Enzyme Immobilisation (Movement) — Biotechnology Unit-1

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→ It refers to the technique of confining or anchoring the enzymes in or on inert support.

→ Materials used for immobilization of enzymes called carriers matrices.

* carriers are of three types :-

Inorganic	Organic	organic Synthetic
• Porous glass	• Cellulose derivative	• Polyvinyl acetate
• Silica	• DEAE-cellulose	• Acrylic polymers.
• Clays	• CM-cellulose	
• Centonite	• Dextron	

Characteristics of Carriers

1) Cost effective
2) Inert
3) Produce No modification in enzymes Molecules.
4) easily available
5) Regeneration Capacity.
6) Avoid micro Contamination.

Advantages of enzyme Immobilisation

1) It is easy to Control the Reaction.
2) It Can be Reuse.
3) Easy Separation of enzyme from RXN Mixture.

Disadvantages

1) Not good all the enzymes.
2) High cost.
3) It affect the stability of enzymes and it also effect the reactivity of enzymes.
4) Not all the industries prefer these technique.

Methods of Enzyme Immobilisation

AE2C2

- Adsorption
- Entrapment
- Encapsulation
- Cross linking
  - Covalent bonding.

(i) **Adsorption** → Simplest, physical binding of an enzymes on the surface of an inert support.

Support material may be → inorganic
→ organic

* **Inorganic** → Alumina, silica gel, calcium phosphate glass.
* **organic** → Starch, carboxy Methyl cellulose DEAE cellulose, DEAE Sephadex.

forces - Vander wall forces, Hydrogen bonding.

Advantages

i) Easy (ii) Cost effective (iii) Simple

Disadvantages

i) adsorbed enzymes can be easily removed by minor changes in pH, ionic strength or temp.

ii) Enzyme linkage from matrix.

(ii) Entrapment

Enzymes are entraped in a support or inside of fibres or polymers membranes. The size of matrix pores are such that the enzymes retained while substrate and product are passed through

Entraping Methods

- Enzyme Inclusion in gel
- In fibres
- In microcapsules

- Inside the gel enzymes entrapped enzymes
- Polyacrylamide gel in a fibre format entrap
- Poly vinyl alcohol gel of the matrix ed in (fibres like cellulose)

Capsules of Polyamine polybasic acid Chloride.

* Material used in Entrapment

- Cellulose
- Rubber
- Glass
- Silicon
- Starch
- Polyacrylamide gel
- Gelatin

[Cross-linked Polymers]

Advantages

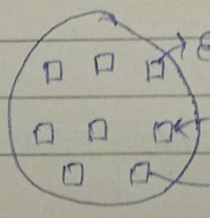
- It has better mechanical stability
- Denaturation is avoided.

Disadvantages

- Chances of Enzyme leakage.
- Deactivation of Enzyme.
- Low loading Capacity.

(iii) Microencapsulation

- Type of entrapment in Spherical particles, formation where in a liquid or suspension is enclosed in a Semipermeable membrane. Membrane may be - polymeric, lipoidal, lipoprotein.



(iv) Covalent bonding

Covalent bonds b/w the chemical group of enzymes and the chemical group of support.

- Functional group - amino (-NH2), Carboxylic (-COOH), Phenolic (), Sulphahydryl -THiol, imidazole, indole, hydroxyl
- Matrices are agarose, Cellulose, Polyacrylamide, Sepharose

The binding procedure of enzymes to the solid support generally goes through two stages -

(a) activation of surface using linkers like glutaraldehyde or carboimide.

(b) enzyme covalent coupling to activated support.

Advantages

1) Strong bonding
2) Little leakage
3) High uniformity

Disadvantages

1) High risk of enzyme denaturation.

2) Huge amount of bioreagent are required to load very less of enzyme

(V) Cross linking

In this, the multifunctional reagents (linkers) are connected with enzyme molecules. In this solid support is absent. 3D- cross linked aggregates are formed.

* Polyfunctional reagents are needed like — glutraldehyde, Diazobenzidine, Hexamethylene diisocyanate, Toluene di- isothiocyanate

Advantages :- 1) Simple Method
2) Minimal enzyme leakage.
3) Cost effective.

Disadvantage

1) enzyme modification
2) Enzyme Conformation change.
3) High risk of denaturation.

* Application of enzyme immobilisation

(i) Industrial production

production of antibiotics, beverages amino acids uses.

(2) Biomedical Applications

→ used in the diagnosis and treatment of many diseases.

. It can be used to overcome inborn metabolic disorders.
used in drug delivery systems especially to encogenic sites.

(3) Food industry

Enzyme like pectinases and cellulases immobilized on suitable carriers are used in the production of jams, jellies, syrups

(4) Production of bio-diesel from vegetable oils.

(5) waste water management treatment of sevage and industrial effluents.

(6) Textile industry

bio polishing and deriving of fabrics.

