Printed, Microwave-based, Transmission-line Sensor for Investigating the Electromagnetic Behavior of Pure Bacteria Culture and Algae in Water

Study the fresh water pathogens (algae and bacterial) electromagnetic behavior using transmission-line printed sensor

Mohammad Russel*, Li Xiaomeng, Qu Meixue
School of Food and Environment
Dalian University of Technology
Panjin 124221,P.R.China
e-mail: mrussel@dlut.edu.cn; 1875212201@qq.com; 2574348720@qq.com

Thomas Mascow
Department of Environmental Microbiology
UFZ, Helmholtz Centre for Environmental Research,
Permoserstr. 15,04318 Leipzig, Germany
e-mail: thomas.maskow@ufz.de

Abstract— **Freshwater ecosystem is playing a key role for maintaining a green environment. It is often subject to anthropogenic and natural hazards, which may adversely affect human health, natural resources and the general ecosystem. Therefore, it is a social urgency to protect the freshwater ecosystem by monitoring the quality of fresh water on regular time intervals. Available methods for monitoring the water quality are mostly laboratory-based, which is time-consuming, laborious and expensive. To solve this issue, we are proposing a printed, microwave-based, transmission-line sensor to better understand the electromagnetic behavior of pure culture of bacteria and algae cells in de-ionized water. This sensing technique is fast, robust, low-cost and requires a very simple sample preparation. We have designed a transmission-line, microstrip sensor that could be used for a wide frequency range. The sensor needs only 50µL of the sample and 60 seconds to analyze it. In this work, we have selected the fresh water algae *Chlorella vulgaris* GIEC-179 and the bacterium *Pseudomonas aeruginosa* to characterize their electromagnetic properties. We investigated their reflection coefficient (S11) resonance peak changes in both low (0.01GHz-1.0 GHz) and high (1.5-2.5 GHz) frequency ranges. The results shows that their S11 resonance peaks are identical with respect to the different concentrations of bacterial, algal and mixture of both in de-ionized water. We also have investigated their S11 parameters of their dead cells. The results indicated that for both alive and dead cells, the S11 peak shifts are significantly different from each other. This method could be a potential approach to real-time monitoring of the pathogenic detection of freshwater quality. Our proposed prototype sensor is able to detect bacterial cells in the range of 100 Cell/mL and algae 2.04 x 10^{-10} g/L, which is sensitive and selective enough for fresh water quality monitoring.**

Keywords- microstrip; transmission line; printed sensing probe; pathogens; microwaves.

I. INTRODUCTION

According to Dr. Tom Waller's quote "You only find what you are looking for and you only find it if it is in concentrations high enough to be detected by the method being used to analyze for it." [1]. To evaluate the quality of various surface water various water management programs, the term "water quality" should be different in terms of protection and restoration of lake, river and marine ecosystem. Water quality is rated with respect to chemical, physical and biological parameters in point-source pollutants (e.g., effluents), non-point-source pollutants (e.g., agricultural runoff, urban), and ambient surface waters [2]. The most common and widespread health-risks associated with drinking-water and infectious diseases are caused by water contamination with pathogenic bacteria, parasites and algae bloom. Therefore, on-line monitoring could assist to understand and manage the risks, especially those associated with waterborne diseases [3].

Surface water is contaminated by pathogens due to multifunctional anthropogenic activity like inadequately treated sewage, faulty or leaky septic systems, runoff from urban areas, boat and marina waste, combined sewer overflows, and waste from pets, farm animals, and wildlife. Therefore, human illnesses are transmitted by drinking or swimming in water that contains pathogens or from eating shellfish harvested from such waters. Sometimes, it is costly and impractical to directly test for pathogens because pathogens are rarely found in waterbodies. Indicator species are usually used to confirm pathogen presence in water that fecal contamination may have occurred. The four most common indicators used today for professional monitoring are total coliforms, fecal coliforms, *E. coli* and *enterococci*. Those are commonly found in the intestine and feces of warm-blooded animals, including wildlife, farm animals, pets, and humans [4].

Currently, available methods for water quality monitoring are plate counting and typical cell culture standards to confirm the presence of pathogens, while they are often costly and take approximately 24-48 hours [5] [6]. Based on this timeline, by the time the analysis results come in, the population may have been already exposed to a serious health hazard. Therefore, there is a need for fast and

 ISBN: 978-1-61208-581-4

reliable detection of contaminants in a broad spectrum of water management situations. To face the current water management challenges, on-line monitoring seems to be the ideal approach for real time detection [7].

Electromagnetic wave sensing methods are gaining more popularity due to the study the materials dielectric properties (complex permittivity) and structural assessment. Materials' dielectric properties always correlate and, by comparing with other material characteristics, we can verify the materials properties, such as moisture content, bulk density, content of biological material, and chemical composition [8]. Electromagnetic wave sensing [9]-[11] has already been proven to be a pragmatic tool for the evaluation of the biomass concentrations of many different microbial strains [12] [13]. It is a straightforward method to measure the magnitude of all intact cells with their β-dielectric dispersion at radio-frequency range. In that case, the cells behave like a tiny capacitor and the signal correlates linearly with the volume fraction of biomass. At the very high levels of biomass concentrations they may lost the linearity. Therefore, the accumulation of lipid droplets, bioplastics, etc. was found to be one of the few exceptions to the rule [14] [15].

The fundamental electrical property through which the interactions are described is the complex relative permittivity of the material (ε_r'). It is mathematically expressed as:

$$\varepsilon_r = \varepsilon_r' + j\varepsilon_r'' \quad (1)$$

whereas the real part of relative permittivity (ε_r') describes how much energy can be stored by the material from the electromagnetic field and the imaginary part of the relative permittivity (ε_r'') shows how lossy the material is under the electromagnetic filed, both being functions of frequency ($\omega = 2\pi f$).

The volume fraction of biomass, the cell size and the membrane capacitance per unit area could be the possible reason to dielectric increment of a cell suspension from high to low frequencies. Also, the conductivity of the suspension has an effect on the permittivity measured at a particular frequency [16], but this effect can be minimized by choosing the right frequencies.

Correct detection and identification of waterborne pathogens based on conventional culturing techniques is very laborious, time-consuming, and must be completed in a microbiological laboratory. These factors make it unsuitable for water quality control if a timely response to possible risks is required.

In this work, a quantitative way is demonstrated, to measure the electromagnetic properties of algae and bacterial cultures by a microwave based, transmission-line, printed sensor, at microwave frequencies. For the exemplification of the method, *Chlorella vulgaris* GIEC-179 (fresh water algae) and *Pseudomonas aeruginosa* (gram negative bacteria) were chosen for our experiments.

II. BASIC THEORY OF THE S-PARAMETER

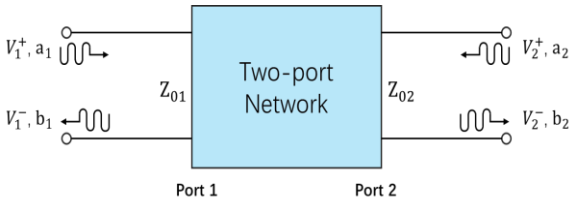

Figure 1. A two-port network with different characteristic impedance.

Measurement systems do not always work under the impedance match condition. Therefore, the general scattering parameter was introduced to describe an impedance mismatch system. A two-port network system is shown in Figure 1. The characteristic impedance at port 1 was Z_{01}, while the characteristic impedance at port 2 was Z_{02} and we assume that $Z_{01} \neq Z_{02}$. The incident power-wave amplitude at port 1 is a_1 and the reflected power-wave amplitude is b_1, while a_2 and b_2 are the incident and reflected power-wave amplitude at port 2, respectively. The incident power-wave amplitude a_1 and the reflected power-wave amplitude b_1 are defined as:

$$a_1 = V_1^+ / \sqrt{Z_{01}} \quad (2)$$

$$b_1 = V_1^- / \sqrt{Z_{01}} \quad (3)$$

The scattering parameter matrix connect the incident wave and reflected wave and are defined as:

$$[b] = [S][a] \quad (4)$$

where $[b] = \begin{bmatrix} b_1 \\ b_2 \end{bmatrix}$, $[a] = \begin{bmatrix} a_1 \\ a_2 \end{bmatrix}$ and $[S] = \begin{bmatrix} S_{11} & S_{12} \\ S_{21} & S_{22} \end{bmatrix}$ while the element in the scattering matrix can be defined as:

$$S_{ij} = \frac{b_i}{a_j} = \frac{V_i^- / \sqrt{Z_{oi}}}{V_j^+ / \sqrt{Z_{oj}}} \quad (5)$$

The generalized scattering parameter describes how the two-port network with the same impedance can be transformed to connect different impedance transmission-line networks [17].

 ISBN: 978-1-61208-581-4

III. FABRICATION OF TRANSMISSION LINE PRINTED PROBE

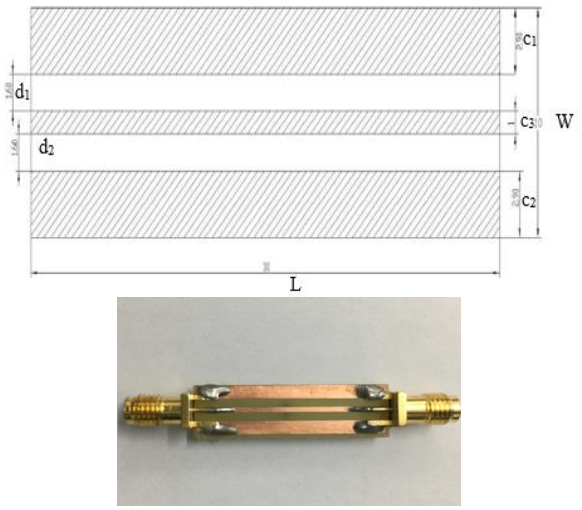

Figure 2. Design of the printed transmission-airline sensor (Up) (L=30mm, W=10mm, d1 & d2 =1.6mm and the copper conductors C1 & C2 = 2.9mm and C3 = 1mm printed on FR4 board). 50Ω SMA connector on both sides to connect to the VNA (Down).

The dual-port printed transmission-airline sensor was designed to be able to get the sample's full S-parameters. The design details are shown in Figure 2. This strip-line probe contains three main sections: one samples holder and two adapters (35 mm SMA connector) to connect the probe to vector network analyzer (VNA). We have used polar Si9000 PCB Transmission line field-solver to design the prototype probe. The length of the sample holder L=30mm, W=10mm is the width of the probe, d1 & d2 =1.6mm of sample holder and conductor C1& C2=2.9mm and C3=1mm of 35 µm thick layer of Cu printed on FR4 board platform. The size of the strip-line was designed to confirm only the transverse electromagnetic (TEM) mode electromagnetic wave translating through the sample under test. To meet the measurement system impedance, the sample holder's size was connected to a Teflon filled 50Ω transmission line section. A no-loss transmission line section can be described by two key parameters: inductance and capacitance [17].

IV. EXPERIMENTAL PROCEDURE

A. Measurement Setup

The measurement instrument VNA HP 8753D (Figure 3) was calibrated with the short, open, 50Ω load and through (SOLT Maury Microwave, model 8050CK11) calibration technique to move the measurement plane from the instrument's ports to the end of the test cables. The printed strip line probe was assembled with two SMA 35mm 50Ω connectors and the probe was connected with the test cables to get the air scattering parameters (Reflection S11 and transmission S12). The samples were carefully layered and compacted in the printed probe sample chamber. A full frequency range from 0.01 GHz to 3GHz was used to get the measurement data and the full two-port S-parameter, both in magnitude and phase form. 201 data points were acquired. A 32-point averaging factor was used to minimize the systematic measurement errors coming from noise.

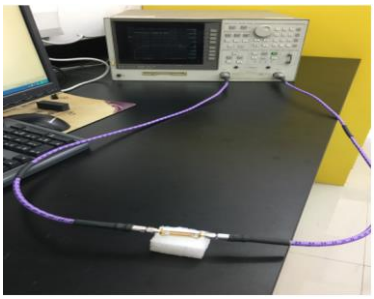

Figure 3. Measurement setup VNA HP 8753D connected to a microwave sensor via coaxial cable.

B. Sample preparation

1) Bacterial Cell sample:

Pseudomonas aeruginosa were inoculated in Luria–Bertani (LB) medium, culture in waterbath shock incubator at 30℃（150 rpm）for 36h. After that, they were centrifuged under 8000 rpm for 5 min and the cell was diluted with de-ionized (DI) water until the absorbance reached approximately 0.5 (CFU is about 10^8/mL). The initial concentration selected was $1x10^8$ CFU/mL for preparing different concentrations of bacterial cell using dilution factor 1:10 with DI water.

2) Algal Cell sample:

The culture medium used for this Chlorella vulgaris GIEC-179 was BG11. The biomass concentration (dry weight per liter) of cultures were measured according to the method reported previously [18] [19]. Microalgae cells were collected, centrifuged and washed with de-ionized water. The washed microalgae pellet was dried at 105 ℃ for 10h and the dry weight was measured. The initial concentration selected was at 2.04 g/L for preparing different concentrations of algal cell using dilution factor 1:10 with DI water.

3) Dead Bacterial and Algal Cell preparation:

Live cells were heated in boiling water (100°C) for 5 minutes, then centrifuged at 8000 rpm for 5 min and washed with DI water.

V. RESULTS AND DISCUSSION

A. Different concentrations of Algae and Bacterial reflection spectra (S11)

Figure 4 illustrates the different concentrations of bacteria ($1x10^8$-$1x10^2$ CFU/mL) *Pseudomonas aeruginosa* and algal (2.04 - $2.04x\ 10^{-10}$ g/L) *Chlorella vulgaris* GIEC-179 cell reflection-spectral distribution in full frequency range of 0.01-3.0GHz, when they set into DI water using the microwave sensor. There were two types of reflection resonance observed. One in lower frequency range 0.01 GHz- 1.0 GHz and the other one in higher frequency range 1.5-3.0 GHz.

 ISBN: 978-1-61208-581-4

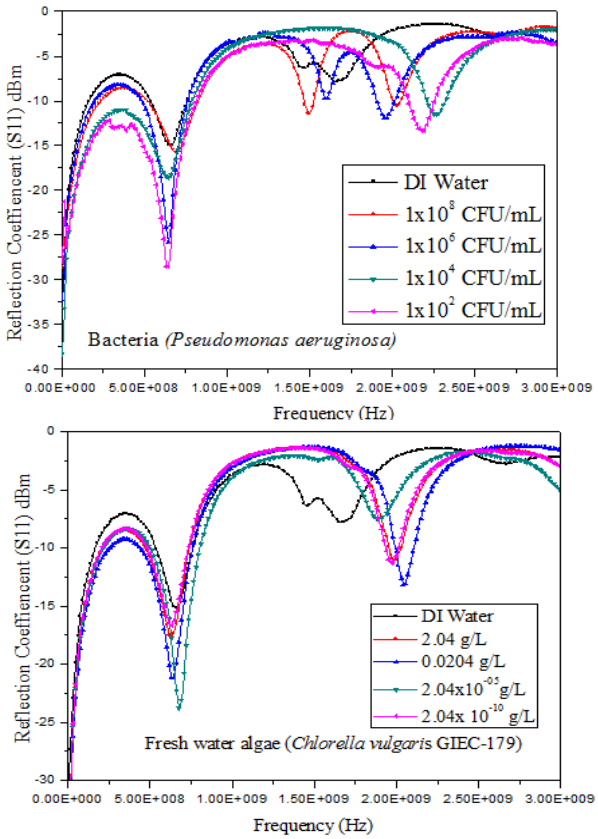

Figure 4. S11 spectra with different concentration of top Bacteria (*Pseudomonas aerugiosa*) and bottom fresh water algae (*Chlorella vulgaris* GIEC-179).

The shift of resonance peaks is quite distinguishable with respect to their different concentrations. If we look at around 0.6 GHz, there is a sharp magnitude drop of almost 30dBm for bacteria and 22dBm for algal cell. On the other hand, in higher frequency range their resonance peak shift is identical with respect to their concentration and the decrease in magnitude around 12dBm for bacteria and 15dBm for algae. It is clear that algae cells reflection resonance peak changes are more unique compared to bacterial cell both in high and low frequency range. Therefore, the microwave sensor is showing significant advantage to determine the bacteria or algae contamination in surface water. It could be a potential sensing method with greater sensitivity and selectivity for real-time monitoring of the water quality.

B. Mixture of both Algae and Bacterial reflection spectra (S11)

Figure 5 shows the sensor's response in a mixture of algae and bacterial cells. Here, the sensor's reflection spectra are behaving in a similar way with the single cell reflection spectra. Again, the resonance of reflection spectra appeared as higher peak changes at lower frequency range (around 45 dBm in 0.01-1.0 GHz) and as lower peak changes at higher frequency range (15dBm). The results show that the electromagnetic signals are dominated by 100% bacterial cell, 25% bacteria+75% algae and 75% bacteria+25% algae. However, it is difficult to understand the 100% algae and mixture of both (50% algae+50%bacteria) electromagnetic behavior. These results are leading us to a better understanding of the microwave sensor sensitivity and selectivity.

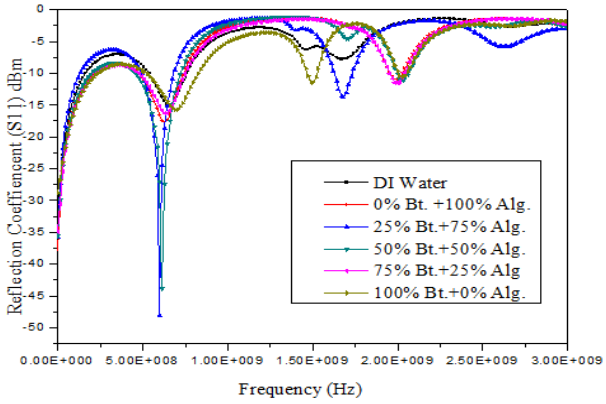

Figure 5. S11 spectra with different concentration of Mixture of both Bacteria (*Pseudomonas aerugiosa*) and fresh water algae (*Chlorella vulgaris* GIEC-179).

C. Dead and alive of Algae and Bacterial Cell reflection spectra (S11)

Another aspect of this work was to understand the differences in electromagnetic behavior of the dead and alive cells. The same experiment was ran with both dead and alive cells. Figure 6 shows the reflection spectra in the full frequency range. It is clearly depicted that there are major differences in the reflection spectra between dead and alive cells. There is a magnitude drop of 25 dBm at low frequency range and a 12 dBm at high frequency range. What is different between the dead and alive cells is the frequency where the magnitude drops at the higher frequency range. The peaks are shifted to lower frequencies for dead cells when compared to the living cells. These results show not only that the sensor can monitor concentration levels and distinguish between different pathogens, but also to assess whether the cells are alive or dead, something that is vital for water risk assessment for pathogen contamination.

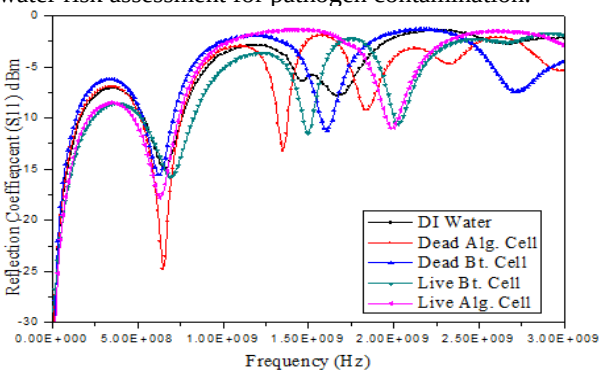

Figure 6. S11 spectral comparison with live and dead bacteria (Bt.)c& algae (Alg.) cells.

 ISBN: 978-1-61208-581-4

VI. CONCLUSIONS AND FUTURE WORK

This research was driven by the industrial need for a novel, real-time method of monitoring the presence of bacteria and algae in water. The printed, transmission-line, microwave-based sensor was developed and tested. The response of the sensor to bacteria and algae in various concentrations, mixture of both and dead & alive cells was investigated with respect to their reflected spectral (S11) analysis. The results clearly confirmed that the sensor is able to accurately determine the concentration of bacteria and algae in water, but to also distinguish between the two and whether the cells are dead or alive. Thus, our proposed method provides both superior sensitivity and selectivity compared to other existing methods. It is important to mention that, the sensor's response returned to its original position, namely air spectrum, after each water sample measurements, confirming that the developed printed, transmission-line sensor is reliable, re-usable and thus, a sustainable solution for water quality monitoring.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (41603069) and the fundamental Research Funds for the Central Universities grants from Dalian University of Technology (Panjin campus) (DUT14QY48, DUT15QY54).

REFERENCES

[1] W. T. Waller and H. J. Allen, "Acute and chronic toxicity", In: Jorgensen SE., Fath BD, eds, Encyclopedia of Ecology, Elsevier, Oxford, UK, pp 32–43, 2008.

[2] W. B. Bryan, et al., "Are Harmful Algal Blooms Becoming the Greatest Inland Water Quality Threat to Public Health and Aquatic Ecosystems?" Environmental Toxicology and Chemistry, vol. 35, No. 1, pp. 6-13, January 2016.

[3] Guidelines for Drinking-water Quality,Fourth Edition, world health organization, "Microbial Aspects", Chapter 7, pp. 117, 2011.

[4] Chapter 17 of the Volunteer Estuary Monitoring Manual, A Methods Manual, Second Edition, EPA-842-B-06-003. The full document can be downloaded from: http://www.epa.gov/owow/estuaries/monitor/ July, 2017.

[5] Federal Register, Drinking water: National primary drinking water regulations; total coliform proposed rule. Federal Register vol. 54, pp. 27544, 1991.

[6] A. E. Greenberg, R. R. T. Russel, L. S. Clesceri, and M. A. H. Franson, "Standard Methods for the Examination of Water and Wastewater", American Public Health Association, Washington, DC, 1992.

[7] L. R. Ramon, T. Pol, C. Sophie, and L. C. Jose, "On-line bacteriological detection in water", Trends in Analytical Chemistry, vol. 44, pp. 46-57, March 2013.

[8] O. Büyüköztürk, T. Y. Yu, and J. A. Ortega, "A methodology for determining complex permittivity of construction materials based on transmission-only coherent, wide-bandwidth free-space measurements", Cement and Concrete Composites, vol. 28, No. 4, pp. 349-359, April 2006.

[9] C. Davey and D. B. Kell in M. O'Connor, RHC Bentall and JS Monahan (Eds.), Emerging Electromagnetic Medicine, Springer, Berlin, 1990.

[10] K. Foster and H. Schwan, CRC Handbook of Biological Effects of Electromagnetic Fields, 1986.

[11] K. R. Foster and H. P. Schwan, Dielectric properties of tissues, Handbook of biological effects of electromagnetic fields, vol. 2, pp. 25-102, 1995.

[12] C. Boulton, P. Maryan, D. Loveridge, and D. Kell, "The application of a novel biomass sensor to the control of yeast pitching rate", Proc 22nd Eur Brew Conv Zurich European Brewing Convention, Oxford University Press, pp. 653-661, 1989.

[13] C. L. Davey, D. B. Kell, R. B. Kemp, and R. J. Meredith, "On the audio-and radio-frequency dielectric behaviour of anchorage-independent, mouse L929-derived LS fibroblasts, Journal of Electroanalytical Chemistry and Interfacial Electrochemistry", vol. 254, pp. 83-98, 1988.

[14] G. Kedia, et al., "Addressing the challenge of optimum polyhydroxyalkanoate harvesting: Monitoring real time process kinetics and biopolymer accumulation using dielectric spectroscopy", Bioresource technology, vol. 134, pp. 143-150, 2013.

[15] T. Maskow, A. Röllich, I. Fetzer, J. U. Ackermann, and H. Harms, "On-line monitoring of lipid storage in yeasts using impedance spectroscopy", Journal of Biotechnology, vol. 135, pp. 64-70, 2008.

[16] C. M. Harris, et al., "Dielectric permittivity of microbial suspensions at radio frequencies: a novel method for the real-time estimation of microbial biomass", Enzyme and microbial technology, vol. 9, pp. 181-186, 1987.

[17] D. M. Pozar, "Microwave Engineering 3e." Transmission Lines and Waveguides, pp. 143-149, 2005.

[18] Y. J. Wang, J. He, Z. Li, and H. Y. Luo, "Determination of Chlorella pyrenoidosa biomass using optical density method", Journal of Northwest University (Natural Science Edition), vol. 42, No. 1, pp. 60-63, February 2012.

[19] S.F. Mohsenpour, B. Richards, and N. Willoughby, "Spectral conversion of light for enhanced microalgae growth rates and photosynthetic pigment production", Bioresource technology, vol. 125, pp. 75-81, 2012.

 ISBN: 978-1-61208-581-4