The EcoChip 2: An Autonomous Sensor Platform for Multimodal Bio-environmental Monitoring of the Northern Habitat

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***Abstract*— This paper presents the EcoChip 2, an autonomous multimodal bio-environmental sensor platform for the monitoring of microorganisms in the northern habitat. The EcoChip 2 prototype includes an array of 96-wells for the continuous monitoring of microbiological growth through a multichannel electrochemical impedance analyzer circuit. In addition, the platform includes luminosity, humidity, temperature sensors and monitoring. The developed electronic board uses an ultra-low-power microcontroller unit, a custom power management unit, a low-power wireless ISM-2.45 GHz transceiver, and a flash memory to accumulate and store the sensor data over extended monitoring periods. When a wireless base station is placed within the transmission range of the EcoChip 2, an embedded low-power wireless transceiver transmits the 96-wells impedance data and the other sensor data stored in the flash memory to the user interface. We present the measured performance of the prototype, along with laboratory test results of bacterial growth measurements inside the 96 wells in parallel. We show that the EcoChip 2 can successfully measure the impedances associated with bacterial growth over several hours using an excitation frequency of 2 kHz with power consumption of 114.6 mW under operating mode.**

I. Introduction

A strong interest is observed within the scientific community to study climate change and global warming as well as to assess the current environmental conditions and its limits. Microbial ecology, for example, investigates the existing relationships between microorganisms and their environment [1]. It is established that the presence of selected microorganisms in a specific environment can predict different environmental factors in a given habitat. These sentinel microbes are called bioindicators. Understanding the microorganisms thriving in these northern regions [2] can greatly advance our knowledge of these habitats and the effects of climate changes. But, as of now, the bioindicator microorganisms that flourish in different habitats that are most affected by climate changes, like those in the septentrional regions of Canada, are still mostly unknown.

This work was supported the Sentinel North Strategy and by the Canada First Research Excellence Fund. B. Gosselin, S. Nazila Hosseini, K. Bouzid, G. Gagnon-Turcotte, K. Ouazaa, P. Sarati Das are with the Dept. of Electr. and Computer Eng., Université Laval, Québec, QC G1V 0A6, Canada (phone: +1(418) 446-4469; e-mail: benoit.gosselin@gel.ulaval.ca). E. Bharucha is with the Sentinel North Platform for instrumentation design. Y. Messaddeq is with the Center for Optics, Photonics and Lasers (COPL), Université Laval. J. Corbeil is with Dept. of Molecular Medicine. D. Tremblay and S. Moineau are with the Dept. of Biochemistry, Microbiology and Bioinformatics, Université Laval.

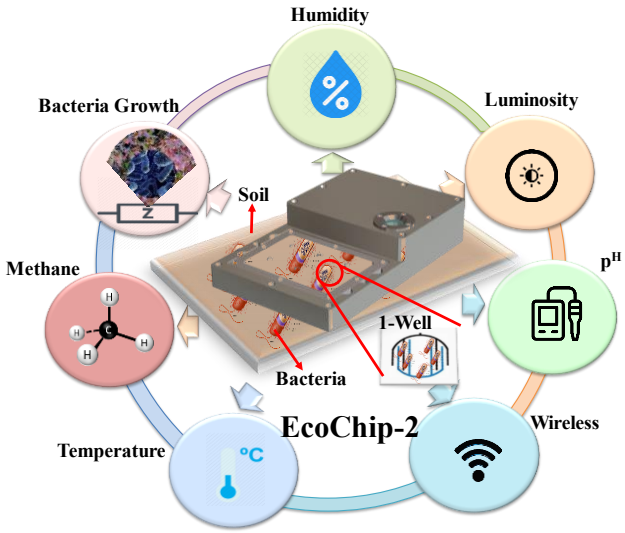

Fig. 1. Overview of the EcoChip 2 autonomous bio-environmental platform.

The transdisciplinary program Sentinel North Strategy at Université Laval strives to develop innovative technologies and increase the collective knowledge of northern habitats and their impacts on human beings and their health. Under the umbrella of the Sentinel North initiative, the EcoChip Project is an ambitious program that aims at developing an autonomous technology for monitoring, comprehending and valorizing microorganisms found in the North [3]. The EcoChip technology is also intended to identify unique molecules that could have potential usage in biological and industrial processes.

