Application of Algae-Biosensor for Environmental Monitoring

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Abstract— **Environmental problems including water and air pollution, over fertilization, insufficient wastewater treatment and even ecological disaster are receiving greater attention in the technical and scientific area. In this paper, a method for water quality monitoring using living green algae (Chlorella Kessleri) with the help of the intelligent mobile lab (IMOLA) is presented. This measurement used two IMOLA systems for measurement and reference simultaneously to verify changes due to pollution inside the measurement system. The IMOLA includes light emitting diodes to stimulate photosynthesis of the living algae immobilized on a biochip containing a dissolved oxygen microsensor. A fluid system is used to transport algae culture medium in a stop and go mode; 600s ON, 300s OFF, while the oxygen concentration of the water probe is measured. When the pump stops, the increase in dissolved oxygen concentration due to photosynthesis is detected. In case of a pollutant being transported toward the algae, this can be detected by monitoring the photosynthetic activity. Monitoring pollution is shown by adding emulsion of 0,5mL of Indonesian crude palm oil and 10mL algae medium to the water probe in the biosensor.**

I. INTRODUCTION

A river is a large natural stream of water emptying into an ocean, lake, or other body of water and usually fed along its course by converging tributaries. Rivers and streams drain water that falls in upland areas. Moving water dilutes and decomposes pollutants more rapidly than standing water, but many rivers and streams are significantly polluted all around the world. A primary reason for this is that all three major sources of pollution (industry, agriculture and domestic) are concentrated along rivers [1, 2].

In Indonesia, the river is a large part of people's lives. As one of the major natural resources, water pollution can eliminate the chances of economic gain for the people in this area incurring social costs. One area of business that contributes to the pollution of the river is palm oil plantations. Processing palm bunch produces wastewater and oil containing a very high organic matter that can cause severe pollution. Additionally, the use of pesticides for crop pests and the inefficiency of fertilization on marginal lands contribute to the pollution of the river and decrease water quality. Monitoring and maintaining water quality is important because it is one of the most important resources and the basis of all life. However, current methods are stationary or obsolete.

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Using green algae as an environmental indicator is a cost-effective solution for river water quality monitoring. A chief advantage of environmental monitoring using algae is the possibility to produce large populations to observe in a relatively short time period. Algae respond rapidly and predictably to a wide range of pollutants and provide potentially useful early warning signals of deteriorating conditions. Because of their nutritional needs and their position at the base of the aquatic food chain, algal indicators provide relatively unique information concerning ecosystem conditions. If the water quality is extremely poor, algae will not be able to grow. Because algae produce oxygen during photosynthesis, an indicator of algal health is the level of dissolved oxygen in the surrounding water.

In this paper, the use of two IMOLA biosensors combined with green algae (living cells) as a signal transducer for water quality is presented. Understanding of cellular evolution and interaction with their micro-environment is useful for environment monitoring. Therefore, we propose to monitor the oxygen production due to photosynthesis of green algae with biosensors.

The IMOLA-IVD utilizes a lab-on-a-chip concept to perform multi parametric measurements of dissolved oxygen (DO), temperature, acidification degree (pH) and impedance simultaneously to simplify the in situ data collection process of the sample [3,4]. In this work, the IMOLA-IVD biosensor utilizes a biological component as biotransducer for pollutant detection. *Chlorella Kessleri* was chosen as a pollutant detector because of its high level of sensitivity [5]. It is a good candidate organism to observe environmental influences based on sensitive response towards insecticides and herbicides [6].

II. MATERIAL AND METHODS

A. Chlorella Kessleri

The biological component in this study is the green algae, *Chlorella Kessleri*. Chlorella Kessleri (stain 211-11h) was purchased from *Sammlung von Algenkulturen Göttingen* (SAG Germany). It is classified as family Chlorellaceae and it is naturally wide spread in freshwater across different climate zones and therefore does not pose a risk of environmental contamination. The cells are shaped spherically, proliferate every 16 to 20 h in optimal conditions and can be cultivated easily under standard lab conditions [7]. Figure 1 shows *Chlorella Kessleri* cells with a diameter of about 2.5-3µm.

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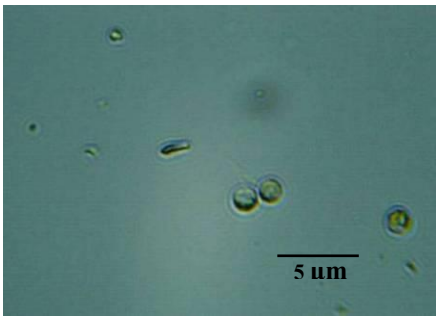

Figure 1. *Chlorella Kessleri* used in the study of water quality monitoring.

B. Biochip and IMOLA System

The cellular respiration or mitochondrial activities of algae is measured using an amperometric dissolved oxygen sensor, which is essentially a Clark-type sensor without a membrane [8]. The primary parameters assessed in the direct vicinity of living cells are extracellular acidification (pH value) and cellular respiration in form dissolved oxygen concentration (pO2). These parameters are modulated by cellular activity and assessed to give information about relative changes in cellular metabolic rates. [4,6].

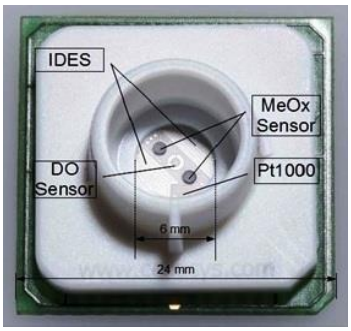

Figure 2. Layout of the four sensor types on the BioChip-C for measuring viability of the algae

The sensor array on the BioChip-C simultaneously collects morphological (two IDES), pH (two MeOx electrodes), temperature (Pt1000 temp sensor), and dissolved oxygen (DO) data (Figure 2) and records it to an external PC [9]. The fluidic perfusion system consists of flasks containing culture medium, waste containers, fluidic head, tubes and a peristaltic pump. The system is controlled remotely with the Data Acquisiton and Link Application (DALiA) software. Cells captured on the chip are fed intermittently with fresh culture medium. At the fluidic head, two light emitting diodes (white 3mm water clear by Lite-On) with 5cd and a light flux of 745 mlm at 20mA serve as artificial light sources to assist in algae photosynthesis. The LEDs are switched on and off via the DALiA software to simulate day and night cycles triggering algal photosynthesis.

C. Kernel Oil and Crude Palm Oil (CPO)

A direct source of contamination that enters river water in Riau Province is wastewater from the CPO industry and Palm Plantation. Samples of kernel oil and palm oil (CPO) from one of the palm oil factory in Riau Province, served as a sample contaminant for experiments (Figure 3).

Figure 3. Palm oil samples from Indonesia palm plantation

D. Measurement Set Up

For simultaneous observation, two parallel IMOLA systems were set up for measurements. 3mL of *Chlorella Kessleri* were stored inside the biochip using filter paper with a 9mm diameter (589/2 of Whatman paper). Algae were fed with Algae Culture Broth (ACB) medium (17124 Fluka) with an osmolarity of 38mOsmol/kg and a pH of 7.0 via a two-phase (ON and OFF) peristaltic pumping protocol. During the ON phase, algae are perfused with broth for 300s (5 minutes) and pumping is suspended during the OFF phase for 600s (10 minutes). In ON mode, the algae gain fresh nutrients and oxygen from the fluidic system. While in the OFF phase; an increase in dissolved oxygen is expected due to photosynthetic processes in the algae.

E. Pollutant Mixtures

Environmental pollution on the biochip was simulated using a mixture of sample pollutant. Several emulsion solutions were made from mixing ACB medium with CPO oil dissolved in a small volume of soap solution consist of 8ml ACB + 0,1mL liquid soap + 0,5ml CPO. A control solution was also mixed that contained soap with no CPO. Solutions were stirred for 2 minutes to emulsify CPO and ACB so it could easily traverse the fluid system and prevent air bubbles.

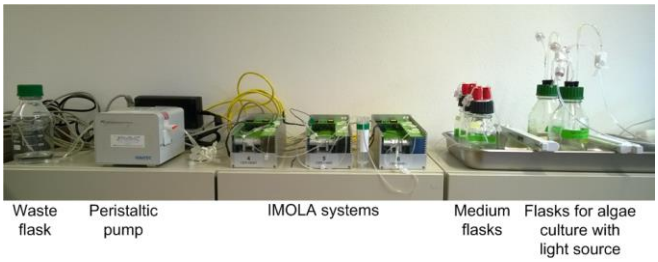

Figure 4. Measurement setup and flasks for algae culture.

III. RESULTS AND DISCUSSION

A. Effect of Artificial Sunlight on Algal Photosynthesis

Two IMOLA were run in parallel to simultaneously measure system changes (IMOLA4) and serve as a reference (IMOLA5) for standard algal behavior.

In figure 4 the measurement setup is depicted. On the right side the flasks for the algae culture are shown. The experimental setup consists of the medium flask (with algae culture broth and oil), three parallel measurement units, the peristaltic pump for medium exchange and the waste flask. The pump and the IMOLA systems are controlled via DALIA client software on a PC (not shown). Figure 5 shows measurements for both IMOLA where an increase in dissolved oxygen is observed on IMOLA4 during the OFF phase. Algal photosynthesis produces excess oxygen in the medium around the sensors resulting in a reduction in the measured voltage. During the measurement procedure, the LED in the fluidic head is kept on, providing a light source to support algal photosynthesis.

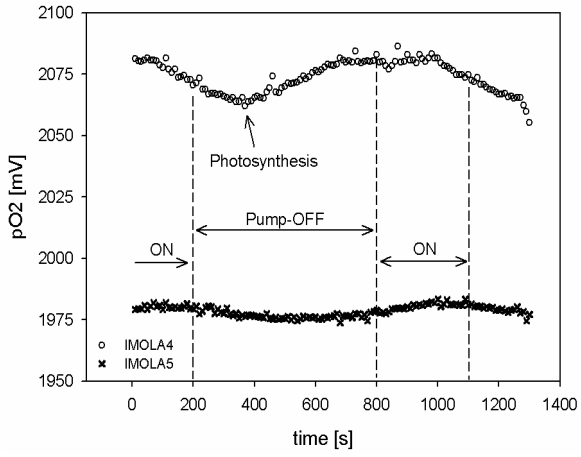

Figure 5. Simultaneous measurement using two active IMOLA and fluidic system

Next, to simulate artificial night and day periods the LEDs in the fluidic head were toggled on and off to assess the impact of light on algal photosynthesis. Results are shown on figure 6.

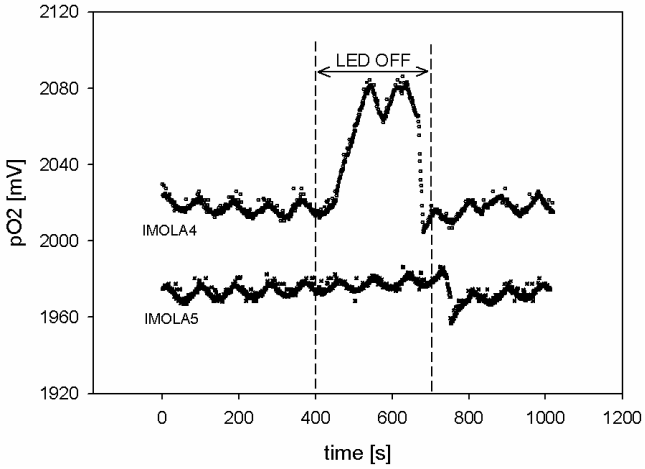

Figure 6. Artificial light to stimulate the algae activity

At the start of the experiment, it appears that the basal $_{P}O2$ for each IMOLA are different, where IMOLA4 has a measured $_{P}O2$ of 2079.68 mV and IMOLA5 1868.46mV. This difference can be attributed to small sensor variations, which causes small deviations in signal voltage between biochips. The LED on IMOLA4 was toggled off, while the LED on IMOLA5 remained on during the measurement. As a result, a stark reduction in dissolved oxygen was detected on IMOLA4, seen as a large spike in the voltage during the LED off cycle. In contrast, IMOLA5 remains constant and the voltage continues to fluctuate normally coinciding with toggling the LEDs on and off. At the end of the extended LED off cycle for IMOLA4 the values return to normal.

B. Pollution Monitoring

Pollution monitoring is done by adding the premixed CPO solution to the biosensor system. Changes in vitality of algae due to the CPO are measured through the oxygen sensor located in the biochip. The cellular respiration of Chlorella kessleri was monitored for about 4 hours with alga culture broth medium. Then 0,5mL of CPO in an emulsion solution was added to the alga culture broth medium for approximately 40 minutes, followed by a recovery period with alga culture broth medium. The record of an amperometric oxygen sensor is shown in Fig. 7.

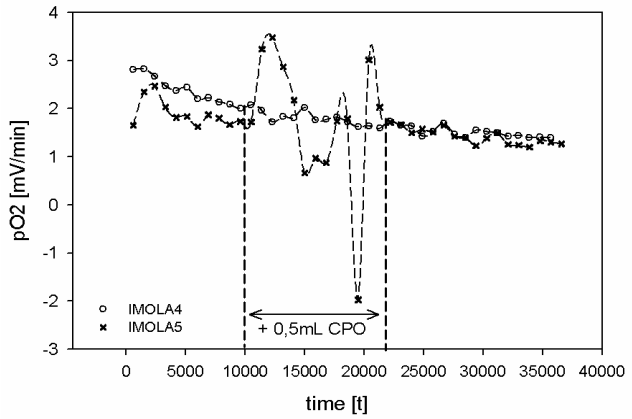

Figure 7. Influence of CPO pollution on photosynthetic activity of algae.

The pump is operated in a stop-and-flow mode as described earlier. During the stop intervals cell metabolic rate is recorded.

When the CPO emulsion is present in solution the photosynthetic oxygen production rate increases. Upon addition of the CPO medium, air bubbles formed in the tubing and were transported onto the chip causing signal instability. Subsequently, at the end of CPO exposure signals on IMOLA5 returned to basal levels indicating that the algae are not permanently damaged by the CPO at this concentration. Oscillations seen in IMOLA5 are caused primarily by air bubbles introduced to the chip by the emulsion.

IV. Conclusion

Water quality monitoring using biosensors IMOLA-IVD with algae *Chlorella Kessleri* as biotransducer was achieved by monitoring the dissolved oxygen via biochip. The changes in dissolved oxygen based on the addition of CPO were due to increased metabolism of the algae *Chlorella Kessleri*, immobilized on the biochip. Increased metabolism of algae caused an increase of photosynthetic activity which is detected by biosensor IMOLA-IVD proportional to decrease of DO rate. The oil pollution is used as an energy source for the algae to increase photosynthetic activity. Further experiments are necessary to fully explore the capabilities of the technology.

V. ACKNOWLEDGEMENTS

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