LuminoTox : A tool for the rapid detection of PAH, BPC and chlorobenzenes in waste water and sediments.

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Introduction

Toxicity control is a major driving force in the efforts to comply with urban and industrial wastewater treatment directives. Toxicity of water samples cannot be determined by chemical analysis. Moreover, the approach using whole organisms is expensive, time consuming and requires considerable effort. The use of small-scale biological tests at various levels of biological organization allows rapid toxicity detection. As numerous toxic molecules inhibit the electron transport chain (**Fig. 1**), many biosensors based on photosynthetic systems have been presented over the past decade[1-6]. Lab_Bell has developed and patented its own method for the stabilization of photosynthetic systems and a novel, portable fluorimetric detection device[7] (LuminoTox Analyzer) enabling the user to assess toxicity in just 10-15 minutes. To increase the number of molecules to be detected, two photosynthetic system arrangements are available: isolated photosynthetic complexes (PECs) and photosynthetic algae (SAPS). Based on chlorophyll fluorescence emission by PECs and SAPS, the toxicity of a sample is indicated by the modification of fluorescence parameters.

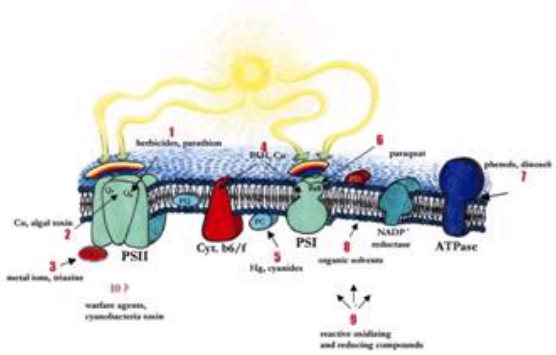

Fig. 1: **Inhibition sites on photosynthetic electron transport chain of PECs and SAPS**

Materials and Methods

PEC isolation was adapted from Boucher and Carpentier[8], stabilized PECs are stored at -80^{o}C, dried and vacuum packed. SAPS are cultivated as described.

In the first commercial model, the LuminoTox Analyzer was designed to detect only molecules inhibiting the PSII complex. An array of 4 LEDs and photodetectors were used for the determination of fluorescence emission from PECs and SAPS. All four LEDs emit at 470 nm and are programmed to flash a saturating pulse. Only the fluorescence emitted over 700 nm is measured. In order to detect molecules acting on the electron transport chain downstream from PSII, modifications have been made to the Analyzer to permit fluorescence kinetic measurements[9]. The saturating pulse of the commercial LuminoTox Analyzer has been modified to a continuous light illumination for 15 sec with the same LED array and photodetector as the first model. The fluorescence data is transferred to a computer for analysis. This kinetic detection mode is under development. Calculations for both detection modes were those suggested by Conrad and coworkers[10].

Standard solutions were purchased from Fisher Scientific. PECs (5 µg/mL) and SAPS (5 x 10^6 cells/mL) supplied by Lab-Bell were incubated 15 and 10 minutes, respectively with toxic molecules.

Sediment extraction protocol

Sediment containing PAH, BPC, chlorobenzene were obtained from National Water Research Institute, National Laboratory for Environment testing, Burlington, Ontario) (supplied by Centre St-Laurent, Montreal, Canada). The extraction protocol is simple: light agitation for 4 hours in water at room temperature at the indicated concentration (W/V). For incubation with PECs and SAPS, the regular protocol was followed: PECs (5 µg/mL) and SAPS (5 x 10^6 cells/mL) were mixed and incubated with 5% wt/v or less of sediment slurries for 15 min (PECs) and 10 min (SAPS). They were then filtered through a 2 µm filter and added to the cuvette for photosynthetic activity measurement with the LuminoTox Analyzer in (pulse) and kinetic modes (curves).

Results

The comparison of sensitivity detection by saturating pulse and kinetic detection modes are presented in Table 1. The results clearly show the comparative efficiency of both models. As expected, the pulse saturating measurements yield lower threshold values with molecules acting on PSII, while the fluorescence kinetic mode yields better sensitivity with molecules acting on the electron transport chain downstream from the photosystem II, *i.e*. substances inhibiting the electron transport chain from the cytochrome b_6/f complex to the PSI complex (for location of inhibition sites, see figure 1).

Table 1: Thresholds obtained for PECs in saturating pulse and fluorescence kinetic modes for toxic molecules inhibiting different sites on the electron transport chain.

Toxic substances	Threshold (mg/L)[1]		Suggested inhibition site on electron transport chain
	Saturating Pulse	Kinetic	
Atrazine	0.002	0.011	Q_B[11]
Copper	0.07[2]	0.07	Oxidizing site of PSII[12]
Methyl viologen	> 1	< 0.005	F_B[13]
PAH (mixture)	> 1[2]	< 0.01	Cyt. b_6/f or PSI[9]
Cyanide	> 1[2]	0.3	PC[14]

[1]: geographic mean calculated by statistic calculation software TOXSTAT, Version 3.5

[2]: incubation time = 15 min; 10 min if not indicated.

Table 2: **Preliminary results obtained for PECs and SAPS by the kinetic mode**

Toxic molecules	**PECs (15 min)**	**SAPS (10 min)**
BCP	0,2 ppm	2 ppm
PAH	0,01 ppm	0,01 ppm
anthraquinone	0,002 ppm	0,002 ppm
naphthalene	0,1 ppm	Non tested
phenanthroline	0,3 ppm	Non-tested
BPC, HAP and chlorobenzenes contaminated sediments (EC1 and EC2)	IC_{50}	IC_{50}
	EC 1: < 5 %	EC 1: > 5 %
	EC 2: < 5 %	EC 2:< 5 %
BPC, HAP and chlorobenzenes	IC_{50}	IC_{50}

contaminated sediments (EC5 and EC6)	EC 5: < 0,6 %	EC 5: < 0,6 %
	EC 6: < 5 %	EC 6:< 0,6 %
Dioxin and furan contaminated sediment	Non tested	Non tested

Discussions

The results obtained with condensed aromatic hydrocarbon derivatives like naphthalene, phenanthroline, anthraquinone indicate that photosystems are affected by these pollutants. Due to industrial activities, the main origin of these pollutants remain the contaminated soils and sediments. To validate the above analysis, Certified Reference Materials from National Water Research Institute (Env. Canada) were used. Analysis was performed on several of these samples, EC1, EC2, EC5 and EC6. The main difference between EC1 and EC2 is the relative quantity of PAH (µg/g) and chlorobenzenes (ng/g). EC1 contains more PAH and EC2 more chlorobenzenes, while the amount of PCB (µg/g) is similar in both. For samples EC5 and EC6, the concentration of PCB and PAH is ten times lower than EC1 and EC2, but similar for chlorobenzenes.

The results for EC1, EC2, EC5 and EC6 show that the water extracted from all four sediment samples display varying levels of toxicity. The IC_{50} values are significantly lower for EC5 and EC6 compared with those obtained for EC1 and EC2. Assays are underway to determine the IC_{50} values obtained with LuminoTox test protocol and results will be compared with control bioassays. Furthermore, 24 hour extractions will be performed in order to establish the optimal protocol for extraction.

We thus conclude that the LuminoTox technology can be applied as a screening test to monitor the presence of PAH, PCB, chlorobenzenes and their derivatives in sediments.

The protocol is simple and satisfies all of the criteria required for rapid *in vitro* toxicity testing:

- Easy to use;
- Inexpensive;
- Fast toxicity response (15 minutes for the incubation time and 10 seconds for the fluorescence reading by the LuminoTox Analyser) providing a very early warning when compared to standard bioassays for which the toxicity response requires between 48 to 96 hours;

§ Sensitive to a wide range of pollutants; toxicity can therefore be detected in complex waste water and slurries;

§ Representative of the studied environment since photosynthetic systems are at the bottom of the trophic chain. The toxic response observed in these systems reveals the potential impact of toxicity at the upper trophic levels of the ecosystem (early warning).

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References

1. Carpentier R. (1997) Current Topics in Biophysics, vol. 6, Biosensors: Advances and Applications. Al. I. Cuza University Press, Iasi, Romania, pp. 261-276.

2. Piletskaya E., Piletsky S., Lavrik N., Masuchi Y., Karube I. (1998) *Anal. Lett.* **31 (15)**: 2577-2589.

3. Laberge D., Chartrand J., Rouillon R., Carpentier R. (1999) *Env. Toxicol. Chem.* **18**: 2851-2858.

4. Naessens M., Leclerc J.-C., Tran-Minh C. (2000). *Ecotoxicol. Environ. Saf. (B)* **46**: 181-185.

5. Koblizek M., Maly J., Masojidek J., Komenda J., Kucea T., Giardi M. T., Mattoo A. K., Pilloton R. (2002) *Biotechnol. Bioeng.* **78 (1)**: 110-116.

6. Podola B., Nowack E.C.M., Melkonian M. (2004). *Biosens. Bioelect.* **19**: 1253-1260.

7. Bellemare, F., Boucher, N., Lorrain,L. , 2001. Method of testing photosynthetic activities. **WO2004/046717**

8. Boucher N. and Carpentier R. (1999) *Photosynth. Res.* **59:** 167-174.

9. Mallakin A., Babu S., Dixon D.G., Greenberg B.M. (2002). *Environ. Toxicol.* **17**: 462-471.

10. Conrad R., Büchel C., Wilhelm C., Arselane W., Berkaloff C., Duval J.-C. (1993) *J. Appl. Phycol.***5**: 505-516.

11. Draber W., Tietjen K., Kluth J. F., Trebst A. (1991). *Angew. Chem.* **30**: 1621-1633.

12. Baron M., Arellano J.A., Gorgé J.L. (1995). *Physiol. Plant.* **94**: 174-180.

13. Fujii T., Yokoyama E.I., Inoue K., Sakari H. (1990). *Biochem. Biophys. Acta* **1015**: 41-48.

14. Rodriguez M. Jr, Sanders C. A., Greenbaum E. (2002). *Biosens. Bioelect.* **17**: 843-849.

15. Dellamatrice, P.M., Monteiro, R.T.R. and C. Blaise (2005). Ista12, Greece.