Yeast-Based Probe for Functional Toxicity of Active Concentrations of Diuron in Water

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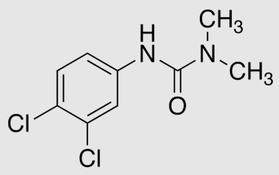
Abstract

A study on short-term and long-term (6 and 24 h respectively) exposures to the herbicide diuron was carried out. The test, basing on a yeast cell probe, investigated the interference with cellular catabolism and possible self-detoxification capacity of *Saccharomyces cerevisiae*. Aerobic respiration was taken as the toxicological end-point. Percentage interference (%ρ) with cellular respiration was measured in water and it was calculated through the comparison of respiratory activity of exposed and non-exposed cells. The test for short-term exposure gave positive %ρ values except that for 10^{-6} M (11.11%, 11.76%, 13.33% and 0% for 10^{-10} M, 10^{-8} M, 10^{-7} M and 10^{-6} M respectively). In the case of long-term exposure the test showed positive %ρ values, but less effect than short-term exposure until 10^{-8} M and much higher at 10^{-6} M (7.41%, 8.82%, 11.76% and 6.06% for 10^{-10} M, 10^{-8} M, 10^{-7} M and 10^{-6} M respectively). The findings of aerobic respiration as toxicological end-point were in agreement with known mechanisms of toxicity and intracellular detoxification for both the doses and exposure times tested. This yeast-based probe is proved to be very sensitive to diuron. Further, it is suited for integration in the patented multiparametric platform BEST for hazard analysis and management in the food chain and the environment.

Aim of the study:

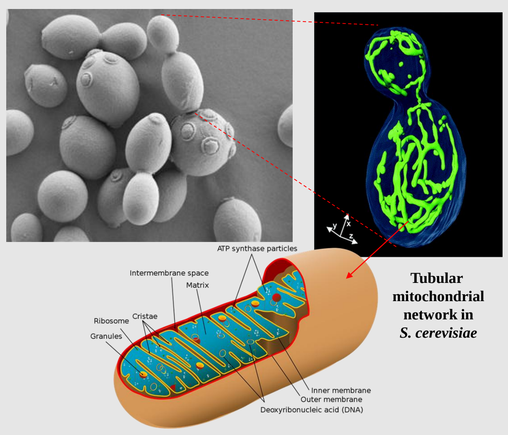
To investigate the effect of **different exposure times** and **different concentrations** of **diuron** on ***Saccharomyces cerevisiae* aerobic respiration** in **aqueous solution.**

Phenylurea herbicide **diuron** (3-(3,4-dichlorophenyl)-1,1-dimethylurea or DCMU)



- **One of the most used pesticides in Italy** [1];
- **One of the most frequently detected** both in **surface** and **groundwater in Italy** [2];
- **Maximum concentration** for **single pesticide** in **drinking water** is **0.1 µg/L** (corresponding to **4.3 ·10^{-10} M** for DCMU) [3].

