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AlgaDec

Development of a rRNA-Biosensor for the Detection of Toxic Algae

Instrument: Cooperative Research (CRAFT)

M 1.2 Final report

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Author:	Thomas Hanken
Author E-Mail:	thanken@isitec.de
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Co-ordinator contact details:

Thomas Hanken
Tel.: + 49 471 92234 11
E-mail: thanken@isitec.de

iSiTEC GmbH
Stresemannstrasse 46
D-27570 Bremerhaven

Project website: **www.algadec.net**

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1 Project execution

1.1 Project contractors:

Partic. Role	Partic. no.	Participant name	Participant short name	Country	Date enter project	Date exit project
CO	1	*ISITEC GmbH*	ISITEC	Germany	Month1	Month 29
CR	2	*Gwent Electronic Materials Ltd*	GEM	UK	Month1	Month 29
CR	3	*Palm Instruments BV*	PALM	Netherlands	Month1	Month 29
CR	4	*CytoBuoy b.v.*	CYTO	Netherlands	Month1	Month 29
CR	5	*Exiqon A/S*	EXIQON	Denmark	Month1	Month 29
CR	6	*Juan José Martín Rodríguez*	JMR	Spain	Month1	Month 29
CR	7	*Skagerrak Skjellmottak AS*	SKJELL	Norway	Month1	Month 29
CR	8	*North Bay Shellfish Ltd*	NBS	UK	Month1	Month 29
CR	9	*Stiftung Alfred Wegener Institut für Polar und Meeresforschung*	AWI	Germany	Month1	Month 29
CR	10	*Centro Oceanográfico de Vigo*	VIGO	Spain	Month1	Month 29
CR	11	*Institute of Marine Research*	IMR	Norway	Month1	Month 29
CR	12	*University of Westminster*	UW	UK	Month1	Month 29

1.2 State of the art

Although algal blooms represent a serious public health and economic problematic, no cost ef-fective device for the specific detection of toxic strains is yet available on the market. Among the methods used for the analysis of phytoplankton (see Table below) only molecular methods, by means of genetic sequences, make a specific recognition of the algae possible.

Nowadays Coastal monitoring requires transportation of samples to specialised laboratories where toxic algae concentration is determined by cell counting. This method is highly time-consuming since it normally involves preliminary identification by inverted light microscopy and further morphological identification through scanning or transmission electron microscopy.

The presence of HABs is also currently detected through monitoring of shellfish for the presence of microalgal toxins, which involves tissue bioassays in mice and rats. These time-to-death bio-assays do not only involve high costs but also obviously raise ethical questions.

Current monitoring methods as above described require well-trained scientific staff and are error-prone since human perception (visual counting and morphological identification) and animal tests play a role in obtaining the results. The whole process including transportation and analysis takes more than one day (results are usually obtained within five working days after receiving the sample) and therefore it does not allow for the main purpose of monitoring: taking preventive measures when coastal areas are threatened by HABs. In addition, the

cost for monitoring represents today a major economic drawback for the European industry, e.g. for Ireland 5 % of the turnover of the Irish shellfish industry.
Up to today there is no technology available for the rapid, in situ and specific recognition and quantification of toxic algal strains.
Electrochemical measurement methods present a cheaper and easier alternative to the fluo-res-cent detection, because no expensive high-density chips are needed and no cell counting is re-quired since the electric current can be easily correlated to the concentration of toxic cells.
Therefore the combination of a method allowing a differentiation at molecular level with an electrochemical reaction is an excellent option to provide quantitative, algae specific results.

1.3 Project objectives

The ALGADEC projects aims at developing a handheld device for the in situ analysis of toxic algae concentrations. The device consists of a housed electric interface equipped with a data recording system and two external units, the fluidic cell and the heating chamber. A whole work package will be devoted to the gathering of specifications to optimise not only the functioning but also the design of the device in order to achieve the maximal degree of compactness and robustness.

Relevant national monitoring organisations and partners of EUROHAB Sciences Initiative [ ] as well as potential end-users have been consulted about the technical and economic requirements that the biosensor must meet in order to satisfy the market demand. The following table contains the technical objectives of AlgaDec and a comparison of planned and actual outcomes, as well as the work packages, deliverables or milestones to which they are related:

Technical objectives	Workpackages	Deliverables and Milestones	Actual outcome
Field analysis and data recording possible	WP4	D 4.1 D 4.2 M 4.1 M 4.2	A warming chamber and fluidic cell which enable for the autonomous functioning of the device has been developed.
	WP5	D 5.2 D 5.3	Development of software for the micro-controller for the automatic control of all de-vice components and permanent data storage. Development of software for PC connection for data management.
Simultaneous detection of area specific groups of toxic algae (8-12 species per region)	WP2	D2.1 M2.1	Selection of species for which new probes should be developed.
	WP3	D3.2 M3.2	Design and development of a multiprobe chip. The sensor array will allow parallel and inde-pendent detection in each sensor.
Reduction of analysis time from 1-2 days to approx. 2 hours	Inherent to method		
Sensitivity of 60 cells/L	WP3	M3.1	Design of ultra high affinity analogues and optimised linker molecules for the probes. Test on detection limits.
Cost per analysis 5-10 Euros	WP3	D3.2 M3.2	Selection of the material of the electrodes. Optimisation of the ratio quality/price
Investment cost per device below 2.500 EURO	WP2	D2.1 M2.1	In the catalogue of specifications the economic requirements will also be taken into account.
	WP5	D5.1	Simplification of the electronic interface to fulfil the requirements and costs of 500 euros

Robustness and adequacy for future implementation into a buoy for remote monitoring	WP6	D6.1	Functional tests of the different components to guarantee the required robustness could nit be conducted within the project time. However, a plan for testing has been elaborated from Cytobuoy.

The prime social objectives of the project are:

- Prediction of dangerous concentrations of algal cells thanks to the rapid in situ measurement and high sensitivity, which will account for the reduction of the health risk caused by presence of algal biotoxins both in swimming waters and in seafood.
- Supporting the economic well being of small coastal fishing communities, which are under threat due to interruptions in fishing activity.
- Preventing potential economic loses in aquaculture and tourist industry.
- Improving the current European monitoring systems.

1.4 Project summary

In the last 29 months intensive work has been invested in the development of a portable device for detecting toxic species. The CRAFT-Project called AlgaDec has been from the EC 6th Framework Programme.

The war on microalgae

Toxin-producing micro algae have been responsible for serious environmental, economic and public health catastrophes today as well as throughout history. For example an outbreak of *Chattonella antiqua* once killed $500 million worth of caged fish in Japan, and in 1981 over 5000 people suffered from diarrheic shell-fish poisoning in Spain. Still today, fish farms remain at risk of from toxin contamination and the problem is aggravated by the effects of human economic activity, such as the use of pesticides and fertilisers in farming. These compounds, which contain phosphates and nitrates, are ferried into the sea by rivers into the sea, where micro algae feed on phosphates and growth is stimulated and blooms result rates included in them. Fish farms are often located in coastal areas where rivers carrying sewage pollutants or agricultural fertilisers reach the sea.

This recurrent toxic phenomenon has made it necessary to devise a cost-effective and simple-to-use device for detecting toxic algae. AlgaDec is developing what will be the first commercially available biological sensor, capable of rapidly detecting the presence of specific harmful algal species before they develop into harmful blooms (HABs). The device will enable aquatic farmers to check for the presence of toxic algae in their waters at an early stage and to take the proper measures to prevent contamination and the economic losses associated with it. Plentiful catches aside, the AlgaDec biosensor is expected to reduce the health risk for humans who eat farm-raised fish and shellfish and even those who collect shellfish personally because warning notices not to collect can be posted earlier.

Good vs. bad algae

Phytoplankton, composed of micro organisms such as algae, form the basis for most aquatic food chains. On the one hand, algae can be used for human benefit, for nutritional purposes

and in cosmetics, or for the production of dyes and even bio diesel, hydrogen and natural oils. However, there are about 100 recognised micro algal species that are toxin-producing.

'A concentration of only a few cells of noxious micro algae per liter can have harmful effects'.

These noxious algae do have severe effects on the environment and humans. A concentration of only a few cells of noxious micro algae per liter can have very harmful side effects. If farm-raised fish and shellfish feed on toxic algae, they can become infected and potentially contaminate consumers. The resulting health impacts can be devastating, ranging from digestive disorders to amnesia or even death. Consumers can also be affected by swimming in infected waters.

The economic repercussions of algae contamination can be very serious. Not only is fish production affected, through stock destruction and consumer mistrust, but there are also ramifications for the tourism sector. Tourists do not like to swim in algal blooms and some blooms cause skin and lung irritations.

Contamination alert

Currently, there is no commercially available product allowing for in situ detection of specific harmful algae. The legal requirement by governments to monitor for toxic algae is by identifying exact species with optical microscopy. This technology has many limitations. For instance, it can only be performed by specialised operators and requires a very lengthy procedure. This morphological observation relies on comparing toxic and non-toxic algae, which can be physically very similar, making them difficult to distinguish. This technique is often complemented by the use of mouse-assays for the detection of toxins in water, a method that raises ethical questions.

In order to overcome the difficulties caused by the visual observation of algae, AlgaDec suggests a new approach, based on a molecular study of algae. The procedure involves DNA testing, giving it the ability to detect noxious algae that undergo hybridisations or genetic evolutions. The developed AlgaDec device will be portable and highly sensitive, and will contain an electromechanical sensor and a multi-probe chip, designed to obtain tailor-made sensors for each species. It will also contain a fluidic cell and warming chamber, controlled by specific software, to achieve a reliable automated detection system.

The AlgaDec biosensor is expected to become an important mechanism widely used by fish-farmers, monitoring agencies and tourist administrations. Its ease of use, rapidity and reliability, not to mention its portability, make it the perfect tool for the detection of dangerous algal contaminations. Early contamination detection is key for the adoption of measures aimed at preventing economic losses and ensuring human health protection, and AlgaDec will make that possible.

1.5 Methodologies and Approaches

Molecular biological methods play an increasingly important role in the detection of algae species, especially toxic ones. Sequences of ribosomic RNA-genes are especially good for the design of species-specific probes (Amman 1995). Alternatively, the distinction between similar species can be achieved by means of mono and polyclonal cell surface antibodies. rRNA and antibody probes are already available for some taxa (reviewed in Medlin et

Simon 1997, Reguera et al., 1998). rRNA probes have been applied in the microscopic detection of microalgae, especially toxic algae (Simon et al. 1995; Lange et al. 1996; Miller & Scholin 1998).
A specific quantification of rRNA in a sample through electrochemical measurements can be obtained by means of sandwich hybridisation. This method consists of three steps:
A taxon specific DNA-probe is immobilised through an avidin - biotin binding on the microchip. This probe is called the capture probe.
RNA-strands from the target organism bind to the corresponding complementary DNA/RNA-strand of the capture probe on the microchip.
The detection of the fixed RNA is carried out by means of a second oligonucleotid, which specifically binds to it. This oligonucleotid sequence contains an epitope, which is recognised by an antibody coupled to an enzyme. The enzyme catalyses in turn the oxidation of a mediator, thus originating an electrochemical reaction and an electric current.

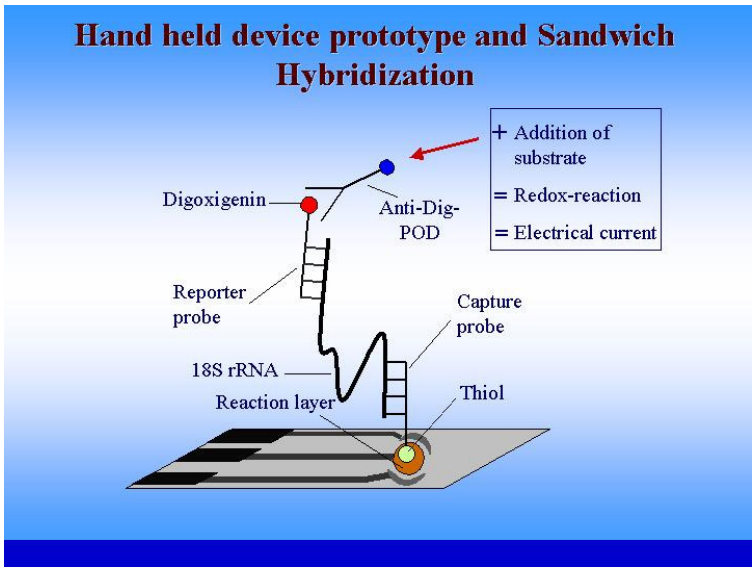

Fig. 1: AlgaDec sandwich hybridization principle

The central part of the sequence-specific electrochemical rRNA sensor consists of a chip carrying three electrodes (working-, anti-, and reference electrode). A potential is applied between the working and the reference electrode to enhance the electro-chemical reaction at the working electrode. The mediator, which is oxidised during the enzymatic reaction, is reduced at the working electrode. The resulting current is proportional to the concentration of peroxidase, which in turn represents the amount of labelled probe and therefore the amount of target rRNA (Figure 2).

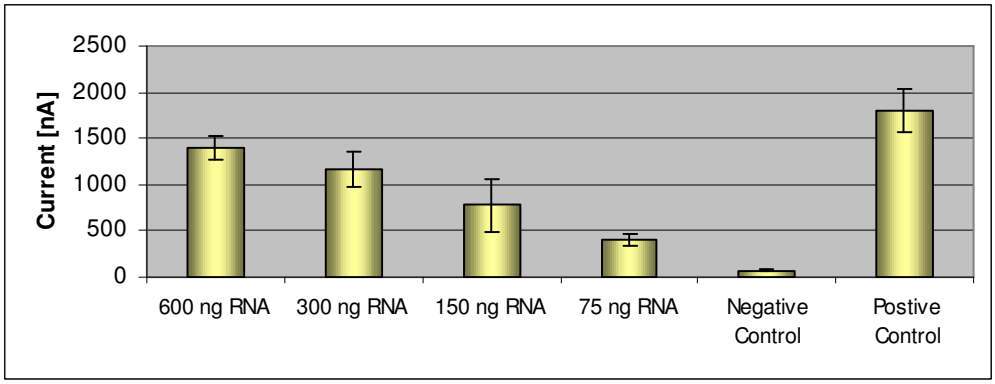

Fig. 2: Decreasing amounts of A. tamarense rRNA hybridized to a A. tamarense probe

Thus the current measured between working and anti-electrode is proportional to the bound rRNA. This signal also shows a good correlation with cell counts from the water column (Figure 3). The sensor to be developed in ALGADEC corresponds to the above-described mechanism of sandwich hybridisation: the rRNA of the toxic algae is specifically recognised and immobilised by the capture probe and the marked oligonucleotid.

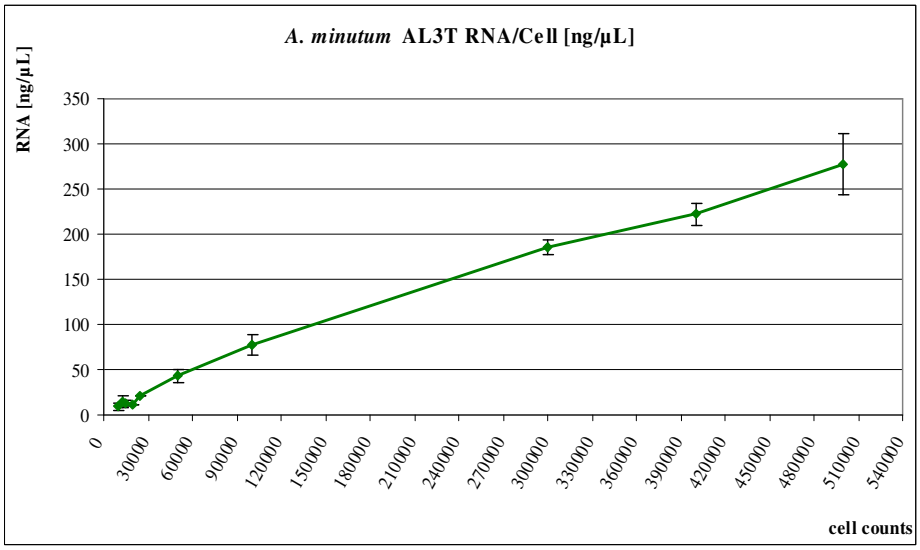

Fig. 3 Concentration curve RNA per cell for *Alexandrium minutum*

In order to obtain free rRNA of the toxic algae, sample pre-processing is needed. Thus, the water sample has to be filtered in order to pre-concentrate the contained cells. After that lysis of the cells must be performed by re-suspending the concentrate in a lysis-solution (with the currently used kit, incubation at 85°C during 5 minutes is needed).

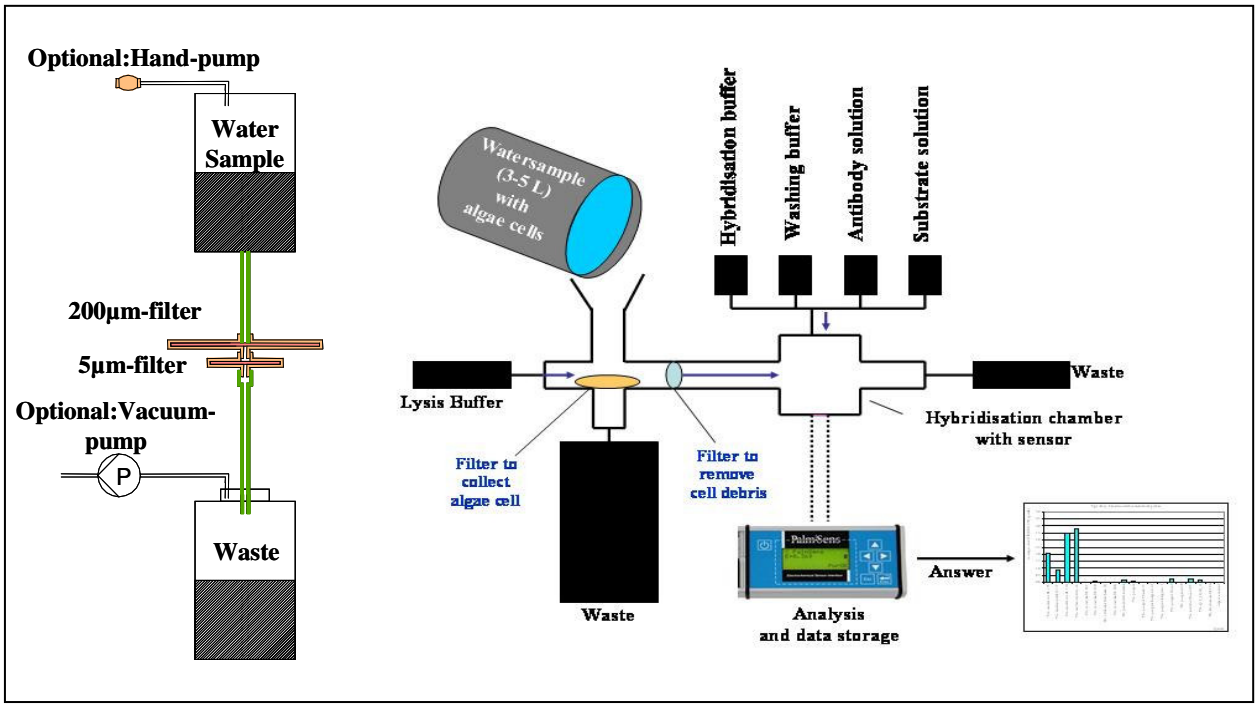

Fig. 4 Flow draft of the new Hand held device

The pre-processed sample can then contact the chip (which has the captured probe already fixed on its surface) and after addition of an adequate quantity of hybridising solution (containing the marked oligonucleotid) and incubation (by the already available probes at 46°C during 2 hours) the electrochemical reaction takes place and the resulting current can be measured. The intensity of the electrochemical reaction is proportional to the amount of bound rRNA.

1.6 Impact

Economic loses generated by fish kills or the closure of seafood farms have been of great impact in recent years. In 1997 and 1998 levels of domoic and isodomoic acid, the ASP toxins, were found to be above the threshold level in scallops (Jacobs mussels) on the West-coast of Scotland. Irish growing areas for the mussel sector such as Banty Bay and Killary Harbour were closed in 2000 due to algal biotoxins. In May 2001 a poisonous algal bloom killed 10.000 tons of mussels in the western Oostershelde, Netherlands. Toxic cyanobacterial blooms appear recurrently in the Baltic Sea, affecting specially the fish farming in Denmark. It has estimated that worldwide, annually, there are some 2000 cases of microalgal-mediated shellfish poisoning, of which 15% are fatal [1].

The AlgaDec biosensor will have an impact on the European seafood industry as it allows facilitated and cost effective analysis of toxic algae of water samples. Based on the recommendation of end-user from three different regions (Orkney Islands, Skagerrak and Galicia) rRNA probes have been developed for the detection of 17 relevant species. The selection of these species can be adopted to these regions or to another European region of interest. The cost for the multianalysis of several toxic algae species will approximately amount to five

1 Hallegraeff et al. 1995

Euros. Moreover, the result of the analysis will be obtained within three hours after water sampling and filtration. Thus, the AlgaDec consortium, in particular the end-user group, considers the output of the projects as sound and recommends the application of the AlgaDec device for routine testing of seafood water samples.

Furthermore, the commercialisation of the portable device will have a positive economic impact for the SME system manufacturers of the AlgaDec consortium, as the partners expect a high market share. The expectations are also founded on the fact that the device could easily be adapted to different pathogen detection applications (e.g. in hospitals or in food).

2 Dissemination and use

The AlgaDec test kit will be commercialised by partner iSiTEC within approximately one year after project completion. It will be an easy to use AlgaDec biosensor for the detection of a wide set of toxic algae. Further research and development activities are necessary before the market launch of the portable biosensor. These activities will mainly involve mainly the final optimisation including reliable calibration of electrochemical signals and algal cells and long term stability of multiprobe chips .

The AlgaDec website (www.algadec.de) will run two further years after project completion and will be updated with relevant information by the coordinator iSiTEC.