

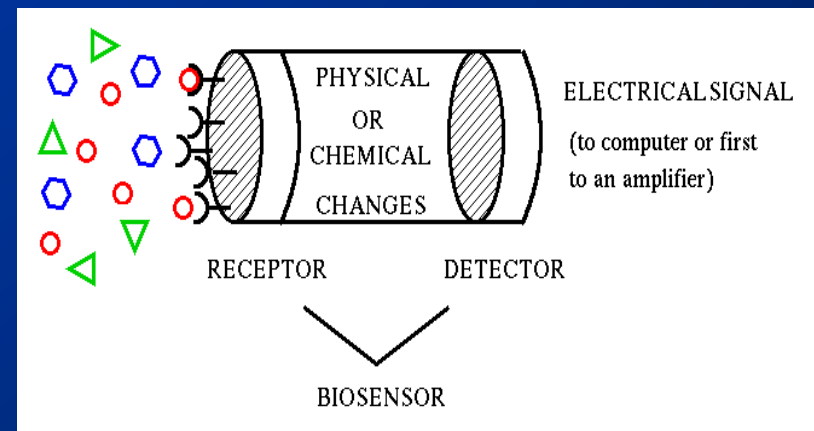
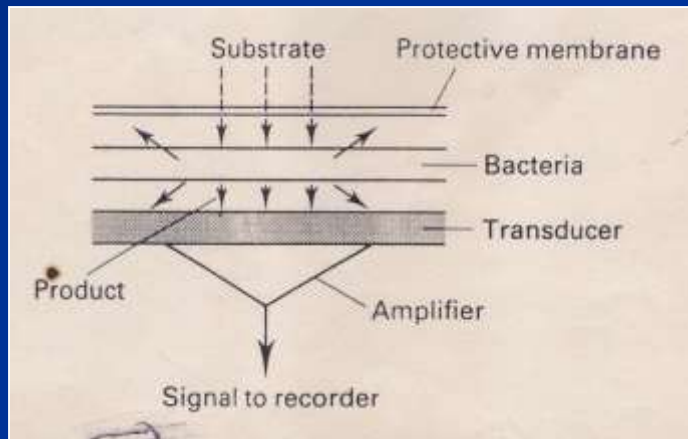
BIOSENSOR AS A TOOL FOR QUALITY CONTROL IN DAIRY INDUSTRY

Introduction

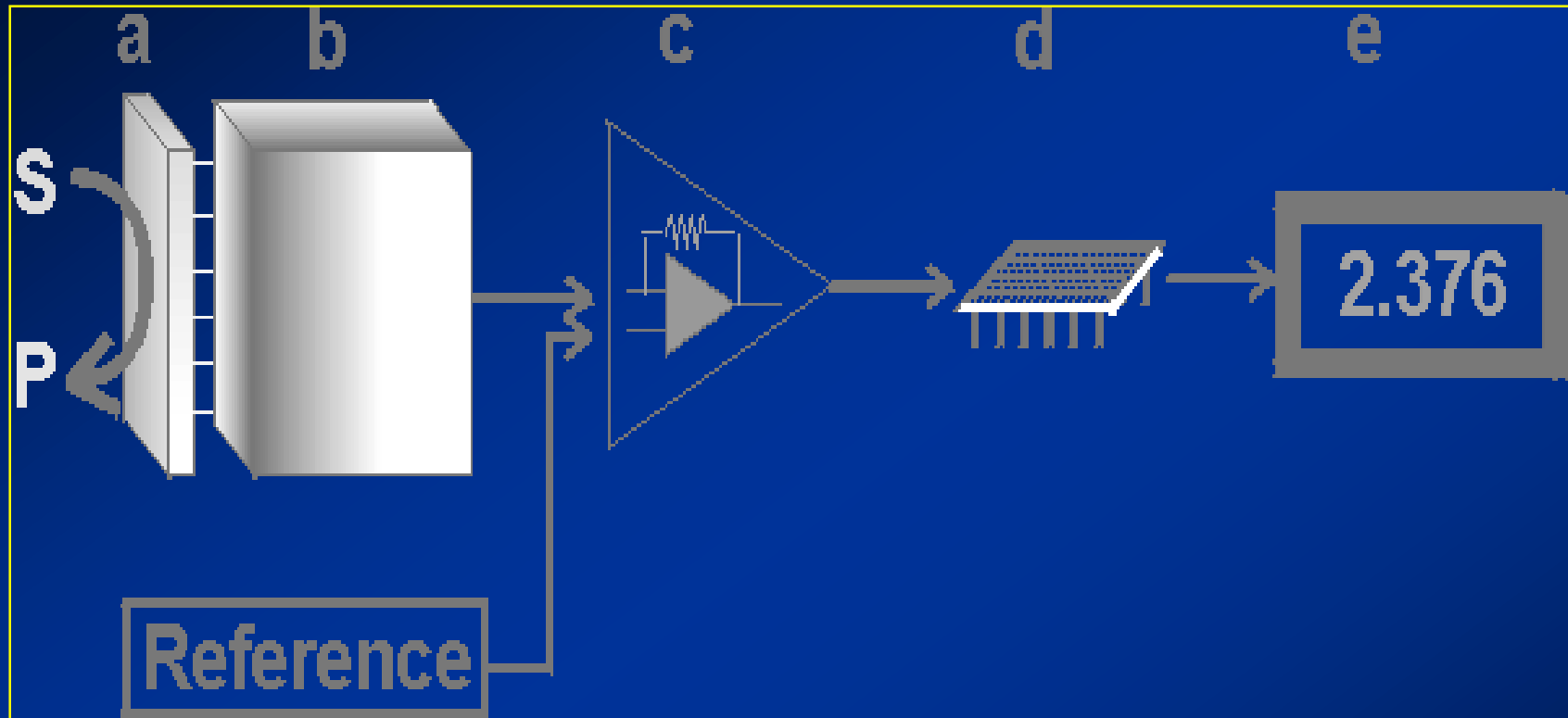
- ❖ Existing inherent problem with continuous analytical techniques used in dairy industry
 - ✓ Specificity
 - ✓ cost
 - ✓ Longer response time
 - ✓ Highly skilled personnel

WHAT IS BIOSENSOR

- “Biosensor is a compact analytical device incorporating a biologically or biologically derived element either integrated with in or intimately associated with a physicochemical transducer”.
- Specific quantitative or semi quantitative analytical information
- Produce discrete, continuous signal ,Proportional to single or related group of analyte



Components of Biosensors



- a → Biocatalyst
- b → Transducer
- c → Amplifier
- d → Processor
- e → Displayer

Brief history of biosensors

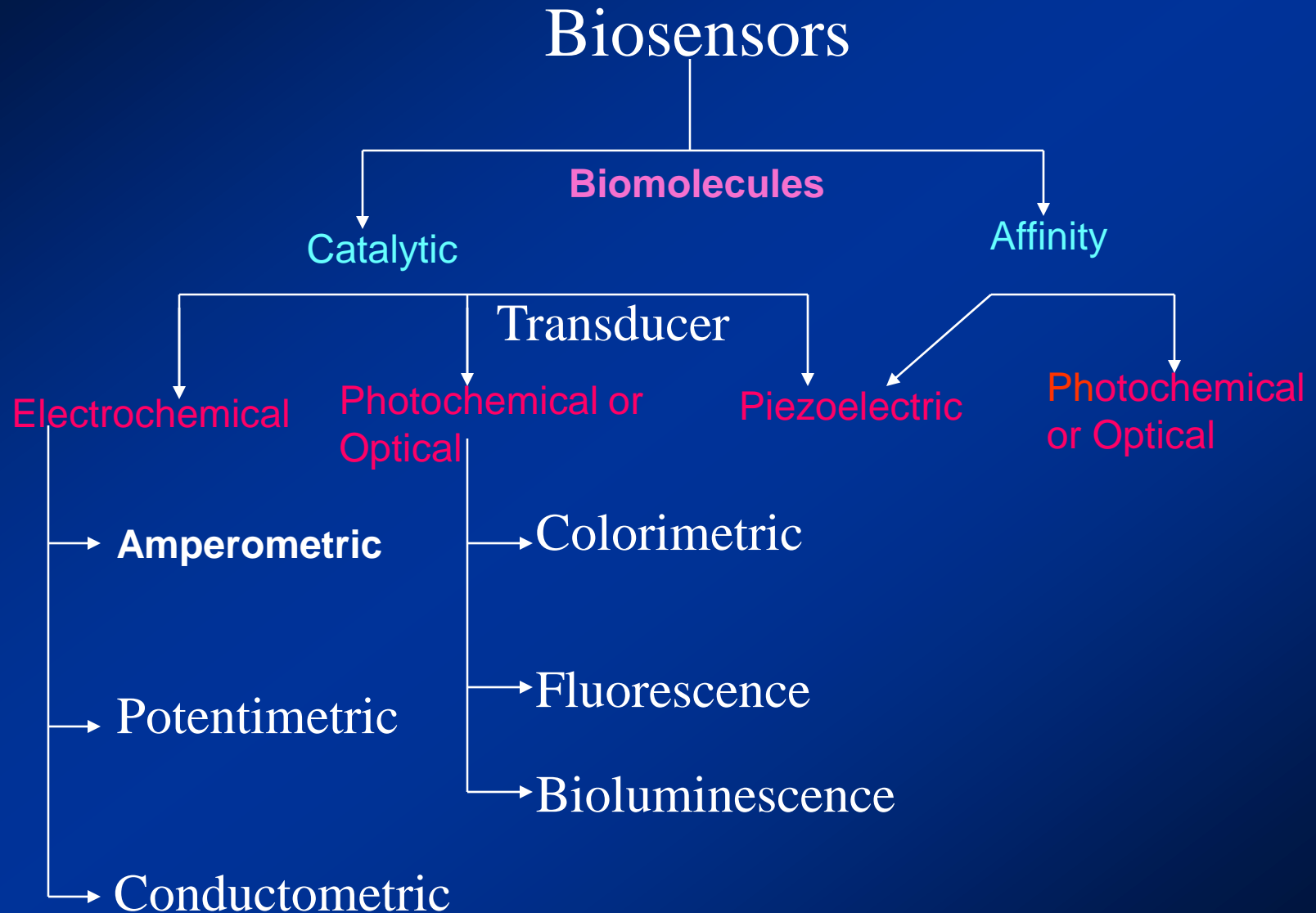


- **Prof. Leland C. Clark** – Father of biosensors concept
- 1956-published oxygen electrode
- 1962 – make electrochemical sensors, coined the term enzyme electrode
(**Clark & Lyons**)
- Guibault & Montalvo – Potentiometric Enz. Electrode
(**urea sensors**)
- 1975 – optode – Lubbers & optiz – fiber optic sensors + bacterial sensors
(**Divie**)
- 1990 -Surface plasmon resonance –launched by BIOcore
(**Liedbera et al**)
- 1998-Launch of LifeScan Fast Take blood glucose biosensor
- NanoSensor
(**Vo-Dinh 2000**)

Ideal biosensors characteristics

- ★ Sensitivity
- ★ Simple calibration (with standards)
- ★ Linear Response
- ★ Background Signal: low noise, with ability for correction
- ★ No hysteresis
- ★ Selectivity
- ★ Long-term Stability
- ★ Dynamic Response

Classification

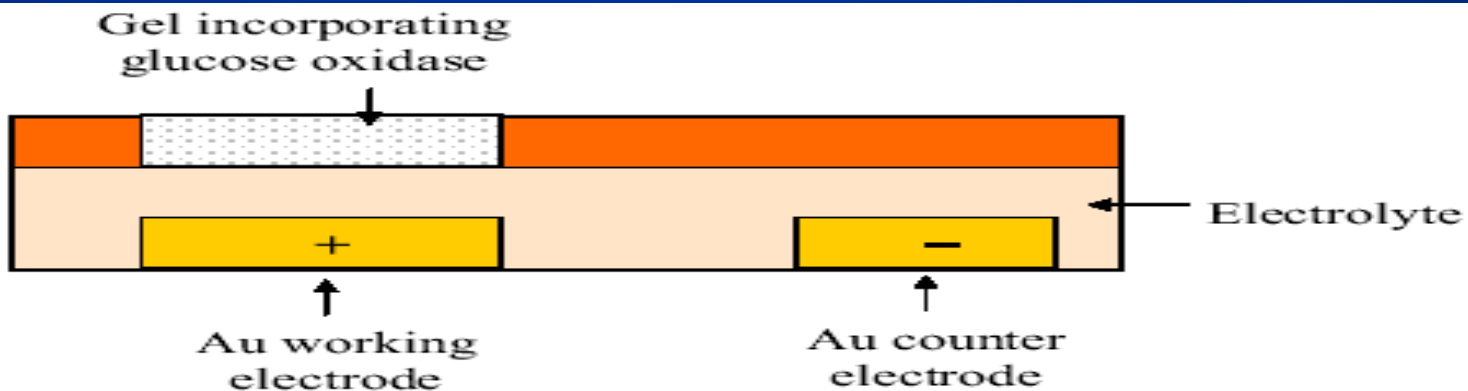
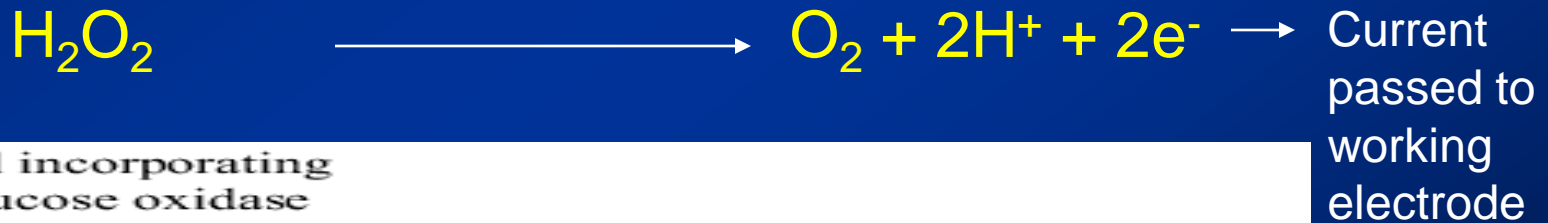


