The EcoChip: A Wireless Multi-Sensor Platform for Comprehensive Environmental Monitoring

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Abstract*— This paper presents the EcoChip, a new system based on state-of-the-art electro-chemical impedance (EIS) technologies allowing the growth of single strain organisms isolated from northern habitats. This portable system is a complete and autonomous wireless platform designed to monitor and cultivate microorganisms directly sampled from their natural environment, particularly from harsh northern environments. Using 96-well plates, the EcoChip can be used in the field for real-time monitoring of bacterial growth. Manufactured with high-quality electronic components, this new EIS monitoring system is designed to function at a low excitation voltage signal to avoid damaging the cultured cells. The high-precision calibration network leads to high-precision results, even in the most limiting contexts. Luminosity, humidity and temperature can also be monitored with the addition of appropriate sensors. Access to robust data storage systems and power supplies is an obvious limitation for northern research. That is why the EcoChip is equipped with a flash memory that can store data over long periods of time. To resolve the power issue, a low-power micro-controller and a power management unit control and supply all electronic building blocks. Data stored in the EcoChip's flash memory can be transmitted through a transceiver whenever a receiver is located within the functional transmission range. In this paper, we present the measured performance of the system, along with results from laboratory tests *in-vitro* and from two field tests. The EcoChip has been utilized to collect bio-environemental data in the field from the northern soils and ecosystems of Kuujjuarapik and Puvirnituq, during two expeditions, in 2017 and 2018, respectively. We show that the EcoChip can effectively carry out EIS analyses over an excitation frequency ranging from 750 Hz to 10 kHz with an accuracy of 2.35%. The overall power consumption of the system was 140.4 mW in normal operating mode and 81 µW in sleep mode. The proper development of the isolated bacteria was confirmed through DNA sequencing, indicating that bacteria thrive in the EcoChip's culture wells while the growing conditions are successfully gathered and stored.

***Index Terms*— Electrochemical impedance spectroscopy, embedded system, microorganisms, bacteria, environmental monitoring, climate changes, DNA sequencing.**

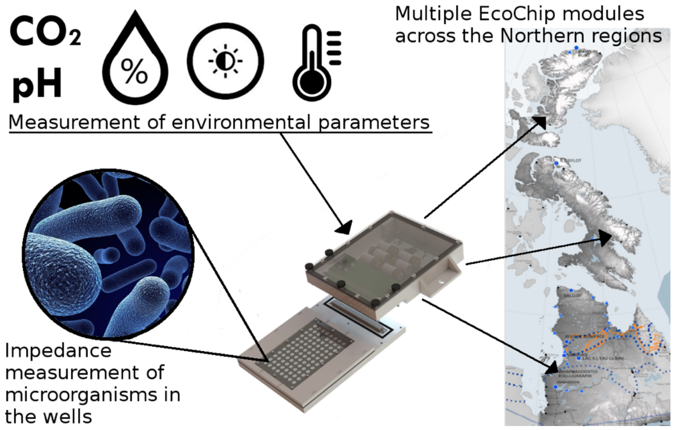

Fig. 1. Overview of the EcoChip system intended for the comprehensive environmental monitoring and valorization of molecules and microorganisms from the Canadian Northern areas. The EcoChip can contribute to identify sentinel microorganisms that are signposts of specific environmental changes across a gradient of Northern regions under the influence of global warming.

I. INTRODUCTION

OVER the past decades, climate change and the way it models the environment has received a lot of attention from researchers and the media. Measuring the actual impact of climate change in a quantitative manner remains, to this day, a challenging issue. Nonetheless, it has been demonstrated that the study of the microorganisms that thrive in a given environment is an efficient way of assessing the impacts of climate change in that particular environment [1]. The problem with this method is that the microorganisms that persist where climate changes are prominent, northernmost areas, are not well known [2]. To resolve this issue, collective knowledge regarding microorganism communities and metabolism should be addressed.

The Sentinel North Strategy allows Université Laval to draw on over a half-century of northern and optics/photonics research to develop innovative new technology and improve our

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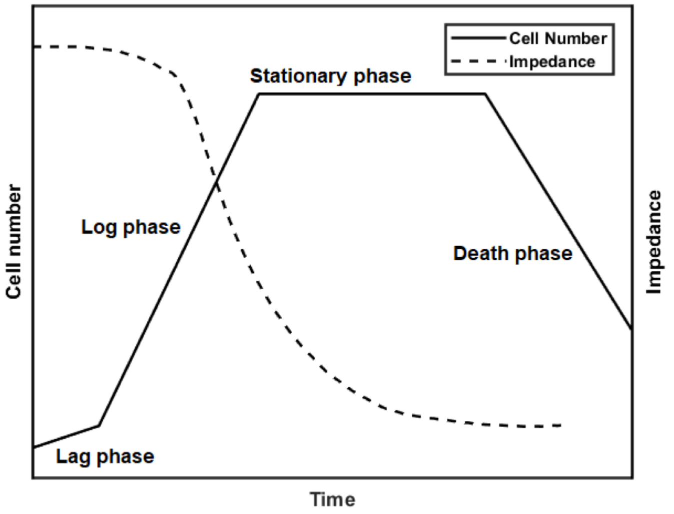

Fig. 2. Variation of impedance relative to cell number in a bacterial colony according to its life cycle, as shown in [7].

understanding of the northern environment and its impact on human beings and their health. One of the main goals of the Strategy is to monitor, understand and valorize microorganisms and molecules in the Canadian North. To do so, we aim at identifying microorganisms that will serve as indicators of environmental changes across a gradient of northern regions currently under the influence of global warming, human interventions and in the presence of contaminants. Another part of the strategy aims at finding and characterizing molecules capable of thriving in extreme environments that could be of interest in industrial and biological technologies. A conceptual representation of the proposed system along with a map of the Canadian northern territory of interest is shown in Fig. 1. For example, it has been found that certain microorganisms found in very cold climates are efficient in degrading petroleum hydrocarbons [3], [4].

One very important aspect of assessing micro-organism's property is measuring their growth rate. Traditional assessment methods for growing undescribed microbes with culture media are time consuming and expensive. Measurement systems based on electro-chemical impedance spectroscopy (EIS) represent an inexpensive and effective alternative method to measure microbial growth [5], [6]. As bacteria grow and consume nutrients, their concentration changes within a medium, causing impedance variations. Ionic conductors, which are at the root of impedance fluctuations, are released by the bacterial metabolism. The typical life cycle of a bacterial colony is characterized by four distinct phases, at which the measured impedance of a colony changes [7]. Fig. 2 illustrates the relationship between the different phases and their corresponding impedance trends. Because of these characteristic phases, EIS technologies are able to identify the life cycle and growth rate of bacterial colonies [8]. Such technologies can also be used to differentiate bacterial species, since the changes in impedance are related to the life cycle and metabolism, which vary from one species to another [9].

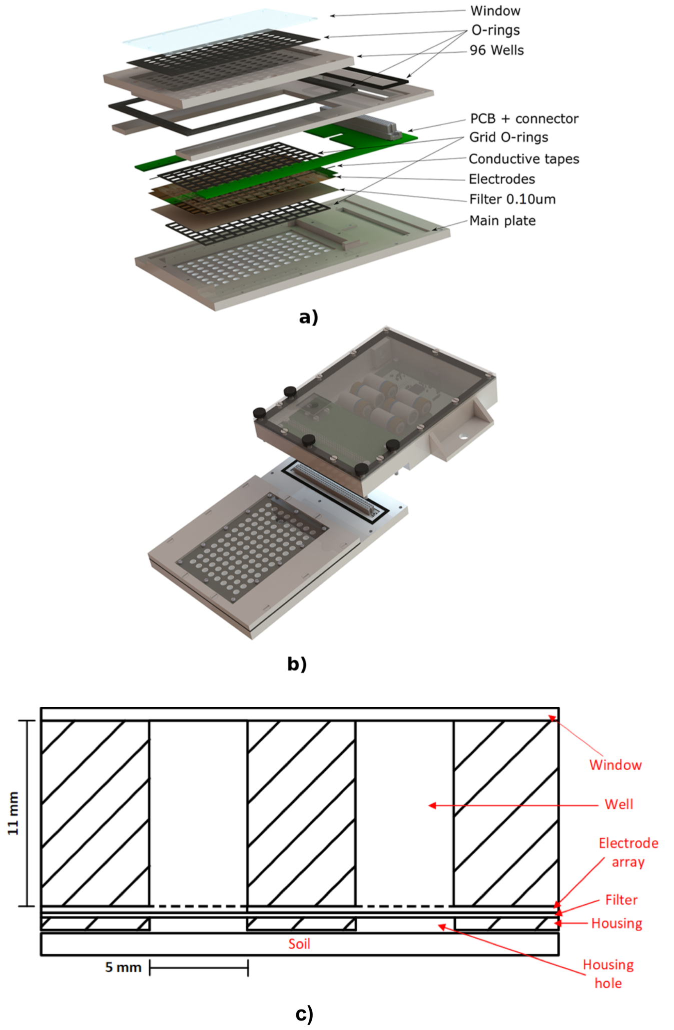

Fig. 3. a) Exploded view of the EcoChip multi-layer structure showing the wells, the electrodes and the filter, b) view of the complete EcoChip including the layered module and the electronic board, and c) a cross-section view of one culture well.

Many research applications and commercial systems are now based on EIS. Hospitals and medical centers use benchtop systems to monitor cell growth and morphology in cell cultures in real time [10]. For example, impedance data is useful to estimate cell counts in any given medium over time. xCELLigence RTCA from ACEA Biosciences Inc. is a versatile benchtop impedance analysis tool that is used for many applications, including drug screening, cell analysis, safety pharmacology, disease studies, and cancer immunotherapy [10]. This device works with typical 96-well plates that are equipped with electrodes to monitor impedance. The complete system has three functional parts: an incubator for culture plates, an analysis station and an associated computer allowing to observe the growth rate in real time. Miniaturized impedance spectroscopy systems including hundreds of channels have also been implemented using CMOS microelectronic technology for high-throughput cell characterization and drug screening [11]. Miniaturized impedance spectroscopy systems are also available with multiple sensors for impedance analysis, optical detection and temperature measurement [12]. Some systems, such as Lab-on-a-chip, are extremely useful in space-limited contexts, but are

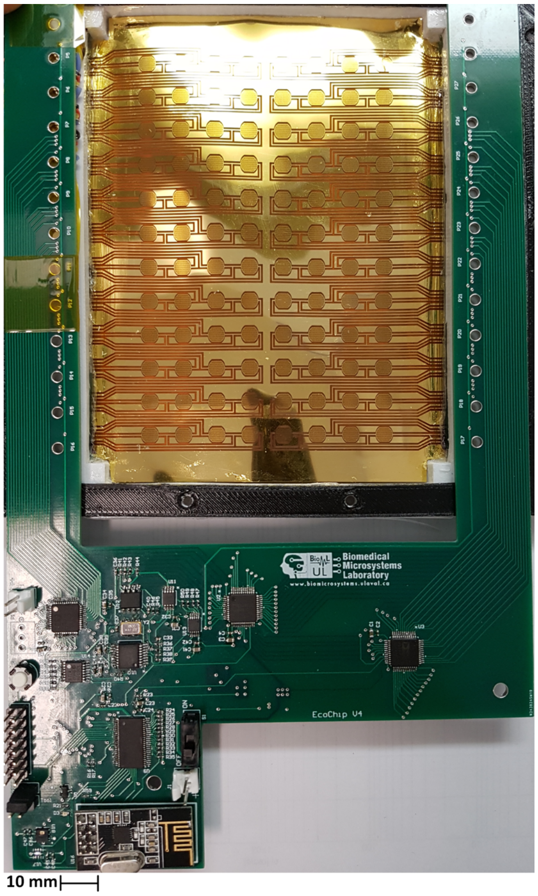

Fig. 4. Picture of the EcoChip printed circuit board along with the microfabricated electrode array located under the wells.

designed to be used in laboratories rather than on the field.

To overcome issues related to cultivation, specialized isolation chambers have been used to cultivate bacteria in situ, in a wide range of environments [13], [14]. One system in particular, called the iChip, which was developed at Northeastern University (Boston, USA) and commercialized by NovoBiotic Pharmaceuticals, enabled the discovery of Teixobactin, among others [15]. Teixobactin is the first commercially viable new antibiotic drug to be developed in 30 years. Applications of a system like the iChip, which allows growing microorganisms in natural environments, are limited by the fact that it does not permit to simultaneously monitor the evolution of the media in which the bacteria are growing. Knowledge on these conditions, including growth rate and environmental monitoring, would lead to a better understanding of the isolated microorganisms and molecules. However, when conducting such studies using electronic systems in remote area and harsh environments, special care needs to be taken in the design of the system. Conditions such as cold temperature, extreme humidity, ice and moisture can be especially rough on the electronics and must be well taken care of. In the case of remotes areas, hardwired power sources are often unavailable. Thus, other options must be considered to insure autonomy.

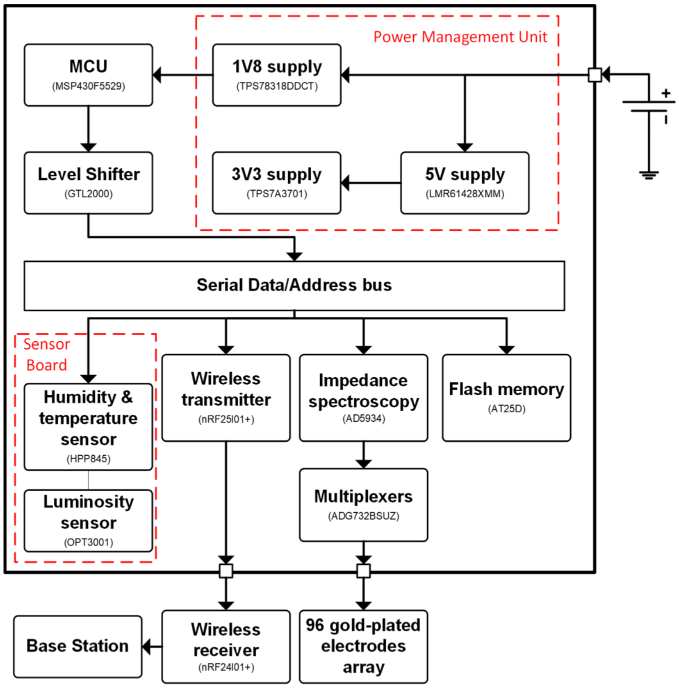

Fig. 5. Block diagram showing the functions and the building blocks of the EcoChip.

We present the development of the EcoChip. With the aim of gaining a better understanding of the microbiomes found in northern climates, Sentinel North's multidisciplinary team from Université Laval developed the EcoChip, a wireless multi-sensor platform. This new system allows to grow microorganisms directly isolated from northern habitats in individual wells. An embedded sensor platform can measure the growth rate of microorganisms as well as the environmental conditions in situ. Unlike currently available systems, the EcoChip allows both the culture of microorganisms directly in the field and the measurement and recording of bacterial growth in a large number of culture wells along with surrounding environmental parameters. The second section of this paper presents an overview of the proposed platform. In Section three, the design of the complete system, including the multichannel impedance spectroscopy module and other sensor interface circuits, the data storage/management unit, the wireless transceiver, the microfabricated electrode array and its custom housing are described. The following section provides experimental results obtained in the laboratory and from two field studies performed in Northern Canada. DNA sequencing results obtained after retrieving the EcoChip from a field study in Kuujjuarapik are also presented along with environmental data. EIS monitoring results obtained from a second field study in Puvirnituq are also presented, and compared to the results obtained *in-vitro*. Finally, in the last section, conclusions are drawn.

II. SYSTEM OVERVIEW

The EcoChip, previously introduced in [16], is a system comprised of two distinct parts, an embedded electronic board which includes different sensor interface circuits, and a layered structure including a microfabricated gold-plated electrode array as well as a 96-well plate. The wells of the EcoChip are

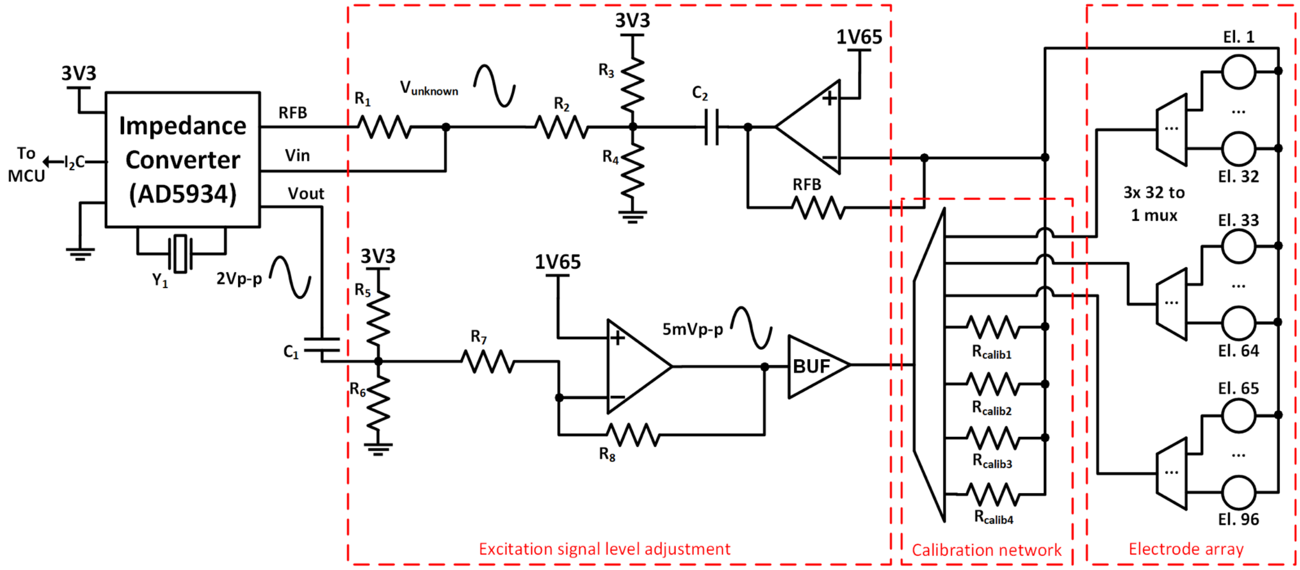

Fig. 6. Circuit schematic of the multichannel impedance spectroscopy circuit. Multiplexers are used to sequentially distribute the signal to the calibration network and the 96 electrodes of the wells. Signal level adjustment circuit is used to lower the signal voltage so that it will not harm microorganisms in the wells, return signal is then amplified to fall back into the AD5934 reading range.

suitable for the culture of single strain microorganisms found from soil or water samples from a given habitat. These two parts of the system are connected together to allow the impedance measurement system contact to the electrodes of the layered structure.

A. *Layered Structure*

A major problem with designing a system that can be left in the field for an extended period of time is the presence of extreme humidity, snow and ice that can produce moisture on the electronic board and cause malfunctions. To solve this problem, the custom enclosure of the electronic system and the wells of the EcoChip are made using 3D printing with ABS (Acrylonitrile butadiene styrene) and rubber to make the complete device as watertight as possible. The result is a layered structure (Fig. 3) which is composed of six parts: a plastic housing, a filter, gold-plated electrodes for 96 wells, a window and O-rings. The window on the top is made of *Plexiglas* to allow the wells to have access to sunlight when possible. The wells are isolated from each other and from the external environment, except at the bottom, where a filter is placed to allow the exchange of nutrients between the culture mediums and the soil the EcoChip is in contact with. Fig. 3 c) presents a cross-section view of one of the culture well. This figure shows that one microfabricated electrode is located under each well, which communicates with the soil and the external environment through laser-drilled holes in the electrode layer. A filter is located between the electrode layer and the holes in the housing to prevent unwanted organisms entering the EcoChip wells, while allowing nutrients from the soil to flow inside. A *MF-Millipore* membrane with pore dimensions of 0.1 µm and is 105 µm thick is utilized for the filter. The 96-electrodes gold-plated electrode layer is electrically connected with the rest of the circuit using an anisotropic conductive tape. The tape is applied between conductive pads under the printed circuit board (PCB) and the electrode layer. These pads are then connected to the electronic circuit with copper traces equally distributed on three different layers of the board to prevent capacitive coupling as much as possible.

B. *Electronic Board*

A photo of the electronic board is shown in Fig. 4. The EcoChip electronic circuit is composed of a multichannel electro-chemical impedance spectroscopy system used to monitor and quantify the growth rate of microorganisms inside the wells using an electrode array, and a flash memory to store and accumulate sensors data for an extended period of time. The system also includes a low-power microcontroller unit (MCU), a power management unit (PMU), and a low-power wireless transceiver that transmits sensors data when a suitable receiver and base station are located nearby the EcoChip. To provide additional information on the growth conditions of the microorganisms in the wells of the EcoChip, various sensors are incorporated to measure various surrounding parameters such as luminosity, humidity and temperature. The four-layer electronic board is controlled by a low-power microcontroller unit, which leverages different energy saving schemes to save as much power as possible, and extend battery lifetime. The EcoChip can operate in 2 modes: 1) a *normal mode*, when impedance analyses and environmental parameters are measured, saved, and sent wirelessly if a nearby receiver is available, and 2) a *sleep mode*, where the system is in most of the time. In this sleep mode most systems on the board are disabled to cut down on power consumption. Once every five hours an interrupt is triggered by an internal timer and the MCU enables the various sensors to conduct analyses and going back to sleep afterwards. The system uses a battery as power source so it can be deployed in remote conditions, and operate autonomously. This allows for the deployment of the system in a large variety of environments in contrast to other systems, which typically need a regular hardwired power source. Humidity can also be damageable to the electronic board by corroding copper traces and causing short circuits. To protect to the electronic board and avoid these problems, a conformal coating is applied on the housing to prevent any moisture inside the 3D printed enclosure. Using a wireless communication to harvest data system also removes the risk of water infiltration due to a connector or a wire pass-through.

III. SYSTEM DESIGN

This section presents the various sensors and systems integrated in the EcoChip, Fig. 5 shows the block diagram of the complete electronic board.

A. Microcontroller unit

The EcoChip is controlled by a MSP430F5529 MCU from *Texas Instrument*. It is an ultra-low-power microcontroller based on a 16-bit CPU which features multiple low-power modes of operation to further reduce power consumption by disabling various peripherals and internal clock signals. This strategy is critical to the project since it allows to drastically reduce power consumption when the device goes into sleep mode. Using these power saving schemes, the EcoChip achieves an average power consumption of 140.4 mW and an idle mode power consumption of 81 µW, which represents less than 0.06% of the power consumption obtained in normal mode, leading to a greater battery life span. The microcontroller is operating on a low-frequency 8-MHz clock to limit power consumption in the MCU and other digital circuits. A UART port is accessible via a connector for debugging and through data harvesting when a wireless communication system is not available.

B. Multichannel Impedance Spectroscopy

The growth of microorganisms inside the culture wells is represented by measuring the impedance inside them. To achieve this goal a multichannel impedance measurement circuit presented in Fig. 6 is used. The core of this circuit is the AD5934 chip from Analog Devices, USA. This 12-bit impedance converter is frequently utilized in such systems since it is easy to use and interface with and also offers a good range of excitation frequencies that can go up to 100 kHz [17], [18]. The AD5934 digital signal processor provides the real and imaginary parts of the measured impedance. These values are calculated with a DFT (digital Fourier transform) performed by the AD5934 on 1024-point of the sampled signal. In order to achieve a good precision however, a calibration process is required. Because of this before every set of measurements the system measures the impedance of a known resistor selected from a calibration network of four resistors depending on the expected impedance range to be measured. The system is programmed to measure these impedance values, and to compare them with known values before running the EIS analysis on the wells. Resistors are used in the calibration process so that the measured phase (ϕ_{calib}) is only due to the AD5934 internal circuits and is not influenced by additional components. The measured impedance values of the culture wells are then corrected by subtracting the phase ϕ_{calib} to the calculated phase value and then using the magnitude and phase to obtain the imaginary and real part of the impedance.

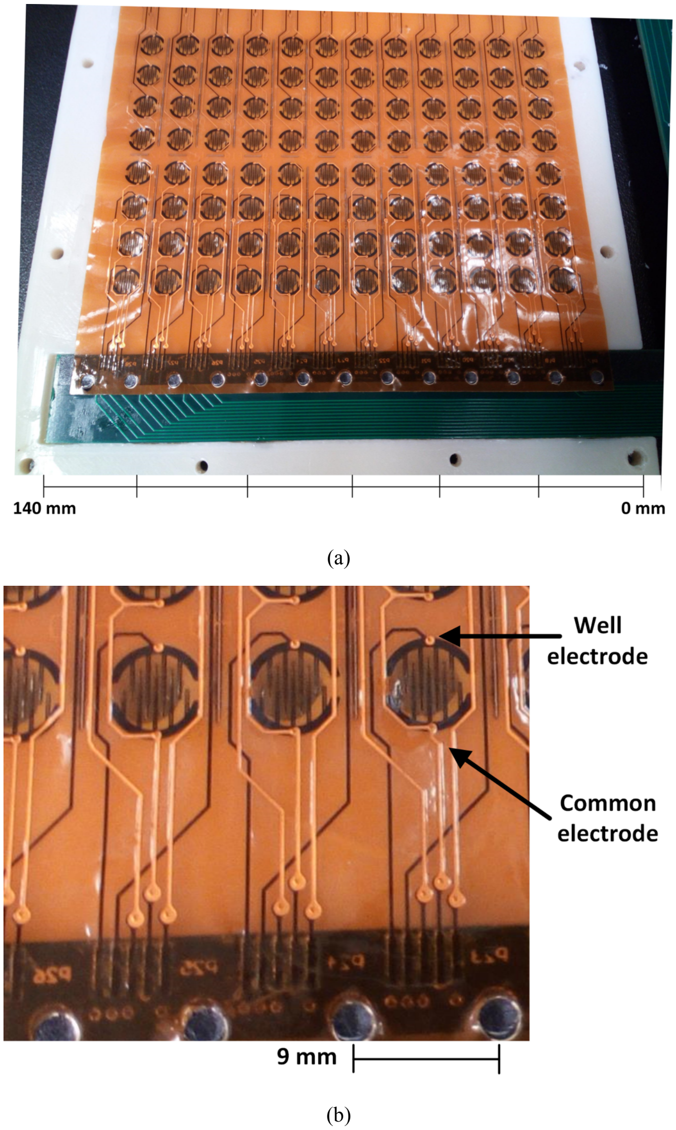

Fig. 7. The electrode array. a) Picture of the 96 microfabricated gold-plated electrodes, and b) On closer view on the electrodes.

The AD5934 is programmed to generate a 2-V_{pp} sinusoidal excitation signal, which is required to be scaled down not to harm the microorganisms found in the wells. Using an operational amplifier in inverting configuration it is reduced to a 5 mV_{pp} signal and it is also re-centered at 1.65V using an AC coupling and a voltage divider. This signal is fed into a buffer and then an analog multiplexer can direct this signal to the calibration network or the next multiplexer layer to the wells. Three 1:32 analog multiplexers are used to sequentially dispatch the excitation to each of the 96 electrodes located inside the culture wells (Fig. 4). The resistance of the signal path, from the AD5934 to the electrode, depends on the PCB trace length and on the multiplexers internal resistances. It has a measured average value of 8 Ω in this design. Currently, this parasitic value is compensated manually in the MCU firmware of the EcoChip, but this procedure can easily be automated in future versions. The measured impedance signal then get amplified so it falls back into the reading range of the AD5934 to allow it to function as intended. When the cycle is completed for each of the 96 wells, data is sent to the MCU via an I_2C bus to be saved in the on-board Flash memory.

A 16 MHz crystal oscillator is supplied to the AD5934 to generate the various excitation frequencies. The time required to perform a complete EIS analysis of the 96 wells depends on the selected excitation frequency. The *time of analysis* decreases with the excitation frequency and as we use a higher frequency the capacitive part of the impedance becomes smaller relatively to the resistive part. Hence, this design uses an excitation frequency of 2 kHz to perform a complete analysis, which provides a good tradeoff, and leads to a time of analysis of 2 minutes and 40 seconds. Since the cold temperature of the northern regions usually results into lower microorganisms growth rates [19], a low sampling rate of 5 hours between each 96 well EIS measurement and other sensor measurement is used in this design. The EcoChip wakes up from idle mode every 5 hours and measures and stores all sensors data, and then falls back into sleep mode between sampling intervals to save energy.

C. Multi-Sensor Interface

Two sensor chips are used to monitor temperature, humidity and luminosity are mounted directly on the electronic board of the EcoChip. The HPP845 temperature and humidity sensor from TE Connectivity, Switzerland is used for its low power consumption and its dual integration of both sensors in a small package. To measure ambient light, the OPT3001 from Texas Instruments, USA is selected since it allows the measurement of light across a wide range of luminosity, going from 0.01 lux to 83k lux, and presents low-power consumption. The light sensor can monitor the luminosity through a small transparent window installed in the housing of the main electronic board. Thus, the location of the sensor within the PCB falls directly under this window. Then, the measured environmental data is transmitted via an I^2C bus to the MCU, and is then stored in the flash memory via a SPI bus, like EIS data. Environmental sensor measurements are performed every five hours, in conjunction with impedance measurements. CO_2 and pH sensors will also be added to the sensor interface in the near future.

D. Wireless Communications

A wireless communication system is implemented on the EcoChip to allow communication with a base station so data can be accessed even during the time the device is in the field. The nRF24l01+ wireless transceiver from Nordic Semiconductor, Norway is selected for this application. The device is communicating in the 2.4-GHz ISM band and offers multiple transmission rate and power along with power saving schemes. A transmission rate of 1 Mbps is used since it allows for better receiver sensitivity. Wireless communications are could become an essential feature in this project considering that in the northern climates snow accumulations may cover the EcoChip. In such a case, the wireless connection can be used to locate and to wake up the EcoChip, and to transmit the sensor data stored in the memory to a base station equipped to receive these data.

E. Microfabricated Electrode Array

The electrodes placed at the bottom of each well are fabricated in two steps (Fig. 7). A sheet of polyimide is placed on a vacuum chamber where a fine layer of gold is deposited on it. A laser is used afterwards to trace the 96 electrodes on this same sheet (Fig. 7a). Electrodes are drawn into a two-comb shape layout (Fig. 7b), holes are also made in this sheet so nutrients can pass through it and the filter membrane also located there. An interdigitated design is used for the electrodes since this configuration provides a better sensitivity than a classic two electrodes system. This is caused by the multiple electrode pairs instead of the single one in a two-electrode configuration [20]. Contact fingers are provided for all electrodes to enable an electric connection with the electronic circuit board. In this configuration, five contact fingers allow the interrogation of 4 wells (one line is common), there are 12 sets of 4 electrodes/wells on each side of the gold-plated electrode layer.

TABLE I. SYSTEM CHARACTERISTICS AND MEASURED PERFORMANCE.

Parameter	Value
Supply voltage (battery)	3.6 V
Battery nominal capacity	19 Ah
EIS excitation voltage	5 mV peak-peak
EIS excitation frequency range	750 Hz to 10 kHz
Impedance measure precision	2.35%
Power consumption (normal)	140.4 mW
Analysis time @2kHz	160 s
Estimated battery lifetime	1009 days
Power consumption (sleep)	81 µW
On-board memory capacity	32 Mb
Wireless transmission rate	1 Mbps
Wireless transmission power	0 dBm
Transmission range	35 m
Temperature accuracy @25°C	±0.3%
Relative humidity accuracy @25°C	±2 RH
Typical channel parasitic resistance	8.0 Ω
Dimensions (electronic board)	221 x 131 mm

F. Power Management Unit

Power is provided to the EcoChip electronic board via a Tadiran TL5930F battery which as a temperature operating range of -55°C to + 85°C. This battery is rated at a nominal 3.6V and is also equipped with a capacitor which is charged by the battery to provide a current boost when it is needed at startup of the device in cold temperature. This 3.6V voltage supply is stepped down to a 1.8-V supply for the MCU using an ultralow power low-dropout regulator (LDO) TPS78318 from Texas Instruments specifically selected to be as energy efficient as possible since it is required to be always enabled to supply the MCU. A LMR61428 from Texas Instruments step-up DC-DC switching regulator is used to step-up the 3.6-V supply into a 5-V supply. This 5-V voltage is then used to produce the 3.3V, which is used to supply all other components of the system, except the MCU. The 5-V supply also supplies the external soil humidity probe. When the EcoChip is in sleep mode, the MCU disables the 3.3-V supply to save power since all the building blocks supplied by this voltage are no longer required in sleep mode. A load switch controlled by the MCU is also implemented ahead of the regulators in order to completely cut the regulators supply when the EcoChip goes into sleep mode. Battery voltage is monitored using one of the analog to digital converter inside the MCU via a voltage divider network. The voltage data is written in the memory along with the EIS and the environmental sensor data after each analysis performed every 5 hours.

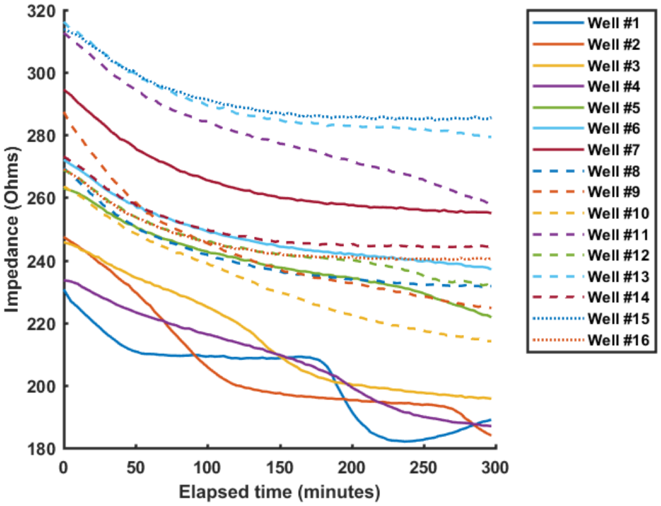

Fig. 8. Impedance spectroscopy measurement results *in-vitro* in controlled laboratory environment at an excitation frequency of 2 kHz.

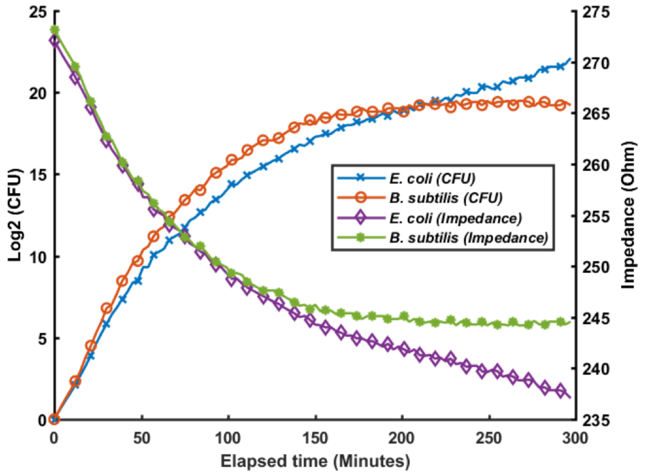

Fig. 9. Evolution of the bacterial concentration over time for *E. coli* and *B. subitilis* in the EcoChip.

From the performance summarized in Table I, it is possible to estimate the battery lifetime. Since the device is waking up every 5 hours to run a complete analysis, we estimate that there will be around 5 analyses every day for a total of 13.33 minutes where the EcoChip is being the active mode every day. Since the system remains in sleep mode the rest of the time, where current consumption is greatly reduced, we can calculate a weighted average of the current consumption, and then estimate the battery lifetime. Doing so, we obtain a lifetime of 2019 days in ideal conditions. We can estimate that this duration can be cut by half to account for the effect of the cold temperature on the batteries, which gives an estimated lifetime of 1009 days in the field [21]. Besides, monitoring the battery voltage inside the EcoChip allows to estimate the device lifetime and will later allow the implementation of advanced energy saving schemes that will adapt to temperature and/or to other varying environmental parameters.

G. *Flash Memory*

The EcoChip is equipped with a 32 Mb on-board Flash memory chip AT25DF321A from Adesto Technologies to store the measured impedance and environmental data. For each impedance analysis performed, the impedance magnitude and phase are stored along with the corresponding well number, so each measured value can be associated with the right well when retrieving the data. At each analysis, the last address used in the memory along with the corresponding sample number is store in a dedicated page in order to keep track of the measurement data and timing, and not lose any measurement values in the event of an unexpected system reset. When the EcoChip exits sleep mode, it first reads this page, and then starts writing data from that point in the memory and so on. Long-term data storage and data integrity are critical in this application, especially when a wireless connection or a base station is not available to retrieve the data. In this case, the data can be retrieved through a UART to USB converter that is installed on the board to transfer the data from the flash memory to a computer user interface, and save it into a *csv* file.

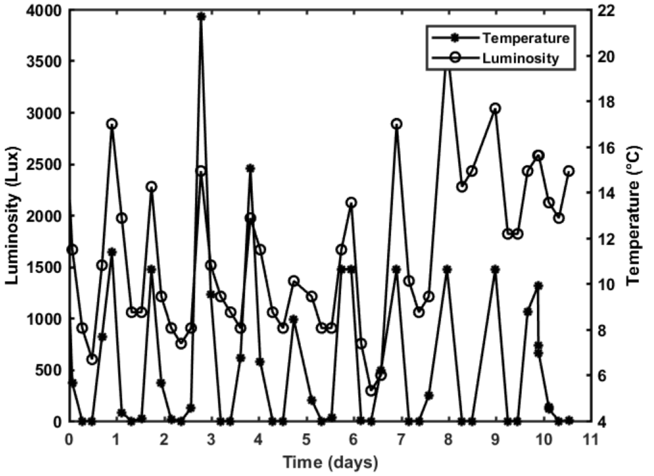

Fig. 10. Field measurements: temperature and luminosity data measured over about 11 days in the village of Kuujjuarapik, in Northern Quebec.

IV. EXPERIMENTAL RESULTS

The EcoChip was fully characterized and tested in the laboratory, and was used to perform bacteria growth measurement *in-vitro* in an incubator. Sensor data and DNA sequencing results were subsequently obtained following a pilot study performed with the EcoChip in the village of Kuujjuarapik, in Northern Canada.

A. *Impedance Spectroscopy Measurements In-vitro*

The EcoChip was validated in a laboratory setting, inside an incubator. Impedance analyses were conducted with known bacterial strains. Wells were populated with *Escherichia coli* B (HER1024) and *Bacillus subtilis* W168 (HER1046) obtained from the *Félix d'Hérelle Reference Centre for Bacterial Viruses* at Université Laval (www.phage.ulaval.ca). Bacterial strains were grown overnight in Trypticase Soy Broth (TSB) with agitation at 37°C. Serial dilutions from cultures were carried out to yield seven dilutions from 10^{-1} down to 10^{-7}. A negative control containing only of sterile growth media was also set aside for later use. Following the dilution steps 100 µL of each serial dilutions and 2 controls, were placed in the microtiter

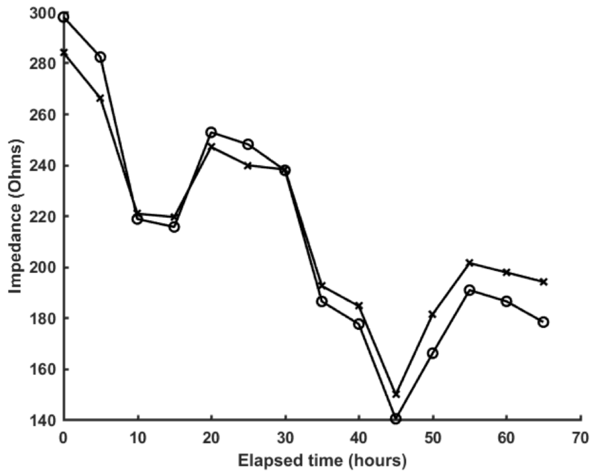

Fig. 11. Impedance measurement for two wells during the field study at the Clearwater Lake Station, Puvirnituq in August 2018.

wells of the EcoChip. The system was then incubated at 37°C for 5 hours until the stationary phase was observed on the most concentrated wells.

For the duration of the five-hour test, impedance was measured in each of the 16 test wells containing bacteria at an excitation frequency of 2 kHz such that the capacitance represents the major part o f the measured impedance, as described in [22] and [23]. Analyses were conducted at regular intervals of 3 minutes and data were directly saved on a computer using UART to USB communication. Impedance results from sixteen wells are presented in Fig. 8. Half of the wells contains *E. coli* (odd numbered wells) while the other half contains *B. subtilis* (even numbered wells). The impedance curves indicate an increase in the bacterial concentration inside the wells as it is characterized by a logarithmic decrease in impedance [23]. One can also observe that at the end of the test, impedance stops varying and reaches a steady state as the bacteria become confluent for most of the wells. These results demonstrate the capabilities of the EcoChip to conduct impedance analyses on multiple wells. The slight variation in the measured impedance between the wells containing the same strains can be explained by a variation in a number of parameters such as the number of initial cells, quantity of nutrients and other small variation in the growth medium between the wells.

Immediately following incubation in the EcoChip, we selected the first well, starting from the most concentrated, that was visually translucent for each strain. This turned out to be the well containing the original 10^{-6} dilution. Post incubation in the EcoChip 50 µL of this 10^{-6} original sample were serially diluted by a factor of 10 down to 10^{-6} again. The second round of dilutions were then plated on sterile TSA (Trypticase Soy Agar) culture medium and incubated over night for a 16-hour period at 37°C.

The following morning, the number of colonies were counted on the TSA plate. Because each colony is derived from a single bacterial cell, the number of colonies can be multiplied by the dilution factors to obtain the total bacterial titer (colony forming units/mL) in each well. The count for *E. coli* was found to be a $4{,}5\text{x}10^6$ cfu/ml and $6{,}1\text{x}10^5$ cfu/ml for *B. subtilis* respectively. These cell counts are then scaled according to a dilution factor and a sampled volume, and used to obtain the CFU curves from the impedance curves shown in Fig. 9. We can show that the initial and final cell count values can be used to linearize the impedance values, and then obtain the growth curves in terms of CFU (Fig. 9), which are traditionally used by microbiologists [24].

B. Field Measurements

A pilot study with the EcoChip was performed at a latitude of 55 degrees in the village of Kuujjuarapik (Nord-du-Québec) in August 2017 at the *Centre for Northern Studies (CEN)* of Université Laval research complex. The protocol was simplified to facilitate utilization outside of the laboratory. A locally sourced 1 g of soil sample was diluted one million-fold in TSB (Tryptic soy broth) culture media and put into the wells of the EcoChip. This dilution factor provides about 6 bacteria per well assuming that 1 g of soil from this northern region should contain about $1\text{x}10^6$ cfu. The diluted solution was sealed between semipermeable membranes in the device and installed in direct contact with the soil to provide the necessary nutrients for bacterial growth during 10 days. Temperature and luminosity were simultaneously measured every five hours during this period (see Fig. 10). Communication problems caused the collected impedance measurements to be invalid and, considering the location of the device, it was impossible to fix on site. However, this field test still provided valuable insight by confirming that microorganisms could survive and grow within the EcoChip.

A second field study was conducted in August 2018, at the Clearwater Lake Station of the CEN, at a latitude of 56 degrees north in in Puvirnituq, Northern Quebec. The pitfalls of the 1st version utilized in the field in 2017 were corrected, and EIS measurements was successfully collected in the field over a time period of 55 hours with two new prototypes. For this new test, soil samples were acquired and prepared according to a similar procedure as for the previous test. Wireless communications were functional this time, and EIS data could be recovered wirelessly from the EcoChip. Once the EcoChip prototypes returned at Université Laval from the field, we could successfully recover the data from the Flash memory. The data retrieved from the memory was consistent with the data transmitted wirelessly a few meters away to the base station during the field test. The EIS data showed that bacterial growth occurred in 11 wells. Fig. 11 presents the EIS data obtained from two of those wells. As can be seen, both curves in Fig. 11 are quite similar considering that they come from the same bacterial strain and same base soil sample, while the growth happened at the same time in the same conditions. The measured EIS values are comparable, and are in the same range of those obtained in the laboratory, which are presented in Fig. 8. However, it can be seen that the growth curve doesn't exactly have the same shape as the ones obtained in ideal conditions in an incubator. Variations in the curves can be due to the changing surrounding environmental conditions. In particular, a temperature in the range of 8°C was measured from hour 15 to hour 30, while a temperature of 12°C to 17°C was measured

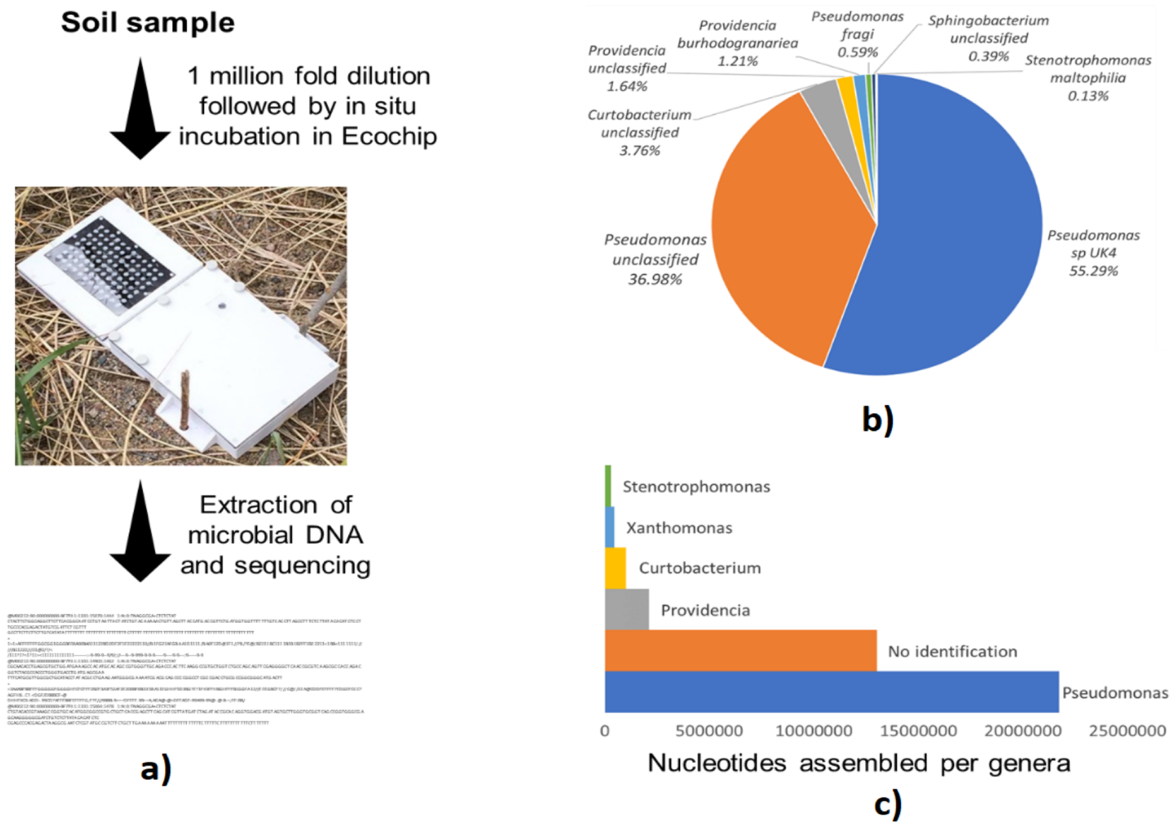

Fig. 12. DNA sequencing results after a pilot study. a) Summary of experimental procedure, including picture of the installed EcoChip. b) Proportion of bacterial species found at more than 0.1% of one sample well. c) Size of the assembly for bacterial genera.

for the remaining of the test. This could also be due to variations in the microorganisms' population between the measurements every five hours. This study allowed to validate the EcoChip in the field, and provided good indications that a sampling period of five hours should be more than sufficient in such cold conditions. Hence, other sampling periods will be tested in future field studies.

C. *DNA Sequencing Results*

In a laboratory setting, microorganisms were collected from the one of the well of the EcoChip that was incubated in Kuujjuarapik. A sterile swab was dipped in 500 µl of sterile water and then used to collect bacteria from the well. The swab was then agitated in the same water to release microbes. Bacterial DNA was extracted from the resulting sample using Purelink Viral RNA/DNA Mini kit (Invitrogen). Sequencing libraries were prepared using the *Nextera XT* protocol (*Illumina*). Shotgun metagenome sequencing was performed on the *Illumina MiSeq* system for 250 nucleotide paired-end sequencing. Metagenomes were assembled using Ray Meta and their microbial content was profiled using MetaPhlAn2. The DNA sequencing results are summarized in Fig. 12. The taxonomical origin of the assembled contigs was determined using Ray Meta as described in [25]. Total assembly size was 42.6 million nucleotides. Overall, 21 million assembled nucleotides were potentially originating from Pseudomonas bacteria, indicating that at least three strains or species of Pseudomonas were present in the sample, since their genome size is usually between 5.5 and 7 million nucleotides. At the time of writing this manuscript, the DNA sequencing results from the second studies were not yet available.

V. CONCLUSION

The EcoChip, a unique system allowing for the monitoring of microorganisms growth used for *in-situ* culture is proposed as an inexpensive method to gain a better understanding of the northern regions microbiome and the impact of climate changes on it. An impedance spectroscopy system is used inside the EcoChip to monitor the bacterial growth inside its 96 wells and other sensors are used to log environmental parameters. Data obtained from these systems are saved on the on-board Flash memory and can be sent wireless to a nearby base station. Laboratory and field tests have shown that the wells of the EcoChip are suitable for the culture of microorganisms and that they can actually grow inside them. The next steps in this project include using different growth media and exposure to different contaminants in the wells to observe their impacts on the growth rates of various microorganisms. The EcoChip will also be used to identify viruses and bacteria isolated from the culture wells. This identification will consist in the characterization, extraction, assembly and annotation of viral and bacterial sequence data, as well as experiments with keystone host-virus systems of particular interest for several areas, such as medicine and pharmacology.

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REFERENCES

[1] American Society of Microbiology. Microbes and Climate Changes. [Online]. Available: https://www.asm.org/images/Colloquia-report/FAQ-Microbes-and-Climate-change-web.pdf.

[2] Environment and Climate Changes Canada. The Science of Climate Change. [Online]. Available: http://ec.gc.ca/sc-cs/Default.asp?lang=En&n=A5F83C26-1).

[3] Y. Wang *et al.*, "Low-temperature degradation mechanism analysis of petroleum hydrocarbon-degrading antarctic psychrophilic strains," *Journal of Pure and Applied Microbiology*, vol. 8, pp. 47-53, 2014.

[4] K. Junge, H. Eicken, J. W. Deming, "Bacterial activity at -2 to -20°C in Arctic wintertime sea ice. *Applied and Environmental Microbiology,* vol. 70, pp. 550-557, 2004.

[5] J. Martinez-Serra *et al.*, "xCELLigence System for Real-Time Label-Free Monitoring of Growth and Viability of Cell Lines from Hematological Malignancies," *OncoTargets and therapy*, vol. 7, pp. 985–994, 2014.

[6] M. Grossi, M. Lanzoni, A. Pompei, R. Lazzarini, D. Matteuzzi and B. Riccò, "A portable biosensor system for bacterial concentration measurements in cow's raw milk," *4th IEEE International Workshop on Advances in Sensors and Interfaces* (IWASI), pp. 132-137, 2011.

[7] Liju Yang, Rashid Bashir, "Electrical/Electrochemical Impedance for the Rapid Detection of Foodborne Pathogenic Bacteria," *Biotechnology advances*, vol. 26, pp. 135-150, 2007.

[8] K.F. Lei, "Review on Impedance Detection of Cellular Responses in Micro/Nano Environment," *Micromachines,* vol. 5, no. 1, pp. 1-12, 2014.

[9] M. Kocanda *et al.*, "Structural differentiation of common bacteria using impedance spectroscopy," *IEEE International Conference on Electro/Information Technology*, pp. 518-523, 2014.

[10] D. Kho *et al.*, "Application of xCELLigence RTCA Biosensor Technology for Revealing the Profile and Window of Drug Resoinsiveness in Real Time," *Biosensors,* vol.5, pp. 199-222, 2015.

[11] C. M. Lopez *et al.*, "A 16384-electrode 1024-channel multimodal CMOS MEA for high-throughput intracellular action potential measurements and impedance spectroscopy in drug-screening applications," *2018 IEEE International Solid - State Circuits Conference - (ISSCC)*, pp. 464–466.

[12] J. S. Park *et al.*, "A high-density CMOS multi-modality joint sensor/stimulator array with 1024 pixels for holistic real-time cellular characterization," *2016 IEEE Symposium on VLSI Circuits (VLSI-Circuits)*, Honolulu, HI, pp. 1-2.

[13] D. Nichols, N. Cahoon, E.M. Trakhtenberg, L. Pham, A. Mehta, A. Belanger, T. Kanigan, K. Lewis, S.S. Epstein, "Use of ichip for high-throughput in situ cultivation of "uncultivable" microbial species," *Appl Environ Micro-biol,* vol. 76, pp. 2445–2450, 2010.

[14] R. T. Sherpa, C. J, Reese, H. M. Aliabadi, "Application of iChip to Grow "Uncultivable" Microorganisms and its Impact on Antibiotic Discovery," *Journal of Pharmacy & Pharmaceutical Sciences*, vol. 18, pp. 303–315, 2015.

[15] Losee L. Ling *et al.*, "A new antibiotic kills pathogens without detectable resistance," *Nature,* vol. 517, pp. 455–459, 2015.

[16] M. Sylvain, F. Lehoux, Morency, F. Faucher, E. Bharucha, F. Raymond, D.M. Tremblay, S. Moineau, M. Allard, J. Corbeil, Y. Messaddeq, and B. Gosselin, "The EcoChip: A Wireless Multi-Sensor Platform for Comprehensive Environmental Monitoring," *IEEE International Symposium on Circuits and Systems* (ISCAS), 2018, pp. 1-5.

[17] S. A. Ghaffari, W-O. Caron, M. Loubier, M. Rioux, J. Viens, B. Gosselin, Y. Messaddeq., "A Wireless Multi-Sens Dielectric Impedance Spectroscopy Platform," *Sensors*, vol. 15, no. 9, pp. 23572–23588, 2015.

[18] S. A. Ghaffari, W-O. Caron, M. Loubier, C-O. Normandeau, J. Viens, M. S. Lamhamedi, B. Gosselin, Y. Messaddeq, " Electrochemical Impedance Sensors for Monitoring Trace Amounts of NO3 in Selected Growing Media," *Sensors*, vol. 15, no. 7, pp. 17715–17727, 2015.

[19] NC. Mykytczuk et al,. "Bacetrial growth at -15°C; molecular insights from the permafrost bacterium *Planococcus halocryophilus* Or1," *The ISME Journal*, vol. 7, pp.1211-1226, 2013.

[20] Liju Yang, Yanbin Li, Carl L Griffis, Michael G Johnson, "Interdigitated microelectrode (IME) impedance sensor for the detection of viable Salmonella typhimurium," *Biosensors and Bioelectronics*, vol. 19, Issue 10, pp. 1139-1147, 2004.

[21] Tadiran Lithium Batteries MODEL TL-5930. Online. Available: http://www.tadiranbat.com/assets/tl-5930.pdf

[22] J. Paredes, S. Becerro, F. Arizti, A. Aguinaga, J.L. Del Pozo, S. Arana., "Interdigitated microelectrode biosensor for bacterial biofilm growth monitoring by impedance spectroscopy technique in 96-well microtiter plates," *Sensors and Actuators* B, vol. 178, pp. 663-670, 2013.

[23] N. Ramirez, A. Regueiro, O. Arias, R. Contreras, "Electrochemical impedance spectroscopy: An effective tool for a fast microbiological diagnosis," *Biotecn. Aplicada*, vol. 26. pp. 72-78, 2009.

[24] M. Grossi, A. Pompei, M. Lanzoni, R. Lazzarini, D. Matteuzzi, and B. Ricco, "Total Bacterial Count in Soft-Frozen Dairy Products by Impedance Biosensor System," *IEEE Sensors Journal*, vol. 9, no. 10, pp. 1270–1276, Oct. 2009.

[25] F. Raymond *et al.*, "The initial state of the human gut microbiome determines its reshaping by antibiotics," *The ISME Journal*, vol. 10, pp. 707-720, 2016