Here, we present the EcoChip 2, the next generation EcoChip technology. The EcoChip is an autonomous system built by our multidisciplinary team to allow and monitor the growth of microorganisms within individual wells in the field [2], [3]. Like its predecessor, the EcoChip 2 can measure the growth rate of microorganisms and their environmental conditions *in-situ* through an embedded sensor platform. The EcoChip 2 improves many aspects of the previous prototype. Among others, it has a gold plated EIS electrode array integrated with the electronic board, a new custom hermetic housing and was manufactured using standard and inexpensive printed circuit board technology. Section II, III gives a system overview and design of the EcoChip 2 platform and explains its improvement over the previous prototype. Section IV presents bacterial growth monitoring results obtained with our prototype in the laboratory and these

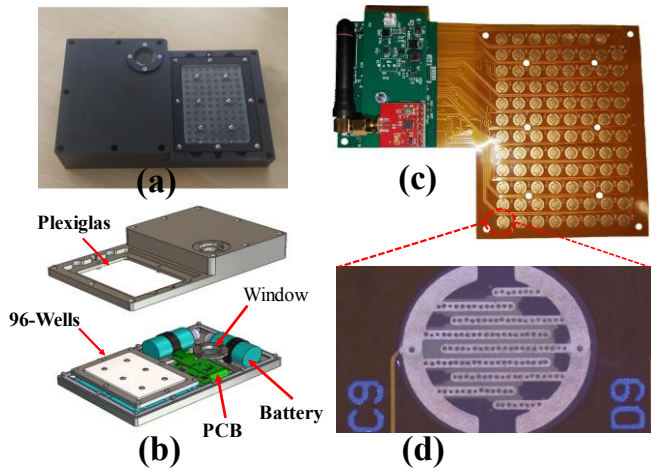

Fig. 2. (a) EcoChip system with encapsulation, (b) Layered view of the proposed system encapsulation, (c) Six layers rigid and flexible electronic board, (d) Enlarged view of one electrode.

results are discussed. Finally, conclusions are drawn in the last section.

II. System Overview

The EcoChip 2 improves the previous prototype on many levels. A complete revision of the electronic board was performed in this new prototype, followed by a revision of the EIS electrodes array. We replaced the original rigid microfabricated electrode array [3] with a flexible electrode array manufactured using standard inexpensive printed circuit board manufacturing technology. Fig. 1 shows a block diagram of the whole EcoChip 2 platform. The system consists of two separate parts, a printed circuit board that contains different bio-environmental sensor interface circuits, and the gold-plated 96-wells printed electrode array. The 96-wells of the EcoChip 2 allow to monitor the microbial growth in each well. The flexible printed electrode array is integrated with the electronic board including all the sensor interface circuits for continuous impedance measurement as well as other sensors for luminosity, humidity and temperature monitoring.

III. System Design

This section describes the different sensors and circuits included into the EcoChip 2 electronic board. Fig. 2(a) and 2(b) show the new multi-layer structure of the EcoChip 2, namely the encapsulation, the wells, the electrode array and the filter. The electrode array is integrated with the six-layer rigid and flexible electronic board, which is enclosed inside the encapsulation with the antenna and the batteries (Fig. 2(c)). The enlarged view of one gold-platted electrode is shown in Fig. 2(d). The characteristics and performance of the EcoChip 2 is summarized in Table 1. We have greatly reduced the size of the PCB as well as power consumption in the current version compared to EcoChip 1 as illustrated in Table 1.

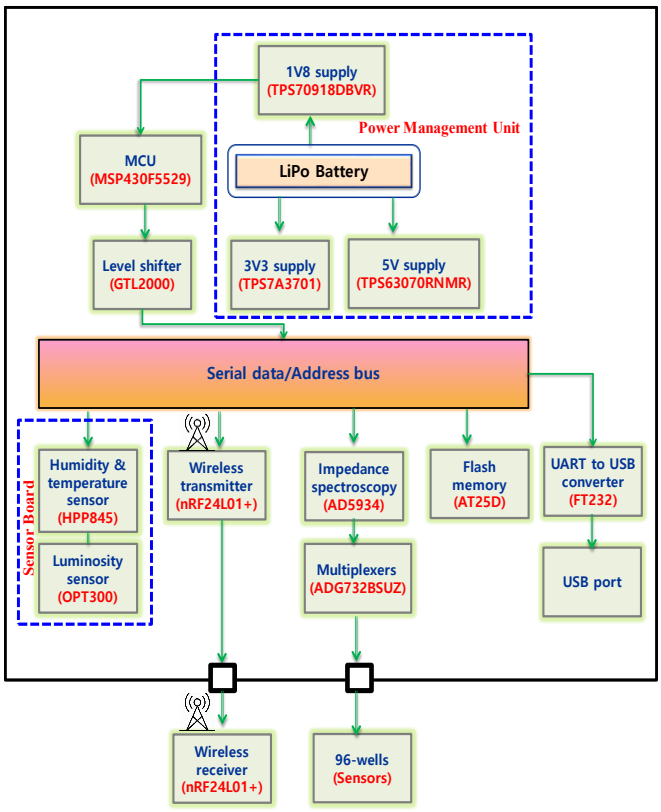

Fig. 3. Functional block diagram of the EcoChip 2.

Table 1: The system characteristics of the EcoChip 2.

Characteristics	EcoChip 2
Supply voltage	3.7V
Number of EIS wells	96
EIS excitation voltage	5 mV
Power consumption	114.6 mW
On-board memory capacity	32 Mb
Wireless transmission rate	1 Mbps
Transmission range	55 metres
Dimensions	240 x 150 x 40 mm
Luminosity accuracy	2%
Temperature accuracy	± 0.3%
Humidity accuracy	± 2 RH

A. 96-channel EIS Analyzer and Calibration Scheme

The modulus of the measured impedance of the culture medium changes in relation to the actual growth of the microorganisms inside the wells. For instance, in a culture of *E. coli*, the measured impedance can be typically around 150 Ω and 30 Ω. The AD5934 chip from Analog Devices is used to measure the impedance of each well. The AD5934 generates an excitation signal, which is connected with an inverting amplifier. The excitation signal is decreased and re-centered through a voltage divider. The transformed excitation signal passes through a buffer and a multiplexer, and through the selected electrode and sample. The EIS analyzer uses a calibration resistance in order to optimize its precision. To reach the highest performance, that calibration resistance must be set as close as possible to the impedance of

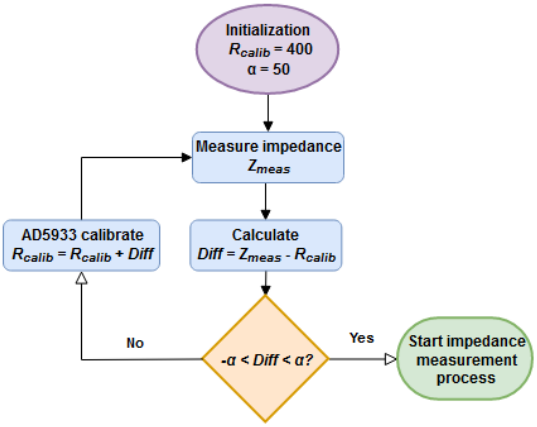

Fig. 4. Calibration scheme of the EIS analyzer.

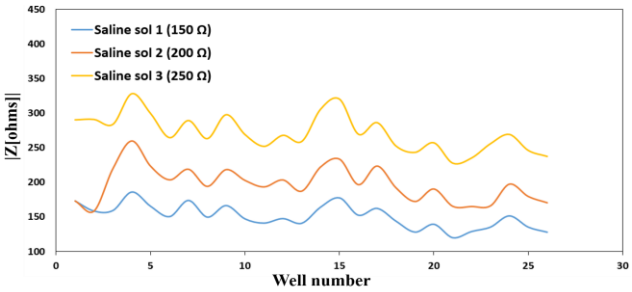

Fig. 5. Impedance modulus of different saline concentrations.

the measured sample. Thus, a recursive scheme has been devised to calibrate this resistor on the fly before performing every measurement. This scheme is described in Fig. 4. First, the calibration resistor R_{calib} is set to an initial value. A convergence value α is chosen. Then, the impedance of the SUT is measured and compared to the calibration resistor. If their difference *Diff* is bounded between the limits of the previously chosen convergence value α, then we proceed with the real measurements; if not, we change the value of the calibration resistor to the value of the previously measured SUT impedance, and we then repeat the process. Since the calibration resistor of this calibration cycle is now set to the measured impedance of the previous cycle, the difference value *Diff* of the current cycle must be enclosed between the convergence value α, supposing that the SUT's impedance has not changed too much in one calibration cycle.

B. *Electrode Array Manufacturing*

The electrodes are fabricated and placed underneath of each well. A piece of Kapton sheet is placed on a vacuum chamber where a thin layer of metals is deposited on it through the Electroless Nickel Immersion Gold (ENIG) process. A laser is utilized in order to trace the 96-wells(electrodes) on the metal-plated sheet so that the nutrients can pass through it. The electrodes array is made into an interdigitated pattern electrode (IDE). We chose IDE because it has multiple pairs of electrodes which give higher sensitivity than other types of electrode system (two or three). The IDE has multiple pairs of electrodes instead of the single one in a two-electrode configuration as indicated in Fig. 2(b).

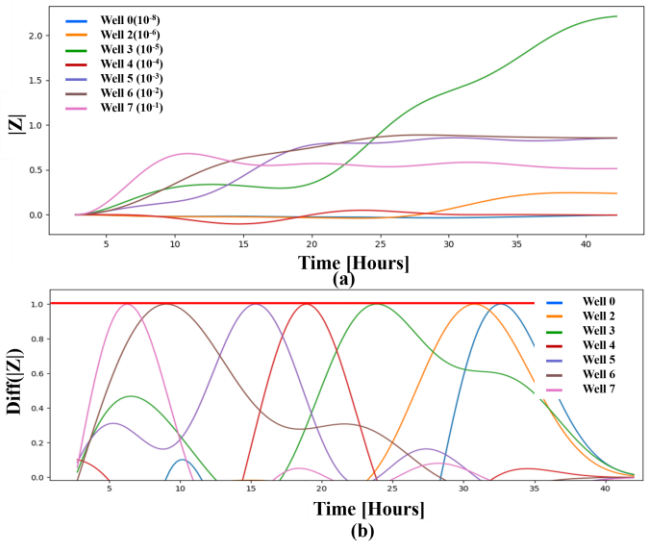

Fig. 6. Impedance values of diluted *E. coli* cultures : (a) Normalized impedances, (b) Derivative of normalized impedances.

C. *Control and Sensor Interface Circuits*

Table 2: Concentrations of *E. coli* in 96-well plate

E. coli	Dilutions
EC1	10^{-8}
EC10	10^{-7}
EC100	10^{-6}
EC1000	10^{-5}
EC10000	10^{-4}
EC100000	10^{-3}
EC1000000	10^{-2}
EC10000000	10^{-1}

The platform uses an ultra-low-power microcontroller unit (MCU) (MSP430F5529, Texas Instruments) to control the whole system and the EIS analyzer, as shown in Fig. 3. It operates at a frequency of 8 MHz to limit power consumption. We used commercial off-the-shelve sensors to monitor several other environmental parameters, like humidity, luminosity, and temperature. These sensor chips are directly mounted on the system PCB (Fig. 3). A HPP845 humidity and temperature sensor (TE Connectivity, Switzerland) was chosen for its low-power and small package. The light sensor OPT3001 from Texas Instruments was chosen to monitor ambient light from 0.01 lux to 83k lux. A circular shape transparent window was installed on the top of the housing to monitor the luminosity. In the Northern habitats, the environmental parameters will be measured every 30 minutes along with the impedances of the 96 wells.

D. *Wireless Communications*

A wireless transceiver module is utilised and mounted on the electronic board to permit communication with a base station. We selected the wireless module (nRF24l01+, nRF25l01+, Nordic Semiconductor, Norway), which communicates in the 2.4-GHz ISM band, as shown in Fig. 3. It gives several transmission rates along with the opportunity to implement different power-saving schemes. To get better receiver sensitivity, a transmission rate of 1 Mbps is utilized.

Wireless transmission is an important feature because snow accumulation can cover the platform and makes it harder to find. The wireless module is used to wake up the platform and download the stored sensor data.

E. Power Management Unit

A Tadiran TL5930F battery is used in the platform to power up the electronic board. The TL5930F has a temperature operating range of 55 °C to + 85 °C as illustrated in Fig. 3. The rated nominal voltage is 3.6 V and decreases according to the temperature (3.0 V at -30 °C). This voltage is stepped down to 1.8 V to power up the MCU using a low-dropout regulator TPS78318 from Texas Instruments. A DC/DC switching regulator LMR61428 from Texas Instruments is used to step-up the 3.6 V voltage to 3.3 V under very cold temperature, when the battery voltage can be very low, to supply all other parts of the electronic board.

IV. Experimental Results and Discussion

The EcoChip 2 prototype was fully characterized and tested in laboratory conditions and was used to perform bacterial growth measurement *in vitro* in a controlled environment. We calibrated the EcoChip 2 with different saline solutions. To prepare different concentrations of saline solutions, we put different quantity of Sodium Chloride into deionized water. Adding different quantities of Sodium Chloride into deionized water allow to adjust the impedance of the solution. Saline solution was put inside the 96 wells for testing the impedance of the solution. The first was made with saline water of concentration bringing the impedance of the saline to around 150 Ω. As can be seen in Fig. 5, the measured impedance was around 150 Ω, with a 15% variation across the wells. The second test uses saline water, the concentration of which brought the impedance to around 200 Ω. As can be seen, the measured impedance was around 200 Ω with a 20% variation across the wells. The third test used saline water the concentration of which brought the impedance to around 250 Ω. As can be seen, the measured impedance was around 250 across the wells with variation of 20%. The EcoChip 2 was then validated in controlled laboratory settings. The wells and the electrode array of the platform were cleaned and sterilized before each impedance test. The 96 wells were populated with different concentrations of *Escherichia coli* (*E. coli*). *E. coli* strain B (HER 1024) was grown overnight at 37˚C in Trypticase Soy Broth (TSB). Serial dilutions of the cultures were carried out to yield eight dilutions from 10^{-1} down to 10^{-8}. The different bacteria concentrations are listed in Table 2. Following the dilution steps, 150 µL of each serial dilution were placed in the microtiter wells of the EcoChip 2 on top of each well. The system was then incubated at room temperature for over 40 hours. We didn't shake during the incubation because the *E. coli* is a facultative anaerobic bacterium. It can grow aerobically if oxygen is present. If the oxygen is absent, it can switch to anaerobic respiration. Data were collected with an excitation frequency of 2 kHz at regular intervals of 15 minutes, stored in memory, and then directly saved on a computer using UART to USB communication [4]. As can be seen in Fig. 6(a), the impedance curves indicate an increase in the bacterial concentration inside the wells, which translated into an increase of the impedance. Furthermore, the humidity (23.79 $g.kg^{-1}$), temperature (20.42 °C) and luminosity (12.8 lux) were simultaneously measured for this test. Then, as the test progressed, impedance stops varying and reaches a steady state because a maximum number of bacteria was reached in the wells. On the other hand, there is a slight variation in the measured impedances as shown in Fig. 6(a). It happened because of the number of metabolites and other small variations in the growth medium between the wells. We can see visually in Fig. 6(b) that the peaks are localized according to the initial concentration of bacteria, ex: the well with the highest initial concentration of bacteria (10^{-1}) had its peak first, while that with the lowest initial concentration (10^{-8}) had its peak last. The effect of bacteria growth on impedance depends on the conductivity of the medium, which is proportional to bacterial activity. Fig. 6 shows a clear change of the impedance which is well correlated with the bacterial concentration.

V. Conclusion

In this paper, we presented an autonomous sensor platform for the monitoring and analysis of several bio-environmental parameters, such as microorganism's growth through EIS, to eventually gain a better understanding of the Northern ecosystems. The EcoChip 2 utilizes EIS to observe the bacterial growth across a 96-well plate as well as other environmental parameters, like luminosity and humidity. The measured sensor data is stored in the platform memory and can be sent wirelessly to a base station for data management and visualization. We conducted laboratory tests, the results of which show that the proposed platform can measure the growth and discriminate between different concentration of microorganisms.

Acknowledgments

The authors would like to thank the Félix d'Hérelle Reference Center for Bacterial Viruses at Université Laval for testing the system in-vitro and the Sentinel North Platform for instrumentation design for supporting the design and the test of the EcoChip platform. The authors also thank the Canada Research Chair in Smart Biomedical Microsystems, Canada Research Chair in Bacteriophages, and the Canada Excellence Research Chair in Enabling Photonic Innovations for Information and Communication.

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