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# Intelligent Mobile Lab for Metabolics in Environmental Monitoring

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**Abstract:** The flexible and modular setup of an intelligent mobile lab (IMOLA) that can be equipped with biosensor chips for monitoring of cellular metabolism is described. An application as an environmental monitoring system is described by recording the extracelluar acidification and cellular respiration of *Chlorella kessleri* after exposure to the herbicide metamitron. The adaptability of the IMOLA system to future developments in the field of biosensors or cellular metabolism analysis is emphasized.

**Keywords:** IMOLA, biosensor, metabonomics, metabolomics, metabolic profiling, biomonitoring, chlorella, environmental early warning system

## INTRODUCTION

For regulatory standards in food quality and the protection of the environment, biosensors are needed to detect potentially hazardous compounds. Depending on the purpose of measurement, these biosensors work with different biological recognition elements such as DNA, enzymes or antibodies. In this work a device for a whole cell biosensor approach is presented, which can be used on one hand to analyze metabolic parameters of living cells [for example tumor cells (Motrescu et al. 2005)] under a given experimental treatment or on the other hand to use cells as signal transducer for potentially hazardous compounds. The scientific area aiming at the analysis of metabolic networks is named metabonomics, metabolomics, metabolics, or metabolic profiling (Robertson 2005).

A sensorchip-based approach to analysis of cell metabolic rates was published previously (Wolf et al. 1998; Brischwein et al. 2003; Wiest et al. 2005b). But it appears that no small and mobile cell-based biosensor system is available. Only a few efforts have been described to develop portable whole cell biosensor systems for field applications (DeBusschere and Kovacs 2001).

A novel device called Intelligent MObile LAb (IMOLA) was developed with an integrated power supply and wireless data transmission. All modules such as connectors, pump system, reference electrode, and software were miniaturized and optimized with regard to minimal energy consumption.

The primary parameters assessed in the direct vicinity of living cells are pH value and dissolved oxygen concentration. These parameters are modulated by cellular activity and assessed to give information about relative changes in cell metabolic rates, i.e., the rate of extracellular acidification and the rate of cellular oxygen exchange. Additionally, there is a sensor to monitor proliferation and morphologic alterations of adherent cells by cell-substrate impedance measurement. The planar sensors are integrated in a chip, which is fabricated either with silicon technology (A) or thin film technology (B). The necessary silicon technology with platin or palladium metallization is established but costly at the required chip sizes and piece numbers. A less expensive alternative is thin film technology on a ceramic substrate. However, ion sensitive field effect transistors (ISFETs) sensors for pH measurement at silicon chips have to be replaced with passive sensor elements such as metal oxide sensors at ceramic based chips. The device for control and read out of the biochips has to fulfill several requirements such as portability and the possibility of long-term and on-line-measurements. Therefore wireless data transfer for remote control, power supply, and a fluidic life-support system were integrated.

## MATERIAL AND METHODS

Cell or tissue probes are directly cultivated on the silicon- or ceramic-based multiparametric biosensor chips. The chips are inserted into the IMOLA and connected to the live-support system (Fig. 1). The measurement setting is defined via the IMOLA software application at a nearby standing laptop. After the start button of the IMOLA is pressed, a wireless connection between the IMOLA and the laptop is established automatically, the IMOLA system receives its configuration, and the measurement starts.

The cells on the chip are confined in a small disk and filled with cell culture medium with a volume of about 7 μL. The active surface of the chip where the living cells are applied or grown directly on the various sensor types has a diameter of 6 mm. The height of the micro-volume disk is adjusted with a spacer ring (or with a rim on the fluid insert) to 0.2 mm (Fig. 2). It is important to achieve a high ratio of cells to the volume of the surrounding culture medium in order to record rates of cell metabolism with a sufficient speed. In case poorly adherent cells, such as yeast cells are used they have to be prevented from being washed out of the chamber. Those cells are trapped by a disk of filter paper (Schleicher & Schuell, Dassel, Germany), laid on top of the spacer ring.

Cellular respiration or mitochondrial activity are measured by dissolved oxygen sensors. Here, a three electrode amperometric dissolved oxygen sensor, which is basically a Clark sensor but without a membrane, or a so-called oxygen field effect transistor ($O_2$-FET) can be used (Wiest et al. 2005a). The $O_2$-FET is able to measure pH and $pO_2$ quasi parallel. The

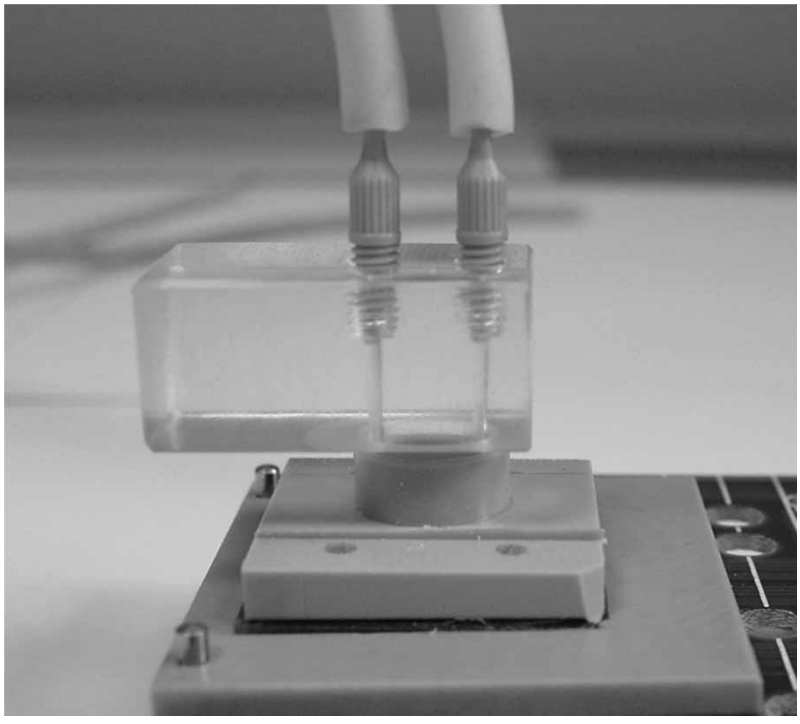

***Figure 1.*** Connector, sensor chip, fluid insert, and fluidic connectors. The sensor chip (middle) is set into the connector socket (height 3,0 mm) to contact the control PCB electronically. The fluidic adapter is pressed into the round cell culture well and seals it. Inflow and outflow tubes are plugged onto standard fluidic connectors (LED inlet not shown). The transparent fluidic adapter has a mechanical extension for easier handling.

sensor can be switched between pH and dissolved oxygen sensor by application of a negative voltage at the noble metal electrode (NME). If zero volts are applied, the $O_2$-FET works as a pH sensor and if $-700$ mV are applied, the $O_2$-FET works as a sensor for dissolved oxygen.

To measure the pH value an ISFET (Bergveld 2002), a metal oxide sensor (MO) (Ardizzone and Trasatti 1996), or again an $O_2$-FET can be used.

An interdigitated electrode structure (IDES) (Ressler et al. 2004) is used for measurement of adhesion forces. At this structure a 100 mV AC signal with 10 kHz is applied and the impedance is determined. For control purposes the temperature at the biosensor chips is monitored also. For this either a PN-diode or a Pt1000 is used. Table 1 summarizes the used sensors depending on the production technologies.

During the development process strict partition into modules was kept to allow maximum adaptability of the IMOLA (Fig. 3) system.

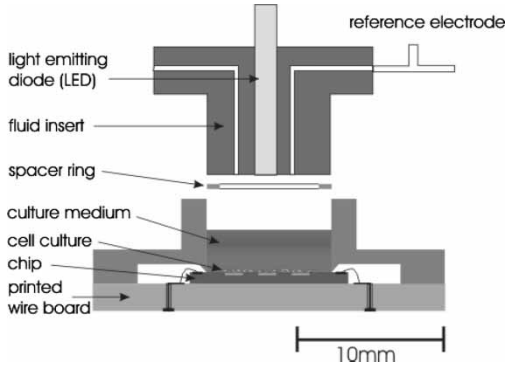


***Figure 2.*** Cross section of the biosensor chip and live support system which was developed at the Heinz Nixdorf-Department for Medical Electronics.

The IMOLA system consists of four exchangeable and adaptable modules. Figure 4 is a scheme of the modular setup of the IMOLA system.

1. Fluidic module: For the life support system standard cell culture flasks and silicon tubes were used. An Ag/AgCl reference electrode (Huang et al. 2003) with an electrolyte barrel (Microelectrodes, Inc., Bedford, NH, USA) filled with Ag saturated 3 M KCl was integrated into the outflow tubing. A piezo micro pump (thinXXS, Mainz, Germany) was integrated that transports maximal 1.8 mL of cell culture media per minute. The fluid insert, a biosensor chip, and the connector interface to the analog module are shown in Fig. 1. To avoid flooding due to leakage or failure the fluidic module is driven in suction mode.
2. Analog module: Two versions of biosensor chips are available: (A) a silicon chip manufactured in silicon technology (Fig. 5) and (B) a ceramic chip manufactured in thin film technology (Fig. 6). The analog module PCB was adapted to the type of sensor chip. Therefore two versions were developed to operate the sensors as described in Table 1.

***Table 1.*** Types of microsensors

| Sensor parameter | Silicon technology (A) | Thin film technology (B) |
|---|---|---|
| Dissolved oxygen | Amperometric oxygen sensor, $O_2$-FET | Amperometric oxygen sensor |
| pH | ISFET, $O_2$-FET | Metal oxide |
| Impedance, bioelectronic potentials | IDES | IDES |
| Temperature | PN-diode | Pt1000 |

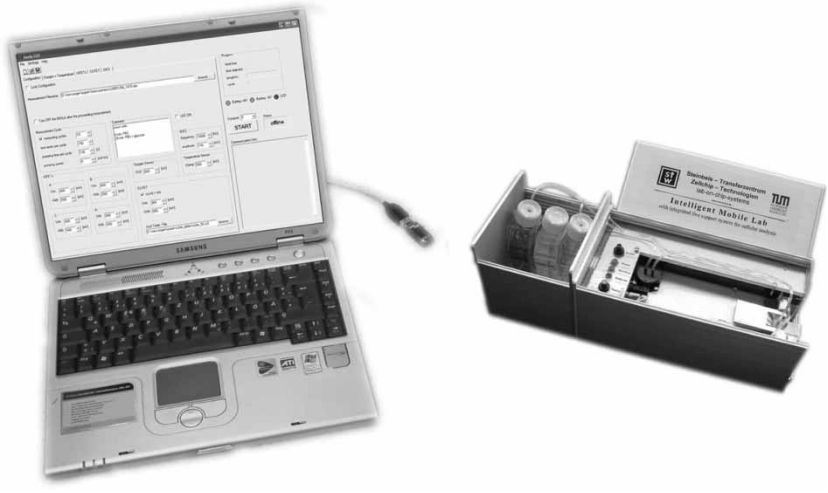


***Figure 3.*** Intelligent Mobile Lab device (produced by Steinbeis Transferzentrum-Lab on Chip Systems, Bernreid, Germany). The front part on the right (size: 210 mm × 105 mm × 90 mm) incorporates the analog and digital electronic circuitry, the biosensor and the live-support system. The back part (size: 90 mm × 105 mm × 105 mm) includes the reservoir and waste tanks and provides an external antenna. The software module is shown on the left running at a laptop.

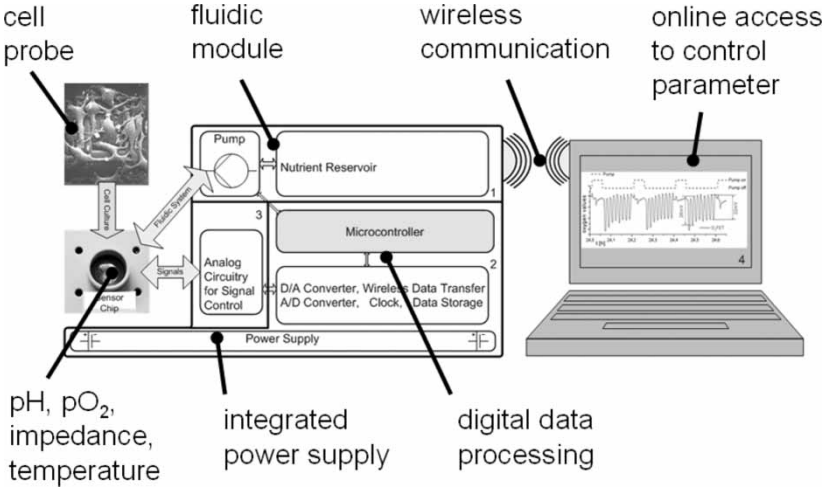


***Figure 4.*** Modular set up of the IMOLA system: 1) fluidic module, 2) analog module including sensor chip with cell probe, 3) digital module including wireless communication and data processing, 4) software module. Furthermore an integrated power supply is included.

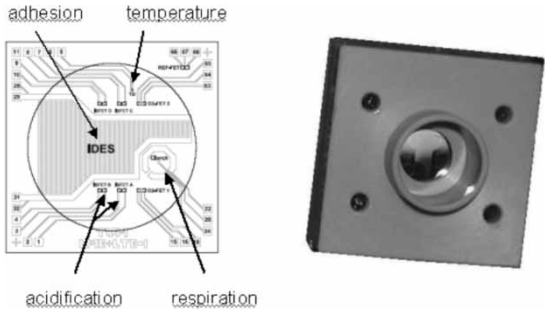


***Figure 5.*** Layout (left) and encapsulated silicon biosensor chip (right) (produced by Heinz Nixdorf-Department of Medical Electronics). An amperometric sensor is used for measurement of $pO_2$, for impedance measurement an IDES sensor is used. pH measurement is performed by ion sensitive field effect transistors (ISFET). Side length of one chip is 24 mm, the cell culture area has a diameter of 6 mm. A temperature diode is integrated for control purposes.

Both versions are equipped with a mezzanine board for control of two IDES structures. To avoid cross talking between the electrochemical active sensors, the pH and oxygen sensors at both PCBs were galvanically isolated.

3. Digital module: The control unit of the IMOLA is the digital PCB. Different types of analog modules can be operated due to defined interfaces. The digital PCB (schematic Fig. 7) contains a microcontroller, four 12-bit D/A converters, two 16-bit A/D converters, a real time clock, a 32 kB EEPROM, a wireless transfer module, and additional electronics. The microcontroller was programmed to adjust the control

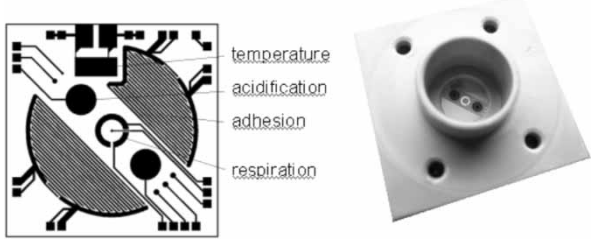


***Figure 6.*** Layout (left) and encapsulated ceramic biosensor chip (right) (produced in cooperation by Heinz Nixdorf-Department for Medical Electronics and Herqeus Sensor Technology). An amperometric sensor is used for measurement of $pO_2$, for impedance measurement an IDES sensor is used. pH measurement is performed by an metal oxide sensor. Side length of one chip is 24 mm; the cell culture area has a diameter of 6 mm. A Pt1000 is integrated for temperature control purposes.

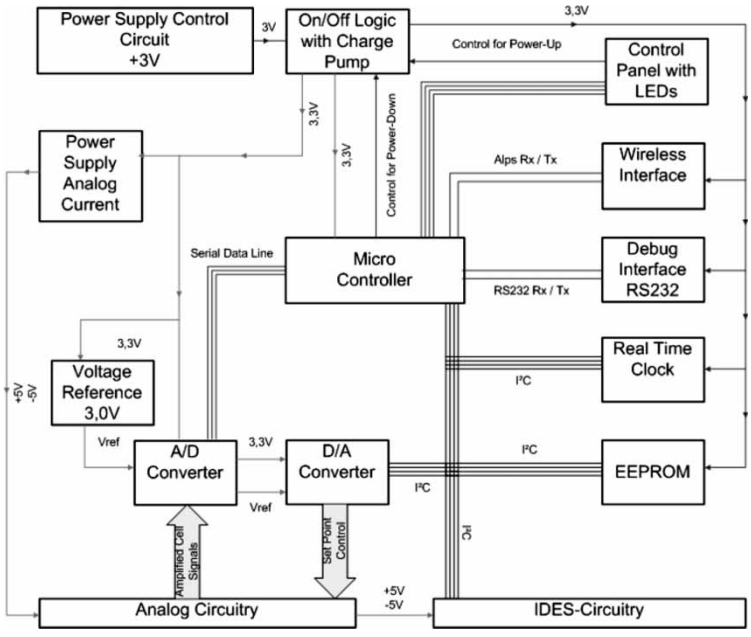


***Figure 7.*** Architecture of digital data management module. Core is the PIC 16F777 microcontroller. The control panels are connected to digital outputs. Two RS232 ports are implemented for connection of the wireless interface and for the debug plug. The D/A converter, the buffer, the clock, and the IDES circuitry are connected via an $I^2C$—bus (Wiest et al. 2005c). The A/D converters are connected directly via a fast serial bus.

parameters for the measurement, to convert and preprocess the analog data, to buffer the data, and to transfer it wirelessly to a laptop or a transfer unit.

4. Software module: The software module is realized at the microcontroller and as a program at the laptop; both are implemented in C/C++. To optimize the lifetime of a wireless measurement, the energy intensive wireless data transfer time was minimized by buffering the data in the EEPROM. If the EEPROM is full, a wireless connection is established, measurement data is transferred to the laptop (optional via a smartphone), new configuration values are received, and the wireless connection is cut. The application software at the laptop has input fields for configuration data (i.e., LED, pump, and measurement cycle time, sensor control wave forms, etc.) as shown in Figure 8, a display unit for online monitoring of the measurement data, post processing tools, and a data storage function.

There are two software routines for the microcontroller available. A) for the silicon chip version and B) for the ceramic chip version. The laptop application software can be switched via the properties menu. So an IMOLA can be

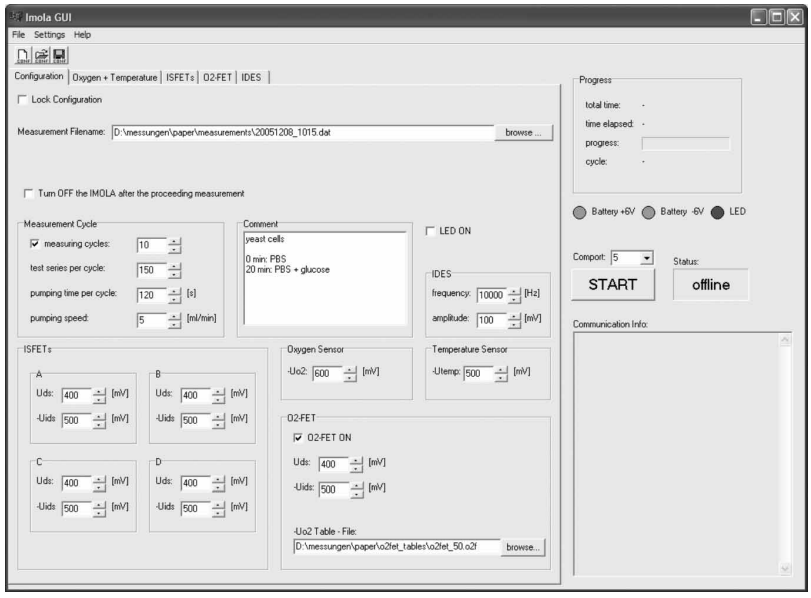


***Figure 8.*** Software configuration menu for measurement cycle adjustment and for control of the microsensors.

switched from a silicon version to a ceramic version by exchanging the analog module PCB and by additional adaptation of the microcontroller software.

After configuration with the application software the measurement runs are autonomous. However, if necessary, the configuration parameters like pump speed and timing, sensor control parameters, and microsensor activation can be adapted on-line during the measurement. To show the application as a biosensor, a measurement was performed for which photosynthetic active algae cells were used as signal transducer.

As test organism *Chlorella kessleri* was used, taken from a monoclonal axenic algal culture. *Chlorella kessleri* is a single-celled green microalgae from the category of chlorophyceae and appears ubiquitous in freshwater. Species of *Chlorella* are well known as biomonitoring organisms due to its sensitivity to contaminations and its efficient uptake mechanism (Rodriguez et al. 2002). As cell culture media a solution with excess of all nutrients as proposed by Zehnder and Gorham (1960) was used, so that any decreases in algal vitality were not generated by nutrient limitation.

In this study a measurement cycle consists of 3 min pump on (to transport fresh cell culture media toward the probe) and 10 min pump off (to monitor the slope of respiration and acidification). This measurement cycle was repeated for more than 10 hours. There was a two-point calibration of the sensors necessary for each measurement. The oxygen sensor was calibrated with a standard liquid containing 100% $pO_2$ saturation and a liquid with a content

of 0% $pO_2$ saturation. The pH-sensor calibration was achieved with a nutrient solution that was adjusted with HCl to pH 9.0 and pH 4.0.

The LED with direct optical access toward the cells was switched on all the time to allow photosynthesis of the used algae. The algae cells were immobilized on a silicon sensor chip similar to the method published by Vedrine et al. (2003). As toxin, 1 mg/L of the herbicide metamitron [4-amino-3-methyl-6-phenyl-1,2,4-triazin-5 (4H)-on] was added to the cell culture media to inhibit photosynthesis.

The herbicide was tested before the measurements, to not influence the oxygen content and the pH in the nutrient solution or cause irritations to the biosensor chip. Therefore, changes in the measured parameters were a response of algae due to the nutrient solution provided with the herbicide.

## RESULTS

The cellular respiration and extra cellular acidification of *Chlorella kessleri* was monitored for about 4 hours with cell culture medium. Then 1 mg/L metamitron was added to the cell culture medium for approximately 1 h, followed by a recovery period with untreated cell culture medium. The record of an amperometric oxygen sensor is shown in Fig. 9. The pump is operated in a stop-and-flow mode as described earlier. During the stop

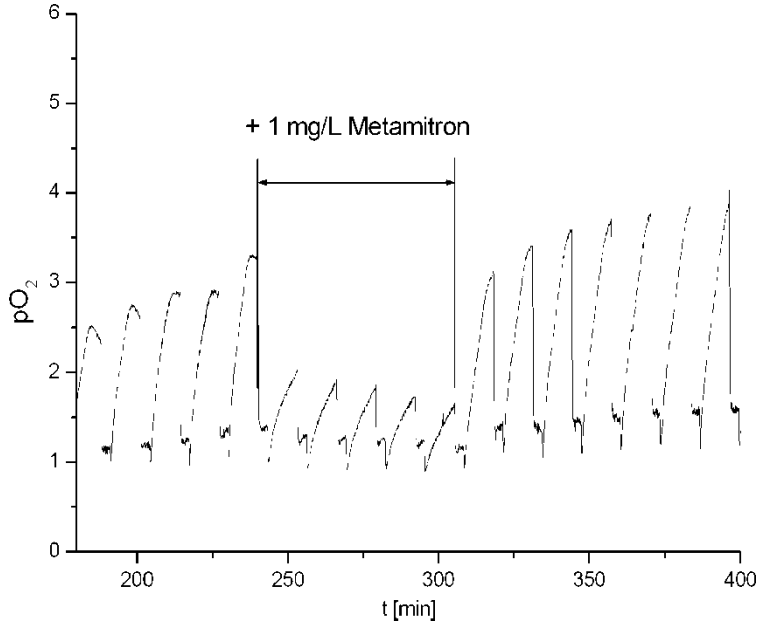


***Figure 9.*** Exemplary measurement of monitoring photosynthesis of algae.

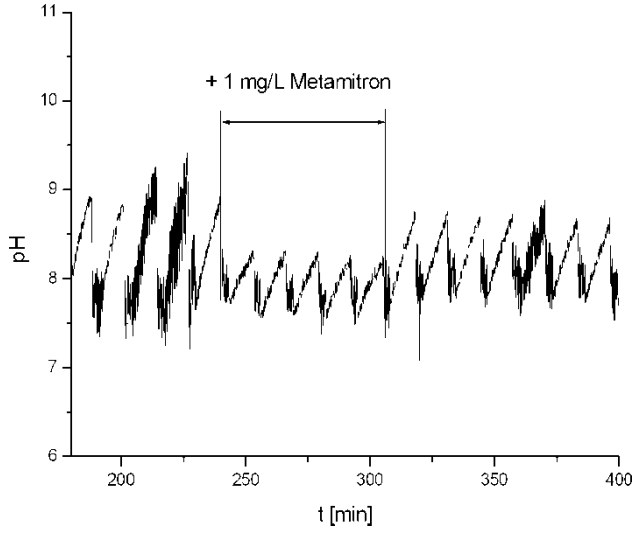


***Figure 10.*** Changes in pH in the microenvironment of algae due to the addition of metamitron.

intervals cell metabolic rate, i.e., essentially the sum of respiration and photosynthesis are recorded, during the flow intervals the medium is regenerated. When metamitron is present in the medium, photosynthetic oxygen production is heavily impaired. Oxygen production rates are calculated from the slope of the graph during the stop intervals. Even though a calibration of the dissolved oxygen sensor was performed, the ordinate is scaled relatively compared to a base value of 1. This reference point is established when 100% oxygen saturated cell culture media is present at the sensor. This was necessary because the linearity of the amperometric oxygen sensor in the range above 100% oxygen saturation is not fully investigated.

Figure 10 shows a record of a pH-ISFET, reflecting the extracellular acidification of the algae. The acidification rate decreases while metamitron is present due to the inhibition of photosynthesis, i.e., less dissolved $CO_2$ is consumed by photosynthesis. One percent of the dissolved $CO_2$ in water forms carbonic acid, which dissociates with water to $HCO_3^-$ and $H_3O^+$. This reaction causes a decrease of the pH value in the solution. Analogously, a $CO_2$ consumption leads to a basification (Schubnell et al. 1999).

## DISCUSSION

A mobile, modular device for measurement with biosensors was developed and tested. The IMOLA can easily be adapted to new chip configurations by merely changing the analog module. The ability of environmental

monitoring was demonstrated by application of the photosynthesis inhibitor metamitron affecting *Chlorella kessleri*. The current work aims at the further miniaturization of the device, the integration of a temperature regulation, and practical field tests for environmental monitoring. Water quality determinations currently measured by physical, chemical, and biological parameters, e.g., composition of diatoms, are time consuming and not cost-effective. Combining a biological component with a biosensor chip is a simple and effective method to monitor freshwater on-line. There is a huge demand for early warning systems that provides the ability to react in case of accidental pollution of the environment and also as the safety fuse of drinking water.

## REFERENCES

Ardizzone, S. and Trasatti, S. 1996. Interfacial properties of oxides with technological impact in electrochemistry. *Adv. Colloid Interfac.*, 64: 173–251.

Bergveld, P. 2002. Thirty years of ISFETOLOGY What happened in the past 30 years and what may happen in the next 30 years. *Sensor. Actuator.*, 88: 1–20.

Brischwein, M., Motrescu, E.R., Cabala, E., Otto, A.M., Grothe, H., and Wolf, B. 2003. Functional cellular assays with multiparametric silicon sensor chips. *Lab. Chip.*, 3: 234–240.

DeBusschere, B.D. and Kovacs, G.T.A. 2001. Portable cell-based biosensor system using integrated CMOS cell-cartridges. *Biosens. Bioelectron.*, 16: 543–556.

Huang, I.-Y., Huang, R.-S., and Lo, L.-H. 2003. Improvement of integrated Ag/AgCl thin-film electrodes by KCl-gel coating for ISFET applications. *Sensor. Actuator.*, 94: 53–64.

Motrescu, E.R., Otto, A.M., Brischwein, M., Zahler, S., and Wolf, B. 2005. Dynamic analysis of metabolic effects of chloroacetaldehyde and cytochalasin B on tumor cells using bioelectronic sensor chips. *J. Cancer. Res. Clin. Oncol.*, 131: 683–691.

Ressler, J., Grothe, H., Motrescu, E.R., and Wolf, B. 2004. New conepts for chip-supported multi-well-plates: Realization of a 24-well-plate with integrated impedance-sensors for functional cellular screening applications and automated microscope aided cell-based assays, *Proceedings of the 26th Annual International Conference of the IEEE EMBC*; 2074–2077.

Robertson, D.G. 2005. Metabonomics in toxicology: A review. *Toxicol. Sci.*, 85 (2): 809–822.

Rodriguez, M., Jr., Sanders, Ch.A., and Greenbaum, E. 2002. Biosensors for rapid monitoring of primary-source drinking water using naturally occurring photosynthesis. *Biosens. Bioelectron.*, 17: 843–849.

Schubnell, D., Lehmann, M., Baumann, W., Rott, F.G., Wolf, B., and Beck, Ch.F. 1999. An ISFET-algal (Chlamydomonas) hybrid provides a system for eco-toxicological tests. *Biosens. Bioelectron.*, 14: 465–472.

Védrine, Ch., Leclerc, J.-C., Durrieu, C., and Tran-Minh, C. 2003. Optical whole-cell biosensor using Chlorella vulgaris designed for monitoring herbicides. *Biosens. Bioelectron.*, 18: 457–463.

Wiest, J., Brischwein, M., Grothe, H., Otto, A.M., and Wolf, B. 2005a. Planar micro-sensors for measurement of cellular respiration. *SENSOR 2005 Proceedings II*, 249–254.

Wiest, J., Brischwein, M., Ressler, J., Otto, A.M., Grothe, H., and Wolf, B. 2005b. Cellular assays with multiparametric bioelectronic sensor chips. *Chimia*, 59 (5): 243–246.

Wiest, J., Schmidhuber, M., Ressler, J., Scholz, A.M., Brischwein, M., and Wolf, B. 2005c. Cell based assays for diagnostic and therapy on multiparametric biosensor chips with an intelligent mobile lab. *IFMBE Proc. 2005*, 10: 132–135.

Wolf, B., Kraus, M., Brischwein, M., Ehret, R., Baumann, W., and Lehmann, M. 1998. Biofunctional hybrid structures—cell-silicon hybrids for applications in biomedicine and bioinformatics. *Bioelectroch. Bioener.*, 46 (2): 215–225.

Zehnder, A. and Gorham, P.R. 1960. Factors influencing the growth of Microcystis aeruginosa Kutz, emend, Elenkin. *Can. J. Microbiol.*, 6: 645–660.