## Enzyme Technology as Analytical Agent

Mata kuliah : Kapita Selekta

Semester 8

**Enzyme Technology** 

Enzyme technology is concerned with the application of enzymes as tools of industry, agriculture and medicine

Enzymes are biological catalysts that fulfil their role by binding specific substrates at their active sites

This **specificity** is one property of enzymes that makes them useful for industrial applications

The value of using enzymes over inorganic catalysts in the technological field is their efficiency, selectivity and specificity **Enzyme Technology** 

Enzymes are able to operate at room temperature, atmospheric pressure and within normal pH ranges (around 7) – all of which create energy savings for industry

Enzymes possess specifically shaped active sites for reacting with one specific substrate thereby generating pure products free from unwanted by-products

Enzymes are biodegradable and, unlike many inorganic catalysts, cause less damage to the environment

### Enzimes in Industry



#### **Products of Enzyme Technology**



Micro-organisms have been used for thousands of years for making products such as wine, beer, vinegar, soy sauce, bread and cheese

The micro-organisms (such as yeast) are really used as a source of enzymes during the manufacture of these products of biotechnology

Many industrial processes now make use of pure sources of enzymes, i.e. the enzymes have been ISOLATED from the micro-organisms before use

#### Microbial enzymes are ISOLATED from a variety of sources and these include bacteria, fungi and yeast cells



Micro-organisms produce enzymes that function inside their cells (intracellular enzymes) and they may also produce enzymes that are secreted and function outside the cells (extracellular enzymes)

#### Isolation and Identification of Proteolytic Bacteria from Pig Sludge and Protease Activity Determination

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Presented on :



#### IOP Conference Series: Earth and Environmental Science

#### PAPER • OPEN ACCESS Isolation and identification of proteolytic bacteria from pig

#### sludge and protease activity determination

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Activate Materials and S

## Result

**Bacteria isolation** 

Isolation of bacteria from 3 types of mud obtained 10 bacterias :

Origin of Mud	Amount of	
	Bacteria	
Farm I	6	
Farm II	2	
Wild pig	2	







## Qualitative Test for proteolytic bacteria



2-3 days at 37°C

protein (casein) are shownthrough clear zones around the colonies

#### *Identification of Proteolytic Bacteria*

## Qualitative test of proteolytic bacteria

Origin of Mud	Bacteria no	Clear zone	Proteolytic Index
	1	-	-
Farm	2	-	-
Ι	3	-	-
	4	±	-
	5	-	-
	6	-	-
Farm	7	-	-
II	8	+ +	0.075
Wild	9	+++	0.625
pig	10	+ +	0.561

## Result



Bacteria no. 8 Bacteria no. 9 Bacteria no. 10

## 16s rRNA sequencing

Macrogen . Inc. South Korea

Universal primer

**Sequencing Primer** 

**PCR Primer** 

785F 5' (GGA TTA GAT ACC CTG GTA) 3' 907R 5' (CCG TCA ATT CMT TTR AGT TT) 3' 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

#### **16S rRNA** *Results*









Phylogenetic Trees Results of alignment of 16srRNA bacteria *Bacillus pseudomycoides* 

Phylogenetic Trees Results of alignment of 16srRNA bacteria *Staphylococcus sciuri*.

#### **Large Scale Production of Enzymes**

#### The large scale production of enzymes involves culturing micro-organisms in chambers called FERMENTERS or BIOREACTORS

Micro-organisms are suitable for use in the large scale production of enzymes in fermenters because:

- They have rapid growth rates and are able to produce larger numbers of enzyme molecules per body mass than many other organisms
- Micro-organisms can be genetically engineered to improve the strain and enhance yields
- Micro-organisms are found in a wide variety of different habitats such that their enzymes are able to function across a range of temperatures and pH
- Micro-organisms have simple growth requirements and these can be precisely controlled within the fermenter
- Micro-organisms can utilise waste products such as agricultural waste as substrates

#### **The Biotechnological Process of Enzyme Production**



#### **Enzymes in Biotechnology**

**Enzymes are used in industrial processes and as analytical reagents in medicine** 



Thermostability and an ability to withstand extremes of pH are essential properties for enzymes used in many industrial processes

**Immobilisation of enzymes is an important technique used** in industry as it enables economical operation of a process and protection of enzymes during their use

Because of their sensitivity and specificity, enzymes are used as analytical reagents in systems such as the detection of glucose in human blood and urine

#### **Immobilised Enzymes**

The costs associated with the use of enzymes for industrial purposes can also be reduced by immobilising the enzymes

**Enzymes for industrial processes are more valuable when** they are able to act in an insolubilised state rather than in solution

Enzymes are immobilised by binding them to, or trapping them in a solid support

Various methods for immobilising enzymes are available





#### Immobilization of *Bacillus megaterium* in Carrageenan from Maluku Sea and Their Effect on Protease Production

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Presented on : 1<sup>st</sup> ISoP 2018











Activity Protease Test Total Plate Count **Proteolitic Test** 

Immobilization

## **Growth Factor**

## Result

Activity Protease Test Total Plate Count Proteolitic Test



**Growth Factor** 





Activity Protease Test Total Plate Count

# Activity Protease Test



MLE

Mik

N-

MLL

N-







#### **Advantages of Immobilising Enzymes**

Compared with free enzymes in solution, immobilised enzymes have a number of advantages for use in industrial processes

The stability of many enzymes is increased when they are in an immobilised state; they are less susceptible to changes in environmental conditions such as temperature and pH fluctuations

> Immobilised enzymes can be recovered and re-used, reducing overall costs

The products of the reaction are not contaminated with enzyme eliminating the need to undertake costly separation of the enzyme from the product

Immobilising enzymes allows for continuous production of a substance with greater automation

#### **Enzymes as Analytical Agents**

The sensitivity and specificity of enzymes makes them useful tools in medicine for the detection and measurement of chemicals in fluids such as blood and urine

Because of their specificity, enzymes will bind to only one substrate – they can therefore be used for the identification of a specific substance in a biological sample

Because of their sensitivity, enzymes are able to detect the presence of specific molecules even when they are present at very low concentrations

The enzyme glucose oxidase is used in an immobilised form for the detection of glucose in biological fluids

## Thank You

Next... BIOSENSOR