Detecting toxic substances in water by Chlorophyll Fluorescence

May, 2018
Photosynthesis and Fluorescence
Photosynthesis – the principle

\[ 2H_2O \rightarrow 4e^- + 4H^+ + O_2 \]

**Carbon dioxide** + **Water** → **Sugars** + **Oxygen**

- **Sun's energy**
- **Chlorophyll**
- **CO\(_2\)** + **H\(_2\)O** → **C\(_6\)H\(_{12}\)O\(_6\)** + **O\(_2\)**

**Energy transfer**: Oxidation of H\(_2\)O

**Light-harvesting pigment (antenna molecules)**

**Reaction center**
Fluorescence – the principle

Absorption

Fluorescence

Reaction center

Light-harvesting pigment (antenna molecules)

Fluorescence F

Absorption (optical density)

Fluorescence (arbitrary units)

Wavelength (nm)
Fluorescence measurement – the principle

**Fluorescence yield, rel. units**

- **F₀**
- **Fᵥ**
- **Fₘ**

**Measurement Light**

**Photosynthetically Active Light**

**Yₘ = Maximum Yield** = Maximum available reaction centres

**ETR = Electron Transfer Rate**

\[
\frac{F'_q}{F'_m} = \frac{F_m - F'_r}{F'_m}
\]

\[
\frac{F'_v}{F'_m} = \frac{F_m - F_0}{F_m}
\]
On-line Chronic and Acute Monitoring for Ecotoxicology with Algae
Different configurations were validated for accuracy and maintainability
CAMEO-A® – the sensor gives two clear values, no scientific background necessary

• Our analyser is based on a cuvette PAM
  – Yield
  – ETR
  → health of the sample
  → determination of toxicity
CAMEO-A – the sensor is easy to use

- No algae culture maintenance necessary
- Continuous monitoring → quick detection
- Algae bead remains separate from the sample or reference liquid
- No interference with suspended solids
- No pre-treatment nor filtering necessary → representative samples
- No interference with the sample colour
- High tolerance for salts (up to 15 mS/cm)
and ecotoxicity
Water is not a commercial product but rather a heritage which must be protected, defended and treated as such!

- Companies are bound to European Legislation 91/27/EEC EC directive concerning urban waste water treatment
  - Planning
  - Regulation
  - Monitoring
    - Information and Reporting

- Monitoring of toxic substances by
  - Group parameters → no information on ecotoxicity
  - Specific analyses → never complete
  - Ecotoxicity testing → Whole Effluent Toxicity
We focus on monitoring of the algae trophic level

- **Pseudokirchneriella subcapitata** (freshwater algae)
  - Reference is made to OECD guideline 201
  - Based on inhibition of growth rate
  - Lab tests / Sampling / 3 days test: snapshot analysis
  - Interpretation of results

**Sample May 2016**

Inhibition growth rate (Lab 1)

Inhibition growth rate (Lab 2)
CAMEO-A – detailed in-depth data compared with analyses by accredited labs

Sample February 2018

Lab 1

Lab 2

I_{ETR} = 20\%
CAMEO-A – detailed in-depth data step-by-step

- Events are shown
- F0 (relative)
- Sample (high) vs Reference medium (low)
CAMEO-A – detailed in-depth data step-by-step

- Events are shown
- F0
- Sample vs Reference medium
- Yield (max. available reaction centres)
CAMEO-A – detailed in-depth data step-by-step

- Events are shown
- F0
- Sample vs Reference medium
- Yield (max. available reaction centres)
- ETR
CAMEO-A – detailed in-depth data step-by-step

- Events are shown
- F0
- Sample vs Reference medium
- Yield (max. available reaction centres)
- ETR
- Inhibition ETR (right axis - up to 40%)
CAMEO-A – Benchmarking test with Zn$^{2+}$ (50 µg/l and 400 µg/l)

- ETR inhibition 400 µg/l
- ETR inhibition 50 µg/l
- Yield inhibition 400 µg/l
- Yield inhibition 50 µg/l
Conclusions

• CAMEO-A can be used in an industrial water treatment for process optimisation and early warnings.

• CAMEO-A is operational friendly.

• CAMEO-A provides an on-line measurement but doesn’t replace the standard methods. Toxicity can be predicted.

• More benchmarking is still planned.
Thank you