Electrochemical Biosensors

❖ Amperometric biosensors

- Fixed potential – reference electrode
- Detection of current – oxidation or reduction species

E.g. Glucose sensor based on oxidation of peroxide & BOD



❖ Potentiometric biosensors

- Ion selective electrode – pH, ammonium, chloride
- Gas sensing electrode – P_{CO_2} , P_{NH_2} - immobilized microbe layer

E.g. Modified glass pH electrode with genetically engg. *E.coli* for detection of organophosphorous

❖ Conductometric biosensors

- Reaction – change in ions – net change in conductivity
- Impedance method for checking quality of Raw milk

(Lei *et al.*,2006)

Photochemical biosensors

Chemical reaction \longrightarrow Photochemical reaction

Measures – light intensity & wave length

Types

❖ **colorimetric biosensors**

measure absorption intensity

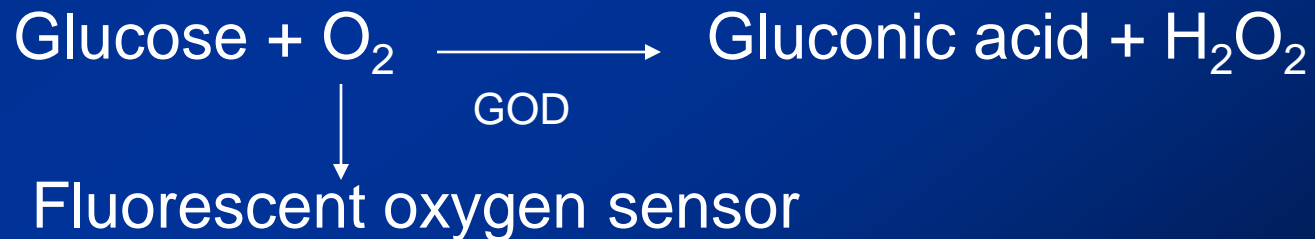
E.g. simple fibre optic biosensor - genetically engg. *E.coli*

organophosphates $\xrightarrow{\text{OPH}}$ on absorbance of PNP

❖ Fluorescence biosensor

- Fluorescent material and green fluorescent protein
- Sensitive technique – low concentration
- Fluorescence emission intensity

Ex: oxidation of glucose



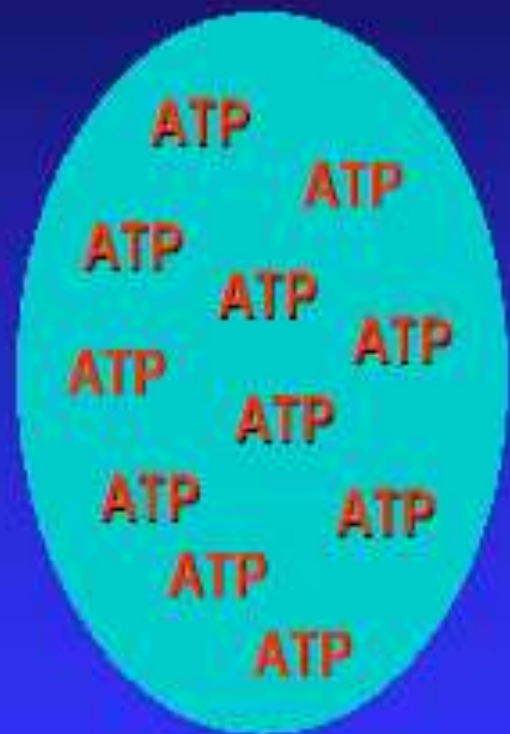
(Lei et al., 2006)

Bioluminescence

➤ Principles of ATP Bioluminescence

- ✓ Adenosine triphosphate (ATP): universal energy donor
- ✓ Living cells have ATP in relatively consistent amounts
- ✓ Enzymes in firefly converts chemical energy (ATP) to light
- ✓ Amount of light is directly proportional to amount of ATP present
- ✓ Not specific for microbial ATP
- ✓ 1 Photon of Light = 1 Molecule of ATP 1
- ✓ Light output is measured in Relative Light Units (RLU)

