# 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 obser
- 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 biotrasformation.
- 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 (eg 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 time.
- 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 biotrasformation 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*

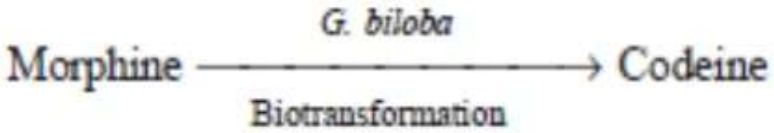
- It has been adequately observed that the most crucial and pivotal biotransformation processes are designed and based upon a variety of chemical reactions which may be classified under several categories, such as : (*a) oxidation ; (b) reduction ; (c) hydrolysis ; (d) condensation ; (e) isomerization ; (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', acetyltropine, 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.

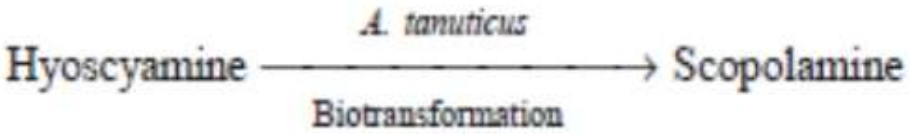
- For example:

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., hydroxydehydrostevic 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 sweetner.

**Codeine from Morphine** : Morphine may be successfully transformed into **codeine** by using the suspension cultures of *Ginkgo biloba* as given below :




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



# 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** an **'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 Hydrogenomonas 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 ; Streptomyces aureofaciens ; Streptomyces 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 grown 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 :