Direct detection of toxic micro-algae *Alexandrium minutum* through high sensitive gravimetric sensors

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PRECODD PRogramme de recherche ECOtechnologies et Développement Durable — Financé par ANR

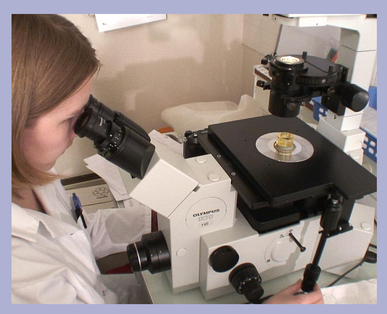
Introduction

Detection of different micro-algae: *Dinophysis, Karenia, Alexandrium, Pseudo-nitzschia*

Approach used now in France:

Map of the network to monitor phytoplankton (REPHY)

Sample of sea water



Numeric determination of the algae concentration by using reverse optical microscope

Problems:

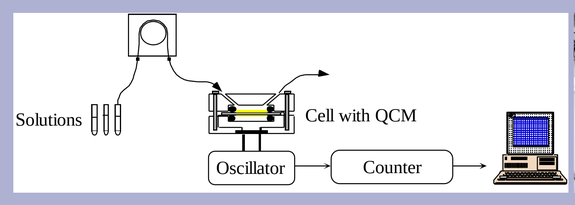
Time consuming, indirect determination, cost, reliability…

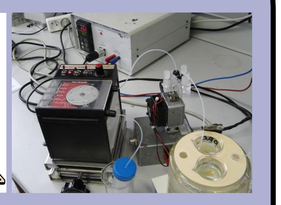
Solution evaluated:

Direct detection by using high sensitive gravimetric DNA sensor

Experimental part

1. Gravimetric device





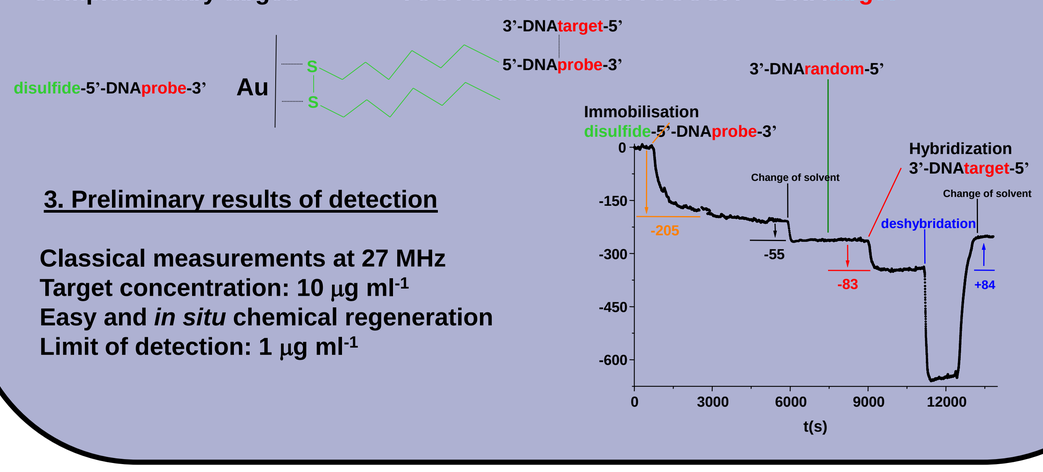
Microcell including the quartz resonator
Inner volume: 50 µl

Complete set up with frequency counter, peristaltic pump and Peltier device

2. DNA probes and targets related to *Alexandrium Minutum*

Probe: AGCACTGATGTGTAAGGGCT DNA**probe**

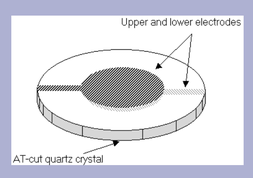
Complementary target: TCGTGACTACACATTCCCGA DNA**target**



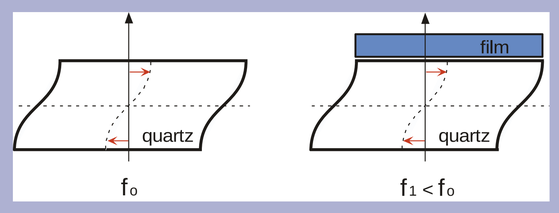
3. Preliminary results of detection

Classical measurements at 27 MHz
Target concentration: 10 µg ml^{-1}
Easy and *in situ* chemical regeneration
Limit of detection: 1 µg ml^{-1}

Concept of microbalance measurements

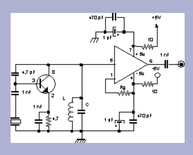


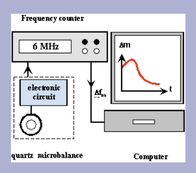
Piezoelectric material
with two active gold electrodes
S=0.2 cm^2



Shear waves generated
Resonant frequency depends on the quartz/film thickness

1. Active mode: classical microbalance approach

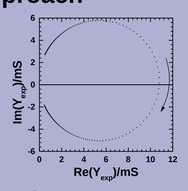


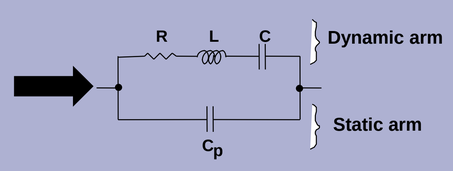


$$\Delta f_m = -k_S^{th} \times \Delta m$$

Direct mass change estimation but necessity to develop complex electronic oscillator

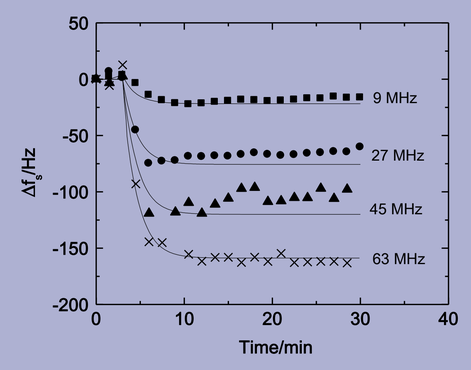
2. Passive mode: electroacoustic approach



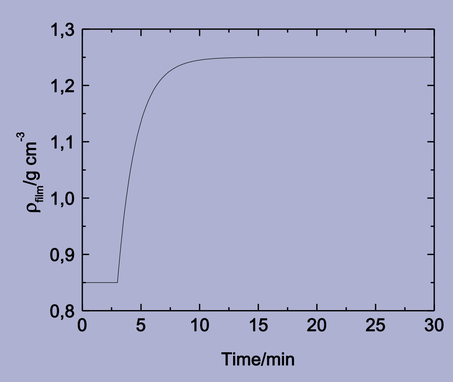


Set up more expensive but more complete and easy measurements

Hybridization at different frequencies



Hybridization at different frequencies
Target concentration: 10 µg ml^{-1}
(experimental and theoretical -)



Evolution of the film density during the hybridization step

Frequency/MHz	Experimental Gain (Hybridization)	Theoretical Gain
9 (fundamental)	1.0	1.0
27 (3rd overtone)	3.0	3.0
45 (5th overtone)	4.8	5.0
63 (7th overtone)	6.5	7.0

Table of the different sensitivities according to the frequencies

Frequency/MHz	Concentration/µg ml^{-1}
9 (fundamental)	0.5
63 (7th overtone)	0.05

Limit of detection between 9 MHz and 63 MHz

QCM calibration at different frequencies

Theoretical mass sensitivity

Sauerbrey equation valid for acoustically thin layers

$$\Delta f_m = -2.26\ 10^{-6}\ \frac{f_n^2}{n}\ \frac{\Delta m}{A} = -k_S^{th}\Delta m$$

Copper electrodeposition under controlled current:

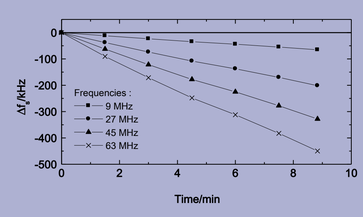
$$Cu^{2+} + 2e^- \rightarrow Cu$$

- microbalance frequency shift is followed: Δf_m

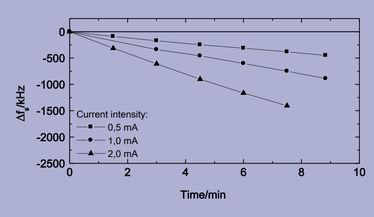
$$k_S^{exp} = \frac{\Delta f_m}{\Delta m_F}$$

- mass deposited estimated through the Faraday law: Δm_F

Electroacoustic measurements at different frequencies: 9, 27, 45 and 63 MHz



Series resonant frequency changes over copper electrogeneration at different frequencies



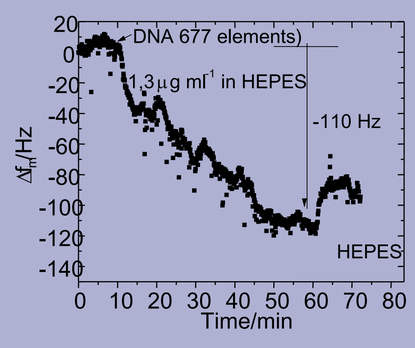
Series resonant frequency changes over different current values
Working frequency: 63 MHz

Frequency/MHz	Experimental (copper)		Theoretical (Sauerbrey)	
	k_S^{exp} (Hz g^{-1} cm^2)	Gain	k_S^{th} (Hz g^{-1} cm^2)	Gain
9 (fundamental)	-1.94*10^{+8}	1.0	-1.831*10^{+8}	1.0
27 (3rd overtone)	-5.88*10^{+8}	3.0	-5.495*10^{+8}	3.0
45 (5th overtone)	-9.41*10^{+8}	4.8	-9.15*10^{+8}	5.0
63 (7th overtone)	-12.4*10^{+8}	6.4	-12.81*10^{+8}	7.0

Comparison of the experimental and theoretical sensitivities

Conclusions

1. **Direct detection of DNA targets up to 0.05 µg ml^{-1}**
2. **Detection of DNA targets from PCR: ss with 677 elements**



3. **Preliminary results with 50 MHz on the fundamental mode**

References:

Lazerges M, Perrot, H, Antoine E, Defontaine A, Compère C. "Oligonucleotide quartz crystal microbalance sensor for the microalgae *Alexandrium minutum* (Dinophyceae)", Biosensors and Bioelectronics, 2006, 21, 7, 1355-1358.

Lazerges M, Perrot H, Antoine E, Compère C. "Layer-by-Layer DNA film synthesis via branched hybridization", 2008, ITBM-RBM, 29: 133-135.