SCIENCE TECHNICS SDN BHD

A BEGINNERS GUIDE TO INDUSTRIAL ENZYMES

Part I : ENZYMOLOGY

August 2017

- There is a sizeable global market for industrial enzymes
- Thousands of tons of industrial enzymes are produced and used annually

- These industrial enzymes are:

i. Powder enzymes sold in boxes or drums

ii. Liquid enzymes sold in Jerry cans or drums

- This presentation is a beginner's guide to industrial enzymes:
  - What are enzymes?
  - How do they act?
  - What are their properties?
  - Historical Development of the Enzyme Industry
  - How are Industrial enzymes produced?
  - How are enzymes used in industry?
- A separate presentation summarises some of the many uses of industrial enzymes. This is Part II.

What are Enzymes?

- Enzymes are natural catalysts produced and used by all living organisms
  - Animals
  - Plants
  - Microorganisms

- Enzymes facilitate or enable living organisms to carry out the many reactions required for life to function

- All living things produce and use enzymes

- Without enzymes life will not be possible

- When we consume food, there are enzymes in our body which digest the food to smaller components

- Example
  1. Enzymes called Amylases digest starch to Glucose
  2. Enzymes called Proteases digest proteins to Amino Acids
  3. Enzymes called Lipases digest fats to Fatty Acids and Glycerol

- The components like Glucose, Amino Acids, Fatty Acids and Glycerol are absorbed in the gut and carried by blood to all parts of the body

- In the body other enzymes utilise the components
- Some of the components are metabolised or oxidised to produce energy for the organism
- Some of the components are used to build up the body mass of the organism

- In a typical organism (Animal or Plant or Microorganism), there will be hundreds of different reactions being carried out
- For each of these reactions there will be a specific enzyme required
- Thus every living organism will produce hundreds of different enzymes to carry out its life functions

What are Enzymes made of?

- Enzymes are made of proteins
- We are familiar with structural proteins that we see as ‘Flesh’ or ‘Meat’
- We are familiar with storage proteins

  Eg.: Proteins in egg white – Albumin

  Proteins in plants like beans

- Proteins are made up of chains of Amino Acids. About 20 Amino Acids are present in Proteins
- Different proteins have different sequences of Amino Acids
- These protein chains can be very long. These are the structural proteins

- But there are also ‘Functional’ Proteins. These are shorter chains of Amino Acids and are soluble

- These Functional Proteins carry out various functions in the body

- Example:

  ANTIBODIES – Give immunity to the body

  HORMONES – Act as ‘Signallers’ in the body

  ENZYMES – Facilitate reactions in the body

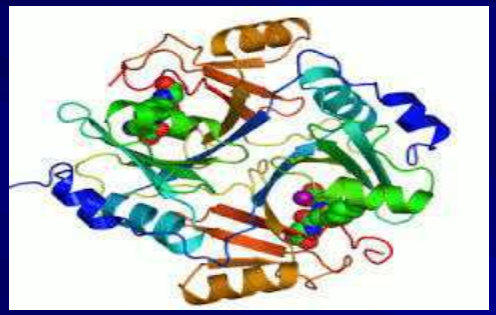
Thus

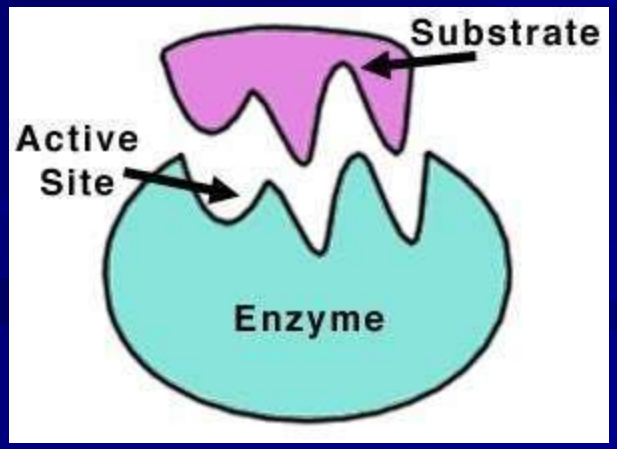
- An enzyme is a soluble protein
- It is a short chain of Amino Acids with a definite sequence
- When an organism has a need for a particular enzyme it produces many molecules or units of the particular protein molecule (enzyme)

- When a particular enzyme molecule is produced in folds itself into a very definite shape

