



University  
of Victoria

Graduate Studies

Notice of the Final Oral Examination  
for the Degree of Master of Applied Science

of

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**“Biofilm Sensing Tool Development for Environmental Monitoring”**

Department of Mechanical Engineering

Friday, January 24, 2020  
10:00 A.M.  
Engineering Office Wing  
Room 502

Supervisory Committee:

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## **Abstract**

In recent years, the treatment of stormwater and surface runoffs using innovatively created natural sites has gained increasing attention. These sites, that are vegetated multi-layered depressions on the ground, named as bioretention cells or rain gardens, are constructed with natural elements like soil, shrubs, grass, and trees. Such facilities are employed to treat stormwater quantitatively and qualitatively via various physical, chemical and biological pathways. The biological treatment of stormwater is carried out mostly by the dense bacterial communities that are existent around the plants of roots stationed in the rain gardens.

These dense bacterial communities, known as biofilms, play a significant role in the biological removal of contaminants through a process called bioremediation. The efficacy of bioremediation processes in bioretention cells is highly dependent on the successful formation and continued presence of root plant-associated bacterial biofilms, also known as rhizospheric biofilms. The availability of rhizospheric biofilms in bioretention cells, therefore, is an important determinant of the contaminant removal efficacy in bioretention cells. The bioremediation process efficacy can be improved by providing the biofilms with their ideal growth and environmental conditions. Being able to discover such conditions is the principal motivation behind the present thesis, the ultimate objective of it being to develop a sensor that monitors the growth of bacterial biofilm. There is, to date, no tool or sensor on the market that could estimate the amount of biomass available in bioretention cells. Gaining knowledge of the biomass availability can lead to further understanding of the ideal environmental and nutritional conditions for rhizospheric biofilms and their presence in subsurface environments.

Developing such a sensor required taking multiple steps, including the evaluation of the past and present methods of monitoring or studying biofilms, assessing the advantages and disadvantages of each method, and finding the best possible method with respect to the application of interest. When it comes to monitoring biofilms in the field or in situ, specific requirements are considered that narrows the choices down to a few methods. The vast majority of classic and innovative techniques of monitoring biofilms lack the required capabilities, such as non-invasiveness, cost-effectiveness and real-time

measurements, to name a few. Impedance microbiology, as a technique that provenly has the capacity of in situ monitoring of microbial metabolism, was chosen as the main premise behind the sensor prototype. The expected capabilities that were considered for the development of the sensor required the employment of a method that can specifically monitor biofilm in a real-time, non-invasive, non-destructive, rapid, simple-to-use, precise, affordable, repeatable, and automatic fashion. These considerations were taken into account within the instrumentation and the proof of concept phases of the monitoring system design and development.

After crafting the prototype, various phases were conducted including the programming, troubleshooting, as well as the testing and verification of the system using a biofilm-forming strain, *Pseudomonas putida*. Promising results were obtained as for the detection of the growth of bacterial samples via real and imaginary impedance monitoring. In addition, optical density measurements were taken from the samples in tandem with the impedance sensing experimentation. Optical density spectroscopy measurements, that were calibrated in terms of the number of cells per volume using hemocytometry, allowed for the estimation of the change in the number of cells per unit of volume respective to the alterations in the impedance properties. Therefore, the real-time biomass estimation of the bacterial samples became possible. The bacterial population estimation range was approximately 9.2 million up to 500 million cells per ml for *Pseudomonas putida*, but further testing and trials could improve and expand the estimation range. Overall, the test results demonstrate the capability of the monitoring system in detecting bacterial proliferation real-time of their growth with high sensitivity. The novelty demonstrated in this work includes but not limited to the affordable manufacturing of the monitoring system together with the calibration using high precision direct counting of biomass. With minor modifications, this sensor can further be improved in terms of different operational capabilities to become commercially available for the monitoring of biofilm in the field, not just in bioretention cells, but also in many other applications where bacterial proliferation is important to monitor, such as biofouling of equipment, food industry, medical and healthcare centers, etc.