(7) Detergent Industry

Immobilization of lipase enzyme for effective dirt removal from clothes

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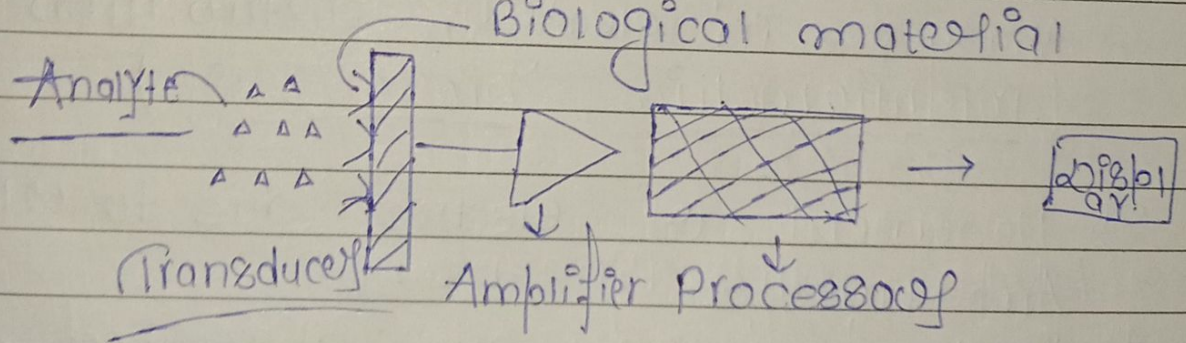
Biosensors → Working and application in pharmaceutical industries.

* These are analytical device which measure conc^n of an analyte. In biosensor biological material interact with an analyte, this interaction produce detectable physical change which is measured and converted in to electrical signal by a transducer
* The electrical signal is amplified interpreted and displayed as analyte conc^n in the soln or prepn.
* Father of biosensor - Leland C-Clark

Detection → Clark electrode (developed first biosensor - for oxygen.



Component of biosensor

Analyte → These are material which are measured molecule, Blood urine Protein, vitamin, Peptide.

(2) Biological molecule

→ These molecule interact with analyte and produce physical changes, antibodies cell, polymers enzymes.

(3) Transducers

→ These molecules generates electrical impulses.

→ They detect following changes:- Thermal, Magnetic, Optical, electrical, electrochemical and so on.

→ The Biological Component interact with analyte which produces physical changes:-

→ The physical changes may be:-

1) Heat released or absorbed by rxn (calorimetric Biosensor).
2) Production of an electric potential due to changes in electrons distribution (Potentiometric biosensor).
3) Movement of electron due to redox Rxn. (Amperometric Biosensor)
4) Light produced or absorbed during the Rxn (optical biosensor).
5) Changes in mass of the biological Component as a result of Rxn. (acoustic wave)

Types of biosensors

(1) Calorimetric biosensor -> They molecule change in temp. due to release (exothermic) or absorption (endothermic) of heat.

(2) Potentiometric biosensor -> They measure current potential difference arising during a redox Rxn e.g. -> urea biosensor.

(3) Amperometric biosensor -> They measure current (flow of electrons) arising during a oxn. ex- glucose biosensor.

(4) Conductometric biosensor -> They measure the changes in electrical conductivity during a oxn ex- urea biosensor.

(5) Acoustic wave biosensor :- They measure electric field developed by piezoelectric effects ex- cocaine biosensors.

(6) Optical biosensor -> They measure light arising from the action of enzyme luciferase.

Signal Characterstics -

1) Linearity - It means detection should be high.

2) Sensitivity - Signals should be very sensitive they show response in low conc^n.

3) Selectivity - They must be very selective not interfact with other chemicals.

4) Response time - less.

Advantages

1) Highly specific
2) Easy to use
3) Durable
4) Required small sample
5) less irritating.

Ex of biosensors

1) Fitness smart band
2) device in ICU
3) Glucometer
4) Pregnancy kit

Application

1) Food analyse
2) Medical diagnosis
3) Study of biomolecules.
4) various analysis of virus and bacteria.
5) Pharmaceutical and drug analysis.
6) Drug adulteration analysis
7) Industrial effluent control
8) Pollution control and monitoring.

(i) Enzyme based electrode

* In enzyme electrode, an enzyme can be combined with an electrochemical sensor.

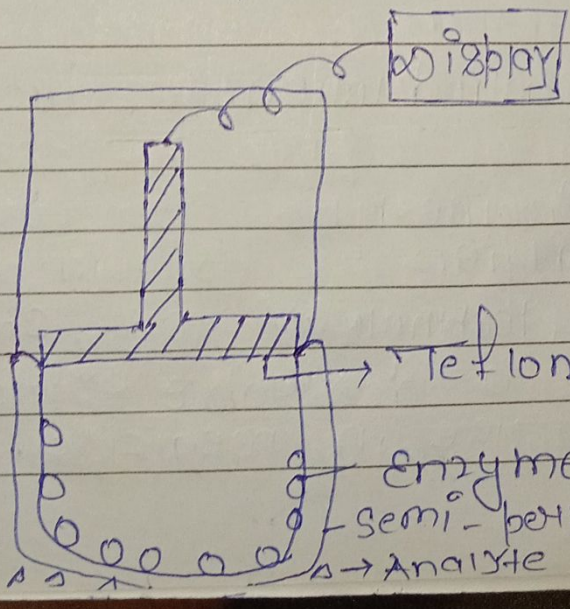
* In E·E is combn of any electrochemical probe (amperometric, potentiometric or conductometric) with a thin layer (10-200 mm) of immobilized enzyme.

Three types of enzyme based electrode

* The enzyme located in the membrane produce such as H^+ ions, O_2, NH_4^+ ions CO_2 or other molecules which are detected by electrode.

For e.g. → In urea electrode, urease enzyme convert the urea into NH_4^+ (ammonium ions). These ions can be detected by ions electrode. This NH_4^+ conc recorded.

→ The potential record is proportional to the conc of urea into sample soln.

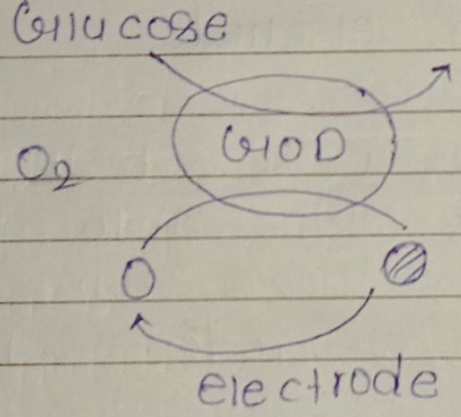


(ii) Glucose Biosensor

Principle → Based on a thin layer of glucose oxidase on an oxygen electrode.
↳ platinum

glucose + glucose oxidase (FAD) (GOD) → gluconic acid + glucose oxidase ($FADH_2$)
reduced form.

Reduced GOD + surrounding oxygen + electrons →
↓ ~~H_2O_2~~ + glucose oxidase (FAD)
$FADH_2$ original form



Result → H_2O_2 conc^n can be detected by platinum electrode.

Result → H_2O_2 Conc^n can be detected by platinum electrode.

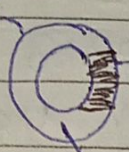
Brief Introduction to protein Engineering

Protein Engineering can be defined as the modification of protein structure with r-DNA technology or chemical treatment to get a desirable function for betterment use in medicine, industry agriculture

ore and other fields also.

P.E is the process of developing useful or valuable proteins.
— Recombinant DNA technology
— Chemical treatment

DNA – RNA – Protein
Central dogma

R.E

Animal DNA
Required piece of DNA
Recombinant DNA
Bacterial DNA

— Restriction Endocrease (RE)
— Ligation (जोड़ना)

• Protein

• AA – A $A_2 - AA_3 - A.A \cdot AA_5 - AA_6$
↓
Peptide bond

• Useful or valuable proteins.

20 AA for ex- Valine
Leucine

Objectives of Protein engineering

1. Improved kinetic properties.
2. Elimination of allosteric inhibition
3. Enhanced substrate and reaction specificity.
4. Increased thermostability

5. Alteration in optimal pH.
6. Suitability for use in organic solvents.
7. Increase / decreased / Optimal temperature.
8. To speed up the process (rate of rxn)
9. Increase protein / enzyme life (Shelf-life).
10. To get high quality of product.

Methods of protein Engineering

1. Site directed mutagenesis
2. Protein Engineering by use of gene families.
3. Protein Engineering through Chemical Modification.

1. **Site directed Mutagenesis** - Mutagenesis means - mutation is induced artificially - by substitution, by insertion, by deletion.
In this mutation is developed at specific site.

Step 1:- (1) In M13 virus or bacteriophage has S.S DNA (plasmid).

(2) The gene which is to be target piece.
- phage D.N.A] DNA. - [ATCGH] phage DNA] DNA
changed whose single stranded copy inserted in M13 phage DNA.

(3) Now this single stranded copy allowed to g

double Stranded.

(4) Now in other ~~strand~~ one nucleotide is substituted by another nucleotide.

(5) Now this mutated DNA allow to enter into host Cell (Bacteria).

(2) Protein Engineering by use of Gene families.

The technique involves isolation of gene from each species and create hybrids. for e.g - Substitution (an enzyme used in detergent industry) genes from 26 Species were mixed so we get is types of substitution enzymes with improved qualities in different aspects.

(3) Protein Engineering through Chemical modifications:-

In this method different chemicals used as "Glutraldehyde" it acts as protein cross linkers. It Stabilises the protein in Soln.

By the "Glutraldehyde" different protein as Haemoglobin, insulin Phosphofructokinase, Lactate dehydrogenase have been Stabilised.

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Different Approaches

(1) Increasing the stability and biological activities.

(a) By addition of disulfide bonds.

for e.g → The lysozyme activity ↑ease by applying disulfide bond at 2 4 and 6 amino acids.

(2) xylanase → This enzymes uses in paper industry of its activity increase at high temp. by introducing disulfide bonds at (1 2 and 3).

(b) By reducing free sulphahydryl group - Some times, free sulphahydryl group decrease the activity of enzyme.

for e.g → In human β-interferon introduce of serine in place of cystine reduce free sulphohydryl group.

(c) Single amino acid changes →

for e.g → (1) α_1-antitrypsin (This enzyme inhibit the activity of elastase, that damage lung tissue. So in α_1-antitrypsin methionine amino acid (358) is replaced by valine so it's activity increased.

ex → (2) Hirudin → (anticoagulant) - by replacing asparagine (47 position) with lysine,

increase the potency of Hirudin.

(2) Improving kinetic properties :- Kinetic properties can change by site directed mutagenesis.

For e.g :- Substitution enzyme. Methionine amino acid is replaced by any other amino acid change its kinetic property.

* Tyrosin → t-RNA synthetase - This enzyme derived from bacteria bacillus Stearothermophillus.

* Replacement of threonine (51) by alanine of proline increase it's kinetic property

Application of protein Engineering

(1) Medical application → use in cancer treatment, Cardiac disease (Hirudin) leukaemia, insulin, tissue plasminogen activator.

(2) Environmental application :- Many pollutants such as phenol, azodyes, pesticides and polycyclic aromatic hydrocarbons can be detoxified using enzyme oxidation.

→ Many protein Engineering used to develop the Production, Stability and detoxification.

(3) Food industry → Many application in food industry as develop the wheat gutin protein. Some large group of enzymes like proteases

amylases and Lipases for both food and detergent industries. Golden Rice Vit-A are upgraded by protein engineering.

Production of Enzymes - General Consideration

→ Enzymes are macromolecules acts as biological

→ They are made up of protein.

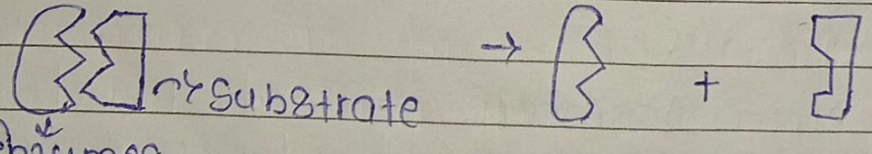
→ accelerate the Rate of Reaction.

→ Enzymes present in every living systems as in Human, microbes (fungi, bacteria etc).

→ Microbial enzymes are the biological catalyst leads to microbial growth and their other metabolic activities as respiration development, reproduction etc.

* Lipid —(Lipase, Enzyme)→ glycerol + fatty acid

Lipid: Substrate



* Enzymes have specific site on which substrate will bind it is called Active site. and then Substrate will convert into the product.

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Micro-organisms

- Bacteria and actinomycetes
  - ex- Rhizobium
  - Bacillus subtilis
  - Azospirillum
  - Pseudomonas
- Fungi, yeast and moulds
  - ex → Aspergillus
  - Penicillin
  - Trichoderma
  - Saccharomyces.
- Algae
  - ex → Chlorella

Steps in product of Enzymes

1) Isolation of Microorganism
2) Strain development
3) Prepn of inoculum
4) Culturing
   - Semi-solid culture
   - Sub merged culture
5) Processing

(1) Isolation of Microorganism

Microorganism & are isolated from different mixture of microorganisms by

- Streak plate technique
- Pour plating technique
- Staining technique
- Selective media (antibiotic Selective media).

(i) Macconkey agar media
- deoxycholate
- Bile salt

(entero bacteria)
ex → Shigella, Salmonella, Vibrio cholarae

Aim for Isolation

* high production
* less time
* Low cost.

. Those substance which produces mutation.

(2) Strain development → By mutagenes, as mutagenic chemicals ie mustard gas, ultra violet rays, x-rays.

(3) Prepn of inoculum → Microorganisms are exposed to culture media. to increase their number and keep for particular time (24-48 hrs).

- Culture media → It may be defined as any substrate or material that enables to grow and multiply the micro'organisms.

For inoculum prepn

- Microbial cell put into culture media and keep the plate incubator at proper temp. and time.

- Now prepare agar petriplate and introduce the culture on petriplate by inoculating loop.

(4) Culturing → (a) Semi Solid culture → In this method (0.5%) or less. Semi solid medium is prepared with agar (0.5% or less). Then the media is sterilized with autoclave and sterilized media is sprayed on metal trays (1-10cm depth). Then culture media is allowed to solidify. Then microbial culture is introduced (for this type of medium

Rice Bran or wheat bran also used].
Medium pH is adjusted according to microorganisms. Then culture trays are kept in incubatory at particular temp. and time.

Ex-> Salmonella Typhi ATCC 539
E. coli ATCC 2592

Semi Solid Media

Advantages

1) Low investment
2) High yield
3) useful for those m'os which can not grow in fermentor

Disadvantages

1) More Space
2) More Labour
3) Manual Handling

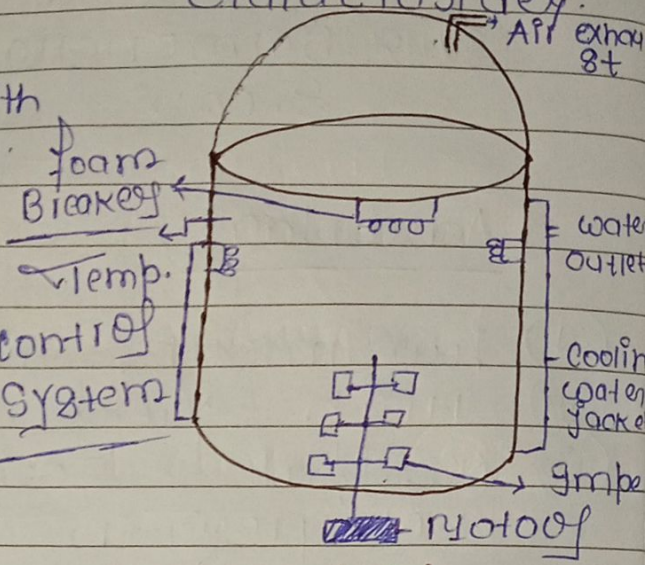
(b) Sub-merged Culturing -> Microorganisms are cultured in fermentor. Fermentation may be defined as the process of growing a culture of microorganisms in a nutrient media and there by converting feed into derived end product.

- Fermentor is a cylindrical device containing nutrient media with different inducers/ inhibitors. for ex->

Enzyme
Invertase
~~Amylase~~
Lipase
β-galactosidase

Induced
Sucrose
Starch
Fatty acids
Galactosides.

- Impellers – to mix liquid with microorganisms.
- ~~Air~~ spargers – to aerate the liquid.
- Water jacket – for temp maintain



fermenter

~~Advantages~~

1) Less labour (2) Low risk of infection
3) Automated process

Disadvantages

(i) High cost
(ii) not suitable for small scale purposes.

(5) Processing

Recovery and purification of products is called down steam processing.

(a) Removal of cell debris → by filtration or centrifugation

(b) Removal of nucleotides :- → Nucleic acid interfere with the recovery and purification of products. They can be precipitated and removed by adding polycations as Polyamines, polyethylinemine etc.

(c) Enzymes precipitation → by using salts ammonium sulfate isopropanol, Ethanol acetone etc.

(d) Liquid-Liquid partition :- To increase conch of desired enzymes, Liquid-Liquid extraction, done by using polyethylene glycol or polyamines.

(e) Separation and purification by → ion exchange, size inclusion, affinity hydrophobic interaction and dye ligand) Chromatography.

(f) Drying → film evaporators or freeze dryers (Lyophilizers.

(g) Packing → Desired product are the packed.

Production of Enzyme - Amylase

* It Catalyses the hydrolysis of starch into sugars.

Starch —amylase→ Glucose

- Present in the saliva of humans.
- Starch - Dextrin → Maltose → Glucose

Types:- (1) α-Amylase (2) β-Amylase (3) γ-amylase

- α-amylase also called 1,4-αD glucan, glucanohydrolase.
- Calcium metalloenzymes.
- hydrolyze internal α-1,4 glycosidic bond found in animal kingdom.
- Presence in saliva, pancreas.
- pH — 6.7 - 7.0
- Starch → amylose and amylopectin
- β-amylase → also called 1,4-αD glucan maltohydrolase.
- Synthesised by bacteria, fungi, plants.

- During ripening of fruit B-amylase breaks Starch into maltose, resulting in sweet flavour of ripe fruit. pH = 4-5. Break Starch into maltose.

- γ-amylase → also termed as 1,4 α-glucosidase. It cleaves α-1,4 and α1,4 glycosidic linkage pH=3. Break amylase and amylopectin to glucose.

Source-→ Bacteria from bacillus species
. Bacillus Subtilis . B. amyloliquefacine
fungi - Aspergillus oryzee, Aspergillus Niger, candida species.

Four steps of production of enzymes

1. Selection of microorganism
2. Formulation of medium
3. Production
4. Recovery and Purification

(1) Selection of Microorganisms

Microorganism preferred which produce maxm amount of enzyme in less time. four Strain improvement different mutagens are used.
ex→ A. niger, A. oryzae preferred.

2) Formulation of Medium :-

Corn starch - 24gm / lit.
KCl — 0.2gm / lit.
Na_2HPO_4 — 4.7gm / lit.
$CaCl_2$ / $CaCO_3$ - 1gm / lit
$MgCl_2 \cdot 6H_2O$ - 0.2 gm / lit.

- Carbon Source :- Maltose, Sucrose, glucose.
- N_2 source :- $(NH_4)_2SO_4$, NH_4Cl, Na_2HPO_4
- Commonly used substrate → starch, cornstrip liquorious, starch hydrolysate, Molasse Yeast extract, whey soyabean meal Malt, Peptone etc.

(3) Production :- Aspergillus niger, A. oryzae

Medium is sterilised

↓

Fermentation is started by inoculating the medium at pH = 5-7 temp 25°-30°C, Time - 3-5 days.
O_2 - aerated (aerobic).

(4) Recovery and purification :- Removal of ~~Cell debris~~ by ~~filtration~~ or Centrifugation

↓

Removal of nucleic acid by adding poly Cations or polyamines Streptomycin and

polyethyleneomine

↓

Enzyme precipitation by ammonium sulphate or NH_4OH - Ammonium Hydroxide.

↓

Precipitate Collect by filtration.

↓

Precipitate are subjected to crystillization. (at ≈4°C)

↓

drying by film evaporaters or freeze dryers.

(5) Application

(a) Production of sweetners e.g. glucose syrup, high mannose sugar & high fructose syrup (by enzymatic Conversion of Starch.

(b) Bakery industry - Break baking.

(c) detergent industry -> In dish washing, Laundry.

(d) Paper industry -> To remove Sizing agent (Starch) used to get Smoothness, Curling elasibility.

(e) Textile industry - To remove Starch used to strong then fiber.

(f) fuel alcohol production

(g) dextin production.

Catalase

* Found in all living organism.
* It catalyse the decomposition of H_2O_2 to H_2O and O_2.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

* Enzyme present in blood and other tissue with antioxidant activity.
* It protect the cell from reactive oxygen species.
* It Contain 4 polypeptide chain (Tetramer) more than 500 amino acid present in each chain.
* Turn over of catalase - highest because one Catalase can Convert million of H_2O_2 to H_2O and O_2.
* Working pH : 6.8 - 7.5

Source → Aspergillus niger (Commercially)

→ Staphylococcus → Pseudomonas adeuginosa

→ Aspergillus fumigated → candide albicans

→ Mycobacterium tuberculosis.

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Types

1. Mono-functional haem catalase (classical catalase)
2. Catalase peroxidase (Atypical cat.)
3. Non haem catalase (Pseudocatalase).

Steps :-

1. Selection of Microorganism
2. Medium Prepn
3. Production
4. Recovery and purification

(1) Selection of Microorganism

Microorganism preferred which produce enzyme in high amount and in less time.

Bacteria - Pseudomonas aeruginosa, Staphylococcus bacillus subtilis, Micrococcus.

Fungi - Aspergillus niger, Candida albicans.

(2) Medium Preparation :-

Carbon Source - glucose, Starch

N2 Source - Ammonium Salt, nitrate Salt, Peptone meat extract.

Mineral - Phosphate, Mg Salt copper Salt, Potassium Salt, Calcium Salt, Cobalt, Molybednum Salt.

pH = 6.5 - 7.5 Temp. - 40 - 60°C.

Media Composition for bacillus Subtilis

- Glucose - 10 gm
- NaNO3 - 5 gm
- Mg.SO4 - 0.5 gm
- K_2HPO_4 - 9.5 gm
- FeSO4 - 0.0026 gm
- Dissolve in 1 litre water

pH = 7 - 7.5

(3) Production

Aerobic Cultivation

- By Submerged fermentation
- Medium is Sterlised at 117°C for 20 min.
- for Seedling - 48 hours of Aspergillus niger grown in Shake flask
- This 48 hours inoculum is used for seedling.
- The culture run in fermenter at 30°C for 72 hours
- pH = 6 - 6.5 (by adding $CaCO_3$)

* Shaking Speed - 400 rpm
* Proteinase innibitors can be added during this fermentation process at 36 hr of fermentation which increase the production of catalase.

4) Recovery -> filtration - Remove the microbial cell from medium.

→ Cells are suspended in 50 mm phosphate buffer (pH=7) mixed with 300 ppm of lysozyme and incubated at 38°C for 1 hour (for cell lysis)

→ Now filtration is proceed.

→ After filtration - filtrate is treated with diatomaceous earth then pass through membrane filter.

→ The resulting soln purified by treating ammonium sulphate precipitation.

→ The eluting the enzyme, treated with 0.5 M NaCl in phosphate buffer.

→ Purified prep can desalted by dialysing against 50 mm phosphate buffer using cellulose tube no. 30.

→ The enzyme - lyophilised (drying).

Application

1) food industry -> Removal of H_2O_2 from pasteurized milk and other dairy product so, used in food wrappers.

→ To determine milk quantity.
→ used with other enzyme as preservative.

② Medical → Treatment of oxidative Stress
→ Removal of H_2O_2 from blood
→ for keeping Contact lenses.

③ Bioremediation :-
→ for phenolic Compound degradation
→ oxygen provider in aerobic bioremediation
→ Treating effluent from textile industry.

④ Pharmaceutical → Biotransformation Reaction.
Preph of Sulfoxide of β-lactones.

Peroxidase

→ These are heme proteins
→ They contain iron Fe(III) proto porphyrin IX as prosthetic group.
→ They convert H_2O_2 in to H_2O

$$H_2O_2 + AH_2 \xrightarrow{\text{Peroxidase}} 2H_2O + A_2$$

AH_2 - oxidizable substrate like oxidizable substrate like polyphenols, ascorbate furosymyl cytochrome c

Peroxidase examples:
- NADH peroxidase
- Horse peroxidase
- Glutathione peroxidase

Steps

(1) Selection of microorganism

Bacteria

- Pseudomonas aeruginosa → Lignin peroxidase, Manganese
- Bacillus megaterium, Pseudomonas stutzeri → Lignin peroxidase

Fungal

- Aspergillus niger - Lignin peroxidase
- Chrysosporium - manganese peroxidase
- Lentinous edodius - Magnese peroxidase

Aspergillus niger - isolated from soil and orange fruits. Pure culture were maintained in potato dextrose agar (PDA) media at 4°C.

→ Four inoculum prepn 10 ml of sterilized distilled water to 5 days PDA slant culture.

(2) For Nutation of medium

Glucose	— 10 gm / litre
Yeast extract	2 gm / litre
NH_4NH_3	0.2 gm / litre
$MgSO_4 \cdot H_2O$	0.5 gm / litre
K_2HPO_4	1gm / litre
$NaH_2PO_4 \cdot H_2O$	— 0.4gm / litre

pH = 6.5

Distilled water - 1000 ml

(3) Production

- by fermentation
- pH = 6.5 , temp = 25°C
- Rotating Shaker - 160 rpm
- Duration - 10 - 12 days.

(4) Recovery

- filtration or Centrifugation
(To remove cell debris)

↓

add Streptomycin, polyamine, Polyethyl amine

(To remove nucleic acid)

↓

Add $(NH_4)_2SO_4$ to Precipitate enzyme.

↓

Ion exchange and Sephadex G-100 gel filtration Chromatographic method

↓

freeze dryer.

Application

(1) Detection of cholestrol level in serum.

→ In this cholestrol esterase hydrolyse cholestrol into cholestrol ester and further oxidize to cholestinone and H_2O_2. This H_2O_2 amount is determined using horse radish peroxidase.

(2) Peroxidase Biosensor - to determine glucose alcohol, glutamate, choline.

(3) In paper industry - Naganese peroxidase in paper industry as bioleaching.

(4) Decolorization of synthetic dyes - Horse redish peroxidase effective in decolorising for paper, printing colour. photographic textile industry.

(5) Lactoperoxidase use as preservative in cosmetics for 2-4 months.

Lipase (ase represent enzyme
(ose represent Sugar

Lipase also termed as triacyl glycerol, Lipase which catalyse the break down of fat into fatty acids and glycerol (lipolysis)

→ Naturally occurring in pancreatic juice, stomach.

- Lipase can be purified and extracted from plant, animal, yeast bacterial and fungal source.

(Fat)

- Triglycerides $\xrightarrow{\text{Lipase}}$ Diglyceride $\xrightarrow{\text{Lipase}}$ Monoglyceride.

Production of Lipase

1. Selection of microorganisms
2. Media preparation
3. Production
4. Recovery and purification
5. Application.

(1) Selection of Microorganism

Bacteria

- Pseudomonas aeruginosa
- Bacillus Stereothermophillus
- Bacillis Subtilis

Fungal

Aspergillus niger

Rhizopus Arrhizus

Penicillin verriocosum

Yeast

- Candida utilis
- Candida cylindreca

→ Microorganism were grown in yeast agar medium and keep at 4°c.

→ Prepn of inoculum → a loop full of cells from freshly grown culture of candida cylindrica was transferred to the flask.

→ Flask is incubated at 30°c on a rotatory shaker at 200 rpm for 36 hrs.

② Media Prepn

Carbon, N2 Source

Chemical	weight gm/litre
• KH_2PO_4	8
• $MgSO_4 \cdot 7H_2O$	1
• urea	4
• $FeCl_3 \cdot 6H_2O$	10 mg
• Thiamine Hydrochloride	Inositol 0.4 mg, 0.2 mg
• Biotin	0.8 mg
• Glucose	10gm
Distilled water	- 1 litre

→ Media autoclave 121°c for 20 min.

Production

→ In production medium glucose as carbon source.

→ Palm oil used as carbon source.

* Formulation - temp 30°C.
→ aeration - 1.0 vvm/min
Stirrer speed - 500 rpm
pH - 6-7 is maintained by addition of 3 N NaOH and 3 NH_2SO_4 soln.

(4) Recovery and purification.

→ Removal by filtration or centrifugation
→ Removal of nucleic acid can be precipitated and removed by polyamines, streptomycin, polyethyleneamine
→ for precipitation of extract treated with ammonium sulfate
→ After this dialysis and ultrafiltration is done.
→ Then concentrated form of lipase applied on Sephadex G-200 column and eluted with 0.1m Tris HCl Buffer.

Application

1. For flavour development in food industry.
2. In cheese ripening process
3. detergent - To remove stains of oils.

(4) Industrial process - used in different industrial reaction like - alcoholysis glycolysis, hydrolysis.

(5) In cosmetics and perfumes, to produce aroma.

(6) Poly unsaturated fatty acids, used as,
- Pharmaceuticals, - Nutraceuticals,
- food additives

(7) Textile industry - In conjugation with other enzyme used in desizing in textile industry in this process to remove the adherive lubricant from the coralp thread that helps in dying.

Protease (protein digesting)

* Belong to the class that Hydrolyse the peptide bond.
* also termed proteolytic enzyme.
* present in nature.

Source type

- Plant proteases - Papane, Bromolin
- Animal protease - Pancreatic, Pepsin, trypsin Renin.

* Animal microbial protease - Bacterial, fungal viral.

* Site of action type

Exopeptidase
act near a terminal of a polypeptide chain

Endopeptidase.
act internally in polypeptide chain.

* on the basis of pH

Acid proteases.
Neutral proteases
Basic or alkaline Proteases

* on their mechanism of action

Serine proteases
Thiol proteases
Metallo proteases
Acid proteases.

Steps

① Selection of microorganism

Bacteria
Bacillus subtilis
amyloliquifaciens

Fungi
Aspergillus nigers
Aspergillus flaveous
" fumingatus

For strain improvement mutagen are introduced. for Ex- X ray, uv ray.

(2) Formulation of medium.

The type of proteases depend on composition of medium.

Bacillus NRRLB 3411 - when grown on a grain medium neutral proteases, and when culture on a fish meal en20 cellulose medium - alkaline protease

→ Peptone - Casein - induce protease

→ Carb source - molasses, Barley, Corn, Wheat, Starch hydrolyses.

→ Protein -> meals of soyabean, Cotton seeds, peanut, whey, corn, steep, liquor, yeast, hydrolysate, Sodium glutamate.

(3) Production process

Both types are used

Submerged fermentation (Bacteria)	Solid state fermentation (fungi)

Submerged form

Microorganisms in closed vessels containing a high broth of nutrient, high concn of O_2.

→ As the microorganisms break down the nutrients, they release the desired enzyme in soln.

Solid state form :- It involves the cultivation of microorganisms on a solid substrate such as grains, wheat bran, sugar beet pulp wheat and corn flour.

* Factors which affect fermentation

(*) Temp, pH, dissolved O_2.

(*) Nature and composition of the medium

(*) Shear rate in fermenter.

- Time - 3-5 days
  Temp - 37°C

→ Bacillus Sp. produces extracellular protease during late exponential growth phase

→ Some species produces during exponenti-
al

growth phase.

④ Recovery :- filtration or Centrifugation
↓
Aqueous portion is Concentrated by evaporation at reduced pressure not less then 40°C.

+ alternate recovery procedure the proteases can be precipitated from aqeous soln by addition of cold acetone ethanol, isopropanol or ammonium sulphate.
↓
for drying use -vacuum or spray drying.

Application

① Dairy industry :- In Cheese prepn.

② Baking industry :- when protease are mixed in flour dough, they reduce mixing time and generate a bread loaf with better texture.

③ Soy Sauce production :- on industrial scale proteases and amylase are used in digestion of Lefflated Soyabean in salt brine.

④ Brewing industry :- To solubilize protein from barley adjuncts.

(5) Pharmaceutical → Treating wounds burns, blood treatment (lymphophytic leukaemia)

(6) Meat tenderization → Exogenous proteases such as collagenase, aspartic proteases thermomobile proteases are being used commercially to improve tenderness of meat.

(7) Industrial production of Aspartame →

Aspartame is artificial non carbohydrate, zero calorie sweetner. Enzymatic synthesis of aspartame is carried out by protease.

(8) Leather industry → Selective hydrolysis of non collagenous constituents of skin and for removal of non fibrillar protein.