ATP Bioluminescence



Living cells
from swab

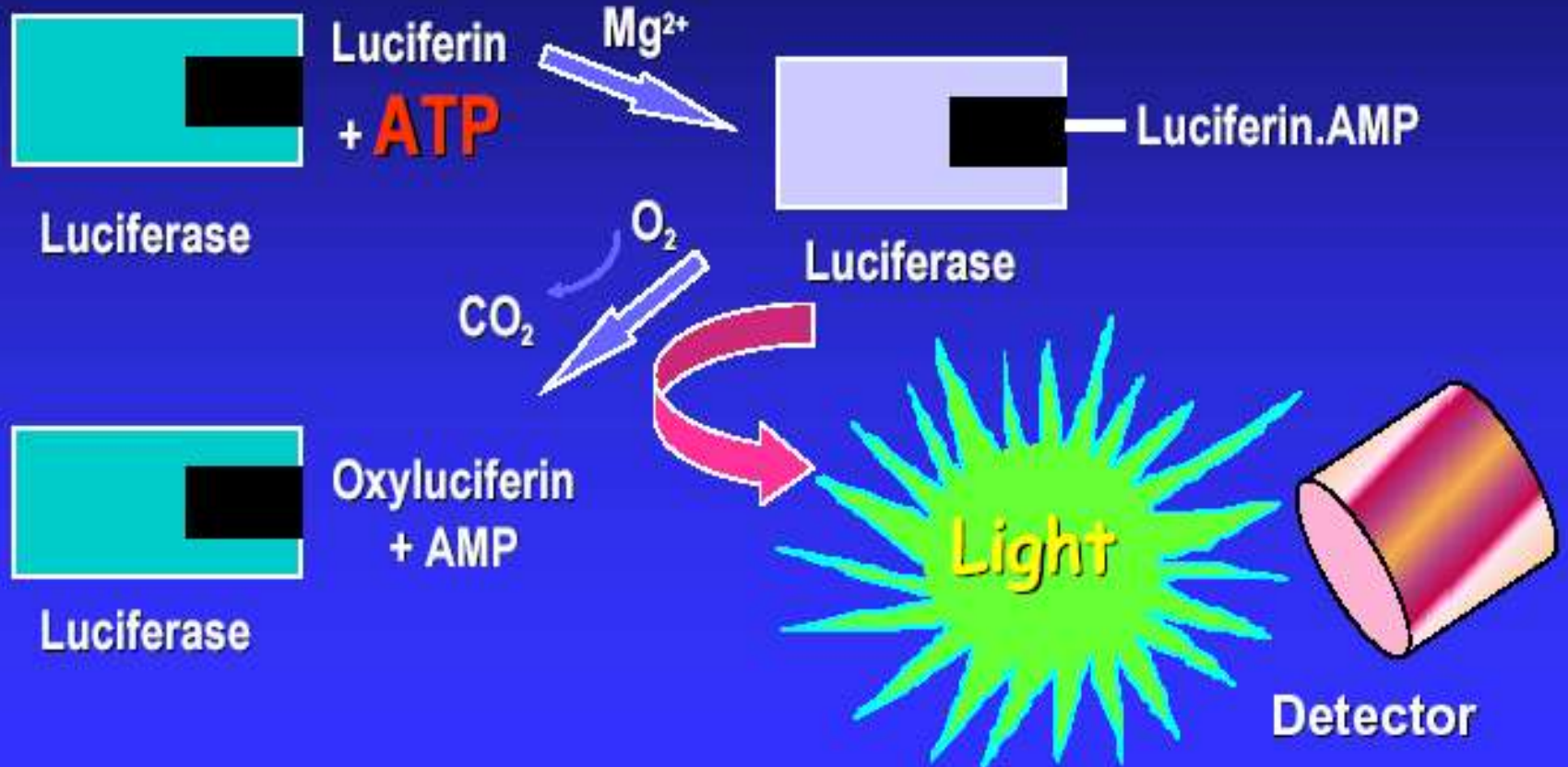


Extractant
ruptures cell



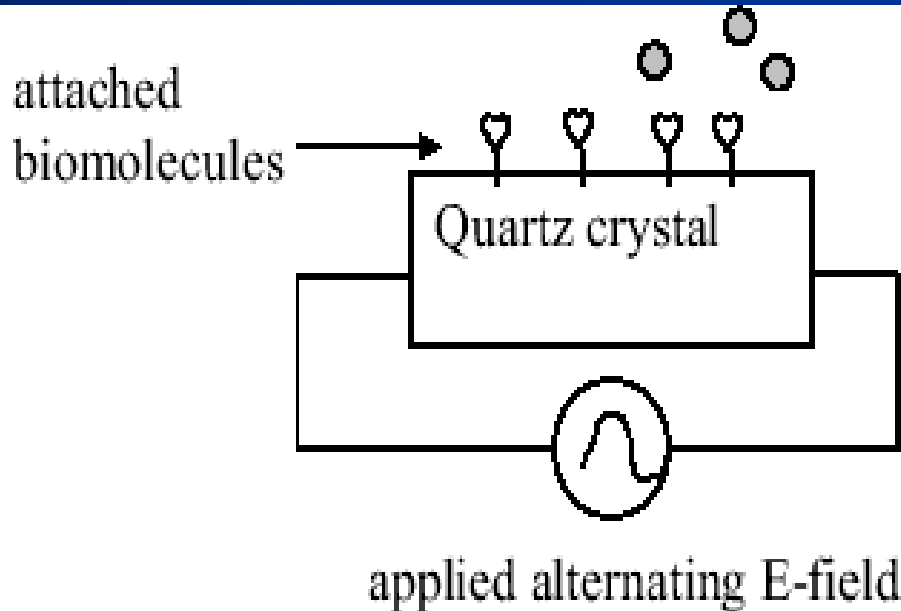
ATP released

The ATP Bioluminescence Reaction



Piezoelectric

- ❖ Mass change from a chemical adsorption in to electrical signal
E.g. : immunosensor for Mycobacterium antigen detection



- Crystal vibrates at resonant frequency parallel to applied field:

$$v = (k/m)^{1/2}$$

typical: 5 MHz;

research grade: 100-200MHz

Immobilization of biomolecules

❖ Physical method

➤ Adsorption

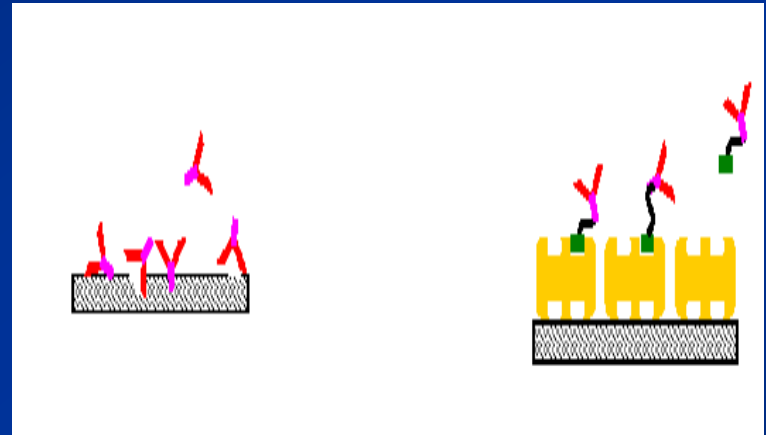


✓ Simplest method

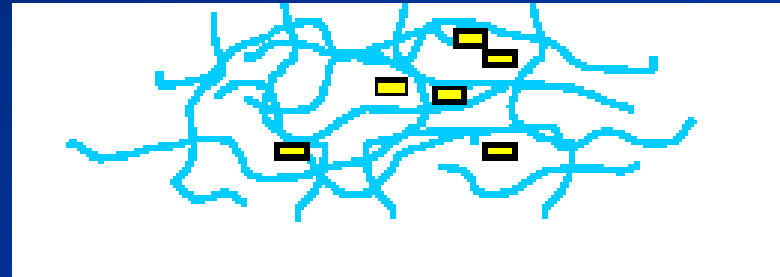
✓ Microbial suspension plus
electrode or immo.matrix incubated

✓ Adsorptive interaction (ionic, polar or H-bonding,
hydrophobic interaction)

✓ Poor stability



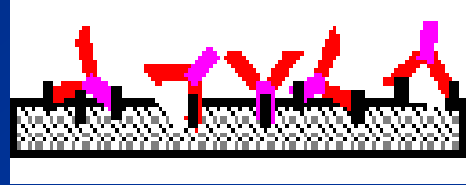
➤ Entrapment



- ✓ Retention cell in contact with transducer surface by using gels or polymers
- ✓ Gels – alginate, PVC, agarose, chitosan, collagen, PV alcohol, polyacrylamide
- ✓ Disadvantage – lower sensitivity & detection limit

❖ Chemical method

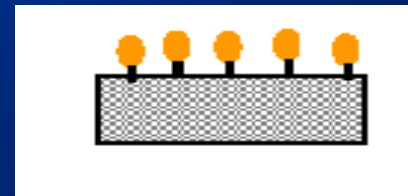
➤ Covalent binding →



✓ Functional groups of the Microbial cellwall components -amines, carboxylic or sulphhydryl

✓ The transducer – amines ,carboxyl, epoxy or tosyl

✓ Loss of biological activity



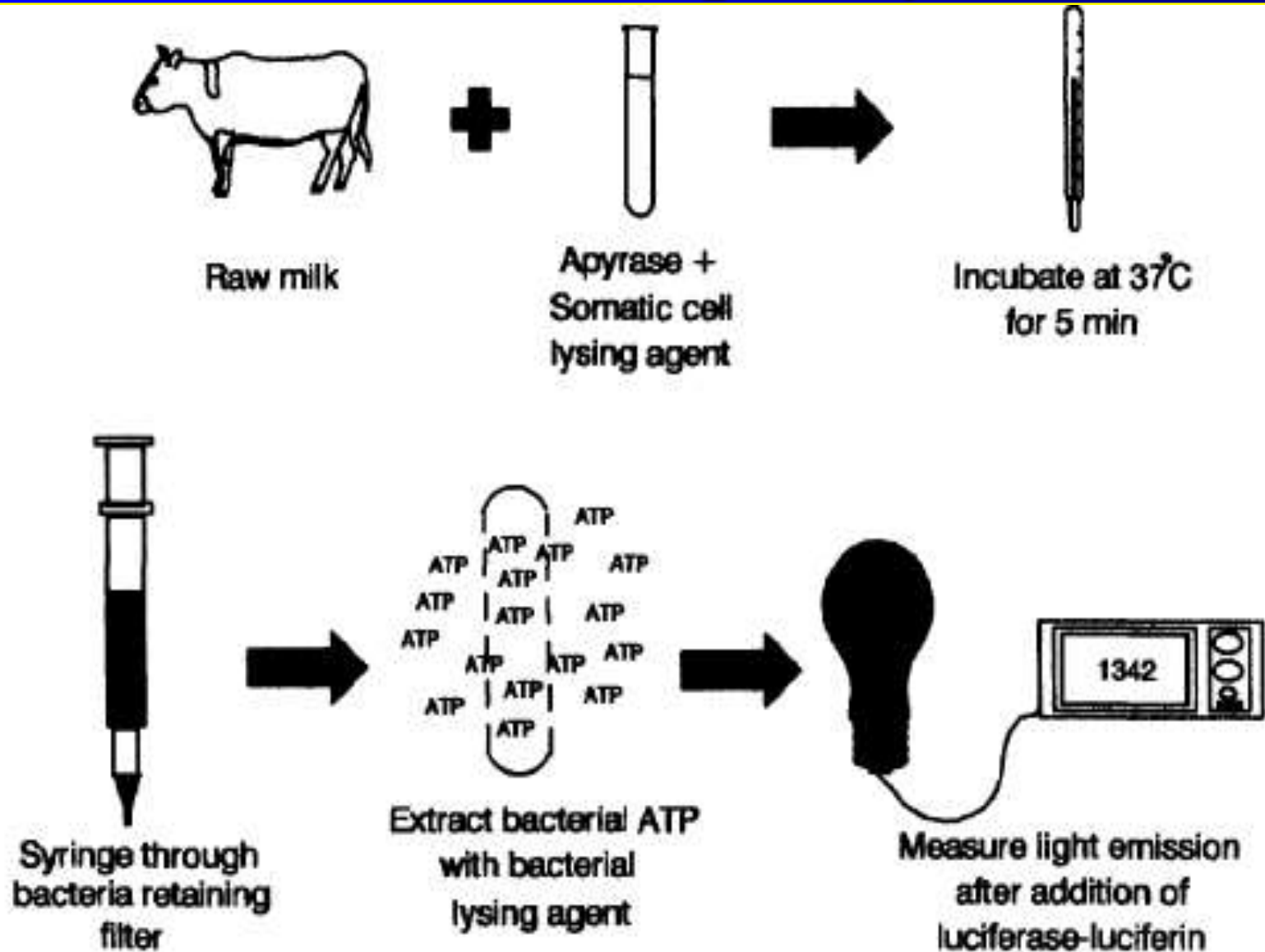
➤ **Cross-linking**

- ✓ Functional groups $\xrightarrow{\text{Bridging}}$ multifunctional reagent
- ✓ Multifunctional reagent – glutaraldehyde ,cyanuric acid
- ✓ Acceptable method

Application of biosensors

- Raw milk quality
- Hygiene monitoring in dairy industry
- Pasteurization efficiency
- Sterility test
- Monitoring of starter activity
- Detergent residues detection
- Evaluation of water quality
- Antibiotics detection
- Fungicides
- Biological oxygen demand
- Pesticides
- Food born pathogen detection

Raw milk quality



Contd...

- ❖ ATP of contaminated bacteria
- ❖ Factor's affecting the sensitivity of bioluminescence
 - Nonbacterial ATP
 - Casein micelles
 - Somatic cells
 - Temperature 25⁰ C
- ❖ Min.detect limit 1 X 10⁴ cells per ml
- ❖ Time : 5-10 min
- ❖ Commercially available tests – Biocrace & lumac

(M W Griffiths.,1993)

Hygiene monitoring

❖ **Cleanness of dairy equipment**

- Swabbing or rinsing
- ATP extraction reagent
- Luciferrin – luciferrase reagent
- Bioluminescence in luminometer

❖ **Swabbing**

RLU - <500 – clean value

>500 – contamination or residue

❖ **Rinsing**

RLU - >200 – residual contamination

>1000 – recleaning

(R K Robinson.,2002)

Pasteurization efficiency

- ❖ Post processing contamination
 - Gram negative Psychrotropic bacteria
 - Preincubation of milk (21⁰ C for 25 hrs)
 - Benzalkon A 50% & crystal violet
 - Penicillin 200 µl & nisin 400U /ml
- ❖ ATP counted by using luminometer

(M W Griffiths et al., 1993)

Evaluation of water quality

❖ ATP bioluminescence

- Total AQUATEST Kit total ATP
- Total ATP = free ATP+ Microbial ATP
- AQUATEST Free Kit free ATP
- Bioluminescence measured in HILYTE Biotrace luminometer
 - ✓ Samples max.150RLU –Good hygienic condition
 - ✓ 300RLU – unhygienic condition (Patricia et al.,2004)

Sterility test

UHT milk & other dairy products

- ❖ Generally determined by PC & pH
- ❖ Time consuming
- ❖ By bioluminescence monitoring growth
 - Storage of the product 30°C for 1 day
 - With apyrase (0.01U)
 - Extraction of ATP
 - Assay
- Understerilized milk detected with in 3 days
- Useful for chocolate milk

Monitoring starter culture activity

- By ATP bioluminescence
 - ATP provides better indication of activity of LAB than pH
 - Strong correlation bet. pH & ATP conc. during growth
 - *L. lactis* & *L. acidophilus*
 - Monitoring of ATP changes
 - Indicate the presence of Antibiotic residues or phage
 - Penicillin Conc. as low as 0.005 U/ml detected with in 90 min

Detergent residues detection

❖ Luminescence

- Cleaning efficiency
- Traces of detergent interfere with the measurement



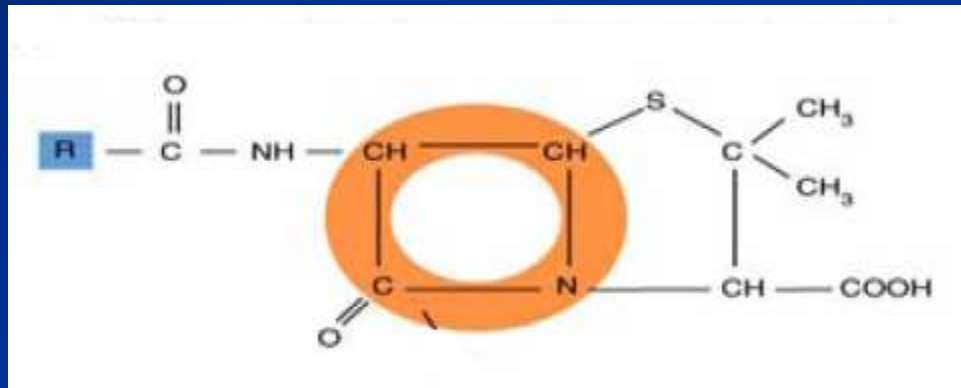
- Calculate the INH % to luminescence activity

$$\text{INH\%} = 100 - \frac{(100 - IT_t)}{IC_t}$$

- < 20% residue is less (detection limit)
- 20-50% less amount
- >50% high amount residue

Detection of Pesticides

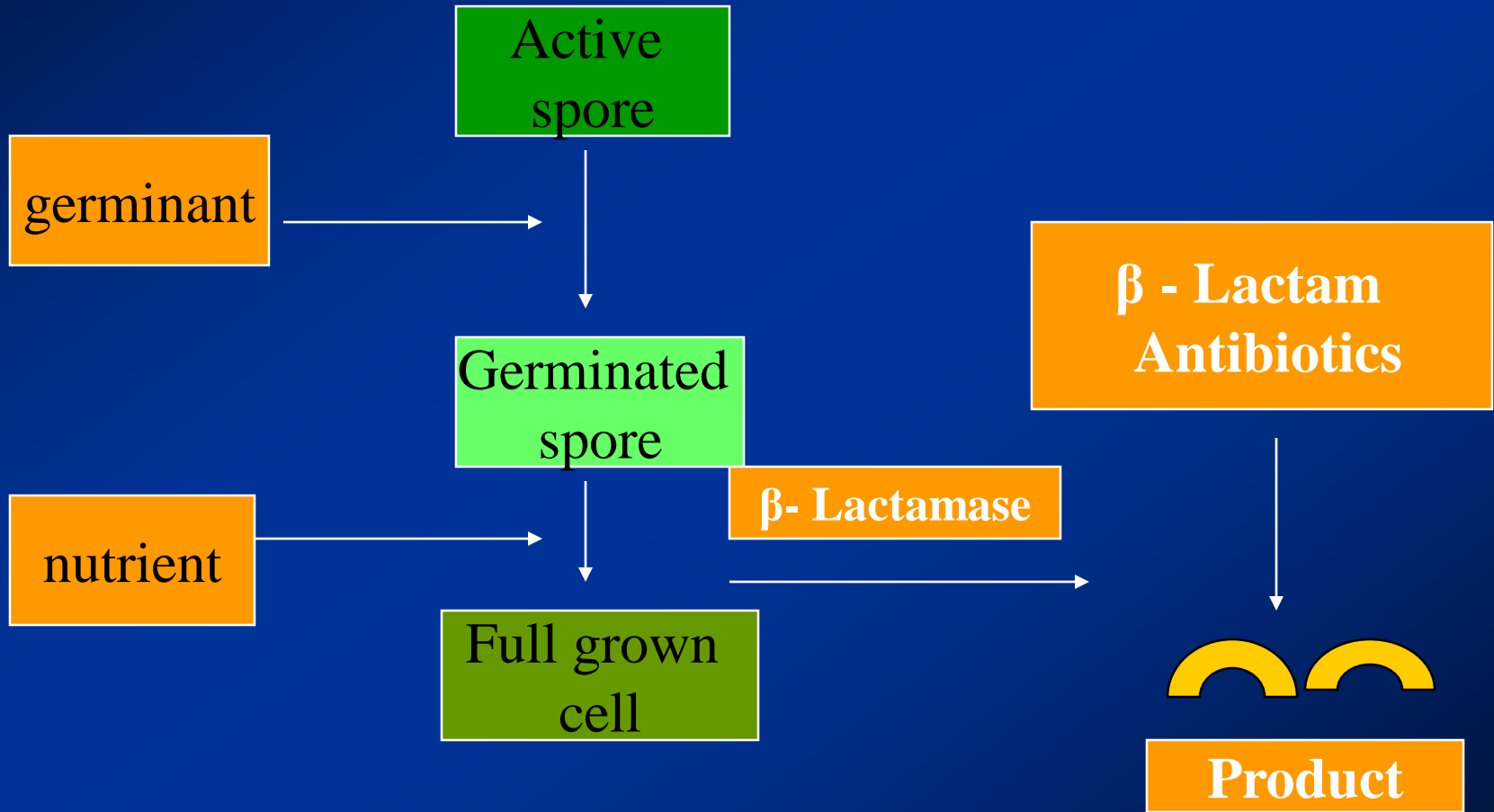
Detection of specific group of β -lactam antibiotics based on β -lactamase enzyme induction by *Bacillus* spp



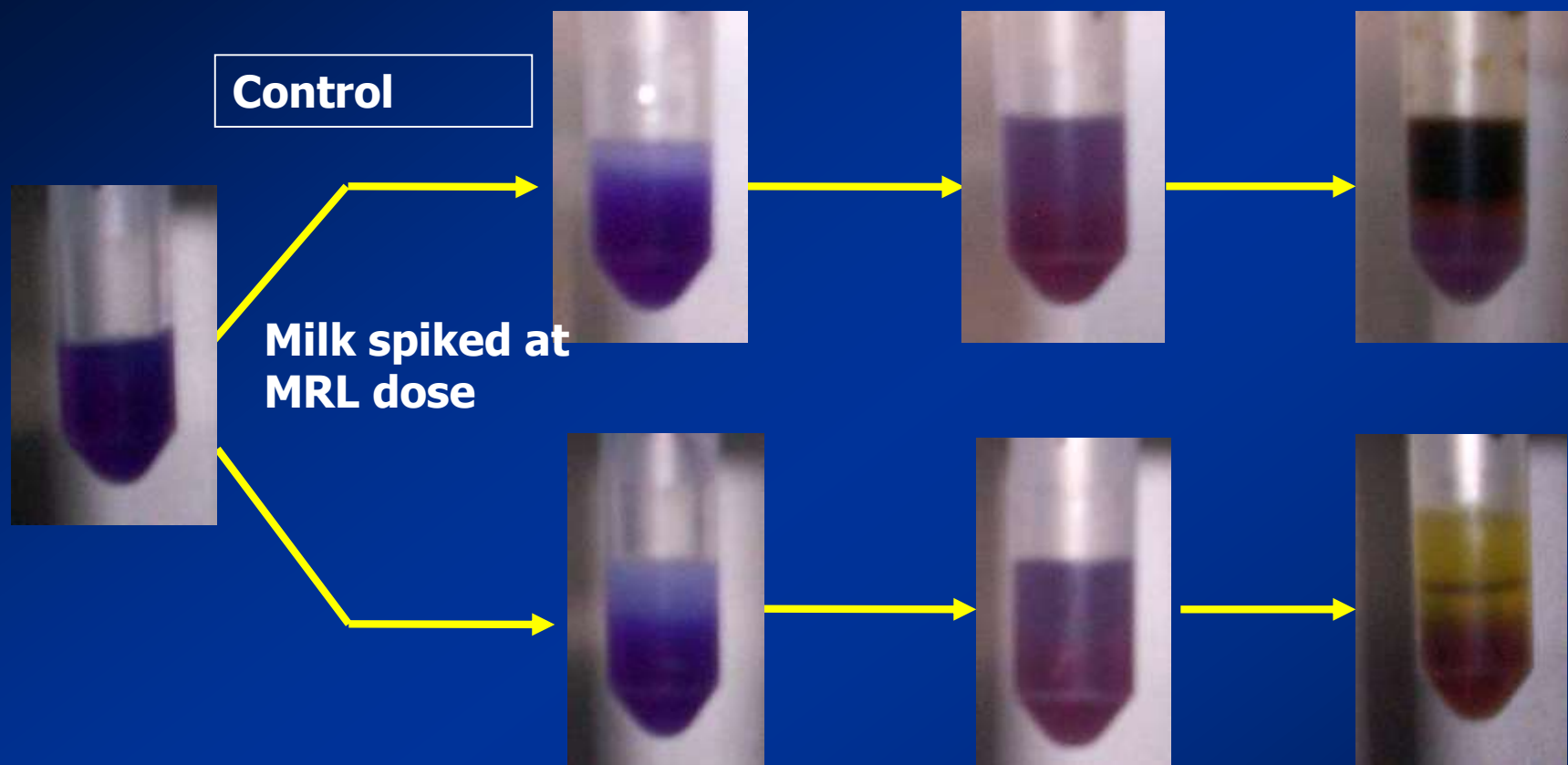
Iodometric method based on starch + iodine mixture

(Das, S. 2006)

Use of spore as biosensor based on targeting specific enzyme



Development of iodometric based analytical process for specific detection of β -lactam group of antibiotics



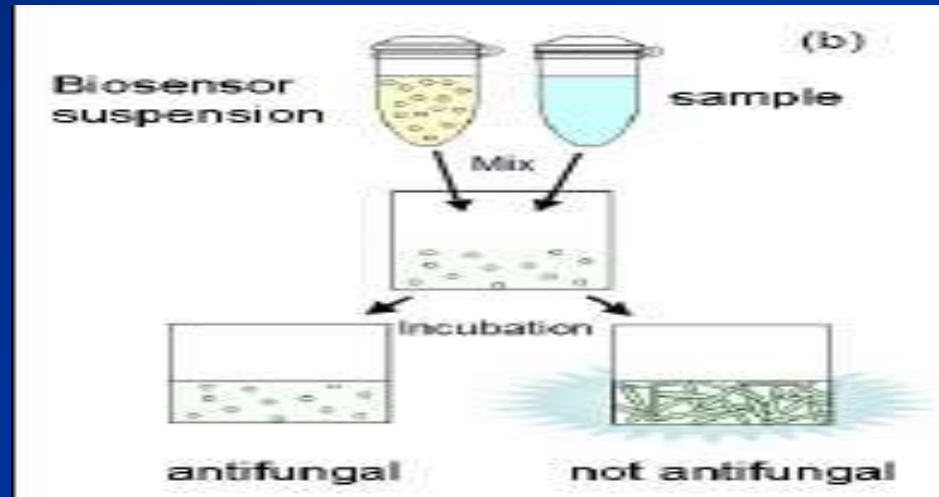
Step 1:
Inoculation of spore suspension and induction with milk at 30°C / 4 hrs

Step 2:
Treatment of Enzyme with antibiotics (30°C/hr)

Step 3:
Treatment with iodine mixture for 30 min

Fungicide

- ❖ iLUX™ antifungal testing KIT
- ❖ Fungus *Neurospora crassa* genetically modified organism



- ❖ **Advantage**

- Sensitive
- Cost effective
- Used for range of matrices – pharmaceutical formulations and food

(Lux biotechnology)

BOD detection by amperometric biosensors

- Biodegradable organic pollutants in aqueous solution
- Based on amperometric oxygen electrode transducer
- Biological sensing element - *T.candida* , *Trichosporon cutaneum*, *Pseudomonas putida*, *K. oxytoca* & Yeast

(Nisshin Denki *et al.*,2006)

Pesticides

- Cholinesterase based biosensors

- Organophosphorus & carbamate insecticides



- Activity measured by amperometric or potentiometric biosensors

- Enzymes inhibited by both

- **Amperometric transducer**

- ❖ **Two biosensors constructed**

- **Choline oxidase**

- **Cholinesterase**

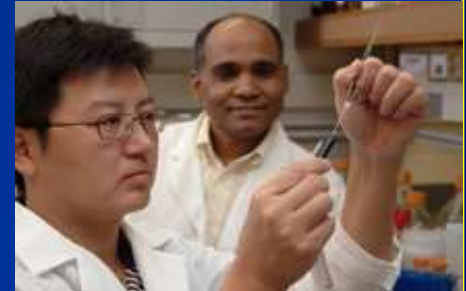
- ✓ Consumption of oxygen measured – oxygen electrode or platinum electrode – monitoring H₂O₂



- ✓ Cholinesterase – acetylthiocholine iodide – electrode
platinum, graphite, graphite epoxy composite entrapped with ChEs

Food borne pathogen detection

❑ Detection of *Listeria monocytogenes* (*Listeriosis*)



❖ Affinity type Optical biosensor

- Light to detect the presence of a target organism or molecule
- Sensor - small piece of optical fiber - a clear, solid, plastic material that transmits light through its core
- Fiber is coated with antibody recognizes *Listeria monocytogenes*
- Capture & binding into the fiber

Contd..

➤ Advantage

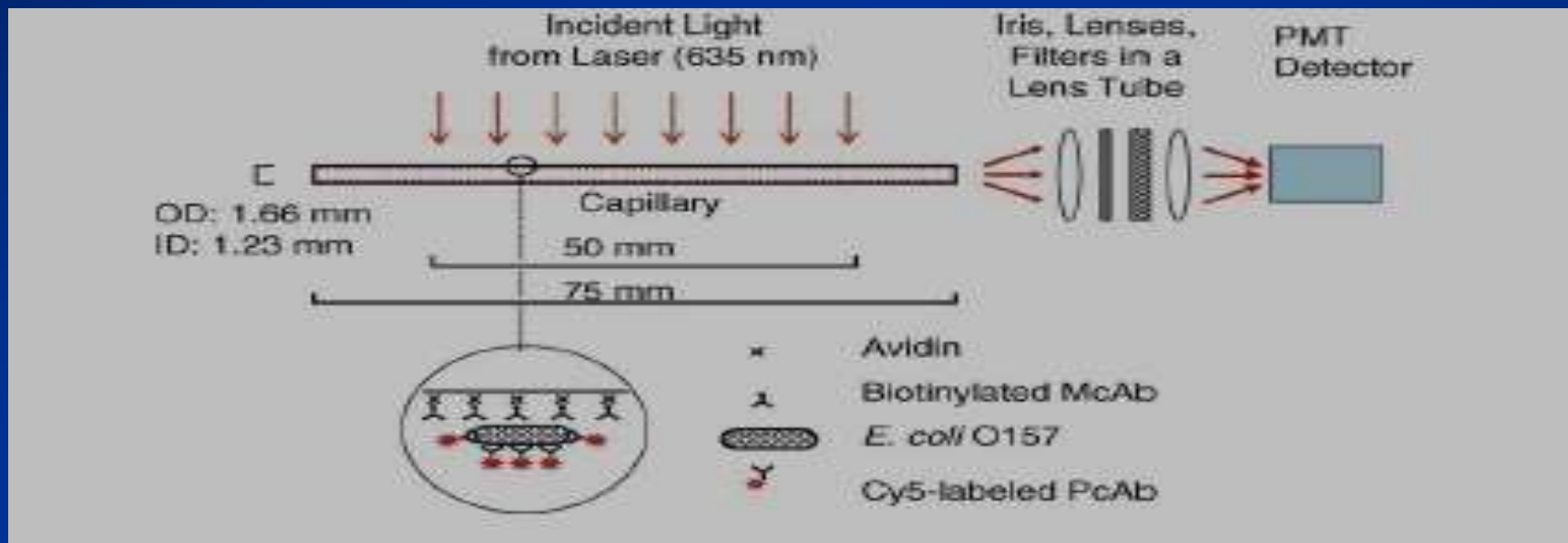
- ✓ Detect with in 24 hrs at a conc. of less than 1000 cells /ml
- ✓ Size is very small
- ✓ Can used for salmonella and *E.coli* detection

(Arun Bhunia *et al.*, 2005)

E. coli O157:H7 Detection

❖ Integrating waveguide biosensor

- Sandwich antibody technique capture & detect antibody
- Captured AB attached on capillary tube
- Illuminated at 90° angle relative to length of wave guide
- Emitted fluorescence collected at the end of wave guide
- Detection limit 40 pg/ml



(Peixuan Zhu *et al.*,2005)

Conclusion

- ❖ Quality as well as safety consideration are important parameter in dairy industry
- ❖ Use of biosensors is a viable alternatives to conventional techniques due to its
 - Specificity
 - Low cost
 - Fast response time
 - Portable ease of use
- ❖ A range of biosensors have been developed based on different combination of bio-receptor and transducer

- ❖ Electrochemical and optical microbial biosensors are two major family of biosensors which are being exploited to use in dairy food analysis
- ❖ In dairy industry biosensors may be used in monitoring of HACCP and detection of raw milk quality, efficiency of pasteurization, water quality, pathogens, antibiotics, BOD, & residues of pesticides, detergent residues in milk
- ❖ In future, biosensors can be used with multianalyte capabilities, integration/miniaturization, implantable devices ,live organisms and tissue element used as biological element & as online detection possible