Microbial Biotransformation
Introduction

• Biotransformations (bioconversion or microbial transformation) refers to the processes in which microorganisms convert organic compounds into structurally related products.

• In other word, biotransformation deals with microbial (enzymatic) conversion of a substrate into a product with limited number (one or few ) enzymatic reactions.

• This is in contrast to fermentation which involves a large number reactions.
• The significance of bioconversion reactions becomes obvious when the production of a particular compound is either difficult or costly by chemical methods.

• Further biotransformations are generally preferred to chemical reactions because of substrate specificity, stereospecificity, and mixed reaction conditions (pH, temperature, pressure).

• The environmental pollution due to biotransformation is almost insignificant or negligible.

• In addition, it is easy to apply recombinant DNA technology to make desired improvements in biotransformations.

• Another advantage of biotransformations is that it is easy to scale up the process due to limited number of reactions.
Types of Biotransformation reactions

- Many types of chemical reactions occur in biotransformations.
- These include oxidation, reduction, hydrolysis, condensation, isomerization, formation of new C-C bonds, synthesis of chiral compounds and reversal of hydrolytic reactions.
- Among these, oxidation, isomerisation and hydrolysis reactions are more commonly observed.
- Many a times biotransformation involves more than one type of reactions.
- The conversion time required for biotransformation is related to the type of reaction, the substrate concentration and the mo’s used.
- In general oxidation, hydrolysis and dehydration reactions are completed in few hours.
Sources of Biocatalysts and techniques for biotransformation

• A wide variety of biological catalysts can be used for biotransformation reactions.

• Includes:
  → Growing cells,
  → Resting cells,
  → Killed cells,
  → Immobilized cells,
  → Cell-free extract,
  → Enzymes and
  → Immobilized enzymes.
Growing cells

- The desired cells are cultivated in a suitable medium.
- As the growth of the cells occurs (6-24 hours), a concentrated substrate is added to the culture.
- Sometimes, addition of emulsifiers (Tween, organic solvents) is required to solubilize substrates and/or products eg. Steroid biotransformation.
- The substrate conversion to product can be monitored by spectroscopic or chromatographic techniques.
- Biotransformation can be terminated when the product formation is optimum.
Non-growing cells

- These are preferred for biotransformation rxn due to following reasons:
  - Very high concentration of substrate can be used (high conc → growth of cells stops usually)
  - Cells can be washed and used thus there will be no contaminating substances.
  - Conversion efficiency of substrate to product is high.
  - Biotransformation can be optimised by creating suitable environmental condition (e.g., pH, temp).
  - Product isolation and its recovery is easy.
Immobilized cells

- Biotransformation can be carried out continuously by employing immobilized cells.
- Further, the same cells could be used for numerous times.
- Several bioconversion with single or multistage reaction are in fact carried out by using immobilized cells. Eg. commercial production of L-analine and malic acid.
Immobilized enzymes

- Cell-free enzyme systems in the form of immobilized enzymes are most commonly used in biotransformation, due to following reasons:
  - No undesirable side reaction.
  - Desired products are not degraded.
  - No transport barrier across the cell membrane for the substrate or product.
  - Isolation and recovery of the product is simpler and easier.
  - Eg. Glucose isomerase, penicillin acylase.
Product recovery in biotransformation

• In most biotransformation reactions, the desired end products are extracellular.
• The product may be either in a soluble or suspended state.
• When whole cells are used, they have to be separated and repeatedly washed with water or organic solvent as required.
• The extracted product can be recovered by employing the commonly used techniques- precipitation by salts, extraction with solvents, adsorption to ion-exchangers, etc.
• Volatile products could be recovered by direct distillation from the medium.
Biotransformation of steroids

A steroid is a type of organic compound that contains a characteristic arrangement of four cycloalkane rings that are joined to each other.
**Design of biotransformation process**

- It has been adequately observed that the most crucial and pivotal biotransformation processes are designed and based upon a variety of chemical reactions which may be classified under several categories, such as: (a) oxidation; (b) reduction; (c) hydrolysis; (d) condensation; (e) isomerization; (f) formation of newer C–C bonds; and (h) introduction of hetero functional moieties.

- In general, the various kinds of biotransformation processes involving typical chemical reactions along with certain specific examples and the percentage efficiency of conversion are summarized in the following. A possible explanation of the reaction(s) involved has been included in order to have a better understanding of these chemical pathways.

- Biotransformation designs have been accomplished with tremendous success for a large number of compounds, namely: cardiac glycoside ‘digoxin’, acetyltyropine, benzylisoquinoline etc. So far, the various typical examples that have been cited in the below table are exclusively related to a variety of chemical reactions in the presence of microorganisms.
• In addition to the above remarkable explicit examples it has been amply demonstrated and adequately substantiated scientifically that ‘plant cells’ are also capable of transforming a wide range of substrates; and, therefore, carry out a large number of reaction(s), for instance: oxidation, hydroxylation, reduction, methylation, glucosylation, acetylation, aminoacylation and the like.

• For example:

1. Glycosylation of salicylic acid by the cultures of *Mallotus japonica* yields a product that possesses an appreciable high analgesic activity, and also exhibits excellent better tolerance in the stomach in comparison to acetylsalicylic acid (*i.e.*, aspirin).

