Spectral Monitoring of the Growth Dynamics of *E. coli* Bacterial Populations in Water Environment

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Abstract: Optical fiber spectroscopy was employed to analyze the development of *Escherichia coli* populations in water environment. By the simultaneous use of optical absorption and Raman spectroscopy, differences among the characteristic growth phases were identified for the strain. The generation time was also determined by turbidity.
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1. Introduction

Human health and food quality are two connected concerns in the modern society. These two fields are intimately associated with a third element: the water. The presence of pathogenic bacteria increases the risk of waterborne contamination in agriculture, leading to human diseases. At the same time, the human consume of non-treated water further increases the risk of infections. In this scenario, development of fast methods for detection and identification of contamination is the key for minimizing harms on human health. Traditional methods for pathogen detection rely on techniques that sometimes suffer from being time-consuming and from requiring laboratorial processes [1]. The need for fast and accurate methods for the detection of contaminated water has driven research in several fields of science.

This work compares two spectroscopic methods for monitoring the growth dynamics of the Gram-negative bacteria *Escherichia coli* (*E. coli*) population in water environment: turbidity and Raman spectroscopy. In one hand, turbidity allows evaluating the different growth phases of a bacterial culture by means of the optical density (O.D.), measured with an UV-VIS spectrometer. On the other hand, the Raman scattering (measured with a dispersive spectrometer) from contaminated water samples provides not only information about the growth phases, but also opens the possibility of the bacterial strain identification by its characteristic fingerprint.

Analysis of turbidity allowed to measure the generation time (the doubling time of a population), close to 0.7 hours for the studied strain. Raman spectra provided information about the time evolution of characteristics bands associated with fingerprints of specific biological components [2], [3]. Results from both techniques allowed identifying the phases lag, exponential and stationary for the studied strains by using Principal Component Analysis (PCA).

2. Methods

Bacterial sample was streaked on EC broth (K25-610063, Kasvi) with a plastic loop and allowed to grow at 37 °C in a thermostatic bath (Lauda Ecoline Staredition E200). The population dynamics for the strain of *E. coli* designed by H3C2/2012 was measured along 24 hours. Fractional samples were removed from the liquid culture medium at regular time intervals and transferred to disposable cuvettes for the spectral measurements. No further treatment (like filtering, centrifuging or concentrating) was applied to the aliquots.

Two optical fiber spectrometers were employed for both optical absorption - turbidity and Raman scattering measurements: UV-VIS (HR4000, Ocean Optics) and Raman (iHR550 Horiba Jobin Yvon), connected to a 4-ports cuvette holder (Ocean Optics, CUV-ALL-UV). Light sources comprised a tungsten halogen lamp (LS-1, Ocean Optics) and a 785 nm diode laser (100 mW).

From the time behavior of the optical density obtained from the UV-VIS spectra, the time generation G can be estimated from the equation $OD600 = OD_0\ 2^{t/G}$, where OD600 is the optical density measured at 600 nm at time t and OD_0 is the initial optical density [4].

Principal Component Analysis (PCA) was applied to the spectral data obtained with Raman spectroscopy to observe possible correlation between the variables.

3. Results and Discussions

Figure 1 shows the OD600 as function of the time. The characteristics phases of bacteria development are identified in the figure: Lag phase, when the bacteria are adapting to the environment; Log phase, when the bacterial cells are doubling at a constant exponential rate; and Stationary phase, when conditions for growth become unfavorable and the cell division stops. In figure 1 is also indicated the generation time G of about 0.7 hours for the studied strain.