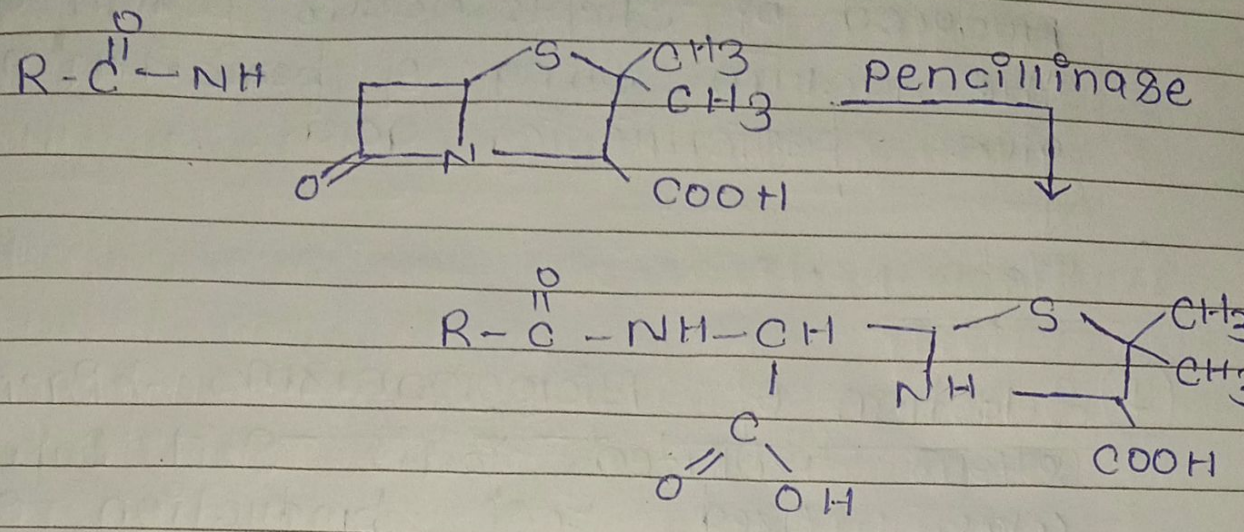
(9) Detergent industry → For cleaning contact lenses to facilitate removal of large variety of stain of oil, blood, grass and body secretion.

Penicillinase

→ Also termed as β-lactomase.

→ This enzyme disrupt the internal

Structure of antibiotic penicillinase and destroy the antimicrobial action of the drug.



→ So these enzymes break β-lactum ring and provide resistance to bacteria towards β-lactum antibiotics like- Penicillin, Cephalosporins and carbapenems Cephamycin.

Penicillinase types

Penicillin amylase or amidase

Produced by Aspergillus. penicillium, mucor

Lactamase

→ They act on amide bond through which the acyl group is attached to the

basic nucleus ie aminopenicillionic acid.

Lactamase

Produced by Streptococcus which attack the lactum ring of penicillin and give penicillionic acid.

Steps

(1) Selection of Microorganisms → Bacillus cereus isolated form soil sample was used for production of penicillinase.

(2) Formulation of medium

→ glucose / glycerol - 2gm / lit

→ Polypeptone - 5gm / lit

→ Yeast extract - 5gm / lit

→ KH_2PO_4 → 1gm / lit

→ $MgSO_4 \cdot 4H_2O$ → 0.2g

PH - 9.0 with $NaHCO_3$

Optimum temp - 30-37°C.

(3) Production → 19 hours old inoculum culture used for fermentation.

→ Glucose or glycerol (0.2%) added.

→ pH, 4, Temp - 30°C for 23 hr on rotatory Shakers.

→ Benzyl penicillin used as an inducer time - 4-5 days.

(4) Recovery → Centrifuged

↓

At pH 6.5, filtrate were stirred in cold for 30 min with 50 gm of Hyposorb gel.

↓

Super gel was suspended in ammonia water with mechanical stirring and elution carried out in cold for 30 min

↙

Add $(NH_4)_2SO_4$ ⟶ Tiny flocules are removed by glass filter.

↓

Lyophilized.

Application

(1) Medicinal use - penicillinase used in treatment of penicillin induced allergies

like – Serum sickness acute arterial and non-arterial etc.

2) Analytical use – for measuring human protection in pharma.

3) Sterility testing of bulk antibiotics – to neutralize the antimicrobial activity of bulk industries.

4) For testing of the microbiological cleanliness of antibiotic manufacturing facilities – Contact plates, flow plates or settle plates containing penicillinase to neutralize the antimicrobial activity.

Basic principles of Genetic Engineering

Genetic Engineering primarily involves manipulation of genetic material (DNA) to achieve desired goal.

Other terms used in Genetic Engineering

* Gene Manipulation
* Recombinant (or D.N.A) DNA technology
* Gene Modification
* New Genetics.

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principles of Genetic engineering (Recombinant D.N.A technology).

1) Generation of DNA fragments and selection of desired piece of D.N.A with the Help of restriction endonuclease enzymes.

2) Inserted of Selected D.N.A into Cloning Vector (e.g - plasmid) to create a recombinant D.N.A or chimeric D.N.A

3) Introduction of recombinant vector into host cell (Bacteria).

4) Multiplication and selection of clones containing recombinant molecules.

5) Expression of the Gene to produce the desired product.

Genetics tools used for G. technology

1. Desired Gene
2. Restriction Endonuclease Enzyme. (for cutting at particular site)
3. Cloning vector
4. Ligase enzyme - for ligation of vector DNA with desired piece of DNA.
5. Host Cell (Bacteria, Bacteriophage virus, Yeast.