- This very definite shape is brought about by
  - The shape of the component Amino Acids
  - The sequence of the component Amino Acids
  - Cross linkages between different Amino Acids

- The shape of a typical enzyme molecule is as follows:





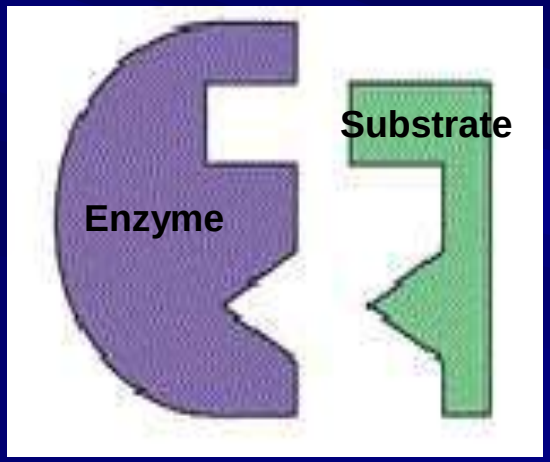
- There is an active site where the reaction takes place

- Adjacent to this is the part of the enzyme that recognises the correct target molecule

- Other parts of the enzyme maintain the structure of the enzyme

Mechanism of Enzyme Action

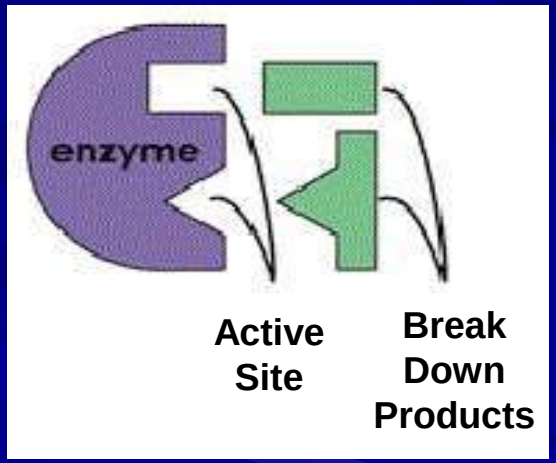
BEFORE REACTION



DURING REACTION

Breaking Down Substrates

AFTER REACTION

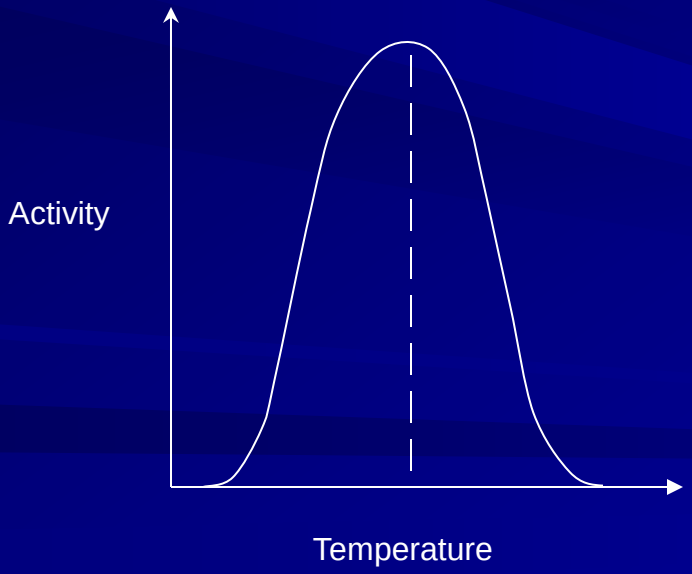


Properties of Enzymes

- Enzymes are proteins produced by living organisms
- Enzymes are not living things
- In this sense enzymes cannot be “killed”

