BACTERIAL BIOSENSORS

Prof A.O. Olaniran

Discipline of Microbiology
University of KwaZulu-Natal
(Westville Campus)

INTRODUCTION

- The increasing pollution of waters and soils is creating a more serious environmental problem.

- Traditionally, pollution risk assessment is based on the measurement of a pollutant's total concentration in a sample.

- The toxicity of a given pollutant in the environment, however, is tightly linked to its bioavailability, which may differ significantly from the total amount.

INTRODUCTION

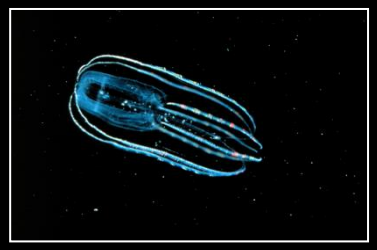
- **Physico-chemical** and **biological** parameters strongly influence pollutant fate in terms of leaching, sequestration and biodegradation.
- Bacterial sensor reporters consist of **living micro-organisms genetically engineered** to produce specific output in response to target chemicals.
- They offer an **interesting alternative** to monitoring approaches.
- Bacterial sensor-reporters detect bioavailable and/or bioaccessible compound fractions in samples.

INTRODUCTION

- The principle of using living cell-based sensing assays have gained impetus and developed into a scientific and technological area only **since the last twenty years**.

- BUT:
  - why would one use living cells and organisms for sensing?
  - what are the specific purposes for basing sensing methods on living cells?
  - what are the advantages that cellular-based sensing can have over other sensing techniques?

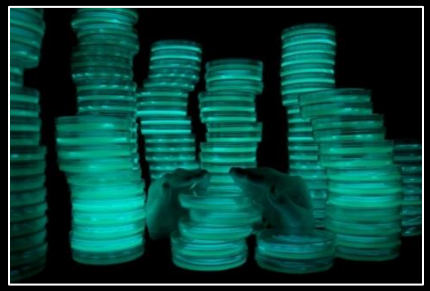


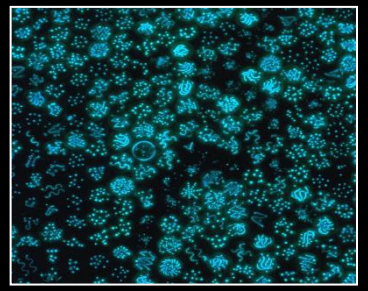




MICROBE-BASED SENSORS (MBS)

- The engineering of microbial cells with the purpose of chemical detection has enormously expanded in the last 20 years.

- The major driving force for this development has been:
  - the advance in genetic engineering techniques;
  - the relative ease to redesign (certain) hardware components in microbial cells and to assemble synthetic genetic circuitry for sensing and producing robust output signals.



MICROBE-BASED SENSORS (MBS)

- In principle, any constituent, product or reaction of living cells can form the basis for a '**sensing device**'.
- However, most research has concentrated on **non-cognate so called "reporter proteins"** that are to be produced by the cell after specific contact or interaction with a target analyte or condition.
- The use of non-cognate proteins as reporters ensures a **low background in the absence of the trigger**, and, **ideally, a highly specific output signal**.
- The choice of a suitable reporter protein is **dependent on the targeted application form**.

MICROBE-BASED SENSORS (MBS)

- Of the several different types of reporter proteins being used in MBS, bacterial and eukaryotic **luciferases** have been particularly popular.
- Mostly because of their **relatively high quantum yields**, luciferases have been the optimal choice for highly sensitive applications.
- Different **spectral variants** have been developed by mutagenesis strategies.
- Eukaryotic luciferases **require substrate addition** and **cell membrane permeabilization in bacteria**, which somewhat limits their practicality for MBS assay configurations.

MICROBE-BASED SENSORS (MBS)

- Bacterial luciferases have been the most applied reporters in MBS.

- Two different configurations have been used;
  - ***luxCDABE***, in which the cells synthesize the substrate for the luciferase
  - ***luxAB***, in which external substrate addition is needed

- Although external substrate addition is somewhat more cumbersome,
  - it avoids false-positive stimulation of luciferase activity by membrane regeneration
  - it is less energy demanding for the cell.

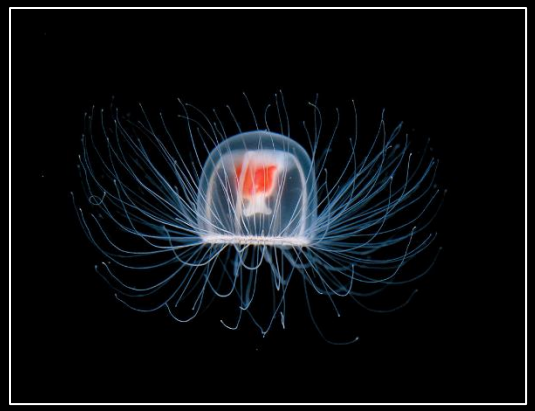
WHOLE-CELL BACTERIAL BIOSENSORS

- Bacteria can be used as biosensors to demonstrate the toxicity of a variety of environmental media including soil, sediment, and water.

- This is done by **coupling bacteria to transducers that convert a cellular response into detectable signals**.

- These bacterial biosensors are engineered by pairing:
  - a **reporter gene** that generates a signal with
  - a **contaminant-sensing component** that responds to chemical or physical change, such as exposure to a specific analyte.

- When the biosensor is exposed to such a change;
  - ➢ the **sensing component stimulates the reporter gene** through a biochemical pathway in the cell.
  - ➢ the **reporter gene then produces a measurable response**, such as emitting visible light, which is indicative of the degree of chemical or physical change.



The **key** part of a biosensor is the **transducer** which makes use of a physical change accompanying the reaction

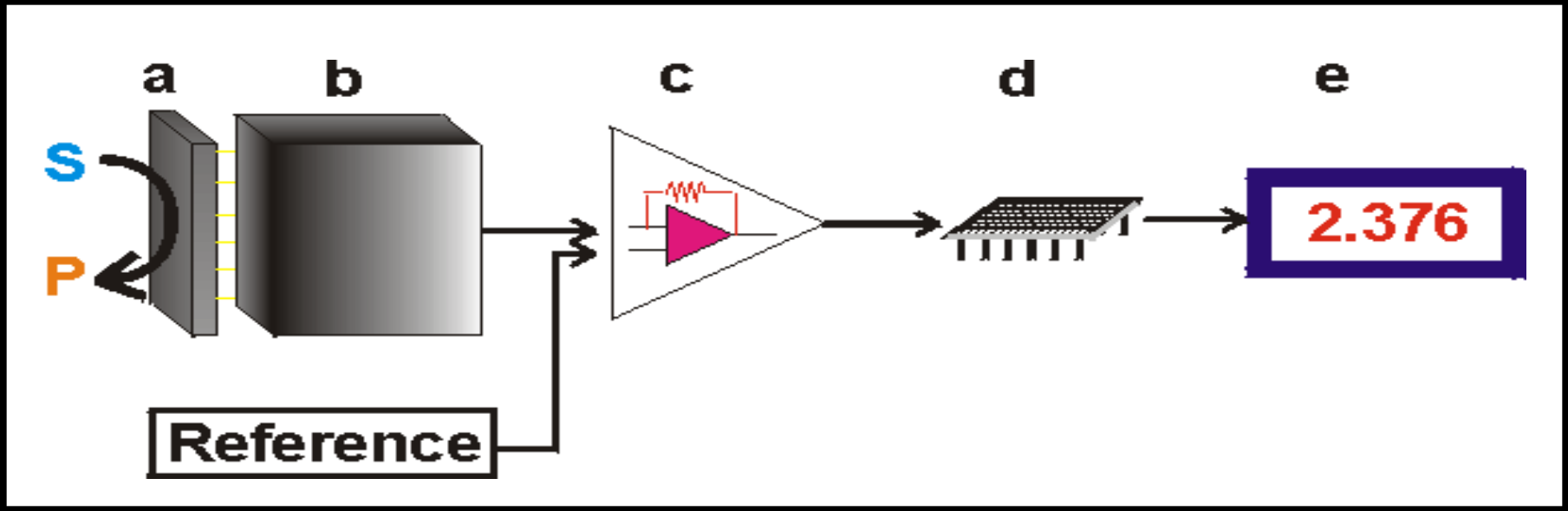


Figure 1: Schematic diagram showing the main components of a biosensor. The biocatalyst (a) converts the substrate to product. This reaction is determined by the transducer (b) which converts it to an electrical signal. The output from the transducer is amplified (c), processed (d) and displayed (e).

The bacterial luminescence reaction involves the oxidation of a long-chain aliphatic aldehyde and reduced flavin mononucleotide ($FMNH_2$) with the liberation of excess free energy in the form of a blue-green light at 490 nm:

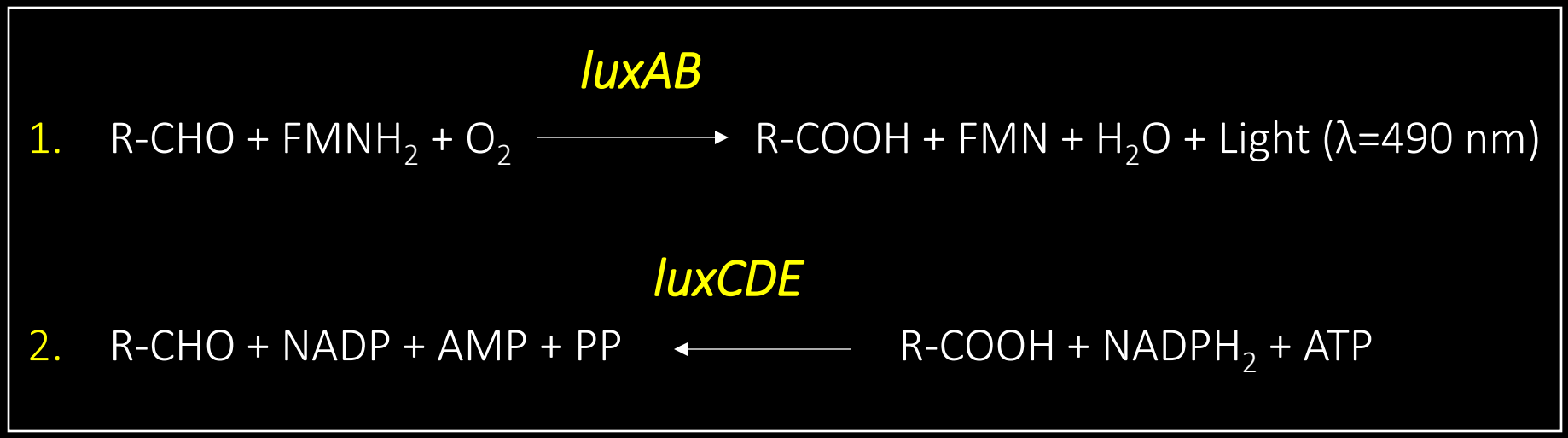


Fig. 1: The bioluminescent reactions encoded for by the luxCDABE operon. The luxAB genes convert an aldehyde substrate to a carboxyl group, generating visible light (Equation 1). The luxCDE genes use $NADPH_2$ and ATP to generate the aldehyde (Equation 2).

REPORTER GENES

- A reporter gene encodes for a mechanism that produces a detectable cellular response.
- It determines the **sensitivity** and **detection limits** of the biosensor.
- The reporter gene must have an **expression or activity** that can be measured using a **simple assay** and **reflects the amount of chemical or physical change**.
- The biosensor must be free of any gene expression or activity similar to the desired gene expression or activity that is being measured to:
  - prevent misinterpretation of the response
  - guarantees the measurement directly reflects the desired chemical or physical change.

REPORTER GENES

luc operon from the firefly *Photinus pyralis*

- The most commonly used reporter gene, widely used as **a monitor of gene expression** and **a reporter** in bacterial biosensors.
- The *luc* operon produces the enzyme, **luciferase**, capable of generating the luminescence of the firefly.
- The activation of the gene results in the transcription or reading of the *luc* operon, which causes the cell to produce luciferase.
- The enzyme luciferase spurs a chemical reaction that produces CO_2 and visible light by catalyzing the oxidation of its substrate, D-luciferin, which binds to the enzyme's active site.
- The visible light produced can be measured with a variety of instruments including a luminometer and optical fibers.

REPORTER GENES

Advantages & Limitations of luc operon

- More efficient at converting chemical energy to light
- The reaction has a high sensitivity level and a broad dynamic range.
- The reporter gene has great versatility
- Can be mutated to produce enzymes that express a range of colors from green to red, which can then be independently controlled for multianalyte assays

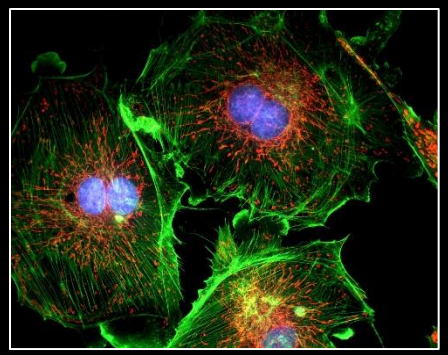
- Complicated by requirements such as:
- the addition of the substrate
- an aerobic environment
- ATP as a source of energy.

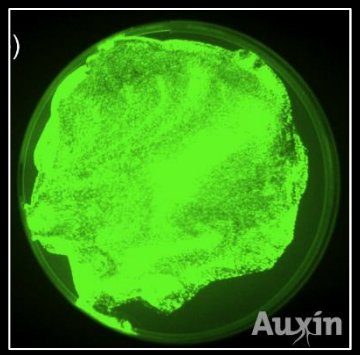
REPORTER GENES

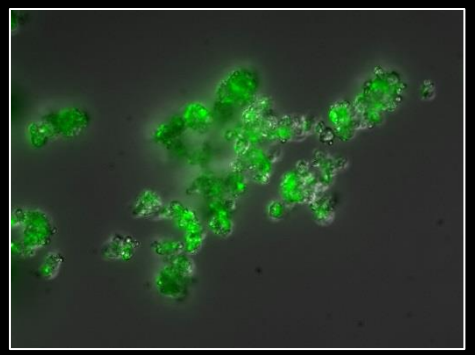
Green fluorescent protein (GFP) from the jellyfish Aequorea victoria

- A photoprotein, GFP, and its encoding gene has been used in biosensors as a reporter gene.

- The production of GFP in the jellyfish results in the emission of a **green fluorescence** that can be measured.







REPORTER GENES

Advantages & Limitations of GFP

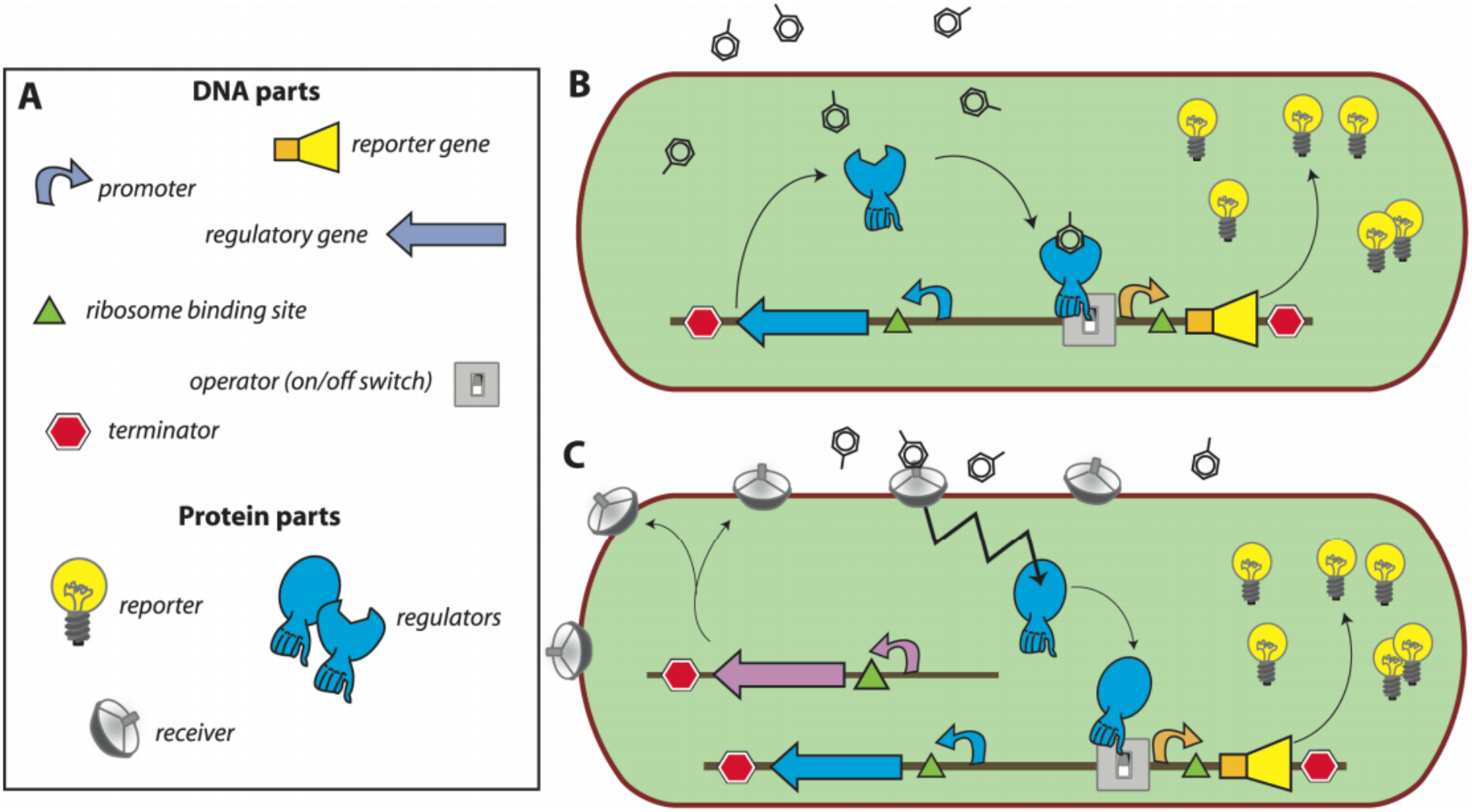
- The GFP system allows real time detection without the addition of substrates and without disrupting the cell's metabolism
- GFP does not rely on internal reducing equivalents being produced by the cell, which may mean that this reporter gene is **not as sensitive to the growth or nutritional status of the biosensor**
- **Ease of detection** and **minimal metabolic cost** to the host cells
- Ability to alter its stability and spectral properties through structural alterations, and thus produce mutants with improved fluorescence intensity, thermostability, and chromophore folding
- Lower sensitivity compared to the *luc* operon
- GFP is a very stable protein, which means it can accumulate in the cell over time which results in background fluorescence
- Results are more stable if the number of proteins is measured instead of their fluorescent activity

Fig 2 (next slide): DNA parts necessary for constructing an inducible sensor-reporter circuit. Parts can be combined and assembled by genetic engineering techniques

(a) Regulatory and reporter genes are necessary for the sensing function and system output, respectively. Promoter, operator(s), terminators, ribosome binding sites, etc. are DNA sequences needed for control of the gene expression.

(b) Set-up in which the sensor function is provided by a single regulatory protein. In this example, the regulator protein binds the target compound and induces the transcription of the reporter gene, leading to the production of reporter proteins (signal amplification).

(c) Set-up for separated sensor and regulator functions. In this configuration, the target compound is sensed by a periplasmic receiver protein that transmits the detection event via a signalling (e.g. phosphorylation) cascade to the regulatory protein (zigzag arrow). The activated regulator then induces reporter gene expression as before

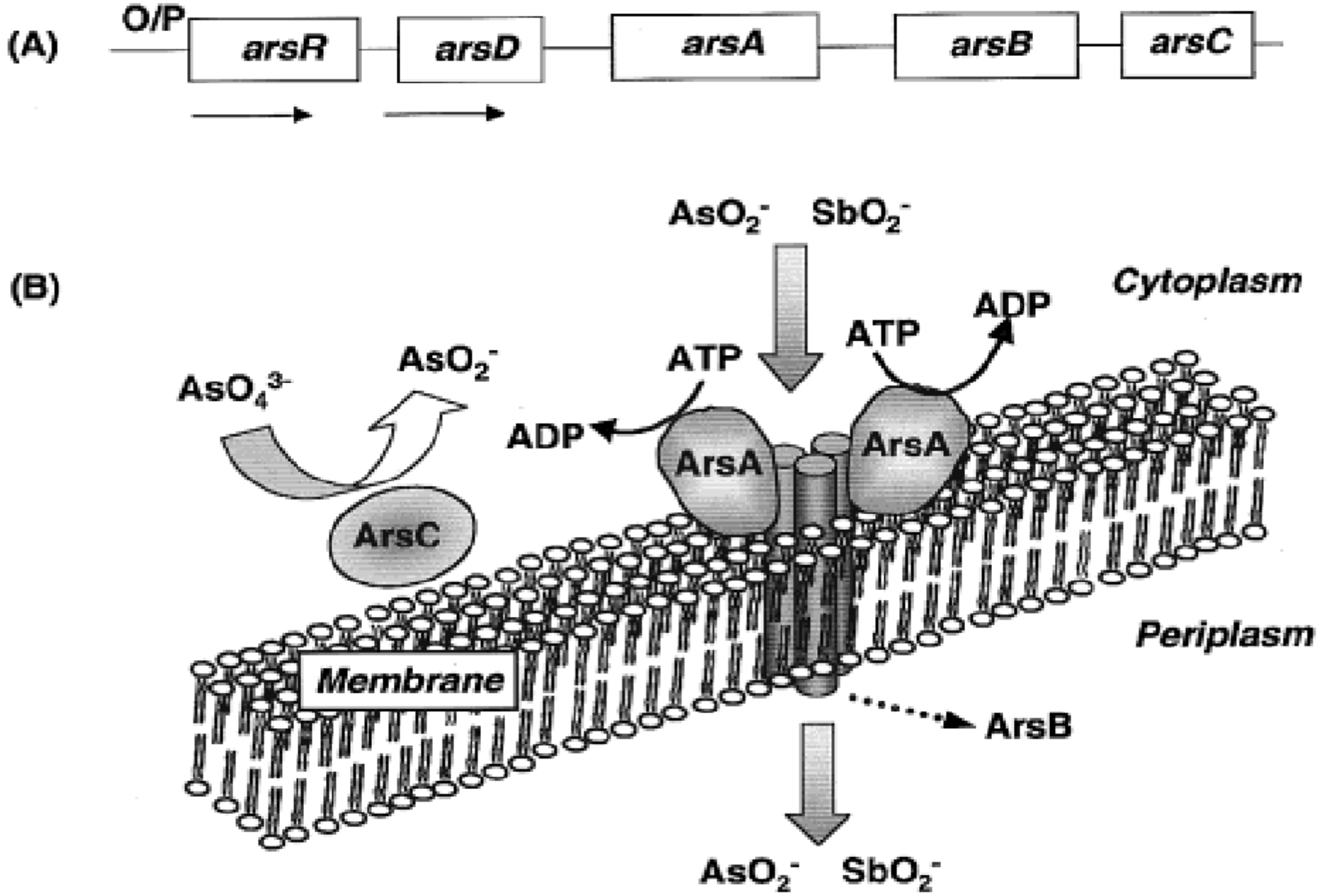

A
DNA parts
reporter gene
promoter
regulatory gene
ribosome binding site
operator (on/off switch)
terminator
Protein parts
reporter
regulators
receiver
B
C

CONTAMINANT-SENSING COMPONENTS

- Microbial systems developed for detoxifying or excreting toxic substances can be used as the **contaminant-sensing component** of the biosensor
- This sensing component **detects the substance** for which it is designed to detoxify or excrete and determines the **specificity** of the biosensor.
- The contaminant-sensing component is combined with reporter genes to create biosensors that can identify toxic substances at very low levels.
- When the contaminant-sensing component detects the substance, it triggers the reporter gene, which produces the luminescent enzyme.

ARSENIC SENSING BIOSENSORS

- Various biosensors have been developed and tested on a research level for detecting bioavailable arsenic.
- To develop the biosensor, the arsenic resistance gene and the reporter gene are cloned and inserted onto one plasmid, which is then inserted into a host bacteria.
- All arsenic sensing biosensors are triggered by arsenic, the analyte, entering the biosensor and **activating the transcription of the resistance gene**, which is **followed by the transcription of the reporter gene**.



(A) Sequence of *ars* Operon (B) Arsenic Resistance Mechanisms.
(Daunert *et al*., 2000)

ARSENIC SENSING BIOSENSORS

- The entire resistance gene is not needed, so many biosensors only use the beginning components such as the promoter.
- It is able to recognize the arsenic and begin the transcription of the plasmid that contains the reporter gene.
- The transcription of the reporter gene produces proteins, which glow in direct correlation to the amount of arsenic entering it (Figure 3).
- Constructed biosensors have used either luciferase or GFP as the reporter gene coupled with various combinations of arsenic resistance mechanism components.
- Various strains of bacteria have served as the host bacteria.

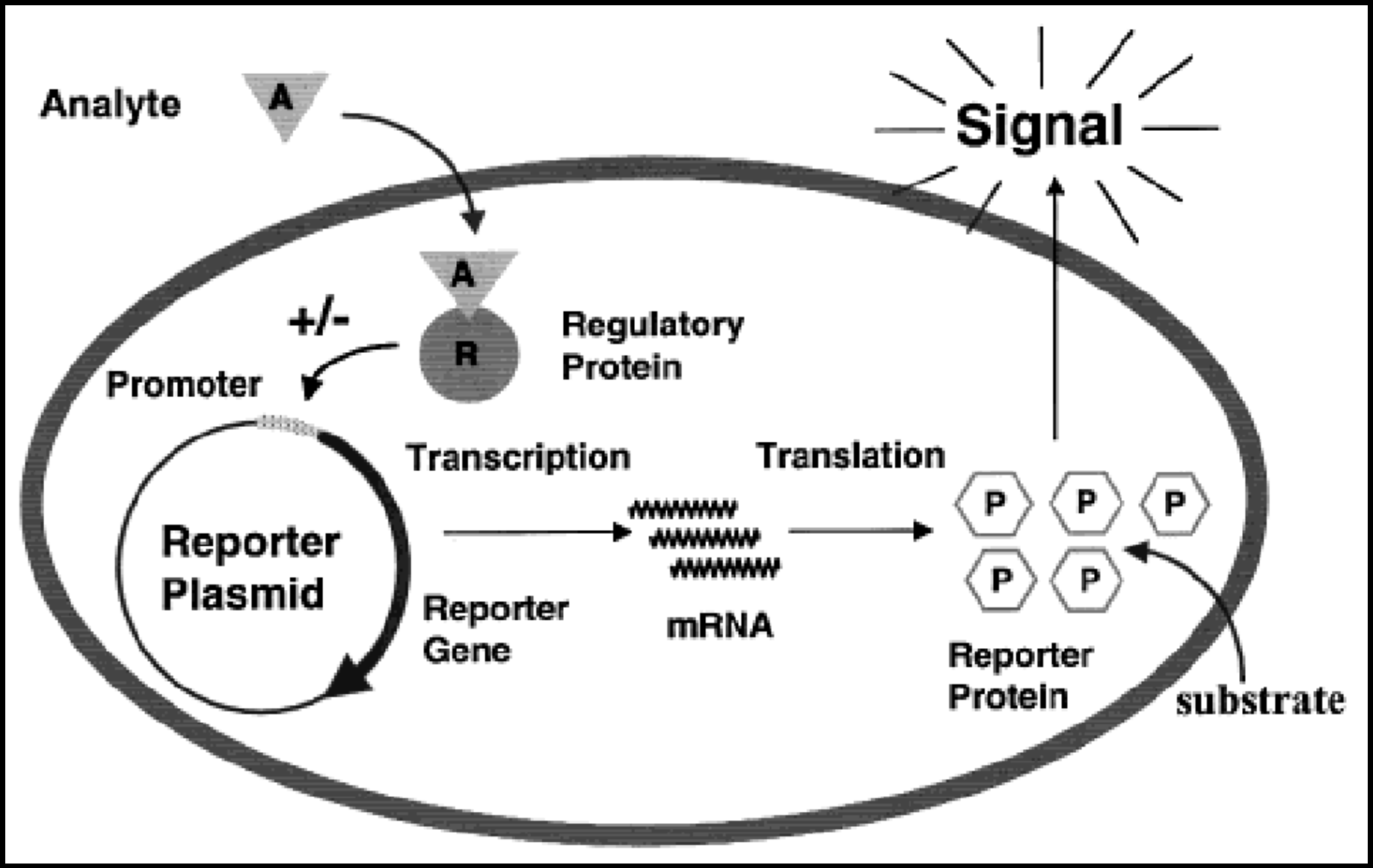
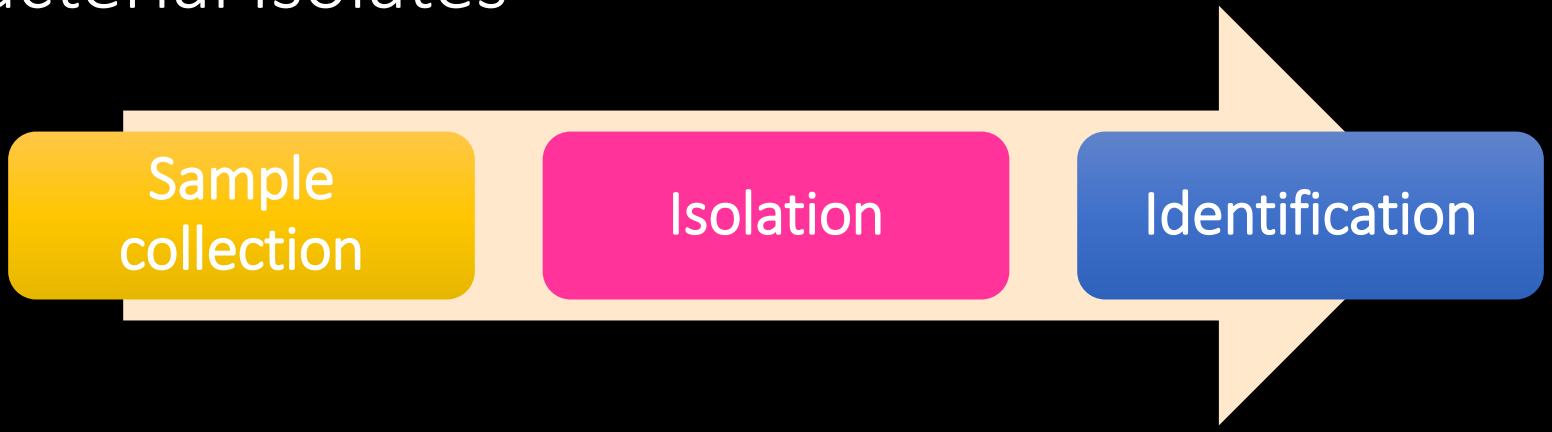


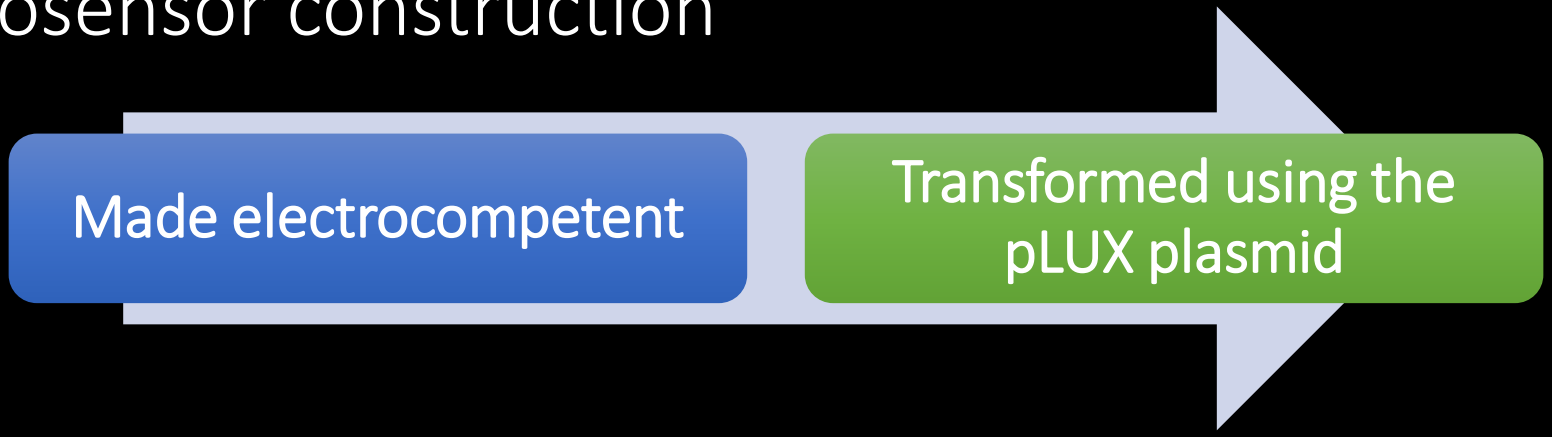
Fig.3: General Mechanisms of Biosensor (Daunert *et al*., 2000)

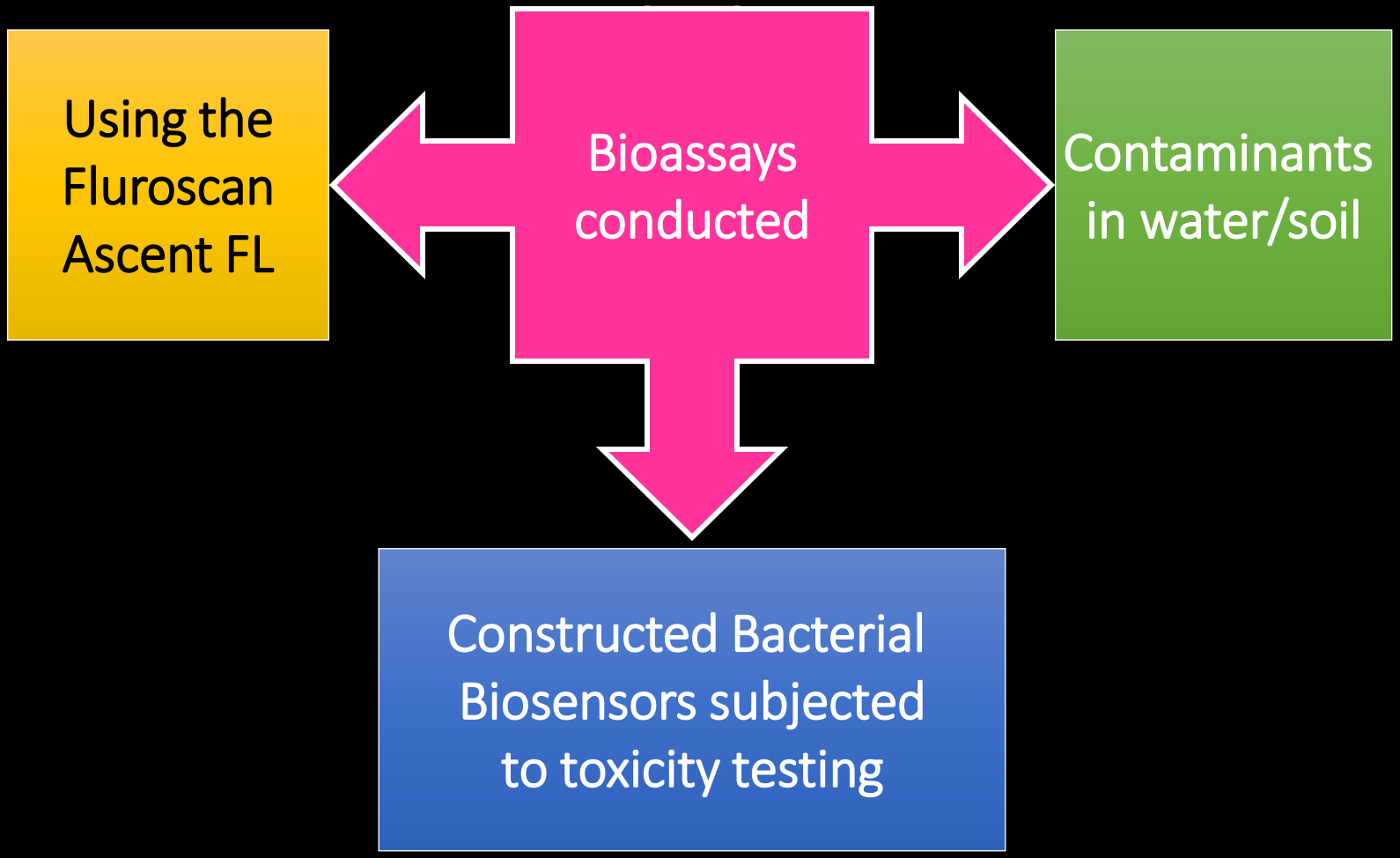
METHODOLOGY

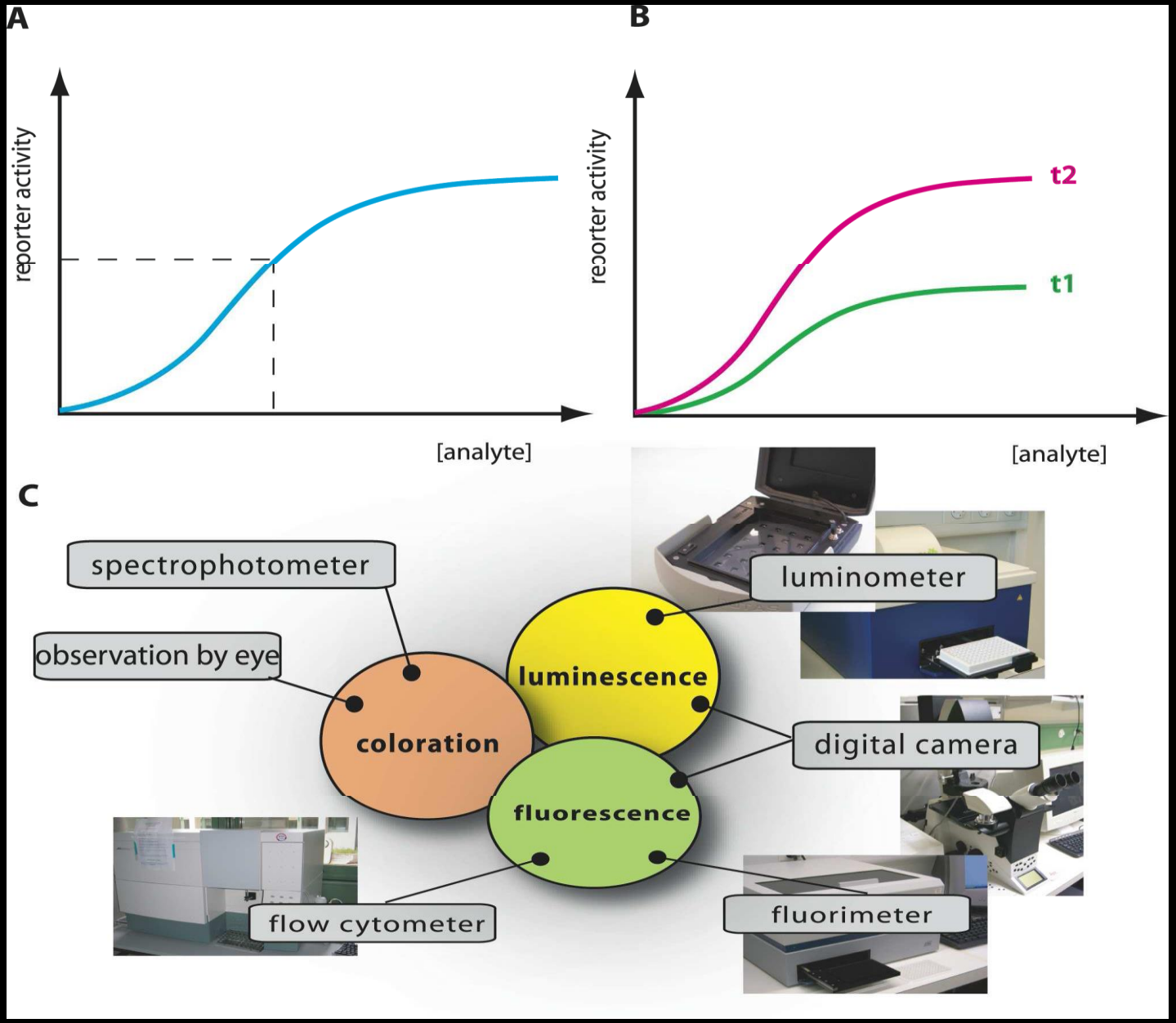
Bacterial Isolates



Biosensor construction




Using the Fluroscan Ascent FL
Bioassays conducted
Contaminants in water/soil
Constructed Bacterial Biosensors subjected to toxicity testing



A:

Typical calibration curve with reporter output as a function of analyte concentration

B:

Time-dependent signal calibration

(C): Various instruments for measuring reporter output

OPTIMIZING RESULTS

- When choosing the host strain, it is important to consider the **natural environment of the bacteria**. For example, those strains native to soil are best for testing soil samples

- **Temperature**, which influences induction time, should correspond to the temperature of the natural environment in which the host bacteria is found

- **Growth phase of the sensors** and **incubation time** have the greatest effect on induction, while luminescence has been found to correlate with the optical density of the culture

- **Preservation** (freeze-drying) and **Storage medium** - Trehalose, skim milk, sucrose etc.

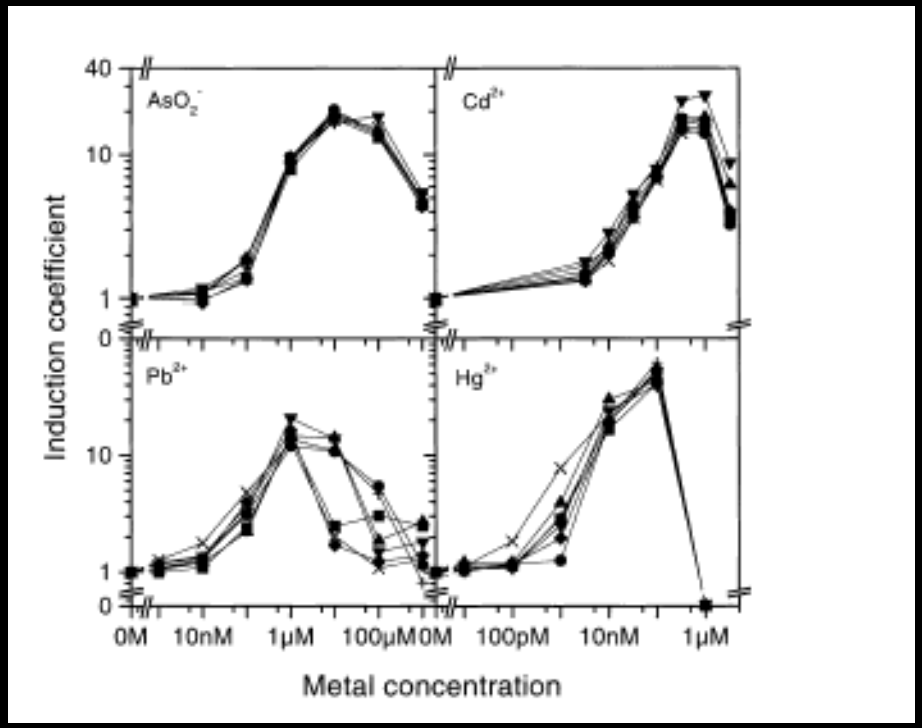
INTERPRETING RESULTS

- In order to correlate the induction coefficient with a concentration of arsenic, a dose-response curve must be established.
- A comparison can then be made between the dose-response curve and a plot of the results from the samples.
- This comparison reveals the bioavailable concentration of the analyte in the unknown sample.

INTERPRETING RESULTS

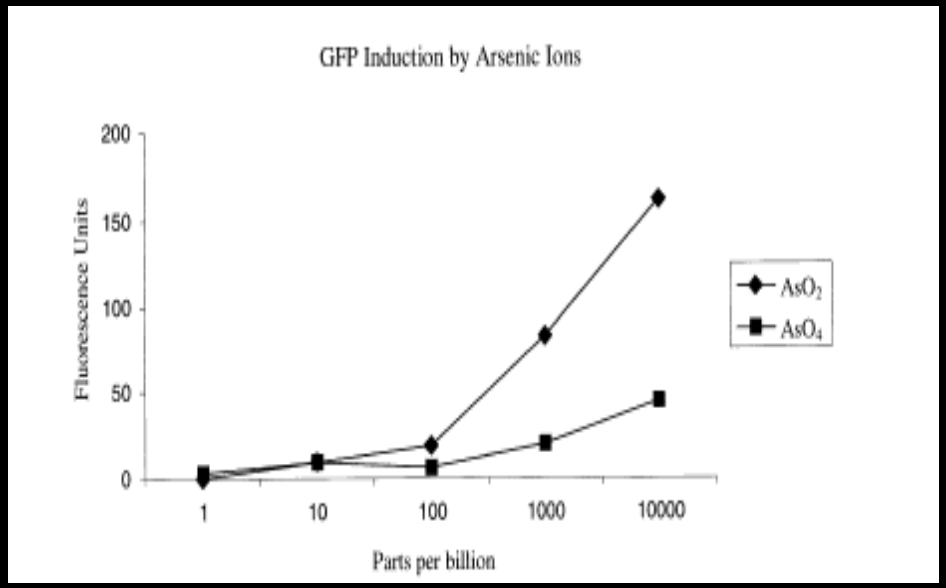
- Typically, the response of the biosensors is nonlinear until a threshold level of concentration is reached, after which the response is linear.

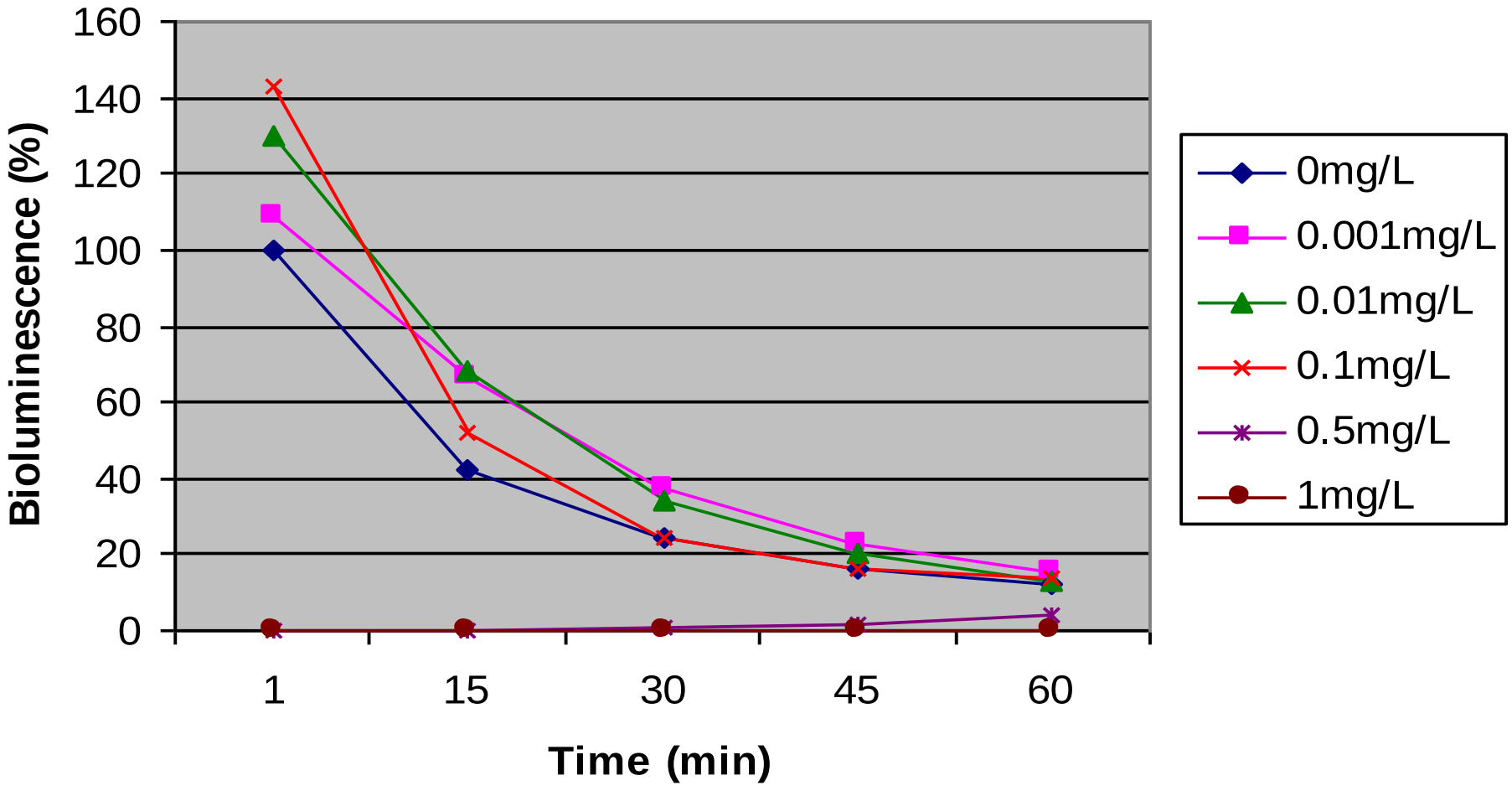
- Once the response peaks, it rapidly decreases due to concentrations so high the cell cannot expel the toxicant and begins to die.



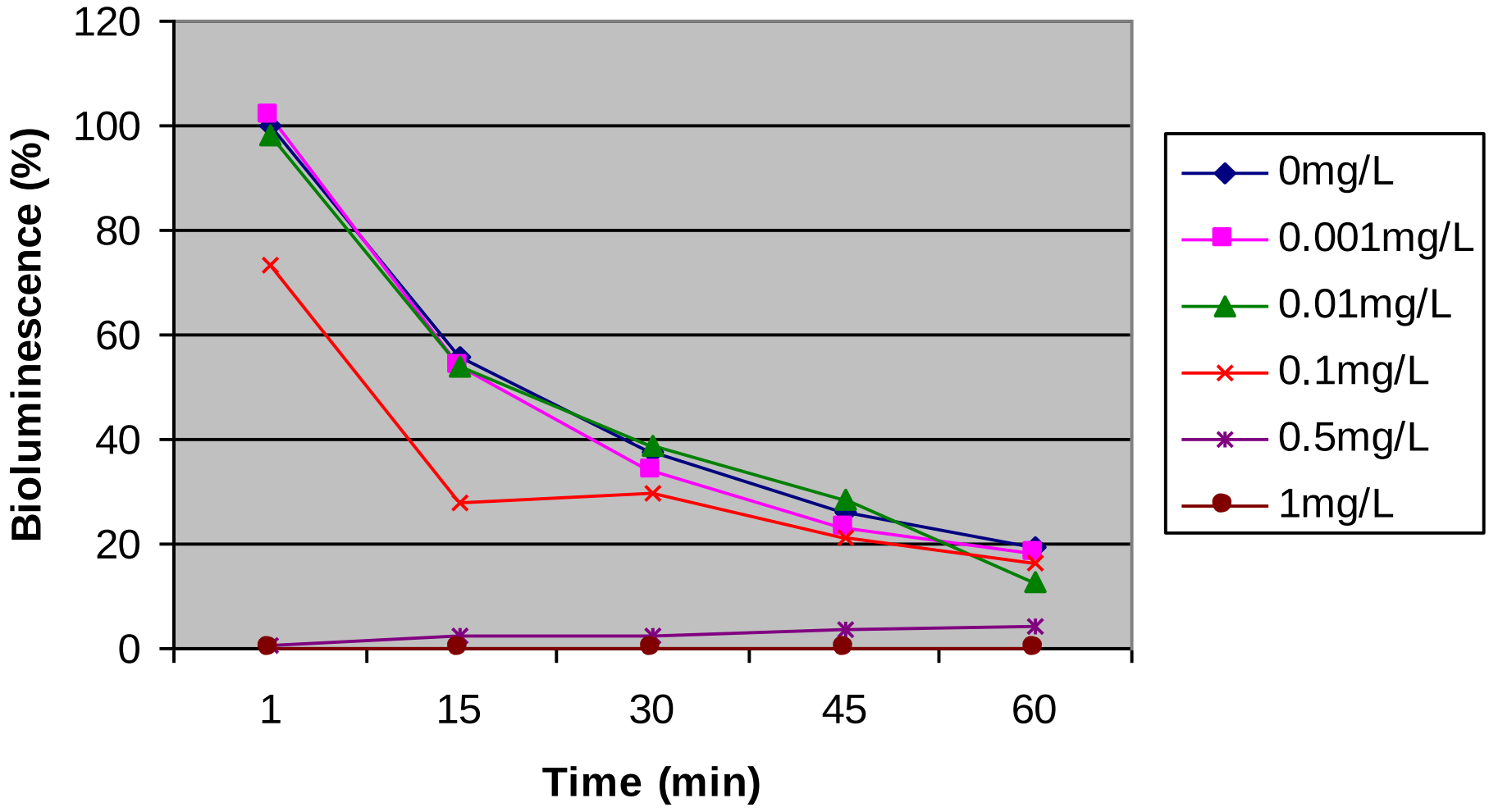
Example of Dose-Response Curve (Tauriainen *et al*., 2000)

Example of Dose-Response Curve (Tauriainen *et al*., 2000)





S. odoriferia pLux biosensor in response to the various concentrations of PCP



S. odoriferia pLux biosensor in response to the various concentrations of DCP

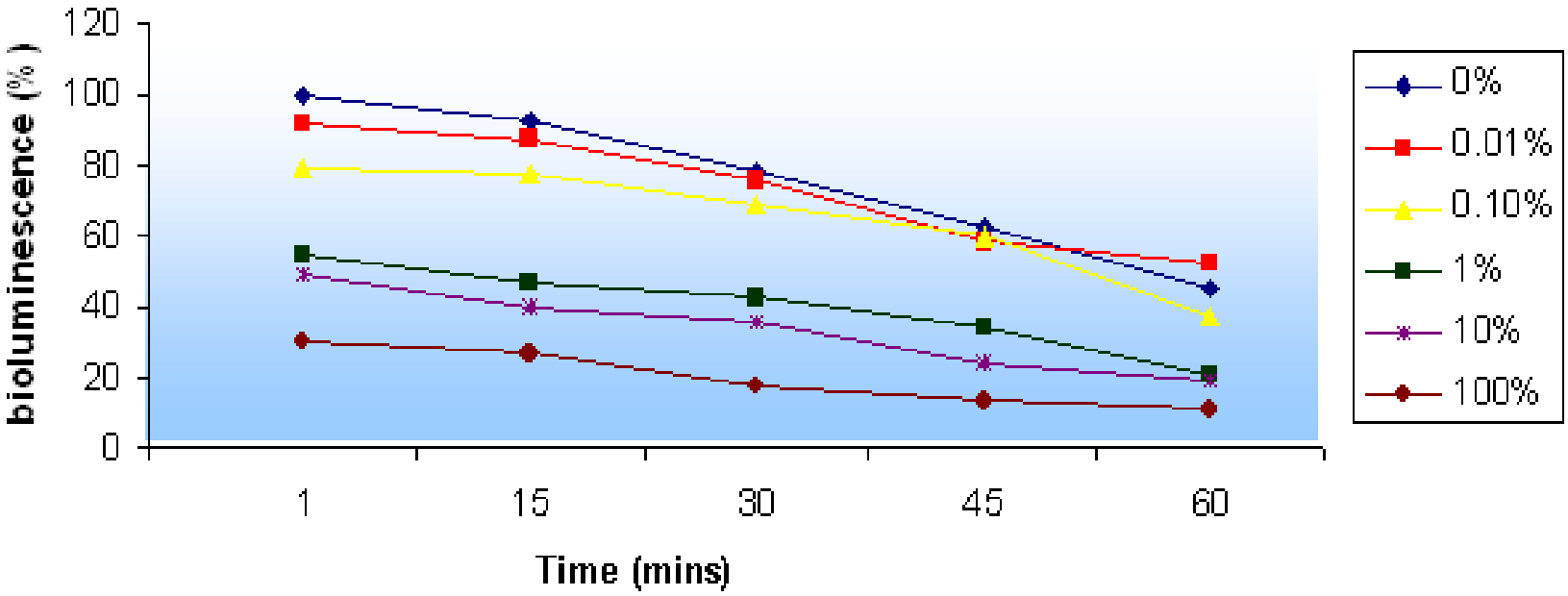
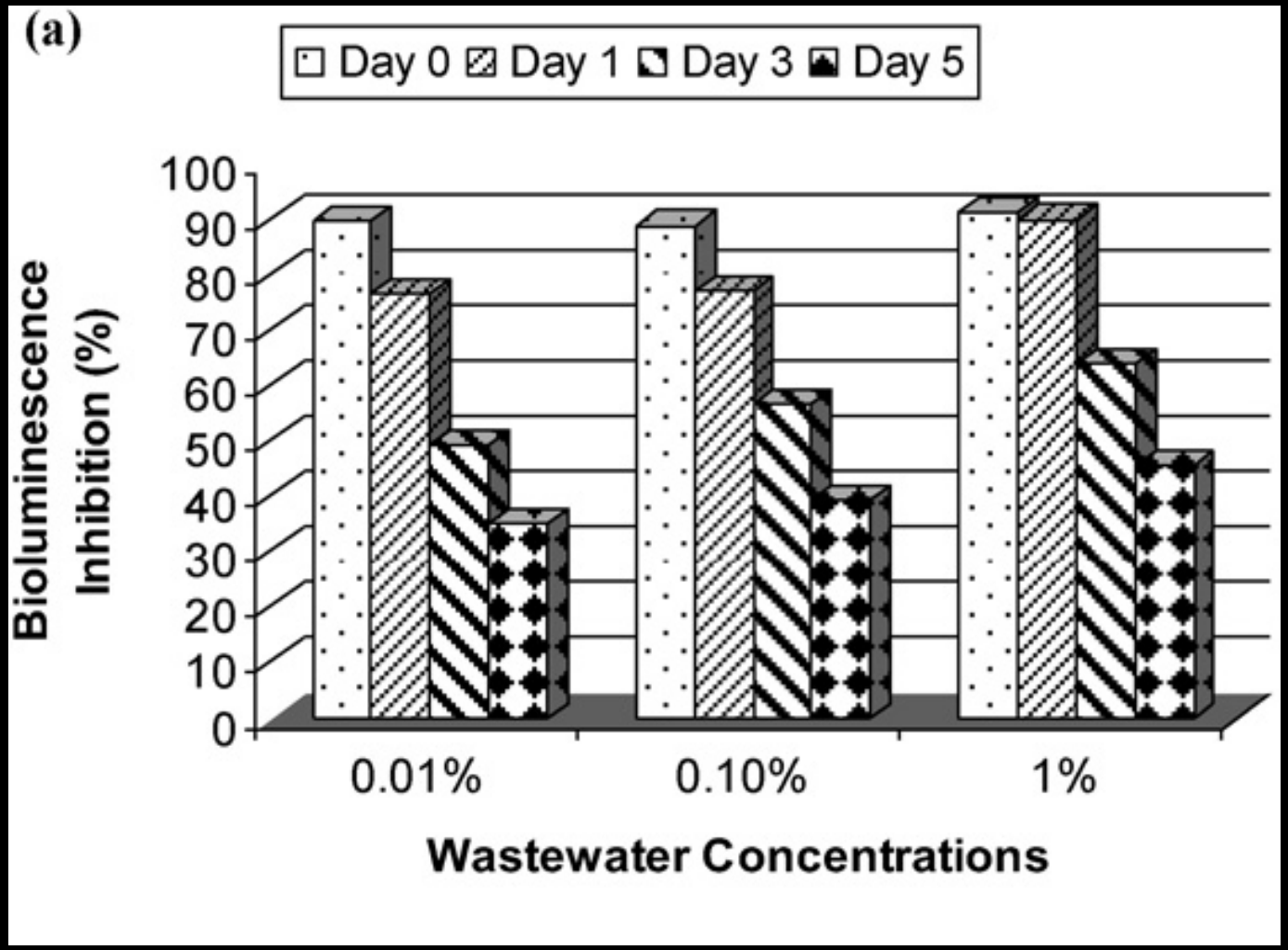
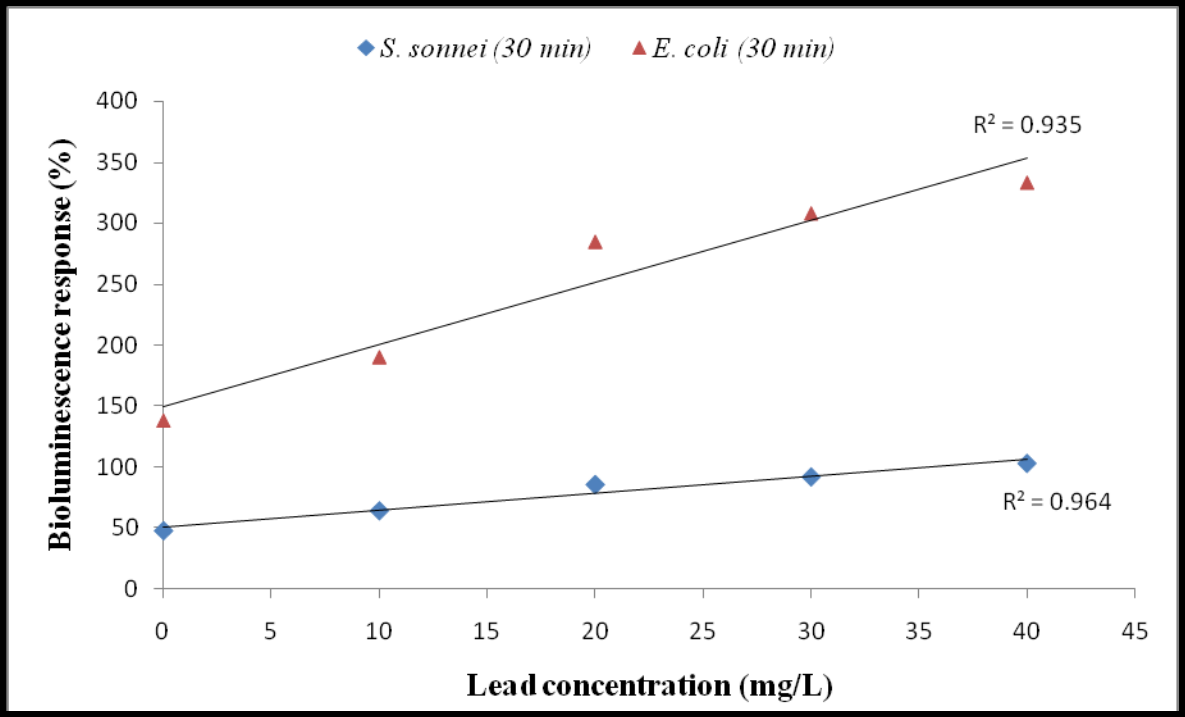
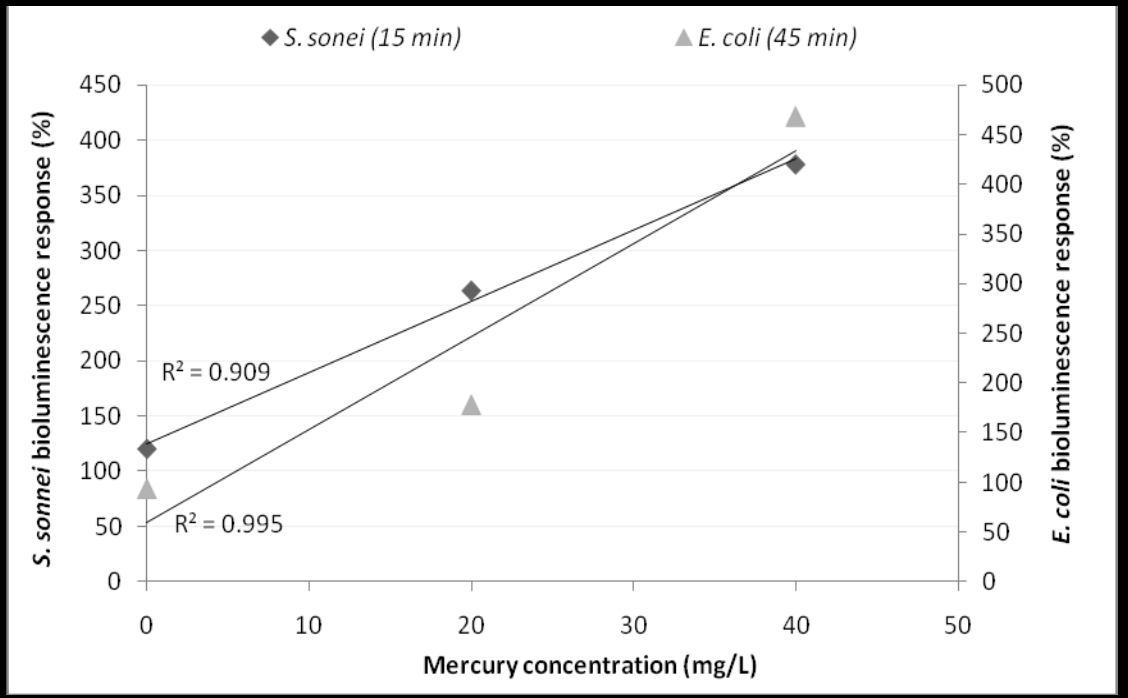


Fig 1.1 Bioluminescent response to various concentrations of Whale dye

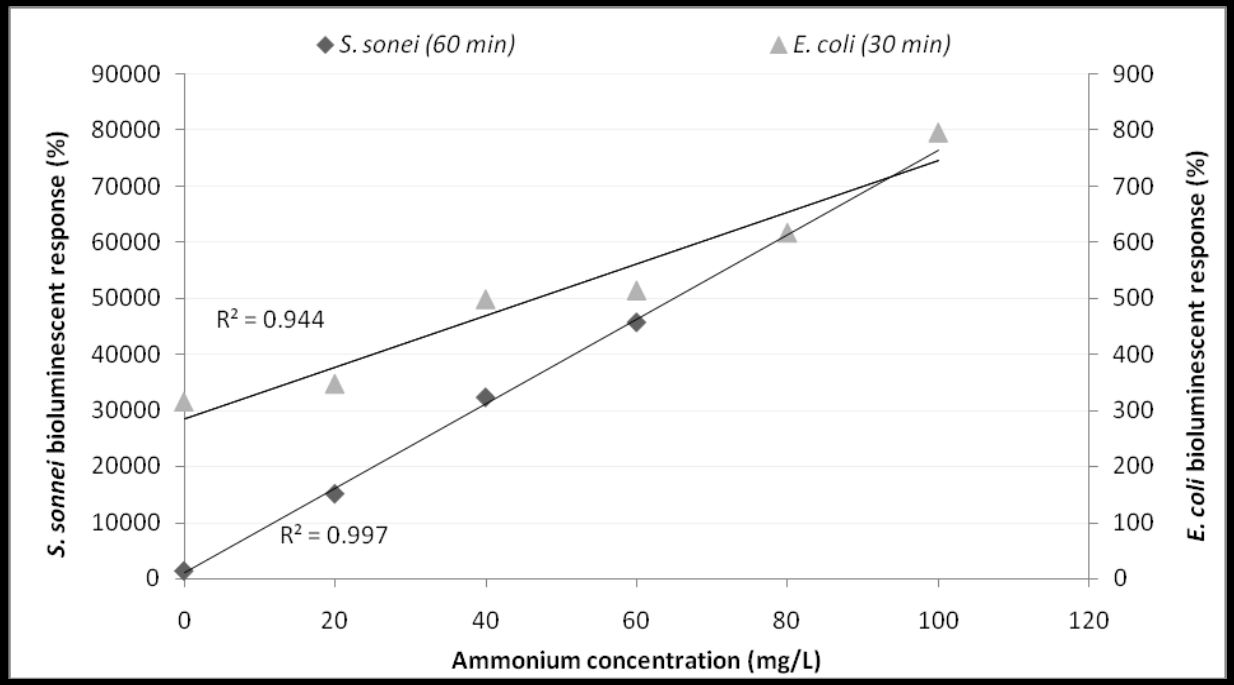


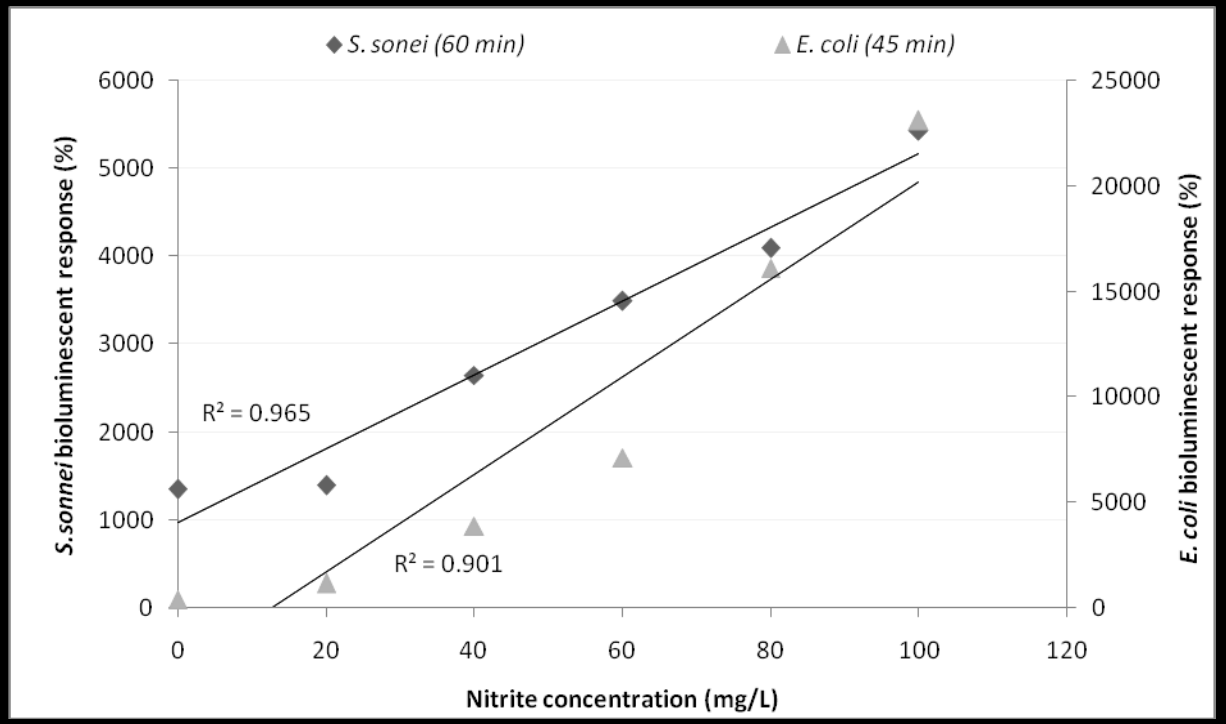
Bioluminescent response of *S. flexneri* pLux biosensor to varying concentrations of wastewater effluent undergoing degradation (Olaniran *et al*., 2008)





Olaniran *et al*. (2011)





Olaniran *et al*. (2011)

TABLE 9. Bacterial biosensors for monitoring petroleum contaminants

Bacterial biosensor	Contaminant	Reporter gene fusion
Pseudomonas fluorescens HK44	Naphthalene	*nahG-luxCDABE*
Pseudomonas putida RB1401	Toluene, xylene	*xylR-luxCDABE*
Pseudomonas putida B2	BTEX	*tod-luxCDABE*
Pseudomonas putida TVA8	BTEX	*tod-luxCDABE*
Escherichia coli DH5α	Alkanes	*alkB-luxAB*
Escherichia coli DH5α	BTEX	*xylR-luc*
Escherichia coli	Benzene derivatives	*xylS-luc*

FEATURES OF A SUCCESSFUL BIOSENSOR

- The biocatalyst must be **highly specific** for the purpose of the analyses
- Be **stable** under normal storage conditions
- The reaction should be as **independent of such physical parameters** as **stirring**, **pH** and **temperature** as is manageable.
- The response should be **accurate**, **precise**, **reproducible** and **linear over the useful analytical range**, without dilution or concentration. It should also be **free from electrical noise**.

FEATURES OF A SUCCESSFUL BIOSENSOR

- The probe must be tiny and biocompatible, having no toxic or antigenic effects if the biosensor is to be used for invasive monitoring in clinical situations,
- If it is to be used in fermenters it should be sterilizable. In either case, the biosensor should not be prone to fouling or proteolysis.
- The complete biosensor should be cheap, small, portable and capable of being used by semi-skilled operators.
- There should be a market for the biosensor.

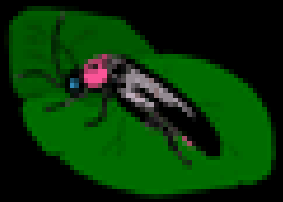
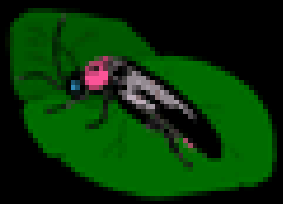
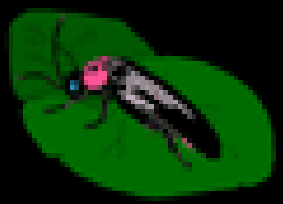
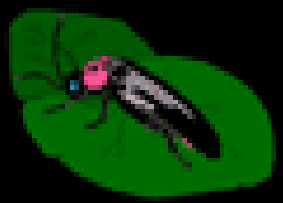
ADVANTAGES & DISADVANTAGES

Advantages:

- Measures bioavailable fraction
- Inexpensive
- Produces real-time data
- Less labor intensive
- More sensitive
- Suitable for field work
- Free of chemical extractions and analytical procedures

Disadvantages:

- Short lifetime
- Lack of genetic stability
- Unknown rates of type I (**luminescence without the presence of the analyte**) and type II (**or no luminescence in the presence of the analyte**) errors
- Limited understanding of applicability to higher organisms
- Performance dependent on environment of procedure
- Non-specificity

Thank You

SHARE YOUR EXPERIENCE OF THE COURSE WITH ME ON:

olanirana@ukzn.ac.za

Kirk et al. (2004). Methods of studying soil microbial diversity. Journal of Microbiological Methods, 58: 169-188.