BIOCATALYSIS- AN EFFECTIVE TOOL FOR A COST EFFICIENT, SUSTAINABLE & GREEN MANUFACTURING

Dinesh Nair; Novozymes
Pharmaceuticals & fine chemicals are one of the most polluting industries

<table>
<thead>
<tr>
<th>Industries</th>
<th>Production Scale (Tons per Year)</th>
<th>E-factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil Refining</td>
<td>$10^6 - 10^8$</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Bulk Chemicals</td>
<td>$10^4 - 10^6$</td>
<td>&lt; 1 - 5</td>
</tr>
<tr>
<td>Fine Chemicals Industry</td>
<td>$10^2 - 10^4$</td>
<td>5 - &gt; 50</td>
</tr>
<tr>
<td>Pharmaceutical Industry</td>
<td>$10 - 10^3$</td>
<td>25 - &gt;100</td>
</tr>
</tbody>
</table>

A sustainable and greener footprint in chemical production is increasingly becoming a global need...

There are existing and emerging solutions to counter pollution

Pharmaceutical manufacture typically has > 100 kg raw material consumption/kg product

- Disposal costs
- Infrastructure costs
- Energy costs

Source: GSK presentation
Biotransformation is the sustainable answer to growing concerns of chemical industry

Nature’s Catalysts

Biodegradable

Renewable sources

Fermentation

Biocatalysis (Isolated Enzymes)

Whole Cells
There are several benefits of using enzymes over chemical reaction to produce APIs & Fine Chemicals.

### General Advantages using Enzymes in API/Fine Chemical Manufacturing

- **Improved Productivity**
  - Higher Yields
  - Shortened synthesis route

- **Greater Savings Potential**
  - Replaces costly chiral resolving agents
  - Significant reduction in waste streams

- **Greater Selectivity**
  - High level of Stereo-, regio-, and chemo-selectivity

- **Milder reaction conditions**
  - Mostly ambient reaction condition

- **Simplified work-streams**
  - Simplified processing and purification
  - Fewer byproducts, reducing impurities
Biocatalysis reactions adhere to green chemistry principles

Parameters of green chemistry principles in relation with biocatalysis

1. Enables new, sustainable routes reducing waste
2. Enables more efficient routes
3. Generally low toxicity
4. Enables designing safer molecules
5. Water as solvent or Class II/Class III solvents
6. Performed at ambient conditions
7. Renewable
8. Chemo, regio & stereo-selective in nature
9. Catalytic with sustainable and greener footprint
10. Flexibility in process design and degradable
11. Real time analysis on impact on pollution possible
12. Minimal (negligible) risks associated with reactions
Biocatalysis gets its principles from several disciplines and applies to a number of industries.
Based on the reaction types, enzymes are grouped into several classes

Hydrolases are the most used enzyme class in biotransformation's followed by oxidoreductases

<table>
<thead>
<tr>
<th>Selected Reactions</th>
<th>Enzyme Class</th>
<th>EC No.</th>
<th>Key Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>▪ C=O and C=C reduction</td>
<td>Oxidoreductase</td>
<td>1</td>
<td>▪ Atorvastatin Intermediate</td>
</tr>
<tr>
<td>▪ <strong>Reductive amination</strong> of C=O</td>
<td></td>
<td></td>
<td>▪ Montelukast</td>
</tr>
<tr>
<td>▪ <strong>Oxidation</strong> of C-H,C=C, C-N and C-O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>▪ Cofactor reduction/oxidation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>▪ Transfer of functional groups such as amino, Acyl, phosphoryl, methyl, glycosyl, nitro &amp; sulphur groups</td>
<td>Transferase</td>
<td>2</td>
<td>▪ Cyclodextrin from Starch</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>▪ Sitagliptin</td>
</tr>
<tr>
<td>▪ Hydrolysis of esters, amides, lactones, lactams, epoxides, nitriles &amp; reverse reactions</td>
<td>Hydrolase</td>
<td>3</td>
<td>▪ Diltiazem Intermediate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>▪ Resolution of rac. phenylethylamine</td>
</tr>
<tr>
<td>▪ Addition of small molecules to double bonds such as C=C, C=N and C=O</td>
<td>Lyase (synthase)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>▪ Isomerisations such as racemizations, epimerizations &amp; rearrangement reactions</td>
<td>Isomerase</td>
<td></td>
<td>▪ HFCS from glucose</td>
</tr>
</tbody>
</table>

Source: Some key examples of enzyme classes and their applications
Biocatalysis is a proven technology for some prominent active pharmaceutical ingredients synthesis

Few important case studies on successful chemo-enzymatic synthesis

- Pregabalin - Lipase from TL Source
Pregabalin Biocatalytic Route

Improvements due to replacing chemical process with chemoenzymatic reaction

Pregabalin Chemical synthesis

- Knovenagel condensation
- cyanation
- KOH

Pregabalin Biocatalytic Route

- Recycling
- Lipolase
- Novozymes Lipase Lipozyme TL 100 L

Inefficient Resolution at rac.pregabalin stage

Undesired (R)-enantiomer

(S)-mandelic acid

Overall 20% yield

45% yield, 98% ee

40% yield after one recycling
Flow Chart of Pregabalin Process

Key Advantages of Biocatalytic Route

- Low protein Loading (0.8%)
- Resolution at first stage - wrong isomer can be recycled
- High throughput
- All reactions conducted in water
- E-factor improved from 86 to 17
- Starting material reduction by 800 Mt (cumulative)
- Drastic reduction in solvent usage
- Mandelic acid usage - 500Mt eliminated (Cumulative)
- Energy usage reduced by 83%
Pregabalin Chemoenzymatic route used >5x less inputs than Chemical Route

### Raw Material Inputs for 1000kg API Output

<table>
<thead>
<tr>
<th>Inputs</th>
<th>Chemical Route Kg</th>
<th>Chemoenzymatic Route Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNDE</td>
<td>6212</td>
<td>4798</td>
</tr>
<tr>
<td>Enzyme</td>
<td>0</td>
<td>574</td>
</tr>
<tr>
<td>S-Mandelic acid</td>
<td>1135</td>
<td>0</td>
</tr>
<tr>
<td>Raney Nickel</td>
<td>531</td>
<td>79.5</td>
</tr>
<tr>
<td>Solvents</td>
<td>50042</td>
<td>6230</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>57920</strong></td>
<td><strong>11681.5</strong></td>
</tr>
</tbody>
</table>

**Reduction**

- CNDE: 23%
- Enzyme: 85%
- S-Mandelic acid: 88%

Future outlook of biocatalysis is very promising

Biocatalysis evolution so far and future outlook

So Far... the first and second wave

First wave
- living cells applied to useful chemical transformations

Second Wave
- Initial protein engineering techniques extended range of enzymes to allow synthesis of unusual synthetic intermediates

The third wave: future outlook

Directed Evolution → Variant Selection or screening → Smarter Libraries of Enzymes

Advanced and Emerging Technologies
- DNA technology & Protein engineering
- Bioinformatics
- Molecular Modelling studies-Docking of substrate with proteins
- Reaction engineering & process intensification using biocatalysts
- High throughput enzyme screening
- Enzyme screening kits
The way forward........

- Biocatalysis likely to be one of the most important tools for Fine Chemicals & Pharma for cost effective & sustainable manufacturing
  - It is likely that biocatalysts would be available for all types of transformations in future
  - Tailor made enzymes by directed evolution & random mutagenesis
- More and more collaborations between fine chemicals/pharmaceutical companies & Enzyme companies are expected to take place to solving complex industrial problems
- Biocatalysis companies will likely evolve into companies providing end to end solutions from screening of variants to optimizing hits to process optimization to intermediate manufacturing

... And Novozymes is adequately positioned and aggressively poised to help the industry in this journey