The yeast *Saccharomyces cerevisiae*

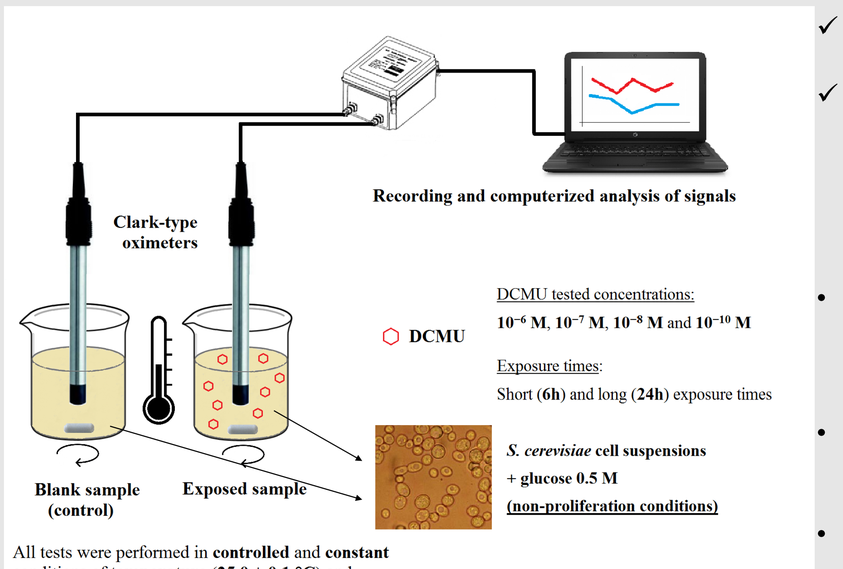


Tubular mitochondrial network in *S. cerevisiae*

Cellular aerobic respiration of *Saccharomyces cerevisiae* is found to be **very sensitive** to the **presence of various toxic chemicals** [4, 5, 6]

Monitoring the **change of dissolved O_2 concentration** (linked to **mitochondrial activities**) exploiting a **yeast-based probe** → **Rapid assessment** of the **presence** of **toxic substances** in **water**

Yeast-based probe



All tests were performed in **controlled** and **constant** conditions of **temperature (25.0 ± 0.1 °C)** and **agitation (stirring speed: 200 rpm)**

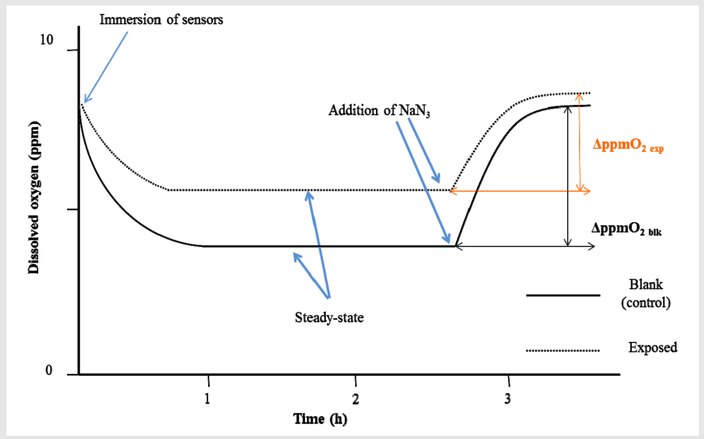
- ✓ Relatively **short running time of tests** (about **2 hrs**);
- ✓ **Amperometry** is one of the most used analytical technique for the development of whole cell-based biosensoristic devices [7]:
  - **linear response → more sensitive than potentiometry** (which response is logarithmic);
  - **low costs of transducers** (compared to optical devices);
  - **measures are unaffected by sample color and/or turbidity** (useful feature for **real samples analysis**).

The **percentage interference of cellular respiration** (**%ρ**) was calculated with the following algorithm:

$$\%\rho = [1 - (\Delta ppmO_{2\ exp} / \Delta ppmO_{2\ blk})] \cdot 100$$

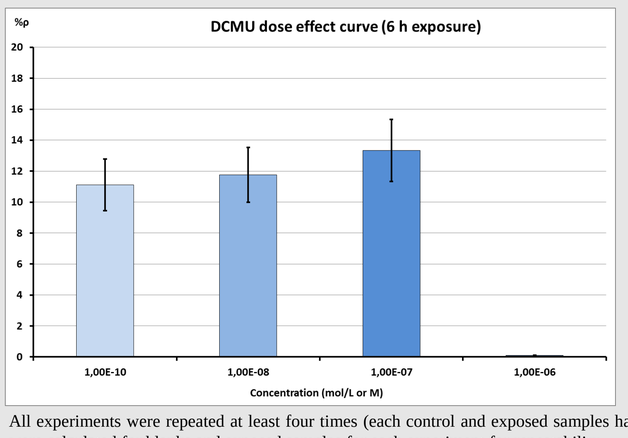
$\Delta ppmO_2$ exp = mean of variations of the dissolved O_2 (in ppm) for exposed samples;
$\Delta ppmO_2$ blk = mean of variations of the dissolved O_2 (in ppm) for blank samples.

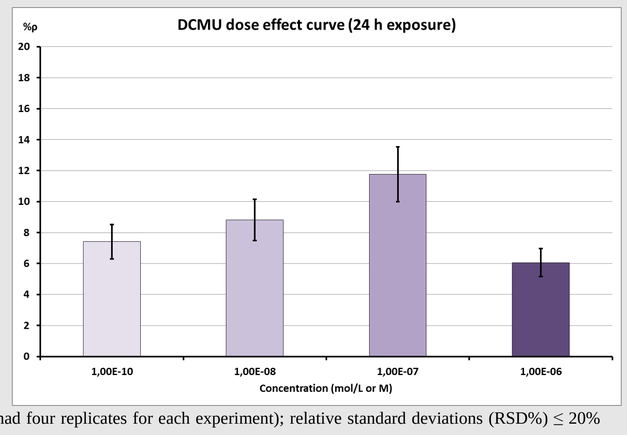
Experimental respirometric curves



The experimental respirometric curves shows the dissolved oxygen (ppm O_2) as a function of time in the case of interference with the respiration of the yeast cells.

Results and discussion





All experiments were repeated at least four times (each control and exposed samples had four replicates for each experiment); relative standard deviations (RSD%) ≤ 20% were calculated for blanks and exposed samples for each experiment for repeatability.
Statistical tests (ANOVA for Randomised Block Design): significant relationship between exposed and non-exposed samples was found in all concentrations for both exposure periods ($p < 0.03$). The only exception was the 6 h exposure experiments using 10^{-6} M of DCMU because under these conditions there is no inhibition (%ρ = 0)

Hypothesis ("*three mechanisms scheme*")
Involvement of mechanisms of:

- ❑ **Toxicity** (**inhibition of Complex III of the mitochondrial electron-transport chain** [8] and subsequent **oxidative stress** due to **reactive oxygen species** (ROS) [9]);
- ❑ **Detoxification** (potential involvement of a known plasma membrane efflux pumps system present in *Saccharomyces cerevisiae* (**ATP-binding cassette** or **ABC transporters**): the role of the ABC transporters in DCMU resistance has been demonstrated [10]);
- ❑ **Cell repair** (adaptive oxidative stress response due to ROS accumulation including **damaged molecules repair/removal systems** [11, 12]).

All these mechanisms are characterized by its own **kinetics of triggering and action** (**presumably dose- and exposure time-dependent**). These kinetics are critical in the interpretation of the results: **%ρ values could originate from the kinetic combination of the rate of all three mechanisms**, like **a snapshot at 6 h and 24 h of exposure**.

Short-term (6 h) exposure:

- **DCMU 10^{-6}M is able to trigger the cellular detoxification/repair mechanisms** (%ρ= 0);
- **At lower doses** (10^{-7} - 10^{-10} M) delaying in trigger due to a **tolerance effect mechanisms →DCMU accumulation in the mitochondria and exertion of toxic effects** (positive %ρ values).

Long-term (24 h) exposure:

- **Exposure to DCMU 10^{-6} M** showed instead a significantly different %ρ value (compared to short term the results obtained under 6h) **→Potential saturation of cellular detoxification/repair mechanisms →DCMU accumulation in the mitochondria and exertion of toxic effects (%ρ= 6.06%)**;
- **Lower doses** (10^{-7} - 10^{-10} M): **attenuation of toxicity mechanism** caused by speed of action of detoxification/cell repair mechanisms.

Conclusion and future perspectives

- ✓ The proposed yeast-based probe is proved to be **very sensitive** and **able to detect herbicide DCMU** at very low concentration (**up to 10^{-10} M i.e. four times below EU limit for drinking water**);
- ✓ The **results** obtained **well match with known mechanisms of toxicity, cellular detoxification and repair described in scientific literature for *Saccharomyces cerevisiae*** at the doses and exposure times employed;
- ✓ This probe is **suited to be automated and integrated** in the **technological platform BEST** (PCT WO/2010/001432): **(Bio) Sensors' System for hazard analysis and management in the food chain and the environment**
  ALERT Project (http://www.alert2015.it/):
  An integrated sensors and biosensors system (**BEST**) aimed at monitoring the quality, health and traceability of the bovine milk supply chain.

ALERT 2015

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efsa European Food Safety Authority

Environmental risk assessment of pesticides: 25 years of scientific advancements since the adoption of Directive 91/414/EEC (EFSA, 15-16 November 2016)