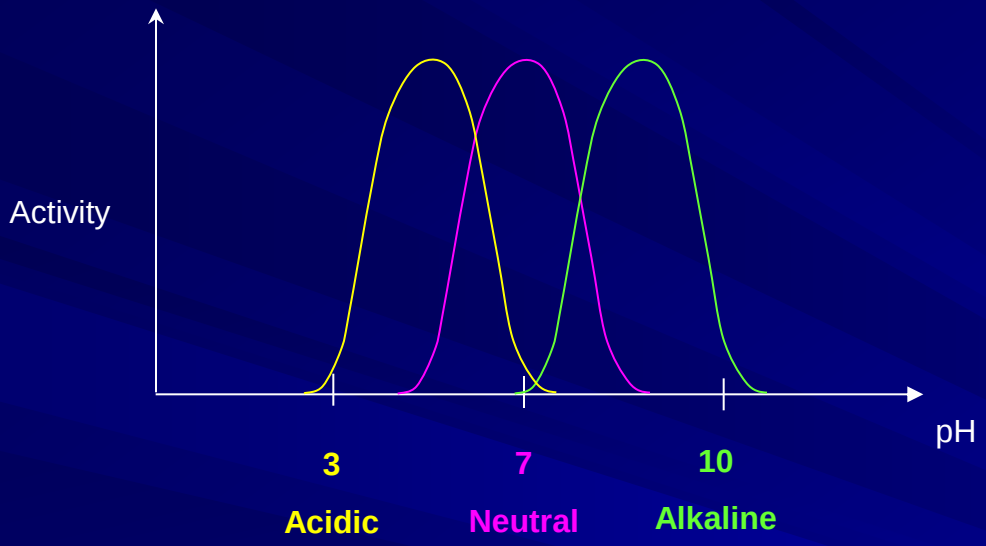
- Enzymes depend on their shape to function
- If an enzyme is subjected to adverse conditions (e.g. high temperature, extreme acidity or alkalinity or chemicals that “poison” the enzymes) the enzymes shape may be changed. The enzyme is “denatured”.
- Denatured enzymes lose their activity and are said to be “inactivated”

- The activity of an enzyme depends on several factors
- Each enzyme has an optimum temperature at which it acts



- Animal based enzymes usually have an optimum temperature near body temperature
- Plant based enzymes can have optimum temperature of over 50-60°C
- Microbial enzymes can have optimum temperature of 80-100°C

- Each enzyme has an optimum pH at which it acts



- **Acid Proteases** hydrolyse proteins under acidic conditions
  - e.g. Pepsin enzyme in stomach
- **Neutral Proteases** break down proteins under neutral conditions
- **Alkaline Proteases** hydrolyse proteins under alkaline conditions
  - e.g. Trypsin in intestines

- Some chemicals help an enzyme to work better. These chemicals are called “enzyme activators”
- Some chemicals reduce or even completely inactivate enzymes. These chemicals are called “enzyme inhibitors” or “enzyme poisons” respectively

Historical Development of Enzyme Industry

- Long before we knew about the structure and mechanism of action of enzymes, mankind has been using enzymes

- Eg:

a) They used an extract from calves stomach which contains an enzyme Rennin to produce cheese

b) They used enzyme from papaya and pineapple to soften or tenderise meat.
These enzymes act on the structural protein of meat.

- About 100-200 years ago enzymes were extracted from animals or plants and were offered for sale

- Eg:
  - Rennin – cheese production
  - Papain – meat tenderising
  - Bromelain – meat tenderising
  - Oropon – Trypsin from animal pancreas produce by Rohm

- This was the beginning of Industrial Enzymes

- Such animal or plant enzymes were a good innovation but of limited scope

  - Only a small number of enzymes was obtained in this way

  - With animals enzymes there were religious issues

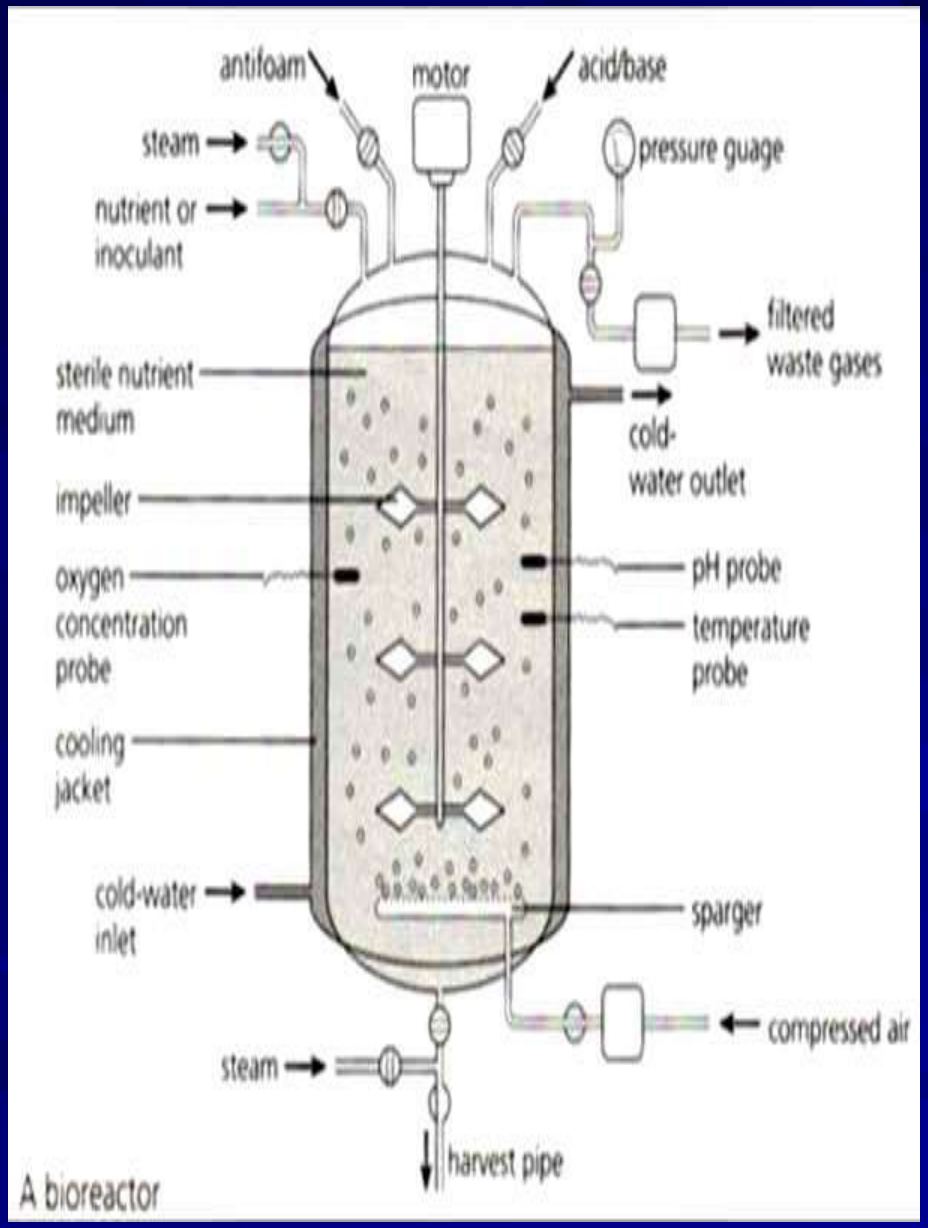
- A Japanese scientist Takamine showed that an amylase enzyme could be extracted from the Koji culture used to produce sake

- This amylase was called Taka-Diastase and was sold as a digestive aid

How are Industrial Enzymes Produced?

- In the period 1940-1960 the production of antibiotics by fermentation was introduced

Today the vast majority of industrial enzymes are produced in similar fermenters or bioreactors.



1. Fermenter is filled with liquid medium suitable for growth of microbe
2. Medium is sterilised by steaming
3. Medium is cooled to microbe cultivation temperature
4. Medium is inoculated with culture of the selected microbe
5. Medium is aerated by sterile air though sparging
6. Medium is agitated by impellers or paddles
7. pH is kept within limits by acid/alkali
8. The microbe secretes the required enzyme into the medium
9. When enzyme level reaches a maximum, the contents of the fermenter are drained for processing

Final Processing of Enzyme Produced

A) Concentrate till a concentrated enzyme solution is obtained. Add stabilisers and preservatives. Pack

B) The enzyme solution is dried to a powder or granules

How are Enzymes Used in Industry?

- Basically enzymes catalyse or promote very specific reactions

- Enzyme companies look for the most appropriate enzyme
- Thousands of microbes are screened for their ability to produce the desired enzyme
- The shortlisted producers are optimised and a final selection of producing microbe made on basis of:

  - Yield and Activity
  - Stability
  - Optimum Temperature
  - Optimum pH
  - Resistance to poisoning
  - Etc.

- There are four variables that affect enzyme performance:
  - Dosage of enzyme
  - Temperature
  - Duration of Reaction
  - pH of the reaction
- In some applications (e.g. hydrolysis of starch to glucose) it will be possible to adjust the reaction pH to the enzymes optimum pH
- In some application (e.g. baking) it will not be possible to adjust pH. Baking is carried out at optimum pH for the yeast. Therefore the enzyme selected must work at the same pH as optimum for yeast work

- As a result often the effectiveness of an enzyme in industrial operations is an interplay of: