

# Chemiluminescence detection of herbicide mediated inhibition of PS II in a $\mu$ -fluidic system

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## Introduction / Background

Taking into account the large amount of samples of industrial and urban effluents, sewage sludge, landfill leak-water, ground water, and irrigation water which have to be monitored, the development of fast and inexpensive tests is very important for large scale environmental screening of specific herbicides and heavy metals.

Biosensors that respond to a range of pollutants can be used for rapid, low cost pre-screening of large numbers of samples to determine samples that will then undergo more detailed analysis.

Photosystem II (PS II) is a multi-enzymatic chlorophyll-protein complex located in the thylakoid membrane of plants. PS II also contains the target site of the most widely used herbicides, which, by binding to PS II, inhibit photosynthesis.

In this work, the possibility to detect herbicide concentrations by measuring the production of hydrogen peroxide by PS II during photosynthesis was investigated using the novel method of chemiluminescence reactions. Combining the detection principle in a fluidic device will lead to the set-up of a field use apparatus, able to reveal specific herbicides in water.

## Chemiluminescence detection of hydrogen peroxide

PS II preparations were made by releasing the thylakoid membranes from chloroplasts of freshly homogenised and filtered spinach leaves. These were illuminated, in order to allow photosynthesis.

The production of  $H_2O_2$  by PS II was then measured using the chemiluminescence reaction with luminol, and Horseradish Peroxidase (HRP) as the catalyst. This reaction emits light ( $\lambda = 430$  nm), which was detected within cuvettes using a bench-top luminometer.

The chemiluminescence signal is proportional to the illumination light intensity and duration – Figure 1.

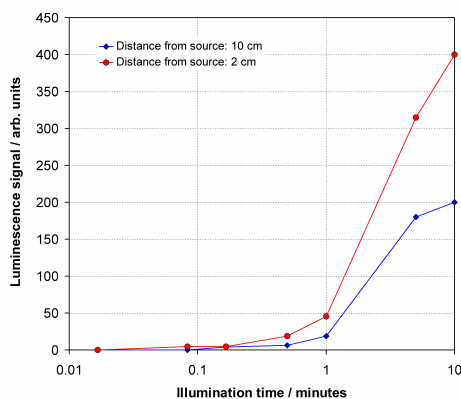


Figure 1. Chemiluminescence light output of light-induced hydrogen peroxide from thylakoids illuminated for different times and distances.

The presence of herbicides in samples reduces the hydrogen peroxide measured in a concentration-dependent manner – Figure 2.

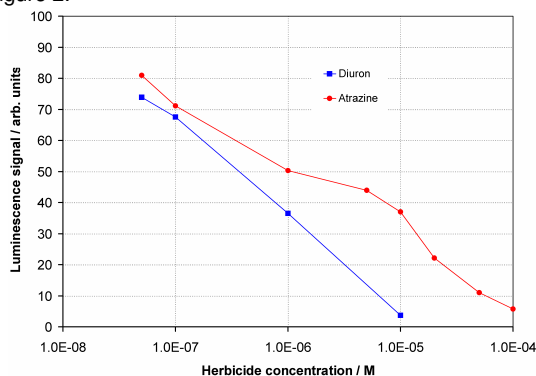


Figure 2. Chemiluminescence light output of light-induced hydrogen peroxide from thylakoids incubated with herbicides.

## $\mu$ -system principles and use

The sensing device proposed combines the production and detection of  $H_2O_2$  in a single flow assay by combining all the individual steps in a compact, portable device that utilises  $\mu$ -fluidic components. A novel feature is the flow-channel in which regions of appropriate reagents are temporarily immobilised using magnetic beads; PS II and HRP will be covalently attached on beads, which will in turn be magnetically immobilised - thus addressing issues of reagent stability and re-generatability – Figure 3.

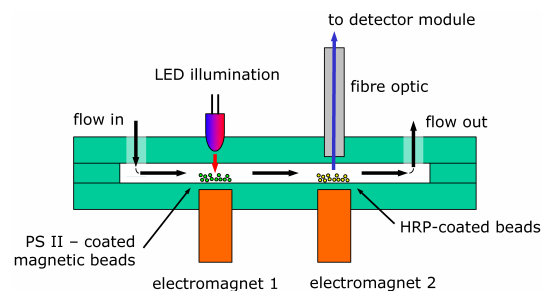


Figure 3. Side-exploded view schematic of the sensing unit

The initial potential of the  $\mu$ -system approach was assessed by performing  $H_2O_2$  detection. The chemiluminescence reaction was performed using HRP immobilised on magnetic beads – Figure 4.

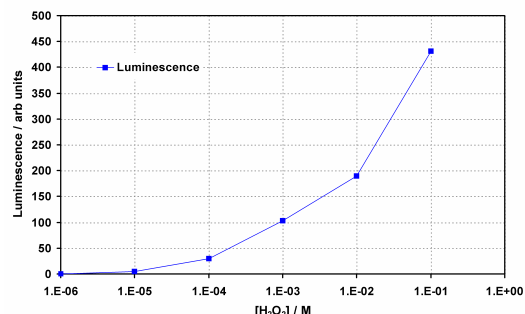


Figure 4. Chemiluminescence light output using the  $\mu$ -system with HRP covalently attached on magnetic beads.

## Conclusions

Demonstration of hydrogen peroxide production by PS II and inhibition thereof by herbicides with clear detection of the latter in the nanomolar region.

Results clarifying uncertainty in literature due to ambiguity of photosynthetic oxygen evolution.

Novel system design introduces ease in regeneration of system, an essential characteristic for potential field use.