2. Transformation of Steviol (aglucon) into Stevioside (glucoside): The transformation of Steviol (*i.e.*, *hydroxydehydrosvestevic acid*) by the cells of *Stevia rebaudiana* (Bert.) Hemsl. (*Eupatorium rebaudianum* Bert.) Compositae, also called yerba dulce (*Habitat: Paraguay*), into a glucoside known as stevioside which is proved to be 300 times sweeter than *sucrose*, and hence used as a sweetener.
Codeine from Morphine: Morphine may be successfully transformed into codeine by using the suspension cultures of *Ginkgo biloba* as given below:

\[
\text{G. biloba} \\
\text{Morphine} \quad \xrightarrow{\text{Biotransformation}} \quad \text{Codeine}
\]

Scopolamine from Hyoscyamine: Hyoscyamine may be conveniently transformed into scopolamine by making use of the suspension cultures of *Anisodus tanuticus* as shown under.

\[
\text{A. tanuticus} \\
\text{Hyoscyamine} \quad \xrightarrow{\text{Biotransformation}} \quad \text{Scopolamine}
\]
Selection of organism

- The selection of strains either from its **natural sources or from the various available cultures** which are solely responsible for catalyzing the desired biotransformation reaction(s) is not only vital and critical but also of great importance.

- It has been observed that there are quite a few microorganisms that usually carry out the desired bioconversions with the help of a related chemical entity.

- In steroid one may encounter a rather difficult problem due to the lack of selective methods so as to identify the colonies precisely which usually perform the appropriate specific activity.
1. **Modified Enrichment Method**:

- The modified enrichment method is invariably used for the isolation of mutants blocked in the substrate dissimilation mechanism.
- In this specific instance, a steroid substrate is normally incorporated as the sole C-source exclusively in a ‘minimal medium’ seeded adequately with the soil dilutions.
- The cells that causes the degradation of the substrate will ultimately *grow*; and are, therefore, subsequently transferred to the same medium but particularly enriched with another C-source, for instance: glucose.
- However, the mutants may be present which are strategically blocked at different stages in the process of degradation of the steroid substrate, but may consume glucose as the C-source.
- It has been profusely established and reported that a fairly large number of microbial strains *viz.*, eubacteria, yeasts, molds, and streptomycetes may be stored and maintained strictly as per the recommended ‘standard methods’, such as: agar slant, soil culture, frozen culture, and lypholized culture preserved at temperatures ranging between – 20°C to – 170°C.
• Besides, the resulting intermediates may get **accumulated**, whereas the lesion-bearing mutants can be isolated conveniently. Furthermore, mutants may also be isolated which are incapable of accumulating a ‘undesirable compound’.
2. Filtration Enrichment Method:

- In this case, after mutagenesis the spores of filamentous organisms e.g., actinomycetes, fungi, are made to develop in a liquid minimal medium.

- The microcolonies of prototrophs thus developed are meticulously separated by filtration, whereby the spores of auxotrophs that were unable to grow left behind in the filtrate.

- The filtrate obtained in this manner in subsequently plated and the resulting colonies are adequately checked for auxotrophic characteristics.
3. **Penicillin-Selection Procedure**: 
- In penicillin-selection procedure the prevailing growing cells are killed selectively by the ‘antibiotic’ treatment, thereby enriching the auxotrophs that are incapable of growing upon the ‘minimal medium’.
- Thus, exclusively based upon their mode of action a plethora of ‘inhibitors’ other than penicillin may also be employed effectively in this procedure, namely **dihydrostreptomycin** for *Pseudomonas aeruginosa*; **nystalin** for *Hansenula polymorpha*, **Penicillium chrysogenum**, *Aspergillus nidulans*, and *Saccharomyces cerevisiae*; **nalidix acid** for *Salmonella typhimurium*; **colistin** for the penicillin-resistant *Hydrogenomononas strain H16*.

4. **Sodium Pentachlorophenolate**: The salt sodium pentachlorophenolate also affords enrichment procedure by virtue of its greater toxicity particularly against the ‘germinating spores’ in comparison to the ‘vegetative cells’.
Example: The above method has been successfully applied with several organisms, such as: *Penicillium chrysogenum*; *Streptomycyes aureofaciens*; *Streptomycyes olivaceus*; and *Bacillus subtilis*.
5. Spraying with Reagents (or Incorporating Indicator Dyes):
One may observe either the presence or absence of specific enzyme activities almost directly in the colonies that are allowed to grow on plates by employing either of the two available common procedures, namely: (a) spraying with appropriate reagents; and (b) incorporating indicator-dyes right into the culture medium.

6. Inhibition of Assay Organisms: In this specific instance the antibiotically-active compounds may be detected quite easily and conveniently by measuring the inhibition of sensitive assay organisms. This procedure allows the precise determination (assay) of the ‘antibiotic content’ of an unknown solution using a reference standard simultaneously.

7. Agar Plug Method: The agar plug method is regarded to be one of the most reliable and precise techniques wherein the agar cylinders having ‘single-colonies’ are transferred to test plates after due incubation preferably in a moist chamber as depicted in figure given below: