Detection of oil in biotope water with a whole-cell biosensor system

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Abstract—Monitoring and maintaining the quality of water is important since it is one of the most important resources and the basis of all life. However, current methods are stationary or obsolete. The IMOLA system is able to measure the vitality of living cells online, marker free and in real time through a multiparametric microsensor chip. In combination with green algae as signal transducers it was elucidated how an emulsion of oil in water affects the vitality of the algae. It was shown that the system using the green algae Parachlorella kessleri as biosensor unit is suitable for mobile, online-detection of changes in water quality.

Index Terms—Environmental monitoring, Biological Cells, Biosensor

I. INTRODUCTION

Economic prosperity and consumer behavior nowadays comes along with sincere ecological risks. This was shown in the Gulf of Mexico, where after the sinking of the mobile oil rig “Deepwater Horizon” on the 20. April 2010 up to 4.7 million liters of crude oil pour out of the bore well [1]. To monitor the occurrence and propagation of oil an easy, effective and fast way to detect oil pollution as early as possible would be necessary. Currently oil pollution is sensed via visual surveillance, either directly via helicopters or airplanes, or indirectly via digital images from drones or satellites [2,3]. This is, however, not possible during bad weather conditions like rain, fog, mist, high waves or at night. A new measurement device for detection of oil should have fast reaction times, the measurement has to be online and the device has to be able to be set up in open water.

The Intelligent Mobile Lab (IMOLA) is a BioChip based measurement device that allows the measurement of temperature, impedance, pH and pO₂ in fluid media. Thereby living cells are used as signal transducers to convert physiological signals from the living cells into physical parameters which are measurable by the BioChip. The cellular acidification rate converts into a change in pH and the cellular respiration into the level of dissolved oxygen [4]. As previously shown the IMOLA can be used for drug development and research with animal cells [5], as well as for environmental monitoring with algae [6]. The IMOLA is especially suitable for environmental monitoring, since an optional GSM module can be implemented. This enables a remote setup as well as an online measurement. Used with algae it is, furthermore, unspecific towards the kind of pollution making it suitable as an early warning system. In this project we were now able to show that remote water pollution with oil is detectable in real time and without the use of markers or labels utilizing the IMOLA using water from a biotope in Munich.

II. MATERIALS AND METHODS

IMOLA

The IMOLA is comprised of four different modules and is shown schematically in Figure 1.

1. Fluidic module: This module contains the tubes, piezo micro pump and flasks of the Live Support System. It supplies fresh medium to the cells on the sensor chip, collects the old and incorporates a Ag/AgCl reference electrode.

2. Analogue module: Consisting of the sensor chip primed with cells. The actual data is obtained here using the living cells as signal transducers.

3. Digital module: All the processes of the IMOLA are controlled by this unit. It includes a 32 kByte EEPROM, a real time clock, four 12-bit D/A and two 16-bit A/D converters implemented, as well as the micro-controller, which converts and processes the analogue data, controls the parameters for the measurement and communicates with a PC or terminal unit.

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Figure 1: Modular setup of the IMOLA with the 1) fluidic module, 2) analogue module, including sensor chip and probe, 3) digital module, including communication and data processing, 4) software module. Moreover, an integrated power supply is included.
4. Software module: This module is realized as a program for a computer, as well as at the implemented micro-controller in the digital module and is written in C/C++. The computer application therefore has input fields to enter parameters for configuration, as well as functions to display, post process and save the measured data.

**Wireless data transfer**

For the wireless data transfer a PCB (Figure 2) was developed containing a GM862-QUAD GSM-module from Telit, for the wireless connection and transfer, and a PIC18F2525 µC, to control the GSM-module and communicate with the IMOLA system. In addition the PCB contains a MIC29312 for a constant power supply and a MAX3222 as signal transducer.

**Figure 2: Layout of the PCB with the four functional sections and their main components: A: Power supply with MIC29312 power regulation (U01); B: Micro-controller with PIC18F4620 (U20); C: GSM-module with GM862- QUAD; D: RS-232 driver with MAX3222 (U30)**

Once the measured data was sent by the IMOLA it was directly forwarded to the GSM-module that sent it to a terminal computer, where a port-listener software received the data and displayed it.

**Algae**

As biological sensor the algae *Parachlorella kessleri* (former *Chlorella kessleri*), a classic green algae, in its stain 211-11h was used. It was obtained from *Sammlung von Algenkulturen Göttingen (SAG)*. *Parachlorella kessleri* is naturally widespread in freshwater and different climate zones and therefore does not pose a risk of environmental contamination. The cells are spherical shaped with a diameter between 2.5 - 8.9 µm, proliferate every 16 to 20 h in optimal conditions and can be cultivated easily under standard lab conditions [7]. It has, furthermore, been shown in combination with the IMOLA, that *Parachlorella kessleri* was very sensitive towards different insecticides and herbicides, what makes it a good organism to observe such environmental influences [8].

**Sensor chip**

The sensor chip used in this project is the BioChip-C (www.cellasys.com). Its edge length is 24 mm and the cell culture has a diameter of 6 mm. The chip contains 2 impedance sensors (IDES), 2 pH sensors (MeOx), a Pt1000 temperature sensor and one dissolved oxygen (DO) sensor (Figure 3).

**Figure 3: Layout of the four sensors on the BioChip-C for measuring viability of the algae. These sensors include the dissolved oxygen (DO) sensor, pH sensors (MeOx), temperature sensor (Pt1000) and interdigitated electrode structures (IDES).**

**LEDs**

Illumination was performed using two LED WHITE 3MM RND WATER CLEAR by Lite-On Inc. They have a candlepower of 5 cd and a light flux of 745 mJm at 20 mA. A diagram of its relative intensity relative versus wavelength (Figure 4) shows that the highest intensity is at a wavelength in a range around 470 nm, correlating with high photosynthesis rates of plants in this range.

**Figure 4: Relative light intensity of the LED versus the wavelength, showing that the highest intensity is reached in a range that correlates with high photosynthesis activity of plants.**
**Measurements**

For the measurements 5 mL algae culture were immobilised on a QUANTITATIV FILTER PAPER WHITE RIBBON, 589/2 by Sigma Aldrich with 6 mm diameter and put on the BioChip-C. The cells were then supplemented in cycles of 10 min pumping at 750 µL/min and 15 min pausing with algae medium first, followed by the test medium. The media were water from a biotope in Munich (48,1713710° N, 11,4955590° E; 08.06.2010), plain, with 5 % DMSO and 5 % DMSO with universal oil from Shell. For analysis the slopes of the change of pH and dissolved oxygen during the pause were calculated.

**Analysis of data**

The analysis of the raw data was done by calculating the slopes of changes during the 15 min pausing (M) of each cycle (see figure 5). Therefore a linear regression was fitted for each 15 min pausing interval of each sensor and the mean slope put into a graph.

**III. RESULTS AND DISCUSSION**

The implementation of the developed PCB into the existing IMOLA system was successful. The data could be received at the terminal PC and further processed.

It could be shown, that the algae react to the pollution with oil by increasing their rates of acidification (Figure 6). This indicates that the cells are not affected negatively, but rather positively by the oil-water-emulsion.

![Figure 6: Slopes of acidification rates of the algae in A: Algae medium and B: Biotope water with 0,5 % DMSO and 1:3.000.000 oil. After the change of medium the acidification rates strongly vary, but generally show higher rates than before.

Furthermore, the control with biotope water and DMSO showed clearly that DMSO affected the vitality of the cells (Figure 7). After the change of medium the acidification rate first strongly decreases, before increasing (i.e. acidification of the medium). This effect is a result of photosynthesis inhibition that results in increased uptake of oxygen to maintain the cell metabolism. The outcome is a lower concentration of dissolves oxygen and a higher concentration of CO₂. CO₂ reacts with water to carbonic acid, increasing the rate of acidification. A similar effect can be seen for biotope water with DMSO and oil, however acidification rates are lower and even slowly decrease again, indicating a increasing vitality of the algae. The very low acidification rates after the change of medium also state a phase of adaption to the new medium, before the actual effect of the additives can be seen.

**IV. CONCLUSION**

It could be shown, that the algae in combination with the IMOLA system can be used as a mobile biosensor system and also an interaction between oil and the metabolism of the algae could be monitored. However, further investigation has to be done to elucidate how the concentration of oil and DMSO affects the cells.
V. REFERENCES

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