- ❑ **Non Toxic**, Non- flammable
- ❑ Can be **Reused** ( Immobilized)

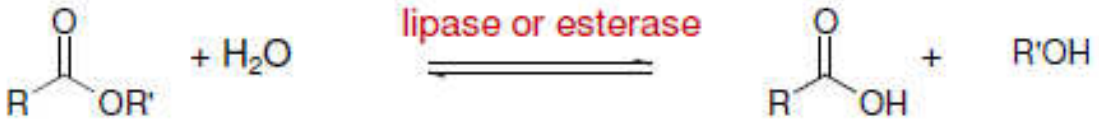
# Types of Enzymatic Reactions

1. Oxidoreductase – catalyze oxidation-reduction reactions



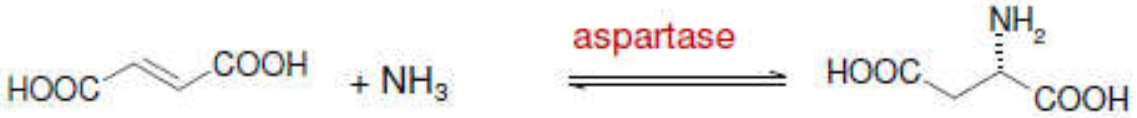

2. Transferases – transfer of groups


3. Hydrolases – formation / breakdown of esters, amides, lactones, etc

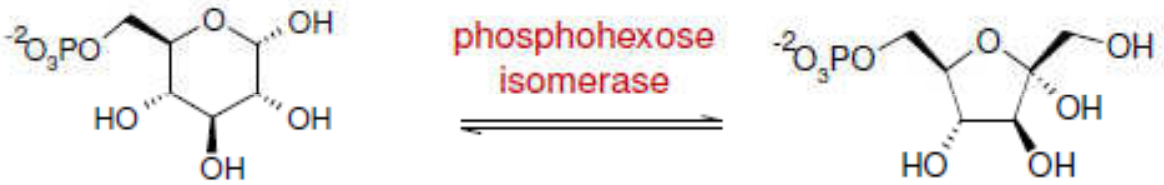


# Types of Enzymatic Reactions

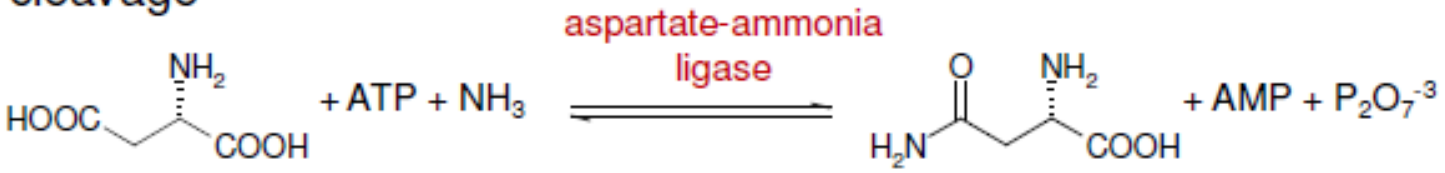
4. Lyases – addition-elimination on C=C, C=N, C=O bonds




5. Isomerases – isomerization such as racematization, epimerization

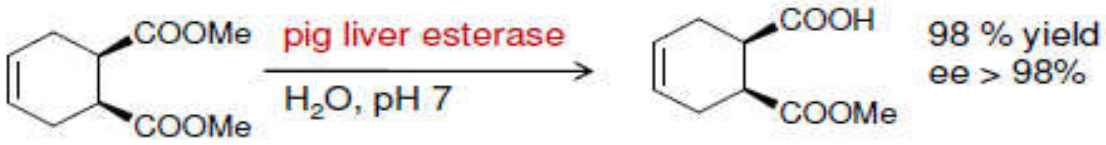



6. Ligases – formation-cleavage of C-O, C-C, C-N bonds with ATP cleavage



# Biocatalysis in Organic Synthesis

- There are lots of examples of application of enzymes outside living system to carry-out organic reactions.
  - Desymmetrization




  - Kinetic Resolution

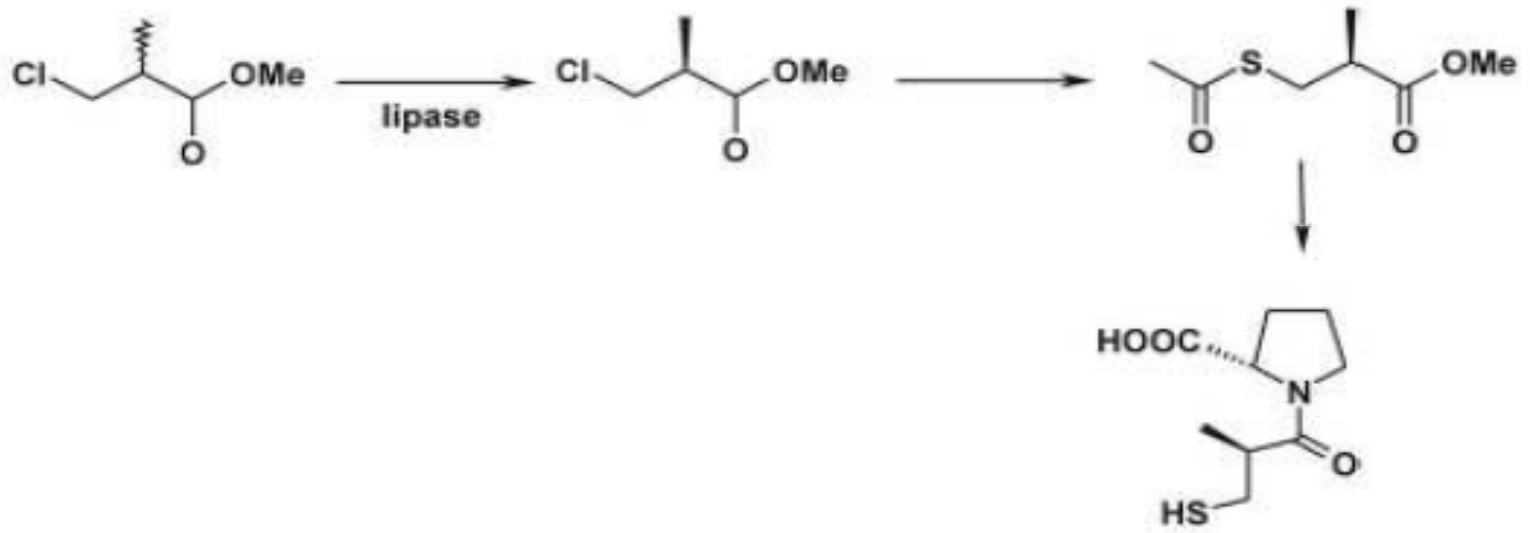

  - Asymmetric synthesis

# Hydrolyses (Lipases)

- Hydrolytic **cleavage of C-O, C-N, C-C** and some other bonds
- **Favorite class of Enzymes** for organic chemistry
- **No co factor** needed
- **Large number** of readily available enzymes
- **Reversible reactions** possible
- Relatively **High  stability** under non –natural conditions
- Often **wide substrate range**

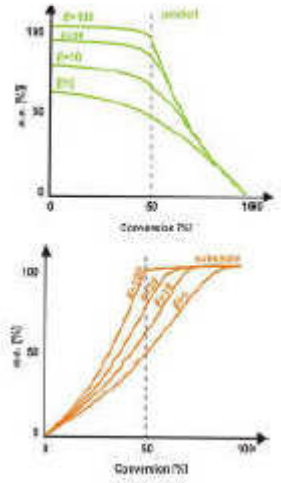
# Resolution of Carboxylic acid

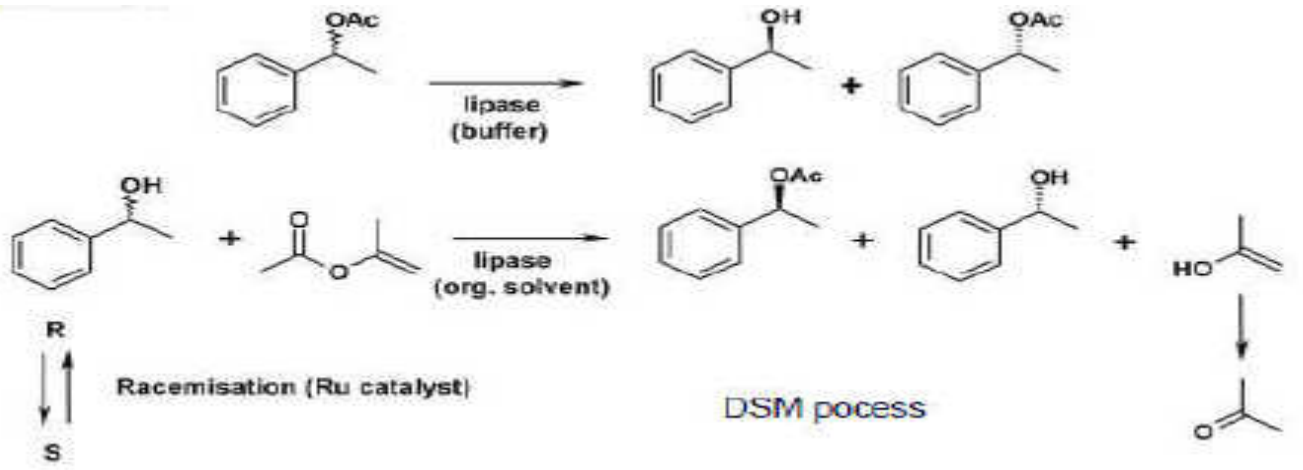
- DSM Process – Using Candida Lipase resolution of building blocks for synthesis of **Captopril** ( treat high BP and heart failure)



# Resolution of  Alcohols

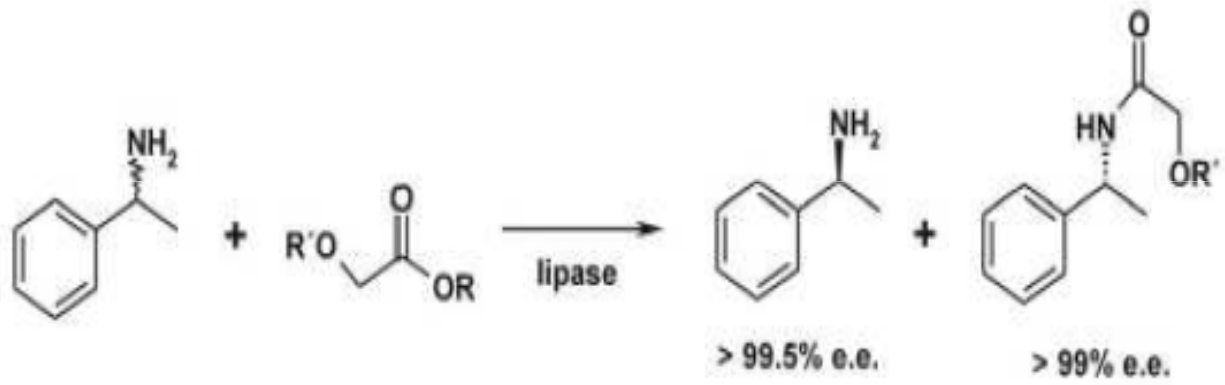
- Versatile building blocks in organic synthesis ( > 1,000 examples with lipases)
- Kinetic Resolution by **Hydrolysis**
- Highly **active in organic solvents**
- Kinetic resolution by **Acylation** – activated acyl donors or enol esters ( e.g. vinyl or isopropenyl acetate) reaction irreversible as alcohol generated undergo keto-enol tautomerism.





# Resolution of Amines

- Accept nucleophiles other than water ( e.g. alcohols, amines)
- BASF process – using lipase in multi ton production of aryl amines, alkyl amines and amino alcohols.



# Enzymes for Biocatalysis

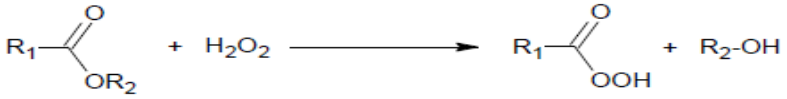
advanced enzymes
Where ENZYME is Life

## Lipases

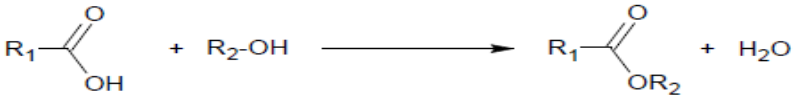
- Addzyme CAL A
- Addzyme CAL B
- Addzyme TL IM
- Addzyme TL RM
- Addzyme *Candida Rugosa*

# Addzyme CAL B Catalysed reactions

**1. Ester hydrolysis:**



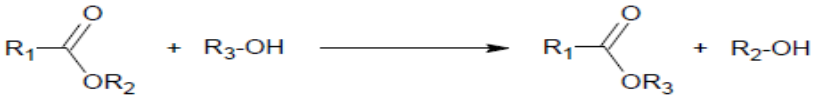
**2. Ester synthesis:**



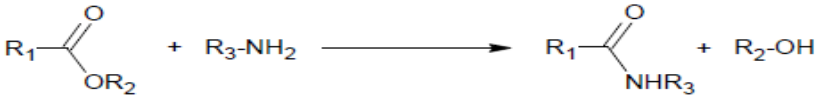
**3. Transesterification:**

**3(i) Acidolysis:**

**3(ii) Alcoholysis:**



**3(iii) Aminolysis:**



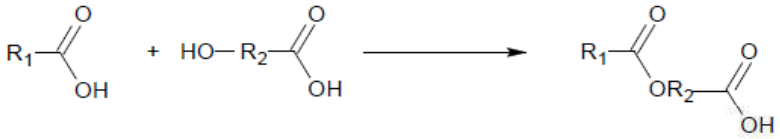
# Addzyme CAL B Catalysed reactions

**4. Interesterification:**

**5. Intramolecular esterification:**

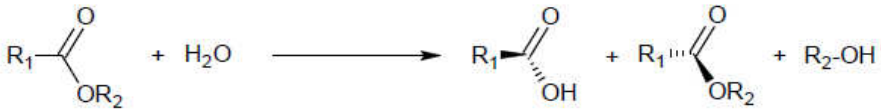
Lactone formation

**6. Synthesis of Estolides and other polymers:**

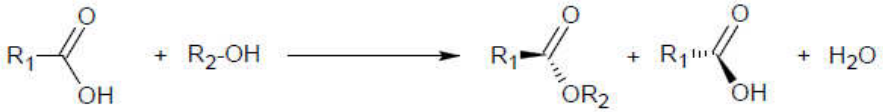


**7. Kinetic resolution:**

**7(i) Enantioselective hydrolysis of ester:**



**7(ii) Enantioselective synthesis of ester:**



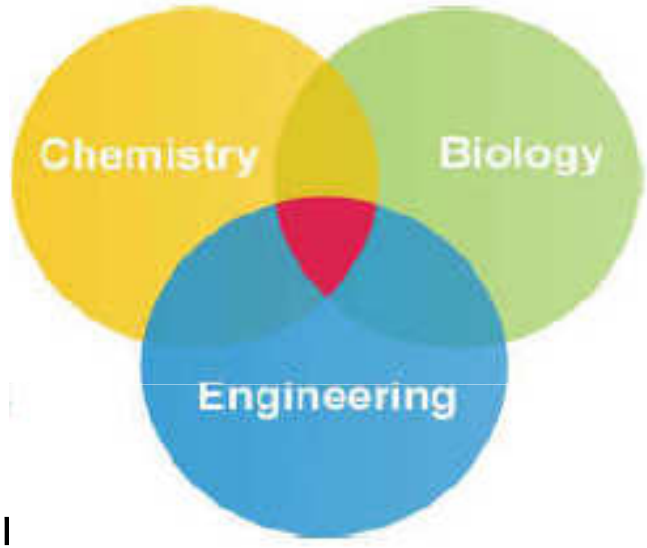
# Current use and future outlook

- ❑ Most of enzyme process introduced during **past 30 years( >100**)
- ❑ **1% of known 3,000** enzymes used in large amounts
- ❑ **Latest process in research**
  - Three dimension structure determination
  - Detailed study of Enzyme reaction mechanism
  - Rational improvement of enzyme properties ( e.g. stability and selectivity)
  - High throughput evolution techniques
- ❑ Number of **Enzymes processes expected to increase** further
  - Optically pure fine chemicals and therapeutics
  - Synthesis of Antibiotics
  - Selective Glycosylation of peptide drugs
  - Environmental technologies

# Current use and future outlook

## Interdisciplinary cooperation

- ❑ **( Bio) Chemistry**
- Biocatalytic properties ( free enzymes)
- Reaction mechanism and kinetics
- Substrate / product properties
- ❑ **Microbiology and Molecular Biology**
- Screen for better enzymes
- Promote molecular in vitro evaluation
- Metabolic ( biochemical pathways) engineer
- ❑ **Engineering**
- Process Engineering
- Scale up the process to production scale
- Improving the later procedure



**Enzymes** are everywhere allowing chemical reaction in a  living organisms, required for their survival and reproduction.

Transfer of the biological solutions to modern technologies- **Enzyme technologies** –create the **future in balance** between economy, cleaner environment and better lives

UNLOCKING THE POWER OF NATURE

# Technological assessment

- We at Advanced Enzymes are manufacturing and exporting the enzymes to the various applications for last 50 years.
- Our technical and application lab is well equipped to develop and resolve the technical queries.
- Supported by the Enzymes experts for the Industrial applications.
- Talk to us for the new development and support.

“Think of Life …

Think of enzymes…

Think of Advanced Enzymes”

Where Enzyme is life